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Phytochemical studies of *Gloriosa superba* L. grown in Terai Region of Uttar Pradesh

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Abstract

The creeper plant *Gloriosa superba* (Glory lily) is a member of the Colchicaceae family. Due to its abundance of valuable phytochemicals, such as colchicines and colchicocides, this plant is also exported from India. Colchicine has been used extensively to treat gout in recent years, and *G. superba* is a common source of colchicine for patients who cannot take non-steroidal anti-inflammatory medicines (NSAIDs). It is a rare plant and does propagate easily. In present study, the phytochemistry of Glory lily, grown in the terai region of Uttar Pradesh studied. The methanolic extract used for the phytochemical evaluation of leaves and tubers of *Gloriosa superba* L. The tuber and leaf extract shows the presence of important secondary metabolites. The quantity of alkaloids, flavonoids and phenols present in *G. superba* leaves are 1.794, 0.499 and 1.687 µg/mL, respectively and the quantity of alkaloids, flavonoids and phenols present in the tubers are 2.789, 0.910 and 1.330 µg/mL, respectively. Glory lily's growth, propagation, yield, and phytochemistry in lower altitude places, such as Terai regions, needed to be well investigated and understood.

Key words : *Gloriosa superba*, Glory Lily, Terai region, phytochemical.

Medicinal plants are a valuable resource for physiologically active chemicals and are crucial in the drug-discovery process. Plants have created the foundation for sophisticated traditional medical procedures that people in China, India, and many other nations have been using for thousands of years[1]. To extract various phytochemical ingredients, several sections are employed, including leaf, root, stem, fruit, seed, and park. Medicinal plants are rich in phytoactive substances like flavonoids, alkaloids, phenols etc. Thirty plants from Indian Ayurveda medicine have demonstrated anticancer properties. Researchers studying natural products are currently paying closer attention to herbal items to extract bioactive components that are significant for medicine. A beautiful climber, *Gloriosa superba* is a member of the

Colchicaceae family. In Hindi, it is referred to as "Kalihari" and in English as "Glory Lily". Around the world, this species is often utilized as an ornamental plant (Figure 1). It is one of the National Medicinal Plants Board (NMPB) of India's top ten prioritized medicinal plants[2]. Africa and tropical Asian nations like Burma, Malaysia, Sri Lanka, and India are the natural habitats of *G. superba*. It occurs in western regions of India, such as Tamil Nadu, Kerala, and Odisha. It is also known as the state flower of Tamil Nadu and the national flower of Zimbabwe. This plant is spotted as a rare plant species in states like Uttar Pradesh. Certain regions of India, including Tamil Nadu, Goa, Chhattisgarh, and Odisha, are also cultivators of it. People have used the Glory lily as a medicinal plant since thousands of years. However, it is an inedible plant that can be fatal if consumed. According to Ayurvedic literature, it possesses abortifacient effects and used to cure many illnesses, including leprosy, asthma, piles, indigestion, fever, snake bites, wound infections, skin infections, and internal parasites. It can also used to decrease labour pain. The seeds and tubers of Glory lily, are commercially used by pharmaceutical industries. The International Union for Conservation of Nature (IUCN) included this species in their "Red Data Book," because this species is on the verge of extinction[3].

Gloriosa superba contains many bioactive substances, including tannins, steroids, alkaloids, flavonoids, glycosides, terpenoids, saponins, phenolics, vitamins, and minerals. The plant's tubers and seeds are rich in colchicine and its derivatives, an essential alkaloid used in medicine; the amount of colchicine in tubers is two to five times lower than in seeds. Up to 0.9% colchicine and 0.8% colchicoside is present in a single plant. The tuber and seed are toxic because of the high amount of colchicine[4]. This plant has a lot of pharmacological activities like anti- arthritic, analgesic, uterotonic, antimicrobial, anti inflammatory, anthelmintic, antioxidant, anti-diabetic activities etc. The medical properties of the glory lily is due to the alkaloids, specifically colchicine, gloriosine, and thiocolchicine, in addition to the few non-alkaloidal chemicals found in it, such as luteolin, chelidonic acid, stigmasterol, and β-sitosterol. Colchicine promotes polyploidy in crops and is utilized as a mitosis-arresting agent, diabetic patient treatment, and cancer treatment[5]. Many Indian tribal societies employ *Gloriosa superba* as an ethnomedical. This plant species' therapeutic qualities are also described in several ancient Indian texts, including Nighantu, Sushruta Samhita, and Charak Samhita. Glory lily has been used ethnomedically for bleeding piles, asthma, conjunctivitis, arthritis, and other diseases. It is taken orally or applied topically[3].

