Chemical, antioxidant and antifungal activities of volatile oil of black pepper and its acetone extract[†]

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Abstract: GC and GC-MS analysis of volatile oil obtained from *Piper nigrum* L resulted in the identification of 49 components accounting for 99.39% of the total amount, and the major components were β-caryophyllene (24.24%), limonene (16.88%), sabinene (13.01%), β-bisabolene (7.69%) and α-copaene (6.3%). The acetone extract of pepper showed the presence of 18 components accounting for 75.59% of the total amount. Piperine (33.53%), piperolein B (13.73%), piperamide (3.43%) and guineensine (3.23%) were the major components. The oil was found to be 100% effective in controlling the mycelial growth of *Fusarium graminearum* in inverted petriplate technique. The acetone extract retarded 100% mycelial growth of *Penicillium viridcatum* and *Aspergillus ochraceus* in food-poisoning technique. Volatile oil and acetone extract were identified as a better antioxidant for linseed oil, in comparison with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).
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Keywords: GC-MS; *Piper nigrum* L; volatile oil; acetone extract; antifungal activity; antioxidant activity

INTRODUCTION

Black pepper (*Piper nigrum*, Piperaceae family) is used as a spice worldwide, and is a generally used food additive for increasing the flavour of different food preparations. It finds extensive use in the Ayurvedic[1] system of medicine, particularly its aqueous extract. Its main constituent, piperine, displays a variety of pharmacological activities.[2] Pepper essential oil plays an important role in the manufacture of perfumery and confectionery products.[3,4] It has been already reported that spice volatile oils and aromatic plant extracts possess strong antioxidant[5–8] and antimicrobial[9–12] activities. Herbs and spices are one of the most important targets to search for natural antioxidants from the point of view of safety, and it is expected that natural antioxidants, which are investigated for basic and applied experiments, will lead to chemoprevention of inflammation, cancer and aging. In preference to natural spices, usage of oleoresin is gaining interest due to its high flavour intensity, low microbial contamination and economy in use. The essential oil and extracts of many plant species have become popular in recent years and attempts to characterize their bioactive principles have recently gained momentum in many pharmaceutical and food-processing applications. In continuation of our studies on essential oils,[13–16] we report antioxidant and antifungal activities of black pepper and its acetone extract along with their chemical constituents in the present communication.

EXPERIMENTAL
Plant material
Black pepper seeds were purchased from local market of Gorakhpur and voucher specimens were deposited at the Herbarium of the Faculty of Science, DDU Gorakhpur University, Gorakhpur.

Isolation of the oil
Black pepper seeds were ground using a domestic electronic mixer and the powdered seeds were subjected to hydrodistillation in a Clevenger type apparatus for 5 h according the procedure recommended by the *European Pharmacopoeia*.[17] A colourless oil with characteristic odour was obtained with yield of 2.2%. It was dried over anhydrous sodium sulphate and the sample was stored at 4 °C before use.

Isolation of the pepper extract
After the isolation of essential oil, the crude material was oven dried at 50 °C for 24 h. Then 20 g of dried material were loaded onto a soxhlet apparatus and the

extraction[18] was carried out with acetone (400 ml) at 90 °C for 3 h. The solvent was removed under reduced pressure and the remaining extract was used for further tests.

Investigation of chemical constituents

The chemical analysis of black pepper oil and its acetone extract was carried out by gas chromatography (GC) and gas chromatography–mass spectroscopy (GC-MS) techniques.

Gas chromatography

A Hewlett Packard (Analytical Technologies SA, Buenos Aires, Argentina) 6890 system equipped with Flame Ionization Detector (FID) and phenyl methyl siloxane column was an HP-5 (length 30 m × inner diameter 0.25 mm × film thickness 0.25 μm), whose injector and detector temperatures were maintained at 250 and 300 °C respectively. Injection volume was 1 μl at 1:80 split and helium was used as the carrier gas at a flow rate of 1.5 ml min^{-1}. The oven temperature was programmed as follows: 60 °C for 1 min, rising at 1.5 °C min^{-1} to 185 °C, held for 1 min, then restarted, rising at 9 °C min^{-1} to 275 °C, held for 2 min.

Gas chromatography–mass spectroscopy

The volatile oil and its acetone 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 interphase, ion source and selective mass detector temperatures were maintained at 280, 280, 230 and 150 °C, respectively. The oven temperature for volatile oil was programmed as follows: 60 °C (1 min), 60–185 °C (1.5 °C min^{-1}), 185 °C (1 min), 185–275 °C (9 °C min^{-1}), 275 °C (2 min) and for its extract it was 70 °C (0 min), 70–280 °C (5 °C min^{-1}) and 280 °C (20 min). Percentages of MS are the means of three runs and were obtained from electronic integration measurements using a selective mass detector.

