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Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability

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Abstract

In the present study, three plant foods, namely, drumstick leaves (*Moringa oleifera*), mint leaves (*Mentha spicata*) and carrot tuber (*Daucus carota*) were extracted with ethanol and analyzed for their antioxidant activity. The antioxidant activity of extracts was evaluated according to the amount of malonaldehyde (MDA) formed by the $FeSO_4$-induced oxidation of linoleic acid and a high PUFA oil (sunflower oil) at 37 °C in Trizma-buffer (pH 7.4). At a concentration of 1.5 mg/ml of linoleic acid ,the extracts from drumstick and carrot had a higher antioxidant activity (83% and 80%) than α-tocopherol (72%). In sunflower oil, the extracts from drumstick leaves and mint leaves were found to exhibit a similar activity(46% and 44%). The extract from drumstick exhibited the highest activity in both lipid systems. In addition, the stability of extracts to pH (4 and 9) and temperature (100 °C, 15 min) was investigated. The antioxidant activity of the extracts from mint leaves and carrot was higher at pH 9 than pH 4, while that of drumstick extract remained the same under both pH conditions. The extract from carrot was more heat-stable than other extracts. The three extracts stored in the dark at 5 and 25 °C after a 15 day period did not show any significant change ($p \leqslant 0.05$) in their antioxidant activity. These data indicate that selected plant extracts are potential sources of dietary antioxidants.

Keywords: Antioxidant activity; Plant extract; Processing; Stability

1. Introduction

Lipid oxidation is a highly deteriorative process in foods, as it leads to unacceptable properties for the customer and a loss in nutritional value. In addition, oxidation leads to health disorders such as atherosclerosis and cancerogenesis among others. Hence the presence of antioxidants in foods is essential for their quality, retention and safety (Koleva et al., 2003; Pizzale, Bortolomeazzi, Vichi, Uberegger, & Conte, 2002). Toxicological effects of synthetic antioxidants and consumer preference for natural products have resulted in increased interest in the application of natural antioxidants (Castenmiller et al., 2002; Kaur & Kapoor, 2001; Koleva et al., 2003; Pizzale et al., 2002). The search for safe and effective naturally occurring antioxidants is now focused on edible plants, especially spices and herbs (Nakatani, 1997). A large number of plants have been screened as a viable source of natural antioxidants including tocopherols, vitamin C, carotenoids and phenolic compounds which are responsible for maintenance of health and protection from coronary heart diseases and cancer (Castenmiller et al., 2002; Javanmardi, Stushnoff, Locke, & Vivanco, 2003; Kaur & Kapoor, 2001). However, food composition tables, which are necessary tools for epidemiological and nutritional studies, are really only representative of food stuffs consumed in their raw state, without considering the fact that concentration of nutrients and their biological activity may be changed by environmental variables as well as processing (Nicoli, Anese, & Parpinel, 1999).

The consequence of food processing and preservation procedure on the overall antioxidant activity of foods are

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generally the result of different events, hence the evaluation of processing factors influencing the antioxidant activity is imperative to increase or preserve their efficacy and bioavailability (Kaur & Kapoor, 2001; Nicoli et al., 1999). In addition, from a nutritional point of view, the understanding of the consequence of food processing is one of the most important steps in correct interpretation and evaluation of study results regarding dietary habits and human health (Kaur & Kapoor, 2001).

The present study addresses the utilization of some plant materials such as drumstick leaves (*Moringa oleifera*), mint leaves (*Mentha spicata*) and carrot tuber (*Daucus carota*) as sources of natural antioxidants. Our objectives were to evaluate the antioxidant activity and stability (pH, heat and storage) of these extracts.

2. Materials and methods

Selected plant materials (drumstick leaves, mint leaves and carrot tuber) were bought in bulk, cleaned, grated (carrot) and dried in a hot air oven at 50 °C. The dried plant materials were ground separately and passed through a 60 mesh sieve and kept in air tight containers at 4 °C until further use.

