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EFFECT OF POLARITY OF SOLVENTS ON *CINNAMOMUM ZEYLANICUM* BARK EXTRACTION

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ABSTRACT

Cinnamomum zeylanicum bark (CZB) powder was extracted in milli-Q-water, 60% methanol and ethyl acetate with polarity 10.2, 8.2 and 4.4 respectively on Tarsons's spinix orbital shaker at room temperature to inspect their various potent antioxidant properties. Extracted polyphenols from CZB in milli-Q-water, 60% methanol and ethyl acetate were 39.03 ± 4.59, 84.31 ± 7.33 and 18.85 ± 2.17mg gallic acid equivalent (GAE)/g dry bark weight respectively as major antioxidant compounds. The flavonoid compounds were 18.35 ± 0.90, 27.12 ± 1.50 and, 9.19 ± 1.08mg quercetin equivalent (QE)/g dry bark in milli-Q-water, 60% methanol and ethyl acetate respectively. Extracts were assayed for DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity and metal induced lipids peroxidation inhibition for the antioxidants properties. Presence of the extract equivalent to 1µg dry bark extract could protect DNA (deoxyribonucleic acid) against damage under H_2O_2 induced oxidative stress. The results of this study indicate that extract in 60% methanol had more polyphenols and exhibited better free radical scavenging, reducing and metal chelating activity, which stand to protect biomolecules like lipids and DNA against oxidative stress.

KEYWORDS: Polyphenols, flavonoids, *Cinnamomum zeylanicum*, DNA, oxidative stress.

INTRODUCTION

Cinnamon is a spice obtained from the inner bark of several trees from the genus Cinnamomum that is used in both sweet and savory foods.[1] The phenolic extracts of plants are always a mixture of different classes of phenols, which are selectively soluble in the solvents. It was reported that *C. zeylanicum* is the richest source of antioxidant.[2] *C. zeylanicum* (cinnamon) and *C. cassia* (cassia) are the source of the oldest spices known to man.[3,4] Polyphenols had been reported in dry bark of *C. zeylanicum* extracted with water, methanol and chloroform using a Soxhlet extractor.[5] Antioxidant composition of aqueous extract of fifteen spices including *C. cassia* has been explored and found that it has high amount of flavonoid and saponin contents compared to other spices.[6] Leafy vegetables, fruits and seeds are good sources of ascorbic acid, vitamin E and phenolic compounds as antioxidants to reduce oxidative damage associated with diseases like arthritis and diabetes.[7] It has been recognized that various solvents affect the phytochemical extractions and their antioxidant activities.[8] Differential antioxidant activity of basil leaves has been profiled in different solvents.[9] It has been demonstrated that polarity of solvents has effect on extraction of polyphenols from Tunisian date seeds.[10] The present study was undertaken to identify and quantify the possible active antioxidant principles in *C. zeylanicum* in varying solvents and to study their effect on the oxidative damage induced in biomolecules.

Chemicals: Chemicals viz. agarose, diphenylpicrylhydrazyl (DPPH), ethyl acetate, gallic acid, hydrogen chloride (HCl), lecithin soya, methanol, sodium hydroxide (NaOH), sodium nitrite ($NaNO_2$), thiobarbuteric acid, bromophenol blue, glycerol, quercetine, tris-base, sodium carbonate, sodium phosphate (monobasic, dibasic), thiobarbituric acid (TBA), xylene cyanol were obtained from HiMedia Laboratories Pvt. Ltd. Ferrous sulfate ($FeSO_4$) and trichloro acetic acid (TCA) obtained from RFCL Ltd. Hydrogen peroxide obtained from Ranbaxy Laboratories Ltd., Folin Ciocalteu's reagent obtained from Loba Chemie Pvt. Ltd., $CuCl_2$ obtained from SD Fine-Chem Ltd. and ethanol was purchased from Bengal Chemicals and Pharmaceuticals Ltd.

MATERIAL AND METHODS

C. zeylanicum bark was purchased from FSTL (Flavourit Spices Trading Limited), Cochin, Kerala, India. Bark of *C. zeylanicum* was dried at 37°C in oven till constant weight is attained. Finely powdered barks (1g/10ml) were extracted for four hours with milli-Q-water, 60% methanol, and ethyl acetate (with decreasing polarity) in triplicate in Tarsons's spinix

orbital shaker at room temperature.[11] Residues were again extracted with relevant solvents for the same time. Collected extracts were filtered through double layered muslin cloth followed by centrifugation at 5000g for 5min to get clear supernatant. Extracts were concentrated in a vacuum evaporator and stored at -20°C for further use.

