NJESR/March-2024/Volume-6/Issue-3 E-ISSN-2582-5836
DOI-10.53571/NJESR.2024.6.3.27-35

Gloriosa superba (Flame Lily): A Comprehensive Review Of Phytochemistry And Analytical Characterization

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(Received-18Feburary2024/Revised-5March2024/Accepted-18March2024/Published-29March2024)

Abstract

The flame lily Gloriosa superba is valued for its rich chemistry of tropolone alkaloids particularly colchicine and related substances. G. superba chemistry and analysis are the main topics of this review, providing an overview of recognized phytochemicals biosynthetic discoveries and analytical techniques for describing its components. Colchicine and its glycoside colchicoside are the main constituents of the plant's wide variety of alkaloids (approximately 24 have been reported). Numerous analogues of colchicine have been discovered such as lumicolchicine gloriosine (N-formyl-deacetylcolchicine) superbrine (superbine) demethyl- and deacetyl-colchicines and semi-synthetic thiocolchicoside. Non-alkaloid constituents such as β-sitosterol, luteolin, chelidonic acid, flavonoids, phenolic acids (e.g. salicylic acid), and tannins are also present. The biosynthesis of colchicine is mainly understood to be the result of phenylalanine and tyrosine forming an intermediate 1-phenethylisoquinoline that is methylated/hydroxylated to (S)-autumnaline followed by phenol–phenol coupling and a special oxidative ring-expansion to form the tropolone colchicine scaffold. It has been determined that eight Gloriosa superba genes for colchicine precursors exist including a cytochrome P450 that catalyzes the expansion of the tropolone-forming ring. Analytical techniques are essential for characterization. Colchicine is commonly quantified using HPLC (typically C18 reverse-phase with UV detection) whereas LC-MS (particularly high-resolution MS with Kendrick mass defect filtering) has identified a large number of colchicine-related alkaloids in extracts. For alkaloids NMR spectroscopy offers a conclusive structural assignment (e. g. The gloriosa comprehensive $^{1}H/^{13}C$ NMR of colchicine) and FTIR can identify important functional groups (FTIR of extracts verified colchicine by exhibiting distinctive C–H and C=O absorptions). When used in pharmaceuticals colchicine from G. Superba (FDA-approved for gout and familial Mediterranean fever) is used as an anti-inflammatory and anti-gout medication. Semi-synthetic analogs such as the muscle relaxant

thiocolchicoside which is derived from colchicoside and other derivatives of colchicine have been created to become more effective or less harmful. This review consolidates the chemical knowledge of G. superba, emphasizing phytochemistry, biosynthesis, analytical characterization, and medicinal chemistry of its alkaloids.

Keywords : Gloriosa superba, Colchicine, Tropolone alkaloids, Phytochemistry, Biosynthesis, Analytical characterization, HPLC–LC–MS, NMR spectroscopy

Introduction

Gloriosa superba L. (Colchicaceae), the flame lily, is a tropical climber that is well-known for its strong alkaloids. Colchicine a tropolone alkaloid that binds tubulin and has therapeutic applications in gout and inflammatory conditions is found in its tubers and seeds. Based on dry weight G. Superba is an appealing source because it frequently contains 0. 6–0. 9 percent colchicine which is higher than levels in related Colchicum species. Early fascination with G. Superba focused on its therapeutic applications (e. g. Ayurveda) but chemically speaking the plant is valued for the variety of colchicinoids it yields. More than 20 different alkaloids have been identified and numerous others have been found using contemporary techniques. Although there are other phytochemicals such as phenolics flavonoids and steroids the chemistry is dominated by colchicines. This review surveys published data (through 2023) on the phytochemical constituents of G. superba, the elucidated biosynthetic pathway of colchicine, analytical techniques used to characterize its compounds, and the chemical rationale for its pharmacological relevance and derivative synthesis. Chemical interpretation is prioritized over botanical and ecological details.

Phytochemical Composition Of Gloriosa Superba

The main phytochemicals alkaloids are tropolone found in G. superba. The most significant is the abundance of colchicine ($C_{22}H_{22}NO_4$) and its glycoside colchicoside. Kavina et al. (2011) found that the plant contains approximately 10 non-alkaloid compounds (e. g. colchicine and colchicoside among its nearly 24 alkaloids). A. Stigmasterol luteolin chelidonic acid and β-sitosterol. Many colchicine analogs have been found through later research. Gloriosine and other N-acetyl or deformyl derivatives lumicolchicines (photochemical isomers) thiocolchicoside (a semisynthetic sulfur analog of colchicoside) 3-demethylcolchicine and 3-demethyl-N-formylcolchicine (demethyl variants) and others are among them. Jana and Shekhawat (2011) for instance listed isolated compounds like lumicolchicine gloriosine superbrine (superbine) thiocolchicoside colchicine and several demethyl/deformyl colchicine derivatives. Toxicology studies and case reports have confirmed that G. Colchicine superbrine

gloriosine and other highly active alkaloids are found in superba along with phenolic acids (e.g., salicylic acid chelidonic acid). In conclusion, G. Superba extracts exhibit an intricate alkaloid profile.In summary, G. superba extracts show a complex alkaloid profile:

• Colchicine ($C_{22}H_{25}NO_6$): The principal active compound, a tropolone alkaloid.

