Review

Research progress on chemical components, pharmacological effects, and quality control of *Crotalaria ferruginea* Graham ex Benth.

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Abstract

***Crotalaria ferruginea* Graham ex Benth. is a commonly used herb among ethnic minorities. Its whole plant is used as medicine for conditions such as heatstroke, tinnitus, hearing loss, phlegm heat cough, gum swelling and pain, lower back and knee pain, vaginal discharge and infantile malnutrition. Modern pharmaceutical research has found that *Crotalaria ferruginea* Graham ex Benth. mainly contains flavonoids, steroids, organic acids and terpenes, which have antibacterial, free radical scavenging, anti-inflammatory and other effects. Quality control mainly focuses on characteristics, identification, content determination, etc. This article summarizes the recent research progress on the chemical composition, pharmacological effects, quality control and extraction process of *Crotalaria ferruginea* Graham ex Benth. on the basis of existing *Crotalaria ferruginea* Graham ex Benth. research reports.**

Keywords: *Crotalaria ferruginea* Graham ex Benth.; chemical composition; pharmacological effects; quality control; extraction process

1 Introduction

Crotalaria ferruginea Graham ex Benth. (abbreviated as *C. ferruginea* hereafter) is the dried whole plant of Crotalaria ferruginea Graham ex Benth., a species belonging to the genus Crotalaria in the legume family (Fabaceae) [1]. The herb was first documented in the Dian Nan Ben Cao (South Yunnan Materia Medica) [2] and has been recorded in various traditional Chinese medicine compendiums since then [3-5]. It is also known with several other names, such as false groundnut, horse ringpod, dog ringpod, dog bell, ringpod bean, wild peanut, wild pea, horse bell herb, false indigo and false peanut. It is widely distributed throughout Yunnan Province and most regions of China [6,7]. The herb is characterized by its sweet, slightly bitter taste and cold nature, and it is associated with the lung, liver, and kidney meridians. It is reputed for the properties to clear heat and dampness, nourish the kidneys and liver, and relieve cough and phlegm. It is usually used to treat conditions such as heat strangury, tinnitus and deafness, phlegm-heat cough, gum swelling and pain, lumbar and knee pain, leukorrhea, and infantile malnutrition [8,9]. It is a

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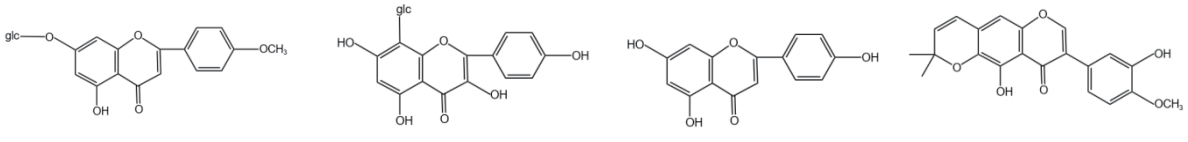
commonly used medicinal herb among the Yi and Bai ethnic minorities in Yunnan Province, China. Modern pharmacological studies have shown that *C. ferruginea* contains various chemical components, including flavonoids, steroids, organic acids, and terpenoids. The herb and its active constituents exhibit a range of pharmacological activities, such as antibacterial, free radical scavenging, and anti-inflammatory effects [10]. Current quality control research focuses on the characteristics, identification, content determination, and some inspection indicators. The extraction process of its chemical components mainly focuses on flavonoids. This study systematically reviews recent domestic research on the chemical composition, pharmacological effects, quality control, and extraction processes of *C. ferruginea*, providing a reference for the further development of its medicinal resources.

