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Chemical composition and antioxidant potential of essential oil and oleoresins from anise seeds (*Pimpinella anisum* L.)†

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Abstract

Recently, interest in plant-derived food additives has grown, mainly because of toxic effects associated with synthetic antioxidants. In the present study, the chemical composition and antioxidant properties of essential oil and oleoresins of the seeds of anise (*Pimpinella anisum*) was investigated. *Trans*-anethole (90.1%) was the major component in anise oil while oleic acid was the major compound in all the oleoresins. The antioxidant activities were assessed by inhibition of linoleic acid peroxidation, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, Fe^{3+} reducing power and various lipid peroxidation assays. The anise oil and its methanol oleoresin showed highest antioxidant activity, even higher than BHA and BHT. However, the antioxidant activities of other oleoresins were somewhat lower. The results obtained from various experiments clearly establish the antioxidant potency of anise oil and its methanol and ethanol oleoresins, which can be utilized for protecting fat-containing foods.

Key words: *Pimpinella anisum*, GC/MS, antioxidant activity, scavenging effect, reducing power, lipid peroxidation.

Introduction

All aerobic organisms produce different forms of activated oxygen species, which include free radicals ($O_2^{\cdot-}$, $HO^{\cdot}$), non-free radical species (H_2O_2), singlet oxygen ($^1O_2$), etc. during their normal body metabolism [1, 2]. These reactive oxygen species (ROS) can react with most of the biological molecules like lipids, proteins and lipoproteins, and damage them. Thus continuous and excessive generation of ROS can cause cell damage which further leads to pathogenesis of various diseases like cancer, arthritis, cardiovascular diseases, diabetes, ageing, etc. [3,4]. To protect cells against oxidative damage, living organisms possess various protective systems, which reduce ROS concentration by scavenging free radicals, chelating catalytic metals and by acting as electron donors. However, under certain conditions, the body's antioxidant system is compromised, leading to accumulation of ROS, resulting in cell damage. In such cases, consumption of antioxidant substances is required to reinforce the body defence system against ROS.

Oxidative processes are also responsible for the degradation of food quality during their processing and storage. To prevent oxidative damage of food, various antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tert-butylhydroxyquinone (TBHQ), etc. are used. However, several nutritional and toxicological studies have linked some synthetic antioxidants such as BHA and BHT to carcinogenesis and hepatic damage [5-7]. In view of this, during the recent years, interest in plant-derived antioxidative substances has grown. Plants and their components are perceived as 'natural' and 'safe' [8]. Many herbs and spices, usually used to flavour food items, are an excellent source of phenolic compounds, which have been reported to show good antioxidant activity by acting as free radical scavengers and metal chelators [9-11].

The commercial anise is the dried ripe seed of *Pimpinella anisum* L. (Apiaceae). The plant is an herbaceous annual

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indigenous to India, Iran, Turkey and other warm countries [12]. It is one of the oldest spices used both for the culinary and medicinal purposes. There are many reports on the antioxidant [11], antimicrobial [13-15] and antipyretic [16] properties of anise oil. However, very few, if any, studies have been done on anise oleoresins. In the present study, chemical composition of volatile oil and four kinds of oleoresins (extracted in ethanol, methanol, *n*-hexane and petroleum benzene) were analyzed by GC/MS. In addition, their antioxidant properties were evaluated in terms of total antioxidant activity, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, reducing power and various lipid peroxidation assays. Furthermore, their antioxidant potentials were compared with a few synthetic antioxidants like BHA, BHT and PG.

Materials and methods

Chemicals

BHA, BHT, PG, glacial acetic acid, potassium ferricyanide, ferrous chloride and ferric chloride were purchased from SD Fine Chemicals, Mumbai, India. NaOH, chloroform, *p*-anisidine, methanol, ethanol, *n*-hexane and petroleum benzene (PB) were purchased from Merck, Mumbai, India. DPPH and linoleic acid were purchased from Fluka Chemicals and trichloroacetic acid (TCA), thiobarbituric acid (TBA) and potassium iodide (KI) from Qualigens Fine Chemicals, Mumbai, India. All the reagents used were of analytical grade.

Extraction of essential oil and oleoresins

The seeds of *Pimpinella anisum* were purchased from the local market of Gorakhpur, India during April and voucher specimens were deposited at the Herbarium of the Science Faculty of DDU Gorakhpur University, Gorakhpur, India. The seeds were washed, sun dried and pulverized into a fine powder. A 100 g of spice powder was subjected to hydrodistillation in a Clevenger's type apparatus for 5 h according to European Pharmacopoeian [17] procedure. The light yellow coloured oil obtained (yield 2.4%) was dried over minimum amount of anhydrous sodium sulphate and stored at 4 ± 1°C.

Oleoresins were produced by extraction of spice powder with a suitable solvent (here, ethanol, methanol, *n*-hexane and PB were used). For this, 30 g of ground spice was loaded on the Soxhlet's apparatus and extracted with the solvent for 5-6 h. After complete extraction, the solvents were distilled off to obtain viscous oleoresins, which were stored at 4 ± 1°C.

