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Effects of Gamma Radiation on Microbial Load and Chemical Constituents from Stem Barks of *Luehea ochrophylla*

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Gamma radiation is an effective method for microbial decontamination of plant materials. However, this treatment can induce changes in the chemical structure of plant constituents. Stem barks of *Luehea ochrophylla* were exposed to different doses of gamma radiation to evaluate decontamination efficiency and changes in chemical composition of plant material including phenolic fraction. The major microbial contaminants of non-irradiated samples were isolated and identified as the fungal species *Eurotium chevalieri* L. Mangin and *Lecythophora decumbens*. The dose of 5.0 kGy was effective to achieve total decontamination of the stem barks of *L. ochrophylla*. The formation of free radicals was verified in the plant material using electron paramagnetic resonance spectroscopy, and was supposed to be related to the *trans*-aconitic acid, a plant constituent. It was the only secondary metabolite to have its concentration significantly altered with radiation in phenolic fraction, as observed by liquid chromatography with diode array detector coupled to mass spectroscopy (LC-DAD-MS). The *trans*-aconitic acid was isolated and exposed to gamma radiation in aqueous medium. Its concentration decreased after exposition to a dose of 3.0 kGy, corroborating the supposition of its degradation. Citric acid was the main radiolytic product formed by irradiation of *trans*-aconitic acid in the presence of water.

Keywords: *trans*-aconitic acid, microbial decontamination, medicinal plant, phenolic compounds, radiolytic products

Introduction

A large proportion of the population of the word depends on plants for primary health care. In developing countries, 80% of the population use medicinal plants as the first therapeutic resource, and the interest in herbal medicine is re-emerging in developed countries.[1] However, several issues must be addressed before the use of medicinal plants, such as reliability of plant identification, side effects, and chemical and microbiological contamination. In special, plant constituents are highly susceptible to microbial contamination from irrigation water, soil, harvesting, storage, or processing.[2] Microbial contamination can reduce the quality of the plant interfering with the efficacy and stability of their bioactive compounds.[3] In extreme

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cases, contaminations with microbial pathogens may cause serious diseases in humans.[4]

Microbial decontamination using ethylene oxide or methyl bromide is prohibited by the regulatory agencies in several countries due to environmental and health risks, such as carcinogenic action.[5] In turn, the decontamination using high temperatures may reduce the bioactivity of plant constituents. On the other hand, gamma radiation provides an efficient and eco-friendly method to reduce or eliminate microbial contamination of these plant materials.[6] Although the ideal dose of gamma radiation for decontamination depends upon the microbial load,[7] doses up to 10.0 kGy are usually accepted by international regulatory agencies.[6] However, gamma radiation can also induce changes in the concentration levels, chemical structure, and physical properties of constituents from plant material like tannins, saponins, phenolic, and flavonoids.[8] These facts have increased the scientific interest of the gamma radiation effects on plant materials.[9]

Luhea species (Malvaceae) are found in Central and South America and are popularly known in Brazil as "*açoita-cavalo*".[10] Barks of some *Luhea* species are usually used by popular medicine for the treatment of gangrenous wounds and a large spectrum of diseases, such as arthritis, rheumatism, tumors, and gastric ulcer.[11,12] Previous chemical investigation[13] on *Luhea ochrophylla* reported the isolation of triterpenes, steroids, and flavonoids. The inflammatory effect of this species was reported,[14] an effect mediated by the high inhibition of T cell proliferation. The potential of this plant species instigated the investigation of microbial contaminants, as well as the suitability of gamma radiation in the processing of *L. ochrophylla* towards industrial applications. Therefore, the present work describes the effects of gamma radiation on microbial decontamination of stem barks of *L. ochrophylla*. The effect of different doses of radiation on the plant material was also evaluated. Moreover, the effect of gamma radiation on *trans*-aconitic acid, which was isolated from aqueous extract of this species, has been also studied using experimental analyses and theoretical investigations concerning its reactivity.

