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Full Length Research Paper

Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils

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Essential oils obtained from the bark of *Cinnamomum zeylanicum* (cinnamon) and the rhizomes of *Zingiber officinale* (ginger) were characterized by analytical TLC and GC/MS, and their antimicrobial and antioxidant compounds were detected by TLC-bio-autography assays. Essential oil of cinnamon bark (CEO) was found to be a unique aromatic monoterpene-rich natural source, with *trans*-cinnamaldehyde (45.62%) as the major constituents. Ginger oil (GEO) was characterized by high content of sesquiterpene hydrocarbons, including β-sesquiphellandrene (27.16%), caryophyllene (15.29%), zingiberene (13.97%), *α*-farnesene (10.52%) and *ar*-curcumin (6.62%). CEO and GEO oils showed significant inhibitory activity against selected strains of bacteria and pathogenic fungi, with MIC values ranging from 20 to 120 µg/ml depending upon the microbial species. Cinnamaldehyde (CA) and eugenol in cinnamon bark oil and β-sesquiphellandrene, caryophyllene and zingiberene in ginger rhizome oil were identified as the most active antibacterial components, with the aid of bioautography on TLC and GC-MS. Also, both oil exhibited appreciable *in vitro* antioxidant activity as assessed by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and β-carotene bleaching methods, compared to α-tocopherol, BHT and BHA. Guided isolation through TLC-autography using 0.05% DPPH and β-carotene/linoleic acid as a detection reagent led to identified CA and eugenol as main active antioxidant compounds in CEO. The significant antimicrobial and antioxidant activities of both oils suggest that it could serve as a source of compounds with preservative phenomenon.

Key words: Antimicrobial, bioautographic assay, antioxidant, essential oils, ginger, cinnamon.

INTRODUCTION

The oxidative stress (OS), induces by reactive oxygen species (ROS), can be described as a dynamic imbalance between the amounts of free radicals generated in the body and levels of antioxidants to quench and or/scavenge them and protect the body against their deleterious effects (Shirwaikar et al., 2006). Excessive amounts of ROS may be harmful because they can initiated biomolecular oxidations which lead to cell injury and death, and create oxidative stress which results numerous diseases and disorders such as aging, cancer, atherosclerosis, cirrhosis and cataracts (Halliwell and Gutteridge, 2000). On other hand, the spread of drug resistant pathogens is one of the most threats to successful treatment of microbial diseases. In addition, the consumption of food contaminated with food-borne microorganisms can pose a serious threat to human health. The existence of microorganisms causes spoilage and results in reduction of the quality and quantity of possessed food (Anwar et al., 2009). Therefore, there has been a growing considerable interest to identify new sources of safe and inexpensive antioxidant and antimicrobial potential of natural origin (Abdel-Baky and El Baroty, 2008; Anwer et al., 2009).

Ginger (*Zingiber officinale*, Zingiberaceae) and cinnamon (*Cinnamomum zeylanicum*, Lauraceae) are widely been consumed as spices and food preservation. They

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Abbreviation: **TLC**, Thin layer chromatography.

are added to food products in the form of essential oils and various extracts (Yu et al., 2007). Also, both spices have been employed as a folk remedy to treat of several diseases, disorders and ailments (Geiger, 2005; Senhaji et al., 2007). Since long time, cinnamon and ginger have been used to treat dyspepsia, gastritis, blood circulation disturbance and inflammatory diseases in many countries (Wang et al., 2009). Also, they show potential antipyretic, antiallergenic, analgesic, antitussive (Gurdip et al., 2008) and chemopreventive activities (Sabulal et al., 2007). This potential activity was believed to be attributed to the major compounds in oils such as cinnamaldehyde and zingiberene, and their activity could be multiple (Ali et al., 2005; Singh et al., 2008; Anwer et al., 2009). However, cinnamon and ginger are locally named karfa and zingabil in several Arabic countries such as Egypt, and are used as common beverages like tea (in tea-bags form) among many people in particular in winter season, due to their protective effect and curative remedy for numerous disorders. Therefore, the aim of this study was to characterize the chemical constituents, and antimicrobial and antioxidant properties of essential oils obtained from two spices, viz. ginger (Z*. officinale*) and cinnamon (*Cinnamomum zeylanicum*). These beneficial characteristics could increase food safety and shelf life of fatty-rich foods and processed food products.

