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Antimitotic Effect of Colchicine from Six Different Species of *Gloriosa* in Onion Roots (*Allium cepa*)

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Colchicine, a chief alkaloid was determined in six different species of *Gloriosa*. Solvent extraction of colchicine with petroleum ether and dichlomethane and quantification through High Performance Liquid Chromatography showed high level of colchicine (0.342 mg g^{-1}) in *Gloriosa planti* amongst the species selected for the study. The mitotic inhibition of colchicine in onion root was standardized using standard colchicine. The effect of colchicine extracted from *Gloriosa* species was studied in onion root tips treated with 30 mg L^{-1} colchicine for 2 h. Mitotic abnormalities have been observed and reported for the extracts from different species of *Gloriosa* under study.

Key words: *Allium cepa*, colchicine, *Gloriosa*, mitosis

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INTRODUCTION

Colchicine is an alkaloid produced by several plants of Liliaceae family. One among Liliaceae, *Gloriosa superba* is the plant containing colchicine (Sarin *et al*., 1974). The species also contains another toxic alkaloid, Gloriosine (Angunawela and Fernando, 1971). Other compounds such as lumicolchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine, 3-demethylcolchicine, N-formyldeacetylcolchicine have been isolated from the plant (Chulabhorn *et al*., 1998). The alkaloid, colchicine is the drug of choice to relieve acute attacks of gout and familial mediterranean fever (Alali *et al*., 2004). At present there is renewed interest in the use of colchicine as a possible cure for cancer related diseases (Evans *et al.,* 1981). Owing to its potent affinity for tubulin, colchicine is used in biological and breeding studies to produce polyploids and in tubulin binding assays as a positive control (Trease and Evans, 1983; Poutaraud and Champay, 1995). Colchicine is believed to interfere with cell division through its disruptive action on mitotic spindle. This inhibition either occurs directly or indirectly, an example of indirect action would be the activation of an enzyme which attacks the spindle while a direct action might involve binding of colchicine to spindle fibers causing them to dissociate into protein subunits.

The minimum concentration required for a mitotic block of animal and plant cells differs by several orders of magnitude. Thus, animal cells are about 100,000 times more sensitive to colchicine than plant cells (Deysson, 1975). Colchicine is usually lethal to dividing animal cells, even at low concentrations necessary to block mitosis, yet plant cells can survive in a state of colchicine induced mitotic arrest (C-mitosis) for several days and return to division after colchicine is removed (Rieder and Palazzo, 1992). Plant microtubules are more resistant to colchicine treatment than vertebrates microtubules, due to the low affinity of plant tubulin dimers for the drug and they can be completely disrupted by extremely high concentrations of colchicine (Morejohn and Fosket, 1991).

The effect of colchicine on higher plant cells is time dependent. Exposure to colchicine for a short time results in disassembly of the interphase microtubule network, prophase band, spindle and phragmoplast (Pickett-heaps, 1967), yet longer treatment leads to formation of tubular arrays with variable ultrastructural organization (Bennett and Smith, 1979).

Macrotubules are another type of a typical tubulin polymer found in plant cells after colchicine treatment (Deysson, 1975). The organization and intracellular location of tubulin containing arrays induced by colchicine vary in different plant species and cell types (Karagiannidou *et al*., 1995). In the root meristem of *Allium cepa*, colchicine triggers the formation of long strands in the cortical cytoplasm of C-mitotic cells (Utrilla *et al*., 1989).

In the present study, colchicine from six different species of *Gloriosa* was extracted and estimated. The antimitotic effects of these extracts have been studied in onion root tips with respect to known colchicine standard.

MATERIALS AND METHODS

Six different species of *Gloriosa* viz., *Gloriosa superba, Gloriosa rothchildiana, Gloriosa planti, Gloriosa lutea, Gloriosa casuariana* and *Gloriosa vuchuria* were grown *in vivo* in nursery at Vellore Institute of Technology during June 2004. One year old corms from each species were collected and sliced into small pieces for freeze drying at -20°C. After seven days, the freeze dried plant material was ground to fine powder and then used for extraction of colchicine.

Extraction method: From the freeze dried samples, 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 the mixture 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 (Alali *et al*., 2004).

Quantification of colchicine: Identification of the colchicine was done by comparing the retention time of the sample with that of the standard obtained from Sigma. Waters HPLC systems equipped with a binary pump1525 (Max.Pressure: 6000 psi.) and a porous silica with 5 μm diameter C_{18} 4.6 X 150 mm column was used for separation. The mobile phase consisted of Acetonitrile: 3% Acetic acid (60:40), at a flow rate of 1 mL min^{-1} and an injection volume of 20 μL. The peaks eluted were detected at 245 nm and identified with authentic standards.