2 Chemical composition

2.1 Flavonoids

Most of the chemical components contained in *C. ferruginea* are flavonoids. Flavonoids are a class of compounds with a C6-C3-C6 skeleton, in which two benzene rings are connected by three carbon atoms. Modern pharmacological studies have shown that flavonoids have functions such as antioxidant, anti-cancer and cancer prevention, antibacterial, anti-inflammatory, antiviral, anti-radiation, anti-cardiovascular disease, hypoglycemic, neuroprotective, and free radical scavenging [11-13]. Long used the medicinal material of *C. ferruginea* [14]. After decocting with water, the sugar was removed by macroporous resin, and then through methods such as repeated silica gel column chromatography, flavonoid compounds cynaroside(5,7-dihydroxy-4-methoxyflavone-7-*O*-*β*-D-glucoside), 5,7,4′-trihydroxy-flavonol-8-C-*β*-D-glucoside, and genistein were isolated from it. Zhang et al. extracted the 95% ethanol extract of *C. ferruginea* with different solvents [15,16]. The extracts were subjected to a silica gel column, and column chromatography separation was carried out repeatedly. The structures of the isolated monomer components were identified by spectroscopic methods. As a result, the flavonoid compounds 5,7-dihydroxy-4-methoxyflavone-7-*O*-*β*-D-glucoside and genistein were isolated. Li et al. reflux-extracted and concentrated *C. ferruginea* with 75% ethanol [17]. After extraction with ethyl acetate, the product was separated by silica gel column chromatography. Through repeated silica gel column chromatography with petroleum ether-ethyl acetate and gradient elution by Sephadex LH-20 column chromatography, the flavonoid compounds 4′,5,7-trihydroxy-6-(2-hydroxy-3-methyl-3-butenyl) isoflavone genistein, orobol, and 3′,5-dihydroxy-4′-methoxy-2″,2″-dimethylpyrano-(5″,6″,6,7)-isoflavone, were obtained respectively. Wu isolated flavonoid compounds such as 4’,7-dimethoxy-5-hydroxyflavone, orobol, genistein, 4’,7-dihydroxyflavone, and 3′,5-dihydroxy-4′-methoxy-2″,2″-dimethylpyrano-(5″,6″,6,7)-isoflavone from the antibacterial active components of *C. ferruginea* [18]. The structure of the specific compound is shown in Fig. 1.



cynaroside(5,7-dihydroxy-4-methoxyflavone-7-*O*-*β*-D-glucoside)

5,7,4′-trihydroxy-flavonol-8-C-*β*-D-glucoside

genistein

3′,5-dihydroxy-4′-methoxy-2″,2″-dimethylpyrano-(5″,6″,6,7)-isoflavone

Fig. 1 Flavonoid compounds in *C. ferruginea*

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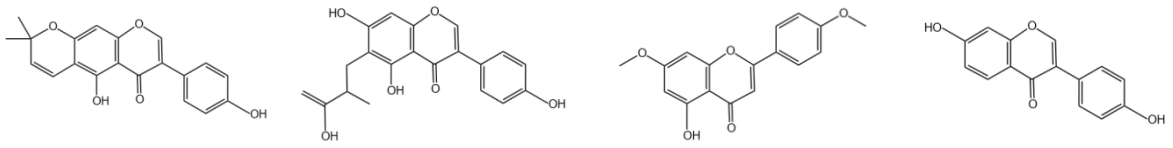
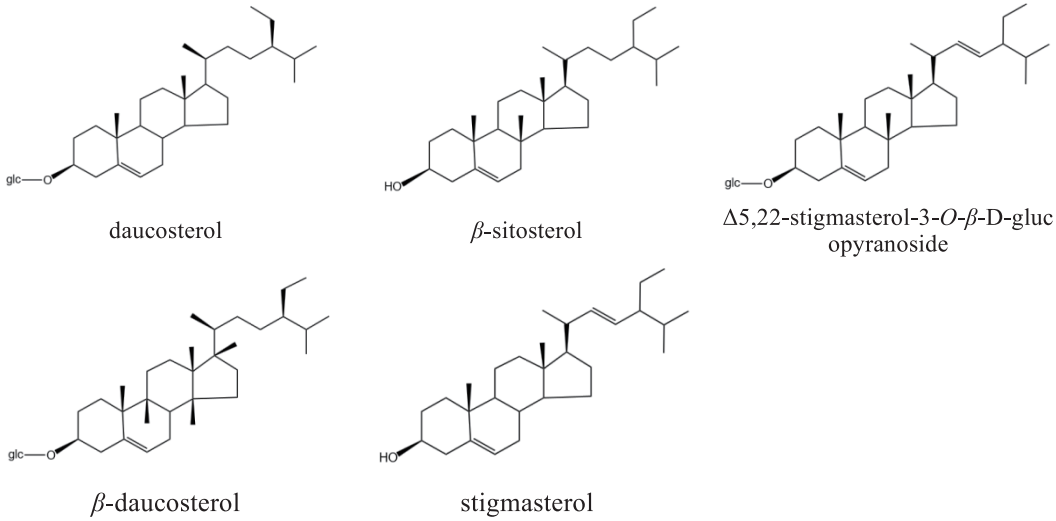


orobol | 4′,5,7-trihydroxy-6-(2-hydroxy-3-methyl-3- | 4',7-dimethoxy-5-hydroxyflavone | 4',7-dihydroxyflavone

