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Research Article

ANTIMICROBIAL ACTIVITY OF *INDIGOFERA BARBERI*, A HIGH VALUED ENDEMIC HERB OF TIRUMALA HILLS

*** R.K. SreeLatha Devi and S.K.M.Basha**
NBKR Medicinal Plant Research Centre, Vidyanagar – 524413, SPSR Nellore (Dt)
**Author for Correspondence*

ABSTRACT
The present study was aimed at antimicrobial potency of *Indigofera barberi* Gamble (Fabaceae) a high valued medicinal plant of Tirumala hills, Andhra Pradesh, India. Information about *Indigofera barberi* was highly recommended and used by ethnic healers and enriches enthomedicobotanical data to provide a new or an additional information regarding efficacy as antimicrobial agent. Our findings provided evidence that the crude aqueous extract of *I. barberi* is a potential source of natural antimicrobial agent and justifies its uses in folkloric medicines. The results of this preformulation study indicates that the powdered extract of *I. barberi* has significant antimicrobial activity when compared with antibiotic Gentamycin.

Key Words*: High Valued, Indigofera barberi, Tirumala Hills, Endemic Herb*

INTRODUCTION
Attention to the discovery of novel plant antimicrobials must be paid in a badly needed new era of chemotherapeutic treatment of infection by using plant-derived principles (Cowan, 1999). Pathogen resistance to synthetic drugs and antibiotics already in use makes search for plants with antimicrobial activity more important, as they can substitute for synthetic antibiotics and drugs (Kothari *et al.,* 2010). Plant antimicrobials also offer potentially new classes of agents to deal with the threat of biowarfare (Gibbons, 2008). Herbal preparations which are comparatively cheaper and have lesser side effects can supplement other systems of medicine for the treatment of diseases caused by bacteria (Archana and Abraham**,** 2011).

Figure 1 A: Habitat

Figure 1 B: Fruiting Stage

Taxonomic Status of Indigofera barberi
Indigofera barberi Gamble of Fabaceae family is high valued endemic herb of Tirumala Hills. Vernacularly known as Adavineelimandu mokka. It is an undershrub grows upto 1 m tall. Its branchlets faintly angled. Leaves 3-foliolate, leaflets ovate-oblong, pubescent, obtuse, mucronate. Flowers pink in

colour arranged in axillary congested racemes. Pods sub-terete, deflexed, appressed, white-tomentose, sharply pointed. seeds 2-4. Flowering and Fruiting season is September to December (Madhava *et al.*, 2013).

Ethnobotanical Usage

Leaf powder (5 g) is taken orally along with butter milk for controlling diabetes. Leaves (50 g), garlic (1 g) and pepper (1 g) made into paste and prepared a pills of peanut size. 5 pills are taken once a day for 5 days to cure jaundice as prescribed by Nakkala and other tribal physicians. It is used as a dye and colouring agent. Devi (2011) documented that Whole plant powder (5 g) is taken along with rice washed water once a day for 10 days to expel interstinal worms and to cure several types of skin diseases and pectic ulcers. Leaf juice is used as an antiseptic to cure wounds, cuts, burns and boils.

According to the literature review, traditionally, the leaf part of *Indigofera barberi* was used for various skin infections. So we thought of proving the activity by the scientific approach it is enlisted to be Red listed endangered plant (Nayar and Sastry, 1990).

MATERIALS AND METHODS

Plant specimens are collected on the waysides of I- and II-Ghats roads and were poisoned, pressed, dried and sticthed on standard herbarium sheets of 28 x 42 cm according to the methodology described by Jain and Rao (1977).

Preparation of Extract

About 400 g of fresh whole plant material (root: stem: leaf: fruit in equal quantity, each 100 gms) were air dried at room temperature. After drying at 37^0C for 24 h the plant material was ground in a grinding machine (Thomas Wiley laboratory mill, model - 4, screen size-1mm). The ground plant material was shaken in distilled water for 48h on an orbital shaker (Digisystem Laboratory, Germany) at room temperature. And was defatted using petroleum ether (40-60°C) for 72 hours using Soxhlet apparatus the extract was filtered using a Buckner funnel and Whatman No 1 filter paper (Harborne, 1998; Gibbs, 1974). Filtrate was concentrated to dryness under reduced pressure at 65-70°C. The thick solution was lyophilized using freeze drying system for antimicrobial investigations. The extract yielded 42.2g. Graded extract was prepared giving a concentration of 25, 50, 75 mg/1 ml.

