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Research Article

ANTIBACTERIAL POTENTIAL OF GLORY LILY, *GLORIOSA SUPERBA* LINN.

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ABSTRACT
The successive Soxhlet extract of *Gloriosa superba,* L. (Liliaceae) was extracted using acetone, dichloromethane, chloroform and methanol in ascending order of the polarity. The extracts were investigated for their antibacterial activity against two Gram positive bacteria *Streptococcus faecalis* and *Enterococcus faecalis* and two Gram negative bacteria *Klebsiella pneumoniae* and *Proteus mirabilis* by using disc diffusion method. Among the four extracts tested, acetone extract had effective antibacterial potential, followed by methanol extract at 25 and 100% concentration against *Enterococcus faecalis*. The acetone extract showed greater activity against Gram-positive than against Gram-negative organisms. The study confirms the antibacterial potential of *Gloriosa superba* leaves extracted using various solvents, and is therefore, a potential drug that requires further studies and development.
KEYWORDS: *Gloriosa superba*, Phytochemicals, Antibacterial activity.

INTRODUCTION

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 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 are a natural choice. The medicinal value of these plants lie in some chemical substances that produce a definite physiological action on the human body[1].

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases[2]. Bacteria for example have shown a remarkable ability to endure and adapt to their environment including the development of different mechanisms of resistance to most old and new antimicrobial agents[3]. Bacterial adaptation to antibiotics has been very successful, and over the years, the increase in antibiotic resistance has generated a considerable worldwide public health problem[4].

Gloriosa superba (Family: Liliaceae) is a glabrous herbaceous climber, which yields different types of troplone alkaloids of medicinal importance. The genus has importance in the ornamental horticulture due to its bright flowers and wiry climbing stem[5]. Glory lily is highly valued in both traditional and modern therapies. Its seeds and tubers (active content Colchicine) are used mainly for treating gout and rheumatism. G. superba is a good abortifacient[6] causing expulsion of foetus from the womb. Roots are purgative, cholagogue, anthelmintic, bitter, acrid, astringent and germicidal. It cures leprosy, swelling, piles, chronic ulcers and colic pain in bladder. Paste is an antidote for snake bite[7]. The alkaloids from the plant (Colchicines and Gloriosine) are used in the treatment of gout and rheumatism[8]. The antipyretic properties of the roots and rhizomes have been mentioned in ancient classics “Charaka”. A paste of the root is applied in bites of poisonous insects, scorpion sting and in parasitic skin diseases.

The objective of the present study was to evaluate the phytochemical constituents and antibacterial activity of *G. superba* to scientifically justify the traditional claims.

MATERIALS AND METHODS

Plant collection and processing

The fresh leaves and tubers of *Gloriosa superba* were collected in the fields of Attur village, Salem, Tamilnadu, India. The plant was identified by Dr. V. Balasubramanian, Associate Professor, PG and Research Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India. A voucher specimen (97/December/2008) was submitted in the Herbarium of Botany Department, Kongunadu Arts and Science College, Coimbatore.

Chemicals, solvents and medium used

Organic solvents used in the present study for the extraction were acetone, dichloromethane, chloroform and methanol. Solvents were purchased from Precision Scientific Company, Coimbatore, Tamilnadu, India. Nutrient agar medium and the nutrient broth used for

culturing the bacteria and for assessing the antibacterial activity were prepared in the laboratory.

Microorganisms used in the present study

Bacteria causing infectious diseases both in animals and human were used in the present study. They were both Gram positive and Gram negative. Two Gram positive bacteria namely *Streptococcus faecalis* and *Enterococcus faecalis* and two Gram negative bacteria such as *Klebsiella pneumoniae* and *Proteus mirabilis* were used in the present study. All the bacterial strains were obtained from the laboratory of Mycology Lab, University of Madras, Chennai, Tamilnadu, India. The cultures were maintained in nutrient broth in the laboratory of Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India.

Extraction procedure

In the present study, the fresh leaves and tubers were used to evaluate their antibacterial activity. The fresh leaves and tubers were collected, washed thoroughly in tap water and dried in room temperature for 10 days. The fully dried plant parts were powdered and weighed. The powdered leaves and tubers (60 gm) were extracted with different solvents in the order of increasing polarity using soxhlet apparatus. The extracts were concentrated to dryness to yield crude residues and were used for further investigation.

Antibacterial assay

The screening of the extracts for antibacterial effect was carried out by determining the zone of inhibition using disc diffusion method [9]. Sterile nutrient agar plates were prepared and inoculated by spread plate method under aseptic conditions. The filter paper discs of 6mm diameter (Whatman's No. 1 filter paper) were prepared and sterilized. The plant extracts to be tested were prepared with various concentrations at 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml and were added to each disc of holding capacity of 10 microlitres. The sterile impregnated discs with plant extracts were placed on the agar surface with framed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. Control discs of Ampicillin were prepared and placed on the agar surface. All the plates were incubated at 37°C for 24 hours. After incubation, the size (diameter) of the inhibition zones was measured.