5,5′-Dithio(bis)nitrobenzoic acid (DTNB) was obtained from Sigma Staishaim, Germany, β-carotene from Sigma chemicals Co. (St. Louis, MO, USA) and α,α′-Bipyridyl from E-Merck Ltd. (Bombay, India). Tocopherol and ascorbic acid were obtained from SD fine chemicals (Bombay, India). Glutathione and linoleic acid were obtained from Sisco Research Lab. Pvt. Ltd. (Bombay, India).

2.1. Determination of antioxidant components

Ascorbic acid was determined according to reduction in the absorbance of 2,6-dichlorophenol indophenol dye on reaction with ascorbic acid (AOAC, 1970).

α-Tocopherol was extracted by direct saponification of dried sample and estimated based on the formation of a red complex from the reaction of α ,α′-bipyridyl with ferrous ion due to reduction of ferric ion by tocopherol (Freed, 1996).

β-Carotene was separated by liquid column chromatography, followed by measuring the absorbance of the eluate at 450 nm against standard β-carotene (Ranganna, 1999). A column of 30 × 1 cm was packed with 6 g of alumina; about 1–2 g of sodium sulphate (anhydrous) was placed on top of the packed column. After loading the sample on top of the column, a mixture of acetone–hexane (3:7, v/v) was used as the developing solvent.

Reduced glutathione was determined based on the development of a yellow compound due to reaction of 5,5′-dithio(bis)nitrobenzoic acid with compounds containing sulphydryl groups (Beutler & Kelly, 1963).

Total phenols were extracted by heating a weighed portion (50–500 mg) of dried sample with 5 ml of 1.2 M HCl in 50% aqueous methanol for 2 h at 90 °C (Vinson, Hao, Su, & Zubic, 1998) and analyzed by Folin-Ciocalteu micro method (Slinkard & Singleton, 1977). Results were expressed as grams of gallic acid per 100 g of dried plant material.

2.2. Preparation of antioxidant extracts

A weighed portion (15 g) of each dried sample was extracted with 50 ml of 95% (v/v) ethanol for 6 h in a mechanical shaker. The extracts were filtered and filtrates were evaporated at 40 °C to dryness in a rotary evaporator (Buchi Laboratoriums-Technik, Flawil/ Schweiz, Switzerland). The dried extract obtained from each plant material was stored in an airtight container at 4 °C until further use.

2.3. Determination of antioxidant activity

(a) *Oxidation system of lipid by ferrous sulphate:* The oxidation of linoleic acid was conducted according to the method of Tamura and Yamagami (1994). An aqueous solution containing linoleic acid (1.5 mg/ml) was poured into a 30 ml test tube and diluted with 4.85 ml of Trizma-buffer solution (0.25 mM, pH 7.4) containing 0.2% sodium dodecyl sulphate (w/v) and 0.75 mM potassium chloride. Trizma buffer was prepared by diluting 6.075 g of Tris(hydroxymethyl)aminomethane and 11.184 g of potassium chloride with distilled water to 1 L after adjusting the pH of the solution to 7.4. Lipid peroxidation was initiated by adding 0.05 ml ferrous sulphate (20 mM). Incubation was continued for 16 h at 37 °C in the dark. The reaction was stopped by adding 50 µl of 1% butylated hydroxytoluene (BHT) in ethanol. The solution obtained (9.9 ml) was used for antioxidant activity assay.

(b) *Antioxidant activity assay:* The dried plant extract (100 µg) in 100 µl of 0.5% ethanol/water was mixed with the solution (9.9 ml) mentioned above when necessary. α-Tocopherol was used as a standard to evaluate the antioxidative activity of samples. The reacted solution obtained (1 ml) was used for TBA assay.

