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# Antioxidant activity of some phenolic constituents from green pepper (*Piper nigrum* L.) and fresh nutmeg mace (*Myristica fragrans*)

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**Abstract**

Antioxidant potential of phenolic compounds from green pepper (*Piper nigrum* L.) and lignans from fresh mace (*Myristica fragrans*) were evaluated for their ability to scavenge 1,1′-diphenyl-2-picrylhydrazyl (DPPH) radical, inhibit lipid peroxidation and protect plasmid DNA damage upon exposure to gamma radiation. $EC_{50}$ values of the major phenolic compounds of green pepper namely, 3,4-dihydroxyphenyl ethanol glucoside, 3,4-dihydroxy-6-(*N*-ethylamino) benzamide and phenolic acid glycosides were found to be 0.076, 0.27 and 0.12 mg/ml, respectively, suggesting a high radical scavenging activity of these phenolics. These results were further confirmed with cyclic voltammetry. Acetone extract of nutmeg mace and its subsequent TLC isolated fractions constituted mainly of lignans as revealed by GC–MS analysis. The major compounds were tentatively identified from their mass spectral fragmentation pattern. DPPH radical scavenging capacity of the acetone extract as well as its fractions was comparatively lower than that of green pepper phenolics. In contrast, these fractions had a greater ability to inhibit lipid oxidation than phenolics from pepper as revealed by β-carotene–linoleic acid assay. A DNA protecting role of these compounds even at doses as high as 5 kGy further suggested the potential use of green pepper and fresh nutmeg mace and their extracts as a nutraceutical in preventing oxidative damage to cells.


*Keywords:* Antioxidant activity; DNA protection; Lignans; Mace; Pepper; Phenolics

## 1. Introduction

Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen have been implicated in degenerative diseases such as cancer, inflammation, atherosclerosis and aging as also in food deterioration (Ames, Shigrnaga, & Hagen, 1993; Halliwell, Gutteridge, & Cross, 1992; Larson, 1997). In foods, the development of rancidity is caused by free radicals that lead to development of off-flavors and undesirable chemical compounds (Horton & Fairhurst, 1987). Antioxidants are compounds that inhibit or delay oxidation of other molecules by terminating the initiation or propagation of oxidizing chain reactions. Restriction on the use of synthetic antioxidants due to their carcinogenic nature (Lindenschmidt, Trika, Guard, & Witschi, 1986) has lead to a growing interest in recent years in natural antioxidants of plant origin for application in food industry to combat food deterioration. The potential value of antioxidants has prompted researchers to look for natural antioxidants with low cytotoxicity.

A great number of spices and aromatic herbs contain chemical compounds exhibiting antioxidant properties (Madsen & Bertelsen, 1995). These properties are attributed to a variety of active phytochemicals including vitamins, carotenoids, terpenoids, alkaloids, flavonoids, lignans, simple phenols and phenolic acids, etc. (Liu &

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Ng, 2000). Antimicrobial and antioxidant properties of spices render them useful as preservative agents. Pepper and nutmeg are one of the most widely used spices in preparation of ayurvedic drugs (Nisamony, 2001). Black pepper is used in intermittent fevers, indigestion, diarrhoea, flatulence, worm infestation, asthma, cough, heart troubles, diabetes, piles epilepsy, elephantiasis, and filaria. Mace, both fresh and dry, is used for flatulence, to allay nausea and vomiting, for convalescents, as an ointment for piles, for leucorrhoea and as a local stimulant to the gastro-intestinal tract. Green pepper, however, does not find much use as an ingredient in ayurvedic preparations.

The presence of two phenolic compounds namely 3,4-dihydroxyphenyl ethanol glucoside and 3,4-dihydroxy-6-(*N*-ethylamino) benzamide in green pepper possessing antibacterial properties has been reported from this laboratory (Bandyopadhyay, Narayan, & Variyar, 1990; Pradhan, Variyar, & Bandekar, 1999; Variyar, Pendharkar, Banerjee, & Bandyopadhyay, 1988). Their complete conversion to black oxidized products during drying resulted in the absence of these compounds in black pepper. Using 4-methyl catechol as a substrate the mechanism of blackening by pepper polyphenol oxidase was demonstrated in our laboratory to occur via quinone intermediate. 4, 4′-Dimethyl diphenylene dioxide-1,2-quinone was identified as a major quinone intermediate in this study (Variyar, 1994). Hydroxy tyrosol (3,4-dihydroxyphenyl ethanol), the major active compounds of extra virgin olive oil has received increased attention as an antioxidant and melanin inhibitor and for its anticancer and anti-platelet aggregation properties (Chimi, Cillard, & Rahmani, 1991; Chlkamatsu et al., 1996; Galli, Petroni, & Visioli, 1994; Galli & Visioli, 1999; Kohyama, Nagata, Fujimoto, & Sehiya, 1997). However, no report exists on the pharmacological action of this compound in green pepper. Except for its antimicrobial properties (Pradhan et al., 1999), no other reports exist so far on the biological properties of 3,4-dihydroxy-6-(*N*-ethylamino) benzamide.

