Antimicrobial and Antioxidant Potentials of Essential Oil and Acetone Extract of *Myristica fragrans* Houtt. (Aril Part)

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ABSTRACT: The antifungal, antibacterial, and antioxidant potentials of essential oil and acetone extract were carried out by different techniques. In poison food medium method, the essential oil showed complete zones of inhibition against *Fusarium graminearum* at the all tested doses. For other tested fungi and bacteria, they gave good to moderate zone inhibition. The antioxidant activity was evaluated by measuring peroxide, thiobarbituric acid and total carbonyl values of rapeseed oil at fixed time intervals. Both the extract and essential oil showed strong antioxidant activity in comparison with butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). In addition, their inhibitory action in linoleic acid system was studied by monitoring peroxide concentration in emulsion during incubation. The results were well correlated with above values. Their radical scavenging capacity was carried out on 2,2′-diphenyl-1-picrylhydracyl (DPPH) radicalm, and they showed strong scavenging activity in comparison with synthetic antioxidants. Their reducing power was also determined, which also proved strong antioxidant capacity of essential oil and extract. Gas chromatographic-mass spectroscopy studies on essential oil resulted in the identification of 49 components representing 96.49% of the total amount, and the major component was sabinene (20.22%), followed by terpinen-4-ol (12.08%), safrole (10.32%), α-pinene (9.7%), β-phellandrene (6.56%), and γ-terpinene (5.93%). The acetone extract showed the presence of 23 components representing 71.66% of the total amount. The major components were isocroweacin (18.92%), elemicin (17.68%), methoxyeugenol (8.13%), linoleic acid (4.12%), dehydrodiisoeugenol (4.06%), palmitic acid (2.8%), and trans-isoeugenol (2.76%).

Keywords: GC-MS, antimicrobial properties, antioxidant potential, scavenging ability, reducing power

Introduction

There is growing interest in the use of natural antioxidants for extending the shelf life of food materials in place of synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone. These food additives, used by the food processing industry to prevent lipid peroxidation, have been reported to possess possible toxic and carcinogenic effects on health (Ito and others 1985). Herbs and spices are amongthe most important targets in which to search for natural antioxidants. Mace is the fleshy orange-red aril around the seeds of nutmeg, *Myristica fragrans* Houtt, which belongs to the family Myristicaceae. Both nutmeg and mace oils were used (whole or ground) for flavor spice and condiments. Various spice essential oils (Lee and others 2002; Mau and others 2003; Singh and others 2004) have exhibited strong antioxidant properties. It has been demonstrated that use of eucalyptus leaf extract as a natural food additive in selected model system (Amakura and others 2002). Hu and others (2003) studied the antioxidant potential of *Aloe vera* extracts by ferric thiocyanate method and 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) method. They observed strong radical scavenging ability of *Aloe vera* extracts in comparison with BHA. Antioxidant property of aroma extract isolated from clove buds was reported (Lee and Shibamoto 2001) by lipid/malonaldehyde and aldehyde/carboxylic acid assays. Both antioxidant and antimicrobial properties are very important in increasing the shelf life of the particular food material. Spice essential oils (Baratta and others 1998; Lambert and others 2001) and their extracts (Vardar-Unlu and others 2003; Singh and others 2004) have strong antimicrobial properties. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander, and eucalyptus essential oils have been reported (Delaquis 2002) by the broth dilution method. There are studies on chemical constituents (Spricigo and others 1999; Ogunwande and others 2003) of nutmeg, but there are very few reports (Pishawekar and Pishawikar 1953; Prakashchandra and Chandrasekharappa 1984) about mace, and they only describes the analytical characteristic of mace fat and its lipid composition studies. Hence, in the present article, we are presenting antioxidant and antimicrobial potentials of mace essential oil and acetone extract (will be called extract in the coming sections) by different assays. The chemical components of both were evaluated by gas chromatography-mass spectrometry.

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Material and Methods

Plant material

The aril parts of the nutmeg seeds were purchased from the local market of Trivandrum, Kerala, India. The voucher specimens were made and kept at the Herbarium of the faculty of science, DDU Gorakhpur Univ., Gorakhpur, India.

The essential oil of mace

The dried aril parts of the nutmeg seeds were ground (750 mesh)

by domestic model electronic mixer and was hydrodistilled in a Clevenger type apparatus (Borosil glass, Ambala, India) for 4 h, according to the method recommended by European pharmacopoeia (1983). Colorless oil, with characteristic odor, was obtained with yield of 4.2%. It was dried over anhydrous sodium sulphate, and the sample was stored at 4 °C before use.

The acetone extract of mace

After the isolation of essential oil, the crude material was oven dried at 50 °C for 24 h. Then, 20 g of dried material was loaded in a soxhlet apparatus, and the extraction was carried out with acetone (400 mL) at 90 °C for 3 h. The solvent was removed under reduced pressure, and the remaining extract was used for further tests.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)

The analysis of mace oil and extract was performed by GC and GC-MS techniques.

GC. A Hewlett Packard 5890(Analytical Technologies, SA, Buenos Aires, Argentina) gas chromatograph fitted with capillary column (5% phenyl methylsiloxane; length 30 m × inner dia 0.32 mm × film thickness 0.25 microns) whose injector and flame ionization detector (FID) temperatures were maintained at 240 and 250 °C, respectively. Injection volume was 1 μL with a split ratio 30:1, and helium was used as the carrier gas with a flow rate of 1.0 mL/min. The oven temperature for both essential oil and extract was programmed as follows: 60 °C for 1 min, rising 1.5 °C/min to 185 °C then held for 1 min then again started rising at 9 °C/min to 275 °C held for 2 min.