Continued Fig. 1

2.2 Steroids

Steroids are another type of chemical components that are relatively abundant in *C. ferruginea.* Long used the medicinal materials of *C. ferruginea* [14]. After decocting in water, the sugar was removed by macroporous resin. Subsequently, through methods such as repeated silica gel column chromatography, the steroid compounds daucosterol and *β*-sitosterol were isolated from the processed materials. Zhang et al. extracted the 95% ethanol extract of *C. ferruginea* with different solvents [15,16]. The extracts were loaded onto a silica gel column, and column chromatography separation was carried out repeatedly. The structures of the isolated monomer components were identified by spectroscopic methods. As a result, the steroid compounds Δ5,22-stigmasterol-3-*O*-*β*-D-glucopyranoside, *β*-sitosterol, *β*-daucosterol, stigmasterol and were isolated. Li et al. reflux-extracted and concentrated *C. ferruginea* with 75% ethanol [17]. After extraction with ethyl acetate, the product was separated by silica gel column chromatography. Through repeated silica gel column chromatography with petroleum ether-ethyl acetate and gradient elution with Sephadex LH-20 column chromatography, the steroid compounds *β*-sitosterol and daucosterol were obtained respectively. Wu isolated steroid compounds such as stigmasterol and *β*-sitosterol from the antibacterial active components of *C. ferruginea* [18]. Lu isolated and identified the steroid compound stigmasterol from the ethyl acetate fraction of the 80% ethanol extract of *C. ferruginea* [19]. The structure of the specific compound is shown in Fig. 2.



daucosterol | *β*-sitosterol | Δ5,22-stigmasterol-3-*O*-*β*-D-glucopyranoside

β-daucosterol | stigmasterol

Fig. 2 Flavonoid compounds in *C. ferruginea*

2.3 Organic acids

Organic acids are also another type of chemical components that are relatively abundant in *C. ferruginea*. Long used the medicinal materials of *C. ferruginea* [14]. After decocting with water, the sugar was removed by macroporous resin, and then through methods such as repeated silica gel column chromatography, the organic acid compound stearic acid was isolated from it. Zhang et al. extracted the 95% ethanol extract of *C. ferruginea* with different solvents [15,16]. The extracts were loaded onto a silica gel column, and column chromatography separation was carried out repeatedly. The structures of the isolated monomer components were identified by spectroscopic methods. As a result, the organic acid compounds *p*-hydroxybenzoic acid and stearic acid were isolated. Li et al. reflux-extracted and concentrated *C. ferruginea* with 75% ethanol [17]. After extraction with ethyl acetate, the product was separated by silica gel column chromatography. Through repeated silica gel column chromatography with petroleum ether-ethyl acetate and gradient elution with Sephadex LH-20 column chromatography, the organic acid compounds *n*-dotetracontanoic acid and *n*-dotriacontanoic acid were obtained respectively. Wu isolated the organic acid compounds *n*-tricosanoic acid, stearic acid (*n*-octadecanoic acid) and *n*-tetracosanoic acid from the antibacterial active components of *C. ferruginea* [18]. The structure of the specific compound is shown in Fig. 3.

