*Chemistry, Antioxidant and Antimicrobial Potentials of White Pepper (*Piper nigrum *L.) Essential Oil and Oleoresins*

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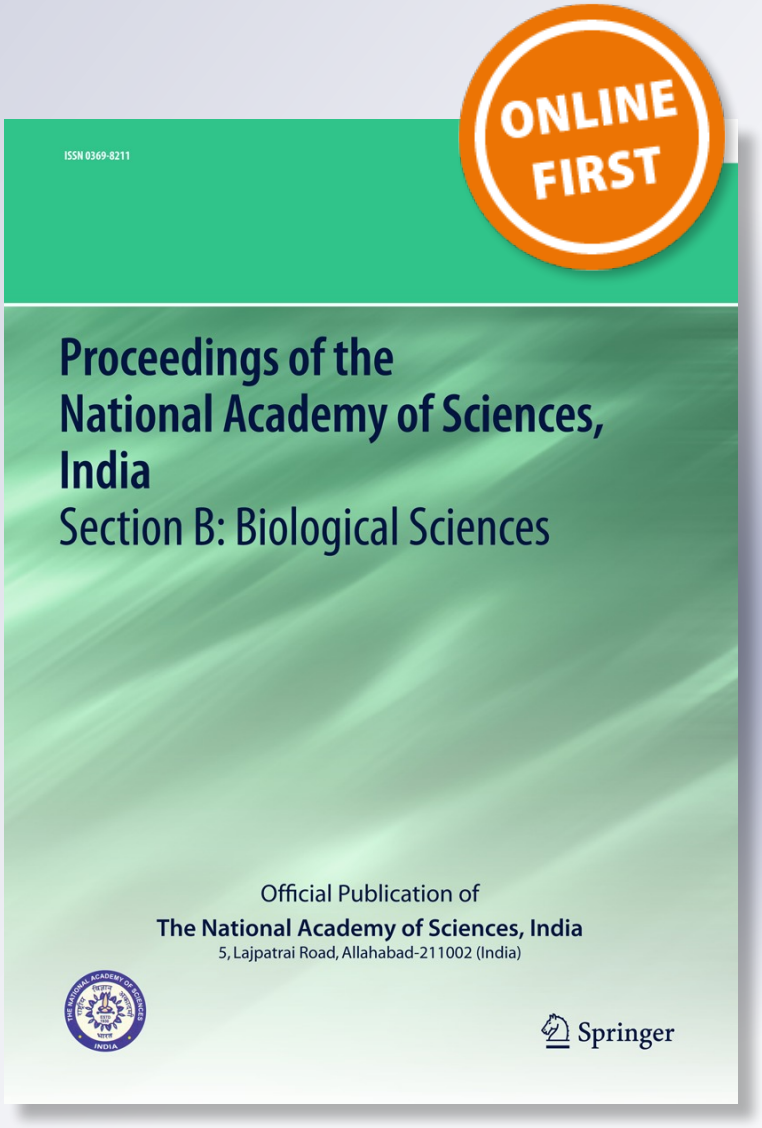
Proceedings of the National Academy of Sciences, India Section B: Biological Sciences

ISSN 0369-8211

Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci.
DOI 10.1007/s40011-012-0148-4



Springer

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RESEARCH ARTICLE

Chemistry, Antioxidant and Antimicrobial Potentials of White Pepper (*Piper nigrum* L.) Essential Oil and Oleoresins

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Received: 21 August 2012 / Revised: 4 December 2012 / Accepted: 13 December 2012

Abstract The antioxidant and antimicrobial potentials of volatile oil and oleoresins of white pepper (*Piper nigrum* L.) was investigated in the present study. The white pepper essential oil has shown strong activity for the inhibition of primary and secondary oxidation products in mustard oil added at 0.02 % concentration which was evaluated using peroxide value and thiobarbituric acid value. Moreover it was further supported by complementary antioxidant assays such as ferric thiocyanate method in linoleic acid system, chelating and scavenging effects on 1,1′-diphenyl-2-picrylhydrazyl radical. In antimicrobial investigations, using inverted petriplate and food poison techniques, white pepper essential oil showed strong inhibition for *Fusarium graminearum* and *Penicillium viridicatum*. The white pepper ethanol and n-hexane oleoresin showed moderate inhibition for all tested fungal strains. Gas chromatography–Mass spectrometry (GC–MS) technique was used to analyze 40 different components constituting approximately 97.7 % of the volatile oil. Among them β-caryophyllene (16.0 %), sabinene (12.6 %), limonene (11.9 %) and torreyol (9.3 %) were the major components with many minor components. Both ethanol and *n*-hexane oleoresins comprise of 26 components having piperine, as the major component.

Keywords Antioxidant · Antimicrobial · Oleoresin · Essential oil · Scavenging effects

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Introduction

Free radicals either formed by cellular metabolism, exogenous chemicals or stress are capable of oxidizing biomolecules which may cause many diseases, including cancer, diabetes, cardiovascular and neurodegenerative diseases. Antioxidants are substances that neutralize these radicals or their actions. Therefore, dietary intake of antioxidant compounds is important for human health. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commonly used to protect oxidative deterioration. However, synthetic antioxidants have shown some toxic effects, such as carcinogenic and hepatotoxic activities [1]. Spices are natural food additives which contribute immensely to the taste of our foods. From ancient times they have been used to enliven our food. Spices possess medicinal as well as nutritional properties. They have been effectively used as one of the most important constituents in the medical field worldwide. They have ability to stimulate digestion and have antioxidant and anti-inflammatory potential [2]. Keeping in mind the potency of spices for medicinal and nutritional uses recent researches are focused on the isolation and identification of compounds from natural products with high antioxidant capacities [3]. One of such natural products is peppercorn, which has culinary applications as well as health benefits.

