Food Chemistry 115 (2009) 1019–1024

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Free radical-scavenging phytochemicals of hot water extracts of *Acacia confusa* leaves detected by an on-line screening method

Yu-Tang Tung [a], Jyh-Horng Wu [b], Ching-Yu Hsieh [a], Ping-Sheng Chen [a], Shang-Tzen Chang [a,*]

[a] School of Forestry and Resource Conservation, National Taiwan University, #1, sec. 4, Roosevelt Rd., Taipei 106, Taiwan
[b] Department of Forestry, National Chung-Hsing University, Taichung 402, Taiwan

ARTICLE INFO

Article history:
Received 15 October 2008
Received in revised form 18 November 2008
Accepted 12 January 2009

Keywords:
Acacia confusa
Leaf
Hot water extracts
On-line RP-HPLC-DPPH
Phenolic compounds

ABSTRACT

Acacia confusa Merr. (Leguminosae), a species native to Taiwan, is widely distributed on the hills and lowlands of Taiwan, and has been traditionally used as a medicine. In this study, phytochemicals and antioxidant activities of hot water extracts from *A. confusa* leaves were investigated for the first time. Among all the fractions from hot water extracts of leaves, the EtOAc-soluble fraction exhibits the best DPPH radical-scavenging activity, superoxide radical-scavenging activity, and reducing power. In addition, a rapid screening method, on-line RP-HPLC-DPPH system, for individual antioxidants in the EtOAc-soluble fraction was developed. Furthermore, following solid phase extraction (SPE) and reverse-phase high-performance liquid chromatography 12 pure phenolic compounds, including five major compounds (gallic acid, (+)-catechin, (−)-epicatechin, myricetin 3-glucopyranoside, and myricetin 3-rhamnopyranoside) were detected using the developed screening method. These results demonstrated that hot water extracts of *A. confusa* leaves have excellent antioxidant activities and thus have great potential as a source for natural health products.

1. Introduction

Acacia confusa Merr. (Leguminosae), a native species in Taiwan, is widely distributed on the hills and lowlands of Taiwan, and has been traditionally used as a medicine (Wu et al., 2005). In our previous studies, it has been found that the crude extracts of *A. confusa* heartwood, flower, and bark clearly have strong antioxidant effects, and contain a wide variety of phenolic compounds (Chang et al., 2001; Tung, Wu, Huang, Kuo, & Chang, 2009; Tung, Wu, Kuo, & Chang, 2007; Wu, Huang, Tung, & Chang, 2008; Wu et al., 2005). In addition, the extract of *A. confusa* leaves is used in Taiwan for wound healing and anti-blood-stasis (Kan, 1978) and has been reported to contain myricetin 3-*O*-(2″-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether, myricitrin, myricetin 3-*O*-(3″-*O*-galloyl)-α-rhamnopyranoside 7-methyl ether, myricetin 3-*O*-(2″,3″-di-*O*-galloyl)-α-rhamnopyranoside, myricitrin 7-methyl ether, myricetin 3-*O*-(2″-*O*-galloyl)-α-rhamnopyranoside, and myricetin 3-*O*-(3″-*O*-galloyl)-α-rhamnopyranoside (Lee, Qiu, Waller, & Chou, 2000). Numerous researches have revealed that some phenolic compounds have anticancer, anticarcinogenic or antimutagenic activities, and these bioactivities of phenolic compounds might be related to their antioxidant properties (Chung, Wong, Wei, Huang, & Lin, 1998; Kaur, Grover, & Singh, 1998; Tung et al., 2007). Therefore, the leaves of *A. confusa* may be a good candidate for further development as an antioxidant remedy. However, to the best of our knowledge there is no prior report on the antioxidant activities of hot water extracts from *A. confusa* leaves. Thus, the aim of this study was to investigate the antioxidant compounds of *A. confusa* leaves, which were detected by a rapid screening method, namely an on-line HPLC-DPPH method. Furthermore, the content, the antioxidant activities, and the structure-activity relationships of these phytocompounds were also investigated.

2. Materials and methods

2.1. Chemicals

1,1-Dipheny1-2-picrylhydrazyl (DPPH), quercetin, nitroblue tetrazolium chloride (NBT), trichloracetic acid (TCA), and (+)-catechin were all purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals and solvents used in this experiment were of analytical grade.

2.2. Sampling

The leaves of *A. confusa* were sampled from the Fu-Chou Moutain in Taipei City. The species was identified by Sheng-You Lu of the Taiwan Forestry Research Institute, and a voucher specimen (AC005) was deposited at the School of Forestry and Resource

* Corresponding author. Tel.: +886 2 33664626; fax: +886 2 23654520.
E-mail address: peter@ntu.edu.tw (S.-T. Chang).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.01.026

Conservation, National Taiwan University. The materials were air dried at ambient temperature (25 °C).

