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Concordance between antioxidant activities and flavonol contents in different extracts and fractions of *Cuscuta chinensis*

Feng-Lin Yen [a,1], Tzu-Hui Wu [a,1], Liang-Tzung Lin [b], Thau-Ming Cham [c], Chun-Ching Lin [c,*]

[a] *Graduate Institute of Pharmaceutical Sciences, School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan*
[b] *Department of Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia, Canada*
[c] *Faculty of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan*

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Abstract

Chinese herbs employed in traditional Chinese medicine (TCM) have been used for centuries in the practice of medicated diet and dietetic therapy. The seed of *Cuscuta chinensis* Lam. (Convolvulaceae), a commonly used traditional Chinese herb, is frequently added in Chinese cooking and preparation of refreshments, including porridge and alcoholic beverages, to nourish the human body. In the present study, we compared the antioxidant activities of water and ethanol extracts from the seeds *C. chinensis* and also of its different organic fractions, including *n*-hexane, ethyl acetate, *n*-butanol and organic water, by assessing their DPPH (1,1-diphenyl-2-picryl hydrazine) free radical-scavenging, superoxide anion scavenging, anti-superoxide anion formation and anti-lipid peroxidation abilities. The flavonol contents of all test samples were analyzed by high-performance liquid chromatography with an ultraviolet (HPLC–UV) detector. The results showed that there is a direct correlation of the flavonol content with the antioxidant activities from the extracts and fractions of *C. chinensis*. Moreover, the ethyl acetate fraction demonstrated significantly better and higher antioxidant effects, and also had a higher flavonol content than had the remaining samples ($P < 0.05$). The water fractions, however, exhibited the weakest antioxidant activity, and had low concentrations of flavonols. Thus, we suggest that the ethanol extract of *C. chinensis*, but not its water extract, could be used as a dietary nutritional supplement to promote human health and prevent oxidation-related diseases, due to its antioxidant properties.

Keywords: TCM; *Cuscuta chinensis*; Flavonol; HPLC–UV; Antioxidant activity

1. Introduction

The balance between prooxidants and antioxidants is an important strategy for maintaining health. Reactive oxygen species (ROS) are well-known prooxidants, such as superoxide anion $O_2^{\cdot-}$, hydroxyl radical ($\cdot OH$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) (Blokhina, Virolainen, & Fagerstedt, 2003; Droge, 2002). Unfortunately, when an excessive amount of prooxidant breaks the balance, this results in cellular oxidative stress that helps aggravate the progression of many clinical diseases, including cancer (Valko, Rhodes, Moncol, Izakovic, & Mazur, 2006), aging (Junqueira et al., 2004), inflammation (Jialal, Devaraj, & Venugopal, 2002), cardiovascular diseases (Suzuki, Jain, Park, & Day, 2006), neurodegenerative diseases (Trushina & McMurray, 2007), and liver injury (Jaeschke et al., 2002). Therefore, dietary supplements, consisting of antioxidants such as flavonols and vitamins, could be used to effectively protect body cells from the attack by oxidative stress and to preserve human body health in general (Rahman, Biswas, & Kirkham, 2006; Sies, Stahl, & Sevanian, 2005).

In traditional Chinese medicine (TCM), it is believed that food and medicine share the same origin but differ in their application and use. Chinese medicated diet

* Corresponding author. Tel.: +886 7 3121101x2122; fax: +886 7 3135215.
E-mail address: aalin@kmu.edu.tw (C.-C. Lin).
[1] These authors contributed equally to this work.

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and dietetic therapy are part of the TCM practices that have been applied for centuries to promote human health and to prevent and cure diseases. One form of Chinese medicated diet is the preparation of medicinal tonics, and this often includes using plants, such as *Angelica sinensis*, *Poria cocos*, and *Lycium chinense*. These herbs are therefore frequently found in the Chinese cuisine and beverages, including porridge, soup and tea (Lin, 2003).

Tu-Si-Zi, the seed of *Cuscuta chinensis* Lam. (Convolvulaceae), is one of the commonly used herbal constituents in Chinese medicinal tonics to nourish the liver and kidney, in both China and Taiwan. It is often added to porridge and alcoholic beverages to improve vision and impotence, and also used to prevent abortion as well as aging in clinical treatment (Zheng, Dong, & She, 1998). Previous studies have indicated that *C. chinensis* possesses anticancer (Nisa, Akbar, Tariq, & Hussain, 1986; Umehara et al., 2004) and immunostimulatory activities (Bao, Wang, Fang, & Li, 2002). In addition, *C. chinensis* glycoside has been demonstrated to exert anti-aging effects and enhance memory by inducing PC12 cell differentiation (Liu, Jiang, Bao, & An, 2003).

