Available Online at

http://www.ijcpa.in

April-June 2018

International Journal of CHEMICAL AND PHARMACEUTICAL ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

Review Article | Volume-5 | Issue-3 | Article ID: 1483

A COMPREHENSIVE REVIEW ON LIQUORICE WITH SPECIAL REFERENCE TO ITS EXTRACTION AND ANALYSIS

Harshal Ashok Pawar

Assistant Professor, Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar, Maharashtra, India

***Corresponding Author:** Email: harshal.pawar@dlhhcop.org*

Received: 5 April 2018 / Revised: 22 June 2018 / Accepted: 26 June 2018 / Available online 30 June 2018

ABSTRACT

Liquorice is one of the most popular ingredients in several traditional herbal medicinal preparations, and glycyrrhizin is the major glycoside present in this plant. Licorice exhibits many important pharmacological activities such as antiviral, anticancer, antiulcer, anti-inflammatory, antioxidant etc. The present review describes the general pharmacognostic features, phytochemistry, biopotential, methods of extraction and analysis of various phytoconstituents of liquorice.

Keywords *– Aloe-emodin, TLC, Isolation, Identification, HPLC Analysis*

1. INTRODUCTION

Licorice or Liquorice is one of the most widely utilized and broadly investigated restorative plants of the world[1,2]. The word liquorice basically gets from Old Greek glykyrrhiza, glykys signifies "sweet," and rhiza is "root"[3]. One of the important phytoconstituent of Licorice is glycyrrhizin, which exhibits a cortisone-like activity. Glycyrrhizin is fifty times sweeter than sucrose[4]. Liquorice is known by various names, for example, sweetwood, licorice, liquorice radix, reglisse (French), lakritzeholz (German), Gan Cao (Chinese), Meyan or Beyan (Turkish), and Solodka (Russian)[5].

As per United States Pharmacopoeia (USP), Licorice consists of the roots, rhizomes, and stolons of *Glycyrrhiza glabra* Linné or Glycyrrhiza uralensis Fisher (Fam. Leguminosae). The botanical characteristics of the Liquorice are described in USP. They are as follows:

i) Macroscopic— The terrestrial stem is nearly cylindrical, 0.5 to 3.0 cm in diameter, and over 1 m in length; it is externally dark brown to red-brown and longitudinally wrinkled. It often has lenticels, small buds, and scaly leaves. The transverse section reveals a rather clear border between the phloem and the xylem, and a radial structure that often has radiating splits.

ii) Microscopic— The transverse section reveals several yellow-brown cork layers, and a layer of phelloderm that is 1 to 3 cells thick. The cortex exhibits medullary rays, and obliterated sieve portions radiate alternately. The phloem exhibits groups of phloem fibers, which are surrounded by crystal cells, with thick but incompletely lignified walls. The vessels are accompanied by xylem fibers, which are surrounded by crystal cells, and by xylem parenchyma cells. The parenchyma cells contain starch grains and often contain single crystals of calcium oxalate.

Fig. 1: Licorice root

Licorice roots **(Fig. 1)** have been utilized worldwide as a medication and flavor in industry for more than 4000 years. Therapeutic employments of licorice are recorded in writings, for example, Assyrian Herbal (2000 BC) and Ebers Papyrus (1600 BC)[6,7]. The most widely distributed species *Glycyrrhiza glabra* is found in Spain, Italy, Turkey, the Caucasus, Central Asia, and the western part of China whereas *Glycyrrhiza uralensis* is distributed form Central Asia to Mongolia and China. Other species includes *G. aspera, G. bucharica, G. echinata, G. eurycarpa, G. iconica, G. inflata, G. korshinskyi, G. lepidota, G. macedonica, G. pallidiflora, G. squamulosa, G. triphylla, G. uralensis, and G. yunnanensis*[8-10].

The present review describes the phytochemistry, biopotential, methods of extraction and analysis of various phytoconstituents of liquorice.