Peppercorns are the berries of *Piper nigrum* and *Piper guineense* and are also known as African black pepper or Ashanti pepper. They are used as spices and preservatives. They also have applications as insecticides and are used in herbal medicine and in the cosmetic industry [4–6]. Peppercorns are usually white or black depending on the time of harvest [7]. The white peppercorn is produced from fully ripe berries, whereas the black peppercorn is produced

Published online: 11 January 2013

from unripe but fully developed berries. Chemically, peppercorn contains lignans, alkaloids, flavonoids, aromatic compounds, and amides [8, 9]. Based on the usefulness and importance among all the spices black pepper is commonly referred as "The King of Spices". It is valued for its flavor, aroma, nutritional and medicinal uses making it an important commodity. No such data is available for white pepper, therefore, it was thought to investigate chemistry, antioxidant and antimicrobial studies on white pepper essential oil and oleoresins.

Material and Methods

Chemicals and Microbial Cultures

The chemicals used TBA (Thiobarbituric acid), DPPH (1,1′-diphenyl-2-picrylhydrazyl radical) and linoleic acid are of Acros (New Jersey, USA). BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene) and PG (propyl gallate) of SD Fine Chem Ltd. (Mumbai, India). Tween 20 and Ferrozine of Merck Pvt.Ltd. (Mumbai, India) and streptomycin of Ranbaxy fine chemicals Ltd. were used. All chemicals and solvents used were of analytical grade. Crude mustard oil was purchased from local oil mill of Gorakhpur, India.

In order to determine the antimicrobial efficacy of the volatile oil and oleoresins, various food-borne fungi were used such as *Aspergillus niger* (*AN,* 2479), *Aspergillus flavus* (*AF,*1884), *Aspergillus oryzae* (*AO*,1846), *Fusarium monoliforme* (*FM,*2088), *Fusarium graminearum* (*FG,*1893) and *Pencillium viridicatum* (*PV,* 2007) and bacteria such as *Bacillus subtilis* (*BS,*1790), *Staphylococcus aureus*(*SA,* 3103), *Escherichia coli* (*EC,* 1672), *Pseudomonas aeruginosa* (*PA,*1942) which were supplied by Microbial Type Culture Collection (MTCC), (Chandigarh, India).

Extraction of Essential Oil and Oleoresins

The fresh and mature berries of white pepper (*Piper nigrum* L.)were washed, sun dried and pulverized into a fine powder. 150 g of spice powder was subjected to hydrodistillation in a Clevenger's type apparatus for 6 h according to method recommended by European Pharmacopoeia [10] to obtain white pepper essential oil (WPEO). 30 g of spice was loaded on the Soxhlet's apparatus and extracted with the solvent viz., ethanol and *n*-hexane (250 mL) for 3 h to obtain an oleoresin. After complete extraction, the solvents were distilled off and viscous white pepper ethanol oleoresin having yield of 5.7 % (WPET) and white pepper *n*-hexane oleoresin having yield of 3.2 % (WPNH) were obtained and stored at 4 ± 1 °C.

Chemical Investigations

WPEO, WPET and WPNH were subjected to the Gas Chromatography (GC) and GC–Mass Spectroscopy (GC–MS) for analysis using Hewlett-Packard gas chromatograph (Model-6890) coupled with a Quadruple Mass Spectrometer 5973 and a Perkins Elmer Elite-5MS capillary column [5 % phenyl methyl siloxane; (30 m × 0.25 mm × 0.25 μm)]. The temperature for interphase, ion source and selective mass detector were maintained at 280, 230 and 150 °C respectively. The carrier gas (He) was at a flow rate of 1.0 mL/min. The oven temperature was programmed as follows:

For essential oil: 60 °C for 1 min; then increased from 60 to 185 °C at the rate of 1.5 °C min^{-1} and held at the rate of 9 °C/min and held at 275 °C for 2 min.

For oleoresins: 60 °C for zero min; then increased from 60 to 300 °C at the rate of 1.5 °C min^{-1} and held at the rate of 5 °C min^{-1} and held at 300 °C for 10 min.

Identification of Components

Most of the components were identified on the basis of comparison of their retention indices and mass spectra with published data [11–13] and computer matching was done with the Wiley 275 and National Institute of Standards Technology libraries (NIST Mass spectral search program) provided with the computer controlling GC–MS systems. The retention indices were calculated using a homologous series of *n*-alkanes C_8–C_{18} and C_8–C_{22} for essential oil and oleoresins respectively which are reported in Tables 1 and 2.

Evaluation of Antioxidant Activity

Sample Preparation

The white pepper oil and oleoresins were added individually at the concentration of 200 ppm (v/v). Synthetic antioxidants such as BHT, BHA and PG were also added to mustard oil at the same concentration, i.e., 200 ppm (w/v). An equal quantity of mustard oil without having any additives was taken as a control sample.

