Extraction and purification of deguelin from *Derris trifoliata* Lour root

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Abstract: This article deals with extraction and purification of deguelin, which is a main rotenoid occurred in the root of *Derris trifoliate* Lour. Deguelin has gained much attention of scientists because of its potential ability to inhibit cancer cell proliferation. The dried root powder was extracted with ethanol by ultrasonic-assisted extraction for 30 min, and then filtrated and concentrated to give pasty concentrate. The concentrate was loaded onto silica gel column chromatography subsequently; and the chemical was eluted using a binary solvent mixture of petroleum ether (60-90ºC)-ethyl acetate (4:1, v/v). All tentative identification was carried out by high performance liquid chromatography (HPLC). Most crystal of deguelin was obtained after the fractions mainly containing deguelin were pooled and placed in the dark at 4ºC for three days. With recrystallization from carbon tetrachloride for three times, the purity of deguelin crystal was 99.15% and the yield was 0.55% of the dried weight of *D. trifoliate* Lour root.

Key words: deguelin, extraction, purification, silica gel column chromatography
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1 Introduction

Derris trifoliate Lour is mainly distributed in humid environment of subtropical and tropical regions[1]. It is widely used as a helminthic, a medication capable of causing the evacuation of parasitic intestinal worms.

Rotenoids, which belong to the isoflavone family, are major bioactivity chemical constituents of *D. trifoliate* root, including tephrosin, deguelin and dehydrodeguelin[2].

Deguelin, one of the rotenoids (Figure 1), was first obtained from the leaves of *Cracco vogelii*, *Derris* root and *Lonchocarpus nicou* root in 1931 by Clark F P, Tephrosin. I [3]. It also occurs in plants of both *Derris* Lour and *Tephrosia* Pers[3-6]. Zeng X N, et al.[6] noted the most abundant content of deguelin was 2.6% in the new leaf of *Tephrosia vogelii* Hook. Furthermore, deguelin has been shown to be present in the root of *D. trifoliate* Lour while absent in its stem and leaf[1].

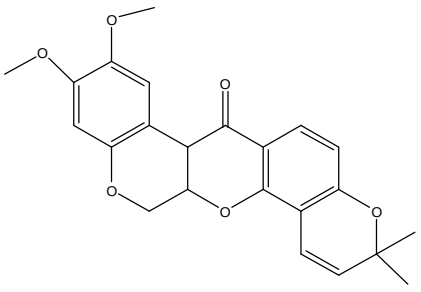

Figure 1 Chemical structural diagram of deguelin

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Deguelin was reported to inhibit the growth of colon cancer cells through the induction of apoptosis and cell cycle arrest[7,8]. It was also reported to inhibit the growth of skin cancer cells[9]. Some data demonstrated that deguelin could inhibit lung cancer cell proliferation effectively, but would not give rise to side effects on normal cells[10-14]. The latest research showed that deguelin could selectively induce apoptosis in breast cancer cells through down-regulation of inhibitor of apoptosis proteins[15].

The liquid extract rotenoids with an organic solvent was usually produced by several methods, such as room-temperature extraction, vibrating-assisted extraction, sohxlet extraction, among othersetc.[16-20]. Of these methods, few studies referred to ultrasonic-assisted extraction. On the other hand, after reviewing technologies for separation and purification of deguelin from other rotenodis by existing literature, it was found that there were few studies referring to silica gel column chromatography separation and crystallization of deguelin except under high performance liquid chromatography (HPLC), which was often high cost[21-27].

In recent years, the pharmacological activities have been more and more interested in deguelin, but new methods for deguelin purification have not been reported. Hence, some new processes or technologies to isolate deguelin from impurities are being sought.

2 Materials and methods

2.1 Chemicals and materials

The analytical standard of deguelin was obtained from the American Sigma Company in the U.S.A., with purity of 98%; the solvents for the HPLC mobile phases were HPLC grade, obtained from the Tianjin Shield Co. in China; doubly distilled water was purified using a Milli-Q system (Milli-pore, Bedford, MA, U.S.); silica gel was from the Qingdao Haiyang Chemical & Special Silica Gel Co. Ltd. in China; and all other reagents were of analytical grade. The *D. trifoliate* Lour root was obtained from the Fujian Haidong Biological Technology Co. Ltd. in China.

2.2 Extraction of deguelin

The root of *D. trifoliate* Lour was dried at 80℃ for 5 h, grinded and passed through a 150-mesh sieve. Dried root (10.0 g) was extracted twice, each time with 100 mL of methanol, ethanol, acetone, chloroform, ethyl acetate and petroleum ether (60-90℃) for 30 min by ultrasonic-assisted extraction using a 600 W ultrasound processor, respectively. Then the extracts were filtrated and concentrated. The concentrate was placed in the dark at 4℃ for further study.