Chemical analysis

Chemical composition of anise oil and oleoresins were analyzed by GC/MS technique using a Hewlett-Packard gas chromatograph (Model 6890) coupled with a quadruple mass spectrometer (Model HP 5973) and a Perkin Elmer Elite-5MS capillary column (5% phenylmethylsiloxane; length 30m x inner diameter 0.25mm x film thickness 0.25 µm). The injector, interphase, ion source and selective mass detector temperatures were maintained at 250, 280, 230 and 150°C, respectively. Helium (He) was used as a carrier gas at a flow rate of 1.0 ml/min for essential oil and at 1.5 ml/min for the oleoresins. For the oil, oven temperature was programmed as: at 60°C for one min; then increased from 60 to 185°C at the rate of 1.5°C/min and held at 185°C for one min; then again increased from 185°C to 275°C at the rate of 9°C/min and held at 275°C for two min. The oven temperature for oleoresins was programmed as follows: 70°C (zero min), increased from 70° to 280°C at the rate of 5°C/min and held at 280°C for 20 min.

Antioxidant properties

Total antioxidant activity (FTC method)

The total antioxidant activity was determined according to ferric thiocyanate method [18] with some modifications. The reaction medium contained 2.5 ml solution of anise oil and oleoresins (1mg/100ml in absolute alcohol), 2.5 ml of 2.51% linoleic acid emulsion and 5 ml of 0.05 M-phosphate buffer (pH 7.0). The mixed solution (10 ml) was incubated at 40°C in the dark. The same solution without any test substance was used as control. The peroxide level of each sample was determined by reading the absorbance at 500 nm in a UV-VIS spectrophotometer (Hitachi-U-2000) after reaction with 0.1 ml of 20 mM $FeCl_2$ and 0.2 ml of 30% ammonium thiocyanate every 24 h. BHA, BHT and PG were used as positive standards. The inhibition of lipid peroxidation was shown in terms of absorbance values. The lower the absorbance, the higher the inhibition.

DPPH free radical scavenging activity

The capacity of anise oil and oleoresins to scavenge the DPPH radical was determined by the method reported earlier [19]. For this, 1 ml of freshly prepared DPPH radical solution (0.1 mM in methanol) was mixed thoroughly with 3 ml of methanolic solution of anise oil and oleoresins (5-20 µg/ml). The reaction mixture was left for 30 min in the dark at room temperature after which the resultant absorbance was recorded at 517 nm. Control (without any additive) and standards (containing BHA, BHT and PG; in place of oil and oleoresins) were also tested. The capability to scavenge the DPPH radical (% inhibition) was calculated using the following equation:

DPPH scavenging effect (%) = $\{1-(A_t-A_b/A_c)\} \times 100$

where, A_t is the absorbance of test sample, A_b is the absorbance of blank and A_c is the absorbance of control sample.

Reducing power

The reducing power of the samples was assessed by the method described by Senevirathne et al [20]. A 1 ml aliquot of each essential oil, oleoresins and synthetic antioxidants (0.5-2.0 mg/ml of absolute alcohol) was mixed with 1 ml of phosphate buffer (0.2 M, pH 6.6) and 1 ml of 1% potassium ferricyanide solution. The mixture was incubated at 50°C for 30 min. After cooling rapidly, it was mixed with 1 ml of 10% trichloroacetic acid and centrifuged at 3000 rpm for 10 min. A 2.5 ml fraction of the supernatant was mixed with 2.5 ml of water and 0.5 ml of 1% $FeCl_3$. Absorbance of the resultant mixture was recorded at 700 nm after 10 min. The higher the absorbance value, the stronger the reducing power.

Oxidation of mustard oil

The unrefined crude mustard oil samples (30 g each) containing 200 ppm (w/v) concentration of anise oil and

oleoresins were prepared. Synthetic antioxidants such as BHA, BHT and PG were also added to mustard oil at the same concentration. Mustard oil without any additive was used as a control sample. All the nine samples were exposed to accelerated oxidation condition by incubating at 70°C in darkness.

Peroxide value

Total peroxide and hydroperoxide oxygen content of the mustard oil samples was determined by measuring peroxide value at regular intervals according to the procedure prescribed by Horwitz [21]. A 5 g of mustard oil sample containing anise oil, oleoresins and/or synthetic antioxidants, dissolved in 30 ml of glacial acetic acid-chloroform (3:2) solution, was mixed with 0.5 ml of saturated KI solution. After one minute, 30 ml of distilled water was added and the mixture was titrated with 0.01 N $Na_2S_2O_3$ using starch indicator. Titration was continued, shaking the flask vigorously until the blue color just disappeared. The peroxide value (meq of peroxide/kg of oil) was calculated as:

$$\text{Meq of peroxide/kg of oil} = \frac{S \times N \times 1000}{\text{wt of sample (gm)}}$$

where, S is ml of $Na_2S_2O_3$ consumed, and N is the normality of $Na_2S_2O_3$.

Anisidine value

The anisidine value of mustard oil samples was estimated by the method described earlier [22]. A 5 g of oil sample was dissolved in isooctane and the volume made up to 50 ml. A 5 ml aliquot of this solution was mixed with 1 ml of 0.25% anisidine reagent and left for 10 min in dark at room temperature. Absorbance of the solution was then recorded spectrophotometrically at 350 nm. A blank test, without anisidine reagent, was also performed. The anisidine value was calculated as:

$$\text{Anisidine value} = \frac{(A_2\text{-}A_1) \times 1.2 \times 50}{\text{wt of oil sample (g)}}$$

where, A_1 is the absorbance of blank, and A_2 is the absorbance of test sample.