2. PHYTOCHEMISTRY

Licorice contains several primary and secondary metabolites. Various biologically active compounds reported in Licorice includes alkaloids, glycosides, flavonoids, phenolics, saponins, tannins, terpenes, anthraquinones, essential oils, and steroids[11-20]. Literature survey revealed that the different species of Licorice contains more than 300 flavonoids[21,22]. *G. glabra* has yellow color due to the flavonoids, e.g., liquiritin and isoliquiritin[23]. A number of licorice flavonoids were identified: liquiritin, liquiritigenin, rhamnolliuiritin, liquiritin apioside, gralbranin, glabrol, licoflavanone, isoliquiritigenin, neoisoliquiritin, licuraside, licochalcone A and B, licoricidin, 7-methillicoricidin, hispaglabridin A and B, liocflavone A and B, liocflavanol, glyzaglabrin, licoisoflavanone, glabroisoflavanone, glabrone, licoricone, and gancaonin [24]. The root of Glycyrrhiza contains triterpenoid saponins (glycyrrhizin, glycyrrhizic acid), which are the major characteristic constituents of liquorice, and they are responsible for the sweet taste[25]. Several coumarins were identified from *G. glabra* including liqcoumarin, glabrocoumarone A and B, herniarin, umbelliferone, and glycyrin[26]. Other secondary metabolites such as fatty acids, phenol, guaiacol, asparagines, glucose, sucrose, starch, polysaccharides, and sterols (β-sitosterol, dihydrostigmasterol) have also been found and reported by Näf and Jaquier[27]. Licorice extract contains sugars, starch, bitters, resins, essential oils, tannins, inorganic salts, and low levels of nitrogenous constituents such as proteins, individual amino acids, and nucleic acids [28,29]. The chemical structure of Glycyrrhizin and Liquiritin are represented in Fig. 2 and Fig.3.

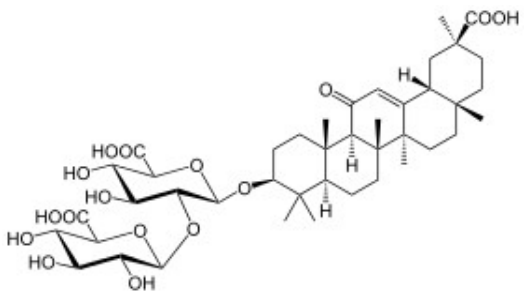

Fig. 2. Chemical structure of Glycyrrhizin (Glycyrrhizic acid)

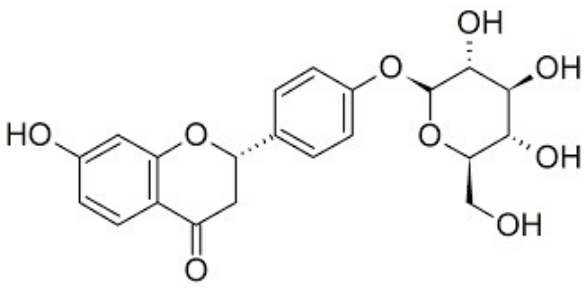

Fig. 3: Chemical structure of Liquiritin

3. Biopotential of Liquorice

In traditional medicine, licorice roots have been used for treating many diseases like lung diseases, arthritis, kidney diseases, eczema, heart diseases, gastric ulcer, low blood pressure, allergies, liver toxicity, and certain microbial infections.

Several therapeutic properties exhibited by licorice root, such as hypocholesterolemic and hypoglycemic, antimicrobial, antiviral, preliminary free radical scavenging, anti-ulcer, cytotoxic, antitumor, antiallergic, antidiabetic, anticarcinogenic, antioxidant, anti-inflammatory and hepatoprotective activities, skin eruptions; dermatitis; and eczema. The licorice can also be used in the management of impaired learning, dementia, Alzheimer's disease, and other neurodegenerative disorders [30-43].

3. Extraction of Liquorice

A lot of extraction methods have been employed to extract glycyrrhizin from licorice which includes analytical, solvent based dipping/percolation/maceration, microwave-assisted, Soxhlet, etc. The various method reported in previously published literature are summarized Table-1[44].

Table 1: Summary of extraction / separation / isolation of various phytoconstituents of licorice roots

