

# Chemical constituents, antifungal and antioxidative potential of *Foeniculum vulgare* volatile oil and its acetone extract <sup>☆</sup>

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## Abstract

GC and GC–MS analysis of *Foeniculum vulgare* volatile oil showed the presence of 35 components containing 96.4% of the total amount. The major component was *trans*-anethole (70.1%). The analysis of its acetone extract showed the presence of nine components accounting for 68.9% of the total amount. Linoleic acid (54.9%), palmitic acid (5.4%) and oleic acid (5.4%) were found as major components in extract. The antifungal and antioxidative potentials were also carried out by different techniques. In inverted petriplate method, the volatile oil showed complete zone inhibition against *Aspergillus niger*, *Aspergillus flavus*, *Fusarium graminearum* and *Fusarium moniliforme* at 6  $\mu$ L dose. It was found to be effective for *A. niger* even at 4  $\mu$ L dose. Moreover, using food poison technique, the volatile oil and extract both showed good to moderate zone of inhibition. The antioxidant value was evaluated by measuring peroxide and thiobarbituric acid values for linseed oil at fixed time intervals. Both, the volatile oil and extract showed strong antioxidant activity in comparison with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). In addition, their inhibitory action in linoleic acid system was studied by monitoring peroxide accumulation in emulsion during incubation through ferric thiocyanate method. The results were well correlated with the above results.

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**Keywords:** *Foeniculum vulgare*; Antioxidants; Peroxide value

## 1. Introduction

There is at present growing interest, both in the industry and in the scientific research, for aromatic and medicinal plants because of their antimicrobial and antioxidant properties. These properties are due to many active phytochemicals including flavanoids, terpenoids, carotenoids, coumarins, curcumines etc. These bioactive principles have also been confirmed using modern analytical techniques (Cao & Prior, 1998; Koleva, Niederlander, & Van Beek, 2001; Mantle

et al., 1998; Soler-Rivas, Espin, & Wichers, 2000). Hence, they are considered to be important in diets or medical therapies for biological tissue deterioration due to free radicals. Herbs and spices are amongst the most important targets to search for natural antimicrobials and antioxidants from the point of view of safety (Brannen, 1975; Ito, Fukushima, & Tsuda, 1985). So far, many investigations on antimicrobial (Beuchat, 1994; Conner, 1993; Shelef, 1983; Singh, Maurya, Catalan, & Lampasona, 2004; Velluti, Sanchis, Ramos, Egido, & Marin, 2003; Zaika, 1988) and antioxidant properties of spices volatile oils and extracts have been carried out. Lee and Shibamoto (2002) studied the antioxidant potential of thyme, basil, rosemary, chamomile, lavender, cinnamon and reported strong antioxidant activity for thyme and basil. According to Ho et al.

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(2000), dried rosemary is a widely used herb in processed foods for lipid stabilization and hence could be considered as a substitute for synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG). These observations have increased the interest in other herbs and spices since some of them remain sensorially more acceptable when added in large amounts (Bandoniene, Venskutonis, Gruzdienė, & Murkovic, 2002).

*Foeniculum vulgare* Mill (Fam. Umbelliferae), commonly known as fennel, is a small genus of annual, biennial or perennial herbs distributed in central Europe and Mediterranean region. It is widely cultivated throughout the temperate and tropical regions of the world for its aromatic fruits, which are used as a culinary spice (Beaux, Fleurentin, & Mortier, 1997; Patra, Shahi, Midgely, & Dikshit, 2002; Tanira, Shah, Mohsin, Ageel, & Qureshi, 1996). Steam distillation of dried fruits yields an essential oil referred as 'Fennel oil', used in western countries for flavouring purposes (Husain, 1994). Although, the chemical constituents and antimicrobial properties of the fruit volatile oil of *F. vulgare* are well studied (Beaux et al., 1997; Coelho, Pereira, Mendes, & Palavera, 2003; Marotti & Pieeaglia, 1992; Muckenturm, Foehchterlen, Reduron, Danton, & Hildenbrand, 1997; Singh, Kapoor, Pandey, Singh, & Singh, 2002; Tanira et al., 1996), potential antioxidative properties have not yet been studied. Moreover, to our knowledge, no work has been reported on the chemistry and activities of its acetone extract. In continuation of our research programme (Singh, Kapoor et al., 2002; Singh, Kapoor, Singh, Leclercq, & Klinkby, 2000; Singh, Pandey, Leclercq, & Klinkby, 1999; Singh, Singh, & Maurya, 2002) on the essential oils, chemical, antifungal and antioxidant studies of *F. vulgare* fruit volatile oil and its acetone extract have been undertaken. The objective of this study is to assess and compare fungicidal as well as antioxidative properties in linseed oil by different methods.