GC-MS. The essential oil and its acetone extract were subjected to GC-MS analysis using gas chromatograph Hewlett Packard (6890) coupled with a quadrupole mass spectrometer (model HP 5973) with capillary column of HP-5MS (5% phenyl methylsiloxane; length 30 m × inner diameter 0.25 mm × film thickness 0.25 microns). The injector, GC-MS interface, ion source, and selective mass detector temperatures were maintained at 280, 280, 230, and 150 °C, respectively. The oven temperature for volatile oil was programmed as follows: 60 °C (1 min), 60 to 185 °C (1.5 °C/min), 185 °C (1 min), 185 to 275 °C (9 °C/min), 275 °C (2 min), and for its extract, it was 70 °C (5 min), 70 to 220 °C (3 °C/min), 220 to 280 °C (5 °C/min) and final hold was 280 °C for 5 min. The percentages of MS are the mean of 3 runs and were obtained from electronic integration measurements using selective mass detector.

Identification of components:

The components were identified on the basis of comparison of their retention indices and mass spectra with published data (Massada 1976; Adams 2001) and computer matching was done with the Wiley 275 and Natl. Inst. of Standards Technology (NIST 3.0) libraries provided with the computer controlling GC-MS system. The retention indices were calculated for the volatile constituents using a homologous series of n-alkanes C_8 to C_{16}. The chemical constituents of essential oil and extract are reported in Table 1 and 2.

Antibacterial assay

The essential oil and extract were individually tested against a panel of microorganisms including 3 Gram positive bacteria, *Staphylococcus aureus* (3103), *Bacillus cereus* (430), and *Bacillus subtilis* (1790), and 3 Gram negative bacteria, *Escherichia coli* (1672), *Salmonella typhi* (733), and *Pseudomonas aeruginosa* (1942). All bacterial strains were procured from Microbial Type Culture Collection (MTCC), Inst. of Microbial Technology, Chandigarh, India, and their reference numbers are coded in parentheses. The bacterial cultures were grown on nutrient agar medium and stored at 4 °C. To prepare bacterial strain for test, initially 1 loopful of bacterial culture from slant was transferred in to nutrient broth solution (10 mL), and it was stored at 37 °C for 24 h. The strain was prepared by adding 1 mL broth to 9 mL Ringers solution. The test samples were prepared by dissolving 2, 4, or 6 μL essential oil or extract in 1 mL dimethyl sulphoxide solution. From which 200 μL was delivered into each well.

Agar well diffusion method

The agar well diffusion method was employed for the determination of antibacterial activity (NCCLS 1997). Briefly, the suspension of the test microorganism (0.1 mL) was spread on the previously prepared, dried nutrient agar plate by using sterile bent rod. The wells 10 mm in dia cut from the agar, and 200 μL of essential oil or extract was delivered into them. After incubating for 24 h at 37 °C, all plates were examined for any zones of growth inhibition, and diameters of these zones were measured in millimeters.

Antifungal assay

The antifungal activity of the volatile oil and extract against various pathogenic fungi *Aspergillus flavus* (1884), *Aspergillus ochraceus* (1810), *Aspergillus terreus* (3374) *Aspergillus niger* (2479), *Fusarium moniliforme* (1893), *Fusarium graminearum* (2088), *Penicillium citrinum* (2553), and *Penicillium viridicatum* (2007) were tested by inverted Petri plate (Rao and others 1994; Castellonus and others 2001) and poison food medium (Ramdas and others 1998; Singh and others 2004) methods. All the fungi cultures were procured from Microbial Type Culture Collection (MTCC), Inst. of Microbial Technology, Chandigarh, India, and their reference numbers are coded in the parentheses. The cultures were maintained in Czapek agar medium. Each test was replicated for 3 times and fungi toxicity was measured in terms of percent mycelial inhibition calculated by the following formula.

$$\text{Percent mycelial inhibition} = [(dc - dt)/dc] \times 100$$

where dc and dt are average diameters of mycelial colony of control and treated sets respectively. In poison food method, calculated quantity (2, 4, 6 μL) of undiluted essential oil (or extract) was mixed with 25 mL medium (approximately 45 °C), and it was poured in previously sterilized and dried Petri plate. In the case of inverted Petri plate method, calculated quantity (2, 4, 6μL) of essential oil (or extract) was soaked with filter paper (10 mm in dia), and it was kept at the lid of the inverted Petri plate. The plates were incubated at 37 °C for 6 d, and the zone of inhibition was measured with the help of vernier caliper.

Evaluation of antioxidant activity

To evaluate antioxidant potential of volatile oil and extract, the crude rapeseed oil was selected, which had initial peroxide value 2.1 meq/kg. In the case of essential oil, an extract (6 μL) was added to rapeseed oil, whereas in the case of synthetic antioxidants, 6 mg (BHA, BHT, and Propyl gallte [PG]) was added at 200 ppm concentration in 100 mL open-mouthed beakers, mixtures were thoroughly homogenized, and samples were incubated at 70 °C in the dark. A blank sample was prepared under the similar condition without any additive. Three replications were performed for each sample. The antioxidant activity of essential oil and extract against rapeseed oil was studied by measuring peroxide, thiobarbituric acid and carbonyl values at fixed time intervals. In addition, their antioxidant potential was studied in linoleic acid system and their individual antioxidant capacity was evaluated by DPPH assay. The reducing power of essential oil and extract has also been evaluated in comparison with synthetic antioxidants.

