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DNA damage protecting activity and antioxidant potential of pudina extract

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Abstract

Pudina extract (*Mentha spicata* Linn.) used as flavoring in culinary preparation throughout the plains of India was examined for its DNA damage protecting activity and antioxidant potential. *n*-Butanol soluble fraction (PE) derived from methanol extract of *Mentha spicata* Linn. at 10 μg/ml exhibited significant protecting activity against DNA strand scission by $^{\cdot}OH$ on pBluescript II SK(–) DNA. IC_{50} concentration of PE to scavenge $DPPH^{\cdot}$, $ABTS^{\cdot+}$ and superoxide radical was 7.47, 4.05 and 57.80 μg/ml, respectively. Inhibition of lipid peroxidation induced with 25 mM $FeSO_4$ on rat liver homogenate as lipid source was noted at 500 μg/ml of PE with Antioxidant Index of 63.43%. Total polyphenol content of one-milligram pudina extract was equivalent to 500 μg of gallic acid. Therefore, this potential bioactivity of pudina extract was associated with its high polyphenolic content.

Keywords: Pudina extract; DNA damage; $DPPH^{\cdot}$; $ABTS^{\cdot+}$; Superoxide radical; Lipid peroxidation inhibition; Polyphenol; Antioxidant activity

1. Introduction

Reactive oxygen species (ROS) are produced by cellular metabolism and by exogenous agents in the cells. These ROS may induce oxidative damage to various biomolecules in cells such as carbohydrates, proteins, lipids and DNA which in turn leads to cardiovascular and neurodegenerative diseases, inflammation and others (Ames, 1983; Stadtman, 1992; Sun, 1990). At least two major human problems aging, and cancer, involve ROS mediated DNA damage (Cerutti, 1994; Wiseman & Halliwell, 1996).

Plant foods and products thereof are rich sources of a variety of biologically active compounds and these phytochemicals have been found to possess a variety of biological activity including antioxidant potential (Craig, 1999). Several epidemiological studies have shown that increased dietary intake of natural phenolic antioxidants correlates with reduced coronary heart disease. Food rich in antioxidants plays an essential role in the prevention of cardiovascular disease, cancer (Gerber et al., 2002; Kris-Etherton et al., 2002; Serafini, Bellocco, Wolk, & Ekstrom, 2002) and neurodegenerative diseases, including Parkinson's and Alzheimer's disease (Di Matteo & Esposito, 2003) as well as inflammation and problems caused by cell and cutaneous aging (Ames, Shigenaga, & Hagen, 1993). Fruits, vegetables and oilseeds are increasingly recognized as sources of natural antioxidants, vitamins, minerals, and soluble/insoluble fibres but also for phenolic compounds including phenolic acids, flavonoids and isoflavonoids and lignans (Shahidi, 1997; Thompson, Robb, Serraino, & Cheung, 1991). Natural antioxidants are also in high demand for application as nutriceuticals as well as food additive because of consumer preferences.

Mints have been used and valued as aromatic herbs for thousand of years (Lange & Croteau, 1999). *Mentha spicata* is characterized by a high carvone (60–70%) and limonene (8–10%) content. Carvone is used in cosmetic, therapeutic, environmental indicator and other applications (De Carvalho & Da Fonseca, 2005). Garden mint or spearmint is widely cultivated throughout the plains of

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India and used for culinary purposes (Ambasta, 1986; The Wealth of India, 2003). The herb also possesses medicinal properties like stimulant, carminative and antispasmodic (Chopra, Nayar, & Chopra, 1956; Medicinal Plant of India, 1987). A sweetened infusion of the herb is used as a remedy in infantile troubles. Leaves are used in fever, bronchitis and increasingly becoming popular as flavorings all over India mainly due to their pleasant flavor. Leaves are also used for flavoring culinary preparations and for making chutney in India. However, little is known about its antioxidant activity. Owing to their diverse biological activities and increasing consumer interest, we investigated the oxidative DNA damage-protecting activity and antioxidant potential of this plant extract in a systematic way in relation to its total phenol and flavonoid content. To the best of our knowledge this is the first report of DNA damage protecting activity of mint.

