



## Anti- Bacterial Potential of Ethanolic Extracts of *Withania somnifera* Root Against Some Broad-Spectrum Bacterial Cultures

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### ABSTRACT

*Withania somnifera* is an important medicinal plant in Ayurveda and ancient medicinal systems. It is widely cultivated as a Kharif crop in various parts of the Indian subcontinent. Madhya Pradesh, Uttar Pradesh, Rajasthan, Gujarat, Punjab, Haryana and Maharashtra are major medicinally important crop producing states. Present investigation indicates that the ethanolic extract of *Withania somnifera* is very effective against some broad - spectrum microbial cultures like *Bacillus subtilis* (BS), *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC), and *Klebsiella pneumonia* (KP). Various concentrations 10 $\mu$ l, 30 $\mu$ l, 50 $\mu$ l, 70 $\mu$ l, 90 $\mu$ l of the ethanolic extracts of *Withania somnifera* were used against these microbial cultures. 90 $\mu$ l concentration (1mg/ml) was found to be most effective against the microbial cultures and average zone of inhibition (in mm) against *Klebsiella pneumonia* (KP) 17.13333 $\pm$ 0.321455 mm followed by *Staphylococcus aureus* (SA) 16.96667 $\pm$ 0.057735 mm, *Pseudomonas aeruginosa* (PA) 16.83333 $\pm$ 0.288675 mm, *Escherichia coli* (EC)-14.86667  $\pm$  0.23094 mm, and *Bacillus subtilis* (BS) 14.13333 $\pm$  0.70946 mm.

**Keywords:** ethanolic extract, Broad spectrum microbes, *Withania somnifera*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

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### INTRODUCTION

Indian subcontinent belongs to 12 diverse countries. Indian agroclimatic zones are divided into 15 regions and further subdivided into 72 subzones. Among the 70000 plant species, 7500 are identified to be medicinally important species. Approximately 7800 small and giant drug manufacturing units consume around 2000 tons of herbs annually (1). Most developing countries especially South Asian countries rely on plant-based medicinal systems also known as the Ayurveda system for primary health care needs (2,3,4). Identification of medicinally important herbs and their key components is tedious and crucial for the development of drugs. It economically strengthens both the farmers and the industries. In India plants are the safer medicinal system used to cure various diseases. *Withania somnifera* also known as Indian ginseng or Ashwagandha is an important medicinal plant that commonly used in Ayurveda, Siddha, Unani and the Homeopathy system against various illness. Roots and leaves are aphrodisiacs, anti-microbial, anti-diabetic, anti-tumour, anti-cancerous, and anti-stress agents. *Withania somnifera* found in various countries like- India, Pakistan, China, Afghanistan, Bhutan, Bangladesh, Egypt, Morocco and Jordan. In India *Withania somnifera* is widely cultivated in Madhya Pradesh, Rajasthan, Gujarat, Uttar Pradesh, Uttarakhand, and some parts of Punjab as a Kharif crop. It grows up to 150 cm height. Roots and leaves have been used in various formulations in Ayurveda as an anti-microbial, anti-cancer, anti-tumour, anti-inflammatory, anti-stress, aphrodisiac, and diuretic agents. It improves the immunity, prevents various infections also it improves reproductive fertility. Previous studies prove that *Withania somnifera* extracts (Leaves, Roots) possess some anti-microbial potential. The anti-microbial activity of *Withania somnifera* was first studied by K in 1956 against the culture of *Salmonella aureus* (5). The major alkaloid of *Withania somnifera* is Withanoloid which possesses the anti-microbial potential. Methanolic extract of *Withania somnifera* was found to be effective against spectrum of bacteria and also showed maximum inhibitory effects [6]. Diethyl ether extracts, methanolic extracts, and hexane extracts of *Withania somnifera* was found to be effective against *Salmonella typhimurium* and *Escherichia coli* [7]. Aqueous fruit extract of *Withania somnifera* was used against *Salmonella* -infected mice, study revealed that the survivability rate of mice was increased in the *Withania somnifera* administered mice (8). Leaves- Methanolic extract of *Withania somnifera* was effective against two Gram-positive isolates -*Staphylococcus aureus* and

