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 Odisha. *G. superba* tuber is considered as a rich source of the alkaloid colchicines. The tubers are useful in treating inflammation, ulcers, scrofula, bleeding piles, white discharge, skin diseases, leprosy, indigestion, helminthes, snake bites, baldness, intermittent fever and debility. It is also considered useful in promoting labor and expulsion of placenta. The *G. superba* tuber with sesamum oil is applied to treat arthritis. 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 plants. 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).

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

Table 1: Colchicine content in wild and cultivated tubers of *Gloriosa superba* from different agro climatic zones of Odisha.
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-Ganjaman), 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
for six hours with solvent methanol by setting
temperature (65°C) for each sample. 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 4:** HPLC chromatogram of standard Colchicine.
Colchicine peak at the retention time 1.2 min detected at a
wavelength of 350 nm.

**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 run by using mobile phase ethyl
acetate: methanol (85:15). 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
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/min. 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 earlier. Colchicine content of 0.15-0.3% in the tubers of G. superba was reported from Karnataka State of India while 37% was estimated in the tubers growing in hilly area of Himachal Pradesh. 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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