B I O D I V E R S I T A S
Volume 10, Number 4, October 2009
Pages: 210-214

ISSN: 1412-033X (printed edition)
ISSN: 2085-4722 (electronic)
DOI: 10.13057/biodiv/d100409

Review: Current Advances in *Gloriosa superba* L.

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Received: 2nd June 2009. Accepted: 7th July 2009.

ABSTRACT

Gloriosa superba L. is an important medicinal plant of Asia and Africa. It is used in diseases, like cancer, gout, scrofula and act as antipyretic, antihelmintic, purgative and antiabortive. It is a source of colchicines and colchicocides, which are very costly, being highly demanded by pharma industries. Due to excessive use of the plant for diverse medicinal purposes the species is on the verge of extinction and included in Red Data Book. The strenuous efforts of botanists, biotechnologists, policy makers and conservationists are required. It is a matter of great concern to conserve this plant otherwise we will be loosing it by 2020.The present review is focused on current status of the genus, source of alkaloids, poisonous nature, the strategies for its conservation and future perspectives of *G. superba*.

Key words: *Gloriosa superba* L., colchicine, endangered plant, red data book, conservation.

INTRODUCTION

From the beginning of human civilization the plants and their products particularly ethnomedicinal plants plays a great role. The antique traditional medicines and prescriptions at a halt exist and largely practiced. The ethnobotanists are trying to ascertain new medicines from the forests by the help of tribal people. In India, the population of tribal people is around fifty three million along with 555 tribal groups or communities, which are reside in forest and srounding. These people have enormous indigenous knowledge which is a possible tool to explore for novel cost-effective plants for food as well as medicine. Several medicinal plants were originally identified and developed through indigenous knowledge, thus ethnomedicines have played key role in the development of drugs used in modern system of medicine. The plant drugs which are originated and developed are cocaine, morphine, quinine, colchicine, atropine, ephedrine, codeine, emetine, caffeine, resurpine, vinablastin, vincristin, and guguline etc. The medicinal plants play a major role in remedial now a days. The entire planet is in search of renovating herbal medicinal plants and its application for the betterment of mankind. World Health Organization (WHO) has previously recognized to re-establish the traditional knowledge of medicine among our conventional theaters. Traditional knowledge since 200 B.C. in Ayurveda is very well recognized especially in India among tribal people.

Gloriosa superba L. (Colchicaceae) also known as Malabar glory lily or "kembang telang" (Java, Indonesia) is a perennial tuberous climbing herb, extensively scattered in the tropical and sub-tropical parts of the India, including the foothills of Himalayas. It is called as 'Mauve beauty', 'Purple prince', 'Modest', 'Orange gem', 'Salman glow' and 'Orange glow' (Bose and Yadav, 1989). It is adapted to different soil texture and climatic variation. The plant grows in sandy-loam soil in the mixed deciduous forest in sunny positions. It is very tolerant of nutrient-poor soils. It occurs in thickets, forest edges and boundaries of cultivated areas in warm countries upto height of 2530 m. (Neuwinger, 1994). *G. superba* is a inhabitant of tropical Africa and now found growing naturally in many countries of tropical Asia including Bangladesh, India, Sri Lanka, Malaysia and Myanmar. In India, it occurs commonly in tropical forests of Bengal and Karnataka (Sivakumar and Krishnamurthy, 2002). The plant thrive from Bundelkhand to humid Assam valley. It is known by different names in India such as 'Kalihari', 'Agnishikha', 'Languliata', and 'Nangulika'. There are several associated species of *Gloriosa* including *G. superba*, *G. simplex*, *G. grandiflora*, *G. lutea*, *G.*

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plantii, *G. lipolidii*, *G. longifolia*, *G. rothschildiana*, *G. virescens*, *G. sudanica,* etc. These species are distributed mainly in Africa. Besides many hybrids popular garden cultivars like the purple prince, African chief, orange gem, orange gift, lavender lady, and orange ball are grown for ample range of flowers and color combination of the perianth, which bloom for long during the autumn. *G. superba* is also known as the national flower of Zimbabwe. Except diverse pharmaceutical products and other therapeutic preparations, it is also a popular plant for providing color in greenhouse and conservatories even immature flowers are gorgeous to behold (Kranse, 1986; Ghani, 2000). *G. superba* is a semi-woody herbaceous branched climber, reaching just about five meters in height. One to four stems arise from a single V-shaped fleshy cylindrical tuber. *Gloriosa superba* is an imperative medicinal plant, all parts are used in the medicine, which contains two important alkaloid, colchicine and colchicoside, leaves are used to treat cancer related diseases, also in ulcer, piles, and scrofula (Evans et al., 1981).

