ISSN: 2320 - 4230

Journal of Drug Discovery and Therapeutics
Available Online at www.jddt.in
CODEN: - JDDTBP (Source: - American Chemical Society)
Volume 3, Issue 29; June-2015; 24-30

Research Article

Comparative HPTLC analysis and invitro antioxidant study of *cinnamon zeylanicum.*

*Makhija Manju [a], Gupta Maheshkumar K [b]

[a]M.Pharm, Rajasthan University of Health Science, Kumbha marg , Pratapnagar, Jaipur, Rajasthan, India

[b]Ph.D, Principal, Kota college of Pharmacy, Kota, Rajasthan, India

Received 03 April 2015; Accepted 05 June 2015

ABSTRACT

Cinnamon (Cinnamomum zeylanicum, Family: Lauraceae) is one of the world's oldest spices. The cinnamon, also known by Cassia, Sweet Wood, and Gui Zhi, is traditionally harvested in Asian countries. It is, perhaps, one of the oldest herbal medicines, having been mentioned in Chinese texts as long as 4000 years ago. Cinnamon contains cinnamaldehyde, eugenol, cinnamic acid etc. EECACC, EEC and AEC were comparatively standardized for the content of Cinnamaldehyde by HPTLC. All the samples were screened for the possible in-vitro antioxidant activity for 1,1-diphenyl-2-picryl-hydrazil (DPPH$^{\cdot}$) free radical scavenging, lipidperoxidation, polyphenolic contents, and reducing power effects. The Cinnamaldehyde content of EECACC, EEC and AEC was found to be 2.78, 2.08, and 0.48% (w/w) respectively. The Polyphenol and flavonoid content of EECACC was found to be 11.35, 9.50 (w/w) respectively. All the tested samples showed marked antioxidant activity in which EECACC showed prominent activity.

Key words: Cinnamon, HPTLC, antioxidant, radical scavenging.

Abbreviations

AEC- Aqueous extract of Cinnamon

EEC - Ethanolic extract of Cinnamon

EECACC - Ethanolic extract of Cinnamon after column chromatography

DPPH- 1, 1-Diphenly-2-picryl-hydrazil

INTRODUCTION

Cinnamon (Cinnamomum zeylanicum, Family: Lauraceae) is one of the world's oldest spices. It is one of the oldest herbal medicines, having been mentioned in Chinese texts as long as 4000 years ago [1].

It has been extensively studied for its anti-diabetic [2], anti-oxidant [3], anti-microbial [4] activities.

The flavor and odour of cinnamon is due to presence of major chemical constituent Cinnamaldehyde.

Cinamaldehyde is proved to have antipyretic, astringent, antimicrobial activity [5], anti - inflammatory activity [6], antibacterial and cytotoxic effect [7-9]. The antioxidative properties of some vegetables and fruits are partly due to the low molecular weight phenolic compounds, which are known to be potent as antioxidants [10]. To evaluate antioxidant activities of compounds and of complex mixtures such as plant extracts various methods have been developed [11]. Just one procedure cannot identify all possible mechanisms characterizing an antioxidant activity [12]. Therefore, the aim of this study is to evaluate the anti-oxidative activity of *Cinnamon* using several different methods, and to evaluate the relationship between the anti-oxidative activity and cinnamaldehyde content of the plants by HPTLC.

Prepared extracts of Cinnamon were standardized using high performance thin layer chromatography for the content of cinnamaldehyde. An attempt was made to compare the content of cinnamaldehyde and the in-vitro antioxidant potential of the prepared cinnamon extracts.

Materials and Methods

Procurement of Cinnamon bark

Authenticated Cinnamon barks were purchased from NRIBAS, Pune, India.

Preparation of Aqueous Cinnamon Extract (AEC)

Aqueous extract (AEC) of finely powdered cinnamon with 100 ml distilled water was prepared by continuous stirring for forty-eight hours by cold maceration method. The extract thus obtained was filtered and dried under vacuum in a rotary evaporator.

