Chapter 14
Glycyrrhiza uralensis 甘草
(Gancao, Licorice)

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14.1 Botanical Identity

Licorice (Gan-Cao) is a perennial herb of Fabaceae family cultivated mainly in Shanxi, Gansu and Xinjiang regions of China. "Gan" of licorice in Chinese means umami taste of licorice, therefore, licorice is an important constituent in many traditional Chinese medicine (TCM) to reduce the bitter taste of other herbal medicine. The height of licorice plant for medicinal use is 30–100 cm. The medicinal part of licorice is the cylinder root and underground stem with a diameter of approximately 3–4 cm. The botanical traits of licorice are axillary raceme flower with purple and white colors, odd number pinnately compound leaf (Fig. 14.1a, b), and legume fruit. *Glycyrrhiza uralensis* (*G. uralensis*) is the most common species for TCM use, although several species of licorice are used in similar ways as TCM including *Glycyrrhiza glabra* L., *Glycyrrhiza inflata*, and *Glycyrrhiza kanscensis* [1].

Licorice is considered as a "Jun" (emperor) herb in TCM prescription composed of several constituents to treat asthma, coughs and peptic ulcers [1]. Cleaned and dried licorice root is sliced obliquely into 3–5 mm thickness with the oval shaped piece as the raw material for market (Fig. 14.1c).

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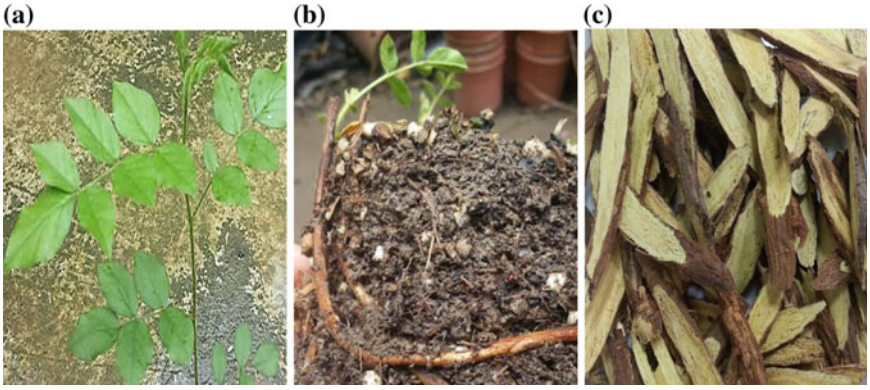
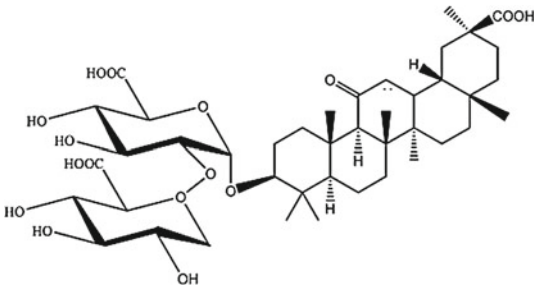


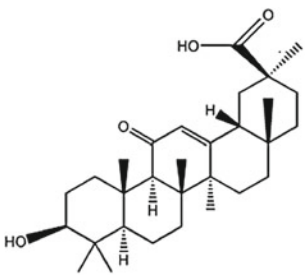
Fig. 14.1 The plant with tender shoots (**a**), roots in the soil (**b**), and crude drug (**c**) of licorice

14.2 Chemical Constituents

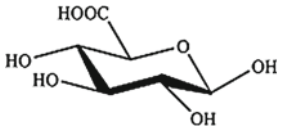
The chemical constituents of licorice were identified and classified into several categories including triterpenoid saponins, flavanoid, coumarin, and alkaloid [1, 2].



Glycyrrhizin or Glycyrrhizic acid, 1



18β-Glycyrrhetic acid, 2



D-Glucuronic acid, 3

Fig. 14.2 Triterpenoid saponins and its components isolated from licorice

14.2.1 Triterpenoid Saponins

Glycyrrhizin (glycyrrhizic acid, GA) (1), indicative compound of licorice and the common name of the major triterpenoid saponin including potassium and calcium salt of glycyrrhizic acid, is composed of one molecule of 18β-glycyrrhetinic acid (2) and two molecules of glucoronic acid (3), shown in Fig. 14.2.