2. Materials and methods

2.1. Chemicals

Fast Plasmid Mini kit from Eppendrof, Hamburg, Germany was used. DPPH (2,2-diphenyl-1-picrylhydrazyl), TBA (thiobarbituric acid), Folin & Ciocalteu's phenol reagent, quercetin dihydrate, butylated hydroxytoluene (BHT), agarose and ethidium bromide (EtBr) were purchased from Sigma-Aldrich, St. Louis, MO, USA. ABTS (2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid), pota ssium persulphate, aluminum chloride, iron (III) chloride (hexahydrate), and 2-deoxy-D-ribose were procured from ICN Biomedicals, Eschwege, Germany. Hexane, diethyl ether, ethyl acetate, *n*-butanol were obtained from Merck, Darmstadt, Germany and are used for extraction of plant material. All other reagents used were of analytical grade.

2.2. Spectrophotometric measurements

Spectrophotometric measurements were performed by using UV-1700 (Pharmaspec) UV–vis spectrophotometer, Shimadzu, Kyoto, Japan.

2.3. Plant material and its extraction

Plant material (*Mentha spicata* Linn.) was purchased from a local vegetable market (Kolkata, India). Voucher specimen was identified from Botanical Survey of India, Shibpur, Howrah, India. Two hundred grams air-dried leaves of *M. spicata* was extracted with one-liter of methanol overnight 3× at room temperature, filtered and concentrated by using a rotary evaporator (Eyela N–N series, Tokyo, Japan) at 40 °C. The concentrated crude extract was defatted with *n*-hexane. The defatted methanol extract was suspended in water and fractionated sequentially into diethyl ether, ethyl acetate and *n*-butanol (Rios, Manez, Paya, & Alcaraz, 1992). The *n*-butanol fraction was concentrated in rotary evaporator to obtain a brown dry powder, which was further dissolved in water (10 mg/ml) and all experiments were performed with this *n*-butanol fraction mentioned as pudina extract or PE.

2.4. Determination of total polyphenol and flavonoid content

The polyphenol content of pudina extract (PE) was quantified by the Folin–Ciocalteau's reagent and was expressed as gallic acid equivalents (Yuan, Bone, & Carrington, 2005). Aliquots of test samples (100 μl) were mixed with 2.0 ml of 2% Na_2CO_3 and incubated at room temperature for 2 min. After the addition of 100 μl 50% Folin–Ciocalteau's phenol reagents the reaction tube was further incubated for 30 min at room temperature, and finally absorbance was read at 720 nm.

A known volume of pudina extract was placed in a 10 ml volumetric flask to estimate flavonoid content according to the method of Zhishen, Mengcheng, and Jianming (1999). Distilled water was added to make the volume to 5 and 0.3 ml $NaNO_2$ (1:20 w/v) was added to this. Three milliliters of $AlCl_3$ (1:10 w/v) was added 5 min later. After 6 min, 2 ml of 1 N NaOH was added and the total absorbance was measured at 510 nm. Quercetin was used as a standard for constructing a calibration curve.

2.5. Effect of pudina extract on pBS plasmid DNA scission induced by hydroxyl radical

DNA damage protective activity of pudina extract was checked on pBluescript II SK(–) vector in *E. coli* XL-1 Blue strain. Plasmid DNA was isolated by Fast plasmid mini kit. Plasmid DNA was oxidized with H_2O_2 + UV treatment in presence of PE and checked on 1% agarose according to Russo et al. (2000) after modifications. In brief, the experiments were performed in a volume of 10 μl in a microfuge tube containing 100 ng of pBS plasmid DNA in 1× TE buffer (10 mM Tris–Cl and 1 mM EDTA), pH 8.0, H_2O_2 was added at a final concentration of 100 mM/ml with and without 1 μl (10 mg/ml) of pudina extract. The reactions were initiated by UV irradiation and continued for 5 min on the surface of a UV transilluminator (Bangalore Genei Ltd., Bangalore, India) with intensity of 8000 μW/cm^2, at 312 nm under room temperature. After irradiation the reaction mixture (10 μl) along with gel loading dye (6×) was placed on 1% agarose gel for electrophoresis. Untreated pBluescript II SK(–) DNA was used as a control in each run of gel electrophoresis along with partial treatment i.e., only UV treatment and only H_2O_2. Gel was stained with ethidium bromide and photographed in Gel Doc. Quercetin (100 μM) was used as positive control.

