

Estimation of Colchicine in Tubers of *Gloriosa superba* L. Originated From Different Agroclimatic Zones of Odisha, India

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ABSTRACT

Colchicine is an important alkaloid used in the treatment of cancer, gout and plant breeding work for inducing polyploidy. Recently there has been an increased demand of this alkaloid obtained from natural sources but no information is available on the colchicine content in the tubers of *Gloriosa superba* L. (glory lily) grown in Odisha. Present study estimated colchicine content in the wild and cultivated tubers of *G. superba* belonging to 4 different locations under 3 agroclimatic zones of Odisha, India. The colchicine content in tubers varied from 0.14% to 0.56% (dry wt.). There is appreciable difference in colchicine content found in both wild and cultivated tubers. The highest colchicine content (0.56%) was observed in the cultivated tubers originated from Ganjam district under East-South-eastern Coastal Plain (ESECP) agro-climatic zone of Odisha, India.

Key words: Alkaloid, Colchicine, *Gloriosa superba*, HPLC, TLC

INTRODUCTION

Colchicine is mostly found in seeds and tubers of *Gloriosa* species. *Gloriosa superba* L., belongs to Liliaceae family and popularly known as 'Glory Lily', 'Kalihari', 'Ognisikha' etc. due to its wavy edged yellow and red flowers. It is a perennial climbing glabrous herb with tuberous rootstock. It is distributed throughout India and also found in open forests in Odisha¹. *G. superba* tuber is considered as a rich source of the alkaloid colchicines^{2,3}. The tubers are useful in treating inflammation, ulcers, scrofula, bleeding piles, white discharge, skin diseases, leprosy, indigestion, helminthes, snake bites, baldness, intermittent fever and debility⁴. It is also considered useful in promoting labor and expulsion of placenta⁵. The *G. superba* tuber with sesamum oil is applied to treat arthritis⁶. Drugs prepared of colchicines are considered effective against several ailments as gout, rheumatism and also in controlling cancer. It is also capable in inducing polyploidy in plants⁷. Since it is obvious to find out and quantify the major active constituent present in desired plant part before domestication or cultivation of any wild medicinal

species, present study focused on estimation of colchicine of *G. superba*, both from wild and cultivated sources found in 4 locations under 3 agroclimatic zones of Odisha.

MATERIALS AND METHODS

Tubers and their wild sources: The plants are generally propagated through its tubers which are usually 'V' & 'L'- shaped and 1.5-2.5 cm thick. Tubers collected from 4 locations under 3 agroclimatic zones of Odisha were grown in the nursery of Regional Plant Resource Center, Bhubaneswar, Odisha during 2009 and 2010 (Table-1).

North Central Plateau (NCP) covers the district Mayurbhanj, major parts of Keonjhar. Climate is hot & moist with sub-humid condition with lateritic, red & yellow and mixed red & black soil types. Mean annual rainfall in this zone is 1534 mm. Temperature range varies in between 36.6-11.1°C. The characteristic features of 3 agroclimatic zones are mentioned here under.

East & South-Eastern Coastal Plain (ESECP) covers the districts Kendrapara, Khurda, Jagatsinghpur, part of Cuttack, Puri, Nayagarh & part of Ganjam. Basically the

Table.1: Colchicine content in wild and cultivated tubers of *Gloriosa superba* from different agro climatic zones of Odisha.

Source/Agroclimatic zones	Colchicine Content in Wild Tubers (<i>in situ</i>) %, d.wt.	Colchicine Content in Cultivated Tubers (<i>ex situ</i>) %, d.wt.
East south-eastern coastal plain (Ganjam Dist.)- ESECP-G	0.49±0.021	0.56±0.12
North central plateau (Keonjhar Dist.)-NCP-K	0.28±0.014	0.35±0.22
Western central table land (Bargarh Dist.)- WCTL-B	0.14±0.032	0.15±0.03
North central plateau (Mayurbhanj Dist.)- NCP-M	0.22±0.16	0.26±0.04

Figure-1: Colchicine content in wild and cultivated tubers of *Gloriosa superba* from different agro climatic zones of Odisha.