The percentage extraction was counted as: the total extraction rate (%) = (weight of *D. trifoliate* Lour root extract/weight of dried root samples)×100%. The extraction yield of deguelin content in extraction was counted as: deguelin content in extract (%) = (weight of extracted deguelin/total weight of *D. trifoliate* Lour root extract)×100%.

2.3 Silica gel column chromatography

The concentrate was dissolved in chloroform at a ratio of 5:1 (V/w) of chloroform-to-the concentrate. The solution was applied to a silica gel column (40 cm× 3.5 cm i.d.), which was preconditioned with petroleum ether (60-90℃). The column was eluted employing chloroform-petroleum ether (60-90℃) (4:1, V/V). The eluent was collected by fraction size of 5 mL, and tentative identification was carried out using HPLC. The fractions mainly containing deguelin were pooled.

2.4 Crystallization and recrystallization

After the pooled fraction mainly containing deguelin was placed in the dark at 4℃ for 3 d, much crystal was gained. With recrystallization from carbon tetrachloride for three times, the crystal was purified.

2.5 HPLC analysis

The HPLC system is consisted of a Waters 2695 High Performance Liquid Chromatography (Waters Cooperation, Massachusetts, U.S.), an automatic sampler, a Waters TM C_{18} reversed-phase column (150 mm× 4.6 mm, i.d., 5 μm) and a Waters 2996 Photodiode Array Detector (Waters Cooperation, Massachusetts, U.S.). System management and hardware interface for data acquisition were performed by the Empower computer sofeware package from the Waters Cooperation. The HPLC separation was performed at column temperature of 25℃ and the injection volume was 15 L. A binary solvent mixture of methanol-water (66:34, V/V) was used

as mobile phase, and measurements were performed at a flow rate of 1.0 mL/min. Detection was done at 240 nm[6].

2.6 FTIR spectroscopy

Fourier transform infrared spectroscopy with a resolution of 2 cm^{-1} were recorded on a NICOLET AVATAR 360 spectrometer in the region from 400 to 4000 cm^{-1} at 25°C. The sample was recorded by scanning 32 times.

2.7 ESI-MS and 1H NMR experiment

ESI-MS experiments were carried out on a Bruker Esquire 3000 plus (Bremen, Germany) in positive ionization mode analyzing ions from 50 m/z up to 2,200 m/z.

Identification of the crystal was performed by a quadrupole time of flight (Q-TOF) Premier system coupled with an ESI source (Micromass, Simonsway, Manchester) and by a Bruker Avance 600 NMR.

2.8 Data analysis

All tests were performed three times, and the mean values are presented in this paper.

3 Results and discussion

3.1 Selection of extractant

The selection of extraction solvent is an important criterion for extracting desired components from plant materials. In this study, methanol, ethanol, acetone, chloroform, ethyl acetate and petroleum ether (60-90℃) were used to extract deguelin. Table 1 compares the total extract of *D. trifoliate* Lour root and the extraction yield of deguelin content in different organic solvent systems. The highest total extract of 20.21% was obtained by methanol, with a relative low extraction yield of deguelin content of 0.95%. The highest extraction yield of deguelin content, as high as 1.19%, slightly higher than in ethanol solution, 1.12%, was obtained by acetone. However, ethanol is a more preferable solvent for this experiment because of its environmentally safe, inexpensive and nontoxic features. Although the extraction yield of deguelin content was 1.05% in chloroform, slightly lower than in ethanol, expensive and toxic features made chloroform an unsuitable solvent in this step of the experiment. Ethyl acetate and petroleum ether (60-90℃) were not effective as solvents for extraction in this experiment because of relatively lower extraction yields, 0.67% and 0.09%, respectively.

Table 1 Extract ability comparison of different solvents on extraction of deguelin

Extractant	methanol	ethanol	acetone	chloroform	ethyl acetate	petroleum ether (60-90℃)
Total extraction rate/%	20.21	14.98	12.56	11.70	9.37	2.05
Extraction yield of deguelin content/%	0.95	1.12	1.19	1.05	0.67	0.09

3.2 Selection of eluent for silica gel column chromatography

After the concentrated extract was loaded onto the silica gel column, petroleum ether (60-90℃) was added as the sample solution layer went down almost to the surface of the column bed. The objective is to keep the crude extract solution in as small a volume as possible to diminish band broadening. After the petroleum ether (60-90℃) reached the surface of the column bed, the authors developed the silica gel column successively with petroleum ether (60-90℃) and then 5%, 10%, 15%, 20%, 25%, 30%, 35% and 40% chloroform in petroleum ether.

