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Antioxidative activities of chromatographic fractions obtained from root, fruit and leaf of Mengkudu (*Morinda citrifolia* L.)

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Abstract

Crude extracts of root, leaf and fruit of *Morinda citrifolia* were fractionated on a Sephadex LH-20 column with ethanol as eluate. Based on UV absorption intensity of phenolic compound (725 mm) the Sephadex LH-20 column was able to separate fruit, leaf and root extracts into six, five and five fractions, respectively. The results showed that all the fractions tested exhibited considerably high antioxidative activity in the ferric thiocyanate assay and thiobarbituric acid test and the activities of some of the fractions were as good as those of either tocopherol or BHT. The fractions from different parts of the plants were found to contain different amounts of total phenolic compounds, which, interestingly, do not correspond to the antioxidative activity measured. This is probably due to the presence of different phenolics in the samples, with different antioxidative activities which involves various mechanisms inhibiting the oxidation process. The study suggested that root, leaf and fruit of *M. citrifolia* might contribute significantly to exogenous antioxidant which is crucial in combating oxidative stress.

Keywords: *Morinda citrifolia*; Antioxidative activity; Chromatographic fractions

1. Introduction

Both artificial and naturally occurring antioxidants have been reported to play major roles in inhibiting free radicals and xenobiotic-induced oxidative damage to membranes and tissues (Burton, 1989; Carini et al., 1990). Most living organisms possess enzymatic and non-enzymatic defence systems against excessive production of reactive oxygen species. However, different external factors (smoke, diet, alcohol, some drugs) and aging, decrease the efficiency of such protecting systems, resulting in disturbances of the redox equilibrium established under healthy conditions. Thus, antioxidants that scavenge reactive oxygen species may be of great value in preventing the onset and propagation of oxidative diseases (Willet, 1994). Recently, more attention has been paid to the role of natural antioxidants, mainly phenolic compounds, which may have higher antioxidant activities than those of conventional vitamins C, E and β-carotene (Vinson, Dabbag, Serry, & Jang, 1995). The antioxidative effects of natural phenolic compounds, in pure forms or in their extracts from different plant sources (vegetables, fruits and medicinal plants), were studied in vitro using different model systems (Meyer, Heinonen, & Frankel, 1998; Pietta, Simonetti, & Mauri, 1998; Yen & Hsieh, 1998). Therefore, antioxidants, which can neutralize free radicals, may be of central importance in the prevention of carcinogenicity, cardiovascular, and neurodegenerative changes associated with aging (Halliwell, 1994; Yu, 1994). Epidemiological studies show that the consumption of vegetables and fruits could protect

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humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species (Ames, Shigena, & Hagen, 1993).

Morinda citrifolia L. (Rubiaceae), locally known as 'mengkudu', has been extensively used in folk medicine by the Polynesians for over 2000 years. It has been reported to have broad therapeutic effects, including anticancer activity, in both human and laboratory animal models. However, the mechanisms for these effects remain unknown. *M. citrifolia* is unique in view of the large number of medical claims that have been made for its efficacy; nevertheless, little is known about its pharmacological potential compared with other popularly used botanicals, and its rapidly evolving commercial success. The chemical components of *M. citrifolia* have not been well studied, and only several anthraquinones and asperuloside were previously isolated (Levand & Larson, 1979; Srivastava & Singh, 1993). The aim of the present work is to evaluate antioxidative activities of various chromatographic fractions from root, fruit and leaf extract of *M. citrifolia* in relation to their total phenolic contents.

2. Materials and methods

2.1. Material

Plant material used in this study include fresh Mengkudu (*M. citrifolia*) root (root with root bark), fruit (seedless without core) and leaf (whole leaf), that were obtained from the Traditional Medicine Plant Plot, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The samples were washed with running tap water before being chopped into pieces. They were then oven-dried at 45 °C for two days and ground to powder.

2.2. Preparation of *M. citrifolia* L. root, fruit and leaf extracts

M. citrifolia root, fruit and leaf were extracted according to the modified method of Chang, Ostric-Matijaseric, Hsieh, and Huang (1977). Samples were defatted with hexane in a shaking water bath for 12 h and extracted with 100 ml of 95% (v/v) methanol for 20 min at 80 °C. The extraction was repeated twice and extracts were combined and evaporated to dryness under vacuum at 40 °C. The dried extracts obtained from several batches of *M. citrifolia* root, fruit and leaf was transferred into air-tight amber bottles and stored at −20 °C until use.

2.3. Sephadex LH-20 column chromatography

The crude root, fruit and leaf extracts were fractionated using a Sephadex LH-20 column {1.5 cm diameter and 83 cm height, particle size 25–100 μm (Pharmacia, Uppsala, Sweden)}. 0.5 Grammes of each extract was dissolved in 3 ml of ethanol and introduced to the column. Fractions collected were measured at 725 nm after colour development for phenols (Shahidi & Naczk, 1995, Chap. 5). Eluates were then pooled into fractions, and solvent removed with a rotary evaporator. Content of total phenolic compounds in each fraction was estimated using Folin–Ciocalteu reagent (Shahidi & Naczk, 1995, Chap. 5).

2.4. Determination of total phenolic compounds

Total phenolic compounds (TPCs) were determined according to the method of Shahidi and Naczk (1995, Chap. 5). A 0.25 ml aliquot of the extract solution was mixed with 0.25 ml of Folin–Ciocalteu reagent (previously diluted with water 1:1 v/v) 0.5 ml of saturated sodium carbonate (Na_2CO_3) solution and 4 ml of water. The mixture was then allowed to stand at room temperature for 25 min, followed by centrifugation at 5000 rpm for 10 min. Absorbance of the supernatant was then measured at 725 nm.

