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Variation in colchicine content in tubers of *Gloriosa superba* L. from Madhya Pradesh for identification of elite chemotypes

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Abstract
Gloriosa superba L. is one of the important species being used in many parts of the world in traditional system of medicines. Colchicine is the major active ingredient and an important drug found in the tubers of this species. The variation in active ingredients according to environmental and edaphic factors have been described for many plant species for evaluation of their quality as well as identification of elite chemotypes. The present study is an effort to find out the variation in colchicine content in tubers of *Gloriosa superba* collected from 14 sites belonging to ten agroclimatic regions of Madhya Pradesh using high performance thin layer chromatography (HPTLC). The results showed significant variation in colchicine content which ranged from 0.164 to 0.489% (dry wt.). The accessions of Katthiwada (Alirajpur) were found the elite chemotypes in terms of its colchicine content (0.489%).

Keywords: *Gloriosa superba*, Tubers, Colchicine, Variations, Elite chemotypes

Introduction
Medicinal plants are used as herbs or traditional medicines for various types of diseases since ancient times and are considered as a rich source of active ingredients which can be used in drug development and synthesis (Manjamalai *et al*., 2011; Farombi, 2003 and Baytop *et al*., 2003) [1-3]. Besides, these plants play a critical role in the development of human cultures around the whole world and according to the World Health Organization (WHO), approximately 80% of the world's population currently uses herbal medicines for their health care (Baker *et al*., 1995) [4]. Medicinal plant industry including drug products and raw materials is growing at the rate of 7-15% annually and total global botanical drug market is estimated as US$62 billion and is expected to grow to the tune of US$ 5 trillion by 2050 (Warude & Patwardhan, 2005 and Anonymous, 2000) [5,6]. To fulfill the increasing demand; over 90 % of the medicinal plants traded in India are harvested from wild only. The increasing demand also resulted in unscientific over exploitation of medicinal plants from forests which resulted many of the prestigious species of Indian medicinal plants threatened in the wild.
Gloriosa superba L. is one of the high traded species in the list of National Medicinal Plant Board (NMPB) in India (Ved & Guraya, 2007) [7]. Overexploitation and unscientific manner of collection from forests made this species vulnerable (VU) in Madhya Pradesh, Maharashtra, Andhra Pradesh, Kerala, Karnataka, endangered (EN) in Odisha and the species of high concerned in other states of the country (Budhiraja *et al*., 2012 and Anonymous, 2010) [8,9]. The widespread use of its tubers for medicinal purposes has led the species in threatened category (Ade & Rai, 2009; Singh *et al*., 2013) [10,11]. Due to its threatened status, it has been placed on negative list of exports by Ministry of Commerce, Govt. of India (Budhiraja *et al*., 2012) [8]. The species belongs to family Liliaceae and popularly known as 'Glory Lily', 'Kalihari', 'Ognisikha' due to its wavy edged yellow and red flowers that appears from November to March every year (Rajak and Rai, 1990) [12]. It is one of the seven upavishas in the Indian medicine, which cure many ailments but may prove fatal on misuse (Joshi, 1993) [13]. It is a perennial branched climbing herb with tuberous rootstock. It is commonly found in low forests almost throughout India including Andaman Islands upto an altitude of 1600 m. The tubers of *Gloriosa superba* are the rich source of an alkaloid, colchicine (Fig. 1) which is used for the treatment of gout, cancer, cirrhosis, rheumatic arthritis, inflammation, ulcers, scrofula, bleeding piles, white discharge, skin diseases, leprosy, indigestion, helminthes, snake bites, baldness, intermittent fever, debility and is also considered useful in promoting labor and

expulsion of placenta (Srivastava and Chandra, 1977; Prajapati *et al*., 2003; Gupta *et al*., 2005; Samy *et al*., 2008; Singh, 1993 and Satyavati *et al*., 1976) [14-19]. With a herbal 'renaissance' occurring across the globe, the plant is also in great demand for commercial formulations of herbal medicines owing to its remarkable pharmacological potentials.

Since, the quality and efficacy of medicinal plants/raw materials depend on their biologically active compounds (Joshi and Uniyal, 2008) [20] which often vary from region to region and localities to localities according to the environmental, edaphic and seasonal factors, the correct identification and quality assurance of the plant materials in terms of their active constituents is, therefore, an essential prerequisite to ensure reproducible quality of herbal drugs for their safety and efficacy (Kushwaha *et al*., 2010 and Goldman, 2001) [21, 22]. WHO and modern herbal pharmacopoeia are also strongly emphasizing for standardization of the quality of medicinal plants with respect to their active constituents (Sharma *et al*., 2010 and Vasudevan, 2009) [23, 24]. Thus, quality evaluation of medicinal plants and its plant parts is the need of the hour to standardize the drug as well as identification of elite chemotypes.

