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Star-anise (*Illicium verum*) and black caraway (*Carum nigrum*) as natural antioxidants

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Abstract

The various solvent fractions of star-anise (*Illicium verum*) and black caraway (*Carum nigrum*), along with their spice powders and volatile oils, were prepared and evaluated for antioxygenic activity, using different methods. Star-anise powder and its ethanol/water (80:20)-soluble fraction showed strong antioxygenic activity in refined sunflower oil while the petroleum ether fraction exhibited marginal antioxygenic activity and the water-soluble fraction was practically devoid of any activity in sunflower oil. The black caraway powder showed marginal antioxygenic activity while its ethanol/water fraction (80:20) showed strong antioxygenic activity and all other fractions showed slight pro-oxygenic activity in refined sunflower oil. Both the spice powders and their extracts were also evaluated for antioxidant activity by linoleic acid peroxidaton, β-carotene-linoleate and 1,1-diphenyl-2-picryl hydrazyl (DPPH) methods. Both the star-anise and black caraway powders, as well as their ethanol/water extracts, exhibited strong antioxygenic activity. Volatile oils from both the spices exhibited antioxygenic activity and the activity did not seem to be concentration-dependent. Volatile oils from star-anise showed relatively higher antioxygenic activity than did those from black caraway. Gas chromatography–mass spectroscopy (GC–MS) studies on star-anise and black caraway volatile oils resulted in the identification of 25 and 22 compounds, respectively, representing 94–97% of the total content.

Keywords: Star-anise; Black caraway; Antioxygenic activity; Solvent extracts; Phenolics; Flavonoids

1. Introduction

In foods, lipid peroxidation and enzymatic hydrolysis cause shelf life problems. The most limiting factor in determining the shelf-life of dehydrated convenience foods is the autooxidation of fats and oils, causing off-flavours (Semwal, Sharma, & Arya, 1999). Rancidity development is an oxidative process that can be blocked by antioxidants, by preventing the formation of free radicals, through the donation of electrons or hydrogen ions. Synthetic antioxidants are widely used to prevent the oxidation of oils and fats and extend the shelf-life of lipid-containing foods. In recent years, their use in foods has suffered severe criticism, as consumers are becoming increasingly conscious of the safety of synthetic chemical additives (Ozcan, 2003). The use of these synthetic antioxidants, however, is restricted because of their toxicity (Kahl & Kappus, 1993; Walton et al., 1999; WHO, 1996). Therefore, the natural products that can act as antioxidants, either alone or synergistically with other additives, have gained importance (Baratta, Dorman, Deans, Biondi, & Ruberto, 1998; Pokorny, Nguyen, & Korzack, 1997). Significant numbers of natural antioxidants have been identified (Bandoniene, Gruzdiene, & Vensutonis, 2001; Bauman, Hadolin, Rizner-Hras, & Knez, 1999; Lee & Shibamoto, 2002; Mau et al., 2003; Moure et al., 2001; Pratt & Hudson, 1990). Among them, rosemary and vitamin E have commercial significance. β-Carotene and many spices, herbs and cereal extracts have been found to be promising natural antioxidants (Patro et al., 2005; Kahkonen et al., 1999; Ozcan, 2003; Semwal et al., 1997). In the present work, two spices, namely star-anise and black caraway, were individually extracted

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with various solvents and the relative antioxygenic activities of these fractions were evaluated, using different assays.

2. Materials and methods

2.1. Materials

Refined sunflower oil (Sunpure brand), without added antioxidants, was supplied by sunflower oil producers (M/S M.K. Agrotech. Srirangapatna, Karnataka, India). Commercially-available good quality whole star-anise and black caraway seeds were procured from the local market, cleaned and powdered in an ultra centrifugal mill (Model Retsch R1, Haan, Germany), using a 1 mm sieve. Linoleic acid, catechin, ammonium thiocyanate, tween 20, tween 40 and DPPH were procured from Sigma chemicals, Mumbai, India. Ferrous chloride, sodium dihydrogen phosphate, disodium hydrogen phosphate, chloroform, methanol, petroleum ether (40–60 °C) and gallic acid were procured from E. Merck, Mumbai, India, while β-carotene was procured from Fluka chemie, Germany. Ethyl alcohol was distilled before use, while all other chemicals were of analytical reagent grade and used as such.