Allium cepa root preparation: *Allium cepa* were germinated in the dark, the lower surface in contact with distilled water. When the roots were grown 2-3 cm long,

the seedlings were transferred to different concentration of standard colchicine viz., 10, 15, 20, 25, 30 mg L^{-1}. Meristematic root tips of length 1 to 2 mm were harvested with a sharp razor blade and placed immediately in fixative ethanol: chloroform: Acetic acid (1:2:1) for 5 min and then washed with distilled water. The tips were transferred to another fixative, Hydrochloric acid: Ethanol (1:1) for 2 min and washed with distilled water and finally fixed in 70% ethanol. Standardization on the effect of colchicine at different concentrations was tried for 1 and 2 h duration of treatment. Colchicine extracts from six different species of *Gloriosa* was used to study its effect on inhibition of mitosis, at the standardized concentration and time.

Sample preparation for microscope: Meristematic root tips of 3 mm long were cut and placed on microscopic slides and gently tapped to make squash. Sufficient acetocarmine stain was added and kept for 8 to 10 min. Coverslips were placed over the squash and flattened the cells by rolling a glass rod over it. Slides were observed under light microscope to observe the different stages of mitosis.

RESULTS

Colchicine extracted in the six different species of *Gloriosa* shows a retention time (RT) of 1.810 min, which was ensured on comparison with the standard colchicine obtained from SIGMA Chemicals at the concentration of 10 μg mL^{-1}. The chromatogram for colchicine extracted from *Gloriosa planti* is represented in Fig. 1. Among the different species of *Gloriosa* considered for the study, G*loriosa planti* exhibited the highest colchicine content

Table 1: Colchicine content in six different species of *Gloriosa* as estimated through HPLC

Gloriosa spp.	Colchicine content (mg g^{-1})
Gloriosa superba	0.211±0.004
Gloriosa rothchildiana	0.162±0.008
Gloriosa planti	0.342±0.047
Gloriosa lutea	0.294±0.019
Gloriosa casuariana	0.246±0.032
Gloriosa vuchuria	0.150±0.030

(0.342 mg g^{-1}) followed by *Gloriosa lutea, Gloriosa casuariana* and *Gloriosa superba* (0.294, 0.246 and 0.211 mg g^{-1} respectively) (Table 1).

The meristematic region of onion roots with out cochicine treatment, used as control shows a normal mitotic distribution represented in Fig. 2a. Most of the actively dividing cells were in prophase and few in metaphase, anaphase and telophase stages of cell division. The metaphase chromosomes were lined up at the equator and were pulled evenly towards spindle poles for the cells at anaphase. No abnormal chromosome was observed. Abnormalities in a metaphase stage were observed with increased concentration of colchicine (Fig. 2b-e). Many cells had abnormal mitotic organization with chromosomes being pulled unevenly at a concentration of 30 mg L^{-1} colchicine treated for 2 h (Fig. 2f). Some cells exhibited C-metaphase with condensed chromosomes arrested in their prometaphase stage for the same treatment.

The colchicine extract from six different species of *Gloriosa* at the concentration of 30 mg L^{-1} shows an inhibition of mitosis in onion root tip comparable with that of standard. The antimitotic effect of colchicine extracted from *Gloriosa* spp. is depicted in Fig. 3.

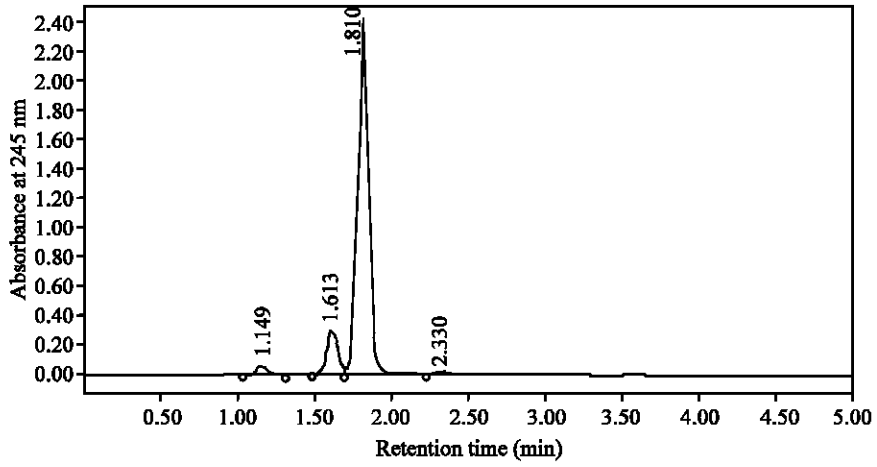

Fig. 1: The chromatogram for colchicine extracted from *Gloriosa planti* showing the RT 1.810 min