The Terai, also known as the Tarai, is a lowland area that is located in sections of southern Nepal and northern India. It is situated north of the Indo-Gangetic Plain and south of the Sivalik Hills and the outer foothills of the Himalayas. Tall grasses, scrub savannah, sal woods, and clay-rich marshes are the features that define this lowland area. The Terai region of India includes the states of West Bengal, Uttarakhand, Uttar Pradesh, Haryana, and Bihar. The Terai region of Uttar Pradesh includes 8 districts including Gorakhpur district and Maharajganj district. The plain region and the Sub-Himalayan foothills meet at the Terai region creating an ecotone. This region is one

of the most diverse eco-parts in the globe and one of the most diverse regions of the nation due to the edge effect, which causes it to display the vegetation of both contiguous regions. Glory lily is found as a rare plant in the terai region of Uttar Pradesh.

Figure 1. An Experiment *G. superba* climber plant grown in pot showing tendrilar leaves and buds.

A defining trait of the genera in the family Colchicaceae is the presence of alkaloids of the colchicines type, which have significant medical use. *G. superba* tubers and seeds have yielded many alkaloids related to colchicine that have been isolated. Comparing tubers to seeds, the tubers have less colchicine. Colchicine has been used extensively to treat gout in recent years, and *G. superba* is a common source of colchicine for patients who cannot take nonsteroidal anti-inflammatory medicines (NSAIDs). Colchicines generally seem to boost the amounts of anti-inflammatory mediators while inhibiting certain pro-inflammatory pathways. Colchicine is used to treat Behçet's illness, prevent heart conditions such as pericarditis, and treat familial Mediterranean fever. Additionally, it shows promise for the palliative care of stomach cancer. It is mostly cultivated as a pharmaceutical plant in the southern state of India[3]. Many studies revealed the concentration of active phytochemical constituents varies with region and altitude. The biochemistry of Glory lily grown in the agroclimate of the terai region of Uttar Pradesh has not explored well yet.

Plant materials :

Plant samples, including leaves, flowers, seeds, and tubers of *Gloriosa superba*, were gathered from the Gorakhpur division of the Terai region. Following identification and confirmation, these plants were present in the Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur, herbarium of the Postgraduate and Research Department of Botany for future use.

Extract preparation :

Fresh flowers, leaves, seeds, and tubers were properly cleaned under running tap water followed by sterilized distilled water, then dried in the shade. They were put through a mechanical pulveriser to become a coarse powder. Every sample, or roughly 100 g of powder, was extracted many times using methanol in a 500 mL round-bottom flask containing 250 mL of solvent. Using Soxhlet equipment, the reflux time for each solvent was 25 cycles for full extraction. A rotating evaporator operating under regulated pressure and

temperature was used to gather and concentrate the filtrate. The crude residue was produced by drying out the extracts. These residues were utilized for phytochemical screening of secondary metabolites and were kept at -20°C.

Phytochemical qualitative analysis :

The primary groups of chemicals (tannins, saponins, flavonoids, alkaloids, phenols, glycosides, steroids and terpenoids) contained in the extracts were determined by confirmatory qualitative phytochemical screening of plant extracts using conventional techniques[6].

1. *A test screening of terpenoids :*

A quantity of 2 mL of $CHCl_3$ was added to 0.5g of extract. Carefully, 3 mL of concentrated H_2SO_4 put on to create a layer. The interface's reddish-brown colouring suggests the presence of terpenoids.

2. *A test screening of flavonoids :*

Five to ten drops of diluted HCl and a small piece of magnesium ribbon were added to a test tube containing 0.5 mL of extract. The mixture was then boiled for a few minutes. The presence of flavonoids is indicated by the appearance of a filthy brown or reddish-pink colour.