MATERIALS AND METHODS

Essential oil distillation

The bark of *C. zeylanicum* and rhizome of *Z. officinale* were purchased from the local spices store, Egypt. About 100 g of each spice were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous Na_2SO_4, filtered and stored at –10°C in a sealed vial until use.

Determination of total phenols

The content of total phenols in the plant essential oils were calculated using the Folin-Ciocalteau reagent method as described by Singleton and Rossi (1965). Eugenol as the reference standard was used for preparation of calibration curve.

Gas chromatography/mass spectrometry (GC-MS) analysis

The analysis of the essential oils were performed using GC/MS system consisted of a HP 5890 series II gas chromatograph, HP 5972 mass detector and Agilent 6890 series auto-sampler (Agilent Technologies, USA). A Supelco MDN-5S capillary column (30 m × 0.25 mm i.d., 0.5 µm film thickness) was used with helium as the carrier gas, at a flow rate of 1 ml/min. GC oven temperature was programmed at an initial temperature of 40°C for 5 min, then heated up to 140°C at 5°C/min and held at 140°C for 5 min, then heated to 280°C at 10°C/min and held for 5 additional minutes. Injector and detector temperatures were 250°C. Diluted sample (1/100, v/v in heptane) of 1.0 µl were injected automatically. Mass spectrometry was run in the electron impact mode (EI) at 70 eV. The components were identified based on the comparison of their GC retention times, interpretation of their mass spectra and confirmed by mass spectral library search using the National Institute of Standards and Technology (NIST) database (Massada, 1976; Adams, 2007).

Antioxidant activity

DPPH scavenging assay

The ability of the essential oil to scavenge DPPH radical was assessed as described by Tagashira and Ohtake (1998). Briefly, different concentrations of tested oil (ranged 5 - 200 µg) in 1 ml of ethanol were added to 25 ml of methanolic DPPH (100 mmol/L^{-1}) solution. The mixtures were incubated in the dark at 27 ± 1°C, then the absorbance was measured at 517 nm for 90 min, at 15 min intervals, against a blank (pure methanol). BHT, BHA and α-tocopherol (2 – 40 µg/ml) were used as reference standards. The radical scavenging activity of the each oil was calculated from a calibration curve. All tests were run in triplicate and averaged. The oil concentration providing 50% inhibition (IC_{50}) was calculated from a graph representing the inhibition percentage against oil concentration.

β-Carotene-linoleic acid bleaching

The ability of the oils to prevent the bleaching of β-carotene-linoleic acid was assessed as described by Abd El Baky and El Baroty (2008). An aliquot, β-carotene (0.2 mg) in chloroform (2 ml), linoleic acid (30 µL) and Tween-20 (200 mg) were transferred into round-bottom flask. Once the solvent was evaporated, 250 ml ultra-pure water was added and the resulting mixture was stirred vigorously. Then, 1 ml of aliquot essential oil (containing 5 – 200 µg oil and 10 mg Tween-20) was added to 50 ml reagent mixture and tested samples were subjected to thermal auto-oxidation at 50°C. At intervals up to 300 min, the absorbances of the reaction mixture (4 ml) were measured at 470 nm against a blank (1% Tween-20 solution). All samples were done in triplicates. Antioxidant capacities of algal extracts were compared with those of BHT, BHA and α-tocopherol (at 2-100 µg ml^{-1}) and control. Inhibition of bleaching β-carotene (I %) was calculated as follows:

Inhibition percentage (%I) = ($Abs_{initial}$ –Abs_{sample} / $Abs_{initial}$) x 100

TLC rapid antioxidant assay: TLC plates were used to detect the most active constituents in both essential oils based on spraying the separated compounds on TLC plates either with β-carotene/ linoleic acid reagent as described by Abdel Baky and El Baroty (2008) or with 0.05 % DPPH radical in methanol (Jaime et al., 2005), to locate the antioxidant compounds. The protection against the bleaching β-carotene gave orange spots and scavenging DPPH radical gave pale yellow colored spots were considered positive results.