Peroxide Value

The peroxide value (PV) measures the total peroxide and hydroperoxides content of the mustard oil samples. The peroxide value was measured at regular intervals of seven days during the incubation period of 28 days, performed according to the procedure prescribed earlier [14]. A 5 g of mustard oil sample was dissolved in 30 mL of glacial acetic acid-chloroform (3:2) and then mixed with 0.5 mL of saturated potassium iodide solution. After one min. 30 mL distilled water was added to titrate against 0.01 N

Table 1 Chemical composition of white pepper (*Piper nigrum*. L) essential oil (WPEO) analyzed by GC–MS

Compounds	%	RI[a]	Identification
α-Thujene	0.8	919	MS, RI
α-Pinene	2.5	928	MS, RI, co-GC
Camphene	Trace	942	MS, RI, co-GC
Sabinene	12.6	967	MS, RI, co-GC
β-Pinene	7.3	973	MS, RI, co-GC
Myrcene	0.9	984	MS, RI
α-Phellandrene	0.4	1,003	MS, RI
3-Carene	0.3	1,006	MS, RI, co-GC
α-Terpinene	0.2	1,012	MS, RI, co-GC
p-Cymene	0.2	1,019	MS, RI, co-GC
Limonene	11.9	1,024	MS, RI, co-GC
β-Phellandrene	2.2	1,025	MS, RI, co-GC
γ-Terpinene	0.4	1,050	MS, RI, co-GC
cis-Sabinene hydrate	0.6	1,063	MS, RI
Terpinolene	Trace	1,078	MS, RI, co-CG
Linalool	1.5	1,096	MS, RI, co-GC
trans-Sabinene hydrate	0.3	1,101	MS, RI
Terpinen-4-ol	3.9	1,172	MS, RI, co-GC
α-Terpineol	0.8	1,188	MS, RI, co-GC
cis-Piperitol	Trace	1,193	MS, RI
β-Elemene	1.0	1,338	MS, RI
α-Terpenylacetate	0.3	1,348	MS, RI, co-GC
α-Copaene	3.4	1,371	MS, RI
β-Cubebene	0.4	1,381	MS, RI
β-elemene	Trace	1,383	MS, RI
β-Caryophyllene	16.0	1,411	MS, RI
cis-Muurola-3,5-diene	Trace	1,446	MS, RI
α-Humulene	0.9	1,450	MS, RI
trans-Farnesene	0.4	1,456	MS, RI
Germacrene-D	Trace	1,482	MS, RI
Epi-cubebol	1.2	1,492	MS, RI
α-Muurolene	1.0	1,597	MS, RI
β-Bisabolene	7.4	1,502	MS, RI
Cubebol	4.4	1,516	MS, RI
δ-Cadinene	1.9	1,524	MS, RI
trans-Nerolidol	Trace	1,558	MS, RI
Caryophyllene oxide	1.6	1,579	MS, RI
T-muurolol	1.7	1,637	MS, RI
Torreyol	9.3	1,641	MS, RI
β-Bisabolol	Trace	1,684	MS, RI
Total	97.7 %		

Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector

Traces <0.05

[a] The retention index was calculated using a homologous series of *n*-alkanes C8–C18; co-GC: co injection with an authentic sample

Table 2 Chemical composition of white pepper (*P. nigrum* L.) oleoresins in ethanol (WPET) and *n*-hexane (WPNH)

Compounds	WPET	WPNH	RI[a]	Identification
Terpinen-4-ol	–	0.1	1172	MS, RI, co-GC
β-Caryophyllene	0.2	0.6	1411	MS, RI, co-GC
β-Bisabolene	0.1	0.4	1502	MS, RI
δ-Cadinene	0.1	0.1	1522	MS, RI
Caryophyllene oxide	Trace	0.1	1578	MS, RI
Curzerenone	Trace	Trace	1606	MS, RI
Torreyol	0.2	0.4	1640	MS, RI
Pellitorin	0.4	0.4	1938	MS
1-Cinnamoyl piperidine	0.2	Trace	2090	MS
Plasticizer	0.3	1.1	–	MS
Piperanine	5.2	2.8	–	MS
Piperlonguminine	2.2	2.2	–	MS
Piperine isomer	1.2	2.1	–	MS
Piperine isomer	0.3	2.6	–	MS
N-Isobutyl-(2*E*, 4*E*, 12*E*)-octadecatrienamide	3.2	5.4	–	MS
N-isobutyl-(2*E*, 4*E*)-octadecadienamide	1.2	3.1	–	MS
Retrofractamide B	0.8	0.8	–	MS
Piperine = *E*,*E*-(*trans*–*trans*)-piperine	43.5	43.0	–	MS
4,5-Dihydropiperettine	1.2	2.4	–	MS
N-Isobutyl-(2*E*, 4*E*, 14*Z*)-eicosatrienamide	3.1	3.6		MS
Piperamide C9:1 (8*E*)	1.3	2.5	–	MS
Piperolein B	6.6	5.0	–	MS
Piperettine	0.4	1.4	–	MS
Sitosterol	0.7	1.4	–	MS
Dehydropipernonaline	2.0	1.5	–	MS
Guineensine	4.0	3.1	–	MS
Total	78.4	86.1		

Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector

WPET white pepper ethanol oleoresin, *WPNH* white pepper *n*-hexane oleoresin

Traces <0.05

[a] The retention index was calculated using a homologous series of *n*-alkanes C8–C18; co-GC: co injection with an authentic sample

sodium thiosulphate solution using starch as an indicator. Titration was continued, shaking the flask vigorously until the blue colour just disappeared. The peroxide value in milli equivalent per kilogram (Meq kg^{-1}) of sample was calculated as:

$$\text{Meq of peroxide/kg of oil} = \frac{S \times N \times 1000}{\text{Weight of oil}}$$

where S is volume of sodium thiosulphate consumed, N is normality of sodium thiosulphate solution and w is weight of sample in gram.