2.2. Solvent extraction procedure

Star-anise and black caraway powders (10 g) were packed in glass columns (25 × 30 cm) and sequentially eluted with 500 ml, each, of petroleum ether (40–60 °C), ethanol/water (80:20 v/v) and distilled water. Each fraction was separately evaporated using a rotary vacuum evaporator (Model Super fit PBU 6, Continental instruments, Mumbai, India) and preserved at 5 °C prior to use. A flow chart for the preparation of various fractions from star-anise/black caraway powders is given in Fig. 1.

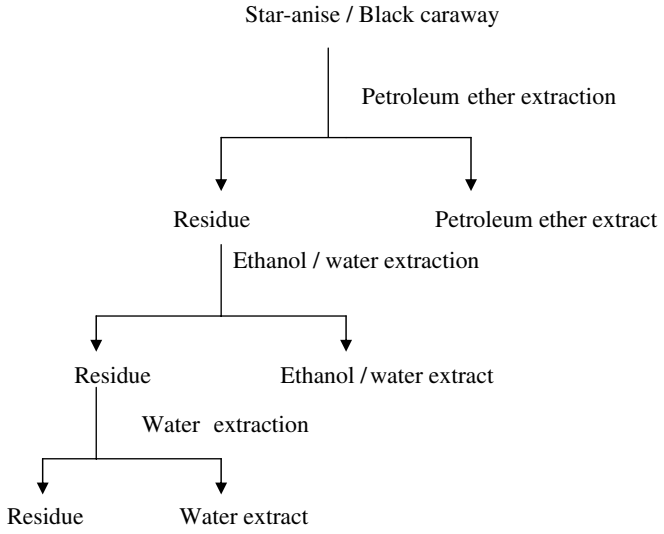

Fig. 1. Scheme for the solvent extraction of star anise/black caraway.

2.3. Preparation of volatile oils

The volatile oils from star-anise and black caraway were obtained by steam-distillation. Spice powders (50 g) from star-anise/black caraway were individually distilled and the distillate (1000 ml) transferred to a separating funnel. The aqueous layer was discarded and the upper volatile oil layer was dried, using anhydrous sodium sulphate.

2.4. Gas chromatography (GC)

Gas chromatographic analysis of volatile oils was carried out using a Chemito GC 1000 HR (M/S Chemito, Chennai, India) system equipped with a flame ionization detector (FID) and BP-5 column (30 m × 0.25 mm i.d.) with 0.25 μm film thickness. Injector and detector temperatures were maintained at 230 °C and 250 °C, respectively. The oven temperature was programmed from 35 °C to 210 °C with an initial hold time of 20 min. Injection volume used was 0.5 μl with split ratio of 1:25. Helium gas (30 cm/s) was used as a mobile phase.

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

Electron impact mass spectrophotometric data were collected on Finnigan Mat GCQ™, UK. GC conditions and column were the same as those used in the GC analysis above. The mass spectrometer was operated at 70 eV, ion source 200 °C and the transfer line temperature 250 °C. Sample components were identified by comparing mass spectral data with the NIST library, as well as published Kovat's indices. Percentage of MS is reported as a mean of three runs.

2.6. Extraction and estimation of total phenolics and total flavonoids

Powders (0.2 g) of star anise and black caraway, as well as their solvent extracts, (0.1 g) were extracted with 25 ml of 70% methanol (70 ml methanol + 30 ml distilled water) for 1 h under sonication, and the extracts were filtered. The residues were re-extracted for 1 h and both the filtrates were combined, and made up to 50 ml with 70% methanol. The total phenolics and flavonoids were estimated according to the method of Gerard and Roberts (2004).

2.7. Test for antioxygenic activity in sunflower oil

The star-anise and black caraway powders and their various solvent fractions and volatile oils were evaluated for antioxygenic activity in refined sunflower oil. Samples (100 g) of sunflower oil, both with and without 0.25 g, each, of star-anise and black caraway and their equivalent amounts of fractions or volatile oils (200–2000 ppm), were incubated in 250 ml glass beakers at 37 ± 1 °C. Initially, and after regular intervals of 15 days, 20 g samples were removed and analysed for peroxide (PV) as well as

thiobarbituric acid (TBA) values by the methods reported earlier (Semwal et al., 1999). Antioxygenic activity was calculated as the ratio between the peroxide value of control and the peroxide value of the sample.

