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Full Length Research Paper

Antibacterial potential of *Withania somnifera* L. against human pathogenic bacteria

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The present study was conducted to evaluate the antibacterial activity of chloroform, acetone, methanolic, and ethanolic crude extracts of stem, leaves, and roots of *Withania somnifera.* It was observed that acetone extracts was the most effective followed by methanolic and ethanolic extracts for all the test organisms used. It was observed that acetone extracts were the most effective in inhibiting the growth of all the pathogenic bacteria used. The zone of inhibition was maximum with acetone extracts ranging between 38 and 10 mm, followed by methanolic extracts ranging between 28 and 10 mm and ethanolic extracts ranging between 25 and 8 mm, respectively. However, *Klebsiella pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA) did not respond to root extracts of both methanolic and ethanolic extracts. The chloroform extracts of stem and leaves showed significant activity against all pathogens with inhibition zone between 20 and 8 mm. The results indicate that acetone, methanolic, and ethanolic extracts of *W. somnifera* might be exploited as natural drug for the treatment of several infectious diseases caused by these organisms.

Key words: *Withania somnifera*, acetone extracts, methanolic extract, ethanolic extract, antimicrobial activity, inhibition zone.

INTRODUCTION

Medicinal plants are a boon of nature to human mankind and have been used for centuries to cure a number of human diseases. In many parts of the world, medicinal plants are used against bacterial, viral, and fungal infections. Evaluation of plants, bearing efficiency in healing various diseases is growing in recent years. Innumerable biologically active compounds of plants are found to possess antibacterial properties. According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Renu Sarin et al., 2010). The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Bandow et al., 2003).

Since the discovery of antibiotics and their uses as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eradication of infectious diseases (Sibanda and Okoh, 2007). However, diseases and disease agents that were once thought to have been controlled by antibiotics are returning in new forms resistant to antibiotic therapies (Levy and Marshall, 2004). Incidents of epidemics due to such drug resistant microorganisms are now a common global problem posing enormous public health concerns (Iwu et al., 1999) of multi-drug resistant bacterial strains is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure of infections (Hancock, 2005). Examples include methicillin-resistant Staphylococci, Pneumococci resistant to penicillin and macrolides, vancomycin-resistant enterococci as well as multi drug resistant Gram-negative organisms (Norrby et al., 2005). Because of this increasing global concern, we are confronted with the need to look for safer phytochemicals. The flora of Saudi Arabia is one of the richest biodiversity areas in the Arabian Peninsula and

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comprises very important genetic resources of crop and medicinal plants (Collenette, 1998; Rahman et al., 2004). Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial agents, a systematic investigation was undertaken to screen the potential antibacterial activity of *Withania somnifera.*

W. somnifera L. *Dunal* is an important medicinal plant, of high ethanobotanical importance grown and distributed widely in Saudi Arabia and used in folk medicine of several other countries. The root of *W. somnifera,* known as Indian ginseng (Ashwagandha), has been described in Ayurvedic folk medicine to have potent aphrodisiac, sedative, and energy-enhancing tonic properties (Williamson, 2002; Misra et al., 2000). Moreover, it is beneficial in the treatment of arthritis, rheumatisms, cough, geriatric problems, stress, and male sexual dysfunctions (Ahmed et al., 2010; Mahesh, 2008). It is traditionally known for its anti-tumor, anti-inflammatory, and anti-septic activities due to its abundance of secondary metabolites (Christina et al., 2004). Several workers have screened the leaves of *W. somnifera* for their antimicrobial activity in Saudi Arabia; however, the potential of its root and stem as an antimicrobial activity has not been explored as such in my knowledge. Hence, the present study was designed to investigate the potential antibacterial effect of leaves, stem, and roots extracts of *W. somnifera* on seven pathogenic bacteria.

MATERIALS AND METHODS

Collection of plant material

Fresh plant material of *W. somnifera*, that is, leaves, stem, and roots were collected from different locations of Riyadh, Saudi Arabia and authenticated by botanist at King Saud University.

