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Research Article

Phytochemical Screening and Antibacterial Activity of *Gloriosa superba* Linn.

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ABSTRACT

The present study was to evaluate the phytochemical and antibacterial properties of different extracts of *Gloriosa superba.* Seeds and tubers contain valuale alkaloids viz., colchine and colchicoside as the major constituents, which are used an antidote for snake bites. The successive Soxhlet extract of seeds and tubers were extracted using hexane, chloroform and methanol in ascending order of the polarity. Preliminary phytochemical screening revealed that the extracts contain Alkaloids, Glycosides, Steriods, Terpenoids and Tannins in all extracts. The extracts were tested for their antibacterial activity against five gram positive bacteria viz., *Bacillus cereus, Bacillus subtilis, Streptococcus cremoris, Streptococcus fecalis, Staphylococcus aureus* and five gram negative bacteria viz., *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia* and *Proteus vulgaris* by well diffusion method *in vitro* condition. Among the three extracts tested, methanol extract had effective antibacterial potential, followed by chloroform extract. The methanol extract showed maximum activity against gram positive than gram negative organisms. In methanol extract showed maximum inhibition of 42.3 mm in *Bacillus cereus* at 250 µl concentration followed by *E. coli* (38.4 mm), *Streptococcus fecalis* (37.2 mm), *Klebsiella pneumonia* (35.6 mm), *S. aureus* (33.3 mm), *P. aeruginosa* (30.5 mm), *S. cremoris* (28.1 mm) *Proteus vulgaris* (26.9 mm) *B. subtilis* (23.5 mm) and least inhibition was observed in *S. typhi* (21.7 mm). Moderate activity was observed in chloroform extract. Minimum activity was observed in hexane at different concentration tested. Compared to synthetic antibiotic Ampicillin (50 mg), solvent extracts recorded significant antibacterial activity. The study confirms the antibacterial potential of *Gloriosa superba* seeds and tubers extracted using various solvents.

Keywords: *Gloriosa superba*, Antibacterial activity, Phytochemicals, Antibiotics, Solvent extract.

INTRODUCTION

Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. India despite its rich traditional knowledge, heritage of herbal medicines and large biodiversity has a dismal share of the world market due to export of crude extracts and drugs. Several plants were known to possess medicinal value including antimicrobial properties. Bacterial and fungal infections were some of the most serious global health issues of the present century[1]. Bacterial diseases accounts for high proportion of health problems in the developing countries. To manage the bacterial diseases, many synthetic antibiotics are regularly used. Due to indiscriminate use of synthetic antibiotics, Bacteria have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created[2]. There are many reports available where Indian medicinal plants and their products are used to control diverse disease[3]. The emergence of antimicrobial resistance pathogens now treats the discovery of potent antimicrobial agents. Antimicrobial resistance has resulted in increased morbidity and mortality as well as health care costs[4]. In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. Plant origin herbal medicines are considered as safe alternatives of synthetic drugs. Plants are used in modern medicine where they occupy a very significant place as raw material for important drugs[5]. Plants specifically herbal medicines have received much attention as source of new antibacterial drugs since they are considered as time-tested and comparatively safe both for human use and for environment[6].

Gloriosa superba Linn. is an important medicinal plant belonging to the family Liliaceae. Which is one of the endangered species among the medicinal plants[7,8]. 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[9]. 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[10, 11, 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

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Table 1. Quantitative phytochemical analysis of various extracts of *Gloriosa superba* seeds and tubers

Bioactive compounds	Hexane extract		Chloroform extract		Methanol extract	
	Seeds	Tubers	Seeds	Tubers	Seeds	Tubers
Alkaloids	30.55 %	42.98 %	60.54 %	65.36 %	80.22 %	94.26 %
Glycosides	12.15 %	20.45 %	62.25 %	68.36 %	67.32 %	85.21 %
Steroids	26.76 %	30.67 %	90.65 %	95.34 %	52.17 %	55.20 %
Tannin	88.22 %	93.15 %	44.30 %	46.20 %	32.16 %	38.65 %
Terpenoids	80.10 %	83.57 %	30.65 %	32.54 %	28.30 %	30.10 %

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]. 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[15]. This plant has gained the importance in medicine in recent years for the production of colchicine in large scale[16]. Several colchicine-related alkaloids have been isolated from tubers and seeds. They are mostly demethyl substitutes and include cornigerine, which is a potent antimitotic, and colchicoside used as a muscle relaxant. A plant can contain up to 0.9% colchicine and 0.8% colchicoside. Colchicine is a powerful antimitotic agent that blocks or suppresses cell division by inhibiting mitosis, the division of a cell's nucleus. The chemical constituents of the tuber are known to be very poisonous to fish[17]. *Gloriosa superba* is widely used as a medicinal plant in south India, despite the fact that the whole plant is very poisonous. The objective of the present study was to evaluate the phytochemicals and antibacterial activity of *G. superba* seeds and tubers.

