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A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents ☆

Gurdip Singh [a,*], Sumitra Maurya [a,1], M.P. deLampasona [b], Cesar A.N. Catalan [b]

[a] *Chemistry Department, DDU Gorakhpur University, Gorakhpur 273 009, India*
[b] *Instituto de Quimica Organica, Universidad Nacional de Tucuman, Ayacucho 471, S.M. de Tucuman 4000, Argentina*

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Abstract

The antioxidant, antifungal and antibacterial potentials of volatile oils and oleoresin of *Cinnamomum zeylanicum* Blume (leaf and bark) were investigated in the present study. The oleoresins have shown excellent activity for the inhibition of primary and secondary oxidation products in mustard oil added at the concentration of 0.02% which were evaluated using peroxide, thiobarbituric acid, *p*-anisidine and carbonyl values. Moreover, it was further supported by other complementary antioxidant assays such as ferric thiocyanate method in linoleic acid system, reducing power, chelating and scavenging effects on 1,1′-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals. In antimicrobial investigations, using inverted petriplate and food poison techniques, the leaf and bark volatile oils has been found to be highly effective against all the tested fungi except *Aspergillus ochraceus.* However, leaf oleoresin has shown inhibition only for *Penicillium citrinum* whereas bark oleoresin has caused complete mycelial zone inhibition for *Aspergillus flavus* and *A. ochraceus* along with *Aspergillus niger*, *Aspergillus terreus*, *P. citrinum* and *Penicillium viridicatum* at 6 μL. Using agar well diffusion method, leaf volatile oil and oleoresin have shown better results in comparison with bark volatile oil, oleoresin and commercial bactericide, i.e., ampicillin. Gas chromatographic–mass spectroscopy studies on leaf volatile oil and oleoresin resulted in the identification of 19 and 25 components, which accounts for the 99.4% and 97.1%, respectively of the total amount and the major component was eugenol with 87.3% and 87.2%, respectively. The analysis of cinnamon bark volatile oil showed the presence of 13 components accounting for 100% of the total amount. (*E*)-cinnamaldehyde was found as the major component along with δ-cadinene (0.9%), whereas its bark oleoresin showed the presence of 17 components accounting for 92.3% of the total amount. The major components were (*E*)-cinnamaldehyde (49.9%), along with several other components.

Keywords: *Cinnamomum zeylanicum* Blume; Eugenol; Cinnamaldehyde; Antioxidant assay

1. Introduction

Free radical reactions occur in human body and food systems. Free radicals, in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology. An over production of these reactive species can occur, due to oxidative stress brought about by the imbalance of bodily antioxidant defence system and free radical formation. These reactive species can react with biomolecules, causing cellular injury and death. This may lead to the development of chronic diseases such as cancers and those that involve the cardio- and cerebrovascular systems. The consumption of fruits and vegetables (Peschel et al., 2006) containing antioxidants has been found to offer protection

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* Corresponding author. Tel.: +91 551 2200745 (R)/2202856 (O); fax: +91 551 2340459.
E-mail address: gsingh4us@yahoo.com (G. Singh).
[1] Present address: Agarkar Research Institute, Pune 411 004, India.

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against these diseases. Dietary antioxidants can augment cellular defences and help to prevent oxidative damage to cellular components (Halliwell, 1989). Besides playing an important role in physiological systems, antioxidants have been used in food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats. These components in food are readily oxidized by molecular oxygen and are major cause of oxidative deterioration, nutritional losses, off flavour development and discoloration. The addition of synthetic antioxidants, such as propyl gallate, butylated hydroxylanisole (BHA), butylated hydroxyltoluene (BHT) and tertiary butylhydroquinone has been widely used industrially to control lipid oxidation in foods. However, the use of these synthetic antioxidants has been questioned due to their potential health risks and toxicity (Kahl and Kappus, 1993). The search for antioxidants from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antioxidants to replace synthetic ones. In addition, these naturally occurring antioxidants can be formulated as functional foods and nutraceuticals that can help to prevent oxidative damage from occurring in the body.

Plants contain a variety of substances called ''Phytochemicals'' (Pratt, 1992), that owe to naturally occurring components present in plants (Caragay, 1992). The phytochemical preparations with dual functionalities in preventing lipid oxidation and antimicrobial properties have tremendous potential for extending shelf life of food products. Several research groups around the world have succeeded in finding and identifying natural antioxidants from herbs and spices using different model systems. The antioxidant activity of Labiatae herbs such as rosemary, sage, summer savory and borage are also well documented (Bandoniene et al., 2002; Djarmati et al., 1991; Ho et al., 2000; Aruoma et al., 1996; Cuvelier et al., 1994; Wong et al., 1995; Chang et al., 1997; Madsen et al., 1996; Gordon and Weng, 1992; Takacsova et al., 1995). However, the aromatic spicy and medicinal plants from Laureceae family are less extensively studied. Cinnamon (*Cinnamomum zeylanicum* Blume, syn *C. verum*, family Laureceae) is a widely used spice and have many applications in perfumery, flavoring and pharmaceutical industries. Although, the chemical constituents of leaf and bark essential oils of cinnamon have been studied (Raina et al., 2001; Simić et al., 2004; Jayaprakash et al., 1997), the potential antioxidant properties have yet not been studied and it seems that investigation on oleoresins are scarce. Hence, in the present work, attempt has been made to explore the possible antioxidant and antimicrobial properties by different methods which can give more comprehensive information especially when the effectiveness of multi component natural oleoresins is investigated. The objective of present investigation is to compare the chemical composition of leaf and bark essential oils and oleoresins as well as demonstrate the possibility of protecting the stored food materials against micro-organism and antioxidative behaviour on mustard oil using as additive by various methods.

2. Materials and methods

2.1. Chemicals

Thiobarbituric acid, pure components eugenol and cinnamaldehyde were received form Merck, Germany. Diphenylpicrylhydrazyl (DPPH), carbendazim were procured from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany) and linoleic acid from Across (New Jersey, USA). BHT, BHA, and 2,4-dinitrophenylhydrazine were purchased from s. d fine-chem Ltd, Mumbai, India. Ampicillin was purchased from Ranbaxy Fine chemicals Ltd., New Delhi, India. Crude mustard oil was purchased from local oil mill, Gorakhpur, India. All solvents used were of analytical grade.

2.2. Sample extraction

Cinnamon leaves and barks were purchased from local market of Gorakhpur, Uttar Pradesh, during January 2004 and voucher specimens were kept at the Herbarium of the Science faculty, DDU Gorakhpur University, Gorakhpur. Cinnamon leaves (250 g) and barks (50 mesh particle size) were hydrodistilled using Clevenger's apparatus to yield essential oils (3.1% and 2.5%, respectively).

