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Antimicrobial Activity of Endangered Medicinal Plant *Gloriosa Superba* L.

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Abstract: ***Indian subcontinent is praised with most varied and diverse soil and climate conditions suitable for the growth of various plant species and endowed with rich wealth of medicinal plants.Gloriosa superba L. has been a source of medicine right from ancient time. Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Screening of antimicrobial activity of Gloriosa superba L. in the leaves was studied. The leaves of the selected medicinal plants were washed, air dried and then powdered. The main objective was to check the presence or absence of the antimicrobial activity of leaves of the selected medicinal plant. In the present study, preliminary antimicrobial activity of Gloriosa superba L.(leaves, stem and flower) exhibited high degrees of antibacterial activity against tested pathogenic microorganisms Bacillus sps., Escherichia coli and Pseudomonas aeruginosa, when studied by the disc diffusion method. The acetone extract of Gloriosa superba L. was found to be effective against all tested microorganisms with the inhibition zone ranging with an average of 1.0 – 2.9 mm. When the result was compared with standard antibiotic, streptomycin, a moderate antibacterial efficiency was observed in the ethanol and acetone extracts of Gloriosa superba L***

Keywords: Endangered medicinal plant, *Gloriosa superba* L., antimicrobial activity, pathogenic microorganisms and disc diffusion method

1. Introduction

Plants have been a valuable source of natural products for maintaining human health especially, for the studies of natural therapies. Since ancient times, people have been exploring the nature for plants in the search of new drugs. Nature has been of material agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Cragg and Newman, 2001 and Alam *et al.*, 2009). This has resulted in the use of a large number of medicinal plants with curative properties that can treat various diseases and it is used in ayurvedic, allopathic and homeopathic treatment. According to the World Health Organization, more than 80% of the world population still relies on herbal medicines as their primary source of heath care. The popularity of using plants for therapeutic purposes has been intensified especially at the onset where traditional health care using traditional medicine is being promoted. Medicinal plants are being used by traditional healers either singly or in combination in the treatment of different types of diseases.Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al.*, 1996, Bibitha *et al.*, 2002 and Mahesh and Sathish, 2008). A wide range of medicinal plant parts is used for extract as raw drugs which possess varied medicinal properties and is reported to have minimal side effects (Maghrani *et al.*, 2005). *Gloriosa superba* L.(Glory lily) is widely distributed in tropical and sub - tropical parts of India including foothills of Himalayas (Kapoor, 2001). It is a national flower of Zimbabwe and also is a state flower of Tamil Nadu state of India.It is known by different names in India, such as Kalihari, Agnishikha, Languliata and Nangulika. The plant thrives from arid Bundelkhand to the humid Assam valley, India. The generic name Gloriosa means “full of glory” and superbameans “superb”, alluding to the striking red and yellow flowers. Its extreme toxicity requires the most cautious of handling.

Gloriosa superb L.is an erect, herbaceous, climbing perennial growing between 3.5- 6 m in length, usually trained at 1.5 m above the ground level. It is adapted to different soil texture and climatic variation. It occurs in thickets, forest edges and boundaries of cultivated areas in warm countries upto height of 253 cm. (Neuwinger, 1994 and Ravindraade and Mahendra raj, 2009). Flowering is noticed from November to December (Swarnapriya *et al.*, 1995). It is one of the most important medicinal plans of Asia and Africa (Sivakumar *et al.*, 2000, Jana *et al.*, 2011). It has been a well known plant in Indian Ayurveda as well as in pharmacological industries (Asolkar *et al.*, 1992). Almost all parts of it find diverse medicinal usage (Kapoor, 2001). Its rhizomes used as a tonic, anti- periodic, anti- helminthic and also against snake bites and scorpion stings.

Gloriosa superb L. show many pharmacological properties like anti-inflammatory (Jomy*et al.*, 2009), Antimicrobial (Hemaiswarya, 2009), Antithrombotic/Anticoagulant potential (Kee*et al.*, 2008), Anticancer activity (Reuter, 2010), Snake bite potential (Haroon, 2008), Hapatoprotactive activity (Mohandass, 2011), Antioxidant activity (Amudha and Shanthi, 2011) and Anthelmintic Activity (Pawar, 2010) etc. 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 (Chitra and Rajamani, 2009).

Environment is the interaction between man and the nature. Human beings are surrounded by plants, animals, and physical objects which are parts of our environment. Many medicinal plants are used to manufacture new antibiotics in pharmaceutical industry. These are resistance to

microorganisms. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs which are utilized as therapeutic agents.(Nascimento *et al.*,2000).

