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Echinacea Genus: An Endless Natural Therapeutic Resource? An Overview

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Abstract

Echinacea spp., Asteraceae family represented one of the important genera of cultivated biologically active plants, well-known in traditional medicine. Its multiple effects were investigated along time, within the research for alternative therapies to synthetic drugs. Due to those effects the *Echinacea* genus represented a valuable resource for medicine in general and for veterinary medicine especially. In the latter, the fight to increase resistance against diseases counter-balances the antimicrobial therapies; further, the potential adjuvant role of *Echinacea* products provide perspectives of an enhanced innate rather than adaptive immunity along with antibacterial effects.

Still, the involvement of plant extracts in therapy and prevention of diseases in general, even by enhancing immunity or increasing the post-vaccination responses needs careful species and age-based tailoring to avoid unwanted or noxious side effects. Could this genus represent more than an alternative? Are its effects more valuable in medicine than thought before? This mini-review is exploring those possibilities.

Key Words: *Echinacea* spp., general biological effects, innate immunity, specific immunity

1. Botanical Description

Compared to other species with medicinal uses, the history of *Echinacea* is relatively short. *Echinacea* includes several plants of the Asteraceae family: Echinacea purpurea (L.) Moench, *Echinacea angustifolia* DC. and *Echinacea pallida* (Nutt.) Nutt. In Methodus Plantas Horti Botanici et Agri Marburgensis, Conrad Moench (1794) accepted that *Echinacea* (*E. purpurea* (L.) Moench) was equivalent to *Rudbeckia purpurea* L. from the family Asteraceae.

The currently accepted taxonomy for *Echinacea* species is based on morphological and anatomical studies by McGregor (McGregor , 1968, American Herbal Pharmacopoeia and Therapeutic Compendium, 2007). According to these data, the genus comprises nine to ten (WFO, 2023) species and two varieties. The ten accepted species are: *Echinacea angustifolia* DC. (Narrow-leaf coneflower), *Echinacea atrorubens* (Nutt.) Nutt. (Topeka purple coneflower), *Echinacea laevigata* (C.L. Boynton & Beadle) S.F.Blake (Smooth

coneflower, smooth purple coneflower), *Echinacea pallida* (Nutt.) Nutt. (Pale purple coneflower), *Echinacea paradoxa* Britton (Yellow coneflower, Bush's purple coneflower), *Echinacea purpurea* (L.) Moench (Purple coneflower, eastern purple coneflower), *Echinacea sanguinea* Nutt. (Sanguine purple coneflower), *Echinacea simulata* McGregor (Wavyleaf purple coneflower) and *Echinacea tennesseensis* (Beadle) Small (Tennessee coneflower)(The International Plant Names Index and World Checklist of Vascular Plants 2023). According to Bauer and Wagner (1990), Rudbeckia serotina Sweet (Table 1) represented just a synonym for *Echinacea purpureea* (L.) Moench.

The *Echinacea* spp. distribution is defined by the natural or introduced status of the plant, broader geographical areas being lately covered by the introduced *Echinacea* (Fig.1).

Corresponding to the Royal Botanic Kew Gardens "Plants of the World Online", native Echinacea spp. are found in the North American continent, while introduced species are spread from Western Europe to South Eastern Asia (Fig.1, https://powo.science. kew.org/).

