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Antioxidant and Antiproliferative Activities of *Desmodium triflorum* (L.) DC

Shang-Chih Lai,*,¶ Yu-Ling Ho,§,¶ Shun-Chieh Huang,† Tai-Hung Huang,† Zhen-Rung Lai,* Chi-Rei Wu,* Kuo-Yuan Lian* and Yuan-Shiun Chang*,‡

*Institute of Chinese Pharmaceutical Sciences

†School of Pharmacy, College of Pharmacy

‡Chinese Crude Drug Pharmacy, China Medical University Hospital
China Medical University, Taichung, Taiwan

§Department of Nursing, Hungkuang University, Taichung, Taiwan

Abstract: This study evaluated the antioxidant and antiproliferative activities of the crude extract and fractions of *Desmodium triflorum* (L.) DC. The total phenolic content, 1,1-diphenyl-2- picrylhydrazyl hydrate (DPPH) free radical scavenging activity, trolox equivalent antioxidant capacity (TEAC), reducing power, total flavonoid content of *D. triflorum* were evaluated for the exploration of its antioxidant activities. Furthermore, its antiproliferative activities were investigated through the MTT method. It was compared with the antioxidant capacities of known antioxidants, including catechin, α-tocopherol, trolox and ascorbic acid. Among all fractions, ethyl acetate fraction was the most active in scavenging DPPH and TEAC radicals, of which 0.4 mg was equivalent to 186.6 ± 2.5 μg and 82.5 ± 2.1 μg of α-tocopherol and trolox respectively. The total phenolic and flavonoid contents of the crude extract were equivalent to 36.60 ± 0.1 mg catechin and 45.6 ± 0.6 mg rutin per gram respectively. In the reducing power assay, 1.25 mg of crude extract was similar to 61.2 ± 0.3 μg of ascorbic acid. For the assessment of the safety and toxicity of *D. triflorum*, LD_{50} of the crude extract was greater than 10 g/kg when administered to mice through gastric intubation. The above experimental data indicated that *D. triflorum* was a potent antioxidant medicinal plant, and such efficacy may be mainly attributed to its polyphenolic compounds.

Keywords: *Desmodium triflorum*; Antioxidant Activity; Free Radical; Antiproliferative; Hep G2; PC3.

Correspondence to: Dr. Yuan-Shiun Chang, Institute of Chinese Pharmaceutical Sciences, College of Pharmacy, China Medical University, 91 Hsueh-Shih Road, Taichung 40402, Taiwan. Tel: (+886) 4-2203-0380, Fax: (+886) 4-2208-3362, E-mail: yschang@mail.cmu.edu.tw
¶The first two authors contributed equally to this work.

Introduction

Solvent extraction is frequently used for isolation in phytochemistry, and both antioxidant activity and extraction yield depend on the solvent, due to different structure of compounds with different polarities (Kang *et al.*, 2003). Plants containing flavonoids have been reported to possess strong antioxidant properties (Raj and Shalini, 1999). Excess amount of free radicals in the human body may lead to advanced aging, cancer, cardiovascular diseases and degenerative diseases, for example chronic arthritis. Certain environmental factors, such as cigarette smoking, stress, excessive exposure to the sunlight, mobile fume and some drugs, can increase free radicals in the human body. Recently, there has been growing interest in evaluating the antioxidant activity of herbs and foods (Cai *et al.*, 2004; Hagen *et al.*, 2002; Halliwell, 2007; Seidman, 2000).

Desmodium triflorum, a medicinal plant from the Fabaceae family and also known assan-dam-jin-cao, is commonly used by traditional Chinese medicine (TCM) clinicians in Taiwan for the treatment of dysmenorrheal, muscle spasm, cough, pain and poisoning. The Taiwanese also call this plant "wings of fly" because of the shape and arrangement of the leaves. Previous phytochemical investigations on *D. triflorum* have been able to isolate astragalin, cosmossiin, tectorigenin (Ogbeide and Parvez, 1992), 2″-O-glucosylvitexin (Adinarayana and Syamasundar, 1982), 2-O-β- xylosylvitexin (Sreenivasan and Sankarasubramanian, 1984), vitexin, genistin, aliphatic alcohols, aliphatic acids, ursolic acid, oleanolic acid, campesterol, stigmasterol, β-sitosterol, campesterol-3-O-β-D-glucose, stigmasterol-3-O-β-D-glucose, sitosterol-3-O-β-D-glucose and (+)-pinitol (Chio and Huang, 1995). Alkaloids such as hypaphorine, N,N-dimethyltryptophan, betaine, choline, β- phenethylamine, and N,N-dimethyltryptamine oxide have also been isolated from *D. trflorum* (Ghosal *et al.*, 1971). In mainland China, three plants in the *Desmodium* genus (*D. styracifolium, D. trifolium, D. gangeticum*) were studied for their affects on cigarette smoke, free radicals and their inhibitory rates on lipid peroxidation (Mao *et al.*, 2007).

