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Research Paper

Qualitative and quantitative analysis of Chinese herb fructus chaenomelis☆

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Abstract

Objective: To establish reliable methods for evaluating the quality of Chinese herb *fructus chaenomelis*. **Methods**: Qualitative analysis by Thin layer chromatography (TLC), reference substances were *Chaenomeles speciosa* (Sweet) *Nakai* and oleanolic acid, a mixed solvent of chloroform-methanol (40 : 1) was employed as the mobile phase, color developing agent was 10% sulfuric acid-ethanol solution. In the system of high performance liquid chromatography (HPLC), a Prontosil Eurobond C^{18} column (250 mm×4.0 mm, 5 μm) was used, the mobile phase was composed of acetonitrile-methanol-0.4% ammonium acetate solution (55 : 25 : 20), the flow rate was 1.0 ml/min with UV detected at 210 nm, the column temperature was maintained at room temperature. **Results**: In the system of TLC, oleanolic acid was separated successfully. In HPLC, the linear ranges of oleanolic acid and ursolic acid were 5.89~13.73 μg (R=0.9990) and 6.84~15.96 μg (R=0.9990), respectively. The average recoveries of oleanolic acid and ursolic acid were 97.52% (RSD=2 .58%), 98.21% (RSD=2.23%), respectively. **Conclusion**: The established TLC method can easily distinguish Chinese herb *fructus chaenomelis* from other commonly used crude drugs of the same family .The HPLC method for determining oleanolic acid and ursolic acid is simple, reproducible, accurate and feasible. The methods reported in this paper can be used scientifically and effectively to evaluate the quality of Chinese herb *fructus chaenomelis*.

Keywords: fructus chaenomelis; TLC; HPLC; oleanolic acid; ursolic acid

INTRODUCTION

The Chinese folk drug, Mugua (*fructus chaenomelis*), is derived from the dried fruit of *Chaenomeles speciosa* (Sweet) *Nakai*. It is specified in Dictionary of Chinese Crude Drugs with the efficacy of removing dampness to restore normal functionating of the stomach, calming the liver and relaxing muscles [1]. Some research results show that it possesses the activities to benefit liver and inhibit bacteria, etc. It can be used for the treatment of hepatitis by local people. Oleanolic acid and ursolic acid are the active constituents from Mugua. Pharmacological study showed that oleanolic acid could significantly inhibit inflammation, enhance immunologic function, prevent hepatocirrhosis, decrease transaminase and so on[2-3]. Recently, medical studies indicated that oleanolic acid and ursolic acid had anti-cancer effects[4-7].

Mugua is a commonly used traditional Chinese medicinal herb. However, its quality evaluation standards in Chinese Pharmacopoeia [8] are not very scientific and reasonable. The maker substance for the TLC differentiation is Chaenomeles speciosa (Sweet) Nakaia and lacks characteristic component for Mugua. Moreover, the quantitative method is to determine the content of oleanolic acid by TLC-scanner[9], which is a relatively rough method.

Oleanolic acid and ursolic acid belong to pentacyclic triterpenoids, which are active extracts from Mugua in protecting the liver. This study estabilished a special qualitative method of TLC and an accurate quantitative method of HPLC to analyze oleanolic

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acid and ursolic acid (***Fig. 1***) from Mugua.

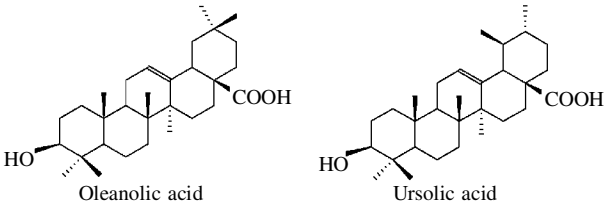


Fig. 1 Structure of oleanolic acid and ursolic acid

MATERIALS AND METHODS

Apparatus and reagents

The analysis was carried out on a HPLC system (Shimadzu,Japan) equipped with a LC-10ATvp pump, SPD-10Avp detector,CTO-10ASvp columm oven, and Peak-ABC chromatography workstation Ver 2.11 (JiTeng Trading Pte Ltd., Singapore). KQ5200 ultrasonic washer (Kunshan Ultrasonic Instrument Co.Ltd.,China) was used.

The acetonitrile and methanol (HPLC/Spectro grade), the distilled water(made in our own laboratory), other reagents (A. R. grade), high performance thin layer silica gel G plates (Qingdao Ocean Chemical Plant, China), *Chaenomeles speciosa* (Sweet) *Nakai* as the reference substance (National Institute for the Control of Pharmaceutical and Biological Products, No: 121003-200303, differentiation), oleanolic acid as the reference substance (National Institute for the Control of Pharmaceutical and Biological Products, No: 110709-200304,determination of content), ursolic acid as the reference substance(National Institute for the Control of Pharmaceutical and Biological Products,No: 110742-200415 determination of content), commercial samples(No.1,2 and 3) of Mugua(purchased from Nanjing, Bozhou and Xuanzhou Chinese Drug Store, respectively) were identified by Dr. Chen Lina and preserved in the Department of Pharmacognosy, Nanjing Medical University. All samples were abraded into powder and sieved with No.4 sieve.