The antioxidant activities and flavonol contents of the water and ethanol extracts from the seed of *C. chinensis* and of its different organic fractions have not yet been reported. In this study, we used different solvents to extract the seed of *C. chinensis* and investigated their antioxidant activities by DPPH free radical-scavenging, anti-superoxide formation, superoxide scavenging and anti-lipid peroxidation assays. High-performance liquid chromatography with an ultraviolet detector (HPLC–UV) was also used to quantitate and characterize the flavonols in the different extracts and organic fractions of the seed of *C. chinensis*.

2. Materials and methods

2.1. Chemicals and reagents

Tris-HCl, thiobarbituric acid (TBA), ferrous chloride, ascorbate, xanthine, xanthine oxidase, cytochrome c, 1,1-diphenyl-2-picryl hydrazine (DPPH), dimethyl sulfoxide (DMSO), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma–Aldrich Chemicals Co. (St. Louis, MO, USA). All other chemical reagents were of analytical grade.

2.2. Plant material

The seeds of *C. chinensis* Lam. were obtained from local Chinese medicine herb store. The authenticity of the plant species was confirmed by a pharmacognosist, Dr. Ming Hong Yen, and stored as a voucher specimen (KMU 0709) in the Herbarium of Herbal Department, Graduate Institute of Natural Products, Kaohsiung Medical University, Taiwan.

2.3. Extraction from the seeds of Cuscuta chinensis

The powder (100 g) from the seeds of *C. chinensis* was extracted with 300 ml of 95% ethanol or water by heat reflux. This procedure was repeated twice. The ethanol (ALC) or water extracts (H_2O) were blended and concentrated by rotary vacuum evaporation and then lyophilized with a freeze-dryer. The curd ALC was re-suspended in 250 ml of water and then successively extracted with the equivalent volume of *n*-hexane (HX), ethyl acetate (EA), and *n*-butanol (NB). The residual water-solvent solution was collected and designated as organic water fraction (OH). The organic solvent of each extract was concentrated and removed under rotary vacuum evaporation. The resulting extracts were subsequently lyophilized until dry and the lyophilized powders were collected, and stored at −20 °C until used.

2.4. Determination of the flavonol contents by HPLC

The chromatographic system (Dionex Softron GmbH, Germering, Germany) consisted of a pump (P-680), an autosampler (ASI-100), and a detector (UVD-170U). All test samples were separated on a LichroCART® Purospher® STAR (250 × 4.6 mm i.d., 5 μm) and the temperature was maintained at an obligatory level of 40 °C. The mobile phase was composed of 1% acetic acid in 25 mM phosphate buffer and acetonitrile (50:50), and the pH value was adjusted to 2.5 with phosphoric acid. The flow rate was set at 0.5 ml/min and the wavelength of the detector was kept at 365 nm. The major constituents in the multiple extracts and fractions were identified by comparing their retention time with those of the flavonol standards, such as quercetin and kaempferol.

2.5. Determination of the antioxidant activity

2.5.1. DPPH assay

DPPH (1,1-diphenyl-2-picryl hydrazine) is a stable free radical which has been used to determine the free radical-scavenging activities of compounds or plant extractions in many studies. The free radical-scavenging effect was evaluated by modified methods from Zou et al. (2005). A concentration of 200 μM of DPPH in ethanol solution was prepared. Series of test sample concentrations were dissolved in DMSO, and 125 μl of DPPH solution was mixed with equivalent aliquots of different samples and then incubated at room temperature in the dark. After 30 min, the absorbance of the reaction solution was measured by an ELISA reader at 517 nm. All determinations were performed in triplicate. The percent scavenging of free radicals by the sample was calculated using the following the equation: % scavenging effect = $[(\text{control}_{517\text{nm}} - \text{sample}_{517\text{nm}})/\text{control}_{517\text{nm}}] \times 100$. The scavenging concentration of each sample at 50% (SC_{50}) was used to compare the levels of free radical-scavenging activity between samples.

