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In vitro antibacterial activity of whole plant extract of *Indigofera barberi* Gamble (Fabaceae)

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ABSTRACT

The study was carried out to ascertain the anti bacterial activity of ethanolic extract of Indigofera barberi. The anti-bacterial testing of whole plant of ethanolic extract was evaluated by disc diffusion method using gram positive bacteria like Staphylococus aureus, Bacillus subtilius and gram negative bacteria like Escherichia coli, pseudomonas aeruginosa. Amongst the test extract, the results as per preliminary phytochemical screening presence of flavonoids, steroids, glycosides, carbohydrates and phenols suggested that ethanol extract showed significant antibacterial activity compared with standard gentamycin drug.

Key words: *I. barberi*, phyto constituents, antibacterial, disc diffusion method, gentamycin.

INTRODUCTION

Plants have always been a major component of traditional system of healing in developing countries, which have also been an integral part of their history and culture [1, 2]. Medicinal plants offer alternative remedies with tremendous opportunities. Many traditional healing herbs and plant parts have been shown to have medicinal value especially in the rural areas and that these can be used to prevent and cure several human diseases [3, 4]. Even today, majority of the world population depends on herbal healthcare practice. The strategic importance of reviving indigenous medical practices to provide safe and affordable primary healthcare to the people of the world is now recognized. During the last two decades or so, WHO's health Assembly has passed a number of resolutions in response to this resurgence of interest in the study and use of traditional medicines and in recognition of the importance of medicinal plants to health care of people in many developing countries. Medicine is as old as life itself. The survival of the species demands that simultaneously with appearance of the disease, all things must have evolved the

means to combat disease. The higher animals are guided by instinct to seek remedies for illness of plants and the herbs [5, 6]. Man with superior intelligence must necessarily have extended the scope of this search for remedies. So if we discount the myth of Garden of Eden which man had to abandon when he fell from the grace "*the thousand ills that flesh is heir to*" must have afflicted man from his birth and the dawn of medicine synchronized with the dawn of disease [7, 8]. Its form and the content are decided by the civilization and the environment it is born *I. barberi* Gamble was shrub, up to 1 to 2 meter tall, branches angular, in young apperssendly pubescent. Leaves 3 centimeter long, tri-foliate, leaflets obovate 1.5-3.5 × 0.5 – 1.5 cm, pubescent, punctuate, base cuneate, margin entire, apex obtuse mucronate. Flowers red, aggregate in 3 cm long axillary racemes. Stamens diadelphous (9+1).Pods sub terete, 1× 0.2 cm, cylindric, deflexed, apperessedly white – villous, torulose, and white pubescent the present investigation was to explore the antibacterial activity of *I. barberi* [9].

MATERIALS AND METHODS

Collection of Plant material:
Plant was collected in the forest regions of Thalakona (Nelakona regions) of Chitoor distict, Andhra Pradesh, India in the month of november. The plant material was taxonomically identified by the taxonomist from Nalgonda. A voucher specimen was certified under Voucher No: **NCOP NLG/ph'cog/2010-11/041**.has been preserved in our laboratory for future reference.

Procedure for Extraction:
The powdered material was subjected to soxhlation using ethanol solvent extraction method. Initially 25gm of crude powder was taken and packed in a packing paper [10, 11]. This pack was placed in a soxhlet extractor for 24 hrs (approx) with ethanol solvent and the temperature was adjusted as per the solvent been used in the extraction. The percentage yield obtained was calculated and reported the obtained extract were subjected to antibacterial screening [12].

Phytochemical screening:
The ethanol extract was subjected to phytochemical screening to detect the which phyto constituents were present [11].

Micro organisms:
The test organisms included for study were gram positive bacteria like *Staphylococus aureus, Bacillus subtilius,* gram negative bacteria like *Escherichia coli, pseudomonas aeruginosa*. All the Bacterial strains were procured from MTCC *(Microbial Type Culture Collection & Gene Bank)* Customer no: 6815, sector 39-a, Chandigarh. The bacteria were grown in the nutrient broth at 37 ^{o}C and maintained on nutrient agar slants at 4 ^{o}C.

Bacterial Media:
Nutrient broth and agar Media was mixed with distilled water individually and then sterilized in autoclave at 15lb Pressure for 15 minutes. The sterilized media were poured into Petri dishes and allowed for Solidification. The solidified plates were using by disc diffusion method. The plates were used for the antibacterial studies.

Antibacterial activity of the plant extract:
Ethanolic extract of whole plant of *I. barberi* at a concentration of 20 mg, 40 mg, 100 mg & 200 mg for *Staphylococcus aureus & Pseudomonas aeruginosa* and concentrations 20mg, 100mg, 200mg, 300mg & 400 mg for *Bacillus subtilis & Escherichia coli* by disc diffusion method.

