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The Source and Extraction of Isoliquiritin

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Abstract:

Licorice, as a traditional herb that belongs to Leguminosae Glycyrrhiza genus, has been used in traditional Chinese medicine for thousands of years. Currently, it was also listed in the *Pharmacopoeia of the People's Republic of China*. Isoliquiritin, as a flavonoid compound, is widely present in the dry roots and the rhizomes of plants that belongs Glycyrrhiza genus (common name as licorice), which includes *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat., and *Glycyrrhiza glabra* L.. It has attracted widespread attention for its various biological activities. Isoliquiritin is one of the active ingredients, demonstrating various potential medicinal values such as antioxidant, anti-inflammatory, antibacterial, and neuroprotective properties. Although isoliquiritin has shown great potential in the field of medicine, its extraction and utilization still face some challenges. At present, the extraction of isoliquiritin mainly relies on plant sources, and the sustainable utilization of plant resources and optimization of extraction methods directly affect its production efficiency and cost. In addition, the biosynthetic pathway of isoliquiritin has not been fully elucidated, which limits the possibility of large-scale production through biotechnological means. This article aims to systematically review the sources and extraction methods of isoliquiritin, explore its potential applications in modern medicine, and analyze the shortcomings and future research directions in current research. This will provide important theoretical basis and practical guidance for the further development and utilization of isoliquiritin.

Keywords: Isoliquiritin extraction, Flavonoid compounds, Biosynthesis techniques

1. Introduction:

Natural medicines (also named crude drug) occupy an important position in medicine. For thousands of years, human ancestors have accumulated a lot of experience in the use of natural medicines. Natural medicines can be divided into animal medicine, phytomedicine, and mineral medicine base on their source. Natural medicines are widely used in traditional medicine, such as Chinese medicine, Ayurve-

da, Native American medicine, Laos traditional medicine, and so on. Phytomedicine accounted for the majority (about 87%) of all source of natural medicine. Among the 616 medicinal herbs recorded in the 2020 edition of the *Chinese Pharmacopoeia*, there are 543 botanical medicines, which involved 624 plants (Huang et al., 2022).

Glycyrrhizae Radix et Rhizoma, also known as licorice or Gan Cao, is widely used in many traditional medicines. It mainly used in the prescript for reconcile medicinal properties, clear heat, detoxify, and dispel phlegm and cough. It belongs to Fabaceae family and the Glycyrrhiza genus which include about 30 species, and more than half of them have potential medical benefits, including *Glycyrrhiza uralensis* Fisch., *Glycyrrhiza inflata* Bat., *Glycyrrhiza glabra* L., and so on (Sharifi-Rad et al., 2021). Traditionally, the dry roots and the rhizomes of these plants are used as medicine (Ye et al., 2022). Many prescriptions included licorice as part of it. In Taiwan, this plant was used in about one third of traditional Chinese medicine prescription (Chien et al., 2013). Licorice, as kind of desert and semi-desert plant, can grow in arid and barren soils of the middle and high latitudes, and the planting environment has a great influence on its properties (Yan et al., 2022). Glycyrrhizae genus was found in most arid areas of Europe, Central Asia, North and South America, Australia, and Africa (Alsaadi et al., 2020; Sharifi-Rad et al., 2021; Gupta and Sarwat., 2022). By the evaluation of Food and Drug Administration (FDA) , Glycyrrhizae was considered as a safe additive in food.

Isoliquiritin is one of the active chalcone type flavonoid glycoside that extracted from licorice (figure 1). It achieves various pharmacological effects in the clinical applications. It mainly which includes anti-tumour (Shi et al., 2014), anti-inflammation (Miao et al., 2024), antioxidation (Liu et al., 2019), and inhibition of angiogenesis (Kobayashi et al. 1995). Although the mechanism is not clear, isoliquiritin also shows anti-fungus (Luo et al., 2016) and antidepressent (Wang et al., 2008) effect.

