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Antifungal and antibacterial activities of crude withanolides extract from the roots of *Withania somnifera* (L.) Dunal (Ashwagandha)

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Abstract

Ashwagandha [*Withania somnifera* (L.) Dunal] is an important medicinal plant and its medicinal properties have been attributed to various classes of withanolide compounds. In the present study, we evaluated antifungal and antibacterial activities of withanolide extracted from roots of ashwagandha, against five pathogenic, fungi and bacteria. The minimal inhibitory concentration (MIC) of withanolides against two fungi, *F. oxysporum* and *A. brassica* were observed to be 4826.25 and 4474.22 ppm respectively. Antibacterial activity of withanolide extract was tested against three bacteria, *E. coli*¸ *Pseudomonas solanacearum* and *Pseudomonas vulgaris*. The maximum zone of inhibition at 4000 ppm of withanolide extract against *E. coli, P. solanacearum* and *P.vulgaris* were observed to be 8.7mm, 12.1mm and 12.5mm respectively. Thus withanolides extract was found to be the inhibitor of pathogenic fungi and bacteria.

Keywords: *Antifungal activity , Antibacterial activity, Ashwagandha, Withanolides*

Introduction

Withania somnifera (L.) Dunal of Solanaceae, is commonly known as Ashwagandha. It is a shrubby bush held in high repute in traditional Indian medicine recommended in Ayurveda (Dash and Junins, 1983).It contains pharmacologically active compounds such as with anolides and alkaloids which attribute to its anti-cancerous, antioxidant, antibacterial, antifungal, aphrodisiac activities (Singh and Kumar, 1998). Withanolides are basically steroidal lactones and various types have been isolated (Kazutoshi and Umehara, 1999). The withanolides are classified according to their structural skeleton (Ray and Gupta, 1994) and the structural variation is responsible for the wide array of pharmacological activities. Withaferin–A, a withanolide, isolated from *Withania somnifera* posses antibiotic activity such as antibacterial activity against acid fast bacilli and gram positive microorganisms (Atta-ur-Rahman and Chaudhary ,1993). Beta epoxywithanolide-I and Beta hydroxywithanolide-K isolated from *Withania coagulance* were found to be active against a number of potential pathogenic fungi (Choudhary *et al.,* 1995). In the present investigation withanolides extracted from the roots of *Withania somnifera* were tested against five pathogenic fungi and bacteria.

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Materials and Method

Pure cultures of various fungi and bacteria were obtained from College of Agriculture and C.B.S.H., G.B.P.U.A.&T., Pantnagar. The cultures were maintained throughout the experiments. The withanolide isolation was based on the method of Gupta *et al.* (1996) with slight modification**.** For bioactivity analysis 100 gm root powder was extracted twice with 500ml methanol. The extract was filtered and evaporated. The extract thus obtained was defatted with n-hexane and then

extracted with 1% sulphuric acid, basified with ammonia. The sulphuric acid insoluble fraction was extracted with diethyl ether. The ether was evaporated and crude withanolide was dissolved in chloroform for checking the bioactivity.To check the bioactivity 10,000 ppm of crude withanolides were prepared. Further dilutions were made from these stock solutions. Poisoned food technique for fungi (Finhold, 1951) and paper disc zone inhibition technique for bacteria (Thornberrry, 1950) were used to screen the withanolide extracts *in vitro*. List of bacteria and fungi used in present experiment are given in Table 1.

For fungi plates were inoculated at the centre by a 5 mm disc of respective fungi, placed with a sterilized needle from the edge of a seven day old fungus culture maintained on PDA medium. For each concentration of withanolide three replicates were taken. Discs were punched out with the help of a 5 mm sterilized steel cork-borer and incubated at 28 ± 0.2^0C and after 7 days diameters of fungal growth were measured from the direction by Vernier Caliper scale. The percent inhibition was determined with the help of mean colony diameter and calculated by using following formulae:

$$\textbf{Percentage inhibition} = \frac{\textbf{X - Y}}{\textbf{X}} \times \textbf{100}$$

Where,

X = Colony diameter in control

Y = Colony diameter in treated medium

For bacteria withanolide extract was suspended in water or suitable organic solvent. Filter paper disc of 10 mm diameter were first dipped in the test liquid and dried in air. The dried disc was then placed on nutrient agar plates. The plates were kept in incubator at 28 ± 0.2^0C. After incubation the inhibition zones about the test organisms were measured.

