Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 1, 2014

Research Article

PHYTOCHEMISTRY AND ANTIMICROBIAL EFFICACY OF *INDIGOFERA LONGIRACEMOSA* (FABACEAE)

DHARMASAMY PREMALATHA[1], RAMASAMY KAMARAJ[1], ARUMUGAME CHANEMOUGAME TANGAVELOU[2]*

[1]Department of Botany, Arignar Anna Govt. Arts College, Musiri 621 201, Tamil Nadu, [2]Bio-Science Research Foundation, 60, Cheran Street, Vengateswara Nagar, Saram, Pondicherry 605 013, South India. Email: actangavelou@hotmail.com

Received: 04 Sept 2013, Revised and Accepted: 01 Oct 2013

ABSTRACT

Objective: An ethnomedicinal plant, *Indigofera longiracemosa* Boiv. *ex* Baill. was investigated for preliminary phytochemical screening and antimicrobial activity.

Methods: Preliminary Phytochemical screening and antimicrobial activity were carried out by standard methods.

Results: Preliminary phytochemical screening of various extracts of the leaves revealed the presence of various classes of compounds such as amino acids, carbohydrates, flavonoids, gum, oil & resins, proteins, phenolic groups, saponins, steroids, tannins and terpenoids. Antimicrobial activity of the petroleum ether, chloroform and ethanolic leaf extract showed concentration-dependent activity against all the tested bacteria with the zone of inhibition at various concentrations.

Conclusion: Thus the findings revealed the medicinal potential of *I. longiracemosa* against various infectious diseases to develop a drug.

Keywords Ethnomedicine, *Indigofera longiracemosa*, Phytochemistry, Antimicrobial, Human pathogens, Drug development.

INTRODUCTION

The genus *Indigofera* L., of family Fabaceae comprises of about 700 species in the world and among them 50 species have been reported from India [1] while 32 species and 2 subspecies were reported from Tamil Nadu state [2]. The species *Indigofera longiracemosa* Baill., is an ethnomedicinal plant used traditionally for treating various diseases. Ethnomedicinally, Kani tribals of Tirunelveli hills, southern western ghats used leaves for the treatment of skin diseases and roots for snake bite. Chemical constituents such as 3-Isopropyl-9a-methyl-1,2,4a,9a-tetrahydroxanthene and rel-(3S, 5R, *6S*, 8R, 8aR, 12aR)- 8- acetoxy- 6- butyl- 3- isothio cyanatodehydropyrido (2,l) quinoline were isolated from stem [3,4]. After the scrutiny of published literature, so far only little work has been done on this selected plant. Hence the basic phytochemical investigation on the extracts for their main phytocompounds is very vital. In order to evaluate the ethnomedicinal information, the present study dealt with phytochemical screening and antimicrobial activity against various human pathogens.

MATERIALS AND METHODS

Plant Material and preparation of the Extracts

The leaves of *I. longiracemosa* were collected from Tirunelveli hills of southern western ghats, Tamil Nadu. The collected plant material was botanically identified and confirmed by the third author (ACT). The herbarium specimens were preserved and deposited at Bio-Science Research Foundation, Pondicherry (Voucher no. ACT77).

The leaves were chopped into small pieces, shade-dried and coarsely powdered by using a pulverizor. The coarse powders were then subjected to successive extraction with organic solvents such as petroleum ether, chloroform and ethanol by Soxhlet method. The extracts were then collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed *in vacuo* and stored at 4˚C. They were used for preliminary phytochemical screening and antimicrobial activity. The graded concentrations (100, 50, 25 and 12.5mg/ml) of different extracts were prepared for the bioassay.

Phytochemical Screening

Phytochemical analysis of the different plant extracts was performed using the standard methods described [5, 6].

Antimicrobial Activity

Test Organisms

All the microbial strains of human pathogens used in the antimicrobial bioassay were procured from Institute of Microbial Technology (IMTECH), Chandigarh. These microbes include the Gram-negative bacteria such as *Escherichia coli* (MTCC 724), *Proteus vulgaris* (MTCC 426), *Pseudomonas aeruginosa* (MTCC 741), *Salmonella typhi* (MTCC 733), *Vibrio parahaemolyticus* (MTCC 451) and *V. vulnificus* (MTCC 1145); the Gram-positive bacteria such as *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96) and *Streptococcus pneumoniae* (MTCC 655) and fungi such as *Aspergillus flavus* (MTCC 277), *A. fumigatus* (MTCC 343), *A. niger* (MTCC 1344) and *Candida albicans* (MTCC 227) respectively.

