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Full Length Research Paper

Microwave assisted extraction and evaluation of *in vitro* antioxidant activity of *Cinnamomum aromaticum*

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Cinnamomum aromaticum **or Chinese cinnamon, belonging to the laurel family (Lauraceae) is an evergreen tree native to southern China, Bangladesh, India, and Vietnam. The dried bark of *C. aromaticum* is used as a spice. In this study, extraction and evaluation of *in vitro* antioxidant activity of *C. aromaticum* bark with ethanol using microwave - assisted extraction and soxhlet extraction were carried out. Antioxidant properties of two extracts were investigated employing various established systems *in vitro* including 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, ABTS assay, total antioxidant activity, hydroxyl radical assay and total reducing power. Total phenolic content of microwave - assisted extract (MCA) and conventional extract of *C. aromaticum* (CCA) reached 546.2±0.45 and 543.9±0.86 mg/g dry weight, respectively, expressed as gallic acid equivalents, which were quantified using Folin-Ciocalteau reagent. Subsequently, total flavonoids content of MCA and CCA extracts were found to be 126±0.18 and 121±0.34 mg/g dry weight, respectively, expressed as rutin equivalents (P < 0.05). MCA and CCA showed excellent antioxidant in all test systems compared to standard ascorbic acid and the antioxidant activities of MCA were all superior to those of CCA. The results obtained in this study clearly indicate that *C. aromaticum* has a significant potential to use as a natural anti-oxidant agent.**

Key words: *Cinnamomum aromaticum*, microwave assisted extract, antioxidant activity, total flavonoids content, total phenolic content.

INTRODUCTION

Many extraction techniques have been used for obtaining bioactive compounds from plant origins nowadays. Conventional extraction is usually performed at reflux temperature for several hours, and this method is very time consuming and requires relatively large quantities of solvents (Proestos and Komaitis, 2008). Over the last decade, the demand for new extraction techniques has encouraged the development of alternative extraction techniques. Microwave-assisted extraction is the simplest and the most economical technique for extraction of many plant derived compounds (Hemwimon et al., 2007). Microwave assisted extraction can reduce both the extraction time and solvent consumption compared to conventional methods, as demonstrated by the drastically accelerated removal of a variety of compounds from solid matrices (Pan et al., 2008). Furthermore, it has the potential to improve extraction quality. Many studies have been undertaken to investigate bioactive compounds of plants deal with microwave-assisted extraction (Pallaroni et al., 2002). China is very rich in endogenous medicinal and aromatic plants that are used as natural health care products in traditional medicine. A number of these plants have been investigated for their biological activities and antioxidant principles (Cai et al., 2004; Pan et al., 2004, 2009). Oxidation is a basic part of the aerobic life and our metabolism. During oxidation, many free radicals are produced which have an unpaired nascent electron. Atoms of oxygen or nitrogen having central unpaired electron are called reactive oxygen or nitrogen species (Finkel and Holbrook, 2000; Halliwell, 2000; Pietta, 2000; Visioli et al., 2000). These are harmful to the body and may cause peroxidation of membrane lipids, aggression of tissue membranes and proteins or damage to DNA and enzyme (Hussain et al., 1987). These can be the cause of some pathology related to arthritis, hemorrhagic

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shock, coronary artery diseases, cancer as well as age related degenerative brain diseases (Parr and Bolwell, 2000). Antioxidants are the vital substances which possess the ability to protect the body from damage caused by the free radical induced oxidative stress (Ozsoy et al., 2008). There is an increasing interest in the study of antioxidant substances mainly due to the findings of the therapeutic effects of free radical scavengers on the organism. A great number of plants worldwide showed a strong antioxidant activity (Baratto et al., 2003; Katalynic et al., 2006) and a powerful scavenger activity against free radicals (Vellosa et al., 2006; Kumaran and Karunakaran, 2007).

Human body posse's defense mechanisms against free radical induced oxidative stress which involves preventive mechanisms, repair mechanisms, physical defenses and antioxidant defenses. Enzymatic antioxidant defenses such as superoxide dismutase (SOD), glutathione peroxidase (GP_X), catalase (CAT) etc. Non enzymatic antioxidants are ascorbic acid (vitamin C), α tocopherol (vitamin E), glutathione (GSH), carotenoids, flavanoids etc. All these act by one or more of the mechanisms like reducing activity, free radical Scavenging, potential complexing of pro-oxidant metals and quenching of singlet oxygen. It is possible to reduce the risks of chronic disease and prevent disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants (Stanner et al., 2004). This is one of the reasons for the interest in the study of the antioxidant activity of the medicinal plants, since the synthetic antioxidants like butylated hydroxyl toluene (BHT) and butylated hydroxyl Anisole (BHA) commonly used have side effects and are also carcinogenic and even toxic (Ito et al., 1983; Branen, 1987).

The medicinal values of plants lie in their component phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which produce a definite physiological action on the human body (Hill, 1952). The beneficial health effects of plants are attributed to flavanoids, a class of secondary metabolites which protect the plant against ultraviolet light and even herbivores (Harborne and Williams, 2000). The protective effects of flavanoids are due to their capacity to transfer electrons to free radicals and to chelate metal catalysts (Ferrali et al., 1997) activate antioxidant enzymes (Elliot et al., 1992), reduce α tocopherol radical (Hancock et al., 2001) and inhibit known free radical producing enzymes, such as myeloperoxidase and NADPH oxidase (Middleton and Kandaswami, 1992) and xanthine oxidase (Nagao et al., 1999). Further flavanoids have demonstrated exceptional cardioprotective effects, essentially because of their capacity to inhibit LDL peroxidation (Mazur et al., 1999).