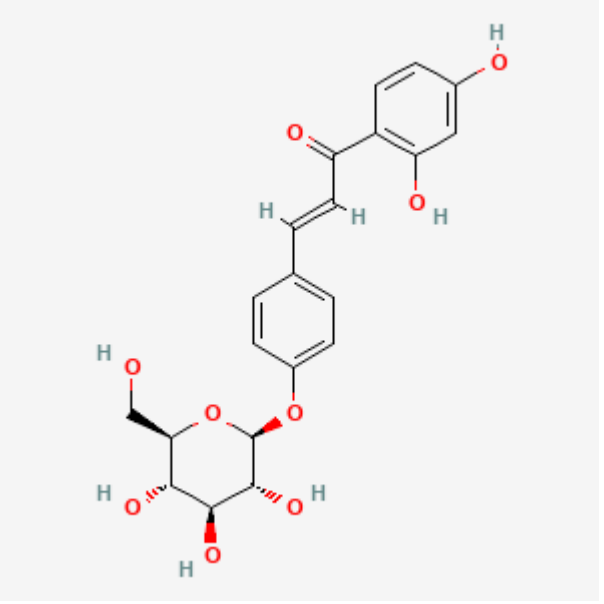

Figure 1: Structure of isoliquiritin (National Center for Biotechnology Information, 2024).

This article aims to systematically review the chemical properties, origin, and extraction of isoliquiritin, and identify its potential applications and development prospects in modern medicine. By analyzing existing research results, we hope to provide new perspectives and ideas for the in-depth study and clinical application of isoliquiritin.

2. Structure and Properties of Isoliquiritin

2.1 Structure of Isoliquiritin

Isoliquiritin is kind of monosaccharide derivative with molecular formulation $C_{21}H_{22}O_9$, the IUPAC name of the chemical is (E) - 1 - (2,4 - dihydroxyphenyl) - 3 - [4 - [(2S,3R,4S,5S,6R) - 3,4,5 - trihydroxy - 6 - (hydroxymethyl)oxan - 2 - yl]oxyphenyl]prop-2 - en - 1 - one. On fourth carbon, and its formula is $C_{21}H_{22}O_9$. A beta-D-glucopyranosyloxy group substitutes a trans-chalcone, and two hydroxy groups substitute the trans-chalcone at the second and fourth position (National Center for Biotechnology Information, 2024).

2.2 Physical and Chemical Properties of Isoliquiritin

Isoliquiritin exists as a yellow crystalline powder at room temperature. The melting point of the chemical is between 185 and 186 ℃, and its density is 1.528 kg/m^3. Molecular weight of isoliquiritin is 418.126385 g/mol. It is not charged, and has 6 rotatable bonds. In vitro, the solubility in ethanol at 25 ℃ is 5 mg/ml, and that in dimethylsulf-

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oxide (DMSO) is 84 mg/ml. Isoliquiritin is not soluble in water (Selleckchem, 2024). Isoliquiritin has 3 rings, and 2 of them are aromatic rings. The Van der Waals molecular volume of isoliquiritin is 370.97 Å^3, and its topological polar surface area is 158.98 Å^2. Isoliquiritin has 30 heavy atoms, 6 hydrogen bond donors, and 9 hydrogen bond acceptors (LIPID MAPS, 2024).

2.3 Classification of Isoliquiritin

Isoliquiritin is kind of phenolic compound, it also belongs to chalcone type flavonoid glycosides. The category of the chemical is polyketide, its main class is flavonoid, and the subclass is chalcone and dihydrochalcone (LIPID MAPS, 2024).

3. Source of Isoliquiritin

3.1 Glycyrrhiza Genus

Plants of the Glycyrrhiza genus are widely cultivated worldwide due to its excellent health benefits and value as a sweetener. they are kind of perennial herbaceous plant with a height of approximately 30 to 150 centimeters. They have odd numbered of feather like compound leaves, with a quantity of about 3 to 19 leaves, which vary among different species. The epidermis sinks to a grayish brown or reddish brown color, and the inner section are yellowish white. The rhizome part of the genus is cylindrical in shape. Glycyrrhiza genus are usually harvested in spring and autumn, with higher quality harvested in autumn (Ye et al., 2022).

