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ORIGINAL ARTICLE

A Comparative Pharmacognostical Evaluation and Simultaneous HPTLC Quantification of Bioactive Alkaloids in Three Species of *Gloriosa*, Collected from Natural Habitat in India

Ankita Misra, Bhanu Kumar, Sharad Srivastava

Pharmacognosy Division, CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India

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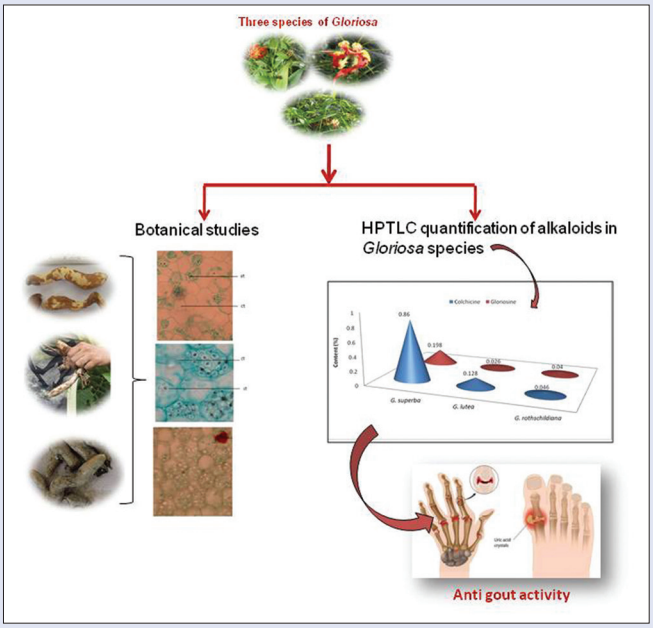
ABSTRACT

Background: The genus *Gloriosa* is commercially valued due to its colchicine metabolite, which is clinically used in gout and as an antimitotic agent. **Objectives:** The study was a comparative pharmacognostical evaluation and simultaneous high performance thin layer chromatography (HPTLC) quantification of bioactive alkaloids in *G. superba*, *G. lutea* and *G. rothschildiana*. *In vitro* anti-gout activity was also established. **Materials and Methods:** Pharmacognostical studies and metabolic variations were analyzed per the Ayurvedic Pharmacopoeia of India and validated HPTLC method. Inhibition of protein denaturation, hydroxyl radical scavenging and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay evaluated the anti-gout activity. **Results:** The morpho-anatomical studies suggest that the target species are similar with no characteristic difference, apart from the flower colour. The pharmacognostic standards were also established as per the Ayurvedic Pharmacopoeia of India to ensure the quality of raw material. Quantification of colchicine and gloriosine content through validated HPTLC method reveals significant variation, ranging from 0.046% to 0.860%, and from 0.040% to 0.198% on dry wt. basis. The maximum content of both the targeted metabolites was in *G. superba*, followed by *G. lutea* and *G. rothschildiana*. The highest *in vitro* anti-gout and radical scavenging activity among the three species was in *G. superba*. **Conclusion:** The pharmacognostic standards of three *Gloriosa* species were established, and this opens avenues for chemotaxonomic studies on *G. lutea* and *G. rothschildiana* from different phytogeographical zones of India for the identification of their elite germplasm. The study also led to the identification of alternate species of *G. superba* which can be explored commercially to meet the industrial demand for colchicine.

Key words: Colchicine, *Gloriosa lutea, Gloriosa rothschildiana, Gloriosa superba*, Gloriosine, HPTLC

SUMMARY

- In the present study, comparative pharmacognostic evaluation and quantification of alkaloid metabolites in three species belonging to the family *Gloriosa* was carried out along with *in vitro* anti-gout activity. The morpho-anatomical characteristics of the three species were found to be similar with no characteristic differentiating feature, apart from the flower colour. The flower colour in *G. lutea* was yellow. The pharmacognostical standards were also established as per the Ayurvedic Pharmacopoeia of India. The content of colchicine and gloriosine metabolite was found highest in *Gloriosa superba* followed by *G. lutea* and *G. rothschildiana*. Similar results were observed in *in vitro* assays, *G. superba* exhibits the highest activity.