Estimation of Antioxidants

Polyphenols: Quantification of Polyphenols was done by The Folin-Ciocalteu method.[12] Aliquot (40µl) of the extracts were mixed with 200µl Folin reagent (1N) and after 5min, 600 µl of 20% (w/v) sodium carbonate was added for colour development, which was read at 765nm after two hours. Standard curve with gallic acid was prepared and results expressed as mg gallic acid equivalent (GAE)/g dry bark.

Flavonoids: These were estimated using $NaNO_2$ and $AlCl_3$ in alkaline medium.[13] Aliquot (0.1ml) of the extract was mixed with 0.03ml of 5% (w/v) $NaNO_2$ at 25°C and after 5min, 0.03ml of 10% (w/v) $AlCl_3$ and 0.2ml NaOH (1mM) was added. Total volume of reaction mixture made 1ml by adding distilled water. Developed colour was read at 510nm. Standard curve with quercetine was prepared and results were displayed as mg quercetine equivalent (QE)/g dry bark.

Estimation of Antioxidant activity

DPPH free radical scavenging activity: Different dilutions of the extracts were incubated with 1.0 ml of DPPH solution ($50x10^{-5}$M) in a final volume of 1.1ml.[14] The decrease in absorbance due to the scavenging of DPPH radicals by the extract was recorded at 517 nm. The percentage of remaining DPPH after 5min with different dilutions of extract was calculated and the concentration at which 50% of the initial DPPH could be scavenged was noted from the graph.

Lipid peroxidatoin inhibition: Inhibition of lipid peroxidation estimated as thiobarbituric acid reactive substances (TBARS).[15] Lipid peroxidation inhibition was monitored as the amount of malonaldehyde (MDA) produced by copper induced soya lecithin peroxidation. Different dilutions of the extracts were added to the reaction mixture containing 2.5mM lecithin and 250mM $CuCl_2$ in 50mM Tris-HCl buffer (pH 7.4) in a total volume of 1ml. After incubation at 37°C for 15min, malonaldehyde produced was monitored as thiobarbituric acid reacting substances by adding 2ml of thiobarbituric acid (TBA) reagent containing 0.37% (w/v) TBA, 15% (w/v) TCA, 0.04% (w/v) BHT and 2% (v/v) ethanol. Mixture was heated at

100°C for 15min and centrifuged at 3000 rpm for 10min. The absorbance of supernatant at 535nm is an index of malonaldehyde concentration. Inhibition of lipid peroxidation estimated as percent dectrease in thiobarbituric acid reactive substances (TBARS) production.

Protection of calf thymus DNA: Hydroxyl radicals generated by Fenton's reaction were used to induce oxidative damage to DNA.[16] The reaction mixture (9µl) containing 1.5µg of calf thymus DNA in 20mM phosphate buffer saline (pH 7.4) and different extracts (equivalent to1µg CZB) was pre-incubated for 15min at ambient temperature. The oxidation was induced by incubating DNA with 1mM $FeSO_4$ and 10mM ascorbic acid for one hour at 37°C. The reaction was terminated by the addition of loading buffer (Xylene cyanol, 0.25% (w/v); bromophenol blue, 0.25% (w/v) and glycerol, 30% (w/v). The mixture was subjected to electrophoresis through 1% (w/v) agarose gel in TAE buffer run at 60Volt. DNA was visualized and photographed by Chemidoc (Biorad) to assess the damage by H_2O_2 and protection by CZB extracts.

RESULT AND DISCUSSION

Spices along with other condiments are used in human diet to impart aroma, taste and colour. The consumption of spices as integral part of food increases the digestion stimulating action, antidiabetic influence, anti-inflammatory and antioxidant potential.[17] The presence of antioxidants in spices imparts them pharmaceutical and medicinal value. Various antioxidants naturaly found in spices can inhibit the propagation of reactions of ROS and/or scavenge them. The present studies analyzed the possible antioxidant properties of bark of *C. zeylanicum*. The antioxidant potential of extracts in various solvent was examined against oxidative damage to biomolecules.

Antioxidants: Quantification of polyphenols and flavonoid content of various extracts was performed and results are shown in the Table 1. It is evident from the observation that the maximum polyphenolic and flavonoid contents from *C. zeylanicum* were extracted in 60% methanolic solvent followed by water and then ethyl acetate.

Table 1: Concentration of various antioxidant compounds in *C. zeylanicum* extract.

