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## Kembang sungsang (*Gloriosa superba* L.): A potential plant as a source of biomutagens

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### Abstract

*Gloriosa superba* (Kembang Sungsang, local name) belongs to the monocotyledoneae class and the Liliaceae family. This plant is known as a medicinal plant and its pharmacological effects have been developed both traditional and modern. All parts of the *Gloriosa superba*, especially the tubers and seeds, contain alkaloids namely colchicine and gloriosine. The content of colchicine and colchicides in tubers and other parts of plants is about 0.1 - 0.9% and 0.8% respectively. Colchicine has a strong affinity for tubulin so it is often used in biological studies and plant breeding to induce mutations that produce polyploidy individuals. Colchicine as a mutagen is used to develop the superior properties of some plants. Polyploidy plants have several advantages including larger cells, higher plants, wider leaves, larger fruits, higher production, and plants becoming more resistant to disease. Considering the role of colchicine above, the prospect of *Gloriosa superba* as a source of biomutagens (natural colchicine) needs to be studied and further developed.

**Keywords:** *Gloriosa superba*; Colchicine; Biomutagen; Kembang Sungsang

### 1. Introduction

Kembang sungsang (*Gloriosa superba* L.) are native to the tropics of the African and Asian continents and also known as Zimbabwe's national flower. Then the plant spreads and is cultivated in other places such as Europe and Australia. This plant grows wild in shrubs, teak forests, and is sometimes planted as ornamental plants that propagate on a fence or pergola because of its beautiful and exotic flowers. *Gloriosa superba* can grow well in the coastal area to a height of 300 m above sea level. In addition, *Gloriosa superba* can grow on sandy soil and is very tolerant of nutrient-poor soils. This plant is one of medicinal plants so its pharmacological effects have been developed both traditionally and in modern pharmacology. In India, these plants have been intensively cultivated and become the main export commodities of non-timber products to developed countries for pharmaceutical use.

### 2. Overview of Taxonomy and Geographic

Distribution Taxonomic changes have been made several times against the family Colchicaceae since it was first described by de Candolle in 1805. Based on variations in fruit structure, stylus, and anthera, Maroyi [1] noted from Baker (1879, 187, 1898), that Colchicaceae, Asparagaceae and Liliaceae included in the suborder or series of familia Liliaceae, namely: subordo A which consists of members of the Liliacea family with the characteristics of loculicidal capsules, undivided stylus, and antheraetrose; subordo B consists of members of the Colchicaceae family with the characteristics of septical capsules, tripartite stylus, and antheraextrose; and subordo C consists of members of the Asparagaceae family with baccate fruit characteristics. The long journey of taxonomic studies on the families of Colchicaceae and Liliaceae, both in terms of their morphology and phylogenetic studies, was successfully traced by Maroyi [1] and obtained various data findings that show the taxonomic position (treatment taxonomy) of several genera in

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Colchicaceae is still considered ambiguous. The Colchicaceae family consists of two genera namely *Colchicum* and *Gloriosa*.

The *Gloriosa* genus has a complicated taxonomy and high morphological variability that is often a barrier to understanding its diversity. Linnaeus formed the genus *Gloriosa* in 1737 based on specimens collected in southwest India (Malabar). In 260 years, more than 40 species of *Gloriosa* around the world were successfully described and published in the International Plant Index [2], although later they were largely reduced to synonyms. The literature study conducted by Maroyi [2] found that *Gloriosa* is considered a monotypic genus, consisting of one very varied species, *Gloriosa superba*. These variations are seen in plant height, shape, size and color of flowers. In the genus *Gloriosa* there are four species. *G. baudii* (Terracc.) Chiov., *G. carsonii* Baker, *G. simplex* L. and *G. superba* L. *Gloriosa* taxa that are the most widely spread is *G. superba*, scattered in South Africa, tropical Africa and Asia. Whereas *G. simplex*, *G. carsonii*, and *G. baudii* spread limitedly on the African continent. *G. baudii* has the most limited distribution range, in dry regions in northern Kenya, Ethiopia and Somalia. *G. carsonii* seems to be phenetically closer to *G. baudii*; and *G. simplex* phenologically close to *G. superba* [3, 1]. Similar opinion came from Raj *et al.* [4] which states that *Gloriosa* taxonomy is confusing, 27 species found in *Gloriosa* and *Gloriosa superba* are the most varied species, while *Gloriosarothschildiana* is the most cultivated *Gloriosa*.