Table 1—Chemical composition of mace oil[a]

Compounds	% MS	RI[b]
n-hexanal	tr	802
Tricyclene	0.01	927
α-thujene	2.64	930
α-pinene	9.70	939
α-fenchene	0.06	953
Camphene	0.20	954
Sabinene	20.22	975
β-pinene	5.56	979
6-methyl-5-hepten-2-one	0.04	984
Myrcene	2.03	991
α-phellandrene	1.11	1007
β-3-carene	2.50	1013
α-terpinene	3.71	1020
p-cymene	1.73	1026
β-phellandrene	6.56	1030
1,8-cineole	tr	1035
cis-β-ocimene	0.02	1040
γ-terpinene	5.93	1064
cis-sabinene hydrate	0.07	1078
Terpinolene	2.81	1088
trans-sabinene hydrate	0.05	1098
Linalool	0.30	1099
1,3,8-*p*-menthatriene	0.01	1115
cis-*p*-menth-2-en-1-ol	0.50	1126
1-terpineol	0.30	1134
Terpinen-4-ol	12.08	1177
α-terpineol	0.93	1189
cis-piperitol	0.16	1198
trans-piperitol	0.12	1210
Iso-bornyl-acetate	0.19	1285
Safrole	10.32	1288
α-cubebene	0.09	1350
α-terpinyl acetate	0.18	1353
Citronellyl acetate	0.07	1358
α-copaene	0.59	1379
Geranyl acetate	0.08	1382
Methyl eugenol	1.88	1407
trans-α-bergamotene	0.08	1439
α-humulene	0.03	1458
α-amorphene	0.01	1479
Germacrene-D	0.10	1480
Bicyclogermacrene	0.04	1496
δ-cadinene	0.15	1527
Isocroweacin <E>	2.68	1558
Elemicin	0.55	1560
Palmitic acid	0.10	—
Total	96.49%	

[a]Percentages are the mean of 3 runs and were obtained from electronic integration measurements using selective mass detector. tr = trace.
[b]The retention index was calculated for all volatile constituents using a homologous series of n-alkanes C8-C16

Table 2—Chemical composition of acetone extract of mace[a]

Compounds	Rt	%	RI[b]
α-terpinene	7.20	1.99	1020
γ-terpinene	8.82	0.96	1064
Safrole	18.96	0.5	1288
Anisole, (p-pentyl)	19.70	0.9	1304
Eugenol	22.15	0.38	1362
α-copaene	22.50	0.79	1379
Methyleugenol	24.13	1.74	1407
trans-β-caryophyllene	24.29	0.29	1420
trans-isoeugenol	26.27	2.76	1453
cis-methylisoeugenol	26.31	1.46	1454
trans-ethylisoeugenol	28.06	1.02	1496
δ-cadinene	28.62	0.62	1527
Isocroweacin	28.99	18.92	1558
Elemicin	30.74	17.68	1560
Guaiol	31.77	0.15	1599
Methoxyeugenol (2,6-dimethoxy-4-allylphenol	32.57	8.13	—
trans-isoelemicin	33.90	0.54	—
Methylpalmitate	43.05	0.15	—
Palmitic acid	45.76	2.80	—
Linoleic acid	51.70	4.12	—
Oleic acid	51.78	1.20	—
Austrobailignan-7[c]	60.64	0.50	—
Dehydrodiisoeugenol	63.42	4.06	—
Total		71.66	

[a]Percentages are the mean of 3 runs and were obtained from electronic integration measurements using selective mass detector.
[b]The retention index was calculated for all volatile constituents using a homologous series of n-alkanes C8-C16
[c]4-hydroxy-3-methoxy-3′,4′-methylenedioxy-7,7′-epoxylignan (7R,7′S,8S,8′S) form.

Peroxide value

The peroxide values of all samples were measured (Horwithz 1980) every 7 d by Schaal oven test (Bandoniene and others 2002). For this purpose, a known weight of edible oil sample (3 g) was dissolved in glacial acetic acid (30 mL) and chloroform (20 mL). Then saturated solution of KI (1 mL) was added. The mixture was kept in dark for 15 min. After the addition of distilled water (50 mL), the mixture was titrated against sodium thiosulphate (0.02 N) using starch as an indicator. A blank titration was done parallel to treated and peroxide value (meq of oxygen/kg) was calculated.

Thiobarbituric acid value

The test was conducted according to the methods described earlier (Ottolenghi 1959; Kikuzaki and Nakatani 1993) with minor modifications. To 10 g of test edible oil sample, 0.67% aq thiobarbituric acid (20 mL) and benzene (25 mL) solution were added. This mixture was shaken continuously for 2 h using mechanical shaker, and the mixture was boiled in a water bath for 1 h. After cooling, the absorbance of supernatant was measured at 540 nm in a Hitachi-U-2000 spectrophotometer (Hitachi High Technologies, America). A lesser value of thiobarbituric acid indicates a higher value of antioxidant activity.

Carbonyl value

The method developed by Frankel (1988) was followed. About 4 g edible oil sample (containing essential oil or extract or synthetic antioxidants) was weighed in a 50 mL volumetric flask and made-up to volume with benzene. Out of this 50 mL, 5 mL was pipetted out, and then 3 mL trichloroacetic acid (4.3% in benzene), and 5 mL 2,4- dinitrophenylhydrazine (0.05% in benzene) were added sequentially. The above mixture was incubated at 60 °C for 30 min to convert free carbonyls in to hydrazones. After cooling, 10 mL potassium hydroxide (4% in ethanol) was added and made-up to volume (50 mL) with ethanol. After 10 min, absorbance measured at 480 nm using UV-visible spectrometer (Hitachi High Technologies, America). Blank was prepared under the same condition substituting 5 mL of benzene instead essential oil or extract. A standard curve was drawn using capraldehyde (50 to 250 μg) in 50 mL of benzene instead of test sample. The total carbonyl values of samples were calculated with the help of standard curve drawn and expressed as milligrams of capraldehyde per 100 g of sample. All the tests were done in triplicate. Carbonyl-free benzene and alcohol were prepared using standard methods and stored as stock solutions in brown bottles, and they were used for all tests.

Determination of antioxidant activity in linoleic acid system

Each sample (1 mL) was added to a solution mixture of linoleic acid (20 μL), 99.8% ethanol (2 mL) and 0.2 M phosphate buffer (pH

= 7, 2 mL). The total volume was adjusted to 6 mL with distilled water. The solution was incubated at 36 °C for 12 d, and the degree of oxidation was measured for every 2 d according to ferric thiocyanate method (Mitsuda and others 1996) with 75% ethanol (10 mL), 0.2 mL sample solution, and 0.2 mL ferrous chloride solution (20 mM in 3.5% HCl) being added sequentially. After stirring for 3 min, the absorbance values of the mixtures were measured at 500 nm in the UV-visible spectrophotometer. The control and standard were subjected to the same procedure except for the control, where the standard 1 mL sample was replaced with 1 mg BHA, BHT, or PG. Lesser the value of absorbance indicates higher the value of antioxidant capacity.

