International Journal of Applied Pharmaceutics
Vol 2 Issue 2, 2010

Research Article

EVALUATION OF ANTIBACTERIAL ACTIVITY OF THE LEAF AND FLOWER ESSENTIAL OILS OF *GLIRICIDIA SEPIUM* FROM SOUTH INDIA

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Received: 19 Jan 2010, Revised and Accepted: 21 Feb 2010

ABSTRACT

Essential oils from leaf and flower of *Gliricidia sepium* were tested for antibacterial activity against ten bacterial strains: *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. Both leaf and flower oils showed significant activity against all the tested microorganisms; however the activity of leaf oil was found to be higher than flower oil. Essential oil extracted from *Gliricidia sepium* leaves showed the highest activity against *Salmonella paratyphi, Streptococcus faecalis, Proteus vulgaris, Serratia marcescens* and *Enterobacter faecalis* and the activity was quite comparable with the standard antibiotics screened under similar conditions. The remarkable antibacterial activity exhibited by the leaf oil can be attributed to the synergic effect of the antimicrobial agents present in it. This study shows that *G. sepium* leaf essential oil can be used as a potential external antiseptic and can be incorporated into the drug formulations.

Key words: *Gliricidia sepium*, essential oil, 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, decoction of 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]. Molykutty et al [17] isolated the essential oils from leaf and flower of *G. sepium* by steam distillation and quantified using GC-MS.

Microbiological assays are used for the quantitative determination of antibiotics and inhibitory chemical agents and also the determination of the sensitivity of the microorganisms to these agents. Synthetic chemicals have their side effects and the development of bacterial resistance to the presently available antibiotics has necessitated the search for new antimicrobial agents. So we look into the nature as an ally and resource in finding new strategies to combat diseases of plants, animals and human beings. Most of the previous chemical investigations on *Gliricidia sepium* have focused mainly on the isolation of potential allelopathic and toxic compounds from heart wood, leaves and roots of the plant. So far no data about the antibacterial activity of G*. sepium* leaf and flower oils has been reported. In this work, the antibacterial property of the *G. sepium* leaf and flower oils was checked against ten pathogenic bacteria namely *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens*.

The Gram-positive bacterium *Staphylococcus aureus* is mainly responsible for post operative wound infection, toxic shock syndrome and food poisoning. The Gram-positive bacteria *Bacillus cereus* cause food borne illness in humans, *Enterobacter faecalis* cause inflammation of inner layer of the heart and *Streptococcus faecalis* is responsible for urinary tract and kidney infections. The Gram-negative bacterium *E. coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicemia. Gram-negative bacteria such as *Klebsiella pneumoniae* cause pneumonia and urinary tract infections. *Proteus vulgaris*, the Gram-negative bacteria, cause wound infections and pneumonia. The Gram-negative *Salmonella paratyphi* causes bacterial enteric fever and *Pseudomonas aeruginosa* causes kidney infection. *Serratia marcescens* is responsible for septicemia, meningitis, wound and eye infections [18].

MATERIALS AND METHODS

Plant material

The leaves and flowers 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.

Antibacterial activity

The essential oils from leaves and flowers of *G. sepium* were extracted by steam distillation and analysed by GC-MS [17] (table 1 and table 2). These oil samples were examined for their antibacterial activity against Gram-positive bacteria such as *Staphylococcus aureus, Bacillus cereus, Enterobacter faecalis* and *Streptococcus faecalis* and Gram-negative bacteria such as *Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Salmonella paratyphi* and *Serratia marcescens*.

Microbial strains

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

Screening for antibacterial activity

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 50 µl of the bark essential oil (at various concentrations) were added in each well. The essential oils of required concentrations 25%, 10%, 5% and 1% were prepared by dissolving the oils into appropriate quantities of DMSO, which did not influence the growth of bacteria was used as a negative control. The plates were then incubated at 37^oC overnight and examined for zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic drugs 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 [17]. All the assays were performed in triplicate and expressed as average values.

The antibacterial spectra of the *G. sepium* leaf and flower essential oils showing zone of inhibition in millimetres, for Gram-positive and Gram-negative bacteria are shown in table 3. In addition, the inhibition zones formed by standard antibiotics and those of negative control are listed in table 4.

RESULTS AND DISCUSSION

Gliricidia sepium leaf and flower essential oils at various concentrations were evaluated for antimicrobial activity against Gram-positive and Gram-negative bacteria strains and the oils exhibited marked activity against all tested microorganisms. As can be seen from table 3, the leaf oil (25%) showed pronounced activity against *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Escherichia coli, Proteus vulgaris*, *Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Streptococcus faecalis* and *Klebsiella pneumoniae* (22-38mm/50µl inhibition zone). The activity of the leaf oil was found to be higher than that of the standard antibiotics such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin (10µg each) screened under similar conditions. The inhibitory effect of 25% leaf oil of *G. sepium* on *Staphylococcus aureus* was comparatively less than that of standard antibiotics. The activities of 10% (18-28mm/50µl inhibition zone), 5% (14-20mm/50µl inhibition zone) and 1% (12-16mm/50µl inhibition zone) of the leaf oil samples were also studied against various pathogenic bacteria and were found to be active on all microorganisms tested.

