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Evaluation of Antibacterial activity of fruit and root extracts of Withania somnifera against Human pathogenic bacteria

Kadudula Prasuna[1] and Yalavarthy Prameela Devi[2]*

[1-2] *Department of Zoology, Kakatiya University, Warangal – 506 009. Telangana State, India.*

Abstract

Withania somnifera, also known as ashwagandha, is an important herb in ayurvedic and indigenous medical systems. The present study was designed to evaluate the antibacterial activities of an 80% aqueous methanolic extract of *W. somnifera* roots and fruits. Antibacterial activities were measured using the agar well diffusion method and MIC values were determined by microdilution method and five pathogenic Gram-negative bacteria: *Escherichia coli, Salmonella typhi, Citrobacter freundii, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The root extracts displayed the highest activity against *S. typhi* (32.00 ± 0.56 mm zone of inhibition), whereas the lowest activity was against *K. pneumoniae* (19.00 ± 1.56 mm zone of inhibition). The lowest minimum inhibitory concentration value was 6.25 mg/ml, which was against *S. typhi*, followed by 12.5 mg/ml against *E. coli.* It will be beneficial to investigate the active compounds present in *W. somnifera* so that its roots can be used to increase the armamentarium of antimicrobial agents and so that other possible therapeutic uses of the plant can be explored.

Keywords: Antibacterial activity, *Withania somnifera,* methanol, MIC, agar well diffusion method,

INTRODUCTION

Medicinal plants are very important in human health. It will act as an antibactericide, \followed from ancient times (Zaika et al, 1988). *Withania somnifera* is an evergreen plant, native to the Indian subcontinent, successfully introduced worldwide, now extensively cultivated in many other countries including India. *W. somnifera* (solanacae) are gaining attentions in various field of research, as they are best suited to the present environmental conditions. *W. somnifera* is used for its anti-inflammatory (Hindawi et al, 1992), antioxidant (Bhattacharya et al, 1997), memory-improving (Schliebs et al, 1997) and analgesic effects (Kulkarni et al, 1997). The leaves are an insect repellant (Buchanan et al, 1987). Development of microbial resistance to antibiotics is a global concern. Isolation of microbial agents less susceptible to regular antibiotics and appearance of increasing resistant isolates during antibacterial therapy is rising throughout the world. *Klebsiella pneumoniae* is a Gram-negative, nonmotile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacteria, found in the normal flora of the mouth, skin, and intestines. *K. pneumoniae* is an important pathogen that causes urinary tract infections (UTIs), pneumonia, and intra-abdominal infections in hospitalized immunocompromised patients with severe underlying diseases (Buchanan et al, 1987).

The aim of this study was to evaluate antimicrobial activity of fruit extracts of *W. somnifera* against human pathogenic bacteria.

MATERIALS AND METHODS

Plant materials

In January 2016, *W. somnifera* fruits and roots were collected from field-grown plants after six months of cultivation in the Botanical Garden of Kakatiya University, Warangal, Telangana State. These plants were identified with the help of the available literature and were authenticated by a botanist, Professor Raju from the Department of Botany, Kakatiya University (Warangal, TS). They were stored at the herbarium lab of the department and the collected fruits of the medicinal plant were cleaned, air-dried in the shade and ground to a fine powder.

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Preparation of plant extracts

The *W. somnifera* fruit and root extracts were prepared according to a modified method described by Kahkonen et al. (1999). Ground dry fruits (500 mg) were weighed in a test tube, followed by the addition of 10 ml of 80% aqueous methanol. The suspension was then gently stirred. The tubes were sonicated for 5 min (45°C) and centrifuged (25°C) for an additional 10 min at 1500 ×g. The resulting supernatants were collected. The extraction procedure was repeated three times, and the supernatants were combined before being evaporated using a rotary evaporator to a volume of approximately 1 ml. The extracts were concentrated under reduced pressure by rotary evaporator and weighed.