2.5. Determination of antioxidative activity of the fractions

2.5.1. Ferric thiocyanate method

The ferric thiocyanate method (FTC) method was adapted from Osawa and Namiki (1981); 4 ml samples in 99.5% ethanol were mixed with 2.51% linoleic acid in 99.5% ethanol (4.1 ml), 0.05 M phosphate buffer, pH 7.0 (8 ml), and distilled water (3.9 ml) and kept in a screwed-cap container in the dark condition at a temperature of 40 °C. To 0.1 ml of this solution have added 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after addition of 0.1 ml of 2×10^{-2} M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of red colour was measured at 500 nm every day until the day after which absorbance of the control reached a maximum.

2.5.2. Thiobarbituric acid test

The test was conducted according to the method of Ottolenghi (1959) and Kikuzaki and Nakatani (1993). The same samples as prepared for the FTC method were used in this test. 20% of trichloroacetic acid (2 ml) and thiobarbituric acid (TBA) solution (2 ml) were added to 1 ml of sample solution. This mixture was then placed in a boiling water bath for 10 min. After cooling, it was then centrifuged at 3000 rpm for 20 min. Absorbance of supernatant was then measured at 532 nm. Antioxidative activity was recorded, based on absorbance on the final day.

2.6. Statistical analysis

All experiments were conducted in triplicate and statistical analysis was done according to the SAS (1990) User's

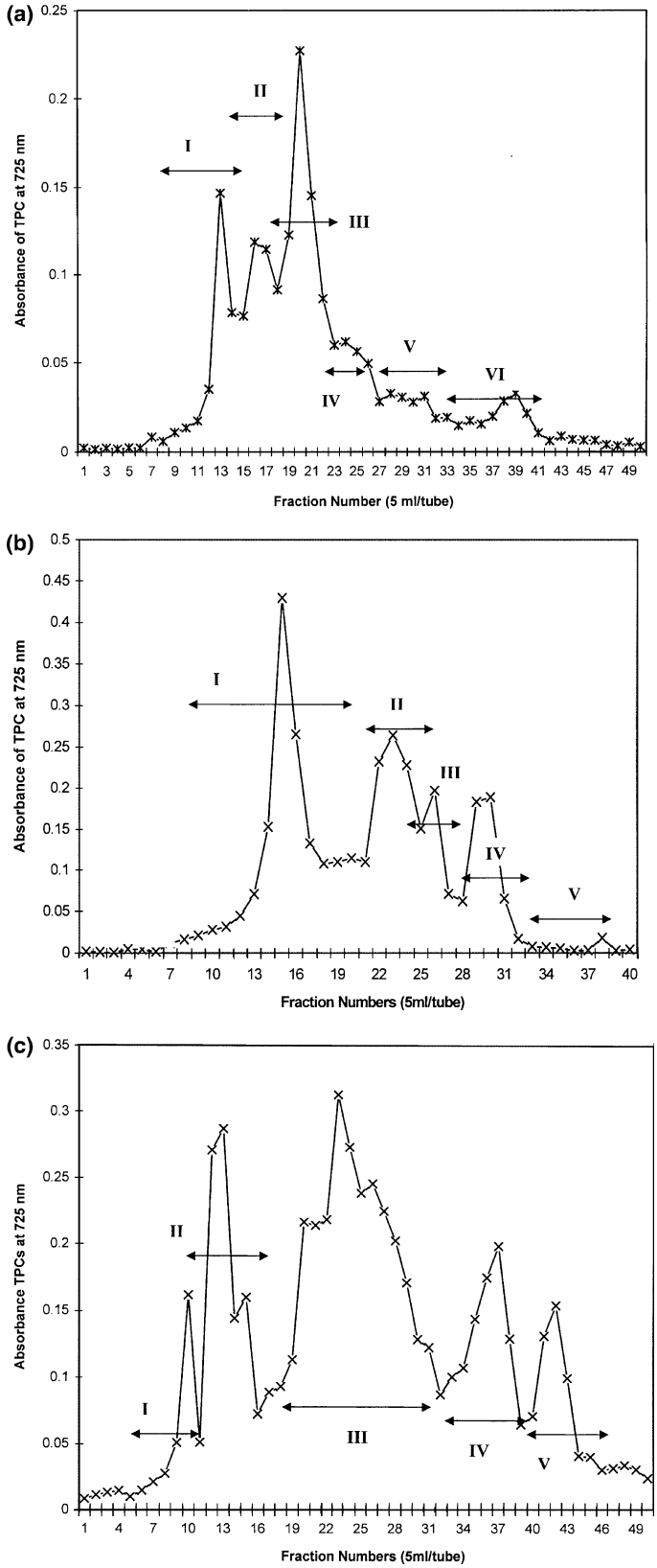

Fig. 1. (a) Eluates, following Sephadex LH-20 column chromatography, of fruit extracts of *M. citrifolia*: samples were separated into six major fraction (I–VI) based on absorbance of phenolics following colour development at 725 nm. (b) Eluates, following Sephadex LH-20 column chromatography, of root extracts of *M. citrifolia*: samples were separated into five major fraction (I–V) based on absorbance of phenolics following colour development at 725 nm. (c) Eluates, following Sephadex LH-20 column chromatography, of leaf extracts of *M. citrifolia:* samples were separated into five major fraction (I–V) based on absorbance of phenolics following colour development at 725 nm.

Table 1
Total phenolic content of fruit extract[a]

Pooled fraction	(%)	Total phenolic content (μg/g) as (+)-catechin equivalents[b]
I	18.4	22.1 ± 10.3 ^{A}
II	14.5	17.4 ± 1.9 ^{AB}
III	15.2	18.2 ± 3.9 ^{AB}
IV	4.29	5.1 ± 0.4 ^{B}
V	23.8	28.5 ± 3.2 ^{A}
VI	23.9	28.7 ± 10.5 ^{A}

[a] Separated on Sephadex LH-20 column.
[b] Values are means ± standard deviation of duplicate analyses. Means with same letter (A, B, C) are not significantly different ($p < 0.05$).