Glycyrrhiza uralensis Fisch., *Glycyrrhiza inflata* Bat., and *Glycyrrhiza glabra* L. are the most often used species for medical in the genus. They are also the only three species in Glycyrrhiza genus were listed in the Pharmacopoeia of the People's Republic of China. The dry roots and rhizomes of the plants are used as the raw material for the treatment and extraction of active chemicals. The pharmacopoeia stipulates that the moisture content of medicinal materials used should be less than 12.0%, the total ash content should be less than 7.0%, and the acid insoluble ash content should be less than 2%. Meanwhile, harmful heavy metal elements such as lead, cadmium, arsenic, mercury, and copper should not exceed 5, 1, 2, 0.2, and 20 mg/kg, respectively (Chinese Pharmacopoeia Commission., 2020).

3.1.1 Glycyrrhiza uralensis Fisch.

Glycyrrhiza uralensis Fisch. mostly grows in areas of China at an altitude of 250-1400 meters. It is found in the Inner Mongolia Autonomous Region, Gansu, Xinjiang Uygur Autonomous Region, Ningxia Hui Autonomous Region, and Shaanxi Province of China (Ye et al., 2022; Cui et al., 2023). Some other region of the central Asia and Europe also have its distribution.

According to the qualitative analysis by ^{1}H-qualitative nuclear magnetic resonance (qNMR), the proportion of isoliquiritin in *G. uralensis* shows significantly difference depending on the place of origin. In which the sample form Longxi is the minimum value (0.01%), while that from Yanchi shows the maximum percentage (0.3%). Other four samples from different region shows similar value within the range (sample from Hangqi: 0.18%; sample from Baotou: 0.09%; sample from Chifeng: 0.29%; sample from Taklamakan desert: 0.13%). The qualitative analysis by high-performance liquid chromatography (HPLC) shows similar results. In *G. uralensis*, the content of Isoliquiritin is significantly lower than that of liquiritin. But the values between isoliquiritin and liquiritigenin are similar (Yu et al., 2021). Compared with other species in Glycyrrhiza genus, *G. uralensis* have the highest value of flavonoids, its isoliquiritin content reaches 4.556±0.1252 mg·g^{-1} (Yang et al., 2018).

3.1.2 Glycyrrhiza inflata Bat.

Glycyrrhiza inflata Bat. is mainly distributed in Xinjiang and Gansu province of China. It is mostly gray brown or gray brown in color, with a hard texture, thick rhizomes, and abundant adventitious buds (Ye et al., 2022).

After identification of species by genetic analysis, Glycyrrhiza genus with genotype tg2, tg4, and tg5 are recognized as *G. inflata*. Seven *G. inflata* from Xinjiang and one specimen from Gansu was analyzed by HPLC. The results shows that the content of isoliquiritin is between 0.011% to 0.068%, and the value of the only sample from Gansu is 0.020%. The mean isoliquiritin contend of *G. inflata* is similar to that of *G. glabra*. But this value is significantly lower than that of *G. uralensis* (Kondo et al., 2007).

3.1.3 Glycyrrhiza glabra L.

Glycyrrhiza glabra L. grows naturally in Xinjiang province of China, Mideast region like Turkey, Europe, and a small portion of mid latitude regions in Africa and the Americas (PlantNet, n.d.). The rhizome of *G. glabra* is hard. Its outer skin is not rough, the pores are small, and there are few cracks in the cross-section.

HPLC, ultraviolet (UV) and fourier transform infrared spectroscopy (FT-IR) fingerprint analysis have been used to test the content of eight compounds (including isoliquiritin) in 36 plants of *G. glabra*. The *G. glabra* came from 9 different locations, and only one fourth of them are cultivated type. The isoliquiritin content is at maximum of 1.03 mg/g from the sample grew in Shaya, Xinjiang. And two samples that cultivated in Uzbekistan shows the minimum

values, which is only 0.08 mg/g. The mean content of all 36 sample is 0.49 mg/g (Yang et al., 2020).

3.2 Biosynthesis

Biosynthesis is the process that using enzymatic reaction to synthesis natural products from simple molecules. This process can be achieved either in vitro or in cells like E.coli (Sosa and Chang, 2024). Research had used these techniques to generate the ingredients of *G. uralensis*, including isoliquiritin in *Saccharomyces cerevisiae*. Gene of the *S. cerevisiae* have been modified, and the different expression of genes will result in changes in the ration of different flavonoids produces. After editing gene GuPAL1, GuC4H1, Gu4CL1, GuCHS1::GuCHR1, GuCHI1 and GuUGT1 into the yeast, the fungal start to synthesis chemical compound of *G. uralensis*. The over-expression of GuCHI1 will improve the production of isoliquiritin, but the production of p-Coumaric acid will also be enhanced and be more than it. And when GuCHI1 and GuCHS1:GuCHR1 are both over expressed in the cell, it will lead to the massive production of isoliquiritin from the it (Yin et al., 2020).