## 2. Materials and methods

### 2.1. Plant material

Fruits of *F. vulgare* were purchased from the local market of Gorakhpur, during July 2002 and voucher specimens were deposited at the Herbarium of the Science Faculty of DDU Gorakhpur University, Gorakhpur.

#### 2.1.1. Isolation of the oil

The powdered fruits (900 mesh size) of *F. vulgare* were hydrodistilled in a Clevenger's type apparatus for 6 h in accordance with European Pharmacopoeia procedure (1983). Yellow colored oil (yield 1.2%), with char-

acteristic odour and sharp taste, was obtained. It was dried over anhydrous sodium sulphate to remove traces of moisture and stored in a refrigerator in the dark at 4 °C until use.

#### 2.1.2. Isolation of the extract

After the extraction of the essential oil, the powdered fruits were dried and then, with the help of Soxhlet apparatus, using acetone as a solvent, a viscous extract (yield 3.7%) was obtained. It was stored at 4 °C until use.

### 2.2. Chemical investigation

The chemical analysis of volatile oil and extract were undertaken by Gas Chromatography (GC) and Gas Chromatography–Mass Spectroscopy (GC–MS) techniques.

#### 2.2.1. GC

Using a Hewlett Packard 5890 series II gas chromatograph equipped with flame ionization detector (FID) and silica column, the gas chromatograms of the oil and extract were obtained. The column was an HP-5 (5% phenyl methyl siloxane, 30 m × 0.32 mm × 0.25 µm) whose injector and detector temperatures were maintained at 250 and 270 °C, respectively. The amount of the samples injected was 0.1 µL (in split mode 85:1). Carrier gas used for oil and extract were He and N<sub>2</sub> respectively with a flow rate 1.1 mL min<sup>-1</sup>. The oven temperature was programmed as follows: 60 °C (1 min), 60–185 °C (1.5 °C min<sup>-1</sup>), 185 °C (1 min), 185–275 °C (9 °C min), 275 °C (2 min) and that for extract was as follows: 100 °C (1 min), 100–280 °C (5 °C min<sup>-1</sup>), 280 °C (2 min).

#### 2.2.2. GC–MS

The volatile oil and extract were subjected to GC–MS analysis using a Hewlett Packard mass detector (model 5973) and a HP-5MS column (length 30 m, inner diameter 0.25 mm, film thickness 0.25 µm). The injector, GC–MS interface, ion source and selective mass detector temperatures were maintained at 270, 280, 230 and 150 °C, respectively. The oven temperature programmed for the volatile oil was as follows: 60 °C (1 min), 60–185 °C (1.5 °C min<sup>-1</sup>), 185 °C (1 min), 185–275 °C (9 °C min), 275 °C (2 min) and that for extract was as follows: 100 °C (1 min), 100–280 °C (5 °C min<sup>-1</sup>), 280 °C (2 min).

#### 2.2.3. Identification of components

The percentages of components were means of three runs obtained from electronic integration measurements using flame ionization detection (FID). The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes C<sub>8</sub>–C<sub>16</sub>. Chemical

