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# Phytochemical composition, health effects and crop management of liquorice (*Glycyrrhiza glabra* L.): A medicinal plant

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## ABSTRACT

Liquorice has been widely appreciated as an important medicinal plant. Its rhizomes and roots have been used for centuries in traditional medicine due to their renowned therapeutic properties. However, increasing market demands and irrational harvesting of wild liquorice plants has rendered the cultivation of the species of major importance. This review, presents aspects related with chemical composition and health effects of the species, and the effect of various cultivation practices. Particular interest is given on glycyrrhizin and its extraction procedures, since it is the main bioactive compound of liquorice roots and its content determines the final product quality.

**KEYWORDS:** *Glycyrrhiza glabra*; Fabaceae; glycyrrhizin; human health; licorice; liquorice; medicinal plant

## Introduction

Wild plants and natural products have been widely used throughout the centuries by primitive societies, for multiple medicinal purposes. Besides the current increasing trend for herbs and spices, it is important to highlight that most of the chemical products and drugs are based on natural compounds, and even the most common chemotherapeutic agents are derived from wild plants.<sup>(1-4)</sup> According to World Health Organization<sup>(5)</sup>, the primary health care intervention in developing countries, is mainly based on natural products and plant-derived drugs.<sup>(6)</sup>

*Glycyrrhiza glabra* L., commonly known as liquorice, licorice or cultivated liquorice, is a traditional plant, to which multiple health benefits have been attributed and its medicinal uses have been dated throughout the centuries. The tapered roots and rhizomes of the plant are widely appreciated and cultivated, since they contain most of the bioactive compounds which are responsible for its medicinal and culinary attributes as flavoring agent and spice. In addition, other *Glycyrrhiza* species such as Chinese liquorice (*Glycyrrhiza uralensis* Fisch), Russian liquorice (*Glycyrrhiza echinata* L.) and *G. inflata* Bat. are also widely used in traditional medicine.<sup>(7-9)</sup>

The economic importance of liquorice in the world trade of spices is very significant, either as raw material or as root extracts after processing. In 2005, the international trade of liquorice was estimated at 42 million US\$, with the main importing countries of liquorice roots being the United States of America (USA), Japan, Republic of Korea and Israel, whereas the leading importing countries of liquorice extracts were Germany, USA, Netherlands, Japan and China. China is also the leading exporting country of liquorice roots, followed by Afghanistan and Uzbekistan, whereas regarding liquorice extracts USA, France, Israel and China are the most representative exporting countries.<sup>(10)</sup>

Most of the marketed final products of liquorice (root and rhizomes extracts and other derivatives) are produced from hand harvested wild plants, a very common practice in the areas where plants grow naturally, which however could threaten sustainability and increase genetic erosion of the species.<sup>(11)</sup> So far, commercial cultivation is mainly based on common practices applied to most of the crops, without relative information being compiled in a thorough text. Considering that commercial cultivation of liquorice is significant for further exploitation of the species, the compilation of a best practice guide, which among other purposes will ensure high yields and final products of high and uniform quality, is of major importance.

This review aims to examine all the aspects related with the therapeutic properties and the human health effects of liquorice, as well with the cultivation practices of liquorice, in order to provide a suitable best practice guide for potential farmers. Special interest is also taken in highlighting those practices that could improve quality in commercial production. Botanical and morphological description, distribution and uses, chemical composition and breeding approaches for quality and yield improvement are also included, as well as extraction procedures of the main bioactive compounds.

### **Botanical description and plant morphology**

*Glycyrrhiza glabra* L. (synonyms: *Glycyrrhiza glandulifera* Waldst. and Kit; *Liquiritiae officinalis* Moench) is a member of the Fabaceae family. The base chromosome number of the species is  $2n=16$ <sup>(5,12,13)</sup>, whereas its name (“Glycyrrhiza”) has its origin from ancient Greece and Dioskouridis who named it after the sweet flavour of plant roots (“glukos” which means sweet and “riza” which means root). The term “glabra” is used to describe its hairless pods.<sup>(11)</sup>

The genus *Glycyrrhiza* includes nearly 30 species<sup>(14)</sup>, and within the *G. glabra* species itself there are various cultivars, such as *G. glabra* var. *typica* Regel & Herder which forms horizontal

stolons brown coloured bark<sup>(15)</sup>; *G. glabra* var. *glandulifera* (Waldst et Kit) Regel & Herder and *G. glabra* var. *violaceae* (Boiss. and Noe) Boiss.<sup>(5,16)</sup>

*G. glabra* is able to fix N<sub>2</sub> from the atmosphere in symbiosis with nitrogen-fixing bacteria. *Mesorhizobium* strains have been reported to induce effective nodules on *G. glabra* and *G. uralensis* species. However, apart from *Mesorhizobium* bacteria, which are true symbionts, other sporadic bacteria belonging to the *Rhizobium*, *Sinorhizobium*, *Agrobacterium* and *Paenibacillus* genera have also been identified and classified as having weak, infrequent or no N<sub>2</sub> fixation capacity.<sup>(17)</sup>

Liquorice (*Glycyrrhiza glabra* L.) is a perennial plant with a height of 0.7-2.0 m and erect growth.<sup>(5,18)</sup> The plant develops a deep root system with a depth of more than 1 m.<sup>(11)</sup> The root system consists of well-developed horizontal stolons and rhizomes<sup>(21)</sup>, while the buds on the underground stolons can expand to form new stems.<sup>(11,19)</sup> The bark of the roots and rhizomes has a brownish green to dark brown colour.<sup>(5)</sup>

Leaves are pinnate<sup>(19)</sup>, and consists of 9-17 alternate oblong to lanceolate leaflets.<sup>(5)</sup> The flowers are small (1 cm long), have purple to whitish blue colour, and the pods (2-3 cm) are flat, with an oblong to linear shape<sup>(5,19)</sup>, have a smooth surface<sup>(11)</sup>, turn brown at maturity and contain 1-7 seeds.<sup>(5,19)</sup> The seeds have dark colour, a reniform shape and small size, with a diameter of about 2.5 mm and a thousand seeds weight of 6.2 g.<sup>(19,20)</sup>