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illness not only because many of them produce toxic reactions but also due to investigate newer drugs with lesser resistance.

The search for antimicrobials of plant origin has been mainly stimulated by the fact that some of the major antibacterial agents have considerable drawbacks in terms of limited antimicrobial spectrum. Now-a-days multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Lakshmi Naidu *et al*., 2006). Resistance in bacteria is most prevalent like methicillin resistant *Staphylococcus aureus* (MRSA) has become a huge problem worldwide to treat nosocomial infections since 1990s (Lee *et al.*, 2007).

The use of plant extract for medical treatments is enjoying great popularity since 1990s when people realized that the effective life span of antibiotic is limited and over prescription and misuse of traditional antibiotics are causing microbial resistance (Cohen, 1992, Eisenberg *et al.*, 1993 and Nascimento *et al.*, 2000).

The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds. Naturally occurring combinations of these compounds can be synergistic and often results in crude extracts having greater antimicrobial activity than the purified individual constituents (Delaquis, 2002 and Alam *et al.*, 2009).Clinical microbiologists have great interest in screening of medicinal plants for antimicrobial activities and phytochemicals as potential new therapeutics (Nascimento *et al.*, 2000). Hence the present investigation attempted the antimicrobial activity of *Gloriosa superba* L. whether it have high antimicrobial activity or not when compared to other medicinal plants.

2. Materials and Methods

Sample Collection and Identification

The study plant *Gloriosa superb* L. (leaf, stem, flower) was collected near Arasan Ganesan Polytechnique College, Sivakasi in Tamilnadu. The collected plant sample was identified in the Department of Botany, The Standard Fireworks Rajaratinam College for women, Sivakasi in Tamilnadu. The sample was stored in shadow places for further analysis.

Preparation of Extracts

The shadow dried *Gloriosa superb* L. samples (leaf, stem, flower) was powdered with the help of electronic blender. Twenty five gram of powdered plant material was taken in clean sterile Soxhlet apparatus and the extraction was done with 250ml of different solvents (low polar to high polar) like as hexane, butanol, ethanol, chloroform, water and acetone. Similarly, another 10 grams of *Gloriosa superb* L. powder was extracted with 100ml of acetone. After extraction the extracts were dried in room temperature until extract reach into solid form. From the solid extract suitable concentrations were made using Dimethyl sulfo-oxide (DMSO) for further analysis.

Anti Microbial Activity-Antibacterial assay

Bacterial strains

The bacterial strains were obtained from Joys Clinical laboratory in Nagercoil, Kanyakumari (District), Tamil Nadu. Three human bacterial pathogens were chosen for the present investigation. Purity of bacterial pathogens was screened using suitable biochemical tests before analysis. The pathogenic strains used in the present study were *Escherichia coli, Pseudomonas aeruginosa, Bacillus* sps. They were sub cultured in nutrient broth and incubated at 37ºC for further analysis.

Antibacterial screening

The antibacterial activity was screened using disc diffusion method.

Disc diffusion method

Filter paper disc diffusion technique in agar was followed to determine antimicrobial activity by the procedure of Garg and Jain, 1998. Whatmann no.1 filter paper discs of 6-mm diameter, placed in dry Petri plates, were autoclaved. The test extracts in measured quantities were dissolved in minimum amount of acetone. Sterile filter paper No.1 discs were loaded with the extracts of *Gloriosa superb* L.(acetone, ethanol and water)using different solvents. The amount of extracts loaded in each disc was in the concentration *viz*., 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml. Similarly discs were prepared for standard antibiotic streptomycin (w/v) and were impregnated in the filter paper discs in different concentrations (50µg/ml).

The pathogenic strains were suspended with nutrient broth (High media) by transferring a loop full of 24hrs growth from agar slopes. The suspensions were vortexes and 0.1ml aliquots were spread over respective agar medium plates. The extracts and streptomycin loaded discs were then placed over the plates seeded with respective microorganisms. The plates were incubating at 37º C for 24hrs. The antibacterial activity was determined by measuring the inhibition zone around the discs. The diameter of inhibition zones (including the diameter to the disc) was measured.

3. Result and Discussion

Antimicrobial Activity

Screening of anti bacterial activity

Antimicrobial screening of the leaf extracts of *Gloriosa superba* L. were analysed in disc diffusion method.

