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Chemical Constituents, Antioxidative and Antimicrobial Activities of Essential Oil and Oleoresin of Tailed Pepper (*Piper Cubeba L*)

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Abstract

The antimicrobial and antioxidant potentials of hydrodistilled essential oil and oleoresin (obtained using acetone as a solvent) of tailed pepper were carried out by different techniques. The results obtained from antioxidant activity measurements of essential oil and oleoresin against mustard oil were measured for duration of 28 days in terms of peroxide, thiobarbituric acid, total carbonyl and p-anisidine values. The results obtained from butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) was similar to both the oleoresin and essential oil. In addition, the inhibitory action in linoleic acid system was studied by monitoring accumulation of peroxide concentration. The radical scavenging capacity of both essential oil and oleoresin on 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical were (71.2%) and (69.77%) respectively at 25 μL/mL. It was relatively lower in comparison with synthetic antioxidants (BHA-96.41%; BHT- 95.91%). The results obtained from reducing power, chelating effect and hydroxyl radical scavenging effect was also supported the antioxidant of essential oil and oleoresin. The tailed pepper essential oil and oleoresin showed 100% mycelial zone inhibition against Penicillium viridicatum at 3000 and 2000 ppm respectively in the poison food method. It is interesting to note that the essential oil revealed 100% clear zone inhibition against Aspergillus flavus at all tested concentrations. The chemical characterization of tailed pepper essential oil by GC and GC-MS resulted in the identification of 44 components accounting for 97.8% of the oil.

KEYWORDS: Piper Cubeba, essential oil composition, antioxidant properties, antimicrobial activity

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1. Introduction

Lipid oxidation, a general term for a multifactorial process that results in the generation of oxidation products, reduces the shelf-life of foods and is responsible for the loss of nutritional and organoleptic quality. The prime cause of this oxidative transformation is the action of free radicals, which initiate the degradation of unsaturated fat producing volatile compounds (Wang et al., 1995). Particularly susceptible to rancidity are foods containing high concentration of prooxidants (transition metals, heme-containing proteins, etc.,) and large amounts of polyunsaturated fatty acids (Kanner, 1994). Traditionally, synthetic compounds such as butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate (PG) are used as antioxidants in lipid and lipid containing food materials. However, some of these compounds have been questioned with regard to their safety (Bran, 1975; Whysner et al., 1994). Therefore, there is an increasing interest in natural food additives such as spices or spice extracts (oleoresins), which can function as natural antioxidants besides seasoning the food. It is well known that herbs and spices possess antioxidant activity (Madsen and Bertelsen, 1995; Schwarz et al., 2001). Plants produce a variety of antioxidants against molecular damage from reactive species which could play a preventive role due to their antioxidant properties. Volatile components of essential oils from both seeds and leaves have been reported to inhibit growth of a number of microorganisms (Delaquis et al., 2002). Among the spices studied, it seems that very little work has been carried on tailed pepper. As a part of our ongoing research programme (Singh et al., 2004; 2005; 2005a) concerning the use of various plant species as a source of biologically interesting compounds, our present research was focused on the antioxidant and antimicrobial activities of the essential oil and oleoresin from tailed peeper. Several analytical methods have been developed to determine the antioxidant capacity of natural substances in vitro (Ragnarsson and Labuza, 1977; Khal and Hilderbrant, 1986; Huang and Frankel, 1997; Robards et al., 1999). However, the total antioxidant activity of plant extracts cannot be evaluated by using a single method due to the complex composition of phytochemicals. So, we used different methods to assess antioxidant as well as antimicrobial properties of the volatile oil and oleoresin of tailed pepper.

2. Materials and Methods

2.1. Chemicals

Diphenylpicrylhydrazyl ($DPPH^{\cdot}$) and carbendazim were bought from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany); linoleic acid from Acros (New Jersey, USA). BHT, BHA, and 2, 4- dinitrophenylhydrazine were purchased from

s. d fine-chem Ltd, Mumbai, India. Thiobarbituric acid from Merck, Mumbai and Cloxacillin was purchased from Ranbaxy Fine chemicals Ltd., New Delhi, India. Crude mustard oil was purchased from a local oil mill, Gorakhpur, India. All solvents used were of analytical grade.

2.2. Extraction of Essential oil and oleoresin

Seeds of tailed pepper were purchased from a local spice market of Gorakhpur, Uttar Pradesh, India, during Jan 2004 and voucher specimens were kept at the Herbarium of the faculty of Science, DDU Gorakhpur University, Gorakhpur. 250g of tailed pepper (100 mesh particle size) seeds were subjected to hydrodistillation using a Clevenger apparatus to yield the essential oil (2.3 %).

Oleoresin was obtained by extracting 30g of powdered spice with 300 mL of acetone for 3h in a Soxhlet extractor. The solvent was evaporated in a vacuum drier under reduced pressure. A viscous extract with 5.9 % yield was obtained. Both essential oil and oleoresin were kept in the refrigerator until further use.

2.3. Analysis of tailed pepper essential oils and oleoresins by gas-chromatography (GC)

A Hewlett Packard 5890 (Analytical Technologies SA, Buenos Aires, Argentina) gas chromatograph fitted with a capillary column (5% phenyl methylsiloxane; length = 30m, inner diameter = 0.32mm and film thickness = 0.25 μm) whose injector and detector (FID) temperatures were maintained at 240 and 250^0C respectively. Injection volume was 1μL with a split ratio 30:1 and helium was used as a carrier gas with a flow rate of 1.0 mL min^{-1}. The oven temperature for both essential oil and oleoresin was programmed linearly as follows: 60^0C to 185^0C at 1.5^0C min^{-1} then held for 1 min then again started at 9^0C min^{-1} to 275^0C.