In another research, biosynthesis technique using methyl jasmonate (MJ) and phenylalanine (PHE) as elicitors to treat the *G. uralensis*, and discovery that the contents of active ingredients in the adventitious root of the plants were improved. The total flavonoids reach 6.43±0.18 mg/g, which is about 1.91 times compared to the control groups (3.37±0.18 mg/g) (Wang et al., 2017).

4. Extraction of Isoliquiritin

4.1 Solvent Extraction (SE)

4.1.1 Introduction and Principle of SE

Solvent extraction is a traditional and most often used extraction methods. It uses organic solvent, to extract the target compounds from crushed plant tissues. Most organic solvent, like methanol and ethanol, come from non-renewable resources, and their usage also bring damage to the environment. And some new solvent, like ionic liquids and natural deep eutectic solvent (NADES) solved this problem (Ling and Hadinoto, 2022). SE uses the principle that different ingredients have different solubilities in different solutions to separate them (Al Momani et al., 2023).

4.1.2 Procedures of SE

SE starts with washing and pulverized of plant tissues. The plant powder will mix with solvent in a certain proportion, then soaking it or heating reflux. This often followed by filtration, condensation, and column chromatography purification, which enhance the purity of isoliquiritin extracted. The different solvent used, and different extraction process will result in huge difference of purity and content (Peng et al., 2021).

4.1.3 Usage of SE in Isoliquiritin Extraction

According to the Pharmacopoeia of the People's Republic of China (Chinese Pharmacopoeia Commission., 2020.), 70% ethanol is often used to extract the ingredients in the licorice for clinical use. While 90% ethanol and 90% methanol will be used to extract the bioactive compounds to verify the quality of the raw materials. The use of solvent extraction do not require large equipment like other methods followed, which enable it to be put into mass production without incurring excessive costs.

4.2 Ultrasound-Assisted Extraction (UAE)

4.2.1 Introduction and Principle of UAE

UAE is kind of green bio-refining technology. Ultrasound refers to the sound that above the threshold (above 20KHz) that human can hear it (National Institute of Biomedical Imaging and Bioengineering, 2024). It enhanced the efficacy by using ultrasound to break the cell structure of the plant tissue, which allow the leakage of bioactive compounds. This also promotes the dissolution of solutes into the solvent, which lower the number of solvents needed to use in the extraction. Since there are no heat involved in the processes of UAE, these methods will be suitable to those thermo-sensitive compounds (Yusoff et al., 2022).

4.2.2 Procedures of UAE

The treatment of plant tissue and mixing in UAE is the same with SE, and the processes is also ended with filtration, condensation, and column chromatography purification. The only difference between UAE and SE is that the container of solution will be put in an ultrasound equip. This allows ultrasound to crush the plant cells (Zhu et al., 2024).

4.2.3 Usage of UAE in Isoliquiritin Extraction

One UAE methods using ionic liquids (IL) as solvent had been verified for flavonids extraction from licorice. This method uses G. as the raw materials. The soaking time for 8 hours and extracting time for 20 minutes. 1.5 M $[C_4MIM]Ac$ aqueous solution had been chosen as the solvent, and the ratio between solvent and solid is 10:1 . The extracted isoliquiritin from the *G. uralensis* is 1.17 ± 0.02 mg/g (Ji et al., 2018).

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4.3 Microwave-Assisted Extraction (MAE)

4.3.1 Introduction and Principle of MAE

Microwaves refers to the electromagnetic waves with frequency range between 300 MHz to 300 GHz (Pournoori et al., 2023). Under the effect of microwave, molecules polarize and generate friction with each other. This friction will increase the kinetic energy between molecules and generate heat (Wypych, 2017). MAE uses heat of solvents and raw materials generated by the microwave to promote the rupture cell wall, which improve the extraction efficiency. Just like UAE, MAE also shorten the time consumed for the extraction and reduce the use of solvent in the process (Bagade and Patil, 2021).

