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ORIGINAL ARTICLE

Constituents of Flavonoids from *Tridax procumbens* L. and Antioxidant Activity

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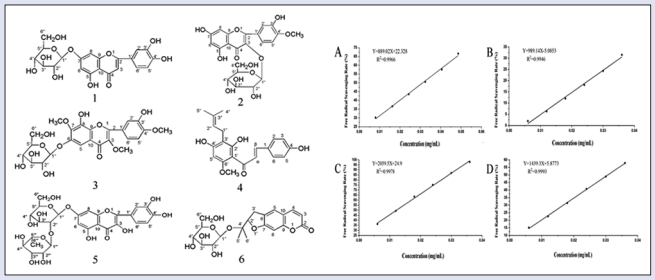
ABSTRACT

Background: To study the chemical constituents and antioxidant activities of flavonoids in *Tridax procumbens* L. **Materials and Methods:** Diaion HP-20, MCI-Gel CHP-20, Sephadex LH-20, and silica gel column chromatography were used to isolate and purify the chemical components of *T. procumbens* L., and the structures of the compounds were identified by physical and chemical properties and spectral techniques (^{1}H-nuclear magnetic resonance [NMR] and ^{13}C-NMR). The antioxidant activities of five flavonoids were evaluated by 2,2-diphenyl-1-picryl-hydrazyl method. **Results:** Six compounds were isolated and identified as luteolin-7-O-β-D-glucoside (1), 5,7,3′-trimethyl-4′-methoxyl-3-O-β-D-flavonoid glucoside (2), 8,3′-dihydroxyl-3,7,4′-trimethoxy-6-O-β-D-flavonoid glucoside (3), 4,2′,4′-trihydroxy-6′-methoxyl-3′-isopentenyl chalcone (4), quercetin-7-O-β-D-glucopyranosyl-(2→1)-α-L-rhamnose (5), and nodakenin (6). **Conclusions:** All compounds except compound 3 were isolated from the plant for the first time. It was found that compounds 1–5 had significant antioxidant and free radical scavenging activities.

Key words: Antioxidant activity, chemical constituents, flavonoids, separation, structures identification, *Tridax procumbens* L.

SUMMARY

- Six constituents of flavonoids in *Tridax procumbens* L. were isolated and identified, and all compounds except compound 3 were isolated from the plant for the first time
- Compounds 1–5 had significant antioxidant and free radical scavenging activities.



Abbreviations used: NMR: Nuclear magnetic resonance; DPPH: 2,2-diphenyl-1-picryl-hydrazyl; TEAC: Trolox equivalent antioxidant capacity; TMS: Tetramethylsilane; TLC: Thin layer chromatography.

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INTRODUCTION

Tridax procumbens L. is commonly known as Ghamra in Hindi.[1] It has been found to have significant medicinal value. Its leaves are used on the treatment of bronchial catarrh, dysentery, and diarrhea and for preventing hair.[2,3] The water decoction of its leaves possesses antiseptic, insecticidal, and parasiticidal properties. It is also used to cure hemorrhages from cuts, bruises, and wounds.[4] An aqueous extract of this plant also has marked depressant action on respiration. In recent years, the chemical components and biological activities of *T. procumbens* L. have been investigated, and the results show that the main chemical components of *T. procumbens* L. are flavonoids and have certain antioxidant activities.[5,6] To explore the pharmacodynamic material basis of *T. procumbens* L. and elucidate its pharmacodynamic effect, and to provide scientific evidence or rational development and utilization of this plant resource, the chemical constituents of flavonoids and biological activities from *T. procumbens* L. whole herb were further studied.