Identification of components

Components were identified by comparison and interpretation of the fragmentation pattern of their 70 eV mass spectra against those stored in the NIST computer database and/or the MS data published in the literature. The retention index was calculated for the volatile constituents using a homologous series of n-alkanes, C8–C16. The results for the volatile oil and its acetone extract are reported in Tables 1 and 2, respectively.

Antifungal activity

The antifungal activity of the volatile oil and its acetone extract against various pathogenic fungi (*Aspergillus flavus* (1884), *Aspergillus ochraceus* (1810), *Aspergillus oryzae* (1846) *Aspergillus niger* (2479),

Table 1. Chemical composition of *P nigrum* volatile oil

Compounds	MS (%)	RI[a]
Tricyclene	1.73	0930
α-Pinene	4.75	0941
Camphene	0.11	0953
Sabinene	13.01	0975
β-Pinene	6.71	0980
Myrcene	0.89	0993
α-Phellandrene	2.14	1007
δ-3-Carene	0.43	1013
α-Terpinene	0.32	1020
p-Cymene	Trace	1026
Limonene	16.88	1031
β-Phellandrene	0.02	1032
trans-β-Ocimene	0.06	1052
γ-Terpinene	0.52	1064
cis-Sabinene hydrate	0.42	1070
Terpinolene	0.21	1088
trans-Sabinene hydrate	0.33	1098
Linalool	0.27	1099
cis-p-Menth-2-en-1-ol	0.14	1126
1-Terpineol	0.05	1139
Camphor	0.08	1147
Terpinen-4-ol	1.99	1177
α-Terpineol	0.19	1189
cis-Piperitol	Trace	1198
Carvone	0.15	1244
δ-Elemene	0.49	1342
α-Cubebene	0.26	1353
Cyclosativene	0.08	1370
α-Copaene	6.30	1376
β-Cubebene	0.52	1390
β-Elemene	0.41	1393
α-Gurjunene	0.12	1413
β-Caryophyllene	24.24	1420
β-Gurjunene	0.14	1432
γ-Elemene	0.04	1434
α-Guaiene	Trace	1440
α-humulene	1.38	1458
trans-β-farnesene	0.28	1469
Germacrene-D	0.40	1480
β-Selinene	0.75	1488
α-Selinene	0.46	1494
Bicyclogermacrene	0.37	1496
α-Muurolene	0.73	1500
β-Bisabolene	7.69	1511
δ-Cadinene	2.37	1527
Cadina-1,4-diene	0.07	1538
Germacrene-B	0.30	1560
Caryophyllene oxide	0.47	1585
α-Muurolol	0.12	—
Total	99.39%	

Percentages are the means of three runs and were obtained from electronic integration measurements using selective mass detector.
[a] The retention index was calculated for all volatile constituents using a homologous series of n-alkanes, C8–C16.

Fusarium moniliforme (1893), *Fusarium graminearum* (2088), *Penicillium citrinum*, (2553), *Penicillium viridcatum* (2007) *Penicillium madriti* (3003) and *Curvularia lunata* (2073)) was tested by inverted petriplate[19] and food-poisoning[20] techniques. All the fungi cultures

Table 2. Chemical composition of acetone extract of *P nigrum*

Compounds	MS (%)	RI[a]
α-Copaene	0.60	1376
β-Caryophyllene	0.39	1420
β-Bisabolene	0.29	1511
δ-Cadinene	0.15	1523
Spathulenol	0.05	1578
Caryophyllene oxide	0.07	1588
Torreyol	0.54	—
Piperylin	0.56	—
Piperine	33.53	—
N-trans-feruloyltyramine	1.45	—
Alkaloid $C_{22}H_{35}NO_3$, MW 361	5.49	—
Alkaloid $C_{22}H_{35}NO_3$, MW 361 (isomer of the previous one)	6.31	—
Retrofractamide A	1.57	—
Hinokinin	1.88	—
Piperamide	3.43	—
Piperolein B	13.73	—
Piperettine	2.76	—
Guineensine	3.23	—
Total	75.59%	—

Percentages are the means of three runs and were obtained from electronic integration measurements using selective mass detector.
[a] The retention index was calculated for all volatile constituents using a homologous series of *n*-alkanes, C8–C16.