2.4. Analysis of tailed pepper essential oils and oleoresins by gas chromatography-mass spectrometry (GC-MS)

Analysis of tailed pepper essential oil and oleoresin were run on a Hewlett Packard (6890)GC-MS system (Analytical Technologies SA, Buenos Aires, Argentina) coupled to a quadrupole mass spectrometer (model HP 5973) with a capillary column of HP-5MS (5% phenyl methylsiloxane, length = 30m, inner diameter = 0.25mm and film thickness = 0.25 μm). The injector, GC-MS interface, ion source and selective mass detector temperatures were maintained at 280, 280, 230 and 150^0C respectively. Injection volume was 1μL with a split ratio 30:1 and helium was used as a carrier gas with a flow rate of 1.0 mL min^{-1}. The oven temperature for essential oil was programmed linearly as follows: 60-185^0C (1.5^0C min^{-1}), 185^0C (1 min), 185-275^0C (9^0C min^{-1}), 275^0C (2 min).The oleoresin was held at 70^0C (5 min), 70-220^0C (3^0C min^{-1}), 220^0C-280^0C (5 ^{0}C

min^{-1}) and held at 280^0C for 5 min. For RI measurements, the oven temperature program suggested by Adams (2001) (60 °C to 246 °C at 3 °C/min) was used.

2.5. Components identification

The components percentage was taken from capillary GC traces with FID. Identification of the individual components of tailed pepper essential oil and oleoresin was based on a) comparison of their mass spectra and retention indices on a HP-5 column with published data (Massda, 1976; Adams, 2001); b) computer matching with the WILEY 275 and National Institute of Standards Technology (NIST 3.0) libraries provided with the computer controlling GC-MS system; c) by co-injection with authentic samples (wherever possible). The retention index was calculated using a homologous series of n-alkanes C_8-C_{16}.

3. Antioxidative activity in mustard oil

3.1. Sample preparation

The essential oil and oleoresin of tailed pepper were added individually to (refined) mustard oil at levels of 0.02 % (v/v). The initial PV value of oil is 1.2 meq of O_2/kg. Synthetic antioxidants (BHA and BHT) were also added to the mustard oil at levels of 0.02 % (v/v). Oxidative deterioration was periodically assessed by the measurement of peroxide (PV), thiobarbituric acid (TBA), total carbonyl (TC) and *p*-anisidine values of the oil substrate.

3.2. PV and TBA values

The rate of oil oxidation was assessed by the increase of peroxide values. 2 ± 0.1 g of each oil sample was weighed and subjected to a previously reported method (AOCS, 1990). The TBA value of the samples was measured as previously described (Kikuzaki and Nakatani, 1993) with minor modifications. 5 ± 0.1 g of oil substrate was weighed and 20 mL of aq. 0.67% of TBA and 20 mL of benzene were added. The mixture was shaken in a mechanical shaker for 2h and boiled in a water bath for an hour. After cooling, the organic layer absorbance was measured at 540 nm using a Hitachi-U-2000 UV-visible spectrometer.

3.3. Total carbonyl (TC)and *p*-anisidine values (AV)

Carbonyl and *p*-anisidine values were assessed according to methods described earlier (Frankel, 1998; AOCS, 1998). A calibration curve was drawn using capraldehyde (50- 250 μg) in 50 mL of carbonyl free benzene instead of the test sample. The TC values of the samples were calculated using the calibration curve and expressed as milligrams of capraldehyde per 100 g of sample. For preparation of solutions, carbonyl free benzene and alcohol were used. In order to assess p-ansidine value of oil substrate 0.6 ± 0.1g of mustard oil was weighed in a 50 mL

volumetric flask and volume was made up with iso-octane; 5 mL of this solution were transferred into a test tube and 1 mL of 0.25 % *p*-anisidine reagent was added. Then it was kept in the dark for 10 min. The absorbance was measured at 350 nm (A_2). In the same manner, without the addition of reagent, the absorbance was measured at 350 nm (A_1). The values were calculated using the following formula

$$AV = [50 \times 1.2 (A_2 - A_1)] / 10 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

4. Antioxidant assays

4.1. Antioxidant activity in linoleic acid system

The test samples of essential oil or oleoresin (20 μL) were pre-dissolved in 1 mL of ethanol (v/v) mixed with linoleic acid (2.5 %, v/v), 99.5% ethanol (4 mL, v/v) and 0.05 M phosphate buffer (pH =7, 4 mL). The solution was incubated at 40^0C for 175 hrs and the degree of oxidation was measured for every day using the ferric thiocyanate method (Sidwell et al., 1954) 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. Lesser the value of absorbance indicates higher the value of antioxidant capacity.

4.2. DPPH assay

The DPPH assay was carried out as described by Singh and his co-workers (2004). 5, 10, 15, 20, 25 μL of essential oil or oleoresin, individually dissolved in 1 mL of methanol were added to 5 mL of 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. The assay was carried out in triplicate and analyses of all samples were run in duplicate and the results are averaged.