Table 2
Total phenolic content of root extract[a]

Pooled fraction	(%)	Total phenolic content (μg/g) as (+)-catechin equivalents [b]
I	75.0	214 ± 13.21 ^{A}
II	12.7	36.3 ± 0.21 ^{AB}
III	3.21	9.15 ± 8.55 ^{B}
IV	8.44	24.1 ± 8.13 ^{AB}
V	0.67	1.9 ± 0.20 ^{C}

[a] Separated on Sephadex LH-20 column.
[b] Values are means ± standard deviation of duplicate analyses. Means with same letter (A, B, C) are not significantly different ($p < 0.05$).

Table 3
Total phenolic content of leaf extract[a]

Pooled fraction	(%)	Total phenolic content (μg/g) as (+)-catechin equivalents[b]
I	16.9	137 ± 2.3 ^{B}
II	13.1	107 ± 20.5 ^{C}
III	9.42	76.6 ± 1.2 ^{D}
IV	46.8	380 ± 12.8 ^{A}
V	13.8	112 ± 6.4 ^{BC}

[a] Separated on Sephadex LH-20 column.
[b] Values are means ± standard deviation of duplicate analyses. Means with same letter (A, B, C) are not significantly different ($p < 0.05$).

Guides. Analysis of Variance was performed by the ANOVA procedure. Duncan's multiple range tests were used to determine significant differences between the means.

3. Results and discussion

3.1. Fractionation of the fruit, root and leaf extracts of M. citrifolia

Recently, more attention has been focussed on the role of natural antioxidants, in particular, phenolic

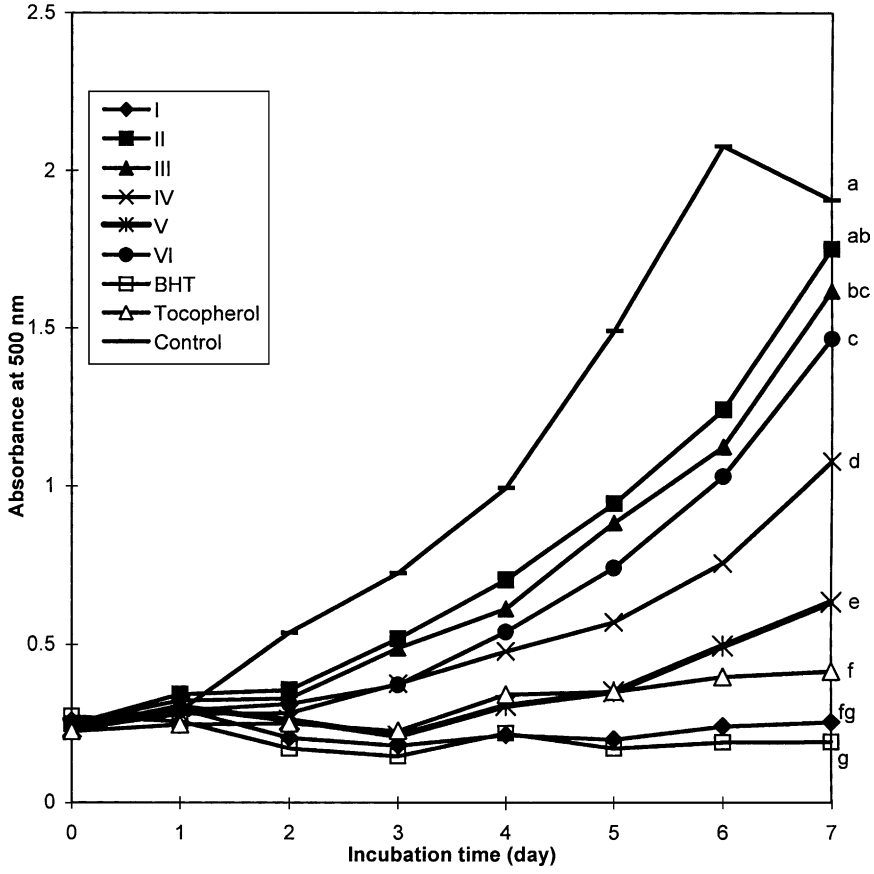

Fig. 2. Antioxidative activities of Sephadex LH 20 column chromatographic fractions obtained from fruit extracts of *M. citrifolia* as measured by FTC method.

compounds, which may act both by reducing the content of toxic compounds in foods and by supplying the human body with exogenous antioxidants. In this study, total phenolic contents and antioxidative activities of chromatographic fractions of *M. citrifolia* were determined. The Sephadex LH-20 column used was instrumental in separating fruit extract into six, fractions, and leaf and root extracts into five fractions, each, based on UV absorption intensity of their phenolic compounds (absorbance at 725 nm) as shown in Fig. 1(a)–(c).

3.2. Total phenolic compounds of the fruit, root and leaf fractions of M. citrifolia

The contents of phenolics, as (+)-catechin equivalents, in each of the six fractions of fruit extract are given in Table 1. Results reveal that the levels of phenolic compounds in different fractions of fruit extract were not significantly ($p < 0.05$) different from each other, except in fraction IV. Phenolic substances that are known to possess high antioxidative activity are common phytochemicals in fruits and leafy vegetables. Most of these phenolics are classified in the two principal groups of phenol carboxylic acids and flavonoids, the latter being the most significant (Bitsch, 1996), and derivatives of flavan (2-phenyl-benzodihydropyran). The main subgroups are the colourless catechins, the red-to blue-coloured anthocyanidins, the light-yellow flavonols and seven flavones, and the colourless proanthocyanidins (Herrmann, 1994). According to Pratt and Hudson (1992), phenolic compounds are found abundantly in all parts of the plant, such as wood, bark, stems, leaves, fruit, root, flowers, pollen and seeds.

