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Antimicrobial activity of *Indigofera glandulosa* (wild)

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ABSTRACT

Our present study is aimed to detect the medicinal uses of the plant Indigofera glandulosa (wild) belonging to the family Fabaceae. The in vitro screening of antimicrobial properties of aqueous, organic solvent extracts (acetone, chloroform, ethanol and dimethyl formamide) and different combinations of extracts were evaluated against the growth of human pathogens viz. Pseudomonas putida, P. aeruginosa, Klebsiella pneumoniae, Aeromonas liquefaciens, Alcaligenes sp., Aspergillus niger, A. flavus, A. fumigatus, A. erythrocephalus and Fusarium sp.. Dimethyl formamide extracts of leaf and root expressed higher activity. Different combination of leaf extracts showed promising antimicrobial activity against tested pathogens. The ethanol and chloroform extracts showed moderate activity.

Key Words: *Indigofera glandulosa*, antimicrobial activity, organic solvents, human pathogens.

INTRODUCTION

Medicinal plants as a group, comprise approximately 8000 species and account for about 50% of all the higher flowering plant species in India. The World Health Organization (WHO) estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs. Also, modern pharmacopoeia still contains atleast 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. Demand for medicinal plants are increasing in both developing and developed countries due to growing recognition of natural products, being non-narcotic, having no side-effects and easily available at affordable prices. Plants have provided Western medicine with an abundance of drugs and treatments for a variety of health problems (Lewis and Elvin-Lewis, 1997; Bruneton, 1999).

The increasing interest on traditional ethnomedicine may lead to discovery of novel therapeutic agents. Antimicrobial drug resistance is also of economic concern with impact on medical practitioners, patients, health care administrators, pharmaceutical companies and the public (Gowan, 2001). The development of new antimicrobial drugs has been used to overcome resistance. However, plant-derived medicines have been part of traditional health care in most part of the world and the antimicrobial properties of plant derived compounds are well documented (Cowan, 1999) and there is increasing interest in plants as sources of antimicrobial agents (Charindy *et al*., 1999).

Indigofera is a large genus of about 700 species of flowering plants belonging to the family *Fabaceae.* They occur throughout the tropical and subtropical regions of the world, with a few species reaching the temperature zone in eastern Asia. The species are shrubs, though some are herbaceous and a few can become small trees up to 5-6 m (16 – 20 ft) tall (Bhalla and Dakwale, 1978; Augustine, 1993). Indigo is an important blue dyestuff, extracted from *Indigofera* species and used in the treatment of epilepsy, bronchitis, liver disease and psychiatric illness (Anand *et al*., 1979). Of the various *Indigofera* species, *Indigofera tinctoria* and *Indigofera suffruticosum* are especially used to produce the dye indigo (Leite *et al*., 2003). Several species of this group are used in anticancer therapy (Vieira *et al*., 2006). The herbs are generally regarded as an analgesic with anti- inflammatory activity. *Indigofera articulate* is used for toothache and swellings. *Indigofera aspalathoides* have also been used as anti – inflammatories (Rajkapoor *et al*., 2005). A patent was grant for use of *Indigofera arrecta* extract to relieve ulcer pain. Hence an attempt has been made to study the antimicrobial activities of *Indigofera glandulosa*.

MATERIALS AND METHODS

Sample collection

The whole plant of *Indigofera glandulosa* were collected from the non-irrigated cultivable lands, in and around Thanjavur, Tamil Nadu. The disease free and fresh plants were selected for the investigation.

Extract preparation

About 2 grams of fresh and healthy leaves and roots were taken for each solvents and surface sterilized with 0.1% mercuric chloride. Again the plant materials were washed throughly with distilled water (three times). The plant extracts were prepared using different organic solvents (acetone, ethanol, dimethyl formamide and chloroform) and aqueous. There are twelve different combination of plant extracts were prepared for antimicrobial efficacy.

Screening for Antimicrobial Assay

Antimicrobial activity was screened by agar well diffusion method (Perez *et al*., 1990). The plant extracts were tested for antimicrobial activity against bacterial pathogens (*Klebsiella pneumoniae, Pseudomonas aeruginosa, P. putida, Aeromonas liquefaciens* and *Alcaligenes* sp.) and fungal pathogens (*Aspergillus niger, A. flavus, A. fumigatus, A. erythrocephalus* and *Fusarium* sp.). The microorganisms were collected from Microbial Germ Plasm Culture Collection Unit at Sri Gowri Biotech Research Academy, Thanjavur and maintained in the laboratory by periodic subculture.

