Use of $^{60}$Co gamma radiation in increased levels of total polyphenol extracts of bark of *Schinus terebinthifolius* Raddi.

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ABSTRACT

Schinus terebinthifolius Raddi (Anacardiaceae) is well known as sources of phenolic compounds. Known as mastic pepper, red pepper tree is a plant native to midsize coast of Brazil. Some of its structures have proven antibacterial, anti-inflammatory, antifungal and healing. The aim of this study was to evaluate the difference in the phenol contents of crude extracts that were measured after irradiating the barks of S. terebinthifolius using gamma radiation from $^{60}$Co. The crude extract were divided into a control group and eight experimental groups, which were separated based on the doses of gamma radiation to which they were exposed: 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0 and 50.0 kGy (Assays were performed in triplicate). The results allow observe that gamma radiation promoted in extracts of bark of S. terebinthifolius, many percents increase (p> 0.05) of total polyphenol content between 2.5 kGy (41.93%) and 50.0 kGy (44.52%) compared to 0 kGy (30.07%), with the same gradual to 10.0 kGy, and reaching peak maximum at 10.0 kGy (68.44%). However, the study puts the process of gamma radiation from $^{60}$Co as an alternative significant increase in the percentage of some natural substances of plant material, and subsequently contribute to the augmentation of various therapeutic applications to which they are assigned.

1. INTRODUCTION

At present, there is a growing interest in medicinal plants as a potential source of new bioactive molecules agents. Thus, the bioactivity of the medicinal plants are inherent to a group of compounds called secondary metabolites. These synthesized from catabolic reactions, anabolic and biotransformation from amino acids, carbohydrates and lipids produced by the plant. Among the major secondary metabolites are phenolic compounds, which are characterized by varied biological activity [1; 2; 3].

Despite advances in the chemical-pharmaceutical industry, the high cost of conventional drugs and their greater aggression to the organism together with its notorious incidence of side effects, the demand for the use of herbal medicines is gaining momentum at present, constituting a growing source of research all medical fields worldwide [4].

The knowledge and research of the benefits of plant species were performed by various civilizations on all continents. Although empirically or intuitive, based on random discoveries, ancient societies used the plants for therapeutic purposes, and later, they formed the basis for Botany, Chemistry and Medicine. And scientifically used as antibiotics. There are, currently, a tendency to return to herbal medicine, attitude
recommended by WHO - World Health Organization The national supported the study and use of medicinal plants as a regional form of lower costs of public health programs, particularly in countries underdeveloped or developing countries, such as Brazil [5].

Among these, Schinus terebinthifolius Raddi, popularly known as mastic, occurs naturally in Argentina, Paraguay, Uruguay and Brazil. In the latter occurs in various regions phytogeological. It has medicinal properties, and its leaves and stem bark decoction used for purposes expectorant, antiseptic, healing and antidiarrheal [6]. Little attention has been given to this species from the pharmacological point of view and phytochemical [7].

Research work by plant species with therapeutic properties originate medicines in less time, lower cost, and therefore more accessible to the population, which, in most cases, is without any financial condition to afford the high costs of procuring drugs that may, be used as part of meeting the needs of primary health [8].

In the past there were exceptions to utilize products of plant origin, have been solved by the device of the process of gamma irradiation with $^{60}$Co source, which is a physical process of heat treatment comparable to pasteurization, freezing or canning. However, the advent provide microbiological control, increased shelf life and storage chemical-physical treatment thereof, and be nontoxic [9; 10; 11; 12; 13; 14].

Using this theory research, the aim of this study is to observe the behavior of crude extracts of Schinus terebinthifolius Raddi subjected to $^{60}$Co gamma radiation at doses up to 50 kGy, assessing the levels of total polyphenols.

## 2 METODOLOGIA

### 2.1 Material Botânico

Bark of Schinus terebinthifolius Raddi were collected in a fragment of savanna vegetation, with about 20 ha, within the "Empresa Pernambucana de Pesquisa Agropecuária" (IPA) Experimental Station, State of Pernambuco, Brazil (08°14’18.2”S and 35°54’57.1”W). The area is located carca 9 km northeast of the city of Caruaru, about 150 km from the state capital of Recife. This area was chosen due to the large number of specimens of the genre and offer good conditions of preservation.

### 2.2 Vegetable Extracts

The crude extract was obtained from the bark of S. terebinthifolius Raddi by maceration for 72 hours on three consecutive extractions with ethanol / water 70%. Subsequently, the hydroalcoholic extracts were filtered and evaporated to dryness on a rotary evaporator Marconi (Model MA-120).