4.3.2 Procedures of MAE

All steps of MAE are the same as UAE. The only difference between these two methods is to exchange the ultrasound equip with microwave reactor (Sparr Eskilsson and Björklund, 2000).

4.3.3 Usage of MAE in Isoliquiritin Extraction

In one research, a method that combine UAE and MAE to extract flavonoids from licorice had been designed. Ultrasonic microwave-assisted micellar extraction (UMAME) is able to enhance the purity of the licorice flavonoids in a sample with ethyl acetate from 36.47% to 90.32%. The process takes only 10 minutes, much quicker than normal solvent extraction (Wang et al., 2021). In another research, natural deep eutectic solvent (NADES) has been proved about its ability to extract isoliquiritin from *G. uralensis* assisted by ultrasound. It found out the best time consumed, and ratio of solvent to extract the ingredient: 30 minutes needed; NADES system, 1,4-butanediol–levulinic acid (1 : 3 molar ratio); NADES-12 water content, 17%; and liquid/solid ratio, 42 ml/g. The extraction results about isoliquiritin are 3.17 mg/g, and the standard deviations were less than 9‰ (Dong et al., 2021). Compared with the value of 1.17 ± 0.02 mg/g isoliquiritin extracted by IL-UAE from the same species, UMAME is a better method.

4.4 Supercritical Fluid Extraction (SFE)

4.4.1 Introduction and Principle of SFE

SFE is kind of green technology, it enables higher selective and quicker extraction. SFE uses solvents at a temperature and pressure above their critical value, which trans the solvents into a state of "supercritical fluid" that combines liquid and gas characteristics. This allows some substances that are gases at room temperature to be used as solvents. Supercritical fluid carbon dioxide ($scCO_2$) is often used as the solvent in SFE (at 31.1℃ and 73.8 atm), because it will not produce pollution and is high available (Wrona et al., 2017).

4.4.2 Procedures of SFE

The system of SFE varies base on the extraction procedure of different ingredients. They often include a pump of the supercritical fluid like CO_2, a sample container with pressure cell, a collecting vessel, a supercritical fluid solvent supply, a cooler, a heater, , a metering valve, a extraction cell, and pressure maintainer (figure 2).

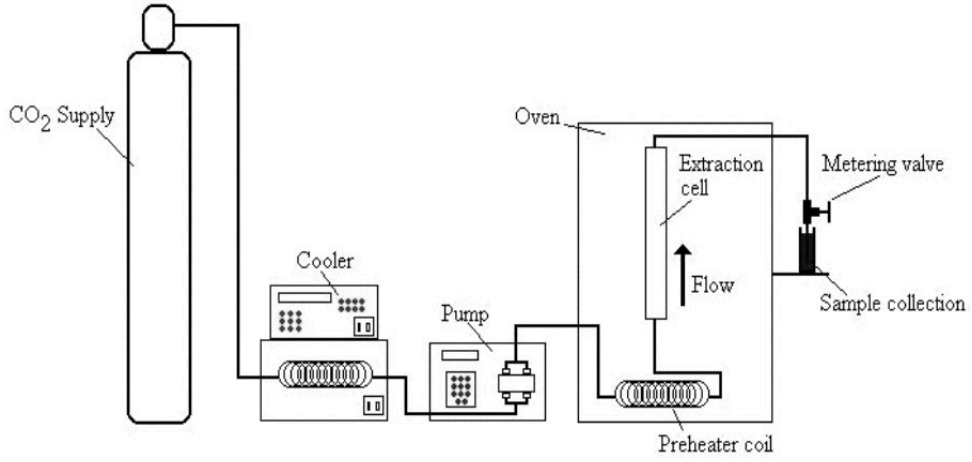

Figure 2: Apparatus of SFE System (Sapkale et al., 2010).

The crushed plant tissue is placed in the extraction cell, and then $scCO_2$ is pumped into the cell to dissolve it. After the ingredients dissolved in the supercritical fluid, $scCO_2$ will be gasified and recovered by lowering the temperature and pressure. And the ingredients will settle and be collected in the collecting vessels (Sapkale et al., 2010). In the process, some polar modifier, like methanol or ethanol, will be added into the $scCO_2$ to improve extraction

rate (Le Floch et al., 1998).

