ACCELERATORS USE FOR IRRADIATION OF FRESH MEDICINAL HERBS

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Abstract

The paper presents the results regarding the electron beam irradiation of fresh *Salvia Officinalis* and *Calendula Officinalis*. Irradiation is already a well-known decontamination method, but it received less attention for medicinal plants, especially on fresh herbs. Microbial load behaviour, antioxidant activity, and enzymatic inhibition activity were measured for doses up to 50 kGy. Herbs are decontaminated without any important alteration in the active principles up to 1 kGy, but they loose their fresh aspect easier than non-irradiated ones. The last effect could be useful for the extracting process in which herbs are stressed anyway.

INTRODUCTION

Microbiological contamination of medicinal herbs is a serious problem in the production of therapeutical preparations. A good quality of the product according to pharmaceutical requirements may be achieved by different methods of decontamination. Decontamination treatments should be fast and effective against all microorganisms [1].

The conventional methods of decontamination were fumigation with gaseous ethylene oxide or methyl bromide, which are now prohibited or being increasingly restricted in most advanced countries for health, environmental or occupational safety reasons. [2, 3]

Treatment by ionising radiation is already a well-known decontamination method [4], but it received less attention for medicinal plants [5], especially on fresh herbs. Irradiation is technically feasible, very effective and friendly enough to environment process.

The paper presents the results regarding the electron beam irradiation of some fresh herbals, *Salvia Officinalis* and *Calendula Officinalis*.

EXPERIMENTAL

Samples

Fresh unwashed green herbs, *Salvia Officinalis* and *Calendula Officinalis* were purchased from Hofigal S. A., which is the main Romanian manufacturer of natural products.

Irradiation

The samples were irradiated in plastic package at room temperature in accelerated electron beams from a linear electron accelerator (ALIN – 10) built in our institute. The ALIN-10 electron accelerator is of travelling-wave type, operating at a wavelength of 10 cm and it generates electron beam pulses of 4 μs duration, 6 MeV mean energy and 75 mA current peak intensity, at a repetition frequency of 50 Hz to 100 Hz. The dose rate at 50 cm from the scattered exit window is up to 4.6 kGy/minute. It was obtained doses up to 50 kGy.

Sample analysis

a. Microbiological analysis: Microbial contamination was measured according to the method described by Romanian Pharmacopoeia [6].

b. Antioxidant activity determination: Antioxidant activity was identified by means of biochemical measurement of lipid peroxidation (LPO) and both enzymatic (superoxide dismutase – SOD) in guinea pig brain homogenate. Inhibition of lipid peroxidation was estimated using a technique based on the measurement of the malonaldehyde concentration using thiobarbituric acid (TBA), which generates a colored product. The spectrophotometrical detection of this colored product was made at $\lambda = 532$ nm. Activity of SOD (EC. 1.15.1.1) was determined by standard method, following the catching action of free radicals in a generating system of them.

c. Inhibition of some enzymes: Inhibition of hyaluronidase was performed by ELISA method using hyaluronic acid as substrat and it is quantified spectrophotometrically at $\lambda = 595$ nm. Phospholipase A_2 (PLA2) inhibition were performed macroscopically using lechitin included in an agarose gel.

RESULTS AND DISSCUSSION

Microbial load determination

Fresh *Salvia Officinalis* had $1x10^4$ microorganisms/g, and these microorganisms were identified as bacteria and moulds (*Rhizopus*, *Mucor* and *Penicilium*). After irradiation with 0.2 kGy, the bacteria were decreased at $1x10^3$/g and moulds were destroyed. No microorganisms were survived after irradiation with 0.5 kGy (see Table 1).

Table 1: Microbial load for *Salvia officinalis*

Dose, kGy	0	0.2	0.5	1	2	5	10	20	50
Microbial load/g	10^4	10^3	0	0	0	0	0	0	0

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Calendula Officinalis had a high microbial load ($1x10^5$ microorganisms/g). 1 kGy irradiation reduced the diameter of colonies and the number of microorganisms at $1x10^3$/g (see Table 2). After 10 kGy dose, the microorganism number did not decrease under $1x10^3$/g, but the colonies had a point-like aspect.

Table 2: Microbial load for *Calendula officinalis*

Dose, kGy	0	1	2	5	10	20
Microbial load/ g	10^5	10^3	10^3	10^3	10^3	10^3

Antioxidant activity analysis

Antioxidant activity of an extract is higher when malonaldehyde quantity is lower. Thus, it is reduced the peroxidation action of ascorbic acid.

For *Salvia Officinalis* extract of 7.5 mg/ml concentration, the LPO decreased suddenly from 74% to 49% at 1 kGy irradiation. The decreasing had small variations for doses up to 50 kGy comparative with 1 kGy (see Fig. 1). The LPO for 3.75 mg/ml concentration had no important decrease after irradiation.