Preparation of nutrient agar medium

Beef extract	-	3 gms
Peptone	-	5 gms
Sodium chloride	-	5 gms
Agar	-	15 gms
Distilled water	-	1000 ml
pH	-	7

All the ingredients were weighed and put into the conical flask containing 1000 ml distilled water. The flask was sterilized by using an autoclave at 121ºC for 20 min at15 lbs pressure.

Preparation of potato dextrose agar medium

Potato	-	200 gms
Dextrose	-	20 gms
Agar	-	15 gms
Distilled water	-	1000 ml
pH	-	5.6

The potato tubers were peeled and weighed for about 200 gms. The tubers were chopped into small pieces with the help of a sterile knife. The chopped potatoes were transferred into a conical flask containing about 1000 ml of distilled water. The contents were boiled for 20 minutes. The supernatant was decanted and filtered through muslin cloth and the filtrate was collected. To this filtrate dextrose and agar were added and shaked well to dissolve the ingredients and made up to 1000 ml by addition of distilled water. Finally, the medium was autoclaved at121ºC for 20 mins at 15 lbs pressure. Streptomycin sulphate (50µg/ml) was added and mixed well to prevent the bacterial contamination. The nutrient agar medium and potato dextrose agar medium were poured into the sterile petri plates and allowed to solidify. The test bacteria and fungal cultures were evenly spreaded over the appropriate media by sterile cotton swabs. Then wells (6 mm) were made in the medium using sterile cork borer. 100 µl of each concentration of extracts were transferred into the separate wells. Streptomycin, Erythromycin, Amphicillin and Amphotericin B (15 mcg/disc) were used as positive control (standard) for bacteria and fungi respectively. The organic solvents were used as negative controls. Then the plates were incubated at 37ºC for 24 hrs and 27ºC for 48 – 72 hrs for bacteria and fungi respectively. After the incubation the plates were observed for formation of clear inhibition zone around the well indicated the presence of antimicrobial activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well.

RESULTS

The antibacterial activity of leaf extracts from *Indigofera glandulosa* was represented in Table 1. The results showed that the ethanolic leaf extract exhibited better inhibitory effect against all the bacterial species followed by chloroform and dimethyl formamide extract. The acetone leaf extracts exhibited moderate antibacterial activity against *Alcaligenes* sp. (9 mm) and *Aeromonas liquefaciens* (13mm). The results of antifungal assay of aqueous and organic solvent extracts were represented in Table 2. The study revealed that the aqueous extract showed maximum zone of inhibition and was followed by dimethyl formamide and ethanolic extracts. The acetone leaf

extract have maximum antifungal activity against *Fusarium* species. Among the different combination of the extracts tested the water:acetone:ethanol (1:1:1) showed maximum activity against the tested bacteria (Table 3). The result of *in vitro* antifungal assay of the combined formulations of the different extracts showed that water acetone mixture showed maximum zone of inhibition against *A. fumigatus* (25 mm) and *A. flavus* (23 mm) (Table 4). The root extracts of *Indigofera glandulosa*, the organic and aqueous extracts were tested against the selected bacterial strains. The dimethyl formamide extract have excellent activity against all test bacterial strains and was followed by ethanol, chloroform and acetone extracts (Table 5).

The results of antifungal properties of aqueous and organic solvents root extracts of *I. glandulosa* were presented in Table 6. The aqueous extract showed moderate inhibitory effect against *Aspergillus niger* (12 mm) and *A. fumigatus* (11 mm). Among the different combinations of root extracts showed maximum activity was observed in ethanol:chloroform:water followed chloroform:ethanol:acetone. The maximum zone of inhibition was observed in *Pseudomonas aeruginosa* (13 mm), *A. liquefaciens* (14 mm) and *Alcaligenes* sp. (15 mm) (Table 7). The antifungal properties of different combination of root extracts of *I. glandulosa* showed maximum activity in dimethyl formamide:chloroform extract. Among the tested spesies *F. oxysporum* showed maximum activity (Table 8).

