

## Antibacterial Activity of *Gymnema kollimalayanum*, A New Plant from Peninsular India

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**Abstract:** *Gymnema kollimalayanum* A. Ramachandran and M.B. Viswan (Asclepiadaceae) is a new plant found in Kolli hills, Eastern Ghats, Peninsular India. The present investigation deals with the antibacterial potentials of the aqueous and organic solvents extracts from powdered leaves of *G. kollimalayanum* were tested against eight Gram-negative (*Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella paratyphi*, *Shigella boydi*, *S. brunci*, *S. dysenteriae*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica*) and two Gram-positive (*Corynebacterium diphtheriae* and *Enterococcus faecalis*) bacterial strains by disc diffusion method. The organic solvents extracts exhibited better antibacterial activity. The aqueous extracts were found to be inactive against the organisms tested. The result of this study supports the use of plants as therapeutic agents for treat several diseases caused by the pathogenic bacterial populations.

**Key words:** *Gymnema kollimalayanum* • New plants • Bacterial stains • Disc diffusion method • Antibacterial assay

### INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value [1]. About three quarter of the world's population relies on plants and their extracts for their healthcare. India represented by rich culture, traditions and natural biodiversity, offers a unique opportunity for drug discovery researchers [2]. The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led to investigate the antimicrobial activity of medicinal plants. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has lead to the screening of several medicinal plants for their potential antimicrobial activity [3-5].

*Gymnema* is a well known herbal medicine due to the therapeutic efficacy of its different species. One of the most important species is *Gymnema sylvestre* R. Br. which has been widely used in herbal medicine. The Leaves of *G. sylvestre* have been used in India for the treatment of diabetes for over 2000 years. Several studies have been published concerning *G. sylvestris* R. Br. activities which have shown that this species has antidiabetic

[6-9], hypolipidaemic and antiatherosclerotic [10-11], insulinotropic [12], anti-inflammation [13], anticancer-cytotoxic [14], anti-oxidant [15], wound healing [16], leishmanicidal [17] and antimicrobial activities [18-22]. On the basis of these results and because of the popular use of the different species of *Gymnema* as diabetic agents in traditional practices, the present research was focused on the antibiotic activity of one new species i.e. *Gymnema kollimalayanum*, an unknown twining shrub found in evergreen forests, Eastern Ghats of India. The scientific knowledge of the plant is least explored. So far, the taxonomic sketch of the plant was drawn [23]. There is no report regarding the biological activity of this taxon. The purpose of present and first-hand investigation was to screen *in vitro* antibacterial properties of different extracts of pulverized leaves of *Gymnema kollimalayanum* were tested against ten bacterial strains.

### MATERIALS AND METHODS

**Plant Material:** The leaves of *G. kollimalayanum* were collected in the month of November 2009, from the evergreen forests (above 1000m in MSL.), Kolli hills, Eastern Ghats of Tamil Nadu, India. The voucher specimens have been deposited in the Herbarium,

Department of Biotechnology, Periyar University, Salem, Tamil Nadu, India. The collected plant materials were shade dried for 15 days.

**Preparation of Plant Extracts:** The organic solvents (acetone, hexane, methanol, ethanol and petroleum ether) and aqueous extracts of the plant materials were prepared as per the method [24] with few modifications. About 20 grams of dried plant material was crushed and blended to be used for each solvent. The blended materials were transferred to beakers and were soaked separately in 100ml of sterile distilled water and organic solvents at room temperature. The extraction was done using rotary shaker (100rpm for 3 days) and the extract was vacuum-dried to a concentration of 1/5 of the original volume. The extracts were diluted in dimethylsulfoxide (DMSO) before testing the antibacterial activity.

**Test Microorganisms:** A total of ten human pathogenic bacterial strains (eight Gram-negative namely *Klebsiella pneumoniae*, *Proteus vulgaris*, *Shigella brunci*, *S. boydi*, *S. dysenteriae*, *Salmonella paratyphi*, *Vibrio parahaemolyticus*, *Yersinia enterocolitica* and two Gram-positive i.e. *Corynebacterium diphtheriae* and *Enterococcus faecalis*) were used in this investigation. All the bacterial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

**Preparation of Bacterial Inoculums:** Test organism was maintained on slants of medium containing 300 mg of manganese sulphate per liter and transferred to fresh slant once a week. Then the slants were incubated at temperature 32°C for 24 h. Organism was then washed by

using 3 ml saline solution from agar slant onto a large agar surface of medium such as Roux bottle containing 250 ml agar and incubated (24 h). Using 50 ml saline solution; the growth from the nutrient surface was washed. Then organism was stored under refrigeration. Inoculum was adjusted at 530 nm, leading to transmission equivalent to  $1 \times 10^8$  cells/ml.