2.3. Extraction and isolation

Leaves were added to boiling double-distilled water and allowed to infuse for 4 h. The extract was decanted, filtered under vacuum, concentrated in a rotary evaporator, and then lyophilised. The resulting crude extract (7.3 g) was fractionated successively with EtOAc, *n*-butanol (BuOH), and water to yield soluble fractions of EtOAc (0.9 g), BuOH (2.0 g), and H_2O (4.1 g). The antioxidative phytochemicals from the EtOAc-soluble fraction were separated and purified by semipreparative HPLC on a model PU-980 pump (Jasco, Tokyo, Japan) equipped with a MD-910 photo-diode array detector (Jasco) and a 250 mm × 10.0 mm i.d., 5 μm Luna RP-18 column (Phenomenex, Torrance, CA). The mobile phase was solvent **A**, 100% acetonitrile; and solvent **B**, ultrapure water. Elution conditions were 0–23 min of 8–50% **A** to **B** (linear gradient); 23–30 min of 50–100% **A** to **B** (linear gradient); 30–35 min of 100% **A** at flow rate of 4 ml/min. ESI-MS data were collected using a Finnigan MAT-95S mass spectrometer, and NMR spectra were recorded by a Bruker Avance 500 MHz FT-NMR spectrometer. The structures of antioxidative compounds **1**–**12** (as shown in Fig. 1) were identified by ESI-MS and NMR, and all spectral data were consistent with those reported in the literature (Chen, Wang, Rosen, & Ho, 1999; Chung, Kim, Takaya, Terashima, & Niwa, 2004; Furusawa et al., 2003; Kazuma, Noda, & Suzuki, 2003; Khallouki et al., 2007).

2.4. 1,1-Diphenyl-2-picrylhydrazyl assay (DPPH assay)

The DPPH radical-scavenging activity of the test extracts or compounds from *A. confusa* leaves was examined according to the method reported by Chang et al. (2001). Briefly, 10 μl of test samples in DMSO were mixed with 90 μl of 50 mM Tris–HCl buffer (pH 7.4) and 200 μl of 0.1 mM DPPH–ethanol solution. After 30 min of incubation at ambient temperature, the reduction of the DPPH radical was measured by reading the absorbance at 517 nm using an ELISA reader. (+)-Catechin and quercetin, well-known antioxidants, were used as positive controls. Three replicates were made for each test sample. The inhibition ratio (percent) was calculated according to the following equation:

$$\%\ \text{inhibition} = [(\text{absorbance of control} - \text{absorbance of sample})/\text{absorbance of control}] \times 100.$$

2.5. Superoxide radical-scavenging assay (NBT assay)

Measurement of superoxide radical-scavenging activity was carried out according to the method of Chang et al. (2001). First, 20 μl of 15 mM Na_2EDTA in buffer (50 mM KH_2PO_4/KOH, pH 7.4), 50 μl of 0.6 mM nitroblue tetrazolium chloride (NBT) in buffer, 30 μl of 3 mM hypoxanthine in 50 mM KOH, 5 μl of the test extracts or compounds in DMSO, and 145 μl of buffer were mixed in 96-well microplates. The reaction was started by adding 50 μl of xanthine oxidase in buffer (1 unit in 10 ml buffer) to the mixture. The reaction mixture was incubated at ambient temperature, and the absorbance at 570 nm was determined every 1 min up to 8 min using the ELISA reader. (+)-Catechin and quercetin were used as positive controls. Three replicates were made for each test sample. The percent inhibition ratio (percent) was calculated according to the following equation:

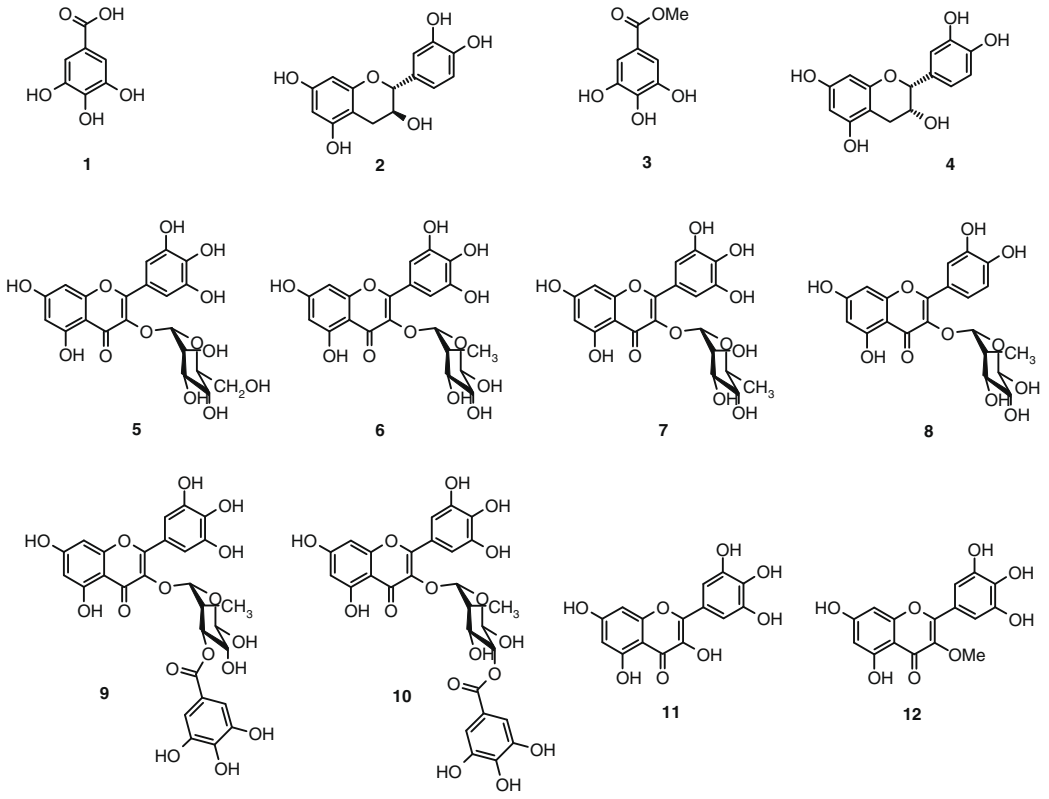

Fig. 1. Phytochemicals isolated from leaves of *A. confusa*. **1**, Gallic acid; **2**, (+)-catechin; **3**, gallic acid methyl ester; **4**, (−)-epicatechin; **5**, myricetin 3-glucopyranoside; **6**, myricetin 3-rhamnopyranoside; **7**, myricetin 3-rhamnoside; **8**, quercetin 3-rhamnopyranoside; **9**, myricetin 3-*O*-(2″-*O*-galloyl)-rhamnopyranoside; **10**, myricetin 3-*O*-(3″-*O*-galloyl)-rhamnopyranoside; **11**, myricetin; **12**, myricetin 3-methyl ether.

$$\% \text{ inhibition} = [(\text{rate of control reaction} - \text{rate of sample reaction})/\text{rate of control reaction}] \times 100.$$

2.6. Reducing power assay

This assay was determined according to the method reported by Oyaizu (1986), with slight modifications. Briefly, 1 ml of reaction mixture, containing 500 μl the test extracts or compounds in 500 μl phosphate buffer (0.2 M, pH 6.6), was incubated with 500 μl potassium ferricyanide (1%, w/v) at 50 °C for 20 min. The reaction was terminated by adding trichloroacetic acid (10%, w/v), and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant solution (500 μl) was mixed with distilled water (500 μl) and 100 μl of ferric chloride (0.1%, w/v) solution, and the absorbance was measured at 700 nm. Three replicates were made for each test sample. Increased absorbance of the reaction mixture indicated increased reducing power. Furthermore, the reducing power of compounds was expressed as (+)-catechin equivalent (CE) in millimoles per millimole of compound.

2.7. Total flavanoid contents

Total flavonoid contents were determined by the $AlCl_3$ method (Quettier-Deleu et al., 2000), using rutin as a standard. The test samples were dissolved in DMSO. The sample solution (150 μl) was mixed with 150 μl of 2% $AlCl_3$. After 10 min of incubation at ambient temperature, the absorbance of the supernatant was measured at 435 nm. Three replicates were made for each test sample. The total flavonoid contents were expressed as rutin equivalents (RE) in milligrams per gram sample.

2.8. On-line DPPH radical-scavenging analysis

The extract of *A. confusa* leaves with the best antioxidant activity (EtOAc fraction) was further monitored by an on-line RP-HPLC-DPPH method. The instrumental setup was applied according to the method reported by Wu et al. (2008). The EtOAc fraction was monitored by analytical HPLC on a model PU-980 instrument (Jasco) with a 250 mm × 4.6 mm i.d., 5 μm Luna RP-18 column (Phenomenex). The mobile phase was solvent **A**, 100% acetonitrile; and solvent **B**, ultrapure water. Elution conditions were 0–62 min of 8–50% **A** to **B** (linear gradient); 62–65 min of 50–100% **A** to **B** (linear gradient) at a flow rate of 0.75 ml/min using a detector, Jasco MD-910 photo diode array, at 280 nm wavelength. As for on-line DPPH radical-scavenging analysis, the flow of DPPH reagent (50 mg/ml in methanol) was set at 0.375 ml/min, and the induced bleaching was detected photometrically as a negative peak at 517 nm.