14.2.2 Flavanoids

Flavanoids and their derivatives in licorice root extract were identified and the representative compounds were shown in Fig. 14.3 as liquiritin (4), isoliquiritin (5), neoliquiritin, neoisoliquiritin, licoricidin (6), licoricone (7), liquiritigenin (8),

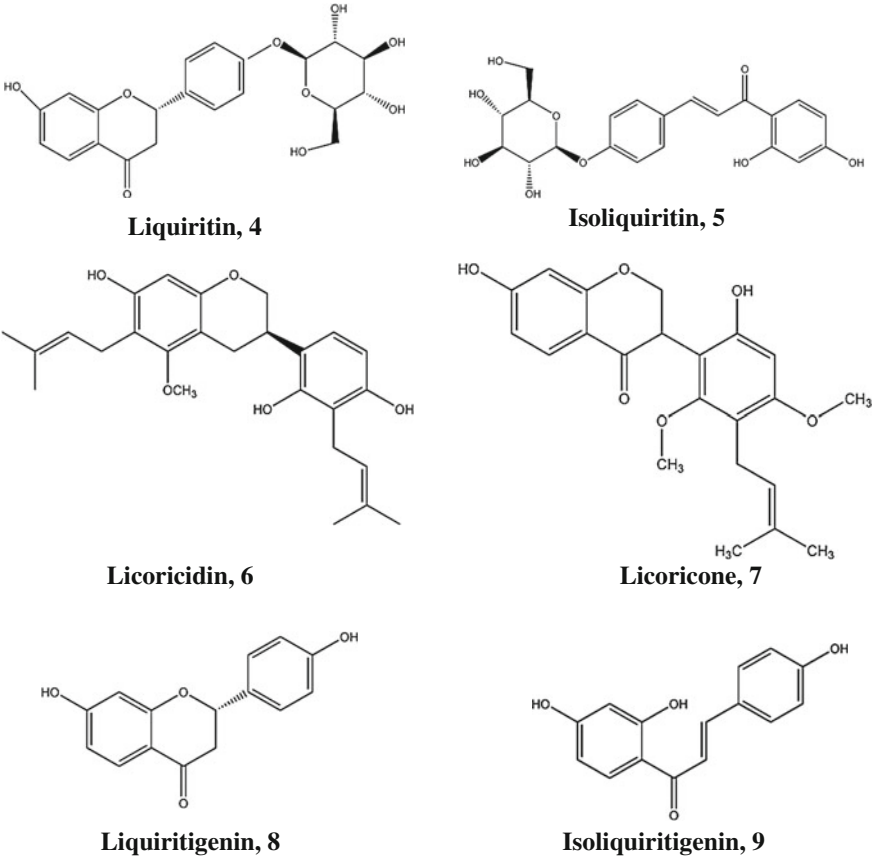
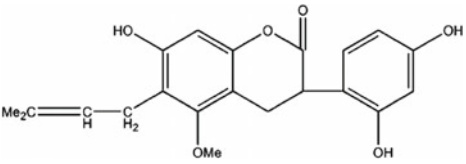


Fig. 14.3 Representative flavanoids isolated from licorice

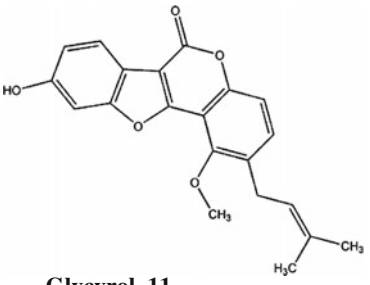
isoliquiritigenin (9), formononetin, 5-O-methyl-licoricidin, liquiritigenin-4′-apiofuranosyl (1 → 2) glucopyranoside, apioliquiritin, apioliquiritigenin-7-4′-diglucoside, vicenin II, isolicoflavonol, isoliquiritigenin-4′-apiofuranosyl (1 → 2) glucopyranoside, licurazid, and apioiso liquiritin.

14.2.3 Coumarins

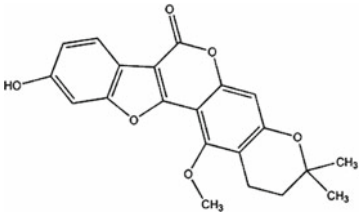
Coumarin derivatives identified in licorice root extracts were shown in Fig. 14.4 including glycycoumarim (10), glycyrol (11), isoglycyrol (12), glycyrin (13), neoglycyrol, licopyranocoumarin (14), and licocoumarione.



