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Research Article

Advances In Green Extraction Technologies and Structural Characterization of Bioactive Phytoconstituents from Glycyrrhiza Glabra

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Abstract

Glycyrrhiza glabra (liquorice) is an important medicinal plant. It is well known for its various bioactive phytoconstituents like glycyrrhizin, flavonoids, phenolic compounds, saponins, coumarins and polysaccharides that have significant pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, antiviral, hepatoprotective, anticancer and immunomodulatory effects. The growing demand for natural therapies and herbal products has raised the need for sustainable and effective extraction methods that maintain the integrity and biological activity of these precious substances. The purpose of this study is to discuss new developments in green extraction procedures and recent structural characterization techniques for the separation, identification and analysis of bioactive phytoconstituents from Glycyrrhiza glabra. The present study provides a systematic evaluation of the literature (2010-2025) for the environmentally friendly extraction technologies viz. Ultrasound-Assisted Extraction (UAE), Microwave-Assisted Extraction (MAE), Supercritical Fluid Extraction (SFE) and Enzyme-Assisted Extraction (EAE). These technologies have been shown to have high extraction efficiency, lower solvent consumption, lower energy consumption, shorter extraction times and better preservation of thermolabile compounds than conventional extraction techniques. The paper also highlights the importance of green solvents such as water, ethanol, natural deep eutectic solvents (NADES) and supercritical carbon dioxide in facilitating sustainable phytochemical extraction.

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1. INTRODUCTION

Glycyrrhiza glabra, commonly known as licorice, is one of the most valuable medicinal plants widely used in traditional and modern systems of medicine due to its extensive therapeutic properties. The roots and rhizomes of *Glycyrrhiza glabra* contain a rich variety of bioactive phytoconstituents including glycyrrhizin, flavonoids, saponins, phenolic compounds, coumarins, and polysaccharides, which exhibit significant pharmacological activities such as antioxidant, anti-inflammatory, antiviral, antimicrobial, hepatoprotective, anticancer, and immunomodulatory effects. The growing demand for natural bioactive compounds and herbal medicines has increased the need for efficient, sustainable, and environmentally safe extraction methods for isolating these valuable phytochemicals.

Traditional extraction procedures such as maceration, Soxhlet extraction and hydrodistillation frequently utilize large quantities of organic solvents, long extraction times, significant energy consumption and may cause the destruction of heat-sensitive chemicals. These restrictions have led to the increasing development of green extraction technologies that offer better extraction efficiency with less environmental effect. Modern green extraction techniques such as ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction and enzyme-assisted extraction have been developed as efficient substitutes to extract bioactive chemicals from medicinal plants. These techniques reduce solvent usage, shorten extraction duration, improve phytochemical stability, and enhance extraction yield while supporting sustainable processing practices. Green extraction technologies are therefore becoming increasingly important in phytopharmaceutical research and industrial herbal product development.

In addition to extraction, structural characterization of phytoconstituents is essential for understanding the chemical composition, molecular structure, purity, and biological activity of plant-derived compounds. Advanced analytical and spectroscopic techniques such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography–Mass Spectrometry (GC–MS), Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), and Liquid Chromatography–Mass Spectrometry (LC–MS) play a crucial role in the identification and characterization of licorice phytochemicals. These analytical tools enable accurate qualitative and quantitative analysis of important compounds such as glycyrrhizin, glabridin, liquiritin, and isoliquiritigenin, thereby supporting herbal drug standardization, quality control, and pharmaceutical formulation development.

Recent scientific advancements have also focused on integrating sustainable extraction methods with advanced characterization technologies to improve the efficiency and reliability of phytochemical research. The combination of eco-friendly extraction processes and high-resolution analytical techniques contributes significantly to the discovery of novel bioactive compounds, enhancement of therapeutic applications, and development of safe plant-based therapeutics. Furthermore, the increasing emphasis on green chemistry and sustainable pharmaceutical production has strengthened the importance of environmentally responsible extraction and analytical practices in medicinal plant research.