Madhya Pradesh is a large state of India, lying between latitude 21°04'N - 26.87°N and longitude 74°02'E - 82°49'E, comprised of 11 agro-climatic regions and endowed with rich and diverse medical plant resources, the variations in active ingredients of medicinal plants is quite evident. As far as *Gloriosa superba* is concerned, the quality standardization of this prestigious species in terms of colchicine content in tubers has not been carried out vigorously in Madhya Pradesh. Since, this prestigious species has already been on the edge of depletion from the forest areas, it is not possible now to fulfill the increasing demand from forest. Therefore, promotion of cultivation of this species, which has already been started at some places in the country (Jana and Shekhawat, 2011) [25], is the need of the hour to meet the market requirement and for this purpose the quality standardization and identification of elite chemotypes is very essential. In the present study, the tubers of *Gloriosa superba* collected from different agroclimatic regions of Madhya Pradesh were assessed for their colchicine content using HPTLC for identification of elite chemotypes and best sites.

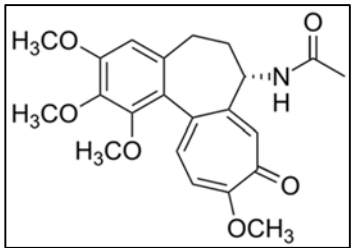

Fig 1: Chemical structure of colchicine

Experimental

Plant materials and chemicals

Gloriosa superba is rare in Madhya Pradesh. Therefore, purposive sampling was followed for collection of tubers of the species from fourteen locations belonging to ten agroclimatic regions of Madhya Pradesh in the month of December. GPS locations of collection sites were recorded. Plants having approximately same ground biomass were selected for collection of tubers. Taxonomic identification of the plant was confirmed by Biodiversity & Sustainable Management Division of Tropical Forest Research Institute, Jabalpur, Madhya Pradesh, India (Identification No. 1758) and the voucher specimen was deposited in the same division. Colchicine standard was purchased from Sigma Aldrich. All chemicals and solvents used were of AR grade.

Processing of plant material

The collected fresh tubers were washed in running water to remove the unnecessary foreign particles, cut into small pieces and dried in shade. The shade dried roots were grinded to make powder and stored in air –tight polythene bags for further analysis.

Quantification of Colchicine in extracts using HPTLC technique

Quantitative evaluation of colchicine in tuber extracts of *G. superba* was carried out by standard method (Anon, 2010) [26].

Statistical analysis

Samples on TLC plates were applied in triplicate and results were expressed as Mean ± SD (n=3). Results were evaluated as significant and non – significant applying analysis of variance.

Results and Discussion

Collection sites, GPS locations and colchicine concentration in tuber samples of *G. superba* are given in Table 1 and Fig. 6. It is clear from the table that colchicine concentration in tubers of *G. superba* collected from fourteen different localities varied from 0.164% - 0.489%. The highest concentration of colchicine was observed in the accessions collected from Katthiwada, Alirajpur (0.489%) and the lowest concentration in the accessions of Pamakhedi, Khandwa (0.164%). The second highest concentration of colchicine was recorded in tuberous samples of TFRI, Jabalpur (0.482%).

The variation in colchicine content within the localities of Madhya Pradesh may be due to various environmental factors viz. temperature, altitude, soil, rainfall, humidity, drought, light intensity, high salinity, supply of water, minerals, freezing temperatures and CO2 which influence the growth of a plant and secondary metabolite production (Morison and Lawlor, 1999; Sandeep *et al*., 2015; Garg *et al*., 1999; Anandaraj *et al*., 2014 and Pothitirat & Gritsanapan, 2006) [27-31]. Many researchers carried out the studies on effect of environmental factors on secondary metabolites in various plants (Payyavula *et al*., 2012 and Rao & Rao, 2012) [32, 33]. Ramakrishna and Ravishankar (2011) [34] also reported the accumulation of secondary metabolites in plants subjected to stresses and help the plants to adapt according to the environment and in overcoming stress conditions. In an another study, the chemical ingredients of turmeric have been reported to vary from location to location due to the influence of environmental, agroclimatic and edaphic conditions (Sandeep *et al*., 2015 and Szakiel *et al*., 2010) [28, 35].

