Antioxidant activity of cinnamon (*Cinnamomum Zeylanicum*, Breyne) extracts

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ABSTRACT

JUSTIFICATION: Lipid oxidation is one of the major changes that can occur during processing, distribution, storage and final preparation of foods. The oxidation could be prevented by adding synthetic or natural antioxidants in spite of safety of synthetic ones has been questioned. This situation promotes increasing demand for food additives of natural origin.

OBJECTIVE: The objective of this study was to evaluate the antioxidant activity of cinnamon extracts.

METHODS: Cinnamon samples were obtained at local market, milled (32 mesh sieve) and submitted to sequential extraction using as solvents: ether, methanol and water. The antioxidant activity in the extracts was measured by the b-carotene/linoleic acid system, at 50°C and absorbances reading at 470 nm every 15 min intervals for 120 min. Two controls were used in this determination: one with synthetic antioxidant (BHT, 100 ppm) and other without antioxidant. The water extract was fraccionated using silica Gel 60 and 60G and through chromatographic processes: thin layer (T.L.C.) and column, using BAW as mobile phase and ethylacetate, petroleum ether, methanol and water as eluent, respectively.

RESULTS: The etheric (0.69 mg), methanolic (0.88 mg) and aqueous (0.44 mg) cinnamon extracts, inhibited the oxidative process in 68%; 95.5% and 87.5% respectively. The BHT control inhibited 80% oxidation. The spray reagents (1) β-carotene/linoleic acid and (2) Fe Cl_3/K_3 Fe $(CN)_6$ 1% sol, showed spots in T.L.C. with antioxidant activity (1) and blue color (2), indicating the presence of phenolic compounds with Rf values of 0.50. Five fractions were obtained by column partition with antioxidant activity and the presence of phenolic compounds .

SIGNIFICANCE: These results suggest that the cinnamon extracts can be used as food antioxidant together with the improvement of food palatability. Further studies are in processing of analysing the sinergic association of extracts with synthetic antioxidant and to identify compounds with antioxidant activity in cinnamon extracts.

KEY WORDS: Antioxidant; Cinnamon; Phenolics

INTRODUCTION

Lipids as food are complex systems with several compounds, consisting mainly of various types of triacylglycerols. The degree of unsaturation of fatty acids molecules in triacylglycerols is related to oxidation in foods provoking changes that affect its nutritional quality, wholesomeness, safety, colour, flavour, and texture [11].

The mechanism of lipid oxidation is a free radical chain reaction and can be described in terms of iniciation, propagation and termination processes. Lipid peroxidation begins with the formation of a free carbon radical. Its rapid reaction with oxygen yields a peroxide radical which can attack a lipid molecule to form a hydroperoxide and a new free radical. Hydroperoxides are not final stable products; they may be further oxidized to give dihydroperoxides, reduced to alcohol, and give various secondary products such as epoxides, ketones, and aldehydes. So, food quality is directly associated with the integrity of unsaturated fatty acids.

Fatty acid peroxidation can be prevented by adding antioxidants to the food. Antioxidants are regarded as compounds which are able to delay, retard, or prevent oxidation processes. They can interfere with oxidation by reacting with free radicals, chelating catalytic metals, and also acting as oxygen scawengers. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgalate (PG) and terc-butylhydroquinone (TBHQ) have been used to control lipid oxidation. These antioxidants, however, may act in some circumstances as deleterious agents to animal organisms [1]. This is the reason why they have been constantly monitored by the, Joint Expert Committee of Food Additives of Food Agricultural Organization JECFA-FAO [14].

Due to the possibility of occuring side effects by the use of synthetic antioxidants, many studies have been carried out in several research centres aiming to identify natural substances which could become eventually a replacement for them [13].

Natural antioxidants can occur in all higher plants and in all of its parts. They are usually phenolic or polyphenolic compounds. Typical molecules of antioxidants are derivatives or isomeric forms of polyphenolics, flavones, isoflavones, flavonols, catechins, eugenol, coumarin, tocopherols, cinnamic acid, phosphatides, polyfunctional organic acids and others [8].