### **Distribution and uses**

Liquorice is widely cultivated as a medicinal plant, but it can also be a troublesome weed in its wild form at warm, temperature climates.<sup>(11)</sup> It is native to the Mediterranean basin and the central and southwest regions of Asia<sup>(7,11)</sup>, and can be found at low or high altitudes, nearly up to 1200 m above sea level.<sup>(21)</sup>

The dried roots and rhizomes (*Glycyrrhiza radix*) are widely used in traditional medicine<sup>(22)</sup>, but liquorice root extracts can be used for other purposes too.<sup>(18,23)</sup> According to US Food and Drugs Administration<sup>(24)</sup>, liquorice extract is defined as the extract from root and rhizome portions, after maceration and extraction with boiling water. Further purification of the extract can be achieved with acid and ethyl alcohol treatments. The final product after extraction and purification can be used as liquid, paste or spray-dried powder.

Glycyrrhizin, the main bioactive compound of liquorice roots, is used as sweetener<sup>(25)</sup>, with its taste being 50 times sweeter than sugar.<sup>(26)</sup> Moreover, liquorice extracts can be used as flavouring agents in baked foods, alcoholic beverages and chewing gums, with the maximum allowed content in glycyrrhizin being 0.05, 0.1 and 1.1% respectively, whereas for other food products the amount varies accordingly (e.g. 0.15% for non-alcoholic beverages, herbs and seasonings and plant protein products, 16% for hard candies, 3.3% for soft candies, 0.5% for vitamins and mineral dietary supplements, and 0.1% for any other food product except sugar substitutes).<sup>(24)</sup> Among the alternative uses of liquorice biomass, bioenergy production, pulp production and the application of liquorice processing by-products as fertilizers, are also included. Yavari et al.<sup>(27)</sup> have proposed the use of liquorice in processing industry waste as an organic substrate that could minimize the use of fertilizers in strawberry cultivation. Apart from the medicinal and therapeutic properties of liquorice, its use as an oil crop has also been proposed. For example, Durak<sup>(21)</sup> reported its application on bio-oil production, since the most commonly used plant part is only the root system and the upper-part is considered as waste. Plant stalks could be used for bio-oil and high added value chemicals production after pyrolysis.<sup>(21)</sup> According to Aysu and Durak<sup>(28)</sup> the highest yield of bio-oil produced by liquorice stalks was achieved at 500 °C in the presence of borax catalyst. There is also a growing demand to the

upcoming use of liquorice extract as a foaming agent.<sup>(29)</sup> Finally, liquorice fibers can be used for pulp and paper production.<sup>(30)</sup>

### **Chemical composition**

Liquorice presents a long history of secular use; moreover, numerous attributes have been increasingly recognized and confirmed through modern scientific research. Considered as an excellent crude drug and plant model by primitive societies, due to its impressive pharmaceutical and culinary (as a sweetener) properties, studies have been carried out in order to determine the main responsible active principles for those benefits and investigate the modes of action. Among liquorice phytochemical constituents, glycyrrhizin (also known as glycyrrhizic or glycyrrhizinic acid, **Fig. 1**), an oleanane-type triterpene saponin, is the major constituent<sup>(25)</sup> In addition, liquiritin apioside is the most abundant flavonoid compound in liquorice roots<sup>(31)</sup> with significant antioxidant properties.<sup>(32)</sup> However, other constituents, not of lesser importance but also contributors to important biological properties are present, namely phenolic compounds. Compounds such as glabridin, hispaglabridin (A and B), 4'-*O*-methylglabridin, isoprenylchalcone, liquiritigenin, isoliquiritigenin, and formononetin are among the most abundant bioactive phenolic compounds that have been identified so far, with several bioactivity properties.<sup>(33-37)</sup> The chemical structure of the main ingredients of liquorice, including glycyrrhizin and glabridin, are shown in **Fig. 1**.

Moreover, 15 different saponins and 49 phenolic compounds (including their glycosides) have been already identified in liquorice roots.<sup>(38)</sup> So, apart from the previously highlighted major components, there are other compounds that in spite of their low content have also significant properties. Among them, many flavonoids (5,8-Dihydroxy-flavone-7-*O*- $\beta$ -*D*-glucuronide (glychionide A), 5-hydroxy-8- methoxyl-flavone-7-*O*- $\beta$ -*D*-glucuronide (glychionide

B), galbrene, glabrone, glabraisoflavanone A, glabraisoflavanone B, isoviolanthin, 5,7-Dihydroxyflavanone, rhamnoliquiritin)<sup>(39-42)</sup>, as well as saponins (licorice saponin A3, licorice saponin G2, licorice saponin J2, licorice saponin C2)<sup>(39)</sup>, coumarins (i.e. glycycomarin)<sup>(43)</sup> and chalcones (isoliquiritin, neolicuroside, licochalcone B) are included.<sup>39</sup>

Various compounds have also been isolated in relative low amounts, including stilbene derivatives from the liquorice leaves<sup>(44)</sup> and derivatives of caffeic acid esters.<sup>(45)</sup> Other constituents, such as fatty acids, phenols (phenol, guaiacol) and some saturated linear  $\gamma$ -lactones have also been identified<sup>(46)</sup>

Among the most commonly known macronutrients, liquorice roots present a substantial amount of sugars (8.10-9.30%) and ash (4.58-7.40%), with their content showing a great variation depending on several factors, such as growth conditions and harvesting time.<sup>(47,48)</sup> Various raw polysaccharides (RPS) have also been detected and a great variation in their content has been observed. For example, Wittschier et al.<sup>(49)</sup> observed that polymers of RPS derived from the aqueous extract after ethanol precipitation, contained a substantial amount of proteins (19%) and 81% of carbohydrates, mainly arabinose, galactose, glucose and glucuronic acid. However, other carbohydrates such as fucose, rhamnose, mannose, ribose, xylose, sucrose, galacturonic acid have also been identified.<sup>(49)</sup>

Regarding the volatile constituents of *Glycyrrhiza* sp. roots, the aroma profile could be used for chemotaxonomic purposes, since significant differences have been observed in volatiles composition among three species of *Glycyrrhiza* sp. (*G. glabra*, *G. inflata* and *G. echinata*).<sup>(50)</sup> According to this study, thymol and carvacrol content can be used as chemotaxonomic marker to identify *G. glabra* from the other two species.



