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Characterization of the phytochemicals and antioxidant properties of extracts from Teaw (*Cratoxylum formosum* Dyer)

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Abstract

The leaves of the Thai vegetable, Teaw (*Cratoxylum formosum* Dyer) were extracted with ethanol to provide an extract that had antioxidant properties. The composition of the extract was studied by high-performance liquid chromatography with a diode array detector, and by electrospray ionization mass spectrometry. The main antioxidant component (peak 1) was chlorogenic acid, which was present at 60% of the extract. Three minor components were present at 7%, 3% and 2%, and other components that were present at lower concentrations were also observed. Treatment of the Teaw extract with 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) caused a similar reduction in peak area of 55.2–58.1% for chlorogenic acid and the three minor components, indicating that these components had common structural features. Component 2 was identified as dicaffeoylquinic acid, and compounds **3** and **4** were identified as ferulic acid derivatives. The radical-scavenging activity of the Teaw extract was compared with α-tocopherol, BHT and chlorogenic acid, using the DPPH$^{\bullet}$ and 2,2′-azinobis (3-ethylbenzothialozinesulfonic acid) radical cation (ABTS$^{\bullet+}$) assays. The Teaw extract scavenged both free radicals more strongly than did α-tocopherol and BHT, and the activity of the extract was consistent with the concentration of chlorogenic acid that was present, confirming that this component is a major contributor to the antioxidant activity. The acute toxicity of the Teaw leaf extract was investigated in mice, and it was found that the LD_{50} of the extract was >32 g/kg. Consequently, this plant is a promising source of a natural food antioxidant.

Keywords: ABTS; Antioxidant; Phytochemicals; Teaw; DPPH; Radical scavenging

1. Introduction

Plants, which are sources of phytochemicals with strong antioxidant activity, have attracted a great deal of attention in recent years. Antioxidants, which inhibit the oxidation of organic molecules, are very important, not only for food preservation, but also for the defence of living systems against oxidative stress (Masuda et al., 2003). Phenolic antioxidants interrupt the propagation of the free radical autoxidation chain by contributing a hydrogen atom from a phenolic hydroxyl group, with the formation of a relatively stable free radical that does not initiate or propagate further oxidation processes (Kaur & Kapoor, 2001).

Phenolic compounds account for most of the antioxidant activity of plant extracts. Plants vary in content and structure of phenolic components (number of phenolic rings, aromatic substitution, glycosylation, conjugation with other phenolic compounds or organic acids) and thus vary in their antioxidant properties. The identification, quantification and characterization of phytochemicals is important for applications of plant extracts as new food additives. This allows the development of efficient quality control measures to ensure the authenticity and standardization of product composition and quality. Identification of the phytochemicals which make a major contribution to the antioxidant activity of plant extracts is often difficult

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because of the large number of phenolic components that are present. An on-line HPLC method for detection of the components which are most active in scavenging the radical species, 2,2-diphenyl-1-picrylhydrazyl (DPPH), has been developed (Bandoniene & Murkovic, 2002; Masuda et al., 2003).

Diverse methods are currently used to assess the antioxidant activity of plant phenolic compounds. Chemical assays are based on the ability to scavenge synthetic free radicals, using a variety of radical-generating systems and methods for detection of the oxidation end-point. 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)- or DPPH radical-scavenging methods are common spectrophotometric procedures for determining the antioxidant capacities of plant components. These chromogens (the violet DPPH radical and the blue green ABTS radical cation) are easy to use, have a high sensitivity, and allow for rapid analysis of the antioxidant activity of a large number of samples. These assays have been applied to determine the antioxidant activity of food, wine and plant extracts and pure components (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003; van den Berg, Haenen, van den Berg, van den Vijgh, & Bast, 2000; Yu et al., 2002). Unfortunately, results from these methods often do not correlate with the ability of a compound to inhibit oxidative deterioration of foods. This is because the antioxidant activity in food and biological systems is dependent on a multitude of factors, including the colloidal properties of the substrates, the conditions and degree of oxidation and the localization of antioxidants in different phases. Hence, it is pertinent to use different assay systems to assess and compare the antioxidant effectiveness in plant extracts (Kaur & Kapoor, 2001).

Cratoxylum formosum Dyer is an indigenous Thai vegetable, mostly grown in the North-East of Thailand. *C. formosum* grows in groves and can withstand barren conditions. Fresh shoots and young leaves of this plant are traditionally consumed in Thailand. The plant tastes sour and a little astringent due to phenolic components. Health benefits of *C. formosum* include applying the leaf to the skin to heal a wound and consuming the flower to remedy a cough. It has been reported that the leaf extract showed strongly antioxidant and antimutagenic properties when compared with 108 species of indigenous Thai plants (Nakahara et al., 2002; Trakoontivakorn et al., 2001). There are no reported data on the composition and properties of phenolic compounds in extracts from this plant. Phenolic compounds of two plants which are in the same family have been reported. *C. pruniflorum* Kurz. leaf contains flavonols and xanthones, including quercetin, hyperoside, 1,3,6,7-tetrahydroxyxanthone, mangiferin and isomangiferin (Kitanov & Assenov, 1988). *C. neriifolium* Kurz. contains biflavanol and stigmasterol in the leaf (Kumar, Brecht, & Frahm, 2004). However, the antioxidant characterization of extracts from *C. formosum* has not been reported. The aim of the present study is to identify the potent radical-scavenging components in *C. formosum* extracts, to determine their contribution to the total antioxidant activity, and to compare the activity of extracts and components with standard antioxidants including, α-tocopherol and butylated hydroxytoluene (BHT), using the trolox equivalent antioxidant capacity (TEAC) value, determined by the ABTS and DPPH radical-scavenging assays.