Preparation of plant extracts

The stems were separated from the roots and the leaves were cut from the stem. These parts were washed individually under running tap water and air dried to a constant weight before extraction. The dried stem, leaves, and root samples were ground well into a fine powder with a mixer grinder. The powder was stored in airtight bottles at room temperature before extraction (Alagesaboopathi, 2011). The method of Alade and Irobi (1993) was adopted for preparation of plant extracts with slight modification. A fixed weight (25 g) of powdered plant material was soaked separately in 150 ml each of acetone, ethanol, and methanol and chloroform in a plugged conical flask and then was kept on a rotator shaker at 180 to 200 rpm for 24 h. At the end of the extraction, each extract was passed through Whatman No.1 filter paper (Whatman, England), and the filtrate obtained was concentrated in vacuum using evaporator. Then, the extracts were used for antibacterial assay or were stored at 4°C for further use.

Growth and maintenance of test microorganisms for antibacterial studies

Bacterial strains used in this study for the evaluation of antibacterial activity were obtained from the Department of Microbiology, King Khaled Hospital, Riyadh, Saudi Arabia. *Bacillus subtilis* ATCC 6633, Methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 12498, *Streptococcus pyogenes* ATCC 19615, *Enterococcus faecalis* ATCC 29212, *Escherichia coli ATCC* 25966, and hospital isolates of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were used as test organisms in the present study. Stock cultures were maintained at 4°C on slopes of nutrient agar.

Screening for antibacterial activity

Agar well diffusion method

Antimicrobial activity of crude extracts in different organic solvents were tested against pathogenic bacteria using agar well diffusion method (Rajendran and Ramakrishna, 2009; Mahesh and Satish, 2008; Kambizi and Afolayan, 2008). Bacteria (200 µl) were aseptically introduced and spread using cotton swabs on surface of gelled sterile Muller Hinton agar plates. The optical density (OD) of the working bacterial culture was measured with the colorimeter and microbial population was confirmed to be within in 10^6 cells/ml. This suspension was used as inoculums (Jaina and Varshney, 2011). A well of about 6 mm diameter with sterile cork borer was aseptically punched on each agar plate. 50 µl of the crude extracts of stem, roots, and leaves of *W. somnifera* were introduced into the wells in the plates. Plates were kept in laminar flow for 30 min for pre diffusion of extract to occur and were then incubated at 37°C for 24 h. The presence of zone of inhibition was regarded as the indicator of antimicrobial activity and was expressed in terms of average diameter of the zone of inhibition in millimeter (means of three replicates ± standard deviation (SD)). Each test was carried out in triplicates.

To evaluate the efficiency of the methodology and to compare the potentiality of the antibacterial effect of the crude extracts tested, a negative control well was made with 50 µl of the extracting solvent and a positive control was made by placing standard antibiotic disc. The standard antibiotic used in this assay are as follows: Tetracycline (T 30 µg, Oxoid), Vancomycin (V 30 µg, Oxoid), Sulphamethoxazole trimetoprine (SXT 25 µg, Oxoid), and Impenem (Im10 µg, Oxoid).

RESULTS

In the present investigation, the antimicrobial activity of acetone, methanol, ethanol, and chloroform extracts of different parts of *W. somnifera* were evaluated against Gram positive and Gram negative bacteria. Our results indicate that all parts evaluated showed positive antibacterial activity against most of the bacteria tested (Tables 1 to 3). The antibacterial activity of crude extract of stem in different polar and non polar solvents is summarized (Table 1). Our findings indicate clearly that all test strains exhibited positive results with acetone extracts. *S. pyogenes* showed a maximum inhibition zone (25.80 ± 0.34), followed by the least in chloroform extract (8.83 ± 0.20 mm). However, *K. pneumoniae* did not show any inhibition with methanolic and ethanolic extracts of the stem. Similarly, MRSA did not respond to ethanolic stem extracts of *W. somnifera.*

Results of antibacterial activity of leaf extracts showed significant inhibition of all the test organisms used for screening except for *K. pneumoniae,* which was not

Table 1. Antibacterial activity of crude stem extracts of *W. somnifera* and their zones of inhibition.