2.8. Determination of antioxygenic activity using the linoleic acid peroxidation method

The antioxygenic activities of star-anise, black caraway and their extracts were determined using the thiocyanate method (Jayaprakasha, Singh, & Sakariah, 2001). The linoleic acid emulsion was prepared by homogenizing 0.28 g of linoleic acid and 0.28 g of tween 20 as an emulsifier and 50 ml of phosphate buffer (0.2 M, pH 7.0). The test samples were prepared in methanol–water mixture (8:2 v/v) and 1 ml of each of them was mixed with 2.5 ml of linoleic acid emulsion, 2 ml of phosphate buffer and incubated at 37 °C for 120 h. The mixture prepared as above without test sample served as a control. Aliquots (0.1 ml) were drawn from the incubated mixtures at intervals of 24 h and mixed with 4.7 ml of 75% ethanol, 0.1 ml of 30% ammonium thiocyanate and 0.1 ml of 0.2 mM ferrous chloride in 3.5% HCl and allowed to stand at room temperature for 3 min. After 3 min, the colour developed was measured at 500 nm. A control was performed using linoleic acid, without the test sample. Synthetic antioxidant TBHQ was used as a control for comparison. The degree of linoleic acid peroxidation was calculated at 96 h using the formula:

$$\text{Antioxygenic activity} = 100 - \frac{\text{Increase in absorbance of sample}}{\text{Increase in absorbance of control}} \times 100.$$

2.9. Determination of antioxygenic activity using the β-carotene-linoleic acid system

The antioxygenic activities of both the spices and their extracts were measured by monitoring the coupled oxidation of β-carotene and linoleic acid with the method described by Peterson, Emmons, and Hibbs (2001). β-Carotene (2 mg) was dissolved in 20 ml chloroform and 3 ml of this solution were added to 40 mg of linoleic acid and 400 mg of tween 40. The chloroform was removed under a stream of nitrogen gas at 40 °C. Oxygenated deionised water (100 ml) was added and the solution mixed well. Three millilitre aliquots of β-carotene/linoleic acid emulsions were mixed with 100 μl of the methanolic extracts (diluted with methanol to obtain the equivalent of 1 mg of starting material/100 μl). Oxidation of the emulsion was monitored spectrophotometrically (Shimadzu UV 1601, UV–Vis spectrophotometer) by measuring absorbance at 470 nm at 15 min intervals, up to 120 min at 50 °C against a blank consisting of 100 μl of water and emulsion without β-carotene. Control contained 100 μl of methanol in place of the extract. Antioxidant activity was expressed as percentage inhibition, relative to the control, in terms of bleaching of β-carotene after incubation for 120 min using the formula

$$\text{AOA} = 100 \times \frac{(\text{DR}_c - \text{DR}_s)}{\text{DR}_c}$$

where AOA is the antioxygenic activity, DR_c is the degradation rate of control, and DR_s is degradation rate of sample.

2.10. Determination of antioxygenic activity by the DPPH radical-scavenging method

The antioxygenic activities of star-anise and black caraway powders, as well as their extracts and TBHQ, were measured in terms of hydrogen-donating or radical- scavenging ability using the DPPH method (Gorinstein et al., 2004). Different amounts of spice extracts (100, 300, 500 and 1000 μl), equivalent to 1, 3, 5 and 10 mg /ml of spices and their extracts, were placed in separate test tubes. The volume was adjusted to 1 ml with methanol; 5 ml aliquots of 0.2 mM methanolic solution of DPPH were added to each tube and shaken vigorously. The tubes were allowed to stand at room temperature (30 ± 0.2 °C) for 30 min. The control was prepared, as above, without any extract and methanol was used for the base line correction. The absorbance of the samples was measured at 517 nm. The radical-scavenging activity was expressed as percentage of inhibition and calculated using the formula:

$$\%\ \text{Radical scavenging activity} = 100 - \frac{\text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100.$$

2.11. Statistical analysis

All the reported results are means of three replicates and were subjected to analysis of variance (ANOVA) using statistical software (Statistica, ver 7.1, series 1205) at $p < 0.01$ and $p < 0.05$ significance levels.