(c) *TBA assay:* The degree of oxidation of oil was measured by thiobarbituric acid (TBA) assay described by Ohkawa, Ohishi, and Yagi (1979).The reacted solution (1 ml) mentioned above was incubated with 0.2% (w/v) thiobarbituric acid (3 ml) and 0.05 M sulfuric acid (2.5 ml) for 30 min in 95 °C water bath. The solution was then cooled in ice for 5 min. The coloured substances were extracted with 4.0 ml of 1-butanol. The absorbance of 1-butanol layer was measured at 532 nm. A calibration curve was constructed by using malonaldehyde-bis-diethyl-acetal and results were expressed as malonaldehayde equivalents. Antioxidant activity (AOA) was expressed as percentage inhibition of lipid peroxidation relative to the control using the following equation:

$$\text{AOA}(\%) = \frac{\text{Absorbance of Control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100.$$

(d) *Heat, pH and storage stability:* The extracts were heated in a boiling water bath for 15 min and the residual antioxidant activity was determined as previously described. For pH stability, antioxidant extracts were pre-incubated at pH 4 and 9 and the residual antioxidant activity was determined. The antioxidant extract from each material was divided into two aliquots. The first aliquot was stored in the dark under refrigeration (5 °C) and the second one was stored in the dark at room temperature (25 °C). Antioxidant activity was determined after 15 days for each aliquot.

3. Statistical analysis

Data were recorded as means ± standard deviation of duplicate measurements. Analyses of variance were performed by ANOVA test and significance differences between the means were determined by Duncan's New Multiple Range Test (Steele & Torrie, 1980).

4. Results

The analyzed antioxidant compounds in dried powder of drumstick leaves (DL), mint leaves (ML) and carrot tuber (CT) are shown in Table 1. Among the samples examined, DL was a rich source of ascorbic acid. All the three samples were found to have appreciable amount of α-tocopherol, β-carotene and polyphenols. The total amount of phenolics (gallic acid equivalents) ranged from 1.68 g% in dehydrated CT-powder to 4.5 g% in dehydrated DL-powder. The estimated value for drumstick is in good agreement with reported data by Siddhuraju and Becker (2003).

A comparison of secondary products of lipid peroxidation measured as malonaldehyde (MDA) equivalents is shown in Table 2. The MDA equivalent values were found to be lower in linoleic acid compared to sunflower oil. In a system comprising of sunflower oil as substrate, the MDA equivalent value was highest for carrot extract, indicating the inability of this extract to inhibit lipid oxidation.

Antioxidant activities (AOA) of the extracts from selected plant materials and α-tocopherol are presented in Fig. 1. The extracts from DL and CT exhibited good AOA in the linoleic acid peroxidation system. At a concentration of 1.5 mg/ml, the two extracts inhibited 80–83% peroxidation of linoleic acid after 16 h incubation, which was significantly ($p \leqslant 0.05$) higher than the value obtained for α-tocopherol (72%). In another system, using sunflower oil as a rich source of PUFA, both DL and ML extracts exhibited similar activities, 46% and 44%, respectively. These results indicated the relative efficiency of extracts in inhibiting lipid oxidation.

The AOA of the three extracts was found to vary with temperature (Fig. 2). Heating at 100 °C for 15 min increased ($p \leqslant 0.05$) the antioxidant potency of ML extract by 15%. Perhaps heat processing improved the bioavailability of the antioxidants present in ML extract. Incubating DL extract at 100°C for 15 min resulted in a significant decrease ($p \leqslant 0.05$) in AOA by 17%. Heat processing may have resulted in degradation of antioxidants present in DL extract, thereby decreasing the activity. However, heat

Table 1
Contents of ascorbic acid, tocopherol, β-carotene, glutathione and total phenols in selected plant materials

Compound	DL[a]	ML[a]	CT[a]
Ascorbic acid (mg)[b]	431.30 ± 15.34[a]	40.25 ± 1.54[b]	20.0 ± 0.81[b]
α-Tocopherol (mg)[b]	24.65 ± 0.21[a]	18.70 ± 0.56[b]	3.98 ± 0.21[c]
β-Carotene (μg)[b]	14,412 ± 898[a]	12,180 ± 668[a]	9667 ± 627[b]
Glutathione (mmol)[b]	129.53 ± 1.91[a]	70.6 ± 0.82[b]	129.53 ± 1.35[a]
Total phenols (g)[b]	4.50 ± 0.25[a]	4.30 ± 0.29[a]	1.68 ± 0.12[b]

Values bearing different superscripts a, b in rows (comparison between samples) differ significantly ($p \leqslant 0.05$).
[a] DL, drumstick leaves; ML, mint leaves; CT, carrot tuber.
[b] Per 100 g dry basis.