TBA value

TBA value of different samples was determined according to the method previously reported by Marcuse (1973) and Pokorny & Dieffebacher [23, 24]. About 100 mg of oil sample was dissolved in 25 ml of 1-butanol. A 25 ml aliquot of above solution was mixed thoroughly with 5.0 ml of TBA reagent (200 mg TBA in 100 ml 1-BuOH) and incubated at 95°C. After 2 h, the reaction mixture was cooled to room temperature under running water and absorbance was measured at 530 nm. At the same time, a reagent blank test (without TBA reagent) was also done. The TBA value (meq of malondialdehyde/g) was calculated as:

$$\text{TBA value} = \frac{50 \times (A\text{-}B)}{M}$$

where, A is absorbance is the absorbance of test sample, B is the absorbance of reagent blank and M is the mass of the sample (mg).

Conjugated diene hydroperoxide (CDH) assay

The CDH content of different oil samples was assessed by the method described by Senevirathne et al [20]. 100 mg of each mustard oil sample was mixed with 10 ml of cyclohexane and vortexed. The absorbance of the resulting mixture was recorded at 234 nm.

Statistical analysis

Experimental results were the means ± standard deviation of three parallel measurements (data are not shown). The results were analyzed by Student's t-test by using Microsoft Excel statistical analysis program and $p \leq 0.05$ was considered as significant.

Results and discussion

Chemical analysis

The data (EM fragmentation and retention indices) obtained from GC/MS analysis were carefully interpreted to identify the various components present in anise oil and oleoresins (Tables 1 and 2). A total of 15 compounds were identified in anise oil representing about 99.9% of the total weight. The major components were *trans*-anethole (90.1%), fenchone (5.0%) and methylchavicol (2.3%). Clark [25] and Kuraali et al [26] have also, independently, reported *trans*-anethole as the major component of anise seed oil. Ethanol oleoresin showed the presence of 19 components constituting about 98.1% of the total amount, the major components being oleic acid (57.9%), *trans*-anethole (14.1%), ethyl ester of oleic acid (11.0%) and palmitic acid (8.5%). In methanol oleoresin, 14 components (96.0%) were found, the major ones were oleic acid (75.5%), palmitic acid (7.9%) and *trans*-anethole (5.7%). Twenty components (88.0%) were present in *n*-hexane oleoresin, of which oleic acid (57.5%), *trans*-anethole (19.7%) and palmitic acid (5.3%) were the most prevelant. In petroleum benzene oleoresin, a total of 16 components were identified comprising 81.5% of the total weight, the major components being *trans*-anethole (6.4%) and palmitic acid (5.7%).

Antioxidant activity

During the initial stages of lipid oxidation, the extent of peroxide was measured using the linoleic acid system. The inhibitory capabilities of different samples against lipid peoroxidation in linoleic acid were assessed by thiocyanate method and the results are given in Figure 1. Anise oil showed higher activity than BHA, but lower than that of BHT and PG. The oleoresins also showed inhibition but their effects are lesser than all the synthetic antioxidants tested. All the results were found to be highly significant ($p \leq 0.01$).

The DPPH scavenging method is based on the reduction of DPPH radicals in the presence of a hydrogen donating substances, resulting in the formation of non-radical form (DPPH-H). Substances which are able to perform this reaction can be considered as radical scavengers and hence antioxidants [27]. The volatile oil and oleoresins of anise showed a dose dependent DPPH radical scavenging effect (Figure 2). The scavenging effect increased with increasing concentration of the test substances. The anise oil showed strongest scavenging power that was even higher ($p \leq 0.2$) than BHA and BHT but lower than that of PG at all the concentrations. Of the four oleoresins, the best activity was shown by methanol oleoresin followed by ethanol oleoresin. Both these oleoresins were found to be better

Table 1. Chemical composition of *Pimpinella anisum* essential oil analysed by GC/MS.

compound	% MS	RI #	identification Φ
α-pinene	0.1	927	MS, RI, co-GC
sabinene	t	964	MS, RI, co-GC
myrcene	t	983	MS, RI, co-GC
α-phellandrene	t	998	MS, RI, co-GC
p-cymene	0.1	1018	MS, RI, co-GC
limonene	0.8	1023	MS, RI, co-GC
1,8-cineole	0.1	1026	MS, RI, co-GC
cis-β-ocimene	t	1029	MS, RI, co-GC
fenchone	5.0	1083	MS, RI, co-GC
camphor	0.2	1138	MS, RI, co-GC
methyl chavicol	2.3	1192	MS, RI
endo-fenchyl acetate	0.1	1224	MS, RI, co-GC
cis-anethole	0.5	1247	MS, RI
p-anisaldehyde	0.5	1253	MS, RI
***trans*-anethole**	**90.1**	**1294**	**MS, RI**
total	**99.9%**		

Trace <0.05; # the retention index (RI) was calculated using a homologous series of *n*-alkanes C_8-C_{18}; Φco-GC: co-injection with an authentic sample. Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector.