2.5.2. Superoxide anion scavenging activity assay

Scavenging effect of $O_2^{\cdot-}$ was estimated by the reduction of cytochrome c method (McCord & Fridovich, 1969). The experimental solution was prepared by adding 2.5 ml of 50 mM KH_2PO_4, 2.5 ml of 0.1 mM EDTA, 2.5 ml of 0.1 mM cytochrome c, and 50 ml of 0.1 mM xanthine. All test samples were dissolved with DMSO and only the water extract was dissolved in water. Subsequently, 530 μl of de-ionized water, 400 μl of experimental solution, 50 μl of test samples solution and 20 μl of 1 unit/ml XO were pipetted into the cuvette and vigorously shaken. Then the mixture was determined for 70 s at 550 nm. All determinations were performed in triplicate. The percent scavenging of $O_2^{\cdot-}$ by the sample was calculated as: $[(\text{control}_{550\ \text{nm}} - \text{sample}_{550\text{nm}})/\text{control}_{550\text{nm}}] \times 100$. The scavenging concentration of each sample at 50% (SC_{50}) was used to compare the levels of free radical-scavenging activity between samples.

2.5.3. Anti-superoxide formation assay

The anti-superoxide ($O_2^{\cdot-}$) formation of the extracts and fractions from the seeds of *C. chinensis* was determined using the xanthine/xanthine oxidase (XO) inhibition test, and the XO activity was evaluated by the formation of uric acid from xanthine (Chang, Chang, Lu, & Chiang, 1994). The water extract was dissolved in water and all other test samples were dissolved with DMSO. An amount of 530 μl of de-ionized water, 400 μl of 50 mM KH_2PO_4, 50 μl of test sample solution, and 20 μl of 1 unit/ml XO were pipetted into a cuvette. The mixture was vigorously shaken and screened for 2 min at 295 nm, using a Hitachi U-2001 spectrophotometer. All determinations were performed in triplicate. The percentage of inhibition was calculated as: $[(\text{control}_{295\text{nm}} - \text{sample}_{295\text{nm}})/\text{control}_{295\text{nm}}] \times 100$. The inhibitory concentration of each sample at 50% (IC_{50}) was used to compare the anti-superoxide anion formation abilities between samples.

2.5.4. Anti-lipid peroxidation assay

The anti-lipid peroxidation ability was evaluated by modified methods from Ohkawa, Ohishi, and Yagi (1979). The liver tissue was homogenized in 150 mM Tris–HCl buffered saline (pH 7.2) with a polytron homogenizer and prepared in 20% homogenate (w/v). Briefly, 50 μl of liver homogenate, 30 μl of test sample, 10 μl of 4 mM $FeCl_2$, and 10 μl of 0.2 mM ascorbic acid were pipetted into a 2 ml microcentrifuge tube and then incubated at 37 °C for 1 h. Subsequently, 100 μl of 0.1 N HCl, 40 μl of 9.8% SDS, 180 μl of de-ionized water, and 400 μl of 0.6% TBA were successively pipetted into each tube and vigorously shaken. These tubes were then heated to 95 °C for 30 min. After cooling, tubes were treated with 1000 μl of *n*-butanol and centrifuged at 1000*g* for 25 min, and the supernatant was subsequently measured with an ELISA reader at 532 nm. All determinations were performed in triplicate. The percentage of lipid peroxidation inhibition was calculated as: $[1 - (\text{induced}_{532\text{nm}} - \text{sample}_{532\text{nm}})/(\text{induced}_{532\text{nm}} - \text{control}_{532\text{nm}})] \times 100\%$. The inhibitory concentration that suppressed 50% of the lipid peroxide production was expressed as IC_{50}.