were procured from Microbial Type Culture Collection (MTCC) Center, Chandigarh, India and their reference numbers are given in parentheses. The cultures were maintained in Czapek (DOX) agar medium. Each test was replicated three times and fungi toxicity was measured in terms of percentage mycelial inhibition calculated by the following formula.

$$\text{percentage mycelial inhibition} = [(\mathrm{d}c - \mathrm{d}t)/\mathrm{d}c] \times 100$$

where d*c* and d*t* are average diameters of mycelial colony of control and treated sets, respectively. In the food-poisoning technique, the calculated quantity (2, 4, 6 µl) of undiluted essential oil (or extract) was mixed with 25 ml of medium ($\sim$45 °C) and in the case of inverted petriplate method, the calculated quantity (2, 4, 6 µl) of essential oil (or extract) was soaked into filter paper (10 mm in diameter) and kept at the lid of the inverted petriplate. The plates were incubated at 37 °C for 6 days and the zone of inhibition was measured with the help of vernier calipers. The results (mean of three replicates) of both volatile oil and its acetone extract obtained by food-poisoning and inverted petriplate techniques are given in Tables 3 and 4.

Evaluation of antioxidant activity

In order to evaluate antioxidant potential of volatile oil and its extract, crude linseed oil was taken, which had initial peroxide value of 4.0 meq kg^{-1}. In the case of essential oil and extract, 6 µl were added to linseed oil, while in the case of synthetic antioxidants, 6 mg (butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG))

Table 3. Effect of *P nigrum* volatile oil and its extract against different food pathogenic fungi by food-poisoning technique

	Percentage mycelial zone inhibition at different doses[a] of sample					
	Pepper essential oil			Acetone extract of pepper		
Fungus	2 µl	4 µl	6 µl	2 µl	4 µl	6 µl
Aspergillus niger	14.3	24.3	41.4	28.6	35.7	45.7
Aspergillus flavus	25.0	13.8	37.5	50.0	56.3	75.0
Aspergillus ochraceus	31.3	40.0	61.3	75.0	87.5	100
Aspergillus oryzae	14.3	21.4	57.1	28.6	35.7	57.1
Fusarium monoliforme	6.3	25.0	27.5	37.5	50.0	56.3
Fusarium graminearum	75.0	81.3	87.5	50.0	60.0	73.8
Penicillium citrium	13.8	33.8	48.8	18.8	25.0	27.5
Penicillium viridicatum	50.0	65.0	81.3	60.0	87.5	100
Penicillium madriti	24.3	42.9	71.4	7.1	14.3	28.6
Curvularia lunata	12.5	56.3	87.5	12.5	62.5	87.5

[a] Average of three replicates.

Table 4. Effect of *P nigrum* volatile oil and its extract against different food pathogenic fungi by inverted petriplate technique

	Percentage mycelial zone inhibition at different doses[a] of sample					
	Pepper essential oil			Acetone extract of pepper		
Fungus	2 µl	4 µl	6 µl	2 µl	4 µl	6 µl
Aspergillus niger	42.9	50.0	74.3	14.3	50.0	71.4
Aspergillus flavus	12.5	18.8	25.0	25.0	33.8	40.0
Aspergillus ochraceus	12.5	18.8	25.0	6.3	12.5	25.0
Aspergillus oryzae	0	42.9	71.4	42.9	57.1	64.3
Fusarium monoliforme	6.3	12.5	20.0	0	0	12.5
Fusarium graminearum	75.0	81.3	100	0	6.3	10.0
Penicillium citrium	6.3	13.8	31.3	6.3	18.8	38.8
Penicillium viridicatum	37.5	56.3	65.0	6.3	13.8	43.8
Penicillium madriti	42.9	71.4	85.7	21.4	30.0	44.3
Curvularia lunata	50.0	56.3	75.0	6.3	13.8	25.0

[a] Average of three replicates.

were added to linseed oil at 200 ppm concentration in 100 ml open-mouthed beakers. Mixtures were thoroughly homogenized and samples were incubated at 80 °C in the dark. A blank sample was prepared under similar conditions without any additives. Three replications were performed for every sample. Three different experimental procedures were employed to evaluate antioxidant activity (AA).