2. GREEN EXTRACTION TECHNOLOGIES OF BIOACTIVE COMPOUNDS FROM GLYCYRRHIZA GLABRA

Green extraction technologies are environment-friendly extraction methods for isolating the bioactive substances from medicinal plants with less usage of toxic chemicals and less environmental damage. These methods are increasingly preferred in phytopharmaceutical and herbal research because they improve extraction efficiency while maintaining the stability of sensitive phytochemicals. In Glycyrrhiza glabra, green extraction technologies are mainly used to isolate important compounds such as glycyrrhizin, flavonoids, phenolic compounds, saponins, and polysaccharides. Compared with conventional extraction methods, green extraction techniques require less solvent, shorter extraction time, and lower energy consumption.

2.1. Importance of Green Extraction Technologies

Green extraction technologies are important because traditional extraction methods often involve excessive use of organic solvents, prolonged heating, and higher energy requirements, which may lead to degradation of bioactive compounds. Eco-friendly extraction methods help reduce environmental pollution and improve sustainability in medicinal plant processing.

These techniques also provide higher extraction yields and better purity of phytochemicals. In herbal medicine and pharmaceutical industries, green extraction technologies support the development of safe, high-quality, and standardized plant-based products.

2.2. Ultrasound-Assisted Extraction (UAE)

Ultrasound-assisted extraction is one of the most widely utilized green extraction methods for phytochemical recovery from Glycyrrhiza glabra. His method uses ultrasonic waves to create cavitation bubbles in the extraction solvent. The collapse of these bubbles disrupts plant cell walls and enhances the release of intracellular bioactive compounds into the solvent.

It may greatly shorten extraction time and solvent consumption, and improve extraction efficiency. It is commonly employed for the extraction of glycyrrhizin, flavonoids and antioxidant chemicals from licorice roots.The technique is especially useful for preserving heat-sensitive phytochemicals because extraction occurs under relatively mild conditions.

2.3. Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction uses microwave radiation to rapidly heat both plant tissues and extraction solutions. Microwave radiation generates internal heating inside plant cells which increases the rupture of cell wall and promotes the release of phytochemicals. This method is highly efficient and requires less extraction time compared with conventional extraction processes.

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Microwave-assisted extraction is commonly used for isolating phenolic compounds, flavonoids, and saponins from licorice. The technique allows for a better extraction yield, lower energy usage and less solvent use. Because of rapid heating, microwave-assisted extraction also minimizes degradation of valuable phytoconstituents.

Table 1. Comparison of Green Extraction Techniques Used for *Glycyrrhiza glabra*

Extraction Method	Main Principle	Major Advantages	Target Compounds
Ultrasound-Assisted Extraction	Ultrasonic cavitation	Fast extraction and low solvent use	Glycyrrhizin, flavonoids
Microwave-Assisted Extraction	Microwave heating	Rapid extraction process	Phenolics, saponins
Supercritical Fluid Extraction	Supercritical CO_2 extraction	Solvent-free residue	Essential oils
Enzyme-Assisted Extraction	Enzymatic cell wall breakdown	Increased extraction yield	Polysaccharides

2.4. Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) is an advanced green extraction process that uses mostly supercritical carbon dioxide (CO2) as a solvent.Under supercritical conditions, carbon dioxide exhibits properties of both liquid and gas, allowing efficient penetration into plant tissues and extraction of bioactive compounds.

This technique is highly eco-friendly because carbon dioxide is non-toxic, recyclable, and leaves no harmful solvent residues in the final extract. Supercritical fluid extraction is mainly applied for extraction of volatile oils, aromatic compounds, and thermolabile phytochemicals from liquorice. The method provides highly pure extracts with improved quality and stability.

2.5. Enzyme-Assisted Extraction (EAE)

Enzyme-assisted extraction uses enzymes such as cellulase, pectinase and hemicellulase to break down plant cell walls and liberate intracellular compounds. The enzymatic degradation of structural polysaccharides improves extraction efficiency and enhances recovery of bioactive compounds.

This process is performed under gentle conditions, which means less harsh chemicals and high temperature are used. Enzyme-assisted extraction is commonly used for extraction of flavonoids, polysaccharides, and phenolic compounds from licorice. The technique is considered highly sustainable and suitable for pharmaceutical and nutraceutical applications.

