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Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITY OF THE BARK, FLOWER AND LEAF EXTRACTS OF *GLIRICIDIA SEPIUM* FROM SOUTH INDIA

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ABSTRACT

The antibacterial activities of petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of the bark, flower and leaf of *Gliricidia sepium* were screened against various pathogenic bacteria such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens* by 'agar well diffusion' method. The plant extracts showed various levels of activity on different test organisms. Methanol extract of *G. sepium* flower was found to be the most potent extract and its activity is quite comparable with the standard antibiotics screened under similar conditions. Ethyl acetate and chloroform extracts of the *Gliricidia sepium* flower as well as leaf and bark methanol extracts and ethyl acetate extract of bark also exhibited pronounced activity whereas petroleum ether extracts were non effective on all organisms tested. The study shows that methanol, ethyl acetate and chloroform extracts of the *Gliricidia sepium* flower, ethyl acetate extract of bark as well as the methanol extracts of bark and leaves of the plant can be used as a potential external antiseptic and can be incorporated into drug formulations.

Keywords: *Gliricidia sepium*, Antibacterial activity, Agar well diffusion method, Standard antibiotics, Drug formulation.

INTRODUCTION

Medicinal plants have a long history of use and their use is widespread in both developing and developed countries. According to the report of the World Health Organisation, 80% of the world populations rely mainly on traditional therapies which involve the use of plant extracts or their active substances[1]. The microorganisms have developed resistance against many antibiotics due to the indiscriminate use of antimicrobial drugs[2]. Antibiotics are sometimes associated with side effects[3] whereas there are some advantages of using antimicrobial compounds of medicinal plants, such as often fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature[4]. All these data high lights the need for new alternative drug regimens.

Gliricidia sepium (Leguminosae family) is a medium sized tree introduced into India from the American continent. This tree is used in Mexico as shade for cocoa and coffee plantations and for this reason it is called 'Madrecacao' (mother of cocoa). It is also used as a poison for rodents and in fact the Latin name *Gliricidia* means rodent poison. It is used as a hedge plant and the flowers are utilized as food in some places in Mexico[5]. In Panama, the decoction of *G. sepium* leaves used in utricaria, rash and also in burns and erysepalas[6]. In Guatemala and Costa Rica, bark decoction is used against bacterial and protozoal infections[7]. Branches of *Gliricidia sepium* is used to reduce fever in children and adults. It has also been used to treat infections produced by *Microsporum canis*, *Trychophyton mentagrophytes* and *Neisseria gonorrohae*[8]. Sharma and Qadry investigated the larvicidal activity of the crude ethanol extract of *Gliricidia sepium* bark and leaves[9]. Various phytochemicals like flavanoids[10], triterpenoid saponins[11], stigmastanol glucoside [12], rhamnogalactoside of kaempferol[13], coumarin, coumaric acid and melilotic acid[14] have been isolated and characterised from various parts of this plant. Allelochemicals from *Gliricidia sepium* leaves were extracted, identified and quantified using HPLC[15]. Rastrelli isolated a new 12a-hydroxy rotenoids from the methanolic extract of *Gliricidia sepium* bark[16].

In the present study antibacterial activities of the crude extracts of *Gliricidia sepium* bark, flower and leaf were investigated for the aim of discovering the medicinal potential of these plant extracts.

MATERIALS AND METHODS

Plant material

Bark, flower and leaf of *Gliricidia sepium* were collected from Kerala, South India and authenticated by Dr. A.K. Pradeep, Dept. of Botany, Calicut University. Voucher specimen is deposited in the specially maintained herbarium, Department of Chemistry, Calicut University.

Preparation of plant extracts

Fifty grams of each of powered plant material were extracted successively with 150ml of petroleum ether, chloroform, ethyl acetate, methanol and water as solvents for 24 hours by Soxhlet equipment[17].

Test microorganisms

The microorganisms used for antibacterial activity evaluation were obtained from Microbial Type Culture Collection and gene bank (IMTECH, Chandigarh, India). They were Gram-positive bacteria such as *Bacillus cereus* (MTCC-1305), *Staphylococcus aureus* (MTCC-96), *Enterobacter faecalis* (MTCC-5112) and *Streptococcus faecalis* (MTCC-439) and Gram-negative bacteria such as *Salmonella paratyphi* (MTCC-735), *Escherichia coli* (MTCC-729), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-647) *Proteus vulgaris* (MTCC-426) and *Serratia marcescens* (MTCC-86).