Peroxide value

The peroxide values (POV) of all samples were measured[21,22] every 7 days using the Schaal oven test.[23] For this purpose, a known weight of edible oil sample (3 g) was dissolved in glacial acetic acid (30 ml) and chloroform (20 ml). Then saturated KI solution (1 ml) was added. The mixture was kept in the dark for 15 min. After the addition of distilled water (50 ml), the mixture was titrated against sodium

thiosulphate (0.02 N) using starch as an indicator. A blank titration was done parallel to treatment and a POV value (meq of oxygen kg^{-1}) was calculated using the following formula:

$$\text{peroxide value} = \frac{1000 \, S \, N}{W} \qquad (1)$$

where S = volume of sodium thiosulphate solution (blank corrected) in ml, N = normality of sodium thiosulphate solution (viz 0.02 N) and W = weight of edible oil sample (in grams). The incubation time vs POV is plotted in Fig 1

Thiobarbituric acid value

The test was conducted according to the methods of Ottolenghi[24] and Kikuzaki and Nakatani[25] with small changes. The same sample as prepared for the POV method was used. To 10 g of edible oil, 0.67% aqueous thiobarbituric acid (TBA; 20 ml) and benzene (25 ml) solution were added. This mixture was shaked continuously for 2 h using a mechanical shaker and the mixture was boiled in a waterbath for 1 h. After cooling, absorbance of supernatant was measured at 500 nm in a Hitachi-U-2000 spectrophotometer. Lesser values of TBA indicate higher values of AA.

The TBA value (meq of malonaldehyde g^{-1}) was calculated using the following formula:

$$\text{TBA value} = \frac{3.2 \times OD}{0.15 \times W} \qquad (2)$$

where OD = absorbance of supernatant solution and W = amount of edible oil sample (in grams). The incubation time versus TBA value is plotted in Fig 2

A standard curve for TBA method was obtained by dissolving 0.22 g of tetra ethoxypropane in 1000 ml of 0.01 hydrochloric acid and 5 ml of this solution were further diluted to 100 ml with 0.01 hydrochloric acid. Aliquots of 2.5, 5, 7.5 and 10 ml were taken and 2 ml of 0.67% TBA in glacial acetic acid were added to each. Finally, the volume was made up to 20 ml with water and the tubes were kept in a boiling water bath for 35 min. Then they were cooled to room temperature and readings were taken at 538 nm in the spectrophotometer. By plotting different aliquot concentrations against the read absorbance equation (2) was obtained.

Determination of AA in linoleic acid system

AA was carried out using the method proposed by Osawa and Namiki[26] with small changes. Each sample (1 ml) was added to a solution mixture of linoleic acid (20 μl), 99.8% ethanol (2 ml) and
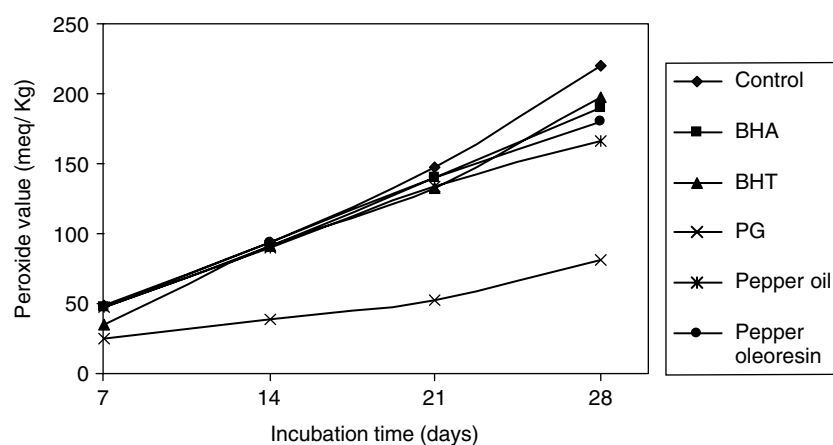

