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ARTICLE

Chemistry and in Vitro Antioxidant Activity of Volatile Oil and Oleoresins of Black Pepper (*Piper nigrum*)†

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Essential oil and oleoresins (ethanol and ethyl acetate) of *Piper nigrum* were extracted by using Clevenger and Soxhlet apparatus, respectively. GC-MS analysis of pepper essential oil showed the presence of 54 components representing about 96.6% of the total weight. β-Caryophylline (29.9%) was found as the major component along with limonene (13.2%), β-pinene (7.9%), sabinene (5.9%), and several other minor components. The major component of both ethanol and ethyl acetate oleoresins was found to contain piperine (63.9 and 39.0%), with many other components in lesser amounts. The antioxidant activities of essential oil and oleoresins were evaluated against mustard oil by peroxide, *p*-anisidine, and thiobarbituric acid. Both the oil and oleoresins showed strong antioxidant activity in comparison with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) but lower than that of propyl gallate (PG). In addition, their inhibitory action by FTC method, scavenging capacity by DPPH (2,2′-diphenyl-1-picrylhydrazyl radical), and reducing power were also determined, proving the strong antioxidant capacity of both the essential oil and oleoresins of pepper.

KEYWORDS: *Piper nigrum*; essential oil; oleoresins; GC-MS; antioxidant activity

INTRODUCTION

Fats and oils present in many foods and cosmetic products may easily deteriorate due to oxidation, in a chain of reactions in which free radicals are formed, propagated, and finally converted into stable oxygenated compounds, which are responsible for off-flavors and other undesirable characteristics (*1*). Antioxidants, when added to lipid-containing foods, can increase shelf life by retarding the process of lipid peroxidation. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) have been used since the beginning of the 20th century in food industries, but there are some arguments about the safty and adverse effects of these substances when used as food additives (*2*). Thus, there is a need for identifying alternative natural and safe sources of food antioxidants (*3*, *4*).

Spices are dietary constituents consumed daily to enhance the flavor or taste of human foods (*5*). Pepper is the most widely used spice throughout the world, and so it is called the "king of spices". Black pepper (*Piper nigrum*) is native to India and the tropical evergreen forest of the Malabar region of southern India. It is an important spice, appreciated for both its aroma and its pungency, belonging to family Piperaceae. It is one of the oldest spices used for both culinary and medicinal purposes (*6*).

Although there are several earlier studies on the chemical constituents (*7*−*9*) antioxidant and larvicidal properties (*10*) of the essential oil of black pepper, no work is reported on the ethanol and ethyl acetate oleoresins of black pepper. The aim of the present study is the comparative study of the chemistry and antioxidant activity of essential oil and oleoresins (ethanol and ethyl acetate) of black pepper by different antioxidant assays to find a new source of natural antioxidants.

MATERIALS AND METHODS

Chemicals. Thiobarbituric acid (TBA), diphenylpicrylhydrazyl radical (DPPH), and linoleic acid were purchased from Acros (Fair Lawn, NJ). BHT, BHA, PG, and 2,4-dinitrophenylhydrazine were purchased from S. D. Fine-Chemicals Ltd., Mumbai, India. Crude mustard oil was purchased from a local oil mill in Gorakhpur, India.

Plant Material. Fruits of black pepper were purchased from the local market of Gorakhpur, India, during September 2008, and a voucher specimen has been deposited in the Herbarium of the Science Faculty of D.D.U. Gorakhpur University, Gorakhpur, India.

Isolation of the Volatile Oil. The powdered fruits of black pepper were subjected to hydrodistillation in a Clevenger-type apparatus for 6 h in accordance with a European Pharmacopoeia procedure (*11*). A colorless volatile oil (yield = 2.6%) with characteristic odor and sharp taste was obtained. This was then dried over anhydrous sodium sulfate to remove traces of moisture, filled into a bottle, and stored in a refrigerator in the dark at 4 °C until use.

Isolation of the Oleoresins. The oleoresins were obtained by extracting 25 g of powdered fruits of black pepper with 250 mL of each solvent (ethanol and ethyl acetate) for 3 h in a Soxhlet extractor, and the solvent was distilled out. The obtained oleoresins (yield = 3.8 and 2.5%) were filled into bottles and stored in a refrigerator in the dark at 4 °C until use.