Antimicrobial assay

Preparation of bacterial cultures

Four species of Gram-positive bacteria (*Bacillus subtilis* ATCC 6633; *Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 27840 and *Micrococcus luteus* ATCC 4698) and two Gram-negative bacteria (*Klebsiella pneumoniae* ATCC 13883 and *Serratia marcescens* ATCC 13880) were routinely used for the antimicrobial assay in our laboratory. These specific strains were recommended for antibacterial screening purpose (NCCLS, 1990). The bacteria were sub-cultured on nutrient agar at 37°C prior to overnight growth in nutrient broth. All overnight cultures were standardized using

sterile saline to produce approximately 1.5 × 10^7 colony forming units (cfu) per ml.

Disc diffusion method

The antibacterial activity of the essential oil was carried out by the disc agar diffusion method. Brief, Mueller Hinton agar (MHA) plates were swabbed with the respective broth cultures of the organisms. Sterile filter paper discs (Whatman's No. 1, 6 mm in diameter) were impregnated with the appropriate equivalent amount of the essential oil dissolved in sterile dimethylsulphoxide (DMSO) at concentrations of 1 - 10 mg/ disc. Control discs impregnated with 10 ml of the solvent DMSO (negative control) and 2 mg/disc chloramphenicol (positive standard antibacterial drug) were used to determine the sensitivity of one strain in each experiment. The plates were incubated at 30°C for 24 h and the antimicrobial activity was evaluated by measuring the inhibition zones expressed millimeters (mm) of inhibition against the tested organism.

Minimal inhibitory concentration

The minimal inhibitory concentration (MIC) values determined for the bacterial strains, as described by Daw et al. (1994). The MIC was defined as the lowest concentration tested samples showing no visible bacterial growth after 24 h incubation period at 37ºC.

Thin layer chromatography (TLC) bioautographic assay

A TLC bioautographic assay was used to detect components in ginger and cinnamon oil as well as the most bio-active constituents (as antibacterial agent). A set of three plates (5 x 20 cm, silica gel G, 60F 254 Merck, Darmstadt, Germany) were used one plate for each bacterial strain and in each experiment, a 5 µl of the undiluted oil was applied to each plate. The plates were then developed with toluene-ethyl acetate (95:5, v/v). The dried TLC plates were then cut with a diamond into three strips. One of the strips was inspected under UV light (254 nm) and also by visualization with 1% vanillin-sulfuric acid reagent and then heated at 110 C for 3 min; the second was used for the bioautography assay, while the active constituents on the third strip were eluted with hexane. TLC bioautography was carried out using the previously identified bacteria (*B. subtilis* and *K. pneumoniae*) that induced highest effectiveness in the disc diffusion assay. Suspensions of the bacteria in Difco-nutrient broth (Augsburg, Germany) containing agar and 0.1% iodonitrotetrazolum chloride media individually distributed over the TLC plate (second) was then incubated at 37°C for 48 h. Inhibition zones were shown as clear areas against a pink background. TLC zones that showed inhibition activities of the third strip of the TLC plats were scraped from the plates and eluted with dichloromethane, filtrated concentrated by nitrogen to a final volume of 50 µl and analyzed by GC/MS as described above (Abdel Baky and El Baroty, 2008).

Antifungal assay

Antifungal activity was assayed as described Daw et al. (1994), based on the determined growth inhibition rates of the mycelia of different mold strains (*Aspergillus niger*, *Penicillium notatum*, *Mucora heimalis* and *Fusarim oxysporum*) in Potato Dextrose broth (PDB, Sigma Co) medium. Under aseptic conditions, one ml of spore suspension (5 x 10^6 cfu/ml) of each tested fungus was added to 50 ml PDB medium in 100 ml Erlenmeyer flask. Appropriate volumes of tested oils were added to produce concentrations ranging from 10 to 100 µg ml^{-1}. Amphotericin B (Sigma Co., St. Louis, MA) was used as a reference antifungal drug. All the flaks were incubated at 27 ± 1°C in the dark for 5 days and then the mycelium was collected on GF/C filter papers (5.5 cm in diameter). The mycelium was washed several times with sterile distilled water and placed in a drying oven at 105°C for 24 h. The filter papers were dried to a constant weight and the level of inhibition, relative to the control flasks was calculated from the following formula:

Percentage of inhibition = (C-T)/C × 100;

where T = weight of mycelium from test flasks and C = weight of mycelium from control flasks.