MATERIALS AND METHODS

Collection of Plant materials: The plant samples such as seeds and tubers of *Gloriosa superba* were collected from Hogenakkal Hills 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 extracts: Fresh 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]. 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 and antibacterial assay.

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, glycosides, steroids, tannins and terpenoids. The phytochemical analyses were carried out using standard procedures[19]. The hexane, chloroform and methanol extracts of *G. superba* were screened for the presence of secondary metabolites using the procedures[20, 21]. The observations were recorded for alkaloids by Mayer's test[22], for glycosides by Biuret and Legal tests[23], for steroids using Salkowski test[24], for tannins using ferric chloride test[25] (Martin and Martin, 1982) and for terpenoids using Salkowski test[24].

Alkaloids test: To 5 g each of the seeds and tubers extracts and 5 ml of honey was stirred with 5 ml of 1% aqueous hydrochloric acid on a steam bath. One ml of the filtrate was treated with few drops of Draggendoff's reagent. Blue black turbidity serves as preliminary evidence of alkaloids.

Glycosides (keller-killiani test): To 5 g of each of the seeds and tubers extracts and 5 ml of honey was dissolved in 2 ml glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1 ml concentrated sulphuric acid. A brown ring of the interface indicates a deoxy-sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a green ring may form just gradually spread throughout this layer.

Steriods test: To 2 ml of acidic anhydride was added to 0.5 g of seeds and tubers extracts and 2 ml of sulphuric acid was added by the sides of the test tube and observed the colour change from violet or blue-green.

Tannins test: To 5 g each of the extracts and 5 ml of honey was stirred with 100 ml distilled water and filtered. Ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate determines the presence of Tannins.

Terpenoids (Salkowski test): To 0.5 g of the extract, 2 ml of chloroform was added: Concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoid.

Microorganisms used in this study: Bacteria causing infectious diseases both in animals and human were used in the present study. They were gram positive bacteria

Table 2. Antibacterial activities of seed extracts of *Gloriosa superba* by well diffusion method.

Bacterial Organisms	Amp (50 µl)	Zone of inhibition (mm)														
		Concentration														
		Hexane					Chloroform					Methanol				
		50 µl	100 µl	150 µl	200 µl	250 µl	50 µl	100 µl	150 µl	200 µl	250 µl	50 µl	100 µl	150 µl	200 µl	250 µl
B. cereus	28.6	-	-	11.5	14.3	17.9	11.1	15.6	21.4	26.5	30.2	17.3	22.6	28.6	31.1	36.4
B. subtilis	15.3	-	-	-	-	10.2	-	-	10.4	14.0	16.8	-	-	10.0	14.3	18.3
S. aureus	22.1	-	-	-	-	10.9	-	10.4	13.4	18.2	23.5	-	11.0	15.2	21.7	27.6
S. cremoris	19.4	-	-	-	-	12.1	-	-	-	12.4	17.0	-	-	12.2	17.1	23.3
S. fecalis	25.4	-	-	-	12.5	17.2	-	11.4	16.4	20.0	26.4	12.4	17.3	21.5	26.9	31.2
E. coli	25.1	-	-	12.6	16.5	21.2	-	13.5	17.6	22.0	27.6	13.0	19.5	26.4	29.4	32.4
K. pneumonia	20.4	-	-	10.1	12.5	18.6	-	-	14.5	21.6	26.3	12.4	18.2	23.4	26.1	30.8
P. aeruginosa	18.3	-	-	-	10.9	13.7	-	-	14.6	19.8	22.7	-	14.3	17.5	22.3	24.4
P. vulgaris	15.7	-	-	-	-	10.2	-	-	11.0	14.2	17.5	-	11.8	14.6	19.5	23.9
S. typhi	13.2	-	-	-	-	-	-	-	-	12.1	16.7	-	-	10.1	13.2	18.4

(zone of inhibition = values are expressed in millimetre (mm), - = Negative results)

viz. *Bacillus cereus, Bacillus subtilis, Streptococcus cremoris, Streptococcus fecalis, Staphylococcus aureus* and gram negative bacteria viz., *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia* and *Proteus vulgaris*. All the bacterial strains were obtained from the Government General Hospital, Dharmapuri. The cultures were maintained in nutrient broth in the laboratory of PG and Research department of Botany, Government Arts College, Dharmapuri, Tamil nadu, India.