TBA Value

TBA value of different samples was determined according to the method reported previously [15]. 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 TBA in 100 mL of 1-butanol) 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 with Hitachi-U-2000 spectrophotometer (Tokyo, Japan). At the same time, a reagent blank (without TBA reagent) was also done. The thiobarbituric acid value (meq of malonaldehyde g^{-1}) was calculated as

$$\text{TBA value} = \frac{50 \times (A - B)}{100}$$

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

Complementary Antioxidant Assays

DPPH Radical Scavenging activity

The DPPH radical absorbs at 517 nm and the antioxidant activity can be determined by monitoring the decrease in this absorbance. The capacity of pepper oil, its oleoresins and synthetic antioxidants to scavenge the lipid soluble DPPH radical was observed at 517 nm by the method reported earlier [16]. For this, 1 mL methanolic solution of white pepper oil and oleoresins at different concentrations (5–20 μg/mL) were mixed with 4 mL of 0.004 % methanolic solution of DPPH. The absorbance was measured at 517 nm after 30 min. Control (without having any additive) and standards (containing synthetic antioxidants, viz., BHA, BHT and PG; in place of oil and oleoresins) were also subjected to same procedure for comparison. The capability to scavenge the DPPH radical was calculated using following equation:

$$\text{Scavenging activity } (\%) = \left(\frac{At - Ac}{Ac}\right) \times 100$$

where Ac is the absorbance of control and At is the absorbance of test samples.

Metal Chelating Activity

The ferrous ions chelating activity of pepper essential oil, its oleoresins and standards was investigated according to the method applied earlier [17]. Essential oil/oleoresin (50–200 μL) was added to the 0.05 mL aqueous of 2 mM $FeCl_2$. The reaction was initiated by the addition of 5 mM Ferrozine (0.2 mL) with shaking vigorously and then left for 10 min. After the mixture reached equilibrium, the absorbance was measured spectrophotometrically at 562 nm. A complex of Ferrozine-Fe^{2+}showed strong absorbance at 562 nm.

Ferric Thiocyanate (FTC) Method

The antioxidant activity of pepper oil and its oleoresins were compared to synthetic standards according to the FTC method in linoleic acid emulsion [18]. The reaction medium contained pepper oil or oleoresins at the concentration of 1 mg/100 mL of absolute ethanol (2 mL), an emulsion of 2.51 % linoleic acid in ethanol (2 mL), 4 mL of 0.05 M-Phosphate buffer (pH 7.0) and 2 mL of distilled water. The solution (10 mL) was mixed and incubated at 40 °C in dark. The same solution, without having any additives (oil or oleoresins) was taken as control sample. Synthetic antioxidants (BHA, BHT and PG) were used for the comparison, in the same manner. At regular intervals during incubation, 0.1 mL aliquot of the mixture was diluted with 9.7 mL of 75 % ethanol followed by the addition of 0.2 mL of 30 % ammonium thiocyanate and 0.1 mL, of 20 mM of $FeCl_2$ in 3.5 % HCl. The peroxide level of each sample was determined by reading absorbance at 500 nm in Hitachi-U-2000 spectrophotometer (Tokyo, Japan). These steps are repeated every 48 h until the control sample reached its maximum. Low absorbance value indicates the efficiency of the test samples to inhibit lipid oxidation.

Antimicrobial Investigations

Antifungal Activity

The selected strains such as *AN*, *AF, AO, FM, FG* and *PV* were grown on Czapeck Dox Agar médium and plates subjected to incubation at 37 °C. The antifungal activity of volatile oil and oleoresins was tested against fungi by inverted petriplate and food poison techniques [19].

In inverted petriplate technique, the required doses (5 and 10μL) of undiluted samples (pepper oil and oleoresins) were aseptically soaked on a pre-sterilized filter paper discs (Whatman No.1, 10 mm, d) and was kept on the lid of petriplate in inverted position at 37 °C for five days. In poison food technique, the required doses of each of the oil and oleoresins were mixed with 25 mL of the sterilized culture medium and the inoculums of fungal strains in the petriplate and were incubated at 37 °C in the control set oil/oleoresins were replaced by equal amount of water. Radial growths of fungal strains in terms of average diameter (mm) were recorded on the 5th day.

Antibacterial Activity

The antibacterial properties of pepper oil and its oleoresins were studied by agar well and disc diffusion methods [20]. The selected bacterial strains such as *BS, SA, EC* and *PA* were inoculated into 10 mL of sterile nutrient broth and incubated at 37 °C for 16–18 h. Using a sterile cotton swab, the broth cultures were swabbed on the sterilized nutrient agar plates. Agar wells were made with the help of sterilized cork borer with 8 mm diameter. Required doses (5 and 10 μL diluted in 1 mL DMSO) were delivered into them.