Experimental

General

Analyses by high performance liquid chromatography (HPLC) in analytical scale were carried out on a Shimadzu liquid chromatograph (Kyoto, Japan), consisting of two pumps (LC-20AT), UV-Vis detector (SPD-20A), and column ODS Hypersil (C_{18}), 250 mm × 4.6 mm × 5 mm (Supelco, Bellefonte, USA). Aliquots of the samples (20.0 µL) were injected at a flow rate of 0.7 mL min^{-1}. Preparative HPLC was performed on a Shimadzu liquid chromatograph (Kyoto, Japan), consisting of a pump (LC-10AV), UV-Vis detector (SPD-10AV), column Dynamax Microsorb (C_{18}) 10C-5250 × 10.0 mm (Varian, California, USA), and a guard column. Aliquots of the samples (1.0 mL) were applied with flow rate at 4.7 mL min^{-1}, according to the specifications "scale-up" linear of the column. After preparative procedure, the fractions were lyophilized using the Thermo Fisher FR-Drying Digital Unit Scientific (Waltham, Massachusetts, USA). Analyses by liquid chromatography diode array detection mass spectroscopy (LC-DAD-MS) of the *trans*-aconitic acid in aqueous solution were carried out on a Shimadzu liquid chromatograph (Kyoto, Japan), consisting of two pumps (LC-30AD), SPD M-20A diode array detector and column Shim-pack® C_{18} column (250 mm × 4.6 mm × 5.0 mm). Flow rate of 200.0 µL min^{-1} was used and the effluent totally directed to the mass spectrometer (Bruker Daltonics, Billerica, Massachusetts, USA) with electrospray ionization (ESI) in negative mode.

Electron paramagnetic resonance (EPR) spectra were recorded at room temperatura on a Miniscope 400 spectrometer (Magnettech, Berlin, Germany) operating at microwave frequency near 9.4 GHz (X-band). ^{1}H nuclear magnetic resonance (NMR) experiments were performed on Bruker DRX 400 spectrometer (Billerica, Massachusetts, USA) using dimethyl sulfoxide (DMSO-d_6) as solvent. Chemical shifts were measured in parts *per* million (δ) relative to tetramethylsilane (TMS) internal standard.

Samples were exposed to gamma radiation using a Gamma Beam-127 irradiator, model IR-214 (Nordion Inc., Ottava, Canada) equipped with a cobalt-60 source. The dose rate was 2.50 kGy h^{-1} with a dose rate error of ± 0.02 kGy. The irradiation dose of each sample, calibrated with a Fricke standard dosimeter, were varied by changing the exposition time to the source radiation.

The statistical analyses were carried out using Statistica 7.0 software.[15] Differences were tested for significance using the analysis of variance (ANOVA) procedure (R Core Team 2013),[16] with a significance level of $p < 0.05$.

Plant

The plant was collected in the Esmeraldas city, State of Minas Gerais, Brazil for authentication purpose. A voucher specimen of *L. ochrophylla* has been deposited at the Herbarium Dendrológico Jeanine Felfili of Departamento de Engenharia Florestal, Faculdade de Ciências Agrárias, Universidade Federal dos Vales do Jequitinhonha e Mucuri (Diamantina, Brazil), registered under code HDJF2043.

The plant was identified by A. Riguetti Corrêa (Escola Nacional de Botânica Tropical - Instituto de Pesquisa Jardim Botânico, Rio de Janeiro, Brazil). To carry out the present work, fresh *L. ochrophylla* was purchased in the central market of Belo Horizonte city, State of Minas Gerais. The stem barks were dried at room temperature until constant weight (about one week). In sequence, the plant material was powdered.

Irradiation conditions

Samples of powdered bark of *L. ochrophylla* (150.0 g each) were packed in polyethylene bags and exposed to different doses of gamma radiation (0.0, 1.0, 3.0, 5.0, 10.0, and 20.0 kGy, named LO_0, LO_1, LO_3, LO_5, LO_{10}, and LO_{20}, respectively) at room temperature (25 ± 1 °C).

Microbial load

Aliquots (100.0 mg) of LO_0, LO_1, LO_3, LO_5, LO_{10}, and LO_{20} were suspended in 10.0 mL of Sabouraud agar (20.0 g L^{-1}) and vigorously shaken on an orbital shaker for 1 h. Aliquots (1.0 mL) of these suspensions, in three replicates, were transferred to tubes containing 9.0 mL of sterile water and the tubes were vigorously shaken again. Serial dilutions were prepared up to 10^{-5}. Aliquots (0.25 mL) of each dilution were spread across the surface of Petri dishes containing sterile Sabouraud agar. The plates remained at room temperature for 67 h. The number of colonies forming units (CFU g^{-1}) of each sample were counted and calculi were used to determine the microbial load *per* gram of plant material.[17]

The microorganisms present in LO_0 samples (control) were isolated and identified using conventional taxonomy and deoxyribonucleic acid (DNA) sequencing analysis.

Presence of free radicals in plant material

Aliquots (1.0 g) of LO_0, LO_1, LO_3, LO_5, LO_{10}, and LO_{20} were immediately placed in capillaries and introduced in EPR quartz tubes in order to register paramagnetic species.