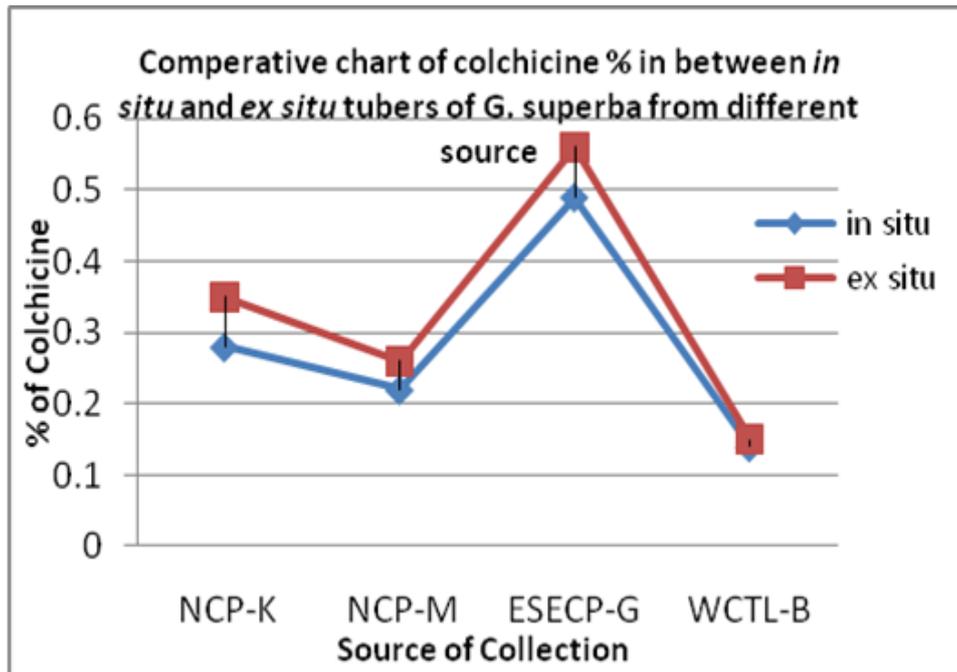


Figure -2 : TLC plates showing colchicine bands of standard and test samples

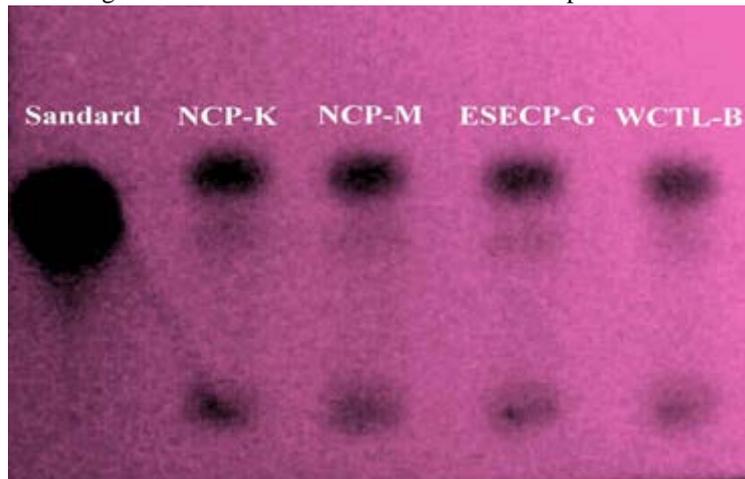
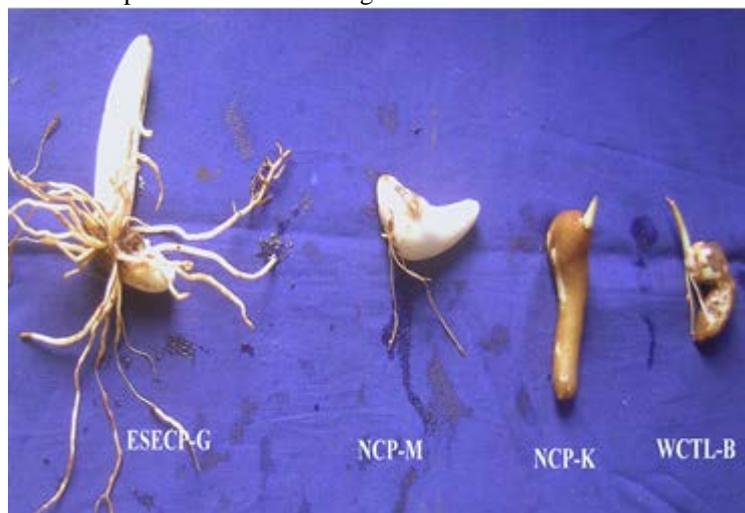


