RESEARCH PAPER Asian Journal of Bio Science, Vol. 4 Issue 2 : 230-234 (October, 2009 to March, 2010)

Studies on antibacterial, phytochemical and pharmacognostical activities of *Indigofera longeracemosa*

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(Accepted : August, 2009)

Antibacterial screening of the crude extracts of *Indigofera longeracemosa* were tested against seven important human pathogenic bacterial strains including gram-negative such as *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Vibrio parahaemolyticus, Vibrio cholerae* and gram–positive namely *Bacillus subtilis* and *Streptococcus pneumoniae*. The results highlighted that most of the bacterial species exhibited better growth inhibitory activity. Aqueous and hexane extracts exhibited good antibacterial properties than other two extracts. Furthermore studies pertaining preliminary phytochemical and pharmagonostic analysis have been investigated.

Key words : Phytochemical and pharmaceutical analysis, Antibacterial tests, Water and solvent extracts, *Indigofera longeracemosa*.

INTRODUCTION

Now a days several synthetic antibiotics are employed in the treatment of infectious and communicable diseases, caused by microorganisms in human as well as animals throughout world. A number of 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; Ahmed *et al.*, 1998; Werner *et al.*, 1999; Perumalsamy *et al.*, 1999; Perumalsamy and Ignacimuthu, 2000). The increasing interest on traditional ethno medicine may lead to discovery of novel therapeutic agents. Branther and Edith Grein (1994) stated that natural products of higher plants may offer a new source of antibacterial agents for external use, e.g., compresses, cataplasms, gargles and ointments.

Antimicrobial drug resistance is also of economic concern with impact on doctors, patients, health care administrators, pharmaceutical companies and the public (Mcgowan, 2001). The development of new antimicrobial drugs has been used to overcome resistance (Monroe and Polk, 2000). However, plant-derived medicines have been part of traditional heath care in most part of the world and the antimicrobial properties of plant derived compounds is well documented (Cowan, 1999). There is increasing interest in plants as sources of antimicrobial agents (Charindy *et al.*, 1999; Palmbo and Semple, 2002).

Indian subcontinent is a vast depository of medicinal plants that are used in traditional medical treatments (Chopra *et al.*, 1956; Kirtikar and Basu, 1991; Ambasta, 1992).

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). Recent studies focus on the several *Indigofera* species have been tested for its 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 and b). The aim of this study was to investigate the further antibacterial, phytochemical and pharmacognostical analysis of aqueous and organic solvent extracts of *Indigofera longeracemosa*.

MATERIALS AND METHODS

Plant collection and extract preparation:

Leaves of *Indigofera longeracemosa* Boiv. ex Baill. (Papilionoideae) were collected from the foothills of Kolli Hills, Salem district of Tamil Nadu during the month of October to December 2004. The collected plants were shade-dried and coarsely powdered by using pulvarisor. The coarse powders were then subjected to successive extraction in various solvents by gradually increasing the polarity such as hexane, chloroform and methanol by using Soxhlet apparatus. The collected

extracts were then taken up for further investigations.

Antibacterial activity study:

Bacteria tested:

A total of seven bacterial strains were used, including gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Vibrio parahaemolyticus, Vibrio cholerae* and gram-positive bacteria namely *Bacillus subtilis* and *Streptococcus pneumoniae*. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

Screening of antibacterial activity:

Antibacterial activity was screened by cup-plate method (Onkar *et al.,* 1995). Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with eight hours old - broth culture of respective bacteria. All the ingredients were accurately weighed and dissolved in distilled water, and transferred to a suitable container and autoclaved at 121^0 C for 15 minutes. It melts at 90^0C and solidified only when cool to about 40^0C. The media was poured in a large sized Petriplates to uniform depth of 4 mm and then allowed to solidify at room temperature. Just prior to use, culture in an incubator was dried at 37^0C with a lid partly opened until the surface was free from visible moisture (15 to 20 minutes).

The plates were inoculated with 15 minutes after preparing the inoculum with a wax pencil, the plate was divided into section according to the number of standard and sample solutions to be used. Sterilized cotton swab was dipped into the nutrient broth, excess fluid was removed by rotating the swabs with firm pressure against the inside of the tube above third level. Using the sterile cork borer, the well about 3mm wide was made. Test and control drugs were added into the cup plate by using micropipette. Then the plates were incubated at 37ºC in incubator. 100 mg/ml, 50 mg/ml 25 mg/ml and 12.5 mg/ml concentration of hexane, chloroform and methanol extracts with respective solvents as control, aqueous in cold, boiled and autoclaved in 100 and 50 per cent concentrations (Ramesh *et al.* 2001) along with respective standard drugs were tested against the pathogens. Diameter of the inhibition zones observed and its values noted. Triplicates were maintained and the experiment was repeated thrice and the average values were calculated for antibacterial activity.