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Table 1. Chemical Composition of Black Pepper Essential Oil

compound	%	RI[a]	identification[b]
α-thujene	0.8	920	MS, RI
α-pinene	**4.5**	**928**	**MS, RI, co-GC**
camphene	0.1	942	MS, RI, co-GC
sabinene	**5.9**	**967**	**MS, RI, co-GC**
β-pinene	**7.9**	**973**	**MS, RI, co-GC**
myrcene	1.0	984	MS, RI, co-GC
α-phellandrene	0.6	1003	MS, RI, co-GC
3-carene	**4.4**	**1006**	**MS, RI, co-GC**
α-terpinene	tr[c]	1012	MS, RI, co-GC
p-cymene	1.2	1019	MS, RI, co-GC
limonene	13.2	1026	MS, RI, co-GC
trans-β-ocimene	tr	1039	MS, RI, co-GC
γ-terpinene	0.1	1050	MS, RI, co-GC
cis-sabinene hydrate	0.2	1063	MS, RI, co-GC
terpinolene	0.1	1078	MS, RI, co-GC
linalool	0.5	1096	MS, RI, co-GC
trans-sabinene hydrate	tr	1101	MS, RI, co-GC
terpinen-4-ol	0.7	1172	MS, RI, co-GC
criptone	tr	1178	MS, RI
α-terpineol	0.2	1188	MS, RI, co-GC
δ-elemene	0.5	1338	MS, RI
α-cubebene	0.1	1348	MS, RI
α-ylangene	0.1	1363	MS, RI
α-copaene	2.8	1371	MS, RI
β-cubebene	0.3	1381	MS, RI
β-elemene	0.9	1383	MS, RI
tetradecane	trace	1400	MS, RI
β-caryophyllene	**29.9**	**1411**	**MS, RI**
β-copaene	0.1	1421	MS, RI
α-humulene	1.9	1443	MS, RI
trans-β-farnesene	0.4	1446	MS, RI
α-amorphene	0.1	1479	MS, RI
germacrene-D	0.3	1482	MS, RI
β-selinene	1.5	1485	MS, RI
α-selinene	1.1	1492	MS, RI
α-muurolene	0.6	1497	MS, RI
β-bisabolene	**3.9**	**1501**	**MS, RI**
cubebol	0.8	1517	MS, RI
δ-cadinene	1.3	1524	MS, RI
cis-calamenene	0.2	1526	MS, RI
elemol	0.2	1549	MS, RI
germacrene-B	0.2	1553	MS, RI
trans-nerolidol	0.4	1557	MS, RI
caryophyllene oxide	**3.9**	**1578**	**MS, RI**
hexadecane	0.2	1600	MS, RI
T-muurolol	0.2	1636	MS, RI
torreyol	1.4	1640	MS, RI
α-bisabolol	0.2	1684	MS, RI
heptadecane	0.3	1700	MS, RI
octadecane	0.4	1800	MS, RI
nonadecane	0.4	1900	MS, RI
eicosane	0.3	2000	MS, RI
heneicosane	0.2	2100	MS, RI
docosane	0.1	2200	MS, RI
total	**96.6**		

[a] Retention index was calculated using a homologous series of *n*-alkanes C8−C22. [b] Co-GC, co-injection with an authentic sample. Percentages were obtained from electronic integration measurements using selective mass detector. [c] Trace, <0.05.

Chemical Investigation. Chemical analyses of volatile oil and oleoresins of *P. nigrum* were undertaken by gas chromatography−mass spectroscopy (GC-MS). A Hewlett-Packard 6890 gas chromatograph coupled to a Hewlett-Packard 5973 quadruple mass spectrometer equipped with a Perkin-Elmer Elite-5MS capillary column (5% phenyl methyl siloxane, length = 30 m, inner diameter = 0.25 mm, film thickness = 0.25 μm) and a selective mass detector was used for GC-MS detection; an electron ionization system with ionization energy of 70 eV was used. Helium was taken as a carrier gas at a flow rate of 1.0 mL/min. Injector and ion source temperatures were set at 230 and 280 °C, respectively. Injection volume was 1 μL with a split ratio 80:1, and the oven temperature for the volatile oil was programmed as follows: 60 °C (1 min), 60−185 °C (1.5 °C/min), 185 °C (1 min), 185−275 °C (9 °C/min), 275 °C (2 min). The oven temperature program for oleoresin was 60 °C (0 min), 60−300 °C (3 °C/min), 300 °C (10 min).

