ICEH CISA PORTO 2014

3rd INTERNATIONAL CONGRESS ON ENVIRONMENTAL HEALTH 2014

PORTO, 24th to 26th SEPTEMBER 2014

PROCEEDINGS BOOK

EMERGING RISKS AND CHALLENGES ON ENVIRONMENT, HEALTH AND SAFETY

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ESTSP | POLITÉCNICO DO PORTO
Escola Superior de Tecnologia da Saúde

Gamma irradiation effects on microbial inactivation and antioxidant activity of *Melissa officinalis*

Authors: Márcia Meneses[1,2], Joana Madureira[1], Rita Melo[1], Amilcar L. Antonio[1,3], Sandra Cabo Verde[1]

1 *Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico, Universidade de Lisboa, Portugal*
2 *Environmental Health RG - Lisbon School of Health Technology - Polytechnique Institute of Lisbon, Portugal*
3 *Centro de Investigação de Montanha (CIMO), ESA, Instituto Politécnico de Bragança, Portugal.*

Presenting Author: Email: marcia17meneses@gmail.com | Tel.: +351 911 880 273 / 963 485 468

INTRODUCTION:

Many herbal products are traditionally being used as medicines and nutraceuticals in different regions of the world (Kumar et al., 2010). This increased consumption of natural products has become a public health problem. The concern in the safety of these products is due, in part, to the possible presence of pathogenic bacteria and fungi producing mycotoxins (Prado et al., 2009). Consequently the evaluations of the hygienic quality of medicinal plants, as well as the use of decontamination methods are significant steps towards the consumer safety and therapeutical efficiency (Soriani et al., 2005).

OBJECTIVES:

The aim of this study is to assess the effects of gamma irradiation on the microbial burden and antioxidant activity of medicinal plants, namely *Melissa officinalis*.

MATERIALS AND METHODS:

Samples of dried *Melissa officinalis* were irradiated in Co-60 experimental equipment (Precisa 22) located at the Campus Tecnológico e Nuclear, Instituto Superior Técnico, Sacavém, Portugal. The applied gamma radiation doses were 1; 2; and 4 kGy at a dose rate of 1.20 kGy/h. Non-irradiated samples followed all the experiments. Regarding the microbiological analyses, dried plant samples were blended on 100 mL of physiological solution with 0.1% of Tween 80. The samples were homogenized in a stomacher equipment during 15 minutes and filtrated using nitrocellulose membranes with a pore size of 45 m. Bacterial and fungal counts were carried out, in triplicate, on Tryptone Soya Agar (TSA) and Malt Extract Agar (MEA) at 30 ºC during 7 days. Microbiological counts were expressed as log colony forming units per gram. Morphological identification of fungi was achieved through macro and microscopic characteristics as proposed by specific atlas for fungal identification. Concerning the mesophilic bacterial isolates, all colonies were macroscopically, microscopically and biochemically typed by gram staining, catalase activity and oxidase test. The frequency of each phenotype was calculated based on the number of isolates and their characterization. Regarding to antioxidant activity, FRAP and DPPH assays were determined. For those experiments, water extract of *M. officinalis* (WEM) and ethanol extract of *M. officinalis* (EEM) were prepared. For preparation of WEM, 80 mg of each irradiated sample was added to 800 L bi-distilled water and the mixture was boiled for 2 min and stirred for 30 seconds. Concerning EEM, 80 mg of each irradiated sample was added to 800 L ethanol and this mixture was stirred for 30 seconds. The FRAP assay was carried out according to the method described by Benzie and Strain (1996). The free radical scavenging activity of the antioxidants of WEM and EEM based on the scavenging activity of stable DPPH free radical was determined according to the method of Brand-Williams et al, 1995. DPPH assay of *M.officinalis* extracts is ongoing.

RESULTS AND DISCUSSION:

The characterization of dried *M. officinalis* microbiota showed an average bioburden value of 10^2 colony-forming units (CFU)/g and a diverse microbial population predominantly composed by 1 morphological type: gram-positive rods (74%). The inactivation studies of the *M. officinalis* mesophilic population indicated linear inactivation kinetics (Figure 1), with a one log reduction of microbial burden (90% inactivation efficiency) for 4 kGy. The survivor microbiota was mainly constituted by gram-negative rods (75%).

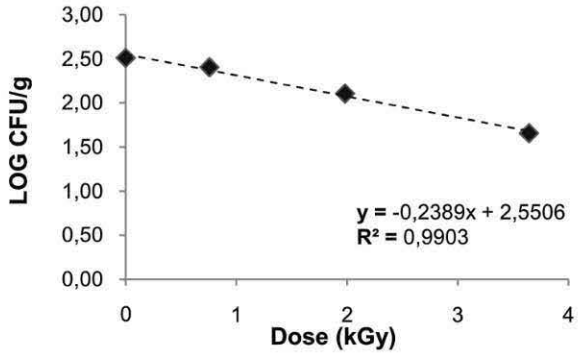

Figure 1 - Gamma radiation inactivation curve for the mesophilic microbial population of dried *M. officinalis.*

Concerning antioxidant activity evaluation, two different methods were applied. FRAP assay express the capability of antioxidants to reduce ferric (Fe^{3+}) ion to ferrous (Fe^{2+}) ion form in ferrous sulphate acidic solution. A higher absorbance at 593 nm indicates a higher ferric reducing power (Koksal et al., 2011). The obtained results are presented in the Figures 2 and 3

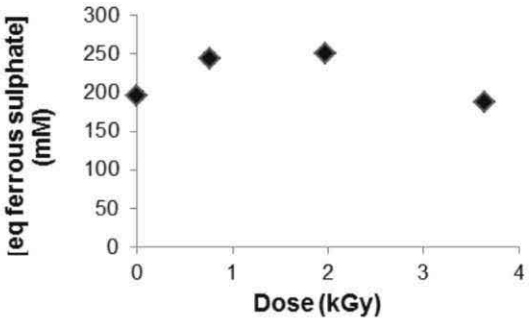

Figure 2 - Reducing power of water extracts of *M. officinalis* by FRAP assays.

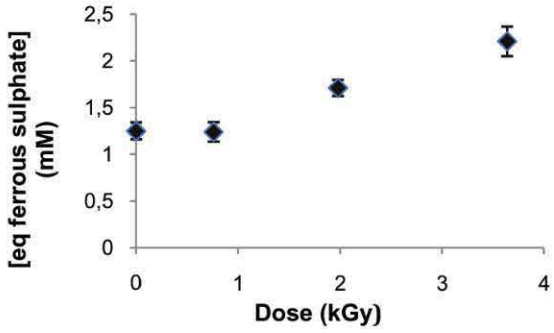

Figure 3 - Reducing power of ethanol extracts of *M. officinalis* by FRAP assays.

As shown in Figure 2 for water *M. officinalis* extract, the antioxidant activity reveals an increase of about 28% at 2 kGy. Regarding ethanol *M. officinalis* extracts, Figure 3 shows an increase of approximately 78% at 4 kGy. However, according to results of the present study, ferric reducing power of WEM was higher than EEM which could be related with antioxidants present in both extracts. This topic as well as DPPH assay is under study.

CONCLUSION:

The obtained results suggested that the gamma irradiation treatment could be advantageous in improving microbial safety of *M. officinalis* with the potential added-benefit of increasing its antioxidant content. The effect of higher irradiation doses on *M. officinalis* will be further investigated, in an attempt to augment the reduction of the microbial population.

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