Table 2
Antioxidant activity of plant extracts in different lipid systems

Lipid system	Antioxidant activity (mmol MDA equivalents/kg oil)		
	DL[a]	ML[a]	CT[a]
Linoleic acid	0.12 ± 0.02[b]	0.23 ± 0.03[a]	0.14 ± 0.03[b]
Sunflower oil	7.63 ± 0.53[b]	7.86 ± 0.65[b]	12.02 ± 0.86[a]

Values bearing different superscripts a, b in rows (comparison between samples) differ significantly ($p \leqslant 0.05$).
[a] DL, drumstick leaves; ML, mint leaves; CT, carrot tuber.

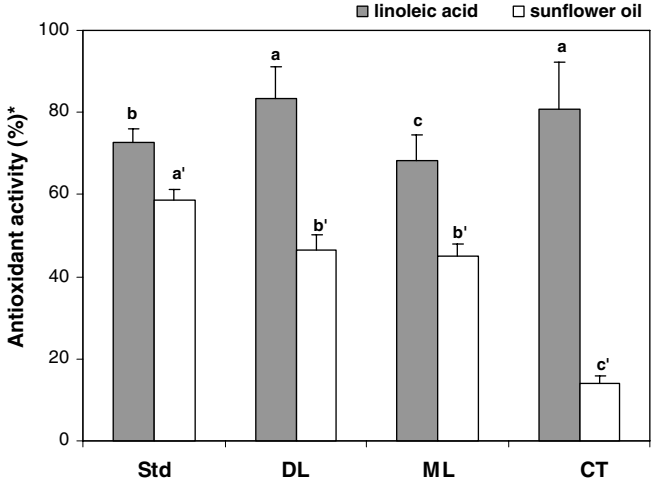

Fig. 1. Antioxidant activity of extracts in different lipid substrates: Std, α-Tocopherol; DL, drumstick leaves; ML, mint leaves; CT, carrot tuber. a, b, c: Values with the same letter in the same lipid system are not significantly different ($p \leqslant 0.05$). *Percentage inhibition of lipid peroxidation relative to control.

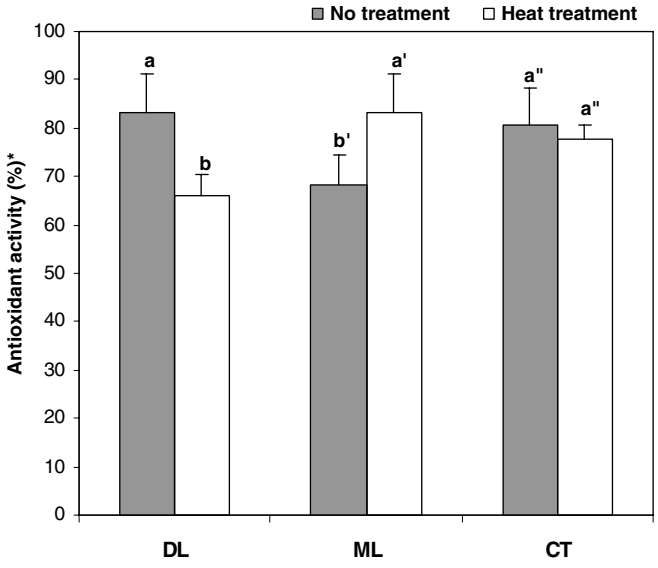

Fig. 2. Effect of heat treatment (100 °C, 15 min) on antioxidant activity of extracts in linoleic acid: DL, drumstick leaves; ML, mint leaves; CT, carrot tuber. a, b: Values with the same letter are not significantly different ($p \leqslant 0.05$). *Percentage inhibition of lipid peroxidation relative to control.

treatment did not make a significant ($p \leqslant 0.05$) change in AOA of CT extract indicating stability of this extract.

The AOA of extracts varied with pH (Fig. 3). Results indicate that the activity of ML and CT extracts was higher at pH 9 than at pH 4, while that of extract from DL was unchanged in both alkaline and acid media.