### **Glycyrrhizin content and extraction**

Glycyrrhizin is one of the most studied chemical constituents derived from the roots of *G. glabra*. According to Fuggersberger-Hein and Franz<sup>(51)</sup>, the highest levels of glycyrrhizinic acid are found in the main roots; lateral roots have significantly lower contents, while the above ground part of the plant does not contain glycyrrhizinic acid and is usually considered as waste. Furthermore, the content of glycyrrhizin in liquorice roots usually ranges from 2% to 25%, depending on the *Glycyrrhiza* species.<sup>(26)</sup> As an example, Račková et al.<sup>(23)</sup> studied various liquorice root extracts and achieved a total glycyrrhizin content of 19% from crude extracts of dried and ground roots and rhizomes with methanol. In addition, the glycyrrhizin content is also highly affected by root depth, with the highest content being detected at the upper 30 cm of soil; by harvesting time, where the highest content is achieved after the second year of establishment; and by climatic conditions, of which low temperatures and drought benefit the glycyrrhizin biosynthesis in *Glycyrrhiza* sp.<sup>(52-54)</sup> Moreover, the fact that the highest glycyrrhizin content is observed in the upper 30 cm of soil, could allow for mechanized harvesting<sup>(52)</sup>, and therefore for further production cost reduction.

Regarding the glycyrrhizin extraction, it has been extensively studied and various effective extraction procedures have been reported so far, including ultrasound, microwave-assisted, supercritical carbon dioxide and multi-stage counter-current extraction methods.<sup>(55-60)</sup> However, the main disadvantage of the several suggested extraction techniques is the use of toxic solvents in the liquid phase during extraction. Solid-liquid extraction procedures, mainly assisted by microwaves (Microwave Assisted Extraction, MAE) result in higher extraction yields, at shorter time periods and also reduce the consumption of organic solvents, comparing with conventional techniques.<sup>(61)</sup> According to Pan et al.<sup>(59)</sup>, 4-5 min of MAE of roots in ethanol-water can result in

higher yields comparing with the conventional extraction for 20-24 h. Other novel procedures implemented for glycyrrhizin recovery, are based on the use of extraction methods assisted by ultrasounds.<sup>(57)</sup> Furthermore, Hedayati and Ghoreishi<sup>(58)</sup> suggested a new method based on the use of supercritical carbon dioxide, in order to minimize the toxic effects of chemical solvents. This supercritical extraction method has better results in terms of extraction efficiency and environmental hazardous effects comparing to conventional techniques; however, further and most detailed studies are needed in order to propose a proper design optimization, prior to the adaptation of innovative techniques by herbal industries.

### **Health effects of *Glycyrrhiza glabra* and its constituents**

Recently, an increasing number of studies, focusing on expanding the knowledge about the main active principles which are responsible for the renowned biological properties of liquorice constituents have been observed. In fact, the majority of the bioactive properties of natural matrices depends on their phytochemical composition and the relative content.<sup>(62,63)</sup> Liquorice is well known for its multiple ethnopharmacological applications, including its uses as anti-inflammatory, antiulcer, antibacterial, antifungal, antiviral, anti-allergic, and immunostimulant.<sup>(35)</sup> Liquorice is also used as a prescription drug for the treatment of various diseases in Japan in various forms [e.g. injectable preparations (Stronger Neo-Minophagen<sup>®</sup> C) or tablets (Glycyron<sup>®</sup>)] while more than 71% of traditional Japanese *Kampo* medicines contain liquorice with an allowed minimum content of 2.5% for glycyrrhizin.<sup>(25)</sup> Liquorice extracts are also ingredients of various drugs in many countries, while glycyrrhetic acid 3- $\beta$ -*O*-hemisuccinate (carbenoxolone), a derivative of glycyrrhetic acid, is being used as a prescription drug.<sup>(25)</sup>

However, not only the content of the individual phytochemicals is important; their biochemical interactions and related synergisms, antagonisms and even polyvalence reactions are also important, since even minor constituents can also contribute to the bioactive properties of natural matrices.<sup>(64)</sup> For example, Singh et al.<sup>(65)</sup> evaluating the effect of polyphenols from olive leaves on their antiplatelet functions, reported that a synergistic effect was observed between various polyphenols. In a similar manner, Uto et al.<sup>(66)</sup> observed that glycyrrhizin has a synergistic effect on the reduction of iNOS expression when coexisting with other constituents in liquorice extracts.

### ***Antioxidant properties***

Liquorice has been shown to have significant antioxidant properties, both through *in vivo*<sup>(67,68)</sup> and *in vitro* studies,<sup>(35,36,69-71)</sup> with the latter being the most commonly assessed. Vaya et al.<sup>(36)</sup> isolated seven compounds that provided anti-oxidant activity against low-dense lipoproteins (LDL) oxidation, with glabridin being the most potent antioxidant compound. The inhibitory effect of glabridin on cholesteryl linoleate hydroperoxide (CLOOH) formation and cholesteryl linoleate (CL) consumption, by using an 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced LDL oxidation system was dose dependent, where a concentration of 40-60  $\mu\text{M}$  of glabridin resulted in 90% inhibition of CLOOH formation, while 20  $\mu\text{M}$  of glabridin was the most efficient dose for the maximum inhibition of CL consumption. Additionally, Martins et al.<sup>(35)</sup> attributed antioxidant potential of hydromethanolic extracts of liquorice roots and rhizomes to apigenin and liquiritin derivatives, a methylated isoflavone and a chalcone, and identified *in vitro* lipid peroxidation inhibition as the main antioxidant effect ( $\text{EC}_{50} = 0.24$  and  $22.74 \text{ mg mL}^{-1}$  for TBARS and  $\beta$ -carotene bleaching inhibition assays, respectively). In another study, antioxidant potential of liquorice extracts from various species referred to six flavonoid

compounds for both lipid peroxidation inhibition and ABTS<sup>+</sup> radical scavenging activity, where especially for lipid peroxidation the effect showed a dose-dependent manner.<sup>(69)</sup> In addition, ethanol and water extracts of both aerial parts and roots of liquorice showed similar antioxidant and radical scavenging activity by inhibiting lipid peroxidation for more than 80% in linoleic acid emulsions, and could be used for enhancing the shelf-life of pharmaceuticals.<sup>(71)</sup>

