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A Review on Comparison of the Extraction Methods Used in Licorice Root: Their Principle, Strength and Limitation

Domnic Maxwell Colvin*

Department of Life Sciences, University of Mumbai - Kalina Campus, Maharashtra, India

***Corresponding author:** Domnic Maxwell Colvin, Department of Life Sciences, University of Mumbai - Kalina Campus, Maharashtra, India, Tel: +918082406864; E-mail: domnicolvin@gmail.com

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Abstract

Medicinal plants are gaining much more interest and importance because of their use in treating many common diseases like cough, cold, fever and other ailments. One such natural herb scientifically called as "*Glycyrrhiza glabra*" and commonly known as "Licorice" is usually recommended for various health disorders. According to the literature, the main part of interest in the plant lies in its rhizomes (roots) which contain the active constituent Glycyrrhizic acid (Glycyrrhizin) a triterpenoid compound, that have been used for thousands of years as a superior expectorant forming a wonderfully effective ingredient in cough medicines and cough drops. Any kind of study on medicinal plants starts with their extraction procedures which plays a critical role to the extraction outcomes (yield and phytochemicals content) and also to the consequent assays performed. A wide range of technologies with different methods of extraction are available nowadays. Hence, this review aims to describe and compare the most commonly used extraction methods for Glycyrrhizin from Licorice roots, based on their principle, strength and limitation to help evaluating the suitability and economic feasibility of the methods. In addition, a novel post-extraction method was formulated to avoid polymerization and contamination of the extracted glycyrrhizic acid which can be combined with the extraction methods to increase the therapeutic efficacy of the licorice root powder.

Keywords: Medicinal plants; Glycyrrhizic acid; Glycyrrhizin; Triterpenoid; Extraction procedures

Introduction

In today's world, a very common and serious problem is cough. Having a cough is one of the most common reasons for seeing doctor. A cough is a sudden, often repetitive spasmodic contraction of the thoracic cavity resulting in a violent release of air from the lungs, and usually accompanied by a distinctive sound. It is normally initiated to clear a buildup of phlegm in trachea. But it often presents as the first and most persistent symptom of many respiratory diseases. Chronic cough of various etiologies is a regular presentation to specialist respiratory clinics and is reported as a troublesome symptom by a significant proportion of the population [1]. Despite this, the treatment options for cough are limited. As climate changes frequently, people are prone to cough which lasts for at least 4-5 days or sometimes even a week causing high fever.

"*Glycyrrhiza glabra*" also known as Licorice and sweet-wood in English, belongs to the family Leguminosae, is a genus of perennial herbs and under shrubs distributed in the subtropical and warm temperate regions of the world. A high number (More than 400) of chemical compounds have been isolated from Glycyrrhiza species, the compound is composed of triterpene saponins, and flavonoids (liquiritoside, isoliquiritoside) are believed to be responsible for the bioactivities of licorice. It is cultivated mostly for its rhizomes (underground stems) that contain the compound glycyrrhizin, which is a triterpenoid saponin and is 50 times more sweetener than sugar. This glycyrrhizin has intensive use in foods, tobacco products and in traditional use and herbal medicine. In the traditional system of medicine, the roots and rhizomes of licorice was an anti-inflammatory agent in the treatment of allergic reactions, antimicrobial, antiulcer, expectorant and anxiolytic activities. It has also been known to relieve rheumatism, osteoarthritis and arthritis, regulate low blood sugar, and was used for Addison's disease. A medicinal use of licorice includes cough suppression, treatment of early Addison disease, treatment of liver disease and dyspepsia also in the prophylaxis and treatment of gastric and duodenal ulcers [2].

Most cough medicines are prepared to treat either dry coughs or "wet" coughs, usually known as productive coughs. Licorice root with glycyrrhizin treats the latter, working as an expectorant to loosen and thin mucus, thus making the productive cough more. The tea form of licorice root is recommended for coughs, but syrup is also available. Its sweetness property has resulted in its use around the world in cough syrups and lozenges as well as candies [2]. Therefore, it has been widely used in folk medicine and is today a highly commercially important target species.

The study of any medicinal plants starts with the pre-extraction and the extraction procedures, which is an important step in the processing of the bioactive constituents from plant materials. Traditional methods such as maceration and Soxhlet extraction are commonly used at the small research setting but significant advances have been made in modern extraction methods which are microwave and ultrasound assisted. With such variety of methods present, selection of proper extraction method needs meticulous evaluation [3]. This review describes the principle, strength and limitations of the commonly used methods in recent years to help in the selection of proper methods for easy, feasible and fast extraction of glycyrrhizin from licorice roots.

Pre-extraction preparation of plant samples

The initial stage in studying any plant extraction method is preparation of plant samples to preserve their biomolecules in their natural condition prior to their extraction. In case of licorice, the roots can be extracted from the fresh or dried plant material. Other pre-preparation of plant materials such as grinding and drying also influences the preservation of phytochemicals in the final extracts.

