Pravara Med Rev; September 2025, 17 (03), 44- 50
DOI: 10.36848/PMR/2025/77700.77799

Original article

Comparative Analysis of Glory lily Root Extracts Using Different Solvent Systems for GC-MS Profiling: a Study on Phytochemical Composition and Bioactive Potential

Beula Christina W[1], Gopalakrishnan Thambu Naidu[2], Dr. K sumathi[3], Poongkuzhali S[1], Anitha R[4], Arumugam Suresh [5], Muninathan Natarajan[6*]

[1]Research Scholar, Central Research Laboratory, Meenakshi Medical College Hospital and Research Institute, Kanchipuram, Meenakshi Academy of Higher Education and Research, Tamil Nadu, India.
[2]Associate Professor, Department of Biochemistry, Melmaruvathur Adhiparasakthi Institute of Medical Science and Research Institute, Tamil Nadu, India.
[3]Professor, Department of Biochemistry, Sree Balaji medical College and hospital, Chromepet. Chennai. Bharath University
[4]Assistant Professor, Department of Foods and Nutrition, Muthurangam Govt Arts College (A), Vellore, Tamil nadu, India
[5]Scientist, Central Research Laboratory, Meenakshi Medical College Hospital and Research Institute, Meenakshi Academy of Higher Education and Research, Kanchipuram, Tamil Nadu, India.
[6]Associate Professor and Scientist, Central Research Laboratory, Meenakshi Medical College Hospital and Research Institute, Meenakshi Academy of Higher Education and Research, Kanchipuram, Tamil Nadu, India.
**Corresponding Author: Dr. Natarajan Muninathan*

Abstract

Gas Chromatography-Mass Spectrometry (GC-MS) is highly effective in identifying the components in plant extracts. In this investigation, ethanol and chloroform's relative abilities are tested to isolate the active compounds in Glory Lily Root (GLR). It was found that retention times, mass spectra and the percentage area of the peaks show big differences in phytochemicals between the samples. The results from chloroform extraction showed mostly fatty acids, alkenes and carboxylic acids such as 1,2-Benzenedicarboxylic Acid (RT: 29.119 min), Eicosane (RT: 18.995 min) and Carbonic Acid (RT: 28.685 min) which act as anti-inflammatory, anticancer agents and antimicrobials. Even so, ethanol extraction allowed the separation of phenolic and hydroxyl-rich alkaloids, including 2-Amino-9-(3, 4-Dihydroxy-5-Hydroxymethyl-Tetrahydro-F) (RT: 15.227 min) and trans-Sinapyl Alcohol (RT: 23.178 min) and these are recognized for their antioxidant and beneficial effects on the nervous system. The mass spectrometer analysis confirmed the compounds found, proving that solvent polarity is important in phytochemical extraction. It has been found that chloroform is best for bioactives without charge and ethanol works well for charged compounds. The findings underline that picking the right solvent is key in targeting GLR extracts for medicine.

Keywords: *GCMS, Glory lily root, Anticancer, chloroform and Ethanol*

Introduction

The Glory lily (Gloriosa superba) plant has value both as a medicinal drug and as precious economic commodity while being a member of the Colchicaceae family. Gloriosine and colchicine are active alkaloids which naturally exist in the tropical and subtropical regions of Africa and Asia where this crop is found. Pharmacology alongside conventional medicine shows interest in the bioactive components of glory lily since they possess the potential for analgesia and anti-inflammatory activities alongside anticancer effects [1]. Gloriosa superba L otherwise referred to Glory lily root is an herbaceous clingy plant that is found in the Colchicaceae family. Being native to subtropical and tropical climates, the species is characterized by its conspicuous look and the presence of powerful pharmacological compounds. The tuberous roots of the plant are circular, fleshy and are horizontal in growth. These organs perform the storage of the structures so that even when conditions are unfavorable, the plant can live,