Phenolic fraction preparation

Aliquots (100.0 mg) of LO_0, LO_1, LO_3, LO_5, LO_{10}, and LO_{20} were suspended in water and submitted to decoction for 4 h at 60 °C. After this period, the samples were filtered and the corresponding aqueous extracts were obtained. These extracts were basified with ammonium hydroxide (until pH 10.0-11.0) and 150.0 mL of a mixture of ethyl acetate:ethyl ether (3:1) solution were added to the aqueous extract. The organic and aqueous phases were separated. Hydrochloric acid (until pH 1.0-2.0) and 150.0 mL of ethyl acetate:diethyl ether (3:1) were added to the aqueous phase. The organic and aqueous phases were separated. The solvent was removed from the organic phase, and the corresponding phenolic fractions (FF) were obtained. Aliquots of FF_0, FF_1, FF_3, FF_5, FF_{10}, and FF_{20}, were subjected to HPLC analyses, during 20.0 min, in isocratic mode, using as mobile phase a mixture of 20% methanol and 80% solution of water:trifluoroacetic acid (99.5:0.5 v/v, pH 4.0).[18]

trans-Aconitic acid

In order to isolate and identify the only chemical constituent (HPLC t_R = 2.88 min) that exhibited concentration changes after the treatment with gamma radiation, stem barks powdered and non-irradiated (1,000.0 g) of *L. ochrophylla* were submitted to decoction with water for 4 h at 60 °C. Subsequently, the same methodology was used to obtain the phenolic preparative fraction (FFp). Aliquots of FFp were subjected by preparative HPLC, in isocratic mode, using as mobile phase a mixture of 20% methanol and 80% solution of water:trifluoroacetic acid (99.5:0.5 v/v, pH 4.0) during 20.0 min. The chemical constituent was isolated and characterized by spectroscopic analysis as *trans*-aconitic acid (Figure S1, presented in Supplementary Information (SI) section).

Effects of the gamma radiation on *trans*-aconitic acid

Samples of powdered *trans*-aconitic acid (1.0 mg) and samples of *trans*-aconitic acid dissolved in water (1.0 mg mL^{-1}) were placed in Eppendorf tubes and also exposed to gamma radiation (0.0, 1.0, 3.0, 5.0, 10.0, and 20.0 kGy). Irradiated samples of powdered *trans*-aconitic acid were named AS_0, AS_1, AS_3, AS_5, AS_{10}, and AS_{20}, according to the radiation dose. Similarly, irradiated samples of *trans*-aconitic acid dissolved in water were named AW_0, AW_1, AW_3, AW_5, AW_{10}, and AW_{20}. After irradiation, samples from AS_0-AS_{20} and AW_0-AW_{20} were immediately placed in capillaries and introduced in EPR quartz tubes in order to register paramagnetic species.

Aliquots (20.0 µL) of AS_0-AS_{20} and AW_0-AW_{20} samples were analyzed by HPLC-UV in isocratic mode, during 20.0 min, using as mobile phase a mixture of methanol (20%) and solution of water:trifluoroacetic acid (99.5:0.5 v/v, pH 4.0) (80%). Aliquots of AW_0-AW_{20} were also analyzed by LC-DAD-MS in gradient mode, using as the mobile phase a gradient starting with methanol (5%) and water (95%) and increasing concentration of methanol up to 100% during

13.0 min. During the following 6.0 min, the concentration of mobile phase was returned and maintained as methanol (5%) and water (95%). Aliquots of AW_0-AW_{20} (5.0 mL) were lyophilized and analyzed by ^{1}H NMR spectroscopy.

Computational studies

Theoretical calculations were performed to investigate the possible mechanisms and products generated from the irradiation of *trans*-aconitic acid. Calculations reported herein were performed at the density functional theory (DFT) level, using Lee, Yang and Parr's correlations functional B3LYP, a hybrid functional including exact HF (Hartree-Fock) exchange in the ratio proposed by Perdew *et al.*[19] and Becke.[20] All calculations employed the 6-31++G(d,p) basis set for all atoms. The stationary points located on the gaseous phase or aqueous solution potential energy surface were characterized as minimum or transition state structures by calculating the Hessian matrices at the B3LYP/6-31++G(d,p) level. Solvent effects were included by means of the polarizable continuum model (PCM), with the molecular cavity computed using the Universal Force Field (UFF) radius.[21,22] All *ab initio* calculations were carried out using the Gaussian09 program.[23]

