Phenols and tannins contents of *Anacardium occidentale* Linn and *Anadenanthera colubrina* (Vell.) Brenan exposed to gamma irradiation.

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ABSTRACT

Anacardium occidentale Linn (cajueiro) and *Anadenanthera colubrina* (Vell.) Brenan (angico) are very know as a source of phenolic compounds, mainly tannins. The aim of this study was to evaluate the difference of phenols and tannins contents of crude extracts were measured after irradiation of barks and leaves of each plant source, using a source of ^{60}Co. The crude extracts were divided into control group and three groups which were separately after exposition to gamma radiation, in doses of 5; 7.5 and 10 kGy. From each group, the total phenols were quantified by the Folin-Ciocalteau method, while the contents of tannins were assessed using precipitation of the casein technique. For all doses, the total phenol and tannin percentages from "cajueiro" barks presented no significant statistical alteration. However, for the leaves of "cajueiro", their chemical composite levels significantly changed with the radiation absorbed dose. On the other hand, the gamma irradiation did not cause alterations in total phenols and tannins content of extracts from "angico".

Keywords: tannins, total phenols, *Anacardium occidentale* L. (cajueiro), *Anadenanthera colubrina* (Vell.) Brenan (angico).

1. INTRODUCTION

However, besides the use of these products as natural source, the chemical characteristics of vegetables allow its application in some medical areas, as in the treatment of cancer and infectious diseases, or in the provision and cosmetic industries, among others [1; 2; 3; 4].

Anacardium occidentale Linn. and *Anadenanthera colubrina* (Vell.) Brenan In Brazil, are widely used by the alternative medicine, mainly against some bacterial infections, These plants are rich in phenols and tannins contents. Obtained from some parts of plants, the tannins are polyphenol compounds of great economic and ecological interest [5; 6; 7] and already found in parts of the *Anacardium occidentale* Linn. and *Anadenanthera colubrina* (Vell.) Brenan [8; 9; 10]. In addition, these compounds can be used in the cleaning of impurities from gasoline; in the manufacture of foodstuff products and drinks, where they are identified by their astringent flavor [11; 12; 13; 14].

The gamma irradiation process, in accordance with the Brazilian legislation, allows the use of this methods in order to eliminate contaminants in the raw material but until the maximum of 15 KGy. The general objective of the present work is to carry out an evaluation of total phenols and tannin contents present in crude extracts from superior vegetables, after the process of gamma exposure with a ^{60}Co source.

2. MATERIALS AND METHODS

2.1. Plant material

The barks and leaves from *Anacardium occidentale* Linn, popularly known as “cajueiro”, and *Anadenanthera colubrina* (Vell.) Brenan, popularly known as “angico” were collected in a “Caatinga” region from the Experimental Station of Caruaru, belongs to the Agronomic Institute of Pernambuco (IPA). This station is situated in the micro region of Ipojuca Valley of Pernambucano (08º14'18.2” S and 35º54'57.1” W) [15].

2.2. Extraction and Irradiation

The crude extracts were obtained from dried and triturated samples were from barks and leaves of *Anacardium occidentale* Linn. and *Anadenanthera colubrina* (Vell.) Brenan, by extraction using etanol/water (70%).

The crude extracts of barks and leaves of *Anacardium occidentale* Linn. and *Anadenanthera colubrina* (Vell.) Brenan were separated in samples of 20g and submitted to exposure of ^{60}Co until the radiation doses (5; 7.5 and 10 kGy). It was used for the irradiation, a Gammacell Co-60 source was used (model 220 Excel-MDS Nordion; dose rate of 10.040 kGy/h).

2.4. Quantification of phenol and tannin levels

It were performed some tests improving the induction of complexes forms to the detection of tannins and the verification of other chemical groups, following the described methodology of Matos et al.[16].

From crude extracts of barks and leaves of *Anacardium occidentale* Linn. e *Anadenanthera colubrina* (Vell.) Brenan extracts were prepared in 80% methanol, with 500 mg of vegetal parts in 5 mL of solvent.

