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Antioxidant constituents in feverfew (*Tanacetum parthenium*) extract and their chromatographic quantification

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Abstract

Medicinal herb feverfew (*Tanacetum parthenium*) has been reported to possess prophylactic properties over migraine and arthritis. However, less attention has been given to its antioxidant activities. In our study the antioxidant activities of the feverfew extract and its bioactive components in terms of their free radical-scavenging activities against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and their Fe^{2+}-chelating capacities were determined. In addition, the bioactive constituents in feverfew were determined by GC–MS and HPLC–UV. The results showed that feverfew powder extracted by 80% alcohol contained camphor, parthenolide, luteolin and apigenin in 0.30 ± 0.08%, 0.22% ± 0.03%, 0.84% ± 0.10% and 0.68% ± 0.07%, respectively. Total phenolic content of the feverfew extract was measured in 21.21 ± 2.11 μg gallic acid equivalent per mg dry material. The feverfew alcoholic extract possessed a strong DPPH free radical-scavenging activity of 84.4% and moderate Fe^{2+}-chelating capacity of 53.1%. Luteolin also showed strong DPPH scavenging activity of approximately 80% at $\geqslant$ 0.52 mg/mL. Parthenolide exhibited weak DPPH scavenging activity of 15% and moderate Fe^{2+}-chelating capacity of nearly 60%. Similar moderate Fe^{2+}-chelating activity (approximately 60%) was observed for luteolin and apigenin at 2 mg/mL.

Keywords: Parthenolide; Feverfew; *Tanacetum parthenium*; Flavonoid; Antioxidant activity; DPPH; Metal-chelating; HPLC; GC–MS

1. Introduction

Feverfew (*Tanacetum parthenium*) has been historically used for the treatment of headache, menstrual irregularities, stomachache and fevers by Greek and European herbalists (Tyler, 1993). However, this aromatic plant was somewhat forgotten by pharmacists until the late 1970s when claims of its efficacy in prevention of migraine were publicized in Britain (Groenewegen, Knight, & Heptinstall, 1992). Since then, feverfew has attracted much more research interest based on several positive clinical trials which has corroborated its anti-migraine activity (Johnson, Kadam, Hylands, & Hylands, 1985; Murphy, Heptinstall, & Mitchell, 1988; Palevitch, Earon, & Carasso, 1997). As a result, feverfew is now among the 50 top-selling supplements in USA according to the *Chemical Market Reporter* (Minkwitz, 1999), which is commercially available like other common herbs in capsules or bag teas. Although the mechanism of migraine prevention by feverfew has not been completely established, many researchers have stated that the bioactive component corresponding for the pharmacological functionality was parthenolide

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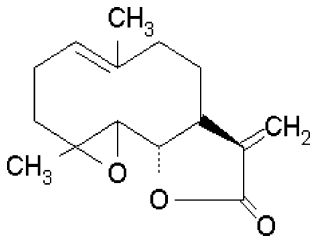

Fig. 1. Structure of parthenolide.

(Groenewegen & Heptinstall, 1990; Groenewegen et al., 1992; Hewlett et al., 1996; Mittra, Datta, Singh, & Singh, 2000), a sesquiterpene lactone (SQL) in feverfew (Govindachari, Joshi, & Kamat, 1964) (Fig. 1). Further studies also showed that parthenolide was effective in inhibition of some tumor cells (Lee, Huang, Piantadosi, Pagano, & Geissman, 1971; Ross, Arnason, & Birnboim, 1999; Wen, You, Lee, Song, & Kim, 2002) and pro-inflammatory transcriptional nuclear factor NF-κB (Bork, Schmitz, Kuhnt, Escher, & Heinrich, 1997; Hehner, Hofmann, Dröge, & Schmitz, 1999; Kang et al., 2002; Patel et al., 2000; Reuter, Chiarugi, Bolay, & Moskowitz, 2002).