TLC methods

Oleanolic acid was accurately weighed 4.010 mg and dissolved in 2 ml anhydrous ethanol to produce a reference solution A.

The powder of *Chaenomeles speciosa* (Sweet) *Nakai* (about 1 g) was put into a 25 ml measuring flask, and treated according to the preparation of test solution to produce a reference solution B.

The sample powder (about 1 g) was put into a 25 ml measuring flask,then dissolved in anhydrous ethanol under ultrasonic oscillation for 30 minutes, diluted to the volume with anhydrous ethanol, mixed well and filtrated. Filtrate of the second filtration was put into a 10 ml measuring flask to prepare test solution.

Procedures

Three microliters of test solutions, reference solution A and reference solution B were separately applied to the plate, which was developed by chloroform-methanol (40 : 1, V/V). After development, the plate was removed, dried in the air, sprayed with 10% sulfuric acid-ethanol solution, then heated at 105℃ for 5 minutes.

HPLC method

Chromatographic condition

Column: Prontosil Eurobond C18 (250 mm×4.0 mm,5 μm); mobile phase: acetonitrile-methanol-0.4% ammonium acetate solution (55 : 25 : 20); flow velocity: 1.0 ml/min;detection wavelength: 210nm; column temperature: room temperature. Under the described chromatographic condition, the theoretical plate number was more than 3 000,and the peak asymmetry factor was 1.03 calculated with oleanolic acid peak. The resolution between the chromatographic peak of analyte(oleanolic acid) and the neighboring peak (ursolic acid peak) was no less than 1.5,and there was no interference in sample c hromatogram.

Preparation of solution

Preparation of reference solution

Oleanolic acid and ursolic acid were accurately weighed 4.905 mg and 5.700 mg, respectively ,and dissolved in 5ml anhydrous ethanol to obtain a reference solution.

Test solution

The sample powder (about 0.5 g) and 20 ml of anhydrous ethanol were put into a 50 ml conical flask, then accurately weighed, extracted under reflux on a water bath for 1 hour, and weighed again. The lost weight of extract solution in the process of reflux was compensated with anhydrous ethanol, then the extract solution was mixed well, and filtered. The successive filtrate through microfilm(0.45 μm) was used as the test solution.

Linearity

0.6, 0.8, 1.0, 1.2, 1.4 ml of the reference solution were separately put into 2 ml volumetric flasks and diluted to volume with anhydrous ethanol and mixed well to obtain a serial solutions.Then 20 μl of the solutions were injected into HPLC system for analysis, respectively.

Precision

The precision was determined with the same refer-

ence solution, 20 μl was injected for 6 times consecutively.

Solution stability

The stability was determined with the same test solution that was allowed to stand at room temperature for 0,1,2,3,4,5 hours, respectively, and 20 μl were injected for 6 times, according to the interval.

Repeatability

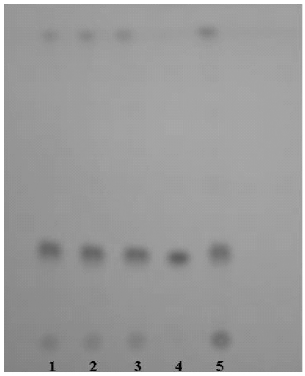
The repeatability was determined with the same sample . Six sample portions of 0.5 g each, were separately extracted according to the method as mentioned in test solution to produce 6 test solutions. Each solution was injected twice, with a volume of 20 μl.

Recovery

Six portions of definite amount of the sample powder (about 0.25 g) were accurately weighed and put into a 50 ml conical flask, then extracted according to the method as mentioned in test solution after adding 1 ml of the reference solution,respectively.

RESULTS

TLC analysis of Mugua obtained from different areas



1–3: Samples; 4: Oleanolic acid; 5: *Chaenomeles speciosa* (Sweet) *Nakai.*

Fig. 2 TLC chromatograms of Mugua obtained from different areas

The results of TLC chromatograms showed that the deep purple-red spots in the chromatograms obtained with the test solutions corresponded in colour and position to the spots in chromatograms obtained with the reference solution A and B. In addition, the chromatograms of all samples of Mugua purchased from different areas showed high similarity (***Fig. 2***).

Determination of oleanolic acid and ursolic acid in samples by HPLC

Linearity The results showed that an excellent correlation existed between the peak area(Y) and the concentration (X) of oleanolic acid and ursolic acid, respectively. The obtained regression equation and linear range were shown in ***Table 1***.

Precision

The peak areas of oleanolic acid and ursolic acid were measured, and the RSD of two compounds were 1.24% and 0.99%, respectively.

Solution stability

The peak areas of oleanolic acid and ursolic acid were invariable and RSD of two compounds were 1.29%, 1.48%,indicating that the solution was stable at least for 5 hours.

Repeatability

According to the peak areas, the contents of oleanolic acid and ursolic acid were calculated. The average contents of two compounds were 0.34% , 0.60% and RSD were 1.24%, 1.16% ,respectively.

