Journal of Applied Pharmaceutical Science
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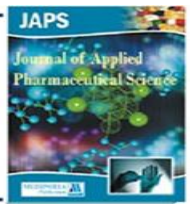

ISSN: 2231-3354
Received on: 19-09-2011
Revised on: 23-09-2011
Accepted on: 26-09-2011

Quantification of Colchicine in Seed and Tuber samples of *Gloriosa superba* by High Performance Liquid Chromatography method.

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ABSTRACT

To quantify the colchicine (alkaloid) present in *Gloriosa superba* by High Performance Liquid Chromatography (HPLC) method. *Gloriosa superba* species are known for their content of colchicine, a valuable alkaloid in the seeds and tubers (corms). HPLC analysis was carried out to confirm the presence of colchicine in *Gloriosa superba.* Treatment of gibberellic acid and *Psedomonas aerugenosa* improved the content of colchicine in seeds and tubers. Seeds of *Gloriosa superba* were rich in colchicines content than the tubers sample.

Key words: Colchicine, Gibberellic acid, *Gloriosa superba*, HPLC, *Psedomonas aerugenosa* treatments.

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INTRODUCTION

Gloriosa superba (Family-liliaceae) is one of the very important exported medicinal plants of India that has become endangered within a very short span of the last 50 years. The root is used as a germicide, to cure ulcers, piles, haemorrhoids, inflammation, scrofula, leprosy, dyspepsia, worm's infestation, flatulence, intermittent fevers, and debility arthritis and against snake poison. (Wealth of India, 1948-76). The tubers which looks like a hoe, It has been the most used in indigenous medical systems of India as well as in Africa. The medicinal importance of the plant is due to the presence of alkaloids (nearly 24 of them) of which colchicines and colchicoside are the principal ones, as well as to the presence of 10 non-alkaloid medicinal compounds including B-sitosterol, chelidonic acid, luteolin, stigmasterol etc (Nautiyal 2011). The major alkaloids are colchicine, 3-demethyl colchicine and colchicoside (Chitra et al., 2009). Seeds and tubers contain alkaloids such as colchicine and colchicoside, which are used to treat gout and rheumatism (Trease and Evans, 1983).

Colchicine is an alkaloid drug, chemically known as N-[(7*S*)-1, 2, 3,10-tetramethoxy-9-oxo-5,6,7,9 - tetrahydrobenzo[*a*]heptalen-7-yl] acetamide, and widely used for the treatment of gout disease (Calogero, 1992). Colchicine has the high market value and consistent demand in the field of medicine (Bharathi et al.. 2006). The alkoloids, colchicines is the drug of choice to relieve acute attack of gout and familial Mediterranean fever (Alali et al., 2004). At present there is renewed interest in the use of colchicines as a possible cure for cancer related diseases (Evans et al., 1981). Considering its significance this study will help in cultivation of *Gloriosa superba* for commercial extraction of colchicine.

MATERIALS AND METHODS

Plant Material

Medicinally important plant species, *Gloriosa superba* L. (Family: Liliaceae) was selected for the present investigation. The plant growth regulator Gibberellic acid (GA3) was purchased from Himedia India Ltd., Mumbai. *Pseudomonas aeruginosa* was obtained from Faculty of Agriculture, Department of Microbiology, Annamalai University, as culture form. During the study, average temperature was 32/26°C (maximum/minimum) and relative humidity (RH) varied between 60-75 per cent. The experimental part of this work was carried out in Stress Physiology Lab, Department of Botany, Annamalai University, Tamil Nadu. The methodologies adopted are described below.

Cultivation methods

The plants were raised in field condition in Udaiyarpalayam (Ariyalur Dist) Tamilnadu India. The tubers were sown during 2010 (September) and 2011 (January) in a completely randomized block design with three replications. The experimental area was tilled and planting furrows (30 cm deep) made at a distance of 1.5 m, 20 days before planting. Plotting mixture (red soil, sand, vermicompost and coir compost in1:1:1:1ratio) was applied in the furrows to ensure nutrient supply to the young plants. Each plot consisted of three 5 m long rows with inter and intra row spacing of 150 cm and 30 cm respectively. The plots were irrigated at weekly intervals. Recommended agronomic and plant protection practices were adopted.

