

# A Systematic Review of the Comparison of Three Medicinal Licorices, Based on Differences of the Types and Contents about Their Bioactive Components

Yong-Jiao Zhang<sup>1</sup>, Yong-An Yang<sup>3</sup>, Hai-Bin Gong<sup>2\*</sup> and Hai-Liang Zhu<sup>1\*</sup>

<sup>1</sup>State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210023, China

<sup>2</sup>Xuzhou Central Hospital, Xuzhou 221009, China

<sup>3</sup>Elionnature Biological Technology Co., Ltd., Nanjing 210038, China

\*Corresponding author: Zhu HL, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210023, China, Tel: 025-89682572; E-mail: zhuhl@nju.edu.cn and Gong HB, Xuzhou Central Hospital, Xuzhou 221009, China, Tel: +86-516-83956601; E-mail: ghbzxh@126.com

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## Abstract

Licorice, derived from the roots and rhizomes of *Glycyrrhiza* genus, is widely distributed all over the world. In China, three *Glycyrrhiza* species, *G. uralensis*, *G. glabra*, and *G. inflata*, are used as licorice without discrimination according to the 2015 edition of the Chinese Pharmacopoeia. Triterpenoid saponins, flavonoids and polysaccharides are considered as bioactive ingredients of licorice. The component properties, types and contents of these chemical compounds, vary significantly in different species and different licorice species have species-specific markers respectively, such as the contents of major flavonoids (liquiritin, liquiritigenin and isoliquiritin) in *G. uralensis* are obviously higher than in *G. glabra* and glycycomarin only exists in *G. uralensis*. To some extent variation in active constituent could affect the therapeutic effects and safety of licorice, thus licorice used for medicinal purposes should be discriminated and chemically standardized according to those component properties in order to promote its reasonable application.

In this review, we describe differences of the types and contents of chemical composition among three licorice species. The result of this review is intended to provides the basis for the accurate identification and reasonable use of licorice.

**Keywords:** Licorice; Triterpenoid saponins; Flavonoids; Polysaccharides; Species-specific markers

## Introduction

Licorice (Gancao in Chinese), one of the oldest and most widely used herbals in the world, has been used by human beings for over 4000 years. As a popular herbal medicine, licorice has been extensively used in the at least 70% of traditional Chinese medicine (TCM) for the effects of tonifying

spleen and replenishing qi, eliminating phlegm and relieving cough, clearing away heat, detoxifying and used for the treatment of coughs, peptic ulcers, inflammation, hepatic disease, viral infections, cancer, and other ailments [1-3]. It is also widely used in food industry and cosmetics industry due to its sweet taste, whitening and anti-inflammatory activity. Modern phytochemical studies have demonstrated that the main active substances of licorice are triterpenoid saponins, flavonoids and polysaccharides, which have a variety of pharmacological activities, including anti-inflammatory, antibacterial, antiviral, antiulcer, antioxidative, antiallergic, anticancer, antidiabetic, anti-depressive, hepatoprotective, immunomodulatory and cardioprotective effects [4-8].

In China, *G. uralensis*, *G. glabra*, and *G. inflata* are considered as being equivalent and are combined and utilized as licorice without discrimination according to the 2015 edition of the Chinese Pharmacopoeia. It is difficult to identify these licorice species accurately based on their root or rhizome morphology [9]. But the contents of these active ingredients may vary significantly due to interspecific differences of licorice, which thus affect their quality, safety and therapeutic effects. In addition, licorices are increasingly being used in the fields of medicine, food, and cosmetics. Therefore, comprehensive quality control and comparative constituent properties to these licorice species are critical to ensure their efficacy and safety.

Quality control analyses in licorice mostly target single compound examination, of which glycyrrhizic acid is the most frequently examined compound. However, with an increasing demand for accuracy and consistency of phytomedicine bioactivity, and the fact that not all pharmacological effects may originate from only one sole constituent, single targeted constituent is an insufficient tool for standardization of licorice [10]. Recently, flavonoids and polysaccharides in licorice are also determined to assess and control the quality of licorice because of their potent activities [11-24].

Numerous studies have established analytical methods to separate and quantify these active ingredients in licorice samples. Although, prior research has attempted to describe the dissimilar composition of the licorice species, these reports most simultaneously compared only one or two classes of ingredients among the triterpenoid saponins, flavonoids and polysaccharides or only simultaneously determined several active chemical compounds [9,10,25,26]. These have been no reports of systematic and comprehensive review for tree active ingredients (triterpenoid saponins, flavonoids, polysaccharides).