Preparation of Ethanolic Cinnamon Extract (EEC)

Alcoholic extract (EEC) of finely powdered cinnamon with 70% v/v ethanol was prepared by continuous stirring for seventy two hours by cold maceration method. The extract thus obtained was filtered and dried under vacuum in a rotary evaporator.

*Corresponding author: Makhija Manju |

Column chromatography:

Prepared ethanol extract was purified by column chromatography. Silica gel was used as stationary phase.

HPTLC

Samples were spotted as bands (width, 6 mm) with a CAMAG microlitre syringe on precoated silica gel aluminium plate 60F-254 (20 cm x 10 cm with 250 µm thickness, E. Merck, Germany) using a CAMAG Linomat IV spotter (Switzerland). A constant application rate (0.1 µl/s) was employed and space between two bands was 6 mm. Mobile phase consisted of Toluene, ethyle acetate, and formic acid (4:6:0.1 v/v/v). Linear ascending development was carried out in twin trough glass chamber saturated with the mobile phase. Optimized chamber saturation time for mobile phase was 20 min at room temperature. The length of chromatogram run was 8 cm. Subsequent to the development; TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 230 nm. Slit dimension was kept at 6 mm x 0.45 mm, and 10mm/sec scanning speed was employed. A model III TLC scanner with CATS IV integration software was used. The source of radiation utilized was deuterium lamp.

Calibration curve

A stock solution containing 52.54 Kg/ml of cinnamaldehyde Reference standard (Central Drug House, New Delhi, India) of 98% purity was freshly prepared in methanol and aliquots of 1-14 µL were applied to the TLC plate to get final concentration of 52.54-735.56 ng/spot. The plates were developed as per the conditions mentioned above. The graph was plotted for peak area versus concentration and the data was treated by linear regression analysis. The regression equation obtained was further used for the quantitative determination of cinnamaldehyde from different extracts.

Sample preparation

Weighed accurately 100 mg each of aqueous extract (AEC) ethanolic extract (EEC) and ethanolic extract after column chromatography (EECACC) and dissolved with 20ml of respective solvents by ultrasonication for 5 min. The above extracts were centrifuged at 5000 rpm for 10 min and the supernatant was filtered and the solution was subjected to chromatographic analysis along with standard solution in triplicates.

Materials and chemicals

Petroleum ether, dichloromethane, formic acid and methanol (GR grade, Merck, Mumbai, India).

Instrumentation

Precoated silica gel aluminium plate 60F-254 (20 cm x 10 cm with 250 µm thickness, E. Merck, Germany) CAMAG microlitre syringe (Capacity100Kl) CAMAG Linomat IV spotter (Switzerland). CAMAG TLC scanner III (Switzerland). CATS IV software

Antioxidant assays

Different biochemical assays were undertaken to assess the antioxidant activity of Cinnamon bark extract

Free radical scavenging activity by DPPH method

The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH$^{\cdot}$) using the method described by Shimada et al. [13]. DPPH (1, 1- diphenyl- 2-picryl- hydrazyl) is stable free radical and methanolic solution of it is used to evaluate the antioxidant activity of several natural compounds. Antioxidants on interaction with DPPH, either transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character, and convert it to 1,1- diphenyl- 2-picryl- hydrazine and the degree of discoloration indicates the scavenging activity of the drug. The change in the absorbance produced at 517 nm, has been used as a measure of antioxidant activity. 1ml Different concentration of extract solution and standard were taken in different vials. To this 5 ml of methanolic solution of DPPH was added, shaken well and mixture was incubated at 37 ^{0} C for 20 min. Measure the absorbance against methanol as blank at 517nm. Take absorbance of DPPH as control.

Percent antiradical activity can be calculated by using following formula.

% Anti-radical activity = (Control Abs - Sample Abs x 100 / Control Abs)

Reducing power assay

Total reduction capability was estimated by using the method of Oyaizu [14].The reducing capability was measured by the transformation of Fe3+- Fe2+ in the presence of different extracts at 700nm as per the reported method**.** Increased absorbance of the reaction mixture indicates increased reducing power.