2.6. Statistical analysis

All data were expressed as means ± standard deviation. Statistical analysis was done with one-way analysis of variance (ANOVA) and the Tukey–Kramer HSD test, using the SPSS software, was applied to calculate the statistical significance. $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. The flavonol contents of C. chinensis seed extracts and of their fractions

Quercetin and kaempferol are the common active polyphenol compounds and their pharmacological effects have also been reported in previous studies (Ackland, van de Waarsenburg, & Jones, 2005; Comalada et al., 2006; Wang et al., 2006). Both flavonol compounds are also present in many plants and herbs, such as *Opuntia cactus* (Kuti, 2004), *Fructus lycii* (Le, Chiu, & Ng, 2007), green tea (Park, Rho, Kim, & Chang, 2006), and *Ginkgo bilob* (van Beek, 2002). In the present study, the different extracts and fractions of the seeds from *C. chinensis* were assayed by chromatographic analysis, using the HPLC–UV, to determine the contents of quercetin and kaempferol. Typical chromatograms of the standard compounds and of the EA fraction are shown in Fig. 1. The calibration curves of quercetin and kaempferol also showed good linearity, and the limits of detection (LOD) were 0.05 and 0.06 μg/ml, respectively (Table 1). As shown in Fig. 2, our results indicated that the quercetin content of EA (54.8 ± 2.44 μg/mg) was highest among the extracts and fractions, followed by ALC (46.8 ± 1.63 μg/mg) as compared with NB (0.77 ± 0.08 μg/mg), H_2O (0.16 ± 0.03 μg/mg), OH (0.14 ± 0.01 μg/mg), and HX (0.02 ± 0.01 μg/mg, 2), respectively ($P < 0.05$). Moreover, EA also had the highest kaempferol content (258 ± 36.4 μg/mg) on comparing it with ALC (48.3 ± 3.78 μg/mg), NB (8.40 ± 1.41 μg/mg), H_2O (0.35 ± 0.02 μg/mg), HX (0.19 ± 0.01 μg/mg) and OH, which showed complete absence of kaempferol ($P < 0.05$). The flavonol contents of *C. chinensis* seed extracts and their fractions ranged in the following descending order: EA > ALC > NB > H_2O > HX > OH.

3.2. DPPH free radical-scavenging effect

DPPH assay has been widely used to determine the free radical-scavenging activity of various plants and pure compounds (Jeller, Silva, Liao, Bolzani Vda, & Furlan, 2004; Murias et al., 2005; Rathee, Patro, Mula, & Gamre, 2006). DPPH is a stable free radical which dissolves in

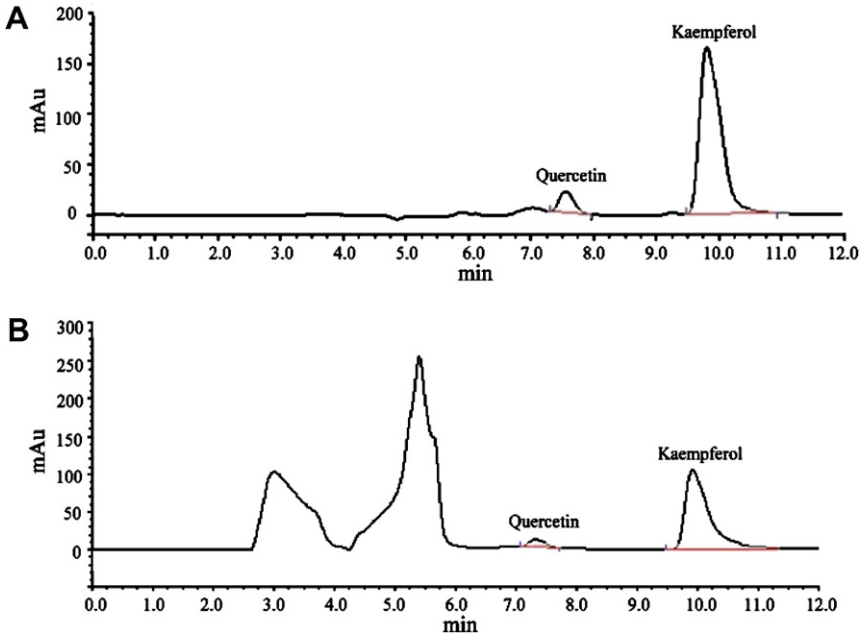

Fig. 1. HPLC chromatogram of (A) quercetin and kaempferol, and (B) ethyl acetate fraction.