Table 1  
Chemical composition of *F. vulgare* volatile oil

Compound	% FID	KI <sup>a</sup>
Ethanol	Tr	–
Acetic acid-ethyl ester	Tr	806
3-Methylbutanal	0.1	831
2-Methylbutanal	Tr	833
<i>Alpha</i> -Thujene	Tr	931
<i>Alpha</i> -Pinene	0.2	941
Camphene	Tr	953
Sabinene	Tr	975
<i>Beta</i> -Pinene	0.2	980
Myrcene	0.1	993
<i>Delta</i> -3-Carene	0.1	1013
<i>Alpha</i> -Terpinene	Tr	1020
<i>p</i> -Cymene	3.1	1026
Limonene	3.1	1031
1,8-Cineole	0.1	1035
<i>trans-beta</i> -Ocimene	0.1	1050
<i>Gamma</i> -Terpinene	2.1	1064
Fenchone	8.6	1088
Linalool	1.2	1099
Camphor	0.3	1144
<i>Beta</i> -Terpineol	Tr	1145
Terpinen-4-ol	0.2	1177
<i>Alpha</i> -Terpineol	0.2	1189
Methyl chavicol	4.7	1195
Fenchyl acetate	0.2	1222
Cuminal	0.4	1246
<i>cis</i> -Anethole	0.4	1254
<i>p</i> -Anisaldehyde	0.5	1256
<i>trans</i> -Anethole	70.1	1287
Thymol	0.1	1291
<i>Alpha</i> -Copaene	0.1	1379
<i>Beta</i> -Caryophyllene	0.2	1420
<i>Alpha</i> -Humulene	Tr	1455
<i>Delta</i> -Cadinene	Tr	1524
Total	96.4%	

Trace (0.01%).

Percentages are the mean of three runs and were obtained from electronic integrations measurements using flame ionization detection (FID).

<sup>a</sup> The retention index was calculated for all volatile constituents using a homologous series of *n*-alkanes C<sub>8</sub>–C<sub>16</sub>.

constituents were identified by comparing their mass spectra with the library (Adams, 1995; Henneberg, Wiemann, & Joppek, 1998) NBS 75 K and/or by co-injection with authentic samples, and the chemical constituents of volatile oil and extract were reported in Tables 1 and 2, respectively.

### 2.3. Antifungal investigations

In order to determine the antifungal efficacy of the volatile oil and its extract, the pathogenic fungi *Aspergillus niger* (AN), *Aspergillus flavus* (AF), *Aspergillus oryzae* (AO), *Aspergillus ochraceus* (AO'), *Fusarium graminearum* (FG), *Fusarium moniliforme* (FM), *Penicillium citrium* (PC), *Penicillium viridicatum* (PV), *Penicillium madriti* (PM) and *Curvularia lunata* (CL) were

Table 2  
Chemical composition of acetone extract of *F. vulgare*

Compounds	% FID
4-Hydroxy-4-methyl-2-pentanone	0.1
Undecane	1.0
<i>cis</i> -Anethole	0.6
<i>trans</i> -Anethole	0.8
Palmitic acid	5.4
Methyl oleate	0.2
Linoleic acid	55.0
Oleic acid	5.4
Stigmast-5-en-3-ol	0.5
Total	68.9%

Percentages are the mean of three runs and were obtained from electronic integrations measurements using flame ionization detection (FID).

undertaken. These fungi were isolated from food materials such as onion, vegetable waste, wheat straw, fruits of *Musa* species, sweet potato, decaying vegetation, vegetable, respectively and purchased from Microbial Type Culture Collection (MTCC), Chandigarh, India. The MTCC code No. of these strains are 2479, 1884, 1846, 1810, 1893, 2088, 2553, 2007, 3003 and 2073 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 5 °C. The antifungal activity of the volatile oil and acetone extract against fungi were undertaken using inverted petriplate (Alvarez-Castellanos, Bishop, & Pascual-Villalobos, 2001) and food poison techniques (Ramdas, Suresh, Janardhanan, & Masilamani, 1998). In the inverted petriplate method, the required doses (2, 4 and 6 µL) of undiluted sample were soaked on a small piece (diam. 12 mm) of Whatmann No. 1 filter paper and it was kept on the lid of petriplate which is in inverted position whereas in food poison technique, the required doses (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 and results of both volatile oil and extract obtained by inverted petriplate and food poison techniques are given in the Tables 3 and 4, respectively.

### 2.4. Antioxidant activity

In order to assess the antioxidant activity (Kikuzaki & Nakatani, 1993; Osawa & Namaki, 1983; Zin, Abdul-Hamid, & Osoman, 2002) of *F. vulgare* volatile oil and its extract, crude linseed oil having initial peroxide value 4.2 meq/kg, was taken for present investigation. The oil was selected due to its high degree of unsaturation and generally used as edible oil in Central Europe and Asia. The antioxidant activities have been determined by three different experimental procedures.