4.3. Reducing power

The reducing power was carried out as described before (Oyaizu, 1986). Various amounts (5, 10,15, 20 μL) of essential oil or oleoresin (dissolved in 2.5 mL of methanol) were mixed with 2.5 mL of 200 mM phosphate buffer (pH = 6.6) and 2.5 mL of 1 % potassium ferricyanide, and the mixture was incubated at 50 ^{0}C for 20 min. After adding 2.5 mL of 10 % trichloroacetic acid, the mixture was centrifuged at 200 xg for 10 min in a Sigma 3K30 model centrifuger. The organic layer (5 mL) was mixed with 5 mL of deionised water and 1 mL of 0.1 % ferric chloride and the absorbance read at 700 nm in a UV-visible spectrophotometer.

4.4. Chelating power of ferrous ions

Chelating power was determined according to the method of described earlier (Shimada et al., 1992). To 2 mL of the mixture, consisting of 30 mM hexamine, 30 mM potassium chloride and 9 mM ferrous sulfate were added to 5, 10, 15, 20, 25 μL of essential oil or oleoresin in methanol (5 mL) and 200 μL of 1mM tetramethyl murexide. After 3 min at room temperature, the absorbance of the mixture was determined at 485 nm. A lower absorbance indicates a higher chelating power. EDTA was used as a positive control.

4.5. Hydroxyl radical scavenging activity

It was carried out by measuring the competition between deoxyribose and the sample for hydroxyl radicals generated from the Fe^{3+} / EDTA / H_2O_2 system. The attack of the hydroxyl radical to deoxyribose leads to TBA reactive substances formation (Kunchandy and Rao, 1990). 5, 10, 15, 20, 25 μL of the sample (essential oil or oleoresin) were added to the reaction mixture containing 3 mM deoxyribose, 0.1 mM $FeCl_3$, 0.1 mM EDTA, 0.1 mM ascorbic acid, 1 mM H_2O_2 and 20 mM phosphate buffer (pH 7.4) making up final volume of 3 mL. The reaction mixture was incubated at 37^0C for 1h. The formed TBARS were measured by the method reported earlier (Ohkawa et al., 1979). One mL of 1% TBA and 1 mL of 2.8% trichloroacetic acid were added to test tubes and incubated at 100^0C for 20 min. After cooling, absorbance was measured at 532 nm against a blank containing deoxyribose and buffer. Reactions were conducted in triplicate. The percent inhibition (I) of deoxyribose degradation was measured.

$$I = [(A_0-A_1) / (A_0)] \times 100 \quad (2)$$

A_0- absorbance of the control; A_1- Absorbance of essential oil or oleoresin

5. Antimicrobial activity

5.1. Antifungal assay

The essential oil or oleoresin was individually tested against a panel of food-borne fungi such as *Aspergillus niger* (MTCC 2479), *Penicillium purpurogenum* (MTCC 1786), *Penicillium madriti* (MTCC 3003), *Acrophialophora fusispora* (MTCC 341), *Penicillium viridicatum* (MTCC 2007) and *Aspergillus flavus* (MTCC 1884). The antifungal activity was assessed by two different methods.

5.2. Poison food technique

This method plays an important role in bioassay methods (Ramdas et al., 1998) to evaluate antimicrobial activity. The calculated amount of sample was added to molten Czapek dox agar (CDA) medium (≈45^0C) to yield the desired

concentration. The pathogen of interest from the growing tips (punched in fungal mat grown on CDA media in sterile Petri dishes) was placed at the center of the Petri dish and allowed to grow and all plates were incubated at 37^0C for 24 h. Radial growth in terms of diameter (mm) was examined after 5 days.

5.3. Inverted Petri dish method

This method (Amvam zolla et al., 1998) allows the determination of antifungal activity of compounds in the vapour phase. In this method, calculated quantity of sample or oleoresin was soaked on filter paper (Whatman No. 1, 10 mm, diameter) and kept at the center of the lid of the inverted Petri dish. Control plates were prepared under the same conditions excepting addition of test compound. All the plates were incubated at 37^0C for 24 h and results were measured in terms of diameter (mm) after 5 days.

5.4. Antibacterial assay

The following food-borne bacteria, *Staphylococcus aureus* (MTCC 3103), *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (MTCC 1790) , *Escherichia coli* (MTCC 1672), *Salmonellae typhi* (MTCC 733), *Pseudomonas aeruginosa* (MTCC 1942) procured from Microbial Type Culture Collection (MTCC), Chandigarh, India were used for the present study. Agar well diffusion method is used to investigate antimicrobial properties of both essential oil and oleoresin. The strains were activated on nutrient agar media and stored at 4^0C. The bacterial strain was further diluted using Ringer's solution. 1000, 2000, 3000 ppm concentration solutions of essential oil and oleoresin were prepared in absolute ethanol. 50 µL aliquots of diluted sample were placed into agar wells (9mm, d) and the activity was measured according to method developed by Davidson (Davidson and Parish, 1989). The plates were incubated at 37^0C for 24 h. All the plates were replicated twice and the results were averaged.

6. Statistical analysis

For the essential oil or oleoresin, three samples were prepared for assays of every antioxidant attribute. The data were presented as mean ± standard deviation of three determinations (data were not shown). Statistical analyses were performed using a one-way analysis of variance (Sokal, 1973). A probability value of $p < 0.05$ was considered significant.