Herbs and spices have been used for many centuries to enhance flavour and extend the keeping times of various foods. This last property was the reason why Chipault et al. [2] in 1952 were looking for antioxidant activity in several spices. They examined the antioxidant activity of 32 spices in lard and in an oil-in-water emulsion systems. All tested spices, exibited antioxidant activity in both systems including cinnamon. More recently Nakatani [10] in 1992 also showed that cinnamon possesed antioxidant activity. In his study he observed that methanol extract was more effective than others in preventing the lipid oxidation.

However little is known about the active compounds present in cinnamon, their relative activity and their possibility to be acting concurrently, serially, additively, and synergistically with other substances.
The objective of this study was to evaluate the antioxidant activity of different cinnamon extracts and elucidate the characteristics of the active compounds.

EXPERIMENTAL

Cinnamon samples were obtained from local market in Sao Paulo City, Brazil milled to permit their passage through a 32-mesh sieve and then submitted to extraction with ether, methanol and water, during 60 minutes, under agitation at environmental temperature (approximately 20°C). Etheric, methanolic and aqueous extracts were obtained sequentially from the cinnamon powered obeying the solvent increase polarity [9]. All the extracts were obtained at ambient temperature (± 23°C) and were completed to 50 mL with the correspondent solvent. The amount of dry material in each extract was determined.
Total lipids were extracted according to the Folch et al [4] method, and determined gravimetrically after solvent evaporation. Aliquots of the final lipid extracts were used to prepare the fatty acid methyl esters (FAME) according to Hartman & Lago [6]. Analysis were perfomed in a CG 500 model gas chromatograph with flame ionization detector and a 12 m x 0,25 mm ID capillary column (Carbowax 20 m). The temperature for a programmed operation was initially 80 °C, and programmed to increase to 210 °C, at 5° C/min. A CG 300 model recorder and integrator was used to quantify the fatty acid components. Standard mixtures of fatty acid methyl esters were used to obtain relative retention times and to identify the fatty acids in the cinnamon extracts.
The antioxidant activity of each extract was evaluated by β-carotene plus linoleic-acid system in stoppered tubes placed in a water bath at 50 °C. The absorption was measured at 15 minute-intervals for two hours in a Spectronic 20 D, (Milton Roy Company). The antioxidant activity was calculated as a percentage of the oxidation of b carotene without antioxidant [7]. Two controls were used in this determination: one with synthetic antioxidant (BHT, 100 ppm) and other without antioxidant (blank).
The relationship between antioxidant activity and extract/substrate proportion was determined with different concentrations of each one and using the previously described methodology.
The extracts were fraccionated through thin-layer chromatography using silicagel 60. The mobile phase was BAW (butanol, acetic acid and water) and the spray reagents were: (1) β carotene/linoleic acid to measure antioxidant activity and (2) ferric chloride/potassium ferrocyanide 1% sol to indicate the presence of phenolic compounds.
The cinnamon water extract was fractionated by silica gel 60 in a chomatographic column, using a mixture of the following solvents: petroleum ether, ethylether, methanol and water, with a flow-rate of 2.5 mL/min., 10 mL fractions were collected. The fractions were screened for the presence of phenolic compounds by means of the reaction with $FeCl_3/K_3Fe(CN)_6$ reagent and the absorbance was read in Spectrophotometer at 570 nm. After pooling the fractions with phenolic compounds and concentrating them, the antioxidant activity of each pool was measured as previously described.

