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Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves

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Abstract

The antioxidant properties and total phenolic contents of methanol, acetone and water extracts of mulberry (*Morus indica* L.) leaves were examined. Various experimental models including iron (III) reducing capacity, total antioxidant capacity, DPPH radical scavenging activity and *in vitro* inhibition of ferrous sulphate-induced oxidation of lipid system were used for characterization of antioxidant activity of extracts. The three extracts showed varying degrees of efficacy in each assay in a dose-dependent manner. Methanolic extract with the highest amount of total phenolics, was the most potent antioxidant in all the assays used. In addition, the effect of temperature (50 °C and 100 °C), pH (3, 5, 7, 9 and 11) and storage (5 °C) on the antioxidant activity of methanolic extract was investigated. The antioxidant activity of the extract remained unchanged at 50 °C and was maximum at neutral pH. The extract stored at 5 °C in the dark was stable for 30 days after which the antioxidant activity decreased ($p \leqslant 0.05$) gradually. On the basis of the results obtained, mulberry leaves were found to serve as a potential source of natural antioxidants due to their marked antioxidant activity.
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Keywords: *Morus indica* leaves; Reducing power; DPPH scavenging activity; Antioxidant activity; Lipid peroxidation; Stability

1. Introduction

Autoxidation of polyunsaturated fatty acids not only lowers the nutritional value of food (Farag, Badei, & El Baroty, 1989), but also produces free radicals or reactive oxygen species such as hydroxyl or peroxyl radicals which are associated with membrane damage, aging, heart disease and cancer (Cosgrove, Church, & Pryor, 1987). The human body has several antioxidant defense systems to protect healthy cell membranes from active oxygen species and free radicals (Fridowich, 1995; Halliwell, 1994; Kaur & Kapoor, 2001). The innate defense systems may be supported by antioxidative compounds taken as foods, cosmetics and medicine. Therefore, the antioxidative compounds provided by the diet may enrich the antioxidative status of living cells and thus reduce the damage, particularly in the elderly (Shukla, Wanasundara, & Shahidi, 1997). The most widely used antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been restricted recently because of serious concerns about their carcinogenic potential (Buxiang & Fukuhara, 1997; Hirose et al., 1998). Therefore, there is great interest in finding new and safe antioxidants from natural sources (Gazzani, Papetti, Massolini, & Daglia, 1998; Namiki, 1990).

Recently, natural plants have received much attention as sources of biologically active substances including antioxidants, antimutagens and anticarcinogens (Dillard & German, 2000). Numerous studies have been carried out on some plants such as rosemary, sage and oregano, which resulted in the development of natural antioxidant formulations for food, cosmetic and other applications. However, scientific information on antioxidant properties of various plants, particularly those that are less widely used in culinary and medicine is still scarce. Therefore, the assessment

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of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and nutraceuticals (Miliauskas, Venskutonis, & Beek, 2004).

Mulberry is a fast-growing deciduous plant that grows under different climatic conditions (i.e., tropical, subtropical and temperate) (Srivastava, Kapoor, Thathola, & Srivastava, 2003). Mulberry is valued for its foliage, which constitutes the chief feed for silkworms. The leaves are nutritious, palatable and non-toxic and are stated to improve milk yield when fed to dairy animals (Sastri, 1962). Reports indicate that mulberry leaves contain proteins, carbohydrates, calcium, iron, ascorbic acid, β-carotene, vitamin B-1, folic acid and vitamin D (Bose, 1989). Apart from their use as animal and insect feed, they have been shown to possess medicinal properties such as diuretic, hypoglycemic and hypotensive activities (Kelkar et al., 1996; Sastri, 1962). However, it is only recently that the mechanism of their action has been related to their antioxidant activity. The presence of rutin, quercetin, isoquercetin and other flavonoids in mulberry leaves has been reported (Zhishen, Mengcheng, & Jianming, 1999).

The total antioxidant activity of plant foods is the result of individual activities of each of the antioxidant compounds present such as vitamin C, tocopherols, carotenoids, and phenolic compounds, the latter being the major phytochemicals responsible for antioxidant activity of plant materials (Javanmardi, Stushnoff, Locke, & Vivanco, 2003; Pizzale, Bortolomeazzi, Vichi, Ueberegger, & Conte, 2002). Moreover, these compounds render their effects via different mechanisms such as radical scavenging, metal chelation, inhibition of lipid peroxidation, quenching of singlet oxygen and so on to act as antioxidants. Even if a sample exhibits high activity with one of these methods, it does not always show similar good results with all other methods. Therefore, it is essential to evaluate samples accurately by several methods. On the other hand, the impact of processing on the antioxidant activity of fruits and vegetables is much neglected and little information is available in this area. Accordingly, the aim of this study was to determine the total phenolic contents and the antioxidant properties of methanol, acetone and water extracts of mulberry leaves by various methods including reducing power, total antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and inhibition of lipid peroxidation *in vitro* which are rarely reported. It was also of interest to evaluate the effect of heat treatment, pH and storage on the stability of antioxidant activity of mulberry extract, which has not been investigated previously.

