Antioxidant Activities and Phenolic Components of Ten *Desmodium* Species

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Short title: Antioxidant Activities of 10 *Desmodium* species

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Abstract

The aim of this study was to examine the antioxidant activities and phenolic components of the crude extracts of 10 *Desmodium species* from Taiwan. In this study, DPPH free radical scavenging activity, ABTS radical monocation scavenging, ferric-reducing antioxidant power (FRAP) and reducing power of the 10 *Desmodium* species were evaluated for their antioxidant activities. The results showed that, of all the samples, *D. sequax* was the most active in ABTS, DPPH, FRAP and reducing power assays. The total polyphenol, total flavonoid and total flavonol contents of the crude extract were calculated. The correlation coefficient (R^2) values of TEAC with phenolic compounds indicated strong positive correlations, except for total flavonoid content. Furthermore, HPLC chromatographic fingerprints were established, and chlorogenic acid and vitexin in *D. sequax* were quantified. Chlorogenic acid was confirmed to express strong antioxidant activities in ABTS, DPPH, FRAP and reducing power assays. The present study indicated the antioxidant activities of the 10 *Desmodium* species were related to their phenolic components. *D. sequax* is a potent antioxidant medicinal plant, and chlorogenic acid may be an important factor in the antioxidant activity of this plant.

Keywords: Desmodium; Antioxidant Activity; Chlorogenic Acid; Vitexin

Introduction

As we know, overproduction of reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide, hydroxyl radical ($OH^•$) and peroxyl radical ($ROO^•$) may induce oxidative stress in the human body, in consequence causing degenerative and pathological damages, such as aging, cancer, cardiovascular diseases, Alzheimer's disease and inflammation (Ames, 1983; Smith et al., 1996). Certain environmental factors, such as stress, cigarette smoking, and some drugs are also associated with elevation of free radicals in the human body. Therefore, antioxidants play an important role in protecting the body from oxidative stress by scavenging free radicals. In recent years, antioxidant activities of herbs and health foods are investigated comprehensively (Cai et al., 2004). Some medicinal plants or plant derived chemical compounds with anti-inflammatory, anti-necrotic, hepatoprotective effect or other pharmaceutical activities have also been demonstrated to possess antioxidant and/or radical scavenging mechanisms, at least partly (Jimoh et al., 2010).

The genus *Desmodium*, of the Fabaceae family, includes about 350 species distributed in tropical and subtropical zones worldwide. In Taiwan, there are 18 known species that belong to the *Desmodium* genus, found mostly in regions of medium and low altitudes. Other than being processed into green manures and forages, some of them can be used as herbal medicines. For example, *D. gangeticum* has been demonstrated to possess antioxidant, anti-nociceptive, anti-inflammatory (Govindarajan et al., 2007; Rathi et al., 2004), antiemetic (Joshi et al., 2007), cardio-protective (Kurian et al., 2005), and anti-ulcer effects (Dharmani et al., 2005). Phytochemical studies have progressively isolated gangetin (Purushothaman et al., 1971), salicylic acid, rutin (Mishra et al., 2005), desmodin, gangetinin (Purushothaman et al., 1975), chlorogenic acid and caffeic acid from this plant (Govindarajan et al., 2006). *D. triflorum* has been reported to express antioxidant (Lai et al., 2010), analgesic and anti-inflammatory activities (Lai et al., 2009), and 2''-O-glucosylvitexin, vitexin, isovitexin, apigenin, and (+)-pinitol have been isolated from this plant (Adinarayana et al., 1982). Another plant from this genus, *D. uncinatum*, has been shown to possess genistein, uncinanone A, uncinanone B, uncinanone C (Tsanuo et al., 2003), uncinanone D and uncinanone E (Guchu et al., 2007) with phytochemical studies. However, no studies to date have been able to demonstrate antioxidant effect of any *Desmodium* species other than *D. gangeticum* and *D. triflorum*.

The aim of this study was to investigate the antioxidant activities and phenolic contents of the crude extracts of *D. gangeticum* (L.) DC. (DG), *D. heterocarpon* (L.) DC. (DH), *D. intortum* (DC.) Urb. (DI), *D. microphyllum* (Thunb *ex* Murray) DC. (DM), *D. renifolium* (L.) Schindl. (DR), *D. scorpiurus* (Sw.) Desv. (DSC), *D. sequax* Wall. (DSE), *D. tortuosum* (Sw.) DC. (DTO), *D .triflorum* (L.) DC. (DTR) and *D. uncinatum* DC. (DU). The antioxidant activities of the 10 *Desmodium* species were estimated by various experimental models, such as DPPH, ABTS, FRAP and reducing power assays. Additionally, HPLC fingerprints of the extracts were also established.