Antibacterial properties of *W. somnifera:*

Extract sterility

The extracts were filtered using Millipore nylon membranes (0.45 µm) and then tested for sterility by introducing 2 ml of the extract into 10 ml of sterile nutrient broth. The extracts were incubated at 37°C for 24 hr. A sterile extract was indicated by the absence of turbidity or the clarity of the broth after the incubation period (Atlas et al, 1995).

Bacterial strains

The *in vitro* antimicrobial activities of *W. somnifera* fruit and root extracts were investigated. Five pathogenic bacteria, namely *Escherichia coli, Salmonella typhi, Citrobacter freundii, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, were obtained from the Department of Microbiology, Kakatiya University, Warangal. All of the microorganisms were maintained at 4°C on nutrient agar slants.

Determination of antibacterial activity

The antibacterial activities of *W. somnifera* crude extracts were determined using the agar well diffusion method. For the evaluation of antimicrobial activities, a fresh 24 hr culture of bacteria was suspended in sterile distilled water. The final inoculum size was adjusted to 5×10^5 CFU/ml. Nutrient agar (nutrient broth+1.8% agar) was inoculated with the given microorganism by spreading the bacterial inoculum on the media. Wells (8 mm diameter) were punched in the agar and filled with 200 µl of the plant extracts (5 mg/ml). Negative control wells containing neat solvent (80% aqueous methanol) or a standard antibiotic solution of tetracycline (100 µg/ml) (positive control) were run in parallel on the same plate. The plates were incubated at 37°C for 24 hr. Antibacterial activities were assessed by measuring the diameters of the zones of inhibition for the respective drugs. The relative antibacterial potency of a given preparation was calculated by comparing its zone of inhibition with that of the standard antibiotic tetracycline.

Determination of the minimum inhibitory concentration (MIC) of the extract

The initial plant extract (100 mg/ml) was serially diluted by transferring 5 ml of the sterile plant extract (stock solution) into 5 ml of sterile nutrient broth to obtain the following dilutions: 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and finally 3.125 mg/ml. After obtaining the different extract concentrations, each concentration was inoculated with 0.1 ml of a standardized bacterial cell suspension (approximately 10^6 CFU/ml) and incubated at 37°C for 24 hr. The lowest concentration of the extract that inhibited the growth of the test organism was taken as the MIC. The controls were prepared as follows: (i) nutrient broth only (positive control), (ii) nutrient broth and sterile plant extract, (iii) nutrient broth and a test organism (positive control), and (iv) the standard antibiotic tetracycline (positive control).

Statistical analyses

All analyses were performed in triplicate, and the data are expressed as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test was used to compare the data. Differences of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

As shown in Table-1, the antimicrobial activities of *W. somnifera* fruit and root crude extracts and their

Table-1. Diameters of the zones of inhibition for the 80% aqueous methanolic extracts of root and fruit extract on several species of human pathogenic bacteria

Test organisms	Diameters of zones of inhibition (mm)		
	Root extract	Fruit extract	Tetracycline (100 µg/ml)
Escherichia coli	28 + 0.82^b	12 + 1.20^c	44 + 1.0^a
Salmonella typhi	32 ± 0.56^b	13 + 0.59^c	46 + 1.32^a
Citrobacter freundii	24 + 1.25^b	9 + 1.22^c	38 + 1.33^a
Pseudomonas aeruginosa	27 + 1.09^b	10 + 1.23^c	40 + 0.54^a
Klebsiella pneumoniae	19 + 1.56^b	8 + 1.52^c	36 + 1.17^a

Each value represents the mean ± SD of three different observations. Different letters in each row indicate a significant difference ($p<0.05$).

Table-2. Determination of the minimum inhibitory concentration (MIC) of the methanolic extracts of the root and fruit extract on test organisms

Test organisms	Concentrations (mg/ml)		
	Root extract	Fruit extract	Tetracycline (100 µg/ml)
Escherichia coli	25	25	0.04
Salmonella typhi	50	25	0.02
Citrobacter freundii	50	50	0.06
Pseudomonas aeruginosa	50	50	0.05
Klebsiella pneumoniae	50	50	0.03

potencies were quantitatively assessed by the presence or absence of a zone of inhibition and the zone diameter, respectively. The methanolic extracts (80%) of the fruit displayed antimicrobial activities against all five pathogenic bacteria. The root extract displayed the highest antibacterial activity against all of the pathogenic bacteria tested compared with the fruit extract.

