

High-performance Thin-layer Chromatographic-densitometric Quantification and Recovery of Bioactive Compounds for Identification of Elite Chemotypes of *Gloriosa superba* L. Collected from Sikkim Himalayas (India)

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ABSTRACT

Background: *Gloriosa superba* L. (Colchicaceae) is used as adjuvant therapy in gout for its potential antimetabolic activity due to high colchicine(s) alkaloids.

Objective: This study aimed to develop an easy, cheap, precise, and accurate high-performance thin-layer chromatographic (HPTLC) validated method for simultaneous quantification of bioactive alkaloids (colchicine and gloriosine) in *G. superba* L. and to identify its elite chemotype(s) from Sikkim Himalayas (India). **Methods:** The HPTLC chromatographic method was developed using mobile phase of chloroform: acetone: diethyl amine (5:4:1) at λ_{max} of 350 nm. **Results:** Five germplasm(s) were collected from targeted region, and on morpho-anatomical inspection, no significant variation was observed among them. Quantification data reveal that content of colchicine (R_f : 0.72) and gloriosine (R_f : 0.61) varies from 0.035%–0.150% to 0.006%–0.032% (dry wt. basis). Linearity of method was obtained in the concentration range of 100–400 ng/spot of marker(s), exhibiting regression coefficient of 0.9987 (colchicine) and 0.9983 (gloriosine) with optimum recovery of 97.79 \pm 3.86 and 100.023% \pm 0.01%, respectively. Limit of detection and limit of quantification were analyzed, respectively, as 6.245, 18.926 and 8.024, 24.316 (ng). Two germplasm(s), namely NBG-27 and NBG-26, were found to be elite chemotype of both the markers.

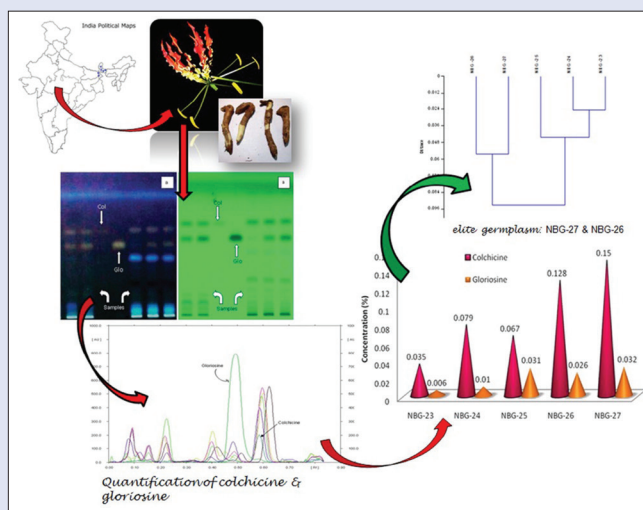
Conclusion: The developed method is validated in terms of accuracy, recovery, and precision studies as per the ICH guidelines (2005) and can be adopted for the simultaneous quantification of colchicine and gloriosine in phytopharmaceuticals. In addition, this study is relevant to explore the chemotypic variability in metabolite content for commercial and medicinal purposes.

Key words: Chemotype(s), colchicine, *Gloriosa superba*, gloriosine, high-performance thin-layer chromatographic

SUMMARY

- An easy, cheap, precise, and accurate high performance thin layer chromatographic (HPTLC) validated method for simultaneous quantification of bioactive alkaloids (colchicine and gloriosine) in *G. superba* L.
- Five germplasm(s) were collected from targeted region, and on morpho-anatomical inspection, no significant variation was observed among them

- Quantification data reveal that content of colchicine (R_f : 0.72) and gloriosine (R_f : 0.61) varies from 0.035%–0.150% to 0.006%–0.032% (dry wt. basis)
- Two germplasm(s), namely NBG 27 and NBG 26, were found to be elite chemotype of both the markers.