The three extracts stored in the dark at 5 and 25 °C after 15 days did not show any significant change ($p \leqslant 0.05$) in their AOA (Fig. 4). Results indicate that the extracts stored at 25 °C, were quite stable compared to those stored at 5 °C, even after 15 days.

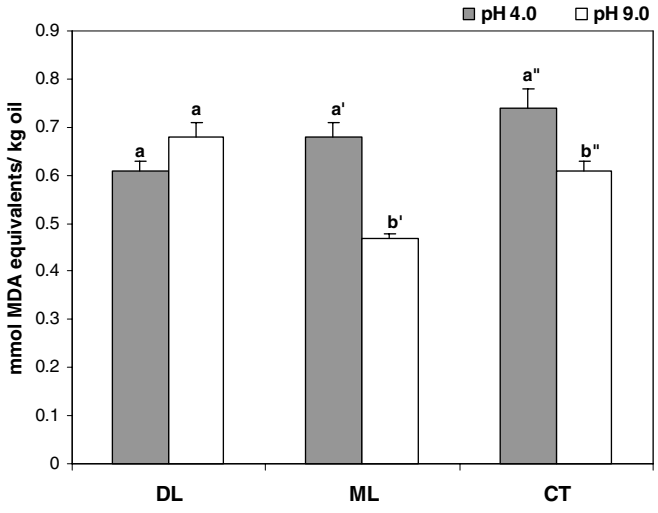

Fig. 3. Effect of pH on antioxidant activity of extracts in linoleic acid: DL, drumstick leaves; ML, mint leaves; CT, carrot tuber. a, b: Values with the same letter are not significantly different ($p \leqslant 0.05$).

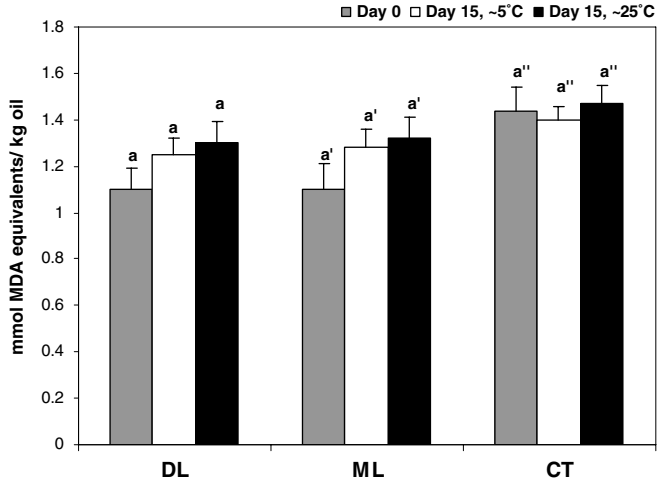

Fig. 4. Effect of storage on antioxidant activity of extracts in sunflower oil: DL, drumstick leaves; ML, mint leaves; CT, carrot tuber. Values with the same letter are not significantly different ($p \leqslant 0.05$).

5. Discussion

In the present study, three plant materials, namely drumstick leaves, mint leaves and carrot tuber, were used as sources of natural antioxidants. Samples were subjected to oven drying (50 °C) before solvent extraction as it was observed that controlled oven drying of the selected samples resulted in maximum retention of antioxidant nutrients such as ascorbic acid, α-tocopherol, β-carotene and glutathione (Reddy, Urooj, & Kumar, 2005; Vishalakshi, 2003).