2.5.1. Densitometric analysis of treated and control pBS plasmid DNA

Gel was scanned on Gel documentation system (Gel-Doc- XR, Bio-Rad, Hercules, CA, USA). Bands on the gels were quantified using discovery series Quantity one 1-D analysis software (Bio-Rad).

2.6. Free radical scavenging ability by the use of a stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical

DPPH (1,1-diphenyl-2-picrylhydrazyl) can make stable free radicals in aqueous or ethanol solution (Kato, Terao, Shimamoto, & Hirata, 1988) and free radical scavenging capacity of pudina extract (3.33–16.6 μg/ml) was noted through the change of optical density of DPPH radicals at 517 nm after 20 min incubation at room temperature. A control (A_0) DPPH (100 μM/ml) was taken without plant extracts under identical conditions. The percent free radical scavenging capacity (%RSC) of test samples was calculated from control and IC_{50} from regression analysis. Quercetin (5–25 μM/ml) was used as a standard antioxidant.

2.7. Free radical scavenging ability by the use of a stable ABTS (2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) radical cation

The free radical scavenging activity of *M. spicata* was determined by ABTS radical cation decolourization assay (Re et al., 1999). ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Because ABTS and potassium persulphate react stoichiometrically at a ratio of 1:0.5 (mol/mol), this will result in incomplete oxidation of the ABTS. Oxidation of the ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 h had elapsed. The radical was stable in this form for more than two days when stored in the dark at room temperature (Katalinic, Milos, Kulisic, & Jukic, 2005). Prior to use, the stock solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C exactly 6 min after initial mixing (A_t). One millilitre of diluted ABTS solution was mixed with 10 μl of pudina extract of different strength (4–8 μg/ml). The percentage decrease of absorbance at 734 nm was calculated for each point and the antioxidant capacity of the test compounds was expressed percent inhibition (%*I*) and IC_{50} Value was calculated from regression analysis. Quercetin (2–10 μM/ml) was used as a standard antioxidant.

2.8. Superoxide anion scavenging activity

Superoxide radical scavenging assay was based on the reduction of nitro blue tetrazolium (NBT) in the presence of NADH and phenazonium methosulphate (PMS) under aerobic condition (Nishikimi, Rao, & Yagi, 1972) with modification. The reaction mixture contained NBT (50 μM), NADH (150 μM) with or without plant extract (16.6–116.6 μg/ml) in a total volume of 3 ml tris buffer (0.02 M, pH 8.0). The reaction was started by adding PMS (15 μM) to the mixture and the absorbance change was recorded at 560 nm after 1 min. Percent inhibition was calculated against a control without the extract. Quercetin (40–80 μM/ml) was used as a standard antioxidant.

2.9. Antilipoperoxidant activity of pudina extract by TBARS assay

2.9.1. Preparation of rat liver homogenate 10% w/v

Male Sprague–Dawley rats of 280–300 g fed on a standard laboratory diet and water ad libitum were used. The liver homogenate was prepared according to the method of Song et al. (2003) with minor modifications. The liver was excised, perfused and homogenized with 120 mM KCl, 50 mM phosphate buffer, pH 7.4 (1:10 w/v). The samples were centrifuged at 700*g* at 4 °C for 10 min and supernatant was kept at −20 °C until use.

2.9.2. Thiobarbituric acid reactive substances (TBARS) assay

In order to quantify the concentration of oxidized lipids the amount of thiobarbituric acid-reactive substances (TBARS) was determined (Ohkawa, Ohishi, & Yagi, 1979). Lipid peroxidation was induced with 25 mM $FeSO_4$ in rat liver homogenate at 37 °C for 3 h. The liver homogenate (100 μl) with different extract (50–500 μg/ml) was added to 2 ml of 0.67% aqueous thiobarbituric acid solution and incubated into a boiling water bath for 20 min. After this incubation samples were cooled on ice, centrifuged at 2300*g* and absorbance was noted at 532 nm. TBARS concentration of control group was expressed as 100% (Song et al., 2003).

$$\%\ \text{Antioxidant Index}\ (\%\text{AI}) = 1 - t/c \times 100$$

Results are expressed as mean value ± standard deviation ($n = 3$). BHT (100–1000 ppm) was used as standard antioxidant, where t is the OD of testing sample and c is the control.