Figure 1. Stabilization of linseed oil by volatile oil and its extract at 80 °C, POV method.
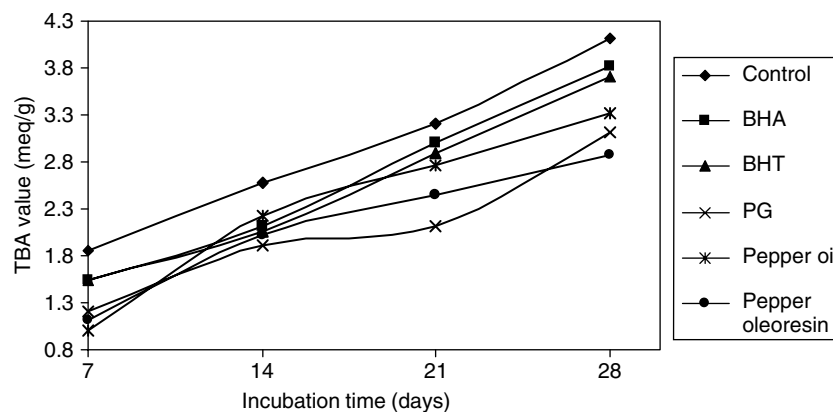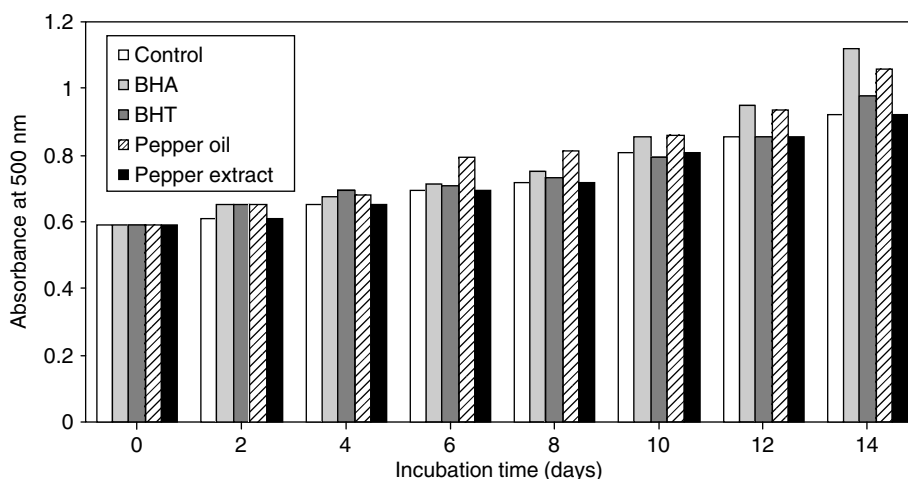

Figure 2. Secondary oxidative effect of volatile oil and its extract against linseed oil, TBA method.

Figure 3. Antioxidant activity in linoleic acid system.

0.2 M phosphate buffer (pH = 7, 2 ml). The total volume was adjusted to 6 ml with distilled water. The solution was incubated at 36 °C for 12 days and the degree of oxidation was measured every 2 days using the ferric thiocyanate method[27] with 75% ethanol (10 ml), 0.2 ml sample solution and 0.2 ml ferrous chloride solution (20 mm in 3.5% HCl) being added sequentially. After stirring for 3 min, the absorbance values of the mixtures measured at 500 nm in the spectrophotometer. The control and standard were subjected to the same procedure except that for the control there was no addition of sample and for the standard 1 ml of sample was replaced with 1 mg of BHA, BHT. The incubation time vs absorbance is plotted in Fig 3. Lesser values of absorbance indicate higher values of AA.

Statistical analysis

All experiments were conducted in triplicate and statistical analysis was carried out using the two-way variance method. Afterwards, the ANOVA method (single-way) was used. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

GC and GC-MS analyses of essential oil and its extract were undertaken using the previously stated HP-5 series. In the volatile oil 49 compounds were identified, representing 99.39% of the total amount. The major component was β-caryophyllene (24.24%) followed by limonene (16.88%), sabinene (13.01%), β-bisabolene (7.69%), β-pinene (6.71%), α-copaene (6.3%), camphene (4.75%), δ-cadiene (2.37%), terpinen-4-ol (1.99%), tricyclene (1.73%) and α-humulene (1.38%), whereas its extract showed the presence of 18 compounds accounting for 75.59% of the total amount. The major component was piperine (33.53%) followed by piperolein B (13.73%), an alkaloid of MW 361 (5.59%) and its isomer (5.49%), piperamide (3.43%), piperettine (2.76%), quineensine (3.23%), hinokinin (1.88%), retrofractamide A

(1.57%) and *N*-trans feruloyltyramine (1.45%), with some other components at lower percentages. Sumathykutty *et al*[28] studied the essential oil constituents of some piper species, and they found a variation of major component among the different species. Tsukamoto *et al*[29] reported three new bisalkaloids, dipiperamides A, B and C, from white pepper along with the known piperine and piperylin.