Results and Discussion

Effects of the gamma radiation on *L. ochrophylla* decontamination

Doses of gamma radiation required to inactivate fungi depend on their chemical constituents and biological characteristics.[7] The content of water mycelial, pigments, amino acids, proteins, and fat acids may be responsible for protection and hence, gamma radiation resistance presented by some microorganisms.[24] Gamma radiation may trigger water radiolysis producing reactive oxygen species (ROS). The radicals formed induce some lesions in DNA and other macromolecules causing damages that alter protein expression, usually resulting in lethal action on microorganisms.[25]

The major microorganisms naturally present in the stem bark from *L. ochrophylla* were identified as the fungi species *Eurotium chevalieri* (synonym: *Aspergillus chevalieri*) and *Lecythophora decumbens*. The initial microbial load present in LO_0 samples (5.04×10^4 CFU g^{-1}) declined after irradiation in relation to samples LO_1 and LO_3 (1.22×10^4 and 8.75×10^3 CFU g^{-1}, respectively). Total decontamination of stem bark of *L. ochrophylla* was verified when doses higher than 5.0 kGy were applied (Table S1, presented in SI section).

The susceptibility of fungi *E. chevalieri* and *L. decumbens* to gamma radiation in plant materials is being described for the first time in the literature. This result is important since *E. chevalieri* is a very common fungal species detected as contaminant of a wide variety of plants, including important commercial crops such as cocoa and is quite resistant to disinfection.[26] *E. chevalieri* produces citrinin, a mycotoxin that can become a contaminant of herbal medicines,[27] and it also causes deterioration in leader and textiles, since *Eurotium* species can survive in low water activity. In foods, these fungi can alter organoleptic characteristics, nutritional quality and decrease shelf life.[28] On the other hand, *Lecythophora* species are also common in wood, bark, and leaves of plants. Some *Lecythophora* species are involved in human infections, often with a fatal outcome.[29,30]

The amount of gamma radiation required for decontamination of stem bark of *L. ochrophylla* is in accordance with described in the literature for elimination of other fungi species, while higher doses are necessary to decontamination of other matrices, in special for mycotoxin-producing species.[31,32]

These results are important since the use of plant material has significantly increased worldwide. Similarly, the number of cases of patients infected by microorganisms found on vegetal materials has also increased due to the high microbial contamination of these materials.[33,34]

Presence of free radicals in plant material

The absorption of gamma radiation by plant materials can induce the formation of free radicals, i.e., paramagnetic species.[35] Figure 1 shows EPR spectra of powdered *L. ochrophylla* stem bark samples (LO_0-LO_{20}), exposed to different doses of gamma radiation (0 to 20.0 kGy). The non-irradiated sample LO_0 presents an almost undetected minimum intensity EPR signal, which is clearly visible for LO_1, LO_3, LO_5, LO_{10}, and LO_{20}, supporting the formation of free radicals by the presence of paramagnetic signals with a g factor of 2.0012. The EPR signals presented by LO_1, LO_3, LO_5, LO_{10}, and LO_{20} vary nonlinearly with the received gamma radiation dose. The intensity of the EPR signal reaches the saturation limit around 50.0 kGy as obtained from the dose response fit shown in Figure 2a. This behavior suggests an increase in response of the formation of free radicals of the stem bark sample and *Luehea ochrophylla* up to doses of 50.0 kGy.

Free radicals are transient and very reactive species, a fact that results in a generally short half-life time.[36] However, in dry samples, the formed radicals may be relatively stable and with sufficient lifetime for their detection.[37] The exponential decay as a function of time of the amount of

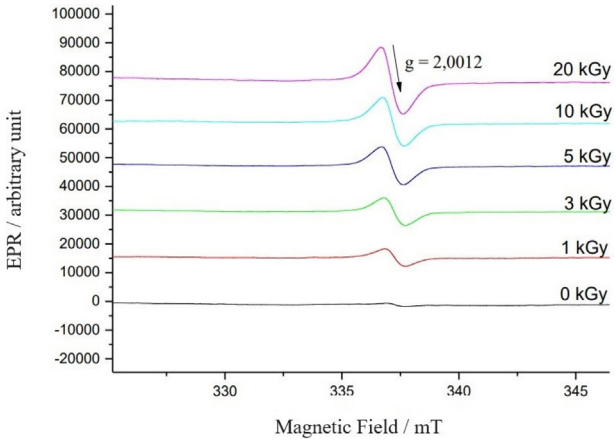

Figure 1. EPR spectra of the powdered *L. ochrophylla* stem bark submitted to different doses of gamma radiation.

free radicals in the stem bark of *L. ochrophylla* is shown in Figure 2b. The half-life time of the radical species in the sample is approximately 33 h (Figure 2b). As shown, the intensity of EPR signals decreased significantly in three days after irradiation.