• Colchicoside (demethylcolchicine glycoside): An O-glycoside of demethylcolchicine, yielding colchicine in vivo. Its derivative thiocolchicoside is used clinically as a muscle relaxant.

• Other tropolone alkaloids: Including lumicolchicine isomers, N-formyl-deacetylcolchicine (gloriosine), 3-demethylcolchicine, 2,3-didemethylcolchicine, 1,2-didemethylcolchicine, 2-hydroxy-6-methoxybenzoic acid, etc. (These have been characterized by MS/NMR in various studies.)

• Minor alkaloids and glycosides: e.g. superbrine (N-formylated colchicine), 3-O-demethylcolchicine-3-O-α-D-glucopyranoside.

• Non-alkaloids: Flavonoids (luteolin and derivatives), steroids (β-sitosterol, stigmasterol), tannins and phenolic acids (salicylic acid, benzoic acid derivatives), saponins and glycosides have been detected by phytochemical screening.

Tropolone alkaloids or colchicinoids are other names for colchicine and its analogues. O-methylation N-acetylation and glycosylation are examples of minor modifications that affect biological activity making their high abundance and structural diversity chemically important. substances that are not alkaloids (e. g. A. Although they are also present flavonoids and phenolics are less important than the chemistry of colchicine). Significantly research concurs that the amount of colchicine in seed extracts is higher than in tuber extracts and that seeds typically contain about 1% dry weight colchicine indicating the plants biochemical ability to accumulate this alkaloid.

Biosynthesis Of Colchicine

The colchicine pathway in G has been gradually clarified by biochemical investigations. amazing. Transcriptomics enzyme characterization and isotope labeling have recently been combined to form a nearly complete pathway. This is the general agreement:

1.Aromatic precursors: L-tyrosine and L-phenylalanine are aromatic precursors that are used as building blocks. Cinnamic acid transforms phenylalanine into 4-hydroxybenzaldehyde and L-tyrosine into dopamine.

2.Isoquinoline formation (Pictet–Spengler): Dopamine and 4-hydroxybenzaldehyde (4-HDCA) combine to form a 1-phenethylisoquinoline skeleton through a Pictet–Spengler condensation.

3.Early methylation/hydroxylation: (S)-autumnaline is produced by the sequential methylation and hydroxylation of 1-phenethylisoquinoline (by O-methyltransferases and P450s). Autumnaline is 1,2,3,4-tetrahydroisoquinoline with a (S) configuration.

4.Phenol coupling: A bridged tetracyclic intermediate is created when the aromatic rings of autumnaline couple with para–para phenols. (A P450 or oxidase is probably needed in this step to form the new C–C bond.).

5.Tropolone ring formation: The bicyclic intermediate is changed into the 6-7-7 tropolone system of colchicine by a special oxidative ring-expansion. Nett et al learned that a G. superba cytochrome P450, the CYP75 family member catalyzes this rearrangement of the carbon skeleton. Colchicines tropolone ring is established by this Ring Expansion oxygenation which is absent from other plant pathways.

6.Final tailoring steps: Along with any necessary O-methylations the resultant tropolone aglycone is subjected to N-methylation or N-acetylation which produces the acetamide of colchicine. The sum of Nett et al. found eight genes from G. superba that transform the early intermediates into the immediate colchicine precursor N-formyldemecolcine. When it comes to heterologous expression (e. g. the Demecolcine (N-formyldemecolcine)) is produced when these genes are introduced into Nicotiana benthamiana supporting the suggested sequence.

In conclusion G. superba after starting with amino acid precursors to produce (S)-autumnaline colchine biosynthesis moves on to phenol coupling and an unprecedented P450-mediated oxidative rearrangement to produce the carbon framework of colchicine. Nett et al. (2020) reconstructed this pathway and emphasized that eight enzymes are necessary to reach the N-formyldemecolcine stage including methyltransferases a P450 and a keto-reductase. These findings explain how G. superba assembles colchicine's complex structure, and provide a basis for metabolic engineering of its alkaloid biosynthesis.