Antimicrobial Studies

Agar well-diffusion method standardized by modified Kirby-Bauer (1996) susceptibility method was followed to determine the antimicrobial activity Muller Hinton agar (MHA) plates were swabbed (sterile cotton swabs) with 8 hour respective bacterial culture. Wells (6mm diameter and about 2cm a part) were made in each of these pates using sterile cork borer. About 100µl of different concentrations of plant aqueous extract were added sterile syringe in to the wells and allowed to diffuse at room temperature for 2hrs.Control experiments comprising inoculums without plant extract were set up (NCCLS, 1997).

The prepared culture plates were inoculated with different selected strains of bacteria using streak plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were loaded into the well using sterile syringe. Thereafter, the plant extracts (25-75 mg/mL) were filled into each well separately. The plates were incubated 18 - 24 hours at 37 ± 2^oC. The plates were observed for inhibition zone formation around the well. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter with MIC Scale (HiMedia, Mumbai).

Table 1: For the present study following pure bacterial were taken

S. No.	Bacterial strian	Type	Culture code
1	*Bacillus subtilis*	Gram Positive	NCIM-1133
2	*Staphylococus aureus*	Gram Positive	NCIM-2773
3	*Escherichia coli*	Gram Negative	NCIM-2047
4	*Klebsiella pneumoniea*	Gram Negative	NCIM-1381

Microorganisms

The Strains of human pathogen microorganisms used in this study were obtained from National Collection of Industrial Micro-organisms (NCIM), NCL, Pune. The bacterial strains were maintained regularly sub cultured on same nutrient agar medium and stored slants at 4^0C.

Test against Standard Controls

Commercially available antibiotics Gentamycin (0.4 µg) of were used as standard control for the entire test microorganism. The sensitivity patterns were recorded and the readings were interpreted according to the critical diameter given by National Committee for Clinical Laboratory Standards (NCCLS, 1997).

The minimum inhibitory concentration (MIC), is scored using MIC scale (Hi-media, Mumbai) by calculating the visible bacterial growth on the culture plates. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

Statistical Analysis

Analysis of data was performed using Microsoft Excel 2012. The 'T' paired test was used to determine if there was any statistically significant between plant extracts and antibiotics; the MIC of the most active extract and the control antibiotics.

RESULTS AND DISCUSSION

Results

The results indicated that the plant extract showed antibacterial activities at variable degrees against *Bacillus subtilis, Escherichia coli, Staphylococus aureus, and Klebsiella pneumoniea.*

The crude extract of *Indigofera barberi* induced important inhibitory activities on tested Gram negative and gram positive microorganisms with inhibition zonediameters (ID) varying from 1.0 mg/ml to 16.5 mg/ml and MIC at <25 mg/ml. These bacteria were found to be sensitive with the zone of inhibition varying from 6mm to 16mm for *Bacillus subtilis*, 5mm to 11mm for *E.coli*, 1.8 mm to 11mm for *S.aureus*, 0.8 mm to 5.6 mm for *Klebsiella.*

Klebsiella pneumoniea is comparatively resistant against extracts. So, the anti-bacterial activity of the *Klebsiella* is very less. The highest IDs 16.2 were obtained with gram positive strain *Bacillus subtilis* at 75 mg/ml. 25 mg/ml was found to be the MIC for plant extract against all the tested bacterial strains.

The antibacterial activity of the *Indigofera barberi* crude extract is depicted in figure 2 and graphical representation in figure 3.

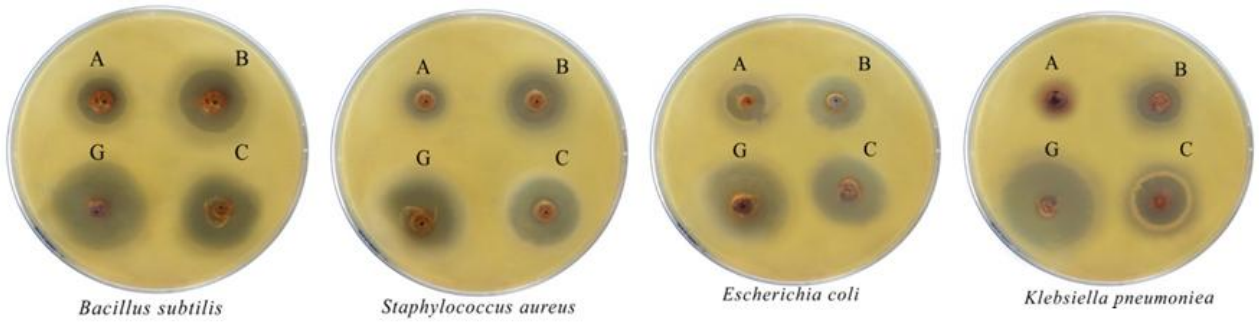

Figure 2: Antimicrobial Activity showing Inhibition Diameter against 4 Bacterial Strains. *Abbreviation:* *A - 25mg/ml; B - 50 mg/ml; C - 75 mg/ml; G – Gentamycin (Antibiotic –Control).*