Treatments

Plots were selected by randomized block design (RBD). 5 µm L-1 GA_3 and 1 slant of *P.aeruginosa* (mixed with 20g peptone, 1.5g $MgSo_4$, 1.5g K_2HPo_4, 10ml Glycerol with 1000 D.W), these concentrations were used for the treatment plants. Treatment plants and control plants, irrigated with tap water. The treatments were given 60 days after planting (DAP) by soil drenching. The plants were taken randomly, separated into tubers and seeds on 120th day, and air dried. A fine powder of this material was used for extraction of colchicine.

Extraction method

In the first method for 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 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). Seeds and tubers of control and treated plants were used to prepare extracts as described above.

Quantification of Colchicine

Identification of the colchicines was done by comparing the retention time of the sample with that of the standard obtained from Sigma. Water HPLC systems equipped with a binary pump 1525 (Max.Pressure: 6000 psi.) and a porous silica with 5µm diameter C_{18} 4.6 × 150 mm column was used for separation. The mobile phase consisted of Acetonitrile: 3% Acetic acid (60:40), at a flow rate of 1mL min^{-1} and an injection volume of 20 µL. The peaks eluted were detected at 245 nm and identified with authentic standards.

RESULT AND DISCUSSION

HPLC analysis of all samples of *Gloriosa superba* indicated the presence of colchicines (**Table 1).**

Table 1: Effect of GA_3 and *Pseudomonas aerugenosa* on colchicine contents (mg/g DW) of *Gloriosa superba* by using HPLC methods.

Plant parts	Control	GA_3	*Psedomonas aerugenosa*
Seeds	0.019 ±0.017[a]	0.022 ±0.003[a] *(114.21%)*	0.025 ±0.002[ab] *(128.94%)*
Tubers	0.0056 ±0.00021[a]	0.0065 ±0.00035[a] *(116.50%)*	0.0073 ±0.00025[b] *(131.00%)*

Values are given as mean ±SD of three experiment in each group. Value that are not sharing a common superscript (a,b.c) differ significantly at P ≤ 0.005 (DMRT). Values in parenthesis indicate percentage over control.

The HPLC profile of the analysis is represented in Figures 1, 2, 3, 4, 5 and 6. The colchicine contents of the tuber and seed samples increased with the age in control and treated plants. There was only slight increase in colchicine content under gibberilic acid treatments when compared to control. But *a Psedomonas aerugenosa* treatment increased in colchicine content when compared to control. The treatment like *P.aerugenosa* and GA_3 increased in colchicine content in *G.superba.* Similarly, Gibberillic acid treatments increased the colchicine content both in tubers and seed samples of *Iphigenia stellata* (Anjali et al., 2011). The alkaloid content increased in treated plants when compared to control. The plant growth regulating properties may be the reason for increase alkaloid content in *Catharanthus roseus* under treatment (Jaleel et al., 2006). Increased alkaloid content were also reported in *Catharanthus* plants by the application of 2,4-D,kinetin and IAA (Amit et al., 1998). In cell suspension culture, if the glucose concentration is increased in the medium, the secologanin got increased simultaneously with increase in terpenoid indole alkaloid production in *Catharanthus* plants (Contin et al., 1998). *Gloriosa superba* corms of all ages have approximately the same Colchicine content, but colchicines levels are highest at the beginning of the growing season (Finnie and Vanstaden 1991).

