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Antibacterial Assay of *Withania somnifera*

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The study was carried out to assess antibacterial bioassay of *Withania Somnifera*. The antimicrobial activities of the butanol, *n*-hexane, chloroform, ethyl acetate and aqueous extracts of *Withania Somnifera*. were investigated. The bacterial strains used were *E.coli, C.albican, B. atropeous*, *Pseudomonas aeruginosa, Klebsiella pneumonia*, *Staphylococcus Aureus and Salmonella*. that highest zone of inhibition 14 mm resulted for the ethyl acetate extract of *Withania Somnifera* against *P. aeruginosa* and Chlorform extract against *E. coli.* Other significant results obtained by different crude extracts are 13 mm, 12mm and 11 mm against *E. coli, C. albican, B. atropeous*, *Pseudomonas aeruginosa, Klebsiella pneumonia*, *Staphylococcus Aureus and Salmonella*. Aqueous, *n*-hexane and chloroform extract were found totally inactive against *Salamonella*

Key words: Antibacterial activity, *Withania Somnifera,* Well diffusion Assay

In recent years, there has been a resurgence of scientific interest in the use of medicinal plants for the development of new pharmacotherapeutic agent. Medicinal plants play an important role for the management of different microbial infections because over medication and long term side effects of synthetic drugs have assumed alarming range. Effective, safe and cheap medicinal agents from plants may appear as potential alternatives for controlling microbial infections particularly the resistant cases[32]. The plants posses bioactive compounds and are screened for different activities like antifungal, antibacterial many other activities of beneficial effects throughout the world and play an important role in the discovery of new compounds for diagnosis and treatment of various diseases[1,2].Antibiotic resistance is increasing throughout the world[27].Pharmaceutical companies are always in search of new antimicrobials[3]. One possible source for new antimicrobials can be plants[4].

Withania somnifera contains more than 80 chemical compounds, mainly alkaloids and steroids (withanolides). Numerous studies have been published on the activities of these compounds, mostly obtained from the leaves and roots. These studies have demonstrated antibiotic, anti-inflammatory, cytotoxic, anti-tumour and cholesterol-lowering activities. This is an important plant in the traditional medicine of Africa and Asia In traditional medicine in southern Africa the leaves are used to heal open as well as septic, inflamed wounds, abscesses, inflammation, haemorrhoids, rheumatism and syphilis; a paste of leaves is applied or ointments are made with fat or oil [5]

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EXPERIMENTAL

General Notes

All studies were carried out at Pakistan Council of Scientific and Industrial Research (PCSIR) laboratory Peshawar.

Solvents

All commercial grade solvents used were distilled before use and in some occasions analytically grade solvents were used.

Plant Material

Withania somnifera plant weight of 5 kg was collected from District Peshawar Khyber Pukhtoon Khwa in June 2011, in flowering season and was identified by Zafar Iqbal chairman of botany deportment Kohat University of Science and Technology

Methodology

The air dried plant material (1 kg) was crushed and soaked in Ethanol (500 ml) for one week. The ethanolic extract was combined and evaporated under reduced pressures to afford a gummy residue (300 g). The concentrated residue was suspended in a mixture of water and defatted with n-hexane (500 ml) to afford fraction weighed (0.50 g). It was again fractionated with chloroform (500 ml) to afford fraction weighed (3.00 g). Similarly it was fractionated with ethyl acetate (500 ml) to afford fraction weighed (4.5g) At last water fraction was obtained of weighed (76g).

Antibacterial activity

Preparation of the test compound

400mg of the compounds were dissolved in Di Methyl Sulfoxide (DMSO). It use as a solvent because it does not show any activity against bacteria therefore DMSO was selected for the present study[6,7]. Anti-microbial activities are better to perform in winter season because in cool weather the growth of these micro organisms are very slow so there is less chance of any disease or harm from these micro organisms and also less chance of contamination. Antibacterial activity of crude extract and fractions were determined by using well assay methods. Seven bacterial strains *E.coli, C.albican, B. atropeous*, *Pseudomonas aeruginosa, Klebsiella pneumonia*, *Staphylococcus Aureus and Salmonella* were used in antibacterial assay. Muller Hinton agar (Oxoid, UK) media was prepared in conical flask according to directions of manufacturer. The media along with petri dishes, pipette and metallic borer were sterilized in autoclave for 15 minutes at 121 °C and 15 psi pressure. The media was poured into petri dishes under aseptic conditions and allowed to solidify.

