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Analysis of the Primary Phytochemical Profile of *Gloriosa superba* Linn. Leaves, Tubers, and Seeds from Dharwad Region of Karnataka, India

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Abstract: Sophisticated analytical techniques such as high-performance liquid chromatography and gas chromatography coupled with mass spectrometry and tandem mass spectrometry are now available for efficient quantitative and qualitative analysis of desired compounds in samples. However, traditional biochemical assays remain widely popular as simple and reliable methods for qualitative screening of major phytochemical classes present in the extracts of medicinal plants. These chemical reactions are generally universal for all the compounds categorized under a given broad group but do not reveal the individual identities of the reacting entities. *Gloriosa superba* is a valued medicinal herb, widely distributed in the Western Ghats of India. It is a traditional remedy for a number of health-related issues. Compounds isolated from the plant also hold importance as precursors to many drugs in modern medicine. The present study was undertaken to establish the broad phytochemical composition of leaves, tuberous roots, and seeds of *Gloriosa superba* from Dharwad region of Karnataka. All parts of the plant were found to contain significantly high levels of alkaloids, followed by polyphenolic compounds including flavonoids and tannins. Carbohydrates and phytosterols were the other major chemical classes present.

Keywords: Phytochemical screening, glory lily, secondary metabolites, alkaloids, qualitative analysis

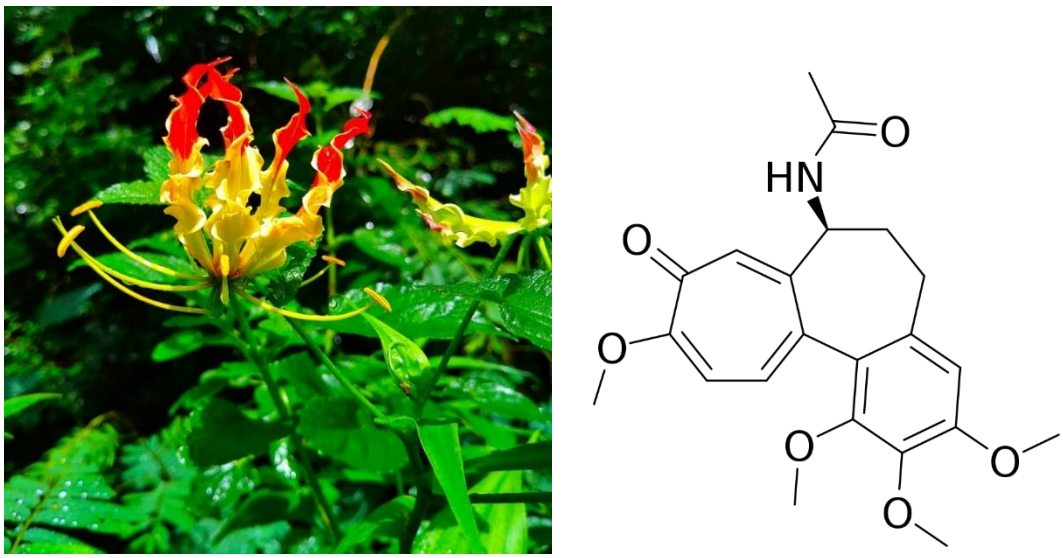
I. INTRODUCTION

Plants with therapeutically active phytochemical compounds have been the traditional source of drugs for the treatment of human ailments. Herbal preparations have been prescribed by ancient as well as contemporary medical practitioners as remedies for various diseases and health-related issues. Nearly 80 percent of the global population, mostly the indigenous and tribal people, are still largely dependent on natural floral resources to meet their healthcare requirements (Sasidharan et al., 2011). The Indian subcontinent is home to between 3,000 and 5,000 species of medicinal plants, and nearly 1,000 of these face the threat of extinction due to uncontrolled exploitation and habitat loss (Schippmann et al., 2005; Pullaiah et al., 2017 Chapter 6). Of the total plant species, more than 2,400 have been well-documented in traditional literature for medicinal use in some form (Tushar et al., 2010; Sajem and Gosai, 2006).