The decrease in the amount of free radicals in the sample is related to radical termination reactions. These reactions promote the combination of free radicals to produce diamagnetic compounds, and therefore, not detectable by EPR.[38]

The results are consistent with data found in the literature for some spices such as *Capsicum annuum* (paprika), *Piper nigrum* (black pepper), *Brassica juncea* (mustard), and *Cinnamomum verum* (cinnamon) irradiated at doses up to 20.0 kGy.[37]

Phenolic fraction

HPLC analysis of the phenolic fraction of *L. ochrophylla* recorded eight intense peaks, considered as a mixture rich in phenolic compounds. The compound with retention time 2.88 min (peak 2) revealed significant change in its relative area with different doses of gamma radiation (Figure 3).

Exposure to oxidative stress conditions induce plants to produce reactive oxygen species (ROS), such as $O_2^{\bullet-}$, H_2O_2 and $HO^{\bullet}$ in the cells. However, plant cells were found to tolerate ROS by endogenous mechanisms of enzymatic and non-enzymatic protection.[39] Decrease in concentration of phenolic compounds in irradiated samples is assigned to the radioprotective and antioxidant effects provided by these compounds.[18,40] Phenolic compounds, when reacting easily with ROS, act as a nonenzymatic defense system and, therefore, their concentration may decrease in the medium.[41] Previous studies[17] with *Cuscuta chinensis*, a medicinal plant with anti-aging and anti-inflammatory effects also showed a decrease in phenolic content when an extract of this species was submitted to gamma radiation.

Effects of gamma radiation on *trans*-aconitic acid

trans-Aconitic acid frequently occurs in higher plants, although data on its distribution are rare.[42] Combined with vitamin C, gallic and caffeic acids, *trans*-aconitic acid showed synergistic antioxidant effect,[43] and the mechanism of the reaction between 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and *trans*-aconitic acid is already described.[43] Nematocidal, anti-Leishmanial, fermentation inhibitory and other activities of this acid were recently reviewed.[44]

Figure 4a shows the EPR spectra of irradiated and non-irradiated *trans*-aconitic acid in powdered solid phase (anhydrous conditions). Radicals in AS_5, AS_{10}, and AS_{20} confirm that gamma radiation led to paramagnetic species. As previously described in literature, irradiated samples in anhydrous conditions provide radicals that can be relatively stable enough to be detectable.[37]

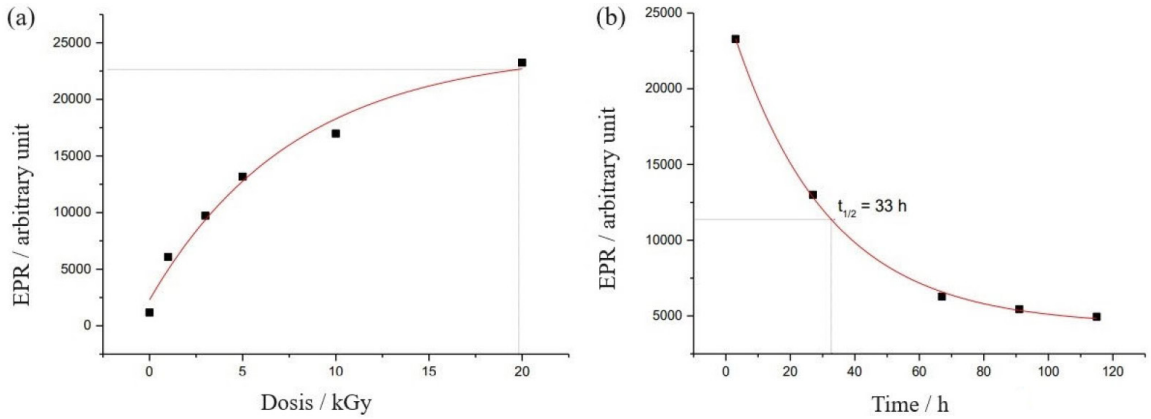

Figure 2. (a) Free radical concentration in the stem bark of *L. ochroplylla* as a function of gamma radiation dose. (b) Decay curve with time (h) of the EPR signal for *L. ochroplylla* stem bark irradiated at 20 kGy-LO_{20}.