MATERIALS AND METHODS

Materials and reagents

The whole plant was collected from Sanya, Hainan Province, China, and identified as *T. procumbens* L. by Shi-Man Huang, Professor of Medicinal Botany, Hainan University. Specimens are kept in our laboratory. Column chromatographic filler: Diaion HP-20, MCI-Gel CHP-20 (Mitsubishi Corporation, Japan), and Sephadex LH-20 Gel (Pharmacia Biotech). Silica gel for column chromatography (160–200 mesh), silica gel G for thin layer chromatography (TLC), and GF_{254} are all products of Qingdao Marine chemical plant. Trolox and 1,1-diphenyl-2-picryl-hydrazyl radicals (DPPH, Sigma Company). The reagents used in the experiments were chemical or analytical pure.

Instruments

WRS-1b digital melting point tester (Shanghai Precision Scientific Instrument Co., Ltd.); Bruker DPX-400 Nuclear Magnetic Resonance (NMR) Instrument (tetramethylsilane is the internal standard); vacuum

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film concentrator (self-assembly in laboratory); rotary evaporation apparatus (Shanghai Yarong Biological Technology Co., Ltd.); SHZ-D (β) circulating water vacuum pump (Yingyu Yuhua of Gongyi City to the China Factory Production); Electronic Balance of PL203 (Mettler Toledo Production); DGG-9240 drying oven with electric heating, constant temperature, and blowing function (Shanghai Senxin Experimental Instrument Co., Ltd.); KQ-250B Ultrasonic Extractor (Jiangsukunshan Ultrasonic Instruments Co., Ltd.); Infinite M2000 Microplate Reader (Tecan, Switzerland); and Ultraviolet (UV)-2102PCS-UV-Visible Spectrophotometer (Shanghai Uniko Instrument Co., Ltd.).

Extraction and separation

After natural drying, 5.5 kg of the whole plant of *T. procumbens* L. was weighed and crushed into coarse powder, and 75% ethanol of the equivalent to three times the dry weight of the raw materials was used as solvent for cold-soak extraction[7] for three times, each time for 24 h. Filter by vacuum method and the filtrate is then combined. Vacuum thin film concentration device[8] was used for concentration to one-fifth of the original volume. An appropriate amount of activated carbon is used to decolorize and remove the activated carbon by vacuum filtration. Then, the concentrate solution is concentrated continuous to no alcohol taste by reducing pressure, a total of 0.65 kg of the concentrated extract was obtained, and the extraction rate was 11.82%. Ultrasonic dispersion was carried out by adding appropriate amount of water to the total extract, petroleum ether, and ethyl acetate were successively used for extraction,[9] the obtained extracts were concentrated by vacuum thin-film concentration device to obtain petroleum ether fraction (53 g), ethyl acetate fraction (146 g), n-butanol fraction (186 g), and water site (152 g), and the yield of each part was 8.15%, 22.46%, 28.62%, and 23.38%, respectively.

The obtained ethyl acetate extract fraction (146 g) was eluted by silica gel (160–200 mesh) column chromatography with gradient elution of petroleum ether–ethyl acetate solvent system, from which compound 4 (25 mg) was isolated and obtained. The obtained n-butanol parts of 136 g were ultrasonic dispersed in water, and then, elution was conducted with H_2O, 10%, 20%, 40%, 60% methanol, and 70% acetone by means of column chromatography with macroporous adsorption resin of Diaion HP-20. The 40% methanol eluting part of Diaion column was separated and purified reduplicative by Sephadex LH-20 and MCI-gel CHP-20 column chromatography with gradient elution of methanol–water solvent system. Five compounds were isolated and obtained: compound 1 (58 mg), compound 2 (56 mg), compound 3 (32 mg), compound 5 (35 mg), and compound 6 (64 mg).