Free radical scavenging is a popular way to evaluate antioxidant activity of foods and herbs in recent studies (Wu *et al.*, 2007; Soong and Barlow, 2004). For such evaluation, DPPH is one of the more widely utilitized assays because it is easy and rapid for measuring the quantity of antioxidants in the tested material which can provide protons or electrons to stabilize DPPH free radicals. Trolox equivalent antioxidant capacity (TEAC) is the ability to reduce free radicals by proton-donation; measurement of this capacity was also involved in this study. In the reducing power assay, the efficiency of the antioxidants is evaluated by measuring the amount of ferricyanide complex that has been reduced into the ferrous form, and such reducing action can be indicated by a color change into Prussian blue. The phenolics assay is used to evaluate the total phenolic compound content in the plant extract and fractions because the more phenolic compounds, the more potent its anti-oxidation activity.

Although *D. triflorum* has been used extensively in folk medicine, we have found no scientific references for its toxicity and fingerprint of this plant. The acute toxicity of *D. triflorum* extract was studied on mice; additionally, HPLC fingerprints of the extract and fractions were also established in this paper.

Materials and Methods

Plant Materials

Mature whole plants of *D. triflorum* were collected in gardens and grasslands in Taichung in August 2004 as described in Flora of Taiwan (Huang, 1998). The plants were identified by Professor Chung-Chuan Chen, Graduate Institute of Chinese Pharmaceutical Sciences, China Medical University, Taichung, Taiwan. A plant specimen was deposited in the institute. The plants were dried in room temperature for one week and then crushed with a machine mill into coarse powder.

Extraction and Fractionation

The coarse powder of *D. triflorum* (1800 g) was extracted with 2 L of methanol each time for three times. The methanol extract was evaporated under reduced pressure using a rotavapor, and 253 g (14.0% net gain) of crude methanol was yielded. The crude methanol extract (253 g) was in turn dissolved and suspended in 500 ml of water in a separatory funnel and then partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol in sequence (300 ml each for three times). Under reduced pressure, fractions were yielded and collected in the order of *n*-hexane fraction (67 g, 26.4% in total extract), chloroform fraction (2.8 g, 1.1%), ethyl acetate fraction (9.3 g, 3.7%), *n*-butanol fraction (65.2 g, 25.8%) and aqueous fraction (108.2 g, 42.8%). The extracts were stored in the refrigerator before the experiment.

Chemicals

All solvents used were purchased from Merck (Darmstadt, Germany). Folin and Ciocalteu's phenol reagent, sodium carbonate, (+)-catechin, 1,1 dipheny l-2-picrylhydrazyl radical (DPPH?), potassium ferricyanide, ferric chloride, trichloroacetic acid, ABTS, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, potassium persulfate (di-potassium peroxdisulfate), potassium iodo bismuthate and carboxymethyl cellulose (CMC), vtexin were purchased from Sigma Aldrich Ltd. (Steinheim, Germany). Trolox (6-hydroxy-2,5,7,8- tetramethychroman-2-carboxylic acid), α-tocopherol, ascorbate were used as antioxidant standards, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and vitexin were also purchased from Sigma Aldrich Ltd.

Experimental Animals

Male ICR mice (20–25 g) were purchased from BioLasco Charles River Technology, Taipei, Taiwan. They were kept in the animal center of China Medical University at 22 $\pm$ 1°C, relative humidity 55 $\pm$ 5%, with a light and dark cycle of 12-hours (08:00 to 20:00) for 1 week before the experiment. Animals were provided with rodent diet and clean water *ad libitum*. All studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The experimental protocol was approved under the code 96-195-S by the Committee on Animal Research, China Medical University.

