

Abasyn Journal of Life Sciences

Open Access



DOI: 10.34091/AJLS.3.2.5

Gloriosa superba L: A critical Review of Recent Advances

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Abstract

Gloriosa superba is a flowering plant belongs to Colchicaceae. It refers to full of glory', and the superba refers 'superb' referring to the outstanding red and yellow flowers. G. superba is a highly toxic plant, due to the presence of an alkaloid, 'Colchicine'. Adequate ingestion of this alkaloid could cause fatalities to humans and animals. Despite its toxicity, G. superba considered as a medicinal plant because of its medicinal constituents and properties such as colchicine, gloriosine, pungent, bitter, acrid, heating, anthelmintic, laxative, alexiteric, and abortifacient. G. superba utilized for the treatment of snakebite, scorpion stings, parasitic skin disease, urological pains, colic, chronic ulcers, piles, gonorrhoea, gout, infertility, wounds, arthritis, cholera, kidney problems, itching, leprosy, cancer, sexually transmitted disease, and countless other diseases. This plant listed under the threatened category due to its indiscreet reaping from the wild as medicinal industries widely use it for its colchicine content. Field study and secondary sources were used to abridge the information on the recent advancement study of G. superba. In the present review, the information regarding the occurrence, botanical description, propagation, medicinal application pharmacological, ethnomedicinal, biological, and toxicological studies of *G. superba* and its recent advancement has been summarized. The detailed study of *G. superba* with current trends helps the researcher to focus on the future development needed for its conservation.

Keywords: *Gloriosa superba*, pharmacognosy, ethnomedicine, toxicity.

Article Info:

Received:
May 14, 2020
Received Revised:
December 10, 2020
Accepted:
December 25, 2020
Available online:
December 31, 2020

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How to cite:

Umavathi S, Gopinath K, Manjula MS, Balalakshi C, Arumugam A. *Gloriosa* superba L: A critical Review of Recent Advances. *Abasyn* Journal of Life Sciences 2020; 3(2): 48-65.

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1. INTRODUCTION

Gloriosa superba L. belongs to the family Colchicaceae, according to the APG III system (Angiosperm Phylogeny Group system) of classification ^{1,2,3}. It is broadly distributed in the tropical and sub-tropical parts of Africa, and Southeast Asia, including India, Myanmar, Sri Lanka, and Malaysia ⁴. The distribution of *G. superba* is spread throughout Senegal, Somalia, South Africa, and Zambia in Africa. Whereas, in India, it is spread throughout Himachal Pradesh, Jammu Kashmir, Haryana, Bengal, Uttar Pradesh, Maharashtra, Chhattisgarh, Orissa, Andhra Pradesh, Karnataka, Kerala, Goa, and Tamil Nadu (Fig. 1). It is the national flower of Zimbabwe and Tamil Eelam and the state flower of Tamil Nadu in India. In Tamil Eelam, they use these flowers on the Maaveerar Day or Heroes' Day celebrated on 27th November. So far more than 17 countries have been released *G. superba* stamp (Fig. 2).

G. superba is an important medicinal plant and cultivated for various economic reasons. It contains a rich source of colchicine, colichicoside and gloriosines content. In pharmacological companies, over 75% of this plant rhizome is collected from the wild source. Overexploitation of this plant occurs due to insufficient scientific knowledge of the local people. In this human-made ecological imbalance, this plant could not further survive in a rough condition. Therefore, it is becoming an endangered plant species and listed out by IUCN Red Data Book⁵. The plant species must be conserved; otherwise, it will be considered as extinct.

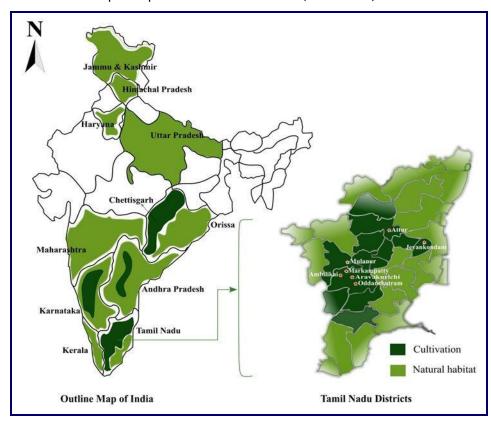


Fig. 1. Natural habitat and cultivation places of G. superba in India



Fig. 2. G. superba postal stamps issued by various countries

2. BOTANICAL DESCRIPTION

The *G. superba* is an amazingly beautiful semi-woody herbaceous lasting creeper with an average height of 6m. It develops from tuberous underground stem annually during the rainy season ⁶. The genus *Gloriosa* comprises of 15 known species, among them, the most important species reported in India is *G. superba*. The plants grow in river edges, spars savannah woodlands, grasslands, semi-evergreen forests, dunes, sandy – loam soil, and nutrient-poor soil. It is very lenient of nutrient-poor soils and is seemed to be more dependent and abundant on *Prosopis juliflora* and *Borassus flabellifer*, as recorded from Tamil Nadu.