In disc diffusion technique, filter paper discs (Whatman No.1) of 8 mm diameter were prepared. These discs were aseptically placed over nutrient agar plates seeded with tested microorganisms. Pepper volatile oil and oleoresins (5 and 10 μL) were aseptically transferred to these discs. For standard, 0.2 mL of aqueous solution of streptomycin (10 mg/mL in DMSO) was used. All the plates were incubated in an upright position at 37 °C for 24 h. The diameters of inhibition zones (in mm) were purchased and averages of three replicates are reported.

Statistical Analyses

For the essential oil or oleoresin, three samples were prepared for assays of every antioxidant and antimicrobial attribute. The data are presented as mean (standard deviation of three determinations) (data are not shown). Statistical analyses were performed using a one-way analysis of variance [21]. A probability value of $p < 0.05$ was considered to be significant.

Results and Discussion

Chemical Composition

The analyses of essential oil and oleoresins obtained from white pepper (*P. nigrum* L.) by GC–MS were presented in Tables 1 and 2. A total of 40 components accounting for 97.7 % of the total amount of WPEO. β-caryophyllene was the major component in WPEO accounting for 16.0 % followed by sabinene (12.6 %), limonene (11.9 %), torreyol(9.3 %) and β-bisabolene (7.4 %). In volatile oil, β-bisabolol, germacrene-D, camphene, terpinolene and *cis*-piperitol was found in trace amount. The chemical composition (Table 2) of WPET and WPNH showed the presence of 26 components accounting for 86.1 and 78.4 % of total amount of oleoresins respectively. In both oleoresins, piperine is the major constituent having a fraction of 43.5 and 43.0 % respectively. Piperanine, *N*-isobutyl-(2*E*, 4*E*, 12*E*)-octadecatrienamide, piperolein B and guineesine are present in minor amount in both the oleoresin extract. Singh et al. [22]. has also observed that β-caryophyllene (29.9 %)and piperine (39.0–63.9 %)were the major component in the essential oil and oleoresins of black pepper respectively. The results of chemical investigations were slightly different from the previous reported work on black pepper where limonene (35.06 %) [23] and germacrene (11.01 %) [9] were the major components. The differences in components were found to be due to geographical factors [24], post crop processing and different nutritional status of the plant.

Antioxidative Assays in Mustard Oil

Figure 1 shows changes of the peroxide value in the mustard oil system with additives. The mustard oil oxidation was measured with storage for 28 days. During this time, the peroxide value of the control sample increased to 181.8 $meqkg^{-1}$by the final day which is significantly higher than for the samples containing essential oils and oleoresins. WPEO, WPET and WPNH showed a strong antioxidative effect ($p < 0.05$) in mustard oil, which could be comparable to BHA and BHT effects at the 6 mg level. However, it was less effective in comparison with PG at the6 mg level. During the oxidation process, peroxides are gradually converted into lower molecular weight compounds such as aldehydes and ketones. One such aldehyde, malondialdehyde, an index of lipid peroxidation was measured by the TBA method. Both essential oils and oleoresins were able to control the secondary oxidation process, which could be comparable to the effect of synthetic antioxidants at the 6 mg level (Fig. 2). The results obtained were in good agreement [22, 25] with the previous work reported in which black pepper essential oil and its oleoresins showed strong inhibition on the formation of secondary oxidation products in mustard oil.

Complementary Antioxidant Assay Evaluation

The decrease in absorbance of the DPPH radical owing to the scavenging capability of WPEO, WPET, WPNH and synthetic antioxidants (BHA, BHT and PG) is illustrated in Fig. 3. DPPH is a stable free radical and accepts an electron or hydrogen radical to convert into a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the absorbance at 517 nm in dose dependent manner. From Fig. 3, it is clear that at 20 μL concentration scavenging activity of essential oil reaches to maximum value, WPEO (92.45 %) which is much better than its oleoresins, BHT (41.2–73.4 %), BHA(75.0–92.1 %) but lower than that of PG (89.3–98.7 %) at all concentrations.

Lipid peroxidation contains a series of free radical mediated chain reaction processes. The ferric thiocyanate method measures the amount of peroxide during the initial stages of oxidation. In this assay, hydroperoxide produced

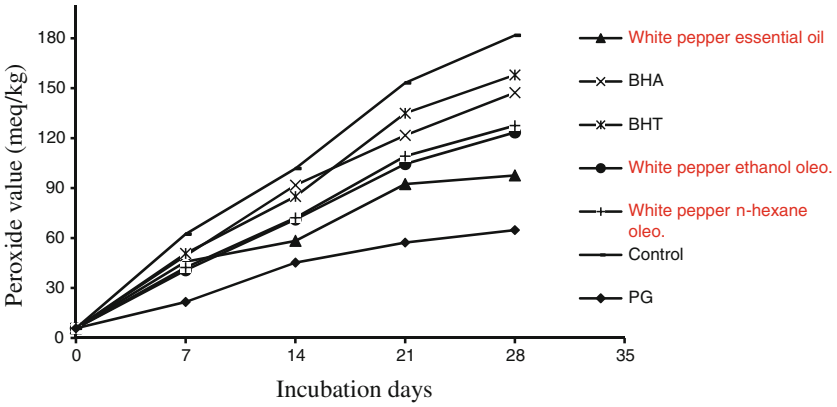

Fig. 1 Stabilization of mustard oil by white pepper essential oil (WPEO), white pepper ethanol oleoresin (WPET) and white pepper *n*-hexane oleoresin (WPNH) at 200 ppm with standards at 70 °C in terms of peroxide value