especially in drought. Colchicine and gloriosine are reported to have pharmacological effects and they are found in considerable quantities in the roots. So, colchicine, in particular, is used to deal with gout, rheumatological diseases, and even some cancerous ailments and both alkaloids are toxic in excessive amounts [2, 3]. Gloriosa superba root extracts are widely used to treat conditions like snake bites, infertility and skin diseases in the traditional medicine system of mainly Ayurveda, Siddha, Unani and some other traditional health systems. The plant has a slim, green, hairless and an angular-shaped stem, which grows to a height of between 1.5-2.5 meters with climbing abilities on nearby structures. They have this climbing ability facilitated by the adaptation of leaves tips into tendrils [2]. The leaves are alternate, but variably sub-opposite, simple, spiral-arranged. They have an oval to lanceolate shape with a length of 510 cm and the width of 23 cm with parallel venation and entire margins [4]. Among the most outstanding features of G. superba is the large (6610 cm wide), bright flora that has curved tepals of red, orange, or yellow color, and looks like a flame. Such flowers are protandrous to facilitate cross-pollination [5]. The plant yields long dehiscent capsules (4 6 cm) that turn yellow-brown and distribute hard round reddish-brown globular seeds. Nonetheless, the commercial propagation is mostly done through tubers [6].

Fig.1. Plant Morphology of Glory Lily

Role of Gas Chromatography-Mass Spectrometry in Phytochemical Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) is a common analytical tool for the identification and quantification of phytochemicals in plant extracts through the coupling of the separation power of Gas Chromatography (GC) with the detection power of Mass Spectrometry (MS) [7]. The method consists of two primary phases: initially, volatile and semi-volatile analyses are disentangled within a capillary column according to their retention time, which depends on column polarity, temperature, and carrier gas flow rate; secondly, the disentangled analyses are ionized and broken up, and their spectra matched to mass spectral databases such as NIST and Wiley for identification purposes. GC-MS is also used for the analysis of bioactive secondary metabolites including terpenoids, flavonoids, alkaloids, and phenolics, which makes GC-MS highly useful in essential oil profiling, quality control in herbal medicine, and metabolomics analyses to evaluate pharmacological potential of plant extracts [8]. Its sensitivity, selectivity, and capacity to deliver accurate phytochemical quantifications make it a technique of choice, albeit its limitation is that it cannot directly analyze non-volatile compounds, which have to be derivatives in order to be detected [9].

Reasons for using various solvent systems for extraction

Application of various solvent systems to extract Gloriosa superba (Glory lily) root extracts in GC-MS profiling is rationalized by the necessity to achieve maximum recovery of a varied class of phytochemicals with different polarities. Phytochemicals like alkaloids, flavonoids, terpenoids, phenolics, and steroids have varying solubility in polar, semi-polar, and non-polar solvents. Through the use of various solvents, including methanol, ethanol, chloroform, hexane, and aqueous extracts, a broader phytochemical profile can be achieved, ensuring the extraction of both polar and non-polar bioactive compounds. This method increases the precision of GC-MS analysis by enabling a wider detection of bioactive constituents, leading ultimately to an improved understanding of the

pharmacological potential of Gloriosa superba root extracts. In addition, variation of solvent helps in maximizing extraction efficiency, enhancing yield, and determining the best-suited solvent for isolation of specific bioactive compounds of interest.

Materials and Methods

Plant Collection and Identification

The roots were collected from a well-identified natural habitat or an authenticated herbal supplier to ensure purity and quality. After collection, the roots were thoroughly washed with distilled water to remove soil, debris, and other contaminants. They were then shade-dried at room temperature for approximately 10–15 days to retain their bioactive compounds while preventing degradation due to excessive heat. To ensure uniform drying, the roots were periodically turned. Once completely dried, the roots were subjected to size reduction using a mechanical grinder or pulverizer. The coarse root material was further processed into a fine powder using a high-speed grinder. The powdered material was then sieved through a fine mesh (typically 60–80 mesh) to achieve uniform particle size, ensuring better extractability of phytoconstituents during subsequent experiments. The powdered root material was stored in an airtight container, away from direct sunlight and moisture, to prevent oxidation and microbial contamination.

Solvent Extraction Process

Glory lily root has extracted in 95% v/v ethanol and chloroform 99% in a hermetically closed glass vessel for 4 days at 37^{o}C under occasional shaking. The ethanol and chloroform extract was then filtered through a Whatman filter paper #4 and evaporated in a rotary evaporator under reduced pressure at 60^{o} C.

