

Antibacterial property of methanolic extract *Gliricidia sepium* against some human pathogens

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Abstract

Plant kingdom harbours a wide variety of active compounds. Medicinal plants would be the best source to obtain a variety of drugs. The use of plant extracts and phytochemical both with known antimicrobial properties can be of great significance in therapeutic treatments. Search for a new antimicrobial agent is a par. In the present study an attempt in this regard was carried out on methanolic leaf extracts of *Gliricidia Sepium* at different concentration, to investigate the antimicrobial activity against human pathogens. The results of the present study revealed that, the methanolic extract showed antimicrobial activity against all tested bacteria. At 50% concentration, the maximum zone of inhibition (23mm) was observed.

Keywords: *Gliricidia Sepium*, Antibacterial activity, Zone of inhibition, Methanol extract

1. Introduction

Plants have been a good source of drugs for humanity from the most primitive time. The development of the modern medicine remains rooted to the medicinal plants. The search for newer source of antibiotics is a global challenge in pharmaceutical companies. Since many infectious agents are becoming resistant to synthetic drugs [1]. Developments of microbial resistance to the available antibiotics have led scientists to investigate the antimicrobial activity of medicinal plants [2]. Medicinal plants as a plant in which one or more organs contain substances that can be used for therapeutic purposes or which precursors for manufacturing of drugs are useful for disease therapy [3]. Local use of natural plants, acts as primary health remedies, due to their pharmacological properties, is quite common in Asia, Africa and Latin America [4]. *Gliricidia sepium* is a leguminous tree and belongs to the family fabaceae [5]. The plant is originated in Central America and is used in many tropical and sub-tropical countries. The research on *Gliricidia sepium* was first carried out in Pakistan especially in the area of mosquito repellent and nematicidal characteristics of this plant.

Leaves of *Gliricidia sepium* have a high feeding value with crude protein comprising 20-30 % of the dry matter, a crude fiber content of about 15% and invitro dry matter digestibility of 60-65% [6]. Traditionally used medicinal plants produce a compound of known therapeutic properties. *Gliricidia sepium* leaf extracts were tested for antimicrobial properties and was found effective against bacteria causing certain infections [7]. Also the plant extracts has formed the basis for many applications, including preservatives, pharmaceutical, alternative medicine and natural therapies [8]. The purpose of this study was therefore, an attempt to carry out pre-clinical evaluation, like antibacterial activity of *Gliricidia sepium* methanolic leaf extracts against human pathogenic bacteria.

2. Materials and Methods

2.1 Collection of Plant Materials

Fresh leaves of *Gliricidia sepium* were collected from in and around Kerala, India.

2.2 Processing of the Collected Leaf

Leaves were thoroughly washed in running water to clean the adhering soil and dust particles and then rinsed with distilled water, shade dried, coarsely powdered and stored in air tight containers for further use.

2.3 Human Pathogenic Bacterial Species

The human pathogenic bacteria such as *Pseudomonas aeruginosa*, *E.coli*, *Serratia marcescens*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were obtained in pure form from the PSG pharmacy college, Coimbatore, Tamil Nadu and were maintained in nutrient agar slant at 4°C for experimental studies.

2.4 Media Preparation for Bacteria

36gm of Muller Hinton media (Hi-media) was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into Petri dishes. The solidified plates were used for antimicrobial studies.

2.5 Preparation of Plant Extract

The methanolic extract of *Gliricidia sepium* were prepared using soxhlet apparatus by dissolving 10 gm of fine powder in 50 ml of methanol for 48 hrs. The extract were concentrated to dryness and the residues were stored under refrigeration at 4°C for further studies.

2.6 Assay of Antimicrobial activity using Agar Well Diffusion method

Antibacterial activity of the plant extract was tested using well diffusion method. The prepared culture plates were inoculated with selected bacteria using streak plate method. Wells were made on the agar surface with 5mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37°C ± 2°C for 24 hrs. The plates were observed for the zone formation around the well and were measured in millimeter (mm). For each treatment three replicates were maintained. The results were recorded.