Spices and herbs are known not only for their taste, aroma and flavour, but also for their medical properties and value. Both spices and herbs have been used for centuries in traditional medical systems to cure various kinds of illnesses such as common cold, diabetes, cough and cancers (Gallo et al., 2010). *Cinnamomum aromaticum* or Chinese cinnamon, belonging to the laurel family (Lauraceae) is an evergreen tree native to southern China, Bangladesh, India, and Vietnam. Like its close relative *Cinnamomum verum*, also known as "*Ceylon cinnamon*" (Suppapitiporn and Kanpaksi, 2006), it is used primarily for its aromatic bark, which is used as a spice. As with these species, the dried bark of *C. aromaticum* is used as a spice. *C. aromaticum*'s flavour is less delicate than that of *C. cinnamon*; for this reason, the less expensive *C. aromaticum* is sometimes called "bastard cinnamon" (Dugoua et al., 2007). A systematic review of research indicates that cinnamon may reduce fasting blood sugar, but does not have an effect on hemoglobin A1C, a biological marker of long-term diabetes. *C. aromaticum's* effects on enhancing insulin sensitivity appear to be mediated by type-A polymeric polyphenols (Slinkard and Singleton, 1977). Despite these findings, *C. aromaticum* should not be used in place of anti-diabetic drugs, unless blood glucose levels are closely monitored, and its use is combined with a strictly controlled diet and exercise program.

MATERIALS AND METHODS

Chemicals

Folin ciocalteu Reagent, Sodium carbonate, Ammonium peroxysulphate, Ascorbic acid, Sodium Nitroprusside, Sodium dihydrogen phosphate, Orthophosphoric acid, Ethanol L.R. grade, Ferric chloride, Thiobarbituric acid, Acetic acid, EDTA were purchased from Merck Pvt Limited, Mumbai. Gallic acid, DPPH, 2 deoxyribose, ABTS were purchased from Himedia, Mumbai. Sulfanilamide, Naphthyl ethylenediamine dihydrochloride were purchased from Loba Cheme Pvt. Limited, Mumbai.

Plant material

The dried barks of *C. aromaticum* were collected from local market of Berhampur, Ganjam District, Odisha, India. Further taxonomic identification was conducted by Dr. A. K. Panigrahi, Professor, Department of Botany, Berhampur University, Berhampur, Odisha, India. A voucher specimen of the plant (RIPS/H/0211) has been deposited in the herbarium at the Department of Botany, Berhampur University.

Microwave and soxhlet extraction of *C. aromaticum*

The powdered *C. aromaticum* bark was used for the preparation of antioxidant extracts employing microwave and soxhlet extraction. Five grams of *C. aromaticum* bark powder were put into a 250 ml extraction vessel and extracted with varied amount of solvent (50 to 150 ml) under different microwave extraction conditions. Microwave power (140 to 320 W), ethanol concentration (65 to 99.9%, v/v), irradiation time (5 to 30 min) and ratio of solvent to material (10 to 30 ml/g), were evaluated for the extraction of flavonoids from *C. aromaticum* bark. The soxhlet extraction was performed in the optimum condition. In brief, 5.0 g of ground material were extracted with 150 ml 95% ethanol in a soxhlet apparatus for 2 h. The

mixtures were filtered and the solvent was removed. Extracts were finally dried in a vacuum drier and dry extracts were stored in a freezer until use.

Determination of total phenol content

Total phenolic content was determined by Folin-ciocalteau reagent method in which gallic acid was used as a standard phenolic compound (Slinkard and Singleton, 1977), 5 ml of the reagent was mixed with 1 ml of Gallic acid at different concentrations and 3 min later 4 ml of 2% Sodium carbonate was added to each of the solutions. Thirty minutes later the blue colour that was developed was read at 760 nm.

Similarly, 50 and 100 µg/ml concentrations of ethanolic extract were treated in the similar manner as that of the standard Gallic acid. The concentrations of total phenols were expressed as mg/g of dry extract (Kim et al., 2003). All the determinations were performed in triplicate. Total content of phenolic compounds were expressed as gallic acid equivalents (GAE) calculated by the following formula:

C = c. V/m

Where: C is total content of phenolic compounds (mg/g plant extract, in GAE); c is the concentration of gallic acid established from the calibration curve (mg/ml); V is the volume of extract (ml) and M is the weight of pure plant extract.