2. Materials and methods

2.1. Materials

α-Tocopherol, formic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), chloroform, and butanol were purchased from Fluka Co. (Buchs, Switzerland). Ethanol, 2-propanol, potassium persulfate, and chlorogenic acid (HPLC grade) were purchased from Sigma (Milwaukee, USA). HPLC grade methanol and water were purchased from Fisher Scientific (Leicestershire, United Kingdom). Acetonitrile (HPLC grade) and BHT were purchased from BDH (Poole, United Kingdom). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt were purchased from Sigma–Aldrich Company Ltd. (Gillingham, UK).

One batch of Teaw leaves (*C. formosum* Dyer) was purchased from a cultivated place in Saraburi province during the harvest season in April 2004. Immediately, when the Teaw leaves were received after harvesting, they were cleaned, and sound leaves were selected to develop the sample preparation method for further extraction.

2.2. Preparation of plant extracts

The fresh plant leaves (80 g) were blended for 1 min with ethanol at −20 °C and the containers were then flushed with nitrogen and shaken for 4.5 h in the dark at 25 °C. The supernatant, after filtration through cheesecloth and Whatman No. 4 filter paper, was evaporated under vacuum. Samples were dried in a freeze dryer and stored in aluminium foil after flushing with nitrogen at −20 °C.

2.3. Toxicity of plant extracts

The acute toxicity of Teaw leaf extract was investigated in mice by the Medicinal plant research institute, Department of Medicinal Science, Ministry of Public Health, Thailand. The extract was dissolved in distilled water and the concentration was adjusted to 0.8 g/ml. Acute toxicity was investigated in 10 mice (five of each sex) by oral administration of two doses of 16 g/kg body weight in two equal amounts, 6 h apart. The extract solution was replaced by distilled water for the control group (10 mice). After consuming the first dose, the mice showed decreased respiration and spontaneous motor activities. Following the second dose, the animals suffered from dyspnea and 5 of the 10 mice showed signs of somnolence. These signs continued

to be observed for the test group until day 10 of the experiment, whereas the control group showed no abnormality. At the end of the 14-day-observation period, all animals survived and necropsy revealed no abnorminality of visceral organs. Hence LD_{50} of the extract is >32 g/kg.

2.4. Analysis of radical-scavenging components by HPLC-DAD

Dry plant extract (1 mg) was dissolved in methanol (3 ml) and passed through a Sep-Pak C18 cartridge (Waters, Milford, MA.). The C-18 cartridge was first conditioned by suction with 1 column volume of methanol, followed by 2 column volumes of a 3% HCl solution (v/v) in HPLC grade water. The cartridge was not allowed to dry out during conditioning. The aqueous sample extract was then transferred to the cartridge. The cartridge bed was then rinsed with HCl (3%, 5 ml) and air-dried under vacuum for ~10 min. Phenolic compounds were eluted with HPLC grade methanol (2 ml) and analysed by HPLC, both before and after reaction with DPPH.

The analytical solution (100 μl) of eluted phenolic compounds was mixed with freshly prepared 5 mM DPPH methanol solution (100 μl). The mixture was stirred well and allowed to stand at 25 °C for 5 min. Samples were filtered through a 0.20 μm Millipore filter (type HA) into a 2 ml autosampler vial for subsequent analysis by HPLC. The solution (10 μl) was injected into the HPLC and analyzed according to the following conditions: column, Synergi Hydro RP column (150 × 4.6 mm i.d., 4μm, Phenomenex), fitted with a Allsphere ODS-2 guard column (10 × 4.6 mm i.d., Alltech). Solvent A was 100% acetonitrile. Solvent B was 1% formic acid in water. The programme was isocratic at 10% A, 90% B for 10 min and then a linear gradient from 10% to 40% A for 39 min and finally it was isocratic at 10% A, 90% B for 10 min. The flow rate was 0.5 ml/min^{-1}. The HPLC system was equipped with a diode array detector (Dionex PDA 100 photodiode array, USA) controlled by Chromeleon software version 6.60 Build 1428 (Dionex Corporation, Sunnyvale, USA). Chromatograms were recorded at 260, 280 and 320 nm. The identification of peak 1 as chlorogenic acid was confirmed by co-injection of chlorogenic acid with the plant extract. The dominant phenolic peak quantification was expressed as chlorogenic acid equivalents.

2.5. Identification of extract components by HPLC-ESI-MS

The phenolic components with strong radical-scavenging ability were characterized by comparison of the HPLC retention time and UV spectrum with standards and also by HPLC-ESI-MS chromatographic separation. The HPLC system consisted of a Shimadzu HPLC, Model LC-10ADvp two pumps, DGU-14A degasser, SIL-10ADvp autosampler, CTO-10ASvp column heater, and a SPD-10Avp detector controlled by SCL-10Avp for LC. The mass detector was an LCMS-2010A trap, equipped with an electrospray ionization (ESI) system and controlled by LCMS solution software. Nitrogen was used as nebulizing gas at a pressure of 6 bars, and the flow was adjusted at 1.5 l/min. The heated capillary and voltage were maintained at 230 °C and 1.7 kV, respectively. The full scan mass spectra of phenolic compounds were measured *m/z* from 650 to 1000. Mass spectrometry data were acquired in the positive and negative ionization mode. The HPLC condition was the same as described above.

2.6. Assessment of free radical-scavenging activity

The total free radical-scavenging capacity of the plant extract was determined and compared to that of α-tocopherol, BHT and chlorogenic acid by using the DPPH and ABTS methods.

2.6.1. DPPH radical-scavenging activity

The stable DPPH radical-scavenging activity was measured using the method of Masuda et al. (1999). DPPH radical in methanol (5 mM) was prepared and this solution (100 μl) was added to extract or antioxidant sample solutions in methanol (4.9 ml) at different concentrations. After 30 min, absorbance was measured at 517 nm. The percentage of DPPH radical-scavenging activity of each plant extract was calculated as

$$\text{DPPH radical-scavenging activity}(\%) = [A_o - (A_1 - A_s)]/A_o \times 100, \quad (1)$$

where A_o is the absorbance of the control solution (containing only DPPH); A_1 is the absorbance of the DPPH solution containing plant extract, and A_s is the absorbance of the sample extract solution without DPPH.

The DPPH radical-scavenging activity (%) was plotted against the plant extract concentration (μg/ml) to determine the concentration of extract necessary to decrease DPPH radical-scavenging by 50% (called EC_{50}). The EC_{50} value of each extract was estimated by sigmoid non-linear regression, using SigmaPlot 2000 Demo (SPSS Inc., Chicago, IL, USA). These values were changed to antiradical activity (A_{AR}), defined as $1/EC_{50}$, since this parameter increases with antioxidant activity. All determinations were performed in triplicate.

2.6.2. ABTS radical-scavenging activity

The ABTS radical-scavenging activity was determined according to Re et al. (1999). This method is based on the ability of antioxidants to quench the long-lived ABTS radical cation, a blue/green chromophore with characteristic absorption at 734 nm, in comparison to that of trolox, a water-soluble vitamin E analogue. The ABTS radical cation was prepared by reacting an aqueous solution of ABTS (7 mM) with potassium persulfate (2.45 mM, final concentration), which was kept in the dark at 25 °C for 12–16 h. The solution was diluted in ethanol to an absorbance of 0.70(±0.020) at 734 nm before use. Aliquots of trolox or

sample in water (20 μl) were added into 2.0 ml of this diluted solution, and the absorbance at 734 nm was determined at 30 °C, exactly 6 min after initial mixing. Appropriate solvent blanks were run in each assay. The antioxidant solution reduces the radical cation to ABTS, which reduces the color. The extent of decolorization is calculated as percentage reduction of absorbance, and this is determined as a function of concentration and calculated relative to the equivalent trolox concentration. The activity of each antioxidant was determined at three concentrations, within the range of the dose–response curve of trolox, and the radical-scavenging activity was expressed as the trolox equivalent antioxidant capacity (TEAC), defined as mMol of trolox per gram of sample.

2.7. Statistical analysis

Each experiment, from sample preparation to analysis, was repeated in triplicate, and the data were then analyzed by SPSS software programme (SPSS Inc., Chicago, IL, USA). The general linear model procedure was applied and Duncan's multiple range test was used to compare the mean values at $P < 0.05$. Mean values and pooled standard errors of the mean (SEM) were calculated.

3. Results and discussion

3.1. Analysis of radical-scavenging components by HPLC

The HPLC chromatogram of the Teaw extract is shown in Fig. 1. Four main peaks were detected and the areas of these peaks were greater at 320 nm than at 260 or 280 nm. The component with the greatest peak area eluted at 11.84 min (peak 1) in the more hydrophilic region (short retention time). The other three main components eluted at 30.98 min (peak 2), 34.07 min (peak 3) and 37.66 min (peak 4) in the more hydrophobic region (longer retention time).

The identity and purity of the main peak (peak 1) in the HPLC chromatogram of the extract of *C. formosum* Dyer were determined by comparison of the retention time and UV spectrum (Fig. 2) with that of pure chlorogenic acid. The identification of the main peak as chlorogenic acid was confirmed by co-injection of chlorogenic acid with the plant extract. HPLC-ESI-MS confirmed the identification of this compound. Negative-ion MS gave a high mass peak with *m/z* 353 $[M - H]^-$ (parent peak). Positive-ion MS showed a base peak of *m/z* 377, due to the sodium adduct ion $[M + Na]^+$. A potassium adduct ion was

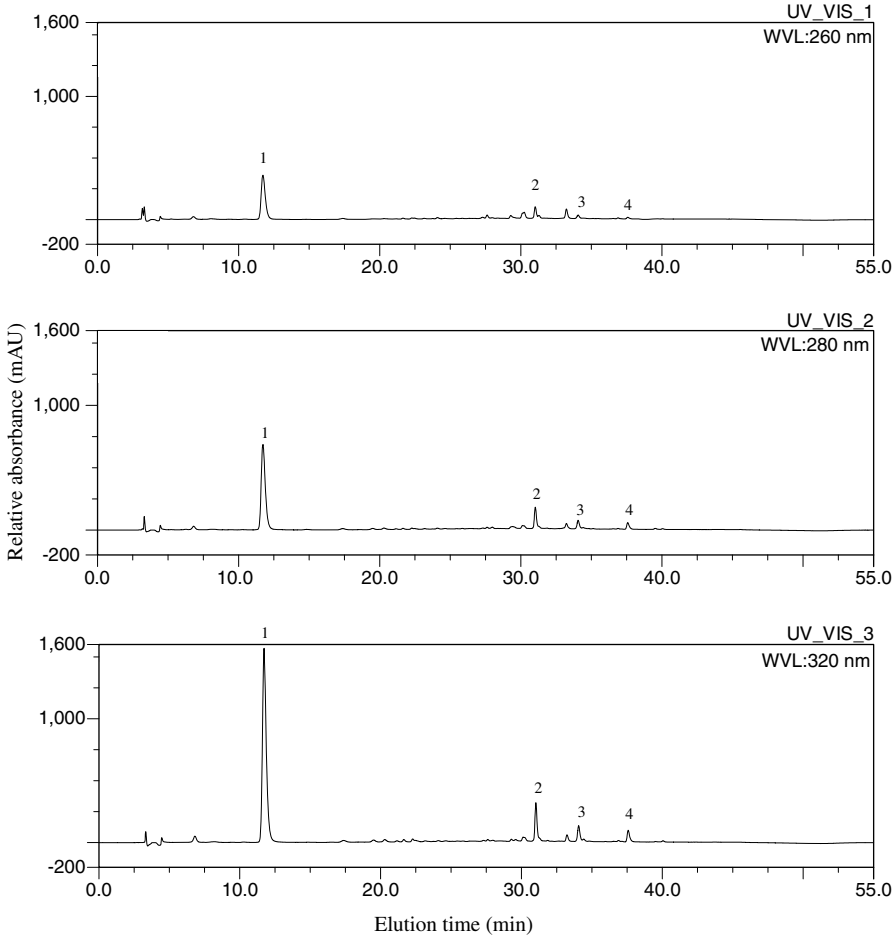

Fig. 1. HPLC chromatogram for the extract from *C. formosum* Dyer detected at 260, 280 and 320 nm.

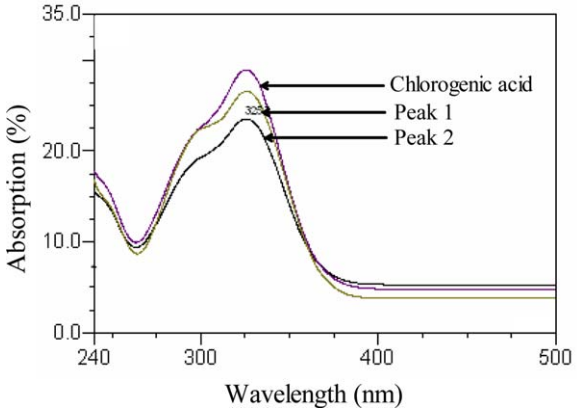

Fig. 2. UV spectrum of peaks 1 and 2 of *C. formosum* Dyer and chlorogenic acid.

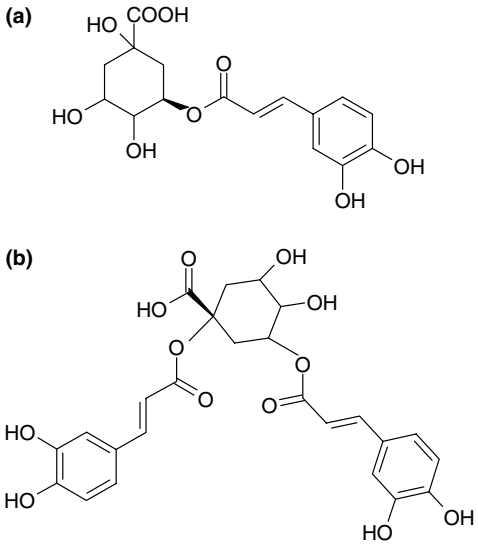

Fig. 4. Structure of: (a) chlorogenic acid and (b) dicaffeoylquinic acid (shown as 1,3-diCQA).

present at *m/z* 393 $[M + K]^+$ (Fig. 3), confirming the molecular weight of 354 for chlorogenic acid (Fig. 4). The compound from peak 2 had a similar UV spectrum to that of peak 1, characteristic of molecules with the caffeic acid moiety, such as chlorogenic acid. Compound 2 had a negative-ion mass spectrum with a peak at *m/z* 515.1 (M + H) indicating a molecular weight of 514.1(Fig. 5a). This is consistent with the compound being dicaffeoylquinic acid (diCQA). The mass spectrum of 1,3 dicaffeoylquinic acid (1,3-diCQA) was reported as showing only the peak *m/z* 515.1 at high mass number in the negative-ion mode. Parejo et al. (2004) reported that chlorogenic acid showed a shorter retention time (8.22 min) than did 1,3-diCQA (21.09 min) under similar HPLC conditions to those used in the study. In this study, the retention time of chlorogenic acid was 12 min and that of peak 2 was 31 min, and these values are consistent with the provisional identification of peak 2 as due to dicaffeoylquinic acid (Fig. 4b). The negative ion mass spectra of 1,3-, 1,4-, and 1,5-diCQA isomers are similar, so the positions of the caffeic acid substituents cannot be identified from these data (Clifford, Knight, & Kuhnert, 2005). The wavelengths for maximum absor-

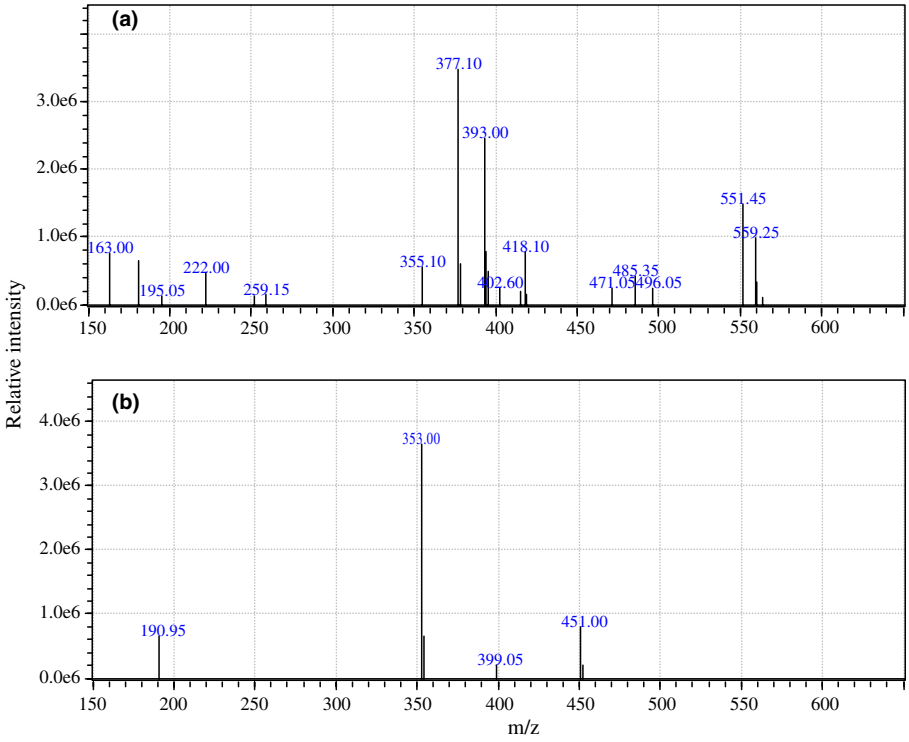

Fig. 3. Positive ion (a) and negative ion (b) LC-MS spectra of peak 1 of *C. formosum* Dyer.

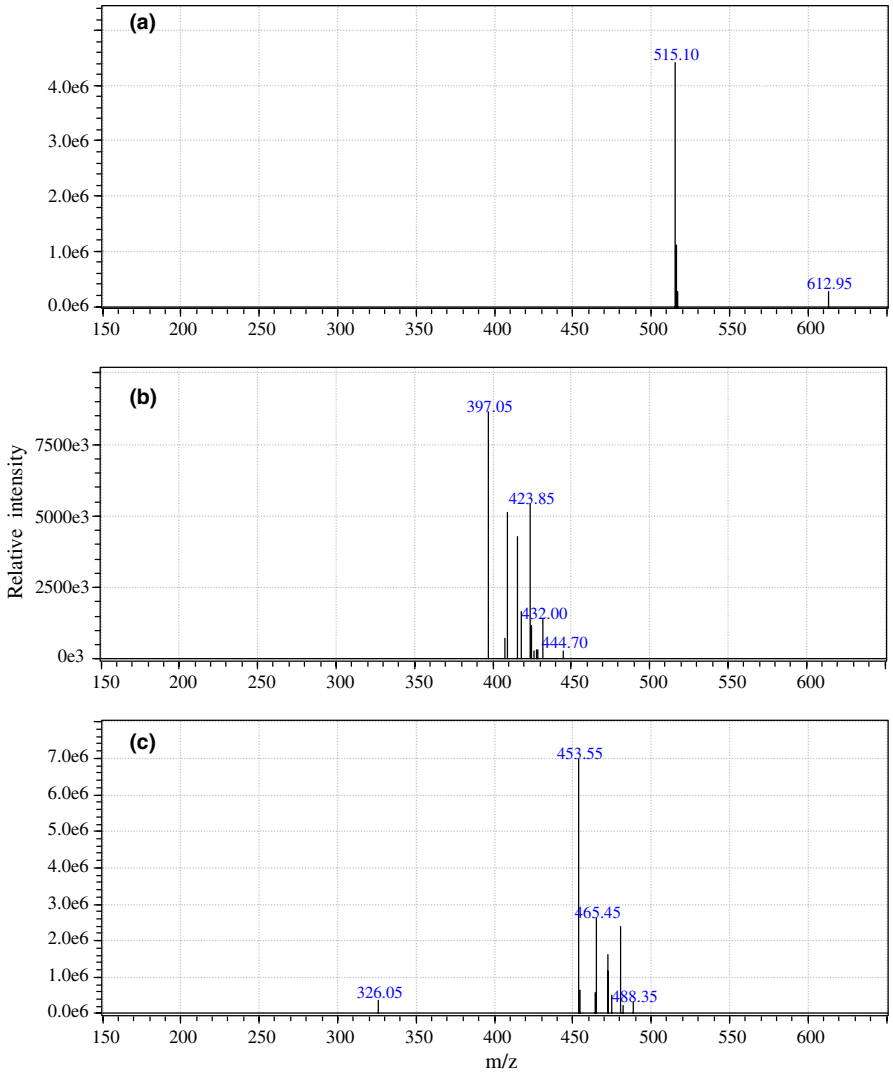

Fig. 5. Negative ion LC-MS of (a) peak 2 and positive ion LC-MS of (b) peak 3 and (c) peak 4 from *C. formosum* Dyer.

bance (λ_{max}) in the spectrum of peak 2 at 325 nm with a shoulder at 296–298 nm are consistent with this identification.

Compounds **3** and **4** had similar UV spectra, characteristic of ferulic acid structures (λ_{max} at 312–315 nm with a shoulder at 298 nm) (Fig. 6). Only positive-ion mass spectra of compounds **3** and **4** were determined. A match for the mass spectrum of compound **3** could not be found from the literature. The mass spectrum of compound **4** is consistent with a molecular weight of 452 since it has the $[M + H]^+$ ion at *m/z* 453.55. This compound could be identical with the ferulic acid hexose derivative with a molecular weight of 452 reported in the literature as a component of berries of the *Ribes* species (Määttä, Kamal-Eldin, & Törrönen, 2003).

Conventional separation of all constituents of plant extracts to identify the radical-scavenging components is very time-consuming and may cause decomposition of phenolic components during repeated fractionation. Addition of DPPH solution to the Teaw extract was used to detect which components reacted readily with the DPPH radical. The reduction in area of peaks 1, 2, 3 and 4 when reacted with DPPH were 58.1%, 55.2%, 56.7% and 58.0%, respectively,

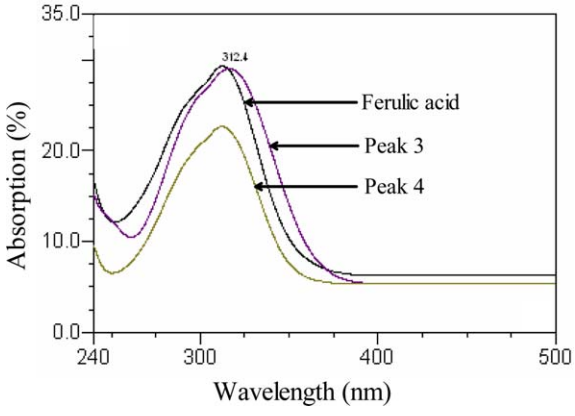

Fig. 6. UV spectrum of peaks 3 and 4 of *C. formosum* Dyer and ferulic acid.

with detection at 320 nm, and detection at 260 and 280 nm showed similar changes (Fig. 7). The similarity in the wavelength of maximum absorbance of peaks 1–4, and the similar change in peak area due to a 5 min reaction with DPPH, indicates that chlorogenic acid and components eluting as peaks 2–4 share common structural features.

Chlorogenic acid is a caffeic acid derivative that is reported to possess antibacterial, antimutagenic, antitumor and antiviral properties, as well as acting as an antioxidant by radical-scavenging and metal chelation (Armesto, Ferrero, Fernandez, & Gotor, 2003; Tudela, Cantos, Espin, Tomas-Barberan, & Gil, 2002).

Treatment of pure chlorogenic acid with DPPH was compared with treatment of the Teaw leaf extract. The results showed that 1 ml of 5 mM DPPH solution reacted with 0.598 mg of pure chlorogenic acid and with 0.593 mg chlorogenic acid in the Teaw extract. These values are identical within experimental error and they confirm that chlorogenic acid is the main antioxidant in the Teaw extract.

Chlorogenic acid (3-caffeoyl-D-quinic acid) is an ester of caffeic acid and quinic acid, and has an *o*-diphenolic structure. In general, *o*-diphenolic compounds are oxidized to *o*-quinones by free radicals, such as DPPH radical (Dufour, Da Silva, Potier, Queneau, & Dangles, 2002). A new HPLC peak (peak X) appeared with a retention time of 3.05 min after reaction of the Teaw extract with DPPH for 5 min (Fig. 7). This oxidation product absorbed more strongly at 260 nm than at 280 or 320 nm (Fig. 7). The UV spectrum of the oxidation product showed an absorbance maximum at 255.6 nm (Fig. 8). A previous study has shown that oxi-

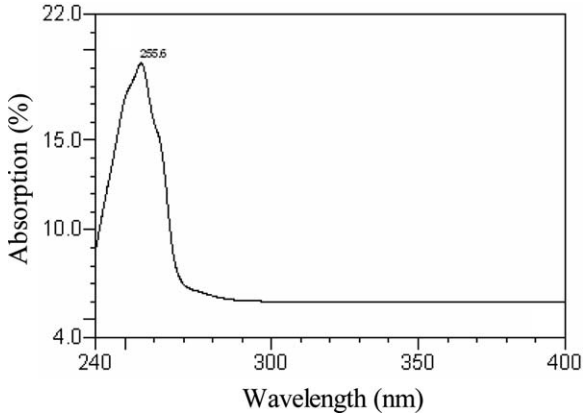

Fig. 8. UV spectrum of oxidation product of *C. formosum* Dyer extract after reaction with DPPH.