Microorganism	Zone of inhibition (mm)				
	Extract				
	Acetone	Methanol	Ethanol	Chloroform	Antibiotic
B. subtilis	18.33 ± 0.57	20.26 ± 0.11	25.26 ± 0.85	10.93 ± 0.05	37.50 ± 0.00 (T)
MRSA	20.40 ± 0.86	16.06 ± 0.15	--	12.96 ± 0.15	26.47 ± 0.15 (V)
S. pyogenes	25.80 ± 0.34	20.10 ± 0.10	8.96 ± 0.05	8.83 ± 0.20	10.26 ± 0.32 (T)
E. feacalis	18.96 ± 0.15	22.53 ± 0.11	20.03 ± 0.15	13.90 ± 0.20	22.60 ± 0.20 (SXT)
E. coli	21.66 ± 0.57	21.96 ± 0.05	15.03 ± 0.15	16.90 ± 0.26	38.56 ± 0.65 (T)
P. aeruginosa	20.83 ± 0.56	21.03 ± 0.11	10.20 ± 0.20	--	8.36 ± 0.11 (Im)
K. pneumoniae	14.86 ± 0.15	--	---	13.23 ± 0.25	29 ± 0.00 (SXT)

Values are mean inhibition zone (mm) ± SD of three replicates. -Indicates no inhibition. Antibiotics used -- Tetracycline (T, 30 µg), Vancomycin (V, 30 µg), Sulphamethoxazole trimetoprine, (SXT, 25 µg), and Impenem (Im, 10 µg). Bacteria used --- *B. subtilis (Bacillus subtilis),* MRSA *(*Methicillin resistant *Staphylococcus aureus*), *S. pyogenes Streptococcus pyogenes)*, *E. faecalis (Eneterococcus faecalis)*, *E. coli (Escherishia coli), P. aeruginosa (Pseudomonas aeruginosa),* and *K. pneumoniae (Klebsiella pneumonia).*

Table 2. Antibacterial activity of crude leaf extracts of *W. somnifera*.

Microorganism	Zone of inhibition (mm)				
	Extract				
	Acetone	Methanol	Ethanol	Chloroform	Antibiotic
B. subtilis	22.53 ± 0.76	20 ± 0.00	15.37 ± 0.42	15.93 ± 0.25	37.50 ± 0.00 (T)
MRSA	16 ± 0.00	28.56 ± 0.40	22 ± 0.00	19.06 ± 0.81	26.47 ± 0.15 (V)
S. pyogenes	34.13 ± 0.75	24.93 ± 0.20	14.20 ± 0.43	10.06 ± 0.11	10.26 ± 0.32 (T)
E. feacalis	24.86 ± 0.61	27.90 ± 0.26	16.46 ± 0.45	15.06 ± 0.35	22.60 ± 0.20 (SXT)
E. coli	38.83 ± 0.15	24.26 ± 0.30	18.13 ± 0.20	16.80 ± 0.10	38.56 ± 0.65 (T)
P. aeruginosa	35 ± 0.00	19.73 ± 0.25	24 ± 0.00	20.30 ± 0.60	8.36 ± 0.11 (Im)
K. pneumoniae	11.10 ± 0.10	-	-	10.76 ± 0.23	29 ± 0.00 (SXT)

Values are mean inhibition zone (mm) ± SD of three replicates. --- Indicates no inhibition. Antibiotics used -- Tetracycline (T, 30 µg), Vancomycin (V, 30 µg), Sulphamethoxazole trimetoprine (SXT, 25 µg), and Impenem (Im, 10 µg). Bacteria used ---MRSA; Methicillin resistant *S. aureus.*

Table 3. Antibacterial activity of crude root extracts of *W. somnifera* and their zones of inhibition.

