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Quantification of colchicine in different parts of *Gloriosa superba* L

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Abstract
Gloriosa superba - a perennial climber and an important medicinal plant is used to cure diseases like gout, rheumatism, arthritis, ulcers and have properties like anthelmintic, purgative, abortifacient, antipyretic *etc*. The medicinal value of the plant is mainly due to the presence of alkaloids in all the parts of plant mainly colchicine which is an amino alkaloid derived from derived from two amino acids phenylalanine and trynosine present in it. In the present study colchicine, a major alkaloid of *Gloriosa superba* was quantified in different parts of plants gown at sub- temperate climate by using HPLC-UV. The seeds were found to contain the highest content of colchicine when compared to other parts under study. The colchicine content ranged from 0.051-0.695 %. The other parts studied were tubers, stems and leaves. The colchicine was detected in all four parts of the plant.

Keywords: Medicinal; alkaloid; colchicine; *Gloriosa superba*; quantification; HPLC-UV

1. Introduction
Gloriosa superba L. (Liliaceae) commonly known as 'Kalihari' in Hindi, 'Malabar Glory Lily' in English and also the trade name as 'Glory Lily' (Ambasta, 1986; Pulliah, 2002) [13, 14]. It is the National flower of Zimbabwe and also, state flower of Tamil Naidu (Kumar *et al.,* 2015) [3]. *Gloriosa superba* is native of tropical Africa and now growing naturally in several parts of tropical Asia as in India, Myanmar, Malaysia and Srilanka (Jayaweera, 1982; Ade & Rai, 2009) [4, 9]. The medicinal value of the plant is mainly due to the presence of alkaloids in all the parts of plant mainly colchicine which is an amino alkaloid derived from derived from two amino acids phenylalanine and trynosine present in it (Sivakumar *et al*., 2004) [5]. Plant is also reported to be used for treating cholera, typhus, Bright's disease, piles, skin diseases, leprosy, gonorrhoea and chronic ulcers by different authors (Chopra *et al.,* 1956; Gupta 1982) [10]. Rootstock is useful in treatment of intestinal worms, bruises, infertility and impotence. Tubers are used in vitiated conditions of kapha and vata. Plants also show medicinal properties like antibacterial, germicidal, antimalarial *etc.* (Jana and Shekhawat, 2011) [12]. The plant has been reported to be used in the treatment of gout, arthritis (Anonymous, 1956) [1], rheumatism (Nadkarni, 1996) [7] and cancer (Chopra *et al.,* 1956) [10]. Due to over-exploitation of this species, this plant has been entered in Red Data Book (Badola, 2002) [2].

2. Experimental
2.1 Plant material
Plants of *Gloriosa superba* were collected from field grown plants at Botanical Garden of Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh, India) which is situated at 1250m altitude, 30˚51'35.85" N latitude and 77˚10'22.66" E longitude. Different parts of plants *i.e.* seeds, tubers, leaves, stems were separated and shade dried.

2.3 Preparation of material for extraction
Shade dried plant parts were grinded mechanically and sieved by mesh size 800 microns sieve to form the uniform part icle size of the plant material, which was used for quantification of colchicine in the samples.

2.4 Determination of colchicine in plant material
The samples (2 gram each) of different plant parts were extracted by soxhlet with methanol for 2 hours duration. After extraction, solvent from each sample was distilled off and the residue was completely dried to constant weight was then subjected to

HPLC analysis for quantification of colchicine. The dilution of the samples was done using mobile phase (acetonitrile: water: 60: 40, v/v), centrifuged at 4000 rpm and then filtrated through 0.45µm membrane prior to injection in the HPLC system.

2.5 HPLC method

The HPLC method development and validation was done by Waters binary HPLC unit with Waters HPLC pump 515 and dual λ absorbance detector 2487 and Sunfire C-18 (4.6 x 250mm, 5µm) column. Isocratic elution of both samples and standards was done for 15 minutes with mobile phase acetonitrile: 3% glacial acetic acid (60: 40 v/v) at a flow rate of 1ml/min and detection at 245nm. The method was validated for linearity which was established for six concentrations ranged from 4.75-95µg/ml with good correlation coefficient 0.999. Limit of detection (LOD) and limit of quantification (LOQ were found 0.003µg/ml and 0.012µg/ml respectively which signifies the limit up to which developed method can detect and quantify the colchicine concentration in samples.