RESULTS AND DISCUSSION

Table I shows the dry matter from extracts that were obtained from 60 g of cinnamon powered. Fractions with specific solubility in apolar and polar mediuns were obtained through the sequential extraction process. The extraction with ethylether gave apolar compounds. Compounds with intermediate polarity were obtained through methanol extraction, while compounds of high polarity were obtained by water extraction [9]. The concentration of a 44.0 mg/mL in methanol extract shows that this solvent is a better extractor than ethylether (34.4 mg/mL) and water (21.8 mg/mL). The final volume of each extract was 50 mL. The total dry material were1,720 mg; 2,200 mg and 1,090 mg for etheric, methanolic and aqueous extracts, respectively.
Total lipids from cinnamon powder was 2.7% and are represented by the following fatty acids: miristic ($C_{14:0}$) 23.13%; palmitic (16:0) 20.11%; oleic ($C_{18:1}$) 7.10%; linoleic ($C_{18:2}$) 35.87% and linoleic ($C_{18:3}$) 1.26% (Table II). The highest concentration of linoleic acid suggests protection mechanisms against lipid oxidation.
To evaluate the system β-carotene linoleic acid is suitable to

Table I. Dry material in etheric, methanolic and aqueous cinnamon extracts.

Solvent	Dry material mg/ml	starting material %
ethylether	34.4	2.9
methanol	44.0	3.7
water	21.8	1.8

Table II. Total fatty acids in cinnamon

Fatty	acid%
$C_{14:0}$	23.13 ±2.14*
$C_{16:0}$	20.11 ± 0.14
$C_{18:0}$	-
$C_{18:1}$	7.10 ± 1.40
$C_{18:2}$	35.87 ± 1.47
$C_{18:3}$	1.26 ± 0.35
ND	11.96 ± 1.82

* Standard Deviation

measure the antioxidant activity of the cinnamon extracts. First the activity of BHT, was tested at different concentrations (Fig. 1). Cort [3] compared the antioxidant activity of spice extracts with that of BHT. The determinations of antioxidant activities concerning etheric, methanolic and aqueous extracts from cinnamon powder are given in Figure 2. It can observed that water extracts showed the greatest proportional antioxidant activity where 0,44 mg of dry material corresponding to 87.5% of protection against oxidation. This result is different from those obtained by Nakatani [10], where methanolic extract showed to be more active than the others. We obtained 95.5% of antioxidant activity with methanolic extract but with 0.88 mg of dry material, this concentration is twice the concentration of aqueous extract. The etheric extract (0.69 mg) was the least activite, with 68% of oxidation protection.

In practical use, rosemary and sage retarded oxidation in plain lard and clone showed extremely high effectiveness in oil-in-water emulsion. Sethi & Aggarwal [12], reported the same antioxidant effects of cinnamon, also in turmeric, nutmeg , clove and other spice extracts using heated peanut oil. Our study with an aqueous cinnamon extract was performed due to its high antioxidant activity and showed that different concentrations of the dry material in the extract have correspondent levels of oxidation protection. Figure 3 shows that the coefficients of correlation $R^2 = 0.9989$ to β carotene oxidation and $R^2 = 0.984$ to β carotene with aqueous cinnamon extract correspond to 0.109 mg. Rela-

Fig. 1. Evaluation of the effectiveness BHT antioxidant activity at different concentrations