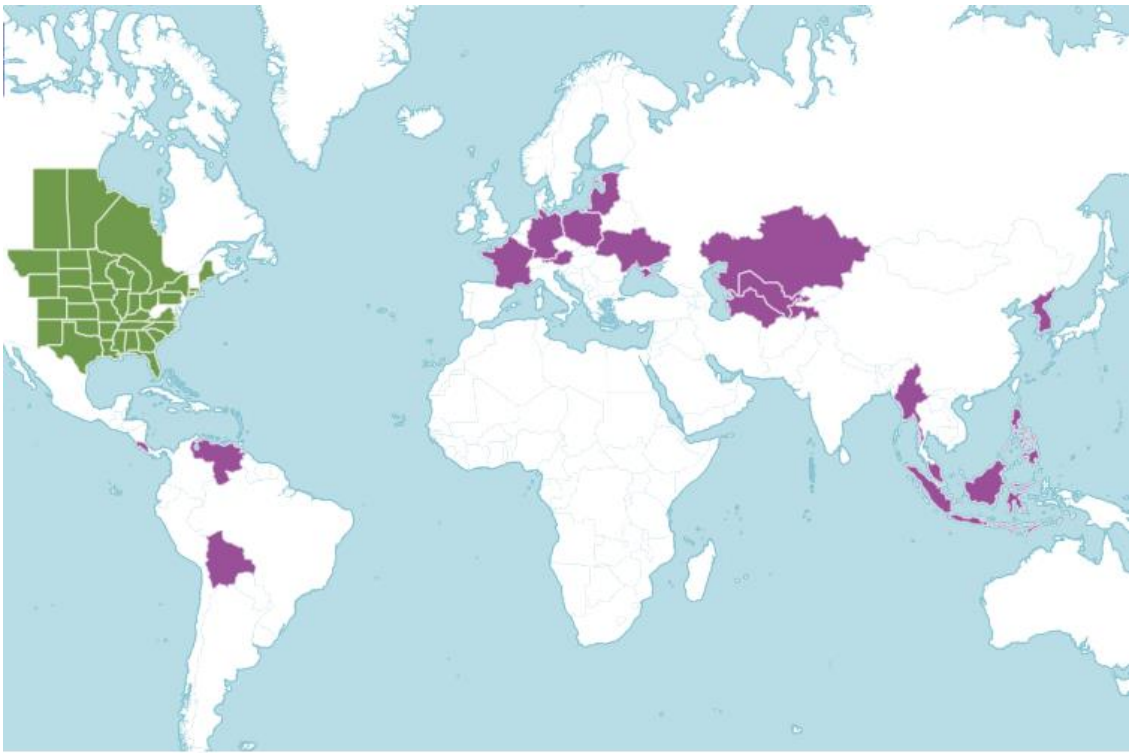


Figure 1. The distribution map of *Echinacea* spp. native or introduced species (http://www.ipni.org , https://powo.science.kew.org/). The green color indicated the native species distribution, while introduced plants are represented by purple color.

Echinacea spp. are perennial herbaceous plants, growing from either tap- or fibrous (E. purpurea) roots in relatively dry climate. The stems are erect up to 1.4 m, with no branches in most species and the rough, hairy leaves are arranged alternately. The leaves decrease in size towards the top of the plant, showing a linear, lanceolate, ovate or elliptic shape, with entire or dentate/serrate margins, showing species specific differences (Belaeva and Butenkova, 2018, WFO 2023). As in all Compositae, the flowers of *Echinacea* spp. are inflorescences, with the outer pink or sometimes yellow florets pointing downward and the middle florets are positioned in a cone-shaped head (cone-flower- WFO 2023).

Table 1. Taxonomy of the genus ECHINACEA, after MCGregor (1968)(Bauer R., Wagner H., 1990)

Echinacea angustifolia DC. var.*angustifolia*
Synonyms: *Brauneria angustifolia, Echinaceea pallida* var. *angustifolia* (DC) Cronq
Echinacea angustifolia DC. Var. *strigosa,* McGregor
Echinacea alrorubens Nutt
Synonyms: *Rudbeckia alrorubens* Nutt.
Echinacea levigata (Boynton & Beadle) Blake
Synonyms: *Brauneria levigata* Boynton & Beadle*, Echinaceea purpureea* (L.) Moench var.*levigata Cronq*
Echinacea pallida (Nutt.) Nutt.
Synonyms: *Echinaceea angustifolia* Hooker*, Rudbeckia pallida* Nutt.*,Brauneria pallida* Britton, *Echinaceea pallida* (Nutt.) Nutt. *F. albida* Steyerm.
Echinacea paradoxa (Norton) Britton var. *paradoxa*
Synonyms: *Brauneria paradoxa* Norton, *Echinaceea atrorubens* Nutt. var*. paradoxa* (Norton) Cronq.
Echinacea paradoxa (Norton) Britton var.*neglecta* McGregor
Echinacea purpureea (L.) Moench
Synonyms: *Rudbeckia purpureea* L., *Rudbeckia hispida* Hoffmgg., *Rudbeckia serotina* Sweet*, Echinaceea purpureea* (L.) Moench var*., arkansana* Steyerm., *Echinaceea purpureea* (L.) Moench f. *ligettii* Steyerm., *Echinaceea speciosa* Paxton, *Echinaceea intermedia* Lindley, Brauneria *purpureea* (L.) Britton*.*
Echinacea simulata **McGregor**
Synonyms: *Echinaceea speciosa* McGregor.
Echinacea sanguinea **Nutt**
Echinacea serotina **(Nutt.)** DC.
Echinacea tennesseensis (Beadle) Small**,** Synonyms: *Brauneria tennesseensis* Beadle, *Echinaceea angustifolia* DC. var*. tennesseensis* (Beadle) Blake