Materials and Methods

Plant Materials

Mature whole plants of the 10 *Desmodium* species were collected from various places in Taiwan. Voucher specimens were deposited in the National Museum of Natural Science (TNM), Taichung. They were authenticated in many aspects, including morphology (flowers, fruits, seeds and pollens), histological microscopic examination (leaves and stems) and ITS (Internal Transcribed Spacer) regions of rDNA. The sequential data was submitted to the National Center for Biotechnology Information (NCBI) genbank.

Preparation of Plant Extracts

Whole plants were dried in a circulating air oven, then ground and crushed into a coarse powder. The powder (500g) was extracted with 2 L of 70% ethanol three times.

The filtrates were collected and concentrated under reduced pressure with a vacuum rotary evaporator. The remaining solution was lyophilized before the final extract was obtained. The extract was stored in -20 ^{0}C before use.

Chemicals

Folin-Ciocalteu's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), sodium carbonate (Na_2CO_3), potassium ferricyanide ($K_3Fe(CN)_6$), ferric chloride ($FeCl_3$), aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$), trichloroacetic acid (TCA), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium peroxydisulfate ($K_2S_2O_8$), sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic (NaH_2PO_4), (+)-catechin, chlorogenic acid, rutin and vitexin were purchased from Sigma Aldrich Ltd (Steinheim, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) and ascorbate were used as antioxidant standards. 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) and all solvents used were purchased from Merck (Darmstadt, Germany).

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Fingerprint Analysis by HPLC

HPLC fingerprint profiles were established for 0.1 mg/ml of the standards (chlorogenic acid, rutin and vitexin) and 10 mg/ml of the 10 samples. The HPLC instruments used in this study consisted of a Hitachi L-7200 autosampler, a Hitachi L-7100 HPLC solvent delivery pump, and a Hitachi L-7455 diode array detector. Analyses were performed with a LiChroCART Purospher Star RP-18e column (250 mm × 4 mm, i.d. 5 μm; Merck, Germany). 20 μl of each sample was filtered through a 0.22 μm Minipore filter and injected into the column. The mobile phase consisted of (A) acetonitrile and (B) 0.2% aqueous formic acid, using a gradient elution of 5-14% A at 0-10 min, 14% A at 10-20 min, 14-28% A at 20-60 min and 28-55% A at 60-85 min. The flow rate was 0.7 ml/min, and the detection wavelength was 330 nm.

Determination of Antioxidant Activity by ABTS Assay

The ABTS assay was performed as reported by Re et al. (1999). The antioxidant abilities of the extracts were indicated as Trolox Equivalent Antioxidant Capacity (TEAC). Briefly, an aqueous solution of ABTS (7 mM) was oxidized with potassium peroxydisulfate (2.45 mM) for 16 hours in the dark. The ABTS solution was diluted with 95% ethanol to an absorbance of 0.75 ± 0.05 at 734 nm (Beckman UV-Vis spectrophotometer, Model DU640B). The reference standard Trolox was diluted into concentrations of 0, 15.625, 31.25, 62.5, 125, 250, 500 μM, and a standard curve was constructed. 20 μl of the samples (125 μg/ml) or Trolox were each mixed with 180 μl of ABTS solution and the absorbance was read. TEAC was expressed as micromole

Trolox / mg dry weight of the extract.

Ferric Reducing Antioxidant Power Assay

The ferric reducing antioxidant power (FRAP) of the crude extracts was evaluated according to the method of Benzie et al. (1999). At 593 nm, the formation of a blue colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ) from a yellow ferric tripyridyltriazine (Fe^{3+}-TPTZ) due to reduction was measured. To prepare the FRAP reagent, 0.1 M acetate buffer (pH 3.6), 10 mM 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) and 20 mM ferric chloride (10:1:1, v/v/v) were mixed. 10 μl of each sample (250 μg/ml) was mixed with 300 μl of FRAP reagent, and the absorbance was read at 593 nm after 15 min. $FeSO_4 \cdot 7H_2O$ was used as the standard reference. A standard curve was established for $FeSO_4 \cdot 7H_2O$ at concentrations of 0, 31.25, 62.5, 125, 250, 500, 1000 μg/ml. The FRAP values were expressed in μmole Fe^{2+}/mg dry weight of the extract.

Determination of Antioxidant Activity by DPPH Radical Scavenging Ability

The capacity of the samples in scavenging 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) stable free radicals was measured according to the method of Yamaguchi et al. (1998). The stock solutions (10 mg/ml) were diluted with 70% ethanol into different concentrations (2000, 1000, 500, 250, 125 and 62.5 μg/ml). The wells in a 96-well plate were pipetted with 25 μl of sample solutions and 175 μl of 0.3 mM DPPH solution, and then left to stand at room temperature for 30 min in the dark before the absorbance was measured at 517 nm. The inhibition percentage (%) of radical scavenging activity was calculated according to the following equation: Inhibition (%) = (Ao − As)/Ao × 100 in which Ao is the absorbance of the control and As is the absorbance of the sample at 517 nm. IC_{50} value was the effective concentration at which 50% of DPPH radicals were scavenged and was obtained by interpolation from linear regression analysis. A lower IC_{50} value indicated a greater antioxidant activity.