7. Results and Discussion

7.1. Chemical Characterization

GC and GC-MS analysis of tailed pepper essential oil (Table 1) led to the identification of 44 components. The oil was characterized by large amounts of sesquiterpene hydrocarbons (48.4%) and monoterpenes (36.3%) accompanied by minor amounts of oxygenated monoterpenoids (7.2%) and oxygenated sesquiterpenoids (5.9%). Sabinene (19.4%), β-cubebene (18.3%), α-copaene (8.8%), β-phellandrene (5.9%), linalool (4.9%) and cubebol (4.7%) were the main components of the essential oil (Table 1). The oleoresin showed (Table 1) the presence of 32 components with cubebol as the major component along with significant amounts of β-cubebene (12.3%), germacrene-D (8.3%), α-copaene (6.2%), sabinene (5.8%) and a cubebol stereoisomer (5.6%). Although chemical studies on several piper species are extent (Srinivas and Madusudana, 1999; Tsukamoto *et al*., 2002; Singh et al., 2004) there is no much detailed investigation on *Piper cubeba* berry oil (Sumathykutty et al., 1999). Our results on the essential oil composition show some resemblance but also significant differences with the composition previously reported (Sumathykutty *et al*., 1999) which most likely are due to the unknown origin of the berries (cultivar, variety, etc.) which were purchased from local markets. On the other hand, we found no reports on the chemical composition of tailed pepper acetone oleoresin.

Table 1. Chemical composition of tailed pepper essential oil and oleoresin analyzed by GC-MS

Compound	Essential oil %FID[Ψ]	Oleoresin %FID[Ψ]	RI[a]	identification
alpha-thujene	4.5	0.6	931	MS, RI, co-GC
alpha-pinene	4.1	0.5	941	MS, RI, co-GC
camphene	trace	---	954	MS, RI
sabinene	19.4	5.8	975	MS, RI, co-GC
beta-pinene	0.7	0-2	980	MS, RI, co-GC
myrcene	0.3	---	993	MS, RI, co-GC
alpha-phellandrene	0.2	---	1007	MS, RI
delta-3-carene	trace	---	1013	MS, RI
alpha-terpinene	trace	---	1020	MS, RI
p-cymene	1.0	0.2	1026	MS, RI, co-GC
beta-phellandrene	5.9	1.2	1030	MS, RI, co-GC
1,8-cineole	trace	0.8	1032	MS, RI
trans-beta-ocimene	---	0.2	1050	MS, RI
gamma-terpinene	0.2	---	1064	MS, RI, co-GC
cis-sabinene hydrate	0.9	0.3	1069	MS, RI
terpinolene	trace	---	1088	MS, RI
trans-sabinene-hydrate	0.5	0.1	1097	MS, RI
linalool	4.9	1.5	1099	MS, RI, co-GC

camphor	trace	---	1144	MS, RI
terpinen-4-ol	0.9	---	1177	MS, RI, co-GC
delta-elemene	0.3	0-6	1335	MS, RI
alpha-cubebene	4.1	3.5	1350	MS, RI
isoledene	trace	---	1372	MS, RI
alpha-copaene	8.8	6.2	1379	MS, RI
beta-cubebene	18.3	12.3	1390	MS, RI
beta-elemene	0.6	1.4	1393	MS, RI
alpha-gurjunene	0.3	0.6	1411	MS, RI
beta-caryophyllene	3.7	3.7	1420	MS, RI, co-GC
aromadendrene	0.1	---	1439	MS, RI
alpha-guaiene	0.2	0.2	1440	MS, RI
alpha-humulene	0.9	1.5	1458	MS, RI, co-GC
allo-aromadendrene	3.1	3.5	1460	MS, RI
gamma-amorphene	2.0	---	1481	MS, RI
germacrene-D	2.6	8.3	1484	MS, RI, co-GC
zingiberene	0.1	---	1490	MS, RI
muurola-4(14), 5-diene trans	---	0.4	1494	MS, RI
Bicyclogermacrene	1.5	0.7	1498	MS, RI
cubebol stereoisomer	0.2	5.6	1502	MS, RI
alpha-muurolene	0.6	1.3	1506	MS, RI
cubebol	4.7	26.1	1516	MS, RI
delta-cadinene	0.9	2.7	1527	MS, RI
cadina-1(2),4-diene-trans	0.2	1.0	1536	MS, RI
germacrene-B	0.1	---	1560	MS, RI
ledol	---	2.9	1569	MS, RI
spathulenol	0.4	---	1580	MS, RI
caryophyllene oxide	0.3	---	1582	MS, RI
viridiflorol	0.3	---	1592	MS, RI
cubenol<1-epi>	---	0.6	---	MS
Alpha-muurolol (=torreyol)	---	0.6	---	MS
TOTAL	97.8%	95.0		

$^{\Psi}$ Percentages were obtained from electronic integrations measurements using flame ionization detection (FID).

[a] The (RI) was calculated using a homologous series of n-alkanes C_8-C_{16}.

7.2. Antioxidant activity in mustard oil

Addition of natural (tailed pepper essential oil or oleoresin) and synthetic antioxidants (BHA or BHT) to mustard oil affected, to different degrees, the peroxide and TBA values during accelerated oxidation at 60^0C for 28 days (Figs 1 & 2). Peroxide value measures primary products of lipid oxidation and TBA value measures the formation of secondary oxidation products, mainly malonaldehyde which may contribute off flavours to oxidized oil (Rossel, 2005). The samples with essential oil and oleoresin were more stable on heating at 60^0C than the control, when assessed by the change in peroxide (Fig. 1) and TBA (Fig. 2)

values. In addition, secondary oxidation products such as carbonyls and 2-alkenals have been measured at the same time interval in terms of total carbonyl (Fig. 3) and *p*-anisidine (Fig. 4) values. The results ($p < 0.05$) were well agreed with PV and TBA values and they proved stabilization effect of essential oil and oleoresin in mustard oil system. Singh *et al*. (2004) have observed that black pepper essential oil which also contains significant amounts of sabinene (13.0%) shows a strong antioxidant activity against linseed oil.