Deguelin could not be eluted by petroleum ether (60-90℃). With lower concentration of chloroform in petroleum, it could be eluted but a large amount of the binary solvent mixture was needed. In the present investigation, 25% chloroform in petroleum ether (60-90℃) was good at separation at this step of the experiment. If the chloroform concentration in petroleum ether (60-90℃) was higher than 25%, deguelin moved down too quickly and could not be separated from many other components. The HPLC analysis of the extract and the pooled fraction mainly containing deguelin after this step of the separation were shown in Figures 2 and 3, respectively.

3.3 HPLC-UV analysis of the crystal

About 55 mg crystal (Figure 4) with the purities of 99.15% was gained after the pooled fraction was placed in the dark for three days at 4℃ and then recrystallized in carbon tetrachloride. The HPLC analysis and ultraviolet

spectra of the recrystallized compounds were shown in Figures 5 and 6, respectively.

As shown in Figure 6, the maximum absorption peak of deguelin was at 271.6 nm, and another two peaks at 300.1 and 320.3 also appeared.

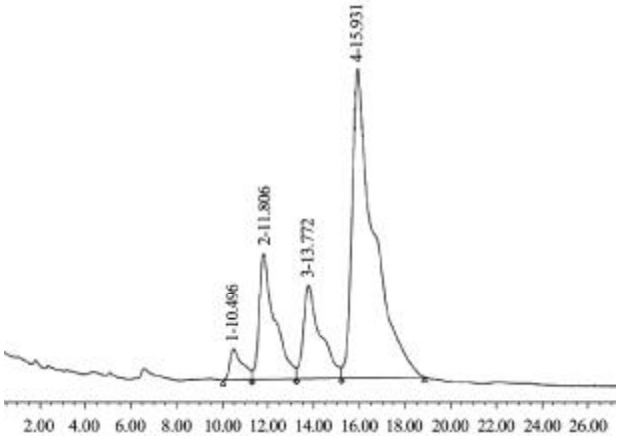

Figure 2 HPLC chromatograms of the extract of *D. trifoliate* Lour root

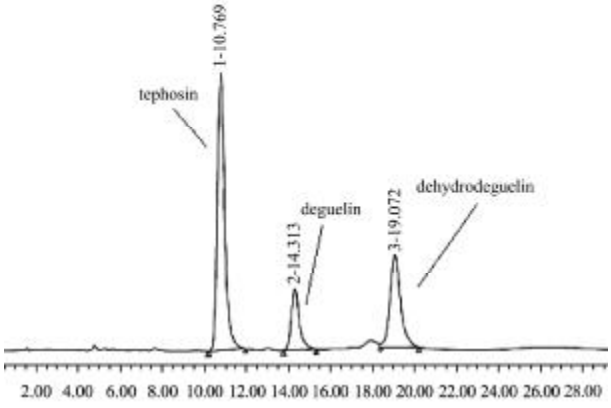

Figure 3 HPLC chromatograms of the mixture containing deguelin, dehydrodeguelin and tephrosin after using silica gel column chromatography for separation.