3. Results and discussion

3.1. Total phenolics and flavonoid contents of star-anise, black caraway and their extracts

Total phenolics and flavonoid contents of star-anise, black caraway and their solvent extracts (Table 1) indicate that star-anise contained higher amounts of both total phenolics (10,025 ppm) and flavonoids (5500 ppm) than did black caraway which contained 4743 and 2437 ppm, respectively.

Table 1
Total phenols and flavonoids in star-anise and black caraway and their extracts ($n = 3$) (ppm)

	Total phenols	Total flavonoids
Star-anise spice powder	10,025 ± 35[a]	5500 ± 25
Petroleum ether extract	–	–
Ethanol/water extract	6823 ± 18	2950 ± 21
Water extract	1952 ± 11	1400 ± 17
Black caraway spice powder	4743 ± 23	2437 ± 42
Petroleum ether extract	–	–
Ethanol/water extract	3065 ± 20	1357 ± 11
Water extract	902 ± 18	520 ± 9

[a] Mean ± SD.

3.2. Antioxygenic activity of star-anise and black caraway powders, their extracts and volatile oils in sunflower oil

Tables 2 and 3 show the effects of star-anise and black caraway powders (2.5 g/kg), as well as their various extracts equivalent to 2.5 g/kg of spice powder obtained by sequential extraction with petroleum ether, ethanol/water (80:20 v/v) and water, on peroxide formation in sunflower oil in comparison to TBHQ. Both star-anise powder and its ethanol/water soluble fraction showed strong antioxygenic effects, whereas petroleum ether extract exhibited only a marginal one. The control gave higher peroxide (PV) and thiobarbituric acid (TBA) values than did samples containing star-anise powder or its ethanol/water fraction (Table 2). The anti-oxygenic activity of spice powder was slightly higher than that of its ethanol/water-soluble fraction (Table 4). The water-soluble fraction was practically devoid of antioxygenic activity. It was interesting to observe that black caraway spice powder exhibited only marginal antioxidant activity whereas its ethanol/water-soluble fraction showed strong antioxygenic activity in sunflower oil (Table 3). The higher antioxygenic activity of the ethanol/water-soluble fraction may be due to the solubility of phenolic and flavonoid compounds, as well as sugars, in the ethanolic medium, which are known to have antioxygenic activity in lipids. On the other hand, both the petroleum ether- and water-soluble fractions exhibited pro-oxygenic activity (Table 4). Both the star-anise, black caraway and their extracts significantly ($p < 0.01$) reduced peroxide and malonaldehyde formation in sunflower oil at 37 °C. Volatile oils from both the spices exhibited significant ($p < 0.01$) antioxygenic activity as compared to the control. However, it was observed that the antioxygenic activity of volatile oils, from both the spices, did not significantly ($p > 0.01$) change, even after increasing the concentration of volatile oil to more than 200 ppm in sunflower oil stored at 37 ± 1 °C. Flavour components in volatile oils from star-anise and black caraway are shown in Tables 5 and 6. The major component in star-anise volatile oil was *trans*-anethole (93.9%), followed by limonene (1.05%) and estragole (1.05%), while caraway volatile oil contained limonene (43.0%), caravone (38.3%), anethole (5.39%) and eugenol (3.85%), with some other components in minor quantities. Inhibitory effects of volatile oils from both the spices may be due to the presence of these essential components, namely α-pinene, β-pinene, 1,8-cineol, eugenol and α-terpineol. Earlier, Ruberto and Baratta (2000) also reported the antioxygenic activity of various essential components in lipid model systems.