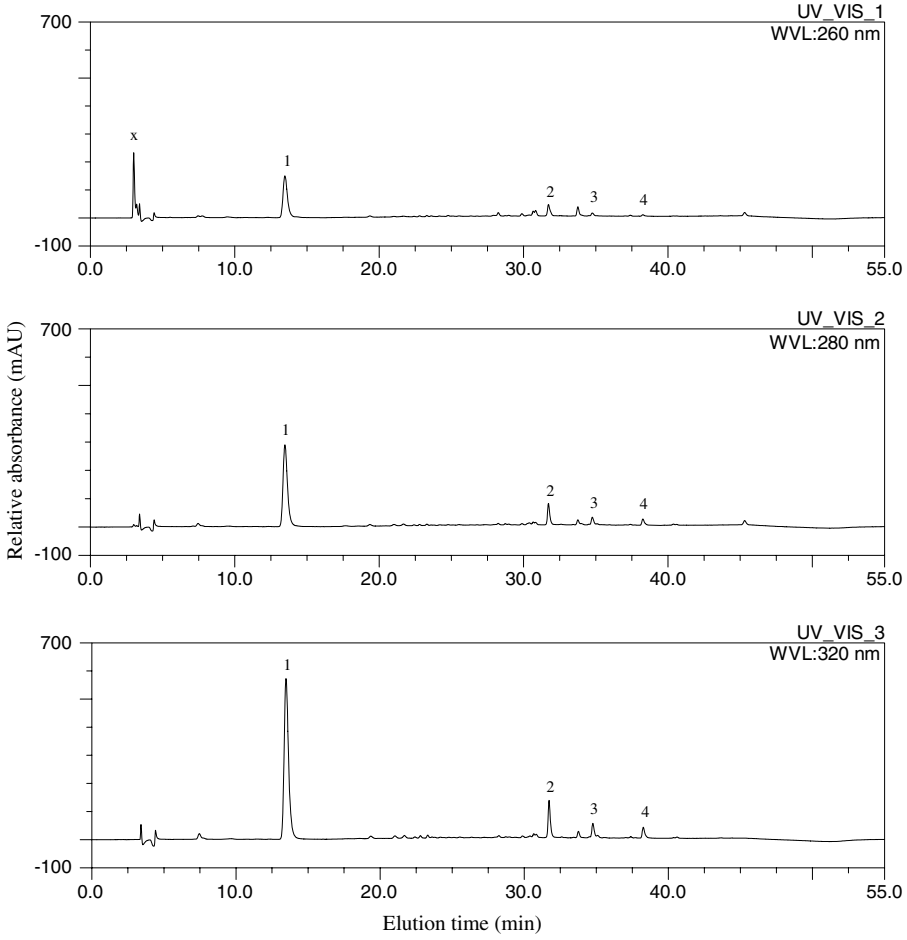

Fig. 7. HPLC chromatogram for the extract from *C. formosum* Dyer after reaction with DPPH.

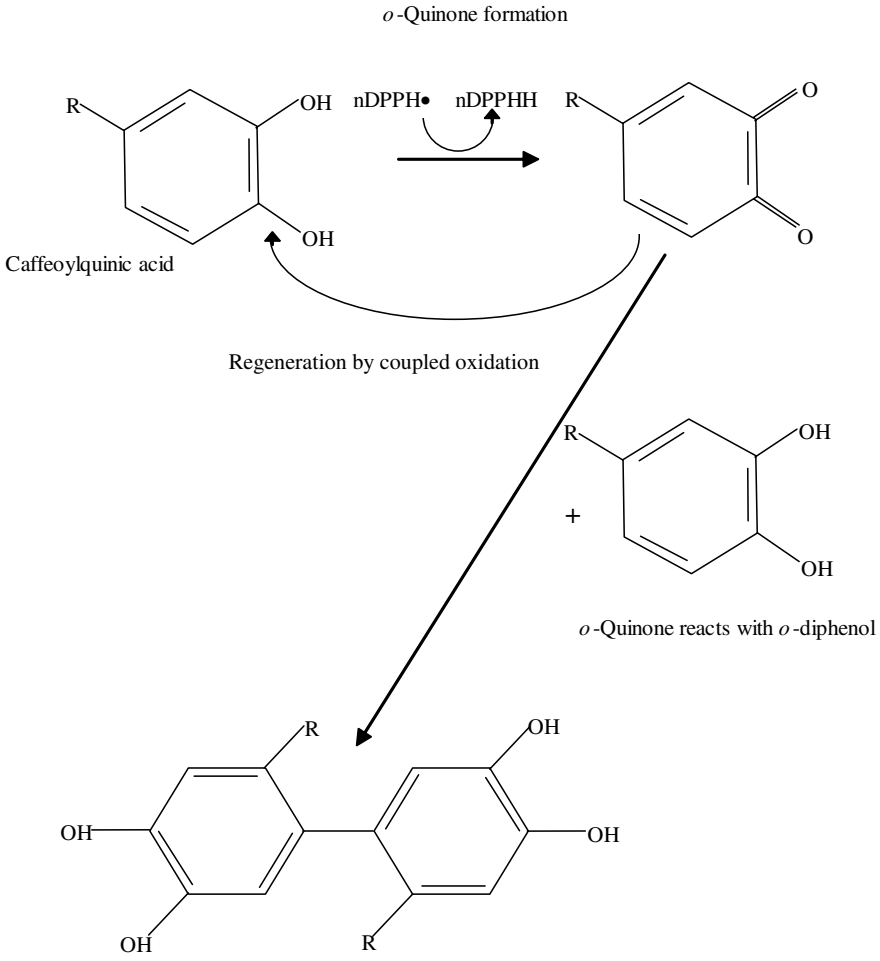

Fig. 9. Possible pathway for the oxidative degradation of caffeoylquinic acid due to reaction with the DPPH radical.

dation of chlorogenic acid with phenolase, or with the reactive oxygen species hypochlorous acid, produces a product with an absorption maximum at about 250 nm which was identified as an *o*-quinone (Kono, Shibata, Kodama, Ueda, & Sawa, 1995). The oxidation of an *o*-diphenol, such as caffeoylquinic acid, to reactive *o*-quinones is well recognized (Le Bourvellec, Le Quéré, Sanoner, Drilleau, & Guyot, 2004). *o*-Quinones are not stable but may polymerize, react with an *o*-diphenol to yield a condensation product, or oxidize other phenols by coupled oxidation with reduction of *o*-quinone back to *o*-diphenol (Le Bourvellec et al., 2004). The mechanism for the oxidative degradation of chlorogenic acid during trapping of DPPH radicals is shown in Fig. 9. The peak at 3.05 min disappeared when the reaction between DPPH and the Teaw extract was allowed to continue for more than 5 min (data not shown). This is consistent with the known reactivity of *o*-quinones.