The *G. superba* is a climbing herb with a leafy stem and possesses tuberous rootstock. The leaves are either alternate, opposite or ternate whorled with a prominent midrib, ovate-lanceolate. The tip is spiral, elongated, and functions like a tendril. The flowers are large, and the inflorescence is axillary or solitary, the pedicels reflexed near the tip. It has six persistent polypetalous perianths with diffused or reflexed, narrow; margins undulate—Stamens-6, hypogynous, six filaments, anthers oblong-linear, versatile, extrorse. Ovary 3-celled, ovules many in each cell, style filiform, sharply deflexed, stigma 3, segments subulate, stigmatose within (Fig. 3). Fruit a large, coriaceous, septicidal capsule, ellipsoid - oblong. Seeds subglobose, testa spongy, embryo cylindric ⁷.

3. PROPAGATION

With the increasing medicinal value, these wild plants are used as raw on a large scale in the pharmaceutical industry. From a single V, L, and comma-shaped fleshly cylindrical corm, around one to four stems produced (Fig. 3). After seed germination, it completes 4 to 5 vegetative cycles to reach the reproductive phase of the flowering (Fig. 4). The mature tuber shows brown colour epidermis, but pure white at young. The mother tuber produces a mature daughter tuber every year ⁸. Besides, the mother tubers were stored in a dormant state under sand or clay/red soil. It becomes the source for the cultivation in the next year. Generally, farmers sell only daughter tubers. Seeds are essential export material. Seeds and tubers can propagate plants. However, crops are grown commercially through the tubers. *G. superba* has reported having a low seed set ⁹. The conventional propagation method has many demerits as the transmittance of soil-borne disease as well as half of the harvest must be well-maintained for raising the succeeding generation. Moreover, the production of corms and tubers seems to be a complicated process as it is acknowledged to be prejudiced by genetic, environmental, and physiological barriers ^{10,11,12,13}. The horticultural propagation practices show results of slow growth in these plants. The genetic enhancement of *G. superba* through advanced breeding technology, micropropagation, and advanced propagation practices is needed to conserve as well as increase the manufacture of this plant species.



Fig. 3. Gloriosa superba: (A) Herbarium and voucher specimen No: KG 001, (B) Habitat, (C) Flower, (D) Different stages of flowering, (E) Fruits, (F) Mature fruits, (G) Seeds, (H) 1^{st} year seedling rhizome, (I) 2^{nd} year germinated rhizome, (J) 3^{rd} year rhizome, (K) $4^{\sim}5^{th}$ year rhizome, (L) Germinated matured rhizomes

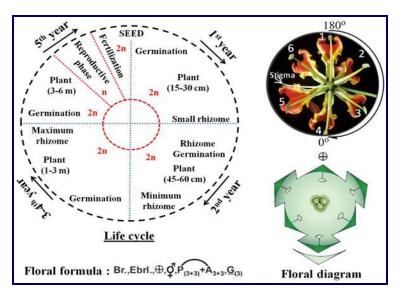


Fig. 4. Floral formula, floral diagram and diagrammatic life cycle of G. superba

4. THE SCENARIO OF PRODUCTION OF G. SUPERBA

In India, the *G. superba* is grown in diverse climatic regions, especially throughout the tropical regions ranging from Northwest Himalayas to Assam, The Deccan Peninsula, Maharashtra, Karnataka, Kerala, Eastern and Western Ghats of Tamil Nadu. There is a massive demand for the medicine extracted from *G. superba* in European countries. In 1972, an Italian pharmaceutical company offered ₹250/kg for *G. superba* tuber. Since then, this plant is grown under contract farming in several places at Dindigul and present Tiruppur districts. The export of *G. superba* was banned in 1980 when it listed as an endangered species. The same year, export was permitted only for the seeds cultivated in the farms. This plant cultivated widely in Ariyalur, Dindigul, Erode, Karur, Namakkal, Nagapattinam, Tiruppur, and Trichy districts of Tamil Nadu. Tamil Nadu considered as a monopoly in the production of *G. superba*, mainly cultivated in and around Moolanur, Oddanchatram, Aravakurichi, Ombilikkai, Dharapuram, Markampatti, Attur, and Jayamkondanin