Identification of Components. The components were identified on the basis of comparison of their retention indices and mass spectra with published data (*12*−*14*), and computer matching was done with the Wiley 275 and National Institute of Standards Technology (*15*) libraries provided with the computer controlling GC-MS systems. The retention indices were calculated using a homologous series of *n*-alkanes C_8−C_{22}, and the results of volatile oil and oleoresins are reported in **Tables 1** and **2**, respectively.

ANTIOXIDANT ACTIVITY

The antioxidant activities of black pepper essential oil and oleoresins were compared to those of BHA, BHT, and PG synthetic antioxidants by different methods such as peroxide, anisidine, thiobarbituric acid value, DPPH radical scavenging, reducing power, and total antioxidant activity by ferric thiocyanate (FTC) methods.

Evaluation of Antioxidant Activity in Oxidation of Mustard Oil. For the present investigation, crude mustard oil, having an initial peroxide value of 5.8 mequiv/kg, was taken. The pepper oil and oleoresins were added individually to unrefined crude mustard oil at the concentration of 200 ppm (w/v). Synthetic antioxidants such as BHA, BHT, and PG were also added to mustard oil at the same concentration, that is, 200 ppm (w/v). An equal quantity of mustard oil without any additives was taken as control for peroxide, *p*-anisidine, and thiobarbituric acid values.

Peroxide Value. The peroxide value measures the total peroxide and hydroperoxide oxygen content of lipids and lipid-containing materials. The peroxide value was determined according to the method prescribed earlier (*16*). For this purpose, 5 g of mustard oil sample was dissolved in a mixture of CH_3COOH/$CHCl_3$ (3:2 v/v), and 0.5 mL of saturated potassium iodide (KI) solution was added. After 1 min, 30 mL of distilled water was added, and the whole solution was titrated against 0.01 N sodium thiosulfate using starch as an indicator. Titration was continued, shaking the flask vigorously until the blue color just disappeared. Peroxide value in milliequivalents per kilogram (meq/kg) of sample was calculated using the formula

$$\text{peroxide value (meq/kg)} = \frac{1000 \times S \times N}{W}$$

where S = volume of sodium thiosulphate used in mL, N = normality of sodium thiosulfate used (viz., 0.01 N), and W = weight of sample in grams.

The results in the form of plot of incubation time versus peroxide value are shown in **Figure 1**.

Anisidine Value. The anisidine value measures 2-alkenals and was determined according to the method described earlier (*17*). The sample (0.5 ± 0.1 g) was dissolved in isooctane, and the volume was made up to 50 mL with isooctane. Five milliliters of this solution was mixed with 1 mL of 0.25% of *p*-anisidine reagent and kept in the dark for 10 min. Its absorbance (A_2) was measured at 350 nm using the same spectrophotometer. A blank test (without the addition of anisidine reagent) was also done (A_1). The anisidine value was calculated as

$$\text{anisidine value} = \frac{(A_2 - A_1) \times 1.2 \times 50}{W(\text{g})}$$

Figure 2 shows *p*-anisidine value versus incubation time.