Table 2 shows total phenolics as catechin equivalents, in each of the five fractions of root extract *M. citrifolia.* Results showed that fraction I of the root extract contained the most (21.4 mg/100 g) phenolic compounds which is significantly more than that found in the fruit extract of the same plant. On the other hand, fraction V was found to have a significantly ($p < 0.05$) lower content of phenolic compounds. Velioglu, Mazza, Gao, and Oomah (1998) found that phenolic compounds were responsible for the antioxidative activity in some selected fruits, vegetables and grains tested. Similarly, Shahidi and Naczk

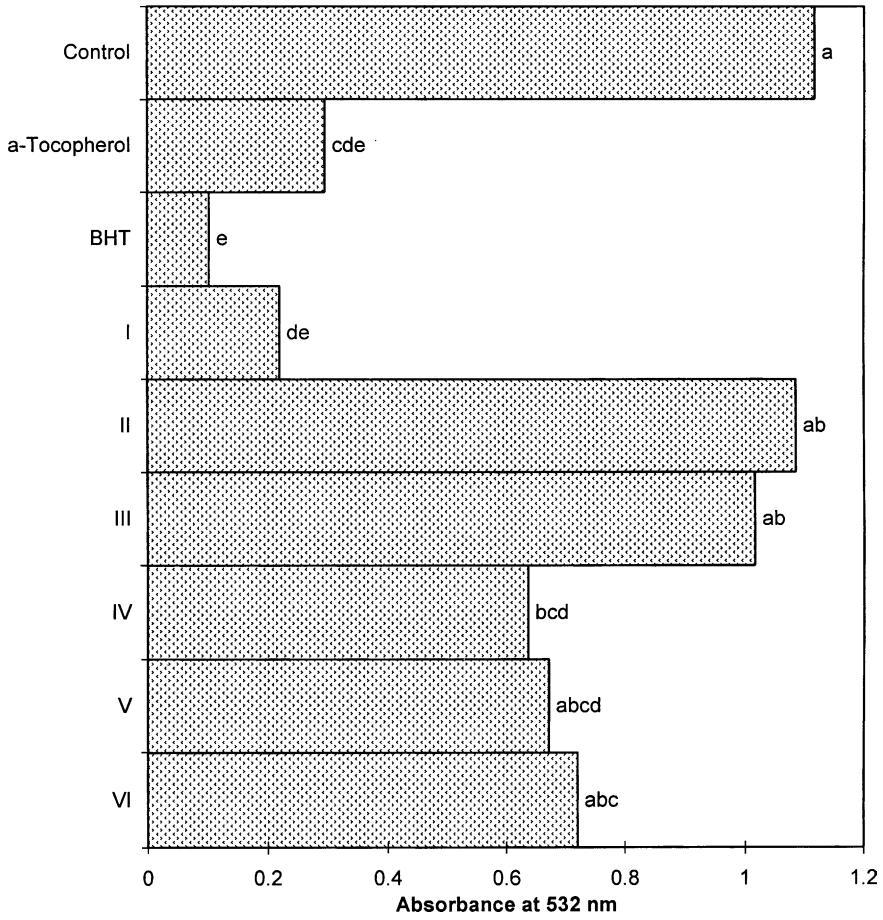

Fig. 3. Antioxidative activities of Sephadex LH 20 column chromatographic fractions obtained from fruit extracts of *M. citrifolia* as measured by TBA method.

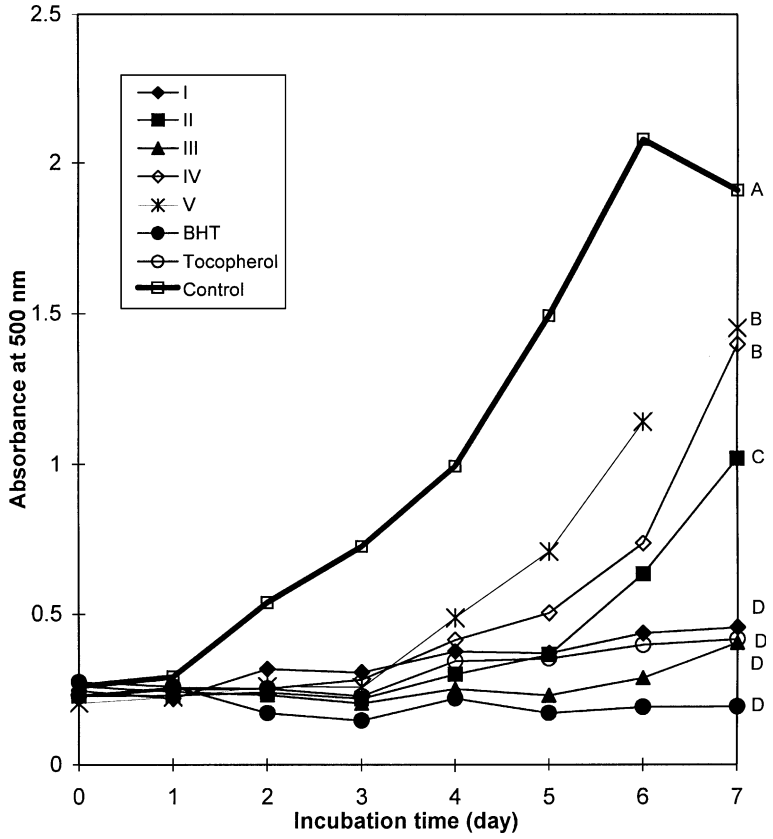

Fig. 4. Antioxidative activities of Sephadex LH 20 column chromatographic fractions obtained from root extracts of *M. citrifolia* as measured by FTC method.

(1995, Chap. 5) reported that naturally-occurring phenolics; exhibit antioxidative activity. Thus, therapeutic properties of *M. citrifolia* root extracts may possibly be attributed to the phenolic compounds measured.