Correspondence:

Dr. Sharad Srivastava,
Senior Principal Scientist, CSIR-National Botanical Research Institute, Rana Pratap Marg, Lucknow, Uttar Pradesh, India.
E-mail: sharad_ks2003@yahoo.com

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INTRODUCTION

Gloriosa belonging to the family Colchicaceae is widely distributed all across the tropical parts of the world. The species under this genus are commonly known as climbing lilies, possessing slender vines with narrow lens-shaped leaves and a bright red-coloured, claw-shaped flower. It grows annually in the rainy season from a dormant mother tuber. The commonly known species in the genus *Gloriosa* are *G. superba*, *G. lutea*, *G. planti*, *G. longifolia*, *G. rothschildiana*, etc. *G. superba* has commercial significance due to its bioactive metabolite colchicine, clinically indicated in gout and Familial Mediterranean fever. Colchicine

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acts as arachidonate release inhibitor, 5-lipoxygenase inhibitor and histamine inhibitor, superoxide anion production inhibitor, and tyrosine phosphorylation.[1–4]

Colchicum autumnale L. was the only source of colchicine, but since its discovery in *Gloriosa superba* in 1915, the species is now commercially exploited for its colchicine content. *Gloriosa superba* is an important medicinal plant of Ayurvedic origin, used in folklore as well as in evidence-based medicine. It has anti-inflammatory, larvicidal, anti-tumor, and thrombotic activity.[1] Traditionally, *Gloriosa* is used in cases of snakebite, to induce labor, abortifacient, gout, and in respiratory disorders.[5,6] The demand for colchicine upsurged in 2005 when the USFDA[7] allowed the clinical use of colchicine in gout; since then, it has been continuously explored for various pharmacological activities. The other metabolites in *Gloriosa superba* are lumicolchicine, gloriosine, superbine, colchicosides, sterols, etc.[1] Literature suggests that *G. superba* is the most studied species among this genus, possibly due to its high colchicine content, that is, 0.9% colchicine and 0.8% colchicosides.[8] Chemotaxonomic studies on *G. superba* collected from various phytogeographical zones of India reveals the significant variation among the intra-specific and inter-specific population.[9–14] *G. superba* is under cultivation in southern states of India like Tamil Nadu, Karnataka, etc., It is a kind of cash crop due to its high returns.[15] Therefore, looking at the industrial relevance of colchicine, it became essential to explore the other species, apart from *G. superba,* which can be commercially used for its colchicine content to meet the location-specific demand.

Hence, in the present study, a comparative pharmacognostical evaluation of three *Gloriosa* species, namely, *Gloriosa superba* L., *Gloriosa lutea* L., and *Gloriosa rothschildiana* O'Brien were carried out, and metabolic variation in bioactive alkaloids, namely, colchicine and gloriosine were quantified through validated HPTLC method. Further, the *in vitro* anti-gout potential of the three targeted species was also compared.

MATERIALS AND METHODS

Chemical and reagents

Colchicine (99.8% w/w), gloriosine (>98%), and bovine serum albumin (BSA) were procured from Chromadex (USA), Toronto Research Chemicals (Canada), and Sigma-Aldrich (St Louis, MO, USA) respectively. The chemical and solvents used in the study were of analytical grade (S.D fine, India). The solvents were filtered (0.45 mm filter, Millipore, Bedford, MA, USA) and sonicated for 15 min before use. HPTLC (20 × 20 cm), precoated silica gel aluminum plates 60 F_{254} (0.25 mm) were purchased from Merck (Darmstadt, Germany).

Plant material

The plant material (tubers) was collected from June to September 2018 after thorough consultation from local/regional floras. The tubers (2–4) were harvested from the flowering vine, keeping in mind that the metabolite(s) synthesis in plants is at its peak during this stage of maturity. The collected samples were authenticated, the passport datasheet of each sample was prepared, and the specimen was deposited in the institute herbarium, CSIR-NBRI (Lucknow). The collection number was assigned to the sample.

