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Validation of the Ethnopharmacological Uses of *Withania somnifera*

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Abstract:
The antimicrobial potential of some solvent extracts of *Withania somnifera* leaves against different strains of bacteria and fungi was investigated. The leaves of *W. somnifera* species were collected from Bacha Khan University, Charsadda, Pakistan, rinsed with distilled water and extracted with chloroform and ethanol. Antibacterial activity of crude extracts of *W. somnifera* was determined by agar well diffusion method against gram positive, gram negative bacterial and antifungal activity was determined by agar diffusion method against fungal strains. All tested plant extracts showed varying zones of inhibition against bacteria and fungi tested. The highest zone of inhibition of 27 mm diameter was noted in *S. aureus* with ethanol extract while the lowest 7 mm zone of inhibition was reported in *Klebsiella pneumonia*. Among the fungal species, promising level of antifungal activity was observed in all fractions against *A. flavus* and *A. niger*. The results obtained exhibit that this plant has good medicinal potential, and it needs further phytochemical exploitation to isolate phytochemical constituents having antibacterial and antifungal activities.
Keywords: Antibacterial, Antifungal, activity, W. Somnifera, leaves, extracts.

INTRODUCTION

Withania somnifera is a common medicinal plant belonging to family Solanaceae known for its ethnopharmacological function, native to Pakistan, Iran, Afghanistan, and India (Ali *et al*., 2017). In Pakistan, it is distributed into various parts of Khyber Pakhtunkhwa (KPK) (Ali *et al.,* 2016), Punjab, and Baluchistan. The local and traditional names are different, because of traditional utilization, the common name is Asghandh in Urdu, Tukhme-Kaknaje-Hidi in Persian. The entire plants are utilized for ethnopharmacological activities (Khodaei *et al.,* 2012). The different parts of the plant are utilized in liver disorder, diuretic, and various other intestinal infections (Alavijeh *et al.,* 2012; Bonjar, 2004; Khodaei *et al.,* 2012; Rasool and Varalakshmi, 2006). *W. somnifera* is an evergreen Shrub 30-75 cm in height. Its leaves are 10cm long, simple and ovate. Flowers are green or yellow in a cyme or axillary. The fruit is globose berries about 8mm in diameter and at maturity orange-red in color also reported. *W. somnifera* is a very important tropical medicinal plant also known as Indian Ginseng because of its wide range of medicinal uses (Rasool and Varalakshmi, 2006; Scartezzini and Speroni, 2000). About 95 medicinal products are made from this plant (Rai *et al.,* 2012).

Many researchers have presented the ethnopharmacological activities of *W. somnifera* which shows the importance of the plant in pharmacology. A large number of the phytochemical extracts are obtained from roots including volatile oil, alkaloids, Amino acid withaniol (Uddin *et al.,* 2012). These chemical extracts from the roots are used as a sedative and hypnotic purpose (Chang *et al*., 2006; Parihaar *et al.,* 2014). Leaves of this plant contain amino acid, chlorogenic acid, and glucose (Chang *et al*., 2006; Agarwal and Prasad, 1999; Bahl and Del Alamo, 1993). *W. somnifera* are widely used for antitumor, antibiotic, anticancer, antistress, anti-inflammatory properties as reported previously (Sharma *et al.,* 2003; Sharma *et al.,* 2004). The chemical profile of its fruits is also very high containing flavonoid, tannins, amino acid, and proteolytic enzymes (Mothana and Lindequist, 2005). The herbal remedy is one, in which the main therapeutic activity rests on plant metabolites (active principle), which it contains (Shahzad *et al.,* 2017). Medicinal plants are an important cure for different diseases by providing ingredients for drug or having played central roles in the drug discoveries (Kalim *et al.,* 2016; Hussain *et al.,* 2016). The current study aims to evaluate the antibacterial and antifungal activity of *W. Somnifera* Leaves.

MATERIALS AND METHODS

Plant materials

Withania somnifera species were collected from Bacha Khan University Charsadda. *W. somnifera* was identified and put in the herbarium, Department of Botany, Bacha Khan University. Leaves of *W. somnifera* were rinsed with distilled water and then kept under shade until complete drying.

Preparation of Extracts

W. somnifera leaves were weighed and grind into a fine powder. After making the powder it was suspended in 80% chloroform and 80% ethanol for one week at 65°C in extraction bottle, after 10 days mixture was filled and filtered 3-time using Whatman-41 filter paper. Chloroform and ethanol were then completely evaporated with the help of rotatory evaporator to get the leaf extracts and were stored at 5°C.