2. Materials and methods

2.1. Chemicals

DPPH and 2-thiobarbituric acid (TBA) were procured from HiMedia Lab. Pvt. Ltd. (Mumbai, India). Butylated hydroxytoluene (BHT) was obtained from Qualigens Fine Chemicals (Mumbai, India). Ascorbic acid and tris-(hydroxymethyl)aminomethane were obtained from SD Fine Chemicals Ltd. (Mumbai, India). All other reagents used were of analytical grade and obtained from usual suppliers.

2.2. Materials

Mulberry (*Morus indica* L.) leaves were harvested in June 2005 from the Department of Sericulture, University of Mysore, Mysore, India. The leaves were washed and dried in a hot air oven at 50 °C for 6–8 h. The dried material was ground to a fine powder, passed through a 60-mesh sieve and kept in an air-tight container at 4 °C until further use.

2.3. Extraction of antioxidants from mulberry leaves

The dried leaves of mulberry (15 g) were extracted overnight with 100 ml each of methanol, acetone or water, respectively, in a mechanical shaker at room temperature. Each extract was filtered with Whatman No. 1 filter paper. The filtrate obtained from methanol and acetone was evaporated to dryness at 40 °C in a rotary evaporator (Buchi Laboratoriums-Technik, Flawil/Schweiz, Switzerland) and the water extract was freeze-dried. The dried sample of each extract was weighed to determine the yield of soluble constituents and stored at 4 °C until use.

2.4. Estimation of total phenolics

Total phenolic content of each extract was determined by the Folin–Ciocalteu micro-method (Slinkard & Singleton, 1977). Briefly, 20 μl of extract solution were mixed with 1.16 ml distilled water and 100 μl of Folin–Ciocalteu reagent, followed by addition of 300 μl of Na_2CO_3 solution (20%) after 1 min and before 8 min. Subsequently, the mixture was incubated in a shaking incubator at 40 °C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve. The phenolic content was expressed as gallic acid equivalents using the following linear equation based on the calibration curve:

$$A = 0.98C + 9.925 \times 10^{-3}, \quad R^2 = 0.9996,$$

where A is the absorbance and C is concentration as gallic acid equivalents (μg/ml).

2.5. Reducing power assay

The ability of extracts to reduce iron (III) was assessed by the method of Yildirim, Mavi, and Kara (2001). The dried extract (125–1000 μg) in 1 ml of the corresponding solvent was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$; 10 g l^{-1}), then the mixture was incubated at 50 °C for 30 min. After incubation, 2.5 ml of trichloroacetic acid (100 g l^{-1}) were

added and the mixture was centrifuged at 1650*g* for 10 min. Finally, 2.5 ml of the supernatant solution were mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3(1\ g\ l^{-1})$ and the absorbance was measured at 700 nm. High absorbance indicates high reducing power.

2.6. Total antioxidant capacity

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Prieto, Pineda, & Aguilar, 1999). An aliquot of 0.1 ml of sample solution (containing 100–500 μg of dried extract in corresponding solvent) was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against a blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples. The antioxidant activity of extracts was expressed as equivalents of α-tocopherol using extinction coefficient of $4 \times 10^3\ M^{-1}\ cm^{-1}$.

2.7. DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the method of Blois (1958). Briefly, 1 ml of a 1 mM methanolic solution of DPPH was mixed with 3 ml of extract solution in methanol (containing 50–400 μg of dried extract). The mixture was then vortexed vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm and activity was expressed as percentage DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.8. Antioxidant activity (in vitro inhibition of lipid peroxidation)

To assess the protective action of mulberry extracts against lipid peroxidation, fresh linseed oil (free from additives) was used as the substrate (Singh, Maurya, Catalan, & Lampason, 2004) for present investigation.

2.8.1. Oxidation system of lipid by ferrous sulphate

Oxidation of oil was conducted according to the method of Tamura and Yamagami (1994). An aqueous solution containing linseed oil (3 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 a dark place. The reaction was stopped by adding 50 μl of 1% BHT in ethanol. The solution obtained (9.9 ml) was used for antioxidant activity assay.

2.8.2. Antioxidant activity assay

The dried plant extract (125, 250 and 500 μg) in 100 μl of corresponding solvent was mixed with the solution (9.9 ml) mentioned above when necessary. BHT was used as a standard to evaluate the antioxidative activity of samples. The reacted solution obtained (1 ml) was used for TBA assay.