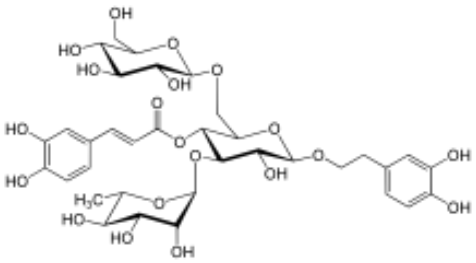
2. **Chemical Composition**

Echinacea spp. has a complex composition. The chemical constituents have been more or less defined over the years, and chemical investigations have even identified different species of Echinacea that are difficult to differentiate botanically. The various plant preparations belonging to the genus Echinacea must be classified according to the plant species (*Echinacea purpurea*, *E. pallida* or *E. angustifolia*), the processed portion of the plant (root, aerial part or whole plant) and the method of processing. Significant pharmacological effects have been described both *in vivo* and *in vitro* for the extract from the aerial parts of E. purpurea and for alcoholic extracts from the roots of *E. pallida, E. angustifolia* and *E. purpurea*. This activity is mainly directed towards the non-specific

cellular immune system. The active components of these plants are polysaccharides, glycoproteins, caffeic acid derivatives (cychoric acid) and alkamides (Bauer et al., 1999, Nyalambisa et al., 2016).

When chemists and pharmacologists started showing interest in Echinacea, numerous constituents were isolated such as polysaccharides, echinacosides, cyclochoric acid, ketoalkenes and alkyl amides (El-Gengaihi et al., 1998). These extracts have shown immune stimulating capacity and were mainly used in prophylaxis and therapy of colds, influenza and septic disorders, however the identity of the active principles was not well known (Hostettmann, 2003).

The main biologically active constituents are: echinacoside (*E. pallida*), verbascoside (*E. angustifolia, E. pallida*)(Fig.2), quinic acid derivatives (cynarin)(*E. angustifolia*, *E. tennesseensis*), chicoric acid (*E. purpureea*)(Fig.3), flavonoids; essential oils, polyacetylenes, alkylamides, alkaloids, polysaccharides and other constituents (resins, acids: oleic, linoleic, cerotic, palmitic, etc.).



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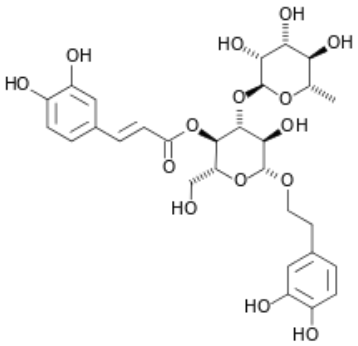

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Figure 2. Echinacoside(a) (*E. pallida*) and verbascoside (b) (*E. angustifolia, E. pallida*) – caffeic acid glycosides from the phenylpropanoid class

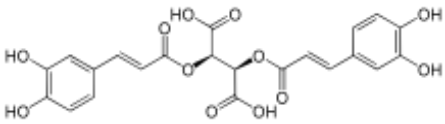

Figure 3. Chicoric acid (*E. purpureea*) a hydroxycinna-mic acid, an organic compound of the phenyl-propanoid class