In addition, feverfew contains other inherent constituents such as aromatic compounds (e.g., camphor) and flavonoids (e.g., luteolin and apigenin) (Christensen & Jakobsen, 1999; Williams, Harborne, Geiger, & Hoult, 1999; Williams, Hoult, Harborne, Greenham, & Eagles, 1995). Apigenin (4H-1-benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxy-phenyl)) was reported to significantly inhibit UV-induced mouse skin tumorigenesis (Lepley, Li, Birt, & Pelling, 1996; McVean, Xiao, Isobe, & Pelling, 2000), while luteolin has shown strong growth inhibition on MCF-7 (Han, Tachibana, & Yamada, 2001) and other tumors (Pettit, Hoard, Doubek, Schmidt, & Pettit, 1996).

Furthermore, leaves and flowers of feverfew contain some lipophilic flavonoids, such as quercetagetin 3,6-dimethyl ether, 6-hydroxykaempferol 3,6-dimethyl ether, and 6-hydroxykaempferol 3,6,4′-trimethyl ether (santin) (Williams et al., 1999). Santin was reported to have anti-inflammatory properties through inhibition of the cyclooxygenase and 5-lipoxygenase pathways in rat peritoneal leucocytes (Williams et al., 1999, 1995).

As well known, many polyphenolic and flavonoid compounds possess antioxidant activities such as metal chelating and free radical scavenging capacities (Bors & Michel, 1999; Boyer & McCleary, 1987; Hertog, Hollman, & Putte, 1993; Pedrielli & Skibsted, 2002) via inactivation of reactive oxygen species (ROS) and other free radicals produced by aerobic metabolism (Lapidot, Walker, & Kanner, 2002; Lee & Shibamoto, 2002; Narayana, Reddy, Chaluvadi, & Krishna, 2001; Oomah & Mazza, 1996). Such quenching capacities are associated with prevention of chronic diseases such as cardiovascular disease and cancer (Cerutti, 1985; Packer, Rimbach, & Virgili, 1999; Sultana & Saleem, 2004). However, to our knowledge, there is no information available for the antioxidant activities of feverfew extract and its major bioactive constituents. Therefore, the objectives of our study were to investigate the antioxidant potential of the feverfew extract and its components, (i.e., parthenolide, camphor, luteolin and apigenin) and to quantify the interested antioxidants by chromatographic techniques.

2. Materials and methods

2.1. Materials and chemicals

Dried feverfew powders (golden parthenium) were generously provided by Dr. James W. Rushing of Clemson University Coastal Research and Education Center, Charleston, SC. Chemical standards parthenolide (99% purity) and camphor (98% purity) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Apigenin (95% purity), luteolin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), α-tocopherol (Vit E) and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO). 2,6-Di-*tert*-butyl-4-methylphenol (BHT, 99%) was purchased from ACROS (NJ, USA). Folin–Ciocalteu's phenol reagent (2 N) was purchased from MP Biomedicals, Inc. (Aurora, Ohio). Anhydrous ferrous chloride was purchased from Riedel-de Häën (Steinheim, Germany), and ferrozine (3-(2-pyridyl)-5, 6-diphenyl-1, 2,4-triazine-4′, 4″-disulfonic acid monosodium salt) was provided by Fluka Co. (Buchs, Switzerland). Reagent alcohol (HPLC grade) was obtained from Fisher Scientific Co. (Fairlawn, NJ).

2.2. Preparation of golden feverfew extracts

Feverfew powder (1.5 g) was weighed and put into a 250 mL glass bottle containing a magnetic stirring bar. The feverfew sample was extracted for 10 min with 100 mL 80% (V/V) alcohol solution with vigorous stirring at room temperature. After filtration through Whatman No. 1 filter paper, clear extracts (16.67 mg/mL) based on dry material weight (d.w.) were obtained and maintained at 5 °C until determinations for total phenolic content, DPPH free radical scavenging and Fe^{2+}-chelating capacities were made.

