Phytochemical Screening of *Gloriosa superba L.* - from different Geographical Positions

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Abstract - *Gloriosa superba* Linn. is one of the important medicinal plant now in endangered list. This plant is widely used for several ethano-medicinal purposes by tribal peoples and traditional practitioners. Seeds and tubers contain valuale alkaloids viz., colchine and colchicoside as the major constituents, which are used an antidote for snake bites, gout and rheumatism. Present study was to evaluate the phytochemicals present in leaves, flowers, seeds and tuber samples were collected from four different places of Dharmapuri District. Preliminary phytochemical analyses were carried out by standard procedures. Methanol extract of all samples were evaluated for protein, starch, total sugar and total phenol. The screening tests also were performed for the presence of the following secondary metabolites such as alkaloid, flavonoids, glycosides, phenols, saponins, steroids, tannin and terpenoids. The results were revealed the presence of various classes of compounds in different parts of the plant. Among the five different geographically collected plant samples, the Hoganekkal Hills sample showed the maximum amount of bioactive compounds than the other samples. The result of the extract from the tuber and seed samples were yields high amount of various biologically active compounds than the leaves and flowers. These compounds could serve as potential source for traditional medicines. Further research on this plant for the specific part could be used for isolation and characterization in large scale production.

Index Terms - *Gloriosa superba*, phytochemicals, enzymes, alkaloids, glycosides, and steroids

I. INTRODUCTION

Gloriosa superba Linn. is an important medicinal plant belonging to the family Liliaceae. It is a semi-woody herbaceous branched climber reaching approximately 5 meters height, with brilliant wavy-edged yellow and red flowers [1]. Which is one of the endangered species among the medicinal plants [2]. It is extensively scattered in the tropical and sub-tropical parts of the India. 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 [3]. Being native form Indian especially Southern India it is known as glory lily and climbing lily- in English. In the world market glory lily considered as rich source of colchicines and gloriosine [4]. The flower has analgesic, anti-inflammatory potential, antimicrobial, larvicidal potential, antipoxviral potential, antithrombotic potential, antitumor potential, enzyme inhibition potential, and also used in treatment of snake bite, Skin disease, respiratory disorders [5, 6, 7, 8, 9].

Medicinal plants have been used as sources of medicine in virtually all cultures [10, 11]. In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs. Thus a search for new drugs with better and cheaper substitutes from plant origin is a natural choice. The medicinal values of these plants lie in some chemical substances that produce a definite physiological action on human body [12]. Different parts of *G. superba* have wide variety of uses especially in traditional system of medicine. The tuber is used for the treatment of bruises and sprains, colic, chronic ulcers, haemorrhoids, cancer, impotence, nocturnal seminal emission, and leprosy and also for including labour pains and abortions [13]. *Gloriosa superba* also used in wounds, skin related problems, Fever, Inflammation, piles, blood disorders, Uterine contractions, General body toner, Poisoning [14, 15]. Roots are acrid, anthelmintic, antipyretic, bitter, digestive, expectorant, highly poisonous and promoting expulsion of the placenta. Root paste is effective against paralysis, rheumatism, snake bite and insect bites [16]. This plant has gained the importance in medicine in recent years for the production of colchicine in large scale [17]. The aim of this study was to identify and determine the phytochemicals present in *G. superba* leaves, flowers, seeds and tubers.

II. MATERIALS AND METHODS

Plant materials

The plant samples such as leaves, flowers, seeds and tubers of *Gloriosa superba* were collected from four different geographical positions such as Hogenakkal Hills (HGS), Mookanur Hills (MGS), Sitheari Hills (SGS) and Vathal Hills (VGS) of Dharmapuri district in Tamilnadu, India. These plants were then identified, confirmed and have been deposited in the herbarium of PG and Research Department of Botany, Government Arts College, Dharmapuri for the future reference.

Preparation of plant extract

Fresh leaves, flowers, seeds and tubers were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. They were ground into coarse powder by using mechanical pulveriser. All the samples, about 100 g of the powder were repeatedly extracted with methanol in a 500 mL round bottom flask with 250 mL solvent. The reflux time for each solvent was 25 cycles for complete extraction using soxhlet apparatus [18, 19]. The filtrate was collected and concentrated by using rotary evaporator under controlled condition of temperature and pressure. The extracts were concentrated to dryness to yield crude residue. These residues were stored at -20°C, used for preliminary phytochemical screening of secondary metabolites.