3.3. Antioxyenic activities of star-anise and black caraway powders and their extracts, using the β-carotene-linoleic acid bleaching method

The antioxygenic activities of star-anise and black caraway powders as well as of their extracts and TBHQ, as measured by the bleaching of β-carotene, are shown in Fig. 2. It can be seen that star-anise and its extracts exhib-

Table 2
Effects of star-anise (0.25%) and its fractions[A] on peroxide value (PV) and thiobarbituric acid value (TBA) of sunflower oil stored at 37 ± 1° C

Spice and its fractions	PV (meq O_2/kg fat)			TBA (mg malonaldehyde/kg)		
	Storage period (Days)			Storage period (Days)		
	15	30	45	15	30	45
Control	33.8	96.4	146.3	0.37	0.78	0.96
TBHQ	20.8[a]	40.7[a]	48.2[a]	0.23[a]	0.4[a]	0.46[a]
Star-anise powder	17.6[a]	43.2[a]	56.5[a]	0.29[a]	0.45[a]	0.51[a]
Petroleum ether extract	30.4[a]	86.0[a]	127.2[a]	0.33[a]	0.71[a]	0.79[a]
Ethanol/water extract	20.0[a]	51.8[a]	73.5[a]	0.25[a]	0.50[a]	0.57[a]
Water extract	33.5	95.5	140.7[a]	0.36	0.77	0.92[a]
Volatile oil (ppm)						
200	24.2[a]	71.5[a]	109.4[a]	0.32[a]	0.61[a]	0.70[a]
500	23.6[a]	68.1[a]	105.2[a]	0.30[a]	0.59[a]	0.69[a]
1000	23.9[a]	69.8[a]	106.1[a]	0.31[a]	0.59[a]	0.69[a]
2000	24.0[a]	70.3[a]	105.7[a]	0.30[a]	0.60[a]	0.70[a]

Initial PV and TBA values were 5.8 and 0.11, respectively.
Values superscripted with 'a' indicate antioxygenic activity significantly different ($p < 0.01$) from control.
[A] Concentrations equivalent to 0.25% of ground spice.

Table 3
Effects of black caraway (0.25%) and its fractions[A] on peroxide value (PV) and thiobarbituric acid value (TBA) of sunflower oil stored at 37 ± 1 °C

Spice and its fractions	PV (meq O_2/kg fat)			TBA (mg malonaldehyde/kg)		
	Storage period (Days)			Storage period (Days)		
	15	30	45	15	30	45
Control	33.8	96.4	146.3	0.37	0.78	0.96
TBHQ	20.8[a]	40.7[a]	48.2[a]	0.23[a]	0.40[a]	0.46[a]
Black caraway powder	29.3[a]	89.2[a]	135.5[a]	0.30[a]	0.67[a]	0.82[a]
Petroleum ether extract	33.6	100.4[b]	159[b]	0.45[b]	0.95[b]	1.08[b]
Ethanol/water extract	20.0[a]	51.8[a]	69.7[a]	0.20[a]	0.41[a]	0.47[a]
Water extract	33.7	112.4[b]	183[b]	0.38	0.87[b]	1.01[b]
Volatile oil (ppm)						
200	30.6[a]	86.4[a]	124.2[a]	0.36	0.71[a]	0.83[a]
500	28.9[a]	84.9[a]	123.4[a]	0.33[a]	0.66[a]	0.81[a]
1000	29.6[a]	85.3[a]	124.1[a]	0.35	0.69[a]	0.84[a]
2000	29.1[a]	85.9[a]	123.9[a]	0.34[a]	0.71[a]	0.82[a]

Initial PV and TBA values were 5.8 and 0.11, respectively.
Values superscripted with 'a' indicate antioxygenic activity significantly different ($p < 0.01$) from control.
Values superscripted with 'b' indicate pro-oxygenic activity significantly different ($p < 0.01$) from control.
[A] Concentrations equivalent to 0.25% of ground spice.

Table 4
Antioxygenic activities[a] of star-anise, black caraway (0.25%), and their fractions,[b] volatile oils and TBHQ in refined sunflower oil stored at 37 ± 1 °C

Spice and its fractions	Anti-oxygenic activity[c] ± SD	
	Star-anise	Black caraway
Spiced powders	2.24 ± 0.34	1.10 ± 0.04
Petroleum ether extract	1.13 ± 0.02	0.96 ± 0.04
Ethanol/water extract	1.85 ± 0.15	1.88 ± 0.21
Water extract	1.02 ± 0.02	0.89 ± 0.10
Volatile oil (ppm)		
200	1.36 ± 0.03	1.13 ± 0.04
500	1.41 ± 0.02	1.17 ± 0.03
1000	1.39 ± 0.02	1.15 ± 0.03
2000	1.39 ± 0.02	1.15 ± 0.03
TBHQ (ppm)		
200	2.35 ± 0.58	2.35 ± 0.58

[a] Values >1 indicate antioxygenic activity and <1 indicate pro-oxygenic activity.
[b] Concentrations equivalent to 0.25% of ground spice.
[c] Mean of three values after 15, 30 and 45 days. Calculated as ratio of peroxide value of control to peroxide value of sample.

