ELSEVIER

Food Chemistry 81 (2003) 575–581

Food Chemistry

www.elsevier.com/locate/foodchem

Antioxidative activity and total phenolic compounds of leaf, root and petiole of four accessions of *Centella asiatica* (L.) Urban

M.K. Zainol[a], A. Abd-Hamid[a,*], S. Yusof[b], R. Muse[c]

[a]*Department of Food Science, Universiti Putra Malaysia, UPM 43400, Serdang, Selangor, Malaysia*
[b]*Department of Food Technology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, UPM 43400, Serdang, Selangor, Malaysia*
[c]*Department of Biochemistry and Microbiology, Faculty of Science and Environmental Studies, Universiti Putra Malaysia, UPM 43400, Serdang, Selangor, Malaysia*

Received 8 April 2002; received in revised form 28 October 2002; accepted 28 October 2002

Abstract

Antioxidative activity and total phenolic compounds of root, leaf and petiole of four accessions of *Centella asiatica* (L.) Urban, namely CA 01, CA 05, CA 08 and CA 11, were evaluated. Antioxidative activity of the extracts was measured using the ferric thiocyanate (FTC) method and thiobarbituric acid (TBA) test. The antioxidative activities were then compared with that of α-tocopherol (natural antioxidant) and butylated hydroxytoulene or BHT (synthetic antioxidant). The results showed that CA 01 and CA 05 had the highest antioxidative activities among the accessions tested. Results also showed that both leaf and root of *C. asiatica* had high antioxidative activity, which was as good as that of α-tocopherol. The total phenolic content, determined according to the Folin–Ciocalteu method, varied from 3.23 to 11.7 g/100 g dry sample, and showed strong association ($r^2 = 0.90$) with antioxidative activity. The results suggest that phenolic compounds are the major contributors to the antioxidative activities of *C. asiatica*.
© 2003 Elsevier Science Ltd. All rights reserved.

Keywords: *Centella asiatica*; Antioxidative activity; Ferric thiocyanate; Total phenolic content

1. Introduction

Centella asiatica or 'pegaga' is one of the local herbs that is claimed to possess various physiological effects. Reports from different places have revealed that *C. asiatica* has been used for wound healing, memory improvement, treating mental fatigue (Goh, Chuah, & Saepadmo, 1995), bronchitis, asthma, dysentery, leucorrhoea, kidney trouble, urethritis (Jaganath & Ng, 1999), antiallergic and anticancer purposes, curing leukorrhea and toxic fever (Kan, 1986). It is also commonly used as a porridge for feeding pre-school children in Sri Lanka in combating nutritional deficiencies (Cox, Rajasuriya, Soysa, Gladwin, & Ashworth, 1993). Even though this precious herb is surrounded with various claims, the underlying mechanisms involved in its physiological effects are lacking. More scientific data are required before recommendation for increase in its consumption/utilization can be given with confidence.

The oxidative deterioration of lipid-containing food is responsible for the rancid odours and flavours during processing and storage, consequently decreasing the nutritional quality and safety of foods, due to the formation of secondary, potentially toxic compounds. The addition of antioxidant is a method for increasing the shelf life of foods. 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 potent scavengers of free radicals and, as such, are potentially useful in the prevention of arteriosclerosis, cancer, diabetes, neurodegenerative diseases, arthritis and others. Protective effects of diets high in fruits and vegetables have been attributed to the presence of these compounds.

Synthetic antioxidants have restricted use in food as various studies have shown them to be carcinogenic

* Corresponding author. Tel.: +60-3-89486101x8374; fax: +60-3-89423552.