Disc diffusion method:
All petridishes and media were heat sterilized in an Autoclave at 121 ºc (15psi) for twenty minutes [13]. All Petric plates were prepared before it reaches room temperature the plates were inoculated with the organisms and poured with an equal thickness of nutrient agar (pour plate) After solidify of the plates a sterile 6 mm diameter disc was taken which were already dipped in different concentrations of ethanolic extract, along with different concentrations of standard gentamycin (5 & 10 µg/ml) and ethanol used as a control [14].

The discs were inoculated and gently pressed by a sterile forceps. Petri dishes were incubated at 37 ºc in the incubator for respective incubation periods [15] based on the organisms, the zones of inhibition of bacterial growth around the discs was measured in millimeters (mm). The experiment was repeated for three times and the average values were recorded.

RESULTS AND DISCUSSION

The results of phytochemical screening of the ethanol extract (Table 1) the presence of flavonoids, steroids, glycosides, carbohydrates, phenols and tannins were present in the ethanolic extract. Flavonoids and tannins have been reported to posses' antimicrobial activity [17].

The antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial wall while that of tannins may be related to their ability to inactive microbial adhesion, enzymes and cell envelop proteins [17].

The ethanol extract showed zone of inhibition of growing from 4- 12 mm against standard gentamycin drug. In that Anti bacterial study was performed at 20 mg, 40 mg, 100 mg & 200 mg concentration of ethanolic extract of *I.barberi*. It shows + ve results against *Staphylococcus aureus & Pseudomonas aeruginosa.*(Table 2).

Anti bacterial study was performed at 20 mg, 100 mg, 200 mg, 300 mg, & 400 mg concentration of ethanolic extract of *I.barberi*. It shows - ve results against *Bacillus subtillis & Escherichia coli.* When compare with gentamycin drug. (Table 3).

As per preliminary phytochemical screening as mentioned phyto constituents responsible for anti bacterial activity. It was suggested that ethanolic extract showed significant antibacterial activity compared with standard gentamycin drug.[11] ,[15], [18].

Preliminary phytochemical analysis:

Table-1: Phytochemical screening of ethanolic extract of whole plant *I. barberi* Gamble

S.No	**Phyto constituents**	**Ethanolic extract**
1	Carbohydrates	+
2	Amino acids	-
3	Proteins	-
4	Alkaloids	-
5	Phenol's & Tannins	+
6	Steroids	+
7	Volatile oils	-
8	Flavonoids	+
9	Saponins	-
10	Cardiac glycosides	+

Table-2: Zone of inhibition of *Staphylococcus aureus* & *Pseudomonas aeruginosa against* gentamycin and ethanolic extract of *I.barberi*

Organism	**Concentration µg/ml**						
Staphylococcus aureus	20 mg	40 mg	100 mg	200 mg	Control	Std- 5 µg/ml	Std- 10 µg/ml
Zone of inhibition in mm	6	7	2	5	--	5	12
Pseudomonas aeruginosa	20 mg	40 mg	100 mg	200 mg	Control	Std- 5 µg/ml	Std- 10 µg/ml
Zone of inhibition in mm	4	5	8	10	--	5	12