Radical scavenging activity

The scavenging effect on DPPH was determined according to methods developed earlier (Cuendet and others 1997; Kirby and Schmidt 1997). Various amount of essential oil or extract (5, 10, 15, and 20 μL) was mixed with 5 mL of 0.004% methanolic solution of DPPH. It was incubated for 30 min in the dark, and the absorbance of the sample was read at 515 nm using the UV-visible spectrophotometer. The control and standard were subjected to the same procedure except for the control, where the standard 5, 10, 15, and 20 μL of sample were replaced with 5, 10, 15, and 20 mg of BHA, BHT, or PG.

Reducing power

The reducing power was carried out as described before (Oyaizu 1986). Various amount of essential oil or extract (20, 40, 60, 80, and 100 μL) was 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 °C for 20 min after 2.5 mL 10% trichloroacetic acid was added, the mixture was centrifuged at 200 × *g* for 10 min in Sigma 3K30 model centrifuger. The upper layer (5 mL) was mixed with 5 mL deionized water and 1 mL 0.1% ferric chloride and the absorbance read at 700 nm in a UV-visible spectrophotometer. The control and standard were subjected to the same procedure except for the control, where there was no addition of sample and for the standard 20, 40, 60, and 80 μL of sample were replaced with 20, 40, 60, and 80 mg of BHA or BHT. A higher absorbance indicates higher reducing power.

Statistical analysis

For the essential oil or extract, 3 samples were prepared for assays of every antioxidant attribute. The data were presented as mean ± standard deviation of 3 determinations (data were not shown). Statistical analyses were performed using a one-way analysis of variance (Sokal and Rohlf 1973). A probability value of $P < 0.05$ was considered significant.

Results and Discussion

Spectral studies

GC and GC-MS analysis of essential oil and its extract were carried out by previously stated HP-5 series. The essential oil resulted in the identification of 49 components representing 96.49% of the total amount. The major component was sabinene (20.22%) followed by terpinen-4-ol (12.08%), safrole (10.32%), α-pinene (9.7%), β-phellandrene (6.56%), γ-terpinene (5.93%), β-pinene (5.56%), α-terpinene (3.71%), terpinolene (2.81%), and α-thujene (2.64%), including some other components in minor quantities whereas extract showed the presence of 23 components representing 71.66% of the total amount. The major component was isocroweacin (18.92%) followed by elemicin (17.68%), methoxyeugenol (8.13%), linoleic acid (4.12%), dehydrodiisoeugenol (4.06%), palmitic acid (2.8%), t-isoeugenol (2.76%) with some other components in minor quantities. Well-researched information on chemical constituents of essential oil and extract of mace meager (Pishawekar and Pishawekar 1953; Prakashchandra and Chandrasekharappa 1984). However, identification of sex in nutmeg was attempted by morphological, chemical, and physiological methods (Chowdhary and others 1957; Flach 1996).

Antimicrobial activity

The results (mean of 3 replicates) of both essential oil and extract obtained are given in the Table 3. Both essential oil and extract showed strong antibacterial activity against *Bacillus subtilis*, while both are ineffective against *Escherichia coli* and *Pseudomonas aeruginosa* at all tested doses. Essential oil and extract showed good antibacterial activity against *Staphylococcus aureus* and *B. cereus*, respectively. The extract showed complete inhibition of growth against *Staphylococcus aureus* at 3000 ppm. The antibacterial activity of standard antibiotics such as ampicillin and cloxacillin (10, 20, and 40 μg/mL) were also tested, and they gave moderate to good antibacterial activity for *S. aureus*, *B. cereus,* and *Salmonella typhi,* whereas they were ineffective against *B. subtilis* and *P. aeruginosa*.

The results (mean of 3 replicates) of both volatile oil and extract obtained by food poison and inverted Petri plate techniques are given in Table 4 and 5. The essential oil was found to be moderatively active against *Aspergillus terreus*, *A. flavus*, *A. niger,* and *Fusarium graminearum* at 6 μL by the inverted Petri plate method. The essential oil was found to be effective against *Penicillium citrinum*, *A. terreus,* and *F. graminearum* even at 2 and 4 μL doses. Extract has also shown considerable zones of inhibition against *A. flavus*, *A. terreus,* and *P. citrium* at the tested level. The extract was not found to be effective in this method, which may be due to its lower volatility. In poison food medium method, the essential oil showed complete zones of inhibition against *F. graminearum* at all tested doses. It also showed the more than 50% zone inhibition against *A. niger*, *A. terreus*, *F. monoliforme,* and *P. citrinum* at 6 μL. The essential oil showed good antifungal activity against *P. citrinum*, *A. niger,* and *A. terreus* at 2 and 4 μL doses. The mace extract showed moderate to good zones of inhibition against *F. graminearum*, *A. terreus*, *A. ochraceus,* and *F. monoliforme* at the tested level. All values are statistically significant ($P < 0.05$). Shridhar and others (2003) reported the antifungal activity of some essential oils by poison food technique, and they found that essential oils of cymbopogan, ajowan, and dill seed exhibited strong antifungal activity against *Colletotrichum lindemuthianum*. Lis-Balchin and Deans (1997) showed that essential oil derived from common botanical sources varied widely in composition and in activity against *Listeria monocytogenes*. Most of the antimicrobial activity in essential oils derived form spices and culinary herbs to derive from phenolic compounds (Lambert and others 2001), whereas other constituents are believed to contribute little to the antimicrobial effects (Shelef 1983; Nychas 1995; Martini 1996). From GC-MS studies (Table 2), it is clear that mace extract contains considerable percentage of phenolic compounds, and hence, the antimicrobial activity could be due to the presence of these phenolic compounds. Bishop and Thornton (1997) showed that terpenoid phenolic and nonphenolic alcohols are the most bioactive to fungi. The lower efficacy of this essential oil and extract against some microorganisms in the present study might have been due to the low activity of their main constituents against particular fungi or bacteria. It is likely that antifungal effects of the essential oil and extract result from the synergistic action of all their components (Dubey and Kishore 1987). Although,