the Trichy district. This plant cultivated in more than 250 acres of land in Thuraiyur and Vaniyambadi areas ^{14, 15}. The cultivation of *G. superba* may yield 1.5 to 3 quintals of seeds and 1.0 to 1.25 quintals of dry rhizomes per hectares annually ^{16, 17}. The international market value for this seed is approximately \$45/kg ¹⁸. India is the leading exporter of *G. superba*, and the annual trade is estimated to be at 100 − 200 million tonnes, and the price ranges from ₹600 to 750/kg. A significant problem in the cultivation of this species is pollination. The peculiar structure of the flower impedes the rate of pollination. To overcome this difficulty, farmers use 'touch pollination' or 'manual pollination' of implanting pollen grains. Touch pollination produces fertilized flowers and yields about 100g of seeds and 1 kg of tubers for each plant and about 500 kg of tuber per acre. The average yield per acre is 100 kg, but some farmers gain about 500 kg of seeds. The price has gone up from 250/kg 30 years ago to 3,430 now, in 2018. Previously, in Tamil Nadu, around 126 farmers were allowed support in 2008–09. Also, 350 farmers have obtained a 50% subsidy at Trichy district in 2013. Nowadays, Tamil Nadu has granted a 50% subsidy for the farmer to cultivate the *G. superba*. Recently, farmers have demand to the government of Tamil Nadu to fix the sales price. Italy is one of the largest buyers of *G. superba* seeds from India.

5. CULTIVATION

For commercial propagation, the underground rhizomes are used. It develops a bi–forked tuber with only one growing bud (Fig. 5). The duration of growth of this plant is about 150 – 180 days—the tubers produced in about 90 days after planting. Weeding is needed at 30, 60- and 90-days intervals for profitable growth of the tuber. Proper water management system and an average rainfall of 70 cm favour the growth. Each tuber will weight of 100g. In the summer the tubers undergo a dormancy period. There are 22 varieties found, among them, the Singaleri selection possesses 0.25% of colchicine. Lutea, Citrina, and Nana were some common varieties in India.

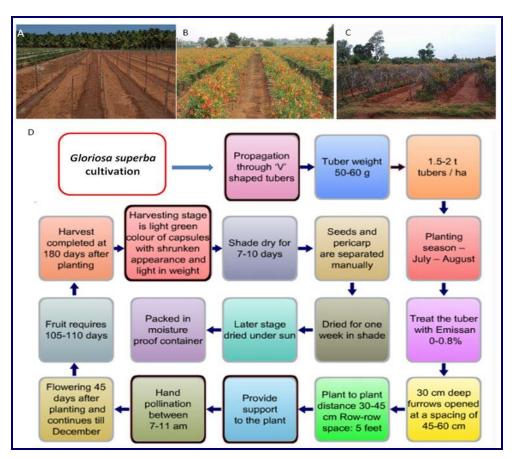


Fig. 5. *G. superba*: (A) Land Preparation (B) Mass cultivation (C) Vine trained over support plant - *Dodonaea viscose* (D) Scheme of *G. superba* cultivation

5.1. Soil and climate

Red loamy soil with an average pH of 6.0 - 7.0 is suitable for the cultivation of G. superba. It can grow up to an altitude of 600 m with a rainfall of 70cm annually.

5.2. Planting

Planting is dispersed from June – July. The field is ploughed 2-3 times with integrated 10 tons of farmyard manure. The tubers are planted in the trenches of 30 cm depth at 30×45 cm spacing. The vines of *Commiphora berryi* and *Dodonaea viscose* trained over to provide support to the plants (Fig. 5). Instead, permanent structures with Galvanized Iron wires can also be used as vines. Irrigation is given approximately after sowing afterward; irrigation is given at five days of intervals. The fertilizer like nitrogen (N), phosphorus (P), and potassium (K) at the ratio of 120:50:75 kg/ha can apply in two divided doses. K, P and half of N are supplied as basal dressing. The left-over quantity of N is used after planting.

5.3. Harvest

The pods can be harvested around 160 - 180 days after the sprouting of tubers. Post-harvest care like trimming must be done to avoid damages while growing rhizomes. The tuber rot can be controlled by the treatment of 0.1 % Carbendazim for 30 min.

5.4. Diseases and pests

G. superba is susceptible to Leaf blight (*Curvularia lunata* and *Alternaria* sp.) and tuber rots (*Sclerotium* sp.)¹⁹. An endophytic fungus called *Pestalotiopsis microspora* is predominantly present in the leaf and seed (Fig. 6). The leaf blight frequency is higher during cloudy weather coupled with humidity. Mancozeb at 0.2 percent concentration or Propiconazole (0.1%) or Hexaconazole (0.1%) at the time of infection is sprayed. 0.5% ZnSO{-4} is sprayed two times at fortnightly interval starting from the 45th day after planting. Major insects like Lily caterpillar and green caterpillar attack foliage and flower buds. Typically, *Polytela gloriosae* pest is the major issue to decrease the crop yield of *G. superba* ²⁰. The larva has eaten fast by the apical meristem, leaves of the *G. superba* emerging corm bud germination, due to the sweetness. However, it is controlled by 5% of neem seed kernel extract spray for better pest management.