2.8.3. TBA assay

The degree of oxidation of oil was measured by the 2-thiobarbituric acid (TBA) assay described by Ohkawa, Ohishi, and Yagi (1979). The reacted solution (1 ml) mentioned above was mixed with 0.2% (w/v) TBA solution (3 ml) and 0.05 M sulphuric acid (2.5 ml). The mixture was heated for 30 min in a 95 °C water bath. After the solution was cooled in ice for 5 min, the coloured substances were extracted by 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 as a standard and results were expressed as malondialdehayde (MDA) equivalents. Antioxidant activity (AOA) was expressed as percentage inhibition of lipid peroxidation relative to the control using the following equation:

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

2.9. Heat, pH and storage stability

The methanolic extract was heated at 50 and 100 °C (60 and 120 min) and the residual antioxidant activity was determined using TBA method as previously described. For pH stability, the extract was pre-incubated at different pH values (3, 5, 7, 9 and 11) and the residual antioxidant activity was evaluated. The extract was also stored in the dark at 5 °C, and the antioxidant activity was determined at intervals of 30 days over a period of 90 days.

2.10. Statistical analysis

Data were recorded as means ± standard deviation of triplicate measurements. Analyses of variance were performed by ANOVA test and significance differences between the means were determined by Duncan's New Multiple Range Test ($p \leqslant 0.05$) (Steele & Torrie, 1980).

3. Results and discussion

3.1. Extract yield and total phenolics

The yield and total phenolics data for different extracts from mulberry leaves are shown in Table 1. The amount of extractable components expressed as percentage by weight of dried leaves ranged from 6.50% (water extraction) to 12.35% (methanol extraction). The amount of total phenolics (gallic acid equivalents) expressed as percentage by weight of dried extract ranged from 7.10% in water extract to 9.32% in methanol extract. Methanol was found to be the most effective solvent in extraction of antioxidants from mulberry leaves. This is in agreement with the reports of Hertog, Hollman, and Van de Putte (1993), and Yen, Wu, and Duh (1996) that methanol is a widely used and effective solvent for extraction of antioxidants.

3.2. Reducing power

Different studies have indicated that the electron donation capacity (reflecting the reducing power) of bioactive compounds is associated with antioxidant activity (Siddhuraju, Mohan, & Becker, 2002; Yen, Duh, & Tsai, 1993). In this assay, the ability of extracts to reduce iron (III) to iron (II) was determined and compared to that of ascorbic acid, which is known to be a strong reducing agent. All the three extracts showed some degree of electron donation capacity in a concentration-dependent manner, but the capacities were inferior to that of ascorbic acid (Fig. 1, Table 2). Methanolic extract containing the highest amount of total phenolics, was the most potent reducing agent, whereas water extract containing the least amount of phenolics, was the weakest in the activity. Similar relations between iron (III) reducing activity and total phenol content have been reported in the literature (Benzie & Szeto, 1999; Gao, Björk, Trajkovski, & Uggla, 2000; Zhu, Hackman, Ensunsa, Holt, & Keen, 2002); however the correlation may not be always linear (Yildirim, Mavi, Oktay, Algur, & Bilaloglu, 2000).

3.3. Total antioxidant capacity

In the phosphomolybdenum assay, which is a quantitative method to evaluate water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity), the three extracts exhibited some degree of activity in a dose-dependent manner; however, the activities were inferior to that of BHT (Fig. 2, Table 2). In this assay, methanol and acetone extracts were found to be rather similar in their action, water extract again being the lowest in activity. The extracts demonstrated electron-donating capacity and thus they may act as radical chain terminators, transforming reactive free radical species into more stable non-reactive products (Dorman, Kosar, Kahlos, Holm, & Hiltunen, 2003).

Table 1
Extract yield and total phenolic contents of different solvent extracts from mulberry leaves

Sample	Yield[↑]	Total phenolics[↕]
Methanol extract	12.35 ± 1.25^a	9.32 ± 0.10^a
Acetone extract	8.25 ± 0.95^b	8.45 ± 0.40^b
Water extract	6.50 ± 1.50^c	7.10 ± 0.25^c

Values in the same column followed by different letters are significantly different ($p \leqslant 0.05$). [↑]Grams of extract per 100 g of dried leaves; [↕]grams of gallic acid per 100 g (dry weight) of extract.

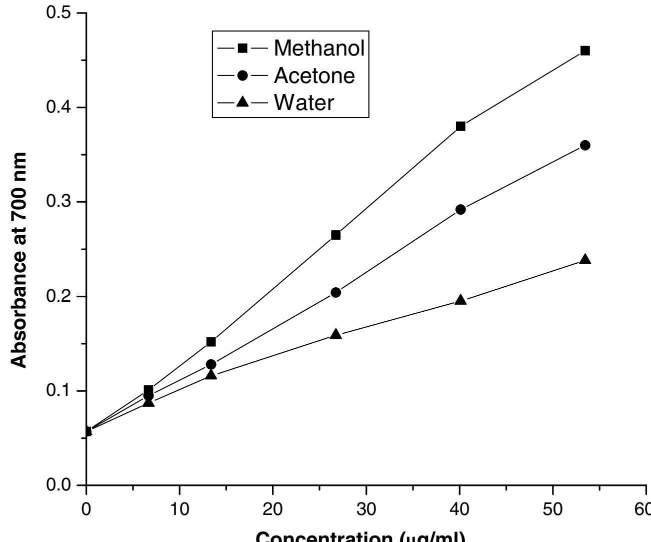

Fig. 1. Reducing powers of methanol, acetone and water extracts of mulberry leaves.