Table 5
Flavour profile of star-anise volatile oil

Name of the compounds	Identity	Peak %
α-Pinene	KI, MS	0.12 ± 0.020
β-Pinene	KI, MS	0.03 ± 0.020
Myrcene	KI, MS	0.02 ± 0.003
α-Phellandrene	KI, MS	0.04 ± 0.001
3-Carene	KI, MS	0.15 ± 0.020
α-Terpinene	KI, MS	0.02 ± 0.001
p-Cymene	KI, MS	0.05 ± 0.003
Limonene	KI, MS	1.05 ± 0.040
trans-Ocimene	KI, MS	0.09 ± 0.010
cis-β-Ocimene	KI, MS	0.01 ± 0.001
γ-Terpenene	KI, MS	0.04 ± 0.001
Terpinolene	KI, MS	0.03 ± 0.003
Linalool	KI, MS	0.29 ± 0.020
γ-Terpineol	KI, MS	0.12 ± 0.030
4-Terpineol	KI, MS	0.09 ± 0.020
α-Terpineol	KI, MS	0.08 ± 0.010
Estragole	KI, MS	1.05 ± 0.120
cis-Anethole	KI, MS	0.14 ± 0.020
trans-Anethole	KI, MS	93.9 ± 1.560
α-Cubenene	KI, MS	0.10 ± 0.010
β-Clemene	KI, MS	0.01 ± 0.001
Carryophyllene	KI, MS	0.10 ± 0.010
Bergamotene	KI, MS	0.01 ± 0.002
Δ-Cardinene	KI, MS	0.04 ± 0.002
α-Cardinol	KI, MS	0.02 ± 0.001

KI, Kovat's index; MS, mass spectra.

ited higher antioxygenic activities than did black caraway and its extracts (Fig. 2). Relatively, ethanol/water extracts, from both the spices, showed the highest antioxygenic activity, followed by water extracts, while the petroleum ether extracts showed the least activity. However, values (65.6–82.2%) were found to be lower than these observed for TBHQ (92.5%). The higher antioxygenic activity of star-anise than of black caraway may be due to the presence of higher concentrations of both phenolics and flavonoids (Table 1). Earlier also, flavonoids and phenolics have been reported as potent antioxidants in β-carotene-linoleic acid bleaching systems (Lu & Foo, 2000; Siddaraju & Becker, 2003).

3.4. Antioxygenic activities of star-anise and black caraway powders, and their extracts in a linoleic acid peroxidation system

The oxidation activities of star-anise and black caraway powders, as well as their extracts, were measured using the ferrithiocyanate method. Fig. 3 depicts the percentage inhibition of linoleic acid after the addition of star-anise and black caraway powders, as well as their extracts and TBHQ. Compared with the control, the spice powders

Table 6
Flavour profile of black caraway volatile oil

Name of the compounds	Identity	Peak %
α-Pinene	KI, MS	0.18 ± 0.060
Sabinene	KI, MS	0.46 ± 0.030
Myrcene	KI, MS	0.31 ± 0.040
α-Phellandrene	KI, MS	0.14 ± 0.010
Δ-3-Carene	KI, MS	0.06 ± 0.010
Cimonene	KI, MS	43.0 ± 1.260
1,8 Cineol	KI, MS	0.76 ± 0.050
(E)-β-Ocimene	KI, MS	0.04 ± 0.010
γ-Terpenene	KI, MS	0.22 ± 0.030
Terpinolene	KI, MS	0.03 ± 0.006
Linalool	KI, MS	0.06 ± 0.020
Terpinen-4-ol	KI, MS	0.06 ± 0.020
α-Terpineol	KI, MS	0.54 ± 0.100
Piperitone	KI, MS	0.18 ± 0.030
Carveol	KI, MS	0.14 ± 0.030
Cuminaldehyde	KI, MS	0.04 ± 0.010
Carvone	KI, MS	38.3 ± 1.210
Anethole	KI, MS	5.39 ± 1.230
δ-Elemene	KI, MS	0.13 ± 0.020
Eugenol	KI, MS	3.85 ± 0.160
α-Cubenene	KI, MS	0.12 ± 0.030
β-Carryophyllene	KI, MS	0.53 ± 0.010

KI, Kovat's index; MS, mass spectra.