0308-8146/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0308-8146(02)00498-3

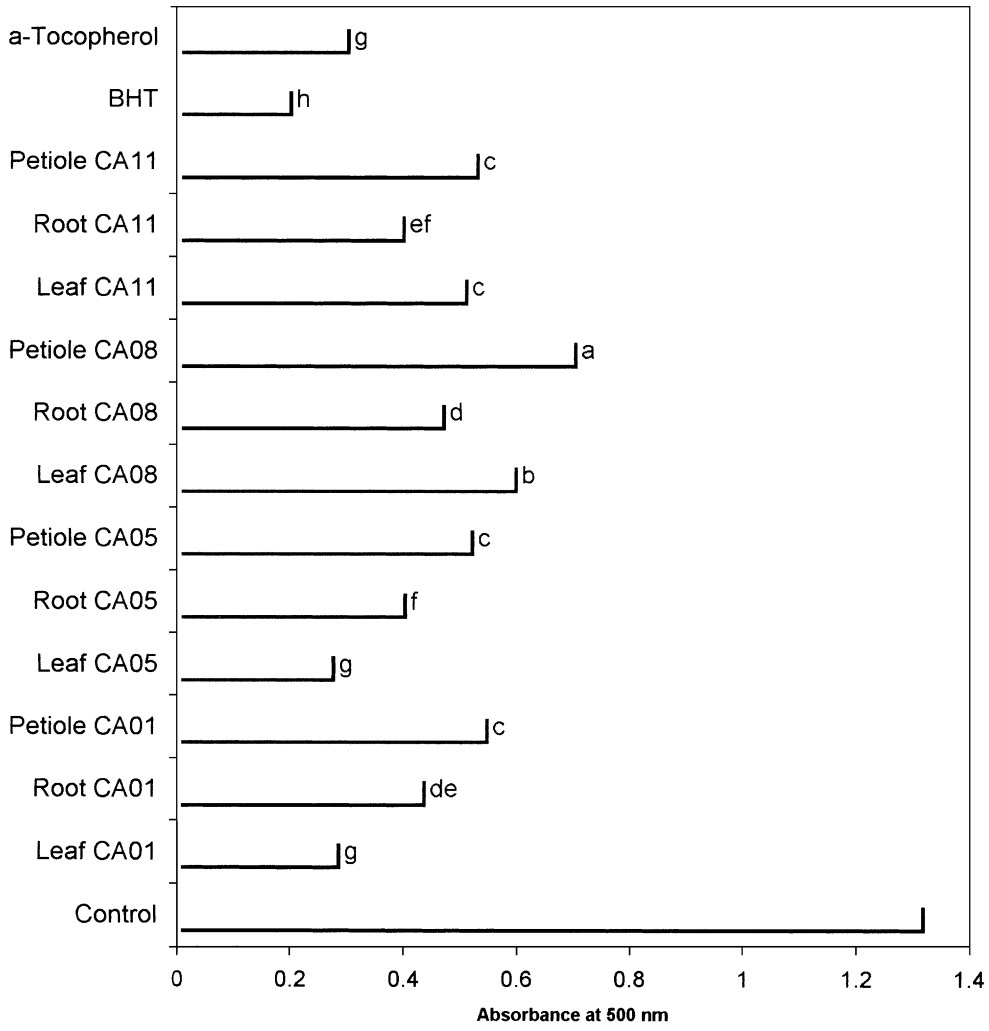

Fig. 1. Antioxidative activities of leaves, roots and petioles of different accessions of *C. asiatica* (L.) Urban, as measured by the FTC method. Absorbance values represent triplicates of different samples analysed. Values with the same letter (a, b, c) are not significantly different ($P < 0.05$) between samples.

(Madavi & Salunkhe, 1995). This is especially true for BHA and BHT. Thus, with increasing consciousness of consumers with regard to safety of food additives and the higher manufacturing cost and lower efficiency of natural antioxidants, a need for identifiying alternative natural and safer sources of food antioxidant is created (Wanasundara & Shahidi, 1998). Therefore, search for natural antioxidants, especially of plant origin, has notably increased in recent years (Löliger, 1991). Antioxidative compounds obtained from natural sources, such as grains, oilseeds, beans, leaf waxes, bark, roots, spices, fruits and vegetables, have been investigated (Chen, Muramoto, Yamauchi, & Nokihara, 1996). According to Pratt and Hudson (1992) most of these active compounds can be found in wood, bark, stem, leaf, fruit, root, flower and seed of many plants.

Thus, the objective of this study was to evaluate the antioxidative activity of four accessions of *C. asiatica* and to determine total phenolic compounds in different parts of different accessions of the plant.