Scavenging of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH assay)

The free radical-scavenging activity of *Illicium verum* ethanol extract was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. In this assay, the purple chromogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine (Blios, 1958). The scavenging capacity is generally evaluated in organic media by monitoring the absorbance decrease at 515 to 528 nm until the absorbance remains constant. 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in ethanol at different concentrations (1 to 35 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical-scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

Percentage inhibition = $(A_0-A_t)/A_0 \times 100$

Where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Scavenging of ABTS radical

The ABTS assay was employed to measure the antioxidant activity of the bark extract. ABTS was dissolved in de-ionised water to 7 mM concentration, and ammonium persulphate added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature overnight (12 to 16 h) in the dark before usage. 0.5 ml of ethanol extract (0.1 to 20 µg/ml) was diluted with 0.3 ml ABTS solution and made up to the volume with ethanol. Absorbance was measured spectrophotometrically at 745 nm. The assay was performed at least in triplicates. Fresh stocks of ABTS solution were prepared every five days due to self-degradation of the radical. The assay was first carried out on ascorbic acid, which served as a standard. The percentage of inhibition was measured by the following formula:

Percentage inhibition= $(A_0-A_t)/A_0 \times 100$

Where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Scavenging of hydroxyl radical

The hydroxyl radical scavenging capacity was measured using modified method as described previously (Halliwell et al., 1987). Stock solutions of EDTA (1 mM), $FeCl_3$ (0.2 mM), ascorbic acid (1 mM), H_2O_2 (10 mM) and deoxyribose (28 mM) were prepared in distilled de-ionized water. The assay was performed by adding 0.1 ml EDTA, 0.1 ml of $FeCl_3$, 0.1 ml of deoxyribose, 0.5 ml of extract (0.1 to 1000 µg/ml) each dissolved in ethanol and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 h. The foregoing reaction mixture was treated with dodecyl sulphate 8.1%, 0.2 ml, thiobarbituric acid 0.8%, 1.5 ml, and acetic acid 20%, 1.5 ml and pH 3.5 and kept in oil bath maintained at 95°C for 1 h and the absorbance was measured at 532 nm. The hydroxyl radical- scavenging activity of the extract was reported as the percentage of inhibition of deoxyribose degradation and was calculated according to the following equation:

Percentage inhibition= $(A_0-A_t)/A_0 \times 100$

Where A_0 was the absorbance of the control (blank, without extract) and A_t was the Absorbance in the presence of the sample of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values. Ascorbic acid was used as a positive control.

Test of total antioxidant activity

The total antioxidant activity was measured using a modified version of the method (Prieto et al., 1999). Sample solution (0.3 ml, 0.5 mg/ml) in 95% ethanol was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C. The total antioxidant capacity was determined using measuring the absorbance at 695 nm each 30 min after the mixture had cooled to room temperature. The mixture prepared as above, without sample was the control. The antioxidant activity is expressed as the absorbance of samples measured at 695 nm. A higher absorbance indicated a higher total antioxidant capacity. Ascorbic acid was used for comparison. All tests were performed in triplicate and mean were centered.

Test of reducing power

The determination of reducing power was performed (Oyaizu, 1986). One millilitre of various ethanol extract solution (0.2, 0.5, 0.8, and 1.2 mg/ml) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). After the mixture was incubated at 50°C for 20 min, trichloroacetic acid (2.5 ml, 10%) was added, and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled

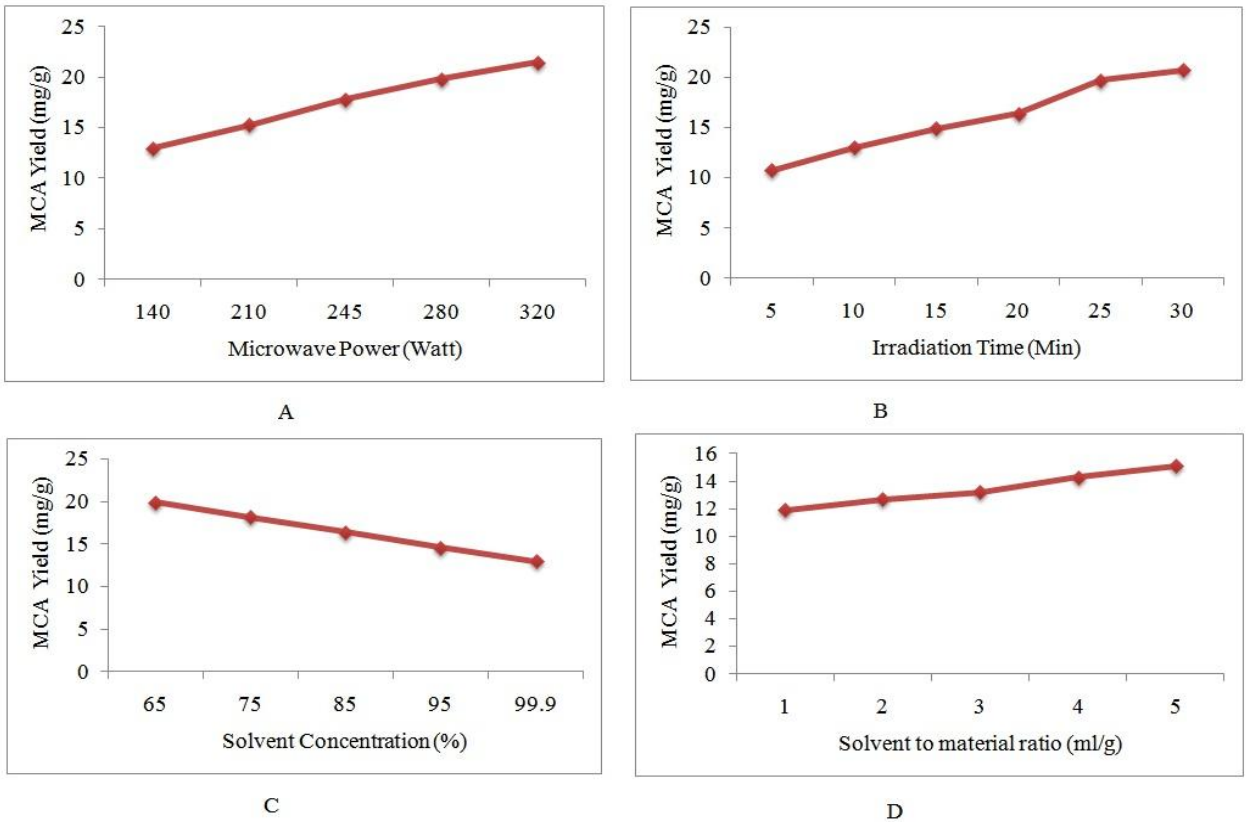

Figure 1. Effect of different factors on MCA yield. (A) Microwave power (95% ethanol, solvent ratio 10 ml/g, 78°C, 10 min, two cycles); (B) Duration of irradiation (140 W, 95% ethanol, solvent ratio 10 ml/g, 78°C, two cycles); (C) Solventl concentration (140W, solvent ratio 10 ml/g, 78°C, 10 min, two cycles); (D) solvent to material ratio (140 W, 95% ethanol, 78°C, 10 min, two cycles).

water (2.5 ml) and ferric chloride (0.5 ml, 0.1%), and then the absorbance was measured at 700 nm against a blank. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used for comparison. All tests were performed in triplicate and mean were centered.

Determination of total flavonoids content

The total flavonoids contents of ethanol extracts were measured (Sultana et al., 2007). Extract solution (1 ml, 0.1 mg/ml) was placed in a 10 ml volumetric flask and then 5 ml of distilled water was added followed by $NaNO_2$ solution (0.3 ml, 5%). After 5 min, $AlCl_3$ solution (0.6 ml, 10%) was added. After another 5 min, sodium hydroxide solution (2 ml, 1 M) was added and volume was made up with distilled water. The solution was mixed thoroughly and absorbance was measured at 510 nm. Total flavonoids amounts were expressed as micrograms of catechin equivalents per gram dry matter. All tests were performed in triplicate and mean were centered.

RESULTS AND DISCUSSION

Microwave and soxhlet extraction of *C. aromaticum*

Figure 1 shows that the yield of *C. aromaticum* bark (MCA) is affected by many factors. In general, the extraction efficiency was higher at microwave power 320 W. The yield increased with the decrease of ethanol concentration significantly when the ethanol volume percentage in the solvent was lower than 65% (v/v). We also found that the increase of duration of microwave radiation or solvent ratio can give increased content of MCA significantly. The optimum conditions of microwave-assisted extraction were obtained by dual extraction with 65% ethanol 30 ml/g material at 320 W for 10 min. The contents of MCA under the optimal extraction conditions were 19.09 mg/g for microwave extract of MCA while that of soxhlet or conventional extract of *C. aromaticum* bark (CCA) was 14.98 mg/g dry weight of extract.

Total phenol content

The content of phenolic compounds (mg/g) in ethanol extract of MCA and CCA was found to be 546.2±0.45 and 543.9±0.86 mg/g plant extract and expressed in gallic acid equivalents. These results suggest that the higher levels of antioxidant activity were due to the presence of phenolic components. The same relationship was also

Table 1. Comparison of total phenolic content, total flavonoids content and free radical scavenging activities of MCA and CCA and ASC A.

Sample	Total phenolic content[a]	Total flavonoid content (mg/g)[b]	DPPH IC_{50} (µg/ml)[c]	ABTS IC_{50} (µg/ml)[d]	Hydroxyl radical IC_{50} (µg/ml)[e]
MCA	546.2±0.45	126±0.18	15.98±0.32	3.99±0.71	18.12±0.27
CCA	543.9±0.86	121±0.34	17.34±0.15	4.27±0,41	18.16±0.21
ASC A	---	---	16.31±0.64	4.23±0.25	18.46±0.43

Values in the same column followed by different letters within each column are significantly different ($p < 0.05$). [a] Milligrams of gallic acid per 1 g (dry weight) of extract. [b] Milligrams of rutin per 1 g (dry weight) of extract. [c] The effective concentration at which DPPH radicals were scavenged by 50%. [d] The effective concentration at which ABTS radicals were scavenged by 50%. [e] The effective concentration at which hydroxyl radicals were scavenged by 50%.

observed between phenolic and antioxidant activity in roseship extracts (Gao et al., 2000). Phenols are very important plant constituents because of their scavenging ability owing to their hydroxyl groups (Hatano et al., 1989). The phenolic compounds may contribute directly to antioxidative action. It is well known that phenolic compounds are constituents of many plants, and they have attracted a great deal of public and scientific interest because of their health promoting effects as antioxidants (Rice-Evans et al., 1997; Hollman and Katan, 1999). The polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up to 1 g daily from a diet rich in fruits and vegetables (Tanaka et al., 1997). The phenolic compounds exhibit considerable free radical scavenging activities, through their reactivity as hydrogen or electron-donating agents, and metal ion chelating properties (Rice-Evans et al., 1996). However, the activity of synthetic antioxidants was often observed to be higher than that of natural antioxidants (Ningappa et al., 2008). Phenolic compounds, at certain concentrations, markedly slowed down the rate of conjugated diene formation. The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Aneta et al., 2007). Therefore, it would be valuable to determine the total phenolic content of the plant extracts.