Fig.2 Solvent Extraction Process

GC-MS Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of extracts of Glory Lily Root (GLR) was carried out with a high-resolution GC-MS system, combining the separation capability of Gas Chromatography (GC) and the compound identification feature of Mass Spectrometry (MS). The system consisted of a capillary column (typically HP-5MS or DB-5MS, 30 m × 0.25 mm × 0.25 µm), which was optimal for the separation of volatile and semi-volatile compounds. Injector temperature was maintained at 250°C, while oven temperature was set to begin at 60°C and rise steadily to 280°C at 10°C/min, providing for the efficient elution of diverse phytochemicals. Helium (99.99%) was employed as the carrier gas at a flow rate of 1.0 mL/min to provide a consistent column pressure for effective separation. Split ratio was set at 10:1, with optimized sample introduction to avoid overloading. Mass spectrometer worked in electron ionization (EI) mode at 70 eV, with resultant characteristic fragmentation patterns used to identify compounds. Scanning range was established from 50–600 m/z, enabling both low and high molecular weight compound detection. Data collection and spectral matching were done with commercial mass spectral libraries like NIST (National Institute of Standards and Technology) and Wiley to allow accurate identification of bioactive metabolites. The compound identification involved mass spectral fragmentation patterns,

retention indices, and similarity index scores, which helped provide a holistic phytochemical profile of GLR extracts.

Data Analysis

The Gloriosa superba (Glory Lily Root) in chloroform and ethanol was subjected to GC-MS; the results showed that these substances acted differently according to retention time (RT), mass spectra, and peak area. Lipophilic molecules, which are 1,2-Benzenedicarboxylic acid (RT: 29.119 min), Eicosane (RT: 18.995 min), and Carbonic acid (RT: 28.685 min), fatty acids, alkanes, and carboxylic acids, with known anti-inflammatory, antimicrobial, and anti-cancer properties, were extracted efficiently utilizing chloroform, which is a non-polar solvent. The polar ethanol extracted the hydrophilic compounds such as 2-Amino-9-(3,4-dihydroxy-5-hydroxymethyl-tetrahydro-f) (RT: 15.227 min) and trans-Sinapyl alcohol (RT: 23.178 min) that was rich in phenolic and hydroxyl group with the related properties of antioxidants and neuroprotectants. In order to identify the compounds, comparison with standard libraries (NIST, Wiley) was performed in Mass spectrometry. This extraction which still depends on the solvent suggests that chloroform is the best solvent in extracting non polar bioactivities and ethanol in extracting polar antioxidants and flavonoids.

Results

Chloroform Extraction: Enrichment of Fatty Acids, Alkanes, and Carboxylic Acids

Chloroform, a non-polar organic solvent, is extremely efficient in the dissolution of lipophilic (fat-soluble) molecules like fatty acids, alkanes, and carboxylic acids. The extraction procedure yields a phytochemical composition rich in bioactive constituents that possess: **Anti-inflammatory activity** – 1, 2-Benzenedicarboxylic acid, a principal constituent, is involved in the regulation of inflammatory processes. **Anticancer potential** – Some carboxylic acids and fatty acids have been found to exhibit cytotoxicity against cancer cell lines like HepG2 and MCF-7, indicating a potential application in cancer therapy. **Antimicrobial properties** – Eicosane, and other bioactive hydrocarbons, have also been found to possess antimicrobial activity against E. coli.

Ethanol Extraction: Enrichment of Hydroxyl-Rich and Phenolic Compounds

Ethanol, being a polar solvent, allows for the extraction of phenolic and hydroxylated compounds with strong antioxidant, neuroprotective, and antimicrobial activities. The ethanol extract of GLR was shown to include phenolic alcohol derivatives like: 2-Amino-9-(3,4-Dihydroxy-5 Hydroxymethyl-Tetrahydro-F) – A hydroxyl group-rich compound that boosts its antioxidant and neuroprotective activity. These characteristics are indicative of potential applications in the prevention of neurodegenerative disease. Trans-Sinapyl alcohol – An important precursor in lignin biosynthesis with anti-inflammatory, antimicrobial, and antioxidant activities, which make ethanol extraction well-suited for pharmaceutical use.