Bioassay

Agar well-diffusion method [7] was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 h old - broth culture of respective bacteria and fungi. Two wells (10mm diameter) were made in each of these plates using sterile cork borer. About 0.3 ml of different concentrations of plant solvent extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 h. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for fungal pathogens. Diameter of the inhibition zones was recorded. Triplicates were maintained and the experiment was repeated thrice and the average values were recorded.

RESULTS

Preliminary Phytochemical screening

The results of preliminary phytochemical screening were given in the Table 1. Flavonoids, quinones, phenolic groups, steroids and terpenoids were present in all the extracts. Aminoacids, proteins and saponins were present in petroleum ether extract but absent in chloroform and ethanol extracts. Carbohydrates were present in petroleum ether and chloroform extracts. Anthraquinones, catechins, coumarins, gum, oil & resins, and tannins were absent in all the three tested extracts.

Antimicrobial activity

The results of antimicrobial activity (Table 2) of the petroleum ether, chloroform and ethanol extract of leaves showed concentration-dependent activity against all the tested bacteria with the zone of inhibition ranged from 15-27 mm at various concentrations and the fungi ranged from 15-23 mm at various concentrations. The solvents used for extraction were used as control and all the solvent control did not show any activity. Standard antibiotics were also used along with the extracts for comparison.

Table 1: Shows the preliminary phytochemical screening of various extracts of the leaves of *I. longiracemosa*.

Phytoconstituents	Leaves extract		
	Petroleum ether	Chloroform	Ethanol
Alkaloids	-	-	-
Amino acids	+	+	-
Anthraquinones	-	-	-
Carbohydrates	+	+	-
Catechins	-	-	-
Coumarins	-	-	-
Flavonoids	+	+	+
Gums, oils and resins	-	-	+
Proteins	+	+	-
Phenolic groups	+	+	+
Quinones	+	+	+
Saponins	+	+	-
Steroids	+	+	+
Tannins	-	-	+
Terpenoids	+	+	+

+ = present ; - = absent

Petroleum ether extract showed the zone of inhibition ranged from 16 to 24 mm against the tested microorganisms. In gram-negative bacteria, maximum zone of inhibition was observed as 24 mm against *P. vulgaris*, 22 mm each against *P. aeruginosa* and *S. typhi* 100mg/ml, 19mm each against *E. coli*, *P. aeruginosa, P. vulgaris* at 50 mg/ml, *V. parahaemolyticus*, *V. vulnificus*, at 100 mg/ml concentration. In fungi maximum zone of inhibition was observed as 21 mm against *C. albicans* at 100 mg/ml concentration.

Chloroform extract showed the zone of inhibition ranged from 15 to 22 mm against the tested microorganisms.. In gram-positive bacteria, maximum zone of inhibition was observed as 22 mm against *S. aureus* at 100 and 50 mg/ml each, 21 mm against B.subtilis at 100mg/ml. In gram-negative bacteria, maximum zone of inhibition as 20 mm each against *E. coli* and *V. parahaemolyticus* at 100mg/ml concentration. In fungi maximum zone of inhibition was observed as 21 mm against *C. albicans* at 100 mg/ml concentration.

Ethanol extract showed the maximum zone of inhibition ranged from 19 to 27 mm against the tested microorganisms. In gram-positive bacteria, maximum zone of inhibition was observed as 27 and 22 mm against B.subtilis at 100 and 50 mg/ml, 24 mm against S.aureus at 100mg/ml, 21 mm each against B. subtilis at 25 mg/ml, against S.aureus at 100mg/ml and *S. pneumoniae* at 100 and 50 mg/ml concentration. In gram-negaive bacteria, maximum zone of inhibition was observed as 26 mm each against *E.coli*, *P. aeruginosa* 100 mg/ml, 24 mm each against *P. vulgaris* and *S. typhi* at 100 mg/ml, 22 mm each against *E. coli*, at 50 & 25 mg/ml, against *V. parahaeomolyticus* at 100 mg/ml, 21 mm each against *P. aeruginosa* at 50 mg/ml, *S. typhi* at 50 & 25 mg/ml concentration. In fungi maximum zone of inhibition was observed as 22 mm against *A. flavus* and 23 and 21 mm against *C. albicans* at 100 and 50 mg/ml concentrations respectively.

Table 2: Shows an antimicrobial activity of various extracts of the leaves of *I. longiracemosa*.