Determination of Total Phenolic Compounds in the Plant Extract and Fractions

Total phenolic compound contents in the plant extract and fractions were estimated by a colorimetric assay (Amarowicz *et al.*, 2004; Soong *et al.*, 2004). The extract and fractions (50 μl, four replicates) were pipetted into wells of a 96 well plate, and for each well, 50 μl of Folin and Ciocalteu's phenol reagent and 125 μl of saturated sodium carbonate solution were added. The contents were shaken for 20 sec and stood at room temperature for 30 min. The absorbance measurements were recorded at 725 nm using ELISA reader (power wave X340, Bio-Tek Instrument, Inc.) and (+)-catechin was used for the construction of standard curves. The total phenolic compound contents were expressed in mg of (+)-catechin equivalents per gram of extract or fractions.

Determination of Total Flavonoid Content

Total flavonoid content of the crude extract was determined according to the method of Lamaison and Carnet (1990). Aliquots of 100 μl of extract and fractions were added to 100 μl of 2% $AlCl_3 \cdot 6H_2O$ (2 g in 100 ml methanol) solutions. The mixture was vigorously shaken, and the absorbance at 430 nm was read after 10 min of incubation. Rutin was used as the standard for the calibration curve. The total flavonoid content was calibrated using a linear equation based on the calibration curve. The total flavonoid content was expressed in mg of rutin equivalent per gram of dry weight.

DPPH Method

DPPH method, described by Kim *et al.* (2003), is a popular way of evaluating antioxidant activity of foods and herbs. The capacity of the prepared extract and fractions to scavenge stable free radicals, 1,1 diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) were compared daily with prepared standard α-tocopherol (final concentration 9.375–75 μg/ml) which served as the control. The samples were diluted with methanol into 0.2 mg/ml, 0.4 mg/ml, 0.8 mg/ml and 1.6 mg/ml of sample solutions. Each well in a 96 well plate was pipetted with 25 μl of sample solution and 175 μl of 0.3 mM DPPH solution. The wells were left to stand at room temperature for 30 min. The absorbance of the solution was measured at 517 nm using ELISA reader (power wave X340). The inhibition percentage (%) of radical scavenging activity was calculated using the following equation: $\text{Inhibition}\ (\%) = (Ao - As)/Ao \times 100$ in which Ao is the absorbance of the control and As is the absorbance of the sample at 517 nm.

Trolox Equivalent Antioxidant Capacity (TEAC)

Trolox equivalent antioxidant capacity was performed as reported by Re *et al.* (1999) and Arts *et al.* (2004). Briefly, ABTS was dissolved in water to a concentration of 8 mM. ABTS radical cation was produced by reacting ABTS stock solution with 8.4 mM potassium persulfate at a ratio of 2:1 and allowing the mixture to stand in the dark at room temperature for 12–16 hours

before use. The crude extract, fractions and vitexin standard were diluted with methanol to prepare sample solutions with concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/ml. The reference standard trolox was diluted with methanol into 125, 62.5, 31.25, 15.63, 7.81 and 3.9 μg/ml. Each well in a 96 well plate was pipitted with 25 μl of sample solution and 175 μl of ABTS radical cation solution. The absorbance of the solution was measured at 734 nm using ELISA reader (power wave X340). The percentage inhibition of absorbance at 734 nm was calculated and a graph of antioxidation efficacy vs. concentration for the samples and Trolox was plotted.

Reducing Power Assay

The reducing powers of the prepared extract and its fractions were determined according to the method described by Wu *et al.* (2007). The reference standard ascorbic acid was diluted with methanol into 250, 125, 62.5, 31.3 and 15.6 μg/ml. The vitexin, extract and fractions were dissolved in distilled water with concentrations of 2.5, 1.25, 0.625, 0.313, 0.156 mg/ml. They were mixed with 50 μl of 50 μM phosphate buffer (pH 6.6) and 50 μl of 0.1 % (w/v) potassium ferricyanide. The mixture was incubated in a 50°C water bath for 20 min. One hundred μl of 1% (w/v) trichloroacetic acid solution was added to stop the reaction. The mixture was centrifuged at 3000 rpm for 10 min. One hundred and seventy five μl aliquot of the upper layer was combined with 25 μl of 5 mM ferric chloride, and the absorbance of the reaction mixture was measured at 700 nm using ELISA reader (power wave X340). Mean values from the independent samples were calculated and plotted as a function of slop ascorbate for the relative reducing equivalent of ascorbate.