India has been home to the Ayurvedic and Siddha systems of traditional medicine, whose practitioners are known as *Vaidyas* and *Siddhars*, respectively. These traditional systems heavily rely on medicinal preparations and formulations largely derived from plant resources (Naik and David, 2023). Apart from the aforementioned systems that are uniform, well-organized, and well-documented, there exist highly localized and not so well documented folk medicinal practices of the aboriginal people and the tribal groups (*Adivasis*). In Ayurveda, the remedies may be prescribed in the form of dry powders (*choorna*), semi-dry pellets (*vati*), juices (*rasa*), individual decoctions (*sattva*, *arka*), or mixed concoctions (*kwatha*) (Abraham et al., 2020). Most of these are taken internally. Some preparations may be applied topically (*lepa*) to relieve pain and treat skin diseases. The preparations vary in their composition, either consisting of a particular part (leaves, roots, inflorescence, fruit, seeds, stem, bark) of a single plant, or different parts of the same plant, or derived from multiple plant species. *Triphala* is one such example of a multi-herb formulation and among the most widely prescribed Ayurvedic remedies. It is derived from fruits of three different species – *amalaki* or amla (*Phyllanthus emblica*), *haritaki* or harad (*Terminalia chebula*), and *vibhitaki* or baheda (*Terminalia bellirica*) (Peterson et al., 2017). However, traditional literature does not provide much information regarding the chemical nature and definite identity of active phytochemical compounds responsible for the therapeutic effect of a given herbal preparation. Some preparations do mention different methods of extraction and the choice of medium (water/milk/oil). As different compounds have differential solubility in different liquids, the choice of medium may be indicative of the chemical nature of the active therapeutic compound.

The Western Ghats mountain range is spread across the Indian states of Maharashtra, Goa, Karnataka, and Kerala. The region is listed as a global biodiversity hotspot and is a habitat for over 1500 endemic species of plants (35% of total floral species found there) (Pullaiah et al., 2017 Chapter 1). *Gloriosa superba* Linn. or glory lily is a perennial plant native to various parts of Africa and Southeast Asia. It is abundantly found in the Western Ghats and is systematically placed in the Colchicaceae family of flowering

plants because of the high concentration of colchicine and colchicine-derived compounds in all its parts, mainly tuberous roots and seeds (Badwaik et al., 2011). The plant is also rich in another compound named gloriosine, a colchicine-like cyclic ketone (Goel et al., 2022). In Ayurveda, it is known by various names including *agnishikha*, *kalikari*, and *langalika*. Its prescription is well-documented for relieving health issues such as indigestion, constipation, abdominal pain, inflammation, skin disorders, and difficult childbirth (Dabire and David, 2021; Augustine et al., 2010). Higher doses of any part are highly toxic and may prove fatal. The tubers are pungent, bitter, acrid, and heating and have laxative, alexiteric, and anthelmintic properties (Jana and Shekhawat, 2011). Colchicine is used in modern medicine for the treatment of rheumatism and arthritis. The compounds found in the plant are also candidate drugs for the treatment of cancer (Goel et al., 2022). The plant has also been described as *garbhaghatini*, which means 'destroyer of the fetus', and is indicated as a potent abortifacient because of its destructive effect on the conceptus when administered orally during pregnancy (Mali et al., 2006). The present study was undertaken to determine the major phytochemical groups present in *Gloriosa superba* by preparing hot solvent extracts (crude aqueous and ethanol extracts) of tubers, leaves, and seeds.



(a) **(b)**

Fig. 1. (a) *Gloriosa superba*, Karnatak University Campus, Dharwad, India **(b)** Chemical structure of colchicine

II. RESEARCH METHODOLOGY

Experimental plant

Fresh leaves, tubers, and seeds of *Gloriosa superba* Linn. were collected from the Karnatak University campus, Dharwad, India (15.27'36 °N, 75.0'37 °E) during September 2022 and authenticated by experts from the Department of Botany, Karnatak University. A voucher specimen (accession no. KUD/Zoo/2020-21/3) was previously deposited in the herbarium of Karnatak University, Dharwad. No rules concerning biodiversity rights were violated in procuring the plant material for the present study.

Extraction

The leaves and tubers were checked for microbial infestation and washed under tap water. The tubers were sliced. The samples were shade-dried at room temperature and separately pulverized using an electronic grinder. The powders were sieved, weighed, and stored. Ethanol extracts of tuber, seeds, and leaf were obtained by extracting 30 g of powder with 300 ml of ethanol in a Soxhlet apparatus (at 50 °C till the solvent ran clear). The obtained extracts were filtered using Whatman Grade 1 filter paper, evaporated in a rotary evaporator, and dried overnight in a laboratory oven at 35 °C. Dried extracts were preserved in air-tight containers at 4 °C until further use. For aqueous extracts, 10 g of each sample was boiled in 100 ml of distilled water for 5 minutes, cooled, and filtered.