2.10. Statistical analysis

IC_{50} values, from the in vitro data, were calculated by regression analysis. Statistical comparisons between groups were performed with Student's *t*-test for independent observations. Differences were considered significant at $p < 0.05$. Each experiment was repeated for three times.

3. Results and discussion

This study is designed to evaluate the DNA damage protecting activity and antioxidant potential of pudina extract in a comprehensive manner employing a variety of in vitro methods. The protective effect of PE on H_2O_2 + UV-induced damage was studied on pBS plasmid DNA. Fig. 1 shows the electrophoretic pattern of DNA after UV-photolysis of H_2O_2 (100 mM) in the presence or absence of pudina extract (10 μg/ml). DNA derived

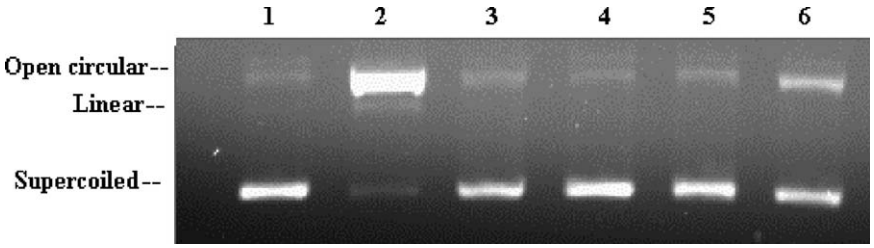

Fig. 1. Electrophoretic pattern of pBluescript II SK (–) DNA after UV-photolysis of H_2O_2 (100 mM) in the presence or absence of pudina extract (10 μg/ml). Lane 1: control, lane 2: treated, lane 3: only UV treated, lane 4: only H_2O_2 treated, lane 5: quercetin (100 μM) and lane 6: PE (10 μg/ml).

from pBS plasmid showed two bands on agarose gel electrophoresis (lane 1) the faster moving prominent band corresponded to the native supercoiled circular DNA (Sc DNA) and the slower moving very faint band was the open circular form (Oc DNA). The UV irradiation of DNA in the presence of H_2O_2 (lane 2) resulting the cleavage of Sc DNA to give prominent Oc DNA and a faint linear (Lin) DNA indicating that $OH^{\cdot}$ generated from UV-photolysis of H_2O_2 produced DNA strand scission. Although both $O_2^{\cdot -}$ and H_2O_2 are potentially cytotoxic, most of the oxidative damage in biological systems is caused by the $^{\cdot}OH$, which is generated by the reaction between $O_2^{\cdot -}$ and H_2O_2 in the presence of metal ions (Guitteridge, 1984). It was noted that only UV treatment and only H_2O_2 treatment (lanes 3 and 4, respectively,) could not induce damage as noted in combined treatment (lane 2). This damage can be reduced in the presence of standard flavonoid antioxidant, quercetin (100 μM) (lane 5) (Russo et al., 2000). The addition of pudina extract to the reaction mixture of H_2O_2 induced the significant protection to the damage of native supercoiled circular DNA (lane 6). Higher concentrations of PE were also checked for the dose dependent activity of PE but there was as much protection as of 10 μg/ml. Hence 10 μg/ml of pudina extract is optimal. Densitometric analysis (Fig. 2) revealed that in pudina extract treated sample the densitometric units of Sc DNA (3502.75) were almost similar to that of the untreated control Sc DNA (3842.19). Whereas the densitometric units (Takahashi & Nagano, 1988) of Sc DNA in H_2O_2 + UV treated control was decreased to 1415.63. H_2O_2 induced damage of human lymphocytes also showed protective effect of pudina extract through the comet assay (data not shown). Further studies are underway in this respect. DNA damage protecting activity of pudina extract is corresponding to its antioxidant potential.

