

# Influence of Irradiation Treatment on Antioxidant Compounds from *Echinacea* species

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*This work studies the concentration change of antioxidant compounds from two species of Echinacea: purpurea and pallida upon treatment with the gamma radiation as compared with the initial state. The plants were supplied by Institute for Research and Development Braşov and were harvested in the fifth year of vegetation. The main goal is to establish the optimal dose of radiation in order to remove the microorganisms that produce alternariosis and fusariosis diseases. The study was carried out on leaves, seeds and aerial part of the plant. The gamma ray irradiation doses were 1 and 10 kGy (energy 1250 keV). Referring to Echinacea purpurea leaves, irradiation process leads to reduction of flavonoids content (decrease of 14.56% irrespective of radiation dose; the total phenols content lowers with 37.86% at 1 kGy and with 40.19% at 10 kGy, respectively. For Echinacea purpurea seeds, the irradiation causes a concentration increase of up to 10.43% in the total phenols content. By irradiation, chlorogenic and coffee acid quantities increase in the extracts obtained from seeds, especially for 1 kGy dose, and considerably decrease in samples of Echinacea purpurea leaves. A major decrease was obtained at 10 kGy for both acids: 93.19% for chlorogenic acid and 83.34% for caffeic acid. The irradiation leads to an insignificant concentration decrease of flavonoids and phenols for both 1 and 10 kGy irradiation doses. The reduction is even less pronounced in the case of Echinacea purpurea. For the plants cultivated in Romania and harvested in the fifth year of vegetation, 1 kGy irradiation dose is optimal for sterilization of Echinacea purpurea leaves and seeds and Echinacea pallida aerial part.*

**Keywords:** *Echinacea purpurea* (L.) Moench, *Echinacea pallida* (Nutt.), radiation doses, antioxidants, flavonoids, total phenols

*Echinacea* species are subject of intensive research studies due to their high therapeutically quality. They originate from North America being also cultivated in our country [1], especially in the lower areas with dry soil, for *E. angustifolia* and *E. pallida*, and in the humid areas, for *E. purpurea* [2].

In the vegetation period, *Echinacea* species are attacked by pathogens from the following groups of harmful organisms: mycoplasma [3-8], bacteria [9] and fungi [10-21]. These harmful organisms continue to live (in seeds, leaves etc.) after the plant is harvested affecting by this both the quality of therapeutical substances, and the new crops health via infested seeds.

The use *Echinacea* for therapeutical purposes requires healthy organic growth, without using herbicides and insecticides, substances which can be found finally in the treated plant. For the same therapeutical use, it must be considered the different composition of the plant organs.

The presence of a microbiological load in the medicinal plants used for obtaining extracts determines the use of a decontamination method, which should have minimal effect on the content of active compound.

Taking into account the growing tendency of doing ecological agriculture, and considering the requirements of European Community standards, in our days the use of insecticides, fungicides and herbicides becomes more and more an issue that is difficult to solve.

This study proposes the use of a decontamination method for the plants from *Echinacea* species, a method which will affect as less as possible the content in active principles and to have a growing efficiency – the decontamination by treatment with gamma radiation method. The use of this method is regulated in EU and

Romania by the organisms accredited in the field of health and nuclear activities [22,23].

The method of sterilisation by irradiation is used for medical product sterilization [24], treating agricultural products [25,26], food cosmetic products and medicinal plants in order to destroy microorganisms.

The efficacy and safety of this method was investigated for a long time and it was established that it doesn't present any danger for consumers [27,28], the organoleptic properties are not affected, the level of perisability decreases and the influence on active principles is minimal.

The radiosensitivity of microorganisms vary very much as function of the nature of microorganisms [29] and of the extrinsic and intrinsic conditions of keeping and preparation of samples. Therefore, the applied dosage must be established taking into account all these factors.

