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Food Chemistry 77 (2002) 465–469

Food Chemistry

www.elsevier.com/locate/foodchem

Characterisation of antioxidative activities of various extracts of *Centella asiatica* (L) Urban

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Received 11 July 2000; received in revised form 21 November 2001; accepted 21 November 2001

Abstract

Antioxidative activity of various extracts from different parts of *Centella asiatica*, including leaves, petioles (stolons) and roots, using three types of solvents (ethanol, water and light petroleum), were evaluated using a linoleic acid model system and the thiobarbituric acid test. Results showed that the ethanol extract of all parts of *C. asiatica* exhibited significantly ($P<0.05$) higher antioxidative activity than the water extract, while the light petroleum ether showed negligible activity. Increasing the concentration of the extract (1000–3000 ppm) resulted in increase in antioxidative activity of both the ethanol and the water extract. From 3000 ppm upward, antioxidative activity of the ethanol extract was not significantly different ($P<0.05$) from that of α-tocopherol. Roots showed the highest activity of the parts tested. The antioxidative activities of the ethanol extracts were found to be stable up to 50 °C and exhibited optimum activity at neutral pH.

Keywords: Antioxidative activity; *Centella asiatica*; Phytochemicals; Natural antioxidants

1. Introduction

Centella asiatica (L.) Urban (Umbelliferae) is a slender, creeping plant, rooting at the nodes. It is native to countries like Sri Lanka, Madagascar, South Africa and Malaysia. It is used in the Ayurvedic system of medicine to treat various diseases. Fresh extracts of this plant have been used by the people of Java and the Malay Peninsula for many years, as both topical and internal agents, for healing of wounds (Kartnig, 1988). In India and Madagascar, this plant was used to treat leprosy (Sahu, Roy, & Mahato, 1989), while the Chinese prescribed the leaves in curing leukorrhea and toxic fever (Kan, 1986). In Malaysia, although this herb is commonly eaten fresh as a vegetable (salad), especially among the Malay communities, it is also said to have beneficial effects in improving memory and in treating mental fatigue, anxiety, and eczema (Goh, Chuah, Mok, & Soepadmo, 1995). However, with all the beneficial effects mentioned above, the underlying mechanism has not been effectively explained and may be antioxidative in nature. Despite the fact that we depend on biological oxidation as a source of energy for survival and activity, the action of oxygen is really two-sided. The powerful reactivity of reactive oxygen species (ROS), such as the superoxide radical (O_2), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\bullet}$), can cause functional damage to man, triggering mutagenesis, carcinogenesis, aging, and circulatory disturbances (Tagi, 1987). Although our bodies have their own systems to destroy ROS, their functions are limited, especially in conditions of severe oxidative stress. Plants produce various antioxidant compounds to counteract ROS in order to survive. Therefore, the objective of this study is to evaluate and characterise the antioxidative activities of *C. asiatica*.

2. Material and methods

2.1. Material

Fresh *C. asiatica* with commercial maturity (2–4 months), was obtained from the Traditional Medicine Plant Plot, Universiti Putra Malaysia, Serdang, Selangor,

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0308-8146/02/$ - see front matter
PII: S0308-8146(01)00384-3

Malaysia. The samples were washed with running tap water and separated into three parts (leaves, roots and petioles) for extraction.

2.2. Extraction of antioxidative compound

Extraction was carried out according to the modified method of Chang, Ostric-Matijasevic, Hseih, and Huang (1977). Ten grams of each freeze-dried part of *C. asiatica* were extracted with three different solvents (ethanol, water and light petroleum) in a shaking incubator at 25 ± 1 °C for 24 h. The extracts were then filtered and rotary-evaporated at 40 °C for 30 min. The flow chart for the preparation of the extract is as shown in Fig. 1.

2.3. Determination of antioxidative activity of the extracts

2.3.1. Conjugated diene method

The linoleic acid model method was carried out according to the modified method of Lingnert, Vallentin, and Eriksson (1979). Linoleic acid methyl ester (10 mM) was mixed with an equal amount of Tween 20 in buffer, pH 7, and homogenised at high power for about 1 min. Different portions of *C. asiatica* extracts (10 μl) were mixed with 5 ml of emulsion and incubated at 50 °C for 20 h. The absorbance at 234 nm was measured before and after incubation. Control without antioxidant was included in each determination. The activity of antioxidant (AOA) was calculated according to the following equation:

$$\mathrm{AOA} = \frac{\Delta \mathrm{A234(C)} - \Delta \mathrm{A234}}{\Delta \mathrm{A234\ nm(C)}}$$

where C = Control.