Table 2. Chemical composition (%) of oleoresins (in various solvents) of *Pimpinella anisum* analysed by GC/MS.

compound	PS2	PS3	PS4	PS5	RI#	identification Φ
p-cymene	---	---	t	---	1028	MS, RI, co-GC
limonene	t	---	0.5	t	1031	MS, RI, co-GC
1,8-cineole	---	---	t	---	1034	MS, RI, co-GC
cis-β-ocimene	---	---	t	---	1039	MS, RI, co-GC
fenchone	1.2	0.3	0.9	0.4	1093	MS, RI, co-GC
undecane	t	---	---	0.3	1100	MS, RI, co-GC
camphor	t	t	t	---	1149	MS, RI, co-GC
methyl chavicol	t	---	0.5	t	1201	MS, RI
endo-fenchyl acetate	t	t	t	t	1226	MS, RI
p-anisaldehyde	t	---	t	t	1258	MS, RI
***trans*-anethole**	**14.1**	**5.7**	**19.7**	**6.4**	**1295**	**MS, RI**
palmitic acid, methyl ester	t	0.3	t	t	1914	MS, RI
palmitic acid	8.5	7.9	5.3	5.7	1968	MS, RI, co-GC
palmitic acid, ethyl ester	1.2	---	---	---	1981	MS, RI, co-GC
linoleic acid, methyl ester	t	0.7	0.1	t	2080	MS, RI, co-GC
oleic acid methyl ester	t	2.8	0.4	0.5	2086	MS, RI, co-GC
oleic acid	**57.9**	**75.5**	**57.5**	**63.5**	**2130**	**MS, RI, co-GC**
oleic acid, ethyl ester	11.0	---	---	---	2146	MS, RI, co-GC
stearic acid	1.9	---	---	---	2157	MS, RI, co-GC
2-oleoylglycerol	2.3	2.8	2.1	3.6	---	MS
squalene	---	t	0.2	t	---	MS
octacosanal	t	t	0.4	0.6	---	MS
stigmasterol	t	t	0.4	0.5	---	MS
sitosterol	---	t	t	---	---	MS
total %	**98.1**	**96.0**	**88.0**	**81.5**		

PS2: ethanol extract; **PS3:** methanol extract; **PS4:** *n*-hexane extract; **PS5:** petroleum benzene extract

Trace: <0.05; # the retention index (RI) was calculated using a homologous series of *n*-alkanes C_6-C_{22}; Φ co-GC: co-injection with an authentic sample. Percentages are the mean of three runs and were obtained from electronic integration measurements using a selective mass detector.

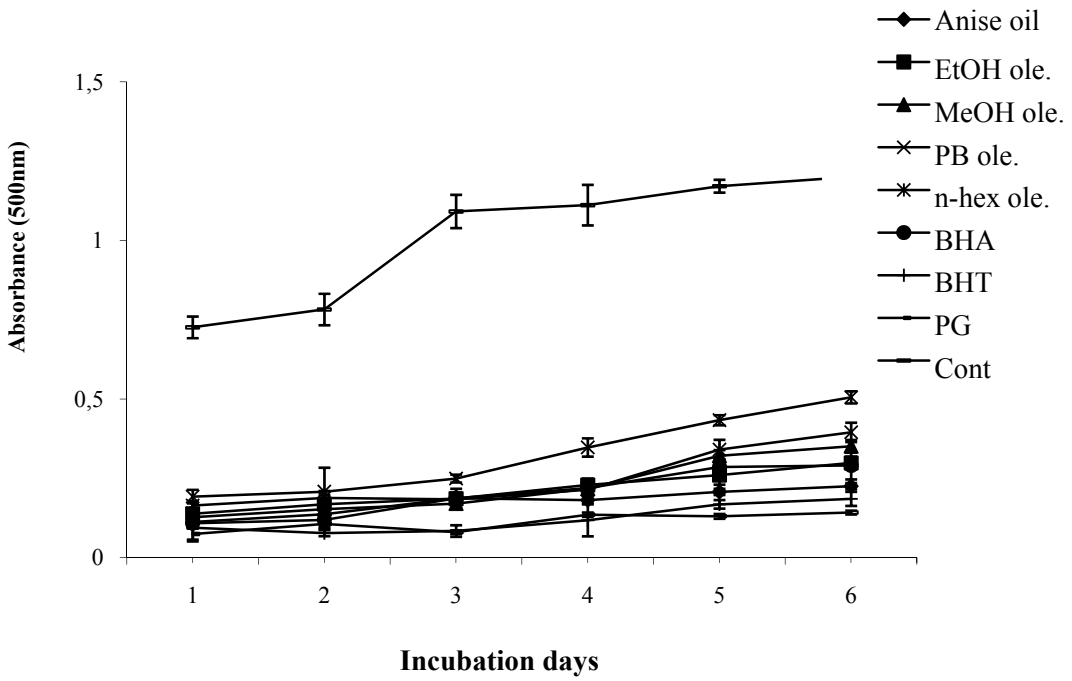

Figure 1. Antioxidant activity of anise oil and oleoresins (ole) in linoleic acid.