Similarly, the contents of phenolics, as catechin equivalents, in the five fractions of leaf extract are given in Table 3. Fraction III had a lower amount of phenolic compounds compared to the other fractions, while fraction IV was seen to have a significantly ($p < 0.05$) higher level of phenolic compounds than other fractions. Phenolic compounds are considered to be the most important antioxidative components of herbs and other plant materials, and good correlation between the concentrations of plant phenolic and the total antioxidant capacities has been reported (Madsen, Nielsen, Bertelsen, & Skibsted, 1996; Pellegrini et al., 2000). Antioxidants interrupt lipid (and protein) oxidation, either in the propagation phase (chain-braking mechanism) or by protecting the oxidation substrates against the first radicals formed in the initiation phase. Accordingly, evaluation of plant material for antioxidative activity should not depend only on a single method, but should include measurement of reactions characteristic of both the initiation and the propagation phase (Schwarz et al., 2000).

3.3. Antioxidative activity of the fruit, root and leaf fractions of M. citrifolia L

Antioxidative activity of phenolic compounds is based on their ability to donate hydrogen atoms to free radicals. Many phenolic compounds, particularly flavonoids, exhibit a wide range of biological effects, including antibacterial, antiviral, and anti-inflammatory, anti-allergic, anti-thrombotic and vasdilatory actions (Cook & Samman, 1996). Studies have also shown that some of these compounds are known potent scavengers of free radicals and, as such, are potentially useful in the prevention of arteriosclerosis, cancer, diabetes, neurodegenerative diseases and arthritis. Protective effects of diets high in fruits and vegetables have been attributed to the presence of these compounds.

In this study, the FTC method was used to measure the peroxides formed during initial stages of lipid oxidation. During the oxidation process, peroxide was gradually decomposed into lower molecular weight compounds. The degradation products were then measured using the TBA method. Thus, in both the TBA and FTC methods, samples with low absorbance values would indicate high antioxidative activity.

Fig. 2 shows the antioxidative activities of the six fractions from fruit extract of *M. citrifolia*, as measured by

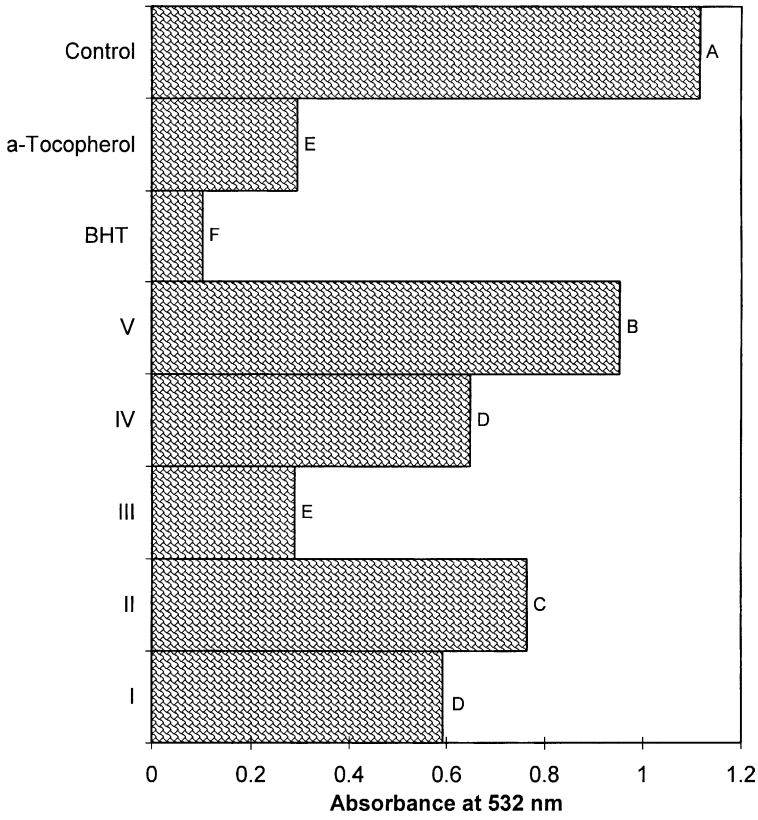

Fig. 5. Antioxidative activities of Sephadex LH 20 column chromatographic fractions obtained from root extracts of *M. citrifolia* as measured by TBA method.

the FTC method. Fig. 3 shows the antioxidative activities of fruit fractions, as measured by the TBA method. The activity in decreasing order was: BHT > I > α-tocopherol > V > IV > VI > III and II. Fraction I displayed the strongest antioxidative activity, with activity that was not significantly ($p < 0.05$) different from those of BHT or α-tocopherol. This is true for both assays. Wang, Cao, and Prior (1996) and Hertog, Hollman, and Vennema (1992) reported that the antioxidant properties of some vegetables and fruits are partly due to low molecular weight phenolic compounds, particularly the flavonoids, which are known to be potent antioxidants. According to Wang et al. (1999), two known glycosides (rutin and asperulosidic acid) and a novel trisaccharide fatty acid ester [2,6-di-*O*-(β-D-glucopyranosyl)-l-*O*-octanoyl-β-D-glucopyranose] were isolated from the *n*-butanol-soluble fraction obtained from the ethanol extracts of the fruit of *M. citrifolia* Interestingly, fraction I, exhibited the highest antioxidative activity, although it contained only 2.21 mg/100 g of phenolic compounds, which was comparatively lower than that found in either fraction V or VI. This is probably due to the more potent antioxidant present in fraction I compared to those of fraction V or VI. The result also showed that lack of correlation occurs between antioxidative activity and their content of phenolic compounds. According to Shahidi and Naczk (1995, Chap. 5), the Folin–Ciocalteu method measures constituents other than phenolics, and its specificity is poor. The Folin–Ciocalteu reagent detects all phenolic groups found in the extract, including those found in the extractable proteins.

These results suggest that factors other than total phenolics may play a role in the antioxidant activity of the fruit fraction of *M. citrifolia.* This also indicates that the amount of phenolic compounds is not the only factor in the consideration of antioxidative activity and that molecular structures play important roles in antioxidant activity. Moreover, all the phenolics do not have the same antioxidant activity; some are powerful and others are weak. They develop antagonistic or synergistic effects in combination with themselves or with other constituents of the extracts (Lien, Ren, Bui, & Wang, 1999; Moran, Klucas, Grayer, Abian, & Becana, 1997; Rice-Evans, Miller, & Paganga, 1996).