Test Microorganisms	Petroleum ether (mg/ml)			Chloroform (mg/ml)			Ethanol (mg/ml)			Standard drug (10 µg/ml)
Gram-positive bacteria	**100**	**50**	**25**	**100**	**50**	**25**	**100**	**50**	**25**	
B. subtilis	19	17	17	21	17	15	27	22	21	31 (A)
S. aureus	18	17	17	22	22	19	24	21	19	30 (A)
S. pneumoniae	19	16	15	19	17	17	21	21	19	31 (C)
Gram-negative bacteria										
E. coli	21	19	16	20	18	17	26	22	22	32 (A)
P. aeruginosa	22	19	16	17	15	-	26	21	18	33 (A)
P. vulgaris	24	19	-	19	18	18	24	20	18	31 (Cl)
S. typhi	22	17	-	16	-	-	24	21	21	30 (Cf)
V. parahaemolyticus	19	17	-	20	16	-	22	19	19	29 (K)
V. vulnificus	19	19	-	-	-	-	19	17	17	32 (K)
Fungi										
A. flavus	20	17	15	20	19	-	22	18	17	34 (N)
A. fumigatus	-	-	-	-	-	-	-	-	-	36 (N)
A. niger	-	-	-	-	-	-	19	17	16	31 (N)
C. albicans	21	19	19	21	20	17	23	21	20	33 (N)

(Measurement indicates the zone of inhibition in mm).

A – Ampicillin; Cl – Clotrimaxazole; Cf – Ciprofloxacin; K – Kanamycin; N – Nystatin

DISCUSSION

Medicinal plants are the richest bio-resources of drugs of traditional medicinal systems, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals, intermediate and chemical entitled for synthetic drugs [8, 9]. In plants, the medicinal value of the secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body [10-13]. In the present study, preliminary phytochemical screening revealed the presence of secondary metabolites such as flavonoids, quinones, phenolic groups, steroids and terpenoids in all the extracts. These bioactive secondary metabolites from plants were utilized more widely as medicines, both in their original and modified forms ([14-16]. Many plants have been used because of their antimicrobial traits and antimicrobial properties of plants have been investigated by a number of workers worldwide [7]. From the results of antimicrobial activity, it was found that the petroleum ether and ethanol extracts exhibited maximum inhibitory activity against the tested human pathogens. In our study, the maximum zone of inhibition against gram negative bacteria such as *E. coli*, *P. vulgaris*, *P. aeruginosa*, *S. typhi* and *V. parahaemolyticus* and against the fungi such as *A. flavus* and *C. albicans* might be attributed to the presence of secondary metabolites such as flavonoids, phenolic groups and steroids as suggested by previous reports [17-19].

The significant activity of the results of ethanol extract against the fungi, *A. flavus* and *Candida albicans* provides additional confirmation to the phenolic compounds and steroidal compounds which are more effective in higher concentration inhibited the

growth of all fungi [18,20,21]. Even in hospitals, majority of disinfectants such as phenols, lysol, cresols used are belonging to phenolic groups. Thus recent findings of antimicrobial activity against *P. aeruginosa*, *P. vulgaris, S. typhi*, and *V. parahaemolyticus* revealed the medicinal potential value of petroleum ether and ethanol extracts against abdominal pain, diarrhea, fever, nausea, septicaemia, urinary tract infections and vomiting, hospital-acquired wound infections, septicaemia and urinary tract infections by *P. vulgaris* and *P. aeruginosa,* typhoid fever by *S. typhi* and diarrheal infections by *Vibrio* species, skin related diseases by *C. albicans* and Aspergillosis and respiratory tract infections by *A. flavus* respectively. Thus the present study provides scientific evidence to claim the ethnomediicnal usages against skin diseases used by the Kani tribals of Tirunelveli hills. McCutcheon *et al*., [22] reported that most of the plants could be active against gram-positive than gram-negative bacteria. But in the present study, ethanol extract showed antimicrobial activity against both gram-positive and gram-negative bacteria. Thus, it revealed the medicinal potential to develop broad spectrum antibiotics of therapeutic interest.

CONCLUSION

Thus from our findings, it is concluded that the *I. longiracemosa* proved to be a potential medicinal to develop broad spectrum antibiotics against various human disease causing microorganisms. It is very essential that the bioactive molecules responsible for the antimicrobial activities against these tested microorganisms should be isolated identified and elucidated its structure to develop a new lead of therapeutic interest to cure various human ailments.

ACKNOWLEDGEMENT

The field man Mr. Sundaresan who helps during the field work are remembered here with thanks.