3.4. DPPH radical scavenging activity

Free radicals which are involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others (Dorman et al., 2003). The DPPH radical has been widely used to evaluate the free radicals' scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids (Porto, Calligaris, Cellotti, & Nicoli, 2000). The scavenging activity of three extracts against DPPH° was concentration-dependent (Fig. 3). Methanolic extract that contained the highest amount of total phenolics, was found to be the most active radical scavenger followed by acetone and water extracts. However, the methanolic extract was not as effective as the positive controls, BHT and ascorbic acid, as the amount of extract required to scavenge 50% of DPPH radicals present in the reaction mixture (EC_{50}) was significantly ($p \leqslant 0.05$) higher than those of BHT and ascorbic acid (Table 2). A high correlation between free radical scavenging and the phenolic contents has been reported for cereals (Peterson, Emmons, & Hibbs, 2001), fruits (Gao, Ohlander, Jeppsson, Björk, & Trajkovski, 2000; Jimenez-Escrig, Rincon, Pulido, & Saura-Calixto, 2001), beverages (Fogliano, Verde, Randazzo, & Ritieni,

Table 2
Comparison of antioxidant properties of mulberry extracts, BHT and ascorbic acid

Sample	Reducing power (absorbance at 700 nm)[†]	TAOC (mmol α-tocopherol/g extract)[†,A]	Antioxidant activity[‡,B]	DPPH° EC_{50} (μg/ml)[C]
BHT	nd	3.921 ± 0.011^a	89.73 ± 0.50^a	41.07 ± 0.52^c
Ascorbic acid	2.555 ± 0.001^a	nd	nd	61.67 ± 0.30^b
Methanol extract	0.265 ± 0.002^b	1.394 ± 0.003^b	81.80 ± 0.52^b	79.53 ± 0.87^a
Acetone extract	0.204 ± 0.007^c	1.386 ± 0.002^b	59.49 ± 1.06^c	nd
Water extract	0.159 ± 0.006^d	0.660 ± 0.002^c	36.92 ± 0.70^d	nd

Values followed by different letters within each column are significantly different ($p \leqslant 0.05$). nd, not determined.
[A] Total antioxidant capacity (phosphomolybdenum assay).
[B] Percentage inhibition of lipid peroxidation relative to control (in linseed oil).
[C] The effective concentration at which DPPH radicals were scavenged by 50%.
[‡] 0.22 mg of BHT or 500 μg of dried extract was used.
[†] 500 μg of dried extract, BHT or ascorbic acid was used.

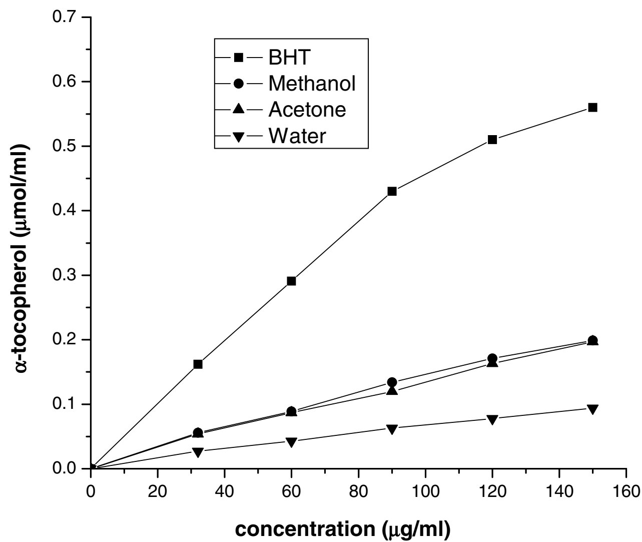

Fig. 2. Total antioxidant activities of methanol, acetone and water extracts of mulberry leaves. BHT was used as positive control.

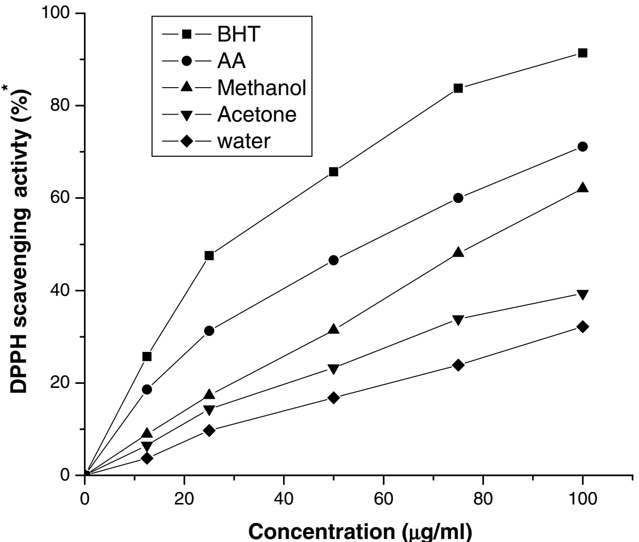

Fig. 3. DPPH radical scavenging activities of methanol, acetone and water extracts of mulberry leaves. BHT and ascorbic acid (AA) were used as positive controls. *Percentage radical scavenging capacity relative to control.