Well Assay

The modified method of Perez *et al.* (1990) was followed. Bacterial culture corresponding to 50 µl was spread on the surface of the solidified media. Then 6 mm disc were on the media. Stock solutions of crude extract and fractions in DMSO at concentration of 6 mg/ml were applied on this disc The zones of inhibition were measured after 24 hours of incubation at 37 °C (Fig 1-7). Ciprofloxacin and was used as standard. The zone of inhibition of water, Ethyl acetate, Chloroform, Ethanol and n-Hexane fractions were compared with zones of inhibition of standard drugs The amount of growth in each petri dish was measured after 24 hours[8] .

RESULT AND DISCUSSION

Our study showed that *Withania somnifera* possesses antibacterial and antifungal

Table 1. Antibacterial activity of different fractions of *Withania somnifera*

Microorganism	Zone of inhibition (mm)					
	Butanol Fraction	*n*-Hexane Fraction	$CHCl_3$ Fraction	Ethyl acetate Fraction	Aqueous Fraction	Ciprofloxacin
E. coli	9	10	14	13	7	32
P. aeruginosa	10	11	12	14	7	34
Salamonella	7	0	0	11	0	27
C.albican	8	11	7	7	8	29
Klebsella	9	12	8	13	7	31
S. aureuses	9	11	8	8	7	30
B. atropeous	7	10	10	11	8	30

activities. The anti-bacterial study was performed against seven bacterial strains i.e *E.coli*, *C. albican*, *P. aeruginosa, Salmonella typhi, Staphylococcus aureuses, B. atropeous* and *Klebsella.* Three fractions are completely inactive on three bacterial strains out of the seven pathogenic bacteria, which were *S. typhi, P. aeruginosa, and Staphylococcus aureuses,* used in the antibacterial assays. All fractions are active against the remaining bacteria (Table 1).

