

THE INFLUENCE OF THE TREATMENT WITH GAMMA RADIATIONS ON THE CONTENT OF THE ACTIVE PRINCIPLES TO THE *HYPERICUM PERFORATUM* L. SPECIES

DANIELA LUMINIȚA ICHIM^{1*}, GOGU GHIORGHITĂ²,
DANIELA NICUȚĂ², ELVIRA GILLE³

Keywords: *Hypericum perforatum* L., gamma radiations, active principles

Abstract: The 100Gy gamma radiations determined the increase of the synthesis of some active principles (hypericin, volatile oils and flavones).

INTRODUCTION

Hypericum perforatum L. is a famous medicinal herb, well-known in ethnobotany for its cholagogue, choloretic, healing and antimicrobial features. Nowadays in the modern medicine, the herb is recommended especially for its antidepressive effect and it is studied for its antiviral and antitumorale effects. We used gamma radiations in order to increase the variability of this species for the test of its radiosensitivity, being known the interest in the use of the physical mutagenic agents in the improvement of medicinal herbs.

Purpose of the researches

The emphases of the effect produced by gamma radiations on the content of active principles to the *Hypericum perforatum* L. species.

MATERIALS AND METHODS

The biological material, represented by seeds of *Hypericum perforatum* L. from the Agricultural and Zootechnic Research Centre of Secuieni-Neamț, the 2000 harvest was irradiated in the Nuclear Department of ICCF, Bucharest. The flow of the irradiation source was 5Gy/min, and the irradiation doses were 10Gy, 30Gy, 50Gy, 80Gy, 100Gy, 120Gy, 150Gy. The seeds were spread in an experimental field in the Botanical garden, in lassy and there were taken samples of the second year of vegetation to determine the active principles. The dose of polyphenols and of flavones was realised in accordance with the methods used in the 10th edition (1998) of the Romanian Pharmacopoeia and the hypericin dose was realised by means of the extraction with ethylic ether and 5% dimethyl sulphoxyde ethanolic solution (DMSO) and with the reading of the absorption to $\lambda=55\text{nm}$. It used the Neo Clevenger method to determine the whole quantity of the volatile oils.

Polyphenols, flavones, and hypericin quantitative estimation was effectuated by thin layer chromatography, on realised on 60 F 254 Kieselgel (Merck)

After etalon and samples spot application, the chromatographic plates were introduced in developing tanks containing special solution for migration.

Chlorogenic acid, caffeic acid, quercitol, rutoside, kaempherol, luteoline, and epigenin were used as standards. The substances identification was realised by comparing, on same chromatogram, of spot fluorescence and of Rf values on analysed samples, comparing to etalon.

RESULTS AND DISSCUSIONS

The phytochemical analysis was represented in *Hypericum perforatum* L's case by dosing the hypericin, the volatile oils, the flavones (rutozid) and the polyphenols (the cafeic acid), taking into consideration the complex composition of the herb which gives variations phytotherapeutical feature.

The gamma radiation had a negative influence to lower doses on the biosynthesis of hypericin and a stimulative effect to higher one can notice an increase to the content of hypericin to the variants 80Gy and 100Gy (36.6%, respectively 42.98%, comparatively to control) and the recording of maximum value to 150Gy (52.77%) comparatively to control.

If the content of hypericin is related to 50Gy to control and the 120 variants, it will record a decrease with 14.47% to the dose of 10Gy, comparatively to control.

The biosynthesis of volatile oils was inhibitor by the irradiation doses used, especially by 150Gy one, were it was recorded a minimum value (28.77%, comparatively to control).

The 100Gy dose has represented an exception, determining an increase in the content of essential oils with 7.78% comparatively to control

As it concerns the content of polyphenols (the cafeic acid) the gamma radiations had an inhibitive effect in the doses used. The decrease of the content of polyphenols has oscillated in the variants treated with doses between 5.2% (30Gy) and 47% (120Gy and 150Gy) with the exception of 10Gy dose were it was recorded an increase of 13.93% comparatively to control.

When analysing the Figure 1, one can notice a decrease in the content of flavones (rutozid) after the action of the physical mutagen agent.

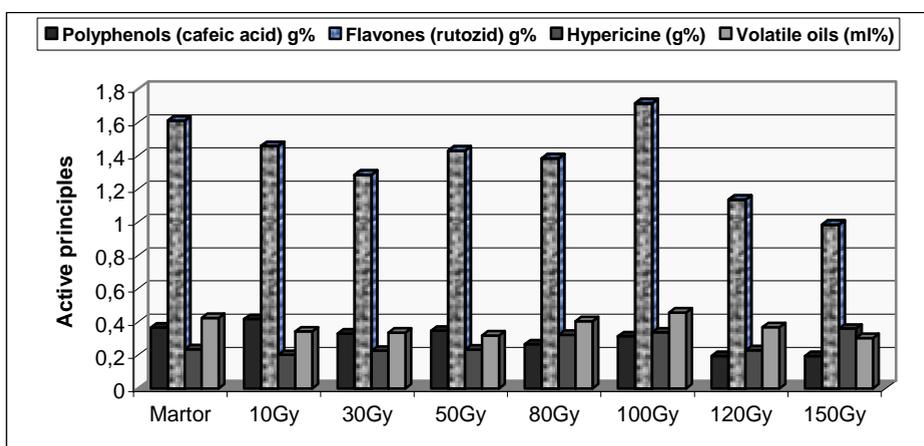


Figure 1. The influence of gamma radiations the treatment with on the content of active principles to herbs of *Hypericum perforatum* L in M₁.

Thus if decrease is only 9.5% comparatively to control in 10Gy dose, it will become important to higher doses of radiation as 120Gy and 150Gy ones (29.55%, respectively 38.86% comparatively to control). It was recorded an increase with 6.52% in the content of flavones, in 100Gy variant, comparatively to control.

We have mentioned, taking in consideration our experimental results of the analysis, the dose of 100Gy for the increase in the content of hypericin, volatile oils and flavones and the dose of 150Gy for the content of hypericin. Tóth et al., 2003 achieved the same results in the same experiments concerning the content in hypericin to 150Gy (0.161g% comparatively to 0.113g% to control).

As it concerns the content of volatile oils, in the same dose (150Gy) it demonstrated a stimulative effect comparatively to our results, the effect was an inhibitive one in the same dose (28.77%, comparatively to control).

The experimental doses presented in here about the content in active principles have demonstrated the fact that it deserves to be taken into consideration the possibility to increase the variability of medicinal herbs by using the physical mutagenic agents.

The layer chromatography was used to evaluate the presence of polyphenols, flavones, and hypericin, in control and in treated variants (10Gy, 50Gy, 80Gy, 100Gy, 120Gy, and 150Gy) (Fig. 2 and 3).