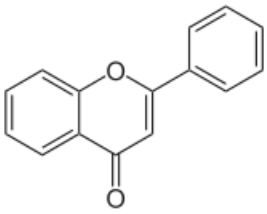

Figure 4. The flavonoid backbone

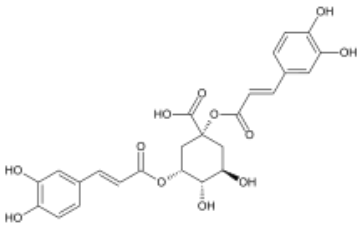

Figure 5. Cynarine)(*E. angustifolia*) hydroxy-cinnamic acid derivative

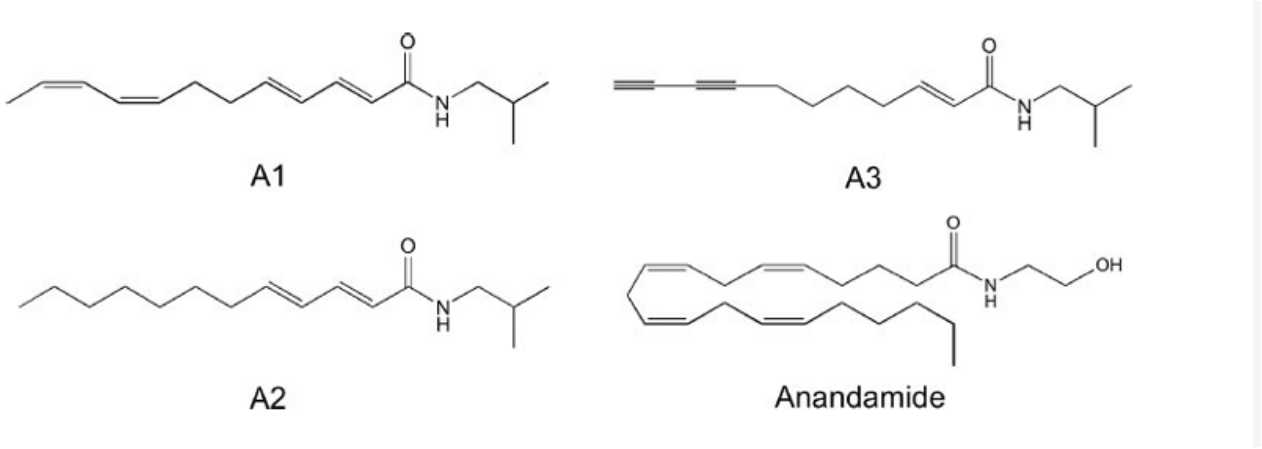

Figure 6. Alkylamides (*E. angustifolia, E. tennesseensis*) hydroxy-cinnamic acid derivative (Raduner et al., 2006)

Chemical evaluation by HPLC showed a differentiated distribution of active compounds in the roots of *E. angustifolia, E. purpurea* and *E. pallida*. Cyclic acid and verbascosides predominated in *E. purpurea* extracts while cynarin and dodeca-2E,4E,8Z,10Z/E-tetraenoic acid isobutylamide represented a major component of *E. angustifolia* extract. Echinacoside and 6-O-caffeoyl-echinacoside were dominant in extracts from *E. pallida* roots. Characteristic alkamides were also examined by tandem electrospray mass spectrometry (MS/MS), finding their characteristic fragmentation. All root and leaf extracts of the three plants showed antioxidant properties in the free radical assay and in the lipid peroxidation assay (Sloley et al., 2001; Kahlos et al., 1989). The flowers of Echinacea spp. contained monoterpenes and monoterpenoids, sesquiterpenes nd sesquiterpenoids, as well as other hydrocarbons, the most prevalent being Sesquiterpenic hydrocarbons identifiable by HS-SPME-GC/MS (Kaya et al., 2018).

3. Biological Effects

The plant is native to North America and has served in the traditional medicine of native Indians. There are records of this plant being used medicinally by the Cheyenne, Dakota, Fox, Kiowa, Crow, Delaware, Comanchee and other North American tribes. It is likely that the *Echinacea* used by them belonged to the species *E. angustifolia, E. purpureea* or *E. pallida.* The earliest archaeological evidence of the use of the plant for therapeutic purposes dates from the 18th century. The first *Echinacea* preparation, known as Meyers Blood Purifier, was marketed around 1880 for rheumatism, neuralgia and snakebites. In the early 20th century, Echinacea was used as the most popular herbal preparation in the US. Commercial cultivation began in Germany around 1939 and since 1950, Vogel has cultivated the plant in Switzerland (Bauer et al., 1988).

For a long time, *Echinacea* species were regarded as esoteric medicinal plants, their use being restricted to a few areas in Germany and the United States. Due to ignorance of its mechanism(s) of action, no relations were sought between its wound healing activity or anti-snake venom effect and modern pharmacology. Until 1930, *Echinacea* extracts were used in experiments of varying scientific accuracy in the treatment of abscesses, puerperal sepsis, septicemia, uremia, malaria, septic shock, typhus, tuberculosis, tetanus or other bacterial toxemias (Bauer and Wagner, 1990).

After 1940, clinical trials became more circumscribed, based on intravenous

administration of *Echinacea* EchinacinR extract or intramuscular administration of Myo-EchinacinR, more rarely oral, drops or external ointments. The main clinical indications of these extracts were shown in Table 2.

Table 2. Clinical results of EchinacinR extracts applied externally or injected (Bauer and Wagner, 1990)

External use
Burns, chemical burns, frostbite, radiation ulcers
Post-operative soft tissue injuries
Atherosclerotic soft tissue and bone wounds, not prone to suppuration, phlegmon, fistulization
Decubital ulcers in elderly, cachectic patients
Ulcus cruris
Eczema (including industrial eczema)
EchinacinR , ampoules
Internal medicine and paediatrics**:** Septic conditions, rheumatoid arthritis, antibiotic resistance. Whooping cough, flu, catarrhal infections. Chronic upper respiratory tract infections.
Gynaecology**:** endodermatitis, parametritis, post-infectious abortion treatment, pelviperitonitis, chronic adnexitis.
Surgery**:** chronic osteomyelitis.
Urology**:** non-specific prostatitis, non-specific urethritis, epididymitis.
Dermatology**:** Psoriasis arthropathica, psoriasis vulgaris, erythroderma of various origins, pemphigus vulgaris, endogenous eczema, atonic skin ulcers (ulcus cruris, irradiation ulcers, decubital ulcers).