Table 3  
Antifungal investigations of *F. vulgare* oil and its acetone extract by using inverted petriplate method

Fungus <sup>a</sup>	% Mycelial zone inhibition at different dose of oil <sup>b</sup>					
	<i>F. vulgare</i> oil			<i>F. vulgare</i> extract		
	2 $\mu$ L	4 $\mu$ L	6 $\mu$ L	2 $\mu$ L	4 $\mu$ L	6 $\mu$ L
<i>Aspergillus niger</i> (AN)	75.0	<b>100.0</b>	<b>100.0</b>	0.0	20.0	50.0
<i>Aspergillus flavus</i> (AF)	87.5	93.7	<b>100.0</b>	0.0	3.5	31.3
<i>Aspergillus oryzae</i> (AO)	20.0	31.2	37.5	6.3	15.0	18.7
<i>Aspergillus ochraceus</i> (AO')	12.5	18.7	50.0	6.3	8.7	12.5
<i>Fusarium graminearum</i> (FG)	87.5	87.5	<b>100.0</b>	37.5	50.0	56.3
<i>Fusarium monoliforme</i> (FM)	75.0	87.5	<b>100.0</b>	37.5	50.0	52.5
<i>Penicillium citrium</i> (PC)	12.5	26.3	56.3	6.3	13.8	43.7
<i>Penicillium viridicatum</i> (PV)	50.0	51.3	87.5	12.5	21.3	26.3
<i>Penicillium madriti</i> (PM)	50.0	75.0	87.5	2.5	12.0	25.0
<i>Curvularia lunata</i> (CL)	12.5	18.7	37.5	0.0	8.8	18.7

<sup>a</sup> For all tested fungi the data was found to be highly significant ( $p < 0.01$ ).

<sup>b</sup> Average of three replicates.

Table 4  
Antifungal investigations of *F. vulgare* oil and its acetone extract using food poison technique

S. No. Fungus <sup>a</sup>	% Mycelial zone inhibition at different dose of oil <sup>b</sup>					
	<i>F. vulgare</i> oil			<i>F. vulgare</i> extract		
	2 $\mu$ L	4 $\mu$ L	6 $\mu$ L	2 $\mu$ L	4 $\mu$ L	6 $\mu$ L
<i>Aspergillus niger</i> (AN)	18.7	26.3	13.7	18.7	43.7	56.3
<i>Aspergillus flavus</i> (AF)	50.0	65.0	87.5	12.5	15.0	26.3
<i>Aspergillus oryzae</i> (AO)	13.7	27.5	46.2	18.7	20.0	56.3
<i>Aspergillus ochraceus</i> (AO')	18.7	26.7	38.7	18.7	43.7	68.7
<i>Fusarium graminearum</i> (FG)	25.0	31.2	37.5	25.0	31.2	50.0
<i>Fusarium monoliforme</i> (FM)	21.3	38.7	65.0	6.3	88.7	13.8
<i>Penicillium citrium</i> (PC)	11.3	21.2	27.5	13.7	21.2	37.5
<i>Penicillium viridicatum</i> (PV)	75.0	81.2	87.5	50.0	62.5	68.6
<i>Penicillium madriti</i> (PM)	18.7	28.7	41.2	16.2	26.3	46.7
<i>Curvularia lunata</i> (CL)	12.5	38.7	50.0	50.0	65.5	70

<sup>a</sup> For all tested fungi the data was found to be highly significant ( $p < 0.01$ ).

<sup>b</sup> Average of three replicates.

#### 2.4.1. Peroxide value (PV) method

For measuring the peroxide value (AOCS, 1998), a modified oven test (Bandoniene, Gruzdiene, & Venskutonis, 2001) was used. The antioxidant activity of volatile oil and acetone extract were compared with synthetic antioxidants, such as BHA, BHT and PG. The calculated quantities of each (200 ppm) were added to 30 g of linseed oil in an open mouthed beaker. The mixtures were thoroughly homogenized and placed into thermostat at 90 °C. The peroxide values (meq of oxygen kg<sup>-1</sup>) were measured in every 7 days and test was replicated for three times. A control sample was prepared under similar condition without any additive. The effects of oil and acetone extract in terms of linseed oil peroxidation at 90 °C are shown in Figs. 1 and 2.

