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In vivo and *In vitro* Estimation of Colchicine in *Gloriosa superba* L. by High Pressure Liquid Chromatography

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Abstract

The presence of Colchicine in *Gloriosa superba* was confirmed by Thin Layer Chromatography (TLC) and High Pressure Liquid Chromatography (HPLC) in which a spot coinciding with the authentic sample of Cochicine in Rf value (Colchicine 0.70) appeared. The maximum amount of Colchicine was found in flower (20.7 mg/gdw) and minimum amount was found in stem (7.4 mg/gdw).The In vitro studies showed the maximum amount of Colchicine in 6 week old cultures (22.6 mg/gdw)) and minimum amount in 2 weeks old cultures (15.3 mg/gdw).

Key words: Colchicine, *Gloriosa superba*, HPLC

Introduction

The plants of *G. superba* are tender, tuberous rooted deciduous perennials, adapted to summer rainfall with a dormant dry season. *G. superba* grows in sandy-loam soil in the mixed deciduous forests in sunny positions. It is very tolerant of nutrient-poor soils. It occurs in thick, forest edges and boundaries of cultivated areas in warm countries up to a height of 2530 m. It is also widely grown as an ornamental plant in cool temperate countries under glass or in conservatories (Neuwinger, 1994). *G. superba* is an emerging industrial medicinal crop in South India for its high colchicines content. Due to over-exploitation and problems faced during field cultivation, this species is now on the verge of extinction (Siva Kumar and Krishnamurthy, 2002). Colchicine is an alkaloid of *Colchicum autumnale.*

Colchicine was isolated in 1820 by Pelletier and Caventou. It is also present in *Gloriosa superba* (Glory Lily) (Gooneratne, 1966; Nagaratnam *et al,* 1973 Thakur *et al,* 1975; Finnie *et al,*1991) Isolation of colchicine from *G. superba* vary in the alkaloid levels of plants grown *in vivo*.

Material and Methods

Collection of plant materials

G. superba was collected (July-August, 2009) from botanical garden of Dr. Y. S. Parmar University of Horticulture & Forestry, Nauni, Solan (Himachal Pradesh). The collected plants were shade dried and finely powdered. Different plant was extracted with constant agitation for 48 h. The extracts were filtered using Whatman filter paper (no. 1) and then concentrated *in vacuo* at 40°C using a Rotary evaporator and stored at 4°C (Harborn, 1984, Harborn and Harborn 1998)

Establishment of tissue culture

For *in vitro* studies terminal shoot tips, stem nodes with single auxiliary bud, dormant and non dormant rhizome were used as explants. The explants were washed with running tap water pre soaked in 0.1% liquid detergent for about 30 min , then the explants were surface sterilized with 0.1% (w/v) mercuric chloride for 3 min. followed by two to three rinses of sterile distilled water.
The basal medium contained MS (Murashige and Skoog, 1962) salts, B5 (Gambrog *et al.,* 1968) vitamins, 3% sucrose and 0.9% agar, Basal medium was supplemented with various concentrations and combinations of growth regulators such as 2,4-D (2,4-dichlorophenoxy acetic Acid), BAP (6-benzylamino purine), NAA (Naphthalene acetic acid), Kinetin , IBA (Indole butyric acetic acid) and IAA (Indole acetic acid). The medium was adjusted to pH 5.8 with NaOH/HCl and dispensed in culture tubes and conical flasks of 100 ml capacity. The media was sterilized by autoclaving at 1.063 Kg/cm² pressure for 20 minutes.

Extraction

Colchicine was extracted from different plant parts of *G. superba* (stem, root, leaf, and flower) (2, 4, 6 and 8 weeks old as well as tissue samples) using a standard method (Hayashi *et al*, 1988). The samples were air dried and powdered, separately. 20 g material extracted for 6 hr in a Soxhlet extractor with methanol. The extract was diluted with distilled water, partitioned against petroleum ether and finally the aqueous phase (containing colchicine) was extracted with chloroform. The chloroform extract was then evaporated.

High-performance liquid chromatography (HPLC)

The identification of colchicine was done by comparing the retention time of the sample with that of the authentic colchicine (Sigma) by High-performance liquid chromatography. The instrument was used an 1100 LC system (Hewlett-Packard, Waldbronn, Germany). A C_{18} column (250×4.6 mm) was used as the stationary phase. The mobile phase consisted of a mixture of methanol and 0.1% acetic acid solution (40: 60) with a flow-rate of 1.0 ml min^{-1}. The wavelength selected for UV detection was 254 nm (Sivakumar *et al*, 2004).

