Quantification of colchicine in various parts of *Gloriosa superba* by HPLC

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ABSTRACT

Gloriosasuperba (Glory lily) belonging to the family of colchicaceae is a valuable source of medicinally important secondary metabolites. Hence, the present investigation was carried out in *Gloriosasuperba* with the aim of extracting colchicine, a medicinally important alkaloid by various methods.Colchicine was quantified in the various extractions (Dichloromethane, Methanol,acetonitrile: water: phosphoric acid (70:30:0.1, v/v/v) and Hot water extraction) of stem, leaf, tuber, pod andflower of *Gloriosasuperba*.Among the various extracts dichloromethane gave maximum and the sonication method gave the minimum extraction of colchicine.

KEYWORDS: Colchicine, *Gloriosasuperba*, Secondary metabolites.

1. INTRODUCTION

There are various types of alkaloids in the plants. Colchicine is the major alkaloid of *Gloriosasuperba* and other members of the Colchicaceae family such as the autumn crocus and is named after Colchicum, a plant containing colchicine (Brossi, 1990). Colchicine can be described as one of the most interesting alkaloids inspections of colchicine structure has shown no obvious relationship to any other known families of plant alkaloids.

All parts of the plant *Gloriosasuperba* contains colchicine.Its content in tuber and seeds varies from 0.15 to 0.3% and 0.7 to 0.9% respectively. The aromatic amino acids like Phenylalanine, Tyrosine and Tryptophan which are derived from the sikhimate pathway are required as building blocks for production of the secondary metabolite Colchicine (Ghosh, 2002). Colchicine is one of the seven Upanishads in the Indian medicine, which cure many ailments like Gout,Famililalmediteeranean fever but may prove fatal on misuse as it is a semi poisonous drug. It also has antibacterial and antimicrobial activity (Rakesh, 2012).

2. MATERIALS AND METHODS

2.1. Collection of plant materials: The plant material *Gloriosasuperba* (leaves, stem, tubers and pods) selected for extraction of colchicine alkaloid was collected from Mr.T.D.Rajendhran, India herbs, Sivakasi and Mr. Mani Raja, Rajapalayam.

2.2. Extraction protocols

2.2.1. Solvent extraction: 0.5 g of powdered plant material was extracted twice with 25 ml of petroleum ether with frequent shaking for 1 h, followed each time by filtration. The solid residues were air dried and then extracted with 10 ml of dichloromethane at room temperature for 30 min with frequent shaking. Then 10% solution of ammonia (0.5 ml) was added to themixture with vigorous shaking for 10 min; the mixture was left undisturbed for 30 min and then filtered. The residue was washed twice with 10 ml of dichloromethane and then combined with the filtrate. The organic phase was evaporated to dryness and then dissolved in 1 ml of 70% ethanol to yield the test sample (Kavina, 2011).

2.2.2. Freeze drying extraction: 20 g of freeze dried material was extracted using 200 ml methanol in a cold room (10°) overnight and the homogenate was centrifuged at 1252 × g for 5 min. The methanolic extract was evaporated to dryness and then the residues redissolved in 50 ml water. The aqueous extract was then centrifuged at 7826 × g for 5 min. The supernatant was first partitioned twice against petroleum ether and then discarded, and then once in diethyl ether, discarding the supernatant each time. The residue was washed five times with equal volumes of chloroform, which was retained and evaporated to dryness. The chloroform residue was redissolved in 95% HPLC grade methanol and then filtered through a 0.2 µm millipore filter to yield the test sample. (Bharathi, 2006)

2.2.3. Sonication method: 0.5 g of plant material was collected and then mixed with 100 ml of acetonitrile: water: phosphoric acid (70:30:0.1, v/v/v). The mixture was sonicated for 5 min and shaken for 10 min and then centrifuged for 5 min. The extract was diluted with 95% HPLC graded methanol, then filtered through a 0.2 µm millipore filter to yield the test sample.