For HPLC quantification of constituent flavonoids in feverfew extract, feverfew powder (5.1 g) was weighed and processed by the procedures mentioned above. The extract was then concentrated to 25 mL (204 mg/mL, d.w.) at 50 °C using a Buchi rotary vacuum evaporator. When using C_{18}–HPLC (high performance liquid chromatography) for collecting fractions for analysis of DPPH free radical scavenging and Fe^{2+}-chelating capacities, feverfew powder (5.6 g) was extracted by the same

procedures aforementioned, and then concentrated to 15 mL (373.33 mg/mL, d.w.) using a rotary vacuum evaporator at 50 °C.

2.3. Measurement of DPPH free radical-scavenging activity

Antioxidant activity of the feverfew alcoholic extract and the standards on scavenging DPPH free radicals was determined according to the method described by Chung, Chang, Chao, Lin, and Chou (2002). An aliquot of 0.2 mL of standards α-tocopherol, BHT, camphor, luteolin, apigenin, parthenolide in a series of concentrations from 0.04 to 1.2 mg/mL, or the same volume of feverfew extract (16.67 mg/mL, d.w.) was mixed with 0.8-mL Tris–HCl buffer (100 mM, pH 7.4), then 1 mL 500 μM DPPH was added into the mixture to a final concentration of 250 μM. The mixture was shaken vigorously and left in the dark at room temperature for 20 min. On the other hand, each C_{18}–HPLC fraction (500 μL) of the feverfew extract (373.33 mg/mL, d.w.) was added to 100 μL of 1.014 mM DPPH in alcohol, shaken vigorously then left in the dark at room temperature for 90 min. Absorbance of the resulting solutions was measured at 517 nm by Spectronic@ 20 GENESYS™ spectrometer from Fisher Scientific Co. (Fairlawn, NJ, USA). The antioxidant capacity to scavenge the DPPH radical was calculated by the following equation:

$$\text{Scavenging Effect } (\%) = \times \left(1 - \frac{\text{absorbance of sample at 517 nm}}{\text{absorbance of control at 517 nm}}\right) \times 100. \quad (1)$$

2.4. Measurement of Fe^{2+}-chelating ability

Fe^{2+}-chelating ability was measured according to the method described by Chung et al. (2002) with minor modification. Fe^{2+} was determined by measuring the formation of ferrous iron–ferrozine complex. Each solution of the feverfew alcoholic extract (16.67 mg/mL, d.w.), α-tocopherol, BHT, camphor, luteolin, apigenin and parthenolide (0.04–2 mg/mL) was added to 2 mM $FeCl_2$ and 5 mM ferrozine at a volumetric ratio of 80:1:2 to a total volume of 0.83 mL. The mixture was vortexed and left at room temperature for 10 min before absorbance was measured at 562 nm. Each collected C_{18}–HPLC fraction (1000 μL) of the feverfew extract was added to 2 mM $FeCl_2$ (20 μL) and 5 mM ferrozine (40 μL), and left in the dark at room temperature for 240 min after vigorous shaking. The mixture absorbance was then measured at 562 nm. A lower absorbance of the reaction mixture indicated a higher Fe^{2+}-chelating ability. The capacity to chelate the ferrous iron was calculated by the following equation:

$$\text{Chelating Effect}(\%) = \times \left(1 - \frac{\text{absorbance of sample at 562 nm}}{\text{absorbance of control at 562 nm}}\right) \times 100. \quad (2)$$

2.5. Determination of total phenolic content

The content of total soluble phenolics was determined according to the modified Folin–Ciocalteau method (Parejo et al., 2002; Torres, Mau-Lastovicka, & Rezaaiyan, 1987). The reaction mixture was composed of 40 μL of extract (16.67 mg/mL, d.w.), 360 μL of distilled water, 200 μL of the Folin–Ciocalteau reagent and 1 mL of 20% sodium carbonate solution. The solution was vortexed and allowed to stand for 2 h. The absorbance was measured at 765 nm in a Spectronic@ 20 GENESYS™ spectrometer from Fisher Scientific Co. (Fairlawn, NJ, USA).