Phytochemical screening

Dried ethanol extracts were redissolved in ethanol (1 g in 10 ml) and diluted with 40 ml of distilled water to get 2% solutions. The extracts were subjected to biochemical tests for the determination of primary phytochemical groups (alkaloids, flavonoids, polyphenols, cardiac glycosides, saponins, tannins, and phytosterols) according to previously described protocols (Shah and Hossain, 2014; Iqbal et al., 2015; Kancherla et al., 2019). The chemicals used were of analytical grade and procured from local suppliers.

1. Tests for carbohydrates

- ***Molisch test*:** To 3 ml of test solution in a test tube, 2-3 drops of freshly prepared Molisch reagent (20% α-naphthol solution in 95% ethanol) was added. Heating may be required. Then 2 ml of concentrated sulphuric acid was added slowly along the wall of the test tube. Formation of a purple/violet ring at the junction of two layers indicates the presence of carbohydrates.
- ***Anthrone test*:** 5 ml of extract was taken in a test tube and 2 ml of anthrone reagent was added to it. Formation of green-blue coloration indicates the presence of carbohydrates.
- ***Benedict test*:** 5 ml of the test sample was mixed with 5 ml of Benedict's qualitative reagent and boiled in a water bath for approximately 5 minutes. The formation of greenish/muddy/brick-red coloration indicates the presence of reducing sugars.

- ***Iodine test*:** to approximately 2 ml of test solution, a few drops of iodine solution were added. Appearance of blue-black coloration indicates the presence of starch.

2. Test for proteins

- ***Biuret test*:** A few drops of Biuret reagent were added to 1 ml of extract solution. The appearance of violet color indicates the presence of proteins.

3. Tests for alkaloids

- ***Dragendorff test*:** To 2 ml of extract solution, 1 ml of Dragendorff reagent was added and mixed well. Formation or orange-brown precipitate indicates the presence of alkaloids.
- ***Hager's test*:** 2 ml of test solution was taken in a test tube. A few drops of Hager's reagent (saturated solution of picric acid) were added to it and shaken well. Formation of a yellow precipitate indicates the presence of alkaloids.
- ***Mayer's test*:** To 2 ml of test solution, a few drops of Mayer's reagent were added and shaken well. Formation of pale-yellow precipitate shows the presence of alkaloids.
- ***Wagner's test*:** To 2 ml of extract solution, a few drops of Wagner's reagent were added and shaken well. Formation of a reddish-brown precipitate indicates the presence of alkaloids.

4. Test for polyphenols

- ***$FeCl_3$ test*:** A few drops of 5% neutral iron (III) chloride/ferric chloride solution were added to 5 ml of extract solution. Appearance of a greenish-brown or blue-black coloration establishes the presence of phenolic compounds.

5. Tests for flavonoids

- ***Shinoda test*:** To 5 ml of extract, 10 drops of dilute hydrochloric acid and a small piece of magnesium metal were added. Formation of pink, red, or brown color in a few minutes indicates the presence of flavonoids.
- ***Pew's test*:** To 5 ml of test solution, approximately 0.1 g of metallic zinc and 5 ml of concentrated sulphuric acid was added. The formation of cherry-red coloration indicates the presence of flavonols.
- ***Alkaline reagent test*:** 2-3 drops of 2% NaOH solution were added to 2 ml of plant extract. Appearance of a deep yellow color that gradually fades away upon the addition of dilute acid (HCl) indicates the presence of flavonoids.

6. Tests for tannins and phlobatannins

- ***Lead acetate test*:** To a small volume of the extract, a few drops of 1% lead acetate solution were added. Formation of yellowish-white precipitate shows the presence of tannins.
- ***Acid test*:** Phlobatannins are tannins that yield phlobaphene with hot dilute acids. A small volume of the extract solution was boiled with a few drops of 1% aqueous hydrochloric acid in a water bath. Formation of a red precipitate indicates the presence of phlobatannins.

