



Pharmacological study

Antibacterial activity of *Withania somnifera* against Gram-positive isolates from pus samples

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Abstract

Background: *Withania somnifera* is an important medicinal plant that has been used in Ayurvedic and indigenous medicine since ancient times. In the view of its varied therapeutic potential, it has also been the subject of considerable modern scientific attention. Attention has been drawn to antibacterial activity of the plant and its metabolites due to the challenge on growing antibacterial resistant pathogens. **Aim:** To examine the antimicrobial potential of leaf extract of *W. somnifera* against Gram-positive cocci. **Materials and Methods:** In this study, leaf extract of *W. somnifera* was used to examine their antimicrobial potential against Gram-positive cocci ($n = 20$) from pus samples of patients admitted in Government Medical College, Haldwani. Agar well diffusion method was used by taking methanolic leaf extract of *W. somnifera*. **Results:** It was observed that the methanolic leaf extract of *W. somnifera* was very effective in inhibiting the test pathogens including methicillin resistant *Staphylococcus aureus* and *Enterococcus* spp., with an average zone of inhibition of 20.6 mm and 19.4 mm at 2 mg/ml (100 μ l) concentration, respectively. **Conclusion:** These results indicate that the antimicrobial property of *W. somnifera* leaf supports the traditional use of the plant in therapeutic use against microbial infections.

Key words: *Enterococcus*, methicillin resistant *Staphylococcus aureus*, *Withania somnifera*

Introduction

Vancomycin-resistant *Enterococcus* and methicillin resistant *Staphylococcus aureus* (MRSA) are associated with increase rates of illness and death. Both these organisms are now endemic in many health care institutions.^[1] These pathogens are now increasingly exhibiting resistance to the commonly used antibiotics due to widespread and inappropriate use of antibiotics in human and veterinary medicine.^[2,3] MRSA is resistant to all beta-lactam antibiotics. Vancomycin had long been considered for the treatment and cure of MRSA infections. However, now vancomycin-resistant strains have been also reported.^[3] To substitute synthetic antibiotics, many of the modern and effective drugs have their origin in traditional folk medicine. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine.^[4]

Withania somnifera (L.) Dunal also known as “*Aswagandha*” belongs to the family Solanaceae and is widely used

in Ayurvedic medicine. It is an ingredient in many formulations prescribed for a variety of musculoskeletal conditions (e.g. arthritis, rheumatism), and as a general tonic to increase energy, improve overall health and longevity, and prevent disease in athletes, and elderly.^[5-7] Recently, the antimicrobial activity of *W. somnifera* was studied by several authors on control strains (microbial type culture collection).^[5,7-9] However, there is no report of antibacterial activity of leaf extract of *W. somnifera* on human pathogenic Gram-positive bacterial strains from soft tissue infection. Hence, this study was planned to examine the antimicrobial potential of leaf extract of *W. somnifera* against Gram-positive cocci ($n = 20$) from pus samples of patients suffering from soft tissue infection.

Materials and Methods

Plant description

Withania somnifera is an erect, herbaceous, and evergreen tomentose shrub. The leaf base is cuneate and is densely hairy beneath. The flowers are yellow and berries orange-red.

Plant collection

The leaves of *W. somnifera* were collected from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Pantnagar (Uttarakhand).

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Preparation of extract

Extraction procedure was followed as Owais *et al.*^[8] with slight modification at the research and development laboratory in CIMAP, Pantnagar. Fresh leaves were washed thoroughly 2–3 times with running water followed by sterile distilled water. Washed leaves were air dried under the shade at room temperature and then pulverized by mortar pestle. Shade dried leaf powder was shaken overnight in methanol and then extracted successively in Soxhlet apparatus. Extract was filtered by Whatman number one filter paper and filtered solution was evaporated under reduced pressure with the help of rotary evaporator. The dried leaf extract was dissolved in methanol to final concentration of 1 mg/ml and 2 mg/ml.