THE CURRENT STATUS

The leaf juice of G*loriosa* is used to kill-lice in hair, tubers contain the bitter principles, superbine and gloriosine, which in large doses are fatal; however, in small doses they are used as tonic, antiabortive, and purgatives. The white flour prepared from the tubers is bitter and used as stimulant. It is given with honey in gonorrhea, leprosy, colic and intestinal worms and for promoting labor pains, a paste of tubers is applied over the suprapubic region and vagina. Its warm-poultice is locally applied in rheumatism and neuralgic pains (Samy et al., 2008). Medicinally, the tuber is used as abortifacient, and in small dose it acts as a tonic, stomachic and anthelmintic. It is also used in the treatment of gout because it contains colchicine. Paste of the tuber is externally applied for parasitic skin diseases.

Samy et al. (2008) conducted ethnobotanical survey of folk plants for the treatment of snakebites in southern part of Tamilnadu, India, in which traditional approach was evaluated methodically with some selected plant extracts which showed potent neutralizing effect against the venom. The conventional method of propagation is through corms, since poor seed germination restricts their use in multiplication. Therefore, propagation by tissue culture technique is necessary (Finnie and van Staden, 1989, 1991). *G. superba* contains about 0.1-0.8% colchicine in bulb which is used in plant breeding to induce mutation and polyploidy and also can solve an important problem in fuchsia breeding. *G. superba* also produces another alkaloid gloriosine. Colchicines affect cell membrane structure indirectly by inhibiting the synthesis of membrane constituent. It binds to tubelin preventing its polymerization into microtubules. This anti-mitotic property disrupts the spindle apparatus that separate chromosomes throughout metaphase, cells with high metabolic rates are most implicated by the arrest of mitosis. Kumar (1953) studied doubling of chromosomes induced by gloriosine isolated from *G. superba.*

Jitpakdi et al. (1999) screened ten plant species including *G. superba* for metaphase chromosome preparation in adult mosquitoes (Diptera: Culicidae) using an inoculation technique for colchicine like substances using a mosquito cytogenetic assay have shown the increased metaphase chromosome. Due to overexploitation for its diverse medicinal applications *G. superba* has been endangered, therefore, there is urgent need to conserve the plant by biotechnological approaches like tissue culture. (Rajgopalan and Khader, 1994). This approach has been very important because it provides complete sterile and virus-free plants by rapid multiplication. *G. superba* is now promising as an industrial medicinal crop in Asia particularly in South India for its high colchicine content. For commercial production of colchicine and its derivatives, natural production from *in vitro* methods of the source plant are thus of great attention. In the past two decades, focus has been on plant biotechnology as a potential alternative production method, using cultured cells rather than plants.

SOURCE OF PRECIOUS ALKALOIDS

Gloriosa superba produces two important alkaloid colchicine and gloriosine, which are present in seeds and tubers while the other compounds such as lumi-colchicine, 3-demethyl-N-deformyl-N-deacetylcolchicine, 3-demethylcolchicine, N-formyl deacetylcolchcine have been isolated from the plant (Sugandhi, 2000; Suri et al., 2001). Suri et al. (2001) reported new colchicine glycoside, 3-O-demethylcolchicine-3-O-alpha-D-glucopyranoside in *G. superba* seeds. Kaur et al. (2007) studied purification of 3-monomeric monocot mannose-binding lectins and their evaluation for antipoxviral activity isolated from *G. superba.* Alkaloids are structurally heterogeneous class of secondary biomolecules derived from basically five amino acids ornithine, lysine, phenylalanine, tyrosine and tryptophan (Thakur et al., 1975). Thakur et al. (1975) reported the substances from plant of the sub-family Wurmbaeoideae and their derivatives along with alkaloids from the *G. superba.*

COLCHICINE

It is conventional drug for gout obtained from corms of *G. superba* and *Colchicum autumnale* (Thakur et al., 1975; Sivakumar and Krishnamurthy, 2002). The term “colchicine” is derived from area

known as Colchis near black sea. *C. autumnale* grows wild in Europe and Africa. Thomson was the first who proposed early idea of action of colchicines in gout treatment. Gout and uric acid metabolism is same way linked and colchicines might act on this and it is caused by deposition of microcrystals of uric acid in joints and may be due to defective regulatory mechanism for endogenous purine synthesis but contradictory result for the action of colchicine on synthesis and extraction of urates have been recorded, colchicines interrupt, the cycle of new deposition which seem to be indispensable for the persistence of acute gout. Distressing side effect has also been recorded sporadically but colchicines remain the drug for acute gout. Modification of the side chain of rings does not abolish anti-gout activity as long as the configuration of C-ring confirms to that of colchicines. It also acts as anti-mitotic and anti-gout agent. It blocks or suppresses cell division by inhibiting mitosis. It inhibits the development of spindles as the nuclei are dividing (spindles are formed by the polymerization of tubuline) from a pool of subunit during a detached phase of cell-cycle and then depolymerized during other phase. It is also used to induce polyploidy initiation, occasionally other mutations also occur like chlorophyll mutations, but frequency is low.

