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Effects of gamma radiation on chemical and antioxidant properties, anti-hepatocellular carcinoma activity and hepatotoxicity of borututu

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ABSTRACT

Borututu is a well-known medicinal plant in Angola for the treatment of liver diseases and for the prophylaxis of malaria. Our research group reported, in a recent study, that its infusion, pills and syrups display significant antioxidant and anti-hepatocellular carcinoma activities. However, during the processing and storage, it can be easily exposed to contamination that can lead to a microbial deterioration or insect infestation compromising its quality, shelf life, and efficiency. Herein, we investigated the effect of gamma irradiation, one of the most promising decontamination methods for many foodstuffs and plant materials, at different doses (1 and 10 kGy) on borututu regarding its nutritional value, lipophilic (fatty acids and tocopherols) and hydrophilic (free sugars and organic acids) compounds, antioxidant and anti-hepatocellular carcinoma activities. In general, the irradiation treatment did not appreciably affect the nutritional value of the studied plant, but the highest energetic contribution (383.83 kcal/100 g), total sugars (8.63 g/100 g), organic acids (3.31 g/100 g dw), total tocopherols (336.72 mg/100 g dw), and PUFA (32.75%) contents were found in the sample irradiated at 10 kGy. Furthermore, this sample presented also the highest levels of total phenolics and flavonoids and, in general, the highest antioxidant activity (EC_{50} values of 0.04 to 0.24 mg/mL for the methanolic extract and 0.03 to 1.34 mg/mL for the infusion). Irradiated samples kept the anti-hepatocellular carcinoma activity, but a decrease was observed in the methanolic extract prepared from sample irradiated at 10 kGy (GI_{50} = 188.97 μg/mL). Overall, gamma irradiation proved to be a suitable technique of preservation of dried herbs without affecting the bioactive compounds.
Industrial relevance: During processing and storage, plants can be easily exposed to contamination that can lead to a microbial deterioration or insect infestation compromising its quality, shelf life, and efficiency. This article highlights gamma irradiation as a suitable technique of preservation of borututu (a widely used dried plant) without significant changes in its bioactive effects.

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1. Introduction

Cochlospermum angolensis Welw. (borututu) is a widespread medicinal plant in Angola, where the barks infusion has been traditionally used by healers for the treatment of many liver diseases and for the prophylaxis of malaria (Poppendieck, 1981; Presber, Herrman, & Hegenscheid, 1991; Silva et al., 2011). Recently, our research group reported that the infusion, pills (Pereira, Calhelha, Barros, & Ferreira, 2013) and syrups (Pereira, Calhelha, Barros, Queiroz, & Ferreira, 2014) of the dry barks display significant antioxidant and anti-hepatocellular carcinoma activities.

However, raw materials from medicinal plants are easily exposed (from the plants themselves, the soil, water, air and dust) to contamination with pathogens during the harvest or the air drying time, and the storage in dried form during long periods, which can lead to a microbial deterioration or insect infestation that decrease herbs quality and shelf life, compromising their efficiency and even posing a public health threat (Pal et al., 2010; Rosa, Medina, & Vivar, 1995). This kind of contamination, particularly with pathogenic non-spore forming, is one of the most significant causes of human suffering all over the world, and according to World Health Organization (WHO), the infectious and parasitic diseases represented the most frequent cause of death (35%) worldwide (Khan & Abrahem, 2010; Loaharanu, 1994). Therefore, to improve the hygienic quality and guarantee the stability of active compounds of the plant materials during storage, making it suitable for human use and commercialization, an adequate technology for decontamination is required (Bhat, Sridhar, & Karim, 2010; Katusin-Razem, Novak, & Razem, 2001; Thomas, Senthilkumar, Kumar, Mandal, & Muraleedharan, 2008).

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Food irradiation arises as one of the most promising decontamination methods for many foodstuffs and plant materials, reducing the reliance on chemical fumigants used by the food and pharmaceutical industries and chemical fumigants like gaseous ethylene oxide or methyl bromide for decontamination or sterilization, that are carcinogenic and increasingly restricted in several countries, due to health, environmental or occupational safety reasons. Among the different types of radiation sources allowed for food processing (gamma, X-ray, UV, electron beam), gamma irradiation represents an effective and environment friendly technology to avoid the re-contamination and re-infestation of the product, since it can be done after packaging (Aouidi, Samia, Hana, Sevastianos, & Moktar, 2011; Khattak, Simpson, & Ihasnullah, 2009). This treatment has been carried out in several plant species and the doses used should guarantee consumer safety without compromising wholesomeness, structural integrity, functional properties, or sensory attributes, being often between 2 and 30 kGy (Khan & Abrahem, 2010; Olson, 1998).