Extensive studies carried out in the last decades highlighted that the plant's active substances have beneficial effects on the non-specific immune system. Establishing, without doubt, the botanical identity of the plant and identifying the effects of isolated components, which was possible both in vivo and *in vitro* experiments, allowed a more precise definition of immune modulating activity (Schranner and Losch, 1986, Schranner et al., 1989). Phylloxanthobilins (PBs) which result from degrading of clorophylle while the plant is aging contained in *Echinacea purpurea* extracts could be ensuring part of the plant's biological activity (Karg et al., 2019).

Traditionally, *Echinacea* has been prescribed externally for various conditions such as wounds, burns, skin or lymph node swellings, insect bites. The roots have been used to combat dental and neck pain. Internally, it has given results in headaches, digestive cramps, coughs, colds, measles, gonorrhea or various intoxications (including snakebite). The roots are the most widely used part of the

plant. The fresh plant has been used as a paste or macerate, less often as an infusion.

4. Biological Activities other than on the Immune System

4.1. Local Tissular Activity: Originally, *Echinacea* extracts were used for their healing properties. Using EchinaceaR, its anti-hyaluronidase action was observed, probably associated with other effects, due to indirect intervention on the hyaluronic acid-hyaluronidase system, expressed by changes in fibroblasts (Koch, cited by Bauer R., Wagner H., 1990). EchinacinR appears to induce synthesis of mesenchymal mucopolysaccharides, suggesting also the pituitary-adrenal axis as a target of influence (Koch cited by Bauer and Wagner, 1990).

Echinacea extract, used in equine feed for 42 days, acts as a haematinic agent, improving blood quality, decreasing haemoglobin concentration and erythrocyte count and thus oxygen transport, thereby increasing physiological parameters of exercise and sports performance (O'Neill et al., 2002).

4.2. The Anti-inflammatory Activity: Factor A, a compound isolated from aqueous extracts of Echinacea purpureea, shows cortisol-like activity and a mixture of polysaccharides from *E. angustifolia* show effects in the rat model of edema disease. An alkylamide fraction has also been found to be responsible for anti-inflammatory effects (Bauer, 2002). Anti-inflammatory effects have also been observed for the isolated polysaccharide fraction from *E. angustifolia* (Tubaro et al., 1988).

Echinacea crude extract (Echinacea B) inhibited auricular edema induced in mice by application of 0.015 ml of a 0.25% croton oil emulsion in water, during its peak (6 h) or decline (18 h), by topical application, the effect being dose-dependent. Moreover, this anti-edema effect has been shown to be stronger than that of benzidamine, a non-steroidal topical anti-inflammatory drug. Intravenous administration one hour prior to inoculation of a 1% carrageenan extract amounting in 0.05 ml, in the posterior plantar perineum of the rat, inhibited edema in the histamine phase as well as the inflammatory process (Tragni et al., 1985, 1988; Tubaro et al., 1988).

Components such as phyllobilins (PBs)(phylloleucobilin, dioxobilin-type phylloleucobilin, phylloxanthobilin, etc.) were also found in E. purpurea. Pharmacological activities such as anti-inflammatory and anti-oxidative have been described for PBs of Echinacea purpurea , including those present in infusions of the plant (Gorfer et al., 2023).

4.3. Antiviral, Antibacterial, Antifungal, Oncolytic and Insecticidal Activities: Echinacosides isolated from *E. angustifolia* strains have anti-staphylococcal activity, at the lower limit of antibiotic activity. Polyacetylenic compounds from the roots of *E. angustifolia* and E. *purpureea* have been shown to inhibit the growth of bacteria (*E.coli, Pseudomonas aeruginosa*) and fungi. High dilutions (1:1000) of an E. angustifolia extract completely inhibited the growth of Epidermophyton interdigitale. Antiparasitic effects of these extracts were also mentioned, thus *in vitro*, an alcoholic extract of E. angustifolia weakly inhibited the growth of Trichomonas vaginalis.

EchinacinR, the already mentioned extract, was shown to be effective against encephalomyocarditis and vesicular stomatitis virus in cell cultures. Extracts of *E. purpureea* reduced the number of plaques in cell cultures treated with influenza virus, herpesvirus and vesicular stomatitis virus respectively (Wacker and Hilbig, 1978). This activity has been described as "interferon-like", but without inducing interferon synthesis.