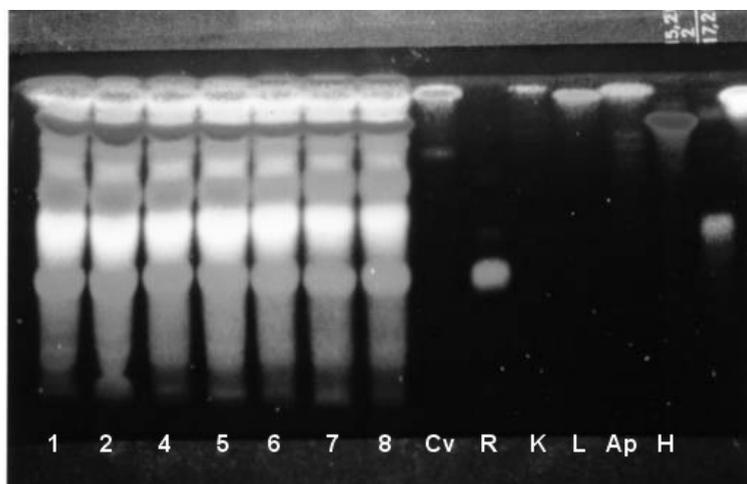


Fig. 2. Chromatogram of flavones and hypericin content in *Hypericum perforatum* L. (1 - control; 2 - 10Gy; 4 - 50Gy; 5 - 80Gy; 6 - 100Gy; 7 - 120; 8 - 150Gy; Cv - quercitol; R - rutoside; K - kaempherol; L - luteoline; Ap - apigenine; H - hypericin)

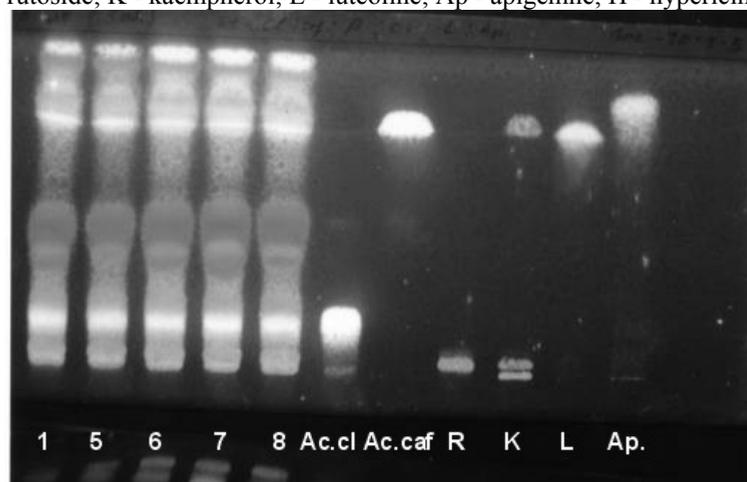


Fig. 3 Chromatogram of polyphenols and flavones content in *Hypericum perforatum* L. (1 - control; 5 - 80Gy; 6-100Gy; 7 - 120Gy; 8 - 150Gy; Ac cl. - chlorogenic acid; Ac caf. - caffeic acid; R - rutoside; K - kaempherol; L - luteoline; Ap - apigenine)

Comparatively to control, in treated variants, the chromatogram analysed evidences differences regarding the colour intensity and spot fluorescence, the differences evaluated by quantitative determinations (Fig. 1)

CONCLUSIONS

The 100Gy dose of radiation stimulated the synthesis of some active principles (the hypericin, the volatile oils, the flavones) to *Hypericum perforatum* L.

The establishment of the content hypericin emphasized a maximum value in 150Gy dose.

Gamma radiations had an inhibitive effect on the content of polyphenols (the caffeic acid) to *Hypericum perforatum* L., excepting the 10Gy dose.

REFERENCES

1. Bombardelli E., Morazzoni P., 1995. *Fitoterapia*, LXVI,1: 43-68.
2. Chatterjee S.S., Bhattacharya S.K. Wonnemann M., Singer A., Muller W.E., 1998. *Life Sci*, 63(6): 499-510.
3. Grigorescu E., Lazăr M. I., Stănescu Ursula Helena, Ciulei I., 2001. *Index fitoterapeutic*, Iași: 191 – 193; 268 – 272.
4. Osińska E., Lotocka B., 2005. XVII International Botanical Congress - Abstracts, Vienna, Austria: 282.
5. Tóth-Tünde Ecaterina, Gille Elvira, Floria Fl., 2003. Proceedings of the 8th national symposium, „Medicinal plants - present and perspectives”, 28 – 29 august. Editura Alma Mater, Bacău: 89-91.

1 – University „Al. I. Cuza” Iași, Faculty of Biology

2 – University of Bacău

3 – „Stejarul” Research Centre, Piatra Neamț

* - danielaluminitaichim@yahoo.com