ROMANIAN CONTRIBUTIONS TO THE STUDY AT SPECIES OF ECHINACEA GENUS WITH A PHARMACEUTICAL IMPORTANCE

DANIELA LUMINIŢA ICHIM*, DIANA MAFTEI2, MIRELA M. CÎMPEANU 1

Nowadays, the species of Echinacea genus are worldwide known during a process of phytotherapy revival, being some of the fewest plants with an immunostimulative effects.

Echinacea genus is a part of Magnolyophytae phylum (Angiospermatophyta), Magnoliatae class (Dicotyledonatae), Asteridae subclass, Asteraceae order, Asteraceae (Compositae) Family, Radiiflorae subclass, Heliantheae tribe, Ecliptinae subtribe (Bawer et Wagner, 1990).

The genus name is derived from the Greek term echinos (= hedgehog) and it refer to thorny fruit of these species, fruit that has a membrane edge its four at its top.

Three of the nine species of the genus are being used as medicinal plants: Echinacea angustifolia (DC) Moench (n=11), Echinacea purpurea (L.) Moench (n=11) and Echinacea pallida Nutt.

There have been controversies about the nomenclature of these species that’s why there are many synonyms for the same species. Thus:

Echinacea angustifolia (DC) Moench -synonyms: Brauneria angustifolia Heller; Echinacea pallida var. angustifolia (DC); Cronq; Echinacea angustifolia (DC) var. strigosa Mc. Gregor.

Echinacea pallida Nutt.-synonyms: Echinacea angustifolia Hooker; Rudbeckia pallida Nutt.; Brauneria pallida Britton; Echinacea pallida (Nutt.) Nult. f. albida Steyerm.

Echinacea purpurea (L.) Moench –synonyms: Rudbeckia purpurea L.; Rudbeckia hispida Hoffmgg; Rudbeckia serotina Swee; Echinacea purpurea (L) Moench var. Arkansana Steyerm; Echinacea purpurea (L) Moench f. ligettii Steyerm; Echinacea speciosa Paxton; Echinacea intermedia Lindley; Brauneria purpurea (L) Britton; Echinacea purpurea (L) (Muntean et Tamaș, 1989)

A feature of Echinaceagenus is represented by the basic chromosome number (x=11), Echinacea pallida having 2n=44, and Echinacea angustifolia and Echinacea purpurea, 2n=22 (Bauer et Wagner, 1990).

Echinacea pallida is a tetraploid one, having 2n=4x=44 chromosomes, its size between 5.87-3.04μm (Muntean et al., 1990).

The karyogram analysis of Echinacea pallidaNutz emphasized some morphological heterogeneity among the basic chromosomal sets, some differences that likely appeared within the evolution process of that species (Muntean et al., 1991).

The karyotype of Echinacea purpurea (L.) Moench have 2n=22 chromosomes of different sizes between 5.94-3.34μm and it shows three types of chromosomes (m-medians, sm-submedian and st-subtelocentric) being an asymmetrical and evolved one (Ichim et al., 2004).

Botanical characterization
The researches from Cluj found as being necessary to study the morphology and the anatomy of the species of Echinacea genus because of the fact that then have not been known as medicinal plants in our country, until 1982. A further reason is represented by the fact that botanical literature mentions some confusion about the medicinal product of different species of Echinacea or with Parthenium integrifolium (Bauer et Wagner, 1990).

Echinacea purpurea (L.) Moench is bush-like shaped, with 80-100 cm height. Its roots have many ramifications which are oblique positioned and they are light-brown coloured. The stem has red coloured basis and ends and oval-lanceolate, 5-nervation leaves, the basic ones being long petiolate and the top ones are sessile and short petiolate.

The flowers are grouped in inflorescences anthodium. These ones are large, having the involucre shaped of conic-oval anthodium, thorn-shaped when they are dried.