2. Materials and methods

2.1. Materials

Centella asiatica (L.) Urban or 'pegaga' used in this study was obtained from the Malaysian Agriculture Research and Development Institute (MARDI), Serdang, Selangor. Four different accessions (obtained from different parts of Malaysia) were used, namely, CA 01, CA 05, CA 08 and CA 11. The whole samples were washed with running tap water and separated into 3 different parts, namely leaf, root and petiole, ready for extraction.

2.2. Extraction of Centella asiatica (L.) Urban

Different parts of *C. asiatica* were extracted according to the modified method of Chang, Ostric-Matijasevic, Hseih, and Huang (1977). Ten grammes of each part were frozen at −20 °C and freeze-dried at −42 °C for 3–4 days (133×10^{-3} psi). The dried sample was then

extracted with absolute methanol for 24 h in a shaking incubator at 37 °C±1 °C.

2.3. Determination of antioxidative activity

2.3.1. Ferric thiocyanate (FTC) method

The FTC method was adapted from the method of Osawa and Namiki (1981). Four milligrammes of samples dissolved in 4 ml of 99.5% (w/v) ethanol were mixed with linoleic acid (2.51% v/v) in 99.5% (w/v) ethanol (4.1 ml), 0.05 M phosphate buffer pH 7.0 (8 ml) and distilled water (3.9 ml) and kept in screw-cap container in the dark at 40 °C. To 0.1 ml of this solution was then added 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% (w/v) ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid to the reaction mixture, the absorbance of the resulting red colour was measured at 500 nm every 24 h until the day after the absorbance of the control reached maximum value.

2.3.2. Thiobarbituric acid (TBA) test

The TBA test was conducted according to the combined method of Kikuzaki and Nakatani (1993) and Ottolenghi (1959). One millilitre of sample from the FTC method was added to trichioroacetic acid (2 ml) and thiobarbituric acid solution (2 ml). This mixture was then placed in a boiling water bath at 100 °C for 10 min. After cooling, it was centrifuged at 3000 rpm for 20 min and absorbance of the supernatant was then measured at 532 nm.

2.3.3. Determination of total phenolic compounds

Total phenolic compounds were determined using a modified version of the Folin–Ciocalteu method (Ragazzi & Veronese, 1973). One millilitre of the extract was added to 10 ml deionized water and 2.0 ml of Folin–Ciocalteu phenol reagent (Merck-Schuchardt, Hohenbrun, Germany). The mixture was then allowed to stand for 5 min and 2.0 ml sodium carbonate were added to the mixture. The resulting blue complex was then measured at 680 nm.

2.4. Statistical analysis

Experimental data was analyzed using analysis of variance (ANOVA) and significant differences among means from triplicate analyses at ($P<0.05$) were determined by Duncan's multiple range test (DMRT) using the Statistical Analysis System (SAS, 1990).