Table 3—Antibacterial activity of essential oil and extract by agar well diffusion method

		Inhibition zone[a] (mm)					
		Gram (+)[b] bacteria			Gram (–)[b] bacteria		
Test	Concentration (ppm)	BS	SA	BC	EC	ST	PA
Mace essential oil	1000	15 ± 2.1	—	—	—	16 ±2.2	—
2000	20 ± 0.1	—	—	—	18 ± 1.5	—	
3000	26 ± 0.2	—	—	—	22 ± 2.0	—	
Mace extract	1000	13 ± 1.9	—	25 ± 0.3	—	—	—
2000	25 ± 0.3	40 ± 0.1	34 ± 0.5	—	—	—	
3000	31 ± 1.7	+	47 ± 0.7	—	—	—	

[a]Average of 3 replicates.
[b](+) indicates complete inhibition; (–) indicates no inhibition.
[c]BS, *Bacillus subtils*; SA, *Staphylococcus aureus*; BC, *Bacillus cereus*; EC, *Escherichia coli*; ST, *Salmonella typhi*; PA, *Pseudomonas aeruginosa.*

Table 4—Effect of mace essential oil and extract against different food pathogenic fungi by poison food medium method

	% mycelial zone inhibition at different dose[a] of sample						
	Mace essential oil			Mace extract			Carbendazim[b]
Fungus	2 µL	4 µL	6 µL	2 µL	4 µL	6 µL	1000 ppm
Aspergillus ochraceus	5.9	11.8	17.6	30.4	35.3	43.5	8.8
Aspergillus niger	33.8	50.0	62.5	20.5	27.0	38.8	0
Aspergillus flavus	2.7	16.0	36.0	6.7	13.3	33.3	28.8
Aspergillus terreus	33.3	60.0	70.6	40.2	43.3	48.0	42.5
Fusarium graminearum	100	100	100	60.5	65.0	73.3	0
Fusarium monoliforme	12.5	25.0	53.3	30.3	43.0	75.9	17.5
Penicillium citrium	50.0	68.8	85.4	12.5	18.8	31.4	44.8
Penicillium viridicatum	14.1	31.3	31.4	14.1	18.8	23.5	22.5

[a]Average of 3 replicates.
[b]Aqueous solution was used.

the major components in essential oil and extract are mostly considered to be mainly responsible for their antimicrobial property, the minor compounds may also play an important role. The synergistic or antagonistic effect of the latter may significantly influence the antifungal action of the former (Daferera and others 2003). Such synergistic or antagonistic action probably occurred with the essential oil and its extract tested in the present study.

Antioxidant activity in rapeseed oil system

Peroxide value is generally used to measure the primary oxidation indicating the amount of peroxides formed in fats and oils during oxidation. The plotted Figure 1 shows peroxide value changes in rapeseed oil system with additives. The oxidation of rapeseed oil samples were assessed at storage of 28 d. During this time, the peroxide value of the control sample increased to 213.6 meq/kg, which is significantly higher than the samples containing essential oil and extract. Both essential oil and extract showed comparable antioxidative activity with BHA and BHT at 6 mg level. Bandoniene and others (2002) studied the antioxidative effects of sage and borage leaf extracts in rapeseed oil by Schaal oven method, and they reported strong antioxidative activity of crude acetone extract from borage leaves. Various studies on antioxidant activity of essential oils and extract were reported earlier (Singh and others 1998; Singh and others 2004) in sunflower and linseed oil system. During the oxidation process, peroxides are gradually converted in to lower molecular weight compounds such as aldehyde and ketones. One such aldehyde, malonaldehyde, was measured by thiobarbituric acid method. Mace extract showed strong antioxidative effect in the secondary oxidation process, which could be comparable with BHA and BHT at 6 mg level (Figure 2). However, both essential oil and extract were not given significant results in comparison with PG in the secondary oxidation process. Zin and others (2002) studied antioxidant activity of root, fruit, and leaf extracts of *Morinda citrifolia* by thiobarbituric acid and ferric thiocyanate methods, and they found that the root extract exhibited higher antioxidant activity. In addition, we also measured (Figure 3) the total carbonyls present in edible oil samples in the same time interval, which also proved the antioxidative effect of essential oil and its extract in the rapeseed oil system. The inhibitory effect of essential oil can be due to the presence of aromatic nucleus containing polar functional groups.

Antioxidant activity in linoleic acid system

To evaluate the antioxidant potential of essential oil and extract, their lipid inhibitory activities were compared with selected standard antioxidants by using the ferric thiocyanate method of measuring the amount of peroxides formed in emulsion during incuba-

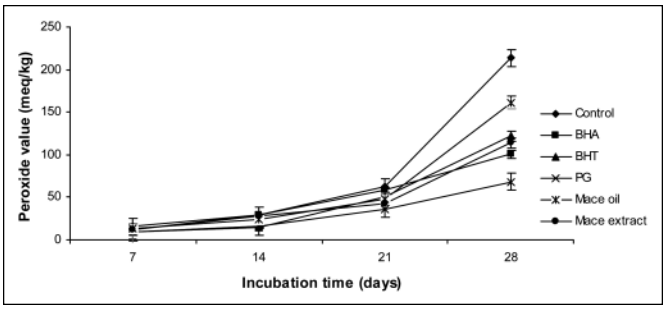

Figure 1—Antioxidant effect of essential oil and its extract in rapeseed oil in terms of peroxide value

Table 5—Effect of mace essential oil and its extract against different food pathogenic fungi by inverted Petri plate method