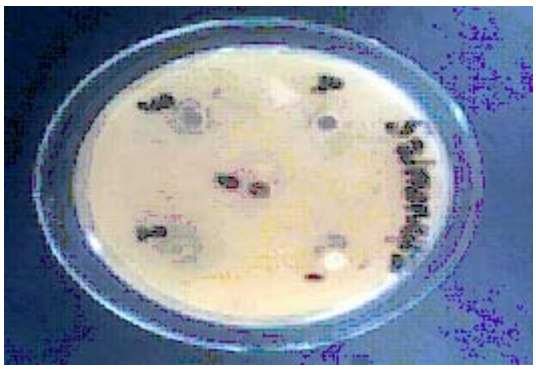

Fig. 1. Picture showing zone of Inhibition against *Salmonella typhi*

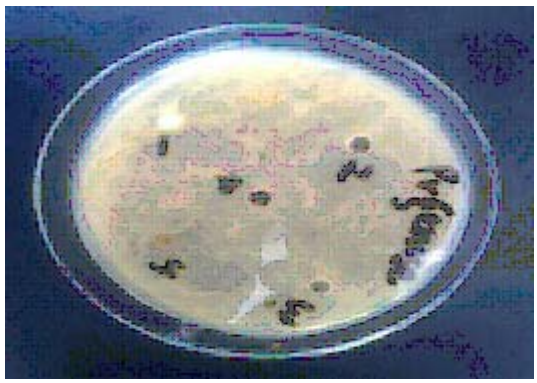

Fig. 2. Picture showing zone of inhibition against *P.aeruginosa*

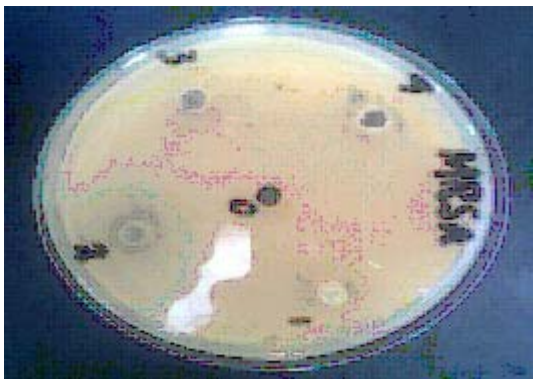

Fig. 3. Picture showing zone of Inhibition against *B. atropeous*

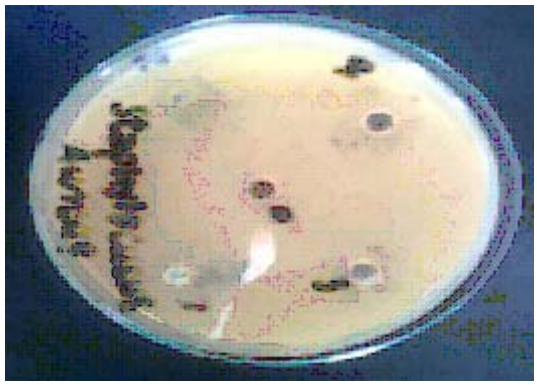

Fig. 4. Picture showing zone of inhibition against *S. aureuses*

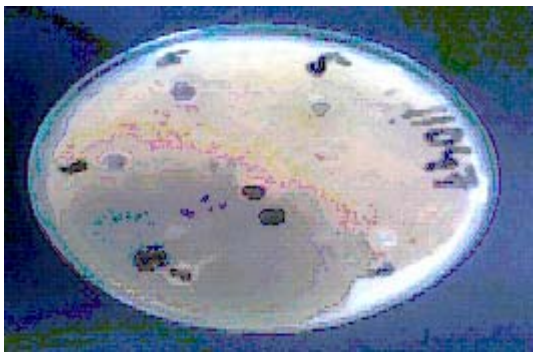

Fig. 5. Picture showing zone of Inhibition against *C. albican*

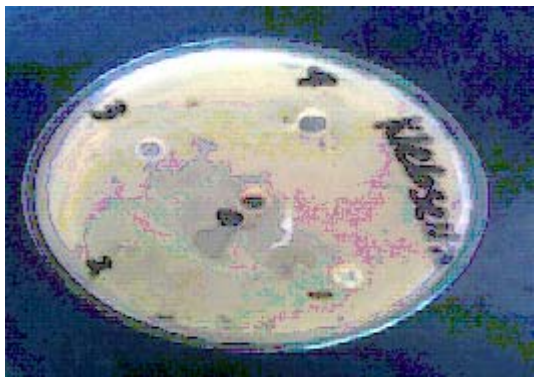

Fig. 6. Picture showing zone of inhibition against *Klebsella*

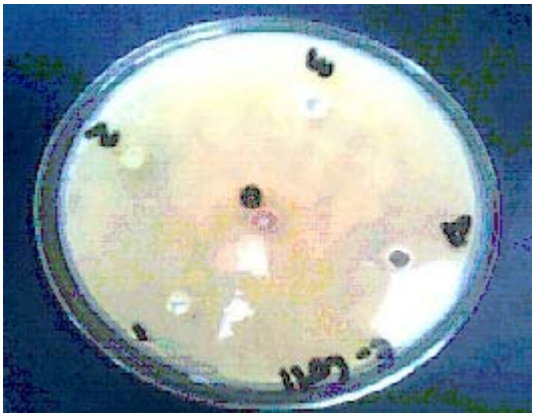

Fig. 7. Picture showing zone of Inhibition against *E.Coli*

Table 1 shows that highest zone of inhibition 14 mm resulted for the ethyl acetate extract of *Withania Somnifera* against *P. aeruginosa* and Chlorform extract against *E. coli.* Other significant results obtained by different crude extracts are 13 mm, 12mm and 11 mm against *E. coli, C. albican, B. atropeous*, *Pseudomonas aeruginosa, Klebsiella pneumonia*, *Staphylococcus Aureus and Salmonella*. Aