

# Evolution of Antimicrobial Activity of Leaf Extract of *Withania somnifera* Against Antibiotic Resistant *Staphylococcus aureus*

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**Background:** Emergence of bacterial resistance is critically an alarming situation in the health care industries. The medicinal plants have been used in the folk medicine to treat diseases without knowing their active compounds.

**Objectives:** The aim of present study was to screening the antibacterial activities of *Withania somnifera* leaf extract against antibiotic resistant strains of *Staphylococcus aureus*.

**Patients and Methods:** In this cross-sectional study, a total of 17 strains of *S. aureus* were isolated from hospitalized patients (Boo-Ali, Nabi-Akram and Imam-Ali hospitals, Zahedan, South-Eastern Iran) suffering from urinary tract infections during the years 2011 - 2012. The extract of *W. somnifera* obtained by rotary and the minimum inhibitory concentrations were investigated to characterize the antimicrobial activities of this extract.

**Results:** Overall, *S. aureus* showed resistance to 6 antibiotics including oxacillin (83.3%), ceftazidime (66.6%), penicillin (50%), trimethoprim-sulfamethoxazol (41.6%), cefixime (33.3%) and vancomycin (8.3%). The highest minimum inhibitory concentrations (MIC) values of extract were found to be 250 ppm against 12 strains and the least value was 63 ppm against 2 strains.

**Conclusions:** Ethanol extract of *W. somnifera* leaf might be exploited as natural drug for the treatment of several infectious diseases caused by this pathogen.

**Keywords:** *Staphylococcus aureus*; *Withania somnifera*; Plant extract; Antibiotic resistant; Minimum inhibitory concentrations

## 1. Background

Recently due to increase in antibiotic resistant strains of clinically important pathogens, a line of new bacterial strains that are multi drug resistant have emerged [1, 2]. Various plant extracts have been examined for their antibacterial activity with the objective of exploring safe alternatives of antibiotics [3]. *Withania somnifera* commonly known as Ashwagandha or Indian ginseng is a major Indian medicinal plant that belongs to family Solanaceae [4]. It can be found growing in Africa, the Mediterranean, and India. It has been used as an antibacterial, antioxidant, aphrodisiac, liver tonic and anti-inflammatory agent [5]. It counteracts the effects of stress and generally promotes wellness. The antimicrobial properties of this plant species have been widely reported in literatures [6, 7]. Previous reports on the activities of methanolic extracts of the whole *W. somnifera* plant against different pathogenic bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans*, have found significant antibacterial properties [8]. *Staphylococcus aureus* is a major cause of nosocomial infections and produces numerous and serious infections in humans [9, 10]. Humans are natural reservoirs for *S. aureus* and asymptomatic colonization is far more common

than infection. Colonization of the nasopharynx, perineum, or skin, particularly if the cutaneous barrier has been disrupted or damaged, may occur shortly after birth and may recur anytime thereafter [11, 12].

## 2. Objectives

Present study was carried out to screen antibacterial activity of *W. somnifera* leaf extract against antibiotic resistance *S. aureus*.

## 3. Patients and Methods

### 3.1. Isolates

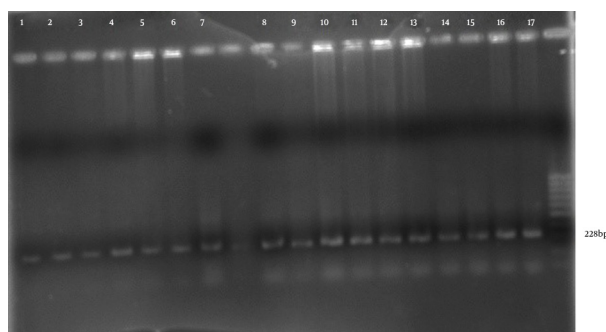
A total of 17 isolates from 50 patients were cultured and screened in the Boo-Ali, Nabi-Akram and Imam-Ali hospitals (Zahedan, south-eastern Iran) during years 2011 - 2012. Isolated Gram and catalase positive cocci were further tested for biochemical characterization including lysostaphin sensitivity, coagulase, clumping factor, thermonuclease and haemolysin tests [13]. Subsequent to biochemical tests, staphylococcal isolates were identified using species-specific gene amplification (16S-rDNA) (Figure 1 and Table 1).

### 3.2. Antibiotics Susceptibility

The susceptibility of the isolates for antibiotics was carried out using disc-diffusion method on Mueller-Hinton agar as recommended by Clinical and Laboratory Standards Institute with minor modification [14]. Briefly, colonies of *S. aureus* were suspended in a tube containing 0.5 mL of saline and bacterial strains were transferred to the surface of the Muller-Hinton agar using sterile cotton swabs. Antibiotic discs aseptically transferred on the surface of inoculated plates. The plates were incubated for 24 hours at 37°C and zones of growth inhibition were recorded. Antibiotics and their potencies were as follow: ceftazidime (30 µg), cefixime (30 µg), trimethoprim-sulfamethoxazol (1.25 + 23.15 µg), penicillin (10 µg), oxacillin (30 µg) and vancomycin (10 µg) (Patan-Teb-Iran).

### 3.3. Plant Materials

The leaves of *W. somnifera* were collected from countryside of Zabol (South-Eastern, Iran) and dried at room temperature. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.



**Figure 1.** PCR for Gene 16sr DNA

**Table 1.** Oligonucleotide Primer for Identification *S. aureus*

| 16sr DNA | Primer Sequence     |
|----------|---------------------|
| Forward  | GTAGGTGGCAAGCGTTACC |
| Reverse  | CGCACATCAGCGTCAG    |

**Table 2.** Antibiotic Resistance of 17 Strains of *S. aureus* <sup>a,b</sup>

|          | CN   | SXT  | V    | CAZ  | P  | OX   |
|----------|------|------|------|------|----|------|
| <b>S</b> | 58.3 | 50   | 50   | 25   | 25 | 0    |
| <b>I</b> | 8.3  | 8.3  | 41.6 | 8.3  | 25 | 16.6 |
| <b>R</b> | 33.3 | 41.6 | 8.3  | 66.6 | 50 | 83.3 |

<sup>a</sup> Values are presented as (%).

<sup>b</sup> Abbreviations: CAZ, Ceftazidime; CN, Cefixime; I, Intermediate; OX, Oxacillin; P, Penicillin; R, Resistant; S, Sensitive; SXT, Trimethoprim-Sulfamethoxazol V, Vancomycin.

### 3.4. Preparation of Extract

The leaves were pulverized into a coarse powder and 20 g grinded powder was soaked in 60 mL ethanol 95%, for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman no. 1 filter paper). Then the filtrates were evaporated using rotary evaporator and 0.97 g of dried extract was obtained and stored at 4°C in air tight screw-cap tube.

### 3.5. Minimum Inhibitory Concentration (MIC) of Plant Extract

The broth micro-dilution method was used to determine MIC using Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged 500, 250, 126, 62 and 31 ppm. To each well, 10 µL of indicator solution and 10 µL of Mueller Hinton broth were added. Finally, 10 µL of bacterial suspension ( $10^6$  CFU/mL) was added to each well to achieve a concentration of  $10^4$  CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates and then they were placed in an incubator at 37°C for 18 - 24 hours. The color change was then assessed visually and the lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by turbidity.

## 4. Results

The isolated strains showed resistance to 6 antibiotics including oxacillin (83.3%), ceftazidime (66.6%), penicillin (50%), trimethoprim-sulfamethoxazol (41.6%), cefixime (33.3%) and vancomycin (8.3%) (Table 2).

Inhibitory effects of extract from *W. somnifera* against *S. aureus* were demonstrated in Table 3. The highest Minimum Inhibitory Concentrations (MIC) values of extract were found to be 250 ppm against 12 strains and the least value was 62 ppm against 2 strains (Table 3).