Solvents (Polarity)	Polyphenol (mg GAE/g dry bark)	Flavonoids (mg QE/g dry bark)
Milli-Q-water (10.2)	39.02± 4.59	18.35 ± 0.90
60% Methanol (8.2)	84.31± 7.33	27.12 ± 1.50
Ethyl Acetate (4.4)	18.85 ± 2.18	09.19 ± 1.08

Antioxidant activities

DPPH free radical scavenging activity: DPPH free radical scavenging assay was performed to determine antioxidant activities of the extracts. Antioxidants present in extracts donate their hydrogen to reduce DPPH, reduced it to hydrazine and colour changes from purple to yellow. The degree of discoloration shows the scavenging potential of the antioxidant compound in terms of hydrogen. CZB extracts exhibited a concentration dependent scvanging of DPPH free radicals in the reaction mixture (Fig.1). It is evident from the observation that maximum DPPH free radical scavenging activity was shown by 60% methanolic extract followed by milli-Q-water and ethyl acetate extract of *C. zeylanicum* respectively. IC50 values of various extracts of CZB in gradient polarity solvents for DPPH free radical scavenging activity were shown in Table 2. The least IC50 value was exhibited by extract in 60% methanol. These results show that 60% methanol has high potential to extract antioxidants from spice bark.

Lecithin peroxidation inhibition: Free radicals and reactive oxygen species oxidize the polyunsaturated lipids throgh the beginning and propagation of oxidative chain reactions. Such reactions leading to the damage of fatty foods. Polyunsaturated fatty acids of cell membrane are more susseptible to oxidation and loss their intigrity and leading to cell death[17]. Oxidation of lipids by heavy metal ions such as iron and copper can induce production of malonaldehyde. The malonaldehyde produced by copper induced oxidation of soya lecithin in presence and absence of gradient concentrations of *C. zeylanicum* extract were determined as thiobarbituric acid reactive substances (Fig.2). IC50 values of various extracts of CZB for lecithin peroxidation inhibition activity were shown in Table 2. The least IC50 value was showen by extract in 60% methanol. Results show that 60% methanol has high extraction potential for antioxidants from spice bark.

Table 2: IC50 values for DPPH free redicle scavenging and Lecithin peroxidation inhibition activity for various antioxidant compounds in CZB extract.

Solvents (Polarity)	IC50 values of CZB dry wt. (mg) for DPPH free radicle scavenging activity.	IC50 values of CZB dry wt. (mg) for Lecithin peroxidation inhibition activity.
Milli-Q-water (10.2)	1.830 ± 0.020	1.693± 0.005
60% Methanol (8.2)	0.040 ± 0.001	1.084± 0.003
Ethyl Acetate (4.4)	0.794 ± 0.251	2.219± 0.115

Protection of DNA: Heavy metal induced oxidative modification in DNA and protection their of by various extracts of *C. zeylanicum* is shown in Fig.3. Calf thymus DNA can be fragmented under oxidative stress developed by Fenton's reaction in vitro. It was observed that all the extracts were effective in preventing DNA oxidative modifications, but maximum protection is there in the pesence of 60% methanol extract. Antioxidants present in extracts of *C. zeylanicum* prevent or delay oxidative damage to DNA under stress condition.

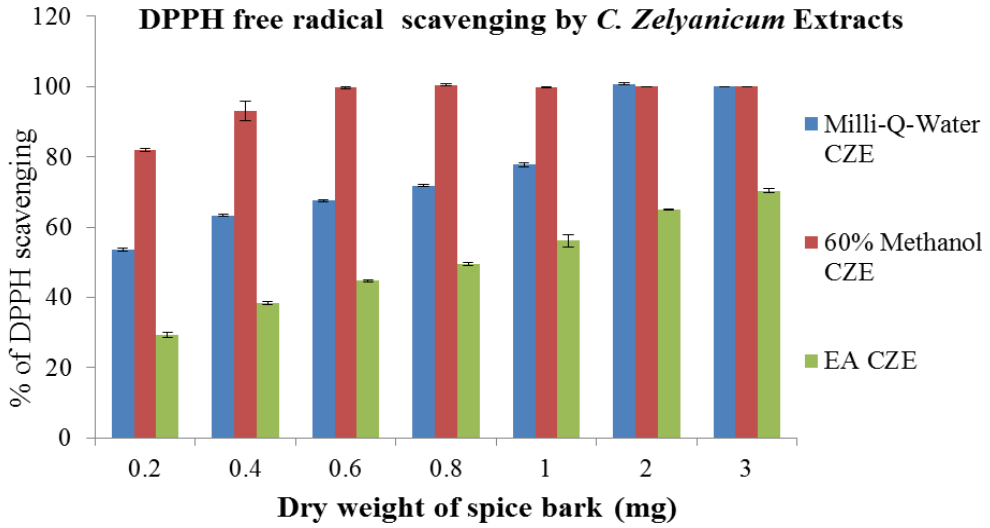

Fig. 1: DPPH free radical scavenging by milli-Q-water, 60% methanol and ethyl acetate extracts of *C. zyelanicum*.