Table 1
The calibration curve and the limits of detection of quercetin and kaempferol

Standard	Regression equation	r	LOD (μg/ml)
Quercetin	$y = 0.8875\ x + 0.1155$	0.9999	0.05
Kaempferol	$y = 2.8561\ x - 0.5217$	0.9998	0.06

methanol or ethanol, and its purple colour shows a characteristic absorption at 517 nm. When an antioxidant scavenges the free radical by hydrogen donation, the colour from the DPPH assay solution becomes light yellow. Fig. 3A shows that all the extracts and fractions from the seeds of *C. chinensis* were able to scavenge DPPH free radical in a concentration-dependent manner (data not shown). The SC_{50} value of the ethanol extract (124 ± 3.19 μg/ml) showed significant DPPH free radical-scavenging and was more effective (2.17-fold) than that of the water extract (269 ± 2.51 μg/ml). EA (50.0 ± 0.76 μg/ml) was the most effective fraction of the ethanol extract and the free radical-scavenging effect of EA was significantly better ($P < 0.05$) than the other organic fractions, including NB (143 ± 5.47 μg/ml), OH (377 ± 12.7 μg/ml), and HX (4130 ± 85.9 μg/ml). In addition, the reference compounds, quercetin (7.11 ± 0.29 μg/ml) and kaempferol (10.2 ± 0.06 μg/ml), exhibited the most effective DPPH free radical-scavenging effect when compared with all test samples ($P < 0.05$). The result suggested that the ethanol extract of the seeds from *C. chinensis* possibly donated hydrogen from phenolic hydroxyl groups in order to discontinue the free radical chain reaction and prevent damage from free radicals, an event in which, particularly, its organic fraction, EA, showed the strongest free radical-scavenging effect in comparison to other extraction fractions. Since the DPPH assay was used as a preliminary free radical-scavenging evaluation, we subsequently performed analysis on superoxide scavenging, superoxide anion formation, and anti-lipid peroxidation to confirm the free radical-scavenging effect from the seed extracts of *C. chinensis*.

3.3. Superoxide anion-scavenging activity

Superoxide anion ($O_2^{\cdot-}$) is a reactive oxygen species (ROS) that can be generated in the human body by auto-oxidative processes. ROS have a causal relationship with oxidative stress (Skulachev, 1998), and the accumulation of oxidative stress over a long period of time can increase the occurrence of many chronic clinical diseases, such as cardiovascular disease, diabetes, liver injury, cancer, and aging, among many others. In the cytochrome c/xanthine oxidase system, xanthine is converted to uric acid by xanthine oxidase; meanwhile, the $O_2^{\cdot-}$ generated would reduce ferricytochrome c to ferrocytochrome c. The reduction of cytochrome c method was used in the present study to determine the $O_2^{\cdot-}$ -scavenging effect. The SC_{50} values of superoxide anion scavenging activity from all test samples from the seeds of *C. chinensis* are shown in Fig. 3B. The results indicated that the $O_2^{\cdot-}$-scavenging effect of the ethanol extract was significantly better than that of the water extract (314 ± 9.80 μg/ml versus 997 ± 76.9 μg/ml, 3.17-fold). The EA fraction was observed to possess the strongest $O_2^{\cdot-}$-scavenging activity (54.3 ± 2.19 μg/ml) among all other organic fractions ($P < 0.05$) with a significant difference of 14.9-fold, 39.6-fold, and 68.3-fold, when compared with NB (811 ± 38.3 μg/ml), HX (2147 ± 130 μg/ml), and OH (3709 ± 8.78 μg/ml), respectively. Moreover, quercetin (1.90 ± 0.07 μg/ml) and kaempferol (2.73 ± 0.25 μg/ml) displayed the highest scavenging effect on $O_2^{\cdot-}$ when compared with all test samples ($P < 0.05$). The results suggest