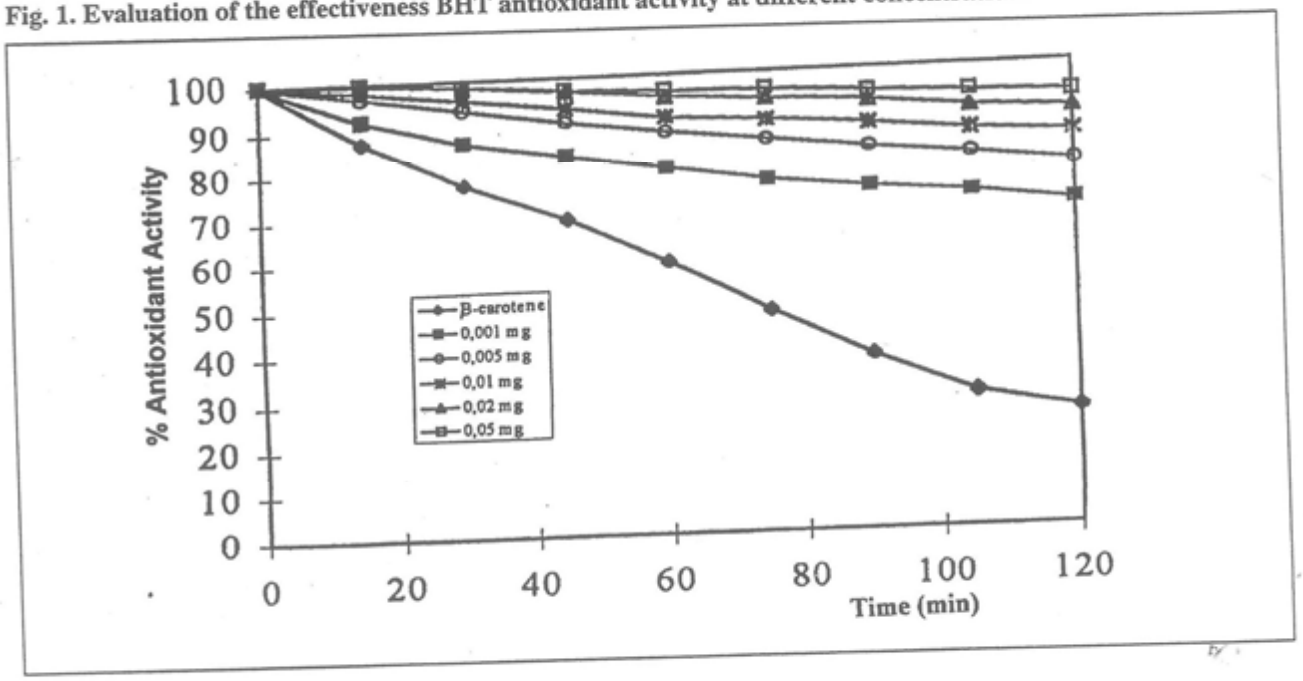
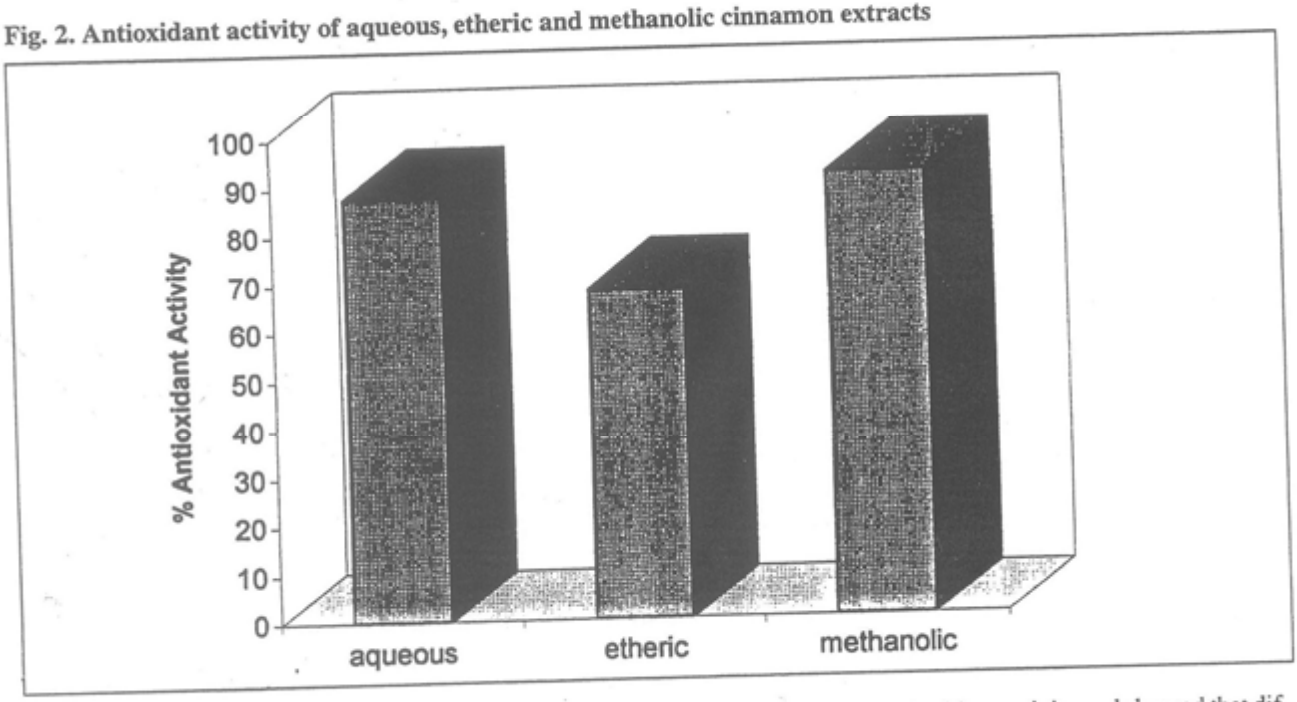


