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IN VITRO ANTIBACTERIAL ACTIVITY STUDIES OF TUBER AND SEED EXTRACTS OF *GLORIOSA SUPERBA* LINN. AGAINST SOME SELECTED HUMAN PATHOGEN

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ABSTRACT

The present study deals with the antibacterial activity of Acetone, Dichloromethane, methanol extracts of the tuber and seed of *Gloriosa superba* L. (Liliaceae) using disc diffusion method against human pathogens, such as *Escherichia coli*, *Klebsiella pneumoniae, Proteus mirabilis*, *Pseudomonas aeurognisoa*, *Staphylocuccus aureus*. In the present investigation all the extracts were found to be effective against five human bacterial species. The Dichloromethane extract showed greater activity against *Proteus mirabilis*.

INTRODUCTION: India is endowed with a rich wealth of medicinal plants which have been a valuable source of natural products for maintaining human health. A large number of these medicinal plants are used in several formulation for the treatment of various disease caused by microbes.

According to world health organization, medicinal plants would be the source of obtaining a variety of drugs. Various societies across the world have shown great interest in curing diseases using plants or plant based drugs. The spread of drug resistant pathogen is one of the most serious threats to successful treatment of microbial diseases.

Bacteria for example have shown a remarkable ability to endure and adapt to their environment including the development of different mechanisms of resistance to antimicrobial agents. 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 (De Esparza *et al.,* 2007).

Gloriosa superba L (Family: Liliaceae) is commonly known a glory lily or climbing lily. This herb is a native of tropical Asia and Africa and found growing throughout tropical India upto an altitude of 2500 m (Chopra *et al.,* 1956). Glory lily is a large glabrous, herbaceous branching climber with narrow leaves ending in spirally twisted climbing leaf tip tendrils. It arises from the perennial, fleshy tuberous rhizome. Glory lily is highly valued both traditional and modern therapies. The tuber and seed is used as a germicide, to cure ulcers, piles, hemorrhoids, inflammation, leprosy, worms, intermittent fevers, cancer and snake bites. The alkaloid from the plant colchicines and Gloriosine is due to mainly present in seed and tuber. They are used in the treatment of Gout and Rheumantism (Nadkarni *et al.,* 1996).

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The present study was to evaluate the antibacterial activity of *Gloriosa superba* to scientifically justify the traditional claims.

MATERIALS AND METHODS:

Collection of plant: The fresh tuber and seed of *Gloriosa superba* were collected in the fields of Jayankondam area, Ariyalur District, Tamil Nadu. The tubers were cleaned of adhering soil/dust in the field by shaking and quick rinsing with tap water. Any remaining particles of soil were removed by use of pressurized air flow and by the use of paint brush and in some cases, by quick rinsing with distilled water. Tuber and seed were placed in paper bag and transferred to the laboratory.

Test organisms and Culture Media: The clinical pathogenic bacterial strains were aseptically collected from Rajah Muthiah Medical College and Hospital, Annamalai University, Chidambaram, Tamil Nadu, India. The bacterial cultures are *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus* were maintained in nutrient broth in the laboratory of Department of Microbiology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu, India.

Extraction of Plant Material: In the present study, the fresh tuber and seed were used to evaluate their antibacterial activity. Plant extract were prepared by cold percolation method. The tuber and seed air dried in room temperature for 10 days. The fully dried plant materials were powdered and weighed. The powdered tubers and seed (5 gm) each was soaked in 50ml of different solvents such as Acetone, Dichloromethane, Methanol and kept for 48 hours with intermittent shaking. The plant extract were filtered through Whatman No. 1 filter paper. The filtrated were collected in separate beaker.

Antibacterial Assay: The screening of the extracts for antibacterial effect was carried out by determining the zone of inhibition using disc diffusion method. Sterile nutrient agar plates were prepared. Then 0.1 ml of test organism was taken from the stock (broth) and swabbed on the agar medium in aspetic condition. The filter paper disc of 2 mm diameter (Whatman No.1 Filter paper) were prepared and sterilized. The plant extracts to be tested were prepared with various concentrations at 50 µ/ml, 100µl/ml, 150 µl/ml and 200µl/ml and were added to each disc of holding capacity of 10 microlitres. The sterile impregnated disc 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. Positive control disc of streptomycin 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.