Fresh vs dried samples

Sometimes few drying procedures are carried out to dry the plant extract isolated from the plant samples. The heat processing can have detrimental effects on natural antioxidants in raw plant materials. Intense and/or prolonged thermal treatment can cause significant loss as a result of the heat instability of compounds in the extract. In contrast to high temperature, Sun-drying and low temperature drying like 40°C oven-drying can increase total polyphenol content and phytochemical activity [4]. Fresh samples are fragile and tend to deteriorate faster than dried samples but still a comparison between fresh and dried *Moringa oliefera* leaves showed no significant effect in total phenolics but with higher flavonoids content in dried sample [5]. In case of licorice, both the fresh and the dried (40°C oven-drying) root powder can be used as the sweetness (glycyrrhizin content) in it remains intact even after drying.

Grinded vs powdered

By lowering the size of the particles, the chances of surface contact of the plant sample with the extraction solvent can be increased. The more surface area is exposed to the solvent, better is the extraction of the target analytes. Conventional mortar and pestle or electric blenders and mills can be commonly used to reduce particle size of sample [6].

In the case of Licorice roots, both the fresh intact roots as well as the dried powdered form can be used for extraction of glycyrrhizin. The composition of this constituent might vary in the dried powder, but its therapeutic application remains unchanged Experimental.

Extraction Methods

Extraction is the separation of the medicinally active component from its parent source using selective solvents through suitable standard procedures. 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. A new technique involving ultra-sound was employed and the product yield was compared with other existing procedures.

Analytical method

Analytical method mainly comprises of extraction of the principle component glycyrrhizin from the licorice roots using the combination of all the three individual analytical extraction methods, namely, acid precipitation, alcohol and ammonia extraction [7]. This method starts with heating of the shredded roots of licorice for 4 to 6 hours in ten times its volume of distilled water at 60°C at a neutral pH. The suspension is centrifuged, and the supernatant is evaporated in vacuum to about 75% of its volume. Crude glycyrrhizic acid is then precipitated by addition of 10% concentrated sulphuric acid (H_2SO_4) at constant stirring. This crude powder is taken up in fresh distilled water and stirred for 4 to 6 hours to bring the pH at 4 by using NH_4OH/ sodium carbonate/potassium carbonate. Stirring is continued followed by centrifugation till a clear supernatant is obtained. Similar washing steps are continued with fresh distilled water to neutralize the pH of the precipitate. Finally, the crude is completely dried and extracted in absolute ethanol which is further evaporated on water bath and dried in vacuum oven to obtain active glycyrrhizic acid.

The recovery of active glycyrrhizic acid by analytical method is 7-8% only [7].

Strength: This method was employed to extract glycyrrhizic acid in a simpler way combining the individual analytical methods using acid, ammonia and alcohol. It was observed that after addition of sulphuric acid, the solid particles of crude glycyrrhizic acid started appearing in the form of precipitate. Hence the extract obtained by this method is very easy to perform and of the utmost pure form.

Limitations: Though analytical method provides purest form of glycyrrhizin, but it is time consuming. The frequent washing steps and incubation period makes it cumbersome and tedious. In addition to the time factor, organic wastes come into major issue as a large number of organic solvents are used in this method for which a proper management of the waste is needed. Also, solvents used in this extraction method must be of very high-grade purity which adds to the cost. Altogether, even if time, cost and solvent purity are not the constrains, the amount of yield obtained is very less.

Studies: As reported in the "Handbook of medicinal plant" and by Mukopadhyay et al. the percentage yield of gycyrrhizin by analytical method is 7 to 8%. But as per our practical studies, we obtained only 0.3% (0.03 gm) yield after using 10 gm and only 0.9% (0.225 gm) after using 25 gm of shredded licorice roots, respectively. This shows that the various disadvantages that occurs during practical handling in this method further reduces the yield.

Microwave-assisted extraction

In this method, microwave energy is used to facilitate the breaking of analytes from the sample matrix into the extraction solvent. Microwave radiation interacts with the dipoles of the polar and polarizable materials in the solvent and the sample causing heating. This heat is transferred through conduction promoting solvent penetration into the sample thereby enhancing migration of the active constituents into the extraction solvent [8].

Strength: This technique reduces extraction time and solvent volume as compared to other conventional methods like Maceration, analytical, sonication. Improved recoveries of the active constituent from the sample can be achieved with constant reproducibility.

Limitations: However, this method is limited to small molecule phenolic compounds such as phenolic acids (gallic acid and ellagic acid), quercetin, isoflavin and trans-resveratrol as they are stable under microwave heating conditions up to 100°C for 20 minutes. Excessive exposure of microwave radiations results in drastic decrease in the yield of phenolics and flavanones, mainly caused due to oxidation of compounds. Also, tannins and anthocyanins may not be suitable for this extraction method as they are degraded at high temperatures [9].