In this review, we describe the variations in constituent properties (types and contents of bioactive ingredients) of licorice species derived from *G. uralensis*, *G. glabra* and *G. inflata* to improve the comprehension of chemical characteristics of licorice and provides the basis for its quality control and reasonable use.

### Triterpene saponins

Triterpene saponins, represented by glycyrrhizic acid and presented as glucuronides mostly, are the major characteristic ingredients in licorice and exhibit extensive biological activities including hepatoprotective, anti-cancer, antiviral, anti-inflammatory [27-29]. However, overconsumption of licorice may result in side effects like sodium and water retention, hypertension, hypokalemia, and suppression of the renin-aldosterone system [30,31]. Therefore, the saponins are of great importance for the efficacy and safety in clinical use of licorice, which makes their analysis of significant interest.

A number of studies have attempted to describe the dissimilarities of the triterpene species in the *Glycyrrhiza* species. For example, Tao W, et al. measured 10 triterpenoid saponins in 82 batches of three licorice species, collected from their main production regions in China and revealed that both the types and contents of the triterpene saponins were significantly different among species [9]. Song W, et al. measured 151 secondary metabolites of three medicinal *Glycyrrhiza* species and noted that the contents of 24 measured triterpenoids in *G. inflata* are higher than which in other two species [32].

### Glycyrrhizin

Glycyrrhizin, a saponin of the pentacyclic triterpene derivative of the oleanane type, is the principal saponin in licorice and generally known for its numerous medicinal benefits at appropriate doses such as anti-inflammatory, anti-allergic, antiviral, hepatoprotective, antiulcer, antiallergy [33,34]. Following hydrolysis, it releases two molecules of D-glucuronic acid and the aglycone glycyrrhetic acid which has been found to exhibit anti-inflammatory, anti-allergic, anti-filarial, anti-cancer, neuroprotective effects [35-37]. In regard to quality control analysis of licorice, glycyrrhizin is often considered as the most frequently monitored metabolite via HPLC [38]. Based on these considerations, the content of glycyrrhizin is a very important ingredient in the dietary and medicinal use of licorice.

Song W, et al. reported that glycyrrhizin was evenly distributed in the three species ( $40.5 \pm 29.1$ ,  $49.3 \pm 15.6$ , and

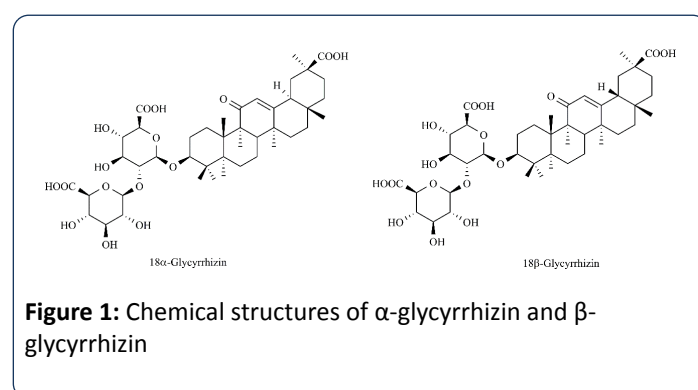
$40.6 \pm 26.4$  mg/g in *G. uralensis*, *G. inflata*, and *G. glabra*, respectively [32], which are identified from their genetic information. Kondo K, et al. also reported that there was no significant discrepancy among the three species regarding the contents of glycyrrhizin [39].

But some studies suggested that the content of glycyrrhizin is different in three licorice species. For example, Tao W, et al. reported that the average ratios of glycyrrhizin in total content of 10 investigated triterpene saponins were 76.8% for *G. inflata*, 83.29% for *G. glabra*, and 51.10–56.15% for *G. uralensis* [9]. Rizzato G, et al. analyzed ten different commercial samples of *Glycyrrhiza* dried roots (samples of *G. glabra* from Europe, samples of *G. uralensis* and *G. inflata* from China) and indicated that glycyrrhizin showed a significantly higher signal intensity in *G. glabra* than in Chinese licorice (*G. uralensis* and *G. inflata*), in which glycyrrhizin did not demonstrated any substantial differences [40]. Whilst Farag MA, et al. noted that the content of glycyrrhizin in licorice root samples derived from different samples were various and Chinese *G. inflata* roots contained the highest levels of glycyrrhizin, lowest levels were found in *G. glabra* (Syria) [10].

