# A New Procedure for Separation and Purification of Scutellarin from *Erigeron multiradiatus*

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**Abstract:** **Objective** To establish a new procedure for isolating scutellarin from *Erigeron multiradiatus*. **Methods** A proposed method was developed by combining macroporous resins with ODS column. Firstly, *E. multiradiatus* was extracted by ultrasound with 80% methanol. Preliminary separation was performed on macroporous resin column. The performance and adsorption characteristics of three macroporous resins, D140, D141, and D605, were compared and the enrichment procedure was optimized. Further purification was carried out by medium pressure liquid chromatography (MPLC) with ODS column. **Results** It was demonstrated that D141 had better extractive effects on scutellarin. The MPLC conditions were optimized as follows: 15% ethanol aqueous as mobile phase with flow rate at 2.5 mL/min. The yield and purity of the isolated scutellarin were 1.20 mg/g and 96.5%, respectively. **Conclusion** The overall procedure is efficient and low-cost, which is considered suitable for the separation and purification of scutellarin from *E. multiradiatus*. The results provide the scientific basis for developing and using scutellarin in clinic.

**Key words:** *Erigeron multiradiatus*; medium pressure liquid chromatography; ODS; scutellarin; separation
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## Introduction

Scutellarin (Fig. 1) is an important bioactive ingredient in *Erigeron breviscapus* (Vant.) Hand. -Mazz. (Asteraceae) (Qu *et al*, 2001; Yang *et al*, 2008). It was also found in *Scutellaria barbata* D. Don (Lamiaceae) (Yao *et al*, 2011). Scutellarin could significantly dilate blood vessel, improve microcirculation, increase cerebral blood flow, suppress the increment of intracellular free calcium in vascular smooth muscle cells, protect brain microvascular endothelial cells injury, and inhibit the platelet aggregation activity (Wang *et al*, 2007; Zhang *et al*, 2009; Zhu *et al*, 2009; Ye *et al*, 2011). In recent years, more and more bioactivity studies as well as official standards chose scutellarin as a chemical marker for the quality evaluation of *E. breviscapus* and its preparation (Wang *et al*, 2011; Su *et al*, 2012; Guo *et al*, 2011). Thus, the need of scutellarin as a reference substance is rapidly increasing.

*Erigeron multiradiatus* (Lindl.) Benth, belonging to the same genus as *E. breviscapus*, is naturally distributed in the northern and southwestern mountain regions of China as well as Sikkim, Nepal, and Afghanista (Zhang *et al*, 1998). *E. multiradiatus* has been widely used for the treatment of hypopepsia, enteronitis, diarrhea, hepatitis, adenolymphitis, rheumatism, and hemiparalysis (Lin and Chen, 1973). Our previous phytochemistry studies reported that *E. multiradiatus* comprised of several kinds of compounds, such as flavonoids, phenolic acids, monoterpenes, sterols, etc. Especially, scutellarin is one of the major flavonoid constituents (Zhang *et al*, 2008).

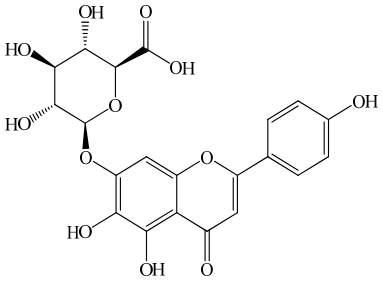


**Fig. 1 Chemical structure of scutellarin**

The aim of this work is to establish a new and low-cost method for the separation and purification of

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scutellarin from *E. multiradiatus.* In this study, preliminary separation was carried out on macroporous resin column and the further purification was performed by medium pressure liquid chromatography (MPLC) with ODS. ODS is based on reversed-phase separation, which may be repeatedly used. The results could not only provide the important and overall information to purify scutellarin, but also could be a powerful method to develop the plants in genus *Erigeron* L.

## Materials and methods

**Solvents and chemicals**

High performance liquid chromatography (HPLC) grade acetonitrile (Tedia, USA), HPLC grade water obtained from a deionized water treatment system (Millipore, USA), and analytical grade acetic acid (Huada, China) were used for the preparation of mobile phase. Methanol and ethanol (Huada, China) of analytical grade was used for the sample preparation and separation.

**Plant material**

Samples of *Erigeron multiradiatus* were collected in the natural growth sites in Luhuo (Sichuan, China) at altitude 4200 m. These samples were authenticated by Prof. ZHANG Hao (West China School of Pharmacy, Sichuan University) using geographical origin identification, macroscopic identification, and microscopic identification. A voucher specimen (E12025) was deposited at Herbarium Center of West China, School of Pharmacy, Sichuan University.