Enterococcus species isolated from the pus samples of humans, leaf methanolic extract showed the zone of inhibition of 20.6 mm and 19.4 mm (9). free and bound flavonoids isolates of *Withania somnifera* used against the bacterial cultures of –*Escherichia coli* MTCC-46, *Proteus mirabilis* MTCC- 3310, *Pseudomonas aeruginosa* MTCC- 1934, and *Staphylococcus aureus* MTCC 3160, against the fungal cultures of *Candida albicans* MTCC 183, *Aspergillus flavus* MTCC 277, and *Aspergillus niger* MTCC- 282, the minimum bacterial and fungal concentrations revealed that *C. albicans* culture was most susceptible followed by *S. aureus*, *P. mirabilis*, *E.coli*, and *P. aeruginosa* (10). Bioactive compounds isolated from Leaf of *Withania somnifera* also showed significant anti-microbial activity against some bacterial cultures (11, 12). Present work performed to check the efficacy of *Withania somnifera* extract against broad spectrum bacteria – both Gram positive and Gram-negative bacteria. *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Enterococcus faecalis*, are common bacteria causes urinary tract infection(UTI). *Pseudomonas aeruginosa* causes various infections such as dermatitis, urinary tract infection (UTI), and respiratory infections in humans. *Proteus mirabilis* and *Escherichia Coli* cause UTI in humans and *Staphylococcus aureus* causes skin and soft tissue infections- wound infections, furuncles and cellulitis also causes food poisoning, endocarditis, pneumonia and osteomyelitis. *Klebsiella pneumonia* is responsible for various infections such as- Urinary tract infection, pneumonia, blood stream infections, abdominal infections and pyogenic liver diseases. *Pseudomonas aeruginosa* causes malignant external otitis, endocarditis, pneumonia, meningitis and septicemia. These bacteria can cause various infections – Urinary tract infection (UTI), blood stream infections, food poisoning, abdominal and liver infections.

## **MATERIAL AND METHODS**

Ethanollic root extracts of *Withania somnifera* were used against the pure microbial cultures of *Bacillus subtilis* (BS), *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC), and *Klebsiella pneumoniae* (KP).

### **Sample Collection**

350 grams of fresh roots of *Withania somnifera* were collected from the field, washed 3 times with sterile-double distilled water to remove the dirt and soil particles, wiped with tissue paper the kept in a wide-mouth glass jar under shade for 7 days at room temperature and a fine powder was made with the help of a grinder. Fine powdered samples were transferred into a conical flask and then ethanol was added. The flask was closed with the cotton plugs. Conical flask containing the sample was placed on a shaker and left overnight. The samples were extracted with the help of a Soxhlet apparatus. Extracts were filtered through the Whatman filter paper number 1. Collected extracts were further evaporated by a rotator evaporator. Collected dried extracts were weighted carefully and stored in the refrigerator for future analysis. For the anti-microbial study 1mg/ml concentration of the sample was prepared in a sterilized Eppendorf tube. *Bacillus subtilis* (BS), *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC), and *Klebsiella pneumoniae* (KP)cultures were procured from King's Institute Guindy Chennai. Gentamycin was purchased from Sigma Aldrich.

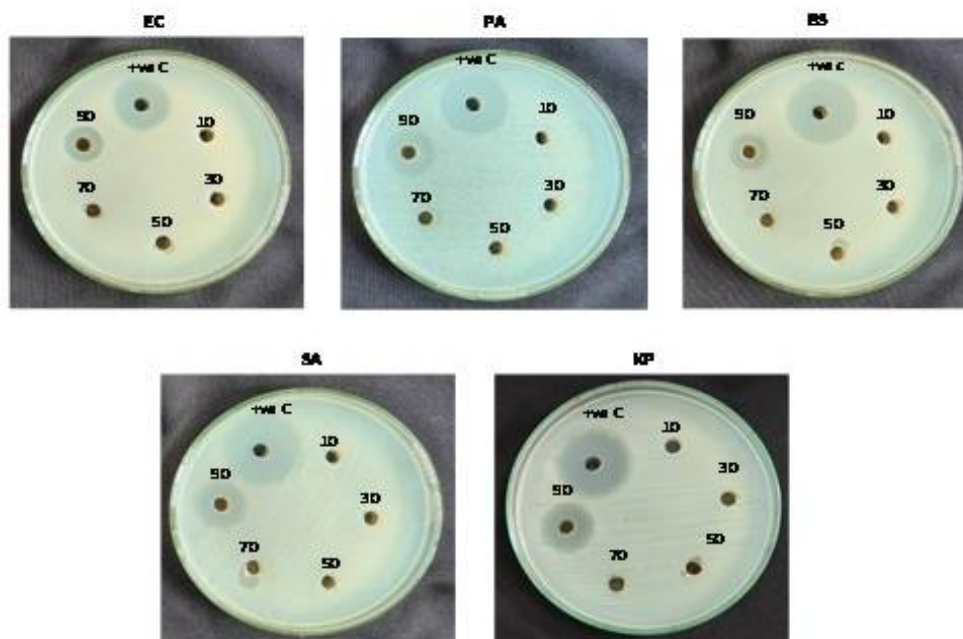
### **Microbial Inocula Preparation**

Pure microbial cultures of *Bacillus subtilis* (BS), *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC), and *Klebsiella pneumoniae* (KP)were obtained and maintained in nutrient agar (NA) slants at 37 °C.