2.9. Statistical analyses

All results were expressed as mean ± SD ($n = 3$). The significance of difference was calculated by Scheffe's test, and values <0.05 were considered to be significant.

3. Results and discussion

3.1. DPPH radical-scavenging activity of A. confusa leaf extracts

DPPH is one of the compounds that have a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers (Yamaguchi, Takamura, Matoba, & Terao, 1998). It is well accepted that the DPPH radical-scavenging by antioxidants is attributable to their hydrogen-donating ability (Chen & Ho, 1995). Accordingly, as shown in Fig. 2, the DPPH radical-scavenging activity of hot water extract and its derived soluble fractions from *A. confusa* leaves, including the soluble fractions of EtOAc, BuOH, and water was shown to occur in a dose-dependent manner. Of these, the EtOAc-soluble fraction showed the strongest activity. Meanwhile, except for the water-soluble fraction, all extracts showed a good inhibitory activity against the DPPH radical. The concentration required to inhibit 50% radical-scavenging effect (IC_{50}) was determined from the results of a series of concentrations tested. A lower IC_{50} value corresponds to a larger scavenging activity. The IC_{50} values of the crude extract, EtOAc fraction, BuOH fraction, and water fraction were 17.6, 8.5, 14.7, and >50 μg/ml, respectively. As for (+)-catechin, a well-known antioxidant compound used as the reference control in this study, its IC_{50} value was 5.9 μg/ml. These results imply that there are abundant antioxidative phytochemicals present in the leaf extracts of *A. confusa*, especially in the EtOAc fraction. Comparison with the results obtained by Wu et al. (2008) shows that *A. confusa* flower extracts were found to possess significant DPPH radical-scavenging activity (IC_{50} = 62.9 μg/ml), indicating that *A. confusa* leaf extracts have better potential source as antioxidants than its flower extracts.

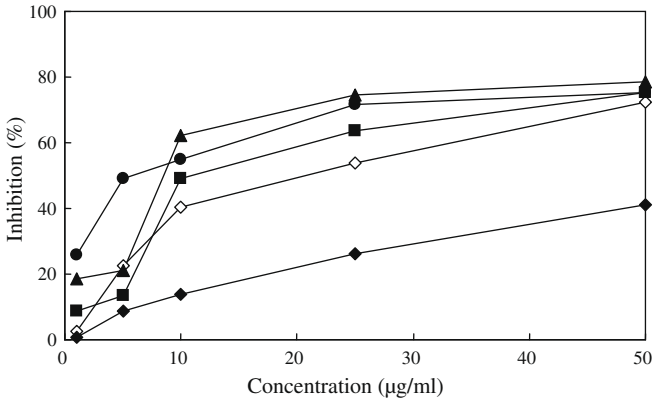

Fig. 2. Free radical-scavenging activity of hot water extracts from leaves of *A. confusa* measured by DPPH assay: (◇) crude extract, (▲) EtOAc fraction, (■) *n*-butanol fraction, (♦) water fraction, (●) (+)-catechin. Results are mean ± SD ($n = 3$).

3.2. Superoxide radical-scavenging activity of A. confusa leaf extracts

Superoxide radical was generated by the hypoxanthine-xanthine oxidase and NBT systems in this assay. Fig. 3 shows the superoxide radical-scavenging activity of hot water extract and

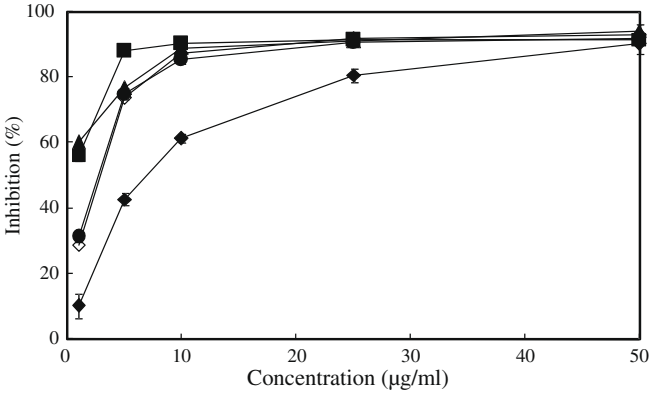

Fig. 3. Free radical-scavenging activity of hot water extracts from leaves of *A. confusa* measured by NBT assay: (◇) crude extract, (▲) EtOAc fraction, (■) *n*-butanol fraction, (♦) water fraction, (●) (+)-catechin. Results are mean ± SD ($n = 3$).