Table 1: Antibacterial activity of leaf extracts of *Indigofera glandulosa*

Solvents Used	Inhibition of Growth (Diameter in mm)				
	Pseudomonas putida	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Aeromonas liquefaciens***	***Alcaligenes* sp.**
Acetone	-	9	-	9	13
Ethanol	16	12	15	13	13
Chloroform	18	14	18	-	11
Dimethyl formamide	11	10	10	10	11
Water	-	-	-	-	-
Streptomycin (15 mcg/disc)	12	-	17	22	22
Erythromycin (15 mcg/disc)	12	11	15	19	19
Amphicillin (15 mcg/disc)	-	14	-	-	-

Table 2: Antifungal activity of leaf extracts of *Indigofera glandulosa*

Solvents Used	Inhibition of Growth (Diameter in mm)				
	Aspergillus niger	***Aspergillus flavus***	***Aspergillus fumigatus***	***Aspergillus erythrocephalus***	***Fusarium* sp.**
Acetone	-	-	8	10	25
Ethanol	15	10	15	10	18
Chloroform	-	-	-	-	10
Dimethyl formamide	20	9	10	13	15
Water	12	15	20	15	21
Amphotericin B (15mcg/disc)	14	15	-	-	15

Table 3: Antibacterial activity of different combinations from leaf extracts of *Indigofera glandulosa*

Different Combinations of Solvents	Inhibition of Growth (Diameter in mm)				
	Pseudomonas putida	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Aeromonas liquefaciens***	***Alcaligenes* sp.**
W: A (1:1)	10	12	8	7	15
W: E (1:1)	11	10	6	10	9
W: D (1:1)	11	9	9	9	13
W: C (1:1)	9	7	11	7	9
A: E (1:1)	11	15	10	10	12
D: C (1:1)	10	9	11	9	8
E: D (1:1)	10	11	10	8	15
W: A: E (1:1:1)	13	10	12	10	15
W: A: D (1:1:1)	12	13	8	8	12
W: A: E (1:1:1)	9	9	10	11	11
C: E: A (2:1:1)	11	12	8	11	9
E: C: W (12:5:3)	13	11	7	11	8

W - Water; A – Acetone; C – Chloroform; D - Dimethyl formamide; E – Ethanol

Table 4: Antifungal Activity of Different Combinations from Leaf Extracts of *Indigofera glandulosa*

Different Combinations of Solvents	Inhibition of Growth (Diameter in mm)				
	Aspergillus niger	***Aspergillus flavus***	***Aspergillus fumigatus***	***Aspergillus erythrocephalus***	***Fusarium sp.***
W: A (1:1)	-	23	25	-	8
W: E (1:1)	-	10	11	10	12
W: D (1:1)	11	10	11	8	10
W: C (1:1)	-	-	20	-	-
A: E (1:1)	-	10	10	10	20
D: C (1:1)	12	-	10	11	-
E: D (1:1)	12	8	10	8	22
W: A: E (1:1:1)	10	-	-	8	11
W: A: D (1:1:1)	11	-	-	10	11
W: A: E (1:1:1)	-	-	-	-	10
C: E: A (2:1:1)	-	10	10	8	11
E: C: W(12:5:3)	-	-	-	-	-

***W** - Water; **A –** Acetone; **C** – Chloroform; **D** - Dimethyl formamide; **E** - Ethanol*

Table 5: Antibacterial Activity of Root Extracts of *Indigofera glandulosa*

Solvents Used	Inhibition of Growth (Diameter in mm)				
	Pseudomonas putida	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Aeromonas liquefaciens***	***Alcaligenes* sp.**
Acetone	-	11	12	7	10
Ethanol	12	15	13	15	15
Chloroform	10	12	13	11	11
Dimethyl formamide	15	14	16	20	10
Water	-	-	-	-	-

Table 6: Antifungal Activity of Root Extracts of *Indigofera glandulosa*

Solvents Used	Inhibition of Growth (Diameter in mm)				
	Aspergillus niger	***Aspergillus flavus***	***Aspergillus fumigatus***	***Aspergillus erythrocephalus***	***Fusarium* sp.**
Acetone	-	-	-	-	7
Ethanol	-	-	7	9	11
Chloroform	-	-	9	10	11
Dimethyl formamide	-	-	-	8	10
Water	12	-	11	-	-

Table 7: Antibacterial Activity of Different Combinations from Root Extracts of *Indigofera glandulosa*