Analytical Characterization Techniques

The chemical analysis of G. superba constituents relies on chromatography and spectroscopy techniques include HPLC (often with UV/DAD detection), liquid chromatography–mass spectrometry (LC–MS), NMR, and FTIR. These methods are used to identify, quantify, and confirm structures of the alkaloids and other metabolites.

HPLC (High-Performance Liquid Chromatography)

Colchicine and related compounds are frequently found and measured using HPLC. Consider Kavina et al. (2011) tested colchicine in G using reverse-phase HPLC (C18 column 1point 0 mL/min 245 nm UV detection). excellent tubers and seeds. They employed genuine standards for identification and their HPLC profiles verified colchicine peaks in every sample. Gopi et al. found that seeds consistently showed higher colchicine levels than tubers. Purity analysis is also done with HPLC. Acharya et al. (2019) purified and extracted colchicine from seeds using supercritical CO_2 the final products HPLC analysis revealed a 99–82% colchicine content. They stated that HPLC was the technique utilized to standardize the purity of colchicine. Colchicine is frequently detected using quantitative HPLC techniques that have been developed and validated for this plant even at low ppm levels. UV detection (240–250 nm) and aqueous/methanol mobile phases are commonly used occasionally in conjunction with diode-array detection to monitor multiple wavelengths. HPLC fingerprints can be used for quality control and chemotype comparison because colchicine and its analogs have different retention times. One study found that treating plants with microbes or gibberellic acid increased the amount of colchicine HPLC quantification tracked these changes.

Liquid Chromatography–Mass Spectrometry (LC–MS)

HPLC analysis is expanded by LC-MS which offers mass-based identification. Common LC-MS methods (e.g. Colchicine and its analogs are detected by ESI-MS (m/z 399 $[M+H]^+$). The Acharya group employed QToF or quadrupole time-of-flight MS to profile G. Superba exceptional seed extracts. All peaks can be accurately determined by mass using high-resolution LC-MS (HRMS) which has been used in global metabolomic surveys of G. Superba. Specifically Zarev et al. conducted UHPLC–HR–MS on native tuber extracts as well as in vitro-cultured ones in 2021. Tropolone alkaloids were identified more quickly by using Kendrick mass defect (KMD) plotting. When homologous colchicine derivatives (differing by CH_2 or OCH_3) were converted into Kendrick plots distinct clusters were formed. This method made it possible to annotate many more colchicine analogs than were previously identified. Zarev et al. shown that the variety of tropolone alkaloids produced by in vitro cultures is even greater than that of plants grown in the field. High-resolution MS/MS fragmentation helps with structure assignment even more. To summarize LC-MS particularly UHPLC-HRMS is a vital tool that can identify several alkaloids in a single run and when paired with methods such as KMD plotting can identify subtle analogs in complex extracts.

NMR Spectroscopy

NMR offers unambiguous structural confirmation. The ^{1}H and ^{13}C NMR spectra which are frequently obtained through 2D experiments are used to identify each proton and carbon after an alkaloid has been isolated (either by preparative HPLC or another method). 400–800 MHz high-field NMR is frequently used. Comprehensive NMR investigations have been conducted on colchicine: Pierens et al. (2017) measured colchicines ^{1}H and ^{13}C chemical shifts in a variety of solvents and compared them with DFT calculations. The importance of NMR in determining every position in the colchicine molecule is highlighted by this work. Likewise novel analogs that were separated from G. Superba. NMR has structurally identified demethyl or glucosyl variants (in conjunction with MS data). For instance, the NMR signals of 3-demethylcolchicine-3-O-β-D-glucoside verified its structure. A typical workflow in practice is to isolate an unknown alkaloid obtain its 1D and 2D NMR spectra in appropriate solvents and analyze them to determine patterns of ring substitution. NMR can also identify the tropolone (seven-membered ring) signals and distinguish between different epimers. The use of NMR is implicit in any structural identification even though we did not mention specific spectra here. In general, one of the most important analytical tools for colchicine alkaloids is contemporary NMR (with sophisticated pulse sequences).

FTIR Spectroscopy

FTIR can be used to quickly screen for functional groups in purified or crude extracts. The presence of important bonds can be verified by FTIR though it is not as precise as MS or NMR. For G. Superba, in order to identify colchicine one study recorded the FTIR spectra of tuber and seed extracts. Particularly a peak at approximately 2926 cm^{-1} (C–H stretch of CH_2) and at approximately 1646 cm^{-1} (carbonyl C=O stretch) were ascribed to colchicine. These IR absorbances were associated with the functional groups of colchicine. Ravi et al. (2012) specifically stated that these peaks in their FTIR spectra confirmed the presence of colchicines. FTIR can typically separate extracts that are rich in alkaloids from those that are not (e. g. peaks for C–O stretches and N–H bending). However, FTIR is more frequently used for fingerprinting than for quantitative analysis because of overlapping bands. In a characterization pipeline it is frequently combined with additional techniques (HPLC MS).