Oleoresins were obtained by extracting 25 g of powdered spice with 250 mL of acetone for 2 h in a Soxhlet extractor. The solvent was evaporated by placing the sample in a vacuum drier under reduced pressure. The viscous oleoresins for leaves and barks, with yield 6.9% and 9.7%, respectively, were obtained. Both essential oils and oleoresins were stored in cold condition and until further use.

2.3. Chemical characterization

2.3.1. Gas chromatography (GC)

A Hewlett Packard 6890 (Analytical Technologies SA, Buenos Aires, Argentina) gas chromatograph equipped with column HP-5 (5% phenyl methylsiloxane, length 30 m × inner diameter 0.25 mm × film thickness 0.25 μm) was used for the analysis whose injector and detector temperatures were maintained at 240 and 250 °C, respectively. The amount of the samples injected was 0.1 μL in split mode (80:1). Carrier gas used was helium with a flow rate of 1.0 mL min^{-1}. The oven temperature for essential oils were programmed linearly as follows: 60 °C (1 min), 60–185 °C (1.5 °C min^{-1}), 185 °C (1 min), 185–275 °C (9 °C min^{-1}), 275 °C (5 min) whereas for oleoresins it was as follows: 70 °C (1 min), 70–170 °C (1.5 °C min^{-1}), 170 °C (1 min), 170–180 °C (9 °C min^{-1}), 280 °C (5 min).

2.3.2. Gas chromatography–mass spectrometry (GC–MS)

Analysis of volatile oils and oleoresins were run on a Hewlett Packard (6890) GC–MS system (Analytical Technologies SA, Buenos Aires, Argentina) coupled to a quadrupole mass spectrometer (model HP 5973) with a capillary column of HP-5MS (5% phenyl methylsiloxane, length = 30 m, inner diameter = 0.25 mm and film thickness = 0.25 μm). The injector, GC–MS interface, ion source and selective mass detector temperatures were maintained at 280, 280, 230 and 150 °C respectively. The oven temperature programmed for the volatile oils were same as provided for GC whereas for oleoresins, it was programmed linearly as follows: 60–185 °C (1.5 °C min^{-1}), 185 °C (1 min), 185–275 °C (9 °C min^{-1}), 275 °C (2 min). The extract was held at 70 °C (5 min), 70–220 °C (3 °C min^{-1}), 220–280 °C (5 °C min^{-1}) and held at 280 °C for 5 min.

2.3.3. Components identification

The components of essential oil and oleoresins were identified on the basis of comparison of their retention indices and mass spectra with published data (Adams, 2001; Massda, 1976) and computer matching with WILEY 275 and National Institute of Standards and Technology (NIST 3.0) libraries provided with computer controlling the GC–MS system. The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phase

reported in the literature (Adams, 2001). The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes C_8–C_{16}.

2.3.4. Antioxidative assays in mustard oil

Oxidative deterioration was monitored under modified Shaal Oven test (Economou et al., 1991). Leaf and bark essential oils and oleoresins along with synthetic antioxidants and major components were added individually to unrefined mustard oil at levels of 0.02% (v/v). The initial PV value of oil is 1.7 meq of O_2/kg. Oxidative deterioration was periodically assessed by measuring the antioxidant parameters such as peroxide (PV), thiobarbituric acid (TBA), *p*-anisidine (*p*-An) and total carbonyl (TC) values.

2.3.5. PV and TBA values

The rate of oil oxidation was monitored by the increase of peroxide values. About 3 g of each oil sample was weighed and subjected to iodimetric determination (AOCS, 1990). TBA values were evaluated according to the methods previously stated by some authors (Sidwell et al., 1954) with small changes. To 10 g of oil sample, 0.67% aq. thiobarbituric acid (20 mL) and benzene (25 mL) solution were added. This mixture was shaken continuously for 2 h using mechanical shaker. After 2 h, supernatant was taken and placed in boiling water-bath for 1 h. After cooling, absorbance of supernatant was measured at 540 nm with Hitachi-U-2000 spectrophotometer.

2.3.6. p-Anisidine value

The test was performed according to the methods (AOCS, 1998,) previously stated by earlier workers (Ottolenghi, 1959; Kikuzaki and Nakatani, 1993). In a 50 mL volumetric flask, 0.6 g of oil sample was taken and volume was made using isoctane solution. From this solution, 5 mL was treated with 1 mL of 0.25% of *p*-anisidine reagent and kept in dark for 10 min and absorbance was measured at 350 nm using a UV–VIS spectrophotometer.

2.3.7. Total carbonyl value

Carbonyl value was evaluated according to the methods as reported earlier (Frankel, 1998). About 4 g of sample was taken in a 50 mL volumetric flask and the volume was made up using carbonyl free benzene. Out of this, 5 mL was pippeted out and mixed with 3 mL of 4.3% trichloroacetic acid and 5 mL of 2,4-dinitrophenyl hydrazine (0.05% in benzene) in 50 mL volumetric flasks. The mixture was incubated at 60 °C for half an hour to convert free carbonyls into hydrazones. After cooling, 10 mL of KOH solution (4% in ethanol) was added and the volume was made with ethanol. After 10 min, absorbance was measured at 480 nm using UV–VIS spectrophotometer. Blank was prepared in the same manner substituting 5 mL of benzene instead of sample. A standard curve was drawn using valeraldehyde (50–250 μg) in 5 mL of benzene instead of sample. The total carbonyl was calculated with the help of the standard curve and expressed as mg of valeraldehyde per 100 g of sample.

2.4. Complementary antioxidant assays

2.4.1. Antioxidant activity in linoleic acid system

Antioxidant activity was carried out using the method proposed by Osawa and Namaki (1983) with small changes. Samples (1 mL) in ethanol were mixed with 2.5% linoleic acid in ethanol (4.1 mL), 0.05 M phosphate buffer (pH = 7, 8 mL) and distilled water (3.9 mL) and kept in screw cap containers under dark condition at 40 °C. This solution (0.1 mL) was added to the solution of 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate. After 3 min, 0.1 mL of 0.02 M ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture, the absorbance of red color was measured at 500 nm in the spectrophotometer, for every two days. The control and standards were subjected to the same procedure except for the control, where there was no addition of sample and for the standard 1 mL of sample was replaced with 1 mg of BHA and BHT.

2.4.2. DPPH and hydroxyl radical scavenging effects

The DPPH assay was carried out as described by Brand-Williams and his co-workers (1995). 5, 10, 15, 20, 25 μL of the sample were added to 5 mL of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. The assay was carried out in triplicate and analyses of all samples were run in duplicate and results are averaged.

