Research Progress on the Chemical Constituents and Quality Evaluation Methods of *Spina gleditsiae*

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Abstract: In this paper, the relevant information on chemical constituents and quality evaluation of Spina gleditsiae were retrieved and summarized. Modern studies show that Spina gleditsiae mainly contains flavonoids, phenols, triterpenoids, coumarins, sterols, lactones, organic acids and other compounds. Then the quality evaluation methods of Spina gleditsiae were introduced. In the future research, it is necessary to explore the extraction and separation technology of its chemical constituents and active constituents, and establish appropriate models to promote its development and application. It provides a reference for the further establishment of scientific and reasonable studies on the effective components of Spina gleditsiae.

1 INTRODUCTION

Spina gleditsiae has a long history of application in China, and also be used as compatible drug in combination with other traditional Chinese medicines to treat cancer, because which can inhibit oncogene expression.

Spina gleditsiae is a traditional Chinese medicine with low price and wide sources. Modern studies show that Spina gleditsiae mainly contains flavonoids, phenols, triterpenoids, coumarins, sterols, lactones, organic acids and other compounds. A large number of literature studies believe that the triterpenoid saponins and flavonoids in Spina gleditsiae are the active material basis of anti-cancer, antibacterial and main medicinal value of this plant medicine. Flavonoids or flavonoid glycosides in Radix Spina gleditsiae have a high content. Most modern studies focus on the extraction process of total flavonoids in Spina gleditsiae, while the extraction process and content determination of other components in Spina gleditsiae are relatively backward, and there is a lack of quality evaluation system for Spina gleditsiae in Chinese Pharmacopoeia.

2 FLAVONOIDS

Flavonoids are the main active components of Spina gleditsiae, which can inhibit the growth of human tumor cells. At present, 14 kinds of flavonoids have been reported in Spina gleditsiae (Table 1).

Table 1: Flavonoids in Spina gleditsiae reported.

No.	Compound
1	Quercetin
2	Fustin
3	Taxifolin
4	Aromadendrin
5	Rutin
6	Isoquercitrin
7	Quercitrin
8	Eriodictyol
9	Fisetin
10	Epicatechin
11	trans-2R,3R-3`,4`,5,7-tetrahydroxyflavanonol
12	8-C-glucopyranosyl-3,4`,7-trihydroxyflavone
13	8-3,3`,5,5`,7-pentahydroflavanone
14	5-7,3`,5`-trihyroxy-5-methoxyflavanonol

The content and monomer of flavonoids in Spina gleditsiae are closely related to its quality. At present, high performance liquid chromatography (HPLC), high performance liquid chromatography mass spectrometry (HPLC-MS) and ultraviolet spectroscopy (UV) are often used for quantitative or qualitative analysis of its components. Yu et al. analyzed 13 batches of different Spina gleditsiae by HPLC, established the fingerprint of Spina gleditsiae, and calibrated 14 common peaks with similarity of more than 0.90, and quantitatively determined three flavonoids, which made up the blank of quality evaluation of Spina gleditsiae in Chinese pharmacopoeia. Jing et al. studied flavonoids in Spina gleditsiae by HPLC-MS. Eight flavonoids were detected and identified by mass spectrometry, and compared with the published studies. Li et al. obtained the extract by column chromatography, after extracting YSpina gleditsiae with methanol, petroleum ether and other solvents, and identified which contained two kinds of flavonoids. Finally, the chemical structures of two flavonoids were determined (Figure 1).

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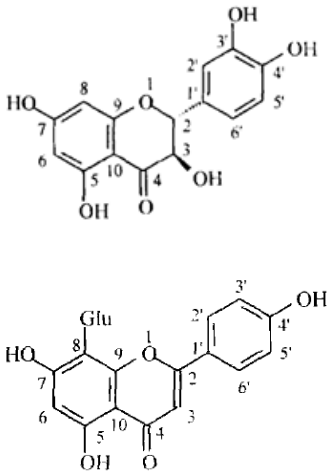

Figure 1 Chemical structure of flavonoids in Spina gleditsiae determined by Li.