Table 1: Chemical composition of the leaf essential oil of *Gliricidia sepium*

Identified components	Percentage
Propylene glycol	25.1
Coumarin	18.2
(Z)-3-Hexenol	17.7
β-Farnesene	14.2
(E)-2-Hexenol	6.5
Thymol	3.6
Benzyl alcohol	3.5
Caryophyllene	2.3
α-Farnesene	2.0
2-Pentene-1-ol	<1
Isovanillin	<1
Isobutyl alcohol	<1
Phenylethyl alcohol	<1
Phenol	<1
Crotonic aldehyde	<1
5,6-Dihydro-4H-cyclopenta-(6)-furan	<1

Table 2: Chemical composition of the flower essential oil of *Gliricidia sepium*

Identified components	Percentage
Benzyl alcohol	0.35
Nonanol	0.62
Maltol	4.42
2-Butyl-2-hexanol	1.03
Myrtenol	12.73
Eucarvone	0.88
Geraniol	0.72
Nonanoic acid	0.55
Myrtenal	0.78
Hydroquinone	21.64
p-Mentha-1,8-dien-1-ol	1.83
p-Mentha-1,4-dien-2-ol	0.73
p-Mentha-1,4-dien-7-ol	0.71
Decanoic acid	0.31
γ- Nonalactone	1.31
Coumarin	43.07
Dodecanoic acid	0.64
Tetradecanoic acid	0.46

Table 3: The antibacterial activity of the leaf and flower oils of *Gliricidia sepium*

	Diameter of inhibition zones(mm/50µl)							
Microorganisms	*G. sepium* leaf oil				*G. sepium* flower oil			
	25%	10%	5%	1%	25%	10%	5%	1%
1. *Bacillus cereus*	32	20	16	14	22	18	14	12
2. *Enterobacter faecalis*	36	24	18	15	19	16	13	11
3. *Salmonella paratyphi*	38	28	20	15	18	15	12	11
4. *Staphylococcus aureus*	22	18	16	13	17	14	12	10
5. *Escherichia coli*	34	20	14	12	28	24	21	16
6. *Streptococcus faecalis*	38	25	18	16	24	19	14	12
7. *Proteus vulgaris*	38	28	16	14	22	18	16	14
8. *Klebsiella pneumoniae*	34	25	20	15	21	18	16	13
9. *Pseudomonas aeruginosa*	28	20	16	14	18	16	14	12
10. *Serratia marcescens*	36	27	18	14	22	18	14	11

Used concentrations: 50µl of 25%, 10%, 5% and 1% of the leaf and flower essential oils of *G. sepium* in DMSO

Table 4: Inhibition zones formed by the standard antibiotics—tobramycin, gentamicin sulphate, ofloxacin, ciprofloxacin and negative control

	Diameter of inhibition zones (mm/50µl)				
Microorganisms	**Tob 10µg**	**Gen 10µg**	**Oflo 10µg**	**Cip 10µg**	**Control DMSO**
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	--

Tob: tobramycin, Gen: gentamicin sulphate, Oflo: ofloxacin, Cip: ciprofloxacin

Gliricidia sepium flower essential oil at various concentrations 25% (17-28mm/ 50µl inhibition zone), 10% (14-24mm/50µl inhibition zone), 5% (12-21mm/50µl inhibition zone) and 1% (10-16mm/50µl inhibition zone) were also exhibited pronounced activity against the tested bacteria; however it showed less activity compared to the leaf oil. It was observed that 10%, 5% and 1% of the *G. sepium* flower oil had more inhibitory effect on *Escherichia coli* than leaf oil with same concentration. Leaf and flower essential oils at a concentration of 5% and 1% performed same activity against *Proteus vulgaris* with inhibition zones 16 and 14mm respectively. The MIC (minimum inhibitory concentration) of the leaf and flower essential oils was found to be 10mg/ml.

As the leaf oil exhibited pronounced antibacterial activity comparable with standard antibiotics, it can be used as an external antiseptic in prevention and treatment of bacterial infections. The major component of the leaf essential oil propylene glycol [19, 20], coumarin [21], hexenol [22], thymol [23] and benzyl alcohol [24] are reported to have antimicrobial activities. The remarkable antibacterial activity exhibited by the *G. sepium* leaf oil can be attributed to the synergic effect of the antimicrobial agents present in the oil.

CONCLUSION

The essential oils from the leaf and flower of *Gliricidia sepium* showed varying degrees of antibacterial activity on the microorganisms tested. The variation of the susceptibility of microorganisms towards the essential oils could be attributed to their intrinsic properties that are related to the permeability of their cell surface to the essential oils. Due to the emergence of the antibiotic resistant pathogens, plants are being looked upon as an excellent alternate to combat the spread of multi drug resistant microorganisms.