Lignans are an important class of plant derived compounds known to possess a variety of biological activities such as antitumor, antimitotic, antiviral and antiatherosclerotic activities (MacR & Towers, 1984). Mace is an important spice of commerce known to possess appreciable amount of lignans. Some of these have been reported to possess antimicrobial property (Orabi, Mossa, & Farouk, 1991). Although lignans from other sources are known to have antioxidant property (Filleur, Le Bail, Duroux, Simon, & Chulia, 2001) no report exists on the antioxidant properties of mace lignans.

Property of natural phenolic antioxidant to scavenge free radicals renders them useful as radioprotective agents reducing DNA damage induced by UV and ionizing radiation (Kumar, Devasagayam, Jayashree, & Kesavan, 2001). Radiation treatment can produce a variety of lesions in DNA resulting in both single and double strand breaks, alteration of bases, destruction of sugar moiety and cross-linking and formation of dimers (Kada, Kaneko, Matsuzaki, & Hara, 1985). Hydroxyl radicals are mainly implicated in these damages (Armitage, 1998). Antioxidants can bind to free radicals before the free radicals cause harm. Radioprotective effects of hydroxytyrosol on X-ray induced chromosomal damage have been reported earlier (Benavente-Garcia, 2002). No report, however, exists on the role of pepper phenolics and mace lignans in reducing DNA damage induced by ionizing radiation such as γ-radiation.

The objective of the present work thus was to evaluate the antioxidant activities of green pepper phenolics and mace lignans for their possible utilization as a food preservative, as nutraceuticals and in therapeutic applications.

## 2. Materials and methods

### 2.1. Materials

Green pepper berries (variety, Panniyur-I) and fresh green nutmeg fruits were obtained from Kottakal Aryavaidya Sala, Kottakal, Kerala, India and stored at 0–2 °C until use. All chemicals used were of analytical reagent grade and were procured from EMerck India Ltd. (Mumbai, India). DPPH, β-carotene and linoleic acid were purchased from Aldrich chemical company (WI, USA) while 2,2′-azobis-3-ethylbenzthiazoline-6-sulfonic acid diammonium salt was from Sigma chemical company (St. Louis, MO, USA).

### 2.2. Preparation of phenolic extracts

#### 2.2.1. Green pepper

Phenolic compounds were extracted according to the method previously described (Variyar et al., 1988; Bandyopadhyay et al., 1990). Green berries (200 g) were added in small lots to 500 ml boiling methanol: water (80:20) under reflux for 45 min. Refluxing was continued for 1 h and the contents were then extracted with 80% aqueous methanol in an omnimixer (Sorval Inc. New town, CT, USA) until the extracts were pale yellow. The extract was concentrated to remove methanol and the remaining aqueous solution was successively extracted with diethyl ether, ethyl acetate and *n*-butanol. The *n*-butanol fraction containing phenolic compounds of interest was chromatographed on a silicagel column using diethyl ether followed by increasing proportion of methanol in ether. The major compounds of interest namely, 3,4-dihydroxyphenyl ethanol glucoside, 3,4-dihydroxy-6-(*N*-ethylamino) benzamide and phenolic acid glycoside mixture were isolated and dissolved in 80% methanol to obtain a 1% solution.

#### 2.2.2. Mace

Fresh red mace arils obtained from nutmeg samples (100 g) were extracted with acetone as above. The extract (500 ml) thus obtained was evaporated to dryness under vacuum in a Buchi rotavapor (R-114) and the residue

was made to 10% solution in methanol. The extract thus obtained was subjected to preparative thin layer chromatography (TLC, silicagel, EMerck, Germany, 0.5 mm thickness) using toluene:ethyl formate:formic acid (5:4:1) as developing solvent system. The major bands at Rf 0.78 (Band I), 0.64 (Band II), 0.51 (Band III) were scrapped, eluted with chloroform and methanol–chloroform mixture, evaporated to dryness and then made to 10% in methanol. The individual fractions were directly subjected to gas chromatography–mass spectrometry (GC–MS) analysis in order to identify the major lignans. UV–Vis spectrum of the acetone extract was carried out on a Shimadzu UV-240 UV–Vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

### 2.3. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis was carried out on a Shimadzu GC–MS instrument (Shimadzu Corporation, Kyoto, Japan) equipped with a GC-17A gas chromatograph and provided with a DB-5 (J & W Scientific, CA, USA) capillary column ((5%-Phenyl)-methylpolysiloxane, length, 30 m, id., 0.25 mm and film thickness, 0.25 μm). The operating conditions were: column temperature programmed from 60 to 200 °C at the rate of 4 °C/min, held at initial temperature and at 200 °C for 5 min and further to 280 °C at the rate of 10 °C/min, held at final temperature for 20 min, injector and interface temperatures, 210 and 230 °C, respectively, carrier gas helium (flow rate, 0.9 ml/min), ionization voltage, 70 ev, electron multiplier voltage, 1 KV. Analyses were carried out in the splitless mode. The major peaks in each fraction were tentatively identified by comparing its mass fragmentation pattern with that of standard spectra available in the spectral library (Wiley/NIST Libraries) of the instrument as well as from the literature data (Hada, Hattori, Tezuka, Kikuchi, & Namba, 1988; Kasahara, Miyazawa, & Kameoka, 1995; Lu & Ralph, 1998; Simonelt et al., 1993; Zacchino & Badano, 1991).