Table 2. Chemical Composition of Black Pepper Oleoresins

compound	ethanol oleoresin	ethyl acetate oleoresin	RI[a]	identification[b]
α-thujene		0.1	924	MS, RI
α-pinene		0.2	932	MS, RI, co-GC
sabinene		0.3	971	MS, RI, co-GC
β-pinene		0.4	977	MS, RI, co-GC
3-carene		0.3	1009	MS, RI, co-GC
p-cymene		0.1	1023	MS, RI, co-GC
limonene		0.7	1028	MS, RI, co-GC
β-phellandrene		0.1	1029	MS, RI, co-GC
δ-elemene		0.2	1337	MS, RI
α-copaene		0.6	1373	MS, RI
β-elemene		0.3	1386	MS, RI
β-caryophyllene	1.0	6.7	1416	MS, RI
α-humulene	0.1	0.5	1451	MS, RI
β-selinene	0.1	0.4	1485	MS, RI
α-selinene	tr[c]	0.3	1492	MS, RI
α-muurolene	tr	0.1	1495	MS, RI
β-bisabolene	0.2	0.7	1505	MS, RI
δ-cadinene	0.2	0.4	1515	MS, RI
elemol	tr	0.1	1544	MS, RI
trans-nerolidol		0.1	1557	MS, RI
caryophyllene oxide	0.2	0.4	1575	MS, RI
dill-apiole		0.1	1612	MS, RI
torreyol	0.4	0.5	1640	MS, RI
1-octadecene		0.1	1793	MS, RI
octadecane		0.1	1800	MS, RI, co-GC
nonadecane		0.1	1900	MS, RI, co-GC
pellitorin	0.3	1.4	1938	MS, ref (*1*)
plasticizer		0.2	1951	MS
palmitic acid		0.2	1967	MS, RI, co-GC
1-eicosene		tr	1992	MS, RI
eicosane		0.1	2000	MS, RI, co-GC
1-cinnamoylpiperidine		0.1	2090	MS, ref (*2*)
heneicosane		0.1	2100	MS, RI, co-GC
2,4-decadienoic acid piperidide	tr	0.7	2140	MS, ref (*3*)
docosane		0.1	2200	MS, RI, co-GC
plasticizer	0.2	2.5		MS
piperanine	**4.0**	**5.1**		**MS, ref** (*4*)
N-isobutyl-(2*E*,4*E*,12*E*)-octadecatrienamide	2.7	2.9		MS, ref (*5*)
N-isobutyl-(2*E*,4*E*)-octadecadienamide	2.5	2.5		MS, ref (*6*)
retrofractamide B	0.7	0.7		MS, ref (*1*)
piperine	**63.9**	**39.0**		**MS, ref** (*4*)
4,5-dihydropiperettine	1.9	2.0		MS
N-isobutyl-(2*E*,4*E*,14*Z*)-eicosatrienamide	2.0	2.2		MS, ref (*1*)
piperamide C 9:1 (8*E*)	0.4	0.7		MS, ref (*7*)
piperolein B	**5.3**	**5.5**		**MS, ref** (*8*)
dehydropipernonaline	0.2	1.1		MS, ref (*4*)
guineensine		1.7		MS, ref (*4*)
total	**83.8**	**82.7**		

[a] Retention index was calculated using a homologous series of *n*-alkanes C8−C22. [b] Co-GC, co-injection with an authentic sample. Percentages were obtained from electronic integration measurements using selective mass detector. [c] Trace, <0.05.

Thiobarbituric Acid Value (TBA). The TBA value of different samples was determined using an earlier method (*18*). The test was performed according to the method previously reported with small changes (*19*). About 100 mg of oil sample was dissolved in 25 mL of 1-butanol. A 25 mL aliquot of the above solution was mixed thoroughly with 5.0 mL of TBA reagent (200 mg of TBA in 100 mL of 1-butanol) and incubated at room temperature, and absorbance was measured at 530 nm with a Hitachi-U-2000 spectrophotometer (Tokyo, Japan). At the same time, a reagent blank (without TBA reagent) was also done. The thiobarbituric acid value (mequiv of malonaldehyde/g) was calculated as

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

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

The effect of volatile oil and oleoresins on mustard oil in terms of incubation time versus TBA value is shown in **Figure 3**.

Complementary Antioxidant Activity in Linoleic Acid System (FTC Method). The antioxidant activities of pepper oil, oleoresins, and synthetic antioxidants were determined according to the ferric thiocynate method (*20*) with some modifications. The reaction medium contains a 2.5 mL solution of pepper oil and oleoresins (1 mg/100 mL in absolute alcohol), 2.5 mL of a 2.51% linoleic acid emulsion, and 5.0 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 additives was used

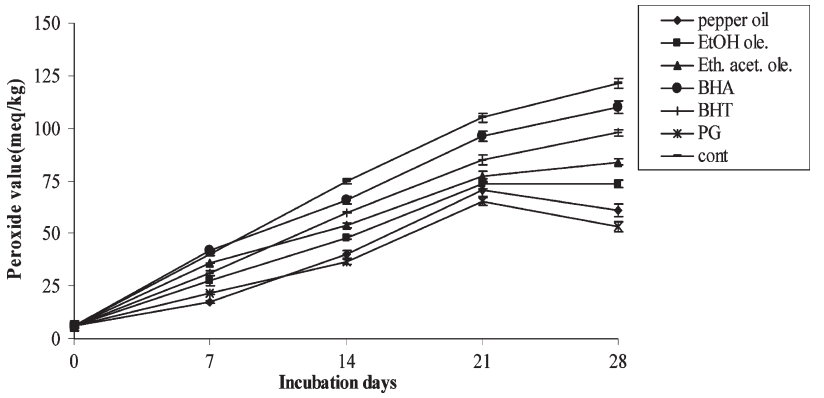

Figure 1. Antioxidant activity of pepper oil and oleoresins in terms of peroxide values.