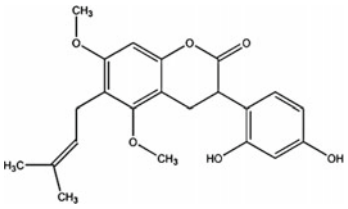
Glycycoumarim, 10



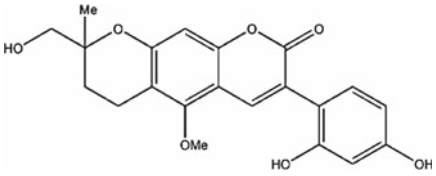
Glycyrol, 11



Isoglycyrol, 12



Glycyrin, 13



Licopyranocoumarin, 14

Fig. 14.4 Representative coumarins isolated from licorice

14.2.4 Alkaloid

Alkaloids in the licorice extract were identified including 5,6,7,8-tetrahydro-4-methylquinoline, 5,6,7,8-tetrahydro-2,4-dimethylquinoline, 3-methyl-6,7,8-trihydropyrrolo[1,2-]pyrimidin-3-one.

14.3 Pharmacological Studies

Pharmacological studies of licorice root extract, including anti-virus, anti-inflammation, anti-oxidation, hepatoprotection, anti-tumor, and anti-asthma [3–7], were extensively documented due to its common use in TCM prescription.

14.3.1 Anti-inflammatory Effects

β-glycyhrritinic acid (GA) has been shown to inhibit glucocorticoid metabolism leading to the accumulation of glucocorticoid with anti-inflammatory effects [2]. GA inhibited reactive oxygen species (ROS) generated in neutrophils which was suggested to be the modulator of anti-inflammation tissue [3]. *G. glabra* and glyderinine, a derivative of GA showed an anti-inflammation and reduced myocardial inflammatory edema [4]. Inhibition against interleukin-1β(IL-1β)-induced prostaglandin E_2 (PGE_2) production in normal human dermal fibroblasts by derivatives of glycyrrhetinic acid has been reported [5]. GA was suggested as the active antiviral component of *G. uralensis* against enterovirus 71 and coxsackievirus A16 infection for hand, food and mouth disease [6]. Nano- or micronized GA were prepared by using the supercritical anti-solvent (SAS) procedure, inhibited by PGE2 and TNF-α production, which was induced by LPS more effectively than that of unprocessed GA [7].

G. uralensis extract (GUE) at a dose of 400 mg/ml cures rotaviral enteritis by coordinating antiviral and anti-inflammation related cytokines (IL8, IL10, IFN-β, INF-γ and TNF-α) [8]. Glycyrrhetic acid is also effective on anti-inflammatory functions [9].

14.3.2 Hepatoprotection

Glycyrrhetic acid or 18β-glycyrrhetinic acid was documented to cure the rotavirus infection [10]. GA was reported to exhibit direct protection of hepatocyte from apoptosis through an IL-6 dependent way and indirect inhibition of T-Cell mediated inflammation through an IL-10 dependent way.

Hepatoprotective function of licorice extract was proved in CCL_4-induced hepatitic rat model and GA being the main component responsible for this effect [11].

14.3.3 Immunomodulatory Effects

Immunomodulatory effects of licorice are attributed to its active compounds, glycyrrhizin and glycyrrhetinic acid [12, 13]. GA selectively activated extrathymic T cells and enhanced Fas-mediated apoptosis without alteration of caspase-3 like activity [14, 15].

Flavonoids isolated from *G. uralensis* revealed anti-asthma effect by reducing eosinophilia pulmonary inflammation, serum IgE, IL-4 and IL-13, and increasing IFN-γ in lung cell culture of allergic asthma model [16].

Liquiritigenin inhibited LPS-induced NF-κB DNA binding activity and suppressed the production of tumor necrosis factor-α (TNF-α), Interleukin-1β (IL-1β) and interleukin-6 (IL-6) from Raw264.7 cells after LPS treatment [17].

14.3.4 Antitumor Effects

The ethanol extract of *G. uralensis* root induced apoptosis and GI cell cycle arrest in MCF-7 human cancer cells [18]. Glycyrrhetinic acid triggered the mitochondrial permeability transition which may lead to the apoptosis of tumor cells [19, 20].