From several research results ranging from biogeographic to molecular patterns of phylogeny of Liliales order, Maroyi [2] obtained data that Colchicaceae originated in Australia, for the first time spread to Asia, then to North America, Africa, Europe, and again spread to Australia. Alemu and Daba [5] explain the distribution of *Gloriosa superba* in Africa, ranging from Eastern Senegal to Ethiopia, Somalia and South Africa. Likewise Dounias [6] said that *G. superba* grew naturally in Africa, India and Southeast Asia, and is now widespread throughout the world and various tropical regions of the world as ornamental plants.

### 3. Vernacular Kembang sungsang (*Gloriosa superba* L.)

*G. superba* has 279 vernacular names, with the countries of India, Kenya and Senegal having the highest number of vernacular names, shows that local people in the country are very interested in this plant. The flower structure and also the flower color of red, purple, or yellow make the shape of the *G. superba* flower compared to the shape of a cow's tongue, rainbow, feet of crows and roosters by locals in several African countries. The Manding-Bambara people of Senegal refer to it as the donontulu species, the Ndebele people in South Africa call it amagugulume, and the Ndebele and Shona people in Zimbabwe call it amakukhulume and kajongwe. All these vernacular names are a common feature of their unification of descriptions of the color of *G. superba* which resembles a chicken's head [7] in India, Kalihari, Kathari, Kulhari, Languli (Hindi); Kannoru (Tamil); Kariari, Mulim (Punjabi). Common names known in the world for this plant are flame lily, glory lily, gloriosa lily, tiger claw, isimiselo, vlamlelie, or ririvavaimoa, "Salman glow" [6,8,9,10,11]. In Indonesia, Maroyi and van de Maesen [7] have found various articles (van Steenis-Kruseman, 1953; Prawiroatmodjo, 1976; Maradjo, 1977; Jessop, 1983; Bunyapraphatsara and Valkenburg, 1999) on the names of vernacular in Indonesian local communities and its utilization solely as a hair lice killer. In Maradjo's report (1977) also Maroyi and van de Maesen [7] found that indigenous people in Indonesia were inventive and imaginative; they recognize the irregular nature of the flowers of *G. superba* as well as the invaluable diagnostic features of this species. They describe that the shape and arrangement of flowers are unusual, so the flower of the *G. superba* is named 'kembangsungsang' (local name) which translates to "reverse flower". Local name variations are also found in Indonesia, namely kembangjonggrang or tiger nails (Jakarta); ketongkat or kembangsungsang (Sunda), kembangtelang, dongkelsungsang, mondalika, pacing tawa (Java); Mandalika (Bali), mandhalika (Madura), and poncoworno (Situbondo).

In Vietnam, there were no reports of the use of *G. superba*. However, this scarcity of information does not necessarily reflect that no one has studied or reported the utilization of *G. superba* in the country [7]. The absence of information about *G. superba* was also reported in one area in Indonesia. Search results of researchers who joined the RISTOJA program in 2012 reported that *G. superba* was found in almost all regions in Indonesia (found in 24 provinces of 26 provinces in Indonesia) except in one province in Java and in the Province of Bali. The name *G. Superba* in the two provinces turned out to appear in RISTOJA's 2015 search, but there was no information on which community in the two provinces knew it and the type of utilization. This condition explains that *G. superba* has not attracted the interest of local Indonesian people in recent years [12,13]. This is in line with the opinion of Maroyi and Van de Maesen [7] who said that in the past people might have relied on *G. superba* for some treatments, before other medicinal plant products on the market were available which gave better treatment results. The development of the field of medicine that occurs in the human race causes some of the use of *G. superba* to be no longer valued and this threatens the loss of knowledge about the benefits of *G. superba* as a medicinal plant.