Low temperatures are increasing the resistance of bacteria to gamma radiations [28],  $D_{10}$  (absorbed dose) for *Salmonella typhimurium* in phosphate buffer is 210 Gy and, for the same bacteria, sampled from fish meat, have a  $D_{10}$  value of 1.74 kGy, i.e. almost ten times higher. The energy of radiation source also have influence on the bacterial population reduction [30].

Since the industry producing food supplements like tea, powder, boxes etc. uses the medicinal plants (or its organs, seeds) in natural condition (dried and minced, eventually), an accesible, i.e. simple and cheap, method of reducing the load in pathogen germs, without affecting the chemical composition, is desirable. The microbiological control of raw material should be also cheap and, if possible simple and fast as well.

The present study establishes the effects of decontamination procedure on the content in active principles with antioxidant activity from plant organs of *E.*

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*purpurea* and aerial part of *E. pallida*. The aim of present work is to find the optimal irradiation dose in order to remove the pathogen microorganisms which leads to diseases like alternariosis and fusariosis.

### Experimental part

The vegetal material was harvested in April 2006, in the fifth year of vegetation, by the National Research and Development Institute for Potato, Sugar Beet and Medicinal Plants Brasov. The study refers to the following two species: seeds and leaves of *E. purpurea* (L.) Moench and aerial part of *E. pallida* (Nutt).

For the quantitative determination of flavonoids and total phenol content, samples of vegetal material were crushed and extracted with ethanol 50% (v/v). In order to limit chemical and enzymatic reaction (especially oxidation), the extraction process was performed at low temperature, i.e. on water bath in which ice was added.

1 g vegetal material was frozen and crushed to a fine paste, afterwards 100 mL ethanol 50% is added, which was first refrigerated to  $-20^{\circ}\text{C}$ . The mixture was homogenized using ULTRATURAX TP 18-10 (model IKA WERK), at 20.000 rpm, for 1 minute. After 15 minutes, the homogenised mixture was centrifugated at 5000 rpm for 5 minutes, in order to remove solid parts from the solution. The supernatant was subsequently filtered. The sediment from centrifugal pipes was further treated with 20mL ethanol 50%, prior refrigerated to  $-20^{\circ}\text{C}$ . The mixture obtained was mixed with a glass rod and centrifuged again afterwards. The second supernatant was mixed together with the first one, after a prior filtering. The volume of extractive solution was brought finally to 100 mL with ethanol 50% and concentrated by rotovaporisation to 20 mL. The extracts were sterilized by filtration and kept subsequently at  $4^{\circ}\text{C}$  until their use.

For determination of flavonoids content a spectrometric method was used. This method is based on the property of flavonoids to react with aluminum chloride in alkaline medium (sodium acetate) [31]. After reaction, it results a stable and yellow chelate compound. This chelate has maximum absorbance at 430 nm. For spectrometric determination solutions were prepared as follows: 1 mL extractive solution was mixed with 5 mL sodium acetate solution 10% and 3 mL aluminium chloride 2.5%. Finally, ethanol 50% is added up to a volume of 25 mL, and afterwards the absorbance of the solution is measured. Quantitative determinations were made by standard calibration curve.

For the determination of total phenols a spectrometric method was, as well, used. The method is based on the property of these phenols to reduce the sodium phosphowolframate (Folin Ciocalteu) [32-34] in alkaline medium at blue wolfram oxide. The maximum absorbance of the reaction product corresponds to the wavelength of 750 nm. The procedure for preparing the solutions whose absorbance was further measured is: 1 ml extractive solution was mixed with 1 mL Folin - Ciocalteu reagent and completed afterwards with a solution of sodium carbonate 5%, up to 25 mL. The control sample was prepared by mixing 1 mL extractive solution with 8 ml distilled water and further completed with sodium carbonate solution 5% up to 25 mL final volume. The total phenols content was determined by reporting to caffeic acid standard calibration curve.