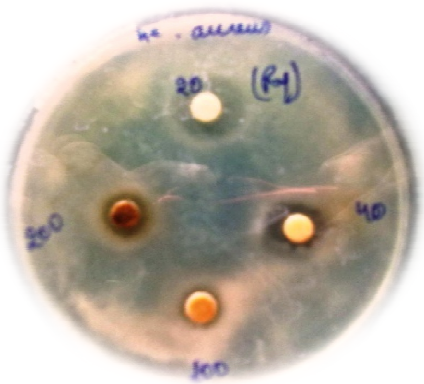

Fig-1: *Staphylococcus aureus*

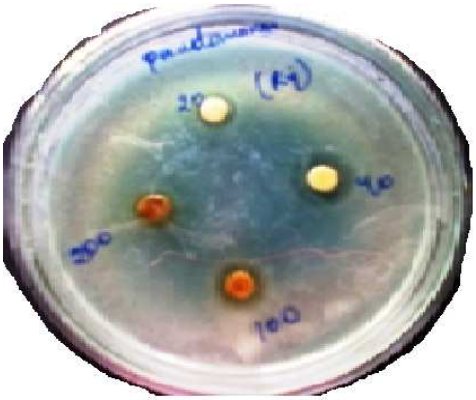

Fig-2: *Pseudomonas aeruginosa*

Figure 3& 4: *Bacillus subtilis*

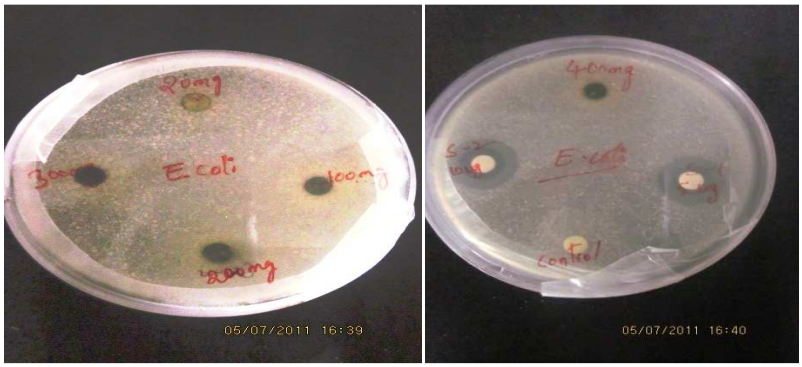

Figure 5& 6: *Escherichia coli*

Table-3: Zone of inhibition of *against Bacillus subtillis* & *Escherichia coli* gentamycin and ethanolic extract of *I.barberi*

Organism	**Concentration µg/ml**							
Bacillus subtillis	20 mg	100 mg	200 mg	300 mg	400 mg	Control	Std- 5 µg/ml	Std- 10 µg/ml
Zone of inhibition	--	--	--	--	--	--	5	12
Escherichia coli	20 mg	100 mg	200 mg	300 mg	400 mg	Control	Std- 5 µg/ml	Std- 10 µg/ml
Zone of inhibition	--	--	--	--	--	--	5	12

CONCLUSION

From the above study, the phyto constituents flavoniods and tannins were responsible for anti bacterial activity. It is concluded that the whole plant of *I.barberi* may represent a new source of anti-bacterial with stable, biologically active components that can establish a scientific base for the use of this in modern medicine. These local ethnomedical preparations of Plant sources should be scientifically evaluated and then disseminated properly. This knowledge about the medicinal plants usage can also be extended to other fields like field of pharmacology.

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REFERENCES

[1] J. N Bhakta, P. Majumdar, Yukihiro Munekage, *The Internet Journal of Alternative Medicine*, **2009**, 7, 1-6.
[2] V. P. Kamboj, *Current Science*, **2000**, 78, 35-51.
[3] J.E. Bandow, H. Brötz, L. I. O. Leichert, H. Labischinski, M. Hecker, *Antimicrobial Agents of Chemotherapy*, **2003**, 47, 948-955.
[4] Rojas, L. Hernandez, R. Pereda-Miranda, R. Mata, *Journal of Ethnopharmacology*, **1992**, 35, 275-283.
[5] N. Benkeblia, Lebensm.-*Wiss. U-Technology*, **2004**, 37, 263-268.
[6] K.R Kirtikar, B.D Basu, Indian Medicinal Plants, 2, International Book Distributors, Dehradun, **2007**, 2509.

[7] C.K Kokate, A.P Purohit, S.B Gokhale, Pharmacognosy, 36, Shri D.K. Furia, Nirali Prakashan, Pune, **2006**, 347.
[8] T.E Wallis, Text Book Of Pharmacognosy, 5, S.K Jain For CBS Publishers and Distributors, New Delhi, **2005**, 574.
[9] K.Madhava chetty, K.Sivaji,K., Tulasi rao.,mentioned in the Book of Flowering plants of chittoor district Andhra Pradesh, II edition, India, 2008, 90 – 95.
[10] W.C Evans, Pharmacognosy, 15, Elseivan, **2005**, 344.
[11] K.R Khandelwal, Practical Pharmacognosy, 17, Nirali Prakashan, Pune, **2007**, 149.
[12] Rajeshwari Shivaraj, Uma Maheswari, *The Antiseptic Journal Of Meicine and Surgery*, **2010**, 7, 34.
[13] J.R Michael J.Pelezar, Microbiology, 5, Library of Congress Cataloging-in-Publication,New Delhi, **2007**, 35.
[14] W. Mackie, Mc Cartney, Practical Medical Microbiology, 13, Churchill Living stone, London, **1989**, 162.
[15] Shibumon Geroge ,Benny PJ, *Int J Pharm sci Bio* **2010**;1 (2), 96-99.
[16] S.Selvakumar and C.M.Karunakaran, *International journal of pharmTech Research*, 2(3), 2054-2059.
[17] Cowan, M.M., *Clin. Micr. Rev*., 12(4); 546-582.
[18] A.M. Musa, G.Abbas, A.B. Aliyu, M.S.Abdullahi and I.N.Akpulu, *Research journal of medicinal plant* **2008**, 2 (2), 74-78.