The tubers were cleaned with distilled water, roughly chopped, and dried under shade. The dried tubers were coarsely powdered (40 mesh sieve), and about 5 g of the powdered sample was defatted using petroleum ether to remove the fatty components. The sample was then macerated with 25 mL methanol for 24 h at room temperature (25°C ± 2) with intermittent shaking, filtered through Whatman no. 4, and the residue was resuspended in fresh methanol. The extraction was repeated three times, and the pooled filtrate was dried in the Rotavapor (Make: Buchi, USA) under reduced conditions of temperature (50°C ± 2) and pressure (40 mbar). The concentrated extract was finally lyophilized to solid residue (Labconco, USA).[16]

Botanical studies and pharmacognostic evaluation

The morphological examination of three *Gloriosa* species was carried out to identify the key morphological characters. The anatomical characters of tubers were also studied in detail.[16] The various pharmacognostical parameters, like foreign organic matter, ash values, and extractive values were evaluated as per the Ayurvedic Pharmacopoeia of India.[17]

Simultaneous quantification of bioactive metabolites

The colchicine and gloriosine metabolites were quantified through the validated HPTLC method developed and calibrated as per guidelines by the International Council for Harmonization.[18] The analysis was carried out at a working solution of 0.1 mg/ml and 10 mg/ml of standards and samples, respectively.

Quantification of polyphenolics and *in vitro* anti-gout activity

The polyphenolic content, i.e. total phenolics[19] and total flavonoid[20] was quantified through the spectroscopic method, and based on the regression curve of standards, values were expressed in percent. The *in-vitro* anti-gout activity was evaluated by inhibition of protein denaturation[21,22] assay with slight modifications. The radical scavenging potential was evaluated by hydroxyl radical scavenging assay,[23] and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.[24]

Statistical analysis

Values were presented as mean ± standard deviation (SD) of three replicates of each observation. Data were subjected to one-way analysis of variance (ANOVA) to test the level of significance (XLSTAT, 2010, Microsoft Corporation, USA).

RESULTS

Botanical studies

The three species, namely, *Gloriosa superba*, *Gloriosa lutea*, and *Gloriosa rothschildiana* were collected from natural populations of Kerala, Sikkim, and Uttar Pradesh states of India respectively [Table 1]. The external appearances of the three species in the natural habitat were found to be similar, except for the flower color. It was observed that there is a slight difference in flower color, although the shape and size remain similar. *G. superba* has a bright red colour flower, whereas *G. lutea* and *G. rothschildiana* have bright yellow and faint reddish-pink flowers. The other common features of three species are as follows: species is a climber, annual, erect herb having L- or V-shaped tubers, and fibrous rootlets are present at the internode. The tubers are pale yellow, and a papery brown sheath forms at the surface with ageing. Fracture is short, non-fibrous, and odorless. However, after detailed macroscopic examination, it is concluded that the species do not exhibit any characteristic difference among themselves [Table 2].

The anatomical features of the three species were also found similar, and no characteristic difference was recorded [Figures 1–3]. The common anatomical characters are the following: the tuber's transverse section (TS) consists of a single layer, rectangular epidermal cell as outermost covering, followed by ground parenchyma. The parenchyma cells were large, thin-walled, and circular to polygonal with visible intercellular spaces. Starch grains are abundantly available throughout

Table 1: Brief passport data sheet of three *Gloriosa* species

Species	Phyto-geographical zone	Collection site	Stage of maturity	Altitude (feet)	Latitude (N)	Longitude (E)	Soil type	Collection no.	Extractive value (%)
Gloriosa superba	Western Ghats	Waynad, Kerala	F	2515	11°47'07.29"	76°07'55.18"	Laterite, loamy and sandy	305306	68.1
Gloriosa lutea	Eastern Himalayas	Sumbuk, Sikkim	F	1180	27°05'54.93"	88°22'1.81"	Red gravel hilly soil	305326	14.7
Gloriosa rothschildiana	Gangetic Plains	Lakhimpur, U.P.	F	485	27°53'59.08"	80°46'43.82"	Loamy soil	305355	2.8

F: Flowering

Table 2: Morphological characters of targeted *Gloriosa* species

	Characteristic common descriptors				
	Shape	Size	Color	Smell	Fracture
Gloriosa species	V- and L-shaped	15 to 20 cm in length and 2.2 to 3.5 cm in width.	Pale yellow inside and papery brown color sheath outside.	Neutral	Short

the TS; however, the density is higher on outer layers. In the cortex, tannin cells and oil globules were observed. Collateral types of vascular bundles were present and scattered throughout the ground tissue. The starch grains are simple, variable shapes, namely, polyhedral, oblong, round with concentric striations and visible hilum. These grains are light-colored, medium in size, and tightly packed.