#### 2.4.2. Thiobarbituric acid (TBA) method

The test was performed according to the methods of Kikuzaki and Nakatani (1993) and Ottolenghi (1959) with small changes. The same samples as prepared for

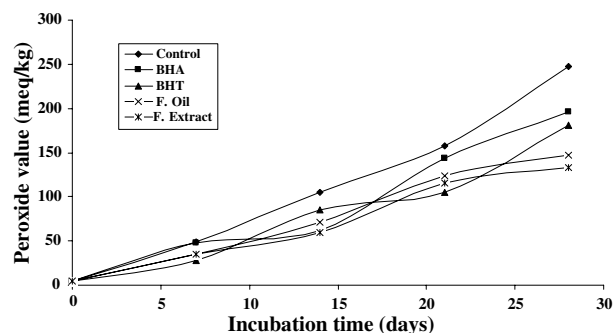


Fig. 1. Inhibitory effect of *F. vulgare* volatile oil and its acetone extract on the primary oxidation of linseed oil as measured using peroxide value method at 90 °C.

the peroxide method were used. To 10 g of 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

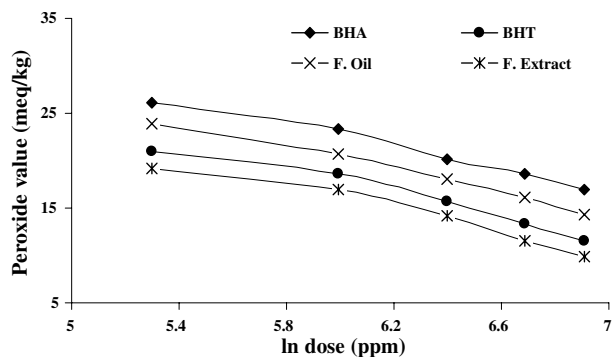


Fig. 2. Logarithmic dose response curve of *F. vulgare* volatile oil and its acetone extract on the primary oxidation of linseed oil as measured using peroxide value method.

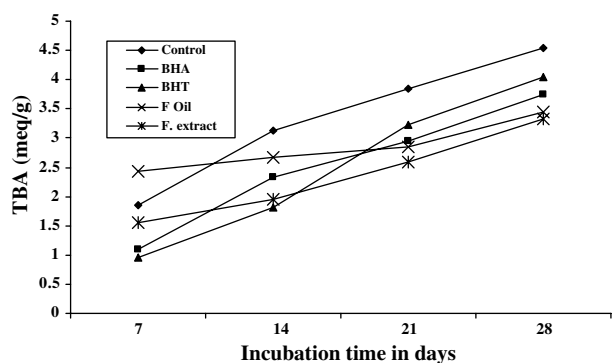


Fig. 3. Inhibitory effect of *F. vulgare* volatile oil and its acetone extract on secondary oxidation of linseed oil at 90 °C measured using TBA value method.

water-bath for 1 h. After cooling, absorbance of supernatant was measured at 540 nm with Hitachi-U-2000 spectrophotometer. The thiobarbituric acid value (meq of malonaldehyde/g) was calculated and the effects of volatile oil and acetone extract on linseed oil in terms of incubation time versus TBA value at 90 °C are shown in Fig. 3.

#### 2.4.3. Ferric thiocyanate (FTC) method

The FTC method reported by Osawa and Namaki (1983) was adopted. Samples (4 mg) in 99.5% ethanol were mixed with 2.5% linoleic acid in 99.5% 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 conditions at 40 °C. To 0.1 mL of this solution was added to 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 colour was measured at 500 nm in the spectrophotometer, for every two days. The control and standard were subjected to the same procedure except for the control, where there was no addition of sample and for the standard 4 mg of sample were replaced with 4 mg of BHA

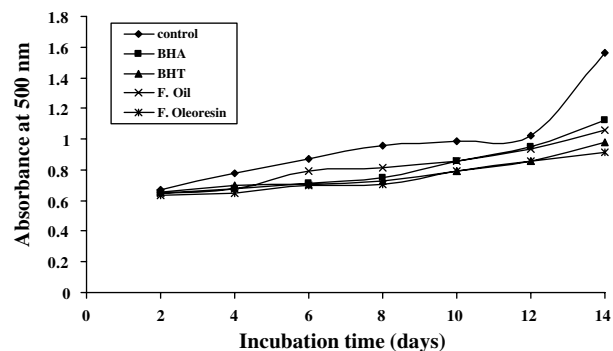


Fig. 4. Inhibitory effect of *F. vulgare* volatile oil and its acetone extract on the oxidation of linoleic acid system measured using ferric thiocyanate method.