Tian et al. established a HPLC method to determine the content of quercetin in Spina gleditsiae. Xu et al. used colorimetry combined with silica gel, glucan gel and other chromatographic methods to separate Spina gleditsiae, in which the active components were tracked. Using spectral data and physical and chemical properties to identify the chemical structure, eight compounds were isolated. Liu et al. studied Spina gleditsiae by chemical composition pre-experiment and HPLC, and HPLC conditions were established: mobile phase as solution A (acetonitrile:formic acid=99.5:0.5) and solution B(water:formic acid=99.5:0.5), detection wavelength as 278nm, column temperature as 25℃, sample quantity as 20μL, the velocity as 1mL•min^{-1}.

3 TERPENOIDS

Li et al. first extracted Spina gleditsiae with different organic solvents, and then separated them by silica gel column chromatography, and obtained two triterpenes which were identified as Echinocystic acid and Gleditsia saponin C. Lim et al. isolated four steroid substances and one triterpenoid substance from Spina gleditsiae. Then, 90% MeOH was used for reflux extraction and the ethyl acetate parts were separated by repeated silica gel column chromatography. A new betulinic triterpene 2-β-carboxyl,3-β-hydroxyl-Norlupa(1)-20(29)-en-28-oic acid and five betulinic triterpenes named zizyberanalic acid, betulic acid, alphitolic acid, 3-0-trans-p-coumaroyl alphitolic acid and 2-hydroxypyracrenic acid were identified, all six compounds were isolated for the first time.

Lim et al. also isolated and identified a triterpene compound D:C-friedours-7-en-3-one in the anti-mutagenic activity screening experiment.

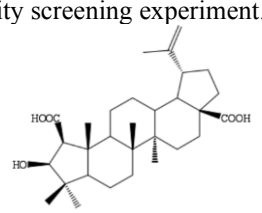

Figure 2 Structure of new terpenne compound from Spina gleditsiae

Xu et al. determined the structure of 8 compounds by 70% ethanol extraction, repeated columnar extraction, NMR and MS, which include 3 β-acetoxy-12-oleanene-28-carboxylic acid, xylem ketone and betulin terpenoids.

It has been reported that saponins are mostly isolated from normal fruit of Spina gleditsiae or pig tooth soap (abnormal fruit of Spina gleditsiae). The aglycones are oleanolic acid or acanthic acid, and the sugar ligand mainly includes rhamnose-arabinose, glucose, xylitol, etc. There were not many saponins isolated from Spina gleditsiae. Li et al. only isolated and identified saponinsC from the n-butanol part of Spina gleditsiae.

4 PHYTOSTEROLS

Xu et al. used organic solvent to extract the ethanol extract of Spina gleditsiae, and then used column chromatography, thin layer chromatography silica gel and other methods to trace the extraction, which separated 8 active components, including β-sitosterol and daucosterol.

Lim et al. extracted Spina gleditsiae by heating reflux with 80% methanol, and isolated 5 compounds conclude four steroids such as stigmasterol, β-sitosterol, stigmast-4-ene-3,6-dione and stigmastane-3,6-dione and the first isolated from a natural product D:C-friedours-7-en-3-one. The five compounds were isolated from Spina gleditsiae for the first time, among which stigmasterol showed the strongest anti-mutagenesis activity.

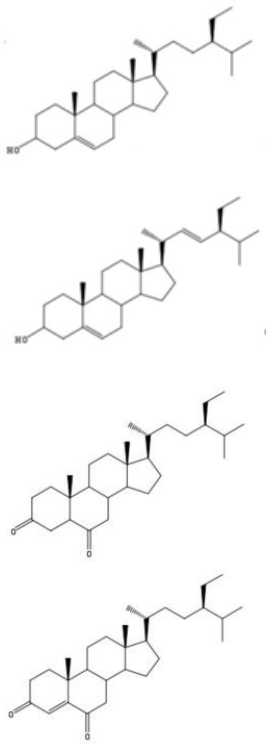

Figure 3 Four structures of steroids from Spina gleditsiae

5 PHENOLIC

Zhou et al. isolated and identified two phenolic compounds ethyl gallate and caffeic acid from Spina

gleditsiae, and caffeic acid showed moderate antibacterial activity.

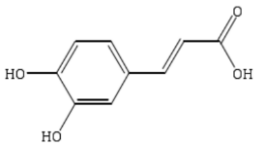

Figure 4 Structures of phenolic compounds from Spina gleditsiae

It was reported that two ellagic sides, 3-o-methylellagic acid-4′-(5"-acetyl)-α-l-furanoside and 3-o-methylellagic acid-4′-o-α-L-rhamnopyranoside, were isolated and identified from the ethyl acetate extract of Spina gleditsiae ethanol extract, which showed significant antifungal activity against rice blast fungus. Both compounds were isolated from Spina gleditsiae for the first time, of which the former was a new compound.