RESULTS AND DISCUSSION: The results showed that all tuber and seed extracts of *Gloriosa superba* showed appreciable antibacterial effect against the tested *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. The result presented in the **tables 1-5, fig. 1-5** showed that the Dichloromethane extract exhibited pronounced inhibition against all the tested organisms. The maximum inhibition was observed on *Proteus mirabilis* in Dichloromethane tuber extract 16 mm and *Escherichia coli* in methanol tuber extract 15 mm and *Staphylococcus aureus* in methanol tuber extract 15 mm.

TABLE 1: ANTIBACTERIAL ACTIVITY OF THE PLANT *GLORIOSA SUPERBA* AGAINST *ESCHERICHIA COLI*

Plant	Plant part	Solvents used	Zone of inhibition (mm)				
			C	50µl	100 µl	150 µl	200 µl
Gloriosa superba	Tubers	Acetone	11	10	11	12	13
		Dichloromethane	10	11	13	10	11
		Methanol	10	15	12	13	12
	Seed	Acetone	11	11	12	13	12
		Dichloromethane	10	10	13	12	11
		Methanol	11	13	14	11	14

TABLE 2: ANTIBACTERIAL ACTIVITY OF THE PLANT *GLORIOSA SUPERBA* AGAINST *KLEBSIELLA PNEUMONIAE*

Plant	Plant part	Solvents used	Zone of inhibition (mm)				
			C	50µl	100 µl	150 µl	200 µl
Gloriosa superba	Tubers	Acetone	13	10	12	10	13
		Dichloromethane	12	10	10	12	11
		Methanol	10	11	14	13	11
	Seed	Acetone	11	11	13	12	14
		Dichloromethane	12	12	11	11	13
		Methanol	11	12	13	12	13

TABLE 3: ANTIBACTERIAL ACTIVITY OF THE PLANT *GLORIOSA SUPERBA* AGAINST *PROTEUS MIRABILIS*

Plant	Plant part	Solvents used	Zone of inhibition (mm)				
			C	50µl	100 µl	150 µl	200 µl
Gloriosa superba	Tubers	Acetone	11	10	13	11	12
		Dichloromethane	15	12	14	13	16
		Methanol	13	11	12	10	12
	Seed	Acetone	13	11	12	11	13
		Dichloromethane	12	10	11	10	12
		Methanol	13	10	12	12	11

TABLE 4: ANTIBACTERIAL ACTIVITY OF THE PLANT *GLORIOSA SUPERBA* AGAINST *PSEUDOMONAS AEUROGINOSA*

Plant	Plant part	Solvents used	Zone of inhibition (mm)				
			C	50µl	100 µl	150 µl	200 µl
Gloriosa superba	Tubers	Acetone	11	12	13	11	12
		Dichloromethane	14	11	10	12	11
		Methanol	11	12	11	13	12
	Seed	Acetone	12	10	13	11	13
		Dichloromethane	11	12	13	11	12
		Methanol	12	10	14	12	13

TABLE 5: ANTIBACTERIAL ACTIVITY OF THE PLANT *GLORIOSA SUPERBA* AGAINST *STAPHYLOCOCCUS AUREUS*

Plant	Plant part	Solvents used	Zone of inhibition (mm)				
			C	50µl	100 µl	150 µl	200 µl
Gloriosa superba	Tubers	Acetone	12	10	12	11	12
		Dichloromethane	13	12	11	12	13
		Methanol	10	11	12	14	15
	Seed	Acetone	11	10	13	12	14
		Dichloromethane	10	10	12	13	12
		Methanol	10	12	13	12	11