Determination of antioxidant activities

With the constant deepening of research on natural antioxidants, more and more methods are used to determine the antioxidant capacity. DPPH assay is a commonly used method.[10,11] In organic solvents, DPPH, which is purple, is composed of three benzene rings and one nitrogen atom. Nitrogen atom has one lone pair electron, so it is a stable free radical with strong absorption at 5l7 nm. In the presence of free radical scavengers, these free radical scavengers will pair with DPPH single electrons, thereby weakening their absorption, so the absorbance has changed and this change also has a quantitative relationship with the number of electrons they accept. Therefore, the scavenging ability of free radical scavengers can be quantitatively analyzed by spectrophotometry.[12-14] Trolox is a water-soluble analog of Vitamin E that is highly reductive and scavenge-free radicals, in the process of determining the free radical scavenging ability of samples. Trolox solution is often used as the positive control to draw the standard curve and convert it into the free radical scavenging ability of samples. The test results are represented by the value of trolox equivalent antioxidant capacity (TEAC), that is, the trolox concentration required for equivalent antioxidant capacity of substances tested at a certain concentration. The method is also known as TEAC method. TEAC value represents the concentration of trolox, and the higher the concentration of trolox, the stronger the free radical scavenging ability. This method is simple, sensitive, and reliable and has become one of the important methods for screening natural antioxidant products.[15-19]

Preparation of sample solution

The petroleum ether, ethyl acetate and n-butanol extracts were weighed respectively 5 mg each, dissolved in anhydrous ethanol and constant volume in a 10-mL volumetric flask, shaken well to obtain the sample solution, and stored in a 4° refrigerator. Before use, they were diluted with 95% ethanol to a concentration of 0.1 mg/mL. DPPH reagent of 36.6 mg was accurately weighed and dissolved it with 95% ethanol and constant volume to 100 mL volumetric flask. After shaking well, DPPH storage solution with a mass concentration of 0.3660 g/L was obtained and placed in the refrigerator for refrigeration. Before use, DPPH storage solution was diluted with 95% ethanol to a diluent with a mass concentration of 0.0723 g/L.

RESULTS AND DISCUSSION

Identification of the structure of the compounds

Compound 1, the yellow amorphous powder, soluble in methanol, at UV 254 nm, showed purple fluorescence, reacted with $FeCl_3$-K_3[Fe (CN)$_6$] reagent and showed blue color, indicating that it contained phenolic hydroxyl, and was positive for hydrochloric magnesium powder test, indicating that it had flavonoid skeleton, and glucose was detected in the TLC after acid hydrolysis, suggesting that the compound was a flavonoid glycoside. ^{1}H-NMR (DMSO, 400 MHz) δ: 7.52 (1H, dd, *J* = 2.4, 8.4 Hz, H-6'), 7.49 (1H, d, *J* = 2.4 Hz, H-2'), 7.25 (1H, d, *J* = 8.4 Hz, H-5'), this proves that there is an ABX system on the benzene ring. δ6.80 (1H, S, H-3), 6.47 (1H, d, *J* = 1.6 Hz, H-8), 6.18 (1H, d, *J* = 2.0 Hz, H-6). ^{13}C-NMR (DMSO, 100 MHz) δ: 181.8 (C-4), 165.2 (C-2), 163.7 (C-9), 163.3 (C-7), 161.6 (C-5), 157.6 (C-4'), 153.2 (C-3'), 148.7 (C-3), 124.9 (C-2'), 118.6 (C-1'), 116.2 (C-6), 113.8 (C-5'), 104.1 (C-10), 101.4 (C-6'), 99.3 (C-1"), 94.3 (C-8), 77.5 (C-5"), 76.1 (C-3"), 73.4 (C-2"), 69.9 (C-4"), 60.9