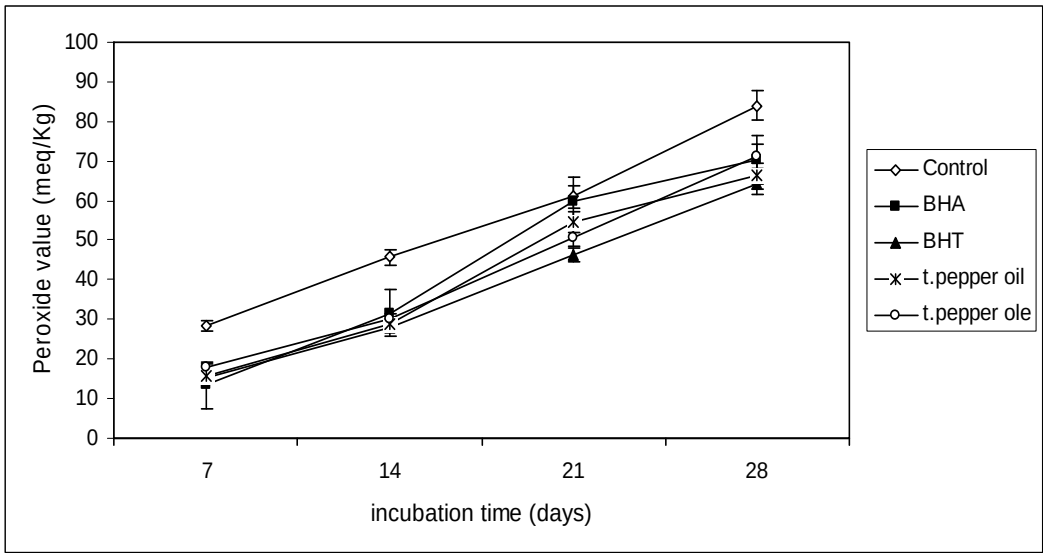

Fig. 1. Antioxidant activities of tailed pepper essential oil and oleoresin in mustard oil in terms of peroxide value

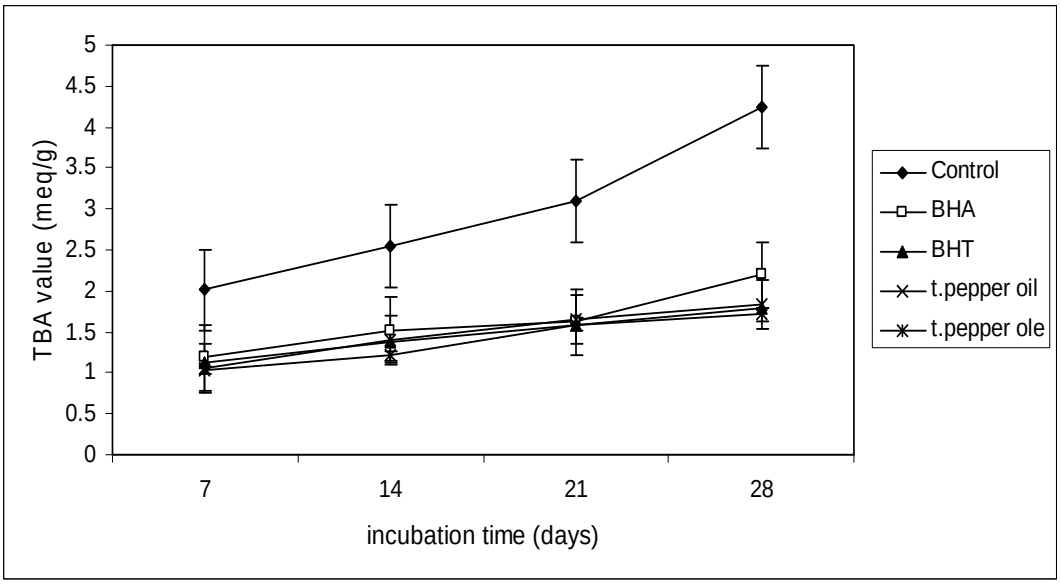

Fig. 2. Antioxidant activities of tailed pepper essential oil and oleoresin in mustard oil in terms thiobarbituric acid value