Culture medium and inoculum

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at 4^0C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10ml of nutrient broth and was incubated at 37^0C for 24hours. On the next day Muller-Hinton agar (MHA) (Merck) sterilized in a flask and cooled to 45-50^0C was distributed by pipette (20ml) into each sterile Petri dish and swirled to distribute the medium homogeneously. About 0.1ml of bacterial suspension was taken and poured into Petri plates containing 20ml nutrient agar medium. Using the L-shaped sterile glass spreader bacterial suspensions were spread to get a uniform lawn culture.

Antibacterial activity assay

The agar diffusion method is used for the antimicrobial evaluations. Wells of 8mm (0.8cm) diameter were dug on the inoculated nutrient agar medium with sterile cork borer and 50µl of the petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of

the bark, flower and leaf of *Gliricidia sepium* were added in each well. Wells introduced with 50µl of pure petroleum ether, chloroform, ethyl acetate, methanol and distilled water served as negative controls. The plates were incubated at 37^{0}C over night and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic drugs such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin were also screened under similar conditions for comparison. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8mm[18]. All the assays were performed in triplicate and expressed as average values.

The antibacterial spectra of the bark, flower and leaf extracts of *Gliricidia sepium*, showing the zone of inhibition in millimeters, for Gram positive and Gram negative bacteria are summarized in table 1. In addition, the inhibition zones formed by standard antibiotics and those of negative controls are listed in table 2.

RESULTS AND DISCUSSION

As can be seen from table 1, the flower methanol extract of *G. sepium* showed pronounced antibacterial activity against all the microorganisms tested (24-32mm/50µl inhibition zone). Ethyl acetate(18-28mm/50µl inhibition zone) and chloroform (15-25mm/50µl inhibition zone) extracts of the flower also exhibited marked activity against all the tested organisms such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa and Serratia marcescens.* Flower aqueous extract was found to be effective on *Escherichia coli, Proteus vulgaris, Bacillus cereus, Enterobacter faecalis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Serratia marcescens* (14-18mm/50µl inhibition zone) where as it had little effect on *Salmonella paratyphi, Klebsiella pneumoniae* and *Streptococcus faecalis.*

Table1: Antibacterial activity of aqueous, methanol, ethyl acetate and chloroform extracts of the bark, flower and leaf of *Gliricidia sepium*

	Diameter of inhibition zones(mm/50µl)											
	***G. sepium* bark**				***G. sepium* leaf**				***G. sepium* flower**			
Microorganisms	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1. *Bacillus cereus*	15	18	12	16	12	--	13	--	24	20	18	14
2. *Enterobacter faecalis*	14	20	--	--	14	14	--	--	30	22	20	15
3. *Salmonella paratyphi*	18	20	--	14	12	--	12	13	28	22	25	11
4. *Staphylococcus aureus*	13	--	--	11	13	--	11	12	32	26	22	14
5. *Escherichia coli*	--	18	14	--	15	--	--	17	28	20	25	18
6. *Streptococcus faecalis*	15	--	--	14	16	--	13	--	26	24	18	13
7. *Proteus vulgaris*	11	20	--	--	14	--	--	--	26	23	20	18
8. *Klebsiella pneumoniae*	--	--	--	13	13	10	12	12	24	18	15	12
9. *Pseudomonas aeruginosa*	17	18	12	11	14	--	--	11	24	18	16	14
10. *Serratia marcescens*	15	16	--	16	--	16	12	16	32	28	21	16

Controls- A: methanol; B: ethyl acetate; C: chloroform; D: distilled water

The bark ethyl acetate extract exhibited significant activity against *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Escherichia coli, Proteus vulgaris*, *Pseudomonas aeruginosa* and *Serratia marcescens* (16-20mm/50µl inhibition zone) whereas it had no effect on *Staphylococcus aureus, Streptococcus faecalis* and *Klebsiella pneumoniae.*

The bark methanol extract was found to be active against *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Streptococcus faecalis, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus* and *Proteus vulgaris* (11-18mm/50µl inhibition zone) while it did not inhibit *Escherichia coli* and *Klebsiella pneumoniae.*

Both chloroform and aqueous extracts of *Gliricidia sepium* bark inhibited the growth of *Bacillus cereus* (12-16mm/50µl inhibition zone). Chloroform extract inhibited *Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa* (12-14mm/50µl inhibition zone). Bark aqueous extract exhibited remarkable activity against *Serratia marcescens, Bacillus cereus, Salmonella paratyphi, Streptococcus faecalis* (14-16mm/50µl inhibition zone) while its activity against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* was less. Petroleum ether extracts of bark, leaf and flower have no antibacterial activity on the test microorganisms.