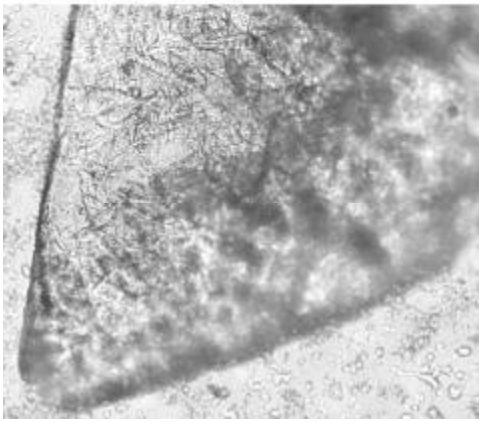

Figure 4 Microstructure of the recrystallized compound

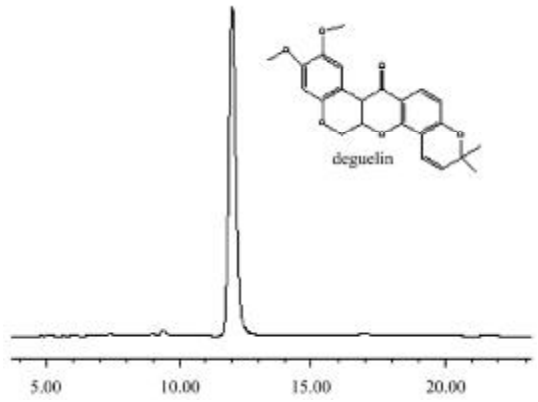

Figure 5 HPLC chromatograms of the recrystallized compound

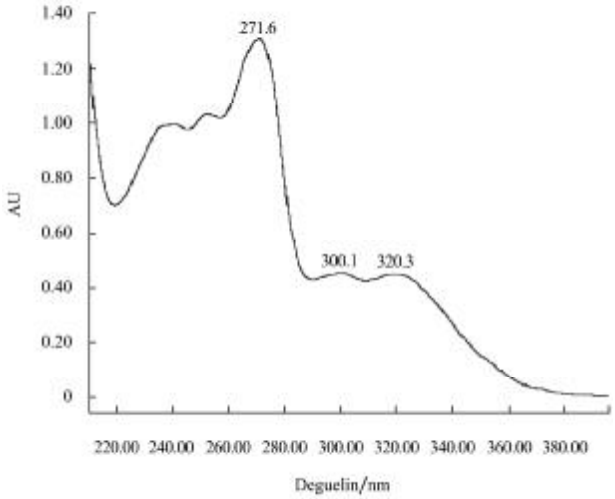

Figure 6 Ultraviolet spectra of the recrystallized compound

3.4 FTIR measurements of deguelin

FTIR spectroscopy is a chemical-microenvironment-sensitive and functional group characteristic technique[28]. The representative spectra of deguelin are shown in Figure 7.

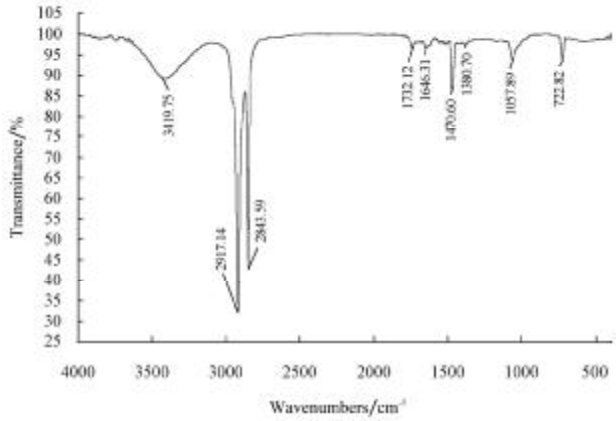

Figure 7 FTIR spectra of the recrystallized compound

In Figure 7, the main peaks of FTIR spectrum at 722.82, 1470.60, 1466.31, 1732.12, 2843.59 and 2917.14 cm^{-1} were attributed to the benzene rings, C-O-C, -C=C, -C=O, $-CH_3$ and $-O-CH_3$, respectively. The peaks in the range of 3150 to 3620 cm^{-1} corresponded to -OH because of a little water in the air mixed with the sample. The above results indicate that there were almost equal contents of $-CH_3$ and $-O-CH_3$ in the compound of deguelin, which agree with the report by Manjary F, et al[29].

3.5 Structure identification

The structure identification of the crystal was performed with ESI-MS (positive ion mode), ^1H NMR, with TMS as the internal standard. Data of the recrystallized compounds was given as follows: ESI-MS (m/z): 395.1 ($[M+H]^+$). ^1H NMR (600 MHz, $CDCl_3$): δ ppm: 8.05 (1H, d, J = 8.7 Hz), 7.95 (1H, s), 7.20 (1H, d, J = 1.9 Hz), 7.06 (1H, dd, J = 1.9 Hz, J = 8.2 Hz), 6.91 (1H, d, J = 8.3 Hz), 6.86 (1H, d, J = 8.8 Hz), 6.81 (1H, d, J = 10 Hz), 3.71 (6H, s), 1.56 (6H, s). Compared with the data given by Shin Y G, et al [27, 30], the recrystallized compounds were identified as deguelin.

4 Conclusions

A means of organic solvent extraction and silica gel column chromatography separation of deguelin from *D. trifoliate* Lour root was set up. The separation was performed by using a silica gel column chromatography, which was then eluted by a binary solvent mixture of petroleum ether (60-90℃)-chloroform (4:1, v/v). The purified solution contains deguelin, dehydrodeguelin and tephrosin. After this mixture was placed in the dark at 4℃ for 3 d, much crystal of deguelin was obtained with the yield of 0.55%.

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