14.3.5 Neurogenesis Protective Effects

G. uralensis exhibited anti-depressive effects by increasing the sum of line crosses and number of rears, decreasing the fecal boli number in the open field, and lowering the immobility time in forced swim and tail suspension tests in a chronic unpredictable stress of depression model rats [21]. *G. Radix* (GR) was noted to prevent the Aβ (25–35) induced neuronal apoptotic death by decreasing the expression of Bax and active caspase-3, and increasing the expression of Bcl-2. Furthermore, GR also significantly inhibited Aβ (25–35) induced elevation of the intracellular Ca^{+2} concentration and ROS generation [22]. An active component isolated from *G. uralensis* isoliquiritigenin (ISL), was demonstrated to reverse the glutamate-induced ROS production and mitochondrial depolarization, and regulate the glutamate-induced changes of Bcl-2 and Bax in HT22 hippocampal neuronal cells. These results suggest that *G. uralensis* plays a therapeutic role for preventing the progresses of neurodegenerative diseases such as Alzheimer's disease [23].

14.3.6 Estrogenic Effects

Licorice was reported to be effective on the prevention of osteoporosis after menopause. Extracts of *Glycyrrhiza* species induced estrogen responsive alkaline phosphatase activity in endometrial cancer cells. Meanwhile, increase of estrogen responses of these extracts were approved using estrogen responsive element (ERE)-luciferase reporter system in MCF-7 cells, and increased *Tff*1 mRNA expression was found in T47D cells [24]. Isoliquiritigenin was demonstrated to be the major estrogenic compound in the licorice extract due to its ERβ selectivity, partial estrogen activity, and non-enzymatic conversion of isoliquiritigenic to liquiritigenin [24].

14.3.7 Anti-metabolic Syndrome

GA, a potential agonist to PPARγ, was investigated for its anti-metabolic syndrome effects. It indicated that oral administration of 100 mg/kg GA for 24 h improved insulin sensitivity and lipid profiles and induced up-regulation of PPARγ and LPL expression in adipose, muscle, liver and kidney tissues [25].

GA, a bioactive component in licorice, significantly reduced the AGEs-induced apoptosis by increasing the SOD activity, decreasing the MDA production, inhibiting ROS over generation, and down-regulating the TGF-β1 and NF-kB protein expressions in human umbilical vein endothelial cells, indicating that GA might be an alternative for the prevention and treatment of diabetic vascular complications [26].

14.4 TCM Applications and Dietary Usage

TCM prescriptions are usually composed of several herbal medicines called "fufang", based on the herbal nature and classified as "Jun-Chen-Zuo-Shi" medicines in a prescription. Licorice is commonly used as a "Jun" (emperor) or "Shi" (courier) medicine to treat the main cause of the disease or to guide the other herbs to the target organs, respectively.

Licorice was documented to treat a lot of diseases in Five-Zang (heart, spleen, liver, lungs and kidneys) and Six-Fu organs (gallbladder, stomach, large intestine, small intestine, bladder and sanjiao). The traditional therapeutic effects of licorice are to clean the evil influence of cold and heat, to strengthen the bones and muscles, and detoxification. Long term treatment of licorice is also beneficial for antiaging [1]. Licorice can be used in TCM either in raw form or prepared form (roasted by honey). Raw licorice is used to clear interior heat; on the other hand, prepared licorice is used for releasing the exterior cold. Combined used of licorice in other prescriptions functions to cure sore throat, tonify spleen and stomach, and nourish the lung.

14.4.1 TCM Applications

One of the most famous Chinese medical book "Treatise on Cold Pathogenic Diseases" described that many cold induced diseases such as cough, sore throat, and omitting could be restored by treating with licorice related prescriptions. Licorice was named as "King of the herbs" to point out the importance of its application in TCM.