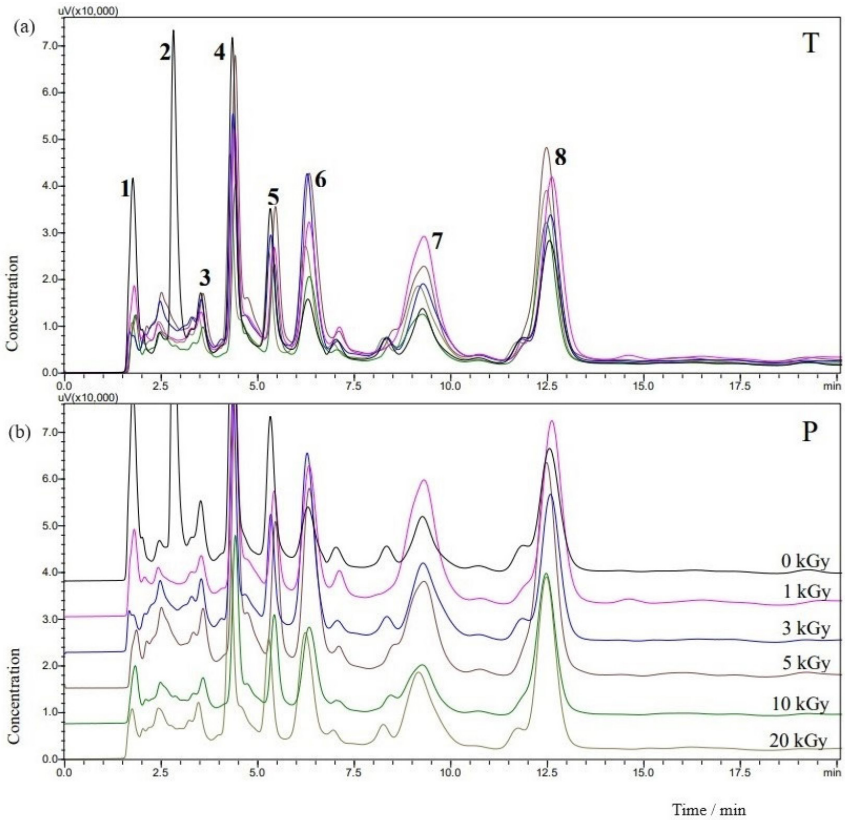

Figure 3. Total (a) and particial (b) overlap of HPLC chromatograms at 280 nm of FF_0, FF_1, FF_3, FF_5, FF_{10} and FF_{20} (t_R between 0.0 and 20.0 min); concentration A:B (1:4), where A = methanol and B = 99.5:0.5 water:trifluoroacetic acid solution.

On the other hand, EPR spectra of irradiated and non-irradiated *trans*-aconitic acid in an aqueous medium did not show signals of free radicals in samples exposed to different doses of radiation (Figure 4b). The literature[45] reports that the presence of water accelerates the disappearance of free radicals generated in the process, since the radiolysis of water molecules, generated by exposition to gamma radiation, produces radicals, aqueous free electrons, and ionized molecules of water. These species easily react with molecules present in the medium. This reaction produces neutral compounds, and therefore they are not detectable by EPR.[35]

Statistical analysis does not suggest significant changes ($p < 0.05$) in the relative proportion of *trans*-aconitic acid irradiated under anhydrous conditions (Figure S2, SI section). On the other hand, the corresponding chromatograms obtained from *trans*-aconitic acid in aqueous medium (Figure S3, SI section) indicate that radiation promotes a sharp decrease in the relative proportion of this acid. Statistical analysis suggests that the concentration of *trans*-aconitic acid in samples dissolved in water is significantly decreased ($p < 0.05$), even at low doses of gamma radiation.

Decrease in concentration of irradiated *trans*-aconitic acid in aqueous medium was also observed by liquid chromatography-mass spectrometry (LC-MS) analysis. The molecular ion peak [M – H] at *m*/*z* 173.0089 was attributed to *trans*-aconitic acid. A new peak at t_R 2.1 min (*m*/*z* 191.0198) was observed in the chromatograms of the compound exposed to doses equal to or greater than 3.0 kGy. This new peak was attributed to the addition of one water molecule (18.0109 mass unit) to *trans*-aconitic acid. The relative proportion of this new peak corresponds to 5.40, 9.34, 15.24, and 28.39% for samples irradiated at 3.0, 5.0, 10.0, and 20.0 kGy, respectively.