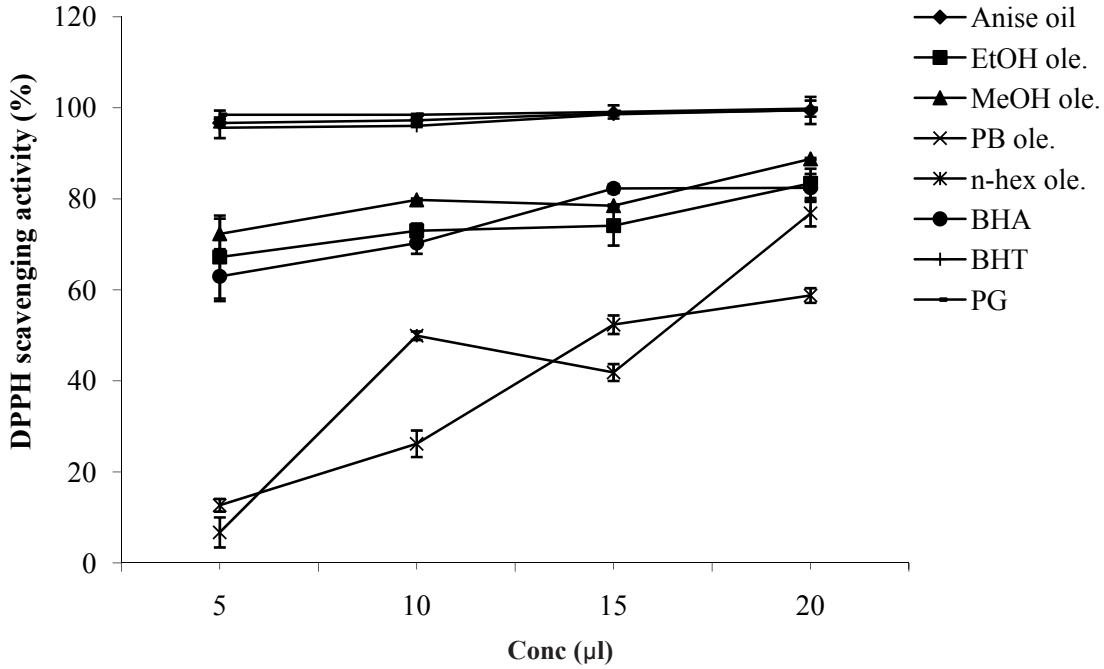

Figure 2. Radical scavenging effect of anise oil and oleoresins (ole) on DPPH.

scavengers than BHA, however, their activity were lesser ($p \leq 0.05$) than BHT and PG. It appears that the anise oil and oleoresins possess some hydrogen donating abilities and hence, were able to reduce the stable DPPH free radical (violet) to yellow coloured diphenylpicrylhydrazine.

For the measurement of reducing ability, we analysed the Fe^{3+} to Fe^{2+} transformations in the presence of various test substances. Measurement of the absorbance at 700 nm can monitor the concentration of Fe^{2+}. Figure 3 shows the reducing abilities of different sample oleoresins and essential oil compared to BHA, BHT and PG. The reducing power of all the samples were found to be significantly higher ($p \leq 0.05$) than the control and depended on the concentration of anise oil and oleoresins in the reaction mixture. Anise oil and its ethanol oleoresin showed better reducing power than the other oleoresins. Their activities were higher than BHA, equivalent to BHT and lower than that of PG. The reducing power of essential oil and oleoresins of anise might be due to their hydrogen donating ability [28] and is generally associated with the presence of reductones [29]. The components present in the anise oil and oleoresins could act as good reductants,