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INTRODUCTION

Gloriosa superba L. (Colchicaceae) is a tuberous climbing herb found throughout the tropical parts of India upto an altitude of 6000 ft and also widely distributed in tropical world. Indigenously, *Gloriosa* is known as “kalihari,” “Agnishikha,” and “Glory lily.”^[1,2] *G. superba* exhibits various medicinal properties such as analgesic, anti-inflammatory, antimicrobial, larvicidal, antipoxviral, antithrombotic, and antitumor, in the treatment of snake bite, gout, and respiratory disorders.^[3-7] In addition, colchicine and its derivative gloriosine is reported to exhibit anti-inflammatory action, tyrosine phosphorylation and superoxide anion production inhibitor, arachidonate release inhibitor, 5-lipoxygenase inhibitor, and histamine

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inhibitor.^[8-11] The species is the potential source of phenethylisoquinoline category of alkaloids, i.e., colchicine, and due to extensive exploitation for the same, *G. superba* is enlisted as threatened species in the IUCN Red data book. A new alkaloid gloriosine was also reported during the isolation of colchicine by Subbaratnam.^[12,13] Other chemical constituent(s) of this tuber are lumicolchicine, 2-demethylcolchicine, 3-demethylcolchicine, N-deacetyl-N-formyl-colchicine, benzoic acid, salicylic acid, sterols, resinous substances, colchicoside, gloriosine, tannins, and superbine. The current thrust of *Gloriosa* tuber in World market is for its colchicine and gloriosine content.^[14]

Colchicines are the largest group of alkaloids among the eight types of distinct alkaloids present within family Colchicaceae.^[15] Colchicine is a major chemical substance present in *G. superba* species with an extraordinary activity against gout. N-formyl-N-deacetyl-derivative of colchicine, also known as gloriosine, is other potential alkaloid having similar therapeutic efficacy as colchicine and contributes synergistically in the pharmacological action of *Gloriosa superba*.^[8,16] These bioactive colchicines (colchicine and gloriosine) are recommended in prophylactic shocks of gout and as an adjuvant to existing therapy in severe cases also. They act as a powerful antimetabolic agent, which blocks suppressed cell division by inhibiting mitosis.^[17] Dated back, the use of colchicine has an associated risk of toxicity, but after the US Food and Drug Administration approval in 1992 for its use in the treatment of gout, the demand and usage of colchicine have upsurge worldwide. *G. superba* contains up to 0.9% colchicine and 0.8% colchicoside.^[18] Our group reported the variations in colchicine content in *G. superba* L., germplasm collected from Central India using overpressured layered chromatography technique.^[19] Few methods for the quantification of colchicine in *Gloriosa* species and pharmaceutical dosage forms were previously reported.^[20-23] However, high-performance thin-layer chromatography (HPTLC) method for simultaneous quantification of colchicine and gloriosine was not reported till date, neither in *Gloriosa* species nor in any formulation. Hence, the study aims to separate and simultaneously quantify the presence of these two highly valuable plant secondary metabolites; colchicine and gloriosine through validated HPTLC method. Analytical quantification of chemical marker through HPTLC has an advantage of combining the chromatographic separation on a silica layer, along with *in situ* densitometric quantification of the separated compounds. This results in an efficient, quick, accurate, and relatively inexpensive method of quantification. Hence, eliminating the possible interference given by other structurally related compounds. Further, with the aid of HPTLC method, the study explores and analyzes the existing phytogeographical variations (in colchicine and gloriosine) among the natural population of *G. superba* L. collected from Sikkim Himalayas of India for identification of its elite chemotype(s).

EXPERIMENTAL

Chemicals and reagent

Colchicine (99.8% w/w) and gloriosine (98.00% w/w) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Toronto research chemical, Canada. Other organic solvents, namely, chloroform, acetone, and diethyl amine are of analytical and high-pressure liquid

chromatography (HPLC) grade, obtained from Merck, Mumbai (India). Solvents were filtered (0.45 mm filter, Millipore, Bedford, MA, USA) and sonicated for 15 min before use. HPTLC plates (20 cm × 20 cm, precoated silica gel aluminum plates 60 F254, 0.25 mm) were purchased from E. Merck (Darmstadt, Germany).

Plant material and extraction protocol

The *G. superba* tubers were collected during the month of September from North-Eastern Himalayan region covering Sikkim and the adjoining area of West Bengal. Five samples were collected from varied condition(s); specimens were authenticated and deposited in repository of CSIR-NBRI with individual voucher number. GPS information of the collected samples is recorded and summarized in Table 1.