Phytochemical analysis

Phytochemical screening were performed to assess the qualitative chemical composition of different samples of crude extracts using commonly employed precipitation and coloration reactions to identify the major secondary metabolites like alkaloids, flavonoids, glycosides, Proteins, phenolic compounds, saponins, starch, steroids, tannins and terpenoids. The phytochemical analyses were carried out using standard procedures [20, 21]. The methanol extracts of *G. superba* were screened for the presence of secondary metabolites using the procedures [22, 23]. The observations were recorded for total starch [24], soluble protein [25], steroids using Salkowski test [26], flavonoids and tannins using ferric chloride test [27], alkaloids by Mayer's test [28], and proteins and glycosides by Biuret and Legal tests, respectively [29, 30] and total phenol [31].

1. Screening for Alkaloids

Meyer's reagent (potassium mercuric iodide) 1.36 gm of mercuric chloride was dissolved in 60 ml of distilled water and 5 gm of potassium iodide was dissolved in 10 ml of water. These two solutions were mixed and diluted to 100 ml with distilled water. To 1 ml of the extract, a few drops of reagent were added. Formation of white or pale precipitate showed the presence of alkaloids.

2. Screening for Flavonoids

In a test tube containing 0.5 ml of extract, 5 to 10 drops of diluted HCl and small piece of ZnCl or magnesium were added and the solution was boiled for few minutes. The appearance of reddish pink or dirty brown colour indicates the presence of flavonoids.

3. Screening for Glycosides

To 5 ml of extract add 5 ml of 5% $FeCl_3$ and 5 ml diluted HCl. Heat for 5 min in boiling water bath. Cool and add benzene or any organic solvent and shake well. Separate the organic layer and add equal volume of diluted Ammonia. Ammonical layer shows pinkish red color indicates the presence of glycosides.

4. Screening for Saponins

In a test tube containing about 5 ml of the extract, few drops of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 minutes. A honeycomb like froth was formed and it showed the presence of saponins.

5. Screening for Steroids

To 2.0 ml of extract, 1.0 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. A red colour produced in the chloroform layer shows the presence of steroids.

6. Screening for Phenols

To 1 ml of the extract 3 ml of distilled water followed by few drops of 10% aqueous Ferric chloride solution was added. Formation of blue or green colour indicates the presence of phenols

7. Screening for Tannins

In a test tube containing about 5 ml of the extract, a few drops of 1% solution of lead acetate was added. A yellow or red precipitate indicates the presence of tannins.

8. Screening for terpenoids

To 0.5g of the extract 2ml of $CHCl_3$ was added. 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

9. Screening for reducing sugars

The aqueous 2ethanol extract (0.5 g in 5 ml of water) was added to boiling fehling's solution (A+B). Brick red precipitate form at the bottom of the test tube indicates the presence of reducing sugars.

Statistical analysis

Phytochemical estimation and quantification were performed in five replicates under standard procedures to ensure consistency of all conclusions. Data of all experiments were statistically analysed and expressed as Mean ± Standard Deviation.

III. RESULTS

In the present study deals with the preliminary phytochemical screening of methanol extracts of *Gloriosa superba* samples from HGS, MGS, SGS and VGS are presented in Table 1 and 2. All the samples of leaves, flowers, seeds and tubers showed the enormous occurrence of phytochemicals. The leaf samples showed the presence of proteins, phenols, tannins, starch and

terpinoides. The flowers, seeds and tubers samples showed the high content of flavonoids. Glycosides and alkaloids were found in maximum amount in seed and tubers in all samples, but they were absent in the leaves and flower extracts. Saponins were found in moderate concentration in all the samples of seeds and tubers extracts but showed the absence in all the sample extracts of leaves. When compare to the all four samples collected from different places in which the HGS sample showed the maximum occurrence of bioactive compounds then VGS samples followed by SGS samples and MGS samples.