The PGPR strains of *Pseudomonas* are known to produce IAA and GA in the rhizosphere of the plants and stimulated the crop growth as evidenced by increased seedling emergence,

Figure 1: HPLC profile of colchicine content in control seed sample of *Gloriosa superba*

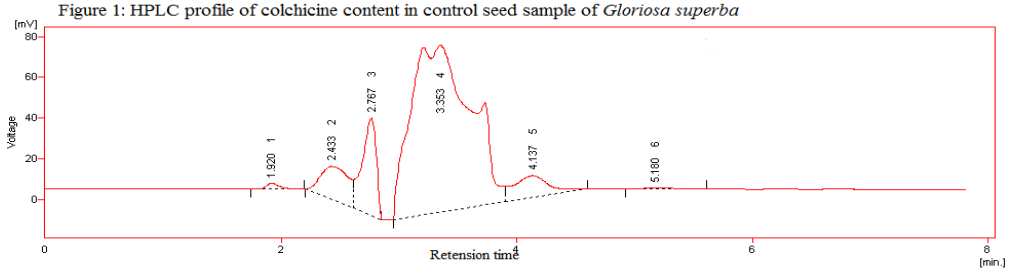


Figure 2: HPLC profile of colchicine content in gibberillic acid treated seed sample of *Gloriosa superba*.

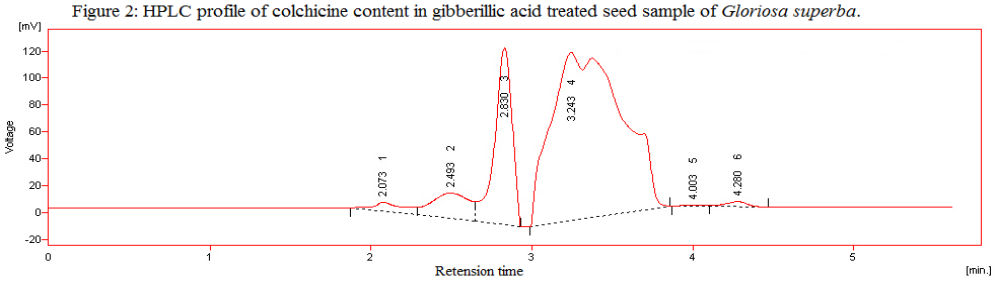


Figure 3: HPLC profile of colchicine content in *Psedomonas aerugenosa* treated seed sample of *Gloriosa superba*.

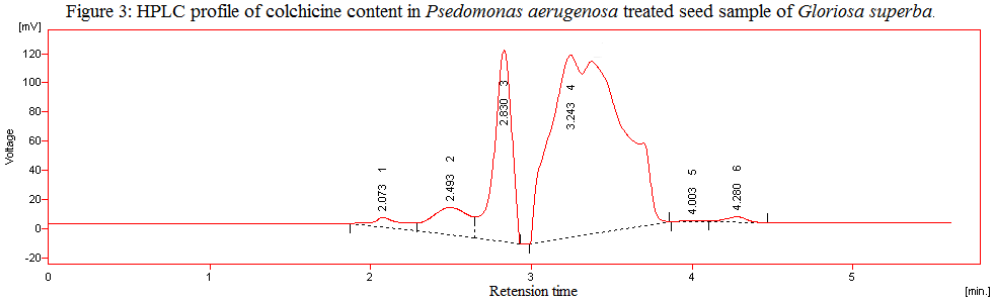


Figure 4: HPLC profile of colchicine content in control tuber sample of *Gloriosa superba*.

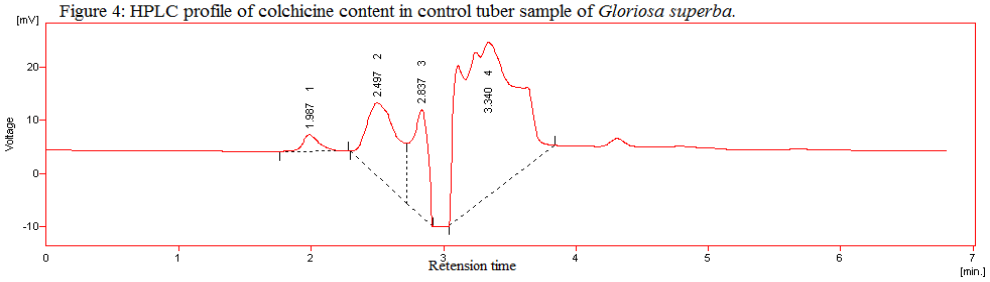


Figure 5: HPLC profile of colchicine content in gibberillic acid treated tuber sample of *Gloriosa superba*.