A standard curve was prepared with gallic acid of known concentrations. The total phenolic content was determined as μg of gallic acid equivalents (GAE)/mg dry material in triplicate.

2.6. GC–MS condition

A Shimadzu GC17A coupled with a QP5050 quadrupole mass spectrometer was used for volatile chemical analysis. Two microliters of each sample was injected into a silica capillary column (DB5 MS, 60 m × 0.25 mm i.d., 0.25 μm film thickness, J &W Scientific, Folsom, CA) using a split mode of 1:10. The injection port was maintained at 180 °C. The oven temperature was programmed from 60 °C for 1 min, and then increased to 300 °C at a ramp rate of 8 °C/min. The interface and ion source were both set at 300 °C. Mass spectra were recorded in the electronic impact (EI) ionization mode at 70 eV. Parthenolide and camphor in feverfew were identified by comparison with the available authentic standards.

2.7. Reverse-phase HPLC condition

Clear concentrated feverfew extract of 204 mg/mL (d.w.) was obtained by filtration through a 0.45 μm PTFE filter. A 30 μL aliquot was analyzed by a Shimadzu HPLC LC-10Avp automated liquid chromatographic system, which consisted of a photodiode array (PDA) detector, a SCLAvp controller, two LC-10ATvp pumps, a SIL-10ADvp autoinjector and a reverse-phase Restek Pinnacle C18 (5 μm, 150 × 4.6 mm, 110 Å) column with a matched guard column. The elution (30 μL) was carried out with a gradient solvent program at a flow rate of 1.0 mL/min at ambient temperature. The mobile phase consisted of water plus 0.2% trifluoroacetic

acid (TFA) (A) and acetonitrile (B) with the following gradient: 0 min, 0% B; 3 min, 30% B; 5 min, 32% B; 25 min, 40% B; 38 min, 50% B; 41 min, 100%B; 45 min, 0%B. The absorbance of the samples was scanned from 190 to 500 nm by the Shimadzu SPD-M10V PDA detector. The analytes were detected at 280 nm. Apigenin and luteolin in feverfew extract were identified by comparison with the available authentic standards. The determination method was modified according to a method of Hertog, Hollman, and Venema (1992).

Fifteen micro liter of the concentrated feverfew extract of 373.33 mg/mL based on dry weight was injected into the same HPLC system with a slightly different solvent program, which used above solvents with the following gradient program: 0 min, 0% B; 3 min, 0% B; 4 min, 15% B; 21 min, 32.4% B; 30 min, 33% B; 31 min, 41% B; 44 min, 50% B; 45 min, 100% B; 46 min, 0% B. Each fraction of the eluant collected at the detector exit was 1 mL. The absorbance of the eluant was scanned from 190 to 500 nm by the Shimadzu SPD-M10V PDA detector.

2.8. Quantification of feverfew constituents by HPLC and GC–MS

Parthenolide standard solutions were prepared from a stock solution and diluted into concentrations of 20, 100, 200 and 400 μg/mL for GC–MS analysis. Each solution was measured three times from a start of the most diluted concentration. Parthenolide content in feverfew extracts was determined by a single point calibration after demonstrating linearity in the detector response to parthenolide concentrations between 20 and 400 μg/mL. Likewise, camphor standard solutions were prepared and analyzed in the same way as parthenolide.

For HPLC analysis of the flavonoids, luteolin standard solutions were prepared from a stock solution into a series of concentrations of 0, 10, 100, 1000 μg/mL. Apigenin standard solutions were prepared in concentrations of 0, 20, 100, 200 μg/mL. Each solution was analyzed beginning from the most diluted concentration. Concentrations of luteolin and apigenin in feverfew extracts were calculated by the induced four point calibration equation.