Different Combinations of Solvents	Inhibition of Growth (Diameter in mm)				
	Pseudomonas putida	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Aeromonas liquefaciens***	***Alcaligenes* sp.**
W: A (1:1)	-	7	-	7	7
W: E (1:1)	11	7	-	9	11
W: D (1:1)	8	7	7	7	-
W: C (1:1)	6	8	6	9	8
A: E (1:1)	10	12	9	10	10
D: C (1:1)	12	10	7	6	10
E: D (1:1)	11	9	9	7	11
W: A: E (1:1:1)	7	10	7	7	11
W: A: D (1:1:1)	7	-	-	-	-
W: A: E (1:1:1)	10	8	-	8	9
C: E: A (2:1:1)	11	12	7	11	10
E: C: W(12:5:3)	12	13	15	14	15

W *- Water;* ***A –*** *Acetone;* ***C*** *– Chloroform;* ***D*** *- Dimethyl formamide;* ***E*** *- Ethanol*

Table 8: Antifungal Activity of Different Combinations from Root Extracts of *Indigofera glandulosa*

Different Combinations of Solvents	Inhibition of Growth (Diameter in mm)				
	Aspergillus niger	***Aspergillus flavus***	***Aspergillus fumigatus***	***Aspergillus erythrocephalus***	***Fusarium* sp.**
W: A (1:1)	-	-	-	-	-
W: E (1:1)	-	7	8	7	12
W: D (1:1)	-	-	-	-	11
W: C (1:1)	7	7	7	8	9
A: E (1:1)	7	8	9	6	10
D: C (1:1)	7	7	12	10	11
E: D (1:1)	7	7	14	6	12
W: A: E (1:1:1)	-	-	-	-	-
W: A: D (1:1:1)	-	7	-	-	10
W: A: E (1:1:1)	-	8	8	10	13
C: E: A (2:1:1)	-	7	8	7	11
E: C: W(12:5:3)	-	8	9	7	17

W *- Water;* ***A –*** *Acetone;* ***C*** *– Chloroform;* ***D*** *- Dimethyl formamide;* ***E –*** *Ethanol*

DISCUSSION

Recent studies focused on the biological activity of *Indigofera* species that is antimicrobial activity *viz. Indigofera oblongifolia* (Dahot, 1999), *Indigofera sedgewickiana* (Alasbahi *et al*., 1999), *Indigofera suffruticosa* (Leite *et al.*, 2006), *Indigofera subulata* (Ramachandran *et al*., 2006) and *Indigofera longeracemosa* (Thangadurai *et al*., 2002).

Mathur *et al*., (2011) reported that the antimicrobial activity of the hydro-alcoholic extracts of leaves, bark and fruits of *Ficus racemosa* L. by well diffusion method. The extracts of each of the parts of the tree were found to be most potent antibacterial agent in comparison to fungal strains. The leaf extracts showed potent activity against *Aspergillus niger* (10 mm) and showed moderate activity against *Candida albicans* (8 mm).

This investigation was comparable to earlier findings, that is, the pronounced antibacterial activity of *Indigofera longeracemosa* was tested against selected bacterial pathogens

(Thangadurai *et al.*, 2002). This study was also supported by Natarajan *et al*., (2010) reported that antibacterial activities of aqueous, hexane, chloroform and methanol extracts from the leaves of *I. caerulea* showed wide spectrum of activity against tested microorganisms namely *Escherichia coli, Klebsiella pneumoniae, Sslmonella typhi, Vibrio parahaemolyticus, V. cholerae, Bacillus subtilis* and *Streptococcus pneumoniae.* The antimicrobial properties of different extracts from the leaves of *I. dendroides* exhibited significant activity against pathogenic microbial populations (Esimone *et al.*, 1999). Recently, Rosy *et al*., (2010) reported that the chloroform extract of *Indigofera aspalathoides* Vahl. exhibited very promising antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella* sp. *Enterobacter* sp., *Proteus vulgaris* and *Proteus mirabilis*. Similarly Sule *et al*., (2010) evaluated the antifungal activity of *Senna alata linn*. Crude leaf extract exhibited moderate activity against *Microsporum canis, Trichophyton jirrucosum, Trichophyton mentagrophytes* and *Epidermophyton jlorrcosum.*

In the present study revealed that the different extracts of root and leaf in *Indigofera glandulosa* showed broad spectrum of antimicrobial activity against tested pathogens. The results from this investigation indicates that the medicinal plant extracts offer significant potential for the development of novel antimicrobial therapies and treatments of several diseases caused by microorganisms. This study supports further research will be needed for identification of the bioactive compounds of the plant which are responsible for the pharmacological action against the disease causing human pathogens.

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