7. Test for cardiac glycosides

- ***Keller-Kiliani test*:** To 5 ml of test sample, 2 ml of glacial acetic acid and a few drops of 2% $FeCl_3$ solution were added. 1 ml of concentrated sulphuric acid was added slowly to the above solution. The appearance of a brown ring at the interface suggests the presence of cardiac glycosides.

8. Test for phytosterols

- ***Salkowski test*:** To approximately 5 ml of test extract in a test tube, 2 ml of chloroform was added and mixed well. To this, 2 ml of concentrated sulphuric acid was added with proper mixing. Appearance of reddish-violet coloration in the upper (chloroform) layer and a greenish color in the acid layer indicate the presence of sterols.

9. Tests for terpenoids

- ***Horizon test*:** 2 ml of trichloroacetic acid was added to 1 ml of test sample. The formation of a red precipitate indicates the presence of terpenoids.
- ***Liebermann-Burchard test*:** 5 mg of dry tuber/leaf extract was dissolved in 2 ml of chloroform and 2 ml of acetic anhydride was added to it. Then, a few drops of concentrated sulphuric acid were added slowly to the above solution and mixed. Formation of green-blue color indicates the presence of terpenoids.

10. Test for saponins

- ***Foam test*:** 5 ml of extract was taken in a test tube and shaken vigorously with addition of 2 ml distilled water to obtain a stable foam. 2 drops of olive oil were added to the foam and mixed well. Formation of a stable emulsion confirms the presence of saponins.

11. Test for anthraquinones

- ***Borntrager's test*:** Approximately 3 g powder of *G. superba* leaves, tubers, and seeds were taken in separate test tubes and 5 ml benzene was added to each test tube and mixed vigorously. The powders were allowed to soak for 10 minutes and then filtered. 5 ml of 10% ammonia solution was added to the filtrate and shaken well. The appearance of pink, violet, or faint red color in the ammonia phase indicated the presence of anthraquinone moiety.

III. RESULTS AND DISCUSSION

In the present study, the leaves of glory lily were found to contain significant amounts of alkaloids and polyphenols (including flavonoids and tannins); moderate amounts of carbohydrates, cardiac glycosides, and saponins; and trace amounts of proteins and sterols. The tubers contained significant levels of alkaloids and polyphenolic compounds; moderate amounts of carbohydrates (mainly starch), saponins, and tannins; trace to moderate amounts of proteins and phytosterols; and trace amounts of cardiac glycosides. The seeds contained the highest levels of alkaloids. Proteins and polyphenolic compounds were moderately present. The carbohydrates were mainly of non-reducing type. Saponins and phytosterols were present in trace to moderate amounts. Cardiac glycosides were not detected. Trace amounts of triterpenoids were also detected in ethanol extracts of all samples. Anthraquinones were not detected in any *Gloriosa* sample in the present study. These findings are summarized in Table 1.

Table 1. Qualitative biochemical tests for identification of phytochemical groups in aqueous and ethanol extracts of *Gloriosa superba* leaves, tubers, and seeds

S. No.	Phytochemical group	Leaves		Tubers		Seeds	
		A	E	A	E	A	E
1.	**Alkaloids**	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
2.	**Polyphenols**	+ + +	+ + +	+ + +	+ + +	+ +	+ +
3.	**Flavonoids**	+ + +	+ +	+ + +	+ +	+ +	+
4.	**Saponins**	+ +	+ +	+ +	+ +	+ +	+
5.	**Phytosterols**	+	+	+	+ +	+	+ +
6.	**Carbohydrates**	+ +	+	+ +	+ +	+ +	+
7.	**Proteins**	+	+	+	+ +	+ +	+ +
8.	**Cardiac glycosides**	+ +	+ +	+	+	–	–
9.	**Tannins**	+ +	+ +	+ +	+	+	+
10.	**Terpenoids**	–	+	–	+	–	+
11.	**Anthraquinones**	–	–	–	–	–	–

The number of '+' symbols represents the intensity of the color reaction for the given extract (+ = nominally present, ++ = moderately present, +++ = significantly high concentration, - = absent). **A**= Aqueous extract; **E**= Ethanol extract.