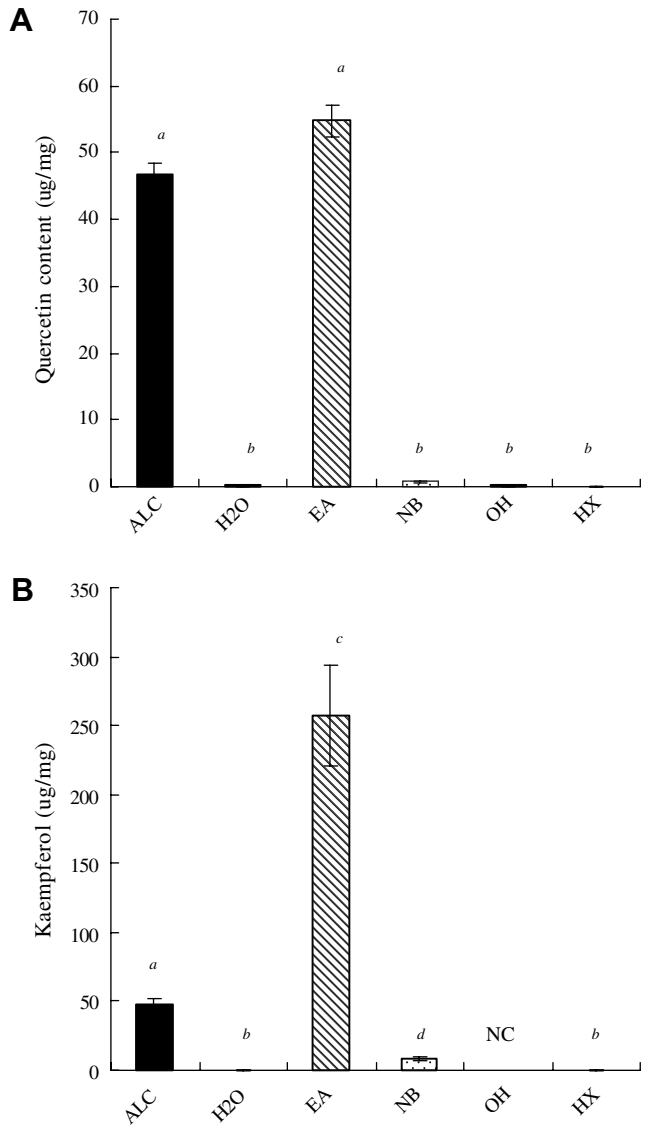

Fig. 2. Determination of the quercetin (A) and kaempferol (B) contents of different extracts and fractions of *C. chinensis* seeds by HPLC analysis. H_2O: water extract; ALC: ethanol extract; EA: ethyl acetate fraction; NB: *n*-butanol fraction; OH: organic water fraction; HX: *n*-hexane fraction. Bars not connected by the same letter are significantly different ($P < 0.05$). NC: no content.

that the *C. chinensis* seed extracts display scavenging effect on $O_2^{\cdot-}$ generation that could help prevent or ameliorate oxidative damage.

3.4. Anti-superoxide anion formation

Xanthine oxidase (XO), a flavoprotein, is widely distributed in the human body, including heart, liver, lung, kidney, brain and gut, as well as blood vessels. XO can catalyze the oxidation of hypoxanthine to xanthine to form, finally, uric acid; meanwhile, this system produces $O_2^{\cdot-}$ (Hille & Nishino, 1995) that also rapidly reacts with nitric oxide or nitrosothiols to form another cytotoxic oxidant, peroxynitrite, which can participate in a chain reaction of oxidative injury process to damage DNA, lipid, and protein (Pacher, Nivorozhkin, & Szabo, 2006). Circulating XO activity plays an important role in many diseases, such as ischemic injuries (Mallick, Yang, Winslet, & Seifalian, 2004), gout (Pacher et al., 2006), inflammatory diseases (Aslan & Freeman, 2002), and chronic heart failure (Berry & Hare, 2004). Therefore, a XO inhibitor will likely help prevent the development of many oxidation-related diseases. As shown in Fig. 3C, the crude extracts and the organic fractions from the seeds of *C. chinensis* displayed anti-superoxide anion activity in the xanthine oxidase inhibitor test. The IC_{50} value of the ethanol extract (859 ± 52.4 μg/ml) indicated more effective inhibition of superoxide anion generation when compared with the water extract (7159 ± 568 μg/ml) ($P < 0.05$). Moreover, the EA fraction (140 ± 4.93 μg/ml) was observed to be an excellent xanthine oxidase inhibitor, presenting a superior anti-superoxide anion formation activity of 15.1-fold, 52.2-fold, and 61.0-fold, when compared with NB (2118 ± 175 μg/ml), OH (7317 ± 612 μg/ml), and HX (8554 ± 462 μg/ml), respectively ($P < 0.05$). In addition, quercetin (4.22 ± 0.09 μg/ml) and kaempferol (6.26 ± 0.18 μg/ml) were the most effective in inhibiting superoxide anion formation when compared with all test samples ($P < 0.05$). These results indicated that *C. chinensis* seed extracts could be a XO inhibitor to decrease $O_2^{\cdot-}$ generation and to further prevent the secondary oxidative damage.