In summary of phytochemical analysis of *G. superba*, alkaloids, glycosides and steroids were showed the maximum occurrence in HGS (90.45%, 82.45%, 56.67%) than VGS (87.25%, 78.54%, 54.25%) followed by SGS (80.45%, 75.21%, 51.31%) and MGS (75.29%, 72.54%, 50.11%) respectively. In moderated amount of flavonoids, starch, terpinoids, proteins and tannins were presence in HGS (55.92%, 47.32%, 47.58%, 40.28%, 36.25%) than VGS (52.47%, 44.68%, 45.72%, 35.22%, 35.65%) followed by SGS (50.87%, 42.35%, 43.14%, 33.11%, 32.45%) and MGS (48.67%, 42.12%, 42.10%, 33.41%, 30.66%) respectively. In lowest amount of phenols and saponins were recorded in HGS (38.64% and 21.08%) than VGS (33.54% and 18.12%) followed by SGS (30.12% and 17.56%) and MGS (30.07% and 12.34%) respectively. The result data were statistically calculated with five replicates of each sample and presented in Table 3.

IV. DISCUSSION

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives [32]. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total [33]. In the present study, methanol extracts of all the samples showed the maximum yield of phytochemicals. However, the methanol extract of *G. superba* showed good results for phytochemicals like phenols, alkaloids, flavonoids and tannins [34]. The plant is a well-known ethnomedicinal use in Ayurveda for its colchicine content which is used to treat arthritis. Phytochemical studies of tubers or dried roots have showed the presence of colchicines, glycoside, gloriosine, long chain fatty acids, flavonoids, tannins, alkaloids, 3-O-demethylcolchicine-3-O-α-D-glucopyranoside, 1,2-didemethyl colchicine, Glucoside, β and γ Lumicolichicines, β silosterol, Flucoside, 2,3-didemethyl colchicine, luterlin, N-formyl deacetyl colchicines, colchicocide, tannins, superbine, 2-hydroxy-6-methoxy benzoic and salicylic acid [35]. According to [36], the first formed assimilate in the plant will be the simple sugars which will be used for the plant metabolic activities and the excess be stored in their reserve organs. Increased total sugars in tubers may be attributed to the high partitioning efficiency and increased efficiency of the sink to accumulate assimilates in the tubers.

The interest of medicinal plants exploration as a source of pharmacologically active compounds has increased worldwide [37]. In most developing countries of the world, plants are the main medicinal sources used in treating infectious diseases. The various phytochemical compounds detected are known to exhibit medicinal activity as well as physiological activity [38]. There are records that show the benefits of these compounds detected from *G. superba*. For example: Many of the previous reports show that the isolated pure compounds with biological activity were alkaloids. Naturally occurring alkaloids are nitrogenous compounds that constitute the basic active principles of flowering plants. Alkaloids are formed as metabolic products and have been reported to be responsible for pharmaceutically active [39]. Some of the simplest bioactive phytochemicals consist of a single substituted phenolic ring. Phenolics are the largest group of and have been said to account for most of the antioxidant activity of plant extracts. Phenolics and alkaloids detected in the extracts are compounds that have been documented to possess medicinal properties and health-promoting effects [40, 41].

Plant steroids are known to be important for their cardiotonic activities; they possess insecticidal and anti-microbial properties. They are routinely used in medicine because of their profound biological activities. Glycosides are nonvolatile and lack fragrance and serve as defense mechanisms against predation by many microorganisms, insects and herbivores [42]. Plant saponins help humans to fight fungal infections, combat microbes and viruses, boost the effectiveness of certain vaccines and knock out some kinds of tumor cells, particularly lung and blood cancers. These compounds served as natural antibiotics, which help the body to fight infections and microbial invasion [43].

Tannins have been traditionally used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, hemorrhoids and diarrhea. Plant tannins have been recognized for their pharmacological properties and are known to make trees and shrubs. Flavonoids are widely distributed group of polyphenolic compounds, characterized by a common benzopyrene ring structure. The biological functions of flavonoids apart from its antioxidant properties include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors. Flavonoids reduced cancers by interfering with the enzymes that produce estrogen [44].

V. CONCLUSION

Plants are natural sources of bioactive compounds to treat life threatening diseases. This plant *Gloriosa superba* has showed various phytochemicals which means that it can use for treating diseases. The plant methanol extracts revealed the presence of different types of phytoconstituents. The result obtained from the present study clearly stated that HGS samples excelled in the accumulation alkaloids, glycosides, flavonoids, starch, soluble protein and total phenol in tubers. Considering all the aspects it can be concluded that the sample HGS might serve as ideal parent for developing hybrids with high seed and tuber yield. Further studies are going on these plants in order to isolate, identify, characterized and elucidate the structure of the bioactive principles to develop new antibacterial and antifungal medications.