The values of minimal inhibitory concentration (MIC) were determined for testes samples, in which no growth was occurred. Each test was conducted three times and fungi growth was determined after 6 days.

Statistical analysis

All data are expressed as mean values ± standard deviation (S.D). Statistical differences between experimental groups were assessed by analysis of variance (ANOVA), using the COSTAT software package (Cohort Software, CA, USA). The main values were compared with LSD test ($P < 0.05$).

RESULTS AND DISCUSSION

The yields (g/100g) and total phenolic content in essential oil of cinnamon (*C. zeylanicum*) and ginger (*Z. officinale)* are given in Table 1. The yields of cinnamon and ginger oils were found to be 0.96 and 0.85%, respectively. These levels were found to be similar to that found in the literature (Massada, 1976). Also, as shown in Table 1, cinnamon oil was characterized by relatively high amounts of phenolic content (18.2% of oil, expressed as eugenol equivalent). While, ginger oil had lower phenolic content (= 1.2% of oil). Our results are in good agreement with the findings of Massada (1976), who reported that the essential oil of cinnamon bark have lower amount of phenolic compounds than that in leaf oil (ranging from 80 to 87% of the oil, as eugenol).

Chemical composition of ginger and cinnamon essential oils are presented in Table 1. Twenty seven and thirty five compounds accounting for 98.58 and 99.42%, respectively of the oils were identified with the aid of TLC and GC-MS analytical methods and literature comparison. Cinnamon oil was characterized with high amounts of oxygen-containing monoterpenes (69.65% of the total oil). Of which, cinnamyl aldehyde (45.13%), cinnamyl alcohol (5.13%), eugenol (7.47%), methyl-eugenol (5.23%), ethyl-cinnamate (3.86%) and dihydro-eugenol (3.31%) were found to be main components of the CEO.

Furthermore, some interesting oxygenated monoterpenic, that is, 1, 8-cineol (1.01%), nerol (1.06%) and geranial (1.79%) were detected in a minor amounts. Ginger oil showed a great chemical homogeneity characterized by relatively high amounts of sesquiterpene hydrocarbon (92.17%), including β-sesquiphellandrene

Table 1. Qualitative and quantitative composition of ginger (rhizomes) and cinnamon (bark) essential oils.

	Relative area %	
Components	**Ginger**	**Cinnamon**
α-Thafone		0.37
α-pinene	0.1	1.12
Benzaldehyde	-	0.25
Heptanol	-	0.79
Sabinene	-	0.52
1-octen-3-ol	-	0.68
β-pinene	-	0.77
Myrcene	-	0.39
p-cymene	-	0.66
Limonene	5.08	1.48
β-phellandrene	0.12	0.37
1,8-cineole	0.63	1.01
γ-terpinene	Tr.	0.99
Octanol	Tr.	0.33
Linalool	Tr.	0.54
terpinen-4-ol	Tr.	0.38
α-terpineol	Tr.	0.51
trans-carveol	Tr.	0.51
Nerol	0.25	1.06
Neral	Tr.	1.16
Geraniol	Tr.	0.78
Geranial	0.23	1.79
neryl acetate	Tr.	0.89
Trans- Cinnamaldehyde	-	45.13
Cinnamyl alcohol	-	5.13
Eugenol	-	7.47
Dihyroeugenol	-	3.31
Ethylcis-cinnamate	-	3.86
t-Methyl cinnamate	-	2.19
Cinnamyl alcohol	-	8.21
Methyl eugenol	-	5.23
Isoeugenol	-	1.59
Cis-Caryophyllene	15.29	Tr.
t-Cinnamic acid	-	0.41
Cinnamyl actate	-	0.21
α-Caryphyllene	Tr.	Tr.
E-ethyl cinnamate	Tr.	0.73
G-epi-Caryophyllene	Tr.	-
γ-Curcumene	Tr.	-
Ar-Curcumene	6.62	-
α-Zingiberene	13.97	-
Apo Farnesal-2-dihydro	5.08	-
α-(Z) Bisabolene	7.84	-
α-(E,E) Farnesene	10.52	-
β-Bisabolene	3.34	-
β-Sesquiphelladrene	25.16	-
γ-Bisabolene (Z)	4.35	-
Total of identified compounds	98.58	99.42
Oil yield (g/100g)	0.96	0.85