REFERENCES

1. Santapau H, Henry AN. A Dictionary of Flowering plants in India. New Delhi: Council of Scientific and Industrial Research; 1973.
2. Nair NC, Henry, AN. Flora of Tamil Nadu. Vol. 1. Coimbatore: Botanical Survey of India; 1983.
3. Thangadurai, D., Ramesh, N., Viswanathan, M.B. and Xavier Prasad, D. 2001b. A novel xanthene from *Indigofera longeracemosa* stem. Fitoterapia. 2001b; 72: 92-94.
4. Thangadurai, D., Viswanathan, M.B. and Ramesh, N.2001a. Characterization of a new decahydropyridoquinoline from *Indigofera longeracemosa* Boiv. ex Baill. (Fabaceae). Nat Prod. 2001a; 15(4): 287-290.
5. Trease GE, Evans WC. Pharmacognosy. London: Bailiere Tindal; 1983.
6. Harborne JB. A Guide to Modern techniques of Plant Analysis. USA: Kluwer Academic Publishers; 1998.
7. Perez C, Paul M, Bazerque P. Antibiotic assay by agar-well diffusion method. Acta Biol Med Exp. 1990; 15: 113-115.
8. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. J Med Plant Res. 2010; 4: 104–111.
9. Mazid M, Khan TA, Mohammad F. Role of secondary metabolites in defense mechanisms of plants. Biol Med. 2011; 3 (2): 232-249.
10. Anpin Raja RD, Prakash JW, Jeeva S. Antibacterial activity of some medicinal plants used by Kani tribe, southern Western Ghats, Tamilnadu and India. In: Trivedi, PC, editor. Ethnic Tribes and Medicinal Plants. Jaipur: Pointer Publishers; 2010. p. 28-45.
11. Nisar M, Ali S, Qaisar M. Preliminary Phytochemical Screening of Flowers, Leaves, Bark, Stem and Roots of *Rhododendron arboretum*. Middle-East J Sci Res. 2011; 10 (4): 472-476.
12. Tirupathi Rao G, Suresh Babu K, Ujwal Kumar J, Sujana P, Rao V, Sreedhar AS. Anti-microbial principles of selected remedial plants from southern India. Asian Pac J Trop Biomed. 2011; 1: 298-305.
13. Maobe, MAG, Gatebe E, Gitu L, Rotich H. Preliminary Phytochemical Screening of eight selected medicinal herbs used for the treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya. European J Applied Sci. 2013; 5 (1): 01-06.
14. Sneader W. Drug Prototypes and their Exploitation, UK: Wiley, Chichester; 1996.
15. Samuelsson G. Drugs of Natural Origin, 5th ed. Stockholm: Apotekarsocieteten; 2004.
16. Salim AA, Chin YW, Kinghorn AD. Drug Discovery from Plants. In: Ramawat KG, Merillon JM, editors. Bioactive Molecules and Medicinal Plants. Heidelberg: Springer-Verlag Berlin; 2008. p. 1-24.
17. Kosalec I, Pepeljnjak S, Bakmaz M, Vladimir-Knezevic S. Flavonoid analysis and antimicrobial activity of commercially available propolis products. Acta Pharm 2005; 55: 423–430.
18. Pereira AP, Ferriera ICFR, Marcelino F, Valento P, Andrade PB, Seabra R, Estevinho L, Bento A, Pereira JA. Phenolic Compounds and antimicrobial activity of Olive (*Olea europaea* L. cv. *cobrancosa*) leaves. Molecules. 2007; 12: 1153- 1162.
19. Lauro Figueroa V, Guillermo Ceballos R, Cedillo FD, Lopez, MDC, Magana, RME, Garcia Fuentes, LV. Evaluation and characterization of antimicrobial properties of pregnenolone-derivatives on *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*. Microbiologia. 2008; 50: 13-18.
20. Winkelhausen E, Pospiech R, Laufenbera G. Antifungal activity of phenolic compounds extracted from dried Olive Pomace. Bull Chem Tech Macedonia. 2005; 24: 41-46.
21. Subhisha S, Subramoniam A. Antifungal activities of a steroid from *Pallavacinia lyellii*, a liverwort. Indian J Pharmacol. 2005; 37: 304-308.
22. McCutcheon AR, Ellis SM, Hancock REW, Towers GHN. Antibiotic screening of medicinal plants of the British Columbian native peoples. J Ethnopharmacol. 1992; 37: 213-223.