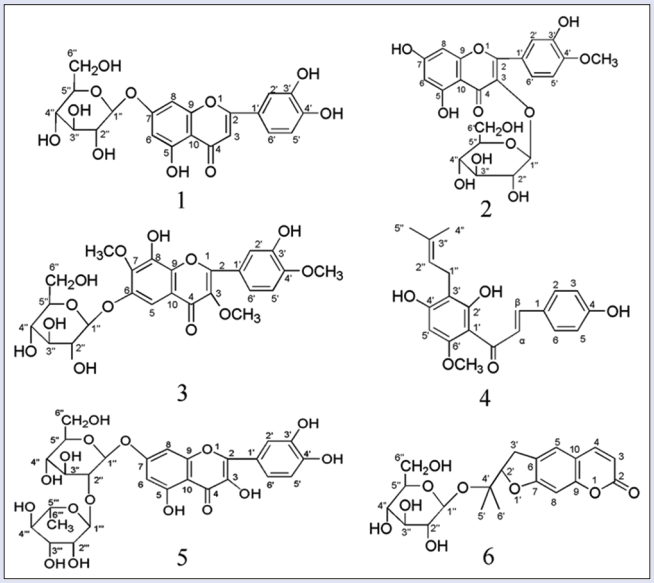

Figure 1: Structures of compounds 1–6

(C-6"). This was basically consistent with the data verified in reference,[20] the compound was determined to be luteolin-7-O-β-D-glucoside, and the structural formula is shown in Figure 1.

Compound 2, the yellow amorphous powder, soluble in methanol, at UV 254 nm, showed purple fluorescence, reacted with $FeCl_3$-K_3[Fe (CN)$_6$] reagent and showed blue color, indicating that it contained phenolic hydroxyl, and was positive for hydrochloric magnesium powder test, indicating that it had flavonoid skeleton, and glucose was detected in the TLC after acid hydrolysis, suggesting that the compound was a flavonoid glycoside. ^{1}H-NMR (DMSO, 400MHz) δ: 7.53 (1H, d, *J* = 2 Hz, H-2'), δ: 7.50 (1H, dd, *J* = 2, 8.8 Hz, H-6'), δ: 7.22 (1H, d, *J* = 8.8 Hz, H-5'), this proves that there is an ABX system on the benzene ring. δ: 6.44 (1H, br. s, H-8), 6.20 (1H, br. s, H-6). ^{13}C-NMR (DMSO, 100 MHz) δ: 178.2 (C-4), 164.6 (C-7), 162.1 (C-3), 161.5 (C-5), 156.7 (C-4'), 155.2 (C-2), 147.9 (C-9), 146.7 (C-3'), 138.5 (C-5'), 124.3 (C-2'), 120.5 (C-1'), 116.1 (C-6'), 115.8 (C-5'), 104.5 (C-10), 101.4 (C-1"), 98.9 (C-8), 94.1 (C-6), 77.5 (C-5"), 76.2 (C-3"), 73.5 (C-2"), 72.5 (C-4"), 70.0 (C-6"), 60.5 (C-4'-OCH_3). This was basically consistent with the data verified in reference,[21] the compound was determined to be 5,7,3'-trimethyl-4'-methoxyl-3-O-β-D-flavonoid glucoside, and the structural formula is shown in Figure 1.

Compound 3, the yellow amorphous powder, soluble in methanol, at UV 254 nm, showed purple fluorescence, reacted with $FeCl_3$-K_3[Fe (CN)$_6$] reagent and showed blue color, indicating that it contained phenolic hydroxyl, and was positive for hydrochloric magnesium powder test, indicating that it had flavonoid skeleton, and glucose was detected in the TLC after acid hydrolysis, suggesting that the compound was a flavonoid glycoside. ^{1}H-NMR (MeOD, 400 MHz) δ: 7.62 (1H, dd, *J* = 2, 8 Hz, H-6'), 7.59 (1H, d, *J* = 2 Hz, H-2'), 7.01 (1H, d, *J* = 8 Hz, H-5'), this proves that there is an ABX system on the benzene ring. δ: 6.85 (1H, s, H-5), 5.11 (1H, d, J = 7.2 Hz, H-1") proved that the structural formula of glucose is to be β-D configuration. δ: 3.92 (3H, s, H-7-OCH_3), 3.87 (3H, s, H-3-OCH_3), 3.79 (3H, s, H-4'-OCH_3). ^{13}C-NMR (MeOD, 100 MHz) δ: 180.4 (C-4), 158.3 (C-2), 157.9 (C-6), 153.7 (C-7), 153.4 (C-9), 151.8 (C-4'), 147.6 (C-3'), 139.8 (C-3), 133.8 (C-8), 124.0 (C-1'), 123.8 (C-5), 122.4 (C-6'), 116.3 (C-10), 112.3 (C-2'), 108.1 (C-5'), 61.5 (C-7-OCH_3), 60.6 (C-3-OCH_3), 56.4 (4'-OCH_3). This was basically consistent with the data verified in reference,[22] the compound was determined to be 8,3'-dihydroxyl-3,7,4'-trimethoxy-6-O-β-D-flavonoid glucoside, and the structural formula is shown in Figure 1.