### 2.4. Total polyphenol estimation

The amount of total polyphenols in the aqueous extract of green pepper and in acetone extract of mace was estimated according to Prussian blue method (Budinin, Tonelli, & Girotti, 1980). For preparation of calibration curve, 0.6–3.0 ml of 100 μM aqueous solution of standard catechin was made to 3 ml in each case followed by addition of 0.2 ml aqueous solution (0.008 M) of $K_3[Fe(CN)_6]$ and 0.2 ml solution of $FeCl_3$ (0.1 M) in 0.1 M HCl. The absorption of the blue colored solution was read after 5 min at 700 nm and the calibration curve was drawn. Three milliliters of each of the samples under study were mixed with same reagents and the absorption was measured as described above. All determinations were performed in triplicate and expressed as milligram of catechin equivalent per 100 g of fresh sample.

### 2.5. DPPH radical scavenging activity

The DPPH radical scavenging activity of pepper and mace extracts was determined according to the previously reported procedure (Brand-Williams, Cuvelier, & Berset, 1995). The initial concentration of DPPH radical was 60 μM in all the antioxidant radical reactions. For each phenolic compound and fractions, seven different concentrations ranging from 0.05% to 0.001% were tested. The absorbance at 517 nm was measured against a blank of pure ethanol. $A_{517}$ value at 15 min of reaction was used to establish the $EC_{50}$ value at experimental conditions. $EC_{50}$ values were calculated from a graph of concentration (mg/ml) of samples vs. %DPPH remaining. The $EC_{50}$ value is the concentration of an antioxidant required to lower the initial DPPH concentration by 50%. Trolox was used as a standard compound.

### 2.6. Cyclic voltammetry

A cyclic voltammeter (Acochemie Autolab, model PGSTAT 20, Netherland) with three-electrode system viz. Ag/AgCl as reference electrode, a glassy carbon electrode as the working electrode and a platinum wire as a counter electrode was used for our experiment. The cell contained 10 ml of the sample solution (1%) and 0.1 mol $dm^{-3}$ KCl. Cyclic voltammetry tracings were recorded from 0.2 to 1.2 V at a scan rate of 50 mV $s^{-1}$. Data were analyzed using GPES software.

### 2.7. ABTS radical scavenging assay

Antioxidant activity of the extracts was also assayed by ABTS radical scavenging by pulse radiolysis technique using a linear electron accelerator. For pulse radiolysis measurements, the absorbed dose was kept to a minimum to avoid decomposition of test extracts. Typical maximum dose per pulse using 50 ns pulses was 15 Gy. The standard pattern of decay with ascorbic acid having four different concentrations of 5, 10, 15 and 20 μg/ml showed typical concentration-dependent curves (Scott, Chen, Bakac, & Espenson, 1993). The ascorbic acid equivalent was computed by extrapolating the results with the standard graph. The reaction scheme after electron beam radiation through the solution is represented by:

$$H_2O \rightarrow e^-_{aq} + {}^{\bullet}OH + H^{\bullet}$$
$$e^-_{aq} + N_2O + H_2O \rightarrow {}^{\bullet}OH + N_2 + OH^-$$
$$N_3^- + {}^{\bullet}OH \rightarrow N_3^{\bullet} + OH^-$$
$$N_3^{\bullet} + ABTS^{2-} \rightarrow ABTS^{\bullet -} + N_3^-$$
$$ABTS^{\bullet -} + \text{Scavenger} \rightarrow \text{Scavenger}^{\bullet +} + ABTS^{2-}$$

### 2.8. β-Carotene bleaching assay

The procedure followed was essentially according to the method described by Velioglu and co-workers (Velioglu,

Mazza, Gao, & Oomah, 1998). For a typical assay, β-carotene (2 mg) was dissolved in 20 ml of chloroform. A 4 ml aliquot of this solution was added to a round-bottom flask containing 40 mg linoleic acid and 400 mg Tween-40. Chloroform was removed under vacuum at room temperature. Oxygenated distilled water (100 ml) was added to β-carotene and mixed well. Aliquot (3 ml) of the oxygenated β-carotene emulsion and 0.2 ml of test solution (0.1%) was placed in stoppered tubes and mixed well. The emulsion was incubated in a waterbath at 50 °C for 60 min. Oxidation of β-carotene was monitored by measuring at an interval of 15 min the absorption of the solution at 470 nm on a spectrophotometer. Antioxidant activity (AA) is expressed as percent inhibition relative to control (0.2 ml methanol) using the equation

$$AA\% = R_{control} - R_{sample}/R_{control}$$

Where $R_{control}$ and $R_{sample}$ are the degradation rates of β-carotene in reactant mixture without and with plant extracts.

Degradation rate $= \ln(a/b) \times 1/t$ where $a =$ initial absorbance, $b =$ absorbance at 60 min and $t =$ time in minutes.