2.6 Source of Colchicine standard: Standard compound colchicine was purchased from Sigma Aldrich, USA.

2.7 Calibration curve for linearity

Standard solutions of colchicine 4.75, 9.5, 19, 38, 76 and 95µg/ml were injected thrice and linearity was established. The regression equation was found as Y = 1.08e+005 X + 9.86e+004 (where, X = concentration µg/ml and Y= absorbance) with correlation coefficient 0.999.

2.8 Statistical Analysis

Statistical comparison was performed using OP-STAT software and was considered statistically significant.

Table 1: Colchicine content (%) in different parts of *Gloriosa superba*

Sr. No.	Plant Part	Extracting solvent	Colchicine Content (%)
1	Seeds	Methanol	0.695 (0.833)
2	Tubers	Methanol	0.396 (0.629)
3	Stems	Methanol	0.051 (0.227)
4	Leaves	Methanol	0.164 (0.405)
$CD_{0.05}$			0.009

Values in the parentheses are transformed values using $\sqrt{X}$ transformed values

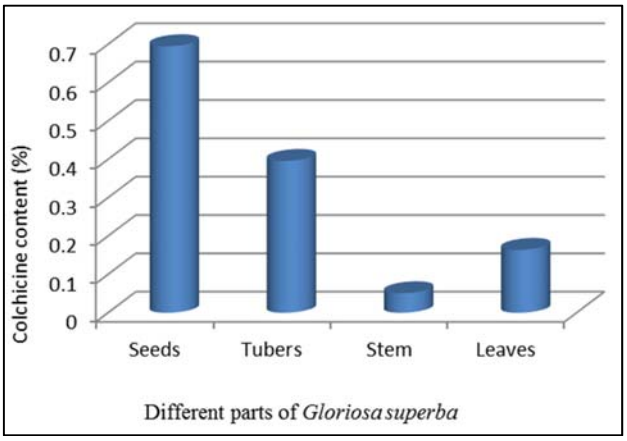

Fig 1: Column chart showing colchicine content (%) in different parts of *Gloriosa superba*