The free radical scavenging capacity of crude methanolic extract of pudina and its butanol fraction was evaluated by means of DPPH and ABTS assays. The results revealed that butanol fraction is more effective than the crude extract with IC_{50} 17.46 and 11.90 μg/ml for crude extract and 7.47 and 4.05 μg/ml for butanol fraction in DPPH and ABTS assays, respectively, which can be compared to that of the quercetin (Table 1). Hence further studies were carried out with butanol fraction derived from methanolic extract of pudina (i.e. PE).

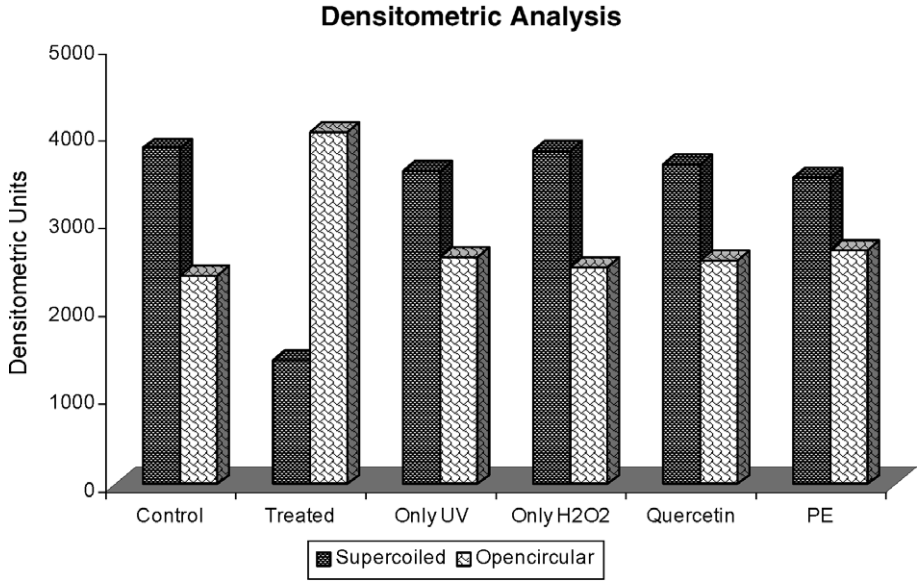

Fig. 2. Densitometry analysis of supercoiled and open circular plasmid DNA after UV-photolysis of H_2O_2 (100 mM) in the presence and absence of pudina extract (10 μg/ml).

The model DPPH provides a method to evaluate antioxidant activity in a relatively short time compared to other methods (Espin, Soler-Rivas, & Wichers, 2000). The concentrations of PE required to scavenge DPPH radical showed a dose dependent response (Table 2). The disappearance of DPPH$^{\cdot}$ is directly proportional to the amount of antioxidant present in the reaction mixture. Decolourization of ABTS$^{\cdot+}$ also reflects the capacity of an antioxidant species to donate electrons or hydrogen atoms to inactivate this radical cation. In ABTS$^{\cdot+}$ decolourization assay potential activity was noted at 8 μg/ml of pudina extract (Table 2). The pudina extract was effective in superoxide radical scavenging assay also in a concentration dependent manner (Table 2). This ability of pudina extract to scavenge superoxide may contribute to its significant antioxidant potential. The pudina extract scavenged superoxide radicals in a dose dependent manner with IC_{50} of 57.80 μg/ml, which is comparable to the IC_{50} of quercetin at 58.33 μM/ml.

Free radical scavenging is a generally accepted mechanism for antioxidants to inhibit lipid peroxidation. The inhibition of lipid peroxidation induced by $FeSO_4$ in rat liver homogenate was assayed by measuring the lipid oxidation products such as TBARS (Fig. 3). Results showed that the pudina extract inhibited TBARS formation in a concentration dependent manner (Ruberto, Baratta, Deans, & Dorman, 2000). Pudina extract (500 μg/ml) was able to inhibit the TBARS production by 63% (AI%), which is better than 100 ppm of butylated hydroxytoluene (BHT, AI% = 60.5%). The antioxidant activity of compounds is often described by their ability to delay the onset of autoxidation by scavenging ROS, or their ability to act as chain-breaking antioxidants by inhibiting the propagation phase of lipid autoxidation (Nawar, 1996).

