Ashwagandha As Antibacterial

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Abstract **- The use of plants in treatment of burns, dermatophytes and infectious diseases is common in traditional medicine. The development of new antimicrobial agents against resistant pathogens is increasing interest. Therefore, the methanolic extracts from different parts of four medicinal plants used locally in folk medicine were evaluated for antimicrobial activity. It was found that most plant extracts studied had antibacterial and antifungal activities. The methanolic extract of leaf of the plant Azadiracta indica, Acacia nilotica and Witania somnifera showed significant antibacterial activity against Bacillus subtilis, Escherchia coli, stphaylocuccus aureus and pseudomonas fluorescence. Azadiracta indica and A.tinolica showed significant antifungal activity against A. flavus, Ziziphus mauritiana. The rhizome extract of curcuma longa showed significant activity against all tested bacteria and showd higher anti-fungal activity against Fusarium verticillioides.**

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 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.*

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 106 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 millimetre (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).

Withania Somnifera:

Fig.1. Withania Somnifera

Withania somnifera (family Solanaceae) is a medicinally important herb used in number of Indian herbal formulations. In India, it is locally known as ‘Ashwagandha’ and is considered as *Indian Ginseng*. Roots of the plant are major source of active chemical substances and are traditionally used to cure ulcers, fever, cough, dyspnoea, consumption, dropsy, impotence, rheumatism, toxicosis and leucoderma. These activities are mainly attributed towards the presence of different withanolides mainly with aferine A and with anolide A.

The plant had been reported to grow in wild and is also cultivated in selective areas of India. Their pharmacological properties are diverse ranging from antiinflammatory, anti-tumor, anti-stress, antioxidant, immunomodulatory, hemopoetic and cardio-protective effects the traditional medical system of India. 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, the elderly, and during pregnancy.

The herbal root extract has been traditionally used as a tonic and as a sedative

Anti-inflammatory agent; it us used to treat rheumatic pain and arthritis in Ayurveda, the berries and leaves of W. somnifera are locally applied to tumors. tubercular glands, carbuncles, and ulcers.

ASHWAGANDHA: AN IMPORTANT MEDICINAL PLANT

Withania somnifera (L.) Dunal, commonly known as Ashwagandha or Indian ginseng or winter cherry, is a renowned medicinal plant in Ayurvedic medicine. The principal active compounds include several withanolide-type compounds. Due to the on-hazardous and great medicinal value, it is commonly used all over the world. Roots, and less often leaves and fruits, have been used as phytomedicines in the form of decoction, infusions, ointment, powder, and syrup. These days, it is cultivated as a crop to maintain the high demand of biomass and a sustainable eminence for the requirements of pharmaceutical industry. Ashwagandha is an important herb in the Ayurvedic and indigenous medicine system for over 3000 years. It belongs to the family *Solanaceae* and possess a chromosome number of 2n=48. In India, only two species of *Withania* are found which includes *W. somnifera* and *W. coagulans*17. This plant has been used as a home remedy for numerous diseases in the India and many parts of the world. It is found in the wild form in many parts of the India and in the Mediterranean region of North Africa. In India, it is grown in Rajasthan, Madhya Pradesh, Himachal Pradesh, Punjab and Uttar Pradesh. It is designated as an herbal tonic and health food in the Vedas and is considered as 'Indian ginseng' in the conventional Indian medicine. It is utilized as a liver tonic, anti-inflammatory, antioxidant, antimicrobial agent and cure for asthma. Withaferin-A has been receiving a good deal of attention because of its antibiotic and antitumor activity. In Unani system of medicine, roots of *W. somnifera* usually known as Asgand are utilized for the medicinal properties. In Ayurveda, Ashwagandha is claimed to have effective aphrodisiac rejuvenating and life extending properties. It has overall animating and regenerative abilities and is used among others, for the treatment of nervous exhaustion, insomnia, memory related conditions, skin problems, tiredness potency issues and coughing. It also increases learning capability and memory capacity. The traditional use of Ashwagandha was to escalate energy, youthful vigor, strength, endurance, health, increase vital fluids, nurture the time elements of the body, muscle fat, lymph, blood, cell production and semen. It helps counteract chronic fatigue, dehydration, weakness, loose teeth, bone weakness, impotency, thirst, premature aging emaciation, muscle tension, debility and convalescence. It aids invigorate the body by rejuvenating the reproductive organs, just as a tree is invigorated by feeding the roots.

CHEMICAL CONSTITUENTS

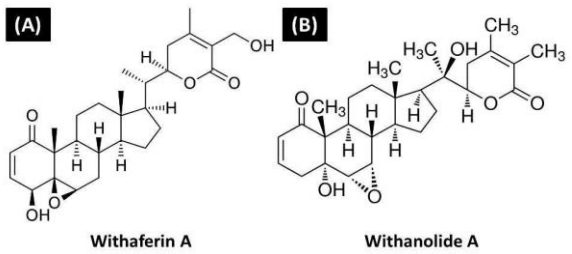

Figure 2: Chemical structure of (A) Withaferin A and (B) Withaolide A

The chemical constituents of *W. somnifera* have always been of great interest to the scientific community. The biologically active chemical constituents are alkaloids (ashwagandhine, anahygrine, cuscohygrine, tropine etc), steroidal compounds i.e. withaferin A, withasomniferin A, ergostane-type steroidal lactones, withanolides A-Y, withasomniferols A-C, withasomidienone, withanone etc22. Withaferin A (Figure 2A), and withanolide A (Figure 2B) are the chief withanolidal active components isolated from the plant. These compounds are chemically similar but varied in their chemical constituents.

PHARMACOLOGICAL ACTIVITIES OF ASHWAGANDHA

W. somnifera possesses various pharmacological activities (Figure 3) viz., antiinflammatory activity, antibacterial activity, antifungal activity, antiviral activity, antitumour activity, immunomodulatory

activity, anti-stress/adaptogenic activity, anticonvulsant activity, neuropharmacological activity, musculotropic activity, anti-oxidant activity, anti-ageing effect, anti-hyperglycaemic effect, macrophage activating effect, hepatoprotective activity, morphine tolerance and dependence inhibiting effect.

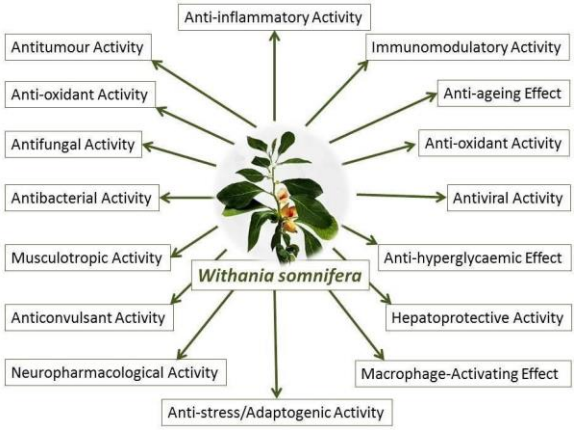

Figure 3: Pharmacological activities of Ashwagandha (*W. somnifera*)

Antibacterial activity of Ashwagandha

Many bacterial species have been used as a test microorganism for the assessment of the antimicrobial activity of extracts and purified compounds of *W. somnifera*. These bacterial strains were *Acinetobacter baylyi, Agerobacterium tumefaciens, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Chlamydophila pneumonia, Citrobacter freundii, Corynebacterium diphtheriae, Enterobacter aerogens, Enterococcus feacalis, Escherichia coli, Klebsiella pnemoniae,* Lactic acid bacterial (LAB) strains, Methicillin Resistance *Staphylococcus aureus, Micrococcus luteus, Neisseria gonorrhea, Proteus mirabilis, Proteus solanacearum, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Raoultella planticola, Salmonella typhi, Salmonella typhimurium, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermis, Streptococcus pyogenes, Xanthomonas axonopodis* pv. *malvacearum*, *Yersinia enterocolitica* and few others. The detailed compilation of information regarding the antibacterial activity of *W. somnifera.*