### 2.9. Preparation of plasmid DNA

pUC18 DNA was isolated from a transformed colony of *E. coli* (DH5α) harbouring this plasmid. Cells were grown overnight in 10 ml Luria–Bertani (LB) medium having ampicillin (100 μg/ml) on a rotary shaker (150 rpm) at 37 °C. An aliquot (2 ml) of the overnight grown culture was sub-cultured into 500 ml LB medium containing ampicillin (100 μg/ml) and incubated on a rotary shaker (150 rpm) at 37 °C. The overnight grown culture was spun at 7000*g* using a centrifuge (Jouan, France) for 10 min. The supernatant was decanted and the pellet was washed twice with 200 ml of wash buffer [Tris (10 mM, pH 8.0), EDTA (1 mM, pH 8.0) and NaCl (100 mM)] by vortexing and spinning. The cell pellet was suspended in 20 ml of GTE [Glucose (50 mM), Tris–Cl (100 mM, pH 8.0), and EDTA (1 mM, pH 8.0)] buffer and mixed properly by vortexing. Cells were lysed using 60 ml of freshly prepared lysis buffer (0.2N NaOH + 1% SDS). The suspension was mixed properly with gentle shaking, and incubated at room temperature for 5 min. To this 30 ml of chilled potassium acetate (3 M, pH 5.2) was added, mixed quickly to avoid localized precipitation and incubated for 5 min on ice followed by centrifugation at 12,000*g* for 10 min. The supernatant was transferred in a separate tube and 66 ml of iso-propanol was added. The suspension was incubated at room temperature for 20 min and then centrifuged at 12,000*g* for 10 min. The supernatant was decanted carefully and DNA pellet was washed twice with 70% ethanol (50 ml each). The pellet was air dried, dissolved in Tris buffer (10 mM, pH 8.0) and used for further experiments.

#### 2.9.1. Testing of radio sensitivity of plasmid DNA

The pUC18 plasmid DNA was distributed as aliquots (10 μl) in sterile microfuge tubes. A 2 μl aliquots of the test samples (stock concentration 1%) was mixed with plasmid aliquots. In controls a 2 μl of milliQ water was used instead of the test samples. In the case of non-irradiated controls similar concentrations of the test compounds were added and incubated for 30 min before loading on the gel to examine the effects of compound as such. γ-Irradiation was carried out in a research irradiator (Gamma cell 220, AECL, Ottawa, Canada) at a dose rate 9.6 Gy/min. The samples were irradiated at doses between 1 and 5 kGy. Treated plasmid DNA aliquotes were subjected to agarose (1%) gel electrophoresis at the voltage of 4 V/cm using Tris acetate–EDTA (TAE) buffer (pH 8.0) (Sambrook, Fritsch, & Maniatis, 1989). Upon completion of electrophoresis gel was stained with ethidium bromide (0.5 μg/ml), visualized using UV-transilluminator and photographed.

### 2.10. Data analysis

Data collected are an average of three independent determinations. Statistical analysis was done using a paired t test and ANOVA (Micrococal Origin 4.1 software), and the results expressed as significant/non-significant at $p \leqslant 0.05$.

## 3. Results and discussion

Total phenolic content of green pepper as estimated by Prussian blue method in the present study was found to be 850 mg catechin equivalent per 100 g of green pepper. Earlier studies (Bandyopadhyay et al., 1990; Variyar et al., 1988) on the blackening of green pepper berries have demonstrated 3,4-dihydroxyphenyl ethanol glucoside, 3,4-dihydroxy-6-(*N*-ethylamino) benzamide (Fig. 1) and phenolic acid glycosides as the major phenolic compounds of the fresh spice. TLC analysis of the extracts from dried green pepper as well as black pepper revealed the absence of the above two compounds in them. This suggests an enzymatic oxidative conversion of the former two compounds to black oxidized product during sun drying. This resulted in a decrease in total phenolic content of black pepper to approximately one-fourth that in green pepper. These compounds were therefore not detectable in the dry spice.

UV–Vis spectra of acetone extract of fresh mace showed absorption maxima at 341 and 275 nm suggesting the presence of phenolic compounds. The three major bands obtained from prep. TLC and accounting for 72% of the mace extract were found to be composed mainly of lignans as evidenced by the mass spectral fragmentation pattern of

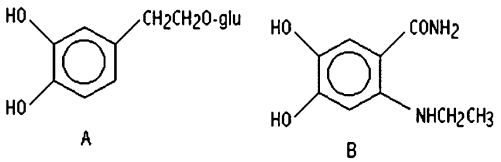


Fig. 1. Structures of (A) 3,4-dihydroxyphenyl ethanol and (B) 3,4-dihydroxy-6-(*N*-ethylamino) benzamide.

the individual peaks resolved on GC–MS. The major compounds tentatively identified and their relative distribution in each of the three bands were:

Band I:

1. Elemicin (13.63%), *m/z*: $[M]^+$ 208 (100), 193 (72.1), 177 (16.0), 150 (14.49), 133 (28.4), 118 (19.45), 105 (23.2), 91 (25.6), 77 (35.21), 65 (16.8), 53 (15.6), 39 (24.8).
2. Erythro-(3,4-methylenedioxy-7-hydroxy-1′-allyl-3′,5′-dimethoxy)-8,0,4′,-neolignan (21.64%), *m/z*: $[M]^+$ 414 (2.89), 221(14.15), 194 (29.70), 179 (14.41), 149 (4.55), 57 (11), 43 (100).
3. 6-Methoxy eugenol acetate (10.30%), *m/z*: $[M]^+$ 236 (5.32) 194 (100), 179 (11.7), 163 (8.1), 147 (8.41), 131 (12.4), 119 (14.45), 91 (14.22).