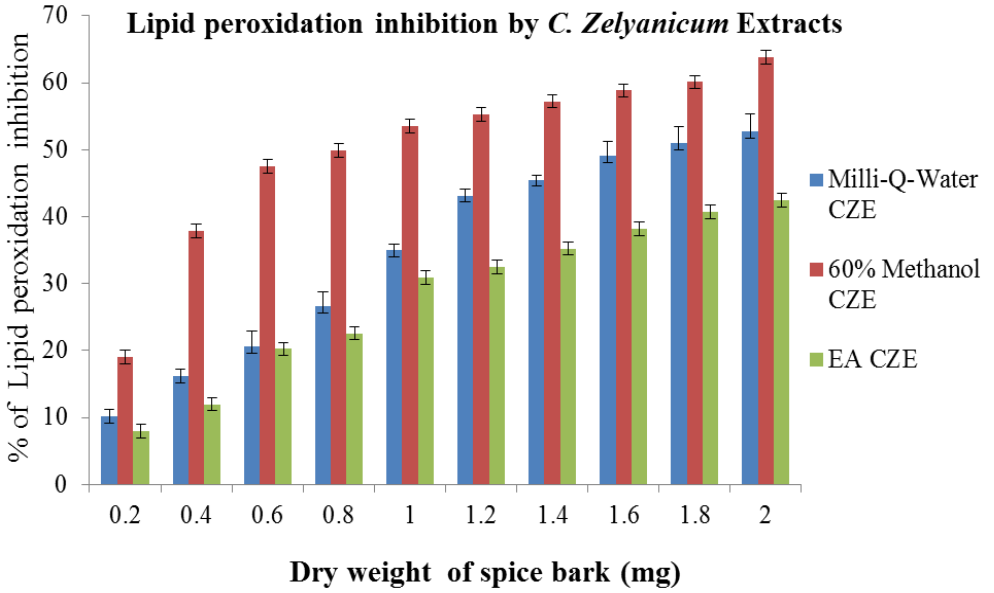

Fig. 2: Lipid peroxidation inhibition by milli-Q-water, 60% methanol and ethyl acetate extracts of *C. zeylanicum*.

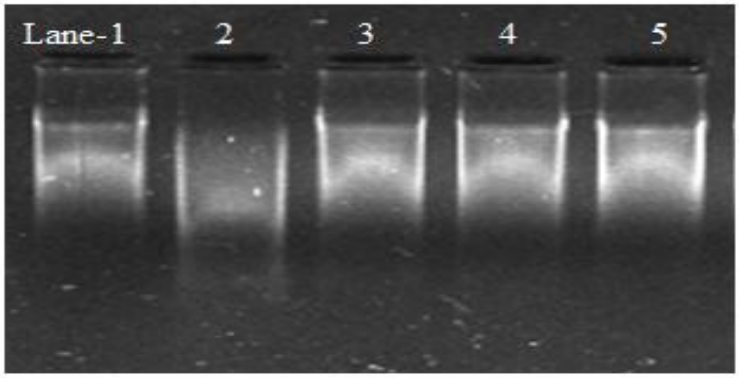

Fig. 3: Calf thymus DNA protection against oxidative stress by extracts. Lane-1: control DNA (1.5 µg); Lane-2: DNA + Fenton's reagent with ascorbic acid; Lane-3: extract in milli-Q-water (1.0 µg) + DNA + Fenton's reagent with ascorbic acid; Lane-4: extract in 60% methanol (1.0 µg) + DNA + Fenton's reagent with ascorbic acid; Lane-5: extract (1.0 µg) + DNA + Fenton's reagent with ascorbic acid.

CONCLUSION

This study supports the hypothesis that the polarity of solvent influenced the polyphenol extraction. 60% methanol with polarity 7.14 is effective in extraction of phytochemical especially polyphenols. The data analysis revealed that polyphenolic compounds and flavonoids were extracted by 60% methanol more effectively compared to other solvents with higher or lower polarity. Since the polyphenols are known to contribute to the antioxidant properties of herbs and spices, further study will be carried out for qualitatively and quantitatively analysis of polyphenols by HPLC/ MS.

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