The samples were washed with water, chopped, and shade dried. The dried tubers were coarsely powdered by passing through 40 mesh sieve (up to 500 mm), and the powdered sample (about 5 g) is defatted using petroleum ether by maceration method (cold) to remove the fatty materials/impurity. The defatted material was then macerated (cold method) with methanol (25 ml) for 24 h at room temperature (25°C ± 2°C) for 3 consecutive days. Extraction was repeated thrice, filtered (Whatman no. 4), and the pooled filtrate was dried in a rotatory evaporator (Buchi, USA) under standard conditions of temperature (55°C ± 2°C) and pressure (40 mbar) and finally lyophilized to solid residue (Labconco, USA). The nature and yield (%) of extract obtained in each germplasm was also documented.

Preparation of standard stock and sample solution for high-performance thin-layer chromatographic analysis

The stock solutions of standard colchicine and gloriosine were freshly prepared by dissolving 1 mg of compound in 1 ml of HPLC grade methanol and were stored at 4°C until analysis. Each day, aliquots of the stock solution (standards) were diluted in 10 ml volumetric flasks with methanol to prepare a working solution of 0.1 mg/ml. Samples were prepared by dissolving the known amount of extract in methanol to obtain a final concentration of 10 mg/ml. Working dilutions of standard and samples were duly filtered through a 0.45 mm Millipore membrane filter (Pall, USA) for HPTLC analysis.

High-performance thin-layer chromatographic instrumentation

Apparatus

A CAMAG Linomat V automated thin-layer chromatography (TLC) sample applicator was used to dispense the aliquots of the standard stock solution and the prepared samples. The plates were developed in Camag ascending twin trough chamber (20 cm × 20 cm). The slit dimensions were 4 mm × 0.45 mm and scanning speed was 100 mm/s. Scanning of bands was performed using Camag TLC Scanner 3 in ultraviolet (UV) absorbance mode by win CATS software (version 3.2.1, Switzerland) using deuterium lamp source.

Table 1: Global positioning system information of collected samples of *Gloriosa superba*

Collection code	Voucher number	Date of collection	Places/location/district	Height (m)	Latitude	Longitude	Soil type
NBG-23	305323	22.8.14	3 rd mile Kalimpong/Darjeeling/West Bengal	1287.8	27°03'28.72" N	88°27'35.47" E	Red gravel hilly soil
NBG-24	305324	22.8.14	6 th mile, Kalimpong/Darjeeling/West Bengal	1287.8	27°03'28.72" N	88°27'35.47" E	Red gravel hilly soil
NBG-25	305325	27.8.14	Kalimpong/West Bengal	1171.6	27°03'36.00" N	88°28'12.00" E	Red gravel hilly soil
NBG-26	305326	27.8.14	Sumbuk/West Sikkim	370.42	27°05'54.93" N	88°22' 51.81" E	Red gravel hilly soil
NBG-27	305327	27.8.14	Jorethang/West Sikkim	339.02	27°07'55.88" N	88°16'53.02" E	Red gravel hilly soil

Chromatographic conditions

Chemical profiling and method optimization for simultaneous quantification of colchicine and gloriosine were carried out on 20 cm × 10 cm TLC aluminum precoated plates with 200 nm layer thickness of silica gel 60 F₂₅₄ (SD. Fine-Chem Ltd., Mumbai, India). Tracks (standard and sample) were applied as 6 mm bandwidth using Camag 100 µl sample syringes (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland) under a flow of N₂ gas. The linear ascending development was carried out with chloroform:acetone:diethylamine (5:4:1 v/v/v) as a mobile phase in a Camag glass twin trough chamber. The saturation time of chamber was conditioned and optimized to 10 min at room temperature (25°C ± 2°C) and relative humidity for better resolution with mobile-phase vapors. The plate was allowed to develop up to a height of approximately 80 mm from the point of application (total length run by mobile phase), and the total run time was standardized to 20 min at room temperature (25°C ± 2°C) and relative humidity of 55% ± 2%. After development, the plates were air-dried for 30 min, and scanning was performed using Camag TLC Scanner 3 at λ_{max} of 350 nm for both colchicine and gloriosine in UV absorbance-reflectance mode operated by winCATS software (version 3.2.1). Quantification was performed using peak area versus concentration of standard marker using regression analysis in the range of 100–400 ng/spot [Table 2]. Images of TLC plate were taken at two wavelengths, namely 254 nm and 365 nm [Figure 1].

