

ANTIMICROBIAL PROPERTY OF *GLIRICIDIA SEPIUM* PLANT EXTRACT

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ABSTRACT:- Quest for the new antimicrobial agent is still there and the present work is an attempt in this regard, Ethanolic extract of *Gliricidia sepium* in different concentration was used to investigate its antimicrobial activity against gram +ve and gram-ve bacteria. The study was also extended to some species of fungi. Results showed that the activity was more pronounced against gram +ve organisms and fungi. Maximum inhibition activity was calculated against all the groups of organisms, which was between 0.5 and 1mg ml⁻¹ against bacteria and 2.5 mg ml⁻¹ against fungi. It is therefore concluded that *G. sepium* provide a lead towards the exploration of new antimicrobial agent.

Key Words: Gliricidia sepium; Antibacterial Activity; Antifungal Activity, Pakistan.

INTRODUCTION

Gliricidia sepium is a leguminous tree and belongs to the family Fabaceae (Chadhokar, 1982). It is originated in Central America and is used in many tropical and sub-tropical countries. This plant was introduced in Philippines and Sri Lanka in 1600s and 1800s, respectively to provide shade to tea plants. Seeds of *G. sepium* were introduced in Pakistan in 1996 from Sri Lanka to provide green manure for coconut plants and shadow for beetle leaf plant in the Plant Research Center of Coastal Research Station, Karachi. Research showed that the plant can be cultivated in the plains of Sindh and Punjab in addition to the coastal areas of Sindh where irrigation facilities are available.

For the first time in Pakistan research on *Gliricidia sepium* is being carried out at PCSIR Labs Complex, Karachi especially in the area of mosquito repellent and nematocidal characteristics of this plant.

The plant is used for fuel wood, animal feed, green manure, shade, living fences and as support plants (Csurhes and Edward, 1998). The leaves of *G. sepium* have a high feeding value with crude protein comprising 20-30% of the dry matter, a crude fiber content of about 15% and in vitro dry matter digestibility of 60-65% (Adejumo and Ademosun, 1985; Gohl, 1981). There are numerous reports of in-

creases in weight and milk production in both large and small ruminants when *Gliricidia* forage is used as a supplement. (Nochebueno and O' Donovan, 1986). *Gliricidia* means mouse or rat killer, which is derived from its bark and leaves which when cooked with grain can be used as poisonous bait for rodents. Though poisonous to rodent and insect, the leaves contain 3-4% dry weight of nitrogen and small amount of phosphorus, potassium, calcium and magnesium, so they can be used as excellent green manure and fodder.

In another study the antimicrobial properties of extracts from the leaves of *Gliricidia sepium* was tested. It was effective against bacteria and fungi causing dermatitis. Plant oils and extracts have been used for various purposes for many thousands of years (Jones, 1996). In particular, the antimicrobial activity of plant oil and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceutical, alternative medicine and natural therapies (Lis-Balchin and Deans, 1997). Traditionally used medicinal plants produce a compound of known therapeutic properties (Iyengar 1981; Chopra et al., 1992, Harborne and Baxter, 1995). The substance that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cell are considered candidate for develop-

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ing new antimicrobial drugs. In the present study *Gliricidia sepium* was selected for screening against some pathogenic bacteria and fungi. The selection of this medicinal plant is based on their traditional uses.

Present work is an attempt to screen antimicrobial agents from plant origin (Clark, 1996). These agents may have many therapeutic effects for the treatment of disease and infections. The side effect associated with these diseases by the usage of synthetic drugs may also be reduced. Antimicrobial agents from plants may also help to reduce the multiple drug resistance burdens.

MATERIALS AND METHODS

Gliricidia sepium plant leaves were collected from PARC-SARC, Karachi. All the leaves samples were preserved in wax quoted paper bags and brought to the laboratory for biological assays.

The fresh leaves of *G. sepium* (5kg) was ground and soaked in ethanol (commercial, doubly distilled 50 l). The filtrate was concentrated under reduced pressure at 40°C to a gum.

Alcoholic extract was dissolved in 6% dimethylformamide(DMF) to make stock solution of 20mgml⁻¹ by which further dilutions were made and used for testing. Ampicillin and Nystatin (1mgml⁻¹) were used as a reference standard. While 6% dimethylformamide was used as negative control. The said activity was assessed against gram +ve and -ve microorganisms. All microorganisms used in the present study were taken from Department of Microbiology, University of Karachi, Karachi. The clinical isolates were biochemically characterized by standard methods. The organisms used in this study were:

Bacillus subtilis, *B. pumilus*, *B. cereus*, *Staphylococcus aureus*, *Streptococcus intermedius*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Fusarium solani*, *Trichophyton rubrum*, *Aspergillus effuses*, *Rhizomucor pusillus*, *Trichophyton sclerosis*, *Macrophomonia phaseolina* and *Rhizoctonia solani*.