It can solve an important problem of fuchsia breeding. Most of the fuchsia species are diploid or tetraploid, a crossing between diploid and tetraploid result often in a triploid, which is mostly sterile because the process of meiosis (cell division for reproduction) requires the coupling of similar chromosomes and there is no mechanism allowing for the alignment of three similar chromosomes, triploid plants are not able to produce fertile reproductive cells. They are, therefore, sterile and unusable as parents. A special problem of colchicines induced ploidy, particularly in vegetatively propagated crops, is the chimerism caused by the instantaneous presence of tissue of different ploidy levels in one plant or plant parts. Colchicine is mostly used in its freshly prepared aqueous form. The range of concentration of colchicine applied varies from 0.006-3%, concentration of about 0.05% is the most commonly used (Milne and Meek, 1998). Kannan et al. (2007) studied optimization of solvents for efficient isolation of colchicines from *G. superba.* The maximum yield of colchicine was obtained when it is extracted with water and alcohol in the ratio of 50:50.

Bellet and Gaignault (1985) reported the production of colchicinic substance from *G. superba.* The colchicines-like activity of *G. superba*-extracted for mosquito (Diptera: Culicide) in which four fractions i.e. hexane fractions, dichloromethane fraction-1, dichloromethane fraction-2 and methanol fraction were investigated. The latter three fractions yielded hopefully high colchicine like activity, whereas hexane fraction yielded very low activity.

Ghosh et al. (2002) studied the root culture of *G. superba* by using direct and indirect precursor of the biosynthetic pathway for the enhancement of colchicine production. They successfully used aluminium chloride as an elicitor, in which they have used root cultures of *G. superba* treated with 5 mM methyl jasmonate and 125 µM $AlCl_3$. The enhancement of intracellular colchicine content was observed in the roots by 50-fold and 63-fold respectively. Ghosh et al. (2006) reported that colchicine can also be applied in the lanolin paste or as a solution, for instance, on a cotton dot, placed in a leaf axil. Khan et al. (2007) evaluated the enzyme inhibition activities of *G. superba* rhizomes extract against lipoxygenase, actylcholinesterase, butyrycholinesterase and urease in which wonderful inhibition was observed on lipoxygenease. Further, Khan et al. (2008) reported antimicrobial potential of *G. superba* extracts in which excellent antifungal activity was confirmed against *Candida albicans*, *C. glabrata*, *Trichophyton longifusus*, *Microsporum canis* and *Staphylococcus aureus.*

Colchicine is synthesized using mainly aromatic amino acids such as tryptophan, phenylalanine and tyrosine. Key enzymes involved in colchicine metabolism are tyrosine ammonia lyase (TAL) and phenylalanine ammonia lyase (PAL). Sivakumar and Krishnamurthy (2004) reported the biosynthesis of colchicine, the *in vitro* supply of exogenous precursor using B_5 medium from *G. superba* calluses. The maximum amount of colchicine i.e. 9.0 mg was detected in the medium fed with 30 M tyrosine. The activity of TAL was higher than that of PAL and a low frequency of tracheary elements was observed.

POISONING NATURE OF PLANT

The colchicine which is a major component of *Gloriosa* is mainly responsible for toxic effect (Vishwanathan and Joshi, 1983). The toxins present have an inhibitory action on cell division, and depressant action on the bone marrow. Just after ingestion of tubers, the symptom develops within two hours; vomiting, numbness and severe effects on throat as well as diarrhea leading to dehydration. Alopecia and dermatitis are the major symptoms develop after two to three weeks after poisoning (Jayaweera, 1982). Colchicine poisoning following ingestion of *G. superba* tubers have been reported by various investigators (Nagratnam et al., 1973; Jose and Ravindran, 1988; Fernando and Fernando, 1990). Mendis (1989) reported the toxicity symptoms as gastroenteritis, acute renal failure, cordiotoxicity and hematological abnormalities. Moreover, there were eight deaths due to *G. superba,* while the gastrointestinal symptoms along with sweating, hypotension jaundice and convulsions were reported after the consumption (Aleem, 1992).