The results are inconsistent, which may because the existences of hybrids among *Glycyrrhiza* species [39] and the content of glycyrrhizic acid is affected not only by their different species but also by geographic area, state of plant maturity, environmental conditions, harvesting and processing [41]. Therefore, glycyrrhizin is inadequate for species distinction, although it is used for the quality control of licorice.

### Glycyrrhizin isomer

Glycyrrhizin exists as 2 isomers viz  $\alpha$ -glycyrrhizin and  $\beta$ -glycyrrhizin with commercial glycyrrhizin products containing mainly the latter (Figure 1).



Both isomers are effective but there are dissimilarities in their biological activities and physicochemical properties.  $\alpha$ -glycyrrhizin appears to have better lipophilicity, higher targeting, greater anti-inflammatory activity and lower incidence of adverse effects.  $\alpha$ -glycyrrhizin has been widely used as a fourth generation of glycyrrhizic acid preparation, such as Magnesium isoglycyrrhizinate is a pure salt of  $\alpha$ -glycyrrhizin which was developed as a new treatment for chronic hepatitis [42].

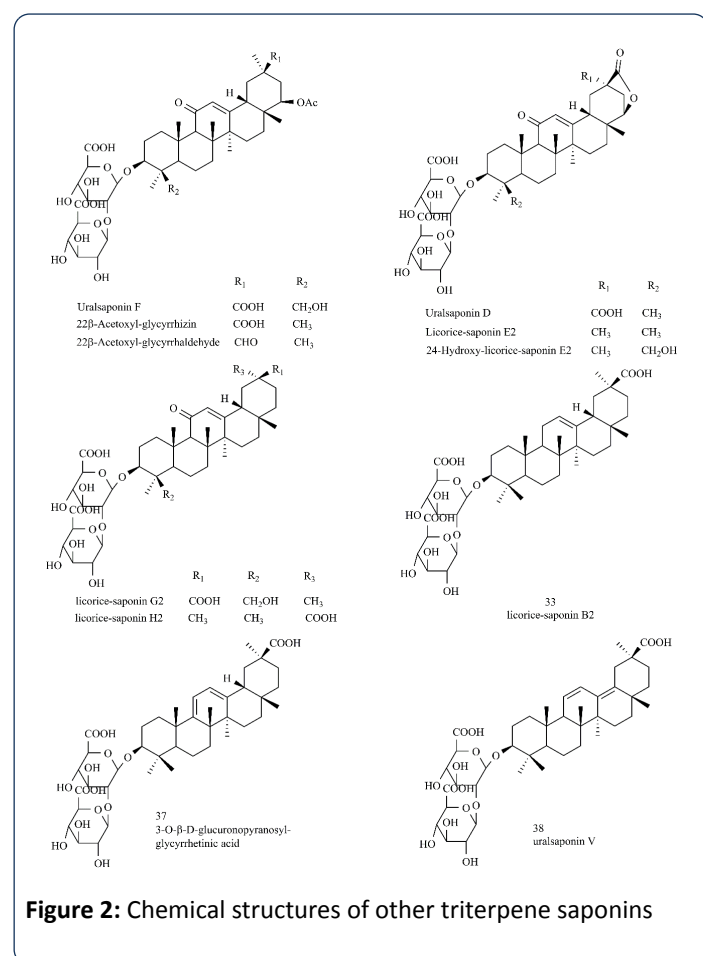
For a long time, both  $\alpha$ -glycyrrhizin and  $\beta$ -glycyrrhizin were considered as total glycyrrhizin in qualitative and quantitative analysis with no separation. Recently, some studies analyzed

glycyrrhizin isomer simultaneously due to the development of quality control analysis techniques.