Reducing Power Assay

The reducing power of the crude extracts was determined according to the method described by Wu et al. (2007). The standard material, ascorbic acid, was diluted with methanol into 250, 125, 62.5, 31.3 and 15.6 μg/ml concentrations. 25 μl of each sample (250 μg/ml) was 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 water bath at 50 ^{0}C for 20 min. Following this, 100 μl of 1% (w/v) trichloroacetic acid solution was added before the mixture was centrifuged at 3000 rpm for 10 min. An aliquot of 175 μl 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. The reducing power was expressed as µg of ascorbate equivalent per mg dry weight of the extract.

Determination of Total Polyphenol Content

The total phenolic compound contents of the extracts were estimated by the Folin-Ciocalteu method (Ragazzi et al., 1973). 20 µl of the sample extracts (250 µg/ml) were added into the wells of a 96-well plate, followed by adding 40 µl of Folin-Ciocalteu's phenol reagent and 200 µl of distilled water into the wells. The mixtures were left to stand at room temperature for 5 min before 40 µl of 20% sodium carbonate was added. The absorbance was recorded at 680 nm. (+)-Catechin was used for the construction of the standard curve. The total phenolic compound contents were expressed in µg of (+)-catechin equivalents per mg of dry weight.

Determination of Total Flavonoid Content

The total flavonoid content of the crude extracts was determined according to the method of Lamaison and Carnet (1990). 100 µl of each sample extract was added to an equal volume of 2% $AlCl3{\cdot}6H2O$ solution (2 g in 100 ml methanol). The mixtures were vigorously shaken, and after 10 minutes of incubation, the absorbance was read at 430 nm. Rutin was used as the standard for the calibration curve, and the total flavonoid content was derived from the linear equation of this calibration curve. The total flavonoid content was expressed as μg of rutin equivalent per mg of dry weight.

Determination of Total Flavonol Content

The total flavonol content of the crude extracts was estimated by the method of Arnous et al (2001). 40 μl of each sample extract (500μg/ml) was added to 200 μl of 0.1% *p*-dimethylaminocinnamaldehyde (DMACA) in methanol/HCl (3:1, V/V). The mixtures were shaken and then left to stand at room temperature for 10 min. The absorbance of the reaction solutions was measured at 640 nm. (+)-Catechin was used as the standard for the calibration curve. The total flavonol content was calibrated using the linear equation based on the calibration curve. The total flavonol content was expressed as μg (+)-catechin equivalent/mg dry weight.

Statistical Analysis

Experimental results were presented as the mean ± standard deviation (SD) of three parallel measurements. The statistical analyses were performed by one-way ANOVA, followed by Dunnett's *t* test. Differences were considered statistically significant when the *p* values were less than 0.05.

Results and Discussions

Extraction Yield

The extraction yields of the 10 *Desmodium* species are shown in Table 1. The extraction yields ranged from 6.74 to 29.62%, and *D. microphyllum* had the highest extraction yield.

HPLC Fingerprint Analysis of the 10 Desmodium Species

HPLC chromatographic fingerprint profiles of the 10 *Desmodium* samples are shown in Figure 1. Variations in fingerprint profiles among the species signify differences in chemical constituents, which in turn indicate discrepant pharmaceutical activities. Furthermore, different fingerprint profiles could help in identifying these species effectively. Regarding the compounds identified, chlorogenic acid and rutin have already been reported to be present in *D. gangeticum*, and vitexin has been isolated from *D. triflorum*. Our chromatographic fingerprinting profiles were in agreement with the findings from previous studies. Additionally, chlorogenic acid and vitexin were found in *D. sequax* by comparing the retention times and UV spectra of authentic standards analyzed under identical analytical conditions, and also confirmed by spiking the extracts with pure standards (standard addition method). The retention times of chlorogenic acid and vitexin were 17.36 min and 35.41 min respectively. The quantitative data of chlorogenic acid and vitexin in *D. sequax* were calculated using their respective concentration vs. peak area calibration curves. According to the calibration curve, the contents of chlorogenic acid and vitexin were 7.96 mg/g of extract and 2.85 mg/g of extract, respectively.