Licorice decotion ("Gancao Tang") and Prepared licorice decotion ("Zhigancao Tang") are two prescriptions that only licorice was used to relief sore throat and anti-cough through nourishing the lung, respectively. "Gancao Xiexin Tang" combined licorice, Huangqin (root of *Scutellaria baicalensis*), Ganjiang (rhizome of *Zingiber officinale*), Banxia (tuber of *Pinellia ternata*), Dazao (fruit of *Ziziphus jujuba*), and Huanglian (rhizome of *Coptis chinensis*) is used to relieve the illness of stomach and nourish stomach. "Gancao Ganjiang Tang" combined licorice and prepared ginger is focusing on the spleen and stomach nourishing. "Gancao Fuzi Tang" combined licorice, prepared Fuzi (daughter root of *Aconitum carmichaeli*), Baizu (*Atractylode smacrocephala*), and Guizhi, (twig of *Cinnamomum cassia*) are prescribed to relieve the pain of Rheumatoid arthritis. "Ganjiang Linzu Tang" combined licorice, Baizhu, dried ginger, and Fuling (sclerotium of *Poria cocos*) are mainly used to remedy the abnormality of renal function caused by cold.

14.4.2 Dietary Usages

Dietary usage of licoriceis mainly as a sweetener in addition to its physiological functions in many soft drinks, food products, candy, chocolate, chewing gums, and herbal medicines. The habitual consumption of licorice derived sweetener is more popular in hot environments. The major compound in licorice used as a sweetener is GA which is 50–100 times sweeter than that of sugar. Although GA and water soluble licorice extract were thought to be nontoxic food additives due to its less bioavailability, accumulated evidences indicated that over ingestion of GA or licorice extract may cause some side effects such as cortisol-induced mineralocorticoid syndrome. Limitations for use of licorice or licorice derivatives in food supplement was stipulated by US FDA (Table 14.1) [27].

Table 14.1 Limitations for the use of licorice and its derivatives in foods, US Food and Drug Administration

Food category	Maximum allowable levels in foods as % glycyrrhizin content	Functional use
Baked goods	0.05	1, 2
Alcoholic beverages	0.1	1, 2, 3
Nonalcoholic beverages	0.15	1, 2, 3
Chewing gum	1.1	1, 2
Hard candy	16.0	1, 2
Soft candy	3.1	1, 2
Herbs and seasonings	0.15	1, 2
Plant protein products	0.15	1, 2
Vitamin or mineral dietary supplement	0.5	1, 2
All other foods, except sugar substituents	0.1	1, 2

1 Flavor enhancer; *2* flavoring agent; *3* surface-active agent

14.5 Clinical Evidences

Scientific clinical evidences of licorice are limited, although use of licorice in TCM is extensively documented in Chinese medical books, especially on anti-hepatitis, anti-hyperglycemia, and GI diseases.

14.5.1 Anti-hepatitis

GA is available in a multiplicity of non-standardized oral formulations [28, 29]. Stronger neominophagen C (SNMC), containing 0.2 % GA, 0.1 % cysteine, and 2 % glycine in physiological solution and administering intravenously 80–200 mg/day, is used in Japan for the treatment of acute and chronic hepatitis [30].

14.5.2 GI Function

The curative effect of deglycyrrhizinized licorice (DGL) in gastric ulcer patients was confirmed during 1970s by clinical trials. Clinically, DGL has been used as a main source for the treatment of GI ulcerative disorders including peptic ulcer, canker sores and inflammatory bowel diseases. A randomized, double-blind, placebo-controlled study indicated that extract of *G. glabra* improved the severity and symptom score of dyspepsia [31]. Liver enzymes, ALT and AST were significantly decreased following administration of 2 g licorice root extract per day for two months in non-alcoholic fatty liver disease patients [32].

14.6 Safety Evaluation and Toxicity Issues

For many years, licorice was believed to be a healthy and safe natural substance without side effects [27]. The major component of licorice is GA with poor bioavailability and low animal toxicity. Oral acute toxicity test showed that LD_{50} of GA was 4320 mg/kg bodyweight in mice. Glycyrrhetic acid, the active inhibitor of 11-ß-hydroxysteroid dehydrogenase enzyme type 2, was generated by intestinal bacterial ß-glucuronidase after GA was ingested. The acute toxicity data showed that LD_{50} of glycyrrhetic acid in mice was 308 mg/kg bodyweight through i.p. injection. Glycyrrhetic acid is further metabolized to glucuronide and sulfate conjugates in liver, then excretes into the bile. Patients ingest GA either long term or periodically, might accumulate glycyrrhetic acid and its derivatives leading to toxicity such as cortisol-induced mineralocorticoid effect, elevated sodium and reduced potassium levels [27] in blood.

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