This test was adopted from a method described by Halliwell et al. (1987). Solutions of the reagents were always prepared freshly. The reaction mixture contained in a final volume of 1.0 mL, 100 μL of 2-deoxy-2-ribose (28 mM in KH_2PO_4–K_2HPO_4 buffer, pH 7.4), 500 μL of various concentrations of the tested oils or the pure compounds in buffer, 200 μL of 1.04 mM EDTA and 200 μM $FeCl_3$ (1:1 v/v), 100 μL of 1.0 mM H_2O_2 and 100 μL of 1.0 mM ascorbic acid. Test samples were kept at 37 °C for 1 h. The free radical damage imposed on the substrate, deoxyribose, was measured using the thiobarbituric acid test (Ohkawa et al., 1979; Shimada et al., 1992). 1.0 mL of TBA (1%), and 1.0 mL tricholoroacetic acid (2.8%) were added to the test tubes and were incubated at 100 °C for 20 min. After cooling, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Reactions were carried out in triplicate.

Inhibition (I) of deoxyribose degradation in percent was calculated in the following way:

$$\mathrm{I}(\%) = 100X(A_0 - A_1/A_0)$$

where A_0 is the absorbance of the control reaction, and A_1 is the absorbance of the test compound.

2.4.3. Chelating effect and reducing power

Chelating effect was determined according to the method of Shimada et al. (1992). To 2 mL of the mixture, consisting of 30 mM hexamine, 30 mM potassium chloride and 9 mM ferrous sulphate were added to 5, 10, 15, 20, 25 μL of essential oil or oleoresin in methanol (5 mL) and 200 μL of 1 mM tetramethyl murexide. After 3 min at room temperature, the absorbance of the mixture was determined at 485 nm. A lower absorbance indicates a higher chelating power. EDTA was used as a positive control.

The reducing power was carried out as described before (Oyaizu, 1986). Various amount (5, 10,15, 20 μL) of essential oil or oleoresin (dissolved in 2.5 mL of methanol) mixed with 2.5 mL of 200 mM phosphate buffer (pH = 6.6) and 2.5 mL of 1% potassium ferricyanide, and the mixture was incubated at 50 °C for 20 min. After adding 2.5 mL of 10% trichloroacetic acid, the mixture was centrifuged at 200 *g* for 10 min in Sigma 3K30 model centrifuger. The organic layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1% ferric chloride and the absorbance read at 700 nm in a UV–visible spectrophotometer.

2.5. Antimicrobial activity

2.5.1. Antifungal investigations

In order to determine the antifungal efficacy of the volatile oil and its oleoresin, the pathogenic fungus *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus terreus*, *Fusarium moniliforme*, *Fusarium graminearum*, *Penicillium citrinum* and *Penicillium viridicatum* were undertaken. These fungi were isolated from food materials such as onion, vegetable waste, wheat straw, fruits of *Musa* species, sweet potato, decaying vegetation and vegetable, respectively and were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The MTCC code No. of these strains are 2479, 1884, 1810, 3374, 1893, 2088, 2553 and 2007, respectively. Cultures of each of the fungi were maintained on Czapek (DOX) agar media with adjusting pH 6.0–6.5 and slants were stored at 4 °C. The antifungal activity of the volatile oil and oleoresin against fungi were undertaken using inverted petriplate (Ramdas et al., 1998) and poison food techniques (Amvam Zolla et al., 1998). In inverted petriplate method, the required dose (2, 4 and 6 μL) of undiluted sample were soaked on a small piece (diameter 12 mm) of Whatmann No. 1 filter paper and it was kept on the lid of petriplate which is in inverted position whereas in poison food

technique, the required dose (2, 4 and 6 μL) of the undiluted sample were mixed with the 20 mL of culture medium. Each test was replicated for three times and fungi toxicity was measured after 6 days in terms of percent mycelial zone inhibition.

2.5.2. Antibacterial investigations

Six pathogenic bacteria *Bacillus cereus* (430), *Bacillus subtilis* (1790), *Staphylococcus aureus* (3103) (gram-positive), *Escherichia coli* (1672), *Salmonella typhi* (733), *Pseudomonas aeruginosa* (1942) (gram-negative) were selected for present study. All the bacterial strains were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. They were sub cultured on nutrient agar broth (Hi-media) and stored at 4 °C. Active cultures for experiments were prepared by transferring one loopful of cells from stock cultures to flask of nutrient agar broth, which were incubated without agitation for 24 h at 37 °C.

In order to determine the antibacterial activity of the essential oils and oleoresins, agar well diffusion method was followed. 0.1 mL of 10^1 time diluted bacterial strain in ringers solution were flood inoculated on to the surface of well settled sterilized culture medium. The wells (10 mm diameter) were cut from agar, and 0.2 mL of sample (2, 4 and 6 μL of essential oil or oleoresin diluted in 1 mL of DMSO) was delivered into them. For standard, 0.2 mL of aqueous solution of ampicillin (1 mg mL^{-1}) was used. After incubation for 24 h at 37 °C, all plates were examined for any zones of growth inhibition according to method developed by Davidson and Parish (1989). All the plates were replicated twice and the results were averaged.

2.5.3. Statistical analysis

For the oil or oleoresin, three samples were prepared for each experiment. The data were presented as mean ± standard deviation of three determinations (data were not shown).The quantitative data of major components of oil and oleoresin were statistically examined by analysis of variance (Sokal, 1973) and significant differences among several groups of data were examined by Ducan's multiple range test. A probability value of $p < 0.05$ was considered significant.

3. Results and discussion

3.1. Chemical analysis

GC and GC–MS analysis of cinnamon leaf volatile oil showed the presence of 19 components accounting for 99.4% of the total amount (Table 1). The major component was eugenol (87.3%) followed by bicyclogermacrene (3.6%), α-phellanderene (1.9%), β-carryophyllene (1.9%), aromadendrene (1.1%), *p*-cymene (0.7%) and 1,8-cineole (0.7%). Moreover, its oleoresin showed the presence of 25 components accounting for 97.1% of the total amount (Table 1). The major components accounting were eugenol (87.2%), spathulenol (1.7%), bicyclogermacrene (1.7%), β-caryophyllene (1.4%) and δ-elemene (1.0%). The analysis of cinnamon bark volatile oil showed the presence of 13 components accounting for 100% of the total amount (Table 2). (*E*)-cinnamaldehyde was found as the major component along with δ-cadinene (0.9%), α-copaene (0.8%) and α-amorphene (0.5%), whereas its bark oleoresin showed the presence of 17 components accounting for 92.3% of the total amount (Table 2). The major components were (*E*)-cinnamaldehyde (49.9%), coumarin (16.6%), δ-cadinene (7.8%), α-copaene (4.6%), (*Z*)-cinnamaldehyde (1.5%), ortho-methoxy cinnamaldehyde (1.5%) and β-bisabolene (1.4%) along with several other components. Recently, Raina et al. (2001) reported eugenol (76.6%), linalool (8.5%) and pipertone (3.31%) as major components from its leaf oil grown in little Andman whereas the steam distilled volatile oil of cinnamon fruit grown at Karnataka and Kerala consists (Simić et al., 2004; Jayaprakash et al., 1997) of hydrocarbons (32.8% and 20.8%) and oxygenated compounds (63.7% and 73.4%) and *trans*-cinnamyl acetate and β-caryophyllene were found to be major component.

Table 1
Chemical composition of cinnamon leaf volatile oil and oleoresin

Compound	Volatile oil		Oleoresin	
	MS[a] %	KI	MS[a] %	KI
α-Thujene	0.1	931	–	–
α -Pinene	0.5	941	–	–
β-Pinene	tr	980	–	–
Myrcene	tr	993	–	–
α-Phellandrene	1.9	1007	0.3	1007
p-Mentha-1(7),8-diene	tr	1011	–	–
p-Cymene	0.7	1026	tr	1026
1,8-Cineole	0.7	1033	–	–
Terpinolene	tr	1088	–	–
α-Terpineol	tr	1191	tr	1191
α-Cubebene	tr	1350	–	–
Eugenol	**87.3**	1358	**87.2**	1358
β-Caryophyllene	1.9	1420	1.4	1420
Aromadendrene	1.1	1441	0.8	1441
α-Amorphene	tr	1490	0.4	1490
Germacrene-D	0.6	1490	0.2	1490
Bicyclogermacrene	3.6	1496	1.7	1496
δ-Cadinene	0.4	1527	0.6	1527
Spathulenol	0.5	1576	1.7	1576
Sabinene	–	–	tr	975
γ-Terpinene	–	–	tr	1064
Terpinen-4-ol	–	–	tr	1177
δ-Elemene	–	–	1.0	1340
Viridiflorol	–	–	0.3	1594
Methoxy-eugenol	–	–	0.1	–
Isospathulenol	–	–	0.3	–
Neophytadiene	–	–	0.3	–
Docosane	–	–	0.1	–
Nonacosane	–	–	0.1	–
Vitamin-E	–	–	0.2	–
Total	99.4%		97.1%	

[a] Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector tr < 0.01.

3.2. Antioxidative assays in mustard oil

The changes of PV in mustard oil of all investigated samples are presented in Fig. 1. The rate of oxidative reactions in mustard oil with additives was almost similar to that of the blank sample. The stability of the mustard oil samples to the formation of peroxides can be ranked in the following descending order:

Leaf oleoresin > BHT > PG ≈ eugenol > Bark oleoresin ≈ BHA > Leafoil > cinnamaldehyde > bark oil

Table 2
Chemical composition of cinnamon bark volatile oil and extract

Compound	Volatile oil		Oleoresin	
	MS[a] %	KI	MS[a] %	KI
α-Pinene	tr	941	–	–
Camphene	tr	953	–	–
Sabinene	tr	975	–	–
β-Pinene	tr	980	–	–
Limonene	tr	1031	–	–
1,8-Cineole	tr	1035	–	–
Camphor	tr	1144	–	–
Z-cinnamaldhyde	tr	1225	1.5	1225
E-cinnamaldhyde	**97.7**	1279	**50.0**	1279
α-Copaene	0.8	1379	4.6	1379
α-Amorphene	0.5	1490	–	–
δ-Cadinene	0.9	1527	7.8	1527
Terpinen-4-ol	–	–	0.1	1177
β-Caryophyllene	–	–	1.0	1420
Coumarin	–	–	16.6	1436
α-Muurolene	–	–	4.4	1500
β-Bisabolene	–	–	1.4	1506
Cadina-1(2), 4-diene	–	–	1.8	1530
Ortho-methoxy cinnamadehyde	–	–	1.5	1532
Cubenol	–	–	0.5	–
1-Heptadecene	–	–	0.2	–
1-Nonadecene	–	–	0.4	–
Tetracosane	–	–	0.1	–
Octacosane	–	–	0.1	–
Nonacosane	–	–	0.2	–
Total	100%		92.3%	

[a] Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector tr < 0.01.

Simultaneously with the measurements of peroxide value, the changes the secondary oxidation products such as malonaldehyde and 2-alkenals, which are measured by thiobarbituric (Fig. 2), *p*-anisidine (Fig. 3) and total carbonyl values (Fig. 4), were also determined after every 7 days. The effects of volatile oils and oleoresins on malonaldehyde formation for mustard oil in terms of incubation time versus TBA value at 60 °C are shown in Fig. 2. The malondehyde formation of all the additives increases with storage time. The oil showed a moderate inhibition at 0.02% concentration, and was comparable to BHA and PG but much lower than BHT. These results were well correlated with *p*-anisidine and total carbonyl values (Fig. 4). However, the sequence is slightly different as compared with the one obtained during measurements of peroxide values. For instance, bark oleoresin had a little greater activity for preventing the formation of secondary oxidation products than primary ones. On contrary, volatile oils were slightly less effective in preventing the formation of secondary oxidation products than primary ones. From the above results, it should be said that the formation of the primary oxidation species, peroxides, were also quite similar with the secondary oxidation products, and the changes of both oxidation characteristics are in a good correlation. Hence, the inhibition activity of leaf and bark oleoresins were excellent among all the additives and there was a significant difference between the blank and antioxidants at the $P < 0.05$ level.

3.3. Antioxidant activity in linoleic acid system

To evaluate the antioxidant potential of volatile oils and oleoresins of leaf and bark, their lipid inhibitory activities were compared with selected antioxidants and their major components by using ferric thiocyanate method of measuring the amounts of peroxides formed in emulsion during incubation. High absorbance is an indication of a high concentration of formed peroxides. The absorbance values of volatile oils and oleoresins of cinnamon along with synthetic antioxidants are shown in Fig. 5. The absorbance

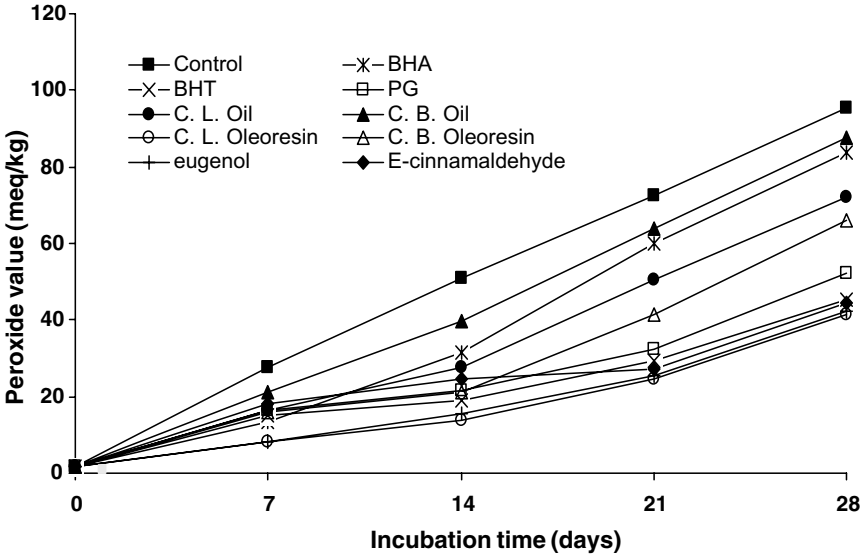

Fig. 1. Inhibitory effect of volatile oil and oleoresin of cinnamon leaf and bark on the primary oxidation of mustard oil measured using peroxide value method.

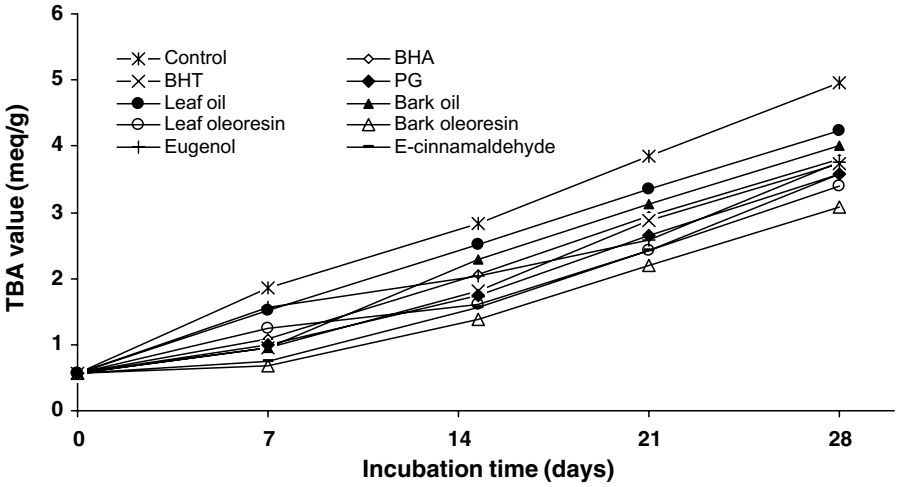

Fig. 2. Inhibitory effect of volatile oil and oleoresin of cinnamon leaf and bark on the malonaldehyde formation in mustard oil measured using TBA value method.

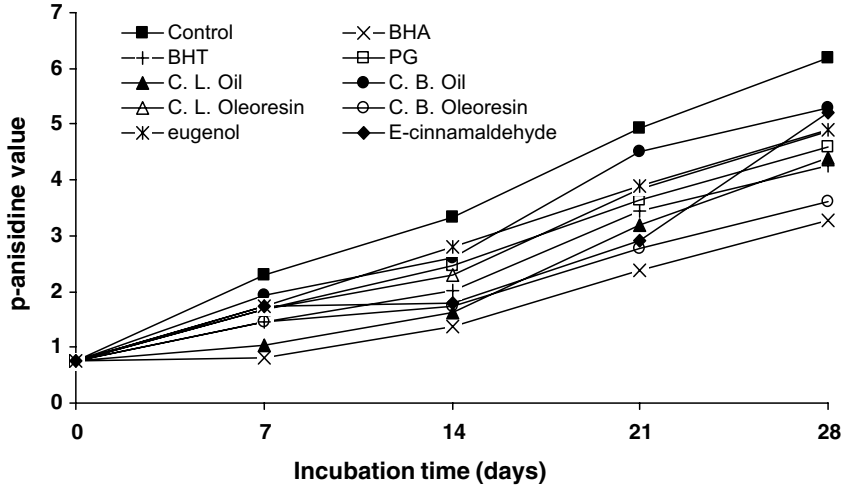

Fig. 3. Inhibitory effect of volatile oil and oleoresin of cinnamon leaf and bark on the formation of 2-alkenals in mustard oil measured using *p*-anisidine method.

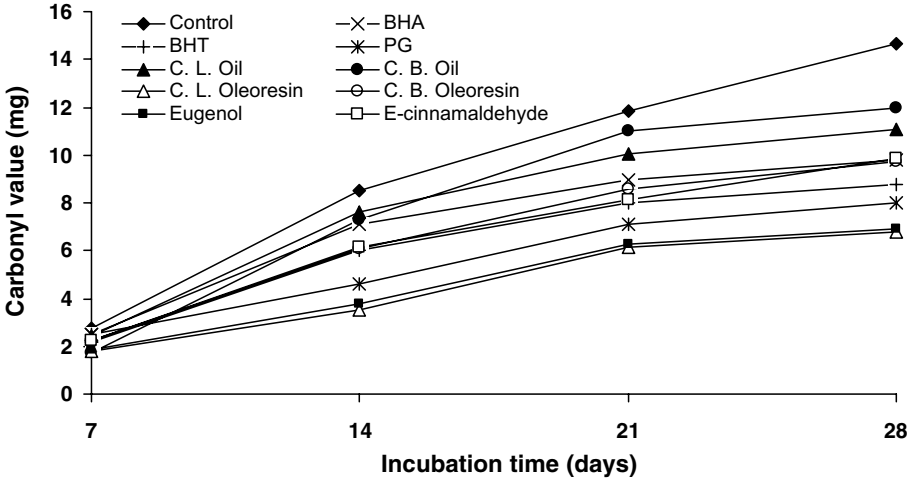

Fig. 4. Inhibitory effect volatile oil and oleoresin of cinnamon leaf and bark on the total carbonyls present in mustard oil.

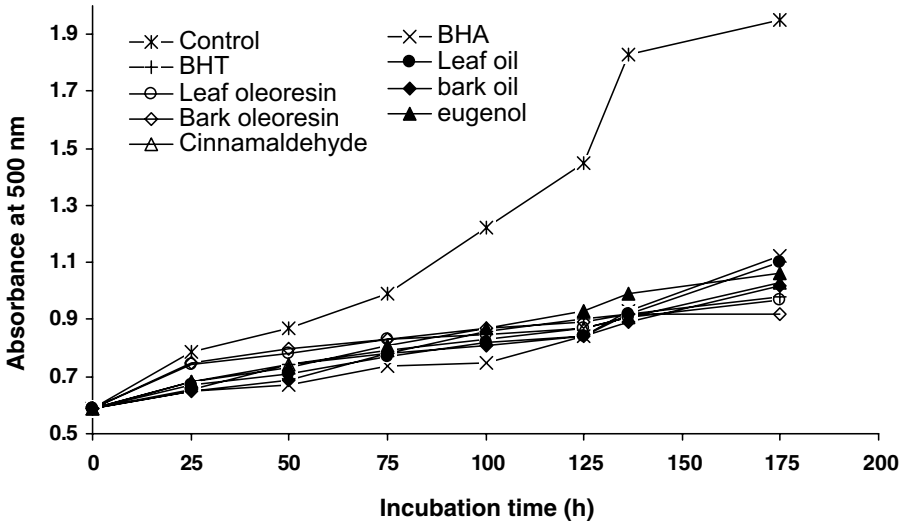

Fig. 5. Inhibitory effect of volatile oil and oleoresin of cinnamon leaf and bark on the primary oxidation of linoleic acid system measured using ferric thiocyanate method.

of linoleic acid emulsion without additive increased rapidly, and there was a significant difference between blank and antioxidants at the $P < 0.05$ level. As can be seen in this fig, bark oleoresin was most effective among all the additives followed by leaf oleoresin. However, there are no significant ($p < 0.05\%$) differences between antioxidative activities of oleoresins, oils, BHA, BHT and PG.