### ***Antimicrobial properties***

Liquorice extracts have been described to have significant antimicrobial properties (antiseptic, antibiotic, antifungal, antibacterial, antiprotozoal and antiviral).<sup>(72-84)</sup> In particular, Chakotiya et al.<sup>(72)</sup> studied *in vitro* the effect of hydromethanolic extracts of liquorice stems and pure glycyrrhizic acid against membrane permeability, efflux activity, and biofilm formation of *Pseudomonas aeruginosa*, as well as their time-killing efficacy comparing to a standard chemotherapeutic drug, and reported significant inhibition of *Pseudomonas aeruginosa* growth for both the extract and the pure compound, while the pure compound was more effective in growth inhibition of bacteria than the extract in terms of time exposure (4 and 12 h, respectively). Moreover, methanolic extracts of liquorice roots have been reported to show significant *in vitro* antibacterial effects against various bacteria, such as *Agrobacterium tumefaciens*, *Bacillus cereus*, *Bacillus subtilis* and *Pseudomonas syringae* pv. *tomato* by using disc diffusion assays and subsequent determination of the minimal inhibitory concentrations, which were as low as 3.90 µg mL<sup>-1</sup>, whereas water extracts did not show such activity.<sup>(75)</sup> The antiviral activity has been suggested to be attributed to various mechanisms, including reduced virus attachment process and inhibition of infectious virus particles release.<sup>(83)</sup> The effect of aqueous extracts of liquorice roots at concentrations of 0.2 mg mL<sup>-1</sup> and various incubation periods on Herpes Simplex Virus 1 has been also reported *in vitro* studies,<sup>(78)</sup> while glabridin

from root extracts at concentrations of 29.16 mg mL<sup>-1</sup> was identified to have an effect against two *Mycobacterium tuberculosis* strains.<sup>(79)</sup> Ethanolic extracts of liquorice leaves at concentrations of 4 and 8 mg have been reported to be effective against *Candida albicans* and gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*,<sup>(81)</sup> while root extracts in ether, chloroform and acetone were not only effective against gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), but also against gram-negative ones (*Escherichia coli* and *Pseudomonas aeruginosa*).<sup>(84)</sup>

### ***Anti-inflammatory properties***

The anti-inflammatory activities of liquorice extracts or individual constituents have been reported in numerous *in vitro*<sup>(69,85)</sup>, as well as *in vivo* studies.<sup>(86,87)</sup> Five flavonoids isolated from liquorice extracts have shown anti-inflammatory potential by reducing the production of nitric oxide, interleukin-6 and prostaglandin E2 in LPS-induced macrophage cells.<sup>(69)</sup> In another study based on the same model (LPS-induced macrophage cells), liquorice extracts at concentrations of 0.2-0.5 mg mL<sup>-1</sup> were found to improve the secreted cytokine profile by reducing tumor necrosis factor-alpha, interleukin-6 and interleukin-10.<sup>(85)</sup> Similar findings were reported by Chu et al.<sup>(88)</sup> by using Licochalcone A (Lico A), a flavonoid isolated from liquorice, which revealed to exert a potent anti-inflammatory potential in *in vitro* and *in vivo* models induced by lipopolysaccharide (LPS). The authors founded that Lico A administered in mice with LPS-induced acute lung injury (ALI), induced a pronounced reduction of the number of inflammatory cells, myeloperoxidase activity, protein leakage and lung wet-to-dry weight (W/D) ratio, at the same time that enhanced the activity of oxidase dismutase. The same molecule was also able to markedly down-regulate both *in vitro* and *in vivo*, the TNF- $\alpha$ , IL-6, and IL-1 $\beta$  levels, at the same time that attenuated alveolar wall thickening, alveolar hemorrhage, interstitial edema, and

inflammatory cells infiltration in mice with ALI.

There are several studies focused on the discovery of the effective modes of action of liquorice extract and its individual compounds, both through *in vitro* and *in vivo* experiments. However, and despite to the recent advances, it still remains unclear which are the effective modes of action of liquorice to several biological activities. In **Table 1** are shown the most common bioactive properties of liquorice, including their most prominent modes of action.

### ***Other therapeutic properties***

Other therapeutic properties related with liquorice include antiulcer, antitussive, anti-allergic, antitumor and hepatoprotective activities, both through *in vitro* and *in vivo* experiments, and using not only liquorice extracts but even isolated bioactive compounds.<sup>(49,70,89-99)</sup> Glycyrrhizin, 18 $\beta$ -glycyrrhetic acid and liquiritigenin were also found to be effective against asthma and allergic diseases.<sup>(94,96)</sup> Liquorice extracts rich in glabridin ( $\geq 3.5\%$  w/w) and other flavonoid compounds were effective against *Helicobacter pylori*, which is considered the main factor that causes peptic ulcers.<sup>(89)</sup> Glycyrrhizin has been reported to have hepatoprotective properties through its antiviral activity against Hepatitis C virus<sup>(90)</sup> and its protective effect against liver oxidative stress<sup>(95)</sup>. The same compound has been described to have antitumor properties since it can inhibit HMGB1 protein, which induces cell proliferation, angiogenesis, inflammation and cell motility,<sup>(97)</sup> while the dried roots and rhizomes of liquorice have antitussive effects.<sup>(93)</sup>