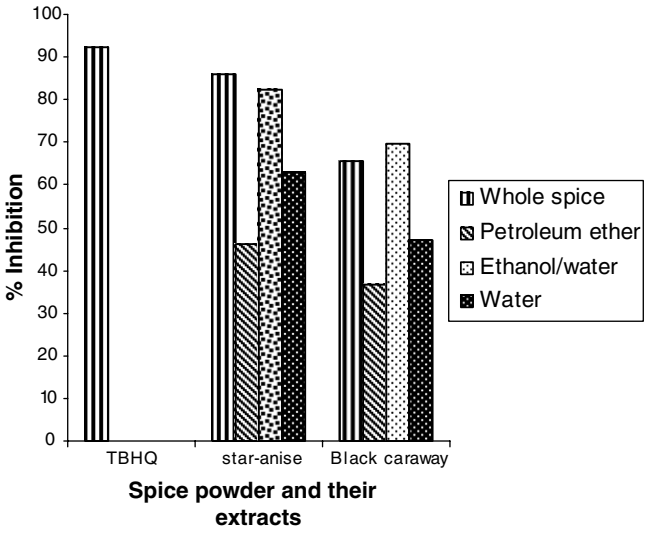

Fig. 2. Antioxygenic activity of spice powders and their extracts using β-carotene bleaching method.

and their petroleum ether, ethanol/water, water extracts and TBHQ gave a lower increase in peroxidation levels over the 96 h of testing. After 96 h, the inhibition percent of star-anise, its petroleum ether, ethanol/water and water extracts were 53.0%, 40.6%, 76.3% and 56.7% whereas the corresponding values for black caraway powder and its extracts were 46.6%, 38.7%, 71.6% and 51.4%, respectively. However, these values were found to be lower than that of the TBHQ (83.9%). Relatively, star-anise powder and its extracts showed slightly higher antioxygenic activity than its black caraway counterparts.

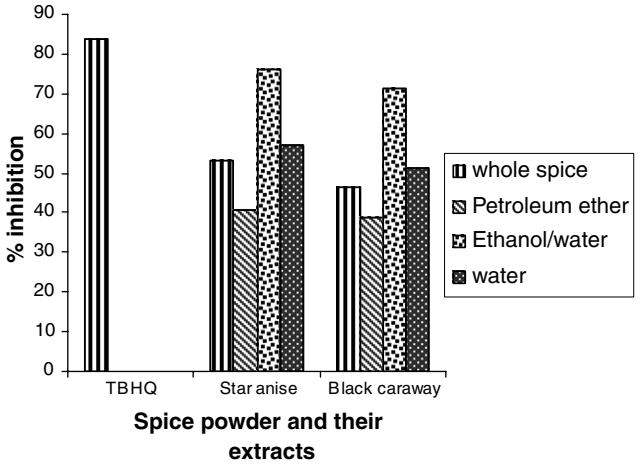

Fig. 3. Antioxygenic activity of spice powders and their extracts using linoleic acid peroxidation method.

3.5. Scavenging effect of DPPH (1,1-diphenyl-2 picryl hydrazyl radical)

It is well known that lipids containing polyunsaturated fatty acids and their esters are readily oxidized by molecular oxygen. On the other hand, antioxidants are believed to scavenge free radicals and suppress free radical chain oxidation by molecular oxygen (Gorinstein et al., 2004). DPPH has been used to evaluate the antioxygenic activity of various natural sources (Brand-Williams, Cuvelier, & Benset, 1995; Sanchez-Moreno, Larrauri, & Saura-Calixto, 1998; Singh, Maurya, Lampasona De, & Catalan, 2005). The scavenging activities of star-anise and black caraway powders, as well as their extracts, were determined and compared with those of TBHQ (Figs. 4 and 5). The order of antioxygenic activity (of 1 mg/ml of star-anise, black caraway and their extracts) was star-anise (ethanol/water extract) > black caraway (ethanol/water extract) >star-anise powder > black caraway powder > star-anise (water extract) > black caraway (water extract) > star-anise (petroleum