Microorganisms

Gram-positive cocci obtained from clinical samples of pus from patients admitted to Government Medical College, Haldwani were used to find out the antimicrobial potential of leaf extract of *W. somnifera*. A total of 20 isolates including *S. aureus* (MRSA and methicillin sensitive *staphylococcus aureus* [MSSA]), *Enterococcus* and *Streptococcus* spp. were examined against methanolic extract of leaf of *W. somnifera*.

Sensitivity testing

The antibacterial susceptibility test was carried out using the agar diffusion method.^[10] Muller Hinton agar (MHA) was used for antibacterial susceptibility testing. For diffusion method, Petri plates were prepared by pouring 20 ml of MHA. Stock bacterial solution was thawed and immediately suspended in peptone water and incubated for 2–3 h at 37°C. After matching the turbidity with 0.5 Mc Farland, the inoculum of each isolate was spread on two MHA plates and was allowed to dry for 10 min. Four wells of 9 mm diameter each were punched in each plate using a sterile borer. Plant extract with concentration of 1 mg/ml and 2 mg/ml with different volumes of 20 µl, 50 µl, and 100 µl were poured in each of the 3 wells of MHA plates containing bacterial inoculum. Fourth (control well) was filled with 50 µl methanol. The plates were kept for 1 h at room temperature to allow the diffusion into the medium and then incubated aerobically at 37°C for 18 h. The inhibition zones formed around the wells were measured in millimeters [Figure 1]. For each concentration, the zones of inhibition produced by different strains of a species were averaged.

Results

Methanolic extract of *W. somnifera* showed potent antibacterial activity against Gram-positive clinical isolates that have been reported to be more resistant against routinely used first line of antibiotics such as ampicillin, co-trimoxazole, and erythromycin. At the concentration of 2 mg/ml (100 µl) average zone size of inhibition for MRSA and *Enterococcus* spp. was 20.6 and 19.8 mm, respectively. Whereas, for MSSA and *Streptococcus* spp. average zone of inhibition were 22.4 and 22.2 mm, respectively. At the concentration of 1 mg/ml (100 µl) average zone size of inhibition for MRSA and *Enterococcus* spp. was 19.2 and 19 mm, respectively. Whereas, for MSSA and *Streptococcus* spp. zone of inhibition were 20.8 and 21.2 mm, respectively.



Figure 1: Zone of inhibition (in mm) by *Withania somnifera* leaf extract in concentration of 1 mg/ml by using different volumes of 20 µl, 50 µl, and 100 µl and 2 mg/ml by using different volumes of 20 µl, 50 µl, and 100 µl on pathogenic isolates of -*Staphylococcus aureus* and *Streptococcus* spp. C-negative control (containing 100 µl methanol)

Overall increase of zone of inhibition was noticed with a higher concentration of leaf extract [Table 1 and Figure 1].

Discussion

W. somnifera leaf extract showed good inhibition of MRSA and *Enterococcus* spp. which were resistant against routinely used first line antibiotics. As in the previous study,^[9] methanolic root extracts of *W. somnifera* revealed 4 mm inhibitory zone against *Escherichia coli* and 10 mm inhibitory zone at 10 µg/ml against *Enterococcus*, which is very less than that observed in our study. This difference may be due to a different part of the plant extract being used. Owais *et al.*,^[8] observed 22 mm zone of inhibition against *S. aureus* at a concentration of 20 mg/ml of *W. somnifera* leaf extract. In another study,^[5] 15 mm zone of inhibition at a concentration of 100 µg/ml against *S. aureus* and *E. coli* by *W. somnifera* leaf extract was reported. In present study good inhibitory zone against *Enterococcus* spp., and *S. aureus* at the concentration of 2 mg/ml was observed. Difference in the zone of inhibition might be attributed to the difference in bacterial strains used, methodology employed, and different geographical area from where the plant was obtained.