Fresh *Centella asiatica*
⇓
Separated into 3 parts; leaves, stems and roots
⇓
Frozen (Liquid nitrogen / dry ice)
⇓
Freeze drier
⇓
Pulverised
⇓
100 ml solvent (ethanol / water/ light pet.) added to 10 g sample
⇓
Shaking water bath (37°C) for 24 h
⇓
Filter by vacuum and concentrate in a rotary evaporator at 40°C
⇓
Crude antioxidant extract
⇓
Frozen for storage in liquid nitrogen
⇓
Diene Conjugation Formation method

Fig. 1. Extraction of different parts of *Centella asiatica*.

2.3.2. 2-Thiobarbituric acid (TBA) method

The thiobarbituric acid (TBA) values were determined according to the AOCS Official Method (1995). The flow chart is shown in Fig. 2.

2.4. Characterization study

The effects of pH and temperature on the antioxidative activities of the extracts were determined for characterization purposes. Phosphate–HCl buffer was used for pH 3 and 5, whereas phosphate–NaOH buffer was used for pH 7, 9 and 11. The range of temperature tested used was 30–90 °C.

2.5. Statistical analysis

All experiments were conducted in triplicate and statistical analysis was accomplished with SAS software, using Duncan's multiple comparison test. The level of significance was set at $P = 0.05$.

3. Results and discussion

3.1. Yield of extracts

The yields of different solvent extracts from 10 g freeze-dried samples are shown in Table 1. The results indicate that the yield of extract is greater with the more polar solvents. Apparently, ethanol is most effective in extracting all parts of *C. asiatica*. This observation is in agreement with that reported by some researchers, that solvents with high polarity are effective for extraction of natural antioxidants (Chang et al., 1977; Duh, Yen, & Yen, 1992; Economou, Oreopoulou, & Thomopoulos, 1991; Tian & White, 1994). Results also show that leaves give the highest yield.

Extracts/ blank/ control
⇓
Heat (30°C)/40 min.
⇓
Storage at 50°C for 5-7 days
⇓
200 mg (samples)
Dissolve with 25 mL1-butanol
⇓
Pipette 5.0 mL of samples
⇓
Add 5.0 mL of TBA reagent
⇓
Place into waterbath (90°C/120 min)
⇓
Cool in water for 10 min
⇓
Measure absorbance at 530 nm (spectrophotometer)

Fig. 2. Thiobarbituric acid method.

3.2. Evaluation of antioixidative activity of C. asiatica extracts

Fig. 3 shows that the ethanol extract had the highest activity. This is true for all parts (leaves, petioles, and roots). Nevertheless, the water extract exhibits appreciable antioxidative activity. On the other hand, the light petroleum extract showed negligible antioxidative activity. These data suggest that the antioxidative property of *C. asiatica* resides in water-soluble compounds. Similarly, Chang et al. (1977) reported that, in extracting antioxidants from rosemary and sage, methanol and ethanol are most appropriate. Our findings also agree with the previous work of De Rosenzweig, Pasquel, and Babbitt (1991), who reported that antioxidant activity of raw shrimp-meat extracts was increased in an ethanol extract while a chloroform extract had very little antioxidant activity and a diethyl ether extract showed no activity.

3.3. Antioxidative property of different parts of C. asiatica

Fig. 3 also shows that different parts of *C. asiatica* plants (leaves, petioles and roots) exhibit different antioxidative activities. Antioxidative activity of the roots was significantly ($P<0.05$) higher than that of either leaves or petioles. This differs from the work of Gordon and An (1995), who reported that powdered licorice root extracted with chloroform possessed high antioxidant activity compared to hexane or methanol extracts. This may be due to the different species being used, different compounds being extracted in the two cases. When the TBA test was employed, roots of *C. asiatica* again exhibited highest antioxidative activity compared to other part tested (Fig. 4).

