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Antioxidative activity of extracts from Mengkudu (*Morinda citrifolia* L.) root, fruit and leaf

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Abstract

This study was conducted to evaluate the antioxidative activity of extracts from different parts of Mengkudu (*Morinda citrifolia* L.), including leaf, fruit and root. Methanol and ethyl acetate were used as solvents and antioxidative effects measured by a ferric thiocyanate method (FTC) and thiobarbituric acid test (TBA). The methanol extract of Mengkudu root exhibited high antioxidative activity that was not significantly ($P<0.05$) different from α-tocopherol or butylated hydroxyl toluene (BHT), while the methanol extracts of fruit and leaf showed negligible activities. On the other hand, the ethyl acetate extract of all parts of Mengkudu exhibited significant antioxidative activity, which is comparable to that of both α-tocopherol and BHT. Similar trends of antioxidant activity were observed using either the FTC or TBA methods. Roots showed the highest activity of the parts tested. The results suggest that several compounds contribute to antioxidative activity of different parts of Mengkudu. Activity in the roots may be due to both polar and non-polar compounds but, in the leaf and fruit, only to non-polar compounds. © 2002 Published by Elsevier Science Ltd.

Keywords: Antioxidative activity; *Morinda citrifolia* Linn; Natural antioxidant

1. Introduction

Plants are potential sources of natural antioxidants. They absorb the sun's radiation and generate high levels of oxygen as secondary metabolites of photosynthesis. Oxygen is easily activated by ultra violet (UV) radiation and heat from the sunlight to produce toxic, reactive oxygen species (ROS). Plants produce various antioxidative compounds to counteract these ROS in order to survive (Lu & Foo, 1995). Recently, peroxidation of unsaturated lipids in living organisms is receiving increasing attention in relation to the possible association between lipid oxidation and a wide range of degenerative diseases, including ageing, cancer, diabetes and cardiovascular diseases. Thus, antioxidants are important inhibitors of lipid peroxidation, not only for food protection but also as a defence mechanism of living cells against oxidative damage (Vimala & Adenan, 1999).

Herbal and natural products have been used for centuries, throughout the world, in every culture. Recently, the scientific community has begun to show interest in *Morinda citrifolia* L. and its products as its benefits become known.

M. citrifolia L., or Indian mulberry, originated in tropical Asia or Polynesia (Abbott & Shimazu, 1985). In the Tropics, it seems to have been much valued medicinally, and the plant is normally cultivated for its roots, leaves and fruits. The roots of these plants are reported to be good sources of anthraquinones, which are usually present as aglycones and, to lesser extent, in the form of glycosides (Thomson, 1971; Zenk, El-Shagy, & Schulte, 1975). Most part of the tree has been widely used, medicinally, for relief of rheumatic and other pains and for its healing effects (Perry & Metzger, 1980).

Consumers, all over the world, are now more conscious of nutritional value and safety of food ingredients. At the same time, there is a preference for natural food and food ingredients that are believed to be safer, healthier, and less subject to contamination than their artificial counterparts. Therefore, it is interesting

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and worthwhile to investigate and identify natural antioxidants from edible plants even though they may not be comparable, in efficiency, to synthetic agents. The objective of this study is to evaluate antioxidative activity of various extracts of different parts of Mengkudu, including roots, leaves and fruits.

2. Material and methods

Plant materials used in this study include fresh Mengkudu (*Morinda citrifolia* L.) leaf (whole leaf), root (root with root bark) and fruit (seedless without core), that were obtained from the Traditional Medicine Plant Plot, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The samples were washed with running tap water and separated before being chopped into pieces. They were oven-dried at 45 °C for 2 days and ground to powder.

2.1. Extraction of antioxidative compounds

Extraction was carried out according to the modified methods of Chang, Ostric-Matijasevic, Hseih, & Huang (1977). The ground powder was extracted with methanol in a water bath at room temperature for 24 h. The solvent was then removed by filtration and fresh solvent was then added to the plant material. The extraction process was twice repeated. The combined filtrates were then evaporated under reduced pressure to give a dark green viscous mass. Antioxidative activity of this methanol crude extract was measured. The remaining methanol crude extract was further extracted with ethyl acetate and water, and then separated using separating funnels. These ethyl acetate-soluble fractions were later evaporated and afforded the ethyl acetate extract. Antioxidative activity of this ethyl acetate extract was then measured.