The flowers which are pink purplish-blue coloured and ligulate are sterile, with three toothed ligule to the external part and the tubular flowers are to the interior part and they are hermaphrodotes and orange-like...
The fruit is an edged and a 2-4 mm long achene. The plant is covered of rigid 1.8-2 mm tector hairs (formed of 2-5 cells, with a filled basis and a sharp top which gives rigidity to plant (Muntean and Tămaș, 1989).

The name of *Echinacea palida* Nutt. Comes from the Greek term *echinos* (=hedgehog) referring to the fact that it has a thorn-like shaped fruit and the same colour of anthodium. The inflorescences are formed of tubular flowers of orange-yellow colour to their internal part and ligulate flowers of pink-purplish blue colour to their external part with three toothed ligules. The whole plant is covered with tector hairs which are shaped of 2-5 cells and which are rigid and 1.8-2 cm height, with a filled basis and a sharp end. Their presence gives rigidity to plant. The fruit is an edged, white coloured, 2-4 mm long achene. (Muntean and Tămaș, 1991).

Hodișan and Tămaș (1984) showed the resemblances and differences in the anatomical study of *Echinacea palida* Nutt. and *Echinacea purpurea* (L.) Moench’s root, stem and leaf.

It emphasized the different feature in studying comparatively the transversal sections in the ligules of the two species (Ilioara, 1997).

When it is multiplied in a hot-bed *Echinacea palida* Nutt. forms a rosette of leaves during its first year of vegetation. Its inflorescences are some sporadic ones (less than 11 of plants). The number of leaves and the plants weight mass have a prominent increase beginning with August.

Herba is 74% and the underground part (radix) is 26% of the total weight of this plant.

*Echinacea purpurea* (L.) Moench forms a rosette of leaves during its first year of vegetation and the first copse of flowers during September and October (to 40% of plants). Herba represents 87% and the underground part is 13% of the whole mass of the plant. The number of leaves and the plant mass have a prominent increase from August.

*Echinacea palida* Nutt. and *Echinacea purpurea* (L.) Moench form a rosette of leaves during the second year of vegetation and the first flower copse appear during June.

The strength of *Echinacea purpurea* (L.) Moench is more significant that *Echinacea palida* Nutt., excepting the roots during the third period of vegetation (Muntean et al., 1991)

**Area, specific feature and biological demands**

All the nine species of *Echinacea* genus (having more varieties) grow spontaneously in the east part of USA, in states as: Arkansas, Oklahoma, Missouri, Kansas, resisting to prairie conditions (dry and sandy soil).

The acclimatization of the species of phytopharmaceutic interest has been done in Europe since XIX\textsuperscript{th} century, nowadays being cultivated in Germany, Switzerland, Italy, Yugoslavia, Slovenia, Spain, Holland and Russian. They have been studied from a technological point of view of cultivation and of that of the content in active principles in all these countries (Muntean, 1990).

*Echinacea* has been recently introduced even in Ukraine (Menshova, 2005).

Species of *Echinacea* genus have been sporadically cultivated in our country, as decorative plants, in some of Botanical Gardens. Since 1982 the species of medicinal importance (*Echinacea palida* Nutt., *Echinacea purpurea* (L.) Moench, *Echinacea angustifolia* (DC) Moench) have been studied to the Medicine and Pharmacy Research Centre Institute of Cluj, being used seeds material from Italy. It was thought from the very beginning that the species introduced in our country was *Echinacea angustifolia* (DC) Moench, but it had been proved to be *Echinacea palida* Nutt. after karyological analyses (Ilioara, 1997).

*Echinacea* species were included in 1988 in the researches schedule of Aromatic and Medicinal Plants Research Centre, Fundulea.

The studies dwelt with establishment of right agrotechnology for each species, species, with the content in active principles and with the use of vegetal products to new pharmaceutical forms.