Physical-chemical analyses were carried out to determination of total phenols usingthe adaptation of Folin-Ciocalteau method and to the tannin determination it was used the casein precipitation methods [17; 18; 19; 20].

The adaptation of the Folin-Ciocalteau method consisted of adding 0.25 mL of the extract in a volumetric balloon of 100 mL containing 75 mL of water adding 5 mL of reagent Folin-Ciocalteau (10% aqueous solution), 10 mL of sodium carbonate solution (7.5%) and volume completed with distilled water. This solution was mixed adequately and later kept at rest for 30 minutes. After this period, the absorbance was measured at 760 nm. The same procedure was adopted for the standard-solution of tannic acid, in 0.1; 0.5; 1.0; 2.5; and 3.75 $\mu g.mL^{-1}$. The relationship between the concentrations and the absorbance measured allowed to get the calibration curve (Figure 1 of Result) which allowed to determine the concentrations of total phenols by the photochemistry tests. The absorbance was measured using the Spectrophotometer - UV-visible Hewlett Packard - HP-8453E.

The tannins were determined by the method of the casein precipitation and residual phenols determined by the Folin-Ciocalteau method [17; 18; 19; 20]. The amount of tannins corresponded to the difference between the value found in this reading and the one obtained in the total phenol determination. The contents of phenols and tannins were expressed in mg of dried matter.

3. RESULTS AND DISCUSSION

3.1. Total phenols and tannins contents

Figure 1 shows the relationship between the concentrations and the measures of absorbance of the tannic acid standard-solution in the following concentrations: 0.1; 0.5; 1.0; 2.5; and 3.75 $\mu g.mL^{-1}$. The graphic obtained is a calibration curve, where it is possible to determine the concentrations of total and residual phenols. The amount of tannins corresponds to the difference between the values found in the two measurements. Total phenols and tannins were expressed in “mg” of dried matter.

The induction of chemical complex shows that the total phenols and tannins presented variations, between barks and leaves extracts, and between the two species: “angico” (Table 1) and “cajueiro” (Table 2).

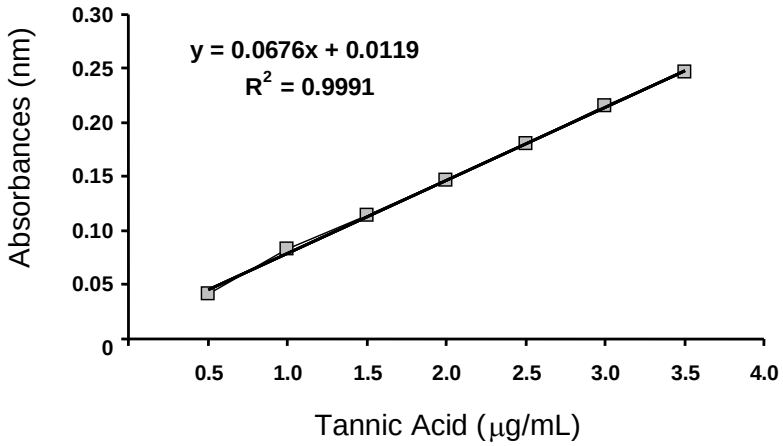

Figure 1. Calibration curve of the concentration of tannic acid in mg/mL^{-1} by absorption. In evidence, find the equation of the line and the coefficient of determination of the adjustment.

In Table 1 the results of the chemical analyses of total phenols and tannin contents for crude extracts of leaves and barks of "angico" are presented. When compared with of "cajueiro", the "angico" presented lower contents of tannins among metabolite compounds: varying between 52 and 57% for barks and 58 and 60% for leaves, however, the percentages of total phenols were comparatively higher. These results are differ from that described by Paes et al [21], when referred the tanning potential of *A. occidentale* ("cajueiro") and *A. colubrina* ("angico"), showing that "cajueiro" has a bigger total phenol content and, a bigger concentration of tannins when compared with the "angico".