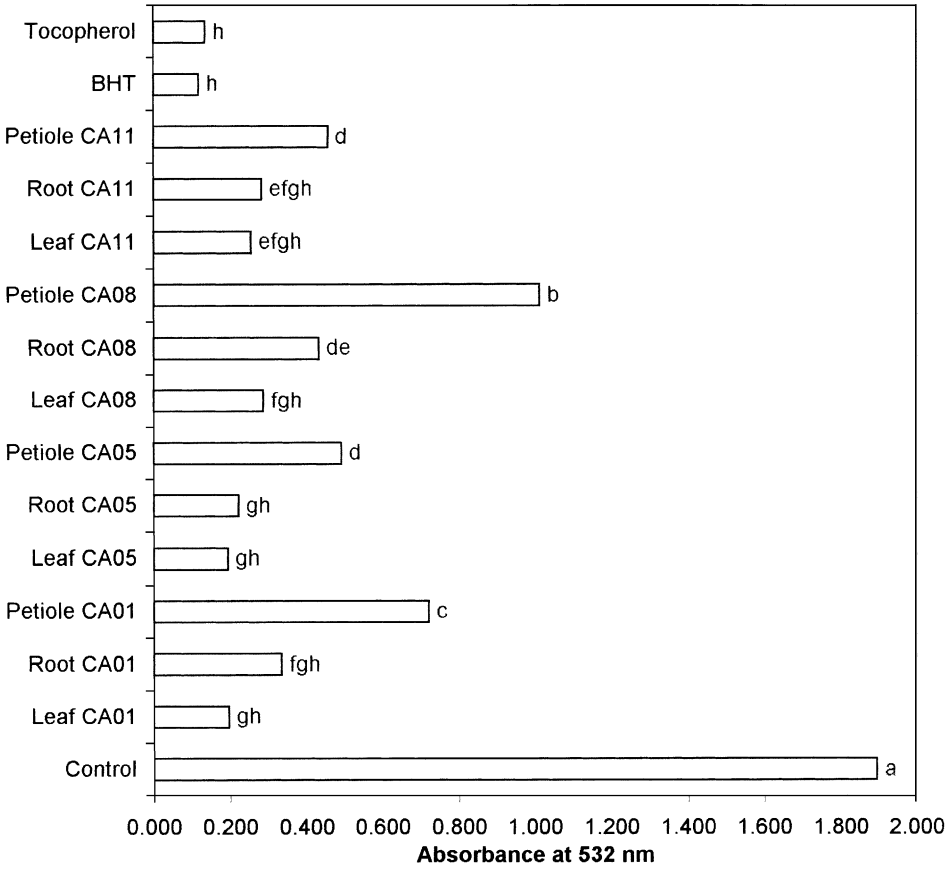

Fig. 2. Antioxidative activity of leaves, roots and petioles of different accessions of *C. asiatica*, as measured by the TBA method. Absorbance values represent triplicates of different samples analysed. Values with the same letter (a, b, c) are not significantly different ($P<0.05$) between samples.

3. Result and discussion

3.1. General

The antioxidative activity of plant parts is mainly contributed by the active compounds present in them. In this study, the antioxidative activity of *C. asiatica* was measured using the FTC and TBA methods. In the FTC method, the peroxides formed during the initial stages of lipid oxidation were measured. As oxidation proceeds, peroxides are gradually decomposed into lower molecular compounds and measured with TBA reagent.

Fig. 1 illustrates the antioxidative activities of different parts (root, leaf and petiole) of four accessions of *C. asiatica* (CA 01, CA 05, CA 08 and CA 11). All accessions tested showed high activities, with CA 05 and CA 01 having the highest, followed by CA 11 and CA 08, respectively. Leaves showed the highest antioxidative activities compared with other plant parts tested. This is especially true for leaves of CA 05 and CA 01, which had exceptionally high antioxidative activities that were not significantly ($P<0.05$) different from that of α-tocopherol. The finding is in accordance with the report of Yen and Hsieh (1998), who found that the antioxidative activity of leaf extract of Du-Zhong (*Eucommia ulmoides*) was higher than the raw and roasted cortex of the plant. On the other hand, Abdul-Hamid, Md. Shah, Muse, and Mohamed (2002) reported that ethanol extract of root of *C. asiatica* exhibited the highest activity though it was not significantly different from the leaves. Similarly, Mohd Zin, Abdul-Hamid, and Osman (2002) reported that ethyl acetate extracts of both root and leaf of *Morinda citrifolia* had high antioxidative activities, which were not significantly different from that of α-tocopherol. The antioxidative activity of different parts of *C. asiatica* may be due to the reduction of hydroperoxides, inactivation of free radicals, chelation of metal ions or combinations thereof.

Fig. 2 shows the antioxidative activities of leaf, root and petiole of different *C. asiatica* accessions, measured using TBA. Results show a somewhat different pattern from that of the FTC method, where root and leaf extracts of all *C. asiatica* accessions showed high antioxidative activities, which were not significantly ($P<0.05$) different from those of both BHT and α-tocopherol, except for the root of CA 08. The differences in antioxidative activities observed here could be ascribed to several factors, including the different mechanisms involved in the two determination methods, structures of the different phenolic compounds, the antioxidative mechanisms exhibited by the compounds and possibly, also, due to the synergistic effects of different compounds.