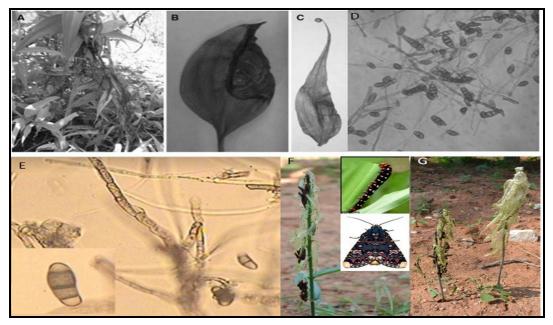


Fig. 6. (A) Leaf blight disease of *G. superba* at an advanced stage, naturally infected with *Alternaria* alternate. (B) Close-up of a naturally infected leaf with clear concentric rings. (C) Blighted leaf at advanced stage. (D) Mycelia and conidia of *A. alternata* isolated from a leaf lesion. (E) *Curvularia lunata* and (F-G) *G. superba* damage by the *Polytela gloriosa* ^{19,20}.

6. ETHNO PHARMACOLOGY

G. superba is the most valued medicinal plant because every part of it is being used for therapeutic determinations, especially in native systems of treatment like Siddha, Ayurveda, Unani. The traditional knowledge of this plant is well recognized by tribes of India, and they are extensively used for remedial applications and as an exchange from the indigenous and foreign dealers.

The leaves and leaf extract are used to cure asthma, ulcers, piles, and also as a smoothening mediator for pimples and skin outbursts. The Kani tribes belonging to South India use the tuber paste to the lower part of the belly for easy childbirth ²¹. The people of Kenya and Tanzania use these plants to treat asthma, for the healing of wounds, and as an antimalarial drug. It has high toxicity, and it is used to treat impotence, abortifacient, and even as a suicidal agent. The whole plant extract acts as an antidote for snakebite as it contains monoethyl ester of 2,6 – dihydroxybenzoic acid.

7. ETHNO MEDICINE

The decoction of tuber in small doses was given as tonic, anti-abortive and purgatives to the patient for treatment. The leaf juice is used to kill head lice. It is given with honey to cure gonorrhoea, leprosy, colic, intestinal worms, and for promoting labor pains. Tubers are tonic and anthelmintic when taken in a dosage of 5 to 10 grains, and the paste is an antidote for snake bite. In the case of induced abortion, rhizome ground with ghee used as orally by Gondi tribes ²². The Similipal tribes use the tuber paste of *G. superba* with long pepper (*Piper longum*) for twenty days to cure rheumatoid arthirits ²³. The Ethnomedicinal application of various plant parts of *G. superba* by tribes of India were summarized in Table 1. Table 2, illustrates the medicinal applications of *G. superba* practiced in the ancient medicinal system in India.

8. PHYTOCHEMISTRY

G. superba possesses a pivotal position in the ancient medicinal system as it is rich in alkaloids like colchicine, lumicolichicine, colichicoside, thiocolchicoside and etc. It has a high concentration of colchicine when compared to the Colchicum species. Colchicine is an amino alkaloid derived from the amino acids phenylamine and tyrosine, which has anti-inflammatory and analgesic effects. All the parts of this plant contain colchicine contents; stem: 0.33%; flower: 1.18%; ovary: 0.08%; rhizome: 0.3% and seeds 0.06%. Apart from colchicine, the root tuber is rich in benzoic acid, salicylic acid, sterols, 1,2 – dimethyl colchicine, 2,3 – didemethylcolchicine, N – deacetylcolichicines, colichioside, tannins, superbine, and resinous substances (Fig. 7). Sucrose crystal was derived from the hot methanol extract of the rhizome powder of *G. superba* ⁴⁰ (Fig. 8). Subbaratnam ⁴¹, extracted a new alkaloid named gloriosine.

Apart from colchicine, many other compounds like colichicoside, gloriosine, lumicolichicine, 3 - dimethyl - N-deformyl-N-deacetyl colchicine, 3 - dimethyl colchicine have also been reported in G. $Superba^{42}$. Suri et al. 43 reported a new colchicine glycoside, 3 - Odemethyl colchicine $-3 - \text{O-}\alpha - \text{D}$ glucopyranoside from the seeds of G. Superba. Maroyi and Van der Mase 44 , studied the purification of 3 - monomeric monocot manure – binding lectins and their evaluation for antipoxviral activity of G. Superba.