6 OTHER INGREDIENTS

Li et al. isolated betulinic acid derivatives from Spina gleditsiae and found that they had anti-HIV activity. Meanwhile, the extraction performance of betulinic acid derivatives from Spina gleditsiae was investigated by ionic liquid.

Liu et al. studied the components of saponin saponin by pretest of chemical composition, and found that flavonoids, lactones, coumarins or their glycosides, sterols, triterpenes, phenols, organic acids, reducing sugars, triterpenes saponins and so on were clearly present in saponin saponin.

Xu et al. isolated palmitic acid from Spina gleditsiae, which is a kind of saturated fatty acid.

7 QUALITY EVALUATION

Li et al. isolated betulinic acid derivatives and triterpenoids from Spina gleditsiae, and found that they had anti-HIV activity. Meanwhile, the extraction performance of betulinic acid derivatives from Spina gleditsiae was investigated by ionic liquid. Liu et al. studied the components of Spina gleditsiae through the chemical composition experiment, and found that flavonoids, lactones, coumarins or their glycosides, sterols, triterpenes, phenols, organic acids, reducing sugars, triterpenes saponins and other components were clearly present in Spina gleditsiae.

Li et al. studied 20 batches of Spina gleditsiae from different producing areas. After comparing the HPLC fingerprint of Spina gleditsiae, evaluating its similarity, and then conducting cluster analysis, it was found that: the chemical constituents of Spina gleditsiae from different producing areas were similar, but the content of Spina gleditsiae was different. There is a big difference between cultivated and wild Spina gleditsiae. This is probably because Spina gleditsiae is a plant of many origins. The differences above were related to light, rainfall, temperature and growth years of different areas.

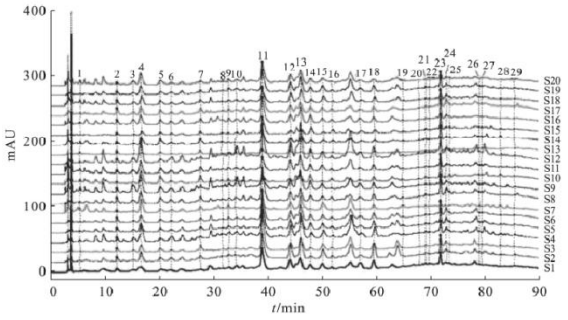

Figure 5 HPLC fingerprint for twenty batches of Spina gleditsiae from different regions

Wang et al. established HPLC chromatographic characteristics of different parts of Spina gleditsiae and methods for the determination of citipine content to evaluate the quality difference of different parts of Spina gleditsiae. The chromatographic column was Waters Symmetry (250 mm × 4.6 mm, 5 μm) with acetonitrile-0.1% formic acid solution as mobile phase. The wavelength was 260 nm, the column temperature was 25 ℃, and the flow rate was 1.0 m L/min. By similarity evaluation, heat map clustering analysis, principal component analysis and variance analysis, the HPLC characteristic chromatograms of different parts of 12 batches of Saponin were analyzed, and the content of citipine was determined.

The similarity between the HPLC characteristic chromatogram and corresponding characteristic chromatogram was greater than 0.990, which contained 12 batches of main prickle root, branch root and tip. Heat map cluster analysis and principal component analysis can distinguish the main prickle root and tip. The results of variance analysis showed that peak 2, citipine and quercetin were the main components that caused the quality difference of different parts of Spina gleditsiae. The content of axisolin in the same radix saponicae was in the order of tip > branch root > main prickle root. This method can reflect the difference of chemical constituents in different parts of Spina gleditsiae and has important significance for quality control and overall evaluation.

8 CONCLUSIONS

In recent years, researches have focused on the pharmacognostical identification and pharmacological action of Spina gleditsiae, and the main chemical components of which has been discovered and separated. The studies showed that flavonoids, terpenoids, sterols and other active ingredients were the most active components in Spina gleditsiae.

As a traditional Chinese herbal medicine, Spina gleditsiae has been shown to have anticancer, anticoagulant and immunological effects. In the future research, it is necessary to explore the extraction and separation technology of its chemical constituents and active constituents, and establish appropriate models to promote its development and application. It is believed that with the deepening of modern Chinese medicine research, the application of Spina gleditsiae will be more and more extensive.

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