Overall, the root extract exhibited the greatest zone of inhibition against all five microorganisms. For the specific plant parts and microorganism, root extract exhibited the highest activity against *S. typhi*, whereas the lowest activity was against *K. pneumoniae*. The largest zone of inhibition for fruit extract was against *S. typhi*, and the smallest zone of inhibition was also against *K. pneumoniae*. For root extract, the largest zone of inhibition was against *E. coli*, whereas the smallest zone of inhibition was against *P. aeruginosa*.

The MICs for root and fruit extracts against the five pathogenic bacteria are shown in . Fruit extract had the lowest MIC against *S. typhi*, whereas its highest MIC values were against *C. freundii, P. aeruginosa* and *K. pneumonia*. The lowest MIC for fruit extract (25.00 mg/ml) was found for *E. coli* and *S. typhi*, whereas MICs of 50 mg/ml were found for *C. freundii*, *P. aeruginosa* and *K. pneumoniae*. For root extract, the lowest MIC (25.00 mg/ml) was found for *E. coli*, whereas its MIC was similar for the four other bacterial species, that is, 50.00 mg/ml.

For the antibacterial activity study, the 80% methanolic extract of all parts of *W. somnifera* displayed activity against five pathogenic Gram-negative bacteria, namely *E. coli, S. typhi, C. freundii, P. aeruginosa* and *K. pneumoniae*, to different magnitudes. *W. somnifera* roots possessed the greatest antimicrobial effects. Phenolics, ascorbic acid and anthocyanins are associated with the antimicrobial efficiency of the plant because they cause hyperacidification at the plasma membrane interface of the pathogen, which potentially results in the disruption of the H+-ATPase required for ATP synthesis (Vatterm et al, 2004). The most susceptible organism was *S. typhi*, indicating that the *W. somnifera* extracts contain active compound that can inhibit the proliferation and growth of *S. typhi*. which can cause diseases such as typhoid fever and foodborne illnesses. This finding may support the traditional uses of *W. somnifera* as a therapeutic agent for diarrhea, dyspepsia and gastrointestinal disorders (Acharyya et al, 2009).

The other tested bacteria also exhibited significant sensitivities against root extract, and fruit extract. These results demonstrate that the methanolic extracts contained the expected compounds for antibacterial activities against the five tested Gram-negative bacteria. *W. somnifera* may be exploited as a natural drug for the treatment of several infectious diseases initiated by these organisms. This finding is important in the quest for new antimicrobial agents because organisms with multidrug resistance are rapidly emerging.

Jain and Varshney (2011) reported on the antibacterial activity of the methanolic extracts of the whole *W. somnifera* plant against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans* and *Candida albicans*, with zones of inhibition of 38, 36, 15, 38 and 32 mm, respectively. These results are similar to our reported zones of inhibition for root extract against two of the same organisms, *Escherichia coli* and *Pseudomonas aeruginosa*, at 28 ± 0.56 and 26 ± 1.08 mm, respectively. However, Jain and Varshney (2011) reported that aqueous extracts of *W. somnifera* had higher antimicrobial activities (a zone of inhibition between 33 and 50 mm) when compared with methanolic extracts. In this study, methanol was used to extract low molecular weight and moderately polar substances because of its wide range of solubility.

Conclusion

The roots of *W. somnifera* possess significant antibacterial properties against Gram-negative organisms, in particular, *S. typhi*. It will be beneficial to investigate the active compounds present in *W. somnifera* so that its roots can be used to increase the armamentarium of antimicrobial agents and so that other possible therapeutic uses of the plant can be explored.

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Competing interests

The authors have declared that no competing interests exist.

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