Title	Year	Method used
Separation of major components in licorice using high-performance liquid chromatography.	1979	Aqueous extract is dissolved in 4% ammonium hydroxide solution for separation of at least eight major components in licorice extract using a gradient, reversed-phase HPLC system.
Microwave-assisted extraction of glycyrrhizic acid from licorice root.	2000	Extraction is performed in microwave-assisted extractor by water, EtOH, EtOH–water, ammonia solution (different concentrations) or ethanol–water–ammonia and analyzed by HPLC.
Purification of glycyrrhizin from *G. uralensis* Fisch with ethanol/phosphate aqueous two-phase system.	2002	Powder is extracted by 0.01% NH_4OH at 90–100◦C, filtered, concentrated, dried under vacuum then added in mixture of EtOH, water and K_2HPO_4. After settling, upper portion collected and precipitation by H_2SO_4.
Isolation and purification of inflacoumarin A and licochalcone A from licorice by high-speed counter-current chromatography.	2004	Roots are extracted with ethanol–water (95:5) by sonication, concentrated by vacuum rotary evaporator; residue is dissolved in 2% NaOH and then filtered. Precipitation takes place by adding HCl in filtrate. Precipitate is washed by water and freeze-dried.
The application of macro-porous resins in the separation of licorice flavonoids and glycyrrhizic acid.	2005	Powder is extracted with EtOH/H_2O (70:30, v/v) by sonication in an ultrasonic bath, solution was centrifuged and supernatant is concentrated to 1/10 of original volume in a rotary evaporator. Then solution is passed though different type of resins.
Separation of glycyrrhizic acid and liquiritin from Glycyrrhiza uralensis Fisch extract by three-liquid-phase extraction systems.	2007	Dried licorice slices are extracted with aqueous NH_3 solution (0.5 vol%) by sonication. Three-liquid-phase systems contained four components that are organic solvent, inorganic salt, polymer and the treated licorice extract.

3.1 Conventional method extraction of glycyrhizic acid and preparation of glycyrrhetinic acid

Glycyrrhetinic acid, a hydrolytic product of glycyrrhizic acid, is a component of licorice and exerts a mineralocorticoid-like effect. Glycyrrhetinic acid is the triterpenoid aglycone of glycyrrhizin.

The conventional way for preparation of 18β-glycyrrhetinic acid from licorice roots included two basic steps.

Step-1: The step 1 involves the procedure the obtain of glycyrrhizic acid from the licorice roots via extraction with using the organic solvents and subsequent purification.

Step-2: The step 2 involves the procedure of hydrolysis of the obtained glycyrrhizic acid to release the glycyrrhetinic acid using the organic solvents and the mineral acids and subsequent purification.

The extraction of glycyrrhizic acid from the Licorice roots included the several stages. 1.0 g sample of the dry the licorice roots (particle size 0.5– 1.0 mm) is boiled four times under reflux. At the first stage, the dry roots are boiled with 30 ml hexane for 120 min. At the next three stages of 25 mL 2% NH_4OH в 70% aqueous solution of ethanol is boiled for 120 min. The obtained extracts are then filtered, combined, and analyzed.

The hydrolysis of glycyrrhizic acid from the Licorice roots is performed using hydrochloric acid. A sample of 0.4 g of extract is dissolved in 4 mL of hot distilled water (60°C) with frequent stirring, and 0.7 mL of concentrated hydrochloric acid (density 1.179) is added. The hydrolysis is carried out for 4 h at 100°C. At the end of the hydrolysis, the obtained precipitate is filtered through paper and washed with distilled water to a neutral pH and then dissolved in 80% ethanol. The aqueous-ethanolic solution was dried at 80°C for 2–3 h[45].

3.2 Isolation of Glycyrhizic acid by supercritical fluid extraction method

Hedayati, Ali and Ghoreishi, S. M. have carried out the extraction of Glycyrrhizic acid (GA) from *Glycyrrhiza glabra* (licorice) root using modified supercritical CO_2 with methanol and water as co-solvents and 30 min of static time. The operating temperature (45–85°C), pressure (10–34MPa), dynamic extraction time (40–120min), CO_2 flow rate (0.8–2ml/min) and methanol concentration in water (0–100% as the binary co-solvent) were considered as the range of operating variables. The high-performance liquid chromatography (HPLC) was used to identify and quantitatively determine the amount of extracted GA[46].

3. ANALYSIS OF LIQUORICE

Various analytical methods have been reported in previously published literatures for qualitative and quantitative analysis of various phytoconstituents of Liquorice. Two simple methods are discussed below:

4.1 Analysis using Thin Layer Chromatography (TLC) as per USP

Test solution— Add 10 mL of a mixture of alcohol and water (7:3) to 2.0 g of pulverized Licorice, heat by shaking on a water bath for 5 minutes, cool, and filter.

Standard solution— Dissolve 5 mg of USP Glycyrrhizic Acid RS in 1 mL of a mixture of alcohol and water (7:3).

Application volume: 2 µL.

Developing solvent system: a mixture of butyl alcohol, water, and glacial acetic acid (7:2:1).