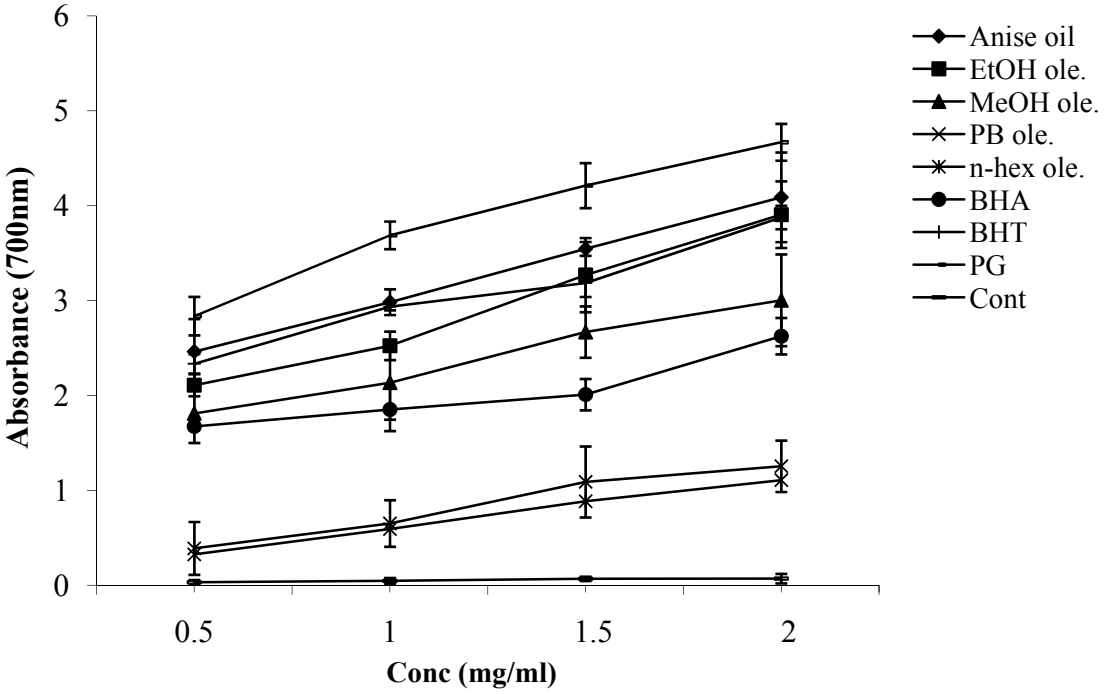

Figure 3. Reducing power of anise oil and oleoresins (ole).

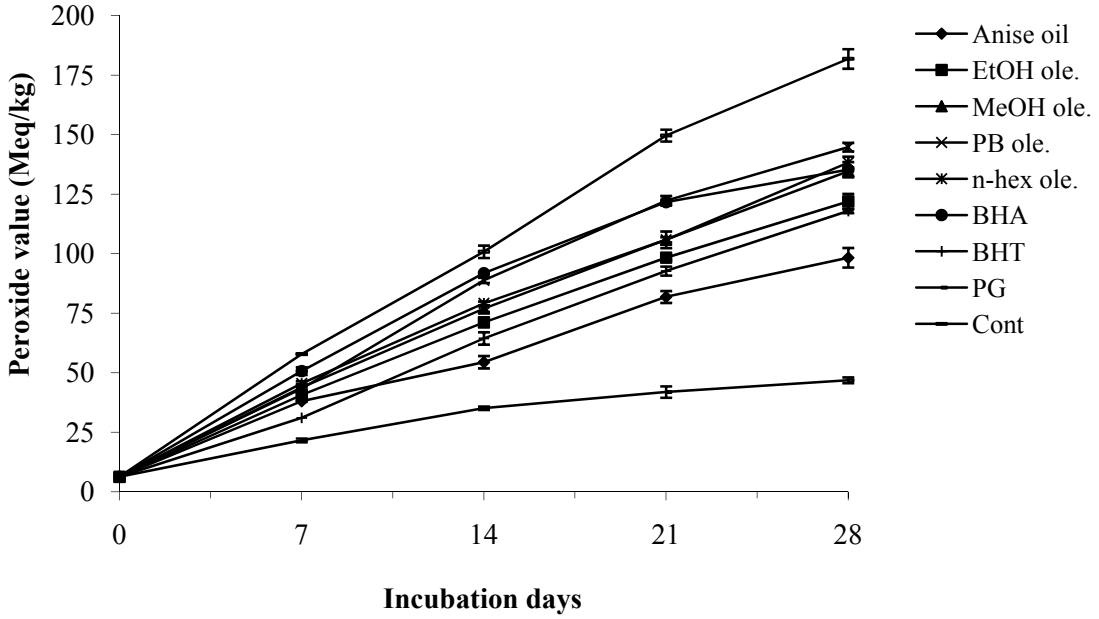

Figure 4. Inhibitory effect of anise oil and oleoresins (ole) on the primary oxidation.

which could stabilise free-radicals and terminate the chain reactions.

Oxidation of mustard oil

The peroxide value assay is commonly used to measure the amount of peroxides and hydroperoxides formed during initial stages of lipid peroxidation. The peroxide values of different mustard oil samples were assessed after every 7 days and the results are shown in Figure 4. The entire test samples showed significantly lower ($p \leq 0.02$) peroxide content than the control for which PV increased from 6.3 meq/kg (day zero) to 181.8 meq/kg (28th day). On the other hand, the PV of anise oil, ethanol and methanol oleoresins were 130, 121.9 and 134.5 meq/kg and that of BHA, BHT and PG were 145.2, 137.9 and 56.8 meq/kg, respectively. Thus, in terms of peroxide inhibitory effect, the relative efficiencies of various samples were: PG > methanol oleoresin > anise volatile oil > ethanol oleoresin > BHT > BHA > *n*-hexane oleoresin > PB oleoresin > control.