CONSERVATION BY MEANS OF *IN VITRO* PROPAGATION

Gloriosa superba usually multiply by corm and seeds but due to low germination capability it restricts for the regeneration. Therefore, in order to safeguard and preserve this important plant biotechnological approaches would be very useful (Sivakumar and Krishnamurthy, 2002). The conventional method of propagation has many disadvantages as 50% of the yield has to be set aside for raising the next crop, transmittance of soil-borne diseases from one crop to the next, and from one location to another and during the 2-3 month storage period between harvest and the raising of next crop (Mrudul et al., 2001). Kala et al. (2004) studied the prioritization of medicinal plants on the basis of available knowledge, existing practices and use value status in Uttaranchal, India in order to understand the pattern of indigenous uses of medicinal plants available in the Uttaranchal state, India and documented 300 species including *G. superba*. Hassan and Roy (2005) reported 92% of the cultures of apical and axillary buds of young sprout from naturally grown *G. superba* plants regenerate four shoots per culture in MS basal medium fortified with 1.5 mg/L BA + 0.5 mg/L NAA. Custers and Bergervoet (1994) reported micropropagation of *G. superba* by shoot cuttings and explants from node, internode, leaves, flowers, pedicels and tubers. *G. rothschildiana* (duphur) vs. *G. rothschildiana* (new accession) and *G. rothschildiana* vs. *G. superba* were cultured on MS basal medium with 3% w/v sucrose, 0-10 mg/L Benzyl Adenine (BA) and 0.1 mg Indole Acetic Acid (IAA) and maintained at 24 days under 16 hours photoperiod. Addition of low level of Benzyl Adenine (BA) (1 mg/L) improved plant growth, whereas the high level of BA (10 mg/L) caused proliferation of multiple shoots, from rhizome meristem, by applying alternatively high and low BA level, a method of continued propagation was achieved which resulted in a 4-7 fold multiplication of qualitatively good plantlets every 18 week. The resulting shoots were incubated on MS medium, with 3% sucrose and 0-1 mg/L IAA or NAA. Transplantation into soil was only possible after the plants had formed.

Samarajeewa et al. (1993) studied clonal propagation of *G. superba* from apical bud and node segment of shoot tip, cultured on solidified agar (0.8% w/v) Gamborg's B_5 medium containing BA, IAA, Kinetin, NAA, IBA or 2,4-D. The cultures were maintained under fluorescent light at 25-27ºC. Primary cultures were initiated in solid B_5 medium containing 0.5 to 1 mg/L BA and 0.01-0.5 mg/L IAA, IBA, NAA when shoot tip of primary cultures were transferred to shoot multiplication media, shoot proliferation occurred via adventitious bud formation within 4-8 weeks.

Somani et al. (1989) reported *in vitro* propagation and corm formation in *G. superba.* The fresh sprouts were excised from corms of *G. superba* and dissected propagules with shoot and root primordia were placed on MS basal medium (Murashige and Skoog, 1962) containing 3% sucrose and 0.6% agar. Explant germinated on the MS medium producing shoot and root, which formed new corm within one month. For shoot and cormlet regeneration, 1-4 mg/L kinetin was added to the medium. Cultures were maintained at 25ºC in white fluorescent light (2500 lux) with an 8-h/day photoperiod. Sivakumar and Krishnamurthy (2002) reported *in vitro* organogenetic responses of *G. superba.* They used MS medium supplemented with ADS and BA, 98%. The callus induction occurred in non-dormant corm bud explants. The maximum number of multiple shoot (57%) was observed in corm-derived calluses.

Gupta (1999) compared the production of different colchicinic substances from *G. superba* and *C. autumnale.* He reported extensive range of these colchicinic compounds like colchicines (0.9%), dimethyl-3-colchicine (0.19%), colchicoside (0.82%) and their formyl derivatives from *G. superba*. While these values were found to be less in case of *C. autumnale* which were reported as 0.62%, 0.9%, and 0.39% respectively. Sivakumar and Krishnamurthy (2002, 2004) studied induction of embryoids from leaf tissue of *G. superba*. The nodular calli were observed on S.H. medium supplemented with 2,4-D and 1 isopentyldene. Gupta et al. (1999) found hepatoprotective activity of *G. superba*. Jha et al. (2005) reported production of forskolin, withanolides, colchicine and tylophorine from plant source by using biotechnological approach.

CONCLUSION

Gloriosa superba is a commercially imperative medicinal plant which has diverse medicinal applications and eventually due to over-exploitation this plant is facing local extinction. It has been affirmed as endangered plant by IUCN and hence there is a pressing need to conserve the plant by *in situ* and *ex situ* multiplication in general and micropropagation in particular so as to meet the ever-increasing demand from the industries. Furthermore, responsiveness should be generated among the common people concerning the importance of *G. superba* and its overexploitation by the people. Peoples participation in conservation of rare and endangered medicinal plants like *G. superba* will also be very useful.

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