2.9. Statistical analysis

Each treatment was repeated three times. Each sample was analyzed by GC/MS and HPLC–UV three times. Microsoft Excel was used to compute means, standard deviation, and the linear regression R^2. Statistical analysis was conducted on a SAS Software for Windows V8 (SAS Institute Inc., Cary, NC). Differences among all sample means were determined by analysis of variance (ANOVA) at $p < 0.05$.

3. Results and discussion

3.1. Quantification of major compounds in the alcoholic feverfew extract

Linear regression analysis of calibration plot gave a correlation coefficient (R^2) of 0.9977 for luteolin, 0.9957 for apigenin and 0.9841 for parthenolide. Camphor, parthenolide, luteolin, and apigenin in the alcoholic feverfew extract were measured by GC–MS and HPLC in amounts of 0.30 ± 0.08%, 0.22 ± 0.03%, 0.84 ± 0.10%, and 0.68 ± 0.07%, respectively, all based on dry weight (Figs. 2 and 3). Camphor, luteolin and apigenin were quantified for the first time for the golden feverfew variety grown in Charleston, South Carolina in the United States. Williams et al. (1999) identified apigenin and luteolin in feverfew but those compounds were not quantified. Parthenolide concentration was in agreement with other published data (Zhou, Kou, & Stevenson, 1999). Total phenolic content in the alcoholic feverfew extract was determined to be 21.21 ± 2.11 μg GAE/mg dry material, which is higher than that detected in tansy (*Tanacetum vulgare*) and many other plant extracts based on dry extract (Kähkönen et al., 1999). This result is in a certain degree in agreement with the following observed strong antioxidant activities of feverfew extract.

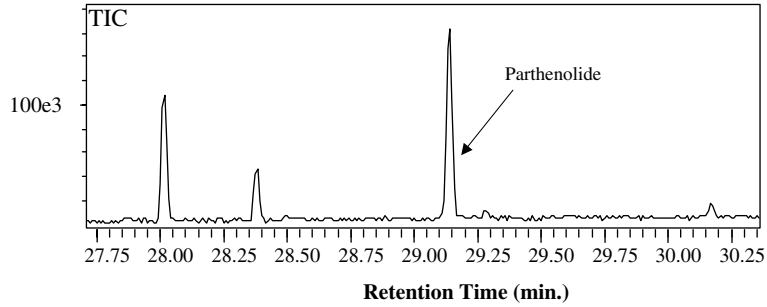

Fig. 2. Parthenolide from alcoholic feverfew extract using a Shimadzu Gas Chromatography/Mass Spectrometry (GC17A-QP5050 MS).

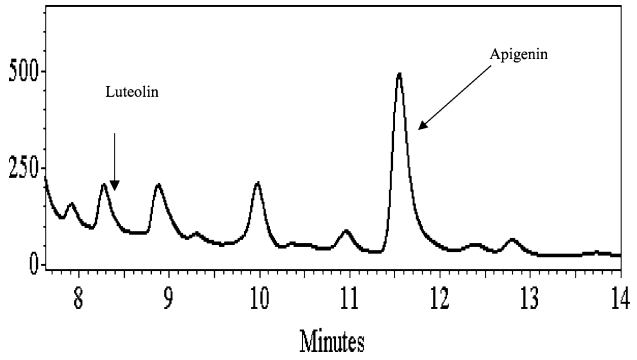

Fig. 3. Apigenin and luteolin in feverfew extract using Shimadzu HPLC LC-10Avp automated liquid chromatographic system for analysis at UV 280 nm.

3.2. DPPH scavenging and Fe^{2+}-chelating activities of four identified compounds

Biological molecules such as DNA and proteins are subject to pro-oxidative stresses induced by free radicals, which can result in various diseases such as cancer, cataract (Parejo et al., 2002) and aging (Finkel & Holbrook, 2000). Therefore, antioxidants that can quench free radicals may be implicated in the prevention of these diseases. To demonstrate the antioxidant capacity of feverfew extract and feverfew inherent bioactive constituents, DPPH assay was employed in our study to determine the proton–radical-scavenging activity based on the reaction that the purple color of DPPH solution fades quickly when it reacts with proton–radical-scavengers.