Due to these strong sets of limitations, microwave assisted method must be avoided for glycyrrhizin extraction.

Ultrasound assisted extraction or Sonication method

This method involves use of ultrasound using a sonicator probe ranging from 20 kHz to 2000 kHz [10]. The vibrational and acoustic

effect from the ultrasound increases the surface contact between the solvent and samples thereby increasing the permeability of the plant cell walls. Thus, the physical and chemical properties of the plant cell wall is disrupted and altered which results in the mass transportation of the solvent into the plant cell. It facilitates release of the active constituent in the desired solvent of extraction [11].

Strength: This procedure is relatively simple and low-cost technology that can be used in both smaller and larger scale of phytochemical extraction. The main benefit of this is mainly due to reduction of time for extraction as well as in solvent consumption.

Limitations: There lies no such vast set of limitations in using this method for glycyrrhizin extraction. Only when an ultrasound of more than 20 kHz is used, it may have some effects on some of the active phytochemicals through the formation of free radicals.

Studies: Due to the various benefits of this method, it was employed on Licorice root extract dissloved in extraction solvent containing ethanol and water (30:70 v/v) in two separate batches, (10 gm and 30 gm). Both the mixtures were then sonicated for about 1 min. and the solvent thus obtained contained glycyrrhizic acid which was monitored by TLC. using the extract of analytical method as a standard. It was observed that the yield of glycyrrhizic acid in both the batches was around 48 to 49%.

Dipping (Maceration) method

Maceration or dipping method involves soaking plant materials (coarse or powdered) in a stoppered container with a desired solvent and allowed to stand in the room temperature for a period of time. This process intends to break the cell wall of plant cells to release the desired phytochemicals in the extraction solvent being used. The choice of solvent used will determine the type of compound extracted from the samples, basically playing the most critical role. In case of licorice roots, the solvent used was ethanol and water (30:70 v/v) for 10 gm of licorice root extract. The root extract was dipped in this extraction solvent for about 60 mins. so that the glycyrrhizic acid gets dissolved in the solvent [10].

Strength: Though being the most traditional and conventional method of extraction, this technique is the easiest and simple. Alteration in the temperature and the choice of extraction solvents can enhance the extraction process. Thus, it also proves to be a flexible method as per our convenience.

Limitations: Being the simplest form of extraction method, there persists no major limitations in this method. But the limitation lies in the choice of the use of organic extraction solvent. The more complex the extraction solvent is, the more purification techniques needs to be employed to obtain the pure form of the active constituent.

Studies: Under the optimum extraction condition, 2.39 mg/g of glycyrrhizic acid was extracted from 10 gm of Chinese licorice root extract using ethanol and water (30:70 v/v). Thus, the maximum recovery was found to be 89.7%.

Discussion

After comparing all the suitable extraction studies for glycyrrhizin from licorice roots and keeping their strengths and limitations into consideration, the sonication and dipping methods were found to be the most conducive, productive and feasible technique. In order to confirm our analysis, practical studies were personally carried out for Analytical, Dipping (Maceration) and Sonication extraction studies. It resulted in proving that the reported authenticity of the glycyrrhizin yield by analytical method is not 7 to 8% but hardly close to 1% considering the practical disadvantages during handling.

With respect to practical handling, it was also observed that during the drying process of the crude glycyrrhizic acid in the analytical method, if proper care is not taken then the precipitate starts polymerizing. Also, it was found that if the extracted glycyrrhizic acid (by any method) is left untreated for a long period of time, it starts polymerizing if it is in solution or gets contaminated by fungal growth if it is in dry state.

In order to avoid such unwanted occurring, a novel approach was employed to safeguard the extracted glycyrrhizin which can be added in its post-extraction method.

Since, glycyrrhizin has been used as a cough expectorant, decoction method is the best suited for its post-extraction method. After employing any of the best suited extraction methods of Sonication or maceration, the resultant solvent containing the active glycyrrhizin was heated on a water bath to reduce its volume to 1/4th and then it was overcoated with 1 gm of fresh original licorice root powder and mixed well and packed.

Such over-coating of the extracted glycyrrhizin on natural edible licorice root powder, enhances its activity which can be boiled in a definite volume of water for a definite period of time for a warm cough decoction.

Conclusion

The different methods of extraction of glycyrrhizin from licorice roots were compared and the most suitable, productive and feasible one was studied practically. It can be concluded from the study that the conventional maceration (dipping) method and the sonication method are the most ideal for fast and efficient extraction of glycyrrhizin with minimum limitations. The analytical method must be avoided due to its low yield and major limitations. Also, a novel approach was found in order to prevent polymerization and contamination of the extracted solvent. This post-extraction method of over-coating the extract on licorice root powder increases the amount of the active cough preventing constituent (glycyrrhizin) and can serve as a direct expectorant or medium for cough decoction.

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