Preliminary Phytochemical Tests

Phytochemical screening of the extract and fractions were performed using the reagents and chemicals described by Hosseinzadeh *et al.* (2000). Alkaloids were screened with Dragendorff's reagent, flavonoids were screened by the use of Mg and HCl.

Fingerprint Analysis by HPLC

HPLC fingerprint profile was established for each of 0.1 mg/ml vitexin and 1 mg/ml of ethyl acetate fraction, *n*-butanol fraction, and methanol crude extract. HPLC analysis was performed on a Waters HPLC 2695 separation module. Chromatographic separation was carried out on LiChroCART RP-18 endcapped column (250×4.6 mm, i.d., 5 μm pore size) with an injection of 10 μl by using an elution of 0.2% formic acid: acetonitrile (80:20) solvent at a flow rate of 0.8 ml/min. Peaks were detected at 336 nm with 2996 PDA detector.

Evaluation of Acute Toxicity

Methanol extract of *Desmodium triflorum* (0.5, 1, 2.5, 5 and 10 g/kg) were orally administered to 10 mice/dose in order to study acute toxicity. The type and initiation of signs of toxicity

during the first hour after the extract administrations were observed and recorded. Ten control animals were given a vehicle of 0.5% CMC. After the acute phase, animals were observed for 14 days, keeping a record of toxicity and mortality (Hosseinzadeh *et al.*, 2000; Rivera *et al.*, 2004). Food and water were provided throughout the experiment.

Cell Culture and Analysis of Cell Viability (MTT Assay)

To evaluate the cytotoxicity of the samples, PC-3, a human prostate carcinoma cell line and Hep G2, a human hepatoma cell line were obtained from BCRC (Food Industry Research and Development Institute, Hsin-Chu, Taiwan). Cells were cultured in F-12K medium supplemented with 10% fetal calf serum, 100 U/ml of penicillin and 100 mg/ml streptomycin mixed antibiotics, and 1 mM sodium pyruvate. All cell cultures were maintained at 37˚C in a humidified atmosphere of 5% CO_2–95% air. The culture medium was renewed every 2–3 days. For sample treatment, stock solutions of the samples were dissolved in dimethyl sulfoxide (DMSO) and sterilized by filtration through 0.2 μm disc filters. Appropriate amount of solution (10 mg/ml DMSO) were added into the culture medium to achieve the indicated concentrations of the sample (Hung *et al.*, 2009). The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was performed to assess cell viability. Briefly, cells were seeded at a density of 3×10^4 cells/ml in a 24-well plate. The cells were treated with the samples at the concentration of 100 μg/ml for 24 hours. Each concentration was repeated three times. After the exposure period, the medium was removed, followed by washing the cells with PBS. Then, the medium was added and incubated with MTT solution (0.5 mg/ml/well) for 4 hours. The medium was removed, and formazan was solubilized in isopropanol and measured spectrophotometrically at 563 nm. The percentage of viable cells was estimated by comparison with untreated control cells.

Results

Determination of Total Phenolic Contents in the Extract and Fractions

The phenolic contents of the fractions are presented in Table 1. It showed that ethyl acetate fraction had the highest phenolic content, 233.33 ± 10.03 mg (+)-catechin equivalents per gram. While the distribution of phenolics in the hydrophobic fractions was low. The total phenolic content of *D. triflorum* methanolic extract was 36.60 ± 0.1 mg/g.

Determination of Total Flavonoid Content

Total flavonoid content was expressed as mg of rutin equivalent per gram of dry weight. The total flavonoid content of 1 g/ml extract of *Desmodium triflorum* was 45.6 ± 0.6 mg of rutin equivalent per gram of dry weight. And the ethyl acetate and *n*-butanol fraction were 273.9 ± 4.9 mg and 72.0 ± 4.0 mg of rutin equivalent per gram of dry weight respectively.