DPPH and ABTS Scavenging Activity

DPPH and ABTS assays are commonly used for the assessment of free radical-scavenging abilities of herbal medicines and health foods due to their simplicity, stability, accuracy and reproducibility (Vijaya Kumar Reddy et al., 2010). ABTS is a synthetic radical that can be used to estimate scavenging activities for both polar and non-polar samples (Shi et al., 2009), and it is often expressed in TEAC values. In this study, TEAC values of the 10 *Desmodium* species were determined from the Trolox calibration curve as shown in Figure 2. The antioxidant activities of the 10 *Desmodium* species were in the following decreasing order: DSE > DH > DM > DU > DI > DG > DSC > DTR > DTO > DR.

DPPH is a stable radical which can accept hydrogen from an antioxidant to form reduced DPPH (Moon et al., 2009). IC_{50} values of the 10 *Desmodium* species were investigated using the DPPH colorimetric method (Table 1). The low IC_{50} values of *D. sequax*, *D. heterocarpon* and *D. microphyllum* indicated that they expressed strong antioxidant activities in the DPPH assay. The above results revealed that ABTS and

DPPH assays were similar. Since both DPPH and ABTS are used to evaluate free radical-scavenging abilities of the samples, our findings revealed consistent results between these two assays.

Previous studies have reported that *D. gangeticum* showed antioxidant activity in the DPPH assay (Govindarajan et al., 2003), and a similar result was obtained in this study. However we discovered that *D. sequax*, *D. heterocarpon*, *D. microphyllum*, *D. uncinatum* and *D. intortum* showed even stronger antioxidant activities in comparison with *D. gangeticum* in both ABTS and DPPH assays. Of all the investigated plant species, *D. sequax* had the highest free radical scavenger activity. *D. sequax* and *D. heterocarpon* are used in folk medicine, and *D. microphyllum* is a traditional Chinese herbal medicine. Therefore, it is worthy to investigate the pharmacological activities of these medicines in the future.

Ferric Reducing Antioxidant Power Assay

As shown in Figure 3, the total antioxidant powers (FRAP) of the 10 *Desmodium* species differed significantly. *D. sequax* expressed the highest FRAP value, followed by DM, DH, DG, DU, DI, DSC, DTR, DTO and DR. Therefore *D. sequax*, *D. microphyllum*, and *D. heterocarpon* expressed strong activities, and *D. sequax* had the strongest activity.

In the present study, FRAP, DPPH and ABTS assays were used to estimate the total antioxidant power. The relationship between FRAP and ABTS and between FRAP and DPPH ($1/IC_{50}$) values of the 10 *Desmodium* species were appraised and expressed as correlation coefficients (R^2). R^2 values of FRAP/ABTS (Figure 4A) and FRAP/DPPH (Figure 4B) were 0.8447 and 0.9224 respectively. The results showed high positive correlations between these assays, and thus revealed high similarity between these assays.

Measurement of Reducing Power

Previous researches have conceived that antioxidant activities of herbal medicines and health foods are probably related to their reducing powers (Wu et al., 2007). The reducing powers of the 10 samples are shown in Table 1, and the results indicated large variations in antioxidant activities. The reducing powers of the 10 samples ranged from 22.34 ± 0.69 to 148.37 ± 3.48 μg ascorbate/mg and were in the following decreasing order: DSE > DH > DM > DU > DI > DG > DSC > DTR > DTO > DR. These results suggested that *D. sequax*, *D. heterocarpon* and *D. microphyllum* had strong abilities to react with free radicals and could convert them into more stable nonreactive forms that eventually led radical chain reactions to termination. *D. sequax* was also demonstrated to possess the highest reducing activity. Similar results were observed for the reducing power assay as compared to the ABTS and DPPH assays, and these were consistent with the free radical scavenging capacities.

Total Polyphenol, Flavonoid, and Flavonol Contents

The total polyphenol, flavonoid, and flavonol contents of the 10 *Desmodium* species are shown in Table 2. The results revealed the total polyphenol contents of the 10 *Desmodium* species extracts varied from 20.92 to 368.05 mg (+)-catechin equivalent/g dry weight, in the following decreasing order: DSE > DH > DM > DU > DG > DI > DSC > DTR > DR > DTO. The total flavonoid contents varied from 42.24 to 387.73 mg rutin equivalent/g of dry weight, in the following decreasing order: DM > DU > DSE > DG > DTO > DTR > DH > DI > DR > DSC. The total flavonol contents of the 10 *Desmodium* species extracts varied from 0.93 to 138.28 mg (+)-catechin equivalent/g dry weight, in the following descending order: DSE > DH > DU > DI > DTR > DM > DSC > DG > DR > DTO.