Ying C, et al. revealed that the content of 18 $\alpha$ -glycyrrhizin and 18 $\beta$ -glycyrrhizin vary significantly, as the content of 18 $\beta$ -glycyrrhizin was much higher than that of 18 $\alpha$ -glycyrrhizin [43]. Yang R, et al. established an HPLC method for simultaneous contents determination of 18 $\alpha$ -glycyrrhizic acid and 18 $\beta$ -glycyrrhizic acid, and compared the contents of these two triterpenoids in licorice derived from the three species. It was demonstrated that the contents of 18 $\alpha$ -glycyrrhizic acid and 18 $\beta$ -glycyrrhizic acid in *G. glabra* were the highest, which in *G. uralensis* were the second and in *G. inflata* were the lowest. Furthermore, the content of 18 $\alpha$ -glycyrrhizic acid was significantly correlated to the content of 18 $\beta$ -glycyrrhizic acid in all three species of licorice [44].

### Other Triterpene Saponins

Some other triterpenoid saponins with less content have been identified and quantified with the use of more sensitive, higher precise and accurate analysis techniques (Figure 2).



**Figure 2:** Chemical structures of other triterpene saponins

Tao W, et al. revealed significant differences among species in both the type of triterpene saponins and their contents and noted that some of the major compounds may serve as chemotaxonomic markers, such as 22 $\beta$ -acetoxy-glycyrrhizin (4) and licorice-saponin E2 (7) were detectable in *G. uralensis* and *G. inflata* only, while 24-hydroxy-licorice-saponin E2 (5) only in *G. uralensis*. And compounds uralsaponin F (2), uralsaponin D

(3), and 22 $\beta$ -acetoxy-glycyrrhaldehyde (8) were present in some *G. inflata*, but not in any *G. glabra* [9].

Song W, et al. found that the three *Glycyrrhiza* species showed distinct biosynthetic preferences. For triterpene saponins, E-ring-substituted saponins (e.g., 22 $\beta$ -acetoxy-glycyrrhizin) and 11-deoxy-glycyrrhizins (33, 37, 38) were relatively abundant in *G. uralensis* and *G. inflata*, respectively [32].

Rizzato G, et al. noted that three saponins—licorice saponin K2/H2 (2), licorice saponin B2 (7) and licorice saponin G2/yunganoside K2 (8) – were higher content in *G. glabra*, as they were not detected with significant intensities in the other two species [40].

### Flavonoids

In recent years, licorice flavonoids have become one of the hotspot of pharmacological studies for their structural diversity and important pharmacological activities of the isolated flavonoids, including chalcones, isoflavones, isoflavans, flavonones, flavanonols, isoflavenes and arylcoumarins [11]. Liquiritin, isoliquiritin and liquiritigenin are considered to be the main flavonoids of licorice, exhibited a variety of biological properties including antitumor, antidepressant, neuroprotective, antioxidant and anti-inflammatory [12-15]. Licochalcone A, an oxygenated chalcone in licorice, was reported to possess antitumor, antimalarial, anti-metastatic, chemopreventive, antibacterial, and anti-spasmodic activity [16-19]. Glycy coumarin, a representative coumarin in *G. uralensis*, showed antithrombotic and antispasmodic activities [3]. Some isoflavans derived flavonoids in licorice, such as glabridin, licoricidin and licorisoflavan A showed anti-inflammatory, antioxidative and antitumor activities [11]. The isoflavone compound dehydroglyasperin C exhibited neuroprotective effects by inducing phase II enzymes [20]. Glycyrol and glycyrin, two major licorice coumarins, showed anti-inflammatory and antihypertension activity, respectively [3]. Furthermore, Quercetin is a flavonol with strong antioxidant activity, the different content of quercetin would affect the radical scavenging activity of *Glycyrrhiza* species.

Numbers of studies have tried to describe the diversity of the flavonoids in the licorice species. It is reported that the types and contents of the flavonoids in different licorice species were obviously various and the content of liquiritin was higher than that of other flavonoids which was used for quality control analysis of licorice.

Liquiritin, liquiritigenin and isoliquiritin are major flavonoids in *G. uralensis*, *G. glabra*, and *G. inflata*. Numbers of studies have shown that the contents of those three compounds in *G. uralensis* are obviously higher than in *G. glabra* and *G. inflata* [25,26,32,39,45].