Table 1. Total Phenolic Content of the Methanol Extract and Its Fraction from *D. triflorum*

Sample (g)	Total Phenolic Compound Content (mg Catechin Equivalent per gram Sample)
Crude extract	36.6 ± 0.1
n-Hexane fraction	18.3 ± 3.0
Chloroform fraction	13.7 ± 4.1
Ethyl-acetate fraction	233.3 ± 10.0
n-Butanol fraction	46.9 ± 3.1
Water fraction	22.8 ± 0.6

(n = 4).

Table 2. Methanol Extract of *D. triflorum* and Its Fractions in Scavenging DPPH Radicals

Samples	DPPH Radical Scavenging μg α-Tocopherol Equivalent 0.4 mg Sample
Crude extract	70.7 ± 1.8
n-Hexane fraction	55.8 ± 4.5
Chloroform fraction	68.5 ± 4.5
Ethyl-acetate fraction	186.6 ± 2.5
n-Butanol fraction	68.4 ± 3.2
Water fraction	30.6 ± 3.0
Vitexin	23.4 ± 2.8

(n = 4).

DPPH Method

The ethyl-acetate fraction of *D. triflorum* exhibited a strong antioxidant activity in DPPH and its equivalent value to α-tocopherol is 186.6 ± 2.5 μg (Table 2). The antioxidant activities of extract and fractions are shown in Fig. 1. A freshly prepared DPPH solution is in dark purple color with a maximum absorption of 517 nm. This color generally fades and disappears when an antioxidant is present in the solution. The reason behind this is that antioxidant molecules can scavenge DPPH free radicals by providing protons or by donating electrons, converting them to colorless products.

Trolox Equivalent Antioxidant Capacity

The ethyl-acetate fraction of *D. triflorum* exhibited a strong antioxidant activity in the trolox equivalent antioxidant assay, and the trolox equivalent value obtained was around 164.2 ± 4.1 μg (Table 3).

Reducing Power Assay

The reducing power of the methanolic extract and fractions were examined with respect to their concentrations. In this assay, yellow color of the sample solution changed to various shades of green and blue depending on the reducing power of the sample. The presence of

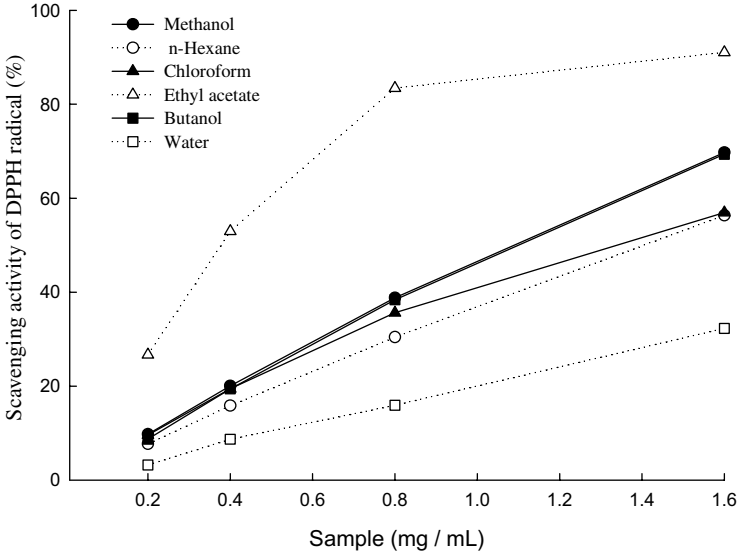


Figure 1. Free radical scavenging activities of the methanol extract and fractions of *D. triflorum* in DPPH.

Table 3. Methanol Extract and Its Fractions from *D. triflorum* in Scavenging ABTS$^{\bullet+}$ Radicals

Samples	TEAC μg Trolox Equivalent per 0.4 mg Sample
Crude extract	51.3 ± 1.6
n-Hexane fraction	33.2 ± 0.6
Chloroform fraction	94.0 ± 2.8
Ethyl-acetate fraction	164.2 ± 4.1
n-Butanol fraction	79.0 ± 3.2
Water fraction	43.6 ± 3.3
Vitexin	15.3 ± 3.9

(n = 4).

antioxidants in the extract and fractions caused the reduction of Fe^{3+}/ferricyanide complex to the ferrous form. Therefore Fe^{2+} could be detected by measuring the formation of Perl's Prussian blue at 700 nm. As shown in Table 4, among all the fractions at the concentration of 1.25 mg, ethyl acetate fraction had the highest antioxidant activity, which was equivalent to 159.2 ± 1.3 μg of ascorbate.