Band II:

1. 7-Oxo dehydro abietic acid (12.53%), *m/z*: $[M]^+$ 328 (100), 255 (48.76).
2. Isoeugenol acetate (29.18%), *m/z*: $[M]^+$ 206 (11.35), 164 (100), 149 (31.29), 133 (15.41), 131 (18.45), 103 (22), 91 (40), 77 (35.11).
3. γ-2-(4′-hydroxy-3′-methoxyphenyl)-c-5-(3″,4″,5″-trimethoxy-phenyl)-t-3,c-4-dimethyltetrahydrofuran (32.43%), *m/z*: $[M]^+$ 388 (50.76), 236(40.15), 224 (7.34), 221 (20.36), 208 (10.67), 196 (10.21), 192 (100), 180 (4.23), 177 (40.32), 164 (10.41), 151 (14.55).

Band III:

1. (+)-Erythro-(7S,8R)-$\Delta^{8'}$-4,7-dihydroxy-3,3′,5′-trimethoxy-8-0-4′-neolignan (100%), *m/z*: $[M]^+$ 374 (8.01), 221 (12.15), 194 (100), 179 (4.1), 164 (6.55), 151 (5), 91 (4), 83 (20).

Total phenolic content in the acetone extract in the present study was found to be 40 mg of catechin eqivalent per 100 g of fresh mace. Polyphenol content was earlier (Anon, 1983–86) shown to decrease with maturity and reported to be negligible in dry mace. Velioglu and co-workers (Velioglu et al., 1998) have demonstrated a highly significant and positive correlation between total phenolic content and antioxidant activity. Thus, a significant decrease in phenolic compounds during drying process could have a considerable impact on the antioxidant activity of dry black pepper and mace of commerce. An interaction of oxidized phenolics/free phenols with cell polysaccharide during sun-drying could also account for the reduction in extractable total phenolics (Chung, Wong, Wei, Huang, & Lin, 1998). It was therefore of interest to determine the antioxidant activity of phenolic fractions of the fresh spices.

### 3.1. DPPH radical scavenging activity

Of the various fractions from green pepper and fresh mace tested for their antioxidant activity, phenolic compounds from pepper showed the lower $EC_{50}$ values (Fig. 2). Among the pepper phenolics, 3,4-dihydroxyphenyl ethanol (hydroxytyrosol) glucoside exhibited the highest activity with an activity approximately 60% higher than standard trolox. Hydroxy tyrosol, the most important and active phenolic compound of olive oil has been reported to possess free radical scavenging activity (Chimi et al., 1991). It was demonstrated to strongly inhibit superoxide production by either a cell free system or activated neutrophils, and to inhibit rat platelet 12-lipoxygenase and rat polymorphonuclear leukocyte 5-lipoxygenase activities (Galli & Visioli, 1999; Kohyama et al., 1997). The phenolic acid fraction constituting mainly syringic, vanillic and ferulic acid as reported earlier (Variyar & Bandyopadhyay, 1994) showed activities comparable to standard trolox. Hydroxycinnamic acid derivatives are known antioxidants of various food products and their antioxidant activity has been extensively studied in vitro (Bakalbassis et al., 2001). 3,4-Dihydroxy-6-(*N*-ethylamino) benzamide a novel phenolic compound reported for the first time in green pepper was the least active component of the phenolic fraction of green pepper. It is known that heteroatom, O or N, at *para* or *ortho* position of OH would enhance antioxidant activity, owing to the resonance between the p-type lone pair of the heteroatom and the radical (Zhang, 1999). Presence of electron-donating groups on the aromatic ring is also expected to increase the antioxidant activity (Zhang, 1999). Recent studies of bond dissociation energies have revealed that electron-donating groups substituted *ortho* and *para* to the phenolic hydroxyl group lower the bond dissociation energies and increase the rate of hydrogen atom transfer to peroxyl radicals (Singh, O'Malley, & Popelier, 2005). 3,4-Dihydroxy-6-(*N*-ethylamino) benzamide with an electron-donating amino group at *para* position to OH groups is thus likely to have a higher antioxidant activity than

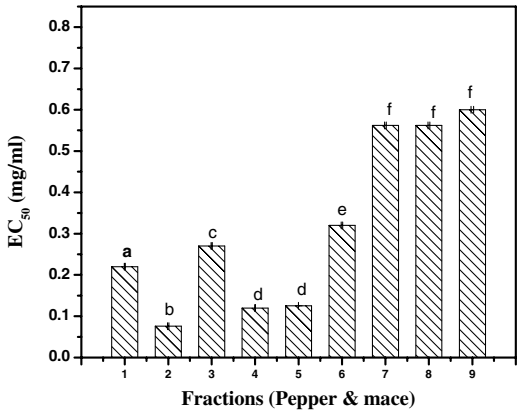


Fig. 2. $EC_{50}$ of fractions from green pepper and mace. The vertical bars represent the SD ($n = 3$) and values marked by same letter are not significantly different ($P < 0.05$). 1 = *n*-butanol extract, 2 = 3,4-dihydroxyphenyl ethanol glucoside, 3 = 3,4-dihydroxy-6-(*N*-ethylamino) benzamide, 4 = phenolic acid glycosides of green pepper, 5 = standard trolox and 6 = acetone extract, 7 = Band 1, 8 = Band II, 9 = Band III of nutmeg mace.