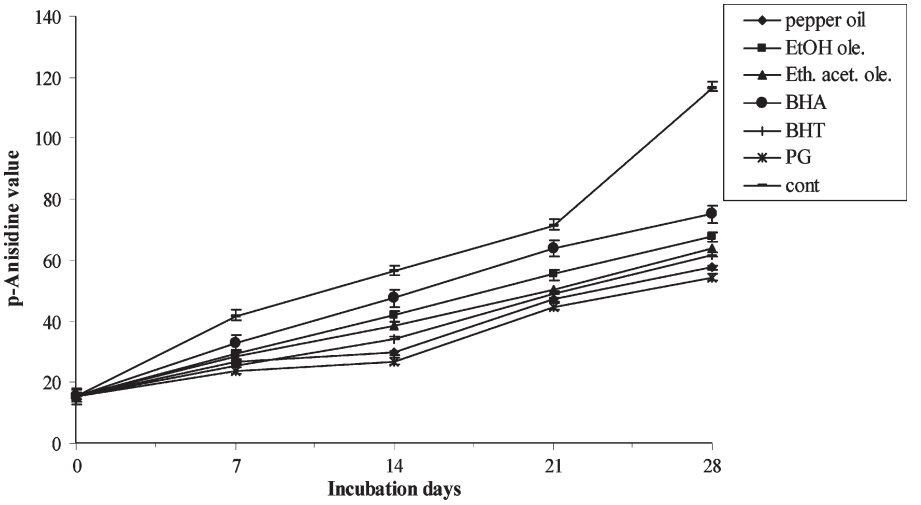

Figure 2. Antioxidative effect of pepper oil and oleoresins in terms of *p*-anisidine values.

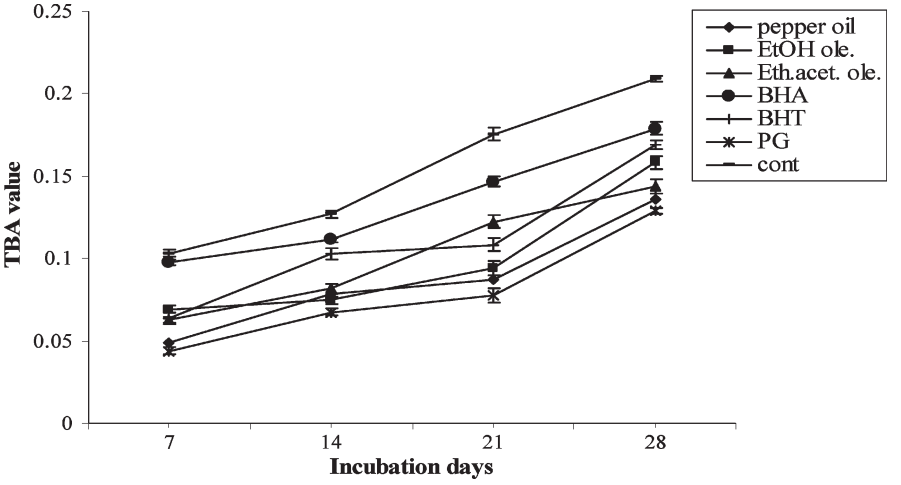

Figure 3. Antioxidative effect of pepper essential oil and oleoresins in terms of thiobarbituric acid values.

as control sample. Synthetic antioxidants (BHA, BHT, and PG) were used for the comparison, in the same concentration. The peroxide level of each sample was determined by reading the absorbance at 500 nm in a Hitachi-U-2000 spectrophotometer after reaction with 0.1 mL of 20 mM $FeCl_2$ and 0.2 mL of ammonium thiocynate every 24 h. BHA, BHT, and PG were used as standard. The inhibition of lipid peroxidation was shown in terms of absorbance value. Lower absorbance showed higher inhibition. The results are reported as incubation time versus absorbance (**Figure 4**).