Preliminary Phytochemical Tests

Preliminary phytochemical testing indicated that both methanol extract and *n*-butanol fraction of *D. triflorum* contained alkaloids. HCl-Mg reaction test indicated that the methanol extract, ethyl acetate and *n*-butanol fractions contained flavonoids.

Table 4. Reducing Power of the Methanol Extract and Its Fractions from *D. triflorum*

Samples	Reducing Power μg Ascorbate Equivalent per 1.25 mg Sample
Crude extract	61.2 ± 0.3
n-Hexane fraction	69.6 ± 0.8
Chloroform fraction	66.1 ± 1.0
Ethyl-acetate fraction	159.2 ± 1.3
n-Butanol fraction	61.8 ± 1.1
Water fraction	17.2 ± 0.9
Vitexin	41.8 ± 1.0

Fingerprint Analysis by HPLC

Vitexin was used as a maker component for the standardization of flavonoid ingredients of the ethyl acetate fraction, *n*-butanol fraction and methanol extract by using HPLC. The retention time of vitexin was at 8.71 min at 336 nm (Fig. 2a). The presence of vitexin was found in HPLC fingerprints of the methanol extract, ethyl acetate fraction and *n*-butanol fraction, as shown in Figs. 2b, c and d. The *n*-buranol fraction had higher vitexin content.

Evaluation of Acute Toxicity

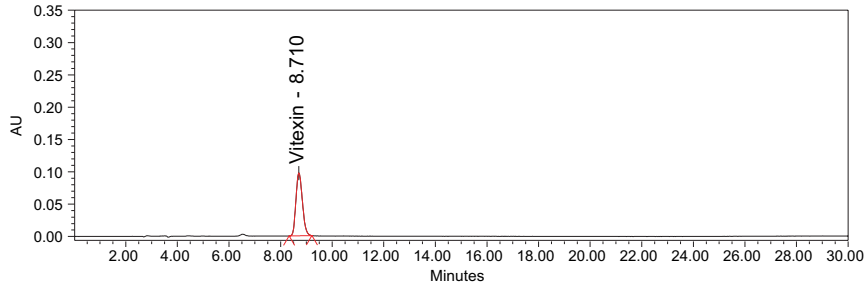
When acute toxicity of oral administration of *D. triflorum* extract was studied, no signs of toxicity were observed in the treated mice. The LD_{50} of *D. triflorum* extract was found to be greater than 10 g/kg when administered at a single dose via gastric intubation.

Cell Culture and Analysis of Cell Viability

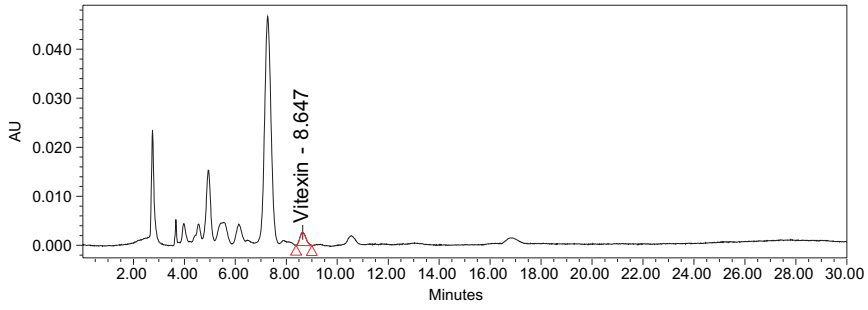
The samples did not affect Hep G2 and PC 3 cell viabilities. The MTT assay was performed to investigate whether the samples affected the growth of Hep G2 and PC 3 cells. The viabilities of human Hep G2 and PC 3 cells treated with 100 μg/ml of these fractions were assayed by MTT. As shown in Fig. 3 and Fig. 4, cell viabilities after treatment with any one of the fractions, except for *n*−hexane fraction, were all above 80% as compared to the control (100%), indicating there is no significant toxic effects.