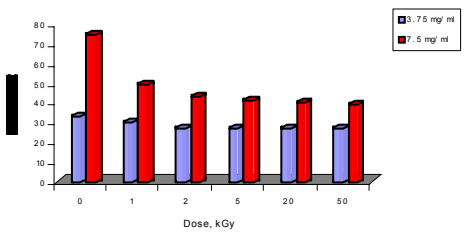

Figure 1: Lipid peroxidation of *Salvia Officinalis* extract

For *Calendula Officinalis* extract of 7.5 mg/ml concentration, the LPO decreased slowly with dose from 68% to 40% at 20 kGy (see Fig. 2). The LPO for 3.75 mg/ml concentration decreased suddenly from 56% to 28% at 1 kGy dose. Then, it decreased slowly with dose.

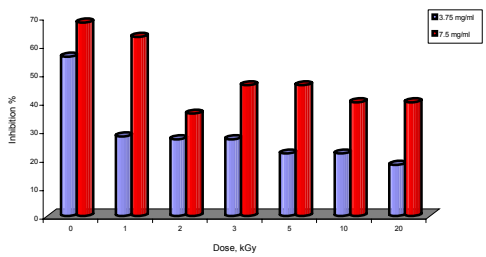

Figure 2: Lipid peroxidation of

Calendula Officinalis extract

Also, certain enzymes from organism are able to capture free radicals being antioxidants, like superoxide dismutase. Such enzymes „like SOD" exist both in *Salvia* and *Calendula*. We observed a significant decrease of SOD for *Salvia* at 1 kGy and after treatment with 20 kGy it had no containt of SOD (see Fig. 3).

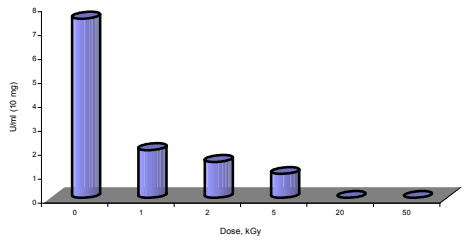

Figure 3: Superoxide dismutase containt of

Salvia Officinalis

Calendula did not show a decrease such significant as *Salvia*, but after 20 kGy dose, we observed a very low SOD containt (see Fig. 4).

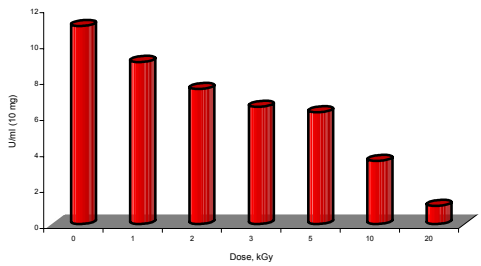

Figure 4: Superoxide dismutase containt of

Calendula Officinalis

Inhibition of some enzymes (hyaluronidase and phospholipase A_2)

Besides the activities mentioned above, both herbals are able to inhibit certain enzymes (hyaluronidase, phospholipase) that appears in some inflamatory processes.

For *Salvia,* the hyaluronidase inhibition is gradually reduced from 43% to 14% at 50 kGy (see Fig. 5).

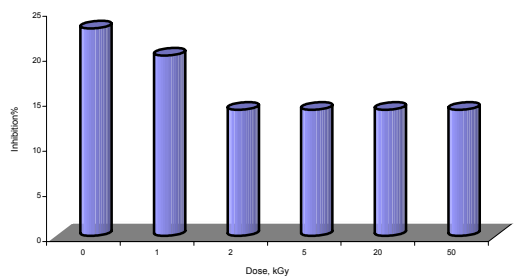

Figure 5: Hyaluronidase inhibition by *Salvia Officinalis*

The hyaluronidase inhibition by *Calendula* is seriously affected after irradiation, being reduced from 40% to 12% even at 1 kGy dose (see Fig. 6).

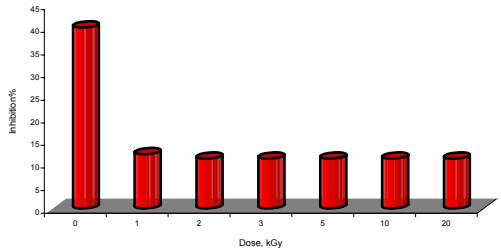

Figure 6: Hyaluronidase inhibition by

Calendula Officinalis

The PLA2 inhibition is observed only for *Salvia Officinalis* and it is significantly reduced with irradiation dose.

CONCLUSIONS

Microbial load was reduced under permissible level according to Romanian Pharmacopoeia for 1 kGy dose for both herbs. *Salvia*, known as a plant with antiseptic activity, was totally decontaminated even after 0.5 kGy.

Antioxidant activity and inhibition of certain enzymes of irradiated *Salvia* and *Calendula* had no significant changes for low doses up to 1 kGy, but these changes can be acceptable for doses up to 3 kGy.

Consequently, we consider that electron beam irradiation can be applied for microbial decontamination of fresh medicinal herbs like *Salvia Officinalis* and *Calendula Officinalis*, keeping their active principles.

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