### 2.3 Irradiation of Extracts

The irradiation of the samples S. terebinthifolius Raddi were placed in glass tubes and taken to Gammacell irradiating with $^{60}$Co source (model 220-Excel MDS Nordion, a dose rate of 10.040 kGy / h) from the Departamento de Energia Nuclear, Universidade
Federal de Pernambuco. Irradiation at doses in kGy following: 2.5; 5.0; 7.5; 10.0; 12.5; 15.0; 20.0; 50.0.

2.4. Determination of total polyphenols

The percentage content of total polyphenols was determined by the Folin-Ciocalteu. The total phenolic content was calculated from 1 ml of the diluted extract (10 mg / ml, v/v), 5 ml aqueous solution of Folin-Ciocalteu reagent (10%, v/v), 10 ml of aqueous sodium carbonate (7.5%, v/v) and 84 ml of distilled water. The solution was allowed to stand in the dark for 30 minutes and the absorbance was measured at 760 nm. The content of total polyphenols were expressed as 1 mg equivalent of tannic acid per gram of extract (mg EAT / g). The samples were analyzed in triplicate. The equation of tannic acid calibration was y = 0.0453x + 0.1594 (R² = 0.9671) [15; 16; 17; 18].

2.5. Statistical Analyzes

The results were evaluated by analysis of variance: a criterion for multiple comparisons followed by Tukey's test at a significance level of 5% (p <0.05). And the Person correlation test, comparing the levels of total polyphenols to gamma radiation doses used (p<0.05).

3 RESULTS AND DISCUSSION

3.1. Calibration curve for the determination of total phenols and tannins

Figure 1 shows the relationships between concentrations and absorbance measurements of standard solutions of tannic acid at the following concentrations: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 μg.mL⁻¹. The graph is obtained from a calibration curve, which, as it was possible to determine the concentrations of total phenols and residual. The amount of tannin is the difference between the values obtained in the two measurements. Phenols and tannins are expressed as "mg" dry matter.

3.2. Total polyphenols

Figure 1 shows the relationship between the concentrations and the measured absorbance of the tannic acid standard-solution at the following concentrations: 1.0, 2.0, 3.0, 4.0, and 5.0 μg.mL⁻¹. This graph is a calibration curve, from which it is possible to determine the concentrations of the total and residual phenols. The amount of tannins corresponds to the difference between the values found in the two measurements. Total phenols expressed in mg of dried matter.
Figure 1. Calibration curve of the concentration of tannic acid in mg/mL\(^{-1}\) by absorbance. In evidence, find the equation of the straight line and the coefficient of determination.

Figure 2 shows the graphs of percentages mean and standard deviation of total polyphenols present in extracts of bark of *S. terebinthifolius* Raddi exposed to gamma radiation from 60Co 0 kGy and 50.0 kGy. The results allow us to observe that gamma radiation promoted the bark extracts of *S. terebinthifolius*, increased levels of total polyphenols (p<0.05), between 2.5 kGy (41.93%) and 50.0 kGy (44.52%) compared to 0 kGy (30.07%), with increased gradual dose-dependent up to 10.0 kGy, and reaching peak at 10.0 kGy (68.44%).

Figure 3 shows the curve and the points of correlation Person that link the content of total polyphenols, which increased between 0 and 10.0 kGy at 91.78% (R = 0.9187, p <0.05). However, between 12.5 and 50.0 kGy showed only 13.43% of correlation (R = 0.1343, p <0.05).
Several studies involving plant extracts show the $^{60}$Co gamma radiation increasing the concentrations of total polyphenols [19; 20; 21; 22; 23; 24] Miranda et al. 2006, Bhat et al. 2007, Stajner et al. 2007, Santos et al., 2011). Principally only Santos et al. (2011), studied extracts of bark and leaves of Anacardium occintale Linn., and found a significant increase ($p < 0.05$), dose-dependent between 0 kGy and 10 kGy only in leaf extracts, with no difference between extracts of bark. However, the $^{60}$Co gamma radiation can act on plant materials peculiar to each species.
3. CONCLUSION

The gamma radiation from $^{60}$Co modified physico-chemical extracts of bark of *Schinus terebinthifolius* Raddi, promoting increased levels of total polyphenols dose-dependent (between 0 and 10 kGy), and peak at a dose of 10 kGy. Thus, gamma irradiation may act by altering specific compounds of each plant species. To confirm this theory, it is necessary to further explore these phytochemicals.

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