Microorganism	Zone of inhibition in mm				
	Extract				
	Acetone	Methanol	Ethanol	Chloroform	Antibiotics
B. subtilis	15.56 ± 0.35	12.33 ± 0.30	10.90 ± 0.17	11.10 ± 0.44	37.50 ± 0.00 (T)
MRSA	10 ± 0.00	-	-	-	26.47 ± 0.15 (V)
S. pyogenes	13.90 ± 0.10	19.53 ± 0.41	17.93 ± 0.30	--	10.26 ± 0.32 (T)
E. feacalis	13.50 ± 0.53	10.66 ± 0.30	13 ± 0.00	11.73 ± 0.25	22.60 ± 0.20 (SXT)
E. coli	16.00 ± 0.12	10.20 ± 0.34	15.96 ± 0.85	--	38.56 ± 0.65 (T)
P. aeruginosa	22.27 ± 0.32	20.80 ± 34	18.86 ± 0.15	---	8.36 ± 0.11 (Im)
K. pneumoniae	18.06 ± 0.11	---	--	13.83 ± 0.25	29 ± 0.00 (SXT)

Values are mean inhibition zone (mm) ± SD of three replicates. --- Indicates no inhibition. Antibiotics used -- Tetracycline (T, 30 µg), Vancomycin (V, 30 µg), Sulphamethoxazole trimetoprine, (SXT, 25 µg), and Impenem (Im, 10 µg). Bacteria used ---MRSA; Methicillin resistant *S. aureus.*

inhibited neither by methanolic nor by ethanolic extracts. Acetone extracts exhibited highest inhibition of *E. coli* (38.83 ± 0.15 mm) followed by *P. aeruginosa* (35 ± 0.00 mm), and *S. pyogenes* (34.13 ± 0.75 mm). MRSA showed positive results with all solvents; however, a maximum zone (28.56 ± 0.40 mm) was exhibited in

Figure 1. Effect of the organic solvents from *W. somnifera* leaf extract on *Bacillus subtilis* and their inhibition zones. Bac: *Bacillus subtilis*; A: acetone; M: methanol; E: ethanol; C: chloroform.