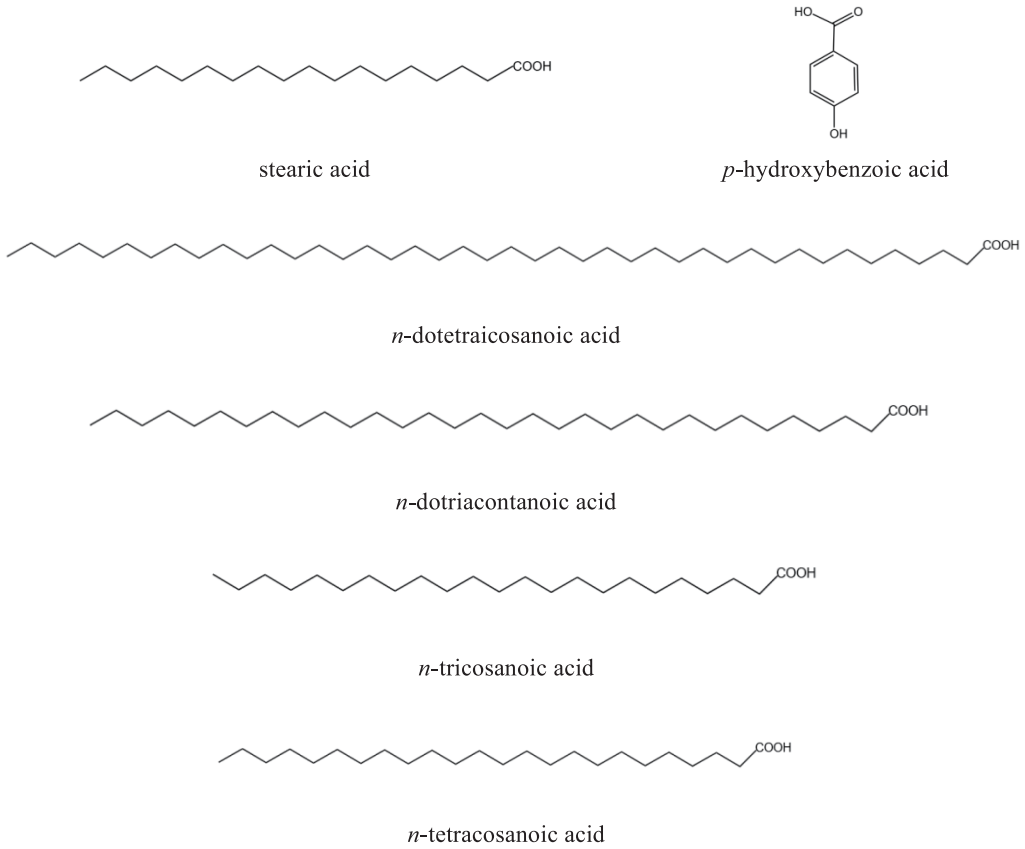
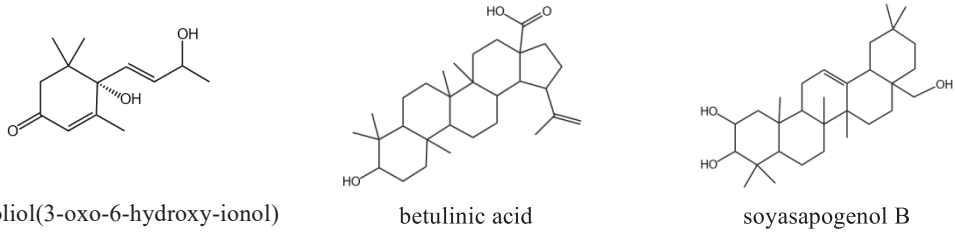


Fig. 3 Organic acids compounds in *C. ferruginea*

2.4 Terpenoids

Long used the medicinal materials of *C. ferruginea* [14]. After decocting with water, the sugar was removed by macroporous resin, and then through methods such as repeated silica gel column chromatography, the terpenoid compound vomifoliol (3-oxo-6-hydroxy-ionol) was isolated. Zhang et al. extracted the 95% ethanol extract of *C. ferruginea* with different solvents [15,16]. The extracts were loaded onto a silica gel column, and column chromatography was carried out repeatedly. The structures of the isolated monomer components were identified by spectroscopic methods, and the terpenoid compound vomifoliol was isolated. Li et al. reflux-extracted and concentrated *C. ferruginea* with 75% ethanol [17]. After extraction with ethyl acetate, the product was separated by silica gel column chromatography. Through repeated silica gel column chromatography with petroleum ether-ethyl acetate and gradient elution with Sephadex LH-20 column chromatography, the terpenoid compounds betulinic acid and soyasapogenol B were obtained respectively. Wu isolated the terpenoid compound betulinic acid from the antibacterial active components of *C. ferruginea* [18]. The structure of the specific compound is shown in Fig. 4.



vomifoliol(3-oxo-6-hydroxy-ionol) betulinic acid soyasapogenol B

Fig. 4 Terpenoids compounds in *C. ferruginea*

2.5 Other compounds

Researchers have also discovered various other chemical components in *C. ferruginea*, including fatty alcohol compounds such as octacosanol and hentriacontanol [15-17], lipid compounds like (2*S*,3*S*,4*R*,12*E*,2′*R*)-2-(2′-hydroxydocosanoylamino)eicosane-1,3,4-triol-12-ene [17], and ester compounds such as 1-octadecyl-2-(9-octadecenoyl)-3-eicosanoyl-glycerol [19].

At present, a variety of chemical components have been isolated and identified from *C. ferruginea*, and the specific chemical components isolated and identified are shown in Table 1.

Table 1 Chemical components isolated from *C. ferruginea*

Category	Serial Number	Compound Name	Reference
Flavonoids	1	Cynaroside (5,7-dihydroxy-4-methoxyflavone-7-*O*-*β*-D-glucoside)	[14-16]
	2	5,7,4′-trihydroxy-flavonol-8-C-*β*-D-glucoside	[14]
	3	genistein	[14-18]
	4	3′,5-dihydroxy-4′-methoxy-2″,2″-dimethylpyrano-(5″,6″,6,7)-isoflavone	[17,18]

(to be continued)

Continued Table 1

Category	Serial Number	Compound Name	Reference
	5	orobol	[17,18]
	6	4′,5,7-trihydroxy-6-(2-hydroxy-3-methyl-3-butenyl)isoflavone	[17]
	7	4′,7-dimethoxy-5-hydroxyflavone	[18]
	8	4′,7-dihydroxyflavone	[18]
Steroids	1	daucosterol	[14,17]
	2	*β*-sitosterol	[14-18]
	3	Δ5,22-stigmasterol-3-*O*-*β*-D-glucopyranoside	[15,16]
	4	*β*-daucosterol	[15,16]
	5	stigmasterol	[15,16; 18,19]
Organic acids	1	stearic acid (*n*-octadecanoic acid)	[14-16,18]
	2	*p*-hydroxybenzoic acid	[15,16]
	3	*n*-dotetraicosanoic acid	[17]
	4	*n*-dotriacontanoic acid	[17]
	5	*n*-tricosanoic acid	[18]
	6	*n*-tetracosanoic acid	[18]
Terpenoids	1	Vomifoliol (3-oxo-6-hydroxy-ionol)	[14-16]
	2	betulinic acid	[17,18]
	3	soyasapogenol B	[18]
Other categories	1	octacosanol	[15,16]
	2	hentriacontanol	[17]
	3	(2*S*,3*S*,4*R*,12*E*,2′*R*)-2-(2′-hydroxy-docosanoylamino)eicosane-1,3,4-triol-12-ene	[17]
	4	1-octadecyl-2-(9-octadecenoyl)-3-eicosanoyl-glycerol	[19]

3 Extraction process of flavonoids

Ye et al. optimized the process of extracting total flavonoids from *C. ferruginea* using ethanol through orthogonal experimental design [20]. Results showed that the optimal extraction process conditions were ethanol concentration of 65%, solid-liquid ratio of 1:30, extraction temperature of 75 °C, and extraction time of 3 h. Under these conditions, the yield of flavonoids was 1.72%. Xue et al. conducted experiments with the L9(34) orthogonal array based on single - factor tests to determine the optimal process for extracting total flavonoids from *C. ferruginea* by the cellulase method [21]. The experimental results indicated that the optimal extraction process conditions were ethanol

concentration of 50%, enzyme mass concentration of 2.0 mg/mL, pH of 4.0, extraction temperature of 55 °C, solid-liquid ratio of 1:30, and extraction time of 120 min. Under these conditions, the extraction rate of total flavonoids from *C. ferruginea* could reach 2.18%. Based on single - factor tests, Li et al. selected extraction time, ethanol concentration, and solid-liquid ratio as influencing factors, with the total flavonoid content as the dependent variable, and optimized the extraction process of total flavonoids from *C. ferruginea* using the Box - Behnken response surface method [22]. After the response surface design, the optimal extraction process was extraction time of 92 s, ethanol concentration of 60%, and solid-liquid ratio of 1:35, and the total flavonoid content in *C. ferruginea* was 21.4142 mg/g. Wang et al. took ethanol concentration, solid-liquid ratio, and extraction time as influencing factors, and the comprehensive score of extract yield, total flavonoid yield, and antibacterial activity as evaluation indexes, and optimized the extraction process through orthogonal experiments [23]. Research results showed that the optimal extraction process was ethanol volume fraction of 70%, solid-liquid ratio of 1:40, and extraction time of 1. 5 h.

4 Pharmacological effects of *C. ferruginea*

At present, it has been proved that *C. ferruginea* has a variety of pharmacological effects, and the specific effects are shown in Fig. 5.