Tubers: Sucrose ⁴⁰, 0.25% of colchicine is present along with sitosterol, glucoside, beta and gamma lumicolchicines, beta sitosterol, flucoside and 2-H-6-Meo benzoicacid.

Root: Sitosterol, flucoside, glucopyranoside.

Leaves: Colchicine, Salicylic acid, sterols

Flowers: Lasteolin and colchicine.

Seeds: The seeds of *G. superba* is rich with thiocolchicoside, a semi – synthetic derivative of occurs naturally as colchicoside. It has been clinically used to cure orthopaedic, traumatic and rheumatological disorders.

9. ACUTE TOXICITY

An alkaloid, colchicine increases the toxicity of *G. superba* ⁴⁵. It slows the division of cell and sedates the bone marrow. After two hours of ingestion of the tubers, the person develops symptoms like nausea, coldness, soreness of the throat, and diarrhoea and leads to extreme dehydration. Along with excessive hair loss has been reported among the patients. Symptoms of Alopecia and dermatitis develop after two to three weeks of poisoning ³¹. Various investigations have reported the poisonous action of colchicine due to tuber ingestion of *G. superba* ^{46, 47, 48} (Fig. 9). The toxicity in *G. superba* leads to symptoms like gastroenteritis, haematological abnormalities, acute renal failure, and cardiac arrest ⁵². Eight deaths have been reported so far due to the consumption of *G. superba* ⁵³.

G. superba most commonly is used to cure skin disease, snake bite, as an abortifacient, an antidote for a scorpion sting, healing of wounds, head lice killer, suicidal agent and as a capable homicide ⁵⁴. The acute toxicity effect of *G. superba* is well documented, and the dominance of poisoning categories are abortifacient, murder due to poisoning and as an antiparasitic ⁵⁵. The study of colchicine on rats and monkeys has been shown to induce epileptic foci in rats, causing generalized seizures and death in animals ⁵⁶.

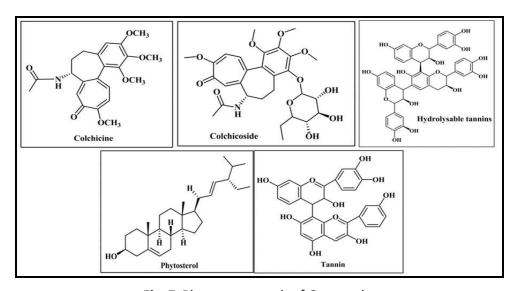


Fig. 7. Phytocompounds of G. superba

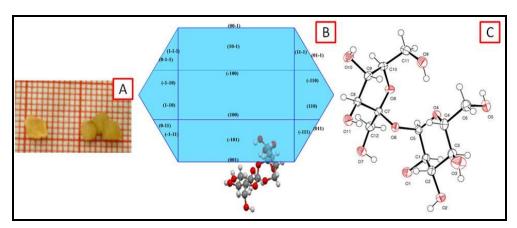


Fig. 8. Sucrose crystal derived from the hot methanol extract of the rhizome powder of *G. superba*. (A) Photograph of sucrose single crystal and (B) Schematic of sucrose crystal using (Mercury 2.4 software) (C) ORTEP diagram of sucrose crystal⁴⁰.

The use of *G. superba* in folk medicine seems to be exploited over the past years due to its poisonous constituents ⁵⁷. Consumption of larger quantities results in poisoning and even death of living beings. The toxicity of *G. superba* is due to the presence of the tropolone alkaloid "colchicine" which is considered as the biological hallmark of the Colchicaceae family.

The Indian Medical System documented colchicine as one among the seven semi-poisonous medicines which cure a variety of ailments but at the same time results in fatal consequences due to misuse ^{58, 59}. So, traditional healers prescribe the medicine in smaller dosages so that the toxic symptoms are minimized. The uses of tubers and seeds have caused numerous deaths in developing countries like India, Srilanka, where the traditional medicinal system is prevalent ⁶⁰.



Fig. 9. A) Massive alopecia 5 days after eating tubers of *G. superba* (B) A patient with C-PAP ventilation at ICU on day 3 of *G. superba* poisoning. Alopecia (C) on admission (D) 23rd day of poisoning, (E) massive hair loss following one month of poisoning, (F-I) Hair loss on 9, 10, 11 and 14 days^{49,50,51}.