The HPLC analysis was performed on Hewlett-Packard (USA) Model 1050 HPLC equipped with the Rheodyne injector according to a standard method well described in the literature [35-37]. The separation column was

Lichrospher RP-18 column (125mm x 4mm i.d., particle size  $5\mu\text{m}$ ), column temperature  $26^{\circ}\text{C}$ , and the detector was an UV-VIS one with variable-wavelength. The chromatograms were recorded at 330 nm. The mobile phase was aqueous formic acid solution 5% (A) and acetonitrile (B). The total flow-rate was  $1.5\text{ cm}^3/\text{min}$  and the injection volume was  $20\mu\text{L}$ . Each sample was injected three times.

*Echinacea* samples were exposed to a standard source of Co-60 with an activity of 125 000 Ci at the IRASM irradiator, SVST Co-60/B, and were irradiated in special containers, which are moving in sequential positions around radioactive source at  $19^{\circ}\text{C}$  temperature. At each position around the source, the samples received a part of total dose [24]. The photon energy of the radiation source is 1250 keV.

The amount of each sample was 1g. The study was performed for two applied doses: 1 kGy and 10 kGy. The highest dose (i.e. 10 kGy) is the maximum allowed for medicinal plants treatment for decontamination with ionizing radiation. This value is specified by EU regulations [22,23]. In USA, the maximum dose of ionizing radiation used for medicinal plants treatment for decontamination purposes is higher, i.e. 30 kGy [38]. In Romania this activity is regulated by laws and rules, which complies with European regulations [39].

The chemical analysis of samples was done 7 days after the irradiation process. Six samples from leaves, seeds and aerial part were exposed to each dose of radiation. Three samples from each king, i.e. leaves, seeds and aerial part were analysed by both spectrophotometric and HPLC methods. The values presented in the results section are average values of these measurements. For all measurements, the SD was less than 10%.

The dosimetric system (IRASM Romania) was based on ECB (ethanol-chlorbenzen) being traceable to the reference laboratory from EU - HDRL RISO, Denmark.

### Results and discussions

The leaves, seeds and aerial part of *Echinacea* contain a variety of chemical compounds from which two classes of compounds (flavonoids and total phenols) are the subject of this study due to their therapeutic activity as antioxidants.

Table 1 presents the results obtained for the total content of flavonoids and polyphenols in leaves of *E. purpurea*, fifth year of vegetation.

Comparing the results obtained from the analysis of total content of flavonoids and total phenols in non-irradiated leaves, which were previously published in [35], with the values obtained after the irradiation, one can observe a decrease in flavonoids and total phenols in the case of *E. purpurea*, for both 1 kGy dose and 10 kGy doses.

In the case of flavonoids, the concentration decrease is roughly the same, irrespective of dose, of about 14.56% vs. non-irradiated sample. The difference in content from 1 to 10 kGy is not significant. For total phenols content, the situation is similar, i.e. the concentration decrease are 37.87% for 1 kGy and 40.19% for 10 kGy doses.

Relative high dose of radiation are used in for plant microbiological sterilisation. Low dose of radiation are used in stimulate growth function. Gamma radiation leads, in the case of *E. purpurea*, to an increase in DNA content at a dose of 120 Gy and in the case of *Hypericum perforatum* similar change takes place at 30 Gy [40].

Concerning the influence of high doses of gamma radiation on antioxidant compounds content, for the seeds of *Echinacea* species, the literature is scarce. The existing data are referring to pholiar mycoflora [41], germination

**Table 1**  
TOTAL PHENOLS AND FLAVONOIDS IN THE SPECIES *E. PURPUREA* (FIFTH YEAR OF VEGETATION) IN LEAVES, BEFORE AND AFTER GAMMA RAY IRRADIATION (1250keV)

Part of plant / <i>E. purpurea</i>	Irradiation dose	Active compound	
		Flavonoids, %(w/w) (as routine)	Total phenols, %(w/w) (as caffeic acid)
Leaves	0K Gy	0.309	1.331
	1K Gy	0.264	0.827
	10K Gy	0.268	0.796

**Table 2**  
THE TOTAL PHENOLS AND FLAVONOIDS FROM SEEDS OF *E. PURPUREA* (fifth YEAR OF VEGETATION), BEFORE AND AFTER DECONTAMINATION WITH 1250KEV GAMMA RADIATION

Part of plant / <i>E. purpurea</i>	Irradiation dose	Active compound	
		Flavonoids, %(w/w) (as routine)	Total phenols, %(w/w) (as caffeic acid)
Seeds	0 kGy	0.530	0.644
	1 kGy	0.184	0.719
	10 kGy	0.159	0.693

**Table 3**  
CONTENT OF PHENOLS AND FLAVONOIDS IN THE SPECIES *E. PURPUREA* (FIFTH YEAR OF VEGETATION) IN LEAVES, AT DIFFERENT IRRADIATING DOSES, BEFORE AND AFTER DECONTAMINATION WITH GAMMA RAY

Part of plant / <i>E. pallida</i>	Irradiation dose	Active compound	
		Flavonoids, % (w/w) (as routine)	Total phenols, % (w/w) (as caffeic acid)
Aerial part	0 kGy	0.198	0.654
	1 kGy	0.127	0.550
	10 kGy	0.124	0.170

process and water content [42,43] or to enzymatic activity of the plant [44].

The irradiation of *E. purpurea* seeds samples leads to a significant decrease in flavonoids content for both 1 and 10 kGy doses (table 2). For 1 kGy dose the decrease is about 65% and for 10 kGy the decrease is  $\approx 78\%$  with respect to non-irradiated samples.

One can observe an increase in total phenols content for both doses ( $\approx 10\%$  at 1kGy and  $\approx 7\%$  at 10 kGy vs. control sample).

In the case of aerial part of *E. pallida*, gamma radiation leads, for both doses, to a decrease in flavonoids content and total phenols content, as compared to non-treated sample.

The flavonoids content decreases with  $\approx 35\%$  and  $\approx 39\%$  at 1 kGy and 10 kGy, respectively, when compared to non-irradiated sample. Regarding total phenols, the decrease is  $\approx 15\%$  for 1 kGy dose and  $\approx 20\%$  for 10 kGy dose, as compared with non-irradiated sample.

As the results show, for the 10 kGy dose one can observe small quantitative changes when compared with the case of 1 kGy: a decrease of only  $\approx 2\%$  for flavonoids content and a decrease of  $\approx 6\%$  for total phenols content, respectively. The content in flavonoids and total phenols is decreasing insignificantly at both radiation doses.

Radiation doses are influencing in a different way the content in chemical substances from plants. Low doses of

radiation (10 Gy) determine the increase of concentrations for compounds like carbohydrates and fats. Upon increasing the radiation dose to 80 Gy, a decrease of these compounds concentration can be observed. Concentration of raw proteins are decreasing regardless of dose used [45].

Irradiation of the samples like cinnamon, oregano, parsley, rosemary, bird pepper, sage, with a dose of 10 kGy causes a decrease of carotenoid content [46]. For similar conditions of treatment, a significant decrease is reported in the case of total ascorbate from black pepper, oregano, sage, nutmeg [46].

The results of HPLC analysis of alcoholic extracts obtained from *E. purpurea* leaves are presented in table 4. In Figures 1 and 2 are presented the chromatograms of alcoholic extracts obtained from leaves irradiated at 1 kGy and 10 kGy doses.

The chlorogenic acid is present in leaves in quantities larger than caffeic acid. This proportion remains roughly the same after irradiation. The treatment with gamma radiation leads to decrease of both chlorogenic acid and caffeic acid content. For 1 kGy dose, the decrease of chlorogenic acid is  $\approx 30\%$ , and for caffeic acid the decrease is  $\approx 58\%$ . A major decrease is observed for 10 kGy dose and both acids:  $\approx 93\%$  for chlorogenic acid and  $\approx 83\%$  for caffeic acid.