3,4-dihydroxyphenyl ethanol glucoside. Lower activity of the former could possibly be due to the high instability of the compound as it undergoes rapid oxidation in solution at pH values above 6.5.

The redox properties are crucial for better understanding of the electron transfer process. Cyclic voltammetry is an established instrumental tool for the measurement of electron transfer efficiency and in turn antioxidant efficacy of a test compound. Thus, we have measured the oxidation potential of the mixture of phenolic acids and the benzamide isolated from green pepper. By and large lower the oxidation potential of a test compound higher the antioxidant efficacy. The low oxidation potential value of 0.66 V (Fig. 3) for the amide further confirmed that this compound was an efficient oxidative radical scavenger.

Mace acetone extract exhibited a higher antioxidant activity compared to the TLC isolated fractions (Fig. 2). Higher activity observed could be accounted for the presence of other components such as lycopene that contributes to the antioxidant activity of the total extract. Activity of the individual fractions was more or less comparable. A lower activity of the total extract and its fractions compared to trolox was noted in the present study. In an investigation on the antioxidant activity of methanolic extracts of sesame cake extract, Suja and co-workers (Suja, Jayalekshmy, & Arumugham, 2004) have compared the antioxidant activities of individual lignans from sesame cake. Presence of methylene dioxy group as well as stereochemistry of the furan–phenyl bond was reported to be responsible for their activity. Chang and co workers (Chang, Yen, Huang, & Duh, 2002) have shown a lower activity of lignans constituents in ethanolic extract of sesame coat compared to α-tocopherol and BHA. Thus, the lower activity of mace lignans compared to trolox in the present study could be a result of their structural characteristics. In a recent study on the radical scavenging potential of phenolic compounds in *Olea europaea*, Nenadis and co-workers (Nenadis, Wang, Tsimidou, & Zhang, 2005) have demonstrated a lower activity of lignans namely pinoresinol and 1-acetoxypinoresorcinol compared to hydroxytyrosol. It was postulated that catechol derivatives with lower bond dissociation energy values (BDE) were more efficient antioxidants compared to monophenols that had higher BDE values. Lower activity of monophenolic mace lignans compared to catechol derivatives (hydroxytyrosol, 3,4-dihydroxy-6-(*N*-ethylamino) benzamide and phenolic acid glycosides) could thus be explained.

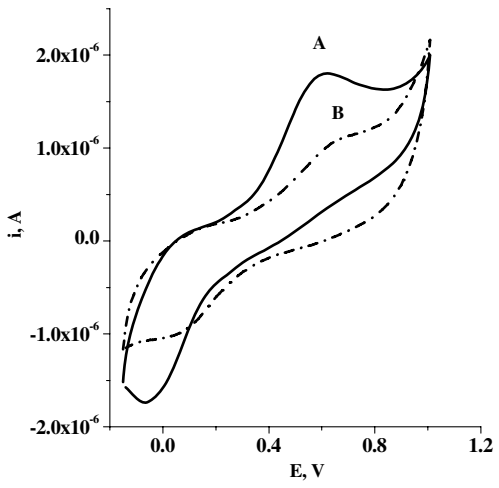


Fig. 3. Cyclic voltammogram of (A) mixture of phenolic acids and (B) 3,4-dihydroxy-6-(*N*-ethylamino) benzamide isolated from green pepper.

DPPH is long-lived nitrogen radical. Many antioxidants that react quickly with transient radicals such as peroxyl radical may react slowly or may be even inert to DPPH (Huang, Ou, & Prior, 2005). Further, reaction of DPPH with eugenol was shown to be reversible (Bondet, Brand-Williams, & Berset, 1997). This would result in erroneously low readings for antioxidant capacity of molecules particularly those containing *o*-methoxy phenol functionalities. These factors could possibly explain the similar $EC_{50}$ values for all the lignan bands in the DPPH assay. The antioxidant activity of these bands was therefore ascertained by their capacity to scavenge short-lived ABTS radical.

Pulse radiolysis study of ABTS radical involves scavenging of primary radicals (·OH) by azide ($N_3^-$) producing azidyl radical ($N_3^{\bullet}$), which in turn generates ABTS radical in solution. In this assay, all the bands from extract of mace showed significant activities (Fig. 4). The sample from Band I showed the highest activity (0.0007% solution of Band I shows an activity of 20 μg ascorbic acid equivalent per ml) followed by Band II (0.0014% solution of Band II shows an activity of 5.7 μg ascorbic acid equivalent per ml) and Band III (0.0014% solution of Band III shows an activity of 1.7 μg ascorbic acid equivalent per ml). A very high radical scavenging efficiency of the three bands is thus demonstrated.