4.4.3 Usage of SFE in Isoliquiritin Extraction

There is insufficient research data and result to discuss the extraction efficiency of SFE for isoliquiritin. But the use of SFE on the purification of other phenolic compounds from herbal medicines had been proved in some research. By adjusting the extracting temperature, pressure in the SFE system, and the use of modifier, specific extraction plan can be designed for specific phenolic compounds (Le Floch et al., 1998; Tyśkiewicz et al., 2018; Buelvas-Puello et al., 2021). So SFE has potential value in extracting isoliquiritin, it may have the ability to efficiently and accurately purify isoliquiritin from other complex ingredients in the dry root and rhizome of licorice.

4.5 High-Performance Liquid Chromatography (HPLC)

4.5.1 Introduction and Principle of HPLC

The basic principle of HPLC is based on the different distribution coefficients of different components in the plant tissue between the stationary phase and the mobile phase. This resulting in different elution times when different bioactive ingredients passing through the chromatographic column. By adjusting the condition of the stationary phase and the mobile phase, HPLC can highly selectively separate the target component from other ingredients in the sample (Nikolin et al., 2004). However, since the cost of HPLC equipment and high-purity solvents is relatively high, this method is normally used for the verification of final products and identification of chemical components in a sample (LabX, n.d.).

4.5.2 Procedures of HPLC

HPLC system is generally made up of a pump, a mobile phase collecting vessels, a chromatographic column with stationary phase, an injection valve, a detector, and a data collection and processing system (figure 3). Some HPLC system also include column oven to maintain a stable temperature, and a mobile phase degassing device to remove bubbles (Meyer et al., 2010; Böttcher et al., 2024).

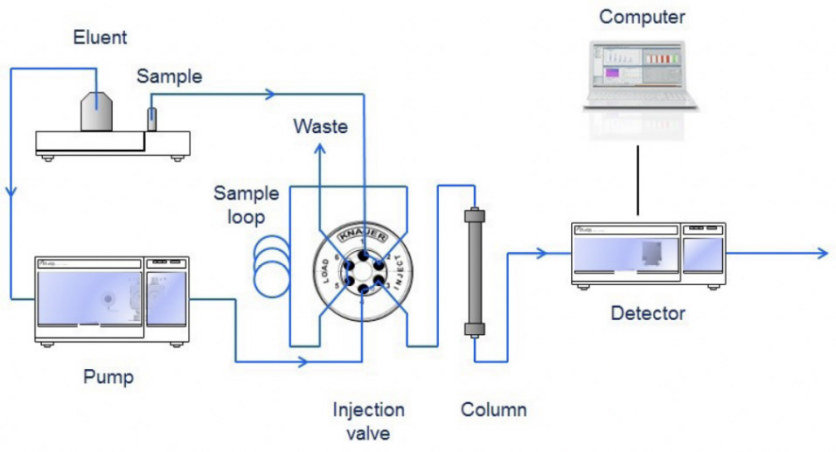

Figure 3: Structure of HPLC system (Böttcher et al., 2024)

Before the start of analysis, the HPLC equips need to be balanced to reach a stable state, which usually cost 30 minutes. The sample will be pumped into the system. And then the detector will collect the data for the computer to analysis and generate chromatogram (Horváth, 1980).

4.5.3 Usage of HPLC in Isoliquiritin Extraction

HPLC is widely used for component identification of various herbs due to its high-precision separation and purification ability. The amount of isoliquiritin in *G. uralensis*, *G. inflata*, and *G. glabra* has also been identified by HPLC (Yang et al., 2018; Kondo et al., 2007). And in much research about isoliquiritin extraction, HPLC is also used as the final verification about these methods (Ji et al., 2018; Dong et al., 2021).

4.6 Liquid Chromatography-Mass Spectrometry (LC-MS)

4.6.1 Introduction and Principle of LC-MS

LCMS is kind of analysis technique that combine HPLC and MS, it can simultaneously achieve separation, identification, and quantification of complex known or unknown samples, and found out the structure and chemical properties of the molecules (Mukherjee, 2019). LC-MS system utilizes the separation ability of HPLC to obtain high-purity samples, and then use MS to analysis it's the physical and chemical property. MS uses the difference of the mass to charge ratio among different charged atoms, molecules, and clusters to separate and analysis them (Murayama et

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al., 2009).