### **Antibacterial activity**

Crude ethanolic root extract was prepared by the Soxhlet method to check the efficacy of *Withania somnifera* against some bacterial cultures by the agar well diffusion method. The pure cultures of i (BS), *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) were obtained and maintained in nutrient agar (NA) slants at 37 °C. The bacterial strains were grown overnight in a nutrient agar liquid medium on a rotary shaker (100 rpm) at 37 °C. The inoculum containing a microbial load of  $1 \times 10^5$  CFU/ml was then swabbed onto the agar plates. Wells of 3 mm diameter were punched aseptically with a sterile cork borer and further loaded with different concentrations- 10 µl, 30 µl, 50 µl, 70 µl and 90 µl of ethanolic root and leaf extracts of *Withania somnifera* (RE) sample, and 20 µl of gentamycin (positive control for bacteria; 10 µg) was used as control. The plates were then incubated for 24 hr at 37 °C. The plates were then analyzed carefully for the zone of inhibition (ZOI; mm) appearing around the isolates which were measured in millimeters

## RESULTS



**Figure 1-** Zone of inhibition by various concentrations- 10µl, 30µl, 50µl, 70µl and 90µl of ethanolic extract of *Withania somnifera* against the pure cultures of *Bacillus subtilis* (BS), *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Escherichia coli* (EC), and *Klebsiella pneumoniae* (KP). 20µl of gentamycin (positive control for bacteria; 10 µg). Ethanolic extract of root samples indicates the significant potential against these broad-spectrum bacterial cultures. 90µl of the extract (1mg/ ml concentration) of *Withania somnifera* showed the average zone of inhibition against *Klebsiella pneumoniae* (KP)  $17.13333 \pm 0.321455$  followed by *Staphylococcus aureus* (SA)  $16.96667 \pm 0.057735$ , *Pseudomonas aeruginosa* (PA)  $16.83333 \pm 0.288675$ , *Escherichia coli* (EC)  $14.86667 \pm 0.23094$ , and *Bacillus subtilis* (BS)  $14.13333 \pm 0.70946$ .

**Table 1:** Zone of inhibition by various concentrations- 10µl, 30µl, 50µl, 70µl and 90µl of ethanolic extract of *Withania somnifera* against the pure cultures of *Bacillus subtilis* (BS),

Name	10µl	30µl	50µl	70µl	90µl	Control
<i>Escherichia coli</i> (EC)	-	-	-	-	$14.86667 \pm 0.23094$	21mm
<i>Pseudomonas aeruginosa</i> (PA)	-	-	-	-	$16.83333 \pm 0.288675$	23mm
<i>Bacillus subtilis</i> (BS)	-	-	-	-	$14.13333 \pm 0.70946$	25mm
<i>Staphylococcus aureus</i> (SA)	-	-	-	-	$16.96667 \pm 0.057735$	26mm
<i>Klebsiella pneumoniae</i> (KP)	-	-	-	-	$17.13333 \pm 0.321455$	25mm

## DISCUSSION

The study indicates that the 90µl of the extract (1mg/ ml concentration) of *Withania somnifera* showed the average zone of inhibition against *Bacillus subtilis* (BS)  $14.13333 \pm 0.70946$ , *Staphylococcus aureus* (SA)  $16.96667 \pm 0.057735$ , *Pseudomonas aeruginosa* (PA)  $16.83333 \pm 0.288675$ , *Escherichia coli* (EC)  $14.86667 \pm 0.23094$ , and *Klebsiella pneumoniae* (KP)  $17.13333 \pm 0.321455$ . Previous studies also support this study and indicate that *Withania somnifera* plant extracts could play an important role in traditional and modern medicine formulations which can be used in various formulations to boost immunity and prevent various microbial infections. These extracts can be used on infected areas directly in the form The present study indicates that *Withania somnifera* possesses a significant anti-microbial activity against the broad-spectrum bacterial culture and multi-drug-resistant microbial cultures. The present study will encourage the use of plat-based medicines against microbial infections also it encourages pharmaceutical sector companies to synthesize naturally existing compounds-based drugs and medicines instead of artificially synthesized compounds.

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