ACKNOWLEDGMENT

The author is thankful to the Principal and Head of the Department, PG and Research Department of Botany, Governemt Arts College, Dharmapuri for providing facilities for this research work.

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Table 1: Preliminary phytochemical tests for the presence of compounds in *Gloriosa superba* leaves, flowers, seeds and tubers of Hogenakkal Hills (HGS) and Vathal Hills (VGS)

S. No.	Compounds	Hogenakkal hills (HGS)				Vathal hills (VGS)			
		Leaves	Flowers	Seeds	Tubers	Leaves	Flowers	Seeds	Tubers
1.	Alkaloids	-	+	++	+++	-	+	++	+++
2.	Flavonoids	+	+++	++	+	+	+++	++	+
3.	Glycosides	-	+	+++	+++	-	+	+++	+++
4.	Protein	+++	++	++	+++	+++	++	++	+++
5.	Phenols	++	++	+++	+++	++	++	+++	+++
6.	Saponins	-	-	+	+	-	-	+	+
7.	Starch	++	+	++	+++	++	+	++	+++
8.	Steroids	+	+	+++	+++	+	+	++	++
9.	Tannin	-	-	++	++	-	-	++	++
10.	Terpenoids	++	+++	+++	+++	++	+++	+++	+++

(- = negative; + = slight; ++ = moderate; +++ = High)

Table 2: Preliminary phytochemical tests for the presence of compounds in *Gloriosa superba* leaves, flowers, seeds and tubers of Sitheri Hills (SGS) and Mookanur Hills (MGS)

S. No.	Compounds	Sitheri hills				Mookanur hills (MGS)			
		Leaves	Flowers	Seeds	Tubers	Leaves	Flowers	Seeds	Tubers
1.	Alkaloids	-	-	+	++	-	-	+	++
2.	Flavonoids	1	++	++	+	+	++	++	+
3.	Glycosides	-	+	++	+++	-	+	++	+++
4.	Protein	+++	++	++	++	++	++	++	++
5.	Phenols	+	+	+	++	+	+	+	++
6.	Saponins	-	-	+	+	-	-	+	+
7.	Starch	++	+	++	+++	++	+	+	+
8.	Steroids	+	+	+	+	+	+	+	+
9.	Tannin	-	-	++	++	-	-	++	++
10.	Terpenoids	+	++	++	+++	+	+++	++	++

(- = negative; + = slight; ++ = moderate; +++ = High)

Table 3: Total percentage of bioactive compounds in *Gloriosa superba* in different places

S. No.	Compounds	HGS	VGS	SGS	MGS
1.	Alkaloids (%)	90.45 ± 7.12	87.25 ± 8.41	80.45 ± 8.12	75.29 ± 7.24
2.	Flavonoids (%)	55.92 ± 4.21	52.47 ± 3.12	50.87 ± 4.45	48.67 ± 4.26
3.	Glycosides (%)	82.45 ± 6.56	78.54 ± 5.66	75.21 ± 7.64	72.54 ± 7.25
4.	Protein (%)	40.28 ± 2.89	35.22 ± 3.85	33.11 ± 3.15	33.41 ± 4.35
5.	Phenols (%)	38.64 ± 3.55	33.54 ± 3.15	30.12 ± 2.85	30.07 ± 3.12
6.	Saponins (%)	21.08 ± 1.86	18.12 ± 1.22	17.56 ± 2.45	12.34 ± 1.17
7.	Starch (%)	47.32 ± 3.73	44.68 ± 4.15	42.35 ± 4.34	42.12 ± 4.65
8.	Steroids (%)	56.67 ± 4.10	54.25 ± 3.95	51.31 ± 5.12	50.11 ± 5.37
9.	Tannin (%)	36.25 ± 2.65	35.65 ± 2.25	32.45 ± 3.64	30.66 ± 3.12
10.	Terpenoids (%)	47.58 ± 3.34	45.72 ± 4.05	43.14 ± 4.11	42.10 ± 3.67

(Mean values for five replicates, n = 5 ± SD)