Fig. 2. Antioxidant activity of aqueous, etheric and methanolic cinnamon extracts



tive results were also obtained with higher concentrations. Thin-layer chromatography permitted the separation of one distinct band with Rf value of 0.50. This band gave a positive test with carotene spray indicating antioxidant activity and blue color with $FeCl_3$ / $K_3Fe(CN)_6$ 1% sol. indicating the presence of phenolic compounds.

The separation of phenolic compounds can be obtained with different adsorbents as sephadex or silica gel [5].

Figure 4 shows the separation of five fractions from cinnamon extract using a silica gel column: fraction I, corresponding to tubes 15 through 20; fraction II, tubes 22 through 30; fraction III tubes 33 through 40; fraction IV, tubes 42 through 51 and fraction V, tubes 52 through 58. The positive reaction to phenolic compounds permitted these separation.The results of antioxidant tests for the five fractions from silica gel column chromatography after the concentration exhibited some degree of activity. The higher activity was obtained in fraction V (Fig. 5). Previous results showed that in MDCK cell culture the influenza growing was diminished by the use of this fraction. Further studies are in processing with these fractions to identify the main compounds directly related to oxidation inhibition.

Works are in progress in Butantan Institute to identify the effects of cinnamon fraction V on development of Influenza virus in cultures cell. Previous results showed that in MDCK culture cells the Influenza virus growing was diminished by the use fraction V from cinnamon.

CONCLUSIONS

- The data reported herein indicate that the etheric, methanolic and aqueous cinnamon extracts possess antioxidant activity that can be measured by the b carotene/linoleic acid system;
- The aqueous extract from cinnamon powered is more antioxidative than the others. This activity correlated well with their total phenolic content.
- The cinnamon extracts can be used as food antioxidant together with the improvement of food palatability.

Fig. 3. Antioxidant activity of aqueous cinnamon extract at different concentrations