2.2.4. Hot water extraction: 5 g of crude plant sample was collected and then washed thoroughly in tap water. The plant material was soaked in 100 ml of water and placed in cooker to maintain the pressure at 15 psi and temperature at 121◦C, then the extract was filtered using filter paper then the sample diluted with 95% HPLC graded methanol, then filtered through a 0.2µm millipore filter to yield the test sample.

2.2.5. High Performance Liquid Chromatography (HPLC): Identification of colchicine was done by comparing the retention time of the sample with that of the standard obtained from Sigma, USA. A Luna C_{18}250 × 4.60 mm column was used as stationary phase. The mobile phase used was 25% HPLC grade methanol with a flow rate of 0.2 ml/min. 100 µl of sample was injected and the peaks were detected at specific wavelength. The colchicine extracted from *Gloriosasuperba* by four different methods was eluted at 12.8 to 13 min.

3. RESULTS AND DISCUSSION

3.1. Colchicine Extraction: The chromatogram obtained for the standard colchicine of different concentrations 0.1 mg/ml, 0.4mg/ml, 0.5mg/ml and 1mg/ml was shown in Figure.1 and Figure 2.

Table.1.Peak area obtained for different concentrations of standard Colchicine

Concentration (mg/l)	Retention time (mins)	Area ($x10^8$)
0.1	12.851	0.0315
0.4	13.538	0.120
0.5	13.254	0.637
1	13.124	1.312

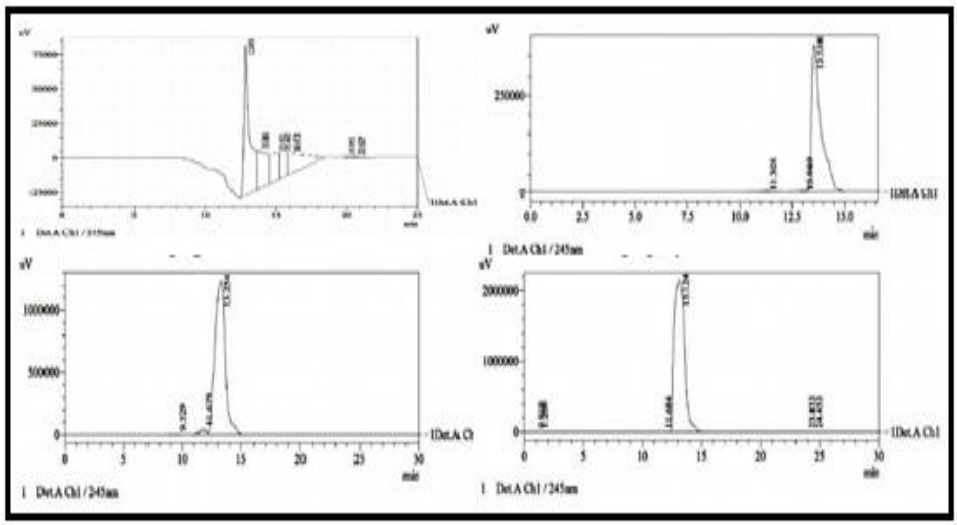

Figure.1.Different concentration of standard colchicine

The standard graph plotted between area vs concentration was shown in figure.2.