	% mycelial zone inhibition at different dose[a] of sample					
	Mace essential oil			Mace extract		
Fungus	2 µL	4 µL	6 µL	2 µL	4 µL	6 µL
Aspergillus ochraceus	6.3	8.8	13.8	5.0	6.3	8.8
Aspergillus niger	25.0	31.3	47.5	2.5	6.3	10.0
Aspergillus flavus	12.5	15.0	37.5	25.0	46.3	68.8
Aspergillus terreus	25.0	50.0	58.8	43.8	51.3	62.5
Fusarium graminearum	31.3	37.5	48.8	0	6.3	11.3
Fusarium monoliforme	5.0	12.5	13.8	12.5	15.0	17.5
Penicillium citrium	33.8	68.8	81.3	25.0	31.3	38.8
Penicillium viridicatum	7.5	12.5	15.0	5.0	8.8	12.5

[a]Average of 3 replicates.

tion. The incubation time compared with absorbance is plotted in Figure 4. High absorbance is an indication of a high concentration of formed peroxides. The absorbance of linoleic acid emulsion without the addition of essential oil, extract, or antioxidants increased rapidly, and there was a significant ($P < 0.05$) difference between the blank and the tested essential oil or extract. As can be seen in Figure 4, both the essential oil and extract were able to reduce the formation of peroxides. Ruberto and Baratta (2000) studied the antioxidant activity of several essential components in 2 lipid model systems, and they found high inhibition of peroxidation of α-pinene, β-pinene, champhene, α-terpineol, 1,8-cineole, camphor, α-humulene, 3-decanone, and 2-undecanone at 1000 ppm in the conjugated diene method. The essential oil of mace also contains considerable percentage of α-pinene (9.7%), β-pinene (5.6%), 1,8 cineole (trace), and α-terpineol (0.5 %), hence the inhibitory effect in the linoleic acid system might be due to the presence of above components.

Radical scavenging activity and reducing power

The DPPH is a stable radical with a maximum absorption at 515 nm that can readily undergo reduction by an antioxidant. Because of the ease and convenience of this reaction, it now has widespread

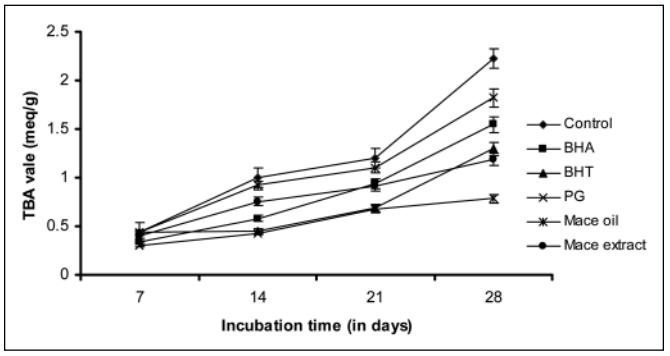

Figure 2—Secondary oxidative effect of essential oil and its acetone extract in terms of thiobarbituric acid value

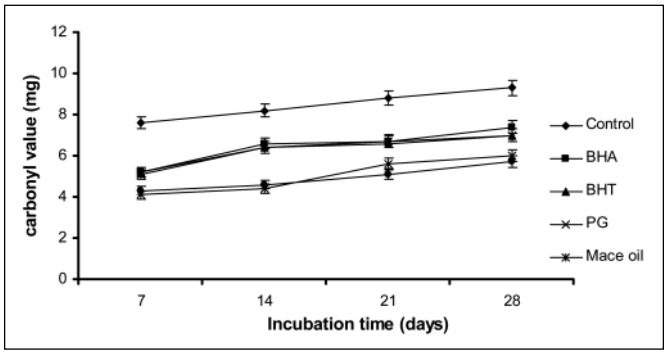

Figure 3—Antioxidative effect of essential oil and its acetone extract in terms of carbonyl value

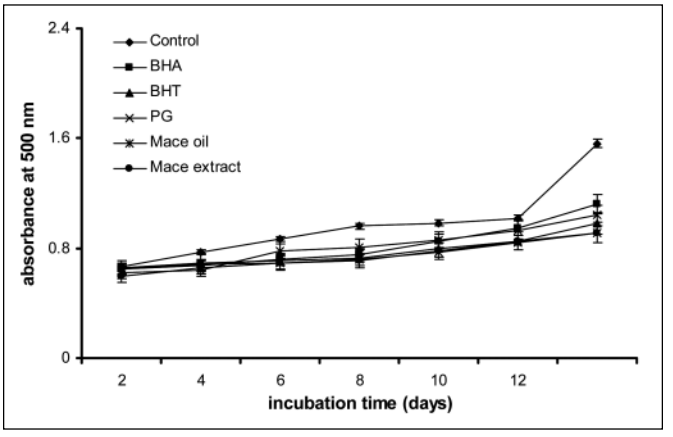

Figure 4—Antioxidative effect of essential oil and its acetone extract with standards assessed by the linoleic acid system method

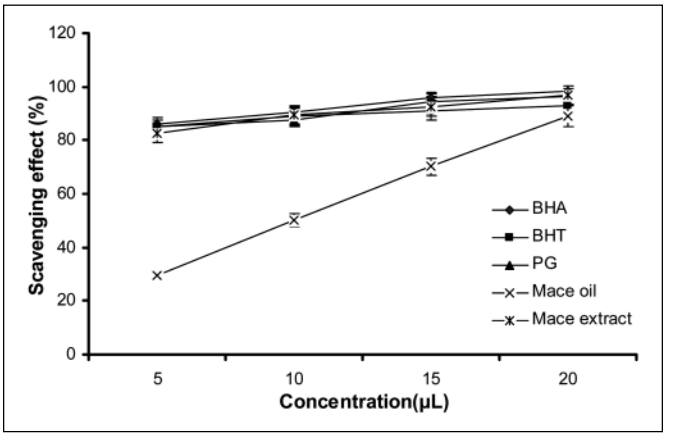

Figure 5—Radical scavenging activity of essential oil and its acetone extract on 2, 2′-diphenyl-1-picrylhydracyl radical