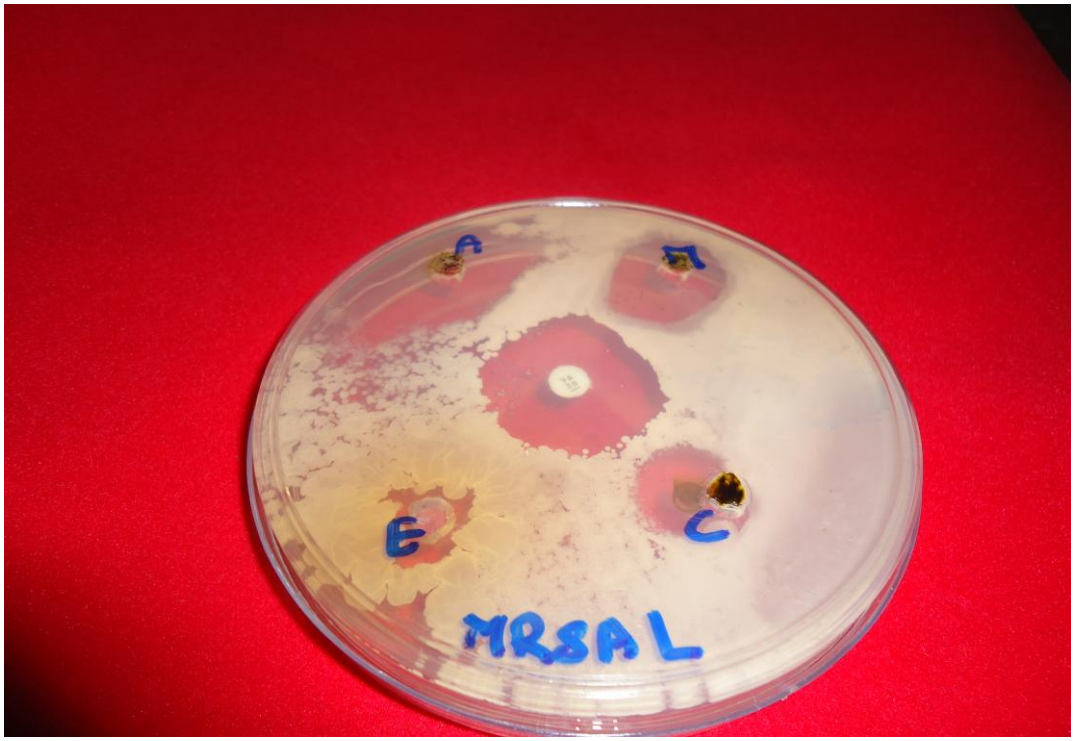

Figure 2. Effect of the organic solvents from *W. somnifera* leaf extracts on MRSA and their inhibition zones in comparison with antibiotic disc Vancomycin 30 µg. MRSA: methicillin- resistant *Staphylococcus aureus*; A: acetone; M: methanol; E: ethanol; C: chloroform.

methanolic extract followed by ethanolic extract (22.56 ± 0.00 mm) (Figures 1 and 2).

The summarized findings of Table 3 state that root extracts of *W. somnifera* show maximum antimicrobial

activity against the test microbes with zone of inhibition lying in the range of 22.27 ± 0.32 to 10 ± 0.00 mm. The acetone extracts of *W. somnifera* roots showed varying range of inhibition of test organisms with a maximum in *P. aeruginosa* (22.27 ± 0.32 mm) and least activity with MRSA (10 ± 0.00 mm). Both *K. pneumoniae* and MRSA did not show any inhibition with root extracts of methanol and ethanol. Our findings show that *K. pneumoniae* did not respond to methanolic and ethanolic extracts of stem, leaves, and root of *W. somnifera* (Tables 1 to 3).

DISCUSSION

The quest for solutions to the global problem of antibiotic resistance in pathogenic bacteria has often focused on the isolation and characterization of new antimicrobial compounds from a variety of sources, including medicinal plants. This has seen several medicinal plants being screened for antimicrobial activities (Sibanda and Okoh, 2007).

Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being (Iwu et al., 1999). Owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial activity in plants are frequent (Betoni et al., 2006; Shibata et al., 2005).

Our results indicate that acetone extracts of stem, leaves and roots showed maximum inhibitory effect on test organisms. The inhibitory zones for the acetone extracts of leaves showed most potent antibacterial activity against test organisms, followed by stem and root extracts with acetone as solvent. Similar investigations have been reported where acetone extracts showed pronounced inhibitory effect on the growth of pathogenic bacteria (Alagesaboopathi, 2011; Abdullahi et al., 2010). Methanolic and ethanolic extracts of stem and leaves of *W. somnifera*, inhibited the growth of

P. aeruginosa, E. coli, B. subtilis, S. pyogenes, and MRSA, except *K. pneumoniae* which did not show any inhibition zone indicating its poor response. The aforementioned results indicate that both alcoholic as well as acetone extracts possessed strong antibacterial activity, while chloroform was not very effective in comparison, this shows the compounds were extracted in polar solvents rather than non-polar solvents (Owais et al., 2005). Similar results have been reported by previous researchers (Mahesh and Satish, 2008; Rajendran and Ramakrishnan, 2009; Sundaram et al., 2011).

According to Mirjalili et al. (2009), the most important compounds, withaferin and withanolides were isolated from methanolic extraction of the roots of *W. somnifera*. The previous findings showed that the aqueous extracts of *W. somnifera* inhibited the growth of Gram negative bacteria, *Neisseria gonorrhoeae,* which also supported the results, because water is the most polar solvent and withanolides can be extracted in water properly (Kambizi and Afolayan, 2008). These findings show that the withanolides steroidal lactones are extracted in acetone, methanol, and ethanol range of polar solvents which are potent inhibitors of bacterial growth.

It can also be concluded that the aforementioned mentioned extracts of *W. somnifera* might be exploited as natural drug for the treatment of several infectious diseases caused by these pathogenic organisms used in our study, especially with MRSA where the results of methanolic leaf extract were more potent than the standard antibiotic, vancomycin (30 µg) used.

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