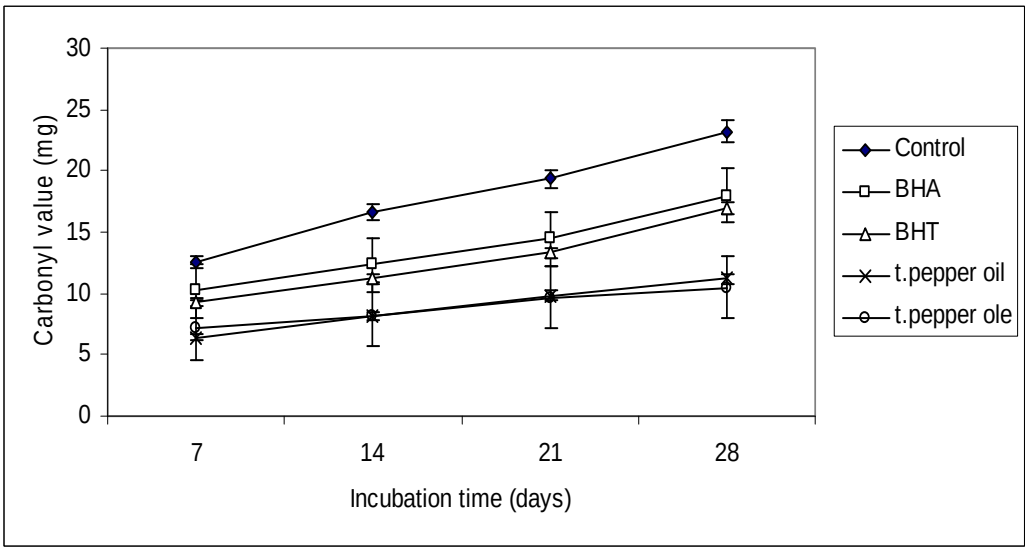

Fig. 3. Antioxidant effect of tailed pepper essential oil and oleoresin at 0.02% level in terms of carbonyl value

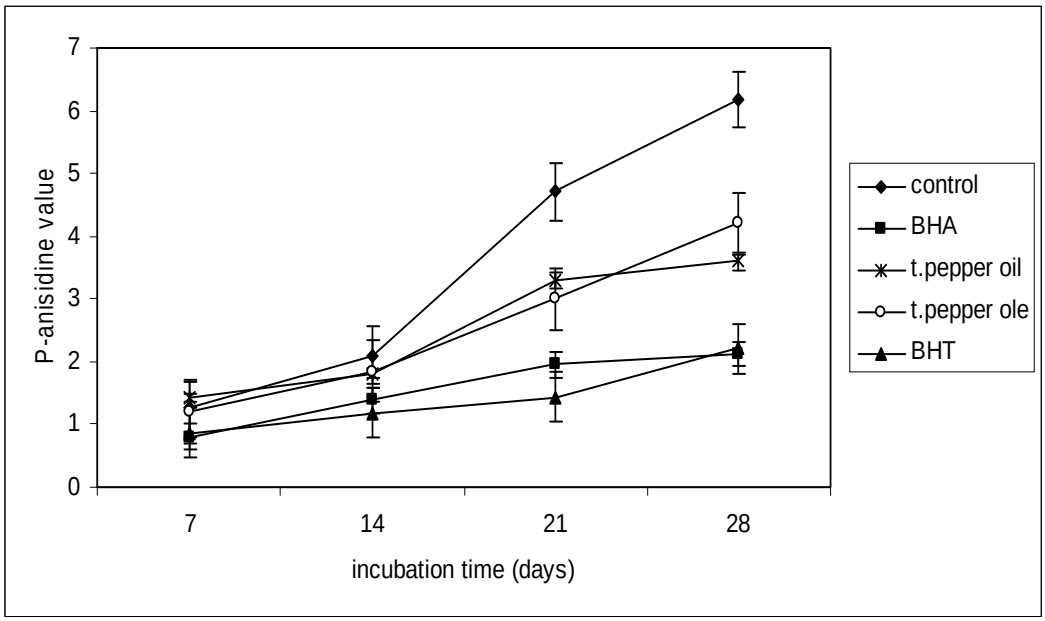

Fig. 4. Antioxidant effect of tailed pepper essential oil and oleoresin at 0.02% level in terms of *p*-anisidine value

7.3. Antioxidant activity by various assays

When linoleic acid was oxidized by incubating at 40^0C for up to 175 h, concentrations of peroxides measured by spectrophotometer at 500 nm increased abruptly in the control sample after 50 h (Fig 5). However, addition of essential oil and oleoresin effectively suppressed the peroxide concentration in emulsion during incubation ($p<0.05$).

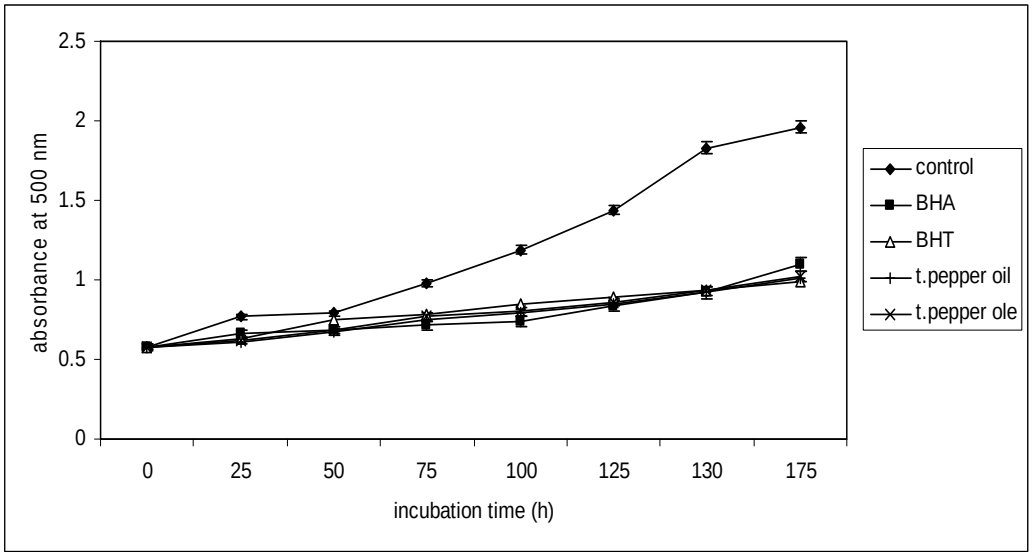

Fig. 5. Antioxidant activities of tailed pepper essential oil and oleoresin compared to standard antioxidants (BHA and BHT) in linoleic system.