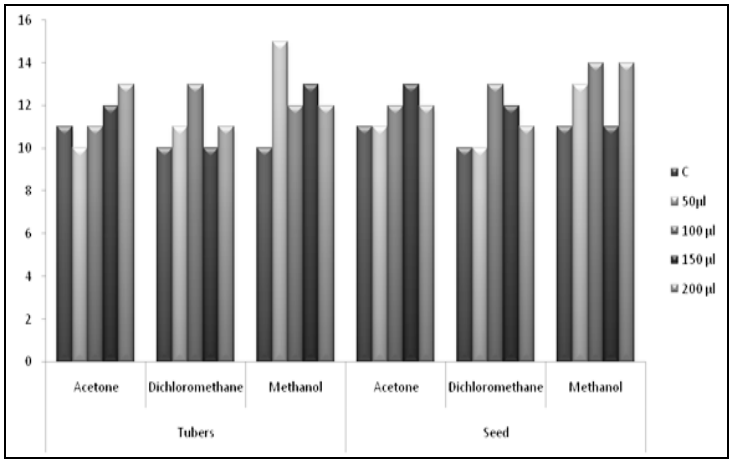

FIG. 1: ANTIBACTERIAL ACTIVITY OF THE PLANT *GLORIOSA SUPERBA* AGAINST *ESCHERICHIA COLI*

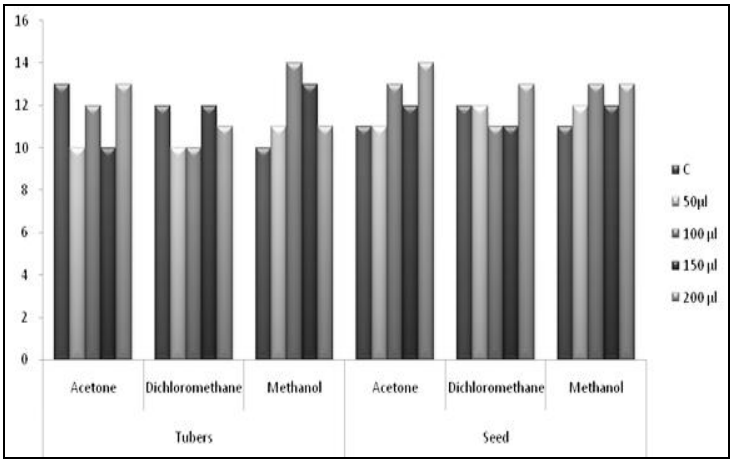

FIG. 2: ANTIBACTERIAL ACTIVITY OF THE PLANT *GLORIOSA SUPERBA* AGAINST *KLEBSIELLA PNEUMONIA*

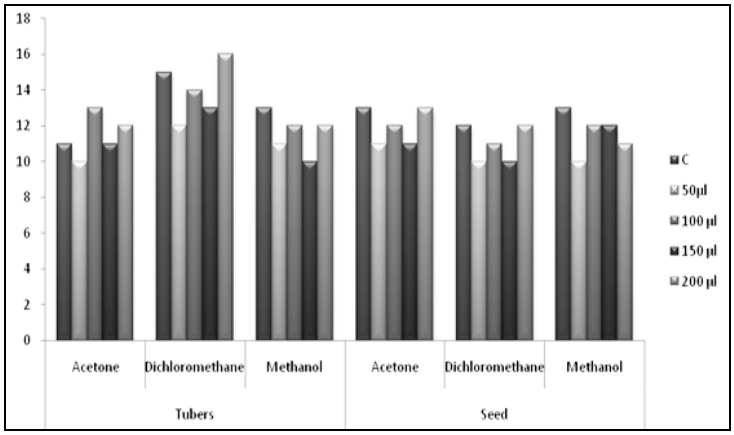

FIG. 3: ANTIBACTERIAL ACTIVITY OF THE PLANT *GLORIOSA SUPERBA* AGAINST *PROTEUS MIRABILIS*

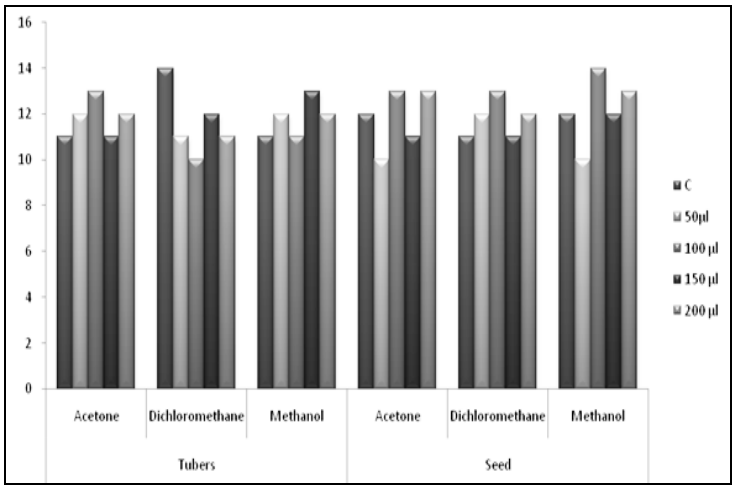

FIG. 4: ANTIBACTERIAL ACTIVITY OF THE PLANT *GLORIOSA SUPERBA* AGAINST *PSEUDOMONAS AEUROGINOSA*

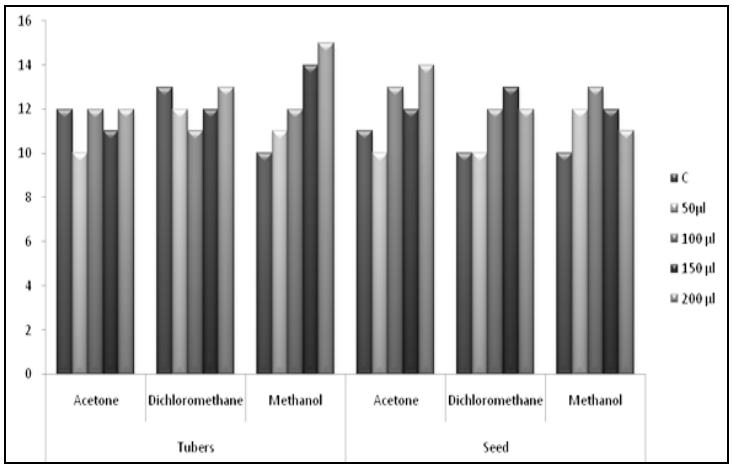

FIG. 5: ANTIBACTERIAL ACTIVITY OF THE PLANT *GLORIOSA SUPERBA* AGAINST *STAPHYLOCOCCUS AUREUS*

The moderate inhibition was observed on *Klebsiella pneumoniae* inhibited by methanol tuber extract 14 mm and acetone seed extract 14 mm. Followed by *Pseudomonas aeuroginosa* methanol seed extract 14 mm.

The positive control, Streptomycine had shown zone of inhibition of 11mm, 13 mm, 15mm, 14mm and 13mm in *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively.

From the study it was observed that all bacteria growth was inhibited by the extracts. It may be due to the reason that the tubers have constant contract with soil. The plants are producing large number of organic compounds as secondary metabolites. These compounds act as chemotherapeutic, bactericidal and bacteriostatics.

In the present study, 100µl methanol extracts of seed showed promising result against *Escherichia coli* (14mm), *Klebsiella pneumonia* (13mm), *Pseudomonas aeruginosa* (14mm), and *Staphylococcus aureus* (13mm).

The acetone extract of seed exhibited more antibacterial activity against 200µl in *Klebsiella pneumoniae* (14mm) and *Staphylococcus aureus* (14mm) followed by 100µl of acetone extracts of seed against *Klebsiella pneumoniae* (13mm), *Pseudomonas aeuroginosa* (13 mm) and *Staphylococcus aureus* (13 mm).

Similarly also reported that methanol extracts of *Gloriosa superba* showed high antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae*.

CONCLUSION: In conclusion, the tuber and seed of *Gloriosa superba* extracts could be potential source of inhibitory substance for certain clinical pathogen. The result may justify the use of plant in the treatment of some diseases as broad spectrum antibacterial agent. This probably explains the use of these plants by the indigenous people against a number of infections since generation.

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