In the food-poisoning technique, the oil was found to be more than 80% effective in controlling the mycelial growth of *Fusarium graminearum, Penicillium viridicatum* and *Curvularia lunata* at 6 μl dose, and its extract retarded the 100% mycelial growth of *Penicillium viridicatum* and *Aspergillus ochraceus* at the same dose. The volatile oil gave good result against *Fusarium graminearum* even at 2 μl dose, and it was found to be less or ineffective for other fungi. In inverted petriplate technique, the oil was found to be 100% effective against *Fusarium graminearum* at 6 μl and it was more than 70% effective against *Aspergillus niger, Aspergillus oryzae* and *Penicillium madriti* at the same dose. Pepper extract was ineffective in this method, since it had no vapour action. Dhananjaya *et al*[30] studied the antifungal activity of *Pogostemon plectranthoides* essential oil by the agar-well diffusion method and they found that the oil was more effective against *fusarium* sp at 2.8 μl concentration. Sridhar *et al*[31] studied the antifungal activity of some essential oils by the food-poisoning technique and they found that essential oils of cymbopogan, ajowan and dill seed exhibited strong antifungal activity against *Colletotrichum lindemuthianum*.

The biodeterioration of the triglyceride constituents of edible oils and fats in foods by bacteria and fungi is the principal cause of rancidity, acidity, soapiness and off-flavours in various fat-containing food materials.[32] The peroxide value is generally used to measure primary oxidation, indicating the amount of peroxides formed in fats and oils during oxidation. The curves in Fig 1 shows peroxide value changes in linseed oil with additives. Linseed oil oxidation was measured at a storage of 28 days. During this time, the peroxide value

of the control sample increased to 220 meq kg^{-1}, which is significantly ($p < 0.05$) higher than the samples containing volatile oil and its acetone extract. The peroxide value of samples containing pepper oil and its extract were always less than BHA and BHT (Fig 1), but both oil and extract were not significantly ($p > 0.05$) better antioxidants for linseed oil compared with PG (Fig 1). Bandoniene et al[8] studied AA of sage and borage leaf extracts by the Schaal oven test, and they reported for the first time strong AA of crude acetone extracts from borage leaves against rapeseed oil. Singh et al[13] studied the AA of some essential oils by measuring peroxide values, and they found that essential oils of ajowain, anise, cumin, saunf and spearmint possess excellent AA against sunflower oil.

During the oxidation process peroxides are generally decomposed to lower molecular weight compounds and one such compound is malonaldehyde, measured by the TBA method. Figure 2 shows that both pepper oil and its acetone extract are better antioxidants for linseed oil at 80 °C compared with synthetic antioxidants BHA and BHT (up to the final day of the experiment), but they have not shown significant ($p > 0.05$) antioxidative effect compared with PG. Hence, the results obtained from peroxide value and TBA value correlated well (Figs 1 and 2).

The initial stage of peroxide level of lipid oxidation was measured using the linoleic acid system. The inhibitor activities against lipid peroxidation in linoleic acid, caused by additives, were evaluated by measuring concentration of ferric thiocynate. The data on linoleic acid peroxidation, determined by the thiocyanate method, are plotted in Fig 3. Mohdzin et al[33] studied the AA of *Morinda citrtifolia* using the TBA and ferric thiocyanate methods and they found that the root extract had better antioxidative effects than fruit or leaf extracts. Figure 3 shows pepper oil and its extract having almost the same AA throughout the experiment as compared with BHA and BHT. It is interesting to note that acetone extract exhibited higher AA than BHA and BHT on the final day of the experiment. Pepper oil and its extract showed considerable antioxidative activities and were significantly ($p < 0.05$) different from the control (Fig 3). These results were well correlated with those obtained previously by peroxide value and TBA (Figs 1 and 2). According to Pratt and Hudson,[34] most natural antioxidants can be found in wood, leaf, fruit and seed. Burits and Bucar[35] studied the AA of *Nigella sativa* essential oil and they reported that the radical scavenging properties of essential oils are due to the presence of *t*-anethole, carvacrol and 4-terpineol. Ruberto and Baratta[36] studied the AA of severel essential components in two lipid model systems and found low inhibition of peroxidation of α-pinene, β-pinene, champhene, α-terpineol, 1,8 cineole, camphor, α-humulene, 3-decanone and 2-undecanone at 1000 ppm in the conjugated diene method. GC-MS studies showed that black pepper contains α-pinene, β-pinene, camphene and camphor in considerable percentages, and they may contribute to the AA activity of black pepper volatile oil. There is enough literature[37–40] regarding AA of various herbs and spices but, to our knowledge there is little literature[41] about AA of pepper. In addition, antioxidative activities observed in volatile oil and its extract could be the synergistic effect of more than two compounds that may be present in the system. It has been reported that most natural antioxidative compounds work synergistically with each other to produce a broad spectrum of antioxidative activities that create an effective defence system against free radical attack.[42] It is difficult to give a definite explanation for all results obtained within the scope of the present study. The composition of the essential oil and its extract is very complex, consisting of various classes of organic compounds and therefore sometimes possessing the opposite effect on the process of lipid oxidation. Based on the results obtained, it is highly possible that several compounds of different polarities may contribute to the AA of the volatile oil and its acetone extract. The stability of linseed oil samples at 200 ppm concentration, in terms of formation of primary and secondary oxidation process, can be ranked in the following descending order: PG > oil > oleoresin > BHT > BHA > control.