Procedure— Proceed as directed in the chapter, except to develop the chromatogram in an unsaturated chamber to a length of about 10 cm. Examine the plate under UV light at a wavelength of 254 nm. The chromatograms show a dark purple zone, among other spots, due to glycyrrhizic acid at an RF value of about 0.4[47].

4.2 HPLC Analysis

4.2.1 Determination of content of glycyrrhizic acid

Solvent: a mixture of alcohol and water (1:1).

Mobile phase: a filtered and degassed mixture of diluted acetic acid (1 in 15) and acetonitrile (3:2).

Standard solution— Dissolve an accurately weighed quantity of USP Glycyrrhizic Acid RS in Solvent to obtain a solution having a known concentration of about 0.25 mg per mL.

Test solution— Transfer about 500 mg of Licorice, reduced to a powder and accurately weighed, to a suitable flask, add 70 mL of Solvent, shake for 15 minutes, centrifuge, and decant the supernatant into a 100-mL volumetric flask. Mix the residue with 25 mL of Solvent, shake for 15 minutes, centrifuge, and add the supernatant to the volumetric flask. Dilute with Solvent to volume, mix, and pass through a membrane filter having a 0.45-µm porosity[47].

4.2.2 Literature method

Andrisano, V.; Bonazzi, D.; Cavrini, V. proposed a reversed-phase HPLC method for the separation of five liquorice triterpenoids, 18 β glycyrrhetic acid (β GA), 18 α glycyrrhetic acid (α GA), 24-hydroxy-18 β-glycyrrhetic acid (24-OH-β GA), α and β liquiritic acid (α and β LA), with potentially different biological activities. The method has been developed by studying the influence of the type of stationary phase, pH, amine modifier and organic modifier on the resolution of the five compounds. The optimized chromatographic conditions were then successfully applied to the analysis of α- and β-GA in pharmaceutical preparations (toothpaste and creams) on a reversed-phase Phenomenex Ultracarb 5 ODS (30) column (150 × 4.6 mm i.d.), using as the mobile phase acetonitrile-THF-0.010 M dioctyl ammonium phosphate buffer (pH 6.5) (25:20:55, v/v/v) at a flow-rate of 0.8 ml min^{-1}. Solid phase extraction methods with diolic and C18 sorbents were developed to isolate and concentrate the analytes and to enhance the sensitivity for the determination of α-GA as an impurity in the β-GA preparations. The method was found to be reliable and suitable for the quality control of β-GA preparations [48].

5. CONCLUSION

This review has presented a comprehensive overview about the botanical features, phytochemistry, biopotential, methods of extraction and analysis of various phytoconstituents of liquorice. This plant has been largely used as a traditional medicine and food industry ingredient, particularly as a flavour and sweetening agent. Different phytochemicals, including glycyrrhizin, 18β-glycyrrhetinic acid, glabrin A and B, or isoflavones, have been identified and associated with the biological activities reported, namely, antioxidant, antiviral, antimicrobial, anticancer, or anti-inflammatory activities as well as hepatoprotection. The compiled information will be useful to the scientists and researchers for extraction and isolation, identification and quantitation of various phytocontituents of liquorice.

6. ACKNOWLEDGEMENT

Author is very much thankful to Dr. Paraag Gide, Principal of Hyderabad Sindhi National Collegiate Boards (HSNCB's) Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar for his continuous support and encouragement.