3.4. Effect of increasing concentration of extract

Fig. 5 shows that increasing concentration of ethanol extracts up to 3000 ppm significantly ($P<0.05$) increases antioxidative activity of all parts of *C. asiatica*. This

Table 1
Yield of extracts from different parts of *Centella asiatica* using various solvents

Solvent	Yield[a] (g)		
	Leaves	Petioles	Roots
Ethanol	2.83±0.33aA	2.33±0.45aA	1.69±0.11bA
Light petroleum	1.13±0.18aB	1.04±0.14aB	0.97±0.16aB
Water	2.02±0.11aA	1.88±0.15abC	1.66±0.19bA

[a] Based on 10.0 g of freeze-dried leaves, petioles or roots. Values are means±standard deviation of three replicate analyses. Means within a column (A,B,C) and means within a row (a,b) marked with different letters are significantly different at $P<0.05$.

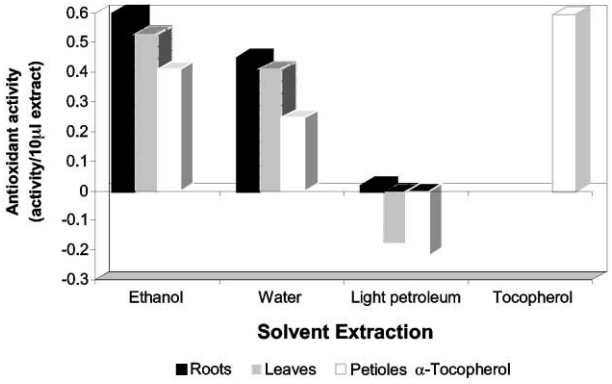

Fig. 3. Antioxidant activity of different extracts of *Centella asiatica* at 3000 ppm in buffer pH 7.

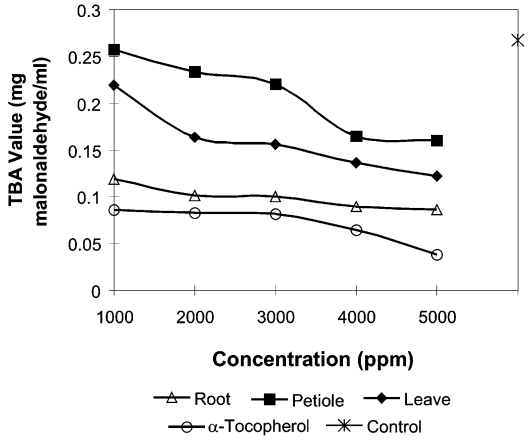

Fig. 4. Thiobarbituric acid (TBA) value of ethanol extracts on different portions of *Centalla asiatica* at different concentrations, and at 50 °C in buffer pH 7.

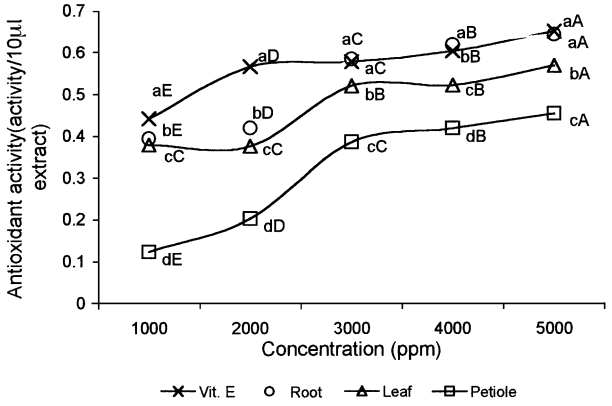

Fig. 5. Antioxidant activity of ethanol extracts of different parts of *Centella asiatica* at 50 °C in buffer pH 7.0. AOA values* represent of triplicates at different level of concentration. Values with same letter (a,b,c) are not significantly different ($P>0.05$) between samples. Values with same letter (A,B,C) are not significantly different ($P>0.05$) between concentrations.