The antioxidative activities of each of the five isolated root fractions and that of both α-tocopherol and BHT, as measured by the FTC method, are presented in Fig. 4. Fraction III of the root extract had the strongest antioxidative activity, which was not significantly ($p < 0.05$) different from those of either BHT, α-tocopherol or fraction I. This is intriguing since fraction III consisted of only 9.15 μg/g of phenolic compounds,

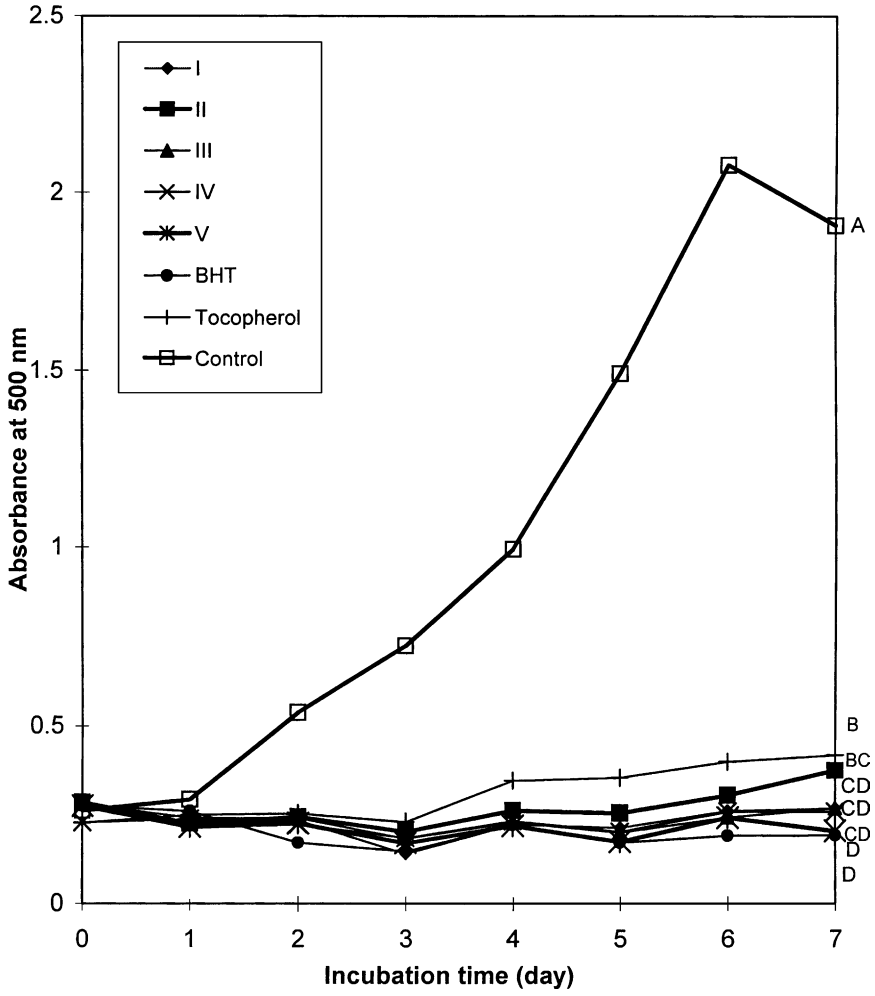

Fig. 6. Antioxidative activities of Sephadex LH 20 column chromatographic fractions obtained from leaf extracts of *M. citrifolia* as measured by FTC method.

which is much lower than that of fraction I with 213 μg/g of phenolic compounds. This suggested that compounds in fraction III were very potent antioxidants, which most probably are different than that found in fraction I or may be also due to synergistic effects different antioxidants.

More recently, the roots have been reported to contain anthraquinones, such as, nordamnacanthal, moridone, rubiadin, rubiadin 1-methylether, and soranjidiol. These anthraquinones are presumably the active principles in the plant, since both antimicrobial activity and other physiological properties have been attributed to such compounds (Jain & Srivastava, 1992; Rusia & Srivastava, 1989). The result suggested that the amount of phenolic compounds is not the only factor in the consideration of antioxidative activity and that the molecular structures of different phenolic compounds play important roles in the antioxidative activity (Zadernowski, Nowak, & Kozlowska, 1991).

Ability of the different root fractions in inhibiting peroxidation of linoleic acid, as measured by the TBA method, is presented in Fig. 5. A trend similar to that obtained previously using the FTC method, was seen, where fraction III displayed the strongest antioxidative activity. But fraction I was significantly ($p < 0.05$) different from those of either BHT or α-tocopherol or fraction III. Again, this shows that different methods gave different results, which is probably due to the different mechanisms of action of the different antioxidants present in the samples.

Figs. 6 and 7 show the antioxidative activities of the five fractions isolated from crude leaf extract. In FTC assayed, all fractions were found to exhibit strong antioxidative activity, comparable to that of α-tocopherol, with fraction V exhibiting the highest activity. The activity, in decreasing order, was: BHT > V > IV > I > III > II and α-tocopherol. However, the results from TBA assay showed a somewhat different pattern compared to the FTC method, where fraction I and II showed high antioxidative activities, which are not significantly ($p < 0.05$) different from those of either BHT or α-tocopherol. The differences in activities