Validation of method

In the employed experimental condition, the HPTLC method for simultaneous quantification of colchicine and gloriosine includes evaluation of the following performance parameters such as specificity, linearity, sensitivity, accuracy, recovery, precision, and robustness.^[24]

Statistical analysis

Results were reported as means ± standard deviation (SD) of each extract in triplicate. Data were subjected to one-way analysis of variance, and the least significant difference between the extracts at *P* < 0.01 was calculated by *post hoc* comparison test (SPSS version 11.5, SPSS Inc. Chicago, IL, USA).

RESULTS

Morphological characteristics of *Gloriosa* germplasm

The studied samples of *G. superba* were collected from five different

locations of Sikkim Himalayas after extensive exploration in its four (East, West, North, and South) regions and adjoining areas in West Bengal, namely, Darjeeling and Kalimpong based on the available literature and flora of the targeted region [Table 1]. On inspecting the foliage part of *Gloriosa*, no distinct morphological features were observed among all the samples. Smooth, fleshy, pale white tubers with scaly/papery sheath were observed in all the germplasms, tubers are divergent in nature, forming a V-shaped structure having one arm shorter than other. Short rootlets are observed on node, from where two arms protrude, and on microscopic evaluation of tubers (tuberous sclerosis), no distinct variation in anatomical features was observed. Starch grains are abundantly distributed throughout the section of tubers in each germplasm.

High-performance thin-layer chromatographic method optimization

Optimization of HPTLC condition(s), namely, selection of mobile phase, absorption maxima, and slit dimensions was standardized to provide an accurate, precise, and reproducible method for simultaneous determination of colchicine and gloriosine. Different combinations of solvent systems were tried based on the chemical nature of targeted alkaloids and finally, chloroform:acetone:diethylamine in the ratio of 5:4:1 (v/v/v) was selected as the best suited system for efficient separation of these metabolites from other unknown markers. Absorption

Table 2: Statistical parameter for linearity validation of colchicine and gloriosine in *Gloriosa superba*

Statistical parameters*	Colchicine	Gloriosine
R _f	0.71	0.61
r ²	0.9987	0.9983
Slope	14.837	18.307
Intercept	417.63	284.32
Linearity range (ng/spot)	100-400	100-400
LOD (ng)	6.245	8.024
LOQ (ng)	18.926	24.316
RSD (%)	0.715	0.816
SD	28.081	44.56
SE	74.13	108.619
Average	3927.495	5166.216
P	0.002	0.058

*n=3, r²: regression coefficient. SD: Standard deviation; SE: Standard error; LOD: Limit of detection; LOQ: Limit of quantification; RSD: Relative standard deviation

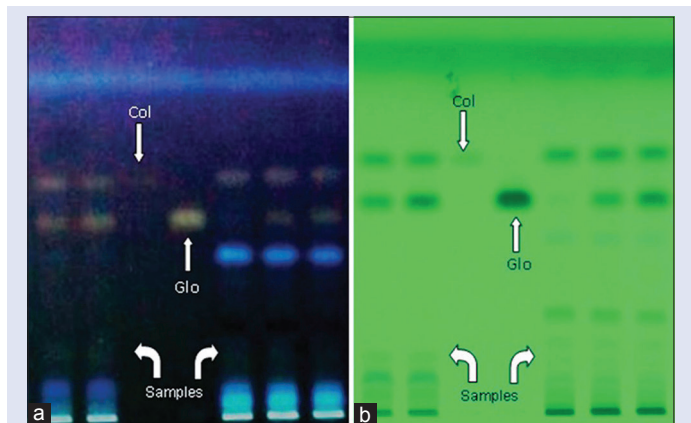


Figure 1: High-performance thin-layer chromatographic plate photograph of *Gloriosa* germplasm and standards (colchicine and gloriosine) at 254 nm (a) and 365 nm (b)