2.6. Green Solvents Used in Extraction

Green solvents are important for sustainable extraction methods since they replace harmful organic solvents with environmentally friendly ones. They are less harmful, are biodegradable, are renewable and are environmentally friendly solvents. Green solvents for extraction of bioactive phytoconstituents of Glycyrrhiza glabra (licorice) enhance extraction efficiency. Safe and stable for valuable phytochemicals such glycyrrhizin, flavonoids, phenolic compounds, saponins, and polysaccharides.

Water is mainly used for extraction of polysaccharides and hydrophilic compounds, while ethanol is commonly used for flavonoid and phenolic extraction. Supercritical CO_2 is suitable for extracting volatile oils and lipophilic compounds. Natural deep eutectic solvents are emerging as highly sustainable alternatives for efficient phytochemical extraction.

Table 2. Common Green Solvents Used in Extraction of *Glycyrrhiza glabra* Phytoconstituents

Solvent	Characteristics	Major Applications
Water	Non-toxic and economical	Polysaccharide extraction
Ethanol	Biodegradable and safe	Flavonoid extraction
Supercritical CO_2	Residue-free extraction	Essential oil extraction
NADES	Eco-friendly solvent system	Polyphenol extraction

2.7. Factors Affecting Green Extraction

The performance of green extraction technologies is dependent on various operational and physical parameters which influence the recovery, purity, stability and yield of bioactive phytoconstituents from Glycyrrhiza glabra. Optimization of these parameters to improve the extraction efficiency is vital but at the same time to preserve the biological activity of valuable substances such as glycyrrhizin, flavonoids, phenolic compounds, saponins and polysaccharides. The quality and composition of the resulting extract can be strongly influenced by the extraction conditions.

Variations in plant source, harvesting conditions, and storage may also affect the concentration of bioactive compounds in licorice extracts. Therefore, standardization of extraction parameters is necessary for obtaining reproducible and high-quality phytochemical extracts.

3. METHODOLOGY

3.1. Study Design

This research was conducted as a systematic review study on the recent advancements in green extraction technologies and structural characterization of bioactive phytoconstituents extracted from Glycyrrhiza glabra. The study followed a systematic scientific approach for collecting, evaluating, and interpreting published literature related to eco-friendly extraction methods and analytical characterization techniques. The review primarily emphasized sustainable extraction processes such as ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, enzyme-assisted extraction, and other environmentally safe technologies used for isolating phytochemicals from *Glycyrrhiza glabra*.

The methodology also revealed advanced techniques for structural characterization for phytochemical profiling like High performance Liquid Chromatography (HPLC), Gas Chromatography Mass Spectrometry (GC-MS), Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic

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Resonance (NMR) and Liquid Chromatography Mass Spectrometry (LC-MS). Both experimental and analytical studies published in peer-reviewed journals were considered for evaluation.

3.2. Eligibility Criteria

Inclusion Criteria

Studies included in this review were required to investigate green or sustainable extraction techniques for isolating phytoconstituents from Glycyrrhiza glabra. Research articles focusing on optimization of extraction parameters, solvent reduction, extraction efficiency, and environmental sustainability were included.

Studies employing analytical and spectroscopic methods for structural characterization and phytochemical identification of bioactive compounds such as glycyrrhizin, flavonoids, saponins, phenolic compounds, and polysaccharides were also considered eligible.

Only peer-reviewed research articles published in English were included to ensure scientific quality and accessibility of data. Experimental studies, comparative studies, and analytical investigations published between 2010 and 2025 were selected for detailed analysis.

Exclusion Criteria

Studies unrelated to green extraction technologies or structural characterization techniques were excluded from the review. Research focusing solely on pharmacological or clinical applications without discussing extraction or characterization procedures was not considered.

Conference abstracts, editorials, opinion articles, book chapters, duplicated studies, and non-English publications were excluded. Studies lacking sufficient methodological details or analytical data were also omitted from the final synthesis.