Antibacterial screening: The agar well diffusion method[26, 27] was employed for the determination of antimicrobial activity of the extracts. The petriplates containing 20 ml of Muller Hinton Agar medium (Himedia) were seeded with 24 h culture of the microorganisms. Sterilized cotton swabs were dipped in the bacterial culture in nutrient broth and then swabbed on the agar plates. Wells of equal size were cut with proper gaps in the medium and the plant extracts were added into it. The wells (6 mm in diameter) were cut from the agar and the extract solutions in different concentration (50 µl, 100 µl, 150 µl, 200 µl and 250 µl) were delivered into them. The control well of Ampicillin was used at 50 µl concentration. The plates were incubated at 37°C for 24 h. Clear inhibition zones around the wells indicated the presence of antibacterial activity. After incubation time, the zone of inhibition was measured precisely in millimeters (mm). The same procedure was followed for standard antibiotics Ampicillin (50 µl) to compare the efficacy of extracts against test organisms. Each experiment was repeated three times, and the average values were calculated.

RESULTS

In the present study, to evaluate the preliminary phytochemical screening of hexane, chloroform and methanol extracts of *Gloriosa superba* seeds and tubers samples are presented in Table 1. All the samples of seeds and tubers showed the abundant occurrence of phytochemicals in varying concentrations. The hexane extract showed the maximum presence of tannin in seeds and tubers (88.22 %, 93.15 %) and terpenoids (80.10 %, 83.57 %) respectively. In chloroform extract showed the maximum presence of steroids in seeds and tubers (90.65 %, 95.34 %) respectively. In methanol extract showed the maximum yield of alkaloids in seeds and tubers (80.22 %, 94.26 %) and glycosides (67.32 %, 85.21 %) respectively. The seeds and tubers samples showed the high content of glycosides and alkaloids followed steroids, tannins and low content of terpenoids. However, the tuber extracts yields more bioactive compounds than seed extracts in all three solvents such as hexane, chloroform and methanol.

The antibacterial activity results of seeds and tubers extracts of *G. superba* showed excellent effect against the five gram positive and gram negative bacteria. The result of seed extracts are presented in Table 2. The maximum zone of inhibition was observed on *B. cereus* (36.4 mm) in methanol 250 µl concentration followed by *E. coli* (32.4 mm), *S. fecalis* (31.2 mm), *K. pneumonia* (30.8 mm), *S. aureus* (27.6 mm), *P. aeruginosa* (24.4 mm), *P. vulgaris* (23.9 mm), *S. cremoris* (23.3 mm), *S. typhi* (18.4 mm) and minimum zone of inhibition observed on *B. subtilis* (18.3 mm). The result of tuber extracts presented in Table 3. The maximum zone of inhibition was

Table 3. Antibacterial activities of tuber extracts of *Gloriosa superba* by well diffusion method.

Bacterial Organisms	Amp (50 µl)	Zone of inhibition (mm)														
		Concentration														
		Hexane extract					Chloroform extract					Methanol extract				
		50 µl	100 µl	150 µl	200 µl	250 µl	50 µl	100 µl	150 µl	200 µl	250 µl	50 µl	100 µl	150 µl	200 µl	250 µl
B. cereus	28.6	10.4	11.7	15.7	19.3	23.1	15.2	20.8	25.4	31.2	35.6	22.1	28.4	32.3	38.5	42.3
B. subtilis	15.3	-	-	-	-	11.0	6.5	8.4	11.2	14.2	18.3	11.5	14.7	17.1	20.1	23.5
S. aureus	22.1	-	-	11.2	14.5	17.6	11.4	14.3	17.8	22.5	26.7	20.7	23.4	26.7	30.7	33.3
S. cremoris	19.4	-	-	10.1	12.6	14.5	8.7	12.1	16.2	20.1	22.8	16.6	19.4	22.2	25.6	28.1
S. fecalis	25.4	-	10.4	13.2	17.5	20.1	14.0	19.4	24.1	28.1	32.1	21.2	26.9	30.1	33.5	37.2
E. coli	25.1	-	12.4	17.6	21.4	25.1	13.7	18.0	22.4	27.8	31.5	18.1	24.1	30.0	34.3	38.4
K. pneumonia	20.4	-	10.7	13.3	17.5	21.2	11.9	13.4	20.1	26.4	30.4	16.4	23.2	27.3	31.2	35.6
P. aeruginosa	18.3	-	-	10.4	12.5	16.4	-	11.2	19.7	23.4	27.8	13.8	18.6	22.1	26.3	30.5
P. vulgaris	15.7	-	-	-	10.0	12.8	-	10.6	14.4	19.5	23.1	11.6	15.0	18.3	22.7	26.9
S. typhi	13.2	-	-	-	-	10.4	-	-	12.4	15.0	18.2	-	10.2	13.5	17.6	21.7