Procedure

1ml of different concentrations of extracts (250-2500 mg/ml) was mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferrocyanide. The mixture was incubated at 500C for 20 min. 2.5 ml of trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 RPM for 10 min. 2.5 ml of upper layer solution was taken and mixed with 2.5 ml distilled water and 0.5 ml of $Fecl_3$ solution and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Anti-lipid peroxidation effect

Lipid peroxidation in rat liver and brain homogenate was evaluated by the TBA method [15].Decomposition of lipid membrane in the body leads to the formation of Malondialdehyde (MDA) along with other aldehydes and enals as the end product.

These react with thiobarbituric acid to form colored complexes. Hence these are called as the Thiobarbituric Acid Reactive Substances (TBARS). The complex of TBA-MDA is selectively detected at 532 nm using UV spectrophotometer.

The mixtures containing 0.5 ml of homogenate, 1 ml of 0.15 M Kcl, and 0.5 ml of different concentrations of drug extract were prepared. Lipid peroxidation was initiated by adding 100 µl of 1 mM ferric chloride. The reaction mixtures were incubated for 30 min at 37°c. After incubation, the reaction was stopped by adding 2 ml of ice-cold 0.25 N HCl containing 15% trichloroacetic acid (TCA) & 0.38% thiobarbituric acid (TBA), and 0.2 ml of 0.05% butylated hydroxy toluene (BHT). These reaction mixtures were heated for 60 min at 80°C, cooled and centrifuged at 5000 g (≈ 6900 rpm) for 15 min. The absorbance of the supernatant was measured at 532 nm against a blank, which contained all reagents except liver homogenate & drug. Identical experiments were performed to determine the normal (without drug and $FeCl_3$) and induced (without drug) lipid Peroxidation level in the tissue. The percentage of anti-lipid Peroxidation effect (% ALP) was calculated by the following formula:

% ALP = ($FeCl_3$O.D.–Sample O. D.) ×100 / $FeCl_3$ O.D. – Normal O.D

Total Phenolic content

The total phenolic content in the samples was measured according to the method described by Harish and shivanandappa [16].

One ml of the extract was added to 10 ml of distilled water and 2 ml of Folin-phenol reagent. The mixture was then allowed to stand for 5 min and 2 ml of sodium carbonate was added to the mixture. The absorbance was measured at 765 nm in a spectrophotometer. Phenolic content was estimated using gallic acid as the standard and expressed as µg of gallic acid equivalent per g of extract.

Total Flavonoid content determination

The total flavonoid content was determined using the method of Meda et al [17]. In brief, 5mL of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the equal volume of the extract solution. After 10 minutes absorption was measured spectrophotometrically at 415 nm against a blank sample consisting of a 5 mL extract solution with 5 mL methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin (100-1000 µg/L) as the standard. The result is expressed as µg of quercetin equivalents/g of extract.

Statistical analysis

All the data are expressed as mean ± standard error of the mean (SEM). IC_{50} values were calculated using linear regression analysis from the regression equation obtained using standard calibration curve. One way ANOVA followed by Dunnett's multiple comparison tests was performed. $p<0.05$ was considered to be significant.

Results

Standardization

Quantitative investigation for cinnamaldehyde content in different cinnamon extracts aqueous (AEC), ethanolic (EEC) and Ethanolic extract after column chromatography (EECACC) were carried out. The study shows that purity of cinnamaldehyde was improved by column chromatography.

DPPH free radical scavenging activity

The extracts of cinnamon showed concentration dependant increase in DPPH free radical scavenging activity. DPPH scavenging activity was found in the order: Ascorbic acid (98.89%) > EECACC (72.15%) > EEC (56.34%) > AEC (35.27%) EECACC showed excellent DPPH free radical scavenging activity as compared to ethanolic (EEC) and aqueous extract (AEC).

Reducing power

All the tested concentrations of the samples showed higher reduction capability. EECACC showed higher reduction capability. The reducing powers of ethanolic extract after column chromatography (EECACC), ethanolic extract and aqueous extracts increased with increasing concentration. The reducing power exhibited by the samples were statistically significant ($p<0.01$) and followed the order EECACC >EEC>AEC respectively.

Anti-lipid peroxidation activity

The extracts of cinnamon showed concentration dependant increase in anti-lipid peroxidation activity. Anti-lipid peroxidation activity was found in the following order:

Ascorbic acid (93.63%) > EECACC (72.15%) > EEC (59.23%) > AEC (33.31%)

Total polyphenol and flavonoid content

The presence of phenolic and flavonoids always correlated with the antioxidant activity. Phenolic compounds and flavonoids are considered to play a major role in the antioxidant activity of the plants. So the extraction of these components is a valuable task in determining the antioxidant property. The total phenolic content and flavonoid content of the samples are shown

in table 2**.** Our findings suggest that EEACC is rich in polyphenol and flavonoid composition. Total polyphenol and flavonoid content was observed to be increased after column chromatography.

Table 1: Quantitative determination of cinnamaldehyde in prepared extracts

Extracts	Cinnamaldehyde Content (%)[a]
AEC	0.48
EECACC	2.78
EEC	2.08

a = average value of three determinations.

Table 2: Total phenolic and flavonoid content of the tested samples.

Extract Type	Total polyphenol Content (mg/g)	Total flavonoid Content (mg/g)
EECACC	11.35 mg/g	9.50 mg/g
EEC	10.20 mg/g	8.15 mg/g
AEC	4.30 mg/g	4.10 mg/g

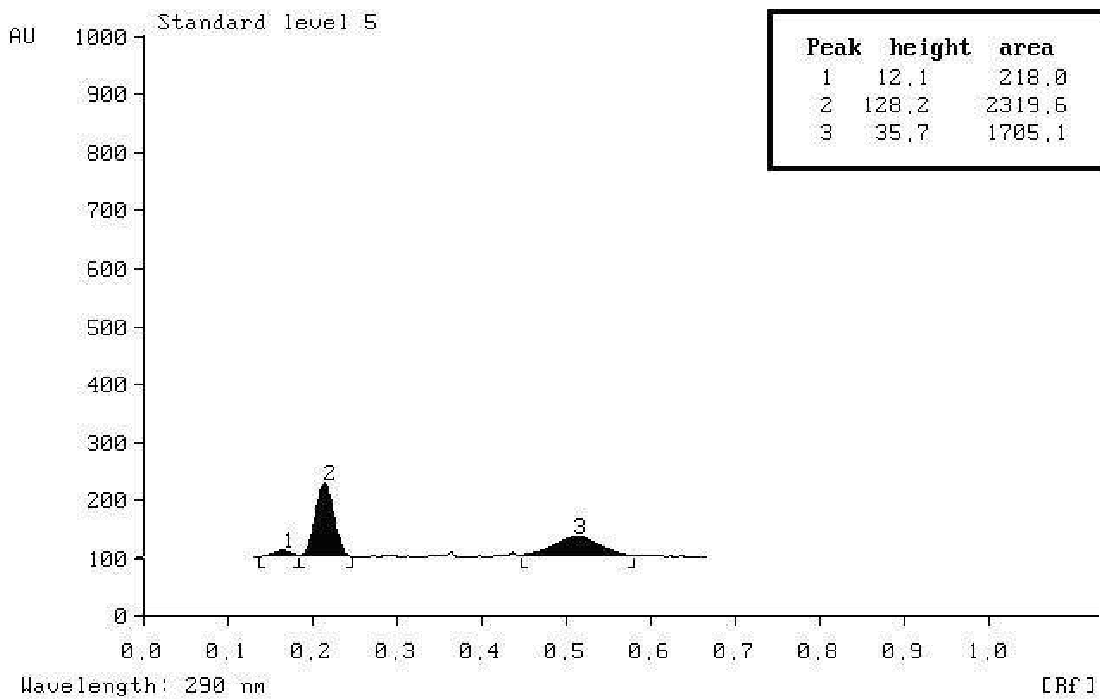

Figure 1: Densitogram of aqueous extract of Cinnamon (AEC).