**Sample extraction**

The dried whole plant (200 g), ground to powder, was extracted for 40 min by ultrasonic extraction (UE) with 80% methanol (1000 mL), evaporated under reduced pressure to remove methanol, and concentrated to afford a dark-brown tarry mass at a temperature below 45 ℃, for three times. Then the dark-brown tarry mass was dissolved in hot water, then cooled and filtered. The filtrate was stored for further research.

**Enrichment of scutellarin from *E. multiradiatus* by macroporous resins**

The filtrate was applied to a glass column (5 cm × 60 cm) wet-packed with 500 g (dry weight) of the pretreated D141 macroporous resins (Chengguang, China), on which adsorption and desorption experiments were carried out. The bed volume (BV) of the resin was 1000 mL. The flow rate of sample solution was 1 BV/h through the glass column. Scutellarin in the eluents was monitored by HPLC analysis of the eluted aliquots collected at 5 min intervals. While it was in adsorptive equilibrium condition, the loading of the sample was stopped. The adsorbate-laden column was washed firstly with deionized water, and then desorbed with ethanol-water (80:20) solution. The desorption solution was concentrated in a rotary evaporator (SBW—1, Shanghai Shenko, China) at 45—50 ℃. and the residue after concentration was lyophilized with a freeze drying system.

**Purification of scutellarin by MPLC with ODS**

For MPLC separation, the frozen residue (2 g) was dissolved in hot water and filtered. This solution was applied to MPLC (Buchi R605 column: 30 cm × 5 cm, Fraction Collector Buchi 684) using ODS as adsorbent and eluted with EtOH-$H_2O$. The elution solvents were collected at 10 min intervals and monitored by HPLC (detected at 280 nm), those exhibiting similar HPLC profiles were combined. The purified scutellarin was identified by IR, ESI-MS, and $^1$H-NMR spectra with the published data, and its purity was determined by HPLC.

**HPLC analysis of extracts and product**

For the change determination of scutellarin content after each step of separation, the analyte (10 mg) was dissolved in methanol-water (80:20) solution and the solution was passed through a 0.45 μm millipore filter prior to chromatographic analysis. Experiments were performed on a Shimadzu LC—6A system (Shimadzu Corp., Japan). For chromatographic analysis, a Shim-pack VP-ODS column (150 mm × 4.6 mm, 5 μm) with a guard column (7.5 mm × 4.6 mm, 5 μm) was used. The mobile phase consisted of methanol-0.4% acetic acid (40:60). The flow rate was 1.0 mL/min and the column temperature was maintained at 35 ℃. The detection wavelength was set at 335 nm for acquiring chromatograms.

## Results and discussion

**Effects of extraction conditions on yield of scutellarin**

In order to obtain the best extraction efficiency, three important factors (the extraction methods, extraction solvents, and extraction time), which might

influence the extraction efficiency of the target constituents, were optimized by using univariate approach.

Firstly, heat-refluxing (4 h, 80 ℃) was compared with UE (30 min, 350 W). The result showed that UE was more effective on the yield of scutellarin with less interference. Hence, the UE method was selected as the optimized method.

Secondly, different solvents combination was another important factor for the efficiency on objective constituents. Therefore, suitable extracting solvent should be selected for the extraction of scutellarin. In this study, water and different concentration of methanol (10%, 30%, 50%, 80%, and 100%) have been used as solvents for the extraction of scutellarin from the powdered whole plant. It could be found that 80% methanol aqueous solution gave a higher yield of scutellarin than others. The results from the present experiments also demonstrated that the scutellarin content of the extract remarkably increased with methanol concentration. This might be due to "like dissolve like" principle. When the methanol concentration is higher than 80%, the extraction yield of scutellarin was decreased, and the reason may be that proteins could be coagulated in methanol with higher concentration, making larger diffusion resistance.

Finally, extraction time is an indispensable parameter for yield of the mark compounds. Therefore, suitable extraction time is necessary to give the higher content of scutellarin. Powdered samples were extracted by UE with 80% methanol for 10, 20, 40, and 60 min, respectively. The results demonstrated that UE for 40 min obtained optimal results, and there was no obvious difference between 40 and 60 min.