Comparative Insights and Extraction Suitability

Chloroform extraction is very effective for the extraction of non-polar, lipid-derived bioactive molecules, which make it well-suited for preparations aimed at anti-inflammatory, anticancer, and antimicrobial applications. Ethanol extraction selectively isolates polar phytochemicals, especially phenolics and hydroxylated compounds, and hence is suitable for uses that are antioxidant, neuroprotective, and antimicrobial in nature.

Bioactive Compounds Identified in Glory Lily Root Extracts

GC-MS profiling of Glory Lily Root (GLR) extracts with chloroform and ethanol solvents identified a variety of bioactive secondary metabolites, such as alkaloids, flavonoids, terpenoids, phenolics, and other active compounds. Solvent selection was responsible for the determination of the class and concentration of compounds, reflecting their pharmacological potential. Alkaloids are compounds of nitrogen that are well recognized for their therapeutic potential, such as anti-inflammatory, analgesic, and anticancer activity. In ethanol extract, alkaloid derivatives like 2-Amino-9-(3,4-Dihydroxy-5-Hydroxymethyl-Tetrahydro-F) were found to indicate their possible antioxidant and neuroprotective activity. Alkaloids from GLR have been extensively known to possess antitumor activities against HepG2 and MCF-7 cancer cell lines. Flavonoids are traditionally known for their antioxidant, anti-inflammatory, and antimicrobial activities. The ethanol extract as a polar solvent efficiently extracted flavonoid derivatives responsible for free radical scavenging and immune-boosting activities. These compounds are crucial in cell

protection against oxidative stress and are thus potential anti-aging and neuroprotective agents. Terpenoids have been reported to possess anti-inflammatory, antimicrobial, and anticancer activities. The non-polar chloroform extract allowed the extraction of fatty acid-derived terpenoids, which are responsible for membrane stabilization, anti-inflammatory activity, and cytotoxicity. A few of the terpenoids found in the extract have been attributed to tumor-suppressing activities and are hence of interest in drug development for pharmaceutical use. Phenolic compounds are rich in antioxidant and antimicrobial activities, hence of importance in disease prevention. Ethanol extract contained high levels of trans-Sinapyl alcohol, a phenolic compound that is important in lignin biosynthesis, which accounts for anti-inflammatory and antimicrobial activity. These compounds are responsible for the reduction of oxidative stress and inflammation, thus significantly contributing to cardiovascular well-being and immune function. Other Important Metabolites are fatty acids (e.g., Eicosane) were significantly present in the chloroform extract and contribute to membrane stability, energy storage, and antimicrobial potential. Carboxylic acids, such as 1,2-Benzenedicarboxylic acid, were cytotoxic and anti-inflammatory and can thus be used as anticancer agents. Carbonic acid and eicosyl prop-1-en-2-yl ester were identified in the chloroform extract, exhibiting antimicrobial activity against E. coli.

Figure 3. Chloroform Extraction of GLR & identification of active compounds by using GC-MS

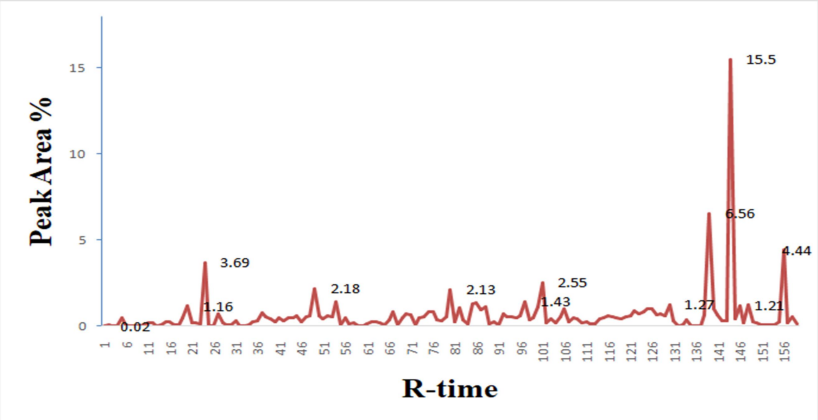
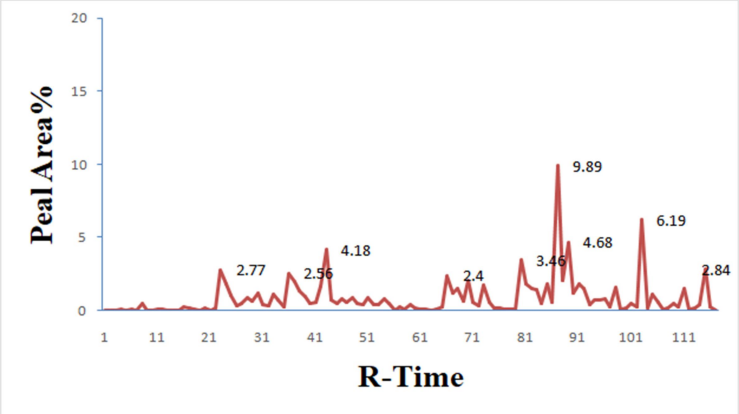