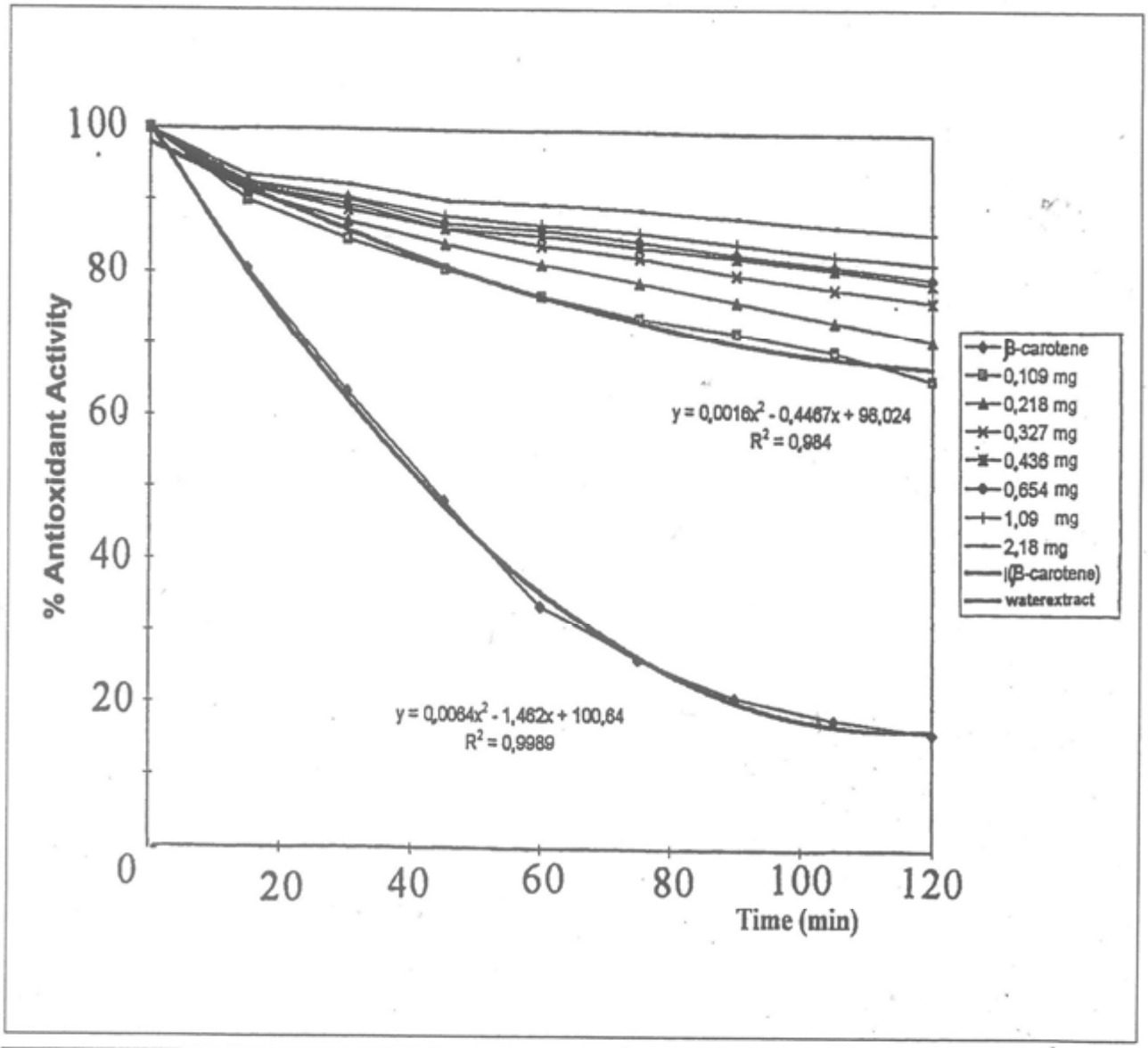


Fig. 4. Column chromatographic fractionation of aqueous cinnamon extract

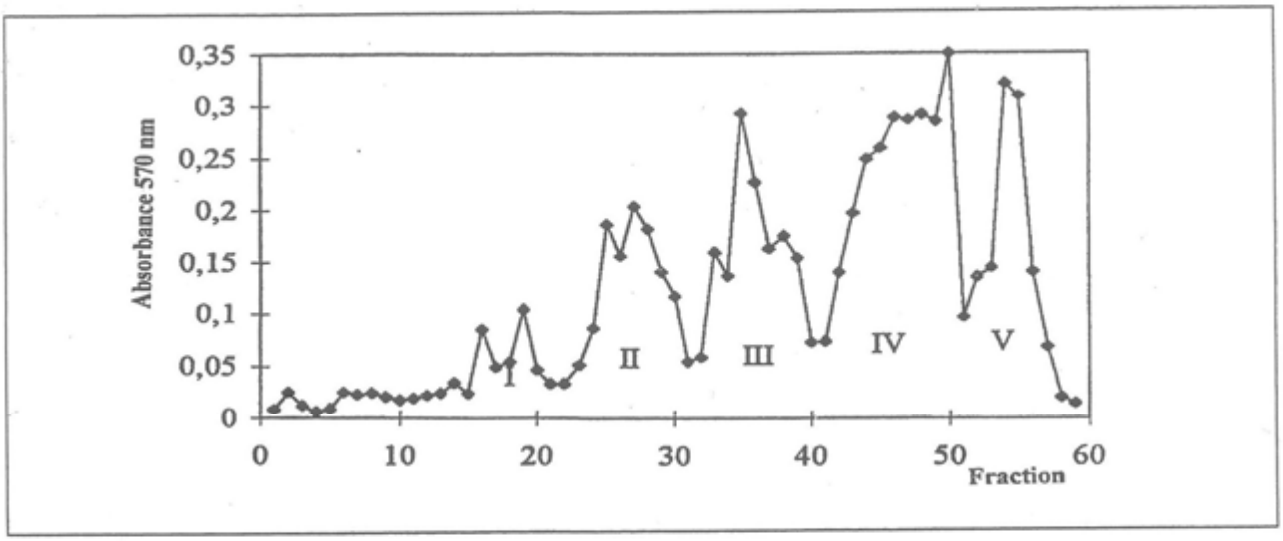