Inhibition zone with diameter less than 12mm were considered as having no antimicrobial activity. Diameter between 5 and 10 were considered moderately active and there with greater than 10 mm were considered highly active.

3. Results and Discussions

The aim of present study was to evaluate the antimicrobial activity of methanolic leaf extract of *Gliricidia sepium*. The result obtained in the present study provides a scientific support to use of plant in the treatment of microbial disease. The antibacterial activity of the extract was quantitatively assessed by the presence or absence of inhibition zone and diameter respectively. Positive and negative control were set

up using methicillin and methanol. *Gliricidia sepium* extract showed activity against all tested organisms. The maximum zone of inhibition (23.25 mm) was observed against *Serretia marcescens* followed by *E. coli* (19.15 mm), *Pseudomonas aeruginosa* (17.05 mm), *Staphylococcus aureus* (15.25 mm), *Klebsiella pneumoniae* (12.75 mm) and *Bacillus cereus* (9.20 mm) and at 50% concentration. Among five concentration (10%, 20%, 30%, 40% and 50%), 50% was found to be the most effective against all six tested pathogens. When compared to the control (22.50), the *Serretia marcescens* showed highest zone of inhibition (23.25 mm). The rest of the pathogens showed less zone of inhibition than the control. (Table 1).

Table 1: Antibacterial activity of different concentrate of methanolic extract of *Gliricidia sepium* leaves

Name of the pathogens	Zone of Inhibition in mm						
	control	methicillin	10%	20%	30%	40%	50%
<i>Pseudomonas aeruginosa</i>	0.00	21.00	-	4.15	9.03	13.02	17.05
<i>Escherichia coli</i>	0.00	20.00	3.00	6.55	10.60	14.55	19.05
<i>Serretia marcescens</i>	0.00	22.50	3.20	7.15	12.42	17.30	23.25
<i>Klebsiella pneumonia</i>	0.00	19.00	-	2.80	5.65	8.90	12.75
<i>Bacillus cereus</i>	0.00	19.00	-	2.02	4.00	6.40	9.20
<i>Staphylococcus aureus</i>	0.00	22.00	1.75	3.25	6.15	10.00	15.25

Various researchers have worked in exploring the antimicrobial potency of leaf extract against infectious bacteria. Their finding were in conforming with few studies who also reported that methanol extract of *Gliricidia sepium* exhibited good antimicrobial activities against *Serretia marcescens*, *Staphylococcus aureus*, *Bacillus subtilis* and, *E. coli* [9]. Reports also show that twenty two Mexican medicinal plants showed antimicrobial activity against *Staphylococcus aureus* and *E. coli* [10]. The use of methanolic leaf extract with known antimicrobial properties can be of great significance for therapeutic treatment [11]. However, some research studies have conducted focusing on the antimicrobial properties of different solvent extracts (chloroform & ethanol) of bark and on essential oils of leaf and flower against different strains of bacteria [12].

4. Conclusion

In the present era, plant and herb resources are abundant. Significant numbers of studies have been used to obtain purified plant chemical, very few screening programs have been initiated on crude plant material. It has been widely observed and accepted that the medicinal value of plant lies in the bioactive substances present in the plants. In the present investigation, the active leaf extract of *Gliricidia sepium* obtained by methanol extraction was tested against six pathogenic bacteria, *Pseudomonas aeruginosa*, *E. coli*, *Serretia marcescens*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus cereus* at five different concentrations. Among five concentration 50% concentration was found to be more effective than rest of the concentration. Among the tested bacteria *Serretia marcescens* showed good zone of inhibition than other bacteria when compared to the control. From the above studies, it was concluded that the methanolic extract of *Gliricidia sepium* have the most active antibacterial components which is stable and bioactive. Further investigation of its activity against a wider range of bacteria,

identification and purification of its chemical constituents and toxicological investigation of the plant extracts should be carried out with a view to develop novel drugs for human consumption.

5. References

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