CONCLUSION
Summarizing these results, it can be concluded that essential oil and acetone extract of pepper possess different AA which may be due to the synergistic effect of polar and non-polar compounds. The volatile oil was found to be 100% effective in controlling the mycelial growth of *Fusarium graminearum* and its extract showed 100% effectiveness against *Aspergillus ochraceus* and *Penicillium viridicatum*. It can also be concluded that the volatile oil and its extract are better antioxidants for stabilizing linseed oil at 80 °C.

ACKNOWLEDGEMENT

REFERENCES
1 Srinivas PV and Rao JM, Isopiperolein B: an alkamide from *piper nigrum*. *Phytochemistry* **52**:957–958 (1999).
2 Bajad S, Singla AK and Bedi KL, Lipid chromatographic method for determination of piperine in rat plasma: application to pharmacokinetics. *J Chromatogr B* **776**:245–249 (2002).
3 Saxena SN, Sharma P, Chopra S and Sahani G, *Cultivation and Utilization of Aromatic Plants*, Ed by Atal CK and Kapur BM. National Institute of Science Communication, New Delhi, pp 217–218 (1982).

4 BACIS, Pepper, in *Volatile Compounds in Food Database*. TNO, Zeist (1999).

5 Yanishileva NV and Marinova EM, Activity and mechanism of action of natural antioxidants in lipids. *Rec Res Dev Oil Chem* **2**:1–14 (1998).

6 Burits M and Bucar F, Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res* **14**:323–328 (2000).

7 Dessi MA, dieana M, Rosa A, Pipredda M, Cottigilia F, Bonsignore L, Deiddn D, Pompei R and Corongiu F, Antioxidant activity of extracts from plants growing in Sardinla. *Phytother Res* **15**:511–518 (2001).

8 Bandoniene D, Venskutonis PR, Gruzdiene and Murkovic M, Antioxidant activity of Sage (*Salvia offcinalis* L.), Savory (*Satureja hortensis* L.) and Borage (*Borago officinalis* L.) extracts in rapeseed oil. *Eur J Lipid Sci Technol* **104**:286–292 (2002).

9 Chinou IB, Roussis V, Perdetzoglou D, Tzakou O and Loukis A, Chemical and antibacterial studies of two *Hedichrysum* species of Greek origin. *Planta Med* **63**:181–183 (1997).

10 Kasali AA and Adio AM, Antimicrobial activity of the essential oil of *Baswellia serraty Roxb*. (Fam. Burseraceae) bark. *JEOP* **5**:173–175 (2002).

11 Vardar-unlu G, Candan F, Sokmen A, Daferera D, Polissiou M, Sokmen M, Donmez E and Tepe B, Antimicrobial and antioxidant activity of the essential oil and methnol extracts of *Thymus pectinatus* Fisch. Et Mey. Var. pectinatus (Lamiaceae). *J Agric Food Chem* **51**:63–67 (2003).

12 Ozcan M and Erkmen O, Antimicrobial activity of the essential oils of Turkish plant spices. *Eur Food Res Tech* **212**:658–660 (2001).

13 Singh G, Kapoor IPS and Pandey SK, Natural antioxidants for sunflower oil. *J Sci Ind Res* **5**:139 (1998).

14 Singh G, Pandey SK, Leclereq PA and Sperkova J, GC/MS analysis of chemical constituents of leaf oil of *Lippia alba (Mill.)* from North India. *J Essent Oil Res* **11**:206–208 (1999).

15 Singh G, Singh OP and Maurya S, Chemical and biocidal investigations on essential oils of some Indian *Curcuma* species. *Prog Crystal Growth Charact* **45**:69–71 (2002).

16 Singh G, Singh OP, Rao GP and Sharma SR, The vapour action of essential oils and monoterpenoids against Patghogenic fungi. *Sugartech* **4**:69–71 (2002).