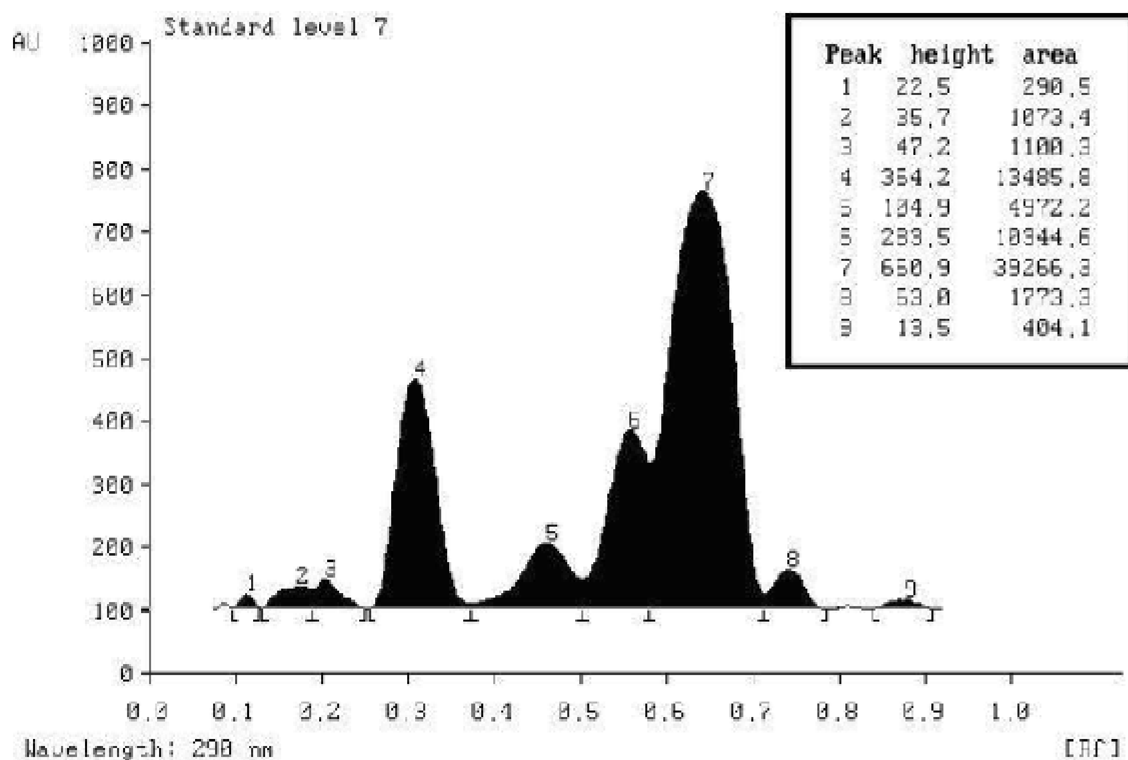

Figure 2: Densitogram for Ethanolic extract of Cinnamon after column chromatography (EECACC)