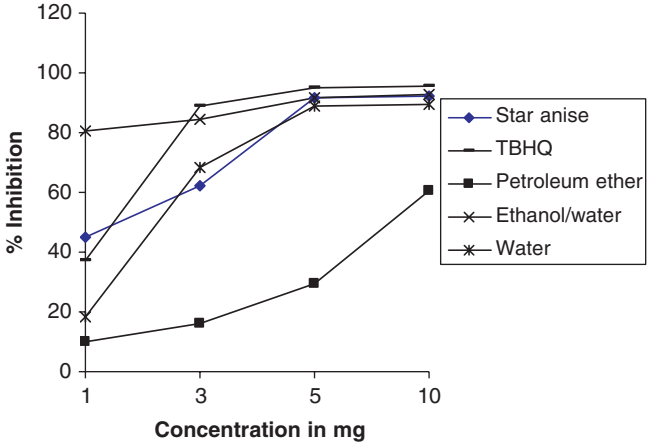

Fig. 4. Antioxygenic activity of star-anise and its extracts using DPPH radical-scavenging method.

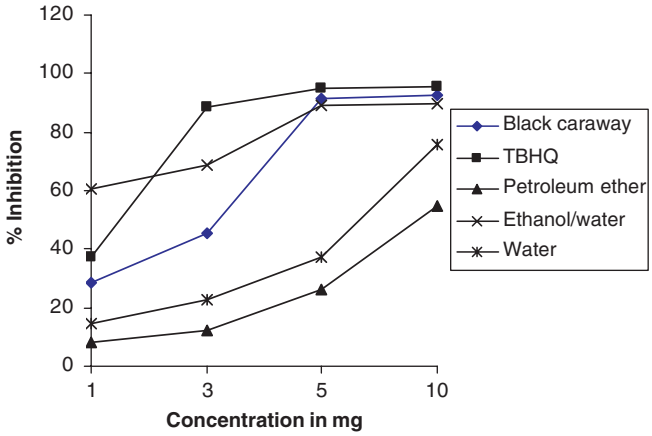

Fig. 5. Antioxygenic activity of black caraway and its extracts using DPPH radical-scavenging method.

ether extract) > black caraway (petroleum ether extract). At 5 and 10 mg/ml both the spice powders and their ethanol/water extracts showed similar scavenging activity to that of TBHQ, while petroleum ether and water extracts showed a moderate scavenging activity. However, the scavenging activity of both the spice powders and their extracts increased with increasing concentrations (Figs. 4 and 5). Relatively, star-anise powder and its extracts showed higher scavenging activities than did their counterparts from black caraway.

3.6. Correlation analysis

Total phenolics and flavonoids from star-anise and black caraway powders showed positive correlation ($r \geqslant 0.38$) with the antioxidant activity determined by β-carotene bleaching and DPPH radical-scavenging methods. The correlation was significant ($p \leqslant 0.05$) between total phenol and β-carotene bleaching ($r = 0.96$), total phenol and DPPH ($r = 0.72$), as well as total flavonoids and β-carotene bleaching ($r = 0.86$) in the case of star-anise. In the case of black caraway there was no significant correlation ($p > 0.05$) found between the antioxygenic activities tested with linoleic acid peroxidation and total phenolics ($r = -0.023$) or total flavonoids ($r = -0.11$). However, total phenol and total flavonoids correlated significantly ($p < 0.05$) with DPPH radical-scavenging ($r > 0.85$), but there was no significant correlation ($p > 0.05$) between the antioxygenic activities tested by β-carotene bleaching and linoleic acid peroxidation methods and the total phenolic and flavonoid contents.

4. Conclusion

The antioxygenic activities of both the spice powders, namely star-anise and black caraway and their extracts, were evaluated by different methods. Ethanol/water extracts from both the spices exhibited higher antioxygenic activities than did other solvent extracts, when evaluated for antioxygenic activity by any of the methods. The isolation and identification of compounds from ethanol/water-soluble extracts of these spices need further investigations. Thus, both the spice powders and particularly their ethanol/water extracts, have great potential as natural antioxidants.

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