Discussion

The result showed that ethyl acetate fraction exhibited the best antioxidant potency in DPPH free radical scavenging activity, trolox equivalent antioxidant capacity and reducing power assays. Among all fractions, ethyl acetate fraction was the most active in scavenging DPPH and ABTS radicals. These activities approached three times the efficacy of the crude extract in scavenging DPPH and ABTS radicals. Although the ethyl acetate fraction accounted for a volume only 3.7% of the total extract and the *n*-butanol fraction accounted for 25.8%, in the total phenolic content assay, they exhibited the highest phenolic contents of about 233.3 mg catechin per gram (24% phenolic content of the total extract) and



(a)



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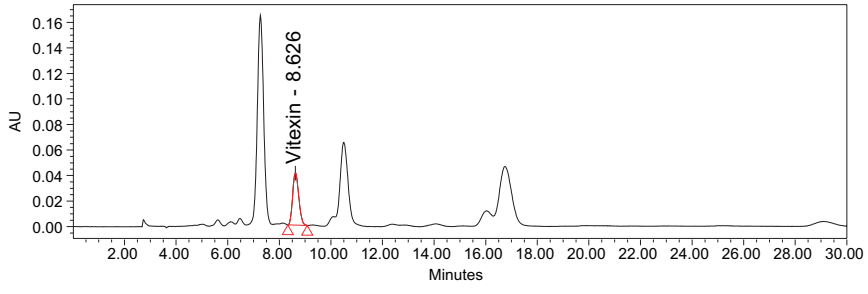

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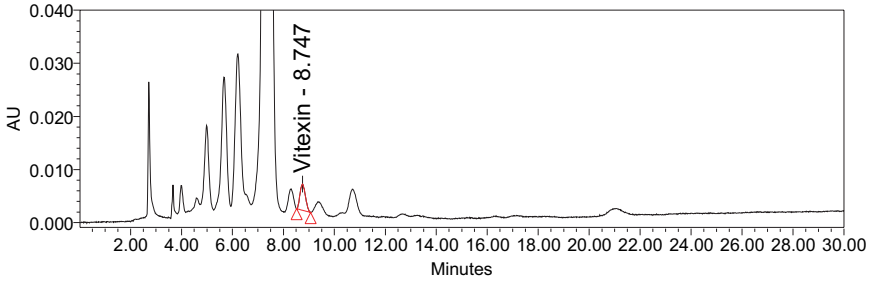

(d)

Figure 2. HPLC chromatography fingerprint profile of vitexin (A), methanol extract (B), ethyl acetate fraction (C) and *n*-butanol fraction (D) at 336 nm.

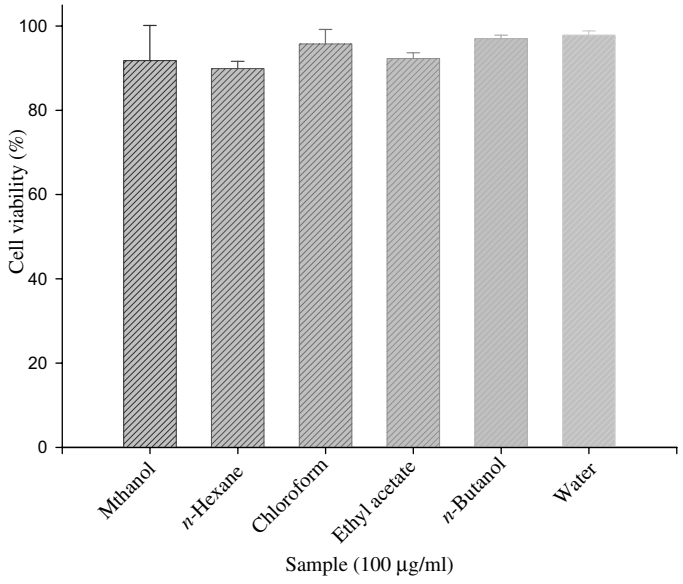

Figure 3. Effect of samples on Hep G2 cell viability. Hep G2 cells were cultured and treated with samples (100 μg/ml) for 24 hours. Cell viability was determined according to the MTT assay as described in the Material and Method sections. Results are expressed as mean ± standard error of three independent experiments.