The colchicine content in *G. superba* tubers originated from four locations of three agroclimatic zones in odisha state ranged from 0.14% to 0.56% (Basak *et al*., 2012) [36]. Colchicine concentration in tubers of *G. superba* has also been reported to the level up to 0.9% (dry wt) (Finnie and Staden, 1991) [37]. In Karnataka state of India, the colchicine percentage in the tubers of *G. superba* has been reported in the range of 0.15 - 0.3% (Farooqi *et al*., 1993) [38]. In hilly area of Himachal Pradesh, the tubers were found to contain 3.7% colchicine content (Gupta *et al.,* 2005) [39]. The

colchicine content was also reported in between 0.42 – 0.46 % for quality standardization of this species in India (Anon, 2010) [26]. In a study, the tubers of *G. superba* from Amarkantak (Chhattisgarh), Bhedaghat (Jabalpur) and Tamia (Chhindwada) were reported to contain the cochicine content of 0.9%, 1.3% and 2.5% respectively (Srivastava *et al*., 2013 [40] while in the present study, the colchicine content in the tubers of Amarkantak (Anuppur), TFRI (Jabalpur) and Tamia (Chhindwada) locations were found as 0.354%, 0.482% and 0.358% respectively. This variation in the colchicine concentration may be due to the collection of tuber samples from different locations of a large region under an agroclimatic zone. Different edaphic conditions under an agroclimatic zone may also be responsible for this variation within the region. Thus, for selection of elite germplasm and to get more fruitful results, more vigorous sampling will be required. The results showed significant variation in colchicine content among the fourteen locations.

Table 1: Colchicine content in tubers of *G. superba*

S. No.	Agro-climatic regions	Districts	Collection sites	GPS locations	Colchicine (%, dry weight)
1.	Vindhyan Plateau	Sehore	Pandado	N 22°46'33.4" E 77°37'23.8"	0.351
2.	Chhattisgarh plains	Balaghat	Rupjhar	N 21°56'59.2" E 80°25'44.1"	0.457
3.	Satpura Plateau	Betul	Baretha	N 22°08'29.4" E 77°53'19.7"	0.339
		Chhindawara	Tamia	N 22°20'40.5" E 78°39'57.4"	0.358
4.	Grid zone	Shivpuri	Chand	N 25°35'42.8" E 77°46'08.8"	0.380
		Gwalior	Audhpur	N 26°10'17.3" E 78°10'33.3"	0.272
		Seopur kalan	Bagwaj	N 25°39'43.2" E 76°49'02.9"	0.362
5.	Kymore plateau & Satpuda Hills	Jabalpur	TFRI	N 23°05'55.3" E 79°59'21.7"	0.482
		Panna	Shyamagiri	N 24°10'35.2" E 80°29'03.6"	0.437
6.	Malwa Plateau	Mandsaur	Navali	N 24º 39' 4.0" E 75º 45'61.6	0.246
7.	Nimar Valley	Khandwa	Pamakhedi	N 21º 53' 28" E 77º 51' 02"	0.164
8.	Jhabua Hills	Alirajpur	Katthiwada	N 22°28'20.10" E 74°08'58.45"	0.489
9.	Central Narmada Valley	Hoshangabad	Panarpaani	N 22°29'11.1" E78°26'08.4"	0.331
10.	Northern Hill Zones of Chhattisgarh	Anuppur	Amarkantak	N 22°40'59.1" E 81°45'07.7"	0.354
CD 0.05					0.135
SE±					0.046

TFRI = Tropical Forest Research Institute

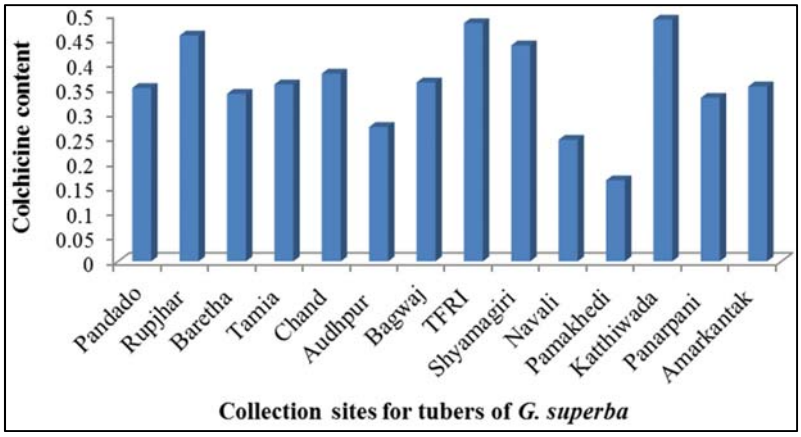

Fig 2: Colchicine content in tubers of *G. superba*

Conclusion

Present study revealed that the colchicine content in the tubers of *Gloriosa superba* varied from one location to another. The colchicine content was varied from 0.164% - 0.489%. The maximum content of colchicine was found in the tubers of Katthiwada, Alirajpur (0.489%) and therefore, the accessions of this location may be considered as existing elite chemotypes and may be *in-situ* conserved. This certainly will help in conservation of such endangered plant species which is on verge of extinction. The tubers of this location may also

be the better source of commercial colchicine and may further be exploited as elite material for propagation purposes.

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