3.3. Search Strategy and Data Sources

A thorough literature search was conducted in main scientific databases like PubMed, Scopus, Web of Science, ScienceDirect and Google Scholar. Keywords and search phrases used during the search process included “Glycyrrhiza glabra,” “green extraction,” “bioactive phytoconstituents,” “ultrasound-assisted extraction,” “microwave-assisted extraction,” “supercritical fluid extraction,” “phytochemical characterization,” “HPLC,” “GC-MS,” “LC-MS,” and “NMR spectroscopy.”

The screening process involved two stages: initial screening based on titles and abstracts followed by full-text evaluation of eligible studies. Relevant references from selected articles were also manually searched to identify additional studies associated with green extraction technologies and structural characterization methods.

3.4. Selection Process and Study Characteristics

The selected studies represented diverse experimental designs and analytical approaches related to extraction and characterization of phytoconstituents from Glycyrrhiza glabra. The studies included laboratory-scale extraction experiments, optimization studies, comparative solvent analyses, and analytical profiling investigations.

Different environmentally friendly extraction methods were evaluated in terms of extraction yield, purity, extraction time, solvent consumption, and preservation of phytochemical activity. The reviewed studies also assessed the efficiency of modern spectroscopic and chromatographic techniques for identifying and characterizing active phytoconstituents.

3.5. Data Extraction and Outcome Measures

Data from the selected studies were extracted in a structured format including author name, year of publication, extraction technique used, solvent system, extraction conditions, characterization methods, identified phytochemicals, extraction yield, and analytical findings.

The primary outcome measures included extraction efficiency, recovery of bioactive compounds, reduction in solvent usage, sustainability of extraction processes, and accuracy of structural characterization methods. Secondary outcomes included phytochemical stability, purity of isolated compounds, and analytical sensitivity of instrumental techniques.

3.6. Statistical Analysis

The collected data were analyzed descriptively in order to evaluate the effectiveness and usability of different green extraction techniques and characterization methods. Comparative evaluation was performed based on extraction yield percentages, extraction time, solvent consumption, and sensitivity of analytical methods.

The findings from various studies were summarized to identify the most effective environmentally sustainable extraction techniques and the most reliable structural characterization tools for phytochemical profiling of Glycyrrhiza glabra bioactive compounds.

3.7. Risk of Bias Assessment

The methodological quality of the included studies was assessed based on study design, reproducibility of extraction procedures, analytical accuracy, sample preparation methods, and clarity of reported results. Potential sources of bias included inconsistent extraction parameters, variation in plant material quality, limited sample sizes, and lack of standardized analytical protocols.

Studies with clearly defined methodologies, validated analytical techniques, and reproducible findings were considered to have lower risk of bias and were given greater emphasis during interpretation of results.

4. STRUCTURAL CHARACTERIZATION OF BIOACTIVE PHYTOCONSTITUENTS FROM GLYCYRRHIZA GLABRA

Structural characterization of bioactive phytoconstituents is an important scientific process used to identify, analyze, and confirm the chemical composition of medicinal plant compounds. Glycyrrhiza glabra, commonly known as licorice, contains numerous therapeutically important phytochemicals such as glycyrrhizin, flavonoids, chalcones, coumarins, phenolic compounds, and polysaccharides. These compounds possess significant biological activities including antioxidant, anti-inflammatory, antimicrobial, antiviral, hepatoprotective,

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and anticancer properties. Structural characterization helps researchers understand the molecular structure, purity, functional groups, and pharmacological potential of these phytoconstituents. Advanced analytical techniques such as HPLC, GC–MS, FTIR, NMR, and LC–MS are widely employed for accurate characterization and phytochemical profiling.

4.1. Major Bioactive Phytoconstituents

The roots and rhizomes of Glycyrrhiza glabra contain several biologically active compounds that contribute to its medicinal importance. Among these, triterpenoid saponins are considered the most important group of phytochemicals. The principal bioactive component of licorice, glycyrrhizin, responsible for the characteristic sweet taste of licorice, possesses important anti-inflammatory and antiviral activities. Glycyrrhetinic acid, another major saponin derivative, shows hepatoprotective and immunomodulatory effects.