Compound 4, the yellow amorphous powder, soluble in methanol, at UV 254 nm, showed purple fluorescence, reacted with $FeCl_3$-K_3[Fe (CN)$_6$] reagent and showed blue color, indicating that it contained phenolic hydroxyl. ^{1}H-NMR (MeOD, 400 MHz) δ: 7.78 (1H, d, *J* = 14.8 Hz, H-β) and δ: 7.67 (1H, d, *J* = 14.8 Hz, H-α) are the signal of H-β and H-α, combined with δ 197.0 of carbonyl signal, this further confirmed the existence of chalcone skeletons. δ 3.87 (3H, s) indicates the presence of the methoxy group. δ 5.19 (1H, t, H-2"), δ 3.14 (2H, d, *J* = 8.4 Hz, H-1"), δ 1.75 (3H, s, CH_3), δ 1.63 (3H, s, CH_3) indicate the presence of the isopentene group. δ 7.50 (2H, d, *J* = 8.0 Hz, H-2,6) and δ 6.81 (2H, d, *J* = 8.0 Hz, H-3,5) means that the B ring has substitution of 4-location. δ 5.99 (1H, s, H-5') indicates the presence of the separate hydrogen in A ring. ^{13}C-NMR indicates that chalcone skeleton has 15 carbon signals, two methoxy signals, and five carbon signals of isopentenyl group. ^{1}H-NMR (MeOD, 400 MHz) δ: 7.78 (1H, d, *J* = 14.8 Hz, H-β), 7.67 (1H, d, *J* = 14.8 Hz, H-α), 7.50 (2H, d, *J* = 8.0 Hz, H-2,6), δ 6.81 (2H, d, *J* = 8.0 Hz, H-3,5), 5.99 (1H, br. s, H-5'), 5.19 (1H, t, H-2"), 3.87 (3H, s,-OCH_3), 3.14 (2H, d, *J* = 8.4 Hz, H-1"), 1.75 (3H, s, CH_3), 1.63 (3H, s, CH_3). ^{13}C-NMR (MeOD, 100 MHZ) δ: 197.0 (C = O), 167.6 (C-4'), 166.0 (C-2'), 160.5 (C-6'), 159.0 (C-4), 144.1 (C-β), 132.7 (C-3"), 132.6 (C-2,6), 128.2 (C-1), 125.2 (C-2"), 123.6 (C-α), 119.0 (C-3,5), 111.1 (C-3'), 102.4.1 (C-1'), 94.1 (C-5'), 58.4 (C-OCH_3), 26.5 (C-1"), δ22.8 (C-4"), 18.4 (C-5"). This was basically consistent with the data verified in reference,[23] the compound was determined to be 4,2',4'-trihydroxy-6'-methoxyl-3'-isopentenyl chalcone, and the structural formula is shown in Figure 1.