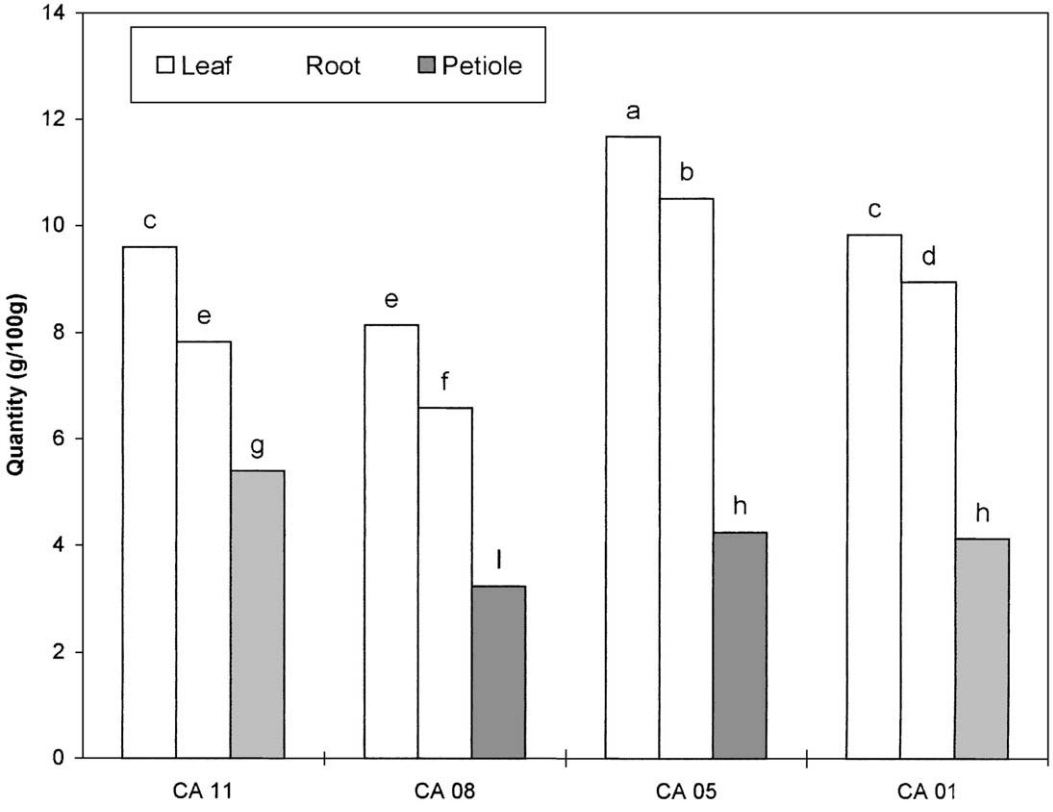

Fig. 3. Total phenolic compounds (as gallic acids equivalents) of leaves, roots and petioles of different accessions of *C. asiatica* (L.) Urban. Absorbance values represent triplicates of different samples analysed. Values with the same letter (a, b, c) are not significantly different ($P<0.05$) between samples.

Antioxidant assay used in this study measures the oxidation products at the early and final stages of oxidation. The antioxidants present in the sample may have different functional properties, such as reactive oxygen species scavenging (quercetin and catechins) (Hatano et al., 1989), inhibition of the generation of free radicals and chain-breaking activity, e.g. *p*-coumaric acids (Laranjinha, Viera, Madeira, & Almeida, 1995) and metal chelation (Van Acker, van Balen, van der Berg, Bast, & van der Vijgh, 1998). These compounds are normally phenolic compounds, which are effective hydrogen donors, such as tocopherols, flavonoids, and derivatives of cinnamic acid, phosphatidic and other organic acids, that are reported to be multifunctional. It was reported that much of the antioxidative potential of tea is ascribed to its phenolic compounds, particularly of catechin derivatives (Gardner, McPhail, & Duthie, 1997). Meyer, Heinonen, and Frankel (1998) reported that catechin was the most potent antioxidant according to the LDL oxidation assay, while Rice-Evans, Miller, and Paganga (1996) reported that quercetin showed the highest antioxidative activity, based on the radical scavenging of $ABTS^+$ test. Thus, Ishige, Chen, Sagara, and Schubert (2001) reported that the mechanisms of protection from oxidative insults by flavonoids are highly specific for each compound. The other factor that determines the antioxidative activity potential of phenolic compound is the stability of the aroxy radical formed in the structure of the compound itself. However, mechanisms, as well as specific compounds, responsible for the observed oxidative properties of *C. asiatica* are still unclear.