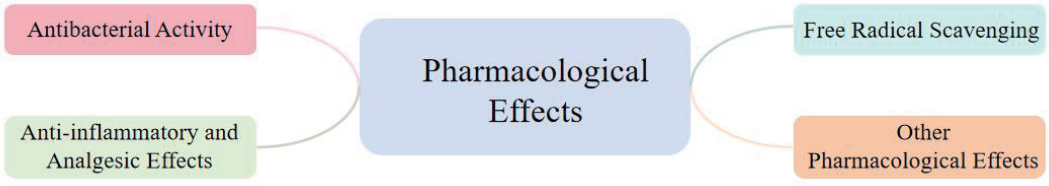


Fig. 5 Pharmacological Effects of *C. ferruginea*

4.1 Antibacterial Activity

In recent years, new studies have shown that compounds such as flavonoids, organic acids, and terpenoids exert antibacterial effects through different mechanisms [24-26]. They can directly inhibit bacterial activity and also can be used in combination with antibiotics to inhibit bacteria. These compounds can enhance the sensitivity of drug-resistant strains, reduce the dosage of antibiotics, and indirectly inhibit bacteria-induced inflammation by regulating the immune response of host cells. In the case of *C. ferruginea*, its active ingredients containing these compound types may play similar antibacterial roles, which is worthy of further in-depth exploration. Under the guidance of antibacterial activity against Escherichia coli and Staphylococcus aureus, Duan carried out component separation and activity research on the extract of *C. ferruginea* using ethyl acetate as the solvent [27]. Results showed that among the components of the *C. ferruginea* extract, the component with the ratio of petroleum ether to ethyl acetate between 3.0:1 and 3.2:1 had the best antibacterial effect against Escherichia coli and Staphylococcus aureus. Zhou et al. extracted *C. ferruginea* with water, alcohol, and ethyl acetate as extraction solvents, and conducted antibacterial experimental studies on the extracts using the agar well-diffusion method and the dilution method [28]. Results indicated that the extracts of *C. ferruginea* with different solvents had obvious *in vitro* antibacterial effects against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Micrococcus luteus, and Penicillium. Wu et al. used

70% ethanol as the extraction solvent to extract the whole-herb powder of *C. ferruginea*, and then separated and purified the extracts [18,29]. The qualitative screening of the antibacterial activity of *C. ferruginea* was carried out by the punch-diffusion method and the minimum inhibitory concentration method. The flavonoid extract of *C. ferruginea* was fully dissolved with 50% DMF to prepare a 100 mg/mL solution. 50% DMF was used as the negative control, and 3 mg/mL penicillin sodium solution (for Escherichia coli) and 2 mg/mL streptomycin solution (for Pseudomonas aeruginosa) were used as the positive controls. Results showed that different extracts of *C. ferruginea* had different antibacterial effects. The total flavonoid extract had a good inhibitory effect on Pseudomonas aeruginosa, Micrococcus luteus, and Staphylococcus aureus, especially on the latter, but had no inhibitory effect on Escherichia coli. The total alkaloid extract had an inhibitory effect on Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus, but had no effect on Micrococcus luteus. The total saponin extract had an inhibitory effect on Pseudomonas aeruginosa and Staphylococcus aureus, but had no effect on the other two bacteria. The MIC of the flavonoid extract against Staphylococcus aureus was 6.25 mg/mL, and its inhibitory effect was stronger than that against other tested bacteria. At the same time, there were significant differences in the antibacterial effects. Whether these differences are related to the choice of the polarity of the extraction solvent and the loss of certain active ingredients during the macroporous resin purification step remains to be studied.

4.2 Free radical scavenging

Ye et al. optimized the process of extracting total flavonoids from *C. ferruginea* with ethanol through orthogonal experimental design, determined the optimal extraction process of total flavonoids from *C. ferruginea*, and investigated the free-radical-scavenging ability and the inhibitory effect on the nitrosation reaction of the total flavonoids from *C. ferruginea* [20]. Results showed that the total flavonoids from *C. ferruginea* had a certain scavenging effect on hydroxyl radicals and superoxide anion radicals, and could effectively scavenge sodium nitrite and block the synthesis of nitrosamines. Lu studied the antioxidant activity of the ethanol extract and extraction parts of *C. ferruginea* [19]. Results showed that the *n*-butanol part of *C. ferruginea* had a certain scavenging effect on DPPH radicals. Li et al. extracted and separated the flavonoid components in *C. ferruginea*, and explored the *in vitro* antioxidant capacity of the total flavonoid extract under the optimal extraction conditions of *C. ferruginea* [22]. The results of the *in vitro* antioxidant test showed that the flavonoid components in *C. ferruginea* had a good free-radical-scavenging ability and a high reducing power, and there was a dose-effect relationship with the total flavonoid content.