Compound 5, the yellow amorphous powder, soluble in methanol, at UV 254 nm, showed purple fluorescence, reacted with $FeCl_3$-K_3[Fe (CN)$_6$] reagent and showed blue color, indicating that it contained phenolic hydroxyl, and was positive for hydrochloric magnesium powder test, indicating that it had flavonoid skeleton, and glucose was detected in the TLC after acid hydrolysis, suggesting that the compound was a flavonoid glycoside. Glucose and rhamnose were detected in the TLC after acid hydrolysis, indicating that the flavonoid glycoside is a glycoside with glucose and rhamnose. ^{1}H-NMR (MeOD, 400 MHz) shows that the aromatic region has five hydrogen protons. δ: 7.66 (1H, d, *J* = 2.2 Hz, H-2'), δ 7.63 (1H, dd, *J* = 2.1, 8.4 Hz, H-6'), and δ 6.86 (1H, d, *J* = 8.4 Hz, H-5') prove that there is an ABX system on the benzene ring. The other two hydrogen signals of δ 6.40 (1H, d, *J* = 2.0 Hz, H-8) and δ 6.20 (1H, d, *J* = 2.1 Hz, H-6) in the aromatic region are a coupling relationship of spacing location. δ 5.10 (1H, d, *J* = 7.5 Hz, H-1") is the end location hydrogen signal for glucose. δ 4.51 (1H, d, *J* = 1.3 Hz, H-1"') is the first location hydrogen signal for rhamnose. A series of peaks from δ 1.11to 3.67 are the hydrogen signal peaks connected to the glucose and rhamnose ring. ^{13}C-NMR (MeOD, 100 Hz) δ 179.4 is the characteristic signal peak of flavonoid 4-carbon, δ 62.3 and δ17.9 are the characteristic signal peaks of glucose 6"-carbon and rhamnose 6"'-carbon. A series of peaks from δ69.7 to 78.2 are the carbon signal peaks connected to the glucose and rhamnose ring. ^{13}C-NMR (MeOD, 100 MHZ) δ: 149.8 (C-2), 135.6 (C-3), 179.4 (C-4), 158.5 (C-5), 94.8 (C-6), 166.0 (C-7), 99.9 (C-8), 163.0 (C-9), 105.6 (C-10), 123.1 (C-1'), 117.7 (C-2'), 159.3 (C-3'), 145.9 (C-4'), 116.0 (C-5'), 123.5 (C-6'), 104.7 (C-1"), 75.7 (C-2"), 78.2 (C-3"), 77.2 (C-4"), 73.9 (C-5"), 62.3 (C-6"), 102.4 (C-1"'), 72.2 (C-2"'), 72.1 (C-3"'), 73.9 (C-4"'), 63.7 (C-5"'), 17.9 (C-6"'). This was basically consistent with the data verified in reference,[24] the compound was determined to be quercetin-7-O-β-D-glucopyranosyl-(2→1)-α-L-rhamnose, and the structural formula is shown in Figure 1.

Compound 6, the amorphous powder, soluble in methanol, at UV 254 nm, showed purple fluorescence, reacted with $FeCl_3$-K_3[Fe (CN)$_6$] reagent and showed blue color. Glucose was detected in the TLC after acid hydrolysis. δ: 7.82 (1H, d, *J* = 9.6 Hz, H-4), 6.28 (1H, d, *J* = 9.6 Hz, H-3) indicate the presence of the cis-form hydrogen of double bond in the construction. ^{1}H-NMR (MeOD, 400 MHz) δ: 7.82 (1H, d, *J* = 9.6 Hz, H-4), 7.78 (1H, s, H-8), 7.44 (1H, s, H-5), 6.28 (1H, d, *J* = 9.6 Hz, H-3), 4.57 (1H, m, H-1"). ^{13}C-NMR (MeOD, 100 MHz) δ: 164.2 (C-7), 160.22 (C-2), 155.53 (C-9), 155.10 (C-5), 146.26 (C-4), 129.89 (C-6), 118.85 (C-10), 114.75 (C-3), 97.88 (C-1"), 97.36 (C-8), 90.68 (C-2'), 78.51 (C-3",5"), 74.82 (C-2"), 71.24 (C-4"), 71.23 (C-4'), 62.51 (C-6"), 28.98 (C-3'), 25.35 (C-6'), 23.72 (C-5'). This was basically consistent with the data verified in reference,[25] the compound was determined to be nodakenin, and the structural formula is shown in Figure 1.