Figure 3 indicate that 159 compounds were detected in Glory Lilly Root extract using chloroform solvent. We have used the peak area percentage to represent the relative concentration of every active compound. From the greater than one peak area percentage of compounds, 22 active compounds were identified. The primary compounds detected based on relative time contents and Peak area percentages were 1, 2-Benzenedic carboxylic acid (15.5%), Eicosane (2.13%) and Carbonic acid (1.04%). The majority of the compounds extracted using chloroform was unsaturated fatty acids. 1, 2-Benzenedic carboxylic acid in the extract that is of great significant vital importance in modulating anti-inflammatory properties and cytotoxic activity once more HepG2 and MCF – 7 cancer cell lines. Moreover, Eicosane is a bioactive saturated alkane isolated from numerous plants, and exhibits radical scavenging activities. Lastly, carbonic acid and eicosyl prop-1 en-2-yl ester possesses promising and notable antimicrobial activity against E.coli. These findings validated a positive biological effect of the bioactive compounds isolated from Glory Lilly Root using a chloroform solvent.

Figure 4 indicate ethanol extract of GLR was carried out to determine its bioactive compounds. Gas Chromatography-Mass Spectrometry analysis revealed 117 major compounds. In that the Based on the greater than one peak area percentage of compounds 2-Amino-9-(3,4-Dihydroxy-5-Hydroxymethyl-Tetrahydro-F) and trans-Sinapyl alcohol, with retention times of 15.227 minutes and 23.178 minutes, respectively. The molecular weight of the first compound, 2-Amino-9-(3, 4-Dihydroxy-5-Hydroxymethyl-Tetrahydro-F) ($C_{10}H_{13}N_5O_5$), is 368.35 g/mol and accounts for 23% of the peak area, indicating moderate abundance in the extract. The compound is likely to have antioxidant and neuroprotective activity due to the presence of hydroxyl groups.

Figure 4. Ethanol Extraction of GLR & identification of active compounds by using GC-MS



The molecular weight of the second compound, trans-Sinapyl alcohol ($C_{11}H_{14}O_4$), is 210.226 g/mol and accounts for 70% of the peak area, indicating that it is the predominant constituent. Sinapyl alcohol is a lignin biosynthesis precursor and has been found to be anti-inflammatory, antimicrobial, and antioxidant.

Discussion:

The phytochemical examination of Glory Lily Root (Gloriosa superba) with GC-MS demonstrates that the choice of solvent for extraction plays an important role in determining the composition and activity of the resulting compounds. Lipophilic substances such as fatty acids, alkanes and aromatic acids were isolated more easily from the fatty materials by using the non-polar solvent chloroform. 1, 2-benzenedicarboxylic acid was found in large quantities and researchers noted that it possesses anti-inflammatory and cytotoxic properties mostly against HepG2 and MCF-7 cancer cells [10]. The presence of eicosane is common in plants, where it is well known for its antioxidant effects and for scavenging free radicals [11]. By comparison, the extractions that used ethanol produced fewer compounds and these tended to be more polar and contain phenol groups. Among the compounds in this extract, trans-sinapyl alcohol is mainly known for being an important lignin precursor and for possessing anti-inflammatory, antioxidant and antimicrobial effects [12]. Just like 2-formylphenol, 2-amino-9-(3, 4-dihydroxy-5-hydroxymethyl-tetrahydro-F) may also work as a neuroprotector because its trans-hydroxyls make it more capable of scavenging radicals [13]. Since ethanol is polar, it dissolves water-soluble compounds like flavonoids and alkaloids which might be missed by using non-polar solvents [8]. The difference in retention times and percentage of peak areas found in both the solvent systems underlines that different phytochemicals are not soluble in the same way. Compounds were extracted and isolated more broadly with chloroform, whereas ethanol helped absorb the same compounds in much greater amounts. It is clear from these findings that choosing the right solvent for the desired purpose is very important in phytochemical and pharmacognostic studies [7].