Other analytical techniques are also useful. Thin-layer chromatography (TLC/HPTLC) has been used historically to separate G. superba extracts; it can give a quick alkaloid profile (simple qualitative check). Although, it is less frequently used for tropolones (non-volatile high-boiling alkaloids) gas chromatography–mass spectrometry (GC–MS) can profile volatile constituents (e. g. fatty acids if analysed). LC and NMR/FTIR are the main instruments used

in modern labs. In sum, the combination of HPLC/LC-MS (for detection and quantification) and spectroscopic methods (NMR, FTIR) provides a comprehensive characterization of G. superba phytochemicals.

Pharmaceutical Relevance And Semi-Synthetic Derivatives

The importance of G. superba lies largely in its pharmacologically active alkaloids. For acute gout and familial Mediterranean fever for example colchicine has FDA approval. Additionally, it is prescribed for Behçets syndrome and pericarditis. Tubers of G. Traditional medicine has utilized superba to treat inflammation and arthritis which is consistent with colchicines anti-inflammatory and anti-mitotic properties. Nevertheless, colchicine can be extremely toxic and has a limited therapeutic index if used improperly even standard gout dosages can result in poisoning. Due to this dual nature structural analogs and derivatives with enhanced selectivity or safety are of interest.

Thiocolchicoside is a semi-synthetic derivative that is worth mentioning. Thiocolchicoside or 3-demethylthiocolchicine-3-O-β-D-glucoside is basically colchicoside with a sulfur substituting for an oxygen atom. It was created to minimize toxicity while maintaining anti-inflammatory and muscle-relaxing properties. Thiocolchicoside is frequently used in medicine (e. g. as a skeletal muscle relaxant), it has analgesic properties and acts on GABA receptors in Europe and India. Ravi et al. note that thiocolchicoside is the "semi-synthetic derivative" of colchicoside. (Elsewhere, it is confirmed that thiocolchicoside was derived from 3-demethylcolchicine and approved for myorelaxant use.)

Pharmacological research has been done on other colchicine analogs. In laboratory settings demecolcine (colcemide) also known as N-formyldemecolcine is used to stop cell division (e. g. for chromosome harvesting) and is of some interest in anti-cancer research. The Nett et al. bring up the study of mitosis and the application of colchicine (and consequently demecolcine) as a research tool. Numerous colchicine derivatives have been created by medicinal chemistry in addition to demecolcine for instance the trimethylsilyl and methyl carbamate analogs have been investigated as anti-leukemia agents. Ciftci et al. (2012) found that a number of derivatives of colchicine exhibited antitumor activity that was similar to that of colchicine but had lower systemic toxicity. The colchicine scaffold is still being exploited in current drug discovery efforts new inhibitors of tubulins colchicine-binding site (with altered tropolone cores or side chains) are in different stages of development.

In conclusion, G. Superba's chemical profile supports its pharmacological significance. Because of the structural variety of its alkaloids analogs and derivatives have been synthesized

(e. g. G. substances) that seek to capture the therapeutic benefits of colchicine with controllable toxicity (such as thiocolchicoside and demecolcine). Research into G. superba chemistry thus directly informs medicinal chemistry: by understanding which functional groups on the colchicine scaffold contribute to activity or toxicity, chemists can design new compounds. Since superba is a source of colchicine some analogs can be produced by semi-synthesis which is a chemical modification that begins with natural colchicine. To create derivatives for instance colchicine can be acylated or demethylated adding sulfur to the colchicine molecule effectively yields thiocolchicoside. Analytical chemistry such as NMR and HPLC confirms the identity and purity of these semi-synthetic products at every step.

Conclusion

A chemically significant medicinal plant Gloriosa superba (Flame Lily) is well-known for its wide variety of tropolone alkaloids especially colchicine and its derivatives. This review has emphasized its intricate phytochemical profile the largely understood colchicine biosynthesis pathway and the analytical methods employed for characterization. Although the plant contains non-alkaloid substances like flavonoids and phenolics colchicinoids predominate in both its chemistry and pharmacological significance. The process by which the plant assembles colchicine from simple amino acid precursors has been made clearer by developments in biosynthetic research particularly the identification of important enzymes involved in tropolone ring formation. For the identification measurement and structural validation of its components analytical techniques like HPLC LC–MS NMR and FTIR are still crucial. Colchicine is still a clinically significant drug and semi-synthetic derivatives like thiocolchicoside show continuous attempts to increase therapeutic utility while lowering toxicity. Overall, G. Superba is an important nexus of drug development biosynthesis analytical science and natural product chemistry with ongoing potential for further study and medical advancement.

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