Preliminary phytochemical study:

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

Pharmacognostical analysis:

The pharmacognostical analysis on *B. floccifera* were carried out by using florescence analysis (Chase and Pradt, 1949), physico-chemical parameters such as total ash, water-soluble ash, acid insoluble ash and loss on drying were determined (Wallis, 1989). The successive extraction with organic solvents in the order of increasing polarity using a Soxhlet apparatus was carried out following the Indian Pharmacopoeia (Anonymous, 1985). The percentage of solubility was calculated.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below:

Preliminary phytochemical and pharmacognostical screening:

The results of preliminary phytochemcial and pharmacognostic analsyis on the leaves of different solvents extracts of *Indigofera longeracemosa* 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

Table 1 : Preliminary phytochemical screening of various extracts of the leaves of *Indigofera longeracemosa*

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

+ = Present; - = Absent

Table 2 : Florescence analysis of aerial part of *Indigofera longeracemosa* under normal and UV light

Chemicals/ Reagents	Normal light	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. 1N NAOH	Purple green	Brown
1N NAOH in methanol	Dark brown	Purple brown
50% HNo_3	Reddish orange	Yellowish orange
50% H_2So_4	Dark brown	Brown

(5.2%), insoluble ash value (3.0%), water insoluble ash (3.4%) extractive value (hexane 2.7%, chloroform 3.9% and methanol 17.5%) and loss of drying (4.7%). Several researchers contributed similar type of investigations on made with different plant species namely *Grewia tilifolia* (Badami *et al.,* 2002), *Tridax procumbens* (Suseela *et al.*, 2002), *Senna uniflora* (Vijai *et al.*, 2004) and *Dysoxylum* species (Parcha *et al.,* 2004).

Antibacterial activity:

The results of antibacterial activities of aqueous, hexane, chloroform and methanol extracts from the leaves of *Indigofera longeracemosa* showed wide spectrum of activity against tested microorganisms namely *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Vibrio parahaemolyticus, Vibrio cholerae* and gram-positive bacteria namely *Bacillus subtilis* and *Streptococcus pneumoniae* (Table 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 *Vibrio 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 remaining bacterial strains did not show any activity.

Hexane 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 (*i.e.* 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*. All the other bacterial strains were not susceptible to the plant extracts tested.

The result highlighted that antibacterial activities of crude extracts of *Indigofera longeracemosa* were tested against seven bacterial strains and better activity was noted in most of the bacterial members namely *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Vibrio parahaemolyticus, Vibrio cholerae* and *Bacillus subtilis*. Aqueous and hexane extracts exhibited good antibacterial properties than other two extracts.

Similarly, Umadevi *et al.* (2003) investigated that the antibacterial activity of chloroform, acetone, methanol and aqueous extracts of *Andrographis echiodes* at different concentrations were tested against seven strains of bacteria. Like wise, Radha *et al.* (2003) studied the

Table 3 : Antibacterial activities of aqueous extracts of *Indigofera longeracemosa*

Name of the bacteria	Aqueous Extract						Standard*
	Cold (%)		Boiled (%)		Autoclave (%)		
	50	100	50	100	50	100	
Escherichia coli	20	24	-	-	-	-	24(A)
Klebsiella pneumoniae	26	31	20	23	16	19	34(A)
Salmonella typhi	26	31	14	18	-	-	36(Cf)
Vibrio parahaemolyticus	27	30	20	24	15	17	24(T)
Vibrio cholerae	23	26	26	30	25	28	26(T)
Bacillus subtilis	25	27	20	25	13	17	30(S)
Streptococcus 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) - = No activity

antimicrobial activity of different extracts (chloroform, ethyl acetate, methanol and water) of *Heliotropium marifolium*. The findings showed potential antimicrobial properties against the organisms tested. This study was 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.

The results from present investigation focused most of the organisms were found to have significant activity against the extracts tested. This study also suggests that crude extracts of such medicinal plant may be used for treatment of several infectious diseases.

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