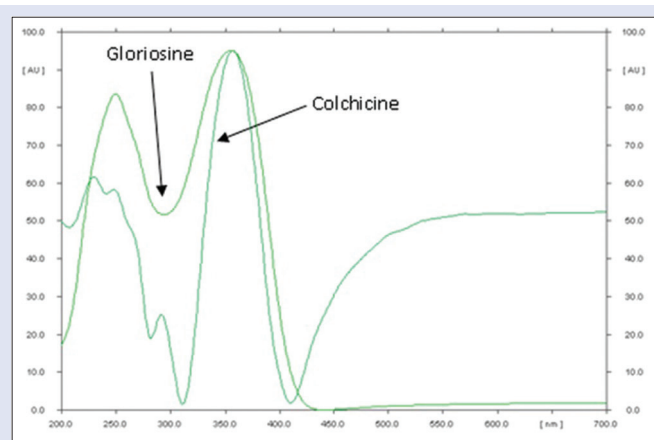


Figure 2: Absorption spectrum of colchicine and gloriosine in the entire ultraviolet range of 200-800 nm

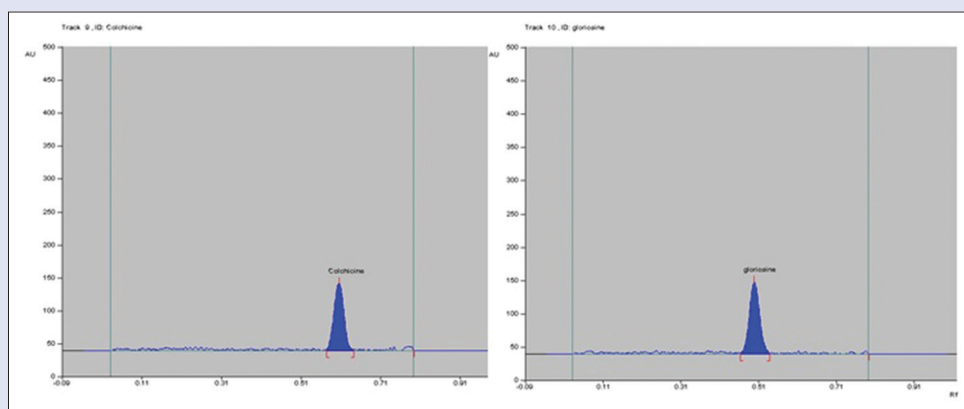


Figure 3: High-performance thin-layer chromatographic densitogram of standards (colchicine and gloriosine)

spectrum of colchicine and gloriosine was obtained at 350 nm [Figure 2] after scanning the entire UV range, from 200 to 800 nm. Specificity of the developed method reflects the clear and complete separation of marker(s) peak [Figure 3] and correspondingly in sample and standard. R_f value of colchicine and gloriosine was obtained at 0.72 ± 0.02 and 0.61 ± 0.01 , respectively. The relationship between concentration of marker compound and its corresponding peak area in sample band was investigated. The linear relationship was also tested and found suitable for simultaneous quantification of both marker(s).

High-performance thin-layer chromatographic method validation

Linearity and quantification

The linearity of the developed method for simultaneous quantification of both markers was achieved at a concentration of 100–400 ng/spot (both marker) with a statistically acceptable regression coefficient (r^2) of 0.9987 and 0.9983 for colchicine and gloriosine, respectively. Other statistical parameters of regression as summarized in Table 2 are within the limit of acceptance and thus confirming the linearity of developed method. In the development of chromatogram [Figure 4], targeted marker(s) in sample was identified by comparing the retention factor (R_f), peak purity, and absorption spectrum with reference marker(s). Quantification data (% dry wt. of sample) reveal that the content of colchicine and gloriosine varies from 0.035%–0.150% to 0.006%–0.032%, respectively. The maximum content of colchicine as well as gloriosine [Figure 5] was found in NBG-27, collected from Jorethang, West Sikkim. The residual plot for calibration curve of standards, namely, colchicine and gloriosine (area vs. concentration) shows the positive random pattern, indicating that a linear model provides a decent fit to the data. In addition to this, a positive, statistically significant correlation (Karl Pearson's correlation coefficient: 0.68) was observed in the content of two metabolites within the species. This suggests the biosynthetic inter-conversion of colchicine and its derivatives.

Specificity

The specificity of method was estimated by evaluating the band of standard (colchicine and gloriosine) in sample solution by comparing the R_f and UV spectra. Peak purity of these compounds was assessed by comparing each spectrum at three different levels, i.e., peak initiation, peak apex, and peak end [Table 3].