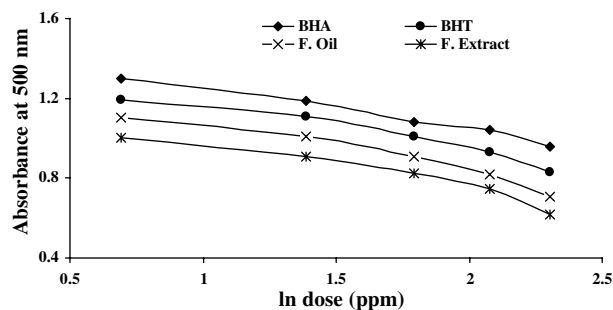


Fig. 5. Logarithmic dose response curve of *F. vulgare* volatile oil and its acetone extract on the oxidation of linoleic acid system measured using ferric thiocyanate method.

and BHT. Results are shown in the form of plot indicating incubation time in days verses absorbance was shown in Figs. 4 and 5.

#### 2.5. Statistical analysis

The statistical analysis was undertaken using one way (antifungal investigations) and two way (antioxidant activity) analysis of variance (Sokal & Rohlf, 1973) were used for locating significant differences within different doses and significant differences among several groups of data were examined by Duncan's multiple range tests.

### 3. Results and discussion

GC and GC-MS analysis of *F. vulgare* volatile oil showed the presence of 35 components accounting for 96.4% of the total amount (Table 1). *trans*-Anethole (70.1%) was found as a major component. Moreover, its extract (Table 2) showed the presence of 9 components. Linoleic acid (55.0%) was found as a major component.

Using inverted petriplate method (Table 3), the oil was found 100% antifungal against *A. niger*, *A. flavus*, *F. graminearum* and *F. moniliforme* at 6  $\mu$ L dose. It

was found to be highly effective even at 4  $\mu\text{L}$  for *A. niger*. Using the same method, extract was found less effective than the volatile oil as only 50% mycelial zone inhibition of *F. graminearum*, *F. moniliforme* and *A. niger* was obtained even at 6  $\mu\text{L}$  dose. Moreover, using the food poison technique (Table 4), the oil was found highly effective against *A. flavus* and *P. viridicatum* as more than 75% mycelial zone inhibition was obtained at 6  $\mu\text{L}$ . For other fungi it was found less or ineffective. The data were found to be highly significant ( $p < 0.01$ ).

Most of the antifungal activity in volatile oils derived from *F. vulgare* appears to derive from phenolic compounds while other constituents are believed to contribute little. Purified compounds derived from volatile oils i.e. anethole have already been reported (Curtis, Shetty, Cassagnol, & Peleg, 1996; De, De, Sen, & Banerjee, 2002; Nychas, 1998) to have antimicrobial activities. This indicates the antifungal activities of the oils are mainly due to the major components. The low activity of extracts as compared to volatile oils can be explained as they have no vapour action and mostly consist of non-volatile compounds.