**Test for Antibacterial Assay:** The antibacterial activity of the plant extracts were tested by the modified disc diffusion method [25]. The bacterial inoculums (20h broth) were uniformly spread over the agar plates using a glass L-rod. A total of 0.2ml of each extract was aseptically added to the discs (0.5mm diameter) and allowed to dry before being placed on the top of the agar plate. The plates were incubated at 37°C for 24h and the diameter of growth inhibition zone was recorded. Vancomycin (30 ig/disc) was used as positive controls. About 5% DMSO was used as negative control. Each extract was tested in triplicate for calculation of standard deviation [26].

## RESULTS AND DISCUSSION

The results of antibacterial activity of aqueous and organic solvents extracts from the leaves of *Gymnema kollimalayanum* are presented in Table 1. Totally ten bacterial strains (two Gram-positive and eight Gram-negative) were screened in this study. It could be observed that the hexane extracts showed good antibacterial activity against all tested pathogens. The acetone and petroleum ether extracts expressed remarkable antibacterial activity against tested bacterial strains. Ethanolic extracts showed significant inhibition of

Table 1: Antibacterial potentials of leaf extracts of *Gymnema kollimalayanum*

Organisms Tested	Extracts Used Zone of growth Inhibition (mm) Mean $\pm$ SD					
	Aqueous	Hexane	Methanol	Acetone	Petroleum ether	Ethanol
<i>Corynebacterium diphtheriae</i>	<sup>a</sup>	13.00 $\pm$ 0.82	<sup>a</sup>	17.00 $\pm$ 0.82	14.33 $\pm$ 0.82	20.00 $\pm$ 0.00
<i>Enterococcus faecalis</i>	<sup>a</sup>	14.00 $\pm$ 0.00	18.66 $\pm$ 1.16	15.33 $\pm$ 1.82	14.00 $\pm$ 0.00	16.33 $\pm$ 0.82
<i>Klebsiella pneumoniae</i>	<sup>a</sup>	14.66 $\pm$ 1.16	13.00 $\pm$ 0.00	15.66 $\pm$ 1.63	12.66 $\pm$ 0.82	14.66 $\pm$ 0.82
<i>Proteus vulgaris</i>	<sup>a</sup>	10.33 $\pm$ 0.82	<sup>a</sup>	11.00 $\pm$ 0.82	12.33 $\pm$ 0.82	<sup>a</sup>
<i>Salmonella paratyphi</i>	<sup>a</sup>	20.00 $\pm$ 0.00	15.00 $\pm$ 0.81	13.00 $\pm$ 0.00	15.33 $\pm$ 0.82	17.33 $\pm$ 0.81
<i>Shigella boydi</i>	<sup>a</sup>	20.33 $\pm$ 2.16	21.33 $\pm$ 0.82	15.33 $\pm$ 0.82	18.66 $\pm$ 1.62	17.00 $\pm$ 0.82
<i>Shigella brunci</i>	<sup>a</sup>	12.00 $\pm$ 0.00	21.66 $\pm$ 0.82	17.66 $\pm$ 1.16	13.00 $\pm$ 0.81	15.33 $\pm$ 0.82
<i>Shigella dysenteriae</i>	<sup>a</sup>	12.33 $\pm$ 0.82	16.00 $\pm$ 0.81	12.00 $\pm$ 0.82	14.33 $\pm$ 0.82	15.66 $\pm$ 1.63
<i>Vibrio parahaemolyticus</i>	<sup>a</sup>	12.66 $\pm$ 1.62	16.66 $\pm$ 1.63	11.00 $\pm$ 0.00	13.66 $\pm$ 1.63	15.33 $\pm$ 0.82
<i>Yersinia enterocolitica</i>	<sup>a</sup>	15.00 $\pm$ 0.81	13.00 $\pm$ 0.82	12.33 $\pm$ 0.82	10.00 $\pm$ 0.82	10.00 $\pm$ 0.82
Vancomycin (antibiotic)	10.00 $\pm$ 0.81	18.33 $\pm$ 0.82	22.66 $\pm$ 1.16	20.33 $\pm$ 0.82	18.33 $\pm$ 0.82	20.66 $\pm$ 0.82

<sup>a</sup> Indicates no activity

various bacteria except *Proteus vulgaris*. The methanolic extracts contributed broad-spectrum of antibacterial activity except two bacterial strains i.e. *Corynebacterium diphtheriae* and *Proteus vulgaris*. The overall diameters of growth inhibition area of extracts studied were in the range 10-21mm. The aqueous extract and negative control DMSO (5%) did not show any activity against the selected bacteria. The previous reports highlighted the antimicrobial/antibacterial properties of aqueous and organic solvents extracts from two species of *Gymnema* namely *G. sylvestre* [18-22] and *G. montanum* [27]. The overall performance of the study shows the plant extracts contributed potent antibacterial activity against the tested pathogens. This study will encourage further research regarding the structural elucidation of bioactive compounds and carryout the clinical evaluation for confirming the therapeutic values.

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