The physico-chemical parameters of the three species were evaluated. As the samples were collected as per good collection practices (GCP) guidelines of the National Medicinal Plant Board (India), the foreign matter was nil. The ash values, namely, total ash and acid insoluble ash and extractive values, that is, alcohol-soluble extractive value and water-soluble extractive value of *G. superba* were within the specified limit of Ayurvedic Pharmacopoeia of India. *G. lutea* and *G. rothschildiana* have at par with *G. superba* and are insignificantly different [Table 3]. The pharmacognostical standards are useful to check and ensure the quality of raw material in the herbal drug industry, especially in cases where dried samples are procured in bulk and there is a high chance of adulteration and substitution practices.

Quantification of alkaloid metabolites

The two major metabolites of *Gloriosa* species, that is, colchicine and gloriosine were quantified through the validated HPTLC method.[13] Densitometric scanning of colchicine and gloriosine at 350 nm reveals that the maximum content (% dry wt. basis) of both the metabolite(s) was recorded in *Gloriosa superba*, followed by *Gloriosa lutea* and *Gloriosa rothschildiana,* respectively [Figure 4]. The metabolite content in these species is insignificant among them but statistically different ($P > 0.05$) from *G. superba*. However, it is noteworthy to mention that our group had mapped the natural population of *Gloriosa superba* from various phytogeographical zones of India. Among the collected 128 samples, elite chemotype was identified from the Western Ghats of India,[13] and was considered in this study. Moreover, in the Indian population, such high content of metabolites in tubers of *Gloriosa superba* was not reported to date. Reversed-phase high performance liquid chromatography (RP-HPLC) quantification of colchicine in six different species of *Gloriosa* shows that the maximum content was recorded in *G. lutea,*[25] although based on localized/conserved population, variability in metabolite content of a species cannot be concluded. And therefore, chemotaxonomic studies on *G. lutea* and *G. rothschildiana* collected from natural habitat the need of time and may lead to the identification of germplasms with high metabolite content. Additionally, exploring the alternate sources of *G. superba* from the natural habitat will reduce the burden on single species and also raise the prospect of cultivation practices (of identified species) in location-specific areas and/or under controlled conditions.

In vitro anti-gout activity

Gout is an inflammatory condition of the joints and is caused due to the deposition of monosodium urate crystals when blood serum uric acid increases in the body. These crystals lead to recurrent, episodic pain in inflamed joints followed by swelling, redness, heat and stiffness during

Table 3: Pharmacognostical standards of three *Gloriosa* species

Species	Total ash (%)	Acid-insoluble ash (%)	Alcohol-soluble extractive (%)	Water-soluble extractive (%)
Gloriosa superba	5.03±0.058	0.94±0.016	6.16±0.032	16.64±0.066
Gloriosa lutea	5.10±0.01	0.53±0.025	6.247±0.215	15.92±0.12
Gloriosa rothschildiana	5.13±0.12	0.49±0.002	5.15±0.150	16.21±0.210

Values are mean±S.D (n=3)

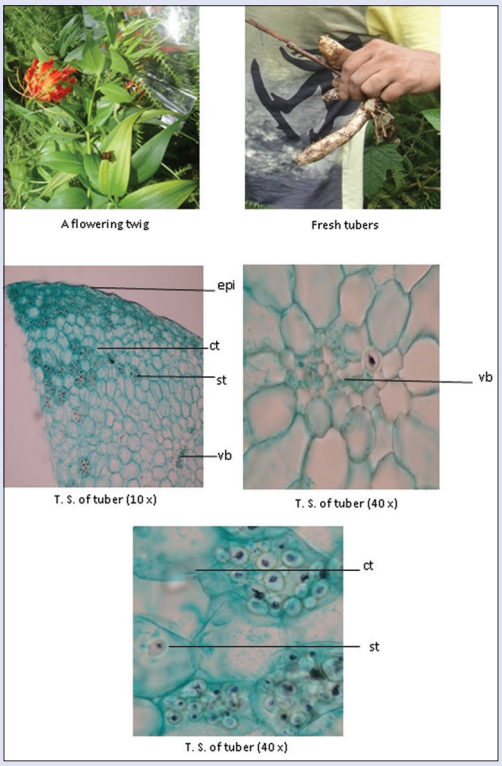

Figure 1: Morphological and microscopical descriptors of *Gloriosa superba*. Abbreviations: epi - epidermis, st - starch grains, vb - vascular bundles, ct - cortex