its derived fractions from *A. confusa* leaves, compared with (+)-catechin. At the 1 μg/ml test concentration, the superoxide radical inhibition of *A. confusa* leaf extract and its derived fractions decreased in the following order: EtOAc fraction (60.4%) > BuOH fraction (56.4%) > crude extract (28.9%) > water fraction (10.2%). The IC_{50} values of (+)-catechin, crude extract, EtOAc fraction, BuOH fraction, and water fraction were 2.1, 2.2, 0.9, 1.5, and 6.3 μg/ml, respectively. These results revealed that the EtOAc fraction possessed the highest antioxidant activity, which was similar to the DPPH radical-scavenging activity results. Results reported by Chua, Tung, and Chang (2008) demonstrated that the crude extract and its BuOH fraction from *Cinnamomum osmophloeum* twigs, a traditional medicinal plant, also showed an excellent inhibitory activity against superoxide radical with IC_{50} values of 6.3 and 4.9 μg/ml, respectively. Comparing these results indicates that *A. confusa* leaf extracts would be an excellent source as a natural antioxidant and merit further investigation.

3.3. Reducing power of A. confusa leaf extracts

Previous reports have demonstrated that the reducing power in plants can be correlated with their antioxidant activities (Tanaka, Kuie, Nagashima, & Taguchi, 1998). The reducing properties have been shown to exert antioxidant action by breaking the free radical chain through the donation of a hydrogen atom (Gordon, 1990). The data in Fig. 4 show the reducing power (as indicated by the absorbance at 700 nm) of the crude extract and its derived fractions, and their ranking order (25 μg/ml) was as follows: EtOAc fraction (optical density (*OD*) = 1.42) > BuOH fraction (*OD* = 0.75) > crude extract (*OD* = 0.52) > water fraction (*OD* = 0.17). The reducing power of the test samples correlated well with increasing concentrations. However, the reducing power of (+)-catechin was relatively more pronounced than that of all the test samples. Shon, Choi, Kahng, Nam, and Sung (2004) reported that red onion, yellow onion, and white onion extracts had absorption values of 0.17, 0.12 and 0.12, respectively at a concentration of 1 mg/ml in the reducing power assay. Comparing the two results shows that the reducing power of *A. confusa* leaf extracts and their derived fractions are much better than that of the onion extracts.

3.4. Total flavonoid contents of A. confusa leaf extracts

Plant flavonoids, in general, are highly effective free-radical scavengers and antioxidants. The total flavonoid contents in the crude extract and its derived fractions were calculated as rutin equivalents (RE) in milligrams per gram sample. Apparently, the total flavonoid content of the EtOAc fraction (355.3 mg/g) was higher than that of the crude extract (55.2 mg/g), BuOH fraction (44.9 mg/g) and water fraction (2.3 mg/g). These results suggested that the antioxidant activity of leaf extracts may correlate with its flavonoid content, and it is proposed here that the phytochemicals from the hot water extracts of *A. confusa* leaves may play an important role in the DPPH radical-scavenging activity, superoxide radical-scavenging activity, and reducing power. These results indicate that antioxidant activity of hot water extracts of *A. confusa* leaves can be effectively enriched in the EtOAc fraction. Thus, the EtOAc fraction was further investigated in this study for its phytochemical characteristics and *in vivo* antioxidant activity.

3.5. On-line RP-HPLC-DPPH method

The on-line RP-HPLC-DPPH method can be used for a rapid assessment of pure antioxidant compounds in complex mixtures, particularly plant extracts (Koleva, Niederländer, & van Beek, 2000; Wu et al., 2008). The more rapidly the absorbance decreases, the more potent the antioxidant activity of the compound in terms of hydrogen-donating ability will be (Gadow, Joubert, & Hansmann, 1997). Combined UV (positive signals) and DPPH· quenching (negative signals) chromatograms under gradient conditions of the EtOAc fraction from *A. confusa* leaves are presented in Fig. 5. Several eluted phytochemicals in the EtOAc fraction were detected and gave positive peaks on the UV detector (280 nm). Among them, gallic acid (**1**), (+)-catechin (**2**), gallic acid methyl ester (**3**), (−)-epicatechin (**4**), myricetin 3-glucopyranoside (**5**), myricetin 3-rhamnopyranoside (**6**), myricetin 3-rhamnoside (**7**), quercetin 3-rhamnopyranoside (**8**), myricetin 3-*O*-(2''-*O*-galloyl)-rhamnopyranoside (**9**), myricetin 3-*O*-(3''-*O*-galloyl)-rhamnopyranoside (**10**), myricetin (**11**), and myricetin 3-methyl ether (**12**) showed hydrogen-donating capacity (negative peak) towards the DPPH radical at the applied concentration. Results revealed that the method can be applied for a quick screening of antioxidant compounds or, more precisely, radical-scavenging activity of compounds. Thus, it is no longer necessary to isolate and purify non-target phytochemicals, leading to very significant reductions in costs and faster results.

3.6. Quantification and antioxidant activities of major active compounds in A. confusa leaf extracts

According to the screening result of the on-line RP-HPLC-DPPH system, gallic acid (**1**), (+)-catechin (**2**), (−)-epicatechin (**4**), myrice-

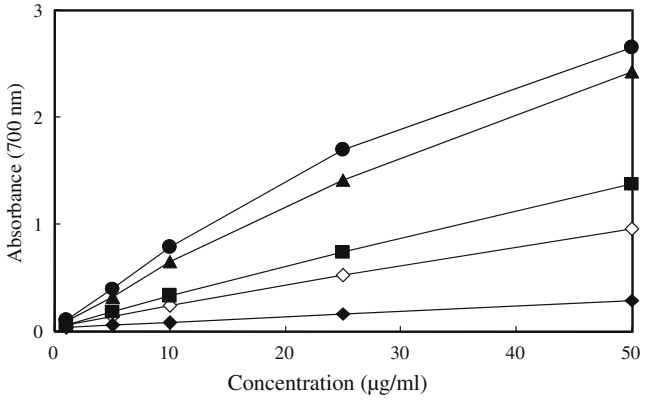

Fig. 4. Reducing power of hot water extracts from leaves of *A. confusa*: (◇) crude extract, (▲) EtOAc fraction, (■) *n*-butanol fraction, (♦) water fraction, (●) (+)-catechin. Results are mean ± SD (n = 3).

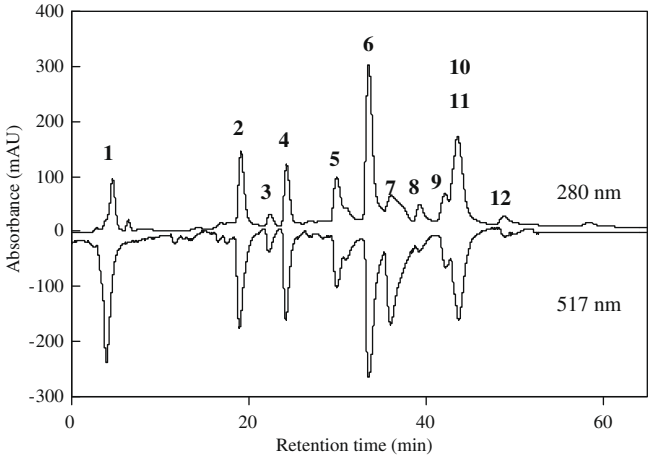

Fig. 5. UV and DPPH radical-quenching chromatograms of EtOAc fraction extracted from leaves of *A. confusa*.

Table 1
Contents, reducing power, and IC_{50} values of major phytochemicals from leaves of *A. confusa* in inhibiting DPPH radical and superoxide radical.

Phytochemicals	IC_{50} (μM)		Reducing power (CE)*	Concentration (mg/g of hot water extract)
	DPPH radical	Superoxide radical		
Gallic acid (**1**)	11.6	12.4	1.81	3.0 ± 0.1
(+)-Catechin (**2**)	19.6	17.9	1.00	10.8 ± 0.0
Gallic acid methyl ester (**3**)	12.9	15.4	1.30	–
(−)-Epicatechin (**4**)	28.8	23.3	0.61	17.4 ± 0.6
Myricetin 3-glucopyranoside (**5**)	12.5	3.1	1.03	12.1 ± 0.5
Myricetin 3-rhamnopyranoside (**6**)	12.9	2.9	1.12	19.1 ± 0.3
Myricetin 3-rhamnoside (**7**)	12.1	3.1	0.95	–
Quercetin 3-rhamnopyranoside (**8**)	19.2	7.4	0.66	–
Myricetin 3-*O*-(2″-*O*-galloyl)-rhamnopyranoside (**9**)	4.0	1.5	1.44	–
Myricetin 3-*O*-(3″-*O*-galloyl)-rhamnopyranoside (**10**)	5.1	1.5	1.47	–
Myricetin (**11**)	8.4	2.6	2.90	–
Myricetin 3-methyl ether (**12**)	11.9	3.2	1.09	–
Quercetin (positive control)	12.7	5.8	1.03	