### 3.2. β-Carotene bleaching assay

The decrease in absorbance of β-carotene in the presence of different extracts and standard trolox is shown in Fig. 5. It is interesting to note that the extracts from mace had a higher antioxidant activity than that from green pepper. This contrasts with the activities observed with DPPH radical scavenging assay. In a study on the structure-antioxidant activity relationship of ferulic acid derivatives, Nenadis and co-workers (Nenadis, Zhang, & Tsimidou, 2003) have demonstrated a lower activity of coniferyl alcohol and ferulic acid compared to less polar ethyl ferulate and isoeugenol in triolene–water emulsion autooxidation studies. The former compounds, however, exhibited higher antioxidant activity in DPPH radical scavenging assay. It was proposed that the higher partition coefficient of less polar compounds resulted in a greater interaction with lipid layer and thus a higher antioxidant activity. Higher antioxidant activity of less polar mace lignans compared to pepper phenolics (Fig. 5) in this assay could thus be accounted.

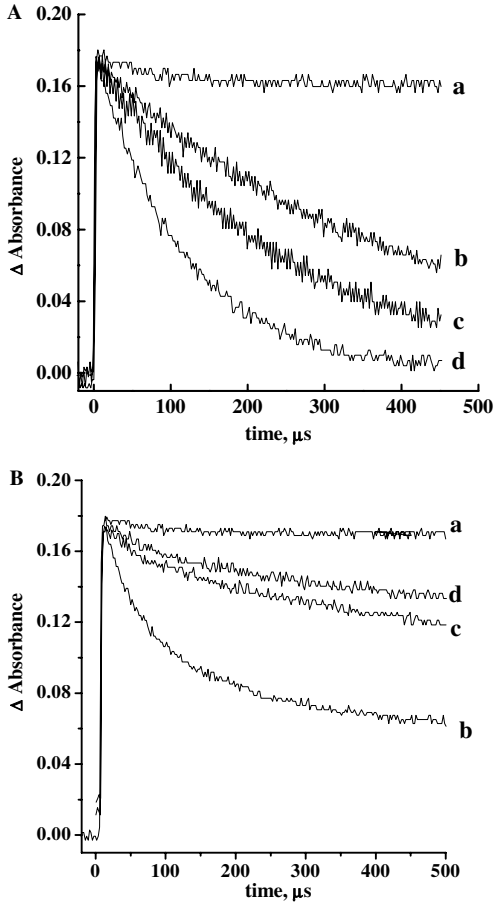


Fig. 4. Antioxidant activity of fractions obtained from nutmeg mace by ABTS assay. (A) ABTS radical scavenging activity of ascorbic acid at different concentration measured at 734 nm: a, ABTS radical decay without ascorbic acid; b, ABTS + 5 μg/ml ascorbic acid; c, ABTS + 10 μg/ml ascorbic acid; d, ABTS + 20 μg/ml ascorbic acid. (B) ABTS radical scavenging activity of nutmeg mace: a, ABTS radical decay without extract; b, ABTS + 0.0007% solution of Band I; c, ABTS + 0.0014% solution of Band II; d, ABTS + 0.0014% solution of Band III.

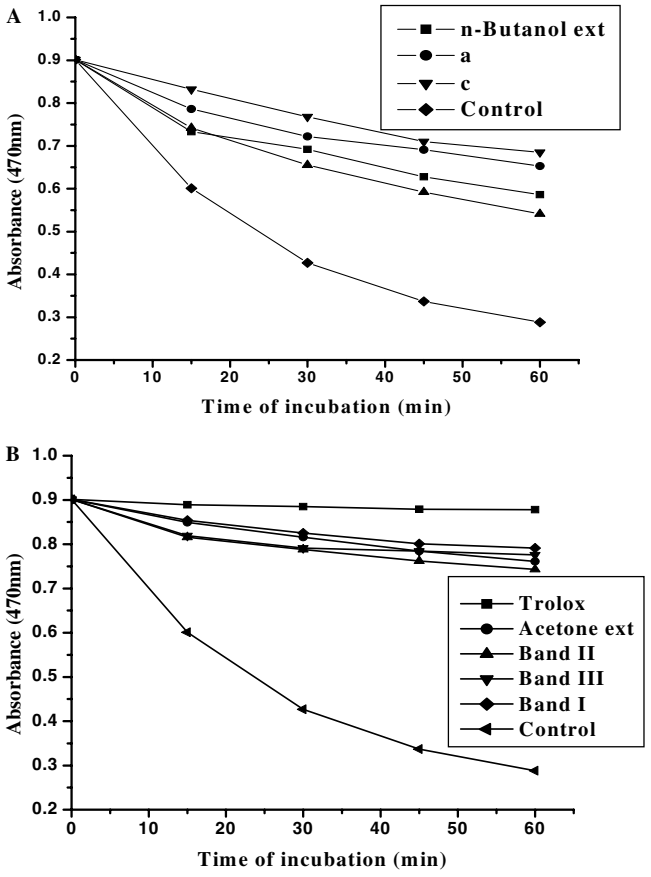


Fig. 5. Antioxidant activity of different fractions obtained using β-carotene linoleate model system. (A) green pepper: a, 3,4-dihydroxyphenyl ethanol glucoside; b, 3,4-dihydroxy-6-(*N*-ethylamino) benzamide; c, phenolic acid glycosides. (B) nutmeg mace.