Reference standard institutes like Clinical Laboratory Standard Institute or European Committee on Antibiotic Susceptibility have guidelines for interpretation of disc diffusion and minimum inhibitory concentration for antibiotics, but there is no such standard zone chart or guideline available for *W. somnifera* extract. In the present study, convincing zone of inhibition was found by *W. somnifera* leaf extract in pathogenic Gram-positive cocci isolates which were resistant to routinely used first line antibiotics (with no zone of inhibition). However, sample size of the present study was low; hence, this requires a much more exhaustive *in vitro* and *in vivo* study involving identification and isolation of specific *W. somnifera* compounds acting against a resistant strain.

Table 1: Zone of inhibition (in mm) for different concentration of *W. somnifera* leaf extract

Bacterial species (total strains)	1 mg/ml concentration of <i>W. somnifera</i> leaf extract in different volumes			2 mg/ml concentration of <i>W. somnifera</i> leaf extract in different volumes		
	20 µl	50 µl	100 µl	20 µl	50 µl	100 µl
MRSA (5)	17.4±0.89	18±1.22	19.2±0.83	18.8±0.83	20.2±1.09	20.6±0.89
MSSA (5)	19.6±0.57	20.2±0.44	20.8±0.83	20±0.00	21.2±0.44	22.4±0.54
<i>Streptococcus</i> spp. (5)	19.8±0.45	20.4±0.54	21.2±0.45	20.2±0.45	20.8±0.45	22.2±0.45
<i>Enterococcus</i> spp. (5)	17.2±1.09	18±1.22	19±1	18.6±0.3	19.4±0.8	19.8±0.7

Values are mean inhibition zone (mm)±SD. MRSA: Methicillin resistant *Staphylococcus aureus*, *W. somnifera*: *Withania somnifera*, SD: Standard deviation, MSSA: Methicillin sensitive *Staphylococcus aureus*

Conclusion

This finding supports the use of leaf extract of *W. somnifera* in the treatment of multidrug-resistant pathogens by alternative systems of medicine. Clinical trials with *Withania somnifera* for its activity against bacterial infections should be conducted.

Acknowledgments

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हिन्दी सारांश

मवाद के नमूनों से पृथक किये गये ग्राम घनात्मक जीवाणु के खिलाफ अश्वगंधा (*Withania somnifera*) की जीवाणुरोधी गतिविधि

पूनम बिष्ट, विनिता रावत

वर्तमान अध्ययन में राजकीय मेडिकल कालेज हल्द्वानी में भर्ती मरीजों के मवाद के नमूनों से पृथक किये गये ग्राम घनात्मक कोकाई के खिलाफ अश्वगंधा की पत्तियों के रस का प्रयोग उसकी जीवाणुरोधी क्षमता का परीक्षण करने में किया गया है। अश्वगंधा की पत्तियों के मेथेनॉलिक रस को लेकर एगार तैल विसरण विधि का प्रयोग किया गया है। यह देखा गया कि, अश्वगंधा की पत्तियों का मेथेनॉलिक रस पृथक किये गये जीवाणु, जिसमें कि मेथिसिलीन प्रतिरोधक स्टेफाइलोकॉक्स ऑरियस (*Methicillin resistant Staphylococcus aureus*) तथा इन्ट्रोकोक्स (*Enterococcus sp.*) शामिल हैं, को अवरोध करने में बहुत प्रभावी है। अश्वगंधा की पत्तियों के रस के 0.2 मि.ग्रा./मि.ली.(100 µl) सान्द्रता का प्रयोग करने पर क्रमशः इनका औसत अवरोध क्षेत्र 20.6 मि.मी. तथा 19.8 मि.मी. प्राप्त किये गये। ये परिणाम यह ईंगित करते हैं कि, अश्वगंधा की पत्तियों के रस के जीवाणुरोधी लक्षण, जीवाणु संक्रमण के खिलाफ चिकित्सकीय प्रयोग में पौधे के पारम्परिक प्रयोग का समर्थन करते हैं।