1999) and culinary herbs (Zheng & Wang, 2001). The results of DPPH radical scavenging assay revealed that the extracts by hydrogen and/or electron donation, might prevent reactive radical species from reaching biomolecules such as lipoproteins, polyunsaturated fatty acids (PUFA), DNA, amino acids, proteins and sugars in susceptible biological and food systems (Halliwell, Aeschbach, Loliger, & Aruoma, 1995).

3.5. Antioxidant activity (AOA)

The reducing power and radical scavenging activity assays are potential indicators of AOA, however, neither these methods utilize a food or biologically relevant oxidizable substrate, so no direct information on the extracts' protective properties can be determined (Dorman et al., 2003). Though PUFA are suspected to play a major role in oxidative deterioration and off-flavour development in foods, it was considered necessary to assess the mulberry extracts in a system consisting of a complex, lipid-rich food, i.e., linseed oil which contains a high proportion of PUFA and generally used as edible oil in central Europe and Asia (Singh et al., 2004).

In the present study, the effect of various extracts from mulberry leaves on inhibition of lipid peroxidation in linseed oil was determined by TBA method, in which the amount of thiobarbituric acid reactive substances (TBARS, expressed as MDA equivalents) formed by oxidation of oil was determined by measuring the absorbance at 532 nm. All the three extracts were capable of preventing the formation of TBARS generated by ferrous sulphate in a dose-dependent manner (Fig. 4). On the basis of estimated percentage of AOA presented in Table 2, the most active extract was found to be methanolic extract, which showed 81% activity; however, the activity was less than the value obtained for BHT (90%) ($p \leqslant 0.05$). The order of activities was correlated with the amount of total phenolics present in the respective extracts. In an earlier study, the AOA of different solvent extracts from *Morus Alba* leaves was evaluated using beta carotene–linoleic acid system and it was found that the AOA of methanolic extract

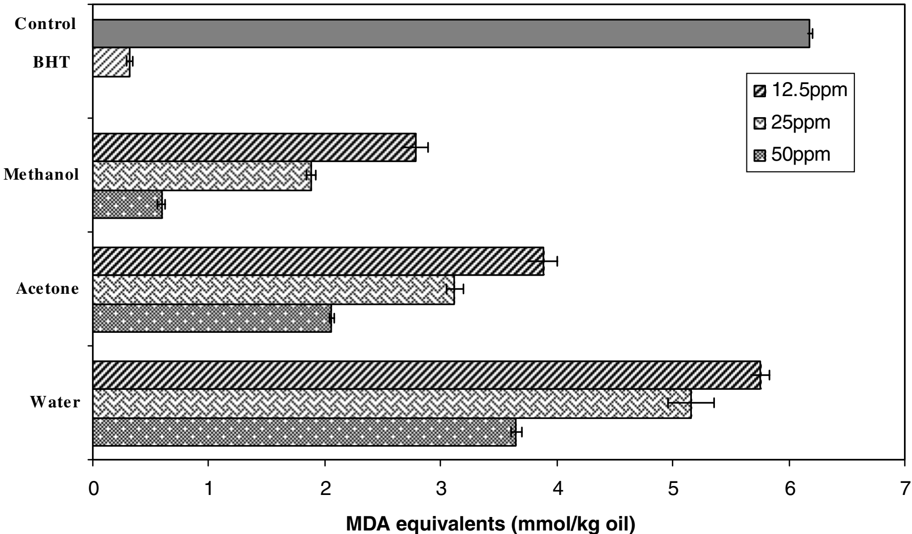

Fig. 4. Effect of mulberry extracts on the production of MDA from linseed oil oxidized by ferrous sulphate. BHT was used as positive control.

was 78.2%, greater than that of α-tocopherol (72.1%) but equal to that of BHA (Yen et al., 1996).