Alkaloids are abundantly present in all the parts of glory lily. These compounds exhibit a diverse range of pharmacological activities from anti-hyperglycemic and anti-tumor to antimicrobial and anti-malarial (*Kittakoop et al., 2014*). Many alkaloids also have analgesic, stimulant, and vasodilatory effects. Of all the plant-derived substances, alkaloids perhaps have the greatest medicinal importance. Many established modern medicines, such as quinine, nicotine, and morphine, belong to this class. Colchicine and colchicine derivatives from *Gloriosa superba* have found applications as precursors for drug discovery. However, large doses of many alkaloids are proven poisons. They can be purified from crude extracts by solvent extraction and column chromatography (*Russo et al., 2013*). Polyphenols are a large family of natural water-soluble phenolic compounds (compounds that contain phenol groups) found abundantly in the plant kingdom. Many drugs used in modern medicine contain phenolic groups. Paracetamol – a widely used analgesic and antipyretic drug – is a phenol. Due to the diverse chemical nature of the compounds classified under the umbrella term polyphenols, their biological effects also greatly vary (Quideau et al., 2011). Flavonoids and tannins are members of the larger polyphenol family. Flavonoids are a common component in the diet of herbivores due to their ubiquitous presence in plants. Compared to other bioactive secondary metabolites of plants, such as alkaloids, flavonoids are perhaps the least toxic group (Ververidis et al., 2007). Significant quantities are ingested by humans in their daily diet in the form of fruits, vegetables, and beverages (Roura et al., 2007). Catechins are the most common flavonoids. Tannins (or tannic acid) are polyphenols commonly found in tree bark and unripe fruits. They have astringent properties. Condensed tannins may comprise as much as up to 50% of the dry leaf weight in some species (Mole, 1993). Phlobatannins are C-ring isomerized condensed tannins (Ferreira et al., 2003).

Saponins are bitter-tasting, generally toxic organic compounds. They produce foam when agitated in aqueous medium. Due to their amphiphilic nature (solubility in both water and oils), they are traditionally used as mild cleansers (Rao and Gurfinkel, 2000). Sterols are alcohols of gonane and are a subgroup of steroids. More than 250 different sterolic compounds have been

identified in plants. Free phytosterols are insoluble in water, partially soluble in oil, and soluble in alcohol (Akhisa and Kokke, 1991). In biological systems, steroids are synthesized from terpenoid precursors. Consequently, the biochemical tests used for the detection of sterols and steroids are extensible for the detection of terpenoids in general. Terpenoids are the largest family of plant secondary metabolites and represent approximately 60% of all known phytochemicals (about 80,000 compounds). They are known for their aromatic qualities, and many of them possess significant biological activity as traditional herbal therapeutics (Ashour et al., 2010).

Cardiac glycosides are commonly found secondary metabolites in several plants such as *Digitalis* (foxgloves). These compounds are named so due to their effects on cardiovascular function; they are used as potent drugs in the treatment of cardiac arrhythmia and congestive cardiac failure (Riganti et al., 2011). However, overdoses may prove fatal due to the induction of cardiac contractions with greater force, and extreme caution is to be followed while using these compounds in medication regimens. Many cardiac glycosides are also reported as potent anticancer drugs. Anthraquinones are aromatic organic compounds poorly soluble in water but soluble in hot organic solvents. They are important phytochemicals found in traditional medicinal herbs, either in glycosylated or free forms (Abdullah and Hussain, 2023). Several significant therapeutic attributes are associated with anthraquinones derived from a number of plants, such as anti-cancer and anti-inflammatory activities and laxative effects (Clementi and Weber-Schöndorfer, 2015).

IV. CONCLUSION

Preliminary phytochemical screening of the leaves, tubers, and seeds of *Gloriosa superba* in the present study confirmed the dominance of alkaloids in all the parts of glory lily. Colchicine, colchicine derivatives, gloriosine, and superbine are the chief alkaloid compounds found in the plant. Of the three plant parts analyzed, seeds contained the richest levels of alkaloids, followed by tubers and leaves in that order. These alkaloids have promising applications as drug precursors in modern medicine. Developing rapid and efficient physicochemical methods for the isolation of *Gloriosa* alkaloids has emerged as a key research interest in recent times. The present study was a qualitative analytical approach only and quantification of alkaloids was beyond the scope of this study. It was noted that ethanol was a better solvent for the separation of alkaloids than water. However, other researchers have reported that chloroform and petroleum ether are the best extraction solvents for the maximum yield of alkaloids from this plant.

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