Results and Discussion

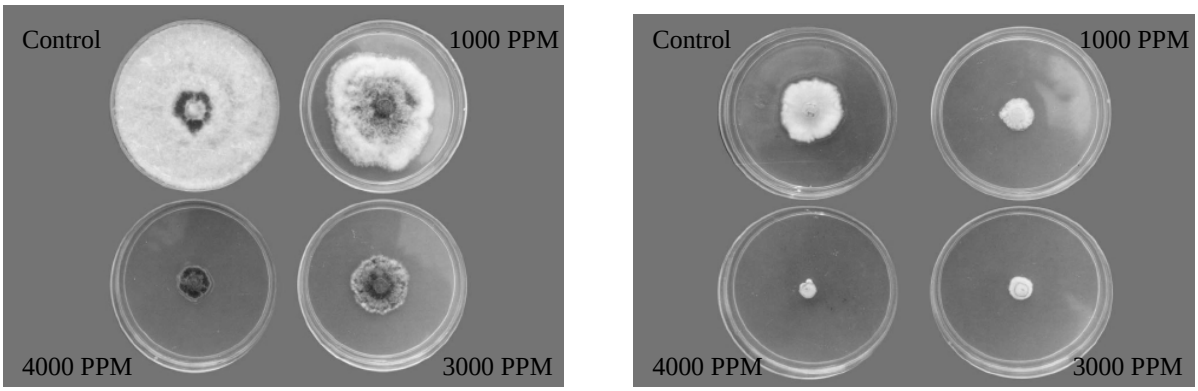
Three concentrations of withanolides, 1000, 3000 and 4000 ppm were used for testing the activity against two pathogenic fungi (Table 2). The minimal inhibitory concentration (MIC) of withanolide against *F. oxysporum* was higher than *A. brassica* with MIC of 4826.25 and 4474.22 ppm respectively. The growth level of both fungi decreased with increase in withanolide concentration. At 4000 ppm of crude withanolide, mean percent inhibition was found to be 95.66 in case of *F. oxysporum* while 86.33 in *A. brassica*. At lowest concentration (1000ppm), withanolides were more effective in *F. oxysporum* with mean percent inhibition of 62.33 as compared to *A brassica* with 20.33 only. At 3000 ppm of withanolides, inhibition was found to be similar in both fungi with mean percent inhibition of 74.66 in case of *F. oxysporum* and 71.33 in *A. brassica* The inhibitory activity was illustrated in Fig.1. The present findings might be supported by the work of Ramteke *et al*. (2003) while studying the antifungal activity of *W. somnifera* root extract against *Fusarium solani*. They found that the extract had higher inhibitory effect on the growth of *F.solani* than clotrimaxole (antifungal).

Table 1: List of Bacteria and Fungi used

S No.	Bacteria & Fungi	Common host	Diseases
1.	*E. coli*	Animals	Diarrhoea
2.	*P. solanacearum*	Potato	Bacterial brown rot of potato
3.	*P. syringae*	Pea, Tomato	Bacterial spot of pea, Bacterial speck of tomato
4.	*A. brassica*	mustard	Alternaria blight of rape seed & mustard
5.	*F. oxysporum*	Tomato, chilli	Damping off of seedlings.

Table 2: Antifungal activity of withanolide extract against pathogenic fungi

Sl. No.	Fungus	Conc. (ppm)	Percent inhibition			Mean %	MIC (ppm)
			R_1	R_2	R_3		
		4000	86	86	87	86.33	
1.	*Alternaria brassica*	3000	72	71	71	71.33	4474.22
		1000	21	20	20	20.33	
		4000	95	96	96	95.66	
2.	*Fusarium oxysporum*	3000	74	75	75	74.66	4826.25
		1000	62	62	63	62.33	