Results are mean ± SD (n = 3).
* CE: (+)-Catechin equivalents in millimoles per millimole sample.

tin 3-glucopyranoside (**5**), and myricetin 3-rhamnopyranoside (**6**) were found to be the five major bioactive phytochemicals in the EtOAc fraction, and their contents were determined to be 3.0, 10.8, 17.4, 12.1 and 19.1 mg per gram of hot water extract, respectively (Table 1). To determine the antioxidant activities of 12 phytochemicals, DPPH, NBT, and reducing power assays were performed. Quercetin was used as a positive control. According to the results of Table 1, compounds **1**, **5**, **7**, and **9**–**12** exhibited greater DPPH radical-scavenging activity than quercetin. On the other hand, the decreasing superoxide radical-scavenging activity order of phytochemicals in NBT assay can be ranked as **9** = **10** > **11** > **6** > **5** = **7** > **12** > **8** > **1** > **3** > **2** > **4**. Among the 12 phytochemicals, except for compounds **1**–**4** and **8**, all the other compounds exhibited an excellent superoxide radical-scavenging activity. Their IC_{50} values were less than quercetin (IC_{50} = 5.8 μM). As for the reducing power, compound **11** ranked as the best, followed by compounds **1**, **10**, **9**, **3**, **6**, **12**, **5**, **2**, **7**, **8** and **4**. Additionally, among them, compounds **1, 3**, **6** and **9**–**12** were more active than quercetin in reducing power assay.

3.7. Structure-activity relationships of flavonoids from A. confusa leaf extracts

The study also investigated the structure-activity relationships of flavonoids in terms of their antioxidant activities. Accordingly, among DPPH, NBT, and reducing power assays, different sugars at the C3 position of flavonoids, such as myricetin 3-glucopyranoside (**5**), myricetin 3-rhamnopyranoside (**6**), myricetin 3-rhamnoside (**7**) exhibited similar antioxidant activities. This result showed that the different sugar moiety on a flavonoid does not notably affect its antioxidant activities. The order of antioxidant activities were as follows: myricetin 3-*O*-(2″-*O*-galloyl)-rhamnopyranoside (**9**) = myricetin 3-*O*-(3″-*O*-galloyl)-rhamnopyranoside (**10**) > myricetin 3-rhamnopyranoside (**6**). According to the results, it is clear that gallate acylation on the glycoside moiety plays an essential role for enhancing antioxidant activities relative to that of the corresponding glycosides. This result is in accordance with the conclusions obtained by Moharram, Marzouk, Ibrahim, and Mabry (2006).

4. Conclusion

It is well-known that free radicals are one of the causes of several diseases. This study demonstrated for the first time that, among the hot water extract and its derived soluble fractions from *A. confusa* leaves, the EtOAc-soluble fraction possessed the highest antioxidant activities and free radical-scavenging activities. On the other hand, the results confirm the feasibility of assessing radical-scavenging activity of specific phytochemicals using the on-line HPLC-DPPH method. This technique could allow rapid detection of natural antioxidants in complex matrices with simple operation. Accordingly, 12 specific antioxidants were detected and identified from the leaf extracts of *A. confusa*. Thus, these results showed that hot water extracts from leaves of *A. confusa* had great potential in preventing diseases caused by the overproduction of radicals and it might be extensively used for the treatment of degenerative diseases. Future studies should focus on the employment of modern medical chemical techniques to modify the structures of specific plant ingredients into better agents with high efficacy and activity. In addition, *in vivo* pharmacological researches should also be conducted.

Acknowledgments

We thank the Forestry Bureau of Council of Agriculture for the financial support (94-00-5-04) and Ms. Shou-Ling Huang (Department of Chemistry, National Taiwan University) for NMR spectral analyses.

References

Chang, S.-T., Wu, J.-H., Wang, S.-Y., Kang, P.-L., Yang, N.-S., & Shyur, L.-F. (2001). Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. *Journal of Agricultural and Food Chemistry, 49*, 3420–3424.

Chen, C.-W., & Ho, C.-T. (1995). Antioxidant properties of polyphenols extracted from green and black tea. *Journal of Food Lipids, 2*, 35–46.

Chen, Y., Wang, M., Rosen, R. T., & Ho, C. T. (1999). 2,2-Diphenyl-1-picrylhydrazyl radical-scavenging active components from *Polygonum multiflorum* Thund. *Journal of Agricultural and Food Chemistry, 47*, 2226–2228.

Chua, M.-T., Tung, Y.-T., & Chang, S.-T. (2008). Antioxidant activities of ethanolic extracts from the twigs of *Cinnamomum osmophloeum*. *Bioresource Technology, 99*, 1918–1925.