Figure-3 : Tubers of *Gloriosa superba* from different agroclimatic zones

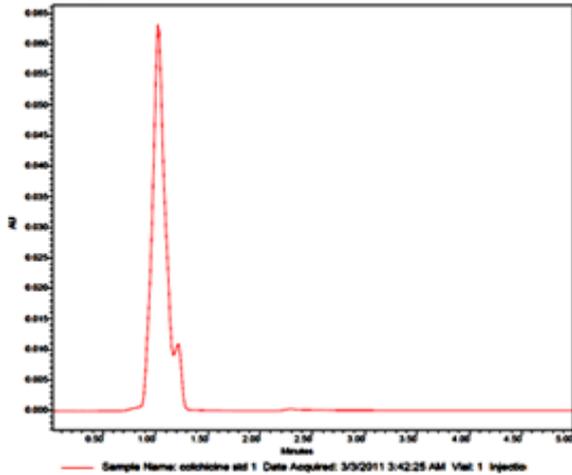


Where, NCP-K(North central plateau-keonjhar), NCP-M(North central plateau- Mayurbhanj), ESECP-G(East south eastern coastal plain-Ganjam), WCTL-B(Western central table land-Bargarh)]

climate condition is hot & humid with alluvial, red & mixed red and black soil. Mean annual rainfall in this zone is 1577 mm. Temperature range varies in between 39.0-11.5°C.

Western Central Table Land (WCTL) covers the districts Bargarh, Bolangir, Boudh, Sonepur, parts of Sambalpur & Jharsuguda. Climate is hot & moist with sub-humid condition. Broad soil groups include red & yellow, red & black, black, brown forest, lateritic. Mean annual rainfall

Figure-4: HPLC chromatogram of standard Colchicine. Colchicine peak at the retention time 1.2 min detected at a wavelength of 350 nm.



for six hours with solvent methanol by setting temperature (65°C) for each sample ⁸. The methanol extract were collected and filtered through 0.45 µm filter. The filtrate thus obtained constituted the test sample for qualitative and quantitative estimation of colchicine.

Qualitative evaluation through TLC: Qualitative estimation or separation of colchicine was performed by TLC. Methanol extract (10µl) and standard colchicine (Sigma 1mg/ml) were loaded over silica gel plates

Figure.5. HPLC chromatogram of Colchicine in cultivated tubers of 'North central plateau' Mayurbhanj (NCP-M).

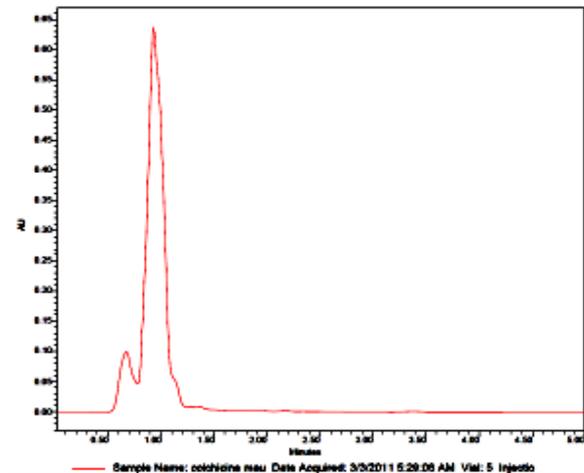


Figure-6:HPLC chromatogram of Colchicine in cultivated tubers of 'North central plateau' Keonjhar (NCP-K).

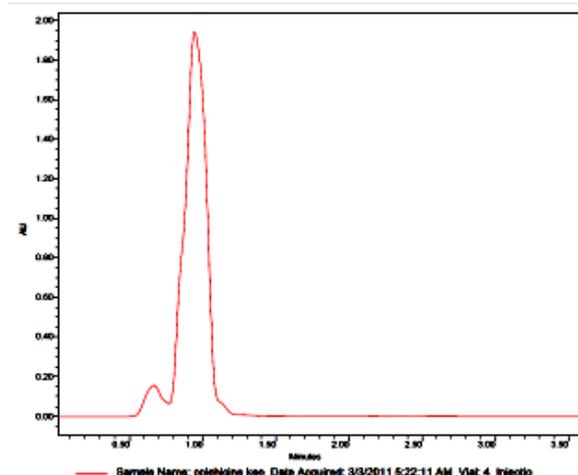
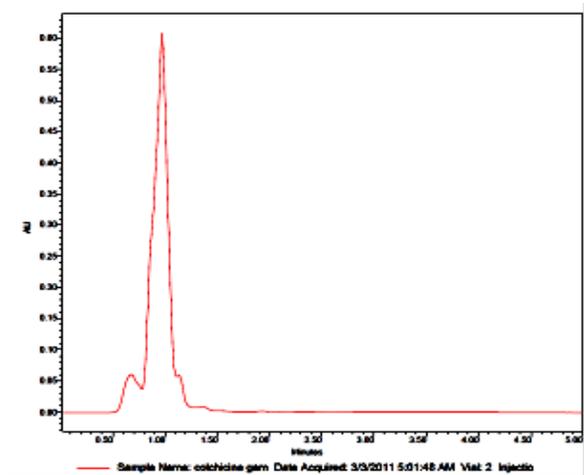


Figure-7:HPLC chromatogram of Colchicine in cultivated tubers of 'East south eastern coastal plain(Ganjam)(ESECP-G).