use in the measurement of free radical scavenging activity (Brand-Williams and others 1995; Sanchez-Moreno and others 1998). The decrease in absorbance of the DPPH radical due to the scavenging capability of essential oil, extract, and antioxidants is illustrated in Figure 5. The scavenging effect of the essential oil (50.2% to 88.9%) and extract (89.5% to 96.7%) on DPPH radical linearly increased with increasing concentration. Both essential oil and extract showed moderate to good percent scavenging activity in comparison with BHA (90.3% to 98.5%), BHT (89.1% to 98.5%), and PG (89.5% to 96.7%) at 10, 15, and 20 μg level. However, the scavenging ability of BHA, BHT, and PG were much more effective at low concentration and were 86%, 85.1%, and 84.9%, respectively. But 5 μL dose essential showed only 29.7% radical scavenging ability. Reducing powers of both essential oil and its extract were strong and increased with increasing concentration (Figure 6), and they showed moderate to good reducing ability in comparison with BHA and BHT. The reducing powers of essential oil and extract might be due to their hydrogen-donating ability. The component present in the essential oil and extract could act as good reductants, which would lead to stabilization and termination of free radical chain reactions.

Phenolic groups play an important role in antioxidant activity (Huang and Frankel 1997; Barata and others 1998). Hence, the presence of phenolic compounds such as methyl eugenol in essential oil and anisole (p-pentyl), eugenol, methyl eugenol, *trans*-isoeugenol, *trans*-methyl eugenol in mace extract are responsible for the antioxidative activity exhibited in all antioxidant assays studied. Strong antioxidative activity of extract might be due to the presence of more number of phenolic components in the extract in comparison with essential oil. In addition, antioxidative activities observed in essential oil and extract could be the synergistic effect of more than 2 compounds that may present in the system. It has been reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidative activities that create an effective defense system against free radical attack (Lu and Foo 1995). The composition of essential oil and extract are very complex. They consist of various classes of organic compounds, which may possess opposite effect on the process of lipid oxidation. Based on the results obtained, it is highly possible that several compounds of different polarity may contribute to the antioxidative activity of essential oil and its extract.

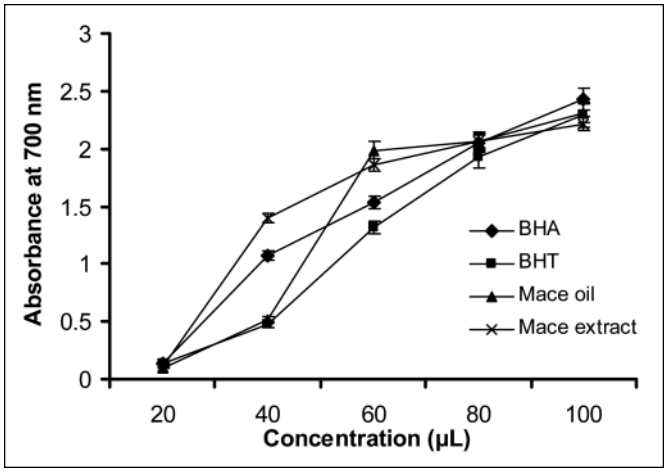

Figure 6—Reducing power of essential oil and its acetone extract with synthetic antioxidants

Conclusions

Summarizing these results, it can be concluded that the both essential oil and its acetone extract of mace exhibited broad spectrum of antimicrobial activity against the tested microorganisms, and it could be a better natural antioxidant for stabilizing rapeseed oil. More studies are needed to clarify the antioxidant mechanisms of the antioxidant activity of essential oil and extract. On the basis of above results, it was observed that both essential oil and extract provided equivalent or higher antioxidative activity when compared with synthetic antioxidants, which provides a way to screen antioxidants for foods, cosmetics, and medicine.