Table 1
Comparative study of IC_{50} values

Sample	DPPH	ABTS
Crude MeOH ext.	17.46 μg/ml	11.90 μg/ml
But-fraction	7.47 μg/ml	4.05 μg/ml
Quercetin	14.00 μM/ml	6.35 μM/ml

One-milligram pudina extract was equivalent to 500 μg of gallic acid and 487 μg of quercetin. The total polyphenol content of the pudina extract was expressed as gallic acid equivalents (Capecka, Mareczek, & Leja, 2005) following confirmation of linearity of the response of the assay using the extract. The total flavonoid content of the pudina extract was determined by using the method of Zhishen et al. (1999) and expressed as quercetin equivalents (Eberhardt, Lee, & Liu, 2000; Luximon-Ramma, Bahorun, Soobrattee, & Aruoma, 2002). Significantly high total flavonoid content in the pudina extract may be corroborated with the traditional use of the plant in India in daily life as well as its reported use in several free-radical mediated diseases since time immemorial (Chopra et al., 1956; The Wealth of India, 2003).

Polyphenols are being increasingly reported to exhibit quality-preserving and antioxidant effects in foods and vegetables (Shahidi & Wanasundara, 1992). Fruits and vegetable are increasingly recognized as sources of phenolic compounds, including flavonoids. Phenolic compounds are effective hydrogen donors, which make them good antioxidants (Rice-Evans, Miller, Bramley, & Pridham, 1995). The free radical inhibitors activity of pudina extract showed a higher efficiency in ABTS with scavenging of 98.8% inhibition using 8.0 μg/ml of PE and in the case of DPPH assay 91.5% RSC inhibition was observed with 16.6 μg/ml of PE. The effect of this extract to inhibit lipid peroxidation in rat liver homogenate 500 μg/ml of PE to show 63% AI. Lamiaceae (Labiatae) family members found to be a rich source of polyphenolic compounds

Table 2
Concentration dependent free radical scavenging activity of PE and standard antioxidant quercetin

	PE (μg/ml)		Quercetin (μM/ml)	
	Concentration	% Activity	Concentration	% Activity
DPPH assay[a]	3.33	24.42 ± 3.41	5	24.54 ± 1.39
	6.66	45.34 ± 3.04	10	55.07 ± 2.40
	10.0	68.95 ± 4.13	15	89.73 ± 2.00
	13.3	85.95 ± 2.03	20	93.09 ± 0.38
	16.6	91.52 ± 0.75	25	93.34 ± 0.34
ABTS assay[b]	4	48.34 ± 2.00	2	15.66 ± 0.87
	5	63.43 ± 0.49	4	35.74 ± 1.13
	6	73.90 ± 2.11	6	44.80 ± 1.30
	7	89.79 ± 0.98	8	61.90 ± 1.20
	8	98.87 ± 0.63	10	78.60 ± 1.63
Superoxide scavenging assay[b]	16.6	30.86 ± 2.24	40	33.38 ± 2.15
	33.3	44.57 ± 2.08	50	43.18 ± 1.69
	66.6	55.05 ± 2.60	60	53.21 ± 1.94
	100	66.85 ± 0.58	70	60.81 ± 1.48
	116.6	68.77 ± 1.73	80	66.46 ± 1.26

[a] % RSC.
[b] % Inhibition.