The scavenging effect of the essential oil (40.20 –71.2%) and oleoresin (38.12–69.77%) on DPPH radical linearly increased with increasing concentration (Table 2). Both essential oil and oleoresin exhibited strong percent scavenging activity in comparison with commercial antioxidants, BHA (82.45 – 96.41%), BHT (85.23 – 95.91%) at 5, 10, 15 and 20 mg. Reducing power (Table 2) of both essential oil and oleoresin were moderate and increased with increasing concentration. However essential oil and oleoresin exhibited low scavenging and reducing effect at lower concentration in comparison with synthetic antioxidants. The reducing power of both essential oil and oleoresin might be due to their hydrogen-donating ability (Hyang-sook et al., 2000; Shimada *et al.,* 1992) and is generally associated with presence of reductones (Duh, 1998). The components present in the essential oil and oleoresin could act as good reductants, which could react with free radicals to stabilize and terminate radical chain reactions.

The chelating effect of the essential oil on ferrous ions was 20.23% at 5 µL and increased to 53.95% at 20 µL and a similar trend was observed for oleoresin (Table 2). However, the chelating ability of EDTA was 96.25 % at 20 µL. Apparently, the essential oil and oleoresin could chelate ferrous ions but were not as effective chelators as EDTA. Since ferrous ions are the most effective prooxidants in food systems (Yamaguchi et al., 1998), the moderate to high chelating effects of tailed pepper essential oil and oleoresin would be beneficial. Hydrogen peroxide scavenging activity of the essential oil and the oleoresin is

presented in (Table 2**)**. The essential oil and oleoresin exerted a concentration-dependent hydroxide radical scavenging activity. Scavenging activities of essential oil and oleoresin at 10 µL were 21.2 and 26.7% respectively. Addition of H_2O_2 to cells in culture can lead to transition metal ion-dependent $OH^{\cdot}$ mediated oxidative DNA damage (Spencer et al., 1996). Phenolic compounds tested in this study were able to form complexes with Fe^{3+} according to the EDTA assay of deoxyribose degradation. The general chelating ability of phenolics is probably related to the high nucleophilic character of the aromatic rings rather than to specific chelating groups within the molecule (Aust and Koppenol, 1991; Becana and Klucas, 1992; Gutteridge et al., 1981).

Table 2. DPPH, OH radical scavenging activity, reducing and chelating power of essential oil and oleoresin in comparison with commercial antioxidants BHA and BHT.

Sample (µL/mL)	**DPPH [a] radical scavenging activity** (in %)			
	BHA	BHT	Tailed pepper oil	Tailed pepper oleoresin
5	82.45	85.23	40.20	38.12
10	88.30	88.02	56.82	46.30
15	91.40	91.22	63.55	55.10
20	94.70	93.71	65.15	61.91
25	96.41	95.91	71.20	69.77
Reducing power[a] (in %)				
5	61.25	64.25	27.61	25.22
10	70.3	69.11	35.55	30.31
15	74.12	76.32	41.12	33.44
20	78.0	81.01	49.55	42.41
25	83.51	84.91	57.09	50.71
OH radical scavenging [a] (in %)]				
5	30.54	33.89	18.1	21.4
10	37.81	41.25	21.2	26.7
15	44.44	51.98	28.88	37.8
20	53.21	58.10	35.6	45.7
25	65.87	67.08	44.35	54.1
Chelating effect[a] (in %)				

Sample (μL/mL)	BHA	BHT	EDTA	Tailed pepper oil	Tailed pepper oleoresin
5	44.72	46.12	50.61	20.23	20.25
10	49.87	49.81	61.23	27.98	29.18
15	54.31	57.21	74.15	36.77	38.70
20	61.56	67.06	85.90	44.98	46.28
25	65.25	71.05	96.25	53.95	55.15

In all cases relative error was below 10%.
[a] Values represents means (DPPH, chelating, OH radical scavenging and reducing power) (n =2).

It has been stated that terpenoids play an important role in antioxidant activity (Madsen *et al*., 1995). GC and GC-MS analysis of tailed pepper essential oil and oleoresin showed that both contain a large number of terpenes. It has been reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidative activities that creates an effective defence system against free radical attack (Lu and Foo, 2001; Vardur Unlu *et al*., 2003).

7.4. Antimicrobial studies

The antifungal activity results obtained by the poison food and inverted Petri dish methods for both essential oil and oleoresin are reported in Table 3. The tailed pepper essential oil and oleoresin showed 100% mycelial zone inhibition against *Penicillium viridicatum* at 3000 and 2000 ppm respectively in the poison food method (Table 3). It is interesting to note that the essential oil revealed 100% clear zone inhibition against *Aspergillus flavus* at all tested concentrations. Both oil and oleoresin showed moderate to strong antifungal activity against the other tested fungal isolates. The essential oil exhibited strong activity against *Aspergillus flavus* and *Penicillium madriti* at 2000 and 3000 ppm respectively in the inverted Petri dish method (Table 3). In general, poison food method gave better results for both essential oil and oleoresin in comparison with inverted Petri dish method. The oleoresin showed poor results in the inverted Petri dish method probably due to its low volatility (Singh *et al*., 2004a, 2005a).