3.2. Association between antioxidative activity and phenolic compounds

Fig. 3 shows the total phenolic compounds found in roots, leaves and petioles of four accessions of *C. asiatica*. It is interesting to note that leaf extract contained the highest amount of phenolic compounds in all accessions tested (8.13–11.7 g/100 g), followed by root (6.46–10.5 g/100 g) while the lowest concentration was in the petiole (3.23–4.91 g/100 g), with a similar order of antioxidative activity as determined previously. In general, the total concentration of phenolic compounds in *C. asiatica* reported in this study was higher than that reported by Velioglu, Mazza, Gao, and Oomah (1998) in fruits, grains and vegetables (0.213–10.6 g/100 g) and Quettier-Deleu et al. (2000) in buckwheat (0.313–0.333 g/100 g). The results were, however, lower than that of the total phenolic compounds in Du-Zhong (*Eucommia ulmoides*) (8.70–21.0 g/100 g), as reported by Yen and

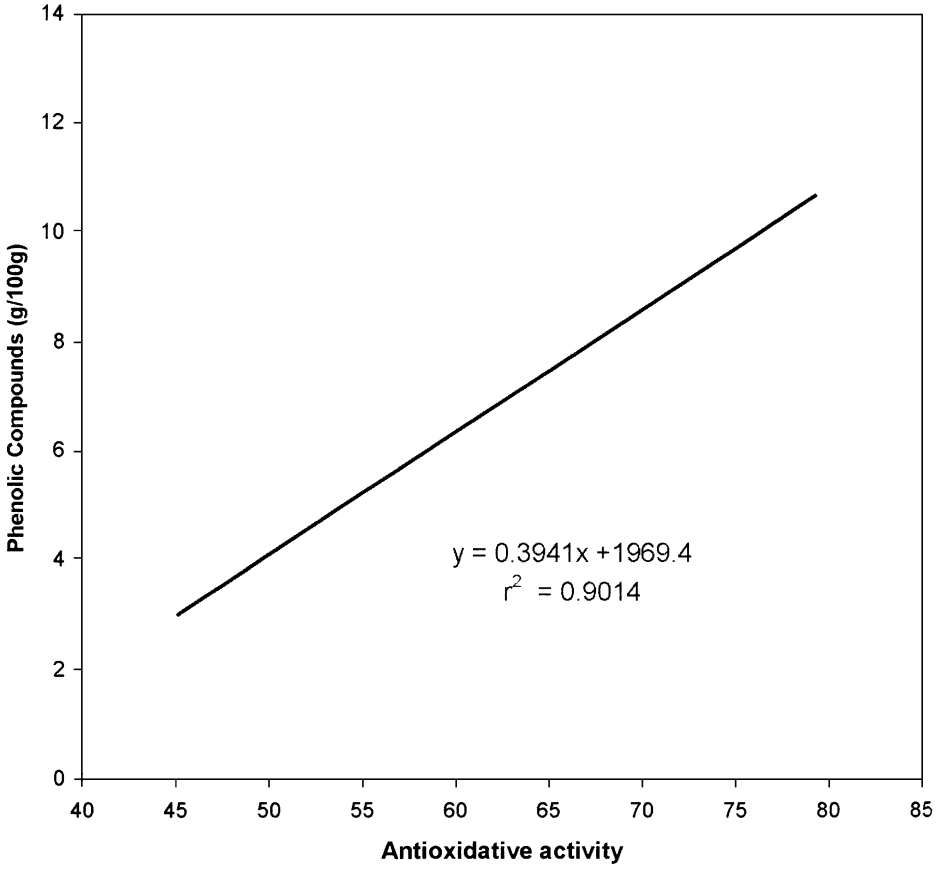

Fig. 4. Regression of antioxidative activity against total phenolic content in *C. asiatica*.