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Relationships between Total Antioxidant Power and Total Polyphenol, Flavonoid, and Flavonol Contents

Phenolic compounds play important roles in stabilizing lipid oxidation and may be attributed directly to antioxidative activities (Yen et al., 1993). Both flavonoids and flavonols are polyphenolic compounds. The correlation coefficients (R^2) between TEAC and total polyphenol, TEAC and total flavonoid, and TEAC and total flavonol of the 10 sample extracts were evaluated in this study. As shown in Figure 5, the R^2 values between TEAC and total polyphenol content and TEAC and total flavonol content of the 10 *Desodium* species extracts were 0.9383 and 0.8199 respectively. However, the R^2 value between TEAC and total flavonoid (Figure 5B) was much lower (R^2=0.1169). The above results suggested strong linear relationships between TEAC and total polyphenol/total flavonol; therefore the higher the TEAC activity, the higher the total polyphenol and flavonol contents in the samples.

Antioxidant Activities of Chlorogenic Acid and Vitexin

In this study, the extract of *D. sequax* was found with the highest antioxidant activity, HPLC analysis also revealed the presence of chlorogenic acid and vitexin. The antioxidant activities of these two compounds were determined. In ABTS assay, the TEAC values of chlorogenic acid and vitexin were 2.04 ± 0.02 mM and 0.14 ± 0.00 mM respectively (Figure 3). In DPPH assay, chlorogenic acid had a lower IC_{50} value in comparison with vitexin (Table 1). In FRAP assay, chlorogenic acid expressed a higher FRAP value than vitexin (Figure 2). In the reducing power assay, we found that chlorogenic acid was more active in comparison with vitexin (Table 1).

Chlorogenic acid has been reported to exhibit antioxidant, anti-bacterial, anti-carcinogenic and anti-inflammatory activities (Shan *et al.*, 2009). Our results of antioxidant activities were in agreement with the previous reports. Therefore, since chlorogenic acid possesses strong antioxidant activities, it may be an essential ingredient in the antioxidant activity of *D. sequax*.

Conclusions

In the present study, the antioxidant capacities of the 10 *Desmodium* species were examined with *in vitro* experiments, including ABTS radical scavenging assay, FRAP method, DPPH radical scavenging assay and reducing power method. The results demonstrated that most samples expressed strong activities and *D. sequax* exhibited the highest antioxidant potency. There are significant relationships between the antioxidant activities and amount of phenolic compounds of the *Desmodium* species, except for flavonoids. The chromatographic fingerprints of the 10 *Desmodium* species were established, and some phytochemicals were found. Furthermore, chlorogenic acid and vitexin were found in *D. sequax*, and the contents of these two compounds were examined by HPLC. Chlorogenic acid has been shown to be strongly antioxidative and may be an important component in the antioxidant activity of *D. sequax*.

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Figure Legend

Figure 1. HPLC chromatographs of the 10 *Desmodium* species and the standards.

Figure 2. TEAC values of the extracts of the 10 *Desmodium* species. Each value represents the mean ± S.D. of three parallel measurements ($P < 0.05$).

Figure 3. FRAP values of the extracts of the 10 *Desmodium* species. Each value represents the mean ± S.D. of three parallel measurements ($P < 0.05$).

Figure 4. Correlation coefficients (R^2) of (A) FRAP and TEAC and (B) FRAP and DPPH of the extracts of the 10 *Desmodium* species.

Figure 5. Correlation coefficients (R^2) of (A) TEAC and total polyphenol content; (B) TEAC and total flavonoids content, and (C) TEAC and total flavonol content of the 10 *Desmodium* species.