Nevertheless, as far as we know, no studies have been performed on borututu irradiated dry barks. The typical doses for insect disinfestation or parasite inactivation are up to 1 kGy and to reduce or eliminate spoilage or disease causing pathogenic microorganisms the common doses used are up to 10 kGy (Molins, 2001). Therefore, this study was undertaken to investigate the effect of different doses of gamma irradiation (1 and 10 kGy) on this plant regarding to its macronutrients composition, lipophilic (fatty acids and tocopherols) and hydrophilic compounds (free sugars and organic acids). Furthermore, due to its benefits in the prevention/treatment of liver diseases, the irradiated plant infusion and methanolic extract were also assessed to evaluate their antioxidant and anti-hepatocellular carcinoma activity depending on the different radiation doses.

2. Material and methods

2.1. Samples and samples irradiation

C. angolensis Welw. (borututu) was obtained from an herbalist shop, Américo Duarte Paixão Lda., in Alcanede (Portugal), imported from Angola, as dry barks material (the taxonomical identification of the plant species mentioned in the label was confirmed). The samples were divided into three groups: control (non-irradiated, 0 kGy), sample irradiated at 1 kGy, and sample irradiated at 10 kGy, where 1 kGy and 10 kGy were the predicted doses.

The irradiation of the samples was performed in a Co-60 experimental chamber (Precisa 22, Graviner Manufacturing Company Ltd., UK) with four sources, total activity 177 TBq (4.78 kCi), in September 2013, and the estimated dose rate for the irradiation position was obtained with Fricke dosimeter. During irradiation process, the dose was estimated using Amber Perspex routine dosimeters (batch V, from Harwell Company, UK), following the procedure previously described by Fernandes et al. (2013), Fernandes et al. (2013). The estimated doses, dose rates and dose uniformity ratios (D_{max}/D_{min}) were, respectively: 1.20 ± 0.07 kGy, 2.57 ± 0.15 kGy h^{-1}, 1.20 for sample irradiated at 1 kGy and 8.93 ± 0.14 kGy, 1.91 ± 0.03 kGy h^{-1}, 1.02 for sample irradiated at 10 kGy. For simplicity, in the text and tables we considered the values 0, 1 and 10 kGy, for the doses of non-irradiated and irradiated samples 1 and 2, respectively.

After irradiation, the samples were reduced to a fine dried powder (20 mesh) using a grinding mill, and mixed to obtain homogenized samples for subsequent analysis or preparation of infusions/extracts.

2.2. Standards and reagents

2.2.1. For irradiation

To estimate the dose and dose rate of irradiation it was used a chemical solution sensitive to ionizing radiation, Fricke dosimeter, prepared in the lab following the standards (ASTM, 1992) and Amber Perspex dosimeters (batch V, from Harwell Company, UK). To prepare the acid aqueous Fricke dosimeter solution the following reagents were used: ferrous ammonium sulfate(II) hexahydrate, sodium chloride and sulfuric acid, all purchased from Panreac S.A. (Barcelona, Spain) with purity PA (proanalysis), and water treated in a Milli-Q water purification system (Millipore, model A10, Billerica, MA, USA).

2.2.2. For chemical analyses

Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Fisher Scientific (Lisbon, Portugal). Fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also were other individual fatty acid isomers, L-ascorbic acid, tocopherol, sugar and organic acid standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (Pleasant Gap, PA, USA).

2.2.3. For antioxidant activity evaluation

2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was from Sigma (St. Louis, MO, USA).

2.2.4. For anti-hepatocellular carcinoma activity and hepatotoxicity evaluation

ellipticine, phosphate buffered saline (PBS), acetic acid, sulforhodamine B (SRB), trichloroacetic acid (TCA) and Tris were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum (FBS), L-glutamine, Hank's balanced salt solution (HBSS), trypsin-EDTA (ethylenediaminetetraacetic acid), nonessential amino acids solution (2 mM), penicillin/streptomycin solution (100 U/mL and 100 mg/mL, respectively) and DMEM (Dulbecco's Modified Eagle Medium) were from Hyclone (Logan, Utah, USA).

Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.3. Chemical composition

2.3.1. Nutritional value

Protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC, 1995). The samples crude protein content (N × 6.25) was estimated by the Kjeldahl method; the crude fat was determined using a Soxhlet apparatus by extracting a known weight of sample with petroleum ether; the ash content was determined by incineration at 600 ± 15 °C. Total carbohydrates were calculated by difference and total energy was calculated according to the following equations: Energy (kcal) = 4 × (g protein + g carbohydrates) + 9 × (g fat).

2.3.2. Lipophilic compounds

2.3.2.1. Fatty acids. Fatty acids were determined after a lipid extraction of the sample (3 g) using a Soxhlet apparatus with petroleum ether; afterwards a transesterification procedure was applied to the lipid extract as described previously by the authors (Barros, Pereira, Calhelha, et al., 2013), using a gas chromatography equipment (DANI 1000), with a split/splitless injector and a flame ionization detector (GC–FID). Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7). The results were expressed in relative percentage of each fatty acid.

2.3.2.2. Tocopherols. Tocopherols (α-, β-, γ- and δ-tocopherols) were determined following a procedure previously optimized and described by the authors (Barros, Pereira, Calhelha, et al., 2013). Analysis was performed by High Performance Liquid Chromatography (HPLC) system that consisted of an integrated system with a pump

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