Peroral treatment for four weeks with *Echinacea* extract in combination with Eupatorium perfoliatum and Thuja

occidentalis (*Echinacea* complex) in patients undergoing surgery for tumour diseases, did not induce the activation of leukocyte or lymphocyte populations or amplification of the secretion of IL-1-α, IL-1-β, IL-2, IL-6, TNF-α, or IFN-γ in supernatants of stimulated whole blood cultures compared to the untreated group of patients (Elsässer-Beile et al, 1996; Bodinet and Freudenstein, 1999; Block and Mead, 2003).

Phytochemical preparations obtained from Echinacea spp., by stimulating non-specific immune effectors (micro- and macrophages), act in the first line of defense against virally infected/transformed cells. Increased levels of these cells in the bone marrow following treatment in rats indicate that at least one of the mechanisms of action of the active compounds is the stimulation of the appearance of new cells in situ (Sun et al., 1999, 2005, Burlou-Nagy et al., 2022).

PBs of *E. purpurea* were proven to bind to actin and inhibit cancer cell migration (Vollmar and Moser, 2023). Further, phylloxanthobilin, induces cell cycle blockage and apoptosis in cancer cells, an increase in anti-proliferative activity being induced by esterification inactive phylloleucobilin and depending on the chain lengths of the alkyl esters (Karg et al., 2020)

4.4.Toxicity: Acute toxicity tests by Lorke (1983) in mice using two of the *Echinacea* polysaccharides resulted in death following intraperitoneal inoculation of over 5000 mg/kg-1, but the nature of the histological lesions (pronounced alveolar and interstitial edema with localized leuko-diapedesis) suggested acute circulatory collapse (decompensated shock) caused by the syrupy nature of the extract and not the plant polysaccharides themselves.

Neutral polysaccharides from *E. purpureea* have been tested for possible genotoxicity in human lymphocyte cultures (Schimmer and Leimeister, 1989). No sister chromatid exchange or chromosomal aberrations occurred in either long or short term experiments.

Experiments carried out recently (Balciunaite et al., 2020) identified by LC-MS/MS glycoproteins were homologous with the lysine motif (LysM) domain containing lectins, common to several Asteraceae plants. Tested in vivo, these induced toxic effects expressed by statistically significant kidney glomerular vacuolization and tubular necrosis.

5. Immunological Investigations

A review of research conducted over the last 40 years (January 1966-July 1999) to confirm the immune stimulating effects of *Echinacea* extracts or purified Echinacea components revealed a lack of agreement between these results obtained by different working groups, with arguments pro and against immunomodulatory activity but not for the safety of using these extracts (Giles et al, 2000).

According to some authors (Văgîi et al., 1996), the immune modulating influence of Echinacea extracts is global, with usefulness in immune deficiencies, particularly in combination with extracts from Epilobii herba, *Usnea barbata*, Urticae folium, Alchemillae herba, Hyperici herba, Crataegi folium cum flores.

By correlating the results obtained with the composition of the different parts of the plant or with that of the different Echinacea species, it could be stated that the immune stimulating effects of alcoholic or aqueous extracts depended very much on the combined action of their constituents. Most chemical analyses have been carried out on Echinacea angustifolia, particularly the older ones, while immune activity has been tested mainly on Echinacea purpurea (Schumacher and Friedberg, 1991).

5.1. Influence on the Complement System: Due to the extremely important physiological role of the complement system, its modulation (inhibition or stimulation) is a target of interest in the development of various drugs. Some plant polysaccharides are known to have complement-modulating activities. However, the classically used hemolysis assay does not allow differentiation between complement inhibitors or activators due to low hemolysis.

Comparative evaluation of the inhibitory and activating effects, respectively, using heparin (inhibitor) and an arabino-galacatan from Echinacea purpurea extract demonstrated that the latter was a complement activator, both in the classical and alternative pathways, by altering the complement incubation periods. Removal of the side chains of the tested arabinogalactan significantly reduced complement stimulating activity, proving that the three-dimensional structure was an essential component of the activating potential. The involvement of arabinogalactan in complement activation could represent one of the attributes of the immune stimulating efficacy traits of Echinacea purpurea extract (Alban et al., 2002).

5.2. Influence on the Phagocytic System: The positive effects of Echinacea extracts on phagocytosis have been followed on isolated rat liver (Bauer, 1996). In order to determine any differences between extracts from plants belonging to different species, the carbon particle inclusion test was performed and extracts of E. purpurea, E. angustifolia and E. pallida were tested. Phagocytosis was amplified in the presence of alcohol extracts by 20-30% at doses of 10-2 to 10-4.

The E. purpurea extract was found to be the most active, and the chloroform-extracted fractions of the ethanolic extract were more effective than the hydrophilic ones (Fig. 7). The results of this *in vitro* assay correlated with those obtained in vivo by oral administration of the alcoholic extract.