A **B**

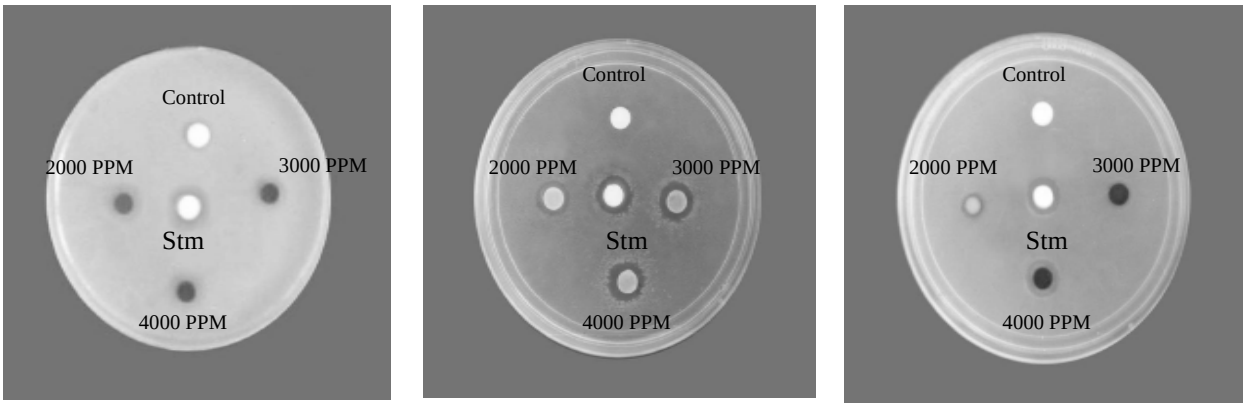
Fig. 1: Antifungal activity of withanolide extract of Ashwagandha against (A) *Alternaria brassica* (B) *Fusarium oxysporum*

Antibacterial activity observed against *P. vulgaris* was found to be maximum among the tested bacteria with zone of inhibition values 8.6, 10.5, 12.5 mm at 2000, 3000 and 4000 ppm of withanolide extract, respectively (Table 3). The maximum zone of inhibition at 4000 ppm of extract in *E. coli*, *P. solanacearum* and *P.vulgaris* were found to be 8.7 mm, 12.1mm and 12.5mm respectively. Withanolides extract was least effective at 2000 ppm in *E. coli* followed by *P. solanacearum* with mean zone of inhibition of 4.8mm and 6.1mm as compared to *P. vulgaris*, 8.6mm. Streptomycin control of 100 ppm was most effective in *P. solanacearum* with zone of inhibition of 12.6 followed by *P. vulgaris* and *E. coli* with 11mm and 8.8mm. In all the tested bacteria zone of inhibition increased with increase in the concentration of crude withanolide extract. The antibacterial activity of withanolides was described in Fig. 2. The present findings are supported by the work of Arora *et al*. (2004) while evaluating the antibacterial/synergistic activity of withanolide extract by agar plate disc-diffusion assay against *S.typhimurium* and *E. coli*. They observed that methanol/hexane extract of roots of Ashwagandha was found to have potent antibacterial activity. Thus, present investigation revealed that withanolide extracts from the roots of *W. somnifera* possess antifungal and anti bacterial activities against pathogenic fungi and bacteria.

Table 3: Antibacterial activity of withanolide extract against various bacteria

S. No.	Bacteria	Conc. (ppm)	Zone of inhibition			Mean
			R_1	R_2	R_3	
1.	*E. coli*	100[C]	9.0	8.5	9.0	8.8
		2000	5.0	5	4.5	4.8
		3000	6.0	5.5	6.0	5.8
		4000	9.0	9.0	8.0	8.7
2.	*P. vulgaris*	100[C]	11.0	11.5	10.5	11.0
		2000	9.0	8.5	8.5	8.6
		3000	11.0	10.0	10.5	10.5
		4000	12.0	12.5	13.0	12.5
3.	*P. solanacearum*	100[C]	13.0	12.5	12.5	12.6
		2000	6.0	6.0	6.5	6.1
		3000	7.0	7.5	7.0	7.2
		4000	12.0	12.0	12.5	12.1

[C] **represent streptomycin (stm) control.**



A **B** **C**

Fig. 2: Antibacterial activity of withanolide extract of Ashwagandha against:
(A) *E. coli*
(B) *P. vulgaris*
(C) *P. solanacearum*

Stm: Streptomycin control

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