Determination of antioxidant activity

DPPH method was used to determine the free radical scavenging ability of samples, which was determined by microplate quantification method combined with 96 microplate and microplate reader.[26] 1, 2, 4, 6, and 8 mL of the prepared positive control solution of trolox was taken out, and the volume was fixed to 10 mL with anhydrous ethanol to obtain trolox solution of different concentrations. 200 μL of sample was reacted with 50 μL DPPH solution (0.36mg/mL) in the dark at 37° for 45min. The absorbance was measured at 517nm. The Trolox solution (0.0326 mg/mL)

was used as authentic standard and the calibration curve was established by plotting the DPPH scavenging. The results were calculated as following formula:

Free radical scavenging rate(%)=1– (Ap – Ac) / Amax × 100 %.

Where Am is the absorbance of DPPH alone, Ap is the absorbance of DPPH and extract and Ac is the absorbance of the extract only. All samples were tested in triplicate. Meanwhile, the standard curve was drawn with the concentration of Trolox (x) as the abscissa and the free radical scavenging rate (y) as the ordinate, and the linear regression equation of trolox as Y = 889.02X + 22.328, R^2 =0.9966, the linear regression equation of petroleum ether fraction as Y = 989.14X − 5.0853, R^2 = 0.9946, the linear regression equation of ethyl acetate fraction as Y = 2059.5X + 24.9, R^2 = 0.9978, the linear regression equation of n-butanol fraction as Y = 1439.3X + 5.8773, R^2 = 0.9993, as shown in Figure 2.

The determination method of free radical scavenging ability in the sample is similar to that of trolox reference solution. It is not necessary to set the concentration gradient of the sample, and the sample concentration should be within the range of 0.00326–0.02608 mg/mL. Taking 0.02 mg/mL sample solution of 200 μL for prepared measurement and mixture it with 50 μL DPPH solution, shake the mixture for 30s, heat preservation for 45 min at 37°C, determining the absorbance value of 517 nm wavelength (Ap), the 0.02 mg/mL sample solution prepared with 200 μL was mixed with 50 μL DPPH solution. At the same time, the absorbance value (Ac) was determined of mixed 200 μL sample solution prepared with 50 μL anhydrous ethanol, and the absorbance value (λ_{max}) of 200 μL DPPH mixed with 50 μL anhydrous ethanol solution was determined and DPPH radical scavenging rate was calculated. According to the standard curve of trolox reference solution, the TEAC value of the sample was calculated to evaluate the free radical scavenging ability of the sample. The experiment was repeated three times in parallel for data analysis, and the results were expressed as mean value ± standard deviation. This paper uses Microsoft Excel 2010 to draw the diagrams.

RESULTS AND DISCUSSION

Flavonoid compounds have strong antioxidant and free radical scavenging ability.[27] The pollution of the external environment, pesticide residues, and unhealthy habits will cause the body organism to produce excessive free radicals, but these radicals cannot be cleared in time, and they could damage other tissues and cells, causing a variety of diseases.[27] Therefore, it has great benefits for the body to supplement a large number of free radical scavengers. In this study, to compare the antioxidant capacity of different extraction fraction of the ethanol extract of *T. procumbens* L., the TEAC value was used to evaluate the free radical scavenging capacity