Of the isolated compounds, chicoric acid induced a significant increase in phagocytosis, confirmed by the carbon particle inclusion assay.

The polysaccharide fraction favored phagocytosis after intravenous injection (EchinacinR), an effect supported by assessing the percentage of lymphocytes and granulocytes labelled with tritiated thymidine in Cohen's experiment (1980) (Table 3).

In vitro investigations using a polysaccharide-rich mixture from the aerial parts of E. purpurea demonstrated its predilect influence on the mononuclear cell subsystem. The same mixture stimulates macrophages to release interleukin-1, but does not induce T lymphocyte proliferation (Lowenthal and MacDonald, 1986; Lowenthal et al., 1999, 2000, Barrett, 2003). Macrophage stimulation and induction of cytokine secretion are due not only to the polysaccharide fraction but also to the glycoprotein fraction (Bauer, 2002).

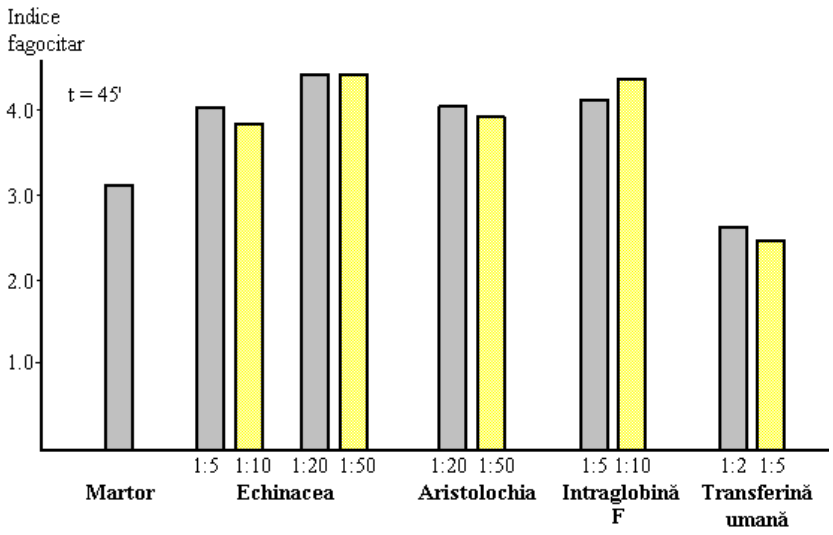

Figure 7. Influence of *Echinacea* extract versus other principles on phagocytic activity in humans (after Bauer R. et al., 1999)

Legend: Echinacea preparations used: 1:5 - Myo Echinacin 5%, 1:20-1:50 - Echinacea angustifolia extract 5:1/30%; Aristolochia - Tardolyt Madaus; Intraglobin F, Ch-B 4111107 Biotest, Human Transferrin - Ch-B 1672 Behringwerke

Echinacea purpurea extracts, stabilised, are well-tolerated, allergic reactions, particularly skin reactions, are reversible and can occur particularly in people with contact hypersensitivity to plants of the Compositae family. Pharmacological data suggested that cold-pressed Echinacea preparations stimulate the non-specific immune system and increase resistance to respiratory infections by stimulating oxidative shunt and modulating monokine secretion (Kligler, 2003, Bauer, 2002; Bauer et al., 1988). These claims are supported by stimulation of natural killer cell activity (Barrett, 2003).

A series of studies in mice using polysaccharides purified from Echinacea cell cultures have shown an immune stimulating effect in cell cultures from mice or in animals injected intraperitoneally. These effects include amplification of phagocytic activity, chemotaxis and respiratory shunting of neutrophils and macrophages respectively. Peritoneal macrophages from treated animals produced increased amounts of tumour necrosis factor (TNF), interleukins (IL-1, IL-6 and IL-10) and were more active in destroying WEHI 164 lineage of tumour cells as well as cells infected with Leishmania enriettii or Candida albicans. Similar effects were observed after administration of Echinacea extracts even in mice suppressed with cyclophosphamide or cyclosporine. These studies highlight the immune stimulating activity of Echinacea polysaccharides in both healthy and immunosuppressed animals (Percival, 2000).

A comparative study carried out to investigate the immune-biological effects of some components of Echinacea extract (cychoric acid, polysaccharides and alkylamides) demonstrated the maximum efficacy of alkylamides at a dose of 12 µg/kg body weight/day in significantly increasing phagocytic activity as well as the phagocytic index of alveolar macrophages. Simultaneously, alveolar macrophages obtained from this group produced significantly more TNF-α and nitric oxide after *in vitro* stimulation with LPS than any other component or the control. None of the concentrations of the investigated components induced TNF-α, IFN-γ and IL-2 release by splenocytes. The immunomodulatory effects of alkylamides appear to be more pronounced on lung

immunocompetent cells than on splenic cells (Goel et al., 2002).