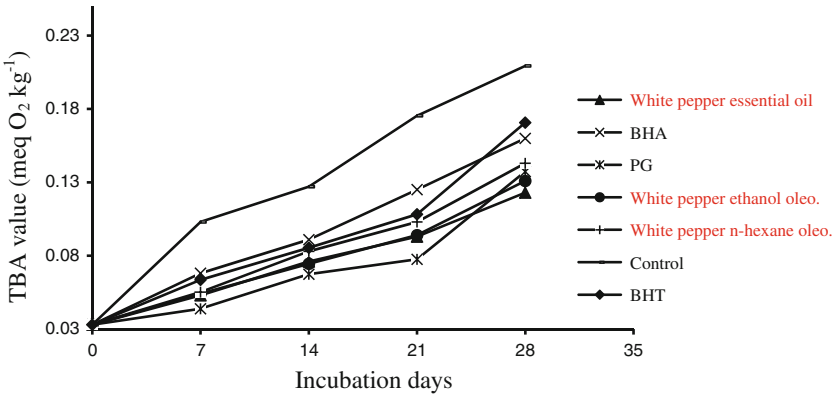

Fig. 2 Secondary oxidative effect of white pepper essential oil (WPEO), white pepper ethanol oleoresin (WPET) and white pepper *n*-hexane oleoresin (WPNH) at 200 ppm with standards at 70 °C in terms of TBA value

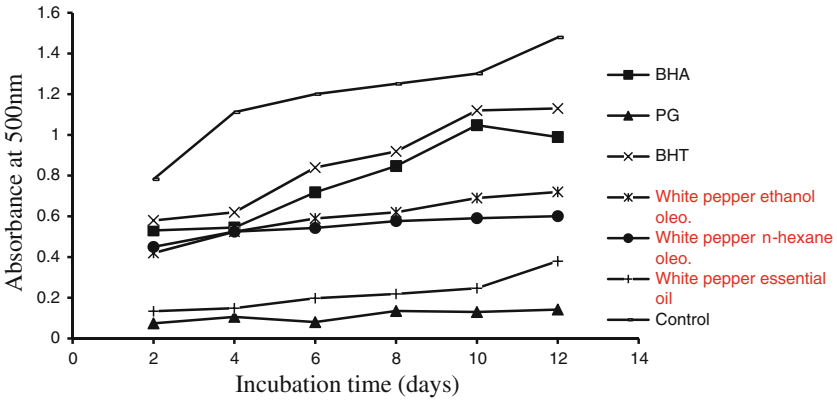

Fig. 3 Antioxidative potential of white pepper essential oil (WPEO), white pepper ethanol oleoresin (WPET) and white pepper *n*-hexane oleoresin (WPNH)on the primary oxidation of linoleic acid system measured using ferric thiocyanate method

by linoleic acid added to the reaction mixture gets oxidized by air during the experimental period, was indirectly measured. Low absorbance value indicates high level antioxidant activity. The control sample showed the highest content of peroxide while the PG sample was at lowest end. The essential oil and both oleoresins of white pepper exhibited effective and significant ($p \leq 0.05$) antioxidant activity than BHT and BHA but lower than PG during whole period of incubation (Fig. 4).

Ferrozine can quantitatively form complexes with Fe^{2+}. However, in the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. The transition metal ion, Fe^{2+} possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals [26].The chelating activity of the extracts was concentration dependent. WPEO exhibited higher chelating activity (up to 78 %) in comparison to the oleoresins but was not effective chelator as EDTA. Maximum chelating of metal ions at 200 µg/mL for WPEO and EDTA was found to be 73.05 and 91.2 % respectively whereas the oleoresins were less

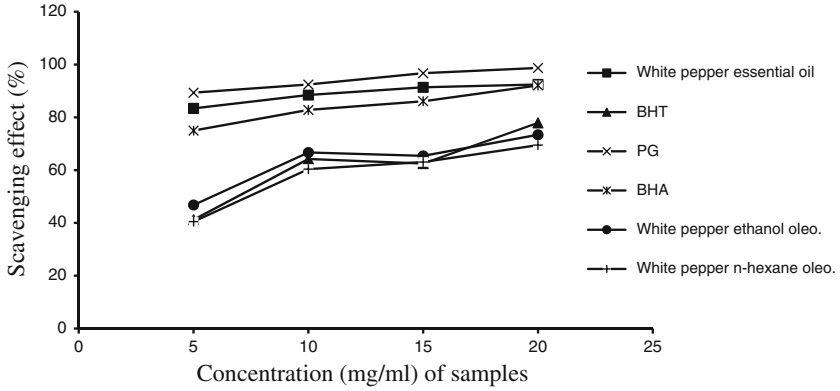

Fig. 4 Radical scavenging ability of white pepper essential oil (WPEO), white pepper ethanol oleoresin (WPET) and white pepper *n*-hexane oleoresin (WPNH) on DPPH radical