3.5. Anti-lipid peroxidation activity

The fluidity and permeability of the cell membrane is maintained by its composition, including phospholipid, glycerides, and fatty acids. In the event of an excess of free radical attacks on the lipids of the cell membrane, and particularly on the polyunsaturated fatty acids, a chain reaction of lipid peroxidation (LPO) ensues that could lead to cell death. Previous studies have demonstrated that iron ions participate in the Fenton reaction and generate superoxide anion and hydroxyl radical as by-products (Neyens & Baeyens, 2003). Furthermore, these free radicals help induce the chain reaction of LPO. Malondialdehyde (MDA) is an end-product and an index of LPO that reacts with thiobarbituric acid (TBA) in the LPO assay (Marnett, 1999). In the present study, the anti-lipid peroxidation was determined, based on iron ions-induced thiobarbituric acid-reactive substances (TBARS) in rat liver homogenate. As indicated in Fig. 3D, all *C. chinensis* seed extract and fraction test samples with the exception of the HX fraction, exhibited anti-lipid peroxidation. The results from the IC_{50} values indicated that the ethanol extract (91.2 ± 7.23 μg/ml) was more effective in inhibiting lipid peroxidation (61.7-fold) than its water extract counterpart (5628 ± 397 μg/ml). In addition, the EA fraction displayed a superior anti-lipid peroxidation IC_{50} value (72.2 ± 18.0 μg/ml) compared with NB (168 ± 40.4 μg/ml) and OH (1272 ± 52.4 μg/ml) ($P < 0.05$). HX did not