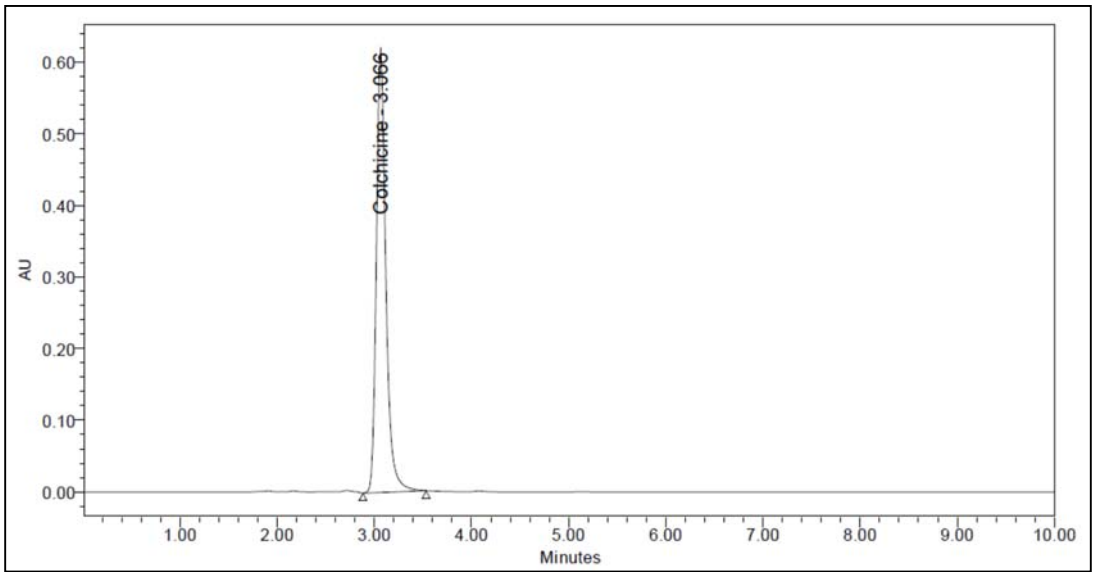

Fig 2: HPLC chromatogram of standard compound colchicine

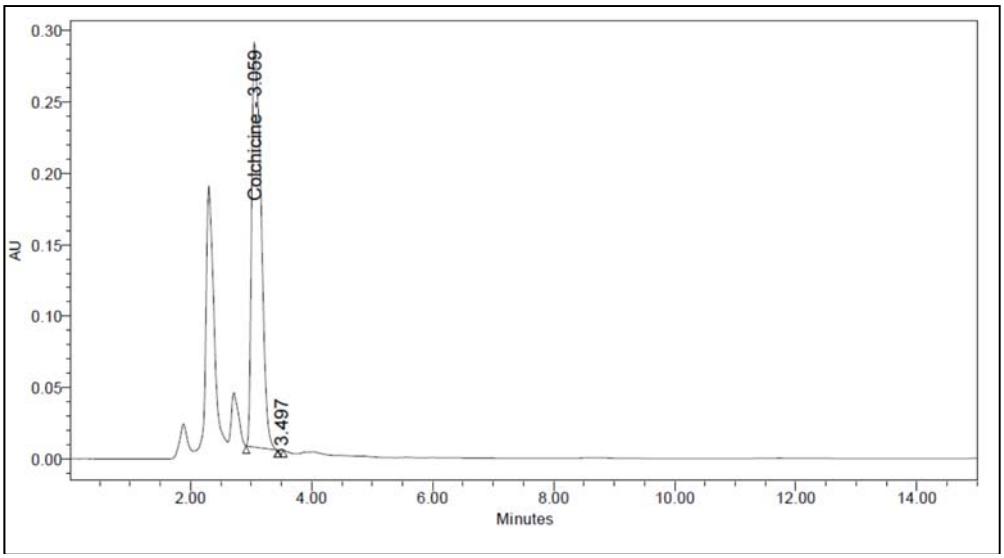

Fig 3: HPLC chromatogram of seed sample of *Gloriosa superba*

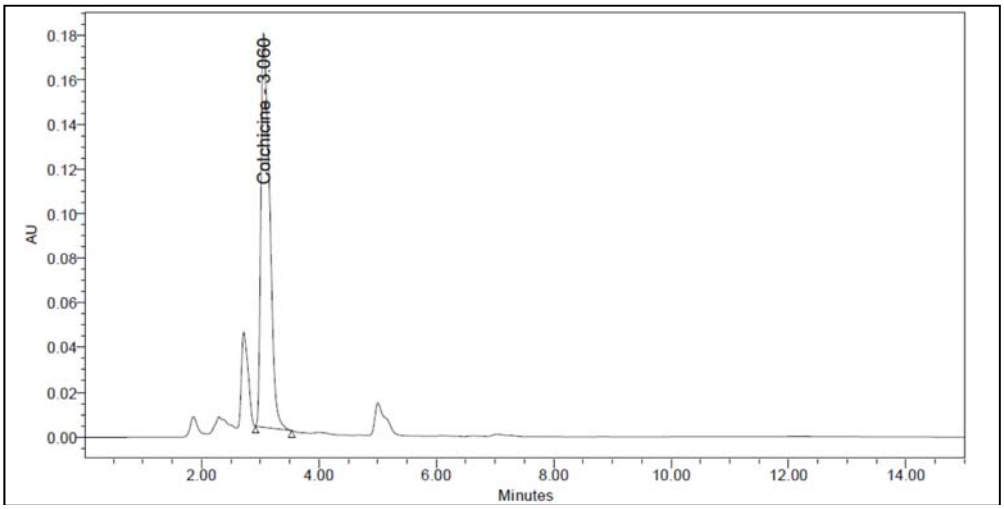

Fig 4: HPLC chromatogram of tubers samples of *Gloriosa superba*

3. Results and Discussion

The results of the study aimed at the quantification of colchicine content in *Gloriosa superba* grown at sub-temperate climate. The results showed that the plants cultivated at sub-temperate climate have appreciable quantity of colchicine. All plant parts *viz.*, leaves (0.164%), stems (0.051%), tubers (0.396%) and seeds (0.695%) contains colchicine. The colchicine content in different parts of the plant found in present study was given in table no. 1 and fig. 1. The HPLC chromatograms of standard colchicine and different parts (seeds and tubers) are given in figure 2-4. Similar type of research work (assesment of colchicine in different parts of *Gloriosa superba*) has been done by Sarin *et al*., 2010 and Raj J. *et al*., 2014 [11, 6].

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