The most important classes of phytochemicals in plant foods are phenolics and there are more than 8000 phenolic phytochemicals (Kuti, 2004). The phenolic compounds have been reported to be highly effective free radical scavengers and antioxidants (Rathee et al., 2007). Probably the most important natural phenolics are flavonoids because of their broad spectrum of chemical and biological activities (Vundac et al., 2007), and their best-described biological property is the inhibition of lipoprotein oxidation (Jiang et al., 2007). So the true antioxidant potential is often more accurately revealed by expressing antioxidant activity in terms of phenolic and flavonoids content. The total flavonoid contents of extracts from *C. aromaticum* were shown in Table 1. The total flavonoid contents of MCA and CCA were 126±0.18 and 121±0.34 mg/g dry weight of extract ($P < 0.05$), respectively, expressed as rutin equivalents.

Inhibition of DPPH radical

The DPPH radical is a stable organic free radical with an absorption maximum band of about 515 to 528 nm and thus, it is a useful reagent for evaluation of antioxidant activity of compounds (Sanchez-Moreno, 2002). In the DPPH test, the antioxidants reduce the DPPH radical to a yellow-colored compound, diphenylpicrylhydrazine, and the extent of the reaction will depend on the hydrogen donating ability of the antioxidants (Bondent et al., 1997). It has been documented that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (for example, hydroquinone, pyrogallol, gallic acid), reduce and decolorize 2, 2-diphenyl-2-picrylhydrazine by their hydrogen donating capabilities.

The ethanol extract of *C. aromaticum* demonstrated a concentration dependent scavenging activity by quenching DPPH radicals. The concentration of *C. aromaticum* needed for 50% inhibition (IC_{50}) was found to be 15.98±0.32 and 17.34±0.15 µg/ml for MCA and CCA respectively, whereas 16.31±0.64 µg/ml was needed for ascorbic acid (ASC A). The different concentrations of ethanolic extract of *C. aromaticum* (0.1 to 100 µg/ml) showed 7.35% ± 0.42 to 92.13% ± 0.56, 6.78% ± 0.28 to 91.42% ± 0.61, and 10.63% ± 0.43 antioxidant activities in a dose dependent manner on the DPPH radical scavenging assay for MCA, CCA and ASC A (Figure 2). A higher DPPH radical scavenging activity is associated with a lower IC_{50} value. The results were also found to be statistically significant and calculated the P value using ANOVA by Bartlett's test for equal Variance (Table 2).

Inhibition of ABTS radical

The reduction capability of ABTS radical was determined by the decrease in its absorbance at 745 nm which is induced by antioxidants. The ethanol extract of *C. aromaticum* bark at quantities of 0.1 to 100 µg/ml scavenged the ABTS radicals in a dose dependent

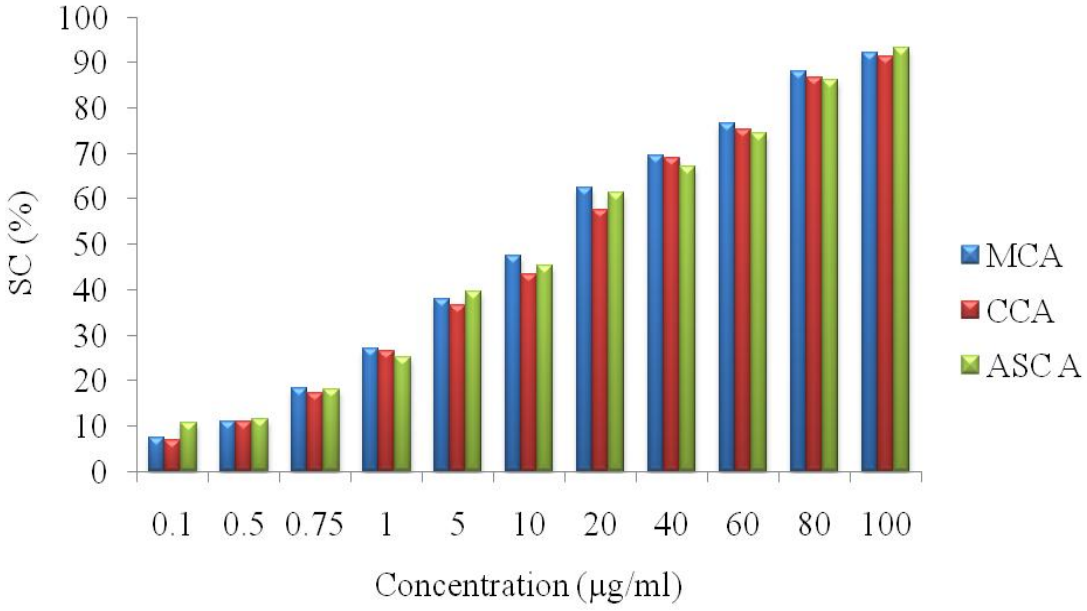

Figure 2. DPPH free radical scavenging activities of MCA and CCA (percentage of scavenging activity on DPPH radical) = [(Acontrol - Atest)/Acontrol] × 100%. Results are mean ± S.D. of three parallel measurements. P < 0.01, when compared to the control.