Figure 1

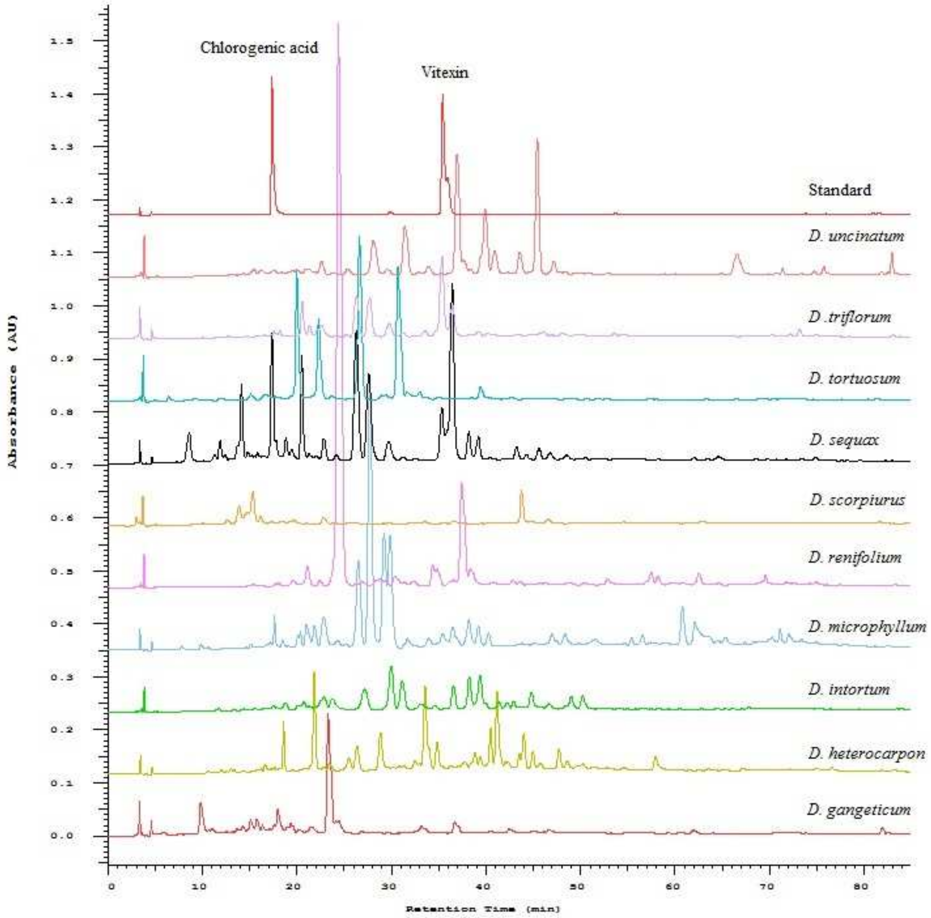
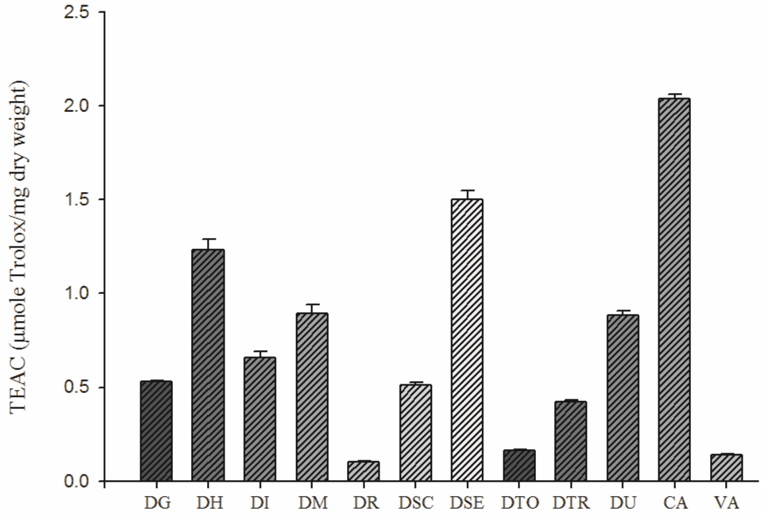
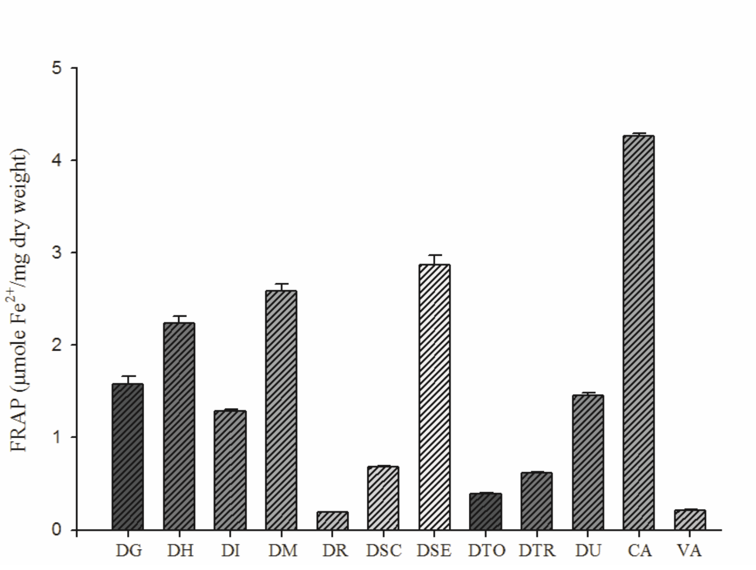

Chlorogenic acid
Vitexin
Standard
D. uncinatum
D .triflorum
D. tortuosum
D. sequax
D. scorpiurus
D. renifolium
D. microphyllum
D. intortum
D. heterocarpon
D. gangeticum
Absorbance (AU)
Retention Time (min)

Figure 2



TEAC (µmole Trolox/mg dry weight)
2.5
2.0
1.5
1.0
0.5
0.0
DG
DH
DI
DM
DR
DSC
DSE
DTO
DTR
DU
CA
VA