2.2. Determination of antioxidant activity of the extracts

2.2.1. Ferric thiocyanate method (FTC)

The FTC method was adapted from Osawa and Namiki (1981). Samples (4 mg or 4 ml) in 99.5% ethanol were mixed with 2.51% linoleic acid in 99.5% ethanol (4.1 ml), 0.05M phosphate buffer, pH 7.0 (8 ml), and distilled water (3.9 ml) and kept in screw cap containers under dark conditions at 40 °C. To 0.1 ml of this solution was 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 the red colour was measured at 500 nm each 24 h until one day after absorbance of the control reached maximum. The control and standard were subjected to the same procedure as the sample except for the control, where there was no addition of sample, and for the standard, where 4 mg of sample were replaced with 4 mg of α-tocopherol or BHT.

2.2.2. Thiobarbituric acid test (TBA)

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

2.3. Statistical analysis

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

Lipid peroxidation has been defined as the biological damage caused by free radical that are formed under oxidative stress (Ferrari et al., 1991). The antioxidative activity of natural sources is due to the active compounds present in the plants. According to Pratt and Hudson (1992), most natural antioxidants can be found in wood, bark, stem, leaf, fruit, root, flower and seed. Most of these compounds are normally phenolic or polyphenolic compounds in nature, e.g. tocopherols, flavonoids and derivatives of cinnamic acid, phosphatidic and other organic acids.

3.1. Antioxidative activity of methanol extracts of Mengkudu

In this study, the antioxidative activities of the root, fruit and leaf extracts of *M. citrifolia* were measured using ferric thiocyanate (FTC) and thiobarbituric acid (TBA). The FTC method was used to measure the peroxide level during the initial stage of lipid oxidation. Low absorbance values would indicate high levels of antioxidative activity. Fig. 1 shows the absorbance values of methanol extracts of root, fruit and leaf of *M. citrifolia*. It is interesting to note that the root extract exhibited higher activity than the fruit or leaf extracts. However, there was no significant ($P<0.05$) difference

between the antioxidative activities of *M. citrifolia* root extract and α-tocopherol or BHT. Results also showed that the methanol extracts of both fruit and leaf of *M. citrifolia* had negligible antioxidative activities, and were not significantly ($P<0.05$) different from the control.

3.2. Antioxidative activity of ethyl acetate extract of Mengkudu

Fig. 2 illustrates the antioxidative activity of the ethyl acetate extract of different parts of Mengkudu.