Table 2. Effect of methanolic bark extract of *Cinnamomum aromaticum* (P values), on different radical scavenging activities.

Parameter	DPPH radical scavenging activity (BTEV)	Hydroxy radical scavenging activity (BTEV)	ABTS radical scavenging activity (BTEV)
MCA	0.0175	0.0091	0.0302
CCA	0.0201	0.0093	0.0138
	P < 0.05	P < 0.05	P < 0.05

MCA: Microwave extract of *Cinnamomum aromaticum;* CCA: Soxhlet extract of *Cinnamomum aromaticum*; BTEV: Bartlett's test for equal variance.

manner. Ascorbic acid at a concentration of 0.1 to 100 µg/ml also found to produce dose dependent inhibition of ABTS radicals. The various concentrations of extract 0.1 to 100 µg/ml showed 11.29±0.77 to 94.65±0.17 for MCA, 10.31±0.26-93.49±0.19 for CCA and 12.24±0.55-94.93±0.17 for ASC A, percentage inhibition on ABTS radical scavenging activity. Results showed the percentage of inhibition in a dose dependent manner (Figure 3). The quantity of *C. aromaticum* bark extract required to produce 50% inhibition of ABTS radical were 3.99±0.71 and 4.27±0.41 µg/ml for MCA and CCA, respectively. Similar effect was produced by ascorbic acid nearly at concentration 4.23±0.25. The results were also found to be statistically significant and the P value was calculated using ANOVA by Bartlett's test for equal Variance.

Inhibition of hydroxy radical

Several *in vitro* methodologies for determination of HO• scavenging capacity are available mostly based on Fe^{3+} EDTA + H_2O_2 + ascorbic acid system to generate a constant flux of HO• radicals. Those radicals attack the sugar 2-deoxy-d-ribose (used as target), degrading it into a series of fragments, some or all of which react upon heating with thiobarbituric acid at low pH to give a pink chromogen. If a HO• scavenger is added to the reaction mixture, it will compete with deoxyribose for HO• radicals, inhibiting the degradation of the target species. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hochestein and Atallah, 1988). Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity (Babu et al., 2001). Among the reactive oxygen species, the hydroxyl radical is the most reactive and induces severe damage to the adjacent biomolecules (Gutteridge, 1984). The potentially reactive hydroxyl radicals can cause oxidative damage to

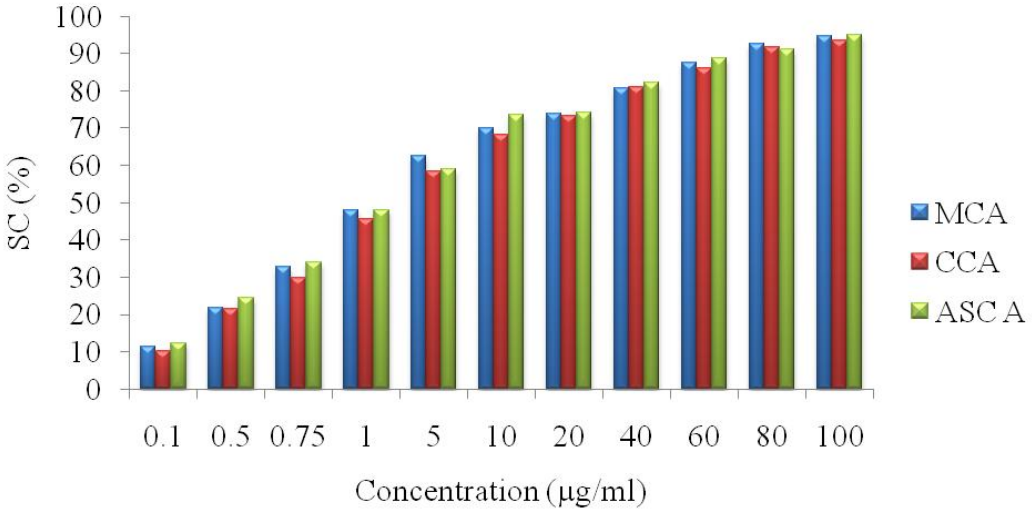

Figure 3. ABTS free radical scavenging activities of MCA and CCA compared to ASC A. SC% (percentage of scavenging activity on ABTS radical) = [(Acontrol - Atest)/Acontrol] × 100%. Results are mean ± S.D. of three parallel measurements. P < 0.01, when compared to the control.