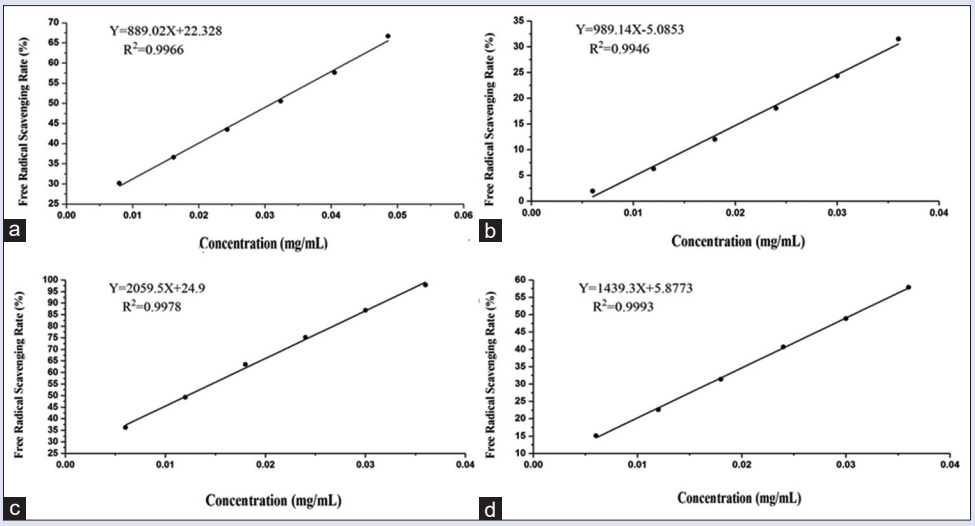

Figure 2: The standard curve of trolox and different extraction fraction on 2,2-diphenyl-1-picryl-hydrazyl scavenging effect. (a) Trolox, (b) petroleum ether fraction, (c) ethyl acetate fraction, (d) *n*-butanol fraction

Table 1: Scavenging rate of different extraction fraction on 2,2-diphenyl-1-picryl-hydrazyl radical and trolox equivalent antioxidant capacity values

Concentration (mg/mL)	Scavenging rate		
	petroleum ether fraction	ethyl acetate fraction	*n*-butanol fraction
0.02	14.721±11.34	72.675±32.65	36.160±21.33
0.016	9.785±12.77	66.876±5.08	29.223±17.08
0.012	6.479±32.12	51.854±8.05	22.010±11.40
0.008	2.635±17.96	40.943±15.13	16.900±19.36
TEAC (mg/g)	0.045±18.38	0.331±32.68	0.643±22.64

TEAC: Trolox equivalent antioxidant capacity

Table 2: Trolox equivalent antioxidant capacity values of antioxidant activity from compounds 1–6

	Compound					
	Compound 1	Compound 2	Compound 3	Compound 4	Compound 5	Compound 6
TEAC (mg/g)	94.31±10.32	121.33±12.77	32.04±10.91	66.54±81.06	87.32±35.53	1.48±11.81

TEAC: Trolox equivalent antioxidant capacity

of the samples. The experiment results of antioxidant and scavenging free radicals ability are shown in Tables 1 and 2. Antioxidant capacity of *T. procumbens* L. was also different in different extraction fractions; Table 1 shows that the antioxidant activity of petroleum ether fraction is not obvious but that of ethyl acetate fraction has certain antioxidant activity, and n-butanol fraction is the strongest; Compounds 1–5 had significant antioxidant and free radical scavenging activities. Flavonoids and flavonoid glycosides are mainly concentrated in n-butanol fraction, which indicates that flavonoids are the main components that play an antioxidant role. The results showed that the n-butanol fraction of the ethanol extract of *T. procumbens* L. was an effective fraction with obvious antioxidant activity.

CONCLUSION

Flavonoid compounds are a kind of important active ingredients in traditional Chinese medicine. Flavonoids have the function of scavenging free radicals and antioxidation. In recent years, especially with the continuous deepening of natural medicine chemical separation technology and the continuous development of spectral and spectral technology means, flavonoid constituents of traditional Chinese medicine have become a research hotspot, which have been widely concerned and have broad application prospects. In this study, six compounds were isolated and identified, among which compounds 1–5 were flavonoids. All compounds except compound 3 were isolated from the plant for the first time. It was found that compounds 1–5 had significant antioxidant and free radical scavenging activities. Strong antioxidant capacity of *T. procumbens* L. flavones provides an important theoretical and material basis for further pharmacological research and clinical applications.

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Conflicts of interest

There are no conflicts of interest.

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