Antibacterial assay

In this assay, a total of five bacterial strains such as *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Klebsiella pneumonia* were chosen to be used. All these strains were sustained on agar slant at 4°C and the slant was allowed to activate at a temperature of 37°C for one day on nutrient agar (NA) before any screening is

carried out. The organisms were kept in Mueller Hinton agar (MHA) in the refrigerator at 4°C prior to subculture. Antibacterial testing was carried out following the already developed agar well diffusion method (Hussain *et al.,* 2016) to study the effectiveness of the *W. somnifera* leaf extracts. Broth media was primed and the test organisms were moved to the broth media from agar plate and were grown-up at 37°C for one day. After 24 hours, 25 ml of MHA was discharged into each Petri plate and cooled in a sterile condition. The fresh culture was primed from day-old culture, after solidification of MHA in the plate, 0.6 ml of a fresh culture of test organism were emptied on to MHA. Wells of 6 mm diameter were dug into the medium by using sterile borer and 10 mg of the *W. somnifera* leaf extracts were used against each organism. DMSO and standard antibiotic (imipenem) were mixed into other wells. The plates were kept in pasteurized inoculation chambers for 60 minutes to facilitate diffusion of the antimicrobial agent into the medium. The plates were then incubated at 37°C for one day and the diameters of the zone of inhibition of microbial progression were measured in millimeters (Carron *et al.,* 1987; Iqbal *et al.,* 2015; Iqbal *et al.,* 2016).

Antifungal assay

Antifungal activity of crude extracts of *W. somnifera* was determined by the agar diffusion method (Kalim *et al.,* 2016) against *Aspergillus niger* and *Aspergillus flavus*. Test samples (400µg/ml, DMSO) were diluted in Sabouraud dextrose agar and retained in a slanting position at room temperature for the whole night. Test fungal cultures were inoculated on the slant and were incubated at 29°C for 3-7 days. Test tubes were observed for linear growth inhibition of fungi in mm upon completion of the incubation period. Percentage inhibition was calculated with reference to negative and positive controls by applying the formula (Nisar *et al.,* 2011). Miconazole was used as a standard antibiotic (Choudhary *et al.,* 1995).

$$\% \text{ Inhibition} = 100 - \frac{\text{Linear growth in test (mm)}}{\text{Linear growth in control (mm)}} \times 100$$

RESULTS AND DISCUSSION

The antibacterial activity of chloroform and ethanolic extracts of *W. somnifera* was checked. The leaf extracts of *W. somnifera* were found active against all bacterial strains. Ciprofloxacin was used as a standard. The high activity was shown by the ethanolic extract followed by the chloroform extract (Table 1). Archana and Namasivayam (1998) reported important chemical constituents which were used for antibacterial activity the same is true in the present report also reported the importance of *W. somnifera* for their important chemical and antimicrobial activity. Similar findings were also reported in other species (Sumbul *et al.,* 2011).

The chloroform and ethanolic extracts of *W. Somnifera* leaves were checked (Table 2) for their antifungal activity against the *Aspergillus flavus* and *Aspergillus niger*. The chloroform extract showed 3.81% activity against *A. flavus* and 8.09% activity against *A. niger*. While the ethanolic extract showed 4.72% activity against *A. flavus* and 6.0% activity against *A. niger.* Alavijeh et al. (2012) and Das et al. (2009) reported the antifungal activity of the *Withania somnifera* plant similar to the findings in the present study. The antimicrobial activity of the root and other vegetative parts like leaves has been shown experimentally. The withaferin A stopped the growth of many gram positive bacteria, aerobic bacilli and other pathogenic

fungi (Abou-Douh, 2002). Withaferin A showed a strong antimicrobial activity due to the presence of a lactone ring which is unsaturated. Lactone showed a strong therapeutic function (Uddin *et al.,* 2012).

Table 1. Antibacterial activity of *W. Somnifera* Leaves.

Bacterial species	Zone of inhibition (mm)		
	Chloroform Extract	Ethanolic Extract	Ciprofloxacin
Bacillus subtilis	16	21	26
Staphylococcus aureus	17	27	35
Pseudomonas aeruginosa	8	20	30
Escherichia coli	13	24	28
Klebsiella pneumoniae	7	17	24

Table 2. Antifungal activity of *W. Somnifera* Leaves.

Fungal species	Inhibition (%)		
	Miconazole	Chloroform Extract	Ethanolic Extract
Aspergillus flavus	20	3.81	4.72
Aspergillus niger	36	8.09	6.0

CONCLUSION

The leave extracts of *W. somnifera* are used for the treatment of many diseases and found to be a big source of many chemical constituents which play strong action against many pathogenic microbes' activities. It is required to determine the bio-efficacy of the compounds in combination with chemicals obtained from other plants for making new drugs. However, it is needed to report the effect and mechanism of these chemical constituents for higher organisms to ensure its safety and potential.

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CONFLICT OF INTEREST

All the authors have declared that no conflict of interest exists.

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