3.4. DPPH and hydroxyl radical scavenging effects

Table 6 shows the DPPH and hydroxyl radical scavenging activity of leaf and bark volatile oils and oleoresins with various concentrations. As positive control, BHA and BHT were also examined. Bark oleoresin showed the best result through all concentrations for DPPH assay. The volatile oils have shown almost equal and moderate radical scavenging activity. At a concentration of 5 μL, significant differences in DPPH scavenging activities was observed between BHA (78.4%), BHT (81.2%) and oleoresins of both leaf (51.3%) and bark (75.6%). However, as concentration increased, the differences in scavenging activities between BHA, BHT and oleoresins become less significant.

For hydroxyl radical scavenging test ·OH radicals were generated by reaction of ferric-EDTA together with H_2O_2 and ascorbic acid to attack the substrate deoxyribose. The resulting products of the radical attack form a pink chromogen when heated with TBA in acid solution (Ohkawa et al., 1979; Shimada et al., 1992). When the oils or oleoresins were incubated with this reaction mixture they were able to interfere with free radical reaction and could prevent damage to the sugar. The results are shown in Table 6. At 5 μL, scavenging effects on hydroxyl radicals were 31.2%, 51.2%, 43.6% and 57.6% for leaf and bark volatile oils and oleoresin. However, at 25 μL BHA and BHT exhibited scavenging activities of 84.9% and 83.2%, respectively. There was a little change in the order of DPPH and hydroxyl radical scavenging activity of leaf oleoresin (86.1%), bark volatile oil (79.6%) and bark oleoresin (78.6%). A close to linear correlation between radical scavenging activity and concentration of polyphenolic compounds in various vegetable and fruits have been reported (Pyo et al., 2004; Robards et al., 1999). These reports indicated that the radical scavenging activity of oleoresins might be mostly affected by position of the phenolic hydroxyl group which is present in eugenol. Yepez et al. (2001) used eugenol as standard which removed 95% of the initial DPPH free radical.

3.5. Chelating effect and reducing power

Chelating effects of the leaf and bark oleoresins on ferrous ions increased from 20.5% at 5 μL to 24.3% at 10 μL and maintained a plateau of 28.2–35.5% at 15–25μL (Fig. 6). The bark oleoresin showed a better chelating effect than those leaf oleoresin and both volatile oils. In addition, chelating effects of oleoresins were relatively parallel and increased from 20.5–23.6% at 5 μL to 38.5–42% at 25 μL. However, at 5 μL, the chelating ability of EDTA was 90.4%. Apparently, the cinnamon leaf and bark oleoresins could chelate ferrous ions but were not as effective chelators as EDTA.

Reducing powers of leaf and bark oleoresins of cinnamon were excellent and were in the range 56.0–58.4, comparable with that of BHA (63.3) and BHT (65.2) at 5 μL (Fig. 7). However, at 25 μL, the reducing power of the leaf and bark oleoresins, BHA and BHT were comparable (78.5–87.9). The reducing powers of the oleoresins might be due to the hydrogen donating abilities (Shimada et al., 1992).

3.6. Antimicrobial studies

The results of volatile oils and oleoresins of cinnamon leaf and bark by inverted petriplate and poison food tech-

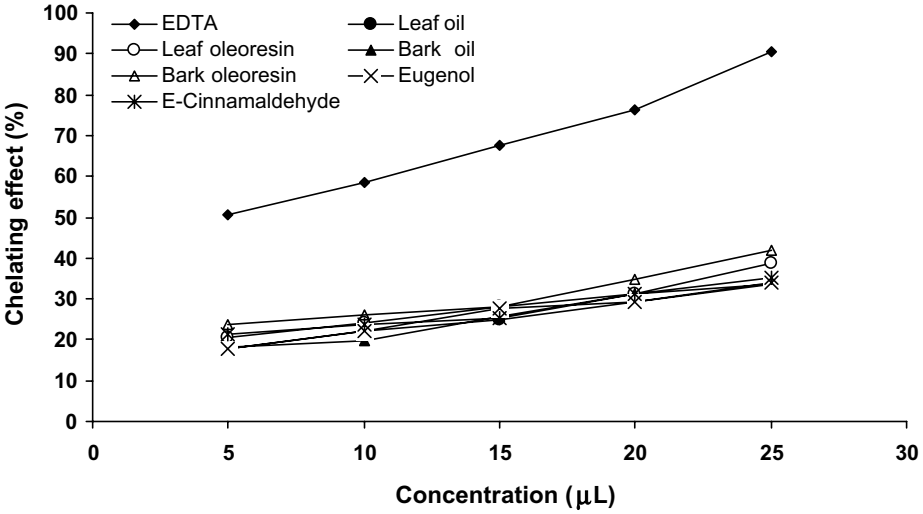

Fig. 6. Chelating effect of volatile oil and oleoresin of cinnamon leaf and bark along with synthetic antioxidants.

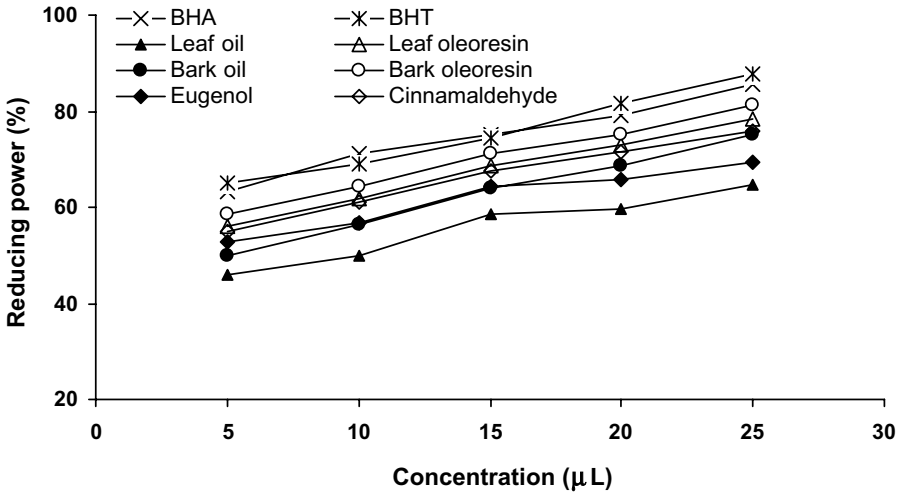

Fig. 7. Reducing power of volatile oil and oleoresin of cinnamon leaf and bark along with synthetic antioxidants.