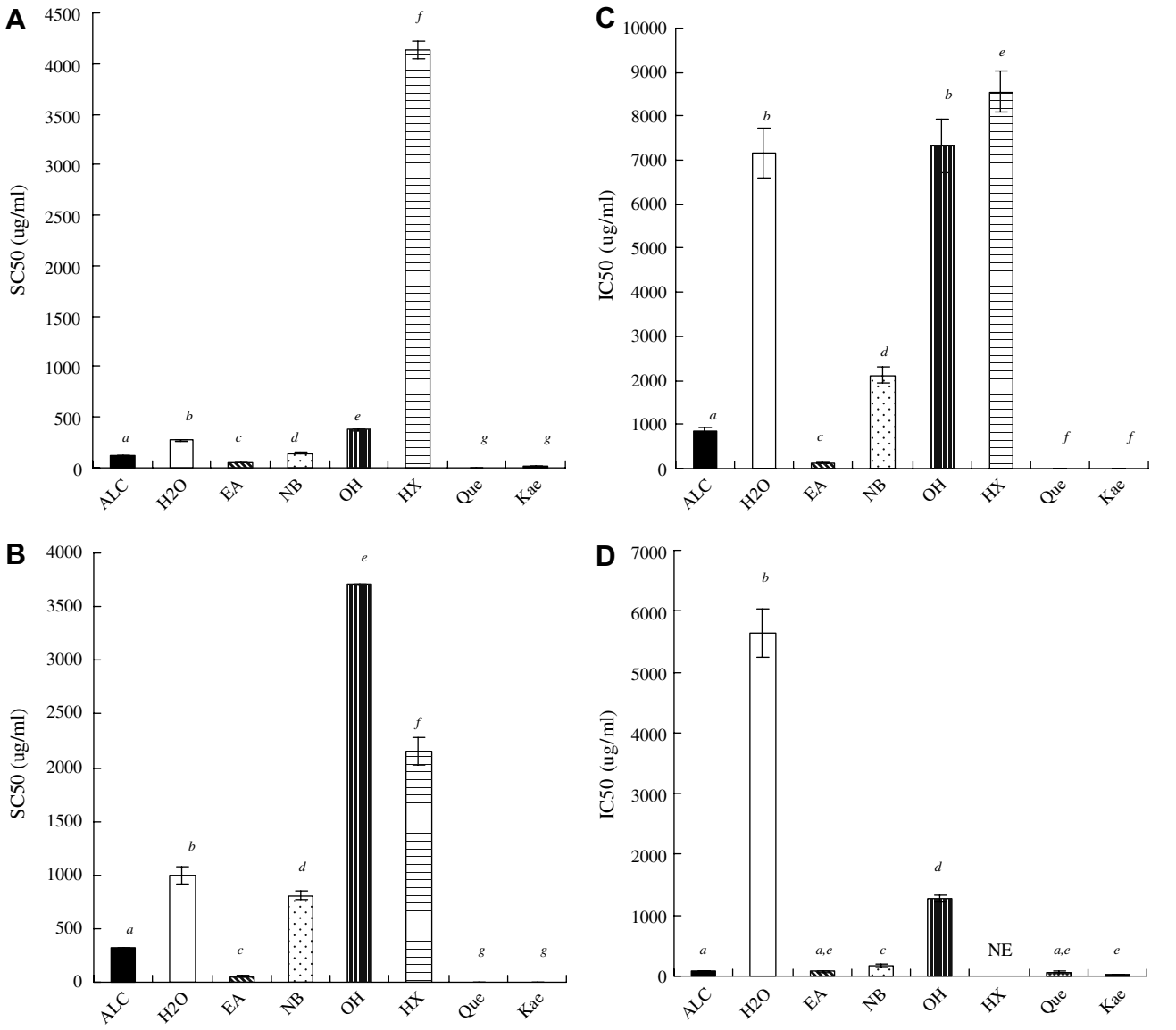

Fig. 3. Antioxidant activities of different extracts and fractions of seeds from *C. chinensis*, using quercetin and kaempferol as positive controls. (A) DPPH assay; (B) $O_2^{\cdot-}$-scavenging effect; (C) anti-superoxide anion formation; (D) anti-lipid peroxidation. H_2O: water extract; ALC: ethanol extract; EA: ethyl acetate fraction; NB: *n*-butanol fraction; OH: organic water fraction; HX: *n*-hexane fraction; Que: quercetin; Kae: kaempferol. Bars not connected by the same letter are significantly different ($P < 0.05$). NE: no effect.

exhibit any effect in this assay. The pure compounds quercetin (64.8 ± 8.49 μg/ml) and kaempferol (33.5 ± 0.69 μg/ml) were the most effective in preventing the lipid peroxidation when compared with all test samples ($P < 0.05$). The above results suggest that *C. chinensis* seed extracts could inhibit the lipid peroxide-induced chain oxidation of the cell membrane to help prevent oxidative damage.

Altogether, the above results demonstrated that the seeds of *C. chinensis* possess antioxidant activities as assessed from the DPPH free radical-scavenging, superoxide anion-scavenging, anti-superoxide anion formation, and anti-lipid peroxidation effects. The overall order of highest efficiency of its extracts and fractions was determined as follows: EA > ALC > NB > H_2O > OH > HX.

The flavonols, quercetin and kaempferol, are the major active chemical compounds of *C. chinensis*, and they also exist in many other plants (Kuti, 2004; Le et al., 2007; van Beek, 2002). Both quercetin and kaempferol have a 3-hydroxy group of the catechol structure in ring B, and they also have a 2,3-double bond in conjunction with a 4-carbonyl group in ring C. The flavonol structure can dramatically increase the resonance stabilization for electron delocalization, as established by its structure–activity relationship (SAR), and this suggests that the SAR of the flavonol structure could enhance the antioxidant activities (Bors, Heller, Michel, & Saran, 1990).

3.6. Concordance between antioxidant activities and flavonol content

The results presented above have indicated that the antioxidant activities of the seeds from *C. chinensis* are likely

attributed to the flavonol compounds and their quantities in each extract and fraction. The order for highest flavonol content was identical to the order of most efficient antioxidant activities determined above: EA > ALC > NB > H_2O > OH > HX. The weaker antioxidant activities exhibited by the water extract from the seeds of *C. chinensis* when compared with its ethanol extract could be explained by the lower contents of these flavonols, due to their poor water solubility (Calabro et al., 2004).

4. Conclusion

In summary, the present results have demonstrated that the antioxidant activities of *C. chinensis* seed extracts and fractions have a direct correlation with and are in proportion to its flavonol content. The EA organic fraction from the ethanol extract was observed to possess strongest antioxidant effects with highest content of the flavonol active compounds quercetin and kaempferol. Moreover, our studies indicate that *C. chinensis* ethanol extract is an effective antioxidant for preventing free radical damage to cell membranes through scavenging of free radicals and by inhibiting the lipid peroxidation process. This could explain its frequent addition to the Chinese medicated diet (beverage and porridge) for promoting human health and for disease prevention.

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