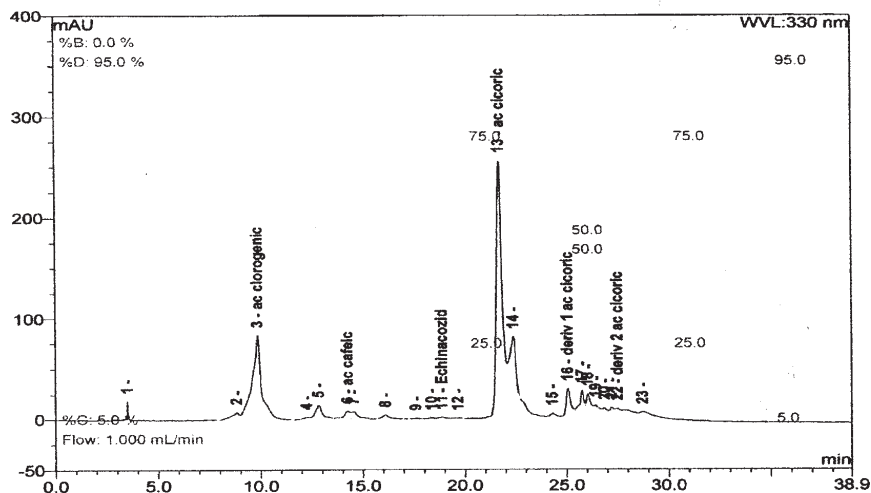


Fig. 1. The chromatogram of the alcoholic extract obtained from *E. purpurea* leaves, irradiated at 10 kGy.

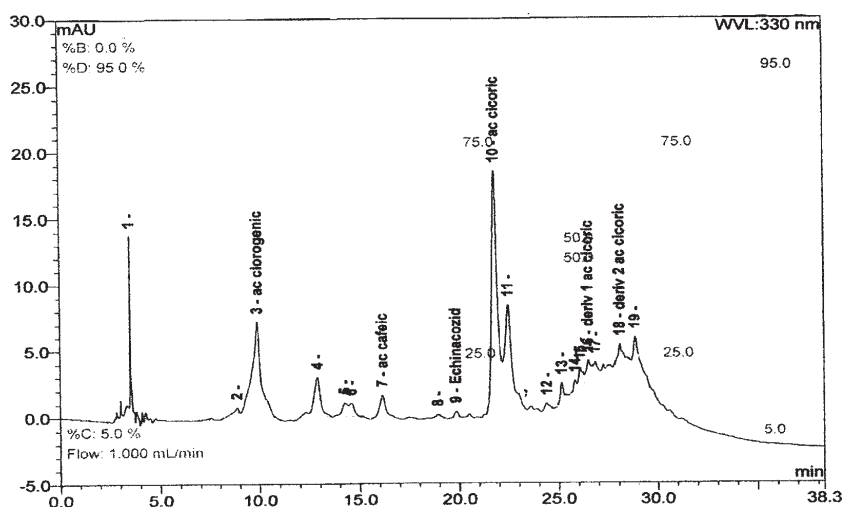


Fig. 2. The chromatogram of the alcoholic extract obtained from *E. purpurea* leaves, irradiated at 10 kGy.

Table 4

THE RESULTS OF HPLC ANALYSIS OF ALCOHOLIC EXTRACTS OBTAINED FROM LEAVES. THE RESULTS CORRESPOND TO SAMPLES FROM *E. PURPUREA* (FIFTH YEAR OF VEGETATION) FOR THE FOLLOWING CASES: NON-IRRADIATED, IRRADIATED AT 1 KGy AND 10 KGy RESPECTIVELY

Vegetative organ/ <i>E. purpurea</i>	Irradiation dose	Active compound	
		chlorogenic acid % (w/w)	caffeic acid % (w/w)
Leaves	0 kGy	0.911	0.024
	1 kGy	0.640	0.010
	10 kGy	0.062	0.004

The results of HPLC analysis on alcoholic extracts obtained from irradiated seeds at 1 kGy and 10 kGy from *E. purpurea* (fifth year vegetation) are presented in table 5.