7. REFERENCES

1. Hoffman D. Easy breathing: natural treatments for asthma, colds, flu, coughs, allergies, sinusitis. Pownal, VT: Storey Books; 2000.
2. Öztürk M, Altay V, Hakem KR, Akçiçek E. Liquorice—from botany to phytochemistry. Basel: Springer Nature; 2017.
3. Schulz V, Hänsel R, Tyler VE. Rational phytotherapy. A physicians' guide to herbal medicine. Berlin: Springer; 1998. pp. 160–187.
4. Brown K. Medicinal plants, indigenous medicine and conservation of biodiversity in Ghana. In: Swanson T, editor. Intellectual property rights and biodiversity conservation. Cambridge: Cambridge University Press; 1995. pp. 201–231.
5. Mills SY, Bone K. Principles and practice of phytotherapy: modern herbal medicine. London: Churchill Livingstone; 2000.
6. Lucas . Nature's medicines. Hollywood, CA: Melvin Powers, Wilshire Book Company; 1976.
7. Reid D. A handbook of Chinese healing herbs. Singapore: Periplus; 2001.
8. Field survey of Glycyrrhiza plants in Central Asia (3). Chemical characterization of G. glabra collected in Uzbekistan. Hayashi H, Hattori S, Inoue K, Khodzhimatov O, Ashurmetov O, Ito M, Honda G. Chem Pharm Bull (Tokyo). 2003 Nov; 51(11):1338-40.
9. Nomura T, Fukai T, Akiyama T. Chemistry of phenolic compounds of licorice (Glycyrrhiza species) and their estrogenic and cytotoxic activities. Pure Appl Chem. 2002;74(7):1199–1206.
10. Fiore C, Eisenhut M, Ragazzi E, Zanchin G, Armanini D. A history of the therapeutic use of liquorice in Europe. J Ethnopharmacol. 2005 Jul 14; 99(3):317-24.
11. Sarker SD, Nahar L. Chemistry for pharmacy students: general, organic and natural product chemistry. London: Wiley; 2007.
12. Varsha S, Agrawal RC, Sonam P. Phytochemical screening and determination of anti-bacterial and anti-oxidant potential of Glycyrrhiza glabra root extracts. J Environ Res Dev. 2013;7(4):1552–1558.
13. Firn R. Nature's chemicals: the natural products that shaped our world. Oxford: Oxford University Press; 2010. pp. 74–75.
14. Kar A. Pharmacognosy and pharmacobiotechnology. New Delhi: New Age International Ltd Publishers; 2007. pp. 332–600.
15. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004 Mar 12; 74(17):2157-84.
16. Puupponen-Pimiä R, Nohynek L, Meier C, Kähkönen M, Heinonen M, Hopia A, Oksman-Caldentey KM. Antimicrobial properties of phenolic compounds from berries. J Appl Microbiol. 2001 Apr; 90(4):494-507.
17. Vashist H, Sharma D. Pharmacognostical aspects of Glycyrrhiza glabra. Asian J Pharm Clin Res. 2013;6(4):55–59.
18. Martinez MJA, Lazaro RM, del Olmo LMB, Benito PB. Anti-infectious activity in the Anthemideae tribe. Stud Nat Prod Chem. 2008; 35:445–516.
19. Maurya R, Singh G, Yadav PP. Antiosteoporotic agents from natural sources. Stud Nat Prod Chem. 2008; 35:517–545.
20. Madziga HA, Sanni S, Sandabe UK. Phytochemical and elemental analysis of Acalypha wilkesiana leaf. J Am Sci. 2010;6(11):510–514.
21. Herz W, Kirby GW, Moore RE, Steglich W, Tamm C. Fortschritte der Chemie Organischer Naturstoffe. New York: Springer; 1998.
22. Li W, Asada Y, Yoshikawa T. Flavonoid constituents from Glycyrrhiza glabra hairy root cultures. Phytochemistry. 2000 Nov; 55(5):447-56.
23. Yamamura Y, Kawakami J, Santa T, Kotaki H, Uchino K, Sawada Y, Tanaka N, Iga T. Pharmacokinetic profile of glycyrrhizin in healthy volunteers by a new high-performance liquid chromatographic method. J Pharm Sci. 1992 Oct; 81(10):1042-6.
24. Zhang Q, Ye M. Chemical analysis of the Chinese herbal medicine Gan-Cao (licorice) J Chromatogr. 2009;1216(11):1954–1969.