When camphor or parthenolide was in the concentration range of 0.04–1.2 mg/mL, their DPPH free radical-scavenging activity remained approximately 15%, which was much weaker than that of Vit E and BHT of the same concentration (Fig. 4). In contrast, luteolin showed an increased DPPH free radical-scavenging activity with increasing concentration in the range of 0.04–0.52 mg/mL. Furthermore, the free radical-scavenging activity of the 500-μM DPPH solution was saturated by luteolin at a concentration of $\geqslant$0.52 mg/mL, resulting in a strong antioxidant activity of approximately 80%. This was comparable to the DPPH free radical-scavenging activity of BHT and Vit E at 0.52 mg/mL (Fig. 4). However, apigenin unlike the other polyphenolics observed, had no DPPH free radical-scavenging activity at concentrations within 0.04–1.2 mg/mL (Fig. 4). This result was not surprising as previous work reported that apigenin might even have pro-oxidative activities (Galati, Sabzevari, Wilson, & O' Brien, 2002).

Free radical-scavenging activity of polyphenolic compounds is believed to be influenced by the number and position of phenolic hydrogen in their molecules (Gao, Huang, Yang, & Xu, 1999). It is also proposed that the higher antioxidant activity is related to the greater number of hydroxyl groups on the flavonoid nucleus (Cao, Sofic, & Prior, 1997). Foti, Piattelli, Baratta, and Ruberto (1996) studied selected flavonoids in a linoleic acid micelle system and proposed that the antioxidant activity was dependent on the presence of *ortho* phenolic functions. For example, configuration of 3′, 4′-dihydroxy in flavones is helpful to increase their antioxidant activities. These hypotheses may explain why the catechol B ring-containing flavonoid luteolin possess stronger free radical-scavenging activity than the phenol B ring-containing flavonoid apigenin.

Fe^{2+} is able to generate free radicals from peroxides by Fenton reactions and may be involved in the progression of human cardiovascular disease. Thus, antioxidants capable of chelating with Fe^{2+} will minimize the ion's concentration and inhibit its capacity to catalyze free radical formation, which will results in protection against oxidative damage. Our tests showed that, among the four identified compounds (i.e., camphor, parthenolide, luteolin and apigenin) in feverfew, only camphor had no Fe^{2+}-chelating activity at the tested concentration range from 0.04–2 mg/mL (Fig. 5). In addition,

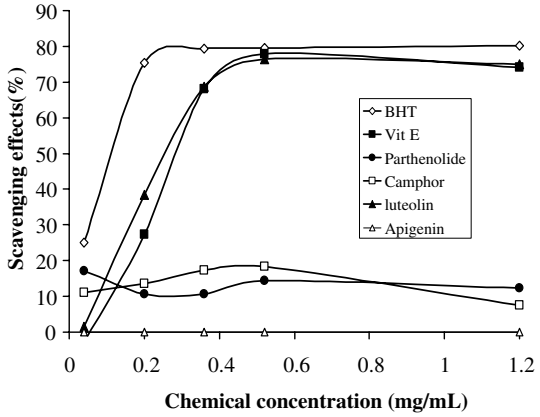

Fig. 4. DPPH free radical-scavenging activities of commercial antioxidants and compounds identified in feverfew extract.