Hsieh (1998). Different levels reported in these studies may be attributed to the different plants and methods used by individual groups of investigator. Wang, Nair, Strasburg, and Booren (1999) and Hertog, Hollman, and Vennema (1992) reported that the antioxidative properties of some vegetables and fruits are partly due to the low molecular weight phenolic compounds, particularly the flavonoids, which are known to be potent antioxidants.

Fig. 4 shows the correlation between antioxidative activity and phenolic compounds in leaves, roots and petioles of different accessions of *C. asiatica*. The results indicate strong association between antioxidative activities and phenolic compound ($r^2 = 0.90$), suggesting that phenolic compounds are probably responsible for the antioxidative activities of *C. asiatica*. A similar finding was reported by Gardner, White, McPhail, and Duthie (2000), who suggested that phenolic compounds are the major contributors to the antioxidative activity of apple, pineapple and vegetable juices. Velioglu et al. (1998) also found that phenolic compounds were responsible for the antioxidative activity in some selected fruits, vegetables and grains tested. Phenolic compounds are also effective hydrogen donors, which makes them good antioxidants (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). Similarly, Shahidi and Naczk (1995) reported that naturally occurring phenolics exhibit antioxidative activity. Thus, therapeutic properties of *C. asiatica* extracts may possibly be attributed to the phenolic compounds present. Although they are found to be the major contributors to the antioxidative activity in *C. asiatica,* the identity of these phenolic compounds still remains unknown.

4. Conclusion

The results revealed that leaves, roots and petioles of different accessions of *C. asiatica* had various antioxidative activities, with CA 05 and CA 01 showing higher antioxidative activity than other accessions tested. The antioxidative activity of the different parts of *C. asiatica* was as good as that of α-tocopherol. Different parts of *C. asiatica* were found to contain high phenolic contents (3.23–11.7 g/100 g dry sample), which exhibit strong association ($r^2 = 0.90$) with antioxidative activities. The results suggest that phenolic compounds are the major contributors to the antioxidative activities of *C. asiatica*.

Acknowledgements

The authors would like to thank The Ministry of Science, Technology and Environment of Malaysia for financing the project and Universiti Putra Malaysia, Serdang, Selangor for the laboratory facilities.

References

Abdul-Hamid, A., Md. Shah, Z., Muse, R., & Mohamed, S. (2002). Characterization of antioxiclative activities of various extracts of *Centella asiatica* (L) Urban. *Food chemistry*, *77*, 465–469.

Chang, S. S., Ostric-Matijasevic, B., Hseih, O. A. L., & Huang, C. L. (1977). Natural antioxidants from rosemary and sage. *Journal of Food Science*, *42*, 1102–1104.

Chen, H. M., Muramoto, K., Yamauchi, F., & Nokihara, K. (1996). Antioxidant activity of design peptides based on antioxidative peptide isolated from digest of a soybean protein. *Journal of Agricultural and Food Chemistry*, *44*, 2619–2623.

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

Cox, D. N., Rajasuriya, S., Soysa, P. E., Gladwin, J., & Ashworth, A. (1993). Problems encountered in the community based production of leaf concentrate as a supplement for pre-school children in Sri Lanka. *International Journal of Food Science and Nutrition*, *44*, 123–132.

Gardner, P. T., McPhail, D. B., & Duthie, G. G. (1997). Electron spin resonance spectroscopic assessment of the antioxidant potential of teas in aqueous and organic media. *Journal of the Science of Food and Agriculture*, *76*, 257–262.

Gardner, P. T., White, T. A. C., McPhail, D. B., & Duthie, G. G. (2000). The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry*, *68*, 471–474.

Goh, S. H., Chuah Mok, J. S. L., & Soepadmo, E. (1995). *Malaysian medicinal plants for the treatment of cardiovascular diseases*. Petaling Jaya: Pelanduk Publication Sdn. Bhd.