As shown in Table 1, the yield and the content of scutellarin were calculated from the formula as follows.

$$Y\,(\%) = \frac{Ws}{Wt} \times 100\% \qquad (1)$$

Where $Y$ is the percentage extraction of scutellarin; $Ws$ and $Wt$ are weights of extracted scutellarin and *E. multiradiatus* samples, respectively

$$C\,(\%) = \frac{Ws}{We} \times 100\% \qquad (2)$$

Where $C$ is the content of scutellarin; $Ws$ and $We$ are weight of extracted scutellarin and total weight of the extract, respectively

**Table 1 Comparison on extraction ability of scutllarin from *E. multiradiatus***

| Extraction | | Extract rate / % | Content of scutellarin / % |
|---|---|---|---|
| method | reflux | 1.23 | 4.78 |
| | UE | 1.48 | 5.22 |
| *t* / min | 10 | 0.74 | 2.37 |
| | 20 | 1.18 | 3.49 |
| | 40 | 1.53 | 5.58 |
| | 60 | 1.55 | 5.64 |
| solvents (methanol-water) | 10:90 | 1.25 | 4.07 |
| | 30:70 | 1.34 | 4.38 |
| | 50:50 | 1.54 | 4.78 |
| | 80:20 | 1.55 | 5.48 |
| | 100:0 | 1.27 | 5.12 |

**Effects of resins type on yield of scutellarin**

Preliminary separation was performed on macroporous resins, which is an ion-exchange group with large pore structure of the polymer adsorbent. It has been successfully applied for the separation of flavonoids, saponion, and alkaids from Chinese materia medica (CMM) or herbs. It was found that the preparative separation of scutellarin could be easily and effectively achieved via adsorption and desorption on D141 resin. The optimized parameters for the adsorption on column packed with D141 resin was as follows: The concentration of scutellarin in sample solution was 0.40 mg/mL, with processing volume of 3 BV and flow rate of 1 BV/h; For desorption: ethanol-water (80:20), 5 BV as eluent, and flow rate of 1 BV/h. Using D141 resin under optimal conditions, the content of scutellarin in the product was increased by 20-fold from 1.55% to 32.80%, and the recovery rate of scutellarin was 88.54% (Table 2). Compared to the conventional separation methods of scutellarin, this adsorption method is superior because of its procedural simplicity, lower cost, and high efficiency. Therefore, further purification is absolutely necessary, which was performed as follows.

**Purification of scutellarin by MPLC with ODS**

Generally, two materials, namely silica gel and ODS for column separation were employed for MPLC,

**Table 2 Adsorption of scutllarin by three kinds of macroporous resins**

| Resin types | Extract rate / % | Content of scutellarin / % |
|---|---|---|
| D140 | 1.24 | 29.63 |
| D141 | 1.42 | 32.80 |
| D605 | 1.17 | 21.44 |

and the former was considered as normal phase separation supports. However, scutellarin might involve in the formation of hydrogen bond between flavonoid glycosides and silanol groups which led to low recoveries, so the separation process is not effective. Compared with the former, ODS could provide the superior separation effect with less trailing effect and high recoveries. In addition, MPLC could provide on-line chromatographic detection. Thus the purification of scutellarin was performed by MPLC with ODS in this study.

In the present purification procedure, sample (2 g) after elution on resins was dissolved in water, and then filtered and injected into the ODS column (5 cm × 30 cm). An optimized mobile phase system consisting of ethanol-water (15:85) was used. The flow rate was about 2.5 mL/min at a pressure of $1.38 \times 10^6$—$2.07 \times 10^6$ Pa. The effluent from the tail end of the ODS column was continuously monitored with a UV detector at 280 nm (Fig. 2). The retention time of scutellarin was about 11 min according to the on-line monitor and HPLC analysis (Fig. 3).

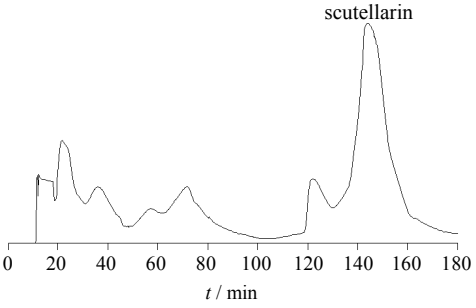


**Fig. 2 MPLC chromatogram of scutellarin**

As a result, the yield of scutellarin was 1.20 mg/g on the air-dry plant and the purity based on HPLC analysis was 96.5% by normalization method. The recovery rate obtained was 78.7%, calculated from the formula as follows.