Degradation of *trans*-aconitic acid was corroborated by ^{1}H NMR data (Figure S4, SI section). The signal at δ_H 6.71, attributed to the alkenyl hydrogen atom of the *trans*-aconitic acid showed intensity decrease and was not observed in the spectrum of the sample exposed to radiation at 20.0 kGy.

Theoretical results showed that, in the absence of gamma radiation, the addition of a water molecule to *trans*-aconitic acid does not occur spontaneously ($\Delta G_{total} = 5.7$ kcal mol^{-1}). Moreover, in the presence of gamma radiation, reactions process with radicals or ions being spontaneous and high favorable.

To determine the most favorable mechanism, theoretical calculations were performed using the density functional theory. The reaction by radical mechanism has as products citric ($\mathbf{P_1}$) and isocitric ($\mathbf{P_2}$) acids from thermodynamically

favorable pathways (Figure 5). $\mathbf{R_1}$ radical is approximately 5 kcal mol^{-1} more stable than $\mathbf{R_2}$ radical (Figure S5, SI section); therefore, the atom of hydrogen attacks preferably the most hydrogenated carbon, giving the $\mathbf{R_1}$. In addition, activation energies of the reaction via mechanisms (a) and (b) are 7.7 and 11.6 kcal mol^{-1}, respectively. According to calculated activation energies, it can possibly estimate the ratio between $\mathbf{R_1}$ and $\mathbf{R_2}$ radicals

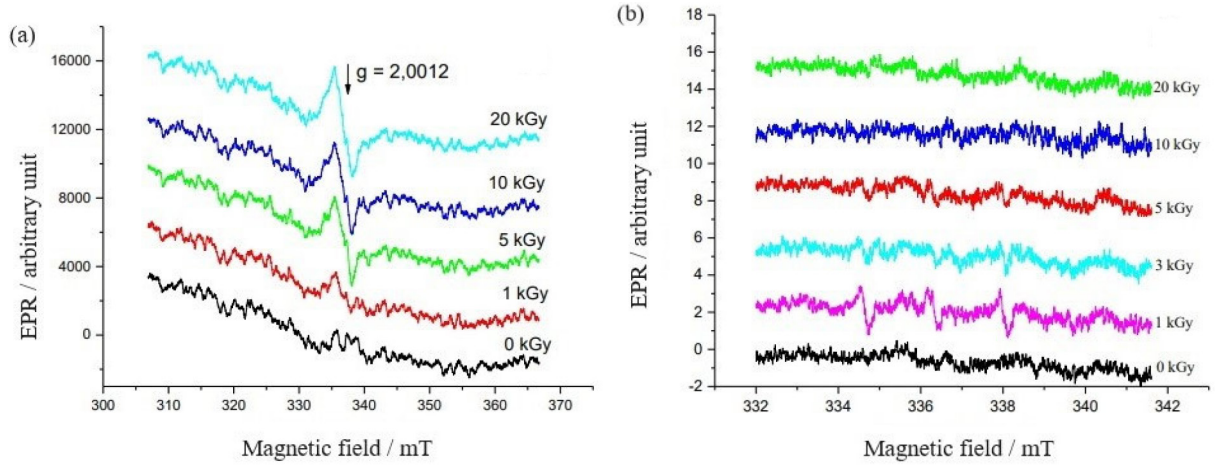

Figure 4. EPR spectra of *trans*-aconitic acid irradiated in anhydrous condition (a) and in aqueous medium (b).