Table 1: Total phenol and tannin contents in the barks and leaves of "angico" (*Anadenanthera colubrina* (Vell.) Brenan), for the control and irradiated samples (5; 7.5 and 10 kGy).

Dose (kGy)	Percentual Values $(\bar{x} \pm \sigma)$ *			
	Barks		Leaves	
	Total Phenols	Relative percent** of tannins	Total phenols	Relative percent** of tannins
0	8.18 ± 0.34	4.64 ± 0.33	5.16 ± 0.23	3.02 ± 0.26
5	8.10 ± 0.21	4.47 ± 0.34	5.18 ± 0.24	3.02 ± 0.28
7.5	8.02 ± 0.54	4.49 ± 0.68	5.16 ± 0.06	3.09 ± 0.09
10	7.81 ± 0.35	4.07 ± 0.23	5.57 ± 0.15	3.33 ± 0.15

* Mean of six repetitions and standard deviation.

** From total phenol content.

Table 2: Total phenol and tannin contents in the barks and leaves of "cajueiro" (*Anacardium occidentale* L), for the control and irradiated samples (5; 7.5 and 10 kGy) and statistical analyses (Different letters indicate that significant statistical difference occurred between data.)

Dose (kGy)	Percentual Values $(\bar{x} \pm \sigma)$ *			
	Barks		Leaves	
	Total Phenols	Relative percent** of tannins	Total phenols	Relative percent** of tannins
0	5.79 ± 0.07 ab	5.34 ± 0.07 a	3.13 ± 0.04 a	2.47 ± 0.06 a
5	5.45 ± 0.18 a	4.92 ± 0.20 a	3.33 ± 0.15 ab	2.77 ± 0.19 b
7.5	5.74 ± 0.28 ab	5.21 ± 0.28 a	3.45 ± 0.07 b	2.83 ± 0.07 b
10	5.96 ± 0.04 b	5.34 ± 0.04 a	3.50 ± 0.08 b	2.93 ± 0.04 b

* Mean of six repetitions and standard deviation.

** From total phenol content.

The analyses of data of contents of "angico" extracts samples showed that there was no statistical significant difference between the control and the irradiated samples of barks and leaves, for both total phenol and tannin contents. The results indicate that there is no influence of the gamma radiation in contents of these compounds (Table 1).

From the data presented in Table 2, about the tannins contents in crude extracts of "cajueiro" it was observed, and was corresponding to 89 to 92% for the barks, and 79 to 84%, of total phenols in leaves. The total phenol and tannin contents in "cajueiro" crude extracts of barks demonstrated that the control sample did not differ statistically (comparison between control and irradiated and between irradiation at different doses). These results suggest that in the "cajueiro" barks the influence of gamma radiation did not occur in contents of secondary metabolites.

Statistical analyses from Table 2 data, suggest that the "cajueiro" leaves extracts, presented an increase of total phenols with statistically significant difference, mainly between the control and the 7.5 and 10 kGy doses and between the irradiated samples which have not presented statistical differences, indicating that the gamma radiation had influenced in total phenol contents only above 5 kGy doses. The tannin contents have statistically significant difference between the control and those irradiated to 5, 7.5 and 10 kGy, indicating that the gamma radiation had influence in the tannin concentration with the increase in absorbed dose, without statistically significant difference between the doses.

These studies showed that, there is a significant increase in tannin percentage with irradiation only in extracts of leaves of *Anacardium occidentale*, which can be explained by the fact that gamma radiation acts as a catalytic agent of the condensation process of polyphenols.

CONCLUSIONS

The gamma irradiation process did not cause alterations in total phenol and tannin contents of extracts of *Anadenathera colubrina*, even using an increase of radiation doses.

However, for *Anacardium occidentale* extracts, the gamma irradiation exposure promoted physical-chemical alterations in the phenolic constituent, increasing total phenol and tannin contents, presenting a dose-dependence behavior mainly in leaves after 7.5Gy.

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