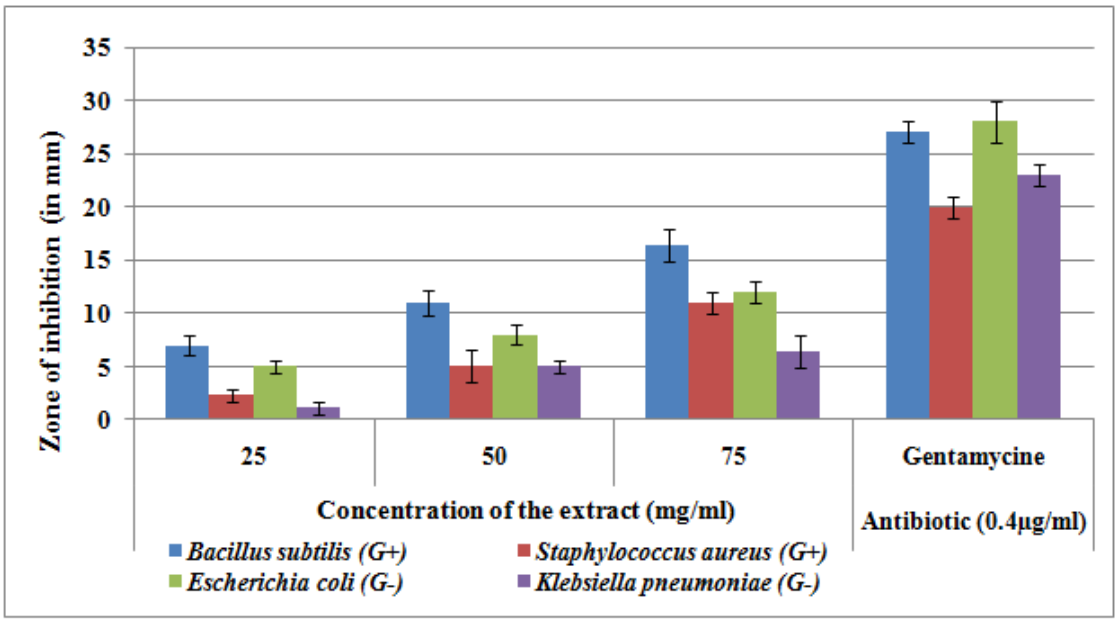

Figure 3: Antibiotic activity of *Indigofera barberi*, whole pant extract

Discussion

As per the traditional belief, we have collected the leaves, identified by local flora, dried under shade, powdered, extracted and assessed for its Antimicrobial activity. A total of four strains were screened for susceptibility to the crude extracts of *Indigofera barberi*. Antibacterial activity of the plant extract was tested using well diffusion method well-variant of the diffusion method was more sensitive and best conditions for the determination of minimal inhibitory concentration (Cleidson *et al.*, 2007).

In this well diffusion assay, all the bacterial strains was found to be susceptible and this endemic herb demonstrated antimicrobial activity with zone diameter ranging from 0.8 - 16mm. The inhibition zones produced by the plant extracts were compared with the inhibition zones produced by commercial standard antibiotic Gentamycin (0.4 µg).

To the best of our knowledge, this is a first report of in vitro antimicrobial activity of crude extract. Due to the presence of phytoconstituents in this investigated species, the observed antimicrobial activity can be directly connected with their constituents. Rojas *et al.*, (1998) analyzed that antibacterial activity is due to different chemical agents in the extract, including essential oils, flavonoids and triterpenoids and other nature phenolic compounds or free hydroxyl groups. Purification of the bioactive components from the extracts has to be investigated which may improve our understanding of possible antimicrobial activities. From this study we can conclude that the traditional use of this plant for the treatment of infectious diseases is promising alternate supplement against pathogenic bacteria.

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