Recovery

The average recoveries of oleanolic acid and ursolic acid were 97.52% , 98.21% , and RSD were 2 . 58%, 2.23%, respectively.

Sample measurement

Each of three commercial samples was extracted according to the method as mentioned in test solution and analyzed. The measured contents were listed in ***Table 2***. Their chromatograms were shown in ***Fig. 3***.

Table 1 **Regression equations , correlation coefficients and linear ranges for two compounds**

Compound	Regression equation	Correlation coefficient	Linear range(μg)
Oleanolic acid	Y = 17128X − 909.81	*R* = 0.9990	5.89 − 13.73
Ursolic acid	Y = 14587.00X + 9802.50	*R* = 0.9990	6.84 − 15.96

Table 2 **Contents of oleanolic acid and ursolic acid in samples** (*n* = 3)

No.	Place of purchase	Content (%)	
		Oleanolic acid	Ursolic acid
020410	Jiangsu, Nanjing	0.26	0.50
020413	Anhui, Bozhou	0.35	0.61
020416	Anhui, Xuanzhou	0.41	0.70

DISCUSSION

Compared with previous study[10], there were larger differences in optimization of chromatographic conditions and selection of determined index.

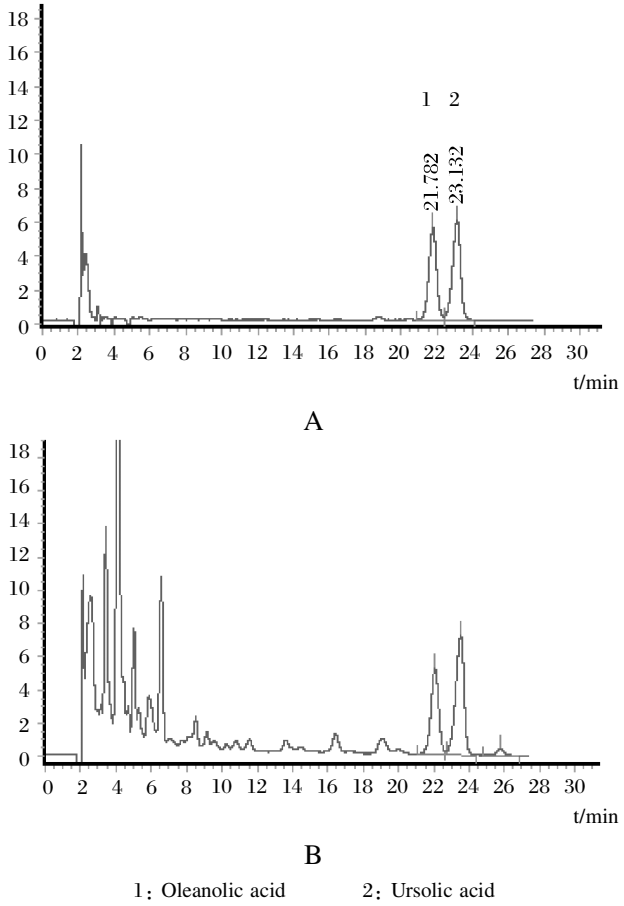


Fig. 3 HPLC chromatograms of reference solution (A) and test solution (B)

Several extraction methods,such as Soxhlet extract,heated in water bath,ultrasionic extraction, and different extract solvents, such as methanol, ethanol, anhydrous ethanol, ethyl acetate and chloroform were used. However, Soxhlet's method extractor took too much time, so that reflux extraction was selected. Furthermore, extracting solvents were also compared. Under reflux with methanol or ethanol, there was interference in sample chromatograms. Eventually,our new method, using anhydrous ethanol as a solvent and reflux extracting on a water bath for 1 hour, was confirmed by the experimental data to be simpler, more economical than the reported method.

Most of previous studies of Mugua were concentrated on flavonoids. However, oleanolic acid which is an active constituent in Mugua was neglected. This study would supply another evidence for TLC analysis of Mugua.

The mobile phases of different systems and proportions were compared,such as methanol-H_2O, acetonitrile-H2O, methanol-acetic acid solution, methanol-phosphoric acid-isopropanol, and acetonitrile-methanol-ammonium acetate solution. The optimized effect for isolating oleanolic acid and ursolic acid was obtained by acetonitrile-methanol-0.4% ammonium acetate aqueous solution (55:25:20) as the mobile phase.

Among the samples tested, the highest level of total content of oleanolic acid and ursolic acid was 1.11% in sample purchased from Xuanzhou, and the lowest one was 0.76% in samples purchased from Nanjing. The quality of Xuan-Mugua is very good (Mugua is mainly produced in Xuanzhou,Anhui Province).

In summary, the proposed TLC method can easily distinguish Mugua from other commonly used crude drugs of the same family.The established HPLC method for simultaneously determining oleanolic acid and ursolic acid is simple, reproducible, accurate and feasible. The methods of TLC and HPLC can be used scientifically to evaluate Mugua both qualitatively and quantitatively.

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