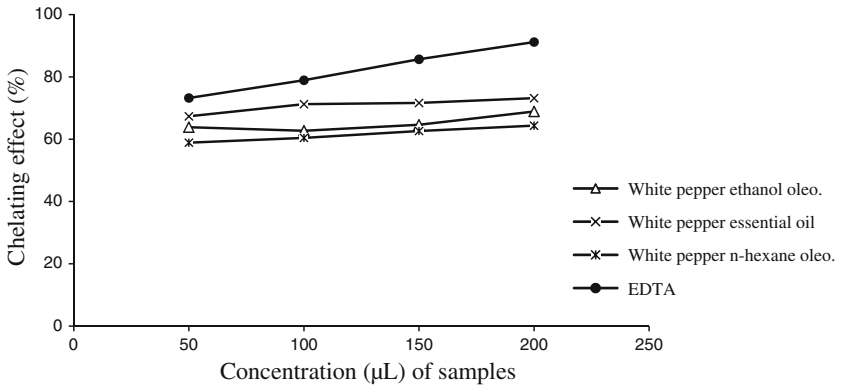

Fig. 5 Chelating effect of white pepper essential oil (WPEO), white pepper ethanol oleoresin (WPET) and white pepper *n*-hexane oleoresin (WPNH)

effective in metal chelation and their metal chelating activity ranges from (58.8 to 68.9 %) (Fig. 5).

The results obtained during the whole antioxidant assays were well correlated with the previous reported work performed by many workers [22, 25]. According to the reported work, the presence of β-caryophyllene, sabinene, β-bisabolene, limonene and α-pinene [27] are responsible for antioxidant activity of essential oil. GC–MS studies revealed the higher percentage of sesquiterpenes and monoterpenes in volatile oil. The essential oil of lemon balm (*Melissa officinalis* L.) showed an antioxidant and free radical scavenging activity with the most powerful scavenging constituents comprising of monoterpenes and sesquiterpenes [28]. Presence of piperine, flavonol glycosides and phenolic amides are responsible for the activity of oleoresins. Earlier studies reported the isolation of phenolic amides including guineesene, piperolein, and *N*-isobutyl-(2E, 4E, 12E)-octadecatrienamide. Piperine is the major component in both oleoresins and has considerable antioxidant activity [29]. The antioxidative activity of separate phenolic amides may not be as effective as when they are combined as a whole in [30] both oleoresins. Previous phytochemical studies of this genus led to the isolation of lignans, amides, alkaloids, flavonoids and aromatic compounds [31]. The phenolic amides which possessed significant antioxidant activities that were more effective than the naturally occurring antioxidant, α -tocopherol in their application in food preservation [32]. Most antioxidative substances work synergistically to produce a broad effect against lipid peroxidation. For the antioxidant activity of essential oil it is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [33].

Antimicrobial Investigations

The results of antifungal evaluations for pepper volatile oils and oleoresins are obtained by food poison and inverted petriplate methods as shown in Tables 3 and 4. In inverted plate technique, WPEO showed strong activity against FG at 10 μL dose concentration. WPEO showed good zone of mycelial inhibition for FG, the inhibition is upto 85 %. WPEO was found to be most effective against the tested fungal strains in both methods. In inverted plate technique, WPEO showed strong activity against FG at 10 μL dose concentration. For FG, zone of mycelial inhibition by WPEO is up to 85 %. Both WPET and WPNH showed moderate to good activity against tested strains. Moreover, in food poisoned method, WPEO was found to be highly effective in controlling the growth of FG and PV as more than 80 % mycelial zone inhibition was obtained.

Table 3 Antifungal investigations (% zone inhibition) of white pepper essential oil and its oleoresins using inverted petri plate method

Samples	Doses (µl)	*Aspergillus niger* (*AN*)	*Aspergillus flavus* (*AF*)	*Aspergillus oryzae* (*AO*)	*Fusarium monoliforme* (*FM*)	*Fusarium graminearum* (*FG*)	*Penicillium-viridicatum* (*PV*)
White pepper essential oil (WPEO)	5	25.7 ± 0.20	35.4 ± 0.30	19.4 ± 0.56	12.7 ± 0.50	65.4 ± 0.14	63.0 ± 0.6
	10	47.3 ± 0.26	47.3 ± 0.36	30.6 ± 0.52	20.1 ± 0.20	81.3 ± 0.17	85.4 ± 0.7
White pepper ethanol oleoresin (WPET)	5	37.1 ± 0.20	25.3 ± 0.20	32.8 ± 0.36	–	47.3 ± 0.20	62.9 ± 0.3
	10	46.4 ± 0.30	42.6 ± 0.30	46.3 ± 0.30	12.7 ± 0.3	52.4 ± 0.14	69.7 ± 0.40
White pepper *n*-hexane	5	32.4 ± 0.44	24.3 ± 0.17	28.0 ± 0.32	–	46.4 ± 0.36	39.3 ± 0.54
oleoresin (WPNH)	10	42.7 ± 0.46	39.7 ± 0.14	42.6 ± 0.40	10.8 ± 0.1	49.3 ± 0.41	44.7 ± 0.6

Average of three replicates

– no inhibition

Table 4 Antifungal investigations (% zone inhibition) of white pepper essential oils and its oleoresins using food poisoned method