Zhu Z, et al. quantified 15 flavonoids in licorice and found that the contents of compounds 2, 5, 11, 12, 14 and 15 (isoliquiritin, isotrifloliol, licoricone, neoglycyrol, glycyrin and licorisoflavan A) in *G. uralensis* were higher than *G. glabra* and *G. inflata* while compound 9 (formononetin) were lower [25]. Qiao X, et al. revealed that the content of compounds glycy coumarin (1),

dehydroglyasperin C (2), and Glycyrol (3) in *G. inflata* and *G. glabra* was lower than those in *G. uralensis* [3]. Fu Y, et al. noted that DTM were not detected in *G. uralensis* and the contents of chalcone derivatives such as licochalcone A, licochalcone B and echinatin in *G. inflata* were higher than in *G. uralensis* [11]. Rizzato G, et al. suggested that a compound belonging to the family of prenylated chalcones, kanzonol C (20), was present in *G. inflata* with statistically significant higher intensities than in *G. glabra* and *G. uralensis*. And a chalcone, identified as glyinflanin D or G (46) and licoflavone B (47), was found only in *G. inflata* and *G. glabra* samples [40]. Additionally, quercetin, a flavonol with strong anti-oxidant activity, was reported that presented in *G. inflata* with higher intensity than in *G. uralensis* and absented in *G. glabra* [10, 40, 46].

Song W, et al. revealed that the contents of 3-aryl-5-methoxy coumarins (79–84) and flavonols (47–54) in *G. uralensis* were significantly higher than those in *G. inflata* or *G. glabra*. Likewise, *G. inflata* contained remarkably more abundant 2'-H chalcones (e.g., licochalcones A, C, E), and *G. glabra* contained more abundant 8-cyclized isoprenyl isoflavanes (e.g., glabridin and 4'-O-methylglabridin) than the other two species [32].

For flavonoid glycosides, Song W, et al. noted that the apiosylation ratio of liquiritin and isoliquiritin was significantly lower in *G. uralensis* ( $47 \pm 19\%$ ) than in *G. glabra* and *G. inflata* ( $74 \pm 13\%$  and  $82 \pm 16\%$ ,  $P < 0.005$ ), whereas the content ratio of flavanone glycosides (1, 4, 5) chalcone glycosides (8, 9, 10) was significantly higher in *G. uralensis* (2–5-fold) [32]. Li G, et al. revealed that the contents of apiosides (liquiritin apioside, isoliquiritin apioside, licuraside) in *G. glabra* root preparations were the highest, which in *G. inflata* were the second and in *G. uralensis* were the lowest [45].

## Polysaccharide

Polysaccharide is one of the main bioactive constituents in licorice. Recently, various studies have shown that *Glycyrrhiza* polysaccharides had many pharmacological activities including immunoregulatory, antioxidant, antitumor, antiviral [21–24]. The determination of monosaccharides in polysaccharides is an important way to explore the quality standard of polysaccharides, study the basic information of structure, properties and structure-activity relationship of polysaccharides. Therefore, the study of polysaccharides should also be used as one of the methods to control the quality of licorice.

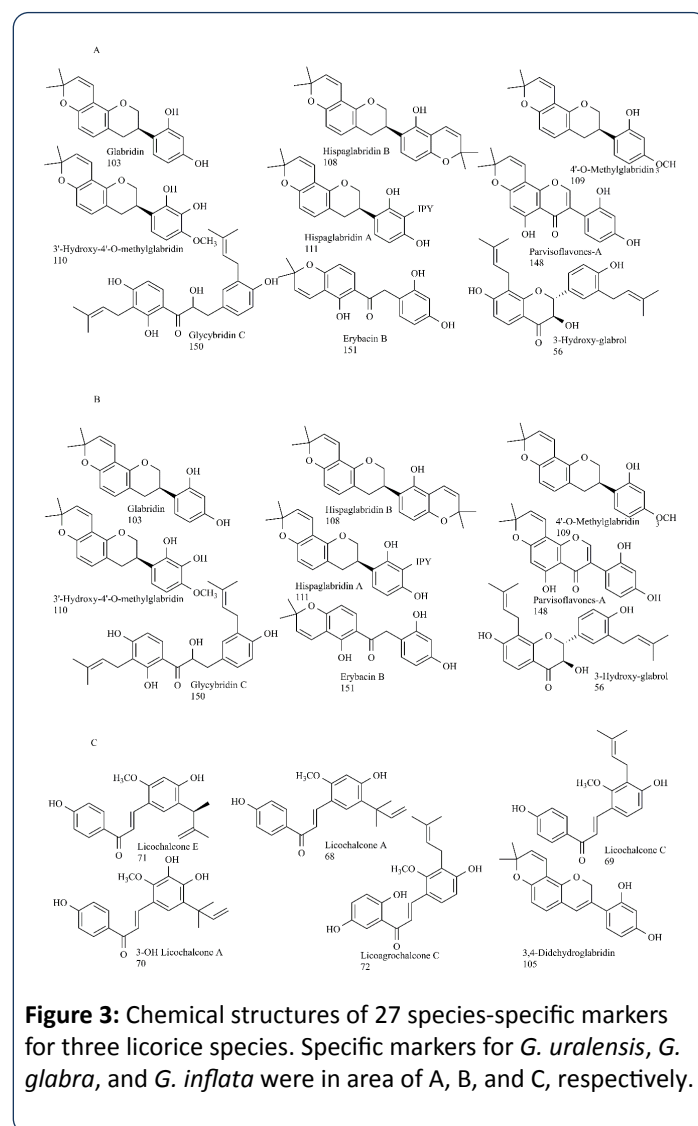
Wei LI, et al. used phenol-H<sub>2</sub>SO<sub>4</sub> colorimetry method measured the contents of the polysaccharide in *Glycyrrhiza* of different species and indicated that polysaccharide content in *G. glabra* was the highest, in *G. inflata* was second, and in *G. uralensis* was lowest [47]. Zhao L, et al. used phenol-sulfuric acid method to measure the polysaccharide content in different *Glycyrrhiza* rootspecies under the same cultivated environment and got the same result, polysaccharide content in *G. glabra* was the highest [48].