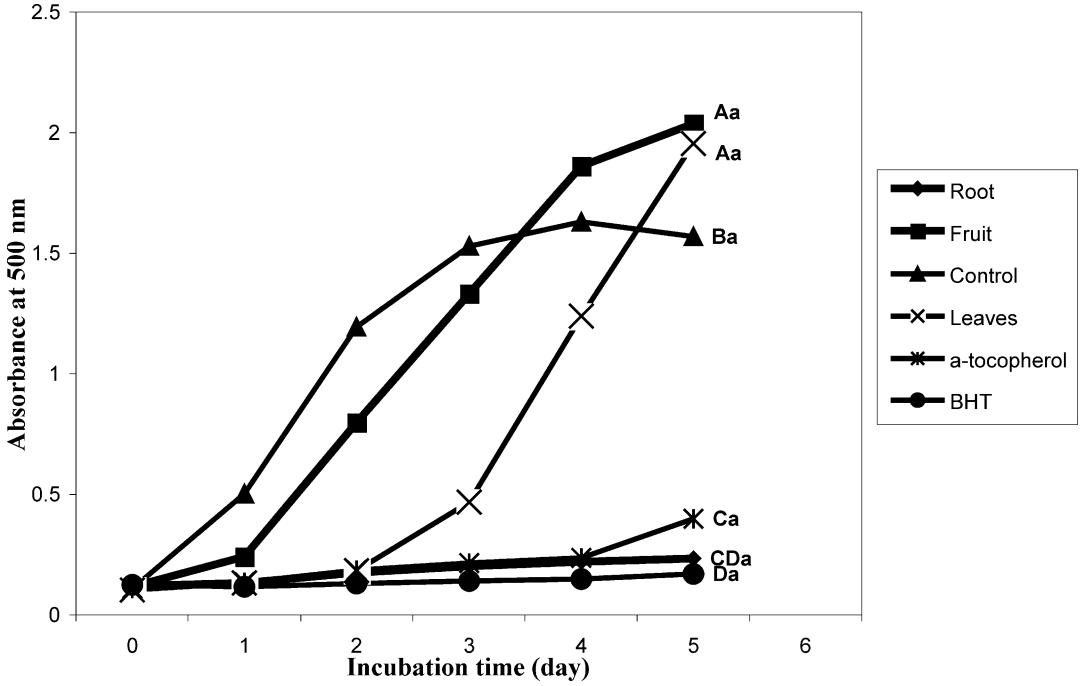

Fig. 1. Antioxidative activity of methanol extract of different parts of *Morinda citrifolia* as measured by the FTC method. Absorbance values represent triplicates of different samples analysed. Values with same letter (a,b,c) are not significantly different ($P<0.05$), between incubation times. Values with same letter (A,B,C) are not significantly different ($P<0.05$), between samples.

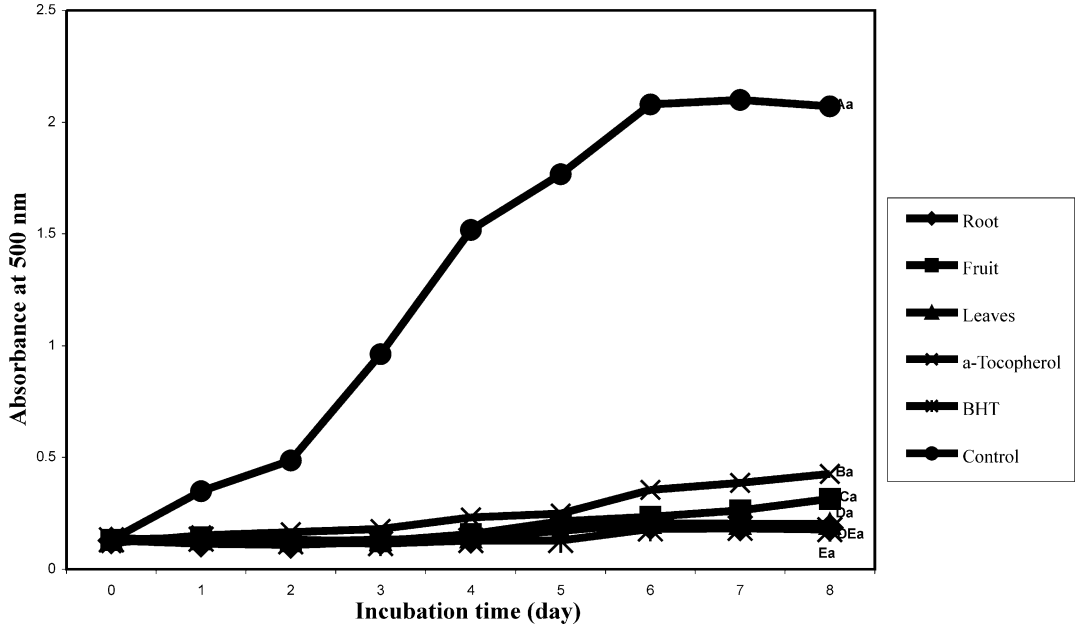

Fig. 2. Antioxidative activity of ethyl acetate extract of different parts of *Morinda citrifolia* as measured by the FTC method. Absorbance values represent triplicates of different samples analysed. Values with same letter (a,b,c) are not significantly different ($P<0.05$), between incubation times. Values with same letter (A,B,C) are not significantly different ($P<0.05$), between samples.