Furthermore, liquorice extracts and their individual bioactive compounds provide several other health benefits for kidney, cardiovascular, immunological, respiratory, endocrine and central nervous systems which need to be underlined, since so far many physiological effects of liquorice on human body systems have been confirmed.<sup>(66,85, 100-114)</sup> The health benefits of liquorice and its constituents have been suggested to be attributed to various physiological

mechanisms, including modulation of vascular injury and atherogenesis,<sup>(111)</sup> inhibition of tyrosinase activity,<sup>(103)</sup> improvement of the brain energy supply,<sup>(110)</sup> increased glucose tolerance,<sup>(112)</sup> activation of the immune system,<sup>(101)</sup> protection against the oxidative stress of the liver,<sup>(95)</sup> memory enhancing effects<sup>(105)</sup> and improvement of the renal function.<sup>(114)</sup> Other physiological effects of liquorice on human body systems are presented in **Table 2**.

### ***Consumption of liquorice: toxicity and administration limits***

Despite the beneficial effects of liquorice, toxicity symptoms have also been reported from frequent and excessive intake.<sup>(4)</sup> After consumption, glycyrrhizin is metabolized by gut bacteria (*Eubacterium* L-8 and *Streptococcus* LJ-22) to 18 $\beta$ -glycyrrhetic acid, as the main product, and to 18 $\beta$ -glycyrrhetic acid-3-*O*- $\beta$ -*D*-glucuronide, as the by-product.<sup>(115)</sup> Glycyrrhetic acid is highly involved in the metabolism of corticosteroids, since it inhibits 11- $\beta$ -hydroxysteroid dehydrogenase and consequently it inhibits the conversion of cortisol to cortisone.<sup>(116, 117)</sup> Therefore, the inhibition of this enzyme due to a high level of exposure to glycyrrhetic acid leads to hypertension and hypokalemia.<sup>(118)</sup>

Based on *in vivo* studies with rats and mice where the consumption of 15-229 mg/kg/day was suggested as safe and after applying uncertainty factors (10 for intraspecies and interspecies differences accordingly), an acceptable daily intake of 0.015-0.229 mg glycyrrhizin kg<sup>-1</sup> body weight per day has been recommended. This quantity is considerably less than the daily consumption in the USA (0.027-3.6 mg glycyrrhizin kg<sup>-1</sup> body weight per day), estimated based on total production of the various liquorice products and their glycyrrhizin content.<sup>(119)</sup> The continuous high level exposure to glycyrrhizin is able to produce hypermineralocorticoid-like effects; however, these effects are reversible upon withdrawal of liquorice and/or glycyrrhizin.<sup>(119)</sup> Both liquorice and glycyrrhizin have been approved for use in food products by

most national and supranational regulatory agencies; however, apart from the previously recommended dosage, the susceptibility to glycyrrhizin toxicity needs to be considered, since it is directly dependent on the general health status of the individuals.<sup>(119,120)</sup> In fact, and despite its poor absorption from gastrointestinal tract, glycyrrhizin is metabolized by intestinal microbiota to glycyrrhetic and monoglucuronyl glycyrrhetic acids, both of them more readily absorbed. Then, through to phase II metabolic pathway, glycyrrhetic acid is primarily excreted into the bile as glucuronate and sulfate conjugates, which allows for the enterohepatic circulation, and consequently takes several days for complete elimination, whereas when liquorice was administered in rats the amount of free glycyrrhetic acid is 2.5 times lower, without however a clear dose-response relationship between them being established in human studies.<sup>(119,121)</sup> According to Sigurjonsdottir et al.<sup>(120)</sup> the patients with essential hypertension and women are more sensitive to 11- $\beta$ -hydroxysteroid dehydrogenase than the healthy individuals or men.

Regarding the use of glycyrrhizin derivatives as flavoring agents in animal feed, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded that the use of ammoniated glycyrrhizic acid is safe when its content is 1 mg kg<sup>-1</sup> feed for all the animal species apart from chickens and hens where the limit is reduced to 0.3 mg kg<sup>-1</sup> feed.<sup>(122)</sup>

Taking into account the previously described health promoting effects, largely stated by ancient citizens and increasingly confirmed by ethnopharmacological data, an exponential number of studies has been carried out towards to properly understand and to clarify the real efficacy of this secular medicinal plant. It is important to point out the fact that these studies are mainly focused on both *in vitro* and *in vivo* models, mostly by using several animal models, as already has been stated. More recently, an increasing number of clinical trials have also been carried out, using patients with multiple health affections, and the application of liquorice



extracts has been revealed to be an upcoming strategy for the treatment of many diseases. However, more than to confirm most of the already known empirical findings, it is crucial to deepen knowledge on the determination of their real modes of action and related pharmacokinetic parameters, as well as to establish safer dose intakes both for prophylactic and therapeutic purposes, in order to doubtless state liquorice as a promissory multiple-health promoting natural product.

### **Cultivation practices**

Liquorice can adapt to a wide range of climates. In its wild form it can be found in regions with an annual rainfall between 400 and 1160 mm, mean air temperature ranging from 5-25 °C and soil pH of 5.7-8.2.<sup>(123)</sup> Root development is enhanced at temperatures of about 15 °C<sup>(124)</sup>, while frosts can severely affect plant establishment during the first year.<sup>(52)</sup> Low temperatures have also an effect on quality, since the roots and rhizomes grown under such conditions have a less tender texture and therefore contain less quantity of juice.<sup>(11)</sup>

The best growth of liquorice occurs in sandy soils, and clay soils should be avoided since they hinder plant establishment and cultivation practices.<sup>(11,17)</sup> Plants prefer deep and well-drained soils,<sup>(26)</sup> but according to Dagar et al.<sup>(125)</sup> and Kushiev et al.<sup>(126)</sup> liquorice may be also cultivated in sodic soils as a means of phytoremediation and soil properties improvement, since most of the conventional crops are sensitive under such conditions. The deep root system allows for more efficient use of water; therefore, liquorice plants could be considered as tolerant in water stress conditions and ideal for dry climates and semi-arid areas with relative lower abundance of irrigation water.