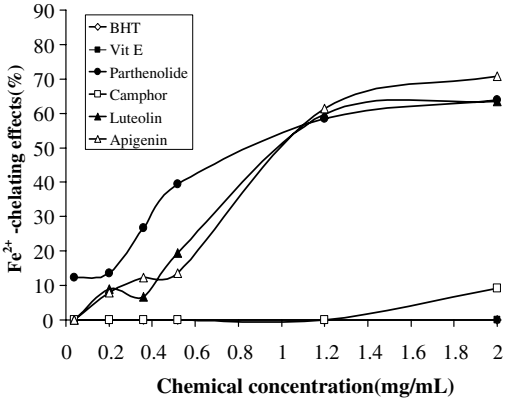

Fig. 5. Fe^{2+}-chelating activities of commercial antioxidants and compounds identified in feverfew extract.

both standard controls, neither Vit E nor BHT, showed Fe^{2+}-chelating activity at concentrations of 0.04–2 mg/mL (Fig. 5).

Parthenolide exhibited moderate Fe^{2+}-chelating activity with increasing concentration of 0.04–2 mg/mL. This activity approached the saturation at $\geqslant 1.2$ mg/mL in the tested system, with approximately 60% relative activity (Fig. 5). This moderately high Fe^{2+}-chelating activity may be attributed to the lactone structure of parthenolide.

Hudson and Lewis (1983) reported that a carbonyl at the 4 position as well as 3- or 5-hydroxyl groups in flavonoids were important for the metal-chelating activity. They proposed that there was cooperation between the 4-carbonyl and the 3- or 5-hydroxyl groups to chelate the copper ion. For example, quercetin, a common flavonoid in some fruits, is a strong binder of metals (Nieto et al., 1993), especially of copper and iron. Luteolin and apigenin also contain the 4-carbonyl and 5-hydroxyl groups, which could explain their similar moderate Fe^{2+}-chelating activity observed at concentrations of 1.2–2 mg/mL, with approximately 60% activity.

3.3. DPPH scavenging and Fe^{2+}-chelating activities of feverfew extract and its fractions

DPPH scavenging activity and Fe^{2+}-chelating capacity of feverfew extract (16.67 mg/mL) were 84.4% and 53.1%, respectively. The strong free radical-scavenging activity might result from the contribution of luteolin which was determined to be $0.84 \pm 0.10\%$ (d.w.) of feverfew extract. The metal-chelating capacity could be attributed to the combined effect of parthenolide at $0.22 \pm 0.03\%$ (d.w.), luteolin ($0.84 \pm 0.10\%$ d.w.) and apigenin ($0.68 \pm 0.07\%$ d.w.) in the feverfew extract. The observed antioxidant properties of the feverfew alcoholic extract may be responsible for some medicinal claims such as the anti-inflammatory activities that are associated with free radicals.

In spite of the above antioxidant observations on feverfew constituents, we were also interested in other potential antioxidant components in the feverfew. Forty-five fractions of the feverfew extract were separated and collected by the C_{18}–HPLC system. Only fractions 7 and 14 exhibited relatively higher DPPH free radical activities (>40%) than any other fractions (Fig. 6(b)). Fractions 7 and 14 correspond to two compounds observed as two separate sharp peaks on the HPLC–UV chromatographic profile (Fig. 6(a)). However, further chromatographic comparison with available standard flavonoids in our lab could not identify these two peaks. Williams et al. (1999) reported that a number of flavone glycosides of feverfew extract such as apigenin 7-glucuronide, luteolin 7-glucuronide, luteolin 7-glucoside and chrysoeriol 7-glucuronide were in the feverfew extract. This implied that