Precision

For intraday precision, three different concentrations of colchicine and gloriosine, namely, 100, 200, and 400 ng/spot were analyzed for 3 times

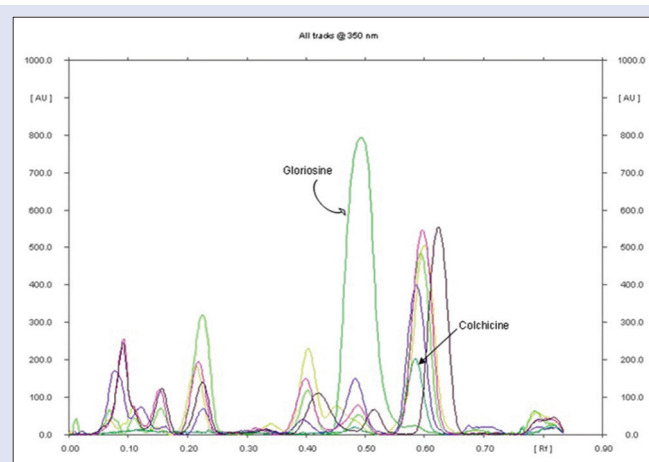


Figure 4: Overlay ultraviolet absorption spectra in sample tract of NBG 23, NBG 24, NBG 25, NBG 26, NBG 27 (from front to back in order) and standards, namely, colchicine (C) and gloriosine (G)

Table 3: Peak purity test for the standard colchicine and gloriosine

Standards	r (s, m) ^x		R (s, m) ^y	
	Standard track	Sample track	Standard track	Sample track
Colchicine	0.9875	0.9885	0.9980	0.9987
Gloriosine	0.9881	0.9997	0.9981	0.9789

s: Refers to start; m: Refers to middle; x: Correlation of spectrum at the start of peak with spectrum at the center of the peak; y: Correlation of spectrum at center of the peak with spectrum at the end of the peak

Table 4: Precision studies of standard compounds (colchicine and gloriosine) by high-performance thin-layer chromatography

Marker	Concentration (ng/spot)	Interday		Intraday	
		Mean (%RSD)	SD	Mean (%RSD)	SD
Colchicine	100	0.091	1.150	0.075	0.943
	200	0.040	1.014	0.067	1.684
	400	0.005	0.3	0.033	1.664
Gloriosine	100	0.076	1.129	0.084	1.238
	200	0.037	1.101	0.044	1.300
	400	0.001	0.115	0.015	0.884

RSD: Relative standard deviation; SD: Standard deviation

a day. In interday precision studies, the same concentrations of 100, 200, and 400 ng/spot were scanned for 5 consecutive days. Results expressed in terms of mean relative standard deviation (RSD) (%) and SD are within the limits of the ICH guidelines (2005) and reflect that the method is precise and reproducible for quantification of targeted metabolites under developed protocols [Table 4].

Accuracy

The accuracy of method was analyzed by recovery studies of both markers at three different levels. Three set of each sample were prepared and spiked with 25, 50, and 100 µg, respectively, and the data were shown in Table 5. The spiked sample was recovered and analyzed again under the same chromatographic conditions of HPTLC. Accuracy test is effective to identify the interference of unknown metabolite with the developed method for quantification of known one(s).

Detection and quantification limit

As per the ICH protocols (2005), SD of response and slope was used to determine the limit of detection (LOD) and limit of quantification (LOQ). The quantification of limit value(s) is based on regression analysis of standard dilutions in the concentration range of 100–400 ng/spot. LOD $\{(3.3 * \sigma)/\text{slope}\}$ and LOQ $\{(10 * \sigma)/\text{slope}\}$ were calculated using SD (σ) of intercept and slope [Table 2].

Repeatability and robustness

Repeatability of method was checked by repetitive application of marker compound ($n = 6$) and measurement of peak area at single concentration level of 100 ng/spot, small mean %RSD (0.091) value represents the good repeatability of method. The method was found to be robust when tested by slight change in mobile-phase composition and saturation time.

