Journal of Medicinal Plants Research Vol. 4(15), pp. 1561-1565, 4 August, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR09.513
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

Screening for antibacterial, phytochemical and pharmacognostical properties of *Indigofera caerulea* Roxb.

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Accepted 8 July, 2010

The purpose of this investigation was to screen antibacterial, preliminary phytochemical and pharmacognostical potentials of pulverized leaf extracts from *Indigofera caerulea* a dye yielding plant. Different aqueous (cold, boiled, autoclaved) and crude organic solvents (hexane, chloroform methanol) extracts of *I. caerulea* were tested against both Gram negative (*Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Vibrio parahaemolyticus, Vibrio cholera)* and Gram positive (*Bacillus subtilis* and *Streptococcus pneumonia*) bacterial strains by performing cup plate method. The results highlighted most of the bacteria exhibited better antibacterial activity. Aqueous and hexane extracts exhibited good antibacterial properties in comparison to others. The results of preliminary phytochemical and pharmacological action of this taxon are discussed. This plant may be used for treatment of several diseases caused by pathogenic microbes. This study recommends future research regarding the pharmacological investigations (drug designs) of this plant.

Key words: *Indigofera caerulea,* antibacterial tests, phytochemical and pharmacognostical analysis, water and solvent extracts.

INTRODUCTION

Nowadays, several synthetic antibiotics are employed in the treatment of infectious and communicable diseases, caused by microorganisms in human and animals throughout the world. Many researches are working seriously to find out substitutes for antibiotics as they cause side effects on the functioning of different parts of the body, organs and systems. Over the last twenty years, intensive efforts have been made to discover clinically useful antimicrobial drugs (Valsaraj et al., 1997; Perumalsamy and Ignacimuthu, 2000). 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).

Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments (Kirtikar and Basu, 1991; Ambasta, 1992). Indigo is an important blue dyestuff, extracted from *Indigofera* species

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and used in the treatment of epilepsy, bronchitis, liver disease and psychiatric illness (Anand et al., 1979). 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 longeracemosa* (Thangadurai et al., 2002) and phytochemical analysis (Hasan et al., 1996; Thangadurai et al., 2001a, b). The main aim of this study was to assess the aqueous and organic solvents extracts of *Indigofera caerulea* (surat indigo) for antibacterial, preliminary phytochemical and pharmacognostical properties.

MATERIALS AND METHODS

Plant materials

The fresh and healthy leaves of *I. caerulea* (Papilionoideae) were collected during the morning hours/flowering season, from the foothills of Pacchaimalai Hills, a part of Eastern Ghats of Tamil Nadu, India. The plant materials were identified by available literature (Matthew, 1993) and matched with herbarium records (Rapinat Herbarium, St. Joseph's College, Tiruchirappalli). The reference specimens were deposited in the Herbarium, Department of Biotechnology, Periyar University, Salem, Tamil Nadu, India.

Extraction of plant materials

The collected leaves were shade-dried and coarsely powdered by using mixer grinder. These coarse powders (25 g) were then subjected to successive extraction in various solvents by gradually increasing the polarity such as hexane, chloroform and methanol (each 250 ml) by using Soxhlet apparatus. The collected extracts were then taken up for further investigations. Simultaneously, three different aqueous (cold, boiled, autoclaved) used for extraction of plant powders and the extractants were stored. The DMSO (Dimethyl sufloxide) is act as dissolved solvents for these extracts.

Screening of antibacterial activity

Bacteria tested

Totally seven bacterial strains were used throughout the investigation namely *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Vibrio parahaemolyticus, Vibrio cholerae* (Gram negative), *Bacillus subtilis* and *Streptococcus pneumoniae* (Gram positive). All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

Antibacterial activity assay

Antibacterial activity was screened by cup-plate method (Onkar et al., 1995) with few modifications. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with eight hours old-broth culture of respective bacteria. The sterilized agar media was poured in a large sized Petri plates (4 mm depth) and then allowed to solidify at room temperature. The broth cultures were swabbed (using sterile cotton or L-rod) on top of medium. Using sterile cork borer, the well (3 mm wide) was made in each Petri dish. The plant extracts (aqueous and different concentration of organic solvents) and negative control drugs were loaded into the well by using micropipette. The respective standard drugs (Tables 3 and 4) were tested for positive control (Ramesh et al., 2001), the plates were incubated at 37°C for 24 h. The diameter of the inhibition zones observed and its values noted (in mm). Triplicates were maintained in each extract/ organism, and the average values were calculated.