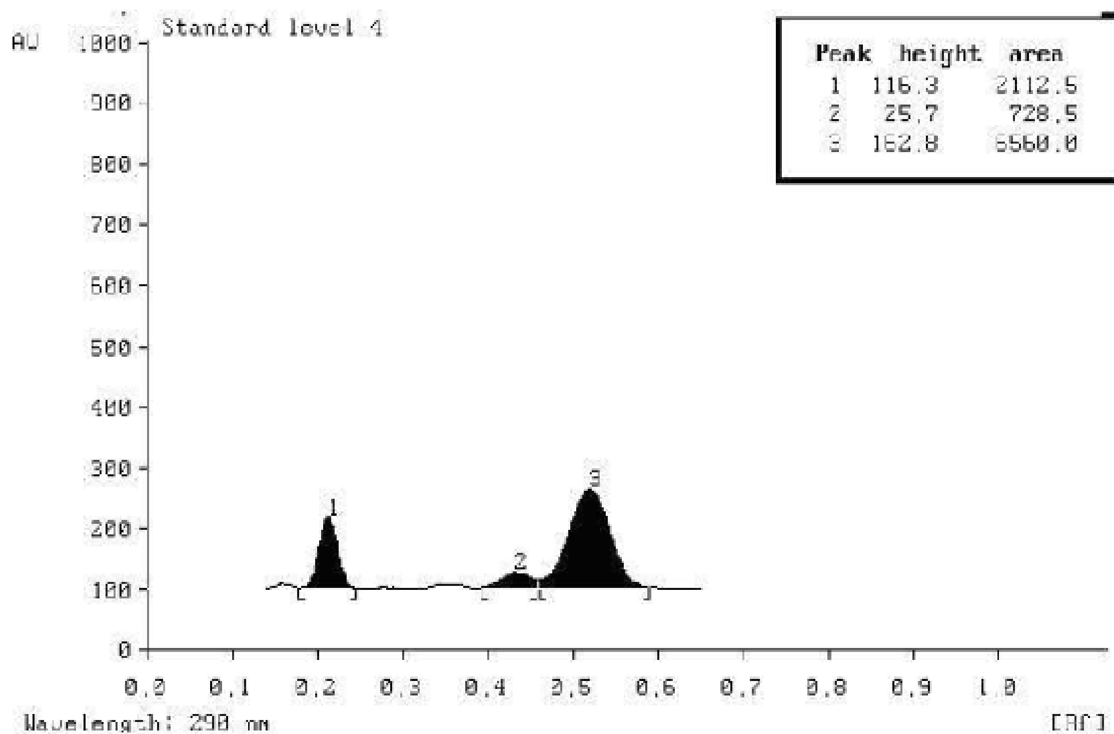

Figure 3: Densitogram for Ethanolic extract of Cinnamon (EEC)

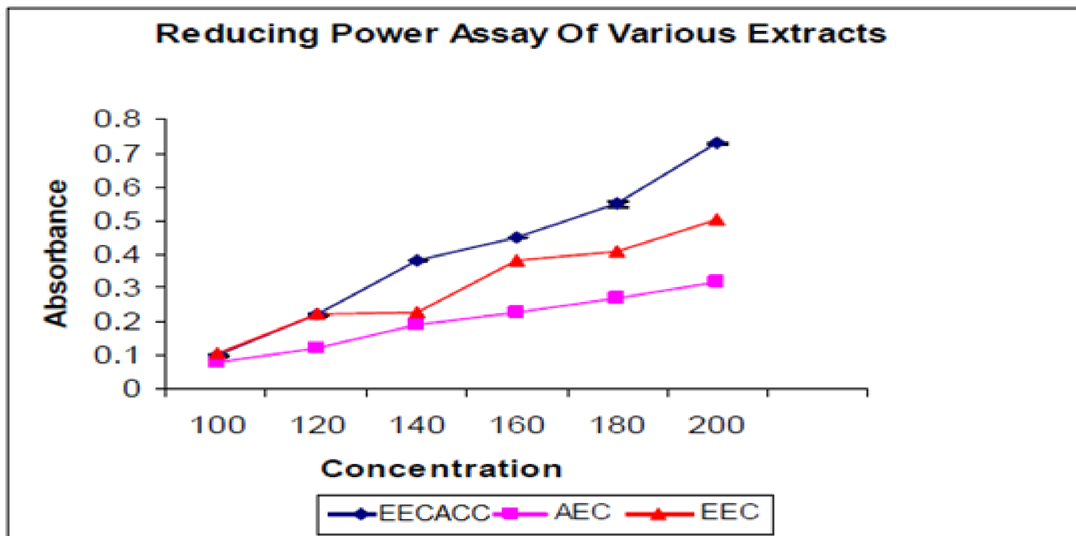

Figure 4: Total reducing power of the tested samples.