Plant is propagated either asexually by using vegetative cuttings (stolons), or sexually with the use of seeds.<sup>(125,127)</sup> For asexual propagation, the optimal size of cuttings is 15-20 cm in

length and 1.5-2.0 cm in diameter.<sup>(124)</sup> On the other hand, for sexual propagation, seed treatment application must be considered prior to seeding, since seed germination percentage is low in *Glycyrrhiza* species, mostly because of the physical dormancy induced by their hard coat.<sup>(128,129)</sup> Various scarification treatments and temperatures have been studied in order to increase germination rate and render sexual propagation applicable for commercial cultivation, since the occurrence of hard seeds is very frequent.<sup>(129)</sup> According to Ghadiri and Torshiz<sup>(130)</sup>, the chemical scarification, by applying sulfuric acid (70%) for 45 min, increases the germination percentage at 25-35 °C, whereas mechanical scarification with a hand rotary scarifier leads to an improvement of germination rates at a broader temperature range (15-35 °C). Moreover, acid scarification with sulfuric acid (98%) for 1 h and incubation at 25 and 10 °C and 12 h/12 h light/dark, respectively, resulted in an increase of germination percentage by up to 95%.<sup>(129)</sup> Seed germination is also affected by salinity, with differences in response being observed among the various species of liquorice, and salinity-inducing ions.<sup>(131)</sup> Moreover, according to Chun-lei<sup>(132)</sup>, seed germination and seedling growth of *G. uralensis* is significantly affected by pH, where the best results were achieved for values of pH=6.

Plant density is essential for total yield, with low densities encouraging rhizome formation and high densities being beneficial for root production.<sup>(52)</sup> Row spacing is usually at 30-80 cm, with 20-50 cm distance within the row and the recommended planting depth is 10-15 cm.<sup>(52,125)</sup> For maximum rhizome and root production, plant density must be at least 24000 plants ha<sup>-1</sup>.<sup>(52)</sup> For planting of cuttings, a similar equipment to the potato planting equipment should be used. In southern Europe and the rest of the world, planting takes place during spring; for example, in India planting is carried out during March.<sup>(125)</sup> *Glycyrrhiza* sp. can also be transplanted; the sowing takes place during spring and young seedlings are transplanted in the field during the

next spring.<sup>(133)</sup> However, in either case (transplanted or directly seeded), a lower plant development is observed when sexual propagation is compared with vegetative propagation, where cuttings are used.

Liquorice plants are not very demanding regarding their water requirements, despite the fact that they respond well to irrigation. Mohammad and Rehman<sup>(134)</sup> reported that irrigation increases the length and thickness of root and rhizomes. Nutrient requirements of liquorice crop are also very low. In India, 40 kg ha<sup>-1</sup> N and 40 kg ha<sup>-1</sup> P are usually applied in liquorice crops.<sup>(124)</sup> Phosphorus and the half of the nitrogen are applied before planting with base dressing. In addition, plant symbiosis with mycorrhizas improves nutrient uptake and plant nutrient status. According to Akhzari<sup>(135)</sup>, the inoculation of liquorice plants with arbuscular mycorrhizal fungi resulted in higher leaf area, shoots length and dry weight comparing with non-inoculated plants, whereas significant differences were also observed in essential oil and protein content. Liu et al.<sup>(136)</sup> studied the effect of colonization of various arbuscular mycorrhizal fungi of the *Glomus* genera and observed a significant increase in leaf and root biomass, as well as in phosphorus and various secondary metabolites content. In addition, Orujei et al.<sup>(137)</sup> concluded that a pronounced improvement on the accumulation of secondary metabolites (glycyrrhizin and total phenolics) in *G. glabra* roots occurs after the inoculation with *Glomus mosseae* and *G. intraradices* mycorrhizal fungi, where inoculation of 6 months old plantlets with *G. mossae* and *G. intraradices* resulted in a 9-fold and 3-fold increase in glycyrrhizin content, and a 9-fold and 6-fold increase in total phenolics content, respectively.

Intercropping or mixed-cropping during the first years of establishment, despite seeming promising for increasing farmers income, should be avoided, since liquorice plants are highly suppressed and root growth is severely affected with a consequent effect on total yield within the

subsequent years.<sup>(138)</sup>

Weed infestation reduces liquorice yield. Once the crop is established, the field should be kept weed-free by hand-hoeing or mechanical cultivation, since no herbicides are registered for liquorice crop use yet. According to Hartley<sup>(139)</sup>, the pre-emergence herbicides trifluralin, chlorpropham plus diuron, cyanazine and metribuzin can be used. Moreover, post-emergence (at the stage where liquorice plants height is up to 10 cm) application of metribuzin and/or bentazone may be also used for weed control, until the foliage develops full canopy, and liquorice plants are able to suppress the weeds on their own. However, late application of post-emergence herbicides should be avoided since severe scorching has been observed.<sup>(140)</sup> Additionally, farmers should also avoid fields severely infested with perennial weeds (i.e. bermudagrass (*Cynodon dactylon* (L.) Pers), purple nutsedge (*Cyperus rotundus* L.), field bindweed (*Convolvulus arvensis* L.) and johnsongrass (*Sorghum halepense* (L.) Pers.), since in this case, weed control is labor intensive and increases production cost.