[a] Identification based on retention time authentic sample and mass spectrum data. [b] Trace: relative area % is less than < 0.05%.

(25.16%), cis-caryophyllene (15.29%), zingiberene (13.97%), α-farnesene (10.52%), α- (7.84%) and β-bisabolene (3.34%) and other. Also, both oils were contained a minor amounts of (1.48 - 5.08%) limonene (unique monoterpene hydrocarbon). Several studies have shown that cinnamon and ginger essential oils are very complex mixtures of compounds and many variations have been found in the chemical composition (Singh et al., 2008; Felipe et al., 2008; Wang et al., 2009). For instance, Jham et al. (2005) reported cinnamaldehyde (75%) as main constituents in cinnamon, while zingiberene (ZB) and β-sesquiterpene were found as major main components in ginger essential oil, ranged from 10 to 60% (Wohlmuth et al., 2006; Felipe et al., 2008).

In general, a literature search revealed cinnamaldehyde , cinnamyl alcohol, eugenol, methyl-eugenol and ethyl-cinnamate and β-sesquiphellandrene, cis-caryophyllene and zingiberene are the main constituents of cinnamon and ginger essential oils, respectively (Kaul et al., 2003; Pauli, 2008; Singh et al., 2008; Wang et al., 2009). On the other hand, based on a quantitative analysis, the amounts of some main compounds calculated in our present results are out the range generally identified in other reports (Vernin and Parkanyi, 1994; Raina et al., 2001; Singh et al., 2007). For example, a relatively high amount of eugenol was found as predominant components (> 85% of the oil) in cinnamon leaf oil, whereas trans-cinnamaldehyde was not detected (Singh et al., 2007). Singh et al. (2008) identified geranial (25.9%) as the major constituent in ginger oil, but it was detected in trace amount (0.78%) in our study.

Such variations in the chemical composition of distilled oils is known to differ considerably not only due to the existence of different subspecies, but also might be attributed to the varied agroclimatic condition (climatic, seasonal, geographic) of the regions, stage of maturity, adaptive metabolism of plants, distillation conditions, the plant part analyzed and some other factors (Anwar et al., 2009; Abd El Baky and El Baroty, 2008; Singh et al., 2008; Wang et al., 2009).

Antioxidant activity

It is known that free radicals are involved in the process of lipid per-oxidation and play a cardinal role in numerous chronic diseases such as cancer and coronary heart disease (Halliwell and Gutteridge, 2000). Thus, the ability to scavenge free radicals is an important antioxidant property in order to minimize oxidative cellular damage. Since, in a series of *in vitro* tests the essential oils from spices and medicinal plants exhibited remarkable antioxidant activity (Abd El-Baky and El-Baroty, 2008; Anwar et al., 2009).

Rapid TLC-screening assay

Antioxidant property of CEO and GEO was tested with a rapid and simple TLC screening based on decolorization of either DPPH radical (DPPH-TLC) or β-carotene/linoleic acid (C-TLC) reagent (Abd El-Baky et al., 2009). In both assays, bands of R_f = 0.56, 0.43 and 0.37 for cinnamon oil, corresponded to authentic volatile compounds of cinnamaldehyde, eugenol and methyl-eugenol exhibited remarkable antioxidant activity. Whereas, for ginger oil bands of R_f = 0.77 and 0.67 corresponded to sesquiphellandrene and caryophyllene showed a moderate antioxidant activity. Therefore, the most antioxidant activity in CEO appears to derive from phenolic components and other constituents are believed to contribute little effect.