10. POLLINATION AND POLLEN INFERTILITY

Pollination by means of small insects was difficult due to the typical structures of the papilloid flowers. The flower consists of six perianth lobes that bent backward. It also has six radiating anthers that are bent 90° near the ovary. The possibility of cross-pollination is determined by wind and also large insects (bees) like *Apis mellifera* and *Bombus pennsylvanicus*, some of the butterflies (Entomophily) *Papilio polytes, Eronia cleodora* ⁶¹ and sunbirds (Ornithophily) like *Nectarinia zeylonica* and *Nectarinia asiatica* with the help of long beaks (Fig. 10). During the fertilization of *G. superba*, several environmental factors will affect the total fertility rate, (i) slower germination of pollen on the stigma, (ii) removal of pollen from anthers by heavy rainfall (or) raindrops, (iii) micronutrient deficiency, (iv) pH of the soil (alkaline) and (v) high-temperature. Gopinath ⁶² reported that *G. superba* tubers grow in two different temperatures and four differents oil pH. As a result, they produced two types of pollen grains that are fertile and infertile. Conclusively, the high temperature and alkaline soil produce infertile pollen grains, which is one of the reasons that inhibit fruit formation. Hence, it cannot survive such environmental conditions and thus undergoes in the end angered plant list. Whereas, temperature and pH are the two significant factors which inhibit the inheritance of paternal characters and promote the infertile pollen grains of *G. superba*.

11. BIOACTIVITY

It has been reported as a potent antimicrobial agent due to the occurrence of diverse types of alkaloids. It seems to have anti-inflammatory activity, mutation property, and has toxicological effects.

The extracts of *G. superba* has significant activity against the bacteria *E. coli, Proteus vulgaris, Salmonella typhi, Bacillus subtilis, Staphylococcus* spp. 63 and antifungal activity on *Candida albicans, C. glabrata, Tichophyton longifusus, Microsporium* spp. 64 . The existence of high phytoconstituents categories is an effective antibacterial as well as an antifungal mediator. It possesses a moderate anti-inflammatory effect, and it is safe up to 2000 mg/kg body weight 65 . Alkaloids isolated from the seeds were tested for the oral administration of colchicine, 2 – demethylcolchicine, 3 – 3- demethylcolichicine and N – formyl – N – deacetylcolchicine at 2,4,6 mg/kg body weight 66 . The colchicine can be used in plant breeding programs like induced mutation and polyploidy.

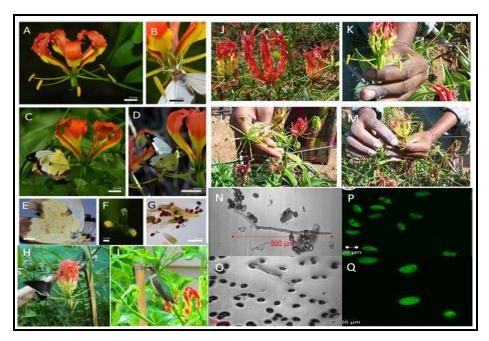


Fig. 10. (A) *G. superba* flower, (B-H) Butterflies accumulate large loads of pollen on their wings, particularly along the veins, (I) Ornithophily, (J-M) Artificial hand pollination (N,O) Pollen germination (P) Fertile pollen grains, (Q) Infertile pollen grains^{61,62}.

12. CONSERVATIONAL APPROACHES

Because of the overexploitation and less production of *G. superba*, it turns out to be an endangered plant and is also listed in Red Data Book. Preventive measures should be taken in order to conserve the species and meet the demand for the crop. The applications of Biotechnology like in-vitro mass multiplication, invitro regeneration, to enhance this plant production (Table 3). In phyto-colchicine production, the callus culture of *G. superba* can be an alternative source of production. The biological process in the natural plant system is understood through the in-vitro propagation of *G. superba*. At a much more superficial level, the critical parameters of biomolecular function can be mimicked through technology since there are no geographical constraints for the production of the plant materials this technology can be availed throughout the year. The technique ensures a pesticide-free product. Moreover, it is time—saving and cost-effective. The major drawback is that the yield of the alkaloid is significantly less as commercial application needs mass callus culture.

12.1. Conservation of G. superba by means of In Vitro Propagation

G. superba generally reproduces by corm and seeds. The low germination capability has restricted regeneration. To protect and preserve this significant plant, biotechnological methods must be used. The drawbacks of the conventional propagation methods like half of the yield to be used for raising the next crop, the next, and from one place to other between storage period and harvest and growth of the next successive generation ^{10,85-86}. Kala et al.,⁸⁷ studied the ranking of medicinally essential plants based on the available knowledge, prevailing practices, and status of use-value in Uttaranchal, India. Hassan and Roy ⁸⁸

reported that 92% of the apical and axillary bud cultures of young sprout from *G. superba* stimulated four shoots per culture in MS basal medium. Custers and Bergervoet ⁸⁹ reported micropropagation of *G. superba* by explants from various parts of this plant.