3. *A test screening of alkaloids :*

Mercuric chloride (1.36 grams) and 5 grams of potassium iodide were dissolved in 60 mL and 10 mL of distilled water, respectively. Distilled water was used to dilute the mixture of both solutions to a volume of 100 millilitres. A few drops of Meyer's reagent (potassium mercuric iodide) were applied to 1 mL of the extract. The appearance of a pale or white precipitate indicates the presence of alkaloids.

4. *A test screening of tannins :*

A few drops of a 1% lead acetate solution were put into a test tube that held around 5 mL of the extract. Tannins are indicated by a precipitate that is either red or yellow.

5. *A test screening of saponins :*

A few drops of sodium bicarbonate were added to a test tube that held approximately 5 mL of the extract. After giving the mixture a good shake for three minutes, it was left. Saponins were visible in the form of a froth that resembled a honeycomb.

6. *A test screening for phenols :*

A few drops of a 10% aqueous ferric chloride solution were added to 1 millilitre of extract and 3 millilitres of distilled water. The formation of a blue or green colour indicated the presence of phenols.

7. *A test screening for steroids :*

Carefully pour 1.0 mL of concentrated sulfuric acid along the test tube's sides into the 2.0 mL of extract. The presence of steroids is shown by the red colour that the chloroform sheet produces.

8. *A test screening for glycosides :*

Add 5 mL of 5% $FeCl_3$ and 5 mL of

diluted HCl to 5 mL of extract. For five minutes, place in a boiling water bath to heat. After cooling, thoroughly shake with benzene or any other organic solvent. After removing the organic layer, apply the same amount of diluted ammonia. The presence of glycosides is indicated by the pinkish-red colour of the ammonical layer.

Phytochemical quantitative analysis :

The quantitative assessment for alkaloids, flavonoids and phenols was performed by methods used by Ghodke and Pandhure[7].

1. *Quantitative assessment of alkaloids :*

The plant sample's obtained supernatant was utilized to estimate the total alkaloids using titrimetric techniques. A 100 mL separating funnel was filled with 10 ml of the supernatant. Add 10 mL of 0.1 (N) HCl, and give it a good shake for two to three minutes. Alkaloids become soluble as a result. Alkaloids neutralized with 0.1 (N) HCl are found in the lower layer, while n-butanol is found in the upper layer. After gathering a portion of 10 millilitres of HCl in a beaker, two to three drops of methyl red were added, giving the solution a faintly crimson hue. The beaker's contents were titrated against 0.1 (N) NaOH until a pale yellow colour changed from red. It was decided what the neutralizing point was. The same process was carried out three times. The following equivalency was taken into account to determine the total amount of alkaloids:

1 mL 0.1N HCl ≡ 0.0162 g alkaloid

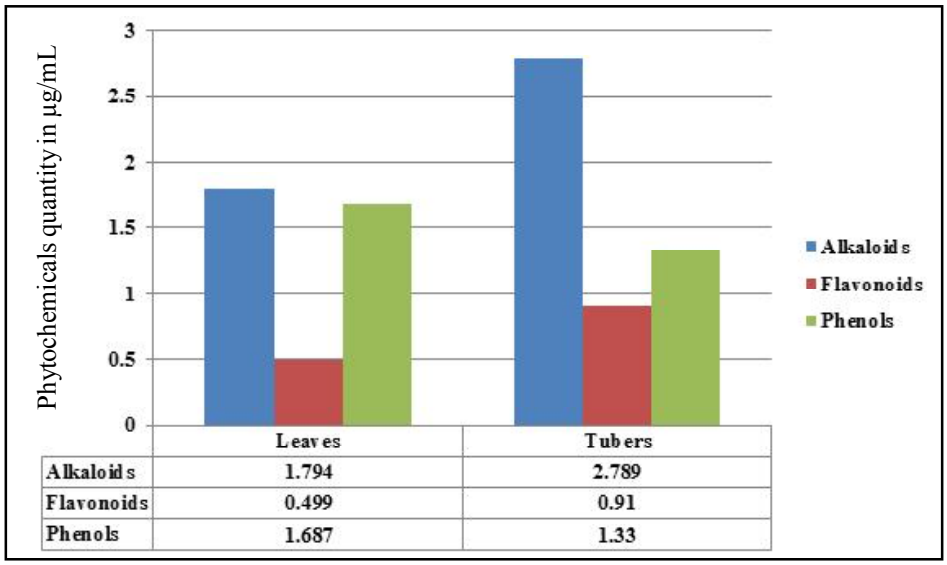
2. *Quantitative assessment of flavonoids :*