4.3 Anti-inflammatory and analgesic effects

Xia et al. screened the anti-inflammatory active parts by the mouse ear swelling method, and the analgesic active parts by the acetic acid writhing method and the hot-plate method, and determined the solvent-extracted parts with anti-inflammatory and analgesic activities of *C. ferruginea* [30]. Research results showed that the n-butanol-extracted part of *C. ferruginea* had a strong anti-inflammatory and analgesic effect on mice.

4.4 Other pharmacological effects

The Deafness Research Group of Shanghai College of Traditional Chinese Medicine conducted a preliminary observation and statistical analysis on the curative effect of *C. ferruginea* in treating

dihydrostreptomycin-induced ototoxicity [31]. Results showed that the effective rate of *C. ferruginea* was 39.2%. Zhang conducted experiments to measure the auricle reflex thresholds and auditory brainstem response thresholds at 2, 4, and 8 kHz of the bilateral ears of guinea pigs in the guinea-pig ear model induced by gentamicin sulfate using the extract of *C. ferruginea* [15]. Research results showed that the water-decocted liquid of *C. ferruginea*, with the ethanol-eluted part of the macroporous adsorption resin and the part obtained by dissolving the ethanol extract in water, had a preventive effect on the hearing loss of animals caused by gentamicin injection. These active parts of *C. ferruginea* are used in treating deafness and tinnitus.

Currently, there are no references to confirm the potential toxicity of *C. ferruginea*. However, as a medicinal plant, its potential toxicity cannot be ignored. *C. ferruginea* contains various chemical components such as flavonoids, organic acids, terpenoids and steroids. The interactions and metabolic changes of these components in the human body remain unknown, and there is a potential risk of generating toxic substances. Moreover, some plants in the Crotalaria genus contain toxic alkaloids like monocrotaline [32]. Although monocrotaline has not been detected in *C. ferruginea*, the presence of other potential toxic components cannot be ruled out. Therefore, it is necessary to conduct acute, sub-acute, chronic toxicity tests, as well as special toxicity tests in mutagenicity, teratogenicity and carcinogenicity in the future to comprehensively evaluate its safety and provide a basis for clinical safe medication and development. The Deafness Research Group of Shanghai College of Traditional Chinese Medicine conducted a preliminary observation and statistics on the treatment of ototoxicity caused by streptomycin sulfate with *C. ferruginea* and found that the effective rate was 39.2%. This indicates that *C. ferruginea* has certain clinical application potential in improving ototoxicity-related hearing loss. However, the relatively low effective rate suggests that there may be multiple factors affecting its efficacy, such as individual patient differences, rationality of dosage and treatment course. Thus, it is necessary to further optimize the treatment plan. Currently, there are few clinical studies on *C. ferruginea*, making it difficult to comprehensively evaluate its clinical efficacy and safety. In the future, large-scale, multi-center, randomized controlled clinical trials are needed. Researchers could expand the patient sample to cover different age groups and disease severity, observe its efficacy on tinnitus, deafness, phlegm-heat cough, lumbar and knee pain and other conditions, monitor adverse reactions, and determine the applicable population, contraindications and safe dosage range to provide a basis for rational clinical medication. With the help of modern medical technologies such as pharmacokinetics, they could explore its metabolic process in the human body and deeply understand its mechanism of action in ordre to promote its clinical application.

5 Quality control

5.1 Characteristics and identification

Yang et al. studied the ethnic medicine name, medication experience, source identification, characteristic identification, microscopic identification and physicochemical identification of *C. ferruginea* [33,34]. Physicochemical identification experiments showed that the main components of *C. ferruginea* were coumarins and alkaloids. The microscopic identification test showed that in the root of *C. ferruginea*, the vessels in the xylem were arranged radially, the cambium was ring-shaped, and the parenchyma cells contained numerous starch grains. In the stem of *C. ferruginea*, non-glandular hairs could be seen