(Zone of inhibition = values are expressed in millimetre (mm), - = Negative results)

observed on *B. cereus* (42.3 mm) in methanol 250 µl concentration followed by *E. coli* (38.4 mm), *S. fecalis* (37.2 mm), *K. pneumonia* (35.6 mm), *S. aureus* (33.3 mm), *P. aeruginosa* (30.5 mm), *S. cremoris* (28.1 mm), *P. vulgaris* (26.9 mm), *B. subtilis* (23.5 mm) and minimum zone of inhibition observed on *S. typhi* (21.7 mm). The positive control, ampicillin (50 µl) had shown zone of inhibition are presented in Table 2 and 3. The maximum zone of inhibition was observed on *B. cereus* (28.6 mm) in methanol 250 µl concentration followed by *S. fecalis* (25.4 mm), *E. coli* (25.1 mm), *S. aureus* (22.1 mm), *K. pneumonia* (20.4 mm), *S. cremoris* (19.4 mm), *P. aeruginosa* (18.3 mm), *P. vulgaris* (15.7 mm), *B. subtilis* (15.3 mm) and minimum zone of inhibition observed on *S. typhi* (13.2 mm).

The *in vitro* antibacterial activity revealed that the methanol extract had significant activity against all the microorganisms tested, mainly *B. cereus*, *E. coli*, *S. fecalis*, *K. pneumonia*, *S. aureus*, and *P. aeruginosa* (zone of inhibition >30 mm) but inactive in lower concentration (50 µl) on *S. typhi*. The chloroform extracts possessed moderate activity against all microorganisms tested, *B. cereus*, *S. fecalis*, *E. coli*, *K. pneumonia S. aureus* and *P. vulgaris* (zone of inhibition >20 mm) but was inactive against *P. aeruginosa P. vulgaris* and *S. typhi*. The hexane extract exhibited only weak activity against *S. typhi*, *P. vulgaris*, *B. subtilis*, *S. aureus*, *S. cremoris* and *P. aeruginosa*. The moderate inhibition activity was observed in antibiotic ampicillin against *B. cereus*, *S. fecalis*, *S. aureus*, *E. coli* and *K. pneumonia* these results showed the similar effect in 100 µl of methanol extract and 200 µl of chloroform extract.

DISCUSSION

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[28]. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. They have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives[29]. 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[30, 31].

In the present study, methanol extracts of seeds and tubers samples showed the maximum yield of phytochemicals. However, the methanol extract of *G. superba* showed maximum growth inhibition on gram positive and gram negative bacteria. The plants have been

producing large number of organic compounds as secondary metabolites. These compounds acts as chemotherapeutic, bactericidal and bacteriostatics[32]. The tuber extracts showed more effect than seeds extract against all bacteria. It may be due to the reason that the tubers have strongly contact with soil and microorganisms[33]. Similar result was reported by[34] that on screening petroleum ether, chloroform and methanol extracts of *M. malabaricum* leaves, antimicrobial activity was shown only by methanolic extract. The present work confirms the antibacterial activity of methanolic extract of seeds and tubers of *G. superba.* There are records that showed 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[35].

Alkaloids have been detected in the extracts are compounds that have been documented to possess medicinal properties and health promoting effects[36, 37]. 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 non-volatile and lack fragrance and serve as defence mechanisms against predation by many microorganisms, insects and herbivores[38] (De *et al*., 1999). These compounds served as natural antibiotics, which help the body to fight infections and microbial invasion[39]. 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[40] (Ogunleye and Ibitoye, 2003). Considering the previous reports and current results, it is clear that the plant possesses antimicrobial property of the hexane, chloroform and methanol extracts of the seeds and tubers of *G. superba*.

CONCLUSION

The results obtained in the present study are clear cut idea about the traditional uses of the plants. Plant produced antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. The plant parts of seeds and tubers of *G. superba* have shown significant activity against the tested pathogens and these can be selected for the further studies and for the determination of minimum inhibitory concentration. Further research is necessary to determine the identity of the antibacterial compounds within these plants parts and also to determine their full spectrum of efficacy.

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