The oxidation of lipids has long been classified as the major deterioration affecting both the sensory and the nutritional quality of foods. Hydroperoxides are the primary oxidation products and they are unstable compounds, which produce a number of secondary products such as alkanes, alcohols, aldehydes and acids some of which smell badly at low threshold values. The primary oxidation products, hydroperoxides were measured by means of peroxide value method. The curves in Figs. 1 and 2 demonstrate PV changes in linseed oil with different additives. Linseed oil oxidation was measured at limited periods (7 days) during 28 days of storage. During this time PV of blank sample increased to 248 meq/kg. The results given in Fig. 1 show that both the oil and extracts reduced the oxidation rate of linseed oil at 90 °C in terms of formation of peroxides. The samples with volatile oil ( $F_{0.01(3,4)} = 727.432$  for days,  $F_{0.01(3,4)} = 16.71$  for dose) and extract ( $F_{0.01(3,4)} = 227.776$  for days,  $F_{0.01(3,4)} = 28.245$  for dose) were found to be significantly more effective than BHA ( $F_{0.01(3,4)} = 147.638$  for days,  $F_{0.01(3,4)} = 22.36$  for dose) and BHT ( $F_{0.01(3,4)} = 12.398$  ns for days,  $F_{0.01(3,4)} = 1.876$  for dose). During the oxidation process peroxides are gradually decomposed to lower molecular weight compounds, one such compound is malonaldehyde, which is measured by the TBA method. Malonaldehyde, the compound used as an index of lipid peroxidation, was determined by selective third order derivative spectrophotometric method previously developed by some authors (Botsoglou et al., 1994). Fig. 2 shows that volatile oil and extract had significantly ( $p < 0.05$ ) lower TBA values than the control up to 28 days of incubation at 90 °C. In both the methods essential oil and extract gave good results comparatively with commercial antioxidants such as BHA and BHT as shown in

Figs. 1–3, respectively. It is interesting to note that, after certain duration, BHA and BHT becomes less effective than extract in stabilizing linseed oil. These results were well correlated with the ferric thiocyanate method (Figs. 4 and 5), which is a measure of the initial stage of peroxide level of lipid oxidation. High absorbance is an indication of a high concentration of formed peroxides. The values obtained without additives were taken for 100% lipid peroxidation. Fig. 4 shows absorbance values measured for oil ( $F_{0.01(5,5)} = 92.650$  for days,  $F_{0.01(5,5)} = 33.947$  for dose), acetone extract ( $F_{0.01(5,5)} = 39.16$  for days,  $F_{0.025(5,5)} = 8.636$  for dose), BHA ( $F_{0.01(5,5)} = 211.74$  for days,  $F_{0.01(5,5)} = 19.72$  for dose) and BHT ( $F_{0.01(5,5)} = 9.7005$  for days,  $F_{0.01(5,5)} = 0.706$  ns for dose) along with control for 12 days. The absorbance of linoleic acid emulsion without the addition of oil, extract, and antioxidants increased rapidly, and there was a significant difference between the blank and antioxidants at the  $p < 0.01$  level. The effectiveness of added materials in stabilizing linseed oil was found to be in the following order:

Extract > Oil > BHA > BHT > Control

The effectiveness of additives depends not only on their structural features but also on many factors such as the character of the lipid system, on the temperature and on the binding of the fatty acids (Sanchez-Mareno, Larrauri, & Sauro-Calixto, 1998; Yanishlieva-Maslarova, 2001). It has been well reported that phenolic compounds are able to donate a hydrogen atom to the free radicals thus stopping the propagation chain reaction during lipid oxidation process (Sanchez-Mareno et al., 1998; Yanishlieva & Marinova, 1998). The volatile oil and extract both contains *trans*- and *cis*-anethole. The lower activity of the volatile oil may also be due to its volatility at higher temperature. In extract, linoleic acid (55.0%) is major component, which do not possess antioxidant activity. Probably other substances, which could be present in, extract and not identified by GC, can contribute to improve antioxidant activity of acetone extract, which needs further investigations.

It is difficult to give a definite explanation for all results obtained within the scope of the present study. The better antioxidant activity of oil and extract may be due to the combinatory effect of more than two compounds, which are present in seed. It has already been reported that most natural antioxidative compounds work synergistically (Kamal-Eldin & Appelqvist, 1996; Lu & Foo, 1995) with each other to produce a broad spectrum of antioxidative activities that creates an effective defence system against free radical attack.

Concluding these results, we can say that *F. vulgare* volatile oil, which is rich in *trans*-anethole, possesses good antifungal activity against *A. niger*, *A. flavus*, *F. graminearum* and *F. moniliforme* whereas its acetone extract, which is rich in linoleic acid, can be used as natural antioxidant for linseed oil. Hence, they are valuable

for increasing shelf life of foodstuffs and protector for highly unsaturated linseed oil, replacing synthetic fungicides and antioxidants such as BHT and BHA, as well as for preventing cellular damage, the cause of aging and human diseases.

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