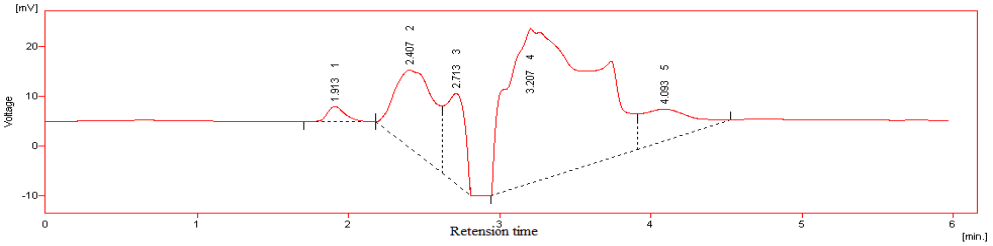
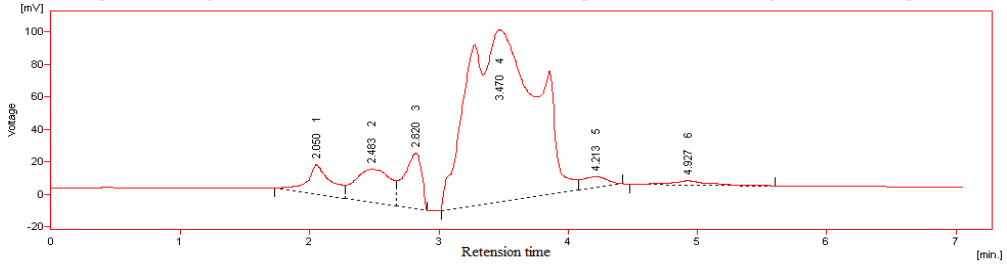


Figure 6: HPLC profile of colchicine content in Psedomonas aerugenosa treated tuber sample of *Gloriosa superba*.



vigour, seedling weight root system development and yield (Loon, 2007). Rhizosphere colonizing fluorescent *Pseudomonas* significant increased growth and yield of crop (Kiely et al., 2006). Gopal (2004) reported that the isolates of *Pseudomonas* from *Withania somnifera* were able to produce IAA. Application of P.fluorescences to seed resulted in yield increase in plants (Iavicoli et al., 2003; Haas and Defago, 2005). Two fold increases in short dry weight and three folds increased in root dry weight was observed due to the inoculation of cucumber with rhizobacteria (Kim et al., 2004). Yan et al., (2003) reported that *Pseudomonas* sp inoculation in tomato significantly increased plant growth, dry matter production and yield. Similar results were observed in *Withania somnifera* (Gopal, 2004). Karthikeyan et al (2007) isolated the *Pseudomonas* sp from the rhizosphere of different medicinal plants namely *Catharanthus roseus, Coleus forskholii, Aloe vera and Ocimum sanctum*. Recently, it has been proved that *Pseudomonas* sp can increase the vegetative growth and ajmalicine production in C.roseus (Jaleel et al., 2007).

CONCLUSION

Gibberellic acid and *Psedomonas aerugenosa* was found useful to increase the colchicine content in seeds and tubers. Seeds contained more amount of colchicine than tubers. Colchicine has the high market value and consistent demand in the field of medicine. Considering its significance this study will help in cultivation of *Gloriosa superba* for commercial extraction of colchicine.

REFERENCE

A. Contin, R.Heijden, A.W.Lefeber, R.Verpoorte, The iridoio glucoscide secologanin is derived from the novel triose phosphate/ pyruvate pathway in *Catharanthus roseus* cell culture, FEBS Lett. 1998; 434: 413 – 416.

Alali, F., K.Tawaha and R.h.Qasaymch, Determination of Colchicines in *Colchicum steveni* and *C. hierosolymitanum* (colchicaceae): comparison between two analytical methods. Photochem. Anal. 2004;15 : 27- 29.

Anjali B. Sabale and Anjali A. Mane. Quantification of Colchicine in Seeds and Corms of *Iphigenia stellata* Blatter by HPLC, Journal of Pharmacy Research. 2011; 4(6):1675-1676.