3.6. Heat, pH and storage stability

It is well known that many factors such as antioxidant concentration, temperature and pH of the media, processing treatment and storage strongly influence the AOA (Gazzani et al., 1998). In the present study, methanolic extract of mulberry leaves which significantly exhibited the highest extraction yield and AOA among the various extracts was subjected to thermal, pH and storage stability studies. Fig. 5 shows the effect of temperature on the antioxidative stability of methonolic extract. The AOA of extract was constant when incubated at 50 °C for 60 min as the amount of MDA equivalents formed by oxidation of oil did not change significantly ($p \leqslant 0.05$). Prolonged heating at 50 °C for 120 min decreased the AOA of extract by 3%. Heating at 100 °C for 60 min reduced ($p \leqslant 0.05$) the AOA of extract by 9%, followed by continuous decrease with increasing the time of boiling. However, the remaining AOA in the extract was about 68% even after 120 min heating at 100 °C. Decline in AOA of extract followed by heating at 100 °C might be related to either loss of naturally occurring antioxidants present in the extract or formation of novel compounds having prooxidant activity. Gazzani et al. (1998) found that the AOA of a number of vegetable juices was stabilized by boiling, suggesting that the initial prooxidant activity was due to prooxidases which are inactivated at high temperatures. Castenmiller et al. (2002) indicated that crucifer extracts exhibit either a prooxidant or an antioxidant activity depending on the thermal processing and variety of the vegetable. It has been reported that the AOA of ethanolic extract of *Mentha spicata* increases due to thermal processing (Arabshahi-Delouee, Devi, & Urooj, 2007).

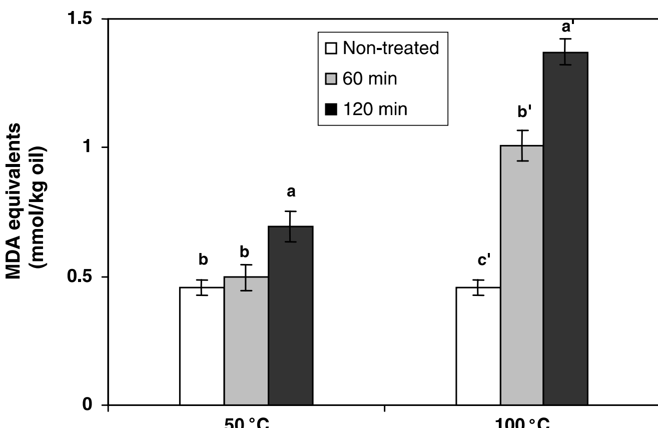

Fig. 5. Effect of heat treatment (50 °C and 100 °C) on the antioxidant activity of methanolic extract in linseed oil. Bars carrying different letters are significantly different ($p \leqslant 0.05$) from each other.

The influence of pH on the stability of methanolic extract is seen in Fig. 6. The amount of MDA equivalents formed by oxidation of oil in presence of extract gradually decreased ($p \leqslant 0.05$) with minimum value at pH 7.0 followed by continuous increase ($p \leqslant 0.05$) at alkaline pH, indicating strong dependence of AOA of extract to the pH of system. The reduction in the activity at alkaline pH might be related to either loss of antioxidant activity of the extract or the enhancement of lipid peroxidation. Liu (1970) reported that hemoprotein-catalyzed oxidation is most active at alkaline pH. Mansour and Khalil (2000) found that the AOA of fenugreek seed and ginger rhizome extracts decrease by increasing the pH of media. The AOA of different extracts from cocoa by-products was found to be higher at alkaline pH (Azizah, Nik Ruslawati, & Swee-Tee, 1999). These differences might be due to different samples used and various compounds being extracted in each case.

The effect of storage on the stability of methanolic extract of mulberry was also studied up to 3 months at

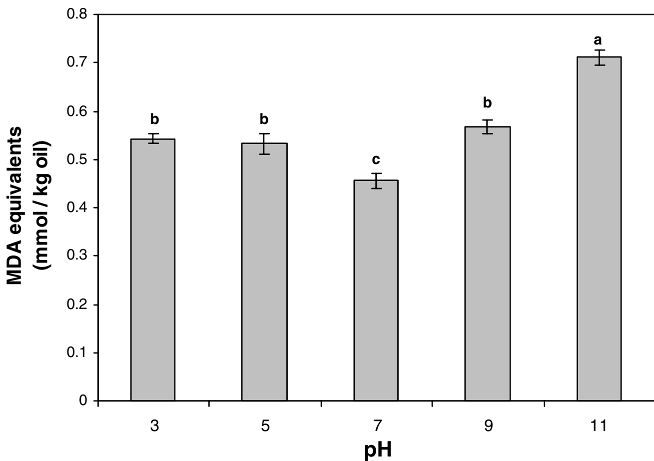

Fig. 6. Effect of pH on the antioxidant activity of methanolic extract in linseed oil. Values with different letters are significantly different ($p \leqslant 0.05$) from each other.

Table 3
Effect of storage (5 °C, 90 days) on the antioxidant activity of methanolic extract in linseed oil

Time(days)	MDA equivalents (mmol/kg oil)
0	0.64 ± 0.03^{c}
30	0.69 ± 0.03^{c}
60	1.05 ± 0.03^{b}
90	1.53 ± 0.04^{a}

Values followed by different letters are significantly different ($p \leqslant 0.05$).

intervals of 30 days (Table 3). The extract stored in a refrigerator (5 °C) over a 30 day period did not show any change in the antioxidant activity. Prolonged storage for 3 months reduced ($p \leqslant 0.05$) the AOA of the extract, however, the remaining activity of extract was about 65% at the end of storage period indicating it can be still considered as a source of natural antioxidants.