Antioxidative activities of the ethyl acetate extract of all parts of Mengkudu had high antioxidative activities, comparable to both α-tocopherol and BHT. It was encouraging to note that the antioxidative activities of both the root and leaf parts were not significantly ($P<0.05$) different from BHT and better than α-tocopherol.

3.3. TBA method

During the oxidation process, peroxides are gradually decomposed to lower molecular weight compounds. One such compound is malonaldehyde, which is measured by the TBA method on the final day of the incubation period (1 day after the control reached maximum). Fig. 3

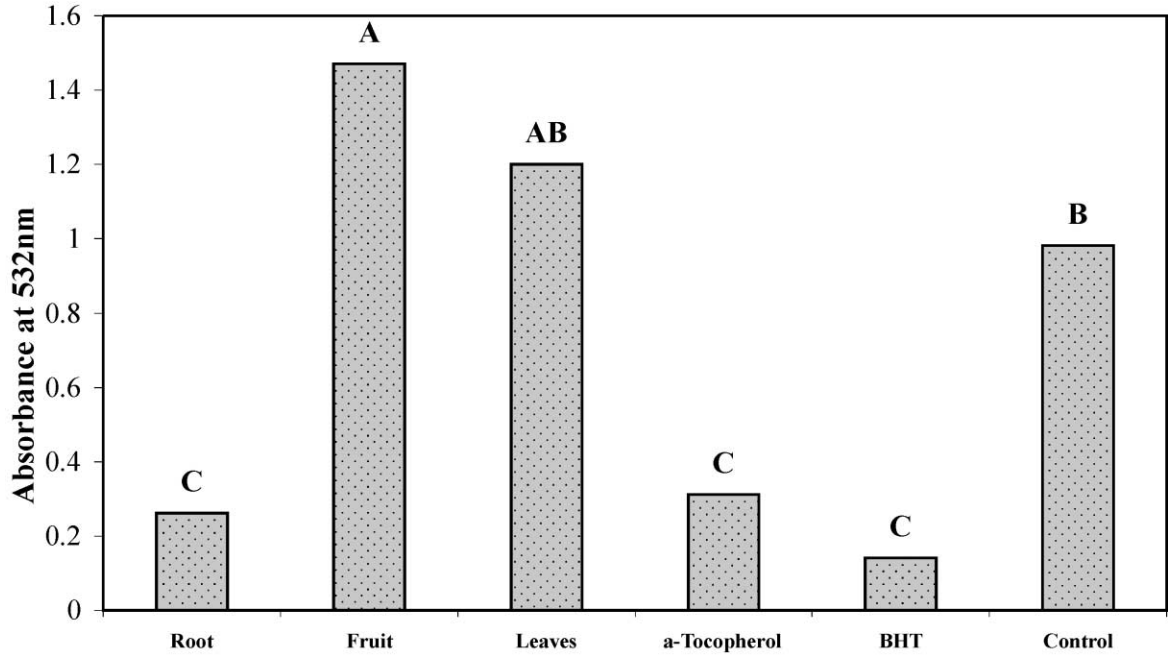

Fig. 3. Antioxidative activity of methanol extract of different parts of *Morinda citrifolia* as measured by the TBA method. Absorbance values represent triplicates of different samples analysed. Values with same letter (A,B,C) are not significantly different ($P<0.05$), between samples.