The anisidine assay measures the high molecular weight saturated and unsaturated carbonyl compounds. The anisidine value represents the level of aldehydes,

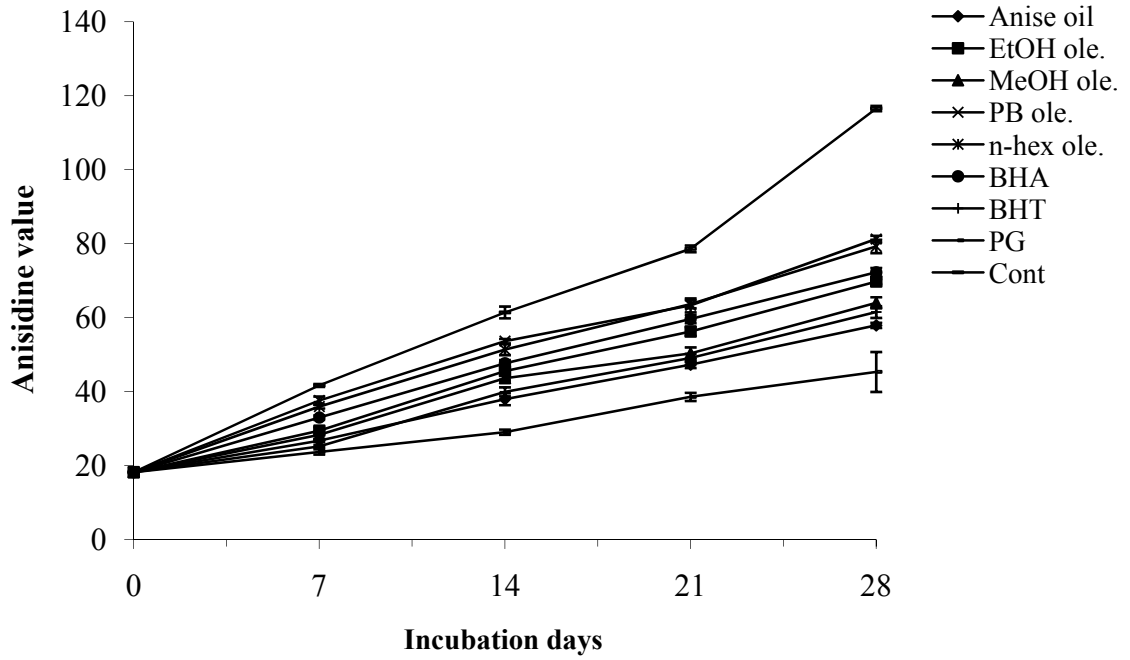

Figure 5. Inhibitory effect of anise oil and oleoresins (ole) on the formation of 2-alkenals.

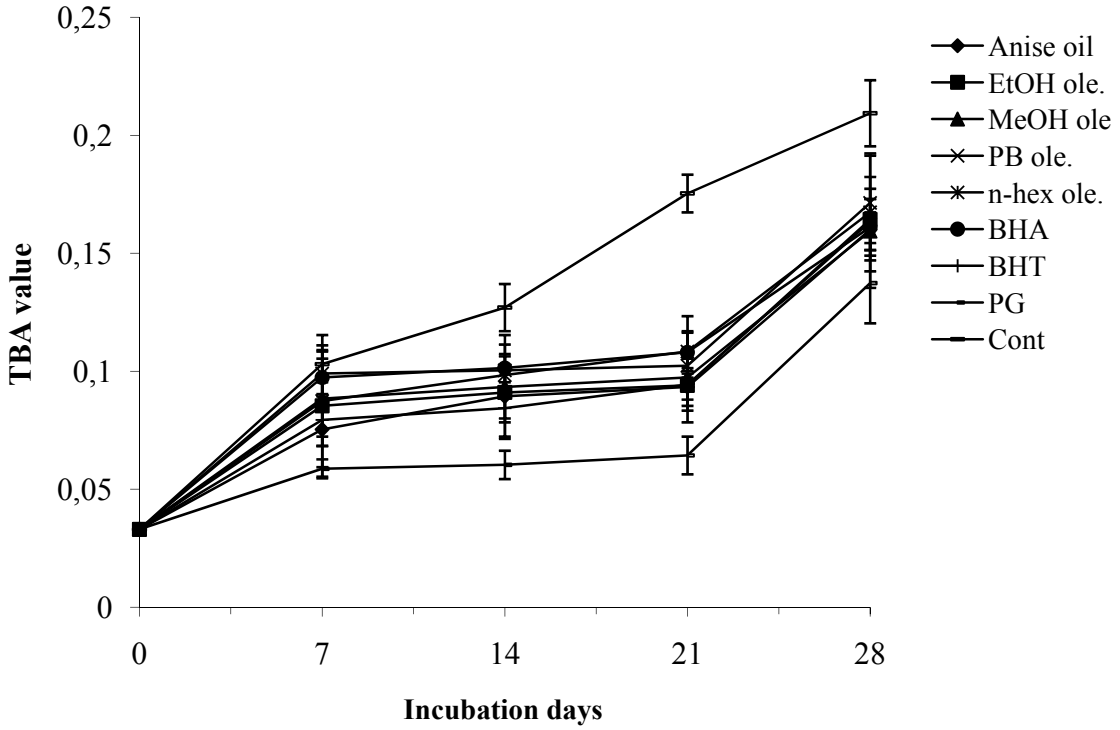

Figure 6. Antioxidative effect of anise oil and oleoresins (ole) in terms of TBA value.

principally 2-alkenals, present in the oils. The anisidine value of different mustard oil samples are given in Figure 5 and the values obtained are in good correlation with the peroxide values of the same mustard oil samples. The essential oil, methanol and ethanol oleoresins had shown significantly higher ($p \leq 0.05$) activities than BHA and BHT. However, their activities were quite less than that of PG. The anisidine values for *n*-hexane and PB oleoresins were lower than all the synthetic antioxidants tested.