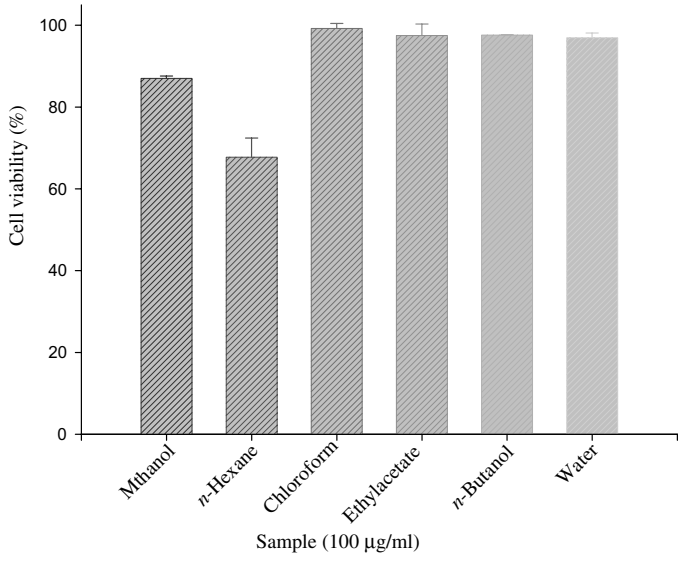

Figure 4. Effect of samples on PC 3 cell viability. PC 3 cells were cultured and treated with samples (100 μg/ml) for 24 hours. Cell viability was determined according to the MTT assay as described in Materials and Methods. Results are expressed as mean ± standard error of three independent experiments.

46.9 mg catechin per gram (34% phenolic content of total extract) respectively. These findings suggested that the antioxidant activity of ethyl acetate fraction may be attributed mainly to its polyphenolic compounds. In addition, the experimental data showed that *n*-hexane fraction had a quite good reducing power in comparison to the other fractions, but did not in the DPPH and TEAC assays. In this study, the DPPH method offered a rapid way to assess the free radical scavenging ability of plant extracts. It is very important to note that the DPPH method is only an evaluation of the antioxidants that can provide hydrogen atoms or donate electrons to quench DPPH free radicals. Furthermore, in the reducing power assay, antioxidants in the *n*-hexane fraction that caused the reduction of the ferricyanide complex into the ferrous form may not result from providing hydrogen atoms or donating electron.

Results indicated that the extract and fractions' scavenging actions of DPPH free radicals were just as efficient and effective as the TEAC assay. The data indicated that ethyl acetate and *n*-butanol fractions contributed to over 50% of the total antioxidant activities in DPPH method, TEAC and reducing power assays. From HPLC fingerprint profile of *D. triflorum*, it was demonstrated that vitexin was distributed in both ethyl acetate and *n*-butanol fractions. Plants containing flavonoids have been reported to possess strong antioxidant properties (Raj and Shalini, 1999). The presence of flavonoids in *D. triflorum* has been reported and has similar efficacies as astragalin, cosmossiin, tectorigenin, vitexin, genistin, etc. The flavonoid compounds in *D. triflorum* can scavenge excess free radicals in the human body and prevent advanced aging, cardiovascular diseases and degenerative diseases. We also found that *D. triflorum* have excellent analgesic and anti-inflammatory activities (Lai *et al.*, 2009). Many studies indicated that vitexin exhibited various biological and pharmacological activities, such as antioxidant, antimicrobial and radioprotective effects (Fu *et al.*, 2007). Therefore, vitexin may also be an active constituent of antioxidant factors in *D. triflorum*. The LD_{50} of the methanol extract of *D. triflorum* indicated that *D. triflorum* is safe to use with low toxicity. Furthermore, people are more and more interested in the understanding of the antioxidative activities of food supplements and herbs which can improve health and keep illnesses away.

In conclusion, from this study, we found that ethyl acetate fraction exhibited good antioxidant activities. These activities may be attributed to the high phenolic contents in the fraction. This study also found that *D. triflorum* had a wide margin of safety among indigenous medicinal plants in Taiwan which also possess high antioxidant effects.

Acknowledgments

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