niques are reported in Tables 3 and 4, respectively. Using inverted petriplate method (Table 3), the leaf volatile oil was found to be 100% antifungal against all the tested fungi except *A. ochraceus* and *A. terreus* at 6 µL. It was interesting to note that complete inhibition against *A. flavus* was obtained only at 2 µL. However, leaf oleoresin has shown complete mycelial zone inhibition only for *P. citrinum.* More than 75% activity was obtained for *P. veridicatum*, *F. moniliforme* and *A. flavus.* Bark volatile oil has shown complete inhibition against the fungi such as *F. graminearum*, *F. moniliforme*, *P. citrinum*, *P. viridicatum* and *A. terreus* at 6 µL. Using poison food technique (Table 4), leaf volatile has caused complete inhibition against all the tested fungi except *P. citrinum* whereas oleoresin has caused complete inhibition only against *P. citrinum.* Bark volatile oil has shown complete inhibition against almost all the tested fungi except for *A. flavus*, *A. ochraceus* whereas its oleoresin has caused complete inhibition for *A. flavus* and *A. ochraceus* along with *A. niger*, *A. terreus*, *P. citrinum* and *P. viridicatum* at 6 µL.

Using agar well diffusion method (Table 5), leaf volatile oil has shown better results in comparison with oleoresin and commercial bactericide, i.e., ampicillin. Complete mycelial zone inhibition was obtained using leaf volatile oil against *P. aeruginosa* and *B. cereus.* However, it has moderate inhibitory effect on *B. subtilis* and *S. aureus* whereas its oleoresin has shown almost 100% activities against *S. typhi* and *B. cereus.* Bark volatile oil has been found to be better than bark oleoresin as it has caused more than 50% inhibition against all the tested fungi. There are several reports (Singh et al., 1995; Hili et al., 1997) stating that *C. zeylanicum* Blume exhibit antimicrobial activity. Their results demonstrate that the leaf oil completely inhibit the growth of *E. coli*, *S. aureus* and *P. aeruginosa* at the

Table 3
Antifungal activity of volatile oils and oleoresins of cinnamon leaf and bark by inverted petriplate method

Test	Dose (μL)	Percent mycelial inhibition zone[a]							
		AN	AF	AO	FG	FM	PC	PV	AT
Leaf volatile oil	2	91.5	100	18.7	50.0	50.0	37.5	37.5	18.7
	4	100	100	56.3	52.5	52.5	56.3	56.3	36.5
	6	100	100	87.5	100	100	100	100	75.0
Leaf oleoresin	2	25.0	45.6	46.3	37.5	57.5	67.8	38.9	46.3
	4	50.0	76.3	56.3	50.0	80.0	93.3	65.5	56.3
	6	58.7	89.3	68.7	56.3	92.5	100	87.5	68.7
Eugenol	2	85.3	100	15.6	36.3	31.2	25.5	28.5	41.3
	4	93.1	100	52.8	45.8	43.2	45.8	47.3	53.2
	6	100	100	85.3	95.2	83.6	86.3	93.7	69.1
Bark volatile oil	2	6.3	6.3	12.5	87.5	75.0	100	100	37.5
	4	38.7	8.8	25.0	87.5	87.5	100	100	56.3
	6	87.2	13.8	37.5	100	100	100	100	100
Bark oleoresin	2	62.5	81.2	54.3	25.0	58.6	100	76.5	87.5
	4	100	100	78.7	50.0	79.5	100	87.5	94.1
	6	100	100	100	58.7	83.3	100	100	100
E-cinnamaldehyde	2	6.3	65.3	12.5	75.0	58.7	100	85.5	56.3
	4	35.1	93.2	25.0	87.5	75.3	100	91.5	85.6
	6	78.3	100	30.8	100	83.8	100	100	100

AN = *Aspergillus niger*; AF = *Aspergillus flavus*; AO = *Aspergillus ochraceus*; FG = *Fusarium graminearum*; FM = *Fusarium moniliforme*; PC = *Penicillium citrinum*; PV = *Penicillium viridicatum*; AT = *Aspergillus terreus*.
[a] Average of three replicates.

Table 4
Antifungal activity of volatile oils and oleoresins of cinnamon leaf and bark by food poisoned method

Test	Dose (ppm)[a]	Percent mycelial inhibition zone[a]							
		AN	AF	AO	FG	FM	PC	PV	AT
Leaf volatile oil	2	100	31.3	50.0	75.0	100	50.0	87.5	18.7
	4	100	87.5	100	100	100	75.0	100	50.0
	6	100	100	100	100	100	87.5	100	56.3
Leaf oleoresin	2	62.5	18.8	35.0	62.5	38.7	35.0	50.0	(–)
	4	77.5	50.0	82.5	77.5	46.3	62.5	65.5	50.0
	6	87.5	100	97.5	87.5	78.7	97.5	70.0	100
Eugenol	2	100	15.6	45.6	63.5	45.6	48.6	73.2	15.5
	4	100	63.2	95.6	82.1	53.6	73.1	85.6	50.0
	6	100	95.6	100	93.8	78.3	82.6	93.6	75.2
Bark volatile oil	2	73.5	(–)	75.0	50.0	75.0	43.7	50.0	32.5
	4	100	51.3	81.2	75.0	83.2	51.3	75.0	45.0
	6	100	87.5	100	87.5	100	65.0	87.5	76.3
Bark oleoresin	2	48.9	88.7	100	65.3	48.7	100	60.0	35.0
	4	65.3	91.3	100	83.2	56.3	100	85.3	76.2
	6	83.6	100	100	100	78.7	100	100	83.7
E-cinnamaldehyde	2	52.3	52.6	100	47.2	63.2	85.2	55.3	42.3
	4	68.7	87.6	100	67.8	65.8	89.7	63.1	45.6
	6	72.3	91.2	100	85.3	87.1	91.2	91.2	89.3
Carbendazim[b]	1000	78.2	85.3	84.2	90.2	97.2	100	100	98.5
	2000	82.2	91.2	91.2	96.3	100	100	100	100
	3000	96.3	96.2	98.4	94.5	100	100	100	100

AN = *Aspergillus niger*; AF = *Aspergillus flavus*; AO = *Aspergillus ochraceus*; FG = *Fusarium graminearum*; FM = *Fusarium moniliforme*; PC = *Penicillium citrinum*; PV = *Penicillium viridicatum*; AT = *Aspergillus terreus*.
[a] Average of three replicates.
[b] Aqueous solution was used.