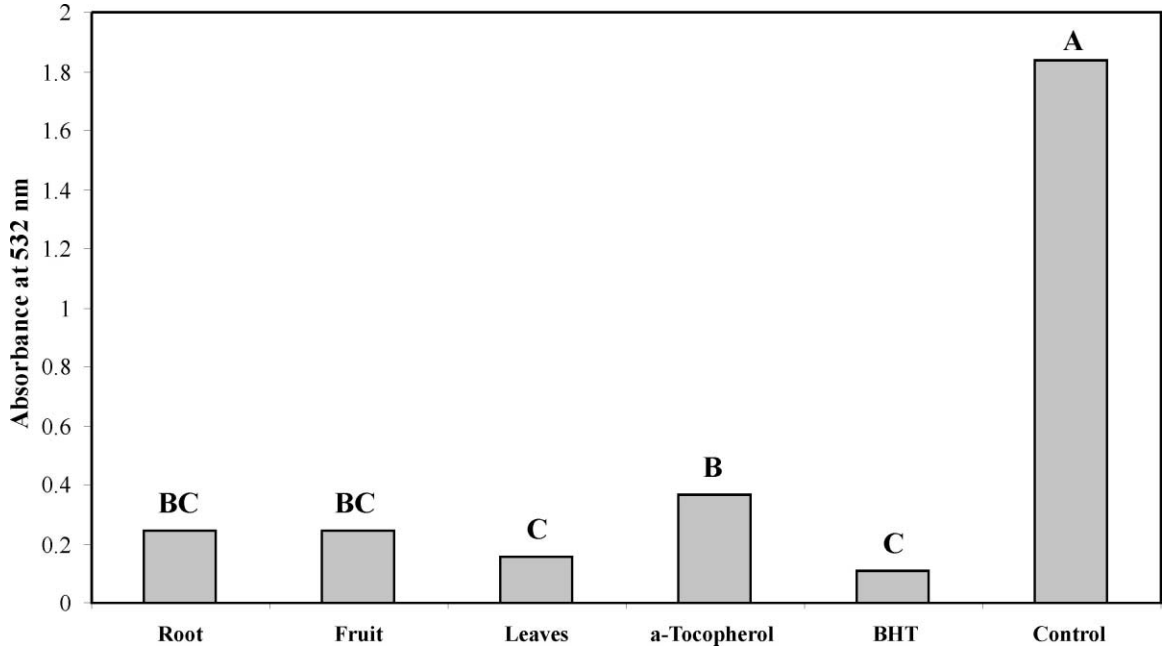

Fig. 4. Antioxidative activity of ethyl acetate extract of different parts of *Morinda citrifolia* as measured by the TBA method. Absorbance values represent triplicates of different samples analysed. Values with same letter (A,B,C) are not significantly different ($P<0.05$), between samples.

reveals that the antioxidative activity of the root methanol extract of Mengkudu was not significantly ($P<0.05$) different from either α-tocopherol or BHT. These results correlated well with those obtained previously, using the FTC method. There was no activity in either fruit or leaf extracts.

Fig. 4 shows antioxidative activities of ethyl acetate extracts, measured on the eighth day, using the TBA method. The antioxidative activities of root, fruit and leaf extracts were not significantly ($P<0.05$) different from either BHT or α-tocopherol.

Based on the results obtained, it is highly possible that several compounds of different polarity may contribute to the antioxidative activity of *M. citrifolia* leaf, fruit and root extracts. Methanol extracts may include phenolic and hydrox-phenolic compounds with acids, alcohols, sugars or glycosides, as reported by Kim and Pratt (1993). Part of the antioxidative activity may be due to flavonoids. However, the responsible antioxidative component present in the ethyl acetate extract may be alkaloid in nature, since alkaloids have been shown to be present in *M. citrifolia* L. (Heinicke, 1985). In addition, antioxidative activities observed in these plants could be the synergistic effect of more than two compounds that may present in the plant. It has been reported that most natural antioxidative compounds often work synergistically with each other to produce a broad spectrum of antioxidative activities that creates an effective defence system against free radical attack (Lu & Foo, 1995). Further studies on the identification and purification of components responsible for the antioxidative activities in different parts of Mengkudu are now in progress.

4. Conclusion

The study has shown that parts of *M. citrifolia* (leaf, fruit and root) have different antioxidative activities. Both polar (methanol extract) and non-polar (ethyl acetate extract) components exhibited appreciable antioxidative activities. It is interesting to note that both polar and non-polar extracts of the root exhibited higher antioxidative activity than either leaf or fruit. On the other hand, ethyl acetate extracts of all parts had high antioxidative activities, comparable to both α-tocopherol and BHT. Active compounds in root of Mengkudu might be both polar and non-polar in nature. However, compounds that contribute to antioxidative activity of both leaf and fruit of Mengkudu are probably non-polar in nature.

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