After irradiation, for both 1 and 10 kGy doses, the chlorogenic and caffeic acids were found in the seed extracts in quantities larger than the case of non-irradiated samples. The increase is bigger for the 1 kGy dose. After irradiation, the content of the both acids decreases in leaves extract and increases in the case of seeds. For seed samples, after irradiation, the amount of both both caffeic and chlorogenic acids increases, the effect being more pronounced for the 1 kGy irradiation dose. Table 6 shows

the results of HPLC analysis of alcoholic extracts obtained from *E. pallida*.

For the aerial part of *E. pallida* irradiated (Table 6), one can observe a decrease in the chlorogenic acid content and an increase in content of caffeic acid. A possible explanation of this fact would be the conversion of a part of chlorogenic acid into caffeic acid upon gamma ray irradiation. Similar studies performed on *Mikania glomerata* can be found in literature [47], where ratio between coumarin and o-coumaric acid was monitored after irradiation [47]. Thus, samples of *Mikania glomerata*

**Table 5**  
RESULTS OF HPLC ANALYSIS OF ALCOHOLIC EXTRACTS OBTAINED FROM SEEDS NON-IRRADIATED, IRRADIATED AT 1 KGy AND 10 KGy DOSES, FOR *E. PURPUREA* (FIFTH YEAR OF VEGETATION)

Vegetative organ/ <i>E. purpurea</i>	Irradiation dose	Active compound	
		% chlorogenic acid (w/w)	% caffeic acid (w/w)
Seeds	0 kGy	0.207	0.014
	1 kGy	0.273	0.170
	10 kGy	0.225	0.130

**Table 6**  
RESULTS OF HPLC ANALYSIS OF ALCOHOLIC EXTRACTS FROM *E. PALLIDA* (FIFTH YEAR OF VEGETATION), OBTAINED FROM AERIAL PART, NON-IRRADIATED, IRRADIATED AT 1 AND 10 KGy RESPECTIVELY

Part of plant / <i>E. pallida</i>	Irradiation dose	Active compound	
		chlorogenic acid % (w/w)	caffeic acid % (w/w)
Aerial part	0 kGy	0.380	0.024
	1 kGy	0.290	0.040
	10 kGy	0.231	0.054

irradiated with 3.5 kGy and 5 kGy doses maintain the same profile of chromatograms [47].

The content in  $\alpha$ -tocopherol,  $\gamma$ -tocopherol,  $\delta$ -tocopherol in olive oil, soy and sunflower decreases gradually as the dose is increases from 1 to 3 kGy [48]. In case of extracts obtained from the root of liquorice, for doses higher than 10 kGy the  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$  concentrations are lower while concentrations in glycyrrhizic acid and maltose increase even at 5 kGy [49].

### Conclusions

Radiation doses used for microbiological sterilization of *Echinacea* species are affecting differently their content in compounds with antioxidant properties. The results of this study shows that the irradiation effect is strongly dependent on the species of plant and on which part of plant is subject of irradiation with gamma ray. Thus, the 1 kGy radiation dose leads to a decrease in flavonoids and total phenols content from leaves and an increase in flavonoids and total phenols content for samples from seeds. The quantities of chlorogenic and caffeic acids are increasing in case of extracts obtained from seeds, especially at 1 kGy, and are considerably decreasing in leaves samples from *Echinacea purpurea*. A major decrease is observed at 10 kGy for both acids. For the aerial part of *Echinacea pallida*, the increase of the radiation dose leads to the decrease of the chlorogenic and caffeic acids content.

1 kGy is the optimal irradiation dose for sterilization of *Echinacea purpurea* leaves and seeds and *Echinacea pallida* aerial part, harvested in Romania, in the fifth year of vegetation.

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