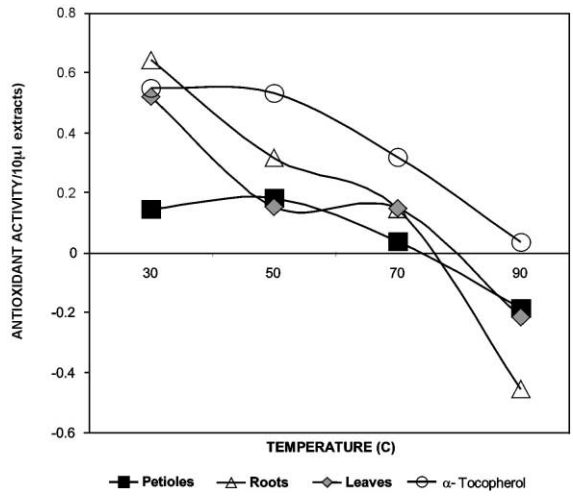

Fig. 6. Effect of temperature on antioxidant activity of ethanol extracts from different portions of *Centalla asiatica* at 3000 ppm and pH 7.

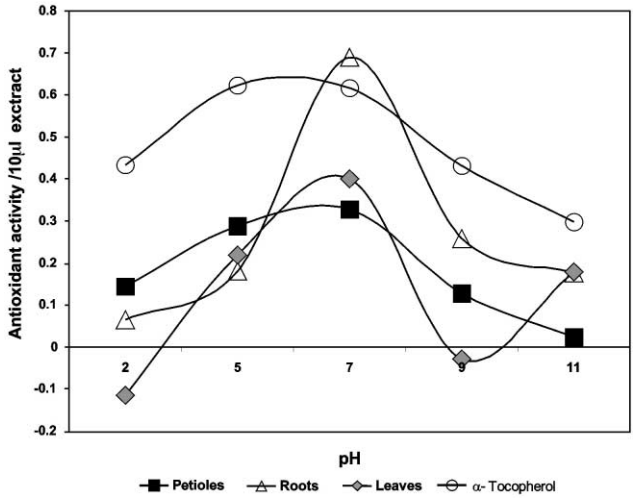

Fig. 7. Effect of pH on the antioxidant activity of ethanol extracts of *Centalla asiatica*, at 3000 ppm, and temperature 50 °C.

result is similar to that reported by Yen, Wing, and Duh (1996), who demonstrated that antioxidative activities of mulberry leaf extracts increased when concentration was increased from 200 to 800 ppm. This study also revealed that, from 3000 ppm upwards, antioxidative activity of ethanol extracts of roots of *C. asiatica* is not significantly different from that exhibited by α-tocopherol. It is not possible to conclude from this study whether antioxidative activity demonstrated by roots, petioles and leaves is due to the same or to different compounds.

3.5. Effect of temperature

The antioxidative activity of ethanol extract incubated at different temperatures is shown in Fig. 6. The study showed that the extracts were stable up to 50 °C, after which antioxidant activities started to decrease significantly for all the samples tested. This result was similar to that reported by Abdul Hamid, Nik Muhammad, and Thed (1999), who noted that the antioxidant activities of cocoa by-product extracts were stable up to 50 °C, the antioxidant activities dropping significantly at 70–90 °C. The antioxidative activity of roots seemed to be less stable than that demonstrated by petioles and leaves.

3.6. Effect of pH

Fig. 7 shows the influence of pH on the stability of the extracts. It is interesting to note that the antioxidative activities of all parts of *C. asiatica* extracts demonstrate the highest activity at pH 7. This finding could be significant, since the optimum pH is similar to physiological pH. A similar result was reported by Yen (1993), who noticed that a methanol extract of peanut hulls exhibited high antioxidative activities at neutral and acidic pH, but showed no effect at pH 9.

4. Conclusion

This study has shown that ethanol is the best solvent for extracting antioxidative compounds from different parts (roots, petioles and leaves) of *C. asiatica*. Roots of *C. asiatica* exhibited higher antioxidative activity than either leaves or petioles with all types of solvent used. Increasing the concentration of extracts to 5000 ppm increased the antioxidative activity of all parts of *C. asiatica*. Both the diene conjugation formation test and the thiobarbituric acid test (TBA) gave similar findings. The antioxidative activity of the ethanol extract, of both roots and leaves of *C. asiatica*, was found to be as good as that of α-tocopherol. Characterization studies revealed that antioxidative activities of ethanol extracts exhibit optimum activity at pH 7 and were stable up to 50 °C. Elucidation of the active compounds responsible for the antioxidative activities of *C. asiatica* is in progress.

Acknowledgements

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

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