Corm formation in G. superba through in vitro propagation was reported 90 . The fresh sprouts are placed on MS basal medium from the corms 91 . Within a month, a new corm with roots and shoots germinated. 1-4 mg/L kinetin medium is preferred for the regeneration of the shoot and cormlet and expose the culture for 8 hours/day under the fluorescent light (2500 lux) at 25° C. Sivakumar and Krishnamurthy 92 reported responses of G. superba on organogenetic propagation in MS medium supplemented with ADS and BA. Gopinath and Arumugam 93 studied micropropagation and in-vitro regeneration of micro-rhizome of endangered medicinal plant G. superba focusing on the conservation approaches (Fig. 11).

Gupta 94 , related the colchicine production from *G. superba* and *Colchicum autumnale*. He reported a widearray of the colchicine compounds like colchicines (0.9%), dimethyl-3-colchicine (0.19%), colchicoside (0.82%), and their formal derivatives from *G. superba*. Subbaratnam 95 studied the initiation of embryoids of *G. superba* from leaf tissues.

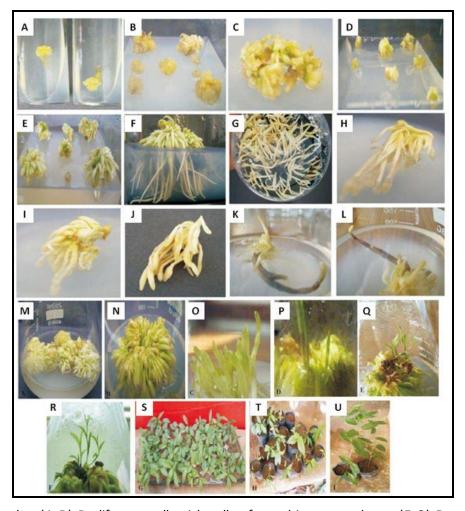


Fig. 11. *G. superba*: (A-D) Proliferate yellowish callus from rhizome explants, (E-G) Root initiated from rhizome callus, (H-J) Microrhizome initiation from rooted callus, (K-L) Well developed micro-rhizome having two germination corm buds. (M-O) Shoot induction from rooted callus on 1/2 MS medium with BAP 2.0 mg/L-1, (P-R) Shoot elongation and multiplication on 1/2 MS medium amended with Kn 1.0 mg/L-1+BAP 1.5 mg/L-1+20% CW, (S) Rooted leaflets were transfer into small plastic nursery tray acclimatization in the mist house for 2-months, (T) Regenerated plantlets were transplanted with polythene bags to hardening for in vivo condition. (U) Well developed plants for after two months ⁹³.

12.2. Applications of G. superba in nanotechnology

Nanotechnology considers as a material of size within the range of 1~ 100 nm with a high surface and volume ratio. The different route has been used for nanoparticle synthesis such as physical, chemical, and green (biological) methods. However, the green synthesis method was widely used due to the low-cost, easy of mass production, less energy, and less time consumption. According to the literature reports, silver, gold, silver/gold bimetallic, ruthenium metal nanoparticles, and cerium oxide (metal oxide) nanoparticles were synthesized using G. superba leaf extract 75,96,97 (Fig. 12). This plant leaf extract leads to controlled morphology in the size and shape of nanoparticles. All the synthesized nanoparticles showed better antibacterial activity without gold nanoparticles which is due to the non-toxicity. Gopinath et al., 98 reported G. superba leaf extract mediated synthesis of gold nanoparticles and its tissue culture application for encapsulating technique (Fig. 13). 1000 μM gold nanoparticles were used for alginate encapsulated root tips of G. superba, which exhibit a positive impact on the root shootlets formation. Gopinath et al., 99 have been reported Terminalia arjuna leaf extract mediated gold nanoparticles tested for Allium cepa root tip cell for mitotic cell division and G. superba pollen grains germination. Moreover, they find the gold nanoparticles have induced diploid cell division and haploid cell growth of A. cepa and G. superba. Similarly, Alharbi et al., 100 reported that *T. arjuna* gum extract mediated gold nanoparticles treated with 100 μM concentration significantly induced G. superba pollen germination. T. arjuna fruit extract mediated gold nanoparticles 1000 µM concentration has the most significant effect on seed germination and vegetative growth of G. superba 101 (Fig. 14). Balalakshmi et al., 102 reported Sphaeranthus induces leaf extract mediated gold nanoparticles at 10% concentration showed excellent pollen germination activity, which is due to the nuclei-metal complex presumably involved in the stimulation of pollen germination. The comprehensive investigation on plant pollen-grain germination activity was gold nanoparticles sizedependent manner. In the coming days, the nanotechnology research approach indeed needs to lead the conservation of endangered plants.