Preliminary phytochemical analysis

The preliminary phytochemical studies were carried out by the methods described by Harborne (1998) and Kokate et al. (2003) with some modifications. The plant extracts was assayed for the presence of alkaloids, proteins, free amino acids, anthraquinone glycosides, flavonoids, tannins, phenolic compounds, carbohydrates, saponins, phytosterol and triterpenes.

Pharmacognostical analysis

The pharmacognostical aspects of extracts were carried out by florescence analysis (Chase and Pratt, 1949) and the physico-chemical parameters such as total ash, water-soluble ash, acid insoluble ash and loss on drying were determined as per the method described by Wallis (1989). The successive extraction with organic solvents, in the order of increasing polarity (using Soxhlet apparatus) was carried out the procedure of Indian Pharmacopoeia (Anonymous, 1985). Finally, the percentage of solubility was calculated.

RESULTS

The results of preliminary phytochemcial and pharmacognostic analysis on the leaves of different solvents extracts of *I. caerulea* showed for the presence of some preliminary phytochemical substances like alkaloids, aminoacids, glycosides, triterpenoids, steroids, phenols, tannins, proteins, glycosides, saponins (Table 1) and pharmacognostic studies such as fluorescence analysis (Table 2), ash value (5.2%), insoluble ash value (3.0%), water insoluble ash (3.4%) extractive value (hexane 2.7%, chloroform 3.9%, methanol 17.5%) and loss of drying (4.7%).

The results of antibacterial activities of aqueous, hexane, chloroform and methanol extracts from the leaves of *I. caerulea* showed wide spectrum of activity against tested microorganisms namely *E. coli, K. pneumoniae, S. typhi, V. parahaemolyticus, V. cholerae* and Gram positive bacteria namely *B. subtilis* and *S. pneumoniae* (Tables 3 and 4). The cold, boiled and autoclaved aqueous extracts (50 and 100%) were tested against seven bacteria (Table 3).

The cold-water extract exhibited significant activity against all the organisms except *S. pneumoniae*. The boiled water showed good activity against *V. cholerae* followed by *B. subtilis, V. parahaemolyticus, K. pneumoniae* and *S. typhi.* Autoclaved water extracts expressed better activity in *V. cholerae, K. pneumoniae, V. parahaemolyticus, B. subtilis* and the growth of the remaining bacterial strains was not affected. Hexane

Table 3. Antibacterial activities of aqueous extracts of *I. caerulea* (Diameter of zone of inhibition in mm).

Name of the bacteria	Aqueous extract (µg/ml)						Standard*
	Cold (%)		Boiled (%)		Autoclave (%)		
	50	100	50	100	50	100	
E. coli	20	24	-	-	-	-	24(A)
K. pneumoniae	26	31	20	23	16	19	34(A)
S. typhi	26	31	14	18	-	-	36(Cf)
V. parahaemolyticus	27	30	20	24	15	17	24(T)
V. cholerae	23	26	26	30	25	28	26(T)
B. subtilis	25	27	20	25	13	17	30(S)
S. pneumoniae	-	-	-	-	-	-	33(Ce)

* A - Ampicillin (30 µg/ml); Cf - Ciproflaxacin (30 µg/ml); T - Tetracycline (30 µg/ml); S - Streptomycin (30 µg/ml); Ce - Cephalosporin (30 µg/ml), - = Nil activity.

Table 4. Antibacterial activity of organic solvents extract of *I. caerulea*.