Various plant parts viz., calyx, fruit, leaves, flower, root and stem of Ashwagandha were used by researchers in the past but leaves and roots were used in most of the studies. Different solvents like, acetone, benzene, butanol, chloroform, chloroform+hexane, deionised water, diethyl ester, distilled water, ethanol, ethyl acetate, glacial acetic acid, hexane, isopropanol, methanol, petroleum ether, toluene and water have been used for the extraction of chemical constituents from various plant parts of Ashwagandha but methanol was the mostly used solvent for the extraction. From the literature survey, we have found that disc diffusion method was the most preferred method for the evaluation of the antimicrobial efficacy of Ashwagandha plant extracts.

Antifungal activity of Ashwagandha

In the past, antifungal activity activity has been evaluated for various extracts of different plant parts of Ashwagandha. The detailed information on the antifungal activity of Ashwagandha is provided in the. Many test fungal species including, *Alternaria brassica, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus oryzae, Candida albicans, Candida kefy, Candida tropicalis, Cryptococcus neoforman, Dreschlera turcica, Fusarium oxysporum* f. sp. *cepae, Fusarium oxysporum, Fusarium verticilloides, Penicillium chrysogenum, Penicillium citrinum* and *Trichoderma viridae* were used for the assessment of the antifungal activity of Ashwagandha.

Various plant parts viz., calyx, flower, fruits, leaves, root and stem were used for the antifungal activity assessment. Mostly used plant part was the root of Ashwagandha. Acetone, benzene, chloroform, ethanol, ethyl acetate, glacial acetic acid, hexane, isopropanol, methanol, petroleum ether, toluene and water (hot and cold) were used as a solvent for the extraction procedure to evaluate antifungal activity of various parts of Ashwagandha. However, methanol was the most preferred solvent for the extraction of phytochemicals from parts of Ashwagandha. Like antibacterial activity assessment, most chosen method for the evaluation was the disc diffusion method. However, agar well diffusion method and poison food technique were also used for the evaluation.

Some common concerns must be established to evaluate the antimicrobial activity of plant extracts, essential oils and the isolated/extracted compounds from them. The greatest relevance is the characterizing and defining common factors, such as plant parts used, methods employed, growth medium and test microorganisms evaluated (Rios and Recio, 2005).

Systematic standards should be used in the selection and collection of the plant parts/materials. Moreover, to avoid unnecessary exercise, the selection of plants and plant parts should be made from an ethnopharmacological perception. The solvent systems and the extraction procedure may alter the final outcome of the study. The solvents and methods for extraction used in folk medicine should be used as they are most appropriate. The active chemical constituents are more soluble in some solvents, which should be used as it may affect the results. The crude extract or essential oil offers variable results as they contain different phytochemicals present in them. The presence of such active phytochemicals depends on their solubility in the solvents used. Sometimes, the presence of phenolic, carboxylic compounds or other impurities in the extract may affect the activity of the active phytochemicals. The experiments may be carried out with a collection of strains, but additional experiments with isolated pathogens would be of importance in the case of purified compounds or active extracts to evaluate their actual effects. According to the literature the evaluation of medicinal plants as antimicrobial agents, understanding medicinal flora and their real value is important, however the use of a standard technique for the research is also crucial. As the reports suggest, *W. somnifera* possesses significant antimicrobial activity, various types of research on the mechanisms of action, interactions of microorganisms with plant extracts and the pharmacokinetic profile of the extracts should be given main concern (Rios and Recio, 2005).

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.*

CONCLUSION

Ashwagandha (*W. somnifera*) owns a tremendous amount of medicinal properties including antimicrobial activity. Many test microorganisms have been used for the assessment of the antimicrobial activity of extracts and purified compounds of various plant parts of Ashwagandha. Still, there are many scopes of the research or the identification and isolation of antimicrobial agents from Ashwagandha. The information provided in this article will provide the platform for the researchers to select plants, plant parts, solvent system, test microorganisms, method of evaluation and other related factors affecting the analysis.

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