**Cultivation technology**

**Culture place:** The cultivation of *Echinacea* species does not represent a problem, being necessary a loose soil, which is well worked and which is not too damp and it is sun exposed (Bauer et Wagner, 1990).

*Echinacea purpurea* (L.) Moench prefers fertile soils, which have no weeds being unadvisable acid, heavy soils which have an extra of humidity and which forms crusts.

It is recommended to cultivate *Echinacea palida* Nutt., in our country, in lower areas of land with good sun exposal, on light and warmer soils and *Echinacea purpurea* (L.) Moench shall be cultivated on higher areas with a good humidity and good proper exposal (Muntean, 1990).

**Preliminary plants:** Preliminary plants are those that leave the soil without any weeds and they have nutritive substances (yearly fodder cultures used for green mass and early cereals). It grows again on the same field in 4-5 years time.

**The use of fertilizers.** The assurance of necessary nutritive elements is made in accordance with the supply degree of the soil with nutritive substances according to agrochemical sorting.

It is necessary to be used well fermented manure on infertile soils, 20-30 t/ha, at the beginning of culture.

The researches done to Agrozootechnical Research Centre of Secuieni (Neamț), during 1999-2001 by Iliescu
and Pânzaru emphasized the positive influence of fertilizers and a more reduced one of that containing phosphorus on the production of *Echinacea purpurea* (L.) Moench herba.

The most increased production of dried herba was recorded to N80 P80 variant (76.26 q/ha) the difference than to the unfertilized control being of 21.8q/ha (40%).

The works of the soil. The basic ploughing is done 20-25 cm deep in soil during autumn until October. The preparation of germination bed is realized in 1-2 days before to seed or to plant the plantlets to 10-15 cm deep, by using the harrow with ditches or by the harrow with adjustable teeth.

Type of growth. Their growth is realized by seeds planted directly in the ground (field) or by plantlets in green houses.

Period of seeding. They are seeded directly on the field: in spring time 1st urgency. To seed 1 ha of *Echinacea* it is recommended a standard of 6-8 kg.

The depth for seeding is 1.5 cm the distance between the rows being of 50 cm and the density is of 6-8 plants/m². It is recommended that seeds purity shall be 90-95% and the germination shall be 75-85%.

The device used had been SUP-21 929) which has distributors for small seeds and limiters for depth to furrow. When it grows by plantlets the period of cultivation is the end of April-the beginning of May, being used developed plantlets (2 mouth from their growth – 4-5 leaves).

The best period of cultivation to *Echinacea purpurea* (L.) Moench, and also to *Echinacea pallida* Nutt., proved to be during May time (Muntean et al., 1999).

Works of interest.

Harvest. It is harvested the upper part (herba) of *Echinacea purpurea* (L.) Moench, from the second year of culture, being used fresh or dried the rate of drying is 1:3.5. There are used even the roots at the end of culture. It is harvested the whole, fresh or dried plant of *Echinacea angustifolia* (the drying rate being 1:3.5) from the third year of vegetation (Muntean and Tâmaș, 1989).

It was noticed at the end of the first year of vegetation that the entire mass of one plant the herb mass and the radix one had more increased values to *Echinacea purpurea* than to *Echinacea pallida*. The production of dried herba was of 3.8q/ha to radix of *Echinacea pallida* in the first year of vegetation and also of 8.4q/ha of herba and 2.2q/ha to radix of *Echinacea purpurea*. The production of herba inflorescence was of 17.4q/ha to *Echinacea pallida* in the second year of vegetation and of 35.6q/ha to *Echinacea purpurea* and the production to radix was between 1.6-6.9 q/ha to *Echinacea pallida* and 1.9-7.8 q/ha to *Echinacea purpurea* (Muntean et. al., 1999).

Storage. It is dried naturally, to shadow or in device with warm air (40-45°C). The production of dried herba is between 4000-5000 kg/ha.

Chemical composition

The first data about chemical composition of *Echinacea* species have been given by Sayre and Lloyd who studied *Echinacea angustifolia* roots.