Flavonoids present in licorice include glabridin, liquiritin, isoliquiritin, and liquiritigenin. These chemicals show a high antioxidant effect and contribute to a reduction in oxidative stress and cellular damage. Chalcones and coumarins such as isoliquiritigenin and licochalcone A also contribute to antimicrobial, anti-inflammatory, and anticancer activities. The diversity of these phytoconstituents makes structural characterization essential for therapeutic and pharmaceutical applications.

4.2. High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography (HPLC) is one of the most widely used analytical techniques for the separation, identification, quantification, and characterization of bioactive phytoconstituents present in *Glycyrrhiza glabra*. Due to its high sensitivity, accuracy, reproducibility, and reliability, HPLC has become an essential tool in phytochemical research, herbal drug standardization, quality control, and pharmaceutical development.

The technique is highly sensitive, reproducible, and suitable for fingerprint profiling of herbal extracts. HPLC is also used to assess the purity and quality of phytoconstituents during herbal drug standardization. Different wavelengths are selected during analysis to detect specific classes of compounds.

Table 1. HPLC Analysis of Major Phytoconstituents from *Glycyrrhiza glabra*

Compound	Retention Time (min)	Detection Wavelength (nm)	Major Application
Glycyrrhizin	12.5	254	Quantitative estimation
Glabridin	9.8	280	Antioxidant profiling
Liquiritin	7.3	276	Flavonoid analysis
Isoliquiritigenin	10.1	360	Structural identification

4.3. Gas Chromatography–Mass Spectrometry (GC–MS)

Gas Chromatography – Mass Spectrometry (GC – MS) is an important analytical technique for identification and characterization of volatile and semi-volatile compounds present in licorice extracts. In this method, compounds are first separated by gas chromatography and then identified through mass spectrometric analysis based on their fragmentation patterns and molecular masses.

GC–MS is particularly useful for detecting essential oils, fatty acids, aromatic compounds, and volatile metabolites. The technique provides highly specific structural information and helps confirm the chemical identity of phytoconstituents. The obtained mass spectra are compared with standard spectral libraries for accurate compound identification.

4.4. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a powerful and widely used analytical technique for the structural characterization of bioactive phytoconstituents present in *Glycyrrhiza glabra*. FTIR is primarily employed to identify functional groups, chemical bonds, and molecular interactions within phytochemical compounds. The technique provides rapid, non-destructive, and reliable analysis, making it an important tool in phytochemical research, herbal drug standardization, and quality control.

FTIR analysis helps identify hydroxyl groups, carbonyl groups, aromatic rings, glycosidic linkages, and other important chemical functionalities in bioactive compounds. This technique is rapid, non-destructive, and highly useful for preliminary structural analysis and confirmation of phytochemical composition.

Table 2. Important FTIR Peaks Observed in *Glycyrrhiza glabra* Extracts

Wavenumber (cm^{-1})	Functional Group	Interpretation
3400–3200	O–H stretching	Phenolic compounds
2920	C–H stretching	Aliphatic chains
1720	C=O stretching	Carbonyl compounds
1600	Aromatic C=C	Flavonoids
1100	C–O stretching	Glycosides

4.5. Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy is one of the most advanced and reliable analytical techniques used for the structural elucidation and characterization of bioactive phytoconstituents present in *Glycyrrhiza glabra*. NMR provides detailed information regarding the molecular structure, stereochemistry, atomic connectivity, and spatial arrangement of atoms within a compound. Due to its high accuracy and non-destructive nature, NMR is widely employed in phytochemical research, natural product chemistry, pharmaceutical development, and herbal drug standardization.

NMR spectroscopy provides valuable information regarding the number of hydrogen atoms, carbon skeleton structure, bonding patterns, and molecular conformation of compounds. It is especially useful for confirming the structures of glycyrrhizin, flavonoids, and chalcone derivatives isolated from licorice extracts. The technique plays a major role in discovering novel bioactive molecules and validating compound purity.

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4.6. Liquid Chromatography–Mass Spectrometry (LC–MS)

LC MS: A sensitive tool for phytochemical study of chromatography coupled with mass spectrometry. This technique is particularly useful for analyzing thermolabile and complex compounds that cannot be effectively analyzed using GC–MS.