Organisms tested	Diameter of zone of inhibition (in mm)												Standard antibiotic*
	Extract concentrations												
	Hexane (µg/ml)				Chloroform (µg/ml)				Methanol (µg/ml)				
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	
E. coli	22	24	-	-	-	-	-	-	20	18	16	14	24(A)
K. pneumoniae	26	31	20	22	-	-	-	-	20	18	14	13	34(A)
S. typhi	24	30	15	19	-	-	-	-	-	-	-	-	36(Cf)
V. parahaemolyticus	30	30	18	21	21	21	20	18	18	16	15	13	24(T)
V. cholerae	24	25	29	29	18	16	15	15	-	-	-	-	26(T)
B. subtilis	25	27	20	27	-	-	-	-	-	-	-	-	30(S)
S. pneumoniae	-	-	-	-	-	-	-	-	-	-	-	-	33(Ce)

* A - Ampicillin (30 µg/ml), Cf - Ciproflaxacin (30 µg/ml), T - Tetracycline (30 µg/ml); S – Streptomycin (30 g/ml), Ce - Cephalosporin (30 µg/ml), -Nil activity.

Table 1. Preliminary phytochemical screening of the leaves of *I. caerulea*.

Constituents	Hexane	Chloroform	Methanol
Alkaloids	+	+	+
Aminoacids	+	+	+
Anthroquinone glycosides	-	-	-
Coumarins	-	+	+
Flavones	-	-	+
Oils	-	-	-
Phenolic groups	+	+	+
Quinones	+	+	+
Saponins	-	-	+
Steroids	+	+	+
Sugars	-	-	+
Tannins	-	-	+
Triterpenes	+	+	+

+ = Present, - = Absent.

Table 2. Fluorescence analysis of aerial part of I. caerulea.

Nature of extracts	Colour perception under normal light	Colour perception under UV light
Powder as such	Bluish green	Black
Benzene	Dark red	Brown
Chloroform	Reddish brown	Brown
Petroleum ether	Purple green	Green
Ethyl acetate	Reddish brown	Brown
Ethanol	Reddish brown	Brown
Water	Dark Brown	Brown
1 N HCL	Bluish green	Brown
Aq. 1 N NAOH	Purple green	Brown
1 N NAOH in methanol	Dark brown	Purple brown
50% HNO_3	Reddish orange	Yellowish orange
50% H_2SO_4	Dark brown	Brown

extracts was found to have better inhibitory effect against *K. pneumoniae, S. typhi*, *V. parahaemolyticus*, *V. cholerae* and *B. subtilis* in all concentrations of the extracts (that is 12.5, 25, 50 and 100 mg/ml). The same extract showed moderate activity against *E. coli* at highest concentration alone. All the other organisms found to be resistant. The chloroform extracts (in varying concentrations) exhibited significant activity against *V. cholerae* followed by *B. subtilis.* The remaining bacterial pathogens found to be inactive. The different concentrations of methanolic extracts showed moderate activity against *E. coli* followed by *K. pneumoniae* and *V. parahaemolyticus* respectively. All the other bacterial strains were not susceptible to the plant extracts tested (Table 4).

DISCUSSION

Herbal plants are nature's gift used to prevent and control the diseases in all over the world. This study was supports *in vitro* screening of antibacterial, phyto-constituents and pharmacognostical approach of the selected taxon. The results highlighted that antibacterial activities of crude extracts of *I. caerulea* were tested against seven bacterial strains and better activity was noted in most of the bacteria (*E. coli, K. pneumoniae, S. typhi, V. parahaemolyticus, V. cholerae* and *B. subtilis).* The organic solvents extracts of medicinal herbs contributed better antibacterial activity because of the easy extraction of bioactive chemical constituents. This investigation was comparable to earlier findings, that is, the antibacterial activity of chloroform, acetone, methanol and aqueous extracts of *Andrographis echiodes* at different concentrations were tested against seven strains of bacteria (Umadevi et al., 2003). This study was also supported by Cimanga et al. (2003) showed two extracts n-hexane and MeOH (80%) from *Mitracarpus scaber* leaves exhibited a pronounced antibacterial activity, based on their concentrations of extracts. The results from pharmacognostical study of *I. caerulea,* showed the fluorescence analysis and percentage of ash content of the plant. This study was correlated with several researchers who have done similar type of investigations on different plant species namely *Grewia tilifolia* (Badami et al., 2002), *Tridax procumbens* (Suseela et al., 2002) and *Senna uniflora* (Vijai et al., 2004) respectively. The overall results focuses the plant extract may be used as potent bacteriostatic/bactericidal agents against bacterial strains.

The results from this investigation indicates that the medicinal plants extracts offer significant potential for the development of novel antibacterial 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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