



ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(3):260-263

**Antimicrobial activity of aqueous and methanolic extracts of
Withania somnifera (Ashwagandha)**

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ABSTRACT

*In the present investigation, the methanolic and aqueous extract of roots of *Withania somnifera* were evaluated for antimicrobial activity against common human pathogens. It was observed that aqueous extract of *Withania somnifera* was more effective in inhibiting all the five test pathogens with zone of inhibition ranging between 33 mm and 50 mm as compared to methanolic root extract (15 to 38mm). The results indicate that the methanolic and aqueous root extracts of *W. somnifera* might be exploited as natural drug for the treatment of several infectious diseases caused by these organisms.*

Keywords: *Withania somnifera*, Methanolic Extract, Aqueous extract, Antimicrobial activity.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and since the beginning of mankind. The application of medicinal plants especially in traditional medicine is currently well acknowledged and established as a viable profession [1]. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. It also facilitates pharmacology studies leading to synthesis of a more potent drug with reduced toxicity [2]. Furthermore, the active components of herbal remedies have the advantages of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [3]. Presently in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulteration and side effects [3]. Therefore, there is the need to search for plants of medicinal value. *Withania*

somnifera (Ashwagandha) is an evergreen plant, native to the Indian subcontinent, successfully introduced worldwide, now extensively cultivated in many other countries including India [4]. In the present investigation, the aqueous and methanolic extracts of *Withania somnifera* were evaluated for antimicrobial activity against common human pathogens.

EXPERIMENTAL SECTION

Collection of plant material

The plant materials were collected from different localities in Kurukshetra district. The samples were washed thoroughly to remove dirt particles present on the surface. The samples were then dried in oven. The dried samples were crushed into powdered form by mortar and pestle.

Preparation of extracts

Twenty five grams of the material was soaked in 100 ml of each of methanol and water and allowed to stand for 72 hrs followed by filtration. The extracts so obtained by filtration were then kept for water bath at 40°C (methanolic extract) and 80°C (aqueous extract) till evaporation of the solvents. The crude extracts left after water bath were scratched against the walls of the beaker and were weighed, after which they were dissolved in DMSO (Dimethyl Sulphoxide) according to the standard of 1ml DMSO/mg of extracts. The extracts were then collected in centrifuge tubes and were stored at 4°C in a refrigerator [5].

Procurement and maintenance of test pathogens

The various human pathogenic microorganisms were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, which included Gram positive bacteria, *Streptococcus mutans* (MTCC 497), *Pseudomonas aeruginosa* (MTCC 2295), *Staphylococcus aureus* (MTCC 7443) and Gram-negative bacterium *Escherichia coli* (MTCC 5704) and a yeast *Candida albicans* (MTCC 3017). The slants of brain heart infusion agar were made to preserve the cultures. All the slants were kept at 40°C in the refrigerator for further studies.

Standardization of inoculum

The microbial inoculum was standardized at 0.5 McFarland. In microbiology, McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range. Original McFarland standards were made by mixing specified amounts of barium chloride and sulphuric acid together. Mixing the two compounds forms a barium sulfate precipitate, which causes turbidity in the solution. A 0.5 McFarland standard is prepared by mixing 0.05 ml of 1.175% barium chloride dehydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$), with 9.95 ml of 1% sulfuric acid (H_2SO_4). The standard could be compared visually to a suspension of bacteria in sterile saline or nutrient broth [6].

Agar well diffusion method

Antimicrobial activity of aqueous and methanolic root extracts were tested using agar well diffusion method [7]. 200µl of bacteria were aseptically introduced and spread using cotton swabs on surface of gelled sterile Muller Hilton agar plates. A well of about 6.0mm diameter with sterile cork borer was aseptically punched on each agar plate. 50µl of the aqueous and methanolic roots extracts *Withania somnifera* were introduced into the wells in the plates. A

negative control well was too made with 50µl of the extracting solvent (DMSO: Dimethyl sulfoxide). A positive control was made by placing antibiotic disc (Ciprofloxacin for bacteria and Ketoconazole for yeast) on agar plate. Plates were kept in laminar flow for 30 minutes for pre diffusion of extract to occur and then incubated at 37°C for 24 hours. Resulting zone of inhibition was measured using a Hi-media zone scale.

RESULTS AND DISCUSSION

Since multidrug resistance of microorganisms is a major medical concern, screening of natural products in a search for new antimicrobial agents that would be active against these microorganisms is the need of the hour [8]. In the present investigation, the antimicrobial activity of aqueous and methanolic root extracts of *Withania somnifera* was evaluated against gram-positive and negative bacteria. Results of the experiment are being concluded in the Table 1, which clearly shows the anti-microbial activity of methanol and aqueous extract of *Withania somnifera*. The summarised findings of the Table.1 states that the aqueous extract of *Withania somnifera* shows maximum antimicrobial activity against the test micobes with zone of inhibition lying in the range of 33 mm to 50 mm. The methanolic extract of *Withania somnifera* also showed significant antimicrobial activity with the zone of inhibition lying in the range of 15 mm to 38 mm. Ciprofloxacin was used as positive control as an antibacterial antibiotic which produced the inhibition zones ranging between 10 and 35 mm whereas *C. albicans* was found to show a zone of inhibition of 22 mm to Ketoconazole which was used as positive antifungal antibiotic. Our findings shown in Table 1, are similar to those reported by Farah *et al.* [9] who also reported the inhibitory activity of methanolic extract of *Withania somnifera* root powder against *Staphylococcus aureus* and *E. coli* whereas our results of methanolic root extract of *Withania somnifera* are better than the results of Babayi and Kolo [10] against *Streptococcus mutans*. Tabulated results of the experiment are shown in Table 1. Negative control showed no formation of zone of inhibition. The encouraging results indicate that the aqueous and methanolic root extracts of *Withania somnifera* might be exploited as a natural drug for the treatment of several infectious diseases caused by these organisms and could be useful in understanding the relations between traditional cures and current medications.

Table 1. Antimicrobial activity of aqueous and methanolic extracts of *Withania somnifera*.

Sample	Solvent	Zone of inhibition (mm)				
		<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>	<i>Candida albicans</i>
<i>Withania somnifera</i>	Methanol	38	36	15	38	32
<i>Withania somnifera</i>	Aqueous	50	41	33	40	41
Positive control	Antibiotic	15	10	17	35	22
Negative control	DMSO	-ve	-ve	-ve	-ve	-ve

Acknowledgement

The authors are grateful to Prof. Dinesh Aggarwal, Director, UIET, Kurukshetra University, Kurukshetra for providing infrastructure to carry out research work.

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