Statistical analysis

The resultant clear zones around the discs were measured in mm. Data of three independent experiments represented by three replicates from each experiment were subjected to statistical analysis (Mean±SE), according to Duncan's Multiple Range Test (SPSS version 2007 WINSAT software).

RESULTS

Antibacterial activity of different solvent extracts

The results showed that all the leaf and tuber extracts of *G. superba* showed appreciable antibacterial effect against the tested Gram positive and Gram negative bacteria. The results presented in the Tables 1 – 4, showed that the acetone and methanol extracts exhibited pronounced inhibition against all the tested organisms. The maximum inhibition was observed on *Enterococcus faecalis* in acetone leaf extract (31±3.21) and methanol tuber extract (30.33±2.40).

The moderate inhibition was observed on *Proteus mirabilis* inhibited by acetone tuber extract (23.33±6.49) followed by *Klebsiella pneumoniae* in acetone tuber extract (23±3.06) and methanol leaf extract (22.67±2.40). All the extracts showed less inhibitory activity against *Streptococcus faecalis*.

The positive control, Ampicillin had shown zone of inhibition of 24.67±2.02 mm, 20.67±4.91 mm, 34.67±1.45 mm and 26±4.16 mm in *Enterococcus faecalis*, *Streptococcus faecalis*, *Klebsiella pneumoniae* and *Proteus mirabilis* respectively.

DISCUSSION

From the study it was observed that both Gram positive and Gram negative bacteria growth was inhibited by the extracts. It may be due to the reason that the tubers have constant contact with soil[10]. The plants are producing large number of organic compounds as secondary metabolites. These compounds act as chemotherapeutic, bactericidal and bacteriostatics[11,12].

Compared to the previous study, the present result coincides with the work of[13] who reported that the ionic fraction of *Curcuma longa* showed promising activity against the gram positive bacteria *Enterococcus faecalis* (3.3 mg/mL^{-1}). In the present study, 25% and 100% acetone and methanol extracts of leaves and tubers showed greater inhibitory effect (31±3.21, 30.33±2.40) on the growth of *Enterococcus faecalis*.

In the present study, 50% and 25% acetone and dichloromethane extracts of tubers showed promising results against *Proteus mirabilis* i.e. (23.33±6.49 and 23±3.21mm minimum zone of inhibition). This finding lends support to that of[14] who demonstrated that acetone and methanol extracts of *Halodule pinifolia* showed maximum activity against the test pathogen.

The 50% methanol extracts of leaves exhibited more antibacterial activity against gram negative bacteria, *Klebsiella pneumoniae* (23±3.06) followed by the 100% acetone extracts of tubers (22.67±2.40). Similarly[15] also reported that methanol extracts of *Gloriosa superba* showed high antibacterial activity against Klebsiella pneumoniae.

CONCLUSION

From the study it can be concluded that the extracts prepared from the leaves and tubers of G. superba are a source of different secondary metabolites which may act in synergy to produce an increased activity against microbes. The results may justify the use of plant in the treatment of certain skin diseases and infected wounds[16,17]. It is also necessary, to study further in order to isolate and purify the antibacterial compounds responsible for controlling the bacterial pathogens tested in the present investigation.

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Table 1. Antibacterial activity of the plant *Gloriosa superba* against *Enterococcus faecalis*

Plant	Plant part	Solvents used	Zone of Inhibition (mm) /Concentration of the extract (%)				
			C	25	50	75	100
Gloriosa superba	Leaves	Acetone	23.33±2.91 c	31±3.21 a	28±3.78 a	26.33±4.63 a	25.33±2.40 a
		Chloroform	30±4.72 a	19±2.08 c	20.33±3.75 c	15.33±2.60 c	22±1.73 b
		Dichloromethane	14.67±2.40 d	13.67±3.28 d	14.67±3.52 d	15.33±2.73 d	20.33±2.96 d
		Methanol	24.67±2.02 b	25.33±1.67 b	26±3.21 b	22.33±3.75 b	21±2.31 c
	Tubers	Acetone	17.67±5.36 c	18.33±4.06 d	18.67±5.93 c	22.33±4.81 a	20±4.36 b
		Chloroform	30±1.73 b	25.67±2.33 a	25±2.08 a	17±3.21 d	19.67±1.45 c
		Dichloromethane	15±0.58 d	19±1.73 c	16±1.73 d	19±2.08 c	16.33±1.45 d
		Methanol	31±1.53 a	25.33±1.76 b	24.33±3.28 b	22±0.56 b	30.33±2.40 a

Values are expressed as Mean ± Standard Error of 3 replicates. Means within a column followed by common letter are not significantly different at 5% level by DMRT.