Under the experimental conditions described, the three selected samples exhibited antioxidant activity. The ability to inhibit lipid oxidation differed according to the type of vegetable extract, oxidizable substrate used, thermal and pH treatment to which the extracts were subjected. In an earlier study, the AOA of both DL leaves extract and powder was evaluated using a linoleic acid–β-carotene system (Reddy et al., 2005). It was interesting to note that the AOA of extract was 98% and that of dehydrated powder was 87% compared to the AOA of synthetic antioxidants butylated hydroxyanisole (85%) and butylated hydroxy toluene (92%). Various solvent extracts of DL from different agroclimatic regions are reported to exhibit marked antioxidant activity (Siddhuraju & Becker, 2003). Also, few reports are available on AOA of different extracts from *M. spicata* (Dorman, Kosar, Kahlos, Holm, & Hiltunen, 2003; Marinova & Yanishlieva, 1997; Tarwadi & Agte, 2003; Triantaphyllou, Blekas, & Boskou, 2001), carrot puree (Talcott, Howard, & Brenes, 2000) and carrot juice (Gazzani, Papetti, Massolini, & Daglia, 1998). However, the effect of processing such as heat, pH and storage on the AOA of plant extracts studied has not been reported so far.

It is well known that many factors such as antioxidant concentration, temperature and pH of the media,

processing treatment and storage strongly influence the antioxidant activity (Gazzani et al., 1998). In the present study, the three extracts were subjected to heat treatment at 100 °C (15 min), which resulted in a significant decrease in AOA in DL extract while no difference was observed in AOA of CT extract. The decrease in AOA of DL extract might be due to the loss of naturally occurring antioxidants present in the extract or formation of novel compounds having prooxidant activity upon heat processing. However, a significant increase in AOA due to thermal processing occurred in ML extract. Thermal processing can induce the formation of compounds with antioxidant properties or improve the AOA of naturally occurring antioxidants (Nicoli et al., 1999). The AOA of a number of vegetable juices is reported to be stabilized by boiling, suggesting that the initial prooxidant activity is due to prooxidases which are inactivated at high temperatures (Gazzani et al., 1998). It is reported that crucifer extracts exhibit either a prooxidant or an antioxidant activity depending on the thermal processing and variety of the vegetable examined (Castenmiller et al., 2002). An increase in AOA of carrot puree due to thermal processing is associated with increased levels of phenolic acids (Talcott et al., 2000).

The influence of pH on the AOA of extracts was also investigated. The AOA of different extracts differed under the two pH conditions studied. No difference was noticed in the AOA of DL extract while a significant increase in AOA of ML and CT extracts was observed with increase in pH from 4 to 9, indicating strong dependence of AOA on the pH of the system. Yen and Duh (1993) reported that a methanol extract from peanut hulls had a higher AOA at neutral and acid pH. The AOA of different extracts from cocoa by-products was higher at alkaline pH (Azizah, Ruslawati, & SweeTee, 1999). These differences might be due to different samples used and various compounds being extracted in each case.

Phenolics are believed to be the major phytochemicals responsible for antioxidant activity of plant materials (Javanmardi et al., 2003; Pizzale et al., 2002). In this study, the total phenolic content of the selected samples was determined, however, the phenolic composition of the extracts was not analyzed as it was not within the scope of present investigation. Further work in this area is currently in progress. The main phenolic compounds in drumstick leaves and its extracts are flavonoid groups such as quercetin and kaempferol (Siddhuraju & Becker, 2003). It is reported that the main phenolic compounds in aqueous Mentha extracts to be the glycoside eriocitrin, caffeic acid dimer rosmarinic acid (Areias, Valentao, Andrade, Ferreres, & Seabra, 2001), chlorogenic acid and 3- or 5-position hydroxylated glycosidic flavonoids (Triantaphyllou et al., 2001).The primary phenolics present in carrot are identified as chlorogenic acid, *p*-OH-benzoic acid, ferulic acid and other unidentified cinnamic acid derivatives (Talcott et al., 2000).

6. Conclusions

In the present study it was found that drumstick, mint and carrot are potential sources of antioxidant components. They exhibit potent antioxidant activity in different lipid systems. The antioxidant activity of extracts varied with pH, heat treatment and storage. These findings confirm that anti- and prooxidant properties of vegetables are strongly influenced by a number of processing factors and by the reaction conditions. Therefore, it is important to consider the optimum technological condition and processing factors influencing activity and bioavailability of plant antioxidants for utilization in food and biological systems. In addition to being consumed as healthy antioxidants, the compounds present in these plants that are responsible for antioxidant activity could be isolated and then used as food additives to delay the oxidative deterioration of foods. Further studies in this area are in progress.

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