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#### 4. Morphological and Habitat Descriptions

*Gloriosa superba* L. is a perennial herb that grows upright and forms bulbs. This plant grows by propagating or climbing using the tip of the leaf which extends to form a convoluted tendril and climbs the surrounding plants. The stem of the plant is soft and can reach heights between 1.8-2.4 m. The leaves are sessile, arranged in a spiral or almost facing. In one plant can have a different phyllotaxis combination with sizes 6-7 x 1.5 - 1.8 cm? The lancet leaves form tapering at the end and the base of the leaf enfolds the stem. The edges of the leaves are flat, the leaves are pale green or dark green. Flower buds appear at the ends of the branches, round, elongated, with long stems, the tip of the pointed buds facing down. Flower crowns are 6, straight or lancet and curly, greenish when young, then turn yellowish-orange and reddish when ripe. The fruit is 2-3 cm long. Fruit contains many seeds, black or orange red. Plant tubers are V or L-shaped, white when they are young and then brown when they are old. Each planting season produces two or more tubers, while the previous season's tubers will shrink. Usually the stem will die in the dry season and the tuber will only grow in the rainy season. The size of tubers will be highly correlated with the dimensions and patterns of branching of plants [3,5,6,8,9,14,15,16, 17].

*Gloriosa superba* can be found on the edge of savanna forest, shrubs, fence houses, hedges, open forests, grasslands, in bushes mixed with other plants, and the boundaries of residential areas to a height of 2530m. This plant thrives at temperatures between 15°C - 30°C, like a place that is somewhat protected until the place is exposed to full sunlight, sandy soil, neutral to acidic soil but must be free from drought, i.e. soil that remains wet during growth and groundwater decreases slightly when the plant begins to flower. Therefore, *G. superba* grow well in areas with a clear rainy season, and do not like humid tropics. These plants are also very tolerant of nutrient-poor soils [2,6,8,9,18]

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#### 5. Plant propagation

Propagation can take place through tubers and seeds. In general, bulb propagation is more often done but takes a long time because the maximum number of tubers per plant per year is two. The Roux and Robbertse [19] study found that 1% hypochlorite was able to shorten the tuber dormancy period to only 4 months. Results of previous studies that showed dormancy period of tubers for 6-9 months. This proves that there is an increase in generation from one or two generations to three generations of seedlings per year. In the same study it was found that there were significant differences in reproduction between the results of plant propagation from hypopodial tubers which were divided compared to the results of normal plant propagation (not divided). The tuber division is able to increase the percentage of flowering stems that grow and develop well by 50%. While the growth of stems from whole bulbs (not divided) produced only one bud per tuber that developed and the development of other shoots was inhibited, or 63% buds developed into mature plants, and 37% formed short herb stems with five to seven leaves and without forming flowers.

However, multiplication using seeds turns out to take longer. The new plant enters the reproductive phase after its age reaches about 3-4 years. Seed germination also takes a long time, which is 3 weeks to 3 months. Plant seeds will grow quickly and produce tubers in the second year and continue into the fourth year. The flower bud takes 17 days to reach the anthesis stage. The stages of flower development can be followed by changes in the color of the perianth (crown) segment. At the beginning of the bud opening, the flower crown looks bright green followed by the development of a receptive stigma which is marked by a red crown at the end, yellow in the middle, and green to the base. Pollination occurs 1 day later and stigma remains receptive for 4 days. Furthermore, the post-anthesis stage is marked by the top of the crown which becomes red from the middle to the end and the bottom is yellow, then finally changes completely to red. Anther will wither a day after pollination. The fruit will mature within 6-10 weeks after pollination. Pollination is assisted by butterflies and sunbirds. Sarcotesta which is red becomes an attraction for animals that helps it to spread seeds [2,6,9,17,18,20].

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#### 6. Pharmacological effects

*Gloriosa superba* is included in medicinal plants so that its pharmacological effects have been developed both traditionally (ethnobotany) and in modern pharmacology. This plant can cause disease or cause death in humans and animals due to intentional or unintentional poisoning. The use of all parts of the plant directly without special treatment is to repel snakes and scorpions by placing them around the door or window. The results of pharmacological studies *in G. superba* indicate the enormous potential of this species in the treatment of inflammation and parasitic and bacterial diseases [2]. Pharmaceutical applications are usually used in the form of pasta, decoction, maceration, powder, and all plants without special treatment. Of the five plant parts: leaves, seeds, raw fruit, tubers, or whole plants, roots/tubers are the most commonly used parts. Water extract of tuber are used for the treatment of abortion, venereal disease, abdominal pain, or general body pain. Decoction and maceration results can be used for internal treatment of the body,