17 *European Pharmacopoeia*, Vol 1. Maissoneue, Sainte Ruffine (1983).

18 Handa SS and Kaul MK, *Supplement to Cultivation and Utilisation of Aromatic Plants*. National Institute of Science Communication, New Delhi, p 394 (1997).

19 Rao GP and Shrivastava AK, Current trends in sugarcane. *Pathogens* **4**:364–374 (1994).

20 Ramdas K, Suresh G, Janardhanan N and Masilamani S, Antifungal activity of 1, 3-disubstituted symmetrical and unsymmetrical thioureas. *Pestic Sci* **52**:145–151 (1998).

21 *Official Methods and Recommended Practices of American Oil Chemist's Society*. AOAC, Champaign, IL (1986).

22 *Official Methods and Analysis of the Analysis of the Association Official Analytical Chemists* (15th edn). AOAC, Champaign, IL (1975).

23 Bandoniene D, Gruzchene and Venkutonis PR, Antioxidant activity of sage extracts in rapeseed oil irradiated with UV rays. *Nahurng/Food* **2**:105–108 (2002).

24 Ottolenghi A, Interaction of ascorbic acid and mictochondrial lipids. *Arch Biochem Biophys* **79**:355–358 (1959).

25 Kikuzaki H and Nakatani N, Antioxidant effects of some ginger constituents. *J Food Sci* **58**:1407–1410 (1993).

26 Osawa T and Namikin M, A novel type of antioxidant isolated from leaf wax of *Eucalyptus* leaves. *Agric Biol Chem* **45**:735–739 (1981).

27 Mitsuda H, Yasumoto K and Iswami K, Antioxidative action of indole compounds during the autooxidation of linoleic acid. *Eiyo to Shokuryo* **19**:210–214 (1996).

28 Sumathykutty MA, Rao JM, Padmakumari KP and Narayanan CS, Essential oil constituents of some *piper* species. *Flavour Fragr J* **14**:279–282 (1999).

29 Tsukamoto S, Cha BC and Ohta T, Dipiperamides A, B and C: bisalkaloids from the white pepper *piper nigrum* inhibiting CYP3A4 activity. *Tetrahedron* **58**:1667–1671 (2002).

30 Dhananjaya S, Singh P and Pant AK, Chemical composition and biological activity of essential oil of *pogostemon plectranthoides*. *Ind Perfum* **45**:35–38 (2001).

31 Sridhar SR, Rajagopal RV, Rajavel R, Masilamani S and Narasimhan S, Antifungal activity of some essential oils. *J Agric Food Chem* **51**:7596–7599 (2003).

32 Eskin NAM, Henderson HM and Townsend RJ, *Biochemistry of Foods*. Academic Press, New York, pp 202–203 (1971).

33 Mohdzin Z, Abdul-Hamid A and Osman A, Antioxidant activity of extracts from Mengkudu (*Morinda citrifolia* L) root, fruit and leaf. *Food Chem* **78**:227–231 (2002).

34 Pratt DE and Hudson BJF, Natural antioxidants not exploited commercially, in *Food Antioxidants*. London, Elsevier Applied Science, pp 171–192 (1992).

35 Burits M and Bucar F, Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res* **14**:323–328 (2000).

36 Ruberto G and Baratta MT, Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem* **69**:167–174 (2000).

37 Lee KG and Shibamoto T, Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. *J Agric Food Chem* **50**:4947–4952 (2002).

38 Takeoka GR and Dao LT, Antioxidant constituents of almond [*Pruns dulcis* (Mill.) D. A. Webb] hulls. *J Agric Food Chem* **51**:496–501 (2003).

39 Duenas M, Hernandez T, Estrella I and Rabanal R, Phenolic composition and antioxidant activity of mocan seeds (*Visnea mocanera* L.f). *Food Chem* **82**:373–379 (2003).

40 Lu F and Foo LY, Antioxidant activities of polyphenols from sage (*Salvia officinalis*). *Food Chem* **75**:197–202 (2001).

41 Calucci L, Pinzino C, Zandomeneghi M, Capocchi A, Ghiringhelli S, Saviozzi F, Tozzi S and Galleschi L, Effects of γ-irradiation on the free radical and antioxidant contents in nine aromatic herbs and spices. *J Agric Food Chem* **51**:927–934 (2003).

42 Lu F and Foo LY, Phenolic antioxidant component of evening primrose, in *Nutrition, Lipids, Health and Diseases*, Ed by Ong ASH, Niki E and Paeker L. AOAC, Champain, IL (1995).