$$R\,(\%) = \frac{Ws \times Ps}{Wt \times Pt} \times 100\% \qquad (3)$$

Where *Ps* and *Pt* are the purity of scutellarin in the extracted sample and in the *E. multiradiatus* samples, respectively. The overall procedure was carried out in replicate. The results demonstrated that it was considered suitable for the preparative separation of scutellarin from *E. multiradiatus*

**Molecular validation for isolated compound**

The isolated compound was identified by IR, ESI-MS, and NMR. IR $\nu_{max}^{KBr}$ ($cm^{-1}$): 3377, 1739, 1665, 1610, 1500. The ESI-MS of the isolated compound indicated a molecular ion at *m*/*z* 461.1 $[M-H]^-$. Under negative ion mode, the glucosidic bond of *O*-glucuronides is easily cleaved in the collision cell to generate product ions of $[M-H-176]^-$. $^1$H-NMR (DMSO-$d_6$) $\delta$: 12.75 (1H, s, $C_5$-OH), 10.39 (1H, s, $C_6$-OH), 8.62 (1H, s, $C_4'$-OH), 7.94 (2H, d, $J$ = 8.6 Hz, H-2′, 6′), 6.91 (1H, s, H-8), 6.83 (1H, s, H-3), 6.97 (2H, d, $J$ = 8.6 Hz, H-3′, 5′), 5.52 (1H, d, $J$ = 6.0 Hz, GlcuA, H-1″), 4.05 (1H, d, $J$ = 9.4 Hz, GlcuA, H-5″). It was identified as scutellarin according to the literature (Zhang *et al*, 1998).

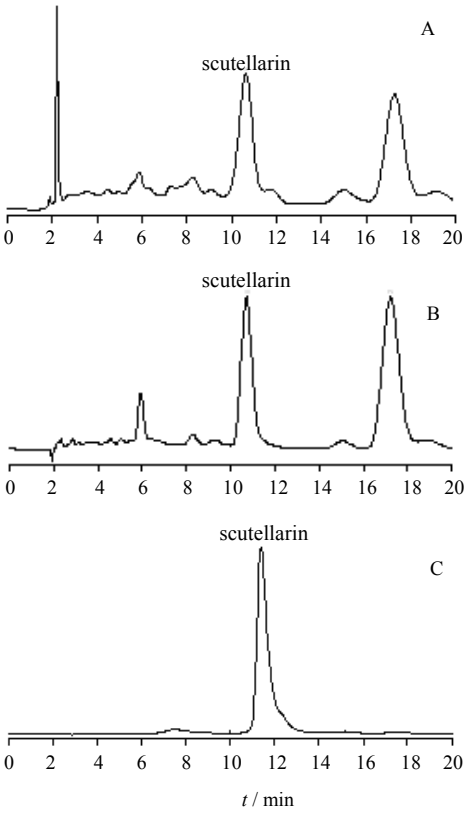


**Fig. 3 HPLC chromatograms of scutellarin in extract from *E. multiradiatus* (A), scutellarin after using D141 macroporous resins column (B), and scutellarin isolated using MPLC (C)**

The separation of natural active compounds is a multi-step and systemic course (Zhang *et al*, 2008; Li and Chen, 2009; Huang *et al*, 2011). In previous study, HPD800 was chosen to separate scutellarin from the crude extracts of *E. breviscapus* and the content of scutellarin in the product was increased by 15.69-fold from 2.61% to 40.96% (Gao, 2007). However, it is impossible to obtain scutellarin with high purity via adsorption-desorption properties of macroporous resins.

Furthermore, scutellarin (95.6%) has been successfully gained from *E. breviscapus* using two-step purification by high-speed counter-current chromatography (HSCCC) (Gao, 2006). However, the HSCCC procedure is considerably expensive to obtain a large amount of scutellarin from the crude extracts for commercial use. Moreover, with the increasing consumption of *E. breviscapus*, the natural resources of *E. breviscapus* gradually decrease and eradicate in some regions (Yang *et al*, 2010). Therefore, the new and efficient procedure for the preparative separation of scutellarin from a new resource is significantly important to the naturally sustainable resource management of *E. breviscapus*.

## Conclusion

A novel preparative separation process of scutellarin is established including extraction of scutellarin from *E. multiradiatus* using 80% methanol and purification by macroporous resins and ODS column. It would probably be an effective method for the preparative separation of scutellarin from *E. multiradiatus*. The overall procedure is efficient, convenient, economically feasible, and repeatable. Moreover, the studies suggest that *E. multiradiatus* could serve as a new drug resource for the preparation of scutellarin.

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