4. Conclusions

Various solvent extracts from mulberry leaves showed varying degrees of antioxidant activity in different test systems in a dose-dependent manner. The antioxidant activity was correlated with the amount of total phenolics present in the respective extracts in each assay. Methanol proved to be the most efficient solvent for extraction of antioxidants from mulberry leaves as the related extract contained the highest amount of phenolic compounds and also exhibited the strongest antioxidant capacity in all the assays used. However, its activity varied with pH, temperature and duration of storage. Therefore, it is important to consider the optimum technological conditions and processing factors influencing activity and bioavailability of plant antioxidants for utilization in food and biological systems. In addition, potential exploitable beneficial effects and safety in humans need to be proven in clinical trials. Consideration of these questions will guide future research work.

References

Arabshahi-Delouee, S., Devi, D. V., & Urooj, A. (2007). Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. *Food Chemistry, 100*, 1100–1105.

Azizah, A. H., Nik Ruslawati, N. M., & SweeTee, T. (1999). Extraction and characterization of antioxidants from cocoa by-products. *Food Chemistry, 64*, 199–202.

Benzie, I. F. F., & Szeto, Y. T. (1999). Total antioxidant capacity of teas by the ferric reducing antioxidant power assay. *Journal of Agricultural and Food Chemistry, 47*, 633–636.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 26*, 1199–1200.

Bose, P. C. (1989). *Genetic resources of mulberry and utilization*. Mysore, India: CSR and TI, pp. 183–190.

Buxiang, S., & Fukuhara, M. (1997). Effects of co-administration of butylated hydroxytoluene, butylated hydroxyanisole and flavonoid on the activation of mutagens and drug-metabolizing enzymes in mice. *Toxicology, 122*, 61–72.

Castenmiller, J. J. M., Linssen, J. P. H., Heinonen, I. M., Hopia, A. I., Schwarz, K., Hollmann, P. C. H., et al. (2002). Antioxidant properties of differently processed spinach products. *Nahrung/Food, 46*, 290–293.

Cosgrove, J. P., Church, D. F., & Pryor, W. A. (1987). The kinetics of the autoxidation of polyunsaturated fatty acids. *Lipids, 22*, 299–304.

Dillard, C. J., & German, J. B. (2000). Phytochemicals: nutraceuticals and human health. *Journal of the Science of Food and Agriculture, 80*, 1744–1756.

Dorman, H. J. D., Kosar, M., Kahlos, K., Holm, Y., & Hiltunen, R. (2003). Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties, and cultivars. *Journal of Agricultural and Food Chemistry, 51*, 4563–4569.

Farag, R. S., Badei, A. Z. M. A., & El Baroty, G. S. A. (1989). Influence of thyme and clove essential oils on cottonseed oil oxidation. *Journal of American Oil Chemists' Society, 66*, 800–804.

Fogliano, V., Verde, V., Randazzo, G., & Ritieni, A. (1999). Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *Journal of Agricultural and Food Chemistry, 47*, 1035–1040.

Fridowich, I. (1995). Superoxide radical and superoxide dismutases. *Annual Reviews in Biochemistry, 64*, 97–112.

Gao, X., Björk, L., Trajkovski, V., & Uggla, M. (2000). Evaluation of antioxidant activities of rosehip ethanol extracts in different test systems. *Journal of Agricultural and Food Chemistry, 80*, 2021–2027.

Gao, X., Ohlander, M., Jeppsson, N., Björk, L., & Trajkovski, V. (2000). Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. *Journal of Agricultural and Food Chemistry, 48*, 1485–1490.

Gazzani, G., Papetti, A., Massolini, G., & Daglia, M. (1998). Antioxidative and pro-oxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. *Food Chemistry, 6*, 4118–4122.

Halliwell, B. (1994). Free radicals, antioxidants and human disease: curiosity, cause and consequence. *Lancet, 344*, 721–724.

Halliwell, B., Aeschbach, R., Loliger, J., & Aruoma, O. I. (1995). The characterization of antioxidants. *Food Chemistry and Toxicology, 33*, 601–617.

Hertog, M. G. L., Hollman, P. C. H., & Van de Putte, B. (1993). Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices. *Journal of Agricultural and Food Chemistry, 41*, 1242–1246.

Hirose, M., Takesada, Y., Tanaka, H., Tamano, S., Kato, T., & Shirai, T. (1998). Carcinogenicity of antioxidants BHA, caffeic acid, sesamol, 4-methoxyphenol and catechol at low doses, either alone or in combination and modulation of their effects in a rat medium-term multi-organ carcinogenesis model. *Carcinogenesis, 19*, 207–212.

Javanmardi, J., Stushnoff, C., Locke, E., & Vivanco, J. M. (2003). Antioxidant activity and total phenolic content of Iranian *Ocimum accessions. Food Chemistry, 83*, 547–550.