After the studies on *Echinacea purpurea*’s roots, Heyl and Saley showed that they do not contain starch but they have in their composition inulin, pentosane (15.6%), reducing sugars, resinous substances, volatile oil, proteins (5.3%), water (18.18%) and ashes (6.93%) (Tâmaș and Hodîșan, 1984).

The active principles identified to the species of *Echinacea* have been: polysaccharides, volatile oil, flavonoids, compound elements of cefeic acid, polycetylenes, alchylamides, alkaloids, etc. there have been isolated two polyurodic fractions of the upper part of *Echinacea purpurea*: PS I-4-O-metylglucoronoarabinoxilan and PS II-ramnoralienogalaactan and there were also isolated a series of polysaccharides to *Echinacea pallida* (Wagner et Proksch, 1981; Wagner et al., 1988; Wagner et al., 1989).

Tâmaș et al., 1989 isolated immunomodulating polysaccharides of *Echinacea angustifolia* identifying a content of 2.24% in the entire plant, 2.6% in roots and much lower values in other parts of the plant. There have been isolated the echinacoside one of the esthers of cefeic acid (of *Echinacea angustifolia* and *Echinacea pallida* roots), cinarin (of *Echinacea angustifolia*’s roots) and cychericnic acid (of *Echinacea purpurea* roots).

There have been isolated izobutylamides of roots and upper parts of *Echinacea* species. The isolated volatile oil of upper parts *Echinacea* species mainly contains borneol, bronil acetate, apoxid caryophylen, D germacran, 8(z)-en-2ona-pentadeca (Grigorecu, 2001; Stânescu et. al., 2004).

After the study about the content in active principles to *Echinacea pallida* Nutt. and *Echinacea purpurea* (L.) Moench in the second year of vegetation, on different parts of the plant, the researchers found a significant quantity of polysaccharides and volatile oil in roots of *Echinacea pallida* and an increased ratio of phenylpropan compounds to upper parts of *Echinacea purpurea* (Muntean et al., 1991).

The content of immunostimulative proteins in the second year of vegetation to two species is more increased in roots, being under 1 g in the other parts of the plant (Muntean et al., 1999).
Illoaara, 1997 analysed compound elements of cafeic acid from a quantitative and a qualitative point of view, with in the study of some polyphenols of Echinacea plants.

The richest in compound of cafeic acid proved to be Echinacea purpurea, Echinacea angustifolia’s leaves and Echinacea pallida’s roots (0.3-2.21%).

The most significant quantity of cichoric acid is found in Echinacea purpurea’s roots. In studying the active principles of the two species of Echinacea, in dynamics, on phases of vegetation to Echinacea pallida Nutt., Vârban, 2001 found an increased content of immunostimulating polysaccharide in roots, followed by inflorescences, leaves and stem and in roots of Echinacea purpurea, in stem, leaves and inflorescences.

The composition of volatile oil brings its contribution to the variety of pharmacological features for which are being known Echinacea species.

Tâmaș et al., 1997, identified in Echinacea angustifolia volatile oil 23 compound elements, the main one supposed to be geranilizibutirat (almost 50.03%), and it was identified the caryophilen as a main element in Echinacea purpurea being followed by mircen beta-pinen.

Schultess et al., 1991, identified the following compound element after the study of Echinacea palida’s achenes of volatile oil: α-pinen, β-pinen, mircen, limonene, 1.8 pentadecadien, germacren D.

Illoaara and Vârban, 1999, found compound elements in Echinacea palida’s volatile oil (the entire plant) which were known even to other authors and there were found 31 elements in fruits, 4 of which (α and β-pinen, α and γ caryophylen) were found also by some other authors (Illoaara et al., 1997).

Phytochemical studies contributed to the establishment of some criteria of Echinacea species identification.