Table 2: Antibacterial activity of the plant *Gloriosa superba* against *Streptococcus faecalis*

Plant	Plant part	Solvents used	Zone of Inhibition (mm) /Concentration of the extract (%)				
			C	25	50	75	100
Gloriosa superba	Leaves	Acetone	14.67±6.06 b	14.67±3.18 a	12±4.58 c	10.67±2.33 a	11.33±1.33 a
		Chloroform	15±5.29 a	13±3.18 c	15±2.91 a	7±1.76 d	6.33±3.61 d
		Dichloromethane	13.67±5.70 c	13±3.79 b	12±3.51 b	7.3±0.88 c	10±2.65 c
		Methanol	10.67±2.91 d	11.3±1.76 d	10.3±1.67 d	9.3±0.88 b	11±1.15 b
	Tubers	Acetone	21±2.52 a	11.33±4.33 b	18±6.69 a	16.67±6.69 a	13±4.93 c
		Chloroform	16±1.53 d	8.67±2.03 c	11±1.20 d	9.67±1.20 d	14±1.15 a
		Dichloromethane	17.33±1.76 c	16.67±2.91 a	11.67±0.67 c	14.33±3.18 b	13.33±3.18 b
		Methanol	20.67±4.91 b	7.33±2.40 d	15.67±3.84 b	12±3.21 c	11.33±0.88 d

Values are expressed as Mean ± Standard Error of 3 replicates. Means within a column followed by common letter are not significantly different at 5% level by DMRT.

Table 3: Antibacterial activity of the plant *Gloriosa superba* against Klebsiella pneumoniae

Plant	Plant part	Solvents used	Zone of Inhibition (mm) /Concentration of the extract (%)				
			C	25	50	75	100
Gloriosa superba	Leaves	Acetone	15.33±3.76 d	15.67±2.60 a	13.33±0.67 c	8.67±1.45 d	13±3.06 c
		Chloroform	16.67±4.48 b	11.33±3.18 d	11.66±3.18 d	14.33±4.41 a	16±4.73 d
		Dichloromethane	18±6.66 a	12.33±5.36 c	15±6.56 b	12.67±5.67 b	16.67±5.36 a
		Methanol	16.33±6.17 c	13.33±2.79 b	23±3.06 a	11±1.15 c	11±2.52 d
	Tubers	Acetone	34.67±1.45 a	22±2.65 a	20±2.65 a	22.33±2.03 a	22.67±2.40 a
		Chloroform	20±5.30 d	12±0.00 c	16.67±1.76 c	14.67±2.03 d	12.67±1.33 d
		Dichloromethane	22±4.04 b	14±1.15 b	18±3.46 b	18±1.00 b	13.67±5.49 c
		Methanol	20.33±5.61 c	9±4.58 d	9.67±1.45 d	14.67±5.81 c	14±2.0 b

Values are expressed as Mean ± Standard Error of 3 replicates. Means within a column followed by common letter are not significantly different at 5% level by DMRT.

Table 4: Antibacterial activity of the plant *Gloriosa superba* against *Proteus mirabilis*

Plant	Plant part	Solvents used	Zone of Inhibition (mm) /Concentration of the extract (%)				
			C	25	50	75	100
Gloriosa superba	Leaves	Acetone	25.33±8.37 a	13.33±1.76 c	13.33±3.48 d	9±0.00 d	12.67±2.33 a
		Chloroform	22.33±9.60 c	13.33±2.19 b	15.33±2.60 a	13±3.46 c	12.33±3.18 b
		Dichloromethane	22.67±9.91 b	17±5.86 a	13.67±5.17 b	15±4.04 b	12.33±4.84 c
		Methanol	21±9.45 d	12.33±4.10 d	13.33±2.91 c	15±3.79 a	11.67±3.18 d
	Tubers	Acetone	24.67±2.60 b	17±2.88 b	23.33±6.49 a	20.33±4.63 b	18.67±5.78 b
		Chloroform	17.33±1.76 c	12.33±0.88 d	15±2.31 d	20.67 ±2.19 a	16.67±4.67 d
		Dichloromethane	11.33±1.20 d	23±3.21 a	21.33±3.28 c	14.33±2.60 d	12.33±2.03 c
		Methanol	26±4.16 a	13.67±1.33 c	22±6.24 b	18±0.58 c	22±4.62 a

Values are expressed as Mean ± Standard Error of 3 replicates. Means within a column followed by common letter are not significantly different at 5% level by DMRT.

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