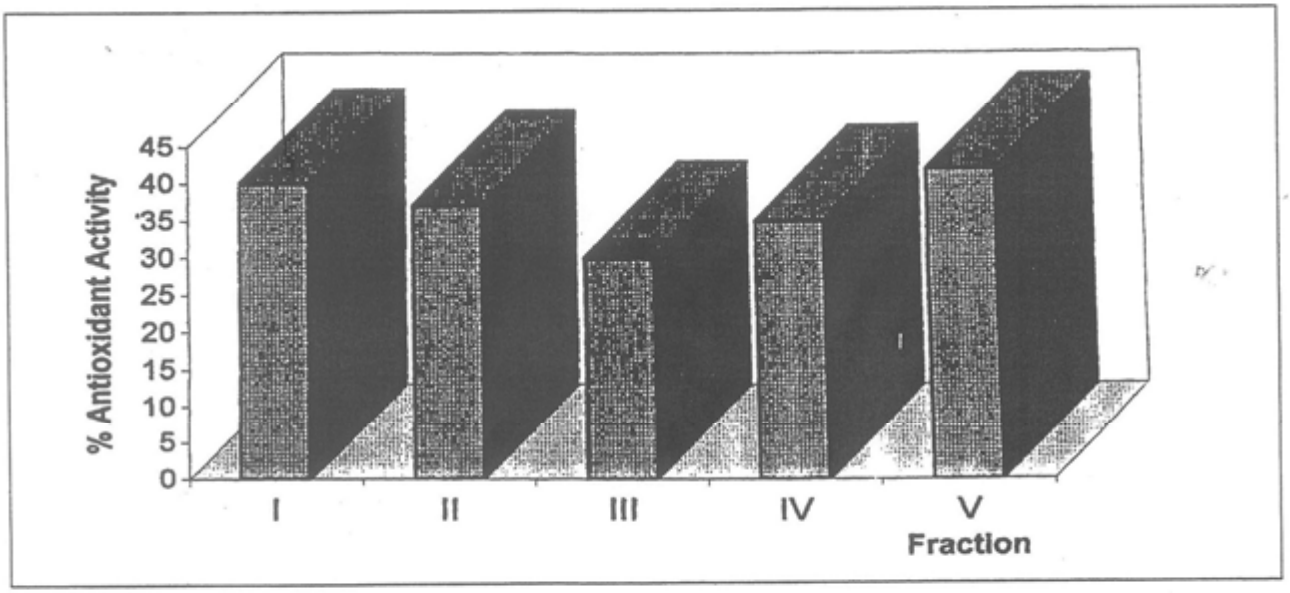


Fig. 5. Antioxidant activity of different fractons from aqueous cinnamon axtract



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