During the oxidation of lipids, various low molecular weight secondary oxidation products are formed. One such compound is malondialdehyde that can be reacted with two molecules of thiobarbituric acid to give a chromogen, which can be detected spectrophotometrically. The TBA values of different samples tested were found to be lower than that of the control (Figure 6), but the differences were not significant. The TBA value is the measure of inhibitory effect of various antioxidative substances on the formation of malondialdehyde. It was observed that the essential oil, ethanol and methanol oleoresins of anise were more

Table 3. Effect of essential oil and different oleoresins (ole) of anise on the formation of conjugated diene hydroperoxides (CDH) in mustard oil during storage.

		conjugated diene hydroperoxides[1] storage time (days)				
sample	**conc.**	**2**	**4**	**6**	**8**	**10**
anise oil	0.1%	0.059 ± 0.04	0.075 ± 0.04	0.121 ± 0.03	0.351 ± 0.04	0.608±0.04
	0.05%	0.067 ± 0.04	0.093 ± 0.01	0.168 ± 0.03	0.419 ± 0.06	0.711 ± 0.05
EtOH ole.	0.1%	0.051 ± 0.03	0.086 ± 0.04	0.135 ± 0.02	0.362 ± 0.04	0.619 ± 0.03
	0.05%	0.059 ± 0.03	0.103 ± 0.03	0.182 ± 0.02	0.423 ± 0.07	0.735 ± 0.04
MeOH ole.	0.1%	0.06 ± 0.03	0.078 ± 0.02	0.115 ± 0.02	0.318 ± 0.03	0.596 ± 0.03
	0.05%	0.071 ± 0.02	0.095 ± 0.04	0.172 ± 0.04	0.406 ± 0.05	0.675 ± 0.05
PB ole.	0.1%	0.084 ± 0.03	0.102 ± 0.02	0.167 ± 0.03	0.422 ± 0.04	0.788 ± 0.04
	0.05%	0.096 ± 0.01	0.188 ± 0.03	0.256 ± 0.03	0.54 ± 0.04	0.856 ± 0.06
n-Hex ole.	0.1%	0.073 ± 0.03	0.092 ± 0.03	0.142 ± 0.03	0.415 ± 0.03	0.775 ± 0.06
	0.05%	0.092 ± 0.02	0.17 ± 0.04	0.208 ± 0.04	0.546 ± 0.05	0.819 ± 0.03
BHA	0.01%	0.07 ± 0.01	0.098 ± 0.02	0.196 ± 0.05	0.493 ± 0.06	0.806 ± 0.05
BHT	0.01%	0.065 ± 0.03	0.096 ± 0.03	0.181 ± 0.03	0.465 ± 0.04	0.795 ± 0.06
control		0.116 ± 0.07	0.208 ± 0.09	0.312 ± 0.1	0.618 ± 0.11	0.931 ± 0.14

[1]CDH values expressed as absorbance at 234 nm.

effective than that of BHA; *n*-hexane oleoresin showed activity equivalent to BHA but lower than BHT and PG while PB oleoresin was least effective. Moreover, all the tested samples were less effective than BHT and PG.

Conjugated diene hydroperoxides (CDH) are formed from unsaturated fatty acids during the process of lipid oxidation. The CDH further undergo radical formation leading to various chain reactions, which increase the lipid rancidity. So, in order to protect lipids/oils from oxidative degradation, CDH formation should also be checked alongwith the other methods of preventing oxidation. Table 3 show the CDH inhibitory potential of volatile oil and oleoresins of anise, which was found to be dose dependent. The anise oil showed strongest CDH inhibition effect followed by methanol and ethanol oleoresins. Anise oil showed significantly higher activity ($p \leq 0.05$) than BHA (0.01%) and BHT (0.01%) at 0.1% as well as 0.05% levels. Ethanol and methanol oleoresins were more effective than BHA at both the concentrations, but less effective than BHT at 0.05% concentration. Furthermore, *n*-hexane and PB oleoresins showed good inhibitory effects, at 0.1% dose, which were nearly equal to that of BHA

The volatile oil contains 90.1% *trans*-anethole, which might be responsible for the observed antioxidant properties. Bruits and Bucar [30] had already reported the free radical scavenging properties of *trans*-anethole. In addition, other compounds present in lesser quantity may also contribute towards the antioxidant activity. In oleoresins, oleic acid was present as the major component followed by *trans*-anethole. The observed antioxidant activities of oleoresins might be due to the combined effect of *trans*-anethole and other compounds that could be present in oleoresins but not identified by GC/MS. It has been reported that most natural antioxidative compounds often work synergistically [31] with each other to produce a broad spectrum of antioxidant activities that creates an effective defence system against free radical attack [27]. It is well known that the effectiveness of added antioxidants varies depending on the food, processing and storage conditions [32]. Moreover, extraction processes, nature of solvents used and several heat, light and moisture induced chemical reactions can also alter the activity of volatile oils and oleoresins.

The results from various experiments revealed that the essential oil and ethanol and methanol oleoresins of anise seeds possess significant antioxidant and radical scavenging properties. As these plant products have no hazardous health effects, they can be promising alternatives of synthetic antioxidants as food additives.

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