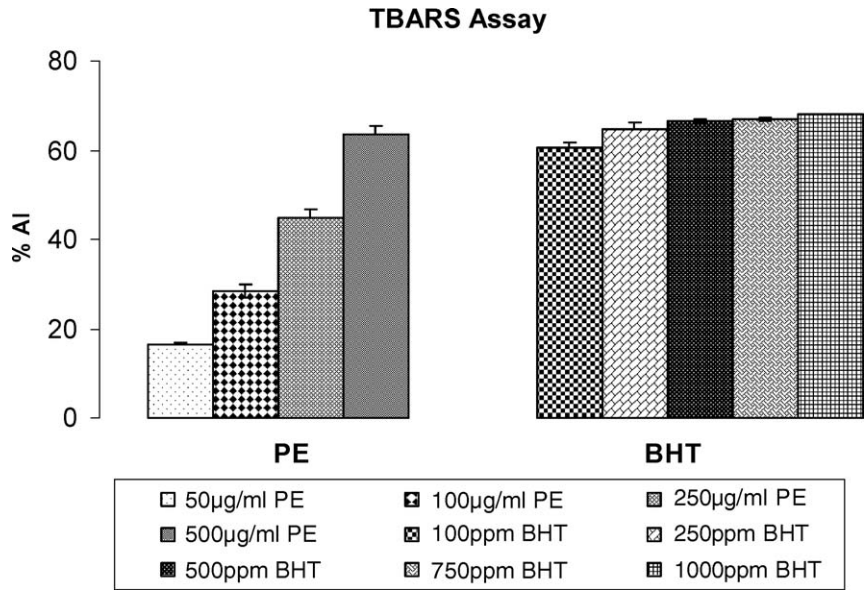

Fig. 3. Inhibition of lipid peroxidation by pudina extract and standard antioxidant BHT.

namely rosmarinic acid (Ellis & Towers, 1970; Zgórka & Glowniak, 2001). Plant derived antioxidant compounds, e.g., rosmarinic acid has drawn considerable attention as dietary antioxidant supplements (Halliwell, Aeschbach, Löliger, & Aruoma, 1995). The antioxidant activity of rosmarinic acid is much higher than that of α-tocopherol and BHT (Chen & Ho, 1997). Therefore, the presence of rosmarinic acid along with other compounds in *M. spicata* (Dorman, Kosar, Kahlos, Holm, & Hiltunen, 2003; Kosar, Dorman, Baser, & Hiltunen, 2004) may be correlated with its oxidative DNA damage protecting activity and antioxidant potential.

In recent years particular attention has been paid to a specific class of antioxidant phytochemicals the flavonoids. Flavonoids are polyphenolic substances naturally present in essentially all plant materials and are prominently ubiquitous in vegetables cereals, fruits, nuts, wine, tea, beer, and cocoa (Bravo, 1998). Voirin, Bayet, Faure, and Jullien (1999) reported the presence of twenty flavonoid aglycones in *M. spicata* and all of them were in the flavone subdivision of flavonoids such as apigenin, acacetin, luteolin and others. The presence of different groups of flavonoids has also been reported from the leaves of *M. spicata* (Subramanian & Nair, 1972). Rare flavone glucoronides have been reported from *M. spicata* (Nair & Gunasegaran, 1981). In agreement with other authors (Rice-Evans, Miller, & Paganga, 1996) it can be concluded that the absence of 3-OH in the B ring of the flavones has an influence on their antioxidant activity. However, the 3-oxo and 2–3 double bond in the B ring, –OH in 5 and 7 position of the A ring and presence of catechol moiety in the C ring (e.g., luteolin, etc.) is making the pudina extract a potential natural polyphenolic dietary antioxidant. Species of the genus *Mentha* have been reported to contain a range of components (Areias, Valentao, Andrade, Ferreres, & Seabra, 2001; Triantaphyllou, Blekas, & Boskou, 2001). In addition, flavanones are also commonly found in aromatic plant like mint (Manach, Scalbert, Morand, Remsey, & Jimenez, 2004). The protective effects of pudina extract rich in polyphenolics comprising flavonoids, against oxidative DNA damage shown here are in accordance with the structure-activity relationship of these compounds (Liopiz et al., 2004). Plant derived polyphenolic flavonoids are well known to exhibit antioxidant activity through a variety of mechanisms including scavenging of ROS, inhibiting lipid peroxidation as well as chelating metal ions (Shahidi, 1997). Earlier studies have shown the antioxidant activity of *Mentha piperita* (Mimica-Dukic, Bozin, Sokovic, Mihajlovic, & Matavulj, 2003). Very recently, *M. spicata* have been reported to have moderate stabilization activity against oxidation of sunflower oil (Marinova & Yanishlieva, 1997). This work has gathered experimental evidence on the most commonly used edible variety of mint (*M. spicata*) as natural antioxidant for its capacity to protect organism and cell from oxidative DNA damage associated with aging, cancer and degenerative diseases. This profound protective effect of pudina extract against oxidative DNA damage, free radical scavenging and inhibition of lipid peroxidation may explain its extensive use in daily life and possible health benefits. Thus, pudina may serve as an ideal candidate for a cost-effective, readily exploitable natural polyphenolic phytochemical.

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