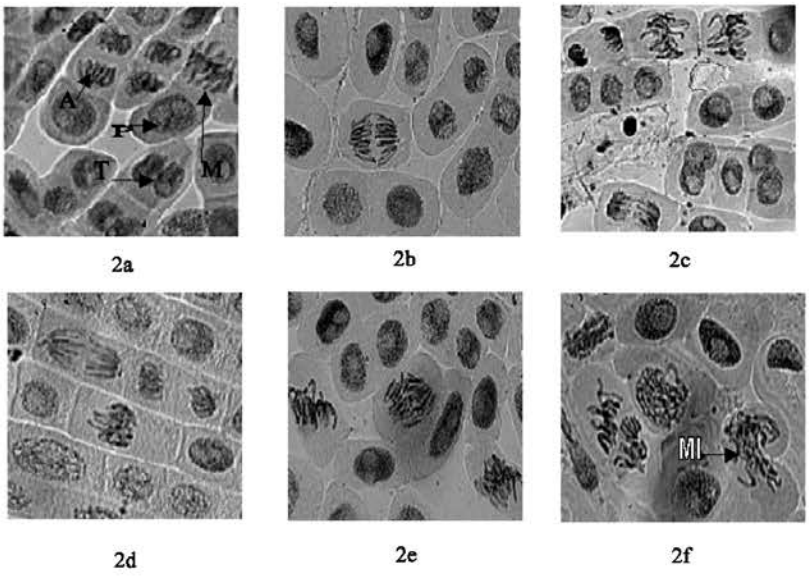

Fig. 2: Mitosis stages in onion root tip - (2a) Control (without colchicine treatment) P-Prophase, M-Metaphase, A-Anaphase, T-Telophase; (2b) 10 mg colchicine/l treated root tip; (2c) 15 mg colchicine/l treated root tip; (2d) 20 mg colchicine/l treated root tip; (2e) 25 mg colchicine/l treated root tip; (2f) 30 mg colchicine/l treated root tip MI-Metaphase inhibition

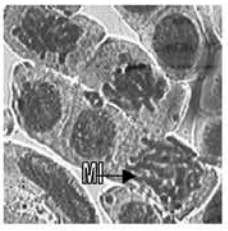

Fig. 3: Antimitotic effect of colchicine extract from *Gloriosa planti* showing the metaphase inhibition, which is similar to MI- Metaphase inhibition effect of colchicine standard in Fig. 2f

DISCUSSION

Finnie and Vanstaden (1991) reported colchicine levels in *Gloriosa superba* corms to the level of around 0.9% (DM). *Gloriosa* species apparently would be a better source of commercial colchicine for *Colchicum autumnale* where the levels of colchicine around 0.2% was reported (Bellet and Gaignault, 1985). Earlier study of *Gloriosa superba* revealed that colchicine levels are highest during the initial growth of plant and these levels decline during maturation, with a slight increase in alkaloid content when corms become dormant at the end of season (Thakur *et al*., 1975). Similar studies in *Colchicum*, showed that the colchicine is higher in one-year-old corms. It is also reported that *Gloriosa superba* corms of all ages have approximately the same colchicine content, but colchicine levels are highest at the beginning of the growing season. (Finnie and Vanstaden, 1991). Comparisons with the previous reports are difficult due to the fact that alkaloid content also varies with locality and season. However the relatively high colchicine content in *Gloriosa planti, Gloriosa lutea* and *Gloriosa casuariana* in this study encourages the cultivation of these species under suitable condition.

Abnormalities in metaphase shows that colchicine extracted from Gloriosa species has an effect on mitosis, at 30 mg L^{-1} treated for 2 h. The effect of colchicine on mitosis is reported to result from inhibition of microtubule assembly, resulting in mitotic arrest at prometaphase in a manner similar to that of podophyllotoxin (Vaughn and Vaughn, 1990). Hillman and Ruthmann (1982) observed similar mitotic

abnormalities with podophyllotoxin, vinblastine and colchicine in beet root tips. In *Allium,* root tips treated with colchicine, resulted in abnormal anaphase configurations along with cells in C-metaphase. (Segawa and Konda, 1978). Other phytotoxic natural products such as the sesquiterpene endoperoxide lactones and quassinoids have similar inhibitory activities for early stages of mitotic cells (Dayan *et al*., 1999).

Colchicine normally prevents the assembly of tubulin into microtubule cap; as a result the chromosomes condense but do not proceed to the next stage after prometaphase due to the lack of spindle microtubule which lead to a polymorphic nucleus (Vaughn *et al*., 1987). Colchicine extracted from *Gloriosa* spp. has antimitotic activity interfering with microtubule organization, resulting in abnormal metaphase. Among the six different species of *Gloriosa* studied *Gloriosa planti Gloriosa lutea* and *Gloriosa casuariana* reported to have higher concentration of colchicine, could be recommended as potent natural source of colchicine for *Colchicum autumnale* in biological and breeding studies.

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