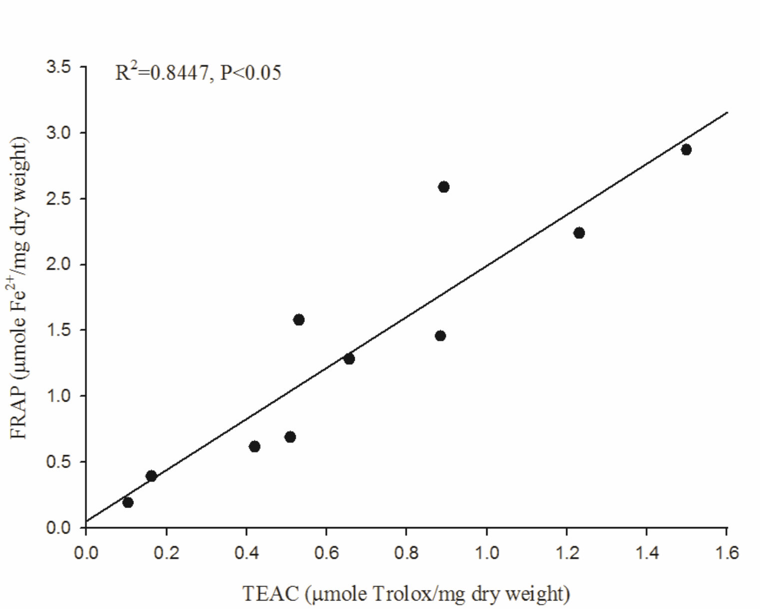
Figure 3



FRAP (µmole Fe2+/mg dry weight)
5
4
3
2
1
0
DG
DH
DI
DM
DR
DSC
DSE
DTO
DTR
DU
CA
VA

Figure 4

A


R²=0.8447, P<0.05
FRAP (µmole Fe2+/mg dry weight)
TEAC (µmole Trolox/mg dry weight)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6

B

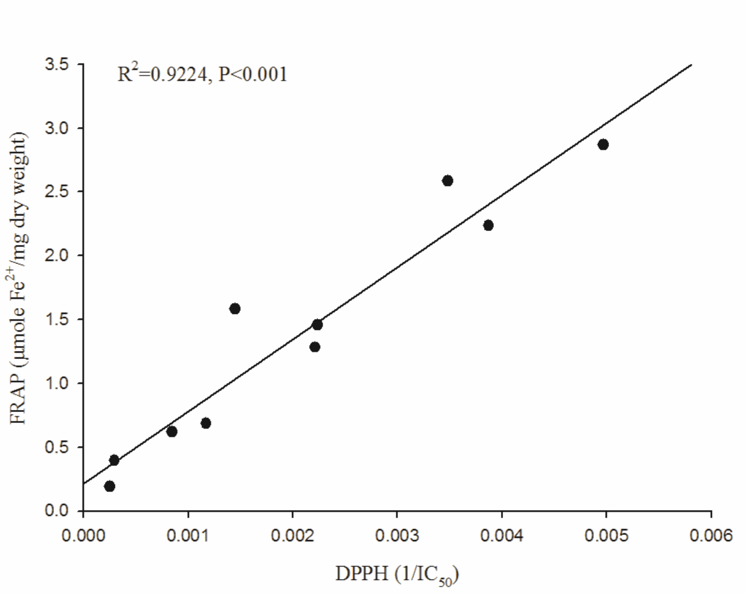
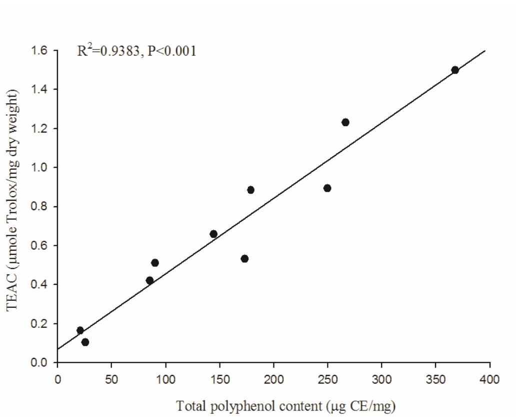

R²=0.9224, P<0.001
FRAP (µmole Fe2+/mg dry weight)
DPPH (1/IC50)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
0.000
0.001
0.002
0.003
0.004
0.005
0.006