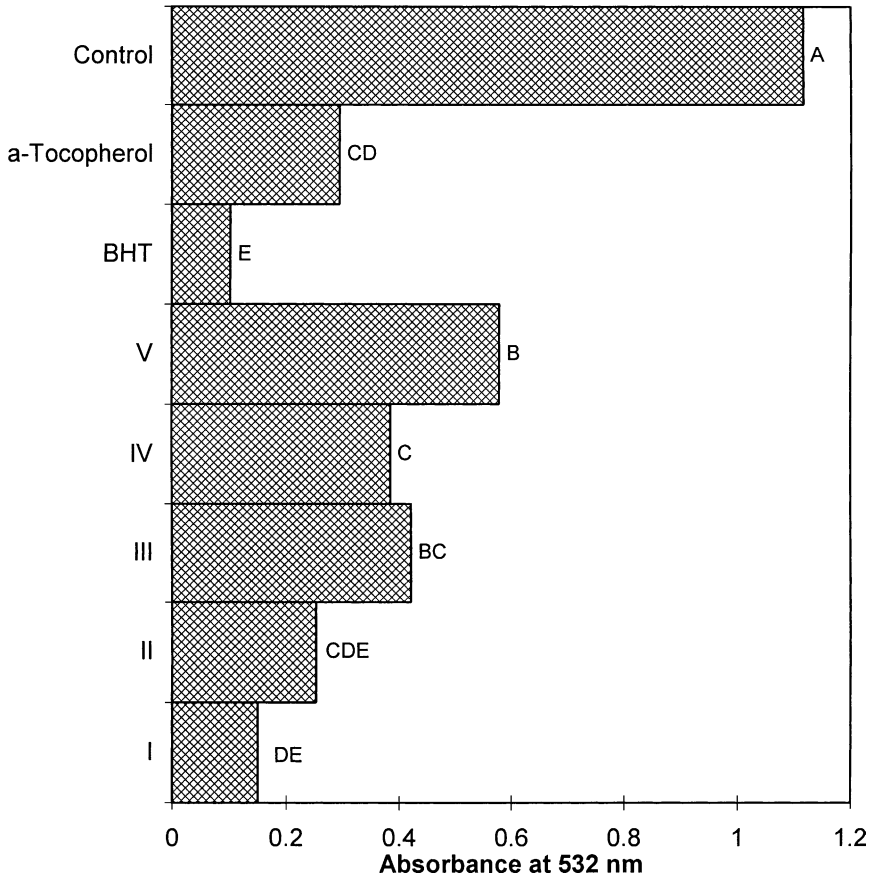

Fig. 7. Antioxidative activities of Sephadex LH 20 column chromatographic fractions obtained from fruit extracts of *M. citrifolia* as measured by TBA method.

observed here could be ascribed to several factors, including the different mechanisms involved in the determinations structures of the different phenolic compounds, differences in antioxidative mechanism exhibited by different compounds and the synergistic effect of the different compounds isolated. The leaves of the *M. citrifolia* plant possessed several interesting compounds that have been recognized for their medicinal properties. Swanholm, John, and Scheuer (1959) determined that alkaloids were present in *M. citrifolia* leaves and stem. Many alkaloids are important pharmaceutical drugs, such as morphine, vincristine, reserpine and scopolamine.

The antioxidative compounds present in the sample may exhibit different mechanisms of action from each other. Rutin and catechins, for example, were reported to be good oxygen species scavenging compounds (Hatano, Yasuhara, Fukuda, Noro, & Okuda, 1989). On the other hand, *p*-coumaric was seen to block the generation of free radical and was thus involved in chain-breaking activity (Laranjinha, Vieira, Madeira, & Almeida, 1995); others act as metal chelators (Van Acker, van Balen, van den Berg, Bast, & van der Vijgh, 1998).

4. Conclusion

Studies have shown that a high consumption of fruits and vegetables containing phenolic antioxidants, inhibit the oxidation of LDL, and thus slow the process of atherosclerosis and also reduce the risk of cancer and many other diseases. It is encouraging to see that all the fractions studied demonstrated high antioxidative activity compared with either BHT or α-tocopherol. The potency of some of these compounds could provide a scientific basis for the health benefits claimed for *M. citrifolia* in folk medicine and warrant further studies to assess their potential as effective natural remedies. The present study suggests that consumption of *M. citrifolia* may have potential health effects. Further work is required to elucidate these results in vivo and to identify compounds, which are responsible for the activities measured.

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References

Ames, B. M., Shigena, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants and the degenerative diseases of aging. *Proceedings of the National Academy of Science of the United States America, 90*, 7915–7922.

Bitsch, R. (1996). Pflanzenphenole und ihre gesundheitliche Wirkung. *Naturw Rdsch, 49*, 47–51.

Burton, G. W., & Ingold, K. U. (1989). Mechanisms of antioxidant action: preventive and chain-breaking antioxidants. In J. Miquel, A. T. Quintanilha, & H. Weber (Eds.). *Handbook of free radicals and antioxidants in biomedicine* (Vol. 2, pp. 29). Boca Raton, FL: CRC Press.

Carini, R., Poli, G., Diazini, M. U., Maddix, S. P., Slater, T. F., & Cheesman, K. H. (1990). Comparative evaluation of the antioxidant activity of a-tocopherol, α-tocopherol poly ethylene glycol 1000 succinate and a-tocopherol succinate in isolated hepatocytes and liver microsomal suspensions. *Biochemical Phamacology, 39*, 1597–1601.

Chang, S. S., Ostric-Matijaseric, B., Hsieh, O. A. L., & Huang, C. L. (1977). Natural antioxidant from rosemary and sage. *Journal of Food Science, 42*, 1102–1106.

Cook, N. C., & Samman, S. (1996). Flavonoids – chemistry, metabolism, cardioprotective effect and dietary sources. *Nutritional Biochemistry, 7*, 66–76.

Halliwell, B. (1994). Free radicals and antioxidants: a personal view. *Nutrition Review, 52*, 253–265.

Herrmann, K. (1994). In pflanzlichen Lebensmitteln vorkommende flavonoide als antioxidantien. *Gordian, 93*, 108–111.

Hertog, M. G. L., Hollman, P. C. H., & Vennema, D. P. (1992). Optimization of the quantitative HPLC determination of potentially anticarcinogenic flavonoidsin vegetables and fruits. *Journal of Agricultural and Food Chemistry, 40*, 1591–1598.