Leaf methanol extract showed appreciable inhibitory activity on all test bacteria (12-16mm/50µl inhibition zone) except *Serratia marcescens.* The leaf aqueous extracts of *Gliricidia sepium* was found to be effective on *Escherichia coli* and *Serratia marcescens* (16-17mm/50µl inhibition zone) whereas it had little effect on *Salmonella paratyphi, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Table 2: Inhibition zones formed by the standard antibiotics—tobramycin, gentamicin sulphate, ofloxacin, ciprofloxacin and negative controls

Microorganisms	**Diameter of inhibition zones (mm/50µl)**				
	Tob	**Gen**	**Oflo**	**Cip**	**Control**
1. *Bacillus cereus*	28	32	34	30	--
2. *Enterobacter faecalis*	26	32	32	26	--
3. *Salmonella paratyphi*	25	30	28	30	--
4. *Staphylococcus aureus*	26	28	24	24	--
5. *Escherichia coli*	30	36	32	34	--
6. *Streptococcus faecalis*	28	34	30	32	--
7. *Proteus vulgaris*	26	30	24	32	--
8. *Klebsiella pneumoniae*	26	32	32	36	--
9. *Pseudomonas aeruginosa*	26	24	32	28	--
10.*Serratia marcescens*	24	32	30	30	--

Controls- A: methanol; B: ethyl acetate; C: chloroform; D: distilled water, Tob: tobramycin, Gen: gentamicin sulphate, Oflo: ofloxacin, Cip:ciprofloxacin

Leaf ethyl acetate extract showed significant activity against *Enterobacter faecalis, Klebsiella pneumoniae* and *Serratia marcescens* (10-14mm/50µl inhibition zone). Chloroform extract of the *Gliricidia sepium* leaves was found to be active against various tested microorganisms such as *Bacillus cereus, Salmonella paratyphi, Staphylococcus aureus, Streptococcus faecalis, Klebsiella pneumoniae* and *Serratia marcescens* (11-13mm/50µl inhibition zone).

The results obtained were compared with standard antibiotics and it was observed that *G. sepium* flower methanol extract at a concentration of 1mg/ml was more active against *Enterobacter faecalis* than 10µg of tobramycin and ciprofloxacin. The activities of flower methanol extract against *Staphylococcus aureus* and *Serratia marcescens* were significantly high compared to all antibiotics tested. Methanol and chloroform extracts showed marked activity against *Salmonella paratyphi* and their activities were quite comparable with that of standard antibiotics such as tobramycin and ofloxacin (10µg). Ethyl acetate and chloroform extracts of *G. sepium* flower exhibited pronounced activity against *Staphylococcus aureus* and their activities were comparable with ofloxacin, ciprofloxacin and tobramycin (10µg each). Methanol extract of *G. sepium* flower was found to be more active against *Proteus vulgaris* than 10µg ofloxacin. The activities of flower methanol extract and 10µg gentamicin sulphate against *Pseudomonas aeruginosa* were same. Flower ethyl acetate extract (1mg/ml) was found to be more active against *Serratia marcescens* than tobramycin (10µg). The Minimum Inhibitory Concentration (MIC) of methanol and ethyl acetate extracts of flower was found to be 0.5mg/ml.

Out of the twelve herbal extracts examined for antibacterial activity, *Gliricidia sepium* flower extract (1mg/ml) showed the highest activity against all the microorganisms studied. Its activity against *Staphylococcus aureus, Salmonella paratyphi, Enterobacter faecalis, Proteus vulgaris, Pseudomonas aeruginosa* and *Serratia marcescens* were quite comparable with that of the standard antibiotics at a concentration of 10µg/ml. Flower ethyl acetate extract showed next highest activity followed by flower chloroform extract. *G. sepium* leaf and bark methanol extracts as well as bark ethyl acetate extract exhibited remarkable activity against the microorganisms studied. The antimicrobial potency of the *G. sepium* plant extracts is due to the presence of saponins[11,] phenolic compounds[19], essential oils[20] and flavonoids[10]. It is interesting to note that even crude extract of this plant showed prominent activity against various pathogenic bacteria where modern therapy has failed. The variation of the susceptibility of the tested microorganisms could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the extracts.

CONCLUSION

Amongst the twelve extracts of *Gliricidia sepium* examined for antibacterial activity flower methanol, ethyl acetate and chloroform extracts, bark ethyl acetate and bark and leaf methanol extracts showed significant activity against the different strains of bacteria. The activities of these extracts are found to be quiet comparable with the standard antibiotics screened under similar conditions. So these extracts can be used as an external antiseptic in prevention and treatment of bacterial infections. The incorporation of these extracts into the drug formulations is also recommended.

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