Result and Discussion

The qualitative and quantitative estimation of identified Alkaloid (Colchicine) from *G. superba in vivo* and *in vitro* has been presented in (Table, 1,2). The presence of Colchicine was confirmed by thin layer chromatography in which a spot coinciding with the authentic sample of Colchicine in Rf value (Colchicine 0.70) appeared. The developed plates when air dried and visualized under UV light and by exposure to ammonia fumes clearly showed the presence of Colchicine in plant parts as well as in tissues. Identification of isolated compound was established by mp (153-157°C) and characteristic HPLC peaks, which were superimposable to respective authentic sample (Fig. 1).

The maximum amount of colchicine was found in flower (20.7 mg/gdw) and minimum amount was found in stem (7.4mg/gdw). The *In vitro* studies showed the maximum amount of colchicine in 6 week old cultures (22.6 mg/gdw) and minimum amount was found in the 2 week old cultures (15.3 mg/gdw) (Table, 1,2)

Table 1. Colchicine content (mg/gdw) in various plant parts of *G. superb*.

S. No.	Plant part	Colchicine content mg/gdw
1	Leaf	8.0
2	Stem	7.4
3	Rhizome	19.8
4	Flower	20.7

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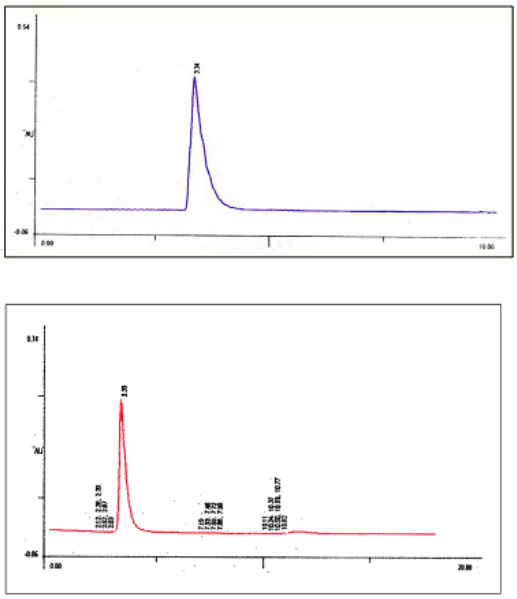
Table. 2. Growth indices (GI) and Colchicine content (mg/gdw) in vitro tissue cultures of *G. superb*.

S. No.	Age of the tissue (in weeks)	Growth indices (GI)	Colchicine content (mg/gdw)
1	2	0.26	15.3
2	4	0.65	18.7
3	6	1.27	22.6
4	8	0.86	19.2

Fig. 1. HPLC Chromatogram of Standard and isolated colchicines.



Isolated Colchicine
Standard Colchicine

References

Finnie, J. F. Van S. and Taden J. (1991): Isolation of colchicine from *G.superba* Lin. Variation in the alkaloid levels of plants grown IN vivo. *J. Plant Physiol.*, 138: 691–5.

Gambrog, O. L. ,R. A. Miller and K. Ojima, (1968): Nutrient requirements of suspension cultures of soyabean root cells. Exp. Cell Res., 50: 151-158.

Gooneratne, B. W. (1966): Massive generalized alopecia after poisoning by *G. superba* Lin. *Br. Med. J.*, 23 1(5494):1023-4.

Harborne, J.B. (1984): *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.* Chapman and Hall, London, UK

Harborne, J.B. and A.J. Harborne, (1998): *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Kluwer Academic Publishers, London, UK

Hayashi, T. Yoshida K. and Sano K. (1988): Formation of alkaloids in suspension cultured *Colchicum autumnale*. Phytochem., 27: 371–1374

Murashige, T. and F. Skoog,. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum 15: 473-497.

Nagaratnam, N. De Silva D.P. De Silva, N. (1973): Colchicine poisoning following ingesgtion of *G. superba* Lin. Tubers. *Trop. Geogr. Med.*, 25(1); 15-7.

Neuwinger H. D. (1994): African ethnobotany poisons and drugs Chemistry. *J. Biol. Chem.*, 24, 213-214.

Sivakumar G. and Krishnamurthy K.V. (2002): *Gloriosa superba* L., a Very Useful Medicinal Plant, *Series Recent Progress in Medicinal Plants, vol. 7, Ethnomedicine and Pharmacognosy, Part II*, Singh, V.K., Govil, J.N., Hashmi, S., and Singh, G., Eds., Texas: Sci. Techn. Publ., 465–482.

Sivakumar, G. Krishnamurthy, K.V., E.J.Hahn and Paek K. Y. (2004): Enhanced *in vitro* production of colchicine in *Gloriosa superba* Lan emerging industrial medicinal crop in South India. *J. Hort. Sci. Biotechnol.*, 79 (4): 602–605.

Thakur RS Potěsilová H and Santavý F. (1975): Alkaloids of the plant *G. superba* Lin. *Planta Med.*, 28 (3): 201-9.

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