**Phytopharmaceutical importance**

There are many preparations based on Echinacea extracts, within therapy and having been used against viral infections, or in those of breathing channels, in urology, in gynaecology and mostly in all the cases when it becomes important to increase body’s self defence system.

External use preparations are used as a healing factor in difficult wounds (Muntean and Tâmaș, 1989).

The main indications of Echinacea extract refer to: the stimulation of self-defense system (by mobilizing the leucocytes and by increasing phagocytary activity), the inhibition of virus multiplication, wounds local treatment, antitumoural action (Tâmaș and Hodişan, 1984).

The first clues about Echinacea’s influence on the nonspecific immunitary system have been given by Büsing, 1958, and quoted by Illoara, 1997, when it was raised the problem of a possible influence on properdinic system.

Bauer et al., 1989, allocated the stimulative action of phagocytes to cichoric acid of Echinacea purpurea (L.) Moench root and Schollhörn et al., 1993, isolated an acid arabinogalactan on cell cultures of Echinacea pallida and which has effects on macrophages and on granulocytes.

Stimpel et al., 1984’s studies dealt which the same idea taking into account a polysaccharide of Echinacea purpurea that stimulated the macrophages.

The researches from Cluj checked up some extracts from an experimental point of view having as a base the existing biological data according to which Echinacea species contain immunomodulatory active principles (Bauer and Wagner, 1998 1990; Colins et al., 1999; Hobbs, 1994; Schar, 2002) and that Echinacea increase properdine level (Bauer, 1994, 1996), that it determines T phagocytes intensification (Bauer, 1994) and it stimulates the production of β interferon and interleukin-1 by macrophages (Luettin et al., 1998).

Mustea et al., 1992, examined the value of renal homogenates breath in considering of Romcis medicine nephrotoxicity and of the protective effect of Echinacea angustifolia extract to rats.

The results obtained showed the value of Echinacea angustifolia extracts, as an auxiliary element in Romcis treatment because of its chemioprotective effect.

Echinacea pallida Nutt. standard extract given to mice in association with cisplatin presents chemoprotective effect which are expressed by a diminished decrease of body’s weigh. The pretreatment with Echinacea pallida Nutt. diminished cisplatin’s nephrotoxicity expressed to the level of renal homogenates breath (Mustea et. al., 1997).

There were noticed the effects of the use of two vegetal extracts of Echinacea purpurea and Echinacea pallida to Wistar rats in an extensive study, the rats being possible immunostimulative agents of some functions of lymphpatic and endocrine system’s organs which are disarranged by cytostatics use that have been widely used within antitumour chemotherapy practice.

The renal of the structure and functions which have been damaged by cyclophosphamide treatment (haemathogen marrow, thymus, spleen and liver) has been a quick one under the influence of Echinacea angustifolia Nutt. extracts comparatively to natural remake a fact that suggests the use of these extracts in chemiotherapy, as auxiliary elements (Hermenean et al., 2002).
It was noticed an increase of neutrophiles phagocitary activity and that of monocytes during treatment to patients with pulmonary problems within clinical attempts concerning. Novastim’s immunostimulative action (tablets obtained of *Echinacea pallida* Nutt., in Pharmacy Faculty of Cluj).

Novastim’s use to immunodepressed patients who has AISD had some positive effects: there were no sign of intolerance, some of the symptoms improved and there had been recorded a slight increase of peripheral neutrophyles (Ilioara, 1997).

It was noticed in a study that assessed the efficiency of auxiliary treatment with Novastim in limiting produced marrow depression by frequent cures of polychimiotherapy the fact that the period of leuko- and thrombocytopeny was considerably reduced in 10 of 18 cases after the treatment allowing to beginning the next cures again (Bojan et al., 2000).

The echinacoside and the derivates of caffeic acid of *Echinacea angustifolia* Nutt.’s roots have an antibacterial action being noticed the fact that 6/3 mg of echinacoside equals to 10 unities of penicillin. Tested bacteria have been: *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Hobbs, 1994).