Reducing Power. The reducing power of the samples was assessed according to a method described by Senevurathne et al. (*21*). A 1.0 mL aliquot of each essential oil, oleoresins, and synthetic antioxidants (0.5−2.0 mg/mL of absolute alcohol) was mixed with 1.0 mL of phosphate buffer (0.2 M, pH 6.6) and 1.0 mL of 1 g L^{-1} potassium ferricyanide, and the mixture was incubated at 50 °C for 30 min. After cooling rapidly, the mixture was mixed with 1.0 mL of 10% trichloroacetic acid and centrifuged at 3000 rpm for 10 min. A 2.5 mL fraction of supernatant was mixed with 2.5 mL of 1% $FeCl_3$. Absorbance of the mixture

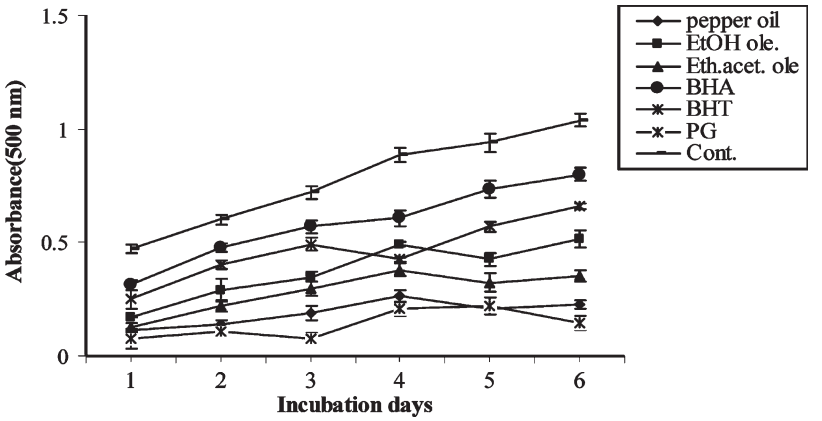

Figure 4. Antioxidative effect of pepper essential oil and oleoresins in linoleic acid system.

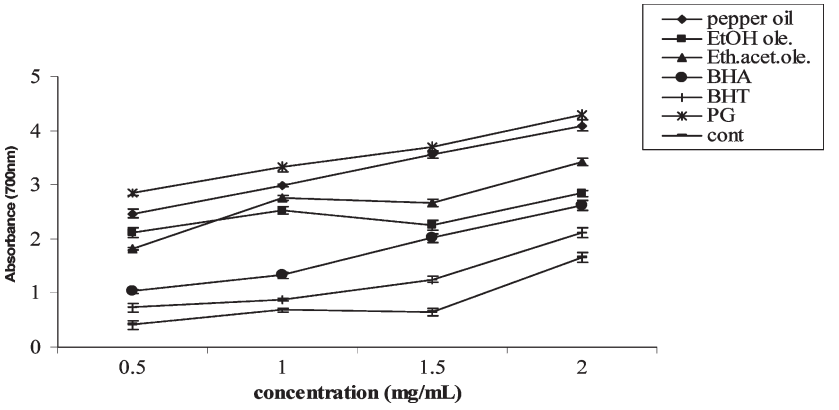

Figure 5. Antioxidative effect of pepper essential oil and oleoresins in reducing power system.