Wei LI, et al. showed that the monosaccharide composition of polysaccharide in different *Glycyrrhiza* was the same, but the proportion of monosaccharide (ManGalUAGlcGalAra) was greatly different [49].

## Species-specific Markers

Many studies have confirmed that glabridin, licochalcon A and glycy coumarin were species-specific markers for *G. glabra*, *G. inflata* and *G. uralensis* respectively [10,32,39,45]. Although Kondo K, et al noted that there was no detection of the three species-specific constituents from 6% of licorices examined [39], they were still important markers for the species identification of licorice.

Some studies identified several novel markers among three licorice species. For example, Song W, et al. discovered a total of 27 species-specific markers (Figure 3), they met the requirements of high interspecies variance, relatively high contents, and nonoverlapped content ranges at 95% confidence interval [32].



**Figure 3:** Chemical structures of 27 species-specific markers for three licorice species. Specific markers for *G. uralensis*, *G. glabra*, and *G. inflata* were in area of A, B, and C, respectively.

Li G, et al. noted that only *G. uralensis* root preparations contained licoricidin (12) [45]. Farag MA, et al. reported that two aryl coumarins, glycyrin isomers (43 and 47) and an indolyl flavanone conjugate identified as licorice glycoside E (21) were identified in *G. uralensis* [10].

Rizzato G, et al. suggested that licochalcone A/C/E and licochalcone D were species-specific markers for *G. inflata* [40].

Farag MA, et al. noted that cadaverine, inflacoumarin A (32), glyinflanin A (51), licochalcone C (41) and D (35) were present exclusively in *G. inflata* extracts [10].

Rizzato G, et al. reported that in addition to kanzonol Y (22) and glabridin (25), which are prenylated flavonoids known to be markers for *G. glabra* species, 2,4,4'-trimethoxychalcone (36), guangsangon F (33), schaftoside (34) and hispaglabridin A (48) were detected only in *G. glabra* [40]. Farag MA, et al. noted that *G. glabra* showed two chemotaxonomic markers including the isoprenylated flavanone derivatives glabrol (50) and 3-hydroxy glabrol (45) [10]. Fang S, et al. noted that 3-hydroxyglabrol, gancaonin H and glyasperin N were present exclusively in *G. glabra* extracts [50].

## Conclusions

In this review, we summarized the interspecific chemical variations in terms of the types and contents of triterpenoid saponins, flavonoids and polysaccharides and species-specific markers among three medicinal licorice species stipulated in Chinese pharmacopoeia. The chemical profiles of licorice vary significantly due to different plant species, which thus affect their quality and therapeutic effects. It is suggested that when used for given medicinal purposes, the licorice specie which was rich in target components could be superior to the other species for the corresponding bioactivities. Therefore, the species of licorice should be selected depending on these different properties when it is used for medicinal purposes.

In addition, because the content of chemical components of licorice is also affected by their geographic area, growth years, growth environment, harvesting and processing, we will further study their relationship to guarantee safe and effective use of licorice.

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