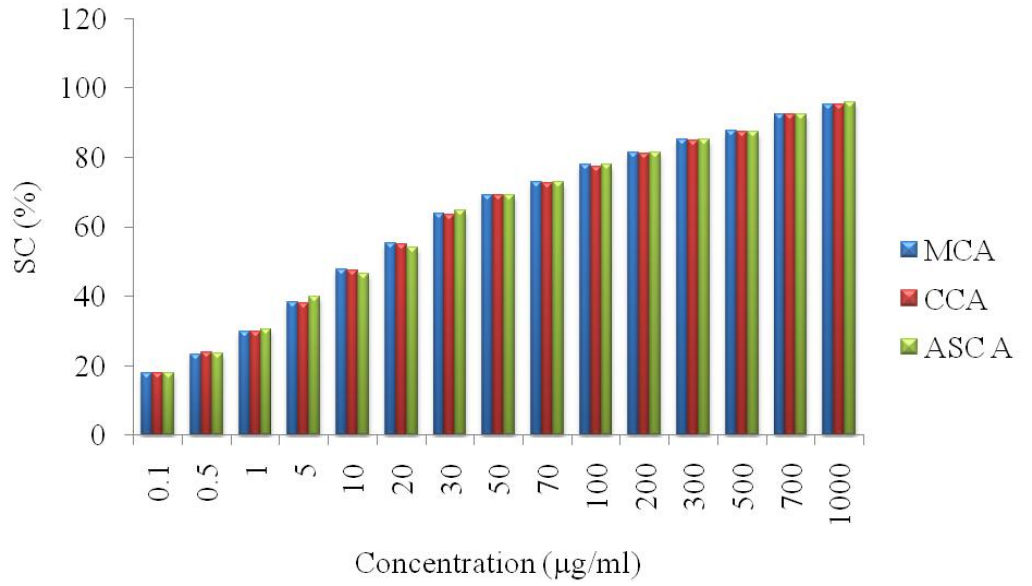

Figure 4. Hydroxyl radical scavenging activities of MCA and CCA compared to ASC A. SC% (percentage of scavenging activity on ABTS radical) = [(Acontrol - Atest)/Acontrol] × 100%. Results are mean ± S.D. of three parallel measurements. P < 0.05, when compared to the control.

DNA, lipids and proteins Ferric EDTA incubated with H_2O_2 and ascorbic acid during which hydroxy radicals were formed in the free solution and were detected by their ability to degrade 2-deoxy-2-ribose into fragments that on heating with TBA form a pink chromogen. When ethanolic extract of *C. aromaticum* bark and the reference compound, ascorbic acid, added to the reaction mixture they removed hydroxyl radicals from the sugar and prevented degradation. The results are shown in Figure 4. The IC_{50} value of ethanolic extract of *C. aromaticum* bark on hydroxyl radical were found to be 18.12±0.27, 18.16±0.21 and 18.46 µg/ml for MCA, CCA and ASC A, respectively. The various concentrations of extract 0.1 to 1000 µg/ml showed 17.96±0.29 to 95.36±0.07 for MCA, 17.54±0.03 to 95.27±0.08 for CCA and 17.85±0.23 to 95.96±0.53 for ASC A, percentage inhibition on hydroxyl radical scavenging activity. Results showed the percentage of inhibition in a dose dependent manner

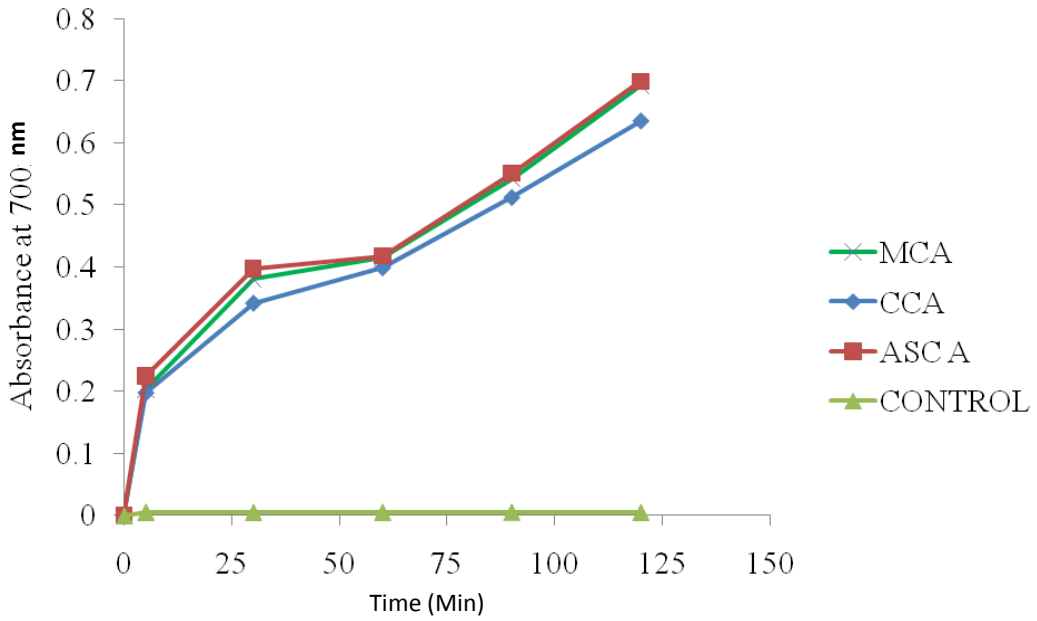

Figure 5. Total antioxidant capacities of MCA, CCA and ASC A. 0.5 mg/ml MCA (X), 0.5 mg/ml CCA (□), 0.5 mg/ml ASC A (□), Control (△). Results are mean ± S.D. of three parallel measurements. P < 0.01, when compared with the control.

(Figure 4) also found to be statistically significant and calculated the P value using ANOVA by Bartlett's test for equal variance. The ability of the previous mentioned extracts to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and seems to be good scavenger of active oxygen species, thus reducing the rate of the chain reaction.