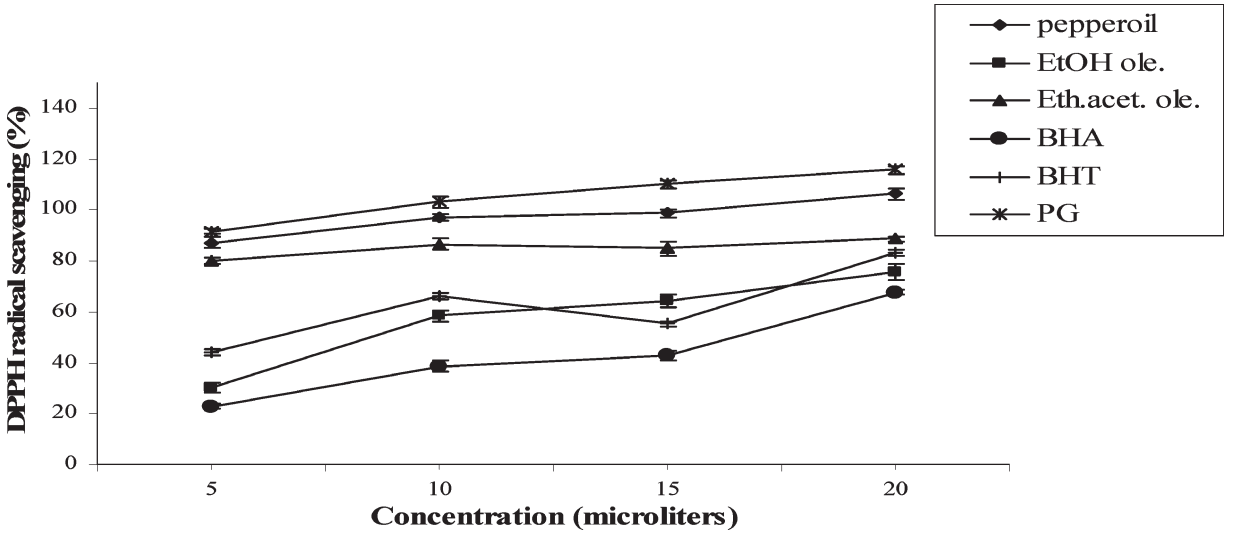

Figure 6. Radical scavenging effect of pepper essential oil and oleoresins on 2,2-diphenyl-1-picrylhydazyl radical.

was recorded at 700 nm after 10 min. The higher the absorbance values, the stronger will be the reducing power. The results are reported as absorbance versus concentration (**Figure 5**).

Scavenging Effect on DPPH. The capacity of pepper oil and oleoresins to scavenge the DPPH radical was determined according to the method reported earlier (*22*). Briefly, 1 mL of 0.1 mM solution of DPPH (in methanol) was mixed thoroughly with 3.0 mL of methanolic solution of pepper oil and oleoresins (0.5−2.0 mg/mL). The reaction mixture was shaken vigorously and incubated at room temperature for 30 min. The absorbance was measured at 517 nm. Control (without any additive) and standard containing BHA, BHT, and PG (in place of oil and oleoresins) were also tested. The free radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the equation

$$\text{DPPH scavenging effect } (\%) = \frac{A_c - A_t}{A_c} \times 100$$

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

The results are reported as scavenging effect (%) versus concentration (**Figure 6**).

Statistical Analysis. Duplicate samples of mustard oil for each treatment/period were taken. Each sample was analyzed individually in triplicate. The data were presented as mean ± standard deviation of three determinations (data not shown). The quantitative data of essential oil and oleoresin were statistically examined by Student's *t* test by using Microsoft Excel statistical analysis program. A probability value of $P \leq 0.05$ was considered to denote the statistically significant differences.

RESULTS AND DISCUSSION

The analyses of essential oil and oleoresins by GC-MS are presented in **Tables 1** and **2**. A total of 54 components were identified in pepper oil, representing about 96.6% of the total weight. The major components were β-caryophylline (29.9%), limonene (13.2%), β-pinene (7.9%), and sabinene (5.9%). Ethanol oleoresin showed the presence of 23 components constituting about 83.7% of the total amount, major components being piperine (63.9%), piperolein (5.3%), piperanine (4.0%), and *N*-isobutyl-(2*E*,4*E*,12*E*)-octadecatrienamide (2.9%). In ethyl acetate oleoresin, 47 components (82.7%) were analyzed, and the major ones were piperine (39.0%), β-caryophyllene (6.7%), piperolein (5.5%), piperanine (5.1%), and *N*-isobutyl-(2*E*,4*E*,12*E*)-octadecatrienamide (2.9%) with many other components in minor amounts (**Tables 1** and **2**). Sumathukutty et al. (*23*) and Singh et al. (*7*) studied the essential oil constituents of some pepper species and found a variety of major components. These differences were found to be due to some geographical factors (*24*), culture conditions (*25*), post crop processing (*26*), and different chemotype and nutritional status of the plants as well as other factors that can influence the composition.