4.6.2 Procedures of LC-MS

LC-MS analysis start with production of sample from the HPLC system. When the molecules in sample enter the MS, they will be ionized by an ion source (like electrospray ionization source, atmospheric pressure chemical ionization source, or atmospheric pressure photoionization). Then a mass analyzer will use electromagnetic field to separate the charged molecules. The data will be collected by a detector and analyzed in the computer (Murayama et al., 2009; Pitt, 2009).

4.6.3 Use of LC-MS in Isoliquiritin Extraction

LC-MS had been used as tool in the research about numerous drug discovery, its effectiveness had been fully validated (Mi et al., 2022; Núñez et al., 2023; Zuo et al., 2023; Li et al., 2024; Zhang et al., 2024). In another research, LC-MS had also been used for quality investigation about three species of Glycyrrhiza and discover the fact that amount of bioactive ingredients are decreased year by year (Jiang et al., 2016). In the further research about isoliquiritin, LC-MS can act as a useful tool for quality identification of components and provide assistance for the development of new drugs based on isoliquiritin structure.

5. Discussion

Compared with other ingredients in licorice, like liquiritin, isoliquiritin has a relatively low natural abundance. And the yield of isoliquiritin from natural sources can vary significantly due to factors such as plant age, environmental conditions, and geographical location. This variability complicates the standardization of isoliquiritin production for research and therapeutic use (Yu et al., 2021). The biosynthetic technology for producing isoliquiritin using MJ and PHE treated *G. uralensis* is not yet mature. Although the production of flavonoids in adventitious root has increased by 1.91 times compared to the control groups, it did not show data about the effect of elicitors in other part of the plants, and it lacks the data to show the acute proportion of each ingredient in the sample (Wang et al., 2017a; Yang et al., 2018).

The production of isoliquiritin through gene edited *S. cerevisiae* also has its limitations. The production mechanism is not absolutely clear, and it is difficult to ensure purity during large-scale production. The impurities like naringenin chalcone are prone to occur. Compared to traditional planting methods to obtain isoliquiritin, biosynthesis can either takes much less time or improves the output. It only takes 37 days to process the seeds and about a week for transgenic expression to obtain the drug, which is much lower than the six-month planting and harvesting cycle. This method has enormous potential in large-scale drug production of isoliquiritin (Wang et al., 2017; Yin et al., 2020). However, the chemical synthesis pathway of isoliquiritin is still unclear. This limits its potential for large-scale industrial production. Future research should delve into this field to provide a deeper understanding of the synthesis and precursor components of isoliquiritin.

The current widely used extraction methods only isolated 1.17 ± 0.02 mg/g isoliquiritin from the *G. uralensis* (Ji et al., 2018), which is a relative low value, it only extracted approximately 25.68% of the total amount of isoliquiritin contained in the *G. uralensis* (Yang et al., 2018). This indicate that large amount of isoliquiritin were wasted in the production process. Although techniques like SFC and HPLC can effectively extract isoliquiritin with high content, the expensive cost of the equips limited their use in industrial production. So further development is needed for the production and separation process of isoliquiritin.

6. Conclusion

Isoliquiritin, as a flavonoid compound, has various biological activities and is derived from the dry roots and rhizomes of plants such as Glycyrrhiza uralensis Fisch., Glycyrrhiza inflata Bat., and Glycyrrhiza glabra L.. Its unique structure contains a beta-D-glucopyranosyloxy group and two hydroxy groups, endowing it with pharmacological properties such as antioxidant, anti-inflammatory, antibacterial, and neuroprotective properties. After extracting and purifying this chemical from plants or get them through biosynthesis, isoliquiritin has demonstrated its potential in treating cancer, neurodegenerative diseases, and microbial infections. Future research should further explore its clinical applications to fully utilize its medicinal value. The low natural abundance of isoliquiritin and the low efficient traditional extraction methods limited its clinical use. Biosynthesis and extraction techniques need to be further explored to solve the dilemmas.

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