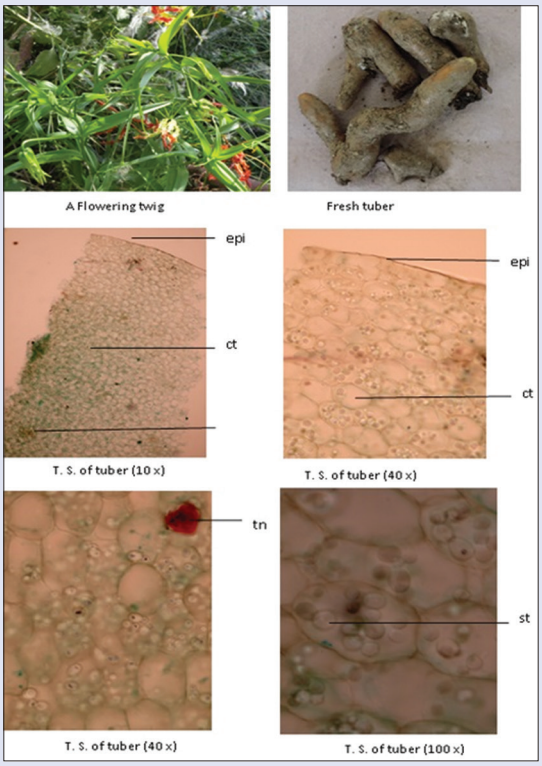

Figure 2: Morphological and microscopical descriptors of *Gloriosa lutea*. Abbreviations: epi - epidermis, st - starch grains, vb - vascular bundles, ct - cortex, tn - tannin

locomotion. The inflammation in gout results from the precipitation of serum urate into monosodium urate crystals (MSU), these MSU crystals are deposited in and around the joints resulting in pain and distortion during locomotion.

It is well evident that *Gloriosa superba* is used in gout due to colchicine exhibiting several biochemical reactions, majorly on inflammatory mediators. The effect is multidirectional and is involved in a series of inflammatory reactions, at the molecular level.[16] Thus, the evaluation of *in-vitro* anti-gout activity via inhibition of protein denaturation model is the ideal method for evaluating the pharmacological potential, specifically in the context of *Gloriosa*. The basic idea behind the selection of this model is to validate the variation in the anti-gout activity of targeted *Gloriosa* species, inherited due to colchicine to control various inflammatory reactions in the body. The competitive binding of colchicine to serum albumin was well established.[26] The evaluation of radical scavenging activity of the *Gloriosa* species was mediated via quantification of polyphenolics (TPC and TFC), HRSA (Hydroxyl radical scavenging assay) and DPPH radical scavenging assay. The radical scavenging potential, as evident from HRSA and DPPH assay, signifies that *G. superba* exhibits the maximum potential followed by *G. lutea and G. rothschildiana* [Table 4]. The phenolic content of each species is higher than its flavonoid content; *G. superba* has the highest content of polyphenolics.

The inhibition of protein denaturation by test extract was analyzed at a single 0.05 mg/ml concentration. It is observed that with an increase in the concentration of the extract, precipitation of protein, that is, denaturation of (bovine serum) decreases. The IC_{50} values of *G. superba*, *G. lutea*, and *G. rothschildiana* were at 0.005 ± 0.006, 0.009 ± 0.05, and 0.012 ± 0.012 mg/ml respectively. Data suggest that *G. superba* exhibits the highest potency since lower the IC_{50} value, the more pronounced is the action. Standard colchicine was also analyzed under the same working protocol at a stock concentration of 0.05 mg/m;, the calibration curve was obtained at five variable dilutions of 0.001–0.005 mg/ml. A linear calibration with a statistically acceptable regression coefficient of 0.991, equation; y = 10531x + 0.561 was obtained, exhibiting IC_{50} at 0.0048 mg/ml.