Hatano, T., Edamatsu, R., Hiramatsu, M., Moti, A., Fujita, Y., Yasuhara, T., Yoshida, T., & Okuda, T. (1989). Effects of tannins and related polyphenols on superoxide anion radical, and on 1,1-diphenyl-2-picrylhydrazyl radical. *Chemical and Pharmaceutical Bulletin*, *37*, 2016–2021.

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

Ishige, K., Chen, Q., Sagara, Y., & Schubert, D. (2001). The activation of dopamine D4 receptors inhibits oxidative stress-induced nerve cell death. *Journal of Neuroscience*, *21*, 6069–6076.

Jaganath, I. B., & Ng, L. T. (1999). *Herbs: the green pharmacy of Malaysia*. Kuala Lumpur: Vinpress Sdn. Bhd.

Kan, W. S. (1986). *Pharmaceutical botany*, p. 416. Taiwan: National Research Institute of Chinese Medicine.

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

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 versus regeneration. *Archives of Biochemistry and Biophysics*, *323*(2), 373–381.

Loliger, J. (1991). The use of antioxidants in foods. In I. O. Aruoma, & B. Halliwell (Eds.), *Free radical and food additives* (p. 121). London: Taylor and Francis.

Madavi, D. L., & Salunkhe, D. K. (1995). Toxicological aspects of food antioxidant. In D. L. Madhavi, S. S. Deshpande, & D. K. Salunkhe (Eds.), *Food antioxidants* (p. 267). New York: Marcel Dekker.

Meyer, A. S., Heinonen, M., & Fankel, E. N. (1998). Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin and ellagic acid on human LD1 oxidation. *Food Chemistry*, *61*, 71–75.

Mohd Zin, Z., Abdul-Hamid, A., & Osman, A. (2002). Antioxidative activity of extracts from mengkudu (*Morinda citrifolia* L.) root, fruit and leaf. *Food Chemistry*, *78*, 227–231.

Osawa, T., & Namiki, M. (1981). A novel type of antioxidant isolated from leaf wax of *Eucalyptus* leaves. *Agricultural and Biological Chemistry*, *45*(3), 735–739.

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

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.

Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, M., Cazin, J. C., Baileul, F., & Trotin, F. (2000). Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of Ethnopharmacology*, *72*, 35–42.

Ragazzi, E., & Veronese, G. (1973). Quantitative analysis of phenolics compounds after thin-layer chromatographic separation. *Journal of Chromatography*, *77*, 369–375.

Rice-Evans, C. A., Miller, N. J., Bolwell, P. G., Bramley, P. M., & Pridham, J. B. (1995). The relative antioxidant activity of plant derived polyphenolic flavonoids. *Free Radical Resources*, *22*, 375–383.

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*(7), 933–956.

SAS Institute, Inc. (1990). *SAS/STAT user's guide*, Version 6 (4th ed). SAS Institute.

Shahidi, F., & Naczk, M. (1995). Method of analysis and quantification of phenolic compound. In *Food phenolics: sources, chemistry, effects and applications* (pp. 287–293). Lanchester, PA. USA: Technomic Publishing Company (pp. 287–293).

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.

Wanasundara, U. N., & Shahidi, F. (1998). Antioxidant and pro-oxidant activity of green tea extract in marine oils. *Food Chemistry*, *63*, 335–342.

Wang, H., Nair, M. G., Strasburg, G. M., Booren, A. M., & Gray, J. I. (1999). Antioxidant polyphenols from tart cherries (*Prunus cerasus*). *Journal of Agricultural and Food Chemistry*, *47*, 840–844.

van Acker, S. A. B. E., van Balen, G. P., van den Berg, D. J., Bast, A., & van der Vijgh, S. A. B. E. (1998). Influence of iron chelation on the antioxidant activity of flavonoids. *Biochemical Pharmacology*, *56*(8), 935–943.

Yen, G. C., & Hseih, C. L. (1998). Antioxidant activity of extracts from Du-Zhong (*Eucoma ulmoides*) toward various lipid peroxidation models in vitro. *Journal of Agricultural and Food Chemistry*, *46*, 3952–3957.