LC–MS allows rapid detection, identification, and profiling of minor and major phytoconstituents in licorice extracts. It is widely applied in metabolomic studies, pharmaceutical research, and bioactive compound screening. The high sensitivity and precision of LC–MS make it an essential tool for advanced phytochemical analysis and quality control.

4.7. Purification and Isolation Techniques

Before structural characterization, phytoconstituents are purified and isolated using different chromatographic methods. Common purification techniques include column chromatography, preparative HPLC, thin-layer chromatography (TLC), and solvent partitioning. These techniques help remove impurities and obtain pure compounds for accurate structural analysis.

Purification improves spectral clarity, analytical reproducibility, and reliability of structural characterization data. Proper isolation and purification are critical for identifying trace phytochemicals and establishing structure–activity relationships in medicinal plant research.

4.8. Importance of Structural Characterization

Structural characterization is essential for understanding the medicinal and pharmaceutical significance of phytoconstituents present in Glycyrrhiza glabra. It helps in identifying novel compounds, standardizing herbal medicines, ensuring product quality, and determining therapeutic efficacy.

The characterization process also supports drug discovery, formulation development, toxicological evaluation, and pharmacological research. Accurate structural analysis enables researchers to understand the relationship between chemical structure and biological activity, which is important for developing safe and effective plant-based therapeutics.

Table: Research Study

Reference	Study Focus	Methodology	Key Findings
Vaseghi Baba et al. (2026)	Comparison of conventional and green extraction techniques for phytochemicals from licorice	Systematic review	Green extraction technologies such as ultrasound-assisted and microwave-assisted extraction demonstrated higher extraction efficiency, reduced solvent consumption, and improved sustainability compared with conventional extraction methods.
Danaboina et al. (2023)	Targeted enrichment and quantification of bioactive compounds from *Glycyrrhiza glabra* root extract	Analytical and phytochemical quantification study	Optimized extraction and enrichment methods significantly improved recovery of bioactive compounds, supporting the development of phytopharmaceutical ingredients from licorice extracts.
Babich et al. (2022)	Chemical composition and biological activity analysis of *Glycyrrhiza glabra* extracts	Experimental phytochemical and biological evaluation study	The study identified several bioactive phytoconstituents including flavonoids and saponins with strong antioxidant and antimicrobial activities.
Jan et al. (2022)	Bioactive characterization of ultrasonicated ginger and licorice freeze-dried extracts	Ultrasound-assisted extraction and bioactive characterization	Ultrasonication enhanced extraction efficiency and improved antioxidant activity and phytochemical stability in licorice extracts.
Sinha et al. (2021)	Identification of bioactive compounds from licorice as potential SARS-CoV-2 inhibitors	Pharmacoinformatics and molecular docking study	Several phytoconstituents from *Glycyrrhiza glabra* showed strong binding affinity against SARS-CoV-2 spike glycoprotein and non-structural protein-15, indicating potential antiviral activity.

5. CONCLUSION

The present study highlights the significant role of green extraction technologies and advanced structural characterization techniques in the isolation, identification, and analysis of bioactive phytoconstituents from Glycyrrhiza glabra. Eco-friendly extraction methods such as ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and enzyme-assisted extraction have demonstrated improved extraction efficiency, reduced solvent consumption, lower energy usage, and better preservation of thermolabile phytochemicals compared with conventional extraction approaches. Furthermore, advanced analytical techniques including HPLC, GC–MS, FTIR, NMR, and LC–MS have enabled precise structural elucidation, qualitative identification, and quantitative profiling of important compounds such as glycyrrhizin, glabridin, liquiritin, and other flavonoids and saponins. The integration of sustainable extraction processes with modern characterization technologies not only supports herbal drug standardization and quality control but also enhances pharmaceutical, nutraceutical, and biomedical applications of licorice-derived bioactive compounds. Overall, the study demonstrates that green extraction and structural characterisation approaches provide an effective and environmentally sustainable foundation for future phytopharmaceutical research and development.

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