Identification of elite chemotype

Cluster analysis (PAST software, version 2.15) Hammer *et al.* 2001 of five samples based on the content of colchicine and gloriosine [Figure 6]

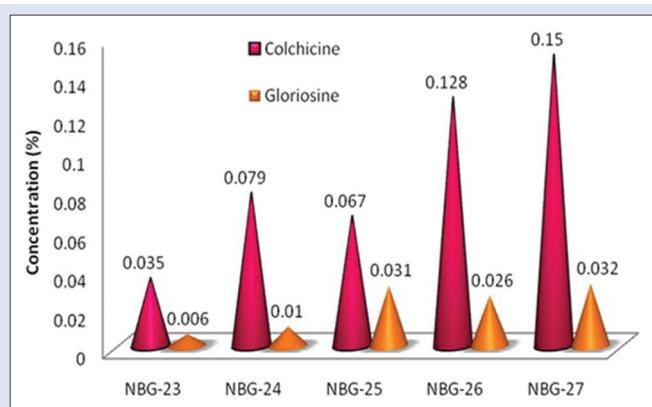


Figure 5: Quantitative estimation of colchicine and gloriosine in the samples of *Gloriosa superba* from eastern Himalayas

segregates into two branches. NBG-26 and NBG-27 of West Sikkim region are clustered together as high-yielding chemotypes into one branch whereas NBG-23, NBG-24, and NBG-25 are grouped together into a second branch.

CONCLUSION

G. superba is a rich source of biologically active alkaloids of colchicines group. Among them, colchicine and gloriosine are therapeutically potential phytochemicals in gout exhibiting antimitotic and other various medicinal effects. Due to importance of these metabolites, species is at the verge of extinction because of overexploitation by local inhabitants and industry. Thus, the chemotaxonomic evaluation of species is essential to encourage its conservation/cultivation to meet the industrial demand. Quantification of active phytochemicals through HPTLC is a method of choice as it is an accurate, easy, and time-saving method. Statistical data suggest that the developed HPTLC method is validated with standardized performance parameters exemplified by linearity, precision, accuracy, reliability, reproducibility, and robustness. This is the first report on simultaneous HPTLC quantification of these two medicinally, industrially valuable alkaloids, colchicine and gloriosine. Cluster analysis among samples reflects that germplasms of West Sikkim region (NBG-27 and NBG-26) were found to be rich (elite) chemotype in both the marker compounds. Thus, the validated method for simultaneous quantification of colchicine and gloriosine in *Gloriosa* developed in this study is accurate, easy, and economical for the detection of targeted marker in various marketed sample(s), formulation(s), and in biological fluid(s) for pharmaceutical industry.

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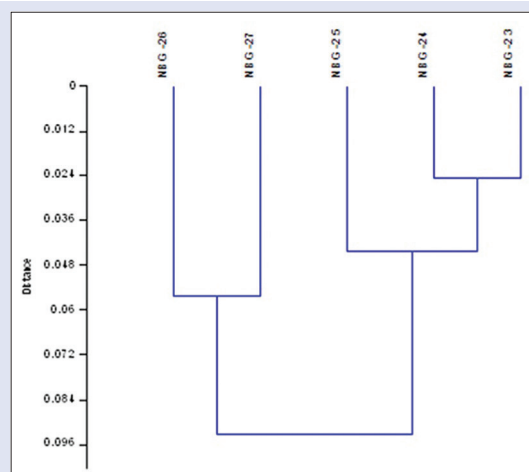


Figure 6: UPGMA dendrogram for collected population of *Gloriosa superba* based on the content of colchicine and gloriosine

Table 5: Recovery studies of standard colchicine and gloriosine in samples of *Gloriosa superba* by high-performance thin-layer chromatography

Standard*	Amount of standard present (µg)	Amount of standard added (µg)	Theoretical value (µg)	Observed value (µg)	Recovery (%)	Mean recovery (%)±SD
Colchicine	0.097	25	25.097	26.89	93.33	97.79±3.86
	0.097	50	50.097	50.04	100.11	
	0.097	100	100.097	100.15	99.94	
Gloriosine	0.035	25	25.035	25.028	100.02	100.023±0.01
	0.035	50	50.035	50.031	100.00	
	0.035	100	100.035	100.01	100.02	