The aluminium chloride colourimetric system was utilized to assess the total flavonoid content in the entire plant extract of *G. superba* L. 0.5 millilitres of plant component extract was taken at various concentrations (10µg, 20µg, 40µg, 60µg, 80µg, and 100µg), and the final volume was increased to 3 millilitres using methanol. Subsequently, the test solution was shaken vigorously while 0.1 mL of potassium acetate, 2.8 mL of distilled water, and 0.1 ml of $AlCl_3$ (10%) were continually added. Absorbance at 415 nm was measured after the incubation periods of 30 minutes. The equivalent of quercetin (QE)/g of sample was used to calculate the flavonoid concentration in test samples. Three replicates of the whole sample were examined.

3. *Quantitative Assessment of Phenols :*

Using an aliquot of diluted extract, the various concentrations of 10µg, 20µg, 40µg, 60µg, 80µg, and 100µg were added to 0.25 mL of Folin Ciocalteu reagent. After adding distilled water to bring the elucidation's final volume down to 3 millilitres, it was well-shaken. The solution was incubated, maintained in a dark environment, and measured at 760 nm using a prepared blank. Milligrams of gallic acid equivalents were used to express the total phenol content of plant parts per gram of dry weight. Three replicates of the entire sample were used for analysis.

Methanolic extract were used for phytochemical evaluation for leaves and tubers of *Gloriosa superba* L. The tuber and leaves extract shows the presence of alkaloid, glycosides, terpenoids, tannin, flavonoids, saponins, steroid and phenols (Table-1).



	Leaves	Tubers
Alkaloids	1.794	2.789
Flavonoids	0.499	0.91
Phenols	1.687	1.33

Figure 2. Quantitative analysis of *Gloriosa superba* L. plant parts in methanolic extract (in µg/mL)

Table-1. Qualitative phytochemical evaluation of *Gloriosa superba* L. leaves and tubers in methanolic extract

Sr.no.	Phytochemical	Leaves	Tubers
1.	Terpenoids	-	+
2.	Flavonoids	+	++
3.	Alkaloids	++	++
4.	Tannins	-	++
5.	Saponins	+	++
6.	Phenols	++	++
7.	Steroids	++	++
8.	Glycosides	+	++

The number of alkaloids, flavonoids and phenols present in *G. superba* leaves are 1.794, 0.499 and 1.687 µg/mL, respectively and the number of alkaloids, flavonoids and phenols present in the tubers are 2.789, 0.910 and 1.330 µg/mL, respectively (Figure 2). The active components are present in high amounts in the *G. superba* grown in the Terai region of Uttar Pradesh.

All parts of *G. superba* contain alkaloids, the primary one being colchicine, an amino alkaloid produced from the amino acids phenylalanine and tyrosine, which accounts for the plant's therapeutic significance. According to Finnie and Staden (1994), colchicine concentrations in *G. superba* stems range from 0.33 to 0.41%, flowers from 1.18% to 0.08%, and ovary from 0.08% [8]. The plant has also produced other chemicals that have been identified, including lumicolchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine, 3-demethylcolchicine, and N-formyl-deacetylcolchicine. Many plant parts are used to treat syphilis, tumours, sores, and spleen issues. The plant extract has a CNS depressive effect[9].

Gloriosa superba L. contains a wide

variety of phytochemicals, including alkaloids, flavonoids, terpenoids, tannins, saponins, phenols and steroids. The methanolic extract of the tuber of exhibits antibacterial properties and has a high intensity and content of phytochemicals. Two secondary metabolites are naturally occurring antioxidants are phenols and polyphenols, which are present in plants. In the present study, we studied the presence of active biochemical components grown in the Terai region of Uttar Pradesh. *G. superba* is a pharmaceutically important plant, is exported from India Tamil Nadu farmers contribute significantly to the 800-1000 tonnes of annual global consumption. In 1996–1997, the plant species' provisional export value was 53 million, and in 2015, it increased by almost 15% [3]. However, it is not easy to grow and propagate. The growth, propagation, yield and phytochemistry of Glory lily in lower altitudes like Terai regions are required to explored well with more depth of understanding in these regions.

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