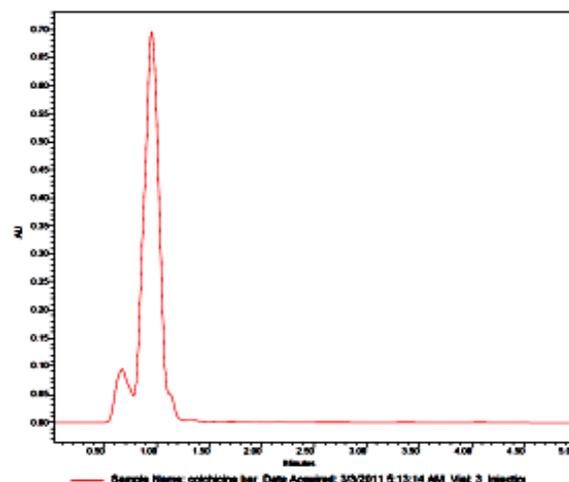
in this zone is 1614 mm. Temperature range varies in between 40.0-12.4°C.

Extraction of crude colchicines: The wild (*in situ*) and newly developed cultivated (*ex situ*) tubers of each location were used to estimate colchicine content. The oven-dried (90°C) and finely powdered tubers (10 gms) were taken in thimble with methanol as solvent for Soxhlet extraction. This extraction process was continued

(MERCK 60 F254) and ran by using mobile phase ethyl acetate: methanol (85:15) ⁴. The sample bands along with standard bands were observed under UV-cabinet at 265nm and stained with Vanillin Sulphuric acid stain for spotting the bands.

Quantitative estimation by HPLC: Quantitative estimation was carried out by Waters make HPLC system equipped with a binary pump and porous silica with 5 µm

Figure-8: HPLC chromatogram of Colchicine in cultivated tubers of 'Western central table land, Bargarh (WCTL-B).



diameters C_{18} 4.6 × 150 mm column. The mobile phase consisted of acetonitrile: 3% acetic acid (60:40), at a flow rate of 1 ml/min⁹. The identification of colchicine in sample extracts was done by comparing retention time of sample extracts with the standard colchicine (Sigma-10 mg/ml) at 350 nm. By this method retention time i.e., 1.2 min was evaluated for each sample and standard. Then the content of colchicine was estimated from standard graph. The standard graph was drawn by taking pick area and different concentration (20µg-100µg) of standard colchicine. Ten samples of each tuber were analysed for estimating colchicines content and value reported as mean.

RESULTS AND DISCUSSION

The different sized tubers collected from 3 agro climatic zones of Odisha are shown in Fig-3 and TLC plates showing colchicine bands of the standard and test samples shown in Fig-2. The estimated colchicine contents of *G. superba* tubers are presented in Table-1 & Fig.1. The chromatograms for standard and sample extracts are shown in Fig-4 to Fig-8.

The colchicine content in *G. superba* tubers ranged from 0.14% to 0.56%. The highest concentration (0.56%) was observed in the cultivated tuber originated in East south eastern coastal plain belonging to Ganjam district. The lowest colchicine content (0.14%) was found in the wild tuber of western central table land of Bargarh district. Colchicine in *G. superba* tubers to the level up to 0.9% (dry wt) has been reported earlier¹⁰. Colchicine content of 0.15-0.3% in the tubers of *G. superba* was reported from Karnataka State of India¹¹ while 37% was estimated in the tubers growing in hilly area of Himachal Pradesh⁴. In the present study, colchicine content was invariably found higher in cultivated tubers than their wild counter parts.

CONCLUSION

Present study revealed that the colchicine content in the *Gloriosa superba* tubers varied from one agro climatic zone to another. Variation in colchicine content was

ranged from 0.14% to 0.56%. The highest colchicine content was found in the tubers of the East south eastern coastal plain (Ganjam dist.) agro climatic zone. The tubers of this region may be a better source of commercial colchicine. As the plants grow naturally in coastal plains and forest areas in Odisha, its domestication through cultivation may improve the socio-economic status of the progressive grower of Odisha.

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