Jimenez-Escrig, A., Rincon, M., Pulido, R., & Saura-Calixto, F. (2001). Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. *Journal of Agricultural and Food Chemistry, 49*, 5489–5493.

Kaur, C., & Kapoor, H. C. (2001). Antioxidants in fruits and vegetables – the millennium's health. *International journal of Food Science and Technology, 36*, 703–725.

Kelkar, S. M., Bapat, V. A., Ganapathi, T. R., Kaklig, G. S., Rao, P. S., & Heble, M. R. (1996). Determination of hypoglycemic activity in *Morus indica* L. (mulberry) shoot cultures. *Current Science, 71*, 71–72.

Liu, H. (1970). Catalysis of lipid peroxidation in meats I. Linoleate peroxidation catalyzed by Metmb of Fe (II)-EDTA. *Journal of Food Science, 35*, 590–595.

Mansour, E. H., & Khalil, A. H. (2000). Evaluation of antioxidant activity of some plant extracts and their application to ground beef patties. *Food Chemistry, 69*, 135–141.

Miliauskas, G., Venskutonis, P. R., & Beek, T. A. V. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry, 85*, 231–237.

Namiki, M. (1990). Antioxidants/antimutagens in foods. *Critical Reviews in Food Science and Nutrition, 20*, 273–300.

Ohkawa, h., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animals tissues by thiobarbituric reaction. *Analytical Biochemistry, 95*, 351–358.

Peterson, D. M., Emmons, C. L., & Hibbs, A. H. (2001). Phenolic antioxidants and antioxidant activity in pearling fractions of oat groats. *Journal of Cereal Science, 33*, 97–103.

Pizzale, L., Bortolomeazzi, R., Vichi, S., Uberegger, E., & Conte, L. S. (2002). Antioxidant activity of sage (*Salvia officinalis* and *S fruticosa*) and oregano (*Origanum onites* and O *indercedens*) extracts related to their phenolic compound content. *Journal of the Science of Food and Agriculture, 82*, 1645–1651.

Porto, C. D., Calligaris, S., Cellotti, E., & Nicoli, M. C. (2000). Antiradical properties of commercial cognacs assessed by the DPPH test. *Journal of Agricultural and Food Chemistry, 48*, 4241–4245.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry, 269*, 337–341.

Sastri, B. N. (1962). *The wealth of India, raw materials*. New Delhi, India: Council of Scientific and Industrial Research, pp. 429–439.

Shukla, V. K. S., Wanasundara, P. K. J. P. D., & Shahidi, F. (1997). Natural antioxidants from oilseeds. In F. Shahidi (Ed.), *Natural antioxidants chemistry, health effects and applications* (pp. 97–132). Champaigu, IL: AOCS Press.

Siddhuraju, P., Mohan, P. S., & Becker, K. (2002). Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chemistry, 79*, 61–67.

Singh, G., Maurya, S., Catalan, C., & Lampason, M. P. (2004). Chemical constituents, antifungal and antioxidative effects of ajwain essential oil and its acetone extract. *Journal of Agricultural and Food Chemistry, 52*, 3292–3296.

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis; automation and comparison with manual methods. *American Journal of Enology and Viticulture, 28*, 49–55.

Srivastava, S., Kapoor, R., Thathola, A., & Srivastava, R. P. (2003). Mulberry (*Morus alba*) leaves as human food: a new dimension of sericulture. *International Journal of Food Science and Nutrition, 54*, 411–416.

Steele, R. G. D., & Torrie, J. H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill.

Tamura, H., & Yamagami, A. (1994). Antioxidative activity of monoacylated anthocyanins isolated from Muscat Bailey A grape. *Journal of Agricultural and Food Chemistry, 42*, 1612–1615.

Yen, G. C., Duh, P. D., & Tsai, C. L. (1993). Relationship between antioxidant activity and maturity of peanut hulls. *Journal of Agricultural and Food Chemistry, 41*, 67–70.

Yen, G., Wu, S., & Duh, P. (1996). Extraction and identification of antioxidant components from the leaves of mulberry (*Morus alba* L.). *Journal of Agricultural and Food Chemistry, 44*, 1687–1690.

Yildirim, A., Mavi, A., & Kara, A. A. (2001). Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *Journal of Agricultural and Food Chemistry, 49*, 4083–4089.

Yildirim, A., Mavi, A., Oktay, A. A., Algur, Ö. F., & Bilaloglu, V. (2000). Comparison of antioxidant and antimicrobial activity of tilia (*Tilia argenta* Desf. Ex. D.C.), sage (*Salvia triloba* L.) and black tea (*Camellia sinensis* L.) extracts. *Journal of Agricultural and Food Chemistry, 48*, 5030–5034.

Zheng, W., & Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry, 49*, 5165–5170.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*, 555–559.

Zhu, Q. Y., Hackman, R. M., Ensunsa, J. L., Holt, R. R., & Keen, C. L. (2002). Antioxidative activities of oolong tea. *Journal of Agricultural and Food Chemistry, 50*, 6929–6934.