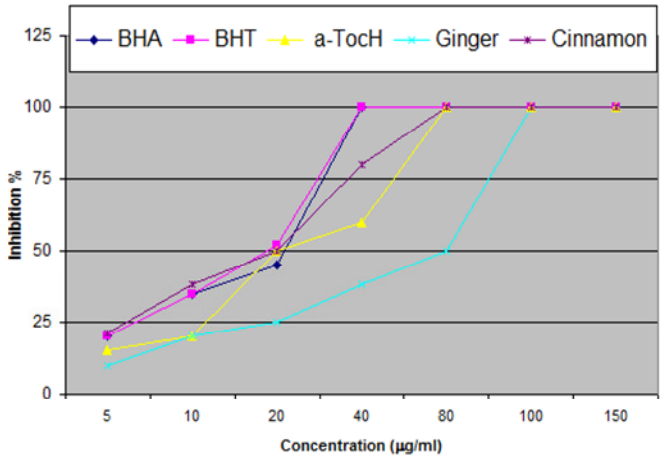

Figure 1. Effect of ginger and cinnamon essential oils on bleaching β-carotene/linoleic acid.

β-carotene bleaching (BCB) in aqueous model system

The potency of the cinnamon (CEO) and ginger (GEO) essential oils on antioxidant activity (AA%) in emulsions, accompanied with coupled oxidation of β-carotene and linoleic acid was determined, by follow up the rate of β-carotene bleaching (Figure 1). GEO, CEO and α-TOC, BHT and BHA possessed an antioxidant activity with AA% values of 66.5, 82.3, 75.4, 74.4 and 81.2%, respectively. Hence, GEO (66.5%) showed a lower antioxidant activity than that shown by CEO (82.3%). Therefore, CEO exhibited an obvious antioxidant activity, by inhibiting the β-carotene bleaching due to retarding/inhibiting of linoleic acid hyderoperoxide-derived, which attack the chromophore-β-carotene. This revealed that the hyderoperoxide produced from linoleic acid auto-oxidation as free radicals will be neutralized by cinnamon oil. Thus, the degradation rate of β-carotene depends on the antioxidant activity of the oils. However, the order of antioxidant activity, expressed as the relative inhibition percentages (1%, of β-carotene oxidation), offered by

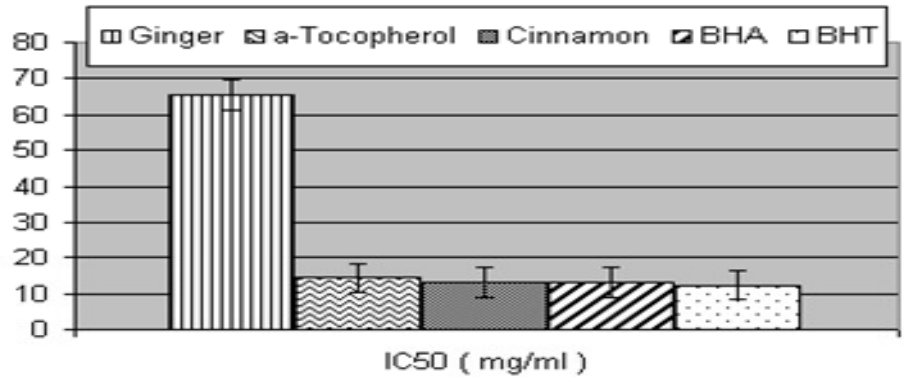

Figure 2. Scavenging activity of ginger and cinnamon essential oils on DPPH• radical.

essential oils and various commercial antioxidant were as follows: BHT = CEO > BHA > α-tocopherol > GEO. Thus, CEO had strong antioxidant capacity compared to synthetic antioxidants (BHT and BHA). These findings indicate that the antioxidant activity of CEO could be mainly attributed to its major compounds, which may be act as chain-breaking antioxidants (Farag et al., 1989 b, c; Abd El-Baky and El Baroty, 2008).