Table 5
Antibacterial activity of volatile oils and oleoresins of cinnamon leaf and bark by agar well diffusion method

Test	Concentration (ppm)	Inhibition zone (mm)[a]					
		Gram (+) bacteria			Gram (−) bacteria		
		Bs	Sa	Bc	Ec	St	Pa
Leaf volatile oil	1000	17.1 ± 0.4	26.1 ± 1.5	43.3 ± 1.7	13.0 ± 0.2	12.5 ± 0.8	25.7 ± 0.6
	2000	20.0 ± 0.6	34.9 ± 1.3	58.0 ± 0.6	18.2 ± 1.1	14.6 ± 1.1	+
	3000	32.6 ± 1.2	48.7 ± 0.5	+	25.8 ± 0.5	17.9 ± 0.2	+
Leaf oleoresin	1000	14.6 ± 1.2	27.1 ± 0.1	64.5 ± 0.6	11.4 ± 0.6	53.6 ± 1.3	20.5 ± 0.1
	2000	19.0 ± 0.2	38.9 ± 0.2	80.4 ± 1.1	13.1 ± 0.7	73.8 ± 0.5	21.4 ± 0.8
	3000	25.4 ± 0.8	49.3 ± 2.2	+	18.5 ± 1.1	78.1 ± 0.8	25.8 ± 0.1
Eugenol	1000	14.3 ± 0.6	23.1 ± 1.1	33.3 ± 1.5	11.3 ± 0.1	12.5 ± 0.8	26.7 ± 0.5
	2000	17.0 ± 0.3	26.9 ± 1.3	56.0 ± 0.8	17.2 ± 1.6	14.6 ± 1.1	+
	3000	29.6 ± 1.2	38.7 ± 0.3	72.3 ± 0.2	21.8 ± 0.3	17.9 ± 0.2	+
Bark volatile oil	1000	14.2 ± 0.5	27.0 ± 0.9	41.3 ± 1.7	28.1 ± 0.2	20.6 ± 1.8	50.2 ± 1.2
	2000	18.3 ± 0.3	44.6 ± 0.8	52.6 ± 1.2	33.2 ± 1.3	32.7 ± 2.0	56.5 ± 0.8
	3000	26.7 ± 0.7	56.7 ± 0.1	56.3 ± 0.5	35.1 ± 0.3	41.3 ± 0.3	60.2 ± 0.3
Bark oleoresin	1000	16.2 ± 1.3	23.1 ± 0.4	38.6 ± 0.2	33.4 ± 0.5	17.2 ± 0.1	40.6 ± 0.4
	2000	20.2 ± 1.1	28.7 ± 0.2	41.3 ± 0.4	35.4 ± 0.3	18.6 ± 0.7	45.3 ± 0.8
	3000	25.3 ± 0.3	33.6 ± 0.3	45.6 ± 0.7	37.1 ± 0.3	19.3 ± 0.5	56.2 ± 0.7
E-cinnamaldehyde	1000	12.3 ± 0.1	23.0 ± 0.7	31.3 ± 1.2	26.1 ± 0.5	18.6 ± 1.4	30.2 ± 1.1
	2000	17.3 ± 0.5	41.6 ± 0.8	48.6 ± 0.2	33.2 ± 1.8	31.7 ± 1.0	48.5 ± 0.6
	3000	23.7 ± 0.6	53.7 ± 0.1	52.3 ± 0.3	34.1 ± 0.2	40.3 ± 0.3	59.2 ± 0.1
Ampicillin	1000	32.5 ± 1.2	29.5 ± 0.6	31.4 ± 0.2	33.6 ± 0.8	21.9 ± 0.5	24.3 ± 0.4
	2000	34.3 ± 0.3	32.6 ± 1.6	34.6 ± 0.1	37.8 ± 1.4	25.6 ± 0.7	26.3 ± 1.5
	3000	41.2 ± 0.2	37.5 ± 0.2	38.2 ± 0.3	39.5 ± 0.6	28.9 ± 1.3	27.3 ± 1.1

Bs = *Bacillus subtilis*; Sa = *Staphylococcus aureus*; Bc = *Bacillus cereus* ; Ec = *Escherichia coli* ; St = *Salmonella typhi*; Pa = *Pseudomonas aeruginosa.*
(+) indicates complete inhibition.
[a] Average of three replicates.

level of 500 μg mL^{-1}. Another report (Smith-Palmer et al., 1998) found the MICs of *C. zeylanicum* against *E. coli* and *S. aureus* were 0.05% and 0.04%, respectively.

To confirm the relationship of the constituents in cinnamon leaf and bark and antimicrobial activity, the major components were tested for antimicrobial activity. The results are shown in Tables 3–5. Among both constituents, E-cinnamaldehyde possessed better activity and these findings are quite similar with the results of Chang et al. (2001). However, eugenol, in spite of being phenolic compound, failed to inhibit the fungal growth by inverted petriplate method but when it was added directly to the growth media in higher concentrations, it appeared to inhibit completely the microbial growth. Nevertheless, it is worth noting that essential oils and oleoresins are very heterogeneous mixtures of a single substances, biological actions are primarily due to these components in a very complicated concert of synergistic or antagonistic effects.

Table 6
Comparison of scavenging effects of cinnamon leaf and bark volatile oils and oleoresins against DPPH and hydroxyl radicals

Sample	Radical scavenging activity[a] (%)									
	DPPH radical					Hydroxyl radical				
	5 μL	10 μL	15 μL	20 μL	25 μL	5 μL	10 μL	15 μL	20 μL	25 μL
Leaf oil	45.2	58.7	69.9	72.1	73.9	31.2	55.7	63.5	68.1	72.2
Leaf oleoresin	51.3	58.9	74.1	76.7	91.2	43.6	57.1	70.4	73.6	86.1
Eugenol	41.3	56.8	65.2	71.3	92.9	39.4	45.1	54.3	61.5	68.2
Bark oil	71.1	73.5	76.2	82.1	83.6	51.2	57.6	73.1	76.9	79.6
Bark oleoresin	75.6	87.5	89.3	91.2	95.3	57.6	62.3	68.9	71.2	78.6
E-cinnamaldehyde	65.3	68.1	72.3	75.1	78.3	49.8	53.6	57.1	65.2	68.3
BHA	78.4	89.3	92.1	94.7	96.4	71.3	75.1	78.5	81.7	84.9
BHT	81.2	85.1	89.2	91.7	94.9	66.2	72.1	75.3	77.5	83.2

[a] Average of three replicates.

4. Conclusion

The present study provided the potential antimicrobial and antioxidant properties of the volatile oils and oleoresins of cinnamon leaf and bark. The oleoresins have shown better effect on primary and secondary oxidation products in mustard oil. The radical scavenging activity and other complementary assays are also in good correlation. Moreover, the potency of the constituents such as eugenol and cinnamaldehyde could provide a chemical basis for some of the health benefits claimed for cinnamon and warrant further studies to assess their potential as effective natural remedies.

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