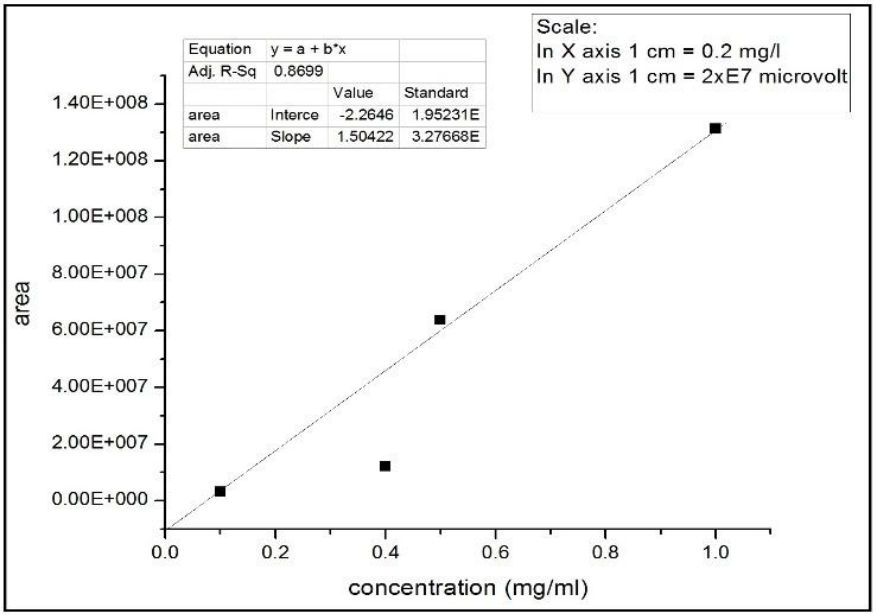

Figure 2: Standard graph

From this standard graph, the slope value was calculated and found to be **$1.504*10^8$**. Using this slope value, unknown concentration of colchicine extracted from *Gloriosasuperba* plant by four methods was calculated and was discussed below.

Table 2: Peak area obtained for colchicine extracted from different plant parts of *Gloriosasuperba*using Different methods

Methods	Sample	Retention time (mins)	Peak area ($x10^8$)	Concentration (mg/ml)
Freeze drying method	Leaf	13.050	0.60	0.39
	Pod	12.904	0.49	0.32
	Stem	13.235	0.61	0.40
	Tuber	13.234	0.67	0.44
	Flower	13.240	0.58	0.38
Dichloromethane method	Leaf	12.996	0.86	0.57
	Pod	13.035	0.73	0.48
	Stem	13.133	0.8	0.53
	Tuber	13.120	1.12	0.74
Hot water extraction method	Leaf	13.308	2.1	1.42
	Pod	13.140	0.79	0.52
	Stem	13.307	0.21	0.14
	Tuber	13.234	0.27	0.18
Sonication method	Leaf	13.415	0.068	0.04
	Pod	12.875	0.31	0.21
	Stem	13.244	0.14	0.09
	Tuber	12.799	0.17	0.11

3.3. Concentration comparison (Table 1 and Table 2)

Freeze drying method - higher concentration of colchicine was extracted from tuber sample and found to be 0.44 mg/ml.
Dichloromethane method - higher concentration of colchicine was extracted from tuber sample and found to be 0.74 mg/ml.
Hot water method - higher concentration of colchicine was extracted from leaf sample and found to be 1.42 mg/ml.
Sonication method - higher concentration of colchicine was extracted from **pod** sample and found to be 0.21 mg/ml.

Among the above four methods of extraction, higher concentration of colchicine was extracted in Dichloromethane **method** and very lower concentration of colchicine was extracted in Sonication method.Also, Hot water method was found to be the cheapest method of colchicine extraction as water was the only solvent used for extraction. Tuber was found to be having higher amount of colchicine in solvent extraction but in hot water method, water cannot extract colchicine from tuber, so increasing the temperature and pressure can extract higher amount of colchicine from tuber samples.

4. CONCLUSION

Extraction of colchicine from different plant parts (tuber, leaf, pod, stem, and flower) of *Gloriosasuperba* was successfully done by Dichloromethane method, Freeze drying method, Hot water method and Sonication method. Yield of colchicine was high in Dichloromethane method of extraction followed by Hot water method. Dichloromethane method of extraction was found to be the optimized method for extraction of colchicine but there was a problem of loss in colchicine content in the plant parts because of using more polar solvents while extraction.

Hot water method of colchicine extraction was economically cheap and less laborious, loss of colchicine was also very less during extraction as water was the only solvent used it can be used for extraction of colchicine from *Gloriosasuperba* commercially. In future, the temperature and pressure used in hot water method should be optimized for improved extraction of colchicine.

Freeze drying method showed considerable yield of colchicine but it is much laborious and takes more time. Though Sonication method was simple it did not have good yield when compared to the other methods of extraction.

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