such as abdominal pain, cough, fever, and malaria. Fruit paste is used externally to treat venereal diseases, injuries, skin diseases caused by parasites and head lice [6]. *G. superba* leaf juice can be used to kill head lice [9]. Roots that are applied to the palm of the hand and foot of a pregnant woman will facilitate the delivery process. Tuber powder mixed with ghee is put into capsules and taken twice a day, effective for abortion. Powder with mustard oil applied to the joints twice a day will reduce pain due to arthritis. Tuber paste can be used externally for various skin diseases, such as wounds and leprosy. Tuber powder can treat stomach disorders in cattle. Flower paste with warm water and applied on the soles of the feet to treat anthrax in cattle. Tubers that are mashed and smeared between the toes of the cow or the boiled water of tubers mixed with the feed grass will increase milk production [21]. From pharmacological studies, traditional ethnobotany use and a significant correlation between the two successfully passed by Maroyi [2] can be concluded that even though further research, clinical trials needed, and product development are still needed, this plant shows the potential of using as an excellent source of medicine in the future

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## 7. Economic Value

India is one of the countries that has intensively cultivated *G. superba* and made it as a main export commodity for non-timber products to developed countries as raw materials in the modern pharmaceutical industry. Bulbs and seeds are an expensive export commodity. Seeds and tubers are traded in the form of: fresh, dry, powder or oil. Plant flowers are harvested as cut flowers or potted plants. Every year, India produces 1.5 - 3 quintals of seeds, 1.0 - 1.25 quintals of dried rhizomes per hectare. Tamil Nadu (India) is one of the central production areas of *G. superba* with an area of 6000 hectares producing 10 tons of seeds per year per hectare. In the international market the price of seeds is around \$45/kg [5,6,8,9,18,21,22,23].

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## 8. Content of Active Compounds

All parts of kembang sunsang (*Gloriosa superba* L.) plant contain active compounds, especially in tubers and seeds which contain lots of alkaloids, namely colchicine and Gloriosin. Alemu and Daba [5] said that the highest colchicine content was found in the early stages of plant growth and would decrease during the aging process with a slight increase when the tubers became inactive at the end of the season. The best source of colchicine is seeds (2-5 times higher than tubers), but other reports from Rwanda say that the highest content of colchicine is in young leaves. Colchicine and colchicides in tubers and other plant parts of breesches were about 0.1 - 0.9% and 0.8% respectively ([6,24], on leaves of 8.0 mg / gdw, stems 7.4 mg / gdw, tubers 19.8 mg / gdw, and flowers of 20.7 mg / gdw [25]; in tubers ranging from 0.14 to 0.56% [26]; the average seed contains 0.541% [10]; on leaves 0.14 mg / ml, seeds 2.29 mg / ml, and tubers 3.38 mg / ml [27]. Chitra and Rajamani [28] examined the colchicine content of 18 *Gloriosa superba* accessions from Tamil Nadu and obtained that the highest content is from the accession of "*andhara wild*" containing 1.32% and the lowest content of 0.02% found in the accession of "*kallimanthayam wild*". Pandey and Banik [29] using various solvents found that the highest content of colchicine was 0.61% obtained from 50% acetone-water extract of tubers. Raj *et al.* [4] used various extraction methods from various parts of the *Gloriosa superba* plant and the results showed that the highest concentration of colchicine obtained from the freeze drying method of tuber extract was about 0.44 mg / l, the dichloromethane method of tuber extract produced about 0.77 mg / l, hot water method in the leaf extract produces about 1.42 mg/l, and from the sonication method in pod extract produces about 0.21 mg/l. Based on these results Raj *et al.* [4] concluded that the hot water method is recommended for commercial extraction of colchicine because it is economically cheap and less tiring.

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## 9. Plant Improvement

This species of plant is close to extinction due to excessive exploitation of tubers and low seed germination percentage. A decade ago, *Gloriosa* was only discovered wild but has now been domesticated for economic and medical purposes in India. To ensure the availability of seeds in the international market and meet high demand, farmers must develop crops that have high yields with high levels of colchicine, dwarf plants, and resistant to leaf blight. For the purpose of high grain yield, the selection is focused on the number of branches per plant, the number of flowers per plant, the number of pods per plant, the number of seeds per plant, and the dry seeds produced. These characters are related and correlate directly with the yield of seeds. Knowledge of genetic diversity / variability is very helpful in exploiting plant diversity directly as cultivars, and indirectly utilizing it as basic information in breeding programs [28,30].

The quality and quantity of production of stable *G. superba* plays an important role in growing the world market. For this reason, high yielding varieties are needed with high quality and yield, such as high levels of colchicine [20]. The use of plant tissue culture technology can be done to produce superior plants. Sarin *et al.* [25] obtained the highest colchicine concentration in 6 weeks old culture (922.6 mg / gdw) and the lowest at 2 weeks culture age (15.3 mg / gdw). Anandhi

*et al.* [10] used physical mutation (Gama) and chemical treatments (EMS and DES) to obtain five *Gloriosa* elite mutants with higher colchicine content than controls. Increasing the content of colchicine in *G. superba* can also be done by administering AM fungi. Plants that were given AM fungi had a colchicine content of 348.9 mg / tan compared to control plants 177.87. Introduction with mycorrhizal technology combined with P fertilizer helps the development of *G. superba* cultivation and colchicine production [22]. In addition, good cultivation patterns also support higher yields of colchicine as Bhasak *et al.* [31] who explained that the colchicine content was found to be higher in the tubers of *Gloriosa* which were cultivated than the contents of the wild plants.

Ernawati's research [32] found that the colchicine compound in kembang sungsang tuber extracts had the same antimitosis effect as standard colchicine from Sigma on root cells of Bombay onion tuber. Similar opinion was expressed by Mukhopadhyay *et al.* [33] who said that the extracted colchicine has better efficacy than standard colchicine from Sigma chemical co.

## 10. The mechanism of action of colchicine

Colchicine is known to affect one of the main constituents of microtubules by binding to tubulin thereby inhibiting microtubule in polymerization. Tubulin is very important in mitosis so tubulin availability is considered as a determinant of the continuity of the mitotic process. Thus colchicine is a very effective for mitotic or spindle poison. Colchicine which is antimitotic, interferes with microtubule organization, causing abnormal metaphase to form cells with multiple chromosomes. Indirectly, colchicine affects the membrane structure by inhibiting the synthesis of the constituent elements of the membrane [9,34].

Colchicine is recommended for cancer treatment. This is based on the characteristics of cancer cells whose mitotic level has increased significantly. And cancer cells are known to be more susceptible to colchicine poisoning than normal cells. Colchicine causes inhibition of spindle thread formation during metaphase. Therefore *Gloriosa superba* which is known having high colchicine content has the potential to be developed as a chemotherapy agent in preventing or inhibiting the growth of cancer cells [9].

The nature of colchicine which has strong affinity for tubulin so that colchicine is often used in biology studies and breeding to induce mutations that produce polyploidy plants and very useful to change the agronomic properties of plants, so that it is widely used in the plant breeding by forming mutant species that change the nature of plants as a result of chromosomal duplication [5,35]. Besides inducing polyploidy initiation it can also cause low frequency chlorophyll mutations. The specific problem of ploidy induction due to colchicine, especially in plants that are propagated vegetatively is chimerism which is caused by a network with different levels of ploidy in one plant or part of the plant [9,34].

## 11. Advantages of Polyploid

Plants In general polyploid plants have several advantages, including having larger cells, taller plants, wider leaves, bigger fruit, higher production, and more resistant to disease attacks [6]. Several subsequent studies have shown that colchicine in kembang sungsang tuber extracts tends to decrease the mitosis index (antimitosis) root cells of bombay onion tuber [36], red chili sprout roots [37], shallots [38], increase plant height, leaf area [39], resulting in 46.6% C-mitosis [40]. Kembang sungsang leaf extract can reduce mitotic index, induce polyploid cells [41,42] Seed immersion in treatment combination of extract of kembang sungsang tuber 60 % an immersion period of 48 hours is the best combination in inhibiting mitosis of root tip cells of red chili [41]; pollen germination, increase in pollen viability, number of fruits [43,44] and reduce the conidial density of fungi *Colletotrichum capsici* causing Antracnosa disease in red chili ([45,46,47].

## 12. Conclusion

The use of colchicine as mutagen for the purpose of plant improvement (plant breeding) is indeed commonly used. Considering this, the tubers, stems, seeds, flowers and leaves of Kembang ungsang which are known to be high in colchicine can be used as a prospective source of biomutagen to induce superior properties in plants.

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## Compliance with ethical standards

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### Disclosure of conflict of interest

The authors declare no conflict of interest.

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