From the above experiment it can be inferred that *Gliricidia sepium* leaf essential oil showed significant activity against Gram-positive and Gram-negative bacteria. The activity of leaf oil was found to be quite comparable with the standard antibiotics screened under similar conditions. So the oil can be used as an external antiseptic in the prevention and treatment of bacterial infections caused by 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*, which have developed resistance to antibiotics. The incorporation of this oil into the drug formulations is also recommended. This study demonstrated that the essential oil from the leaf of *Gliricidia sepium* is as effective as modern medicine to combat pathogenic microorganisms.

ACKNOWLEDGEMENT

One of the authors (Beena Jose), sincerely acknowledge UGC for providing financial assistance to carry out the study.

REFERENCES

1. World Health Organisation. Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram, 1993; 28: 13-14.
2. Ahmad I, Mahmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial propreties. J Ethnopharmacol, 1998; 62:183-193.
3. Cunha BA. Antibiotic side effects. The Med Clin North Am, 2001; 85: 149-185.
4. Vermani K, Garg S. Herbal Medicines for Sexually Transmitted diseases and AIDS. J. Ethnopharmacol, 2002; 80: 49-66.
5. Delizo RL, Del Fierro VF. Vegetative propagation of Madre Cacao, *Gliricidia sepium* (Jack) Steud, by cuttings using Alpha Naphthalene Acetic Acid (ANAA). Araneta Res J, 1974; 21: 20-33.
6. Asolkar LV, Kakkar KK, Chakre OJ editors. Second Supplement to Glossary of Indian Medicinal Plants with Active Principles, Part I (A-K). New Delhi: CSIR; 1992.
7. Berger I, Barrientos AC, Caceres A, Hernandez M, Rastrelli L, Passreiter CM et al. Plants used in Guatemala for the treatment of protozoal infections. II: Activity of extracts and fractions of five Guatemalan plants against *Trypanosoma cruzi.* J Ethnopharmacol, 1998; 62(2): 107-115.
8. Gupta MP. *Gliricidia sepium*. In: Gupta PM, editor. Plantas Medicinales Iberoamericanas. 1st ed. Bogota: Presencia Ltda; 1995. pp. 378-379.
9. Sharma N, Qadry JS, Subramanium B, Verghese T, Rahman SJ, Sharma SK. Larvicidal activity of *Gliricidia sepium* against mosquito larvae of *Anopheles stephansi*, *Ades aegypti* and *Culex quinquefasciatus*. Pharmaceut Biol, 1998; 36(1): 3-7.
10. Manners GD, Jurd L. Additional flavonoids in *Gliricidia sepium*. Phytochemistry, 1979; 18(6): 1037-1042.
11. Rastrelli L, Caceres A, Simone DF, Aquino R. Studies on the constituents of *Gliricidia sepium* leaves and roots: Isolation and structure elucidation of new triterpenoid saponins and aromatic compounds. J Agric Food Chem, 1999; 47(4): 1537-1540.
12. Herath HMTB, Silvia S. New components from *Gliricidia sepium*. Fitoterapia, 2000; 71(6): 722-724.
13. Rangaswami S, Iyer VS. Chemical examination of the leaves of *Gliricidia maculata.* Curr Sci, 1966; 14: 364-365.
14. Griffiths LA. On the co-occurrence of coumarin, o-coumaric acid and melilotic acid in *Gliricidia sepium*. J Expt Bot, 1962; 13: 169-175.
15. Murugesan R, Paliwal K. Allelopathic compounds in the leaves of *Gliricidia sepium* and its effect on *Sorghum vulgare* L. J Chem Ecol, 1993; 19(8): 1691-1701.
16. Rastrelli L, Berger I, Kubelka W, Caceres A, Tommasi DN, Simone DF. New 12a-Hydroxyrotenoids from *Gliricidia sepium* bark. J Nat Prod, 1999; 62(1): 188-190.
17. Molykutty MK, Arif MM, Jirovetz L, Shafi PM. Essential oil composition of *Gliricidia sepium* (Leguminosae) leaves and flowers. Ind J Chem, 2007; 46(B): 1359-1360.
18. Martinko JM, Madigan MT, editors. Brock Biology of Microorganisms. New York : Prentice Hall; 2005.
19. Olitzky I. Antimicrobial properties of a propylene glycol based topical therapeutic agent. J Pharm Sci, 2006; 54(5): 787-788.
20. Faergemann J, Fredriksson T. Antimycotic activity of propane-1, 2-diol. Med Mycol, 1980; 18(3): 163-166.
21. Simone MS, Franco DM, Artur SJ. Antibacterial activity of coumarins. Z Naturforsch, 2005; 60C: 693-700.
22. Togashi N, Shiraishi A, Nishizaka M, Matsuoka K, Endo K, Hamashima H. Antibacterial activity of long-chain fatty alcohols against *Staphylococcus aureus*. Molecules, 2007; 12: 139-148.
23. Didry N, Dubreuil L, Pinkas M. Antibacterial activity of thymol, carvacrol and cinnamaldehyde alone or in combination. Pharmazie, 1993; 48(4): 301-304.
24. Lucchini JJ, Corre J, Cremieux A. Antibacterial activity of phenolic compounds and aromatic alcohols. Res Microbiol, 1990;141(4):499-510.