Free radical scavenging activity (FRSA)

In the DPPH assay the ability of CEO and GEO to act as hydrogen donors for transformation of DPPH into its reduced form DPPH-H was done in comparison with those of BHA, BHT and α-TOC. As shows in Figure (2), all tested samples exhibited good radical scavenging activity (FRSA) with varied degrees. The highest FRSA was shown by CEO, with IC_{50} of 13.1 µg/ml, compared to the values of 14.4, 12.2 and 13.1 µg/ml for α-TOC, BHT and BHA, respectively. GEO offered lower antioxidant activity (IC_{50} = 65.5 µg/ml) compared with the CEO. Thus, cinnamon oil had high potential DPPH radical scavenging activity and was similar to those of synthetic antioxidants. Overall, the protection of β-carotene from oxidation and radical scavenging activity of test samples increased in the order: GEO < α - TOC < BHA = CEO < BHT. Looking at the effects of cinnamon oil in different assays one would conclude that CEO contains relatively high amounts of phenolic compounds (18.2%, of the oil), their phenolic group plays an important role in antioxidant activity, which act as hydrogen donor. There are many reports emphasize that the positive correlation between volatile phenolic compounds in the essential and its antioxidant activity (Farag et al., 1989 [a]). On the other hand, the antioxidant activities observed in ginger oil could be due to the synergistic effect of two or more compounds present in it. However, Singh et al. (2008) identified some phenolic compounds in ginger oil such as shogaols, zingerone, gingerols and gengerdiols, as minor component (< 2 % of oil). Herein, ginger oil consist of a very complex mixture of various classes of organic compounds (Table 1), which may produce either synergistic or antagonistic effects on the process of lipid oxidation or scavenging of radicals.

Antibacterial activity

The antibacterial activity of the essential oils cinnamon and ginger as well as chloramphenictol (antibiotic standard) against selected bacterial strains was assessed (Table 2). The results from the disc diffusion method revealed that both oils showed significant antibacterial activity toward all tested strains. The zone of inhibition was found to be 10 and 18 mm in diameter at doses of 1 and 5 mg/disc of CEO and GEO, respectively, for all tested bacterial strains (data not shown). However, this activity seemed to be lower than chloramphenicol (inhibition zone 14 -18 mm, at 2 mg/disc). Furthermore, the antibacterial activity of both oils quantitatively was assessed by determination of minimum inhibitory concentration. Cinnamon oil exhibited strong inhibitory action against all tested bacteria with MIC values ranged from 20 to 25 µg/ml), followed by ginger oil (MIC 90 to 120 µg/ml). However, the anti`bacterial activity of both essential oils was found to be concentrations depended. In general, antibacterial activity of cinnamon bark oil (MIC= 20 to 25 µg/ml) is comparable with the antibiotic standard, chloramphinicol (MIC= 18 µg/ml).

The essential oils of ginger and cinnamon essential oils revealed a high antibacterial activity against strains *B. subtilis*, *S. aureus* and *K. pneumoniae* as it was characterized by TLC-bioautotography and disc diffusion methods. For cinnamon essential oils, the most active compounds which induce large clear inhibition zones on TLC plate had R_f values of 0.56, 0.43 and 0.37 correspond to cinnamyl aldehyde, eugenol and methyl-eugenol as it was determined by GC/MS. Whereas, for

Table 2. Inhibition % and minimum inhibition concentration of ginger and cinnamon essential oils against the selected fungal strains.

Fungus strains	Inhibition % of fungus growth												
	Ginger oil (µg/ml)						Cinnamon oil (µg/ml)						Amphotericin B
	10	25	50	75	100	MIC	10	25	50	75	100	MIC	MIC
A. niger	32	65	93	100	100	70	26	54	78	93	100	100	30
M. hemalis	34	62	90	100	100	75	27	58	80	95	100	100	30
F. oxysporum	36	60	93	100	100	75	24	53	75	92	100	100	30
R. stolonifer	35	60	90	100	100	75	26	57	82	96	100	100	25

Values represent the mean of three replicates and rebated three times MIC: Minimum inhibition concentration, values given as µg/ml for samples and for amphotericin B.

ginger oil, bands at R_f 0.77, 0.67 and 0.46 were found to correspond to sesqui-caryophellene and limonene.