Tryptic soya agar (Merck) was used to test bacteria and Sabouraud Dextrose agar was used for fungi. Bacterial cultures freshly grown at 37°C and fungal culture at 25°C. Bacterial cultures were appropriately diluted in sterile normal saline solution to obtain the cell suspension at 10⁶CFU ml⁻¹.

Antibacterial Activity

This activity was carried out by agar well diffusion method (Ahmad et al., 1998). According to this method, 0.1 ml of diluted inoculums (10⁶ CFU ml⁻¹) of test organism was thoroughly mixed with 20 ml of molten sterile tryptic soya agar and poured into pre-sterilize Petri dishes under sterile condition. All plates were left to set at 4°C for 30-40 minutes. Holes of 6 mm diameter were made in the center of each seeded plates. Holes were then filled aseptically with 0.1 ml of test solution (various extract in various conc.) reference standard and negative control (i.e., solvent only) respectively and marked accordingly. All plates were then incubated at 37 ± 1°C for 24h and zone of inhibition exhibited by the different extracts in various concentration measured and recorded accordingly. All plates were run in triplicates.

Antifungal Activity

This activity was determined by agar tube dilution method (Paxton, 1991). Test tubes having sterile sabouraud dextrose agar were inoculated with test solution of different concentration and kept in slanting position at room temperature for solidification. Test fungal cultures were inoculated on slant incubated at 25°C for 7 days and growth inhibition were observed after 7 days incubation period (Washington and Sutter, 1980). Nystatin was used as standard antifungal drug.

MIC Determination

The MIC values of ethanolic extract of *Gliricidia sepium* were determined against the gram +ve and -ve bacteria and fungi (10⁶ CFU ml⁻¹) by the serial dilution technique (Reiner, 1982). Nutrient agar and

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Nutrient broth were used as bacteriological media. A set of tubes with different concentrations (0.25, 0.5, 1, 2, 2.5, 5, 10 and 20 mg ml⁻¹) were prepared. The tubes were inoculated with test organisms incubated at 37°C for 24 h. After incubation time, plates were analyzed visually for the presence of growth. Growth is seen to diminish as the concentration of extract increased and eventually that concentration was observed at which growth fails to occur.

Statistics

The data are analyzed as mean \pm S.E. and compared by applying t-test using Sigma Plot software version 11.2. The value less than 0.5% is considered as significant.

RESULTS AND DISCUSSION

In the present study, Ethanol extract of leaves of *Gliricida sepium* were tested against some pathogenic bacteria and fungi. The antibacterial activity of extract was quantitatively assessed by the presence or absence of inhibition zone and diameter, respectively (Figure 1).

G. sepium extract showed activity against all gram-ve organisms at 20mg ml⁻¹ concentration while at 10 mgml⁻¹ and 5 mgml⁻¹ concentration showed good and low activity respectively all these concentration showed significant difference ($p > 0.05$). At 5mg ml⁻¹ concentration *Klebsiella pneumoniae* was found resistant as concentration increases extract showed

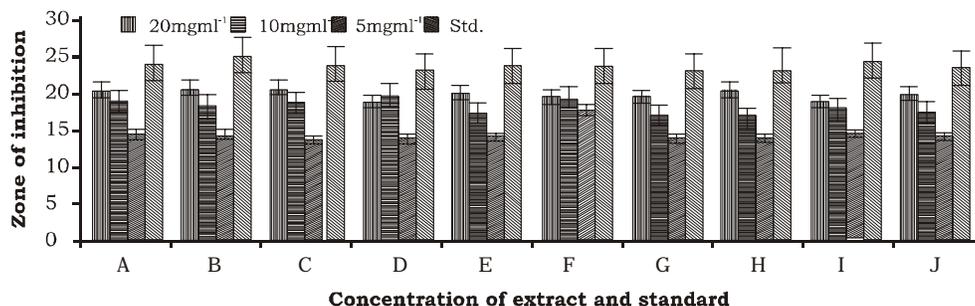


Figure 1. Antibacterial activity exhibited by *G. sepium* gram +ve and -ve organisms (A= *Staphylococcus aureus*, B= *Streptococcus intermedius*, C= *Bacillus pumilus*, D= *Bacillus subtilis*, E= *Bacillus sereus*, F= *Escherichia coli*, G= *Salmonella typhi*, H= *Klebsiella pneumoniae*, I= *Proteus mirabilis*, J= *Shigella flexneri*)