Samples	Doses (µl)	*Aspergillus niger* (*AN*)	*Aspergillus flavus* (*AF*)	*Aspergillus oryzae* (*AO*)	*Fusarium monoliforme* (*FM*)	*Fusarium graminearum* (*FG*)	*Penicillium viridicatum* (*PV*)
White pepper essential oil (WPEO)	5	16.2 ± 0.30	29.1 ± 0.40	18.4 ± 0.36	9.1 ± 0.10	50.0 ± 0.70	48.6 ± 0.42
	10	25.7 ± 0.17	33.2 ± 0.20	22.7 ± 0.42	12.7 ± 0.00	65.7 ± 0.57	67.3 ± 0.36
White pepper ethanol oleoresin (WPET)	5	11.7 ± 0.42	15.6 ± 0.35	11.3 ± 0.17	–	37.2 ± 0.20	30.7 ± 0.17
	10	23.6 ± 0.43	22.1 ± 0.32	13..5 ± 0.40	–	44.1 ± 0.14	41.2 ± 0.30
White pepper *n*-hexane oleoresin (WPNH)	5	9.4 ± 0.44	13.1 ± 0.70	10.5 ± 0.44	–	34.7 ± 0.17	27.3 ± 0.14
	10	21.3 ± 0.40	20.7 ± 0.57	12.8 ± 0.46	–	40.6 ± 0.32	39.6 ± 0.21

Average of three replicates

– no inhibition

Table 5 Antibacterial activity of white pepper essential oil and its oleoresins against a few bacterial species using agar well diffusion method

Diameter of inhibition zone (mm)[a]					
Samples	Doses (µl)	*Bacillus subtilis*	*Staphyllococcus aureus*	*Escherichia coli*	*Pseudomaonas aeruginosa*
White pepper essential oil	5	11.1 ± 0.37	17.5 ± 0.40	9.3 ± 0.31	10.1 ± 0.17
	10	14.5 ± 0.57	19.1 ± 0.44	11.9 ± 0.14	12.7 ± 0.15
White pepper ethanol oleoresin	5	5.7 ± 0.81	8.4 ± 0.36	–	–
	10	8.1 ± 0.20	10.6 ± 0.42	–	–
White pepper *n*-hexane oleoresin	5	4.7 ± 0.31	7.4 ± 0.57	–	–
	10	6.4 ± 0.42	9.2 ± 0.56	–	–
Streptomycin	5	11.4 ± 0.32	12.7 ± 0.36	–	–
	10	13.5 ± 0.2	14.3 ± 0.47	–	–

[a] Average of three replicates

The antibacterial investigation results are given (Tables 5 and 6) using Agar well diffusion and Disc diffusion method, in which tested bacterial strains showed different levels of sensitivity towards WPEO, WPET and WPNH at different doses. In both agar well diffusion and disc diffusion methods, gram positive bacteria including *BS* and *SA* showed good level of sensitivity towards WPEO whereas the level of sensitivity of gram negative bacteria including EC and PA is very less for WPEO, WPET and WPNH. Using both methods, volatile oil has shown better results in comparison with oleoresin and commercial bactericide, i.e., streptomycin. The antibacterial activity of standard streptomycin was also tested and they showed moderate to good antibacterial activity against *BS*, *SA*, *EC* and *PV*. The resistance of gram negative bacteria towards tested samples is related to lipopolysaccharides in their outer membrane [34].

The results obtained from the antimicrobial investigation were in good agreement with the previous reported work

Table 6 Antibacterial activity of white pepper essential oil and its oleoresins against a few bacterial species using disc diffusion method

Diameter of inhibition zone (mm)[a]					
Samples	Doses (μl)	*Bacillus subtilis*	*Staphyllococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*
White pepper essential oil	5	21.1 ± 0.32	27.4 ± 0.40	15.1 ± 0.20	16.7 ± 0.17
	10	25.7 ± 0.42	29.3 ± 0.20	18.7 ± 0.52	18.1 ± 0.15
White pepper ethanol oleoresin	5	23.7 ± 0.42	19.7 ± 0.14	–	–
	10	25.1 ± 0.43	21.2 ± 0.17	–	–
White pepper *n*-hexane oleoresin	5	9.3 ± 0.36	18.7 ± 0.33	–	–
	10	13.1 ± 0.40	20.4 ± 0.14	–	–
Streptomycin	5	13.6 ± 0.44	16.3 ± 0.41	–	–
	10	15.7 ± 0.42	18.4 ± 0.47	–	–

[a] Average of three replicates

[25]. The marked antimicrobial efficacy of WPEO is believed to be due to presence of higher percentage of sesquiterpenes and monoterpenes like β-caryophyllene, sabinene, limonene and torreyol [35].These essential oils are a complex of volatile compounds and may exhibit the potent antimicrobial activity by a single major compound or by the synergistic or antagonistic effect of various compounds [36]. Research into the antimicrobial effects of monoterpenes suggests that they diffuse into cells and damage cell membrane. Earlier reports supported that piperine and various amide bearing isobutyl, pyrrolidone, piperidine moieties present in smaller amount have pharmacological and antifungal activity [37].

Conclusion

Chemical investigations revealed that β-caryophyllene is major component of WPEO and piperine as major component in both oleoresins (WPET and WPNH). WPEO and both oleoresins of white pepper were found to be effective antioxidants. In addition they show remarkable antimicrobial activity. So it is inferred that if applied to food products, they could be used as natural food preservatives.

Acknowledgments The authors are grateful to the Head, Department of Chemistry, DDU Gorakhpur University, Gorakhpur for providing laboratory facilities. The financial support as JRF from UGC to Sunita Singh and Emeritus fellow to Dr. Gurdip Singh is also acknowledged.

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