Total antioxidant capacity assay

In order to examine the total antioxidant capacity, the Mo (VI) to Mo (V) reduction in the presence of the extracts was investigated. Ascorbic acid was also used for comparison purpose. In this assay, a green phosphate/Mo (V) complex will be formed in the condition of acid pH and could be monitored at 695 nm with a spectrophotometer (Prieto et al., 1999). According to the results (Figure 5), MCA and CCA had significant activities. All effects increased with increasing reaction time and increasing concentration. Figure 5 also depicts apparently that total antioxidant activity decreased as follows:

ASC A > MCA > CCA.

Reducing power assay

Measurement of reducing potential can reflect some aspects of antioxidant activity in the extracts. In this assay, the presence of reductants (antioxidants) in the samples would result in the reduction of the Fe^{3+}/ferricyanide complex to its ferrous form and amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Higher absorbance value indicates higher reducing power. Figure 6 shows the reductive capabilities of samples. The concentrations of samples measured were 0.2, 0.4, 0.8 and 1.2 mg/ml. The absorbances of MCA at 700 nm were 0.959, 1.827, 2.376 and 3.761, respectively, while those of CCA were 0.917, 1.813, 2.271 and 3.679, respectively. Similarly, for ascorbic acid the absorbances were 0.472, 0.867, 1.769 and 2.148 respectively.

Conclusion

Free radicals particularly reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in the pathogenesis of several chronical and degenerative diseases such as inflammation, cardiovascular diseases, neurodegenerative diseases, cancer and aging related disorders. We have demonstrated the ethanol extract of *C. aromaticum* bark contained total phenolic compounds and were capable of inhibiting, quenching free radicals to terminate the radical chain reaction, and acting as reducing agents. Furthermore, phenolic compounds present in the plant kingdom are mainly responsible for the antioxidant potential of plants. Accordingly, in this study, a significant and linear relationship was found

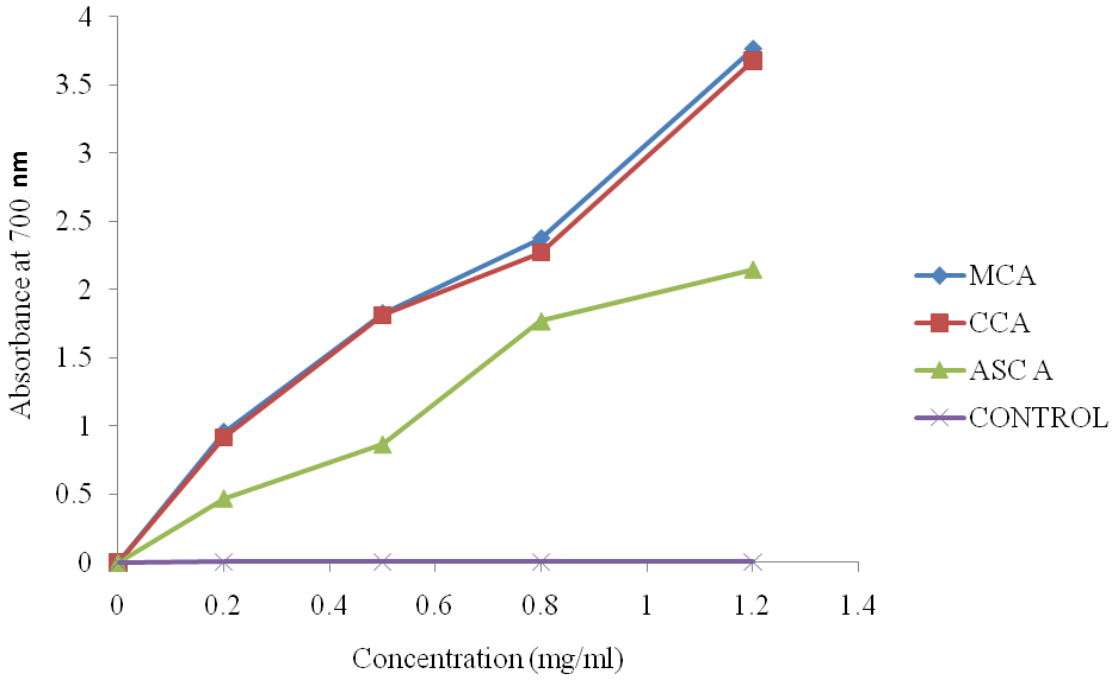

Figure 6. Reducing capabilities of MCA and CCA compared to ASC A. MCA (◻), CCA (□), ASC A (△). Results are mean ± S.D. of three parallel measurements. P < 0.05, when compared with control (x).

between the antioxidant activity and phenolic content, indicating that phenolic compounds could be major contributors to antioxidant activity. The ethanol extract of *C. aromaticum* bark showed strong antioxidant activity by inhibiting DPPH, hydroxyl radical, nitric oxide and ABTS radical scavenging activities when compared with standard ascorbic acid. In addition, the *C. aromaticum* bark found to contain a noticeable amount of total phenols which plays a major role in controlling antioxidants. Although the antioxidant activities found *in vitro* experiment were only indicative of the potential health benefit, these results remain important as the first step in screening antioxidant activity of *C. aromaticum*. The results of the present study would certainly help to ascertain the potency of the crude extracts from *C. aromaticum* as potential source of natural antioxidants and to prove that microwave-assisted extraction is an effective way to obtain bioactive compounds from plants. Thus, it can be concluded that ethanol extract of *C. aromaticum* bark can be used as an accessible source of natural antioxidants with consequent health benefits.

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