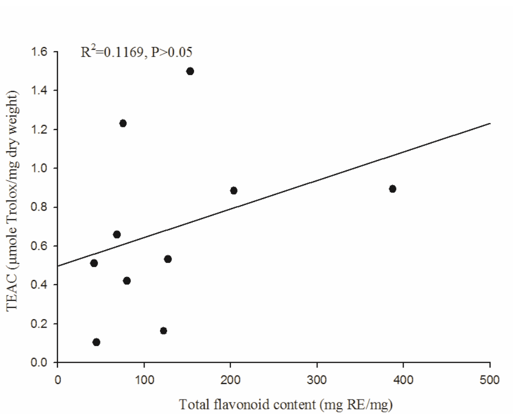
Figure 5

A



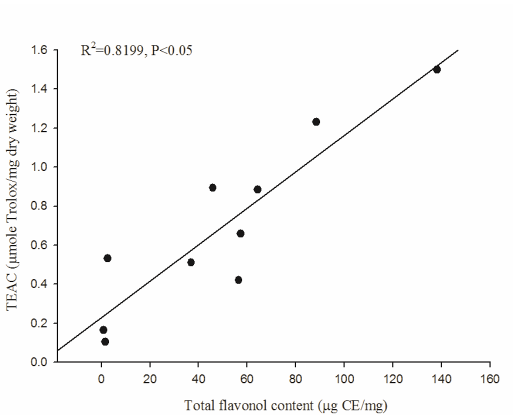
R²=0.9383, P<0.001
TEAC (μmole Trolox/mg dry weight)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 50 100 150 200 250 300 350 400
Total polyphenol content (μg CE/mg)

B



R²=0.1169, P>0.05
TEAC (μmole Trolox/mg dry weight)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 100 200 300 400 500
Total flavonoid content (mg RE/mg)

C



R²=0.8199, P<0.05
TEAC (μmole Trolox/mg dry weight)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 20 40 60 80 100 120 140 160
Total flavonol content (μg CE/mg)

Table 1. Extraction yields, DPPH IC_{50} values, and reducing powers of the extracts of 10 *Desmodium* species.

Species and positive controls	Extraction yield[a] (%)	DPPH[b] IC_{50} (μg/ml)	Reducing power[b] (μg ascorbate/ mg sample)
D. gangeticum (DG)	13.91	688.73 ± 5.67	66.92 ± 2.54
D. heterocarpon (DH)	21.68	258.21 ± 7.31	110.37 ± 2.53
D. intortum (DI)	20.19	451.76 ± 6.48	71.16 ± 2.47
D. microphyllum (DM)	29.62	286.94 ± 5.54	103.88 ± 0.64
D. renifolium (DR)	22.78	>4000	22.34 ± 0.69
D. scorpiurus (DSC)	10.13	852.87 ± 20.21	48.40 ± 1.56
D. sequax (DSE)	8.68	201.19 ± 12.77	148.37 ± 3.48
D. tortuosum (DTO)	14.75	3390.93 ± 53.81	28.84 ± 0.68
D .triflorum (DTR)	13.74	1179.31 ± 21.72	41.30 ± 1.38
D. uncinatum (DU)	6.74	446.56 ± 10.44	80.01 ± 0.39
Chlorogenic acid	—	167.47 ± 5.64	270.48 ± 3.89
Vitexin	—	3537.94 ± 68.72	18.59 ± 0.21

[a] Dried weight basis.

[b]Values represented mean ± S.D. of three parallel measurements (P<0.05).

Table 2. Total polyphenol, flavonoid, and flavonol contents of the extracts of 10 *Desmodium* species[a].

Species	Total polyphenols (μg CE/mg)[b]	Total flavonoids (μg RE/mg)[c]	Total flavonols (μg CE/mg)[b]
D. gangeticum (DG)	173.28 ± 3.07	127.49 ± 2.73	2.62 ± 0.01
D. heterocarpon (DH)	266.50 ± 6.10	75.79 ± 2.66	88.54 ± 0.72
D. intortum (DI)	144.31 ± 5.96	68.64 ± 1.74	57.43 ± 0.18
D. microphyllum (DM)	249.85 ± 3.02	387.73 ±11.58	45.95 ± 0.26
D. renifolium (DR)	25.50 ± 0.42	45.01 ± 0.52	1.56 ± 0.05
D. scorpiurus (DSC)	90.04 ± 2.28	42.24 ± 1.26	37.01 ± 0.50
D. sequax (DSE)	368.05 ± 8.99	153.32 ± 1.50	138.28 ± 0.92
D. tortuosum (DTO)	20.92 ± 0.19	122.52 ± 1.40	0.93 ± 0.01
D .triflorum (DTR)	85.50 ± 0.77	80.33 ± 0.38	56.48 ± 0.14
D. uncinatum (DU)	178.88 ± 3.17	203.93 ± 2.93	64.42 ± 0.09

[a] Values represented mean ± S.D. of three parallel measurements.

[b] Data expressed in μg (+)-catechin equivalent/mg dry weight (μg CE/mg).

[c] Data expressed in μg rutin equivalent/mg dry weight (μg RE/mg).