Western blot analysis of the *in vivo* effects of *Echinacea purpurea* (L.) Moench extract showed that it induced, along with changes in lipopolysaccharide (LPS) response, IFN-γ-induced cyclooxygenase-2 (COX-2) and nitric oxide synthase (iNOS) expression in peritoneal macrophages. Thus, treatment with 100 mg kg(-1) of Echinacea extract reduced only COX-2 expression, demonstrating that the anti-inflammatory effect of the extract may be due to this mechanism (Raso et al., 2002).

Table 3. Percentages of labelled lymphocytes and granulocytes in the total population (after Bauer and Wagner, 1990)

Time for thymidine inoculatin (h)	Lymphocytes	Granulocytes
24	11	0
48	15	6
72 (Echinacin application)	7	34
78	40	89
97	7	59

5.3. Influence on Specific Humoral-Mediated Immunity: Numerous immunomodulatory effects have been attributed to Echinacea angustifolia extracts, however little is known about the stimulation of antigen-specific immunity. Studies in rats shed some light on these issues, indicating increased synthesis of immunoglobulins by enhancing the primary and secondary IgG immune response to antigen (Rehman et al., 1999). Contrary to these results, administration of Echinacea preparations of different origin (standard or officinal products) to male and female rats decreased the concentration of antigen-specific immunoglobulins (standard products) or had no effect (officinal products) in females, but was neutral in efficacy in males (South and Exon, 2001).

5.4. Influence On Specific Cell-Mediated Immunity: Although Echinacea extract acted immune stimulating, not all cell categories were encouraged. Thus, B lymphocytes were not activated nor did they produce antibodies in increased concentrations against a thymus-dependent antigen, sheep red blood cells (Barett, 2003). In some papers, a weak stimulation of T lymphocytes was reported, but those cells did not synthesize increased amounts of IL-2, IFN-β2, or IFN-γ. Delayed hypersensitivity, a T lymphocyte-mediated reaction, was not affected by treatment with Echinacea extract. These data strongly indicate the action of purified polysaccharides from E. purpurea on the non-specific side of the immune response, i.e. phagocytes, rather than on the specific side of immunity (Percival, 2000). Other works (Wagner et al., 1984, 1985; Wagner, 1991; Wagner and Jurcic, 2002) suggest that Echinacea purpurea extract in mixture with extracts from other plants (*Glycyrrhiza glabra*), in tablet form (Revitonil), exerted a remarkable stimulating effect both on phagocytic activity, measured by the carbon particle engulfment test and chemiluminescence, and on T lymphocytes (30-50%) in the CD69 bioassay at a concentration of 100 microg-1/ml.

6. Is There More?

Several reseachers mention that their results open gates for further investigations on the biological effects of Echinacea genus, given the sometimes substantial composition differences, which were found among the species. Similarly, some of the biological effects of these plants supported by traditional medicine uses still remain unexplained. Therefore, several

areas/directions to be tackled by further research and several unanswered questions could be identified.

There is an obvious need for identifying new compounds, whose biological effects remained insufficiently unveiled, i.e., further research on phyllobilins and all the compounds included in this group, where investigations are at their beginnings.

Similarly, not all the effects connected with single components have been revealed, therefore new research techniques, mainly *in vivo* studies with respect to the three R (replacement, reduction, refinement) could be applied to increase the database on the biological efficacy result of these plants.

One of the difficulties in preparing plant products for medicinal use consist of standardization, therefore the use of single purified components seems to be a solution to the problem. But will the overall effects be those expected under these circumstances? Will the patients' reactions be the same? Broadening the variety of subjects, will the "species" factor become a more influential one? Other questions yet to be answered in the future.

Finally, all the available data suggest that the "pharmacy of nature" withholds numerous secrets and to avoid the Pandora box effect only depends on those involved in its research.

7. Conclusion

Due to multiple biological effects, the *Echinacea* genus represents a valuable resource for medicine in general and especially for veterinary medicine, where the fight of increasing resistance against diseases counter-balances the antimicrobial therapies; further, the potential adjuvant role of *Echinacea* products provide perspectives of an enhanced innate rather than adaptive immunity along with antibacterial effects.

Still, the involvement of plant extracts in therapy and prevention of diseases in general, even by enhancing immunity or increasing the post-vaccination responses needs careful species and age-based tailoring to avoid unwanted or noxious side effects.

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Author Contribution

All the authors gave the same effort and contribution to this review and they approved the final version for the submission.

Conflicts of Interest

There is no conflict of interest for any of the authors of this article.

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