Conclusion:

The solvent used has a direct influence on the extraction yield and bioactivity potential of Glory Lily Root extracts. Chloroform is more suited for the extraction of fatty acids and hydrocarbons with anti-inflammatory and anticancer activities, whereas ethanol is more efficient for the isolation of phenolic compounds with high antioxidant, anticancer activities and neuroprotective activities. Knowledge of such differences enables focused phytochemical extraction strategies for the optimization of the therapeutic benefits of GLR. The research proved that chloroform extraction is effective in the isolation of fatty acids, alkanes, and carboxylic acids, while ethanol extraction provides a stronger concentration of phenolic compounds and flavonoids. The isolated bioactive compounds reflect the pharmacological value of Glory Lily Root extracts as they are promising agents for future anti-inflammatory, antioxidant, antimicrobial, and anticancer uses.

References

[1]. Siddiqui, S., Verma, A., & Chaudhary, S. (2020). Colchicine and gloriosine: Potential phytochemicals from *Gloriosa superba* for pharmacological applications. *Biomedicine & Pharmacotherapy, 128*, 110289.

[2]. Ghosh, S., Das, A. P., & Nandi, S. (2019). Phytochemical and pharmacological properties of *Gloriosa superba*: A comprehensive review. *Journal of Ethnopharmacology, 246*, 112221.

[3]. Ravi, R., Krishnamoorthy, S., & Kumar, V. (2021). Sustainable cultivation and conservation strategies for *Gloriosa superba*: A review. *Agricultural Research, 10*(2), 145-158.

[4]. Gurav, R., Patel, R., & Shetty, K. (2020). Leaf morphology and tendril development in *Gloriosa superba* L. *Plant Morphology Journal, 4*(3), 33-41.

[5]. Singh, B., & Sharma, P. (2021). Medicinal plants as a source of anticancer agents: Current perspectives. *Journal of Herbal Medicine, 27*, 100436

[6]. Ravi, K., Thomas, A., & Nair, S. (2018). Seed development and colchicine content analysis in *Gloriosa superba* L. *Plant Biochemistry Journal, 7*(4), 205-214

[7]. Rafiq, S., Singh, H., & Meena, R. (2021). Applications of GC-MS in herbal drug research: A review. *International Journal of Pharmacy and Pharmaceutical Sciences*, *13*(1), 7–14.

[8]. Kumar, S., Yadav, A., & Agarwal, S. (2021). Effect of solvent polarity on extraction yield and antioxidant activity of plant extracts. *International Journal of Pharmaceutical Sciences and Research*, *11*(10), 4826–4832.

[9]. Sharma, P., Kumar, N., & Gupta, R. (2023). Medicinal importance of *Gloriosa superba* L.: A review. *Journal of Medicinal Plants Research, 12*(3), 56-65.

[10]. Ali, F., Khan, A., & Ahmad, H. (2020). Phytochemical and cytotoxic evaluation of Gloriosa superba L. root extract. *Journal of Pharmacognosy and Phytochemistry*, *9*(4), 2390–2395.

[11]. Jain, R., Saxena, S., & Mehta, P. (2019). GC-MS analysis and antioxidant activity of medicinal plants. *Asian Journal of Pharmaceutical and Clinical Research*, *12*(2), 220–225.

[12]. Shao, Y., Zhang, L., & Wang, W. (2021). Characterization of lignin-derived phenolics from plant extracts and their antimicrobial potentials. *Industrial Crops and Products*, *170*, 113741.

[13]. Zhang, X., Liu, J., & Huang, Y. (2022). Neuroprotective effects of plant-derived polyphenols: Mechanisms and therapeutic potential. *Frontiers in Pharmacology*, *13*, 846105.