The basic objectives of *G. glabra* breeding should focus on quality improvement and more specifically on the content in active ingredients, such as glycyrrhizin and liquiritin, which determine the marketable quality of the final product. According to Liu and Lin,<sup>(140)</sup> these two compounds are the most representative secondary metabolites of liquorice, with their relative content being significantly different between the various species of the *Glycyrrhiza* genus. Considering that cultivated plants of liquorice contain less amounts of glycyrrhizin than wild ones, the selection of wild strains with high content in this compound is of major importance.<sup>(141)</sup> The thorough screening of the genetic diversity within the species and its wild relatives, regarding various agronomic traits and the chemical composition, and more specifically the glycyrrhizin and liquiritin content, is essential for further breeding improvement and chemical

fingerprinting of the various species, whereas the use of inter-simple sequent repeat primers could be a useful means for that purpose.<sup>(140)</sup> The wild populations derived from different geographical areas could be used to improve the liquorice crop, since the concentration of glycyrrhizin in roots differs significantly among the various *G. glabra* ecotypes collected from different geographical areas.<sup>(142)</sup> Moreover, Montoro et al.<sup>(143)</sup> have also reported that the contents of glycyrrhizin acid in *G. glabra* roots varied between different geographical areas, with Chinese *G. glabra* roots containing the highest levels (53.9 mg of glycyrrhizin acid g<sup>-1</sup> of dried plant material) comparing to the other tested ecotypes. Kojoma et al.<sup>(141)</sup> studied various strains of *G. uralensis* plants propagated from seeds and detected a great variation in both glycyrrhizin and liquiritin content, whereas they also observed a positive correlation between the content of these compounds. Moreover, liquorice genotypes may also vary on their agronomic vigour, which can be a useful trait for high yield and biomass production.<sup>(144)</sup>

The liquorice roots growth and glycyrrhizin biosynthesis can also be manipulated by mediated transformation with *Agrobacterium rhizogenes*. More specifically, the *G. glabra* plant infection with *Agrobacterium rhizogenes* resulted in a direct induction of hairy roots and subsequent integration of the TDNA fragment from *Agrobacterium* Ri plasmid into *G. glabra* genome.<sup>(145)</sup> The application of controlled drought stress could be also implemented as a means to up-regulate the expression of genes which have a key role in the biosynthetic pathways of liquorice secondary metabolites, such as glycyrrhizin.<sup>(53)</sup>

The harvesting of roots occurs during the autumn and after the foliage senescence and defoliation. 3-4 years after plant establishment, the roots achieve the required marketable size,<sup>(11,52,124)</sup> and harvesting can take place. The harvesting of liquorice roots can be done with a single potato or carrot digger, whereas there is also the traditional way where harvesting is done

by hand.<sup>(123)</sup> Regarding the effect of plant spacing on yield, several authors have confirmed its importance in achieving high total yield. Douglas et al.<sup>(52)</sup> reported that a plant density of 25800 plants ha<sup>-1</sup> resulted in a total yield of 11400 kg ha<sup>-1</sup>, with similar quantities of roots and rhizomes (5460 and 5940 kg ha<sup>-1</sup>, respectively), whereas although a further increase in plant density improved the total yield (15420 kg ha<sup>-1</sup>) it did not significantly increase the yield of rhizomes (6010 kg ha<sup>-1</sup>). Moreover, Marzi et al.<sup>(124)</sup> reported that the highest yield (20.4 t ha<sup>-1</sup>) occurs at a plant density of 42000 plants ha<sup>-1</sup>, whereas despite the determinant effect of plant density on the total yield of roots and rhizomes, the total glycyrrhizin content and its ratio between roots and rhizomes was not significantly affected.<sup>(52)</sup>

Liquorice is little susceptible to pests (such as, insects) and diseases with scarce reports for crop infestation so far. For example, in Italy, Casulli and Ippolito<sup>(146)</sup> identified liquorice rust [*Uromyces glycyrrhizae* (Rab.) Magn.] as a pathogen of *G. glabra*. The infected plants presented pustules on their leaves, stem elongation, early resetting, browning of pith and the formation of many buds in the crown region.<sup>(146)</sup> Foliar sprays with fungicides (i.e. kresoxim-methyl) have been very effective against liquorice rust.<sup>(147)</sup>

Furthermore, liquorice is also susceptible to aphids. In Iran, aphids (*Aphis craccivora* Koch) have been identified as liquorice pests.<sup>(148)</sup> In Italy, Casulli and Ippolito<sup>(146)</sup> have also observed aphid infections in liquorice plants. Nowadays, there are no registered insecticides to use on liquorice crop and only aphid's parasitoids may be used as biological control agents. According to Rakhshani et al.<sup>(148)</sup> *Aphis craccivora* is parasitized by *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Braconidae: Aphidiinae).

## Conclusions

Overall, liquorice is an important medicinal plant, with a widespread use, both in traditional and

contemporary medicinal industries. However, most of the final products (root and rhizomes extracts) derive from wild plants, a common practice that could threaten sustainability and induce genetic erosion in the species. Therefore, cultivation and breeding of liquorice for further exploitation and commercialisation, and the subsequent compilation of a best practices guide, which among other purposes should aim to ensure high yields, and final products with high and uniform quality is of major importance. Finally, further research is needed regarding glycyrrhizin extraction in order to develop an efficient and environmental technique for application in the herbal industry.

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**Table 1.** Bioactive effects of *Glycyrrhiza glabra* L. and its constituents, including their most prominent modes of action.

Bioactive effect	Physiological action	References
Antibacterial	- Suppression of the adhesion of <i>Helicobacter pylori</i> to human gastric mucosa	(49)
Anticariogenic	-Antibacterial activity against <i>Streptococcus mutans</i> causing dental caries	(80)
Antifungal	- Reduction/inhibition of the yeast ( <i>Candida albicans</i> ) growth -Inhibition of the biofilms formation/growth	(76,82)
Anti-inflammatory	-Inhibition of nitric oxide (NO), and prostaglandin E2 (PGE2) production -Inhibition of inflammatory cytokines production (i.e. tumor necrosis factor- $\alpha$ (TNF- $\alpha$ ), interleukin 1 $\beta$ (IL-1 $\beta$ ), interleukin-6 (IL-6)). -Inhibition of reactive oxygen species (ROS) production -Reduction of inflammatory proteins production (i.e. cyclooxygenase-2 (COX-2))	(69,86,87)
Antioxidant	- Inhibition of reactive oxygen species generation - Inhibition of mitochondrial lipid peroxidation - Reduction of the NADPH oxidase activation and increase of cellular glutathione	(35,69,70,87)
Antiprotozoal	- Alteration of the ultrastructure of the parasite mitochondria -Inhibition of <i>Pf</i> LDH (Parasite lactate dehydrogenase) enzyme acting at NADH site. -Induction of oxidative stress in parasites through ROS (Reactive Oxygen Species)/RNS (Reactive Nitrogen Species) generation.	(73,74)