Hatano, T., Yasuhara, T., Fukuda, T., Noro, T., & Okuda, T. (1989). Phenolic constituents of licorice. II. Structures of licopyranocoumarin, licoarylcoumarin and glisoflavone, and inhibitory effects of licorice phenolics on xanthine oxidase. *Chemical and Pharmaceutical Bulletin, 37*, 3005–3009.

Jain, R. K., & Srivastava, S. D. (1992). Two new anthraquinones in the roots of *Morinda citrifolia*. *Proceeding of the National Academy of Science in India Section A Part I, 62*, 11–13.

Kikuzaki, H., & Nakatani, N. (1993). Antioxidant effects of some ginger constituents. *Journal of Food Science, 58*, 1407–1410.

Levand, O., & Larson, H. O. (1979). Some chemical constituents of *Morinda citrifolia*. *Planta Medica, 36*, 186–187.

Lien, E. J., Ren, S., Bui, H. H., & Wang, R. (1999). Quantitative structure-activity relationship analysis of phenolic antioxidants. *Free Radical Biology and Medicine, 26*, 285–294.

Laranjinha, J., Vieira, O., Madeira, V., & Almeida, L. (1995). Two related phenolic antioxidants with opposite effects on vitamin E content in low density lipoproteins oxidized by ferrylmyoglobin: consumption vs regeneration. *Archieves of Biochemistry and Biophysics, 323*, 373–381.

Meyer, A. S., Heinonen, M., & Frankel, E. N. (1998). Antioxidant interactions of catechin, cyaniding, caffeic acid quercetin and ellagic acid on human LDL oxidation. *Food Chemistry, 67*, 71–75.

Madsen, H. L., Nielsen, B. R., Bertelsen, G., & Skibsted, L. H. (1996). Screening of antioxidative activity of spices. *Food Chemistry, 57*, 331–337.

Moran, J. F., Klucas, R. V., Grayer, R. J., Abian, J., & Becana, M. (1997). Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: prooxidant and antioxidant properties. *Free Radical Biology and Medicine, 22*, 861–870.

Osawa, T., & Namiki, M. (1981). A novel type of antioxidant isolated from leaf wax of *Eucalyptus* leaves. *Journal of Agricultural and Food Chemistry, 45*, 735–739.

Ottolenghi, A. (1959). Interaction of ascorbic acid and mitochondrial lipids. *Archieves of Biochemistry and Biophysics, 79*, 355–358.

Pietta, P., Simonetti, P., & Mauri, P. (1998). Antioxidant activity of selected medicinal plants. *Journal of Agricultural and Food Chemistry, 46*, 4487–4490.

Pratt, D. E., & Hudson, B. J. F. (1992). Natural antioxidants not exploited commercially. In B. J. F. Hudson (Ed.), *Food Antioxidants* (pp. 171–192). London: Elsevier Applied Science.

Pellegrini, N., Simonetti, P., Gardana, C., Brenna, O., Brighenti, F., & Pietta, P. (2000). Polyphenol content and total antioxidant activity of Vini Novelli (Young red wines). *Journal of Agricultural and Food Chemistry, 48*, 732–735.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationship of flavonoids and phenolic acid. *Free Radical Biological and Medicine, 20*, 933–956.

Rusia, K., & Srivastava, S. K. (1989). A new anthraquinone from the roots of *Morinda citrifolia* Linn. *Current Science, 58*, 249–251.

Schwarz, K., Huang, S. W., German, J. B., Tiersch, B., Hartmann, J., & Frankel, E. N. (2000). Activities of antioxidants are affected by colloidal properties of oil-in-water and water-in- oil emulsion and bulk oils. *Journal of Agricultural and Food Chemistry, 48*, 4874–4882.

Shahidi, F., & Naczk, M. (1995). *Food phenolics sources, chemistry, effects, and applications*. Lancaster Basel: Technomic Publishing Co, p. 128.

Srivastava, M., & Singh, J. (1993). A new anthraquinone glycoside from *Morinda citrifolia*. *International Journal of Pharmacognosy, 31*, 182–184.

Swanholm, C. E., John, S. H., & Scheuer, P. J. (1959). A survey of alkaloids in Hawaiian plants. *Pacific Science, 13*, 295–305.

Van Acker, S. A. B. E., van Balen, G. P., van den Berg, D. J., Bast, A., & van der Vijgh, W. J. F. (1998). Influence of iron chelation on the antioxidant activity of flavonoids. *Biochemical Pharmacology, 56*, 935–943.

Vinson, J. A., Dabbag, Y. A., Serry, M. M., & Jang, J. (1995). Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *Journal of Agricultural and Food Chemistry, 43*, 2800–2802.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and green products. *Journal of Agricultural and Food Chemistry, 46*, 4113–4117.

Willet, W. C. (1994). Diet and health-what should we eat?. *Science, 264*, 532–537.

Wang, M., Kikuzaki, H., Csiszar, K., Boyd, C. D., Maunakea, A., Fong, S. F. T., et al. (1999). Novel trisaccharide fatty acid ester identified from the fruits of *Morinda citrifolia* (noni). *Journal of Agricultural and Food Chemistry, 47*, 4880–4882.

Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry, 44*, 701–705.

Yen, G., & Hsieh, C. (1998). Antioxidant activity of extracts from Duzhong *(Eucommia ulmoides)* towards various lipid peroxidation models in vitro. *Journal of Agricultural and Food Chemistry, 46*, 3952–3957.

Yu, B. P. (1994). Cellular defencses against damage from reactive oxygen species. *Physiological Reviews, 76*, 139–162.

Zadernowski, R., Nowak, H., Kozlowska, H. (1991). Natural antioxidants from rapeseed. In: *Rapeseed in a Changing World, 8th International Rapeseed Congress, July 9–11 Saskatoon*, Canada, 1991, (pp. 883–887).