It was been noticed that all tested oil have had a bacteriostatic effect on *Staphylococcus epidermides*’ stem after antimicrobial activity testing of volatile oils and of isolated saponins of *Echinacea pallida* and *Echinacea purpurea* on eight bacterial stems.

It is important to notice that none of the tested samples had been active on *Pseudomonas aeruginosa*.

Saponins presented an unsignificant antimicrobial activity (Ilioara, 1997).

The action of *Echinacea* preparation is based on a change of host organism tissues so that hyaluronidase is inhibited.

*Echinacea’s* polysaccharides form a compound with hialuronic acid, a complex that becomes resistant to enzyme’s action (Büsing, 1952, quoted by Ilioara, 1997; Bonadeo et al, 1971).

The test of volatile oil activity obtained of *Echinacea pallida* (roots and the whole plant) proved an intense activity on *Candida albicans* (Ilioara, 1997).

The *in vitro* researches with alcoholic extract of *Echinacea angustifolia* have demonstrated the decrease of growing and increasing ratio to *Trichomonas vaginalis* (antiparasitic effect).

*Echinacea* extracts proved an antiviral action, stopping the virus to enter healthy cells (they do not have a virucide action) (Wacker and Hilbing, 1978).

There have been obtained positive results to all patients within the test of Novastim medicine by local applications, in 150 cases of simplex herpes and also in 20 ones of zoster zones.

The local treatment with Novastim has been associated with a general treatment with vitamins and devo-dopa in order to hasten the effect in zoster zone (Tamaș et al., 1989).

Schar, 2002, demonstrated the antiviral action of one alcoholic extract of *Echinacea purpurea* roots.

*Echinacea purpurea* fresh juice stimulates cells regeneration (Tragne et al., 1985; Tubaro et al., 1987).

The study on the influence of *Echinacea* extract on liver regeneration has demonstrated that the extract of *Echinacea angustifolia* (DC) Moench does not modify the level of serum cholinesterase (ChE) to animal subjects (white rats) but it has induced an increased synthesis of γ-glutamyl-transpeptidase (γGT).

Partial hepatectomy, under *Echinacea* extract protection, is followed by a supra-production of γGT and normal value of ChE. Young hepatocytes are not able to metabolize *Echinacea* extract given excessively (21 days) and they do not control γGT enzymes’ value so that a negative phenomena is realized - the summation of dose effect (Protase et al., 1990).

North Americans use *Echinacea angustifolia* and *Echinacea pallida* in therapy, most of the time and *Echinacea purpurea* is rarely used (Tamaș and Hodișan, 1984).

The three species of medical importance of *Echinacea* genus are used in various preparations (alone or in combination with other plants) because of their immunostimulative, antiviral, antibacterial, healing, antiinflammatory actions.

The well-known preparations which contain *Echinacea purpurea*, *Echinacea angustifolia* and *Echinacea pallida* are: Echinacin, Echinacea, Esberitox, Esberisan, Immunopret, Conrinfekt, Uralyt Echinacea-ratiopharm, Osttriuw etc.

Novastim is a preparation presented as tablets and gel and contains dried extract of *Echinacea pallida* Nutt. and it is obtained in the Institute of Medicine and Pharmacy, Cluj-Napoca.

Ilioara, 1997, analyzed Romanian pharmaceutical forms (Novastim – tincture and tablets) and some foreign ones (Echinatforce, Echinacin, Echinacea forte) from a quantitatively and qualitatively point of view. The result of analytic researches emphasized the presence of main compound elements found to *Echinacea* species in tincture and pharmaceutical forms.
As a conclusion we notice that *Echinacea* genus represents a very well-known name in the process of phytotherapy regeneration and it is also appreciated for its therapeutical qualities, being among the fewest plants which have immunostimulative and antiviral effects.

REFERENCES