Bharathi.P, D.Philomina and S.Chakkarvarthi, Antimitotic effect of colchicine from six different species of *Gloriosa superba* in onion roots (*Allium cepa*). J. Med. Sci. 2006;6 (3):420-425.

Calogero M., (1992): *Ortopedia e Traumatologia* oggi Anno XI (2) aprile.

Chitra Rajagopal and Rajamani Kandhasamy, Genetic variability of *kazhappai kizhangu* (*Gloriosa superba* L.) in Tamil Nadu assessed using morphological and biochemical traits/ Journal of Tropical Agriculture. 2009;47 (1-2): 77-79.

Evans, D.A., S.P.Tanis and D.J.Hart. A convergent total synthesis of (±) colchicines and (±) Deacetoamidoisocolchicine. J. Am. hem. soc. 1981;103:5813 – 5821.

Finnie, J.F. and J.Van Staden. Isolation of colchicines from *Sandersonia aurantica* and *Gloriosa superba* variation in alkaloid levels of plant grown in vivo. J.Plant Physiol. 1991; 138: 691- 695.

Gopal, H. 2004.Development of microbial copsortium for improvement for growth, yield and alkaloid content of Ashwagantha. Ph.D. Thesis, Tamilnadu Agriculture University, Coimbatore.

Haas, D., G.De`fago.. Biological control of Soil- borne pathogens by *fluorescent Pseudomonas*. Nature Rev. Microbiol. 2005;3:3 07 – 319.

Iavicoli ,A, E. Boutet, A.Buchala, J.P.Me`traux , Induced systematic resistances in *Arabidopsis thaliana* in response to root inoculation to root inoculation with *Pseudomonas fluorescence* CHAO. Mol.Plant. Micro. Interact. 2003;16: 851- 858.

Jaleel CA, R.Gopi, G.M.Alagu Lakshmanan, R. Panneerselvam, Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G.Don. Plant science Journal. 2006; 171:271- 276.

Jaleel, C.A., P.Manivannan, B.Sankar, A.Kishorekumar, R.Gopi, R.Somasundaram, R.Panneerselvam. *Pseudomonas fluorescens* enhances biomass yield and ajmalicine production in *Catharanthus roseus* under water deficit stress. Colloids surf.Biointerfaces. 2007;60:7- 11.

K.J.Amit, P.K.Dubey, R.C.Rana, In vitro callus induction and biomass production of *Catharanthus roseus*, Plant Arch. 2005;5:55- 60.

Karthikeyan, B., C.A.Jaleel, R.Gopi, M.Deiveekasundaram. Alterations in seedling vigour and antioxidant enzyme activies in *Catharanthus roseus* under seed priming with native diazotrophs. J.Zhejiang Univer. Sci. 2007;B8:453- 457.

Kiely, P.D., J.M.Haynes, C.H.Higgins, A.Franks, G.L.Mark, J.P. Marrissey, F.O Gara. Exploiting new system – based strategies to elucidate plant bacterial based strategies to elucidate plant bacterial interactions in the rhizosphere. Microbial. Ecol . 2006;51:257 – 266.

Kim, M.S., Y.C.Kim, B.H. Cho. Gene expression analysis in cucumber leaves primed by root colonization with *Pseudomonas chlororaphis* of upon inoculation with *Corynespora cassiicola*. Plant Biol . 2004;6:105 – 108.

Loon. V.L.C. Plant responses to plant growth promoting Rhizobacteria. Eur. J.Plant .Pathol. 2007;119: 243 – 254.

Nautiyal, O.PIsolation of 3-demethylcolchicine from *Gloriosa superba* sludge and coupling with -acetobromoglucose to yield colchicoside and thiocolchicoside /Journal of Natural Products. 2011;4: 87-93.

Trease, S.E. and Evans, D. Colchicum seed and corm.In: *Pharmacognosy*, 12th edn. Balliere Tindall, London, pp. (1983), 593–597.

Yan. Z., M.S. Reddy, J.m.Kleopper.. Survival and colonization of *Rhizobacteria* in a tomato transplant system. Canadian J.Microbiol. 2003;49:383- 389.