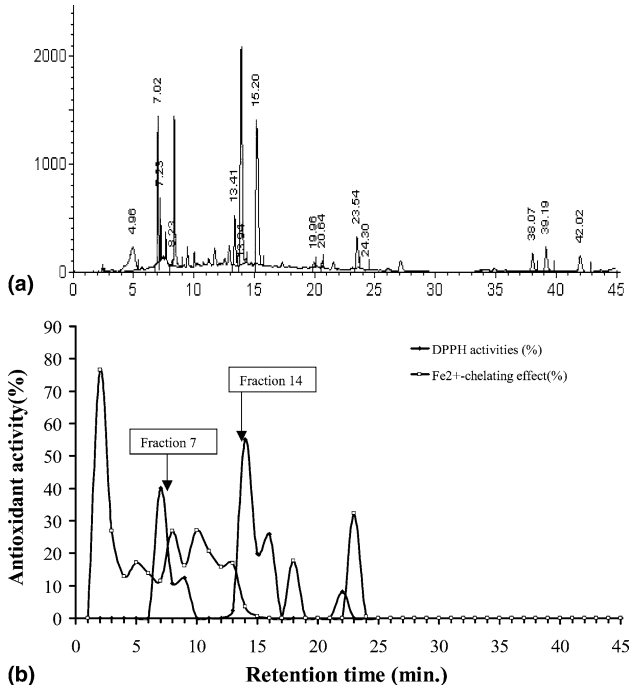

Fig. 6. DPPH free radical scavenging and Fe^{2+}-chelating activities of fractions separated from feverfew extract with C_{18} chromatography (b) with HPLC–UV chromatographic profile of feverfew extract during separation by C_{18} chromatography (a).

those two unidentified peaks on HPLC–UV chromatographic profile might be part of those flavone glycosides. Further studies are needed, using either acidic or enzymatic hydrolysis to break down the suspected conjugated flavonoids or applying other methods such as HPLC–MS or NMR, for further chemical identification.

When compared to the spectrums of the authentic standards, a peak with retention time at 19.96 min (fraction 20) was identified as luteolin, and the peak with retention time at 23.54 min (fraction 23) was identified as apigenin (Fig. 6(a)). However, it is worthy noting that, due to a limited injection volume (15 μL) of feverfew extract on the HPLC column, the luteolin concentration of fraction 20 was equivalent to 0.05 mg/mL, and the apigenin concentration of fraction 23 was approximately to 0.04 mg/mL. Compared to the DPPH activity of their respective authentic standards, luteolin at 0.05 mg/mL and apigenin of 0.04 mg/mL exhibited very low or no DPPH free radical activity (Fig. 4), which is in agreement with the low DPPH free radical activity observed on fraction 20 and 23 (Fig. 6(b)). Based on the DPPH free radical activity of the separated fractions (Fig. 6(b)), the strong scavenging activity could be contributed to the unidentified components (fractions 7 and 14) in the feverfew extract.

Relatively higher Fe^{2+}-chelating activity (76.6%) was observed in fraction 2 (Fig. 6(b)). Except for fractions 8, 10 and 23, no other fractions were observed with a rela-

tively high Fe^{2+}-chelating activity (>20%) to match a peak on HPLC–UV chromatographic profile (Fig. 6(a)). The discrepancy between the antioxidant and HPLC separation profiles of the feverfew extract demonstrates that there are some strong inherent antioxidants in feverfew extracts that need to be identified in the further investigation.

4. Conclusions

Four compounds were identified and quantified using HPLC–UV and GC/MS: (1) camphor at $0.30 \pm 0.08\%$; (2) parthenolide at $0.22 \pm 0.03\%$; (3) luteolin at $0.84 \pm 0.10\%$; (4) apigenin at $0.68 \pm 0.07\%$ based on dry weight. The total phenolics of feverfew extract were determined as 21.21 ± 2.11 μg GAE/mg dry material. Also, this study for the first time reveals that the alcoholic extract of feverfew possesses strong free radical-scavenging activity of 84.4% in a DPPH test and moderate metal-chelating capacity of 53.1%. The strong free radical-scavenging activity and moderate metal-chelating capacity of the feverfew extract might result from the presence of luteolin, parthenolide and other unidentified components observed on the HPLC–UV chromatographic profile. Since many studies have revealed that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of some human diseases, it seems plausible to assume that the consumption of feverfew extract, except the benefits for treating migraine, might be able to confer some health-benefiting values against oxidative stresses. However, more scientific work needs to be done regarding the bioavailability of feverfew bioactive constituents in order to further verify their antioxidant effects in *in vivo* conditions.

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