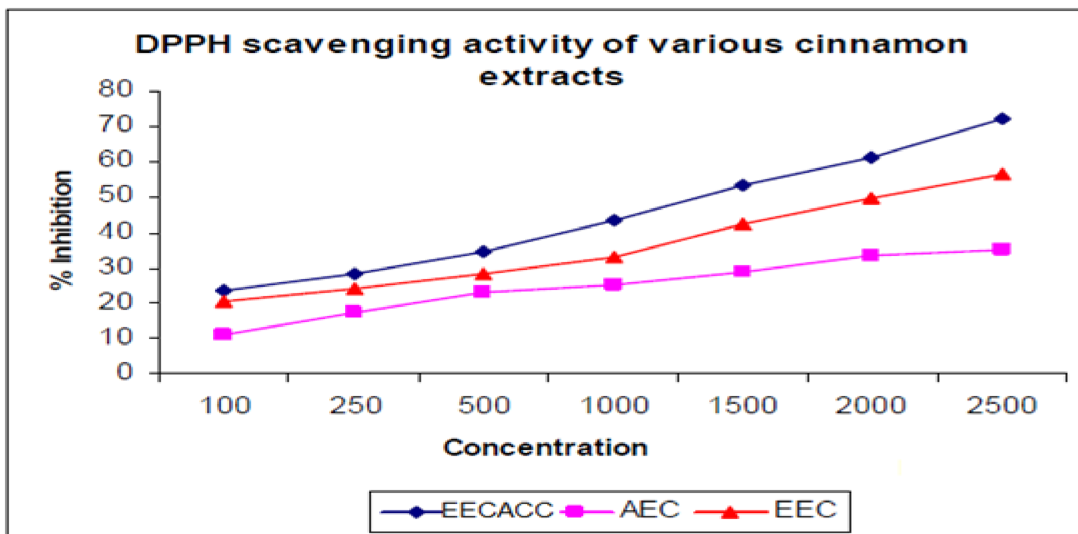

Figure 5: % DPPH scavenging effect of the tested samples.

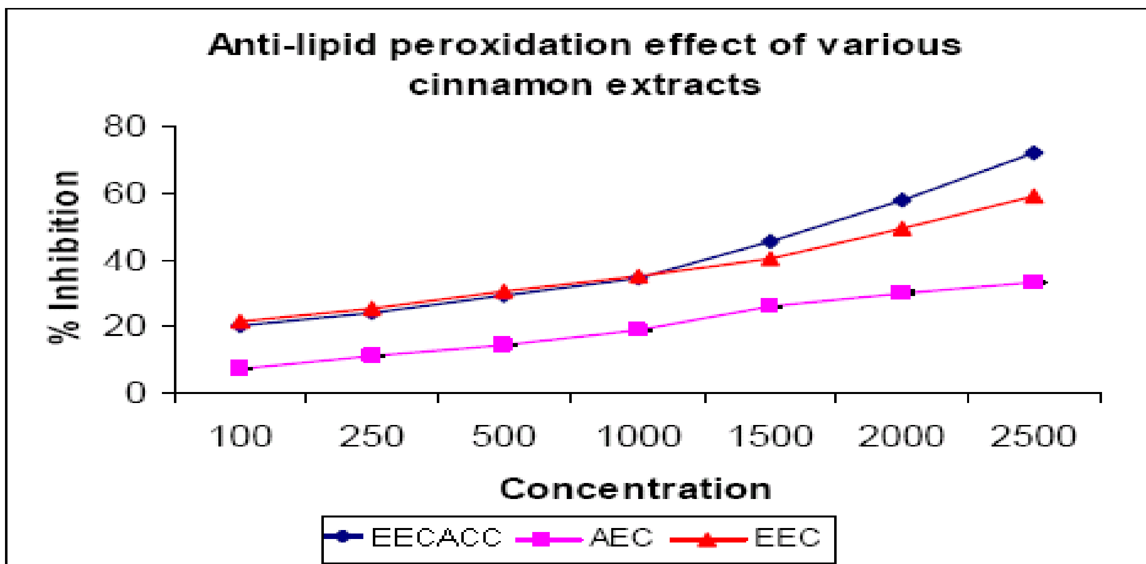

Figure 6: % Antilipid peroxidation of the tested samples.