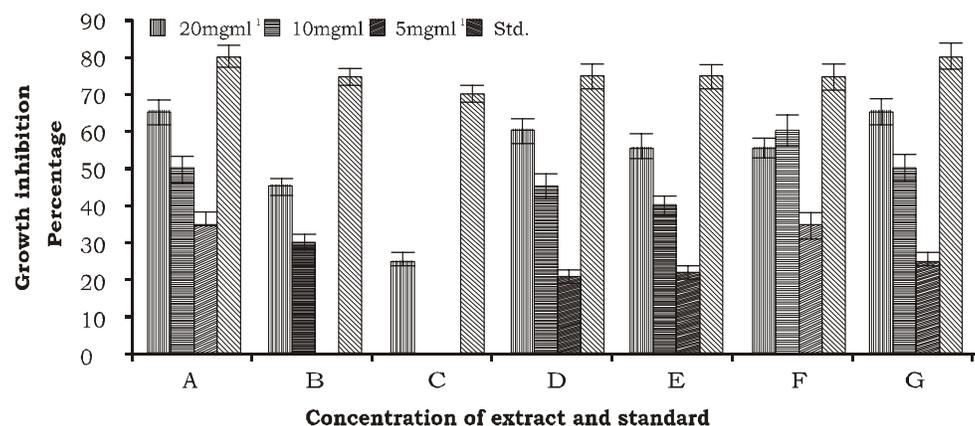


Figure 2. Antifungal activity exhibited by *G. sepium* (A= *Fusarium solani*, B= *Trichophyton rubrum*, C= *Aspergillus effuses*, D= *Rhizomucor pusillus*, E= *Trichophyton sclerosis*, F= *Macrophomonia phaseolina*, and G= *Rhizoctonia solani*)

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significant inhibitory activity. In gram +ve organisms, extract showed significant activity at 20mg ml⁻¹ concentration comparable to standard antibiotic. These findings were in conformity with Jhon et al. (2006) and Abulude and Adebote (2009) who also reported that ethanol extract of *G.sepium* exhibited good antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* (Figure 2). Similarly Kakuko et al. (2005) also reported that 22 Mexican medicinal plants showed antibacterial activity against *Staphylococcus aureus*, and *Escherichia coli*. However some research studies established on different solvents like chloroform and methanol extract of the bark of *G.sepium* and on essential oils of leaf and flower part of *G.sepium* also showed significant activity against different bacterial strains (Salud et al., 2007; Beena and Reddy, 2010).

Among fungi none showed significant

activity at highest concentration 20mg ml⁻¹. At this concentration, *Fusarium solni*, *Rhizomucor pusillus*, *Trichophyton sclerosis*, *Macrophomnia phaseolina* and *Rhizoctonia solani* showed good activity while *Aspergillus effuses* showed low activity at 20mgml⁻¹ and was resistant at 10 and 5mg ml⁻¹. In some studies *G. sepium* showed significant activity against *Candida albican*.

An attempt has therefore been made to determine the minimum inhibitory concentration of ethanol extract against gram + ve and -ve bacteria as well as fungi (Table 1 and 2). In gram + ve bacteria (*Staphylococcus aureus*, *Streptococcus intermedius*, *Bacillus pumilus*, *B. subtilis* and *B. cereus*) MIC value was found 1mg ml⁻¹. Against the gram -ve bacteria (*Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Shigella flexneri*), MIC value was found to be 0.5mgml⁻¹. Against fungi MIC value was 2mgml⁻¹ except *Aspergillus*

Table 1. Minimum inhibitory concentrations of ethanolic extract against gram -ve and+ve bacteria

Test organism	Concentration (mg ml ⁻¹)							
	20	10	5	2.5	2	1	0.5	0.25
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	+	+
<i>Streptococcus intermedius</i>	-	-	-	-	-	-	-	+
<i>Bacillus pumilus</i>	-	-	-	-	-	-	+	+
<i>Bacillus subtilis</i>	-	-	-	-	-	-	+	+
<i>Bacillus cereus</i>	-	-	-	-	-	-	+	+
<i>Escherichia coli</i>	-	-	-	-	-	-	-	+
<i>Salmonella typhi</i>	-	-	-	-	-	-	-	+
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-	-	+
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	+
<i>Shigella flexneri</i>	-	-	-	-	-	-	-	+

Table 2. Minimum inhibitory concentrations of ethanolic extract against gram -ve and +ve yeast and fungi

Test organism	Concentration (mg ml ⁻¹)							
	20	10	5	2.5	2	1	0.5	0.25
<i>Fusarium solani</i>	-	-	-	-	+	+	+	+
<i>Trichophyton rubrum</i>	-	-	-	+	+	+	+	+
<i>Aspergillus effuses</i>	-	+	+	+	+	+	+	+
<i>Rhizomucor pusillus</i>	-	-	-	-	+	+	+	+
<i>Trichophyton sclerosis</i>	-	-	-	-	+	+	+	+
<i>Macrophomnia phaseolina</i>	-	-	-	-	-	+	+	+
<i>Rhizoctonia solani</i>	-	-	-	+	+	+	+	+

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effuses which is somewhat resistant.

It may be concluded safely that ethanol extract of *G.sepium* have the most active antibacterial components than antifungal and can be a good source of chemical compound.

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