The peroxide value is commonly used to measure hydroperoxide and peroxide formed during the initial stage of lipid peroxidation, that is, good indicators of lipid peroxidation (*27*). The peroxide values of different mustard oil samples were determined every 7 days (**Figure 1**) during the storage period of 28 days; the peroxide value of control sample increased from 5.8 to 181.8 mequiv/kg, which is significantly higher than those of the other samples containing pepper oil, oleoresins, and synthetic antioxidants. Thus, in terms of peroxide, the inhibitory effect was found in following order:

PG > essential oil > ethyl acetate oleoresin >

ethanol oleoresin > BHA > BHT > control

The anisidine values of different samples in mustard oil (**Figure 2**) showed that the samples containing essential oil and/or oleoresins had significantly lowers anisidine value than the control set. It is also evident that the antioxidant potential of essential oil and oleoresins is comparable to those of BHA and BHT; however, they are less effective than PG. During the oxidation process, peroxides are generally decomposed to lower molecular weight compounds; one such compound is malondialdehyde, which is measured by the TBA method. Malondialdehyde, used as an index of lipid peroxidation, was determined by a selective third-order derivative spectrophotometric method previously developed by some othors (*28*). From the result (**Figure 3**) it is clear that essential oil, ethyl acetate, and ethanol oleoresins of pepper were more effective than BHA and BHT but less effective than PG. Moreover, essential oil and ethyl acetate oleoresin of pepper were better than other samples except PG.

The initial value of lipid peroxidation was measured using a linoleic acid system. A low absorbance value indicates a high level of antioxidant activity. From the inhibitor activities against lipid peroxidation in linoleic acid caused by additives shown in **Figure 4** it is clear that pepper oil and its ethyl acetate oleoresin showed higher antioxidant activity than BHA and BHT but lower antioxidant activity than PG. The ethanol oleoresin also showed inhibition, but its effect is less than that of all of the synthetic antioxidants except BHA. All of the results were found to be highly significant ($p < 0.02$).

For the measurement of reducing ability of Fe^{3+} to Fe^{2+} transformations in the presence of additives are shown in **Figure 5**. From the figure the reducing power of all samples was found to be significantly higher than ($p < 0.05$) control and depended on the concentration of essential oil and oleoresins. Moreover, pepper oil and its ethyl acetate oleoresin showed better reducing power than ethanol oleoresin. Their activities are higher than those of BHA and BHT but lower than that of PG.

Volatile oil and oleoresins of black pepper showed a dose-dependent DPPH radical scavenging effect. From **Figure 6** it is clear that as the concentration increased, the scavenging effect also increased. Pepper oil showed better scavenging power that was even higher ($p < 0.5$) than those of BHA and BHT but lower than that of PG at all concentrations. In oleoresins, ethyl acetate oleoresin is better than ethanol oleoresin. The scavenging effect of various samples was

PG > essential oil > ethyl acetate oleoresin >

ethanol oleoresin > BHA > BHT > control

The effectiveness of additives (essential oil and oleoresins) depends not only on their structural features but also on many factors such as the character of lipid system, the temperature, and the binding of fatty acid (*29*, *30*). GC-MS studies showed that black pepper essential oil and oleoresins contains β-caryophyllene, limonene, β-pinene, piperine, piperolein, etc., in considerable percentages, and they may contribute to the antioxidant activity of black pepper. Earlier studies (*31*, *32*) also showed that flavonides, piperine, phenolic amides, and several other constituents present in black pepper show specific antioxidant properties. In addition, antioxidant activity observed in volatile oil and oleoresins could be the synergistic effect of more than two components that may be present in the system. It has been reported that most natural oxidative compounds work synergistically with one another to produce a broad spectrum of antioxidant activities that create an effective defense against free radical attack (*33*, *34*). Nevertheless, it is difficult to give a definite explanation for all results.

To summarize these results, essential oil and oleoresins of black pepper have high antioxidant and radical scavenging activities against various antioxidant assays in vitro. These assays have important applications in food industries. Moreover, black pepper can be used as an easily accessible source of natural antioxidant and as a possible food additive in the food industry. The various antioxidant mechanisms of pepper oil and oleoresins may be attributed to strong hydrogen-donating ability, metal chelating, and their effectiveness as good scavengers of free radicals. Therefore, it is suggested that further work could be performed on the isolation and identification of the antioxidant content of oil and oleoresins.

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