Chung, K.-T., Wong, T.-Y., Wei, C.-I., Huang, Y.-W., & Lin, Y. (1998). Tannins and human health: A review. *Critical Reviews in Food Science and Nutrition, 38*, 421–464.

Chung, S. K., Kim, Y. C., Takaya, Y., Terashima, K., & Niwa, M. (2004). Novel foavonol glycoside, 7-*O*-methyl mearnsitrin, from *Sageretia theezans* and its antioxidant effect. *Journal of Agricultural and Food Chemistry, 52*, 4664–4668.

Furusawa, M., Ito, T., Nakaya, K. I., Tanaka, T., Ibrahim, I., Iinuma, M., et al. (2003). Flavonol glycosides in two diospyros plants and their radical scavenging activity. *Heterocycles, 60*, 2557–2563.

Gadow, A., Joubert, E., & Hansmann, C. F. (1997). Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), α-tocopherol, BHT, and BHA. *Journal of Agricultural and Food Chemistry, 45*, 632–638.

Gordon, M. H. (1990). The mechanism of antioxidant action *in vitro*. In B. J. F. Hudson (Ed.), *Food antioxidants* (pp. 1–18). London: Elsevier Applied Science.

Kan, W.-S. (1978). Leguminosae. In W.-S. Kan (Ed.). *Manual of medicinal plants in Taiwan* (vol. 2, pp. 239–240). Taipei: National Research Institute of Chinese Medicine.

Kaur, S., Grover, I. S., & Singh, M. (1998). Antimutagenicity of hydrolyzable tannins from *Terminalia chebula* in *Salmonella typhimurium*. *Mutation Research, 419*, 169–179.

Kazuma, K., Noda, N., & Suzuki, M. (2003). Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. *Phytochemistry, 62*, 229–237.

Khallouki, F., Haubner, R., Hull, W. E., Erben, G., Spiegelhalder, B., Bartsch, H., et al. (2007). Isolation, purification and identification of ellagic acid derivatives, catechins, and procyanidins from the root bark of *Anisophyllea dichostyla* R. Br. *Food and Chemical Toxicology, 45*, 472–485.

Koleva, I. I., Niederländer, H. A. G., & van Beek, T. A. (2000). An on-line HPLC method for detection of radical scavenging compounds in complex mixtures. *Analytical Chemistry, 72*, 2323–2328.

Lee, T. H., Qiu, F., Waller, G. R., & Chou, C. H. (2000). Three new flavonol galloylglycosides from leaves of *Acacia confusa*. *Journal of Natural Products, 63*, 710–712.

Moharram, F. A., Marzouk, M. S., Ibrahim, M. T., & Mabry, T. J. (2006). Antioxidant galloylated flavonol glycosides from *Calliandra haematocephala*. *Natural Product Research, 20*(10), 927–934.

Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition, 44*, 307–315.

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

Shon, M. Y., Choi, S. D., Kahng, G. G., Nam, S. H., & Sung, N. J. (2004). Antimutagenic, antioxidant and free radical scavenging activity of ethyl acetate extracts from white, yellow and red onions. *Food and Chemical Toxicology, 42*, 659–666.

Tanaka, M., Kuie, C. W., Nagashima, Y., & Taguchi, T. (1998). Applications of antioxidative Maillard reaction products from histidine and glucose to sardine products. *Nippon Suisan Gakkaishi, 54*, 1409–1414.

Tung, Y.-T., Wu, J.-H., Kuo, Y.-H., & Chang, S.-T. (2007). Antioxidant activities of natural phenolic compounds from *Acacia confusa* bark. *Bioresource Technology, 98*, 1120–1123.

Tung, Y. T., Wu, J. H., Huang, C. Y., Kuo, Y. H., & Chang, S. T. (2009). Antioxidant activities and phytochemical characteristics of extracts from *Acacia confusa* bark. *Bioresource Technology, 100*, 509–514.

Wu, J.-H., Huang, C.-Y., Tung, Y.-T., & Chang, S.-T. (2008). Online RP-HPLC-DPPH screening method for detection of radical-scavenging phytochemicals from flowers of *Acacia confusa*. *Journal of Agricultural and Food Chemistry, 56*, 328–332.

Wu, J.-H., Tung, Y.-T., Wang, S.-Y., Shyur, L.-F., Kuo, Y.-H., & Chang, S.-T. (2005). Phenolic antioxidants from the heartwood of *Acacia confusa*. *Journal of Agricultural and Food Chemistry, 53*, 5917–5921.

Yamaguchi, T., Takamura, H., Matoba, T., & Terao, J. (1998). HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1,-diphenyl-2-picrylhydrazyl. *Bioscience, Biotechnology, and Biochemistry, 62*, 1201–1204.