*Values in µg/10 ml dry weight basis. SD: Standard deviation

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Pulliah T. Medicinal Plants in India. Vol. 1. New Delhi: Regency Publications; 2002. p. 269-70.
- Warrier PK, Nambiar VP, Ramankutty C. Indian Medicinal Plants. Vol. 3. New Delhi, India: Orient Longman; 1995. p. 76-81.
- Ade R, Rai MK. Review: Current advances in *Gloriosa superba* L. Biodiversity 2009;10:210-4.
- Alagesaboopathi C. Antimicrobial screening of selected medicinal plants in Tamil Nadu. J Microbiol 2011;5:617-21.
- Hemaiswarya S, Raja R, Anbazhagan C, Thiagarajan C. Antimicrobial and mutagenic properties of the root tubers of *Gloriosa superba* linn. (Kalahari). Pak J Bot 2009;41:293-9.
- Banu R, Nagarajan N. Anti bacterial potential of glory lily, *Gloriosa superba* linn. Int Res J Pharm 2011;2:139-42.
- Mathur A, Verma SK, Singh SK, Mathur D, Prasad GB, Dua VK. Investigation of anti-inflammatory properties of *Swertia chirata* and *Gloriosa superba*. Recent Res Sci Technol 2011;3:40-3.
- Jana S, Shekhawat GS. Critical review on medicinally potent plant species: *Gloriosa superba*. Fitoterapia 2011;82:293-301.
- Nadkarni KM. Indian Materia Medica. Mumbai: Popular Book Depot; 1996.
- Peters-Golden M, McNish RW, Davis JA, Blackwood RA, Brock TG. Colchicine inhibits arachidonate release and 5-lipoxygenase action in alveolar macrophages. Am J Physiol 1996;271:L1004-13.
- Ghosh S, Ghosh B, Jha S. Polymorphism in *Gloriosa superba*. Plant Genet Res Charact Util 2009;7:9-15.
- Subbaratnam AV. Alkaloid constituents of *Gloriosa superba* L. J Sci Ind Res 1952;11 B:446-7.
- Subbaratnam AV. Studies on alkaloid constituents of *Gloriosa superba* Linn. J Sci Ind Res 1954;13:670-1.
- Prajapati ND, Purohit SS, Sharma AK, Kumar T. A Handbook of Medicinal Plants. Jodhpur: Agro Bios Publishers, India; 2003. p. 221-34.
- Larsson S, Rønsted N. Reviewing Colchicaceae alkaloids – Perspectives of evolution on medicinal chemistry. Curr Top Med Chem 2014;14:274-89.
- Asolkar LV, Kakkar KK, Chakare OJ. Second Supplement to Glossary of Indian Medicinal Plant with Active Principles. Part-I (A-K). New Delhi: Publications and Information Directorate, Council of Scientific and Industrial Research; 1992.
- Bharathi P, Philomina D, Chakkaravarthi S. Estimation of colchicine in six different species of *Gloriosa* grown *in vivo*. Indian J Pharm Sci 2006;68:806-9.
- Roberge CJ, Gaudry M, Gilbert C, Malawista SE, de Médicis R, Lussier A, *et al.* Paradoxical effects of colchicine on the activation of human neutrophils by chemotactic factors and inflammatory microcrystal. J Leukoc Biol 1996;59:864-71.
- Srivastava S, Misra A, Shukla PK, Kumar B, Lata S, Rawat AK. A validated over pressured layered chromatography (OPLC) method for separation and quantification of colchicine in *Gloriosa superba* (L.) tubers from different geographical regions. RSC Adv 2014;4:60902-6.
- Hadad GM, Badr JM, El-Nahriry K, Hassanean HA. Validated HPLC and HPTLC methods for simultaneous determination of colchicine and khellin in pharmaceutical formulations. J Chromatogr Sci 2013;51:258-65.
- Chauhan SK, Singh AP, Agarwal S. Development of HPTLC method for the estimation of colchicine in different parts of *Gloriosa superba*. Indian Drugs 1998;35:266-8.
- Alali F, Tawaha K, Qasaymeh RM. Determination of colchicine in *Colchicum steveni* and *C. hierosolymitanum* (Colchicaceae): Comparison between two analytical methods. Phytochem Anal 2004;15:27-9.
- Bodoki E, Oprean R, Vlase L, Tamas M, Sandulescu R. Fast determination of colchicine by TLC-densitometry from pharmaceuticals and vegetal extracts. J Pharm Biomed Anal 2005;37:971-7.
- Validation of Analytical Procedures; Text and Methodology Q2 (R1). Geneva: International Conference on Harmonization; 2005.