Conclusion

The EECACC was proved to possess high antioxidant potential than the other extracts. The study shows that cinnamaldehyde content is maximum with EECACC than with other extracts. The polyphenol and flavonoid content is maximum with EECACC than with other extracts. It shows that though polyphennnol and flavonoid are responsible for the antioxidant property of Cinnamon, the high antioxidant activity may be due to synergistic effect by cinnamaldehyde which were better extracted with EECACC. The study shows that purity of cinnamaldehyde was improved by column chromatography.

References:

1. K. Toriizuka. Pharmacological effect of cinnamon. Kampo Med 1998; 11: 431-436.
2. Anderson RA. Isolation and characterization of polyphenol type- A polymers from cinnamon with insulin-like biological activity. Journal of Agricultural and Food Chemistry 2004.
3. Singh G., Sumitra Maurya, M.P. deLampasona, Cesar A.N. Catalan. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food and Chemical Toxicology 2007; 1-35.
4. Matan N., Rimkeeree H, Mawson A.J., Chompreeda P., Haruthaithanasan V., M. Parker. Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. International Journal of Food Microbiology 2006; 107: 180– 185.
5. Shaik Mahaboob Ali., Aleem A Khan., Irshad Ahmed M.Musaddiq., Khaja S Ahmed., H.Polasa., L. Venkateswara Rao., Chittoor M Habibullah., Leonardo A Sechi. And NIyaz Ahmed., 2005. Antimicrobial activities of Eugenol and Cinnamaldehyde against the human gastric pathogen Helicobacter pylori, Annals of Clinical Microbilogy and Antimicrobials, 4(20), 1-7 (2005)
6. Youn H.S., Lee J.K., Choi Y.J., Saitoh S.I., Miyake K., Hwanq D.H. and Lee J.Y.,2008. Cinnamaldehyde suppresses toll-like receptor 4 activation mediated through the inhibition of receptor oligomerization, Biochem Pharmacol, 75(2), 494-502.
7. Chang S.T., Chen P.F. and Chang S.C., 2001. Antibacterial activity of leaf essential oils and their constituents from Cinnamomum osmophloeum, Journal of Ethnopharmacology, 77(2), 123-127.
8. Fang H., Rao Y.K. and Tzeng Y.M., 2004. Cytotoxic effect of trans-Cinnamaldehyde from Cinnamomum osmophloeum Leaves on Human Cancer Cell Lines, Int. J. Appl. Sci. Eng, 2(2), 136-137.
9. Lee H.S.,2002. Inhibitory activity of Cinnamomum cassia bark-derived component against rat lens aldose reductase, J. Pharm. Pharmaceut. Sci, 5(3), 226-230.
10. H Wang, M.G. Nair, G.M. Strasburg, A.M. Booren, J.I. Gray, 1999, Antioxidant polyphenols from tart cherries (Prunus cerasus), Journal of Agricultural and Food Chemistry, 47(3), 840-844.
11. M Antolovich, P.D. Prenzler, E. Patsalides, S. McDonald, and K. Robards, 2002, Methods for testing antioxidant activity, The Analyst, 127(1), 183-198.
12. EN Frankel, and A.S. Meyer, 2000, The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants, Journal of the Science of Food and Agriculture, 80(13), 1925-1941.
13. Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T. 1992. Antioxidative properties of xanthone on the auto oxidation of soybean in cylcodextrin emulsion. J. Agric. Food Chem. 40, 945 – 948.
14. Oyaizu M. (1986) Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr 44: 307 – 15.
15. Ohkawa H, Ohishi N, Yagi K, 1979. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem, 95, 351–358
16. Harish, R and shivanandappa, T.,2006. Antioxidant activity and hepatoprotective potential of Phyllanthus niruri. Food Chemistry 95, 180-185.
17. Meda, A., Lamien, C.E., Romito, M., Millogo, J., Nacoulma, O., G. 2005. Determination of the total phenolic, flavonoid and proline contents in burkina fasan honey, as well as their radical scavenging activity. Food Chemistry, 91, 571–577.