### 3.3. Radioprotection of plasmid DNA

Figs. 6A and 6B illustrates the agarose gel profile of plasmid pUC 18 DNA irradiated with and without the extracts of green pepper and mace at 5.0 kGy, respectively. The pUC 18 is a 2.69 kb plasmid with an ampicillin resistant marker on it. Phenolic compounds isolated from green pepper showed similar radioprotective properties (Fig. 6A). However, among these, 3,4-dihydroxy-6-(*N*-ethylamino) benzamide was found to have comparatively less protective effect (Fig. 6A, lane-6). This could possibly be due to the unstable nature of this compound as detailed earlier. These results indicated the significant protection of plasmid DNA from radiation damage by the compounds from mace and green pepper, correlating well with their antioxidant properties. Radioprotection by aqueous extracts obtained from chilli, black pepper and turmeric to plasmid DNA has been reported earlier (Sharma, Gautam, & Jadhav, 2000). Curcumin, piperine and capsanthinin in turmeric, black pepper and chilli, respectively, known antioxidants in these spices, were shown to offer protection to DNA against damage by gamma radiation up to a dose of 5 kGy. Phenolics from *O. europaea* (rutin, oleuropein, hydroxytyrosol, verbascoside, luteolin) were also earlier demonstrated to exhibit protective action against X-ray induced chromosomal damage (Benavente-Garcia, 2002). It was proposed that the presence of specific functional groups, mainly catechol groups, resulted in such protective action. Four different lignan bands isolated from TLC plate of acetone extract of mace were assessed for their radio-protective efficacy. It is clearly noted that the components present in each of the four bands significantly protected the plasmid DNA from radiolytic damage up to a dose of 5 kGy (Fig. 6B, lanes 3–6). The unprotected plasmid bands were however substantially degraded by radiation treatment (Fig. 6B, lane 2). The protective action of isolates from green pepper and mace against radiation induced DNA damage could thus be ascribed to the phenolic nature of the constituents currently investigated.

The present work has demonstrated the efficacy of green pepper and fresh mace phenolics as antioxidants. Although black pepper finds wide application as a flavorant, preservative and as drug ingredient, green pepper has no

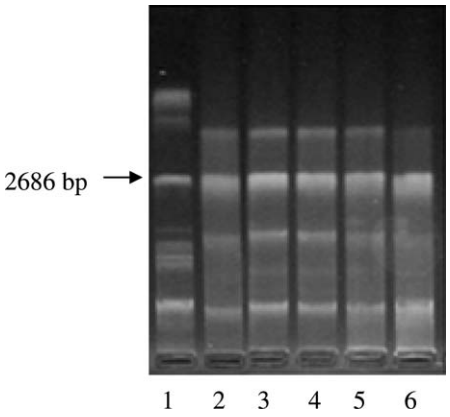


Fig. 6A. Agarose gel electrophoresis profile depicting the extent of damage of pUC18 plasmid DNA due to gamma irradiation (5 kGy) in the absence and presence of phenolic compounds extracted from green pepper. Lanes: 1, non-irradiated control; 2, irradiated control; 3, *n*-butanol extract; 4, 3,4-dihydroxyphenyl ethanol glucoside; 5, phenolic acid glycosides and 6, 3,4-dihydroxy-6-(*N*-ethylamino) benzamide.

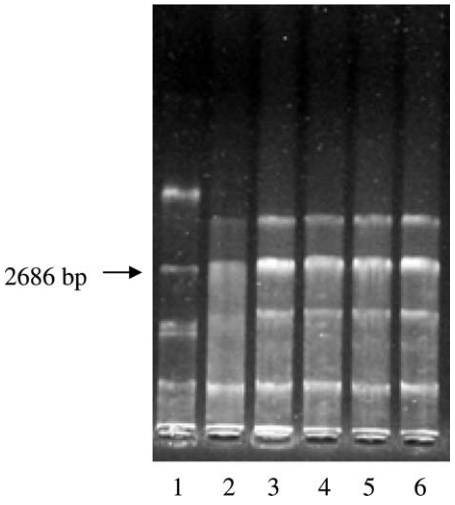


Fig. 6B. Agarose gel electrophoresis profile depicting the extent of damage of pUC18 plasmid DNA due to gamma irradiation (5 kGy) in the absence and presence of lignans extracted from Mace. Lanes: 1, non-irradiated control; 2, irradiated control; 3, acetone extract; 4, Band I; 5, Band II and 6, Band III.

commercial significance. Presence of phenolics with high antioxidant capacity that are lost during preparation of black pepper suggests the potential use of the fresh spice and its extracts as preservative. Although mace lignans show lower antioxidant activity in vitro, it is known that lignans and their glycosides are metabolized to biologically active compounds having catechol structural moiety after absorption into the body (Nakai et al., 2003). This would further enhance their activity making them effective antioxidants. Further, a radioprotective action of the above compounds towards DNA suggests the use of green pepper and mace extracts as a nutraceutical and as therapeutic agent in the treatment and prevention of human diseases.

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