3.2. Assessment of free radical-scavenging activity

Free radical-scavenging is the main mechanism by which antioxidants inhibit lipid oxidation. Two common methods, DPPH and ABTS radical-scavenging, were used to assess the antioxidant activity of the Teaw extract compared to that of the major component, chlorogenic acid, and α-tocopherol and BHT as reference standards.

Table 1
Antioxidant activity of compounds determined by the DPPH method ($1/EC_{50}$) and the ABTS decolorization assay[A]

Compound	EC_{50} (μg/ml)	EC_{50} (μg/μg DPPH)	Antiradical activity ($1/EC_{50}$)	TEAC (mmol of trolox/g sample)
Cratoxylum formosum Dyer	8.96 ± 0.07^{b}	0.23 ± 0.00^{b}	4.42 ± 0.04^{c}	2.67 ± 0.03^{c}
Chlorogenic acid	6.26 ± 0.06^{a}	0.16 ± 0.00^{a}	6.31 ± 0.07^{d}	3.06 ± 0.04^{d}
α-Tocopherol	15.0 ± 0.23^{c}	0.38 ± 0.01^{c}	2.68 ± 0.04^{b}	2.30 ± 0.03^{b}
BHT	15.4 ± 0.21^{d}	0.39 ± 0.01^{c}	2.57 ± 0.04^{a}	0.73 ± 0.01^{a}

[A] Note: Data followed by different letters within each column are significantly different according to Duncan's multiple range test at $P < 0.05$. Data obtained from at least three replicates for the DPPH method and nine replicates for the ABTS assay.

The loss of absorbance of the DPPH· at 517 nm in the presence of the selected antioxidants was studied in methanol and the calculated EC_{50} values are presented in Table 1. Chlorogenic acid, which is the main phenolic compound in the Teaw extract, showed the greatest radical-scavenging activity but the Teaw extract was more active than α-tocopherol and BHT. According to Brand-Williams, Cuvelier, and Berset (1995), the antiradical power of caffeic acid is ~2.2× that of BHA and BHT, and chlorogenic acid has similar activity to caffeic acid.

The antioxidant activities of the extract and pure compounds were also determined by the ABTS radical-scavenging method. The advantages of this radical are its water-solubility and high absorption coefficient at long wavelengths, allowing the determination of its rate of consumption with minimal interferences (Campos & Lissi, 1997). In addition, scavenging of the ABTS radical is less susceptible to steric hindrance when bulky antioxidants are studied. In the present study, it was found that the relative ranking of the four additives, using the ABTS radical, was the same as in the DPPH assay (Table 1). However, the differences between chlorogenic acid, the Teaw extract and α-tocopherol were less than in the DPPH assay. The relative order of activity of different samples assessed by the two radical-scavenging methods is more relevant than absolute values for comparing activities. Generally, the two methods (ABTS and DPPH) correlated strongly with each other (Awika et al., 2003; Leong & Shui, 2001). In this study, a correlation was found ($R = 0.76$, $P < 0.01$) between the values from the two methods (Fig. 10). The fact that α-tocopherol and BHT are not water-soluble contributes to their low value in the ABTS assay, and reduces the correlation coefficient.

The DPPH radical-scavenging activity of the Teaw extract was about 70% of that of chlorogenic acid, whereas the TEAC value of the Teaw extract, determined by the ABTS method, was about 87% of that of chlorogenic acid. The HPLC analysis showed that the four main peaks in the Teaw chromatogram corresponded to phenolic compounds with concentrations of 0.60, 0.07, 0.03 and 0.02 CAE/g, respectively, when expressed as grams of chlorogenic acid equivalents per gram of plant extract (CAE/g). This indicated that the Teaw extract contained ~0.72 CAE/g from the main peaks. Since the radical-scavenging activity of the extract was found to be 70–87% of that of chlorogenic acid, it appears that the compounds eluting as peaks 2–4 had activities similar to chlorogenic acid. Peak 2 has been identified as dicaffeoylquinic acid, and the chlorogenic acid isomers, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid, were reported to have almost the same antioxidant activity as chlorogenic acid (Chun, Kim, Moon, Kang, & Lee, 2003). The small difference in antioxidant activities of the Teaw extract obtained from the ABTS and DPPH assays when compared to the value for pure chlorogenic acid reflects differences in the structure and reactivity of the radical species.

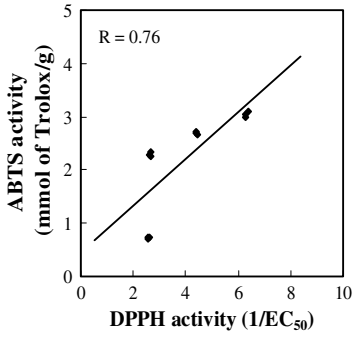

Fig. 10. Correlation between radical-scavenging activity determined by the ABTS and DPPH methods for *C. formosum* Dyer extract, chlorogenic acid, α-tocopherol and BHT.

4. Conclusions

The present study demonstrates, for the first time, that chlorogenic acid is the main antioxidant component in the ethanolic extract of Teaw leaves. Dicaffeoylquinic acid, and two ferulic acid derivatives were identified as minor components with radical-scavenging activities similar to chlorogenic acid that were present at small concentrations in the extract.

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