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References

Adams RP. 2001. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Allured Publishing Corp. Illinois.
Amakura Y, Umino Y, Tsuji S, Ito Hatano T, Yoshida T, Tonogai Y. 2002. Constituents and their antioxidative effects in eucalyptus leaf extract used as a natural food additive. Food Chem 77:47–56.
Bandoniene D, Venskutonis PR, Gruzdiene D, Murkovic M. 2002. Antioxidant activity of sage (*Salvia officinalis* L.), savory (*Satureja hortensis* L.), and borage (*Borago officinalis* L.) extracts in rapeseed oil. Eur J Lipid Sci Technol 104:286–92.
Baratta MT, Dorman HJD, Deans SG, Figueiredo AC, Baroso JG, Ruberto G. 1998. Antimicrobial and antioxidant properties of some commercial essential oils. Flavour Frag J 13:235–44.
Bishop CD, Thornton IB. 1997. Evaluation of the antifungal activity of the essential oils of *Monadra citrriodora* var. *citriodora* and *Melaleuca alternifolia* on postharvest pathogens. J Essen Oil Res 9:77–82.
Brand-Williams W, Cuvelier ME, Benset C. 1995. Use of free radical method to evaluate antioxidant activity. Lebensm Wissens Technol 28:25–30.
Castellonus PPA, Bishop CD, Maria J, Villalobos P. 2001. Antifungal activity of the essential oil of flowerheads of garland chrysanthemum *(Chrysanthemum coronarium*) against agricultural pathogens. Phytochem 57:99–102.
Chowdhary RS, Garg OK, Borah PC. 1957. Physiological changes in relation to sex in papaya. Phyton 9:137–41.
Cuendet M, Hostettmank K, Potterat O. 1997. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. Helv Chim Acta 80:1144–52.
Daferera DJ, Ziogas BN, Polissiou MG. 2003. The effectiveness of plant essential oils on the growth of *Bortytis cinerea*, *Fusariunm* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Protect 22:39–44.
Delaquis PJ, Stanich K, Girard B, Mazza G. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Intl J Food Microbiol 74:101–9.
Dubey NK, Kishore N. 1987. Fungitoxicity of some higher plants and synergistic activity of their essential oils. Tropical Sci 27:23–7.
European pharmacopoeia. 1983. 1. Maissoneue SA, Sainte Ruffine, Canada.
Flach M. 1996. Nutmeg cultivation and sex problems. Eng sum Meded Landh Hogesh 66:1–5.
Frankel EN. 1998. Lipid oxidation. Dundee, United Kingdom: The Oily Press. p 301.
Horwithz W. 1980. AOAC official methods of analysis. 13th ed. Washington, D.C.: AOAC.
Hu Y, Yu J, Hu Q. 2003. Evaluation of antioxidant potential of *Aloe vera* (*Aloe barbadensis*. Miller) extracts. J Agric Food Chem 51:7788–91.
Huang SW, Frankel EN. 1997. Antioxidant activity of tea catechins in different lipid systems. J Agric Food Chem 40:3033–8.
Ito N, Fukushim S, Tsuda H. 1985. Carcinigenicity and modification of carcinogenic response by BHA, BHT and other antioxidants. Crit Rev Toxicol 15:109–50.
Kikuzaki H, Nakatani N. 1993. Antioxidant effects of some ginger constituents. J Food Sci 58:1407–10.
Kirby AJ, Schmidt RJ. 1997. The antioxidant activity of Chinese herbs for eczema and of placebo herbs. Intl J Ethnopharmocol 56:103–8.
Lambert BJW, Skandamis PN, Coote PJ, Nychas GJE. 2001. A study of minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol 91:453–62.
Lee KG, Shibamoto T. 2001. Antioxidant property of aroma extracts isolated from clove buds (*Syzygium aromaticum* L. Merr. et perry). Food Chem 74:443–8.
Lee KG, Shibamoto T. 2002. Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. J Agric Food Chem 50:4947–52.
Lis-Balchin M, Deans SG. 1997. Bioactivity of selected plant essential oils against *Listeria monocytogens*. J Appl Bacteriol 82:758–62.
Lu F, Foo LY. 1995. Phenolic antioxidant component of evening primrose. In: Ong ASH, Niki E, Packer L, editors. Nutrition, lipids, health and diseases. Cham-

paign, Ill.: American Oil Chemists Society Press.
Martini H, Weidenborner M, Adams S, Kunz B. 1996. Eugenol and carvacrol: the main fungicidal compounds in clove and savory. Ital J Food Sci 1:63–67.
Massada Y. 1976. Analysis of essential oils by Gas chromatography and mass spectrometry. New York: Halsted/Wiley.
Mau JL, Lai YC, Wang NP, Chen CC, Chang CH, Chyau CC. 2003. Composition and antioxidant activity of the essential oil from curcuma zedoaria. Food Chem 82:583–91.
Mitsuda H, Yasumoto K, Iswami K. 1996. Antioxidative action of indole compounds during the autooxidation of linoleic acid. Eiyo to Shokuryo 19:210–4.
[NCCLS] National Committee For Clinical Laboratory Standards. 1997. 6th ed. Performance standard for antimicrobial disc susceptibility test, approved standard, M2-A6. Wayne, U.S.A.
Nychas GJE. 1995. Natural antimicrobials from plants. New York: Chapman & Hall.
Ogunwande IA, Olawore NO, Adeleke KA, Ekundayo O. 2003. Chemical composition of essential oil of *Myristica fragrance* Houtt. (Nutmeg) from Nigeria. J Essen Oil Bearing Plants 6:21–26.
Ottolenghi A. 1959. Interaction of ascorbic acid and mitochondrial lipids. Arch Biochem Biophysic 79:355–8.
Oyaizu M. 1986. Studies on products of browning reactions: antioxidative activities of products of browning reactions prepared from glucosamine. Japanese J Nutri 44:307–15.
Pishawikar AO, Pishawikar DG. 1953. The chemistry of nutmeg aril (Jay-patri). Curr Sci 22:81–5.
Prakashchandra KS, Chandrasekhrappa G. 1984. Lipid profile and fatty acid composition of fat extracted from arils of *Myristica fragrance* and seeds of *Artocarpour hirsutus*. J Food Sci 21:40–2.
Ramdas K, Suresh G, Janardhanan N, Masilamani S. 1998. Antifungal activity of 1, 3- disubstituted symmetrical and unsymmetrical thioureas. Pestic Sci 52:145–51.
Rao GP, Gillaspie JR, Upadhayay PP, Pergaminfilho A, Agnihotri VP, Chen CT. 1994. Current trends in sugarcane pathology. 4th ed. Delhi: Intl. books and periodical supply service. p 364–74.
Ruberto G, Baratta MT. 2000. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem 69:167–74
Sanchez-Moreno C, Larrauri JA, Saura-Calixto F. 1998. A procedure to measure the antiradical efficiency of polyphenols. J Sci Food Agric 76:270–6.
Shelef L. 1983. Antimicrobial effect of spices. 1983. Antimicrobial effect of spices. J Food Saf 6:29–44.
Shridhar SR, Rajagobal RV, Rajavel R, Masilamani S, Narasimhan. 2003. Antifungal activity of some essential oil. J Agric Food Chem 51:7596–9.
Singh G, Kapoor IPS, Pandey SK. 1998. Studies on essential oils, Part-13: Natural antioxidant for sunflower oil. J Sci Industr Res 57:139.
Singh G, Maurya S, Catalan C, Lampasoma MP. 2004. Chemical, antifungal, antioxidative studies of ajwain oil and its acetone extract. J Agric Food Chem 52:3292–6.
Sokal RR, Rohlf FJ. 1973. Introduction to biostatics. San Francisco: WH Freeman and company. p 164–85.
Zin MZ, Abdul-Hamid A, Osman A. 2002. Antioxidant activity of extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit, and leaf. Food Chem 78:227–231.