Disc diffusion method

All solvent extracts of *Gloriosa superb* L. used in the present study exhibited degrees of antibacterial activity against tested pathogenic microorganisms *Bacillus sp., Eschericia coli* and *Pseudomonas aeruginosa*, when studied by the disc diffusion method. The acetone extract of *Gloriosa superb* L. was found to be effective against all tested micro organisms with inhibition zone ranging with an average of 1.0 – 2.9 mm in extracts prepared by mortar and pestle extractions (Table 1.1 and Plate 2.1 - 2.3). When the result was compared with standard antibiotics streptomycin a moderate antibacterial efficiency was observed in the ethanol, acetone and water extracts of *Gloriosa superb* L.

Table 1.1: The Antibacterial Effect of *Gloriosa Superba* L. Using Acetone Extracts Against Human Pathogenic Bacterial Organisms in Disc Diffusion Method

Antimicrobial Activity of Acetone

Solvent	Conc. (µg/ml)	Diameter of inhibition (mm)								
		Bacillus sps.			*Eschericia coli.*			*Pseudomonas aeruginosa*		
		S	L	F	S	L	F	S	L	F
Acetone	25	0.8± 0.08	0.7 ± 0.06	0.8 ±0.08	0.5± 0.06	0.56 ± 0.12	0.5 ±0.08	0.6± 0.08	0.6 ± 0.22	0.6 ±0.08
	50	1.7± 0.08	1.73± 0.05	1.63± 0.05	1.16± 0.05	1.26± 0.05	1.2± 0.08	1.2± 0.08	1.2± 0.08	1.2± 0.08
	75	1.8± 0.08	1.8± 0.08	1.73± 0.12	1.46± 0.05	1.43± 0.65	1.43± 0.12	1.5± 0.16	1.5± 0.08	1.43± 0.12
	100	2.3± 0.16	2.36± 0.21	2.4± 0.08	1.9± 0.08	1.86± 0.12	1.9± 0.16	1.83± 0.12	1.83± 0.12	1.86±0.17

Antimicrobial Activity of Water

Solvent	Conc. (µg/ml)	*Bacillus sps.*			*Eschericia coli.*			*Pseudomonas aeruginosa*		
		S	L	F	S	L	F	S	L	F
Water	25	0.36±0.09	0.36± 0.05	1.4 ±0.17	0.3 ± 0.06	0.3 ± 0.08	0.36+0.12	0.4 ± 0.08	0.4 ± 0.08	0.46±0.21
	50	0.8± 0.08	0.7± 0.06	0.8 ±0.08	0.5± 0.06	0.56 ± 0.12	0.5 ±0.08	0.6± 0.08	0.6 ± 0.22	0.6 ±0.08
	75	1.7± 0.08	1.73± 0.05	1.63± 0.05	1.16± 0.05	1.26± 0.05	1.2±0.08	1.2± 0.08	1.2± 0.08	1.2±0.08
	100	1.8± 0.08	1.8± 0.08	1.73± 0.12	1.46± 0.05	1.43± 0.64	1.43±0.12	1.5± 0.16	1.5± 0.08	1.43±0.12

Anti Microbial Activity of Ethanol

Solvent	Conc. (µg/ml)	Diameter of inhibition (mm)								
		Bacillus sps.			*Eschericia coli.*			*Pseudomonas aeruginosa*		
		S	L	F	S	L	F	S	L	F
Ethanol	25	1.7± 0.08	1.73±0.05	1.63± 0.05	1.16±0.05	1.26±0.05	1.2± 0.08	1.2± 0.08	1.2±0.08	1.2±0.08
	50	1.8± 0.08	1.8± 0.08	1.73± 0.12	1.46±0.05	1.43±0.65	1.43± 0.12	1.5± 0.16	1.5±0.08	1.43±0.12
	75	2.3± 0.16	2.36± 0.21	2.4± 0.08	1.9±0.08	1.86±0.12	1.9± 0.16	1.83± 0.12	1.83±0.12	1.86±0.17
	100	2.86±0.48	2.8± 0.08	2.86± 0.48	2.4±0.08	2.4± 0.08	2.43± 0.12	2.5± 0.08	1.83±0.12	2.56±0.48

Anti Microbial Activity of Streptomycin

Solvent	Conc.(µg/ml)	Diameter of inhibition (mm)		
		Bacillus	*Eschericia coli.*	*Pseudomonas aeruginosa*
Streptomycin	50	6.5±0.14	7.3±0.17	8.4±0.24

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