**Table 3.** Antimicrobial Susceptibility (as MIC) of *W. somnifera* Extract for *S. aureus* (ppm)

| Bacterial Code | MIC for Extract, ppm |
|----------------|----------------------|
| 1              | 250                  |
| 2              | 250                  |
| 3              | 250                  |
| 4              | 250                  |
| 5              | 250                  |
| 6              | 250                  |
| 7              | 250                  |
| 8              | 250                  |
| 9              | 126                  |
| 10             | 250                  |
| 11             | 250                  |
| 12             | 62                   |
| 13             | 250                  |
| 14             | 250                  |
| 15             | 126                  |
| 16             | 126                  |
| 17             | 62                   |

## 5. Discussion

As our results, all isolates of *S. aureus* showed resistance to the antibiotics including oxacillin (83.3%), ceftazidime (66.6%), penicillin (50%), trimethoprim-sulfamethoxazol (41.6%), cefixime (33.3%) and vancomycin (8.3%). Emergence of bacterial resistance is critically an alarming situation in developing and developed countries. According to the study of Duran et al. the staphylococcal isolates showed resistance to tetracycline (41%), penicillin (92%) and trimethoprim-sulphamethoxazol (32.2%) [15]. Also in one study in Mashhad, staphylococcal isolates were highly resistant against ceftazidime (94%), followed by penicillin (91%), ampicillin (82%), cefotaxime (65%), erythromycin (60%) and oxacillin (43%) [16]. In another study, the overall susceptibility of isolated *S. aureus* strains to antimicrobial agents was 100% for vancomycin, 49.4% for amikacin, 43.8% for gentamicin, 36.8% for trimethoprim-sulfamethoxazole and tetracycline, 36.3% for cefazolin, 30.6% for cephalixin, 24.4% for oxacillin, 23.8% for erythromycin and 3.1% for penicillin [17]. Ghasemian et al. showed that the resistance of *S. aureus* to other antibiotics (by the disc diffusion test) are as follows: cephalotin (87.0%), gentamicin (26.0%), trimethoprim-sulfamethoxazole (19.3%), clindamycin (12.9%) and ciprofloxacin (9.7%) [18]. In the study of Rahimi et al. the resistance pattern was as follows: ampicillin (100%), ciprofloxacin (93%), methicillin and oxacillin (88%), kanamycin (66%), cephotaxim (65%), tetracycline (64%), erythromycin and sulphamethoxazole-trimethoprim (41%), chloramphenicol (40%), clindamycin (38%), gentamicin (20%) and vancomycin (0%) [19].

In our study, the highest MIC values of extract were found to be 250 ppm against 12 strains and two MIC values were 63 ppm. In a similar study, the ethanol extract of *W. somnifera* showed more activity against *S. aureus* (zone of diameter  $20.1 \pm 0.1$  mm) compared with the ethanol extract [20]. In another study, on the other hand, maximum activity was observed in water solvent (zone of diameter  $21.8 \pm 0.2$  mm) against *R. planticola* followed by benzene (zone of diameter  $19.8 \pm 0.2$  mm) and chloroform solvent (zone of diameter  $15.8 \pm 0.2$  mm) against *S. aureus* [20]. In the study of Srinu et al. *W. somnifera* plant extract showed more inhibitory activity on Gram positive organisms (*S. aureus* and *B. cereus*) [21]. Jain and Varshney reported the antibacterial activity of the methanolic extracts of the whole *W. somnifera* plant against *E. coli*, *P. aeruginosa*, *S. aureus*, *S. mutans* and *C. albicans* with zones of inhibition of 38, 36, 15, 38 and 32 mm, respectively [8]. As the results of Pandit et al. methanol extract of *W. somnifera* demonstrated a broad antibacterial range against *Streptococcus mutans* and *Streptococcus sobrinus* (MIC 0.125 - 2 mg/mL) [22]. According to the study of Alam et al. methanol extract of the plant displayed the highest activity against *S. typhi* ( $32.00 \pm 0.75$  mm zone of inhibition), whereas the lowest activity was against *K. pneumoniae* ( $19.00 \pm 1.48$  mm zone of inhibition) [23]. Different parts of this plant show antimicrobial effects against wide range of microorganisms including Multiple Drug Resistant (MDR) strains. In one study, root extract of *W. somnifera* was effective against all the MDR *S. aureus* strains isolated from local and patient sources and different root extracts showed different degree of effectiveness against the isolates [24]. Flavonoid or other phenolic compounds may participate in selective inhibitory action of the extract. Lee and Kim have previously isolated flavonoids, including quercetin glycosides from the leaves of *W. somnifera* [25].

In conclusion, the present study demonstrated that the ethanol leaf extract of *W. somnifera* hold an excellent potential as an antibacterial agent. The leaf extract of *W. somnifera* was found on the other hand, to be more effective in inhibiting the antibiotic resistant *S. aureus* strains.

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## Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

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