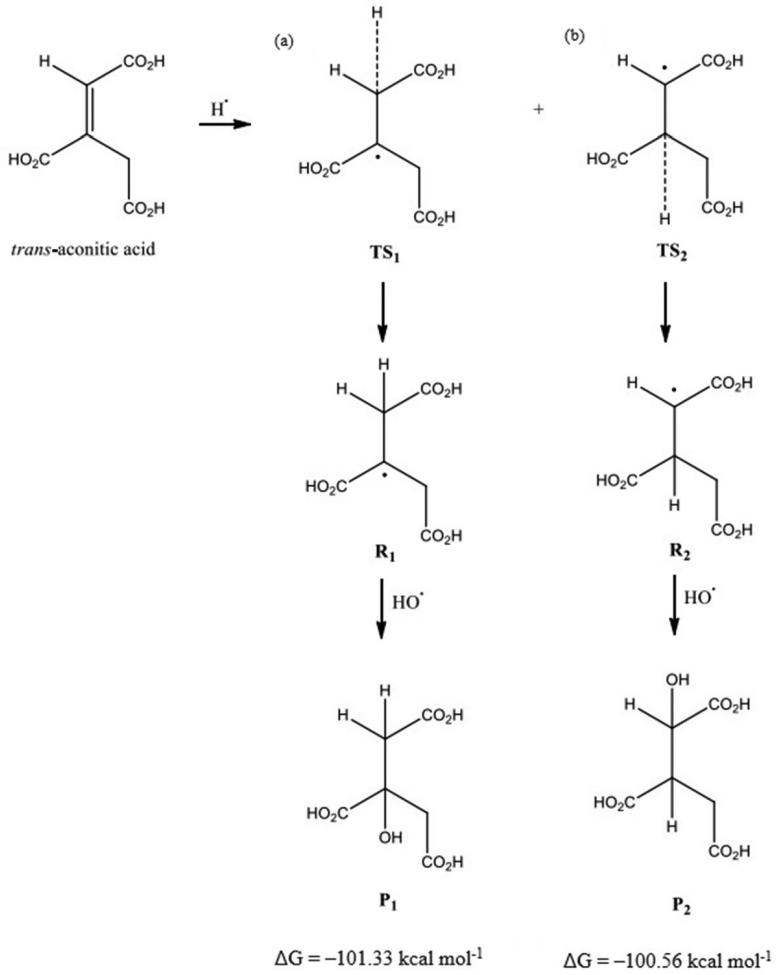

Figure 5. Additional of radical hydrogen and the radical hydroxyl to the tertiary (a) and secondary (b) carbocation of the *trans*-aconitic acid with formation of citric and isocitric acid, respectively.

formation constant rates. Using Arrhenius equation and assuming pre-exponential factor approximately the same for both mechanisms, the formation rate of $\mathbf{R_1}$ radical is ca. 722 higher compared to $\mathbf{R_2}$ radical. All energy values are shown in Table S2 (SI section).

In a second moment, the reaction was also studied via an ionic mechanism. In this way, it was carried out considering the addition of a proton and a hydroxyl ion to carbo-cation. Citric ($\mathbf{P_1}$) and isocitric ($\mathbf{P_2}$) acids were formed by paths thermodynamically favorable. Energy values showed that the reaction via ionic mechanism is more favorable than a radical mechanism (Table S3, SI section). As the tertiary carbo-cation ($\mathbf{R_1}$) is more stable than secondary carbo-cation ($\mathbf{R_2}$), the main product of the reaction is $\mathbf{P_1}$ (citric acid).

Citric acid is the main radiolytic product of *trans*-aconitic acid in an aqueous medium. This is common in plant species and is related with getting energy to the activities developed by cells.[46] This information is important since it allows to state that the absorption of gamma radiation, for doses up to 20 kGy, by stem bark of *L. ochrophylla*, dry or in the presence of water, does not lead to the formation of toxic radiolytic products. This result suggests *trans*-aconitic acid as a possible radioprotein agent since it seems to suppress radiation-induced damage through free radical scavenging.

Conclusions

In this work, the optimal dose of gamma radiation for inactivating microorganisms in stem barks of *L. ochrophylla* was studied. The radiation dose of 5.0 kGy was sufficient to eliminate *Eurotium chevalieri* L. Mangin and *Lecythophora decumbens*, the major microorganisms detected in the total load. *trans*-Aconitic acid, isolated from a phenolic fraction obtained from *L. ochrophylla*, suffers a significant decrease in its relative proportion after subjected to gamma radiation. It was observed that this acid in the presence of water originates the citric acid, the main radiolytic product. Determining the suitable method and the necessary dose to eliminate microbial contamination as well as the study on the effects of gamma irradiation on constituents of *L. ochrophylla* is a crucial requirement for consumer safety.

Supplementary Information

Supplementary information (^{1}H NMR spectrum and structure of *trans*-aconitic acid, HPLC chromatograms, values of CFU g^{-1} for stem bark of the *L. ochrophylla,* and tables with calculated geometry and energy parameters) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Clináscia R. R. Araújo, Thiago M. Silva and Antônio F. C. Alcântara were responsible for the conceptualization, data curation, investigation, project administration, analysis and data interpretation, and drafted the manuscript; Ezequias P. Siqueira and Markus Kohlhoff for investigation by LC-DAD-MS of the *trans*-aconitic acid; Márcio T. Pereira for irradiation of samples; Klaus Krambrock for EPR investigation; Jacqueline A. Takahashi for biological analysis and writing-review; Dalva E. C. Ferreira and Willian R. Rocha for conceptualization of theoretical calculations, resources, and writing-review. All authors have read and agreed to the published version of the manuscript.

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