25. Blumenthal M, Goldberg A, Brinckmann J. Herbal medicine: expanded commission E monographs. Newton, MA: Integrative Medicine Communications; 2000.
26. Williamson EM. Potter's cyclopedia of herbal medicine. Saffron Walden: C.W. Daniel; 2003. pp. 269–271.
27. Näf R, Jaquier A. New lactones in liquorice (Glycyrrhiza glabra L.) Flavour Fragr J. 2006;21:193–197.
28. Hoffmann D. The new holistic herbal. 2. Shaftesbury: Element; 1990.
29. Isbrucker RA, Burdock GA. Risk and safety assessment on the consumption of Licorice root (Glycyrrhiza sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. Regul Toxicol Pharmacol. 2006 Dec; 46(3):167-92.
30. Sitohy MZ, el-Massry RA, el-Saadany SS, Labib SM. Metabolic effects of licorice roots (*Glycyrrhiza glabra*) on lipid distribution pattern, liver and renal functions of albino rats. MS. Nahrung. 1991; 35(8):799-806.
31. Ambawade S, Kasture VS, Kasturi SB. Anxiolytic activity of Glycyrrhiza glabra Linn. J Nat Remedies. 2001; 2:130–134.
32. Patil SM, Patil MB, Sapkale GN. Antimicrobial activity of Glycyrrhiza glabra Linn. Roots. Int J Chem Sci. 2009;7(1):585–591.
33. Cinatl J, Morgenstern B, Bauer G, Chandra P, Rabenau H, Doerr HW. Glycyrrhizin, an active component of liquorice roots, and replication of SARS-associated coronavirus. Lancet. 2003 Jun 14; 361(9374):2045-6.
34. Toshio F, Kazue S, Taro N. Preliminary evaluation of anti-nephritis and radical scavenging activities of glabridin from Glycyrrhiza glabra Linn. Fitotherapia. 2003; 74:624–629. doi: 10.1016/S0367-326X(03)00164-3.
35. Da Nagao Y, Sata M, Suzuki H, Tanikawa K, Itoh K, Kameyama T. Effectiveness of glycyrrhizin for oral lichen planus in patients with chronic HCV infection. J Gastroenterol. 1996 Oct; 31(5):691-5.
36. Hossain MS, Hossain MA, Islam R, Alam AH, Zahan K, Sarkar S, Farooque MA. Antimicrobial and cytotoxic activities of 2-aminobenzoic acid and 2-aminophenol and their coordination complexes with magnesium (Mg-II) Pak J Biol Sci. 2004; 7:25–27. doi: 10.3923/pjbs.2004.25.27.
37. Ram A, Mabalirajan U, Das M, Bhattacharya I, Dinda AK, Gangal SV, Ghosh B. Glycyrrhizin alleviates experimental allergic asthma in mice. Int Immunopharm. 2006;6(9):1468–1477.
38. Kroes BH, Beukelman CJ, van den Berg AJ, Wolbink GJ, van Dijk H, Labadie RP. Inhibition of human complement by beta-glycyrrhetinic acid. Immunology. 1997 Jan; 90(1):115-20.
39. Satomi Y, Nishino H, Shibata S. Glycyrrhetinic acid and related compounds induce G1 arrest and apoptosis in human hepatocellular carcinoma HepG2. Anticancer Res. 2005; 25(6B):4043-7.
40. Belinky PA, Aviram M, Mahmood S, Vaya J. Structural aspects of the inhibitory effect of glabridin on LDL oxidation. Free Radic Biol Med. 1998 Jun; 24(9):1419-29.
41. Wu YT, Shen C, Yin J, Yu JP, Meng Q. Azathioprine hepatotoxicity and the protective effect of liquorice and glycyrrhizic acid. Phytother Res. 2006 Aug; 20(8):640-5.
42. Akhtar N, Khan MS, Iqbal A, Khan BA, Bashir S. *Glycyrrhiza glabra* extract cream: effect on skin pigment melanin. Proceeding book of international conference on bioscience, biochemistry and bioinformatics IPCBEE. Singapore: IACSIT Press; 2011.
43. Chakravarthi KK, Vadhani RA, Narayan RS. Effect of *Glycyrrhiza glabra* root extract on learning and memory in wistar albino rats. Int J Biol Med Res. 2012;3(3):2059–2064.
44. Mukhopadhyay, Mamata; Panja, Palash. A novel process for extraction of natural sweetener from licorice (Glycyrrhiza glabra) roots. Separation and Purification Technology, 2008, 63.3: 539-545.
45. Lekar, Anna V., et al. New fast "One-pot" Technique for the Production of Glycyrrhetinic Acid from the Roots of licorice (Glycyrrhiza glabra). Natural Product Communications, 2018, 13.7: 1934578X1801300709.

46. Hedayati, Ali; Ghoreishi, S. M. Supercritical carbon dioxide extraction of glycyrrhizic acid from licorice plant root using binary entrainer: experimental optimization via response surface methodology. The Journal of Supercritical Fluids, 2015, 100: 209-217.
47. https://www.drugfuture.com/pharmacopoeia/usp32/pub/data/v32270/usp32nf27s0_m45050.html
48. Andrisano, V.; Bonazzi, D.; Cavrini, V. HPLC analysis of liquorice triterpenoids—applications to the quality control of pharmaceuticals. Journal of pharmaceutical and biomedical analysis, 1995, 13.4-5: 597-605.