outside the epidermis, and the cortical cells near the epidermis contained chloroplasts. In the leaf, non-glandular hairs could be seen outside the epidermis, and at the main vein part, collenchyma tissues were present on the outer sides of both the phloem and the xylem. The powder characteristics were consistent with the tissue characteristics. Long summarized the plant morphological characteristics and crude drug characteristics of *C. ferruginea*, and studied the microscopic identification of *C. ferruginea* [14]. The results of the microscopic identification were consistent with those of Yang et al. [33,34]. Liu et al. studied the quality control of *C. ferruginea* medicinal materials [35]. Through microscopic identification, starch grains, vessels, non-glandular hairs and cork cells were found in *C. ferruginea*. A thin-layer identification method was established using genistein as the reference substance, toluene-ethyl formate-formic acid as the developing agent, silica gel G thin-layer plate as the stationary phase, and 3% aluminum trichloride ethanol solution as the color-developing agent. Shi et al. established a thin-layer identification method for *C. ferruginea* using genistein, kaempferol, acacetin, and apigenin as reference substances, cyclohexane-ethyl acetate-water as the developing agent, silica gel GF_{254} thin-layer plate as the stationary phase, and visual inspection under a UV lamp at 254 nm [36].

5.2 Content determination

Long extracted *C. ferruginea* by ultrasonic extraction method, used genistein as the reference substance, and determined the content of genistein in *C. ferruginea* by high-performance liquid chromatography (HPLC) [14]. The determination results showed that when the mobile phase was methanol-2% glacial acetic acid, the detection wavelength was 254 nm, the flow rate was 1.0 mL/min, the column temperature was 30 °C, and the standard product peak was completely separated from other impurity peaks. Genistein had a good linear relationship in the range of 4.07-40.72 μg/mL, with a repeatability RSD of 1.47%. The average recovery rate of sample addition was 102.34%, and the RSD was 0.76%. Liu XQ and Liu WQ et al. extracted *C. ferruginea* by ultrasonic extraction method, used genistein as the reference substance, and determined the content of genistein in *C. ferruginea* by HPLC [35,37]. Results showed that when the chromatographic column was Boston C_{18} (4.6 mm × 150 mm, 5 μm), the mobile phase was acetonitrile-1% glacial acetic acid aqueous solution, the detection wavelength was 260 nm, the flow rate was 1.0 mL/min, and the column temperature was 30 °C. Genistein had a good linear relationship in the range of 0.65-13 μg, with a repeatability RSD of 0.76%. The average recovery rate was 99.67%, and the RSD was 0.86%. When studying the determination methods and content changes of total flavonoids and three flavonoid aglycones in *C. ferruginea* medicinal materials, Tang et al. established a method using rutin, quercetin, genistein, and kaempferol as reference substances [38]. The absorbance of rutin was measured at a wavelength of 500 nm by ultraviolet spectrophotometry, and the amount of total flavonoids in *C. ferruginea* was calculated based on rutin. The contents of quercetin, genistein, and kaempferol in *C. ferruginea* were determined by HPLC. This method had a good linear relationship, and the methodology such as precision, accuracy, and repeatability was verified. Li et al. used quercetin as the internal standard, determined the relative correction factor (RCF) of quercetin and kaempferol, and established a quantitative analysis of multi-components by single-marker (QAMS) method for determining the contents of quercetin and kaempferol in *C. ferruginea* by HPLC [39]. Quercetin and kaempferol both had good correlations in the range of 0.01-0.20 μg, with repeatability RSDs of 1.38% and 1.42% respectively. The average recovery rates were 98.47% and

97.88% respectively, and the RSDs were 1.19% and 1.52% respectively.

5.3 Inspection indicators

Liu et al.explored the quality control of *C. ferruginea* medicinal materials and detected the total ash and acid-insoluble ash of 6 batches of *C. ferruginea* [35]. The average value of the total ash was 6.8%, and the average value of the acid-insoluble ash was 1.7%.

6 Conclusion

Despite preliminary investigations into the chemical constituents, extraction methodologies, pharmacological properties, and quality assessment protocols of *C. ferruginea* in recent decades, current scientific understanding remains fragmented and requires systematic characterization. The active ingredients of *C. ferruginea* have not been fully determined, and the research on the mechanisms of pharmacological activities is not deep enough. It is necessary to further study its active substances and make a more in-depth and systematic study on the relationship between chemical components and pharmacological activities. At the same time, the research on the quality control and the separation and extraction of chemical components of *C. ferruginea* is far from enough. *C. ferruginea* is widely distributed in China, rich in resources and has good application value. The separation and extraction more chemical components, the development of pharmacological activities and commercial value are still important research topics. This can also better guide clinical medication and new drug research and development.

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