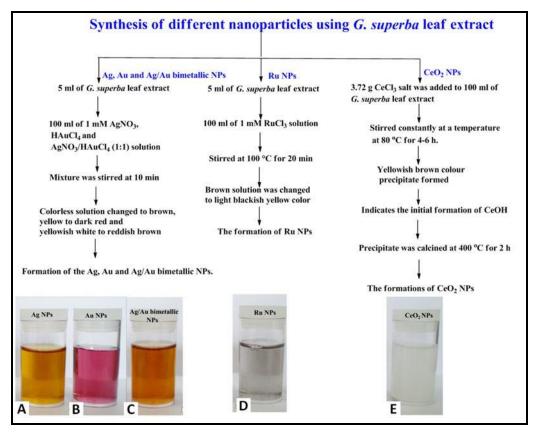


Fig. 12. Synthesis of different nannopaticles using *G. superbα* leaf extract: (A) Silver nanoparticles, (B) gold nanoparticles, (C) Silver/Gold bimetallic nanoparticles, (D) Ruthenium nanoparticles and (E) Cerium oxide nanoparticles⁹⁶⁻⁹⁸.

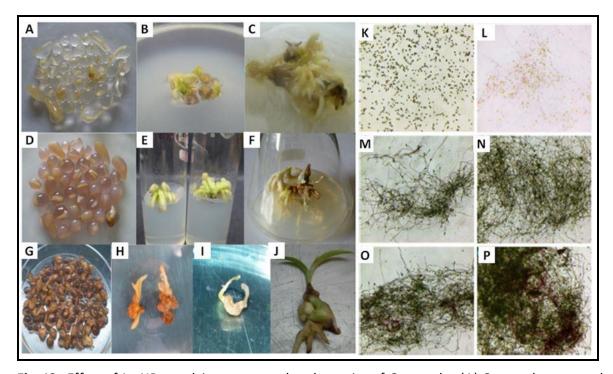


Fig. 13. Effect of Au NPs on alginate encapsulated root tips of *G. superba*: (A) *G. superba* encapsulated root tip cells without Au NPs treated. (B, C) Without Au NPs encapsulated root tip cells produced root initiation on MS medium. (D) 500 μM Au NPs encapsulated root tip cells. (E) 500 μM Au NPs treated beads induced root initiation. (F) 500 μM Au NPs treated beads induced the multiple roots with single shootlet. (G) 1000 μM Au NPs encapsulated root tip cells. (H-J) Regenerated alginate beads to produced root and shoot. *G. superba* pollen grains exposed to Au NPs at different concentrations: (K) control, (L) 1%, (M) 3%, (N) 5%, (O) 7%, and (P) 10%. 99 .

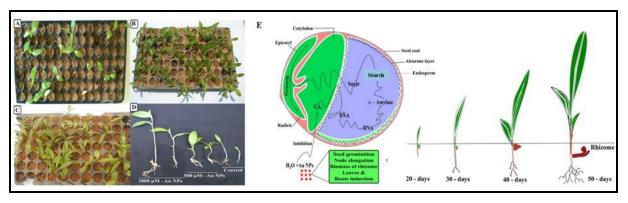


Fig. 14. Effect of Au NPs on *G. superba* seed germination: (A) Control, (B) 500 μ M gold nanoparticles, (C) 1000 μ M gold nanoparticles for a duration of 30 day, (D)- Induction of node elongation, biomass of rhizome leaf, and root initiation of gold nanoparticles -treated samples for a duration of 40 day, (E) - Schematic representation; effect of gold nanoparticles induced the *G. superba* seed germination, node elongation, biomass of rhizome, leaf and root initiation ¹⁰¹

13. CONCLUSIONS

G. superba is a commercially authoritative medicinal plant with various curative uses, and ultimately, the plant is facing local extinction. G. superba has been claimed for its various pharmacological actions and their applications in Ayurvedic medicine. The low seed germination, tuber dormancy, demand for

traditional remedies, over-harvesting from the natural forest, habitat destruction and over-exploitation leads *G. superba* in the world record of endangered plants. There is a crucial need to preserve this plant using biological tactics. Emphasis should be given to increase productivity and enhancement of colchicine by *G. superba* to encounter the industrial bids. Hence, there is demand; it will be needed to conserve the plant by *in situ*, *Ex-situ* multiplication and micropropagation and nanotechnology approaches in particular so as to meet the ever-increasing demand from industries. Even though various Biotechnological techniques are done to improve the productivity and chemical constituent of *G. superba*, but a satisfactory improvement wasn't reported about colchicine content. The colchicine constituents are varied between wild and micro propagated plants. In this review, distribution, cultivation, phytochemical constituents, biological and toxicological activities were explained. So, the present review can help researchers to explore this plant to a further extent.

ACKNOWLEDGMENT

This work was supported both by the MHRD-RUSA 2.0 [F.24/51/2014-U, Policy (TNMulti-Gen), Dept. of End. of India and University Grants Commission (UGC), New Delhi, for major research project (37-14/2009-SR).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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