Furthermore, those compounds were confirmed with GC-MS analysis. Thus, these findings emphasize the main compounds were mainly contributor to the antibacterial property of these oils. Our results also are comparable with the several investigations (Baratta et al., 1998; Senhaji et al., 2007; Ali et al., 2005). They found cinnamon and ginger essential oils exhibited an inhibitory effect against a wide range of pathogenic bacterial and fungi, and their effect were probably due to their main components in both oil.

Antifungal activity

The inhibitory effect of cinnamon (CEO) and ginger (GEO) essential oils on four fungal strains *A. niger*, *P. notatum*, *M. heimalis* and *F. oxysporum* were assessed. The results are presented in Table 2. CEO and GEO (in parentheses) at dilutions 10, 25, 50, 75 and 100 µg/ml inhibited the growth of the common spoilage fungus *A. niger* with 26 (32), 54 (65), 78 (93), 93 (100), 100 (100%), respectively. Thus, CEO and GEO completely inhibited (MIC=100%) the growth of *A. niger* at 75 and 100 µg/ml doses, respectively. Moreover, CEO and GEO oils showed similar inhibitory effect against *P. notatum*, *M. heimalis* and *F. oxysporum*. In general, on the bases of MIC values, CEO had stronger antifungal activity against tested fungi than that of GEO. However, antifungal activity of cinnamon and ginger essential oils is comparable with the standard drug, amphotericin B.

It appears that there is a relationship between the chemical constituents of oils and its antimicrobial activity. The cinnamon oil containing high amounts of phenolic compounds (18.2%), while ginger oil was rich in sesquiterpene hydrocarbons. Because both oils had a different chemical profiles, difference in antimicrobial activity could be expected. It has been reported that cinnamon (rich in eugenol and cinnamaldehyed) and ginger (rich in sesquiterpenes) essential oils possessed a wide spectrum of antimicrobial activity (Baratta et al., 1998; Singh et al., 2005; Ali et al., 2005; Senhaji et al., 2007, Anwer, 2009). These results corroborated with the earlier reported data of Daw et al., (1994) and Farag et al. (1989b). They stated that the antimicrobial activity of volatile constituents was decreased in the decreasing order: phenols (highest active)> alcohols > aldehydes > ketones > ethers > hydrocarbons. Generally speaking, the extract of antimicrobial mechanism of essential oils has not been completely elucidated. However, it has been proposed that lipophilicity or hydrophobicity and chemical structure of essential oils or their main compounds such as the presence of functional polar groups and aromaticity could play an important role for the antimicrobial activity (Farag et al., 1989b; Daw et al., 1994), which enable them to partition between lipids of the bacterial or fungal cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable, which will lead to cell death (Sikkema et al., 1994). Thus, as can be seen from Table 1, some of the major components present in cinnamon (CA and eugenol) and ginger oils can penetrate the membrane of the microorganisms and react with the membrane enzymes and proteins as well as phospholipids bilayer, which cause an impairment of microbial enzyme system and/or disturb genetic material functionality (Farag et al., 1989 b, c; Abd El-Baky and El-Baroty, 2008; Conner, 1993). Bang et al. (2000) reported that cinnamon oil contain high amount of cinnamaldehyde (CA), which inhibited the fungal-cell-well synthesizing systems through the reaction with sulfhydro groups present in active site of these enzymes. Therefore, the bioactivity of essential oils is dependent not only on the major compounds but also on the chemical structures of these compounds (Farag et al., 1989 b, c).

This study high light on the potential use of cinnamon and ginger essential oils in ethno-medicine as a preventer of cellular damage, and in food industries as preserver of foodstuffs against spoilage bacteria and fungi. Also, the both oils and bioactive components could be employed as natural food preservatives, preventing lipid peroxidation, which could cause food spoilage (at concentration levels 20 - 100 µg/ml), besides its traditional uses.

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