CONCLUSION

In this study, three species of *Gloriosa*, namely, *G. superba*, *G. lutea*, and *G. rothschildiana* were collected from their natural habitat and two bioactive metabolites were quantified through the validated HPTLC method. The targeted metabolites, that is, colchicine and gloriosine, content vary from 0.046% to 0.860% and from 0.040% to 0.198%, respectively; the highest content of both the metabolites was in *G. superba*. Morpho-anatomical studies suggest that the target species are similar and no characteristic difference was observed among them,

Table 4: Radical scavenging assay and polyphenolic content in *Gloriosa* species

Species	Polyphenolic content (%)		Radical scavenging assay (IC_{50}, mg/ml)	
	Total phenolic content	Total flavonoid content	Hydroxyl radical scavenging assay	DPPH radical scavenging assay
Gloriosa superba	2.25±0.001	0.096±0.003	0.0043±0.002	0.228±0.0012
Gloriosa lutea	2.28±0.001	0.028±0.008	0.0054±0.05	0.312±0.005
Gloriosa rothschildiana	0.275±0.005	0.009±0.004	0.12±0.005	0.415±0.02

Values are Mean±S.D, *n*=3

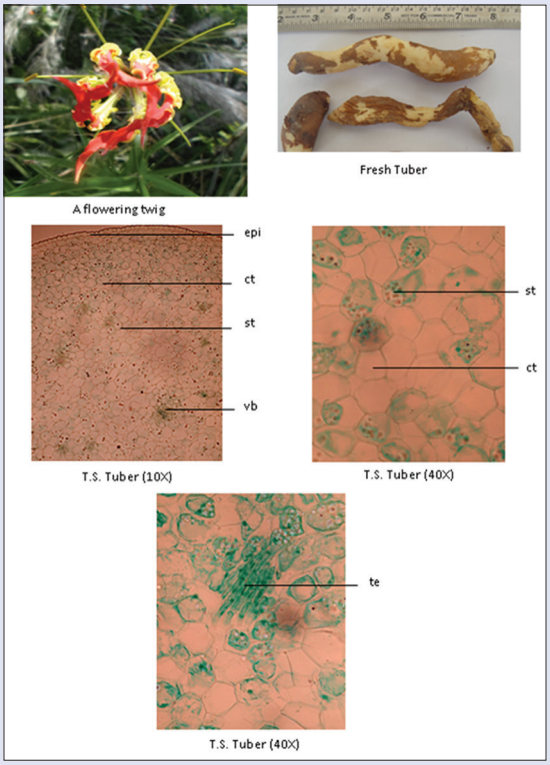

Figure 3: Morphological and microscopical descriptor of *Gloriosa rothschildiana*. Abbreviations: epi - epidermis, ct - cortex, st - starch, vb - vascular bundle, te - tracheids

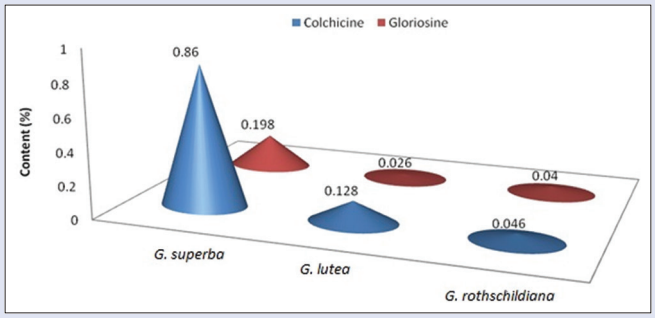

Figure 4: Quantification of alkaloid metabolite(s) in *Gloriosa* species. Values are mean (*n* = 3)

apart from the flower colour which is characteristically yellow in *G. lutea*. Pharmacognostical standards were established to ensure the quality of raw material; values were statistically insignificant among the three species and within the limit of the Ayurvedic Pharmacopoeia of India. Further, *in vitro* anti-gout and radical scavenging activities of species suggest that *G. superba* has the highest potential, followed by *G. lutea* and *G. rothschildiana*. In conclusion, the pharmacognostic standards of three *Gloriosa* species were established and this opens avenues for chemotaxonomic studies on *G. lutea* and *G. rothschildiana* from different phytogeographical zones of India for the identification of their elite germplasm. The study will also lead to the identification of alternate species of *G. superba* which can be explored commercially to meet the industrial demand for colchicine.

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Authors' contribution

AM: Collection, experimental (*in vitro* biological assay and HPTLC quantification), data acquisition, statistical analysis, preparation of the manuscript draft.

BK: Collection, botanical studies, data acquisition, preparation of the manuscript draft.

SS: Designing, conceptualization of study design, manuscript editing and manuscript review, and guarantor.

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Conflicts of interest

There are no conflicts of interest.

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