Antitumor	<ul style="list-style-type: none"> <li>- Protection against oxidative damage induced by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)</li> <li>-Inhibition of tumor cells proliferation and migration</li> <li>- Inhibition of cyclooxygenase (COX)-2 overexpression</li> <li>- Suppression of the activity of phosphoinositide 3-kinase (PI3K)</li> <li>-Inhibition of tumor cell growth and migration via blocking AKT (serine-threonine protein kinase)/mTOR(mammalian target of rapamycin)/STAT3 (signal transduction and activator of transcription) pathway</li> </ul>	(91,92,97,98,108)
Antiulcer	<ul style="list-style-type: none"> <li>- Inhibition of adhesion of <i>Helicobacter pylori</i> to human gastric mucosa</li> </ul>	(49)
Antiviral	<ul style="list-style-type: none"> <li>- Inhibition of virus attachment process and replication</li> <li>- Inhibition of infectious virus particles release</li> </ul>	(78,83)

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**Table 2.** Physiological effects of *Glycyrrhiza glabra* L. and its constituents on each human body system.

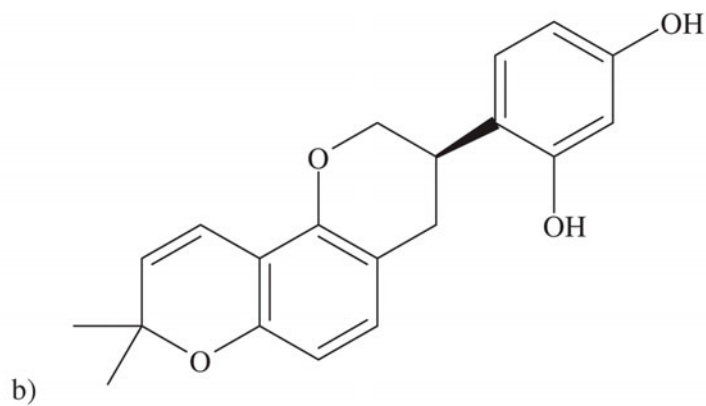
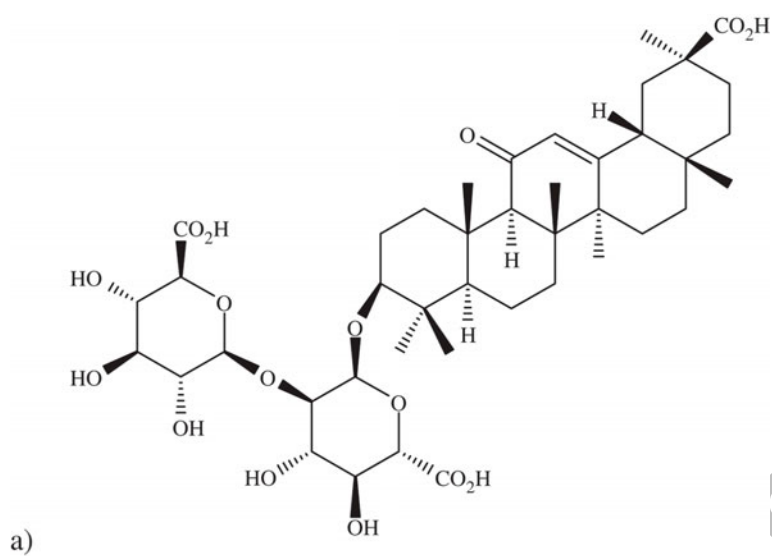
Body system	Physiological effect	References
Cardiovascular	<ul style="list-style-type: none"> <li>- Reduction in the atherosclerotic lesions.</li> <li>-Inhibition of LDL(low-density lipoprotein) oxidation</li> <li>-Action as thrombin inhibitor</li> <li>- Decrease in tube formation in vascular endothelial cells</li> <li>- Modulation of vascular injury and atherogenesis</li> <li>-Protection against the development of arrhythmia</li> </ul>	(67,104,111,113)
Cerebral/ Nervous	<ul style="list-style-type: none"> <li>- Inhibition of serotonin re-uptake</li> <li>- Memory enhancing effects</li> <li>- Increase of succinate dehydrogenase (SDH) activity in several parts of the brain</li> <li>- Improvement of brain energy supply</li> <li>- Amelioration of the effect of vibration</li> </ul>	(100,105,109,110)
Endocrine	<ul style="list-style-type: none"> <li>- Reduction of the glucose levels</li> <li>-Increase in antioxidants enzyme activity in pancreas</li> <li>- Action as natural estrogen agonists</li> <li>-Inhibition of tyrosinase activity</li> </ul>	(103,111,112)
Immune	<ul style="list-style-type: none"> <li>-Action against immune function dysfunction</li> <li>- Improvement of antioxidant defense system.</li> <li>-Activation of immune cells-Stimulation of the expression of CD69 glycoprotein on granulocytes and NK (Natural killer) cells</li> <li>-Enhancing of dendritic cells maturation</li> <li>- Regulation of iNOS expression</li> </ul>	(85,101,102,107)

Hepatic	- Protection against oxidative stress of the liver (70,95,99) -Reduction of liver enzyme levels -Changes in antioxidant enzymes (SOD: super oxide dismutase and CAT: catalase) activities in the liver -Inhibition of hepatic lipid peroxidation -Protection of liver mitochondria against oxidative stress
Renal	- Anti-nephritis effects (106,114) - Ameliorates renal defects
Respiratory	- Antitussive effects (93)

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**Figure 1.** Structures of the main active constituents of liquorice (*Glycyrrhiza glabra*): a) glycyrrhizin ( $C_{44}H_{62}O_{16}$ ) and b) glabridin ( $C_{20}H_{20}O_4$ ).



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