Table 3. Antifungal effect of tailed pepper essential oil and oleoresin against different food-borne pathogenic fungi by poison food medium and inverted Petri dish method.

Test	Concentration (ppm)*	Percent zone inhibition [#] **Various food -borne fungi (Poison food medium method)**					
		2479	**1786**	**3003**	**341**	**2007**	**1884**
tailed pepper essential oil	1000	30.6	50.0	60.7	16.7	71.9	100.1
	2000	58.9	56.3	66.3	21.6	85.7	100.3
	3000	73.0	62.5	74.1	26.8	100.0	100.4
tailed pepper oleoresin	1000	35.3	50.0	26.7	16.8	81.8	71.7
	2000	53.0	55.7	34.8	24.7	100.1	84.8
	3000	76.5	62.5	43.9	31.7	100.2	100.5
carbendazim	1000	8.2	-	24.2	30.2	-	-
	2000	12.4	-	31.2	36.4	12.6	16.7
	3000	16.5	30.2	15.4	43.1	17.4	21.8
Test	Concentration (ppm)*	**Various food pathogenic fungi (Inverted Petri dish method)**					
		2479	**1786**	**3003**	**341**	**2007**	**1884**
tailed pepper essential oil	1000	9.3	50.0	60.4	28.6	50.0	87.5
	2000	36.4	56.3	87.6	27.1	68.8	100.5
	3000	45.3	60.0	100.1	71.4	37.5	100.5
tailed pepper oleoresin	1000	6.7	52.5	16.7	24.0	31.3	37.5
	2000	26.7	60.1	24.8	31.6	37.6	43.8
	3000	46.7	65.4	37.9	40.0	63.8	62.5

[#]Average of three replicates

*DMSO was used for dilution

2479- *Aspergillus niger*, 1786- *Penicillium purpurogenum*, 3003- *Penicillium madriti*, 341- *Acrophialophora fusispora*, 2007-*Penicillium viridicatum*, 1884- *Aspergillus flavus*.

Table 4. Antibacterial activity of tailed pepper essential oil and oleoresin by agar well diffusion method

Test	Concentration (ppm)*	Inhibition zone (mm)#					
		Gram (+) bacteria			**Gram (-) bacteria**		
		BS	**SA**	**BC**	**EC**	**ST**	**PA**
tailed pepper essential oil	1000	15.1±0.2	25.8±0.5	+	15.5±0.1	-	-
	2000	21.2±0.6	36.5±0.3	+	20.1±0.1	14.2±0.1	-
	3000	26.5±1.1	41.2±1.5	+	25.6±1.5	17.0±0.2	-
tailed pepper oleoresin	1000	20.6±1.1	60.2±0.5	35.5±1.6	15.4±2.1	21.0±2.3	-
	2000	25.0±0.4	+	46.4±0.1	17.1±1.7	26.7±1.5	-
	3000	32.1±0.8	+	53.2±1.5	19.4±1.2	33.2±1.1	-
cloxacillin	1000	-	22.7±0.6	12.1±0.2	-	-	-
	2000	-	26.4±1.1	13.2±1.1	-	12.4±0.8	-
	3000	-	30.2±1.0	15.4±0.5	-	16.4±0.4	-

BS- *Bacillus subtils* ; **SA** - *Staphylococcus aureus* **; BC** -*Bacillus cereus* ; **EC**- *Escherichia coli* ; **ST**- *Salmonellae typhi;* **PA**- *Pseudomonas aeruginosa.*

Average of three replicates
(+) indicates complete inhibition
(-) indicates no inhibition
*****DMSO was for dilution

The results obtained for the antibacterial activity of essential oil and oleoresin is summarized in Table 4. Tailed pepper essential oil exhibited complete inhibition against *Bacillus cereus* and at the same time was totally ineffective against *Pseudomonas aeruginosa* at all tested concentrations. Both oil and oleoresin displayed good to moderate activity against the other tested bacterial strains. There is increasing evidence that specific compounds such as p-cymene (Soliman and Badeaa, 2002), linalool (Zambonelli et al., 1996), caryophyllene oxide (Magiatis et al., 2002) and β-pinene (Soliman and Badeaa, 2002) play an important role in the antifungal activity. In the present study, the lower efficacy of this essential oil and oleoresin against some microorganisms might be due to the low activity of their main constituents against particular fungi or bacteria. It is likely that the antifungal effects of the essential oil and oleoresin result from the

synergistic action of all their components (Dubey and Kishore, 1987). The strength of inhibition and the spectrum of antimicrobial activity suggest that complex interactions among the many components of the tailed pepper essential oil and oleoresin led to the overall activity. A study on antibacterial activity (Pattnaik et al., 1997) showed that linalool was the most effective compound in retarding bacterial growth followed by cineole, geraniol and citral. Disruption of the membrane by terpenes has been shown in gram positive (Ultee et al., 1999; Cox *et al*., 2000) and gram negative bacteria. (Helander et al., 1998) This type of disruption might be occurring in the present study.

7.5. Conclusion

In terms of peroxide, thiobarbituric acid, total carbonyl values, it can be concluded that both tailed pepper essential oil and oleoresin are good antioxidants for mustard oil. They have shown moderate to strong antioxidant activity by DPPH and hydroxyl radical scavenging assays. In addition they have also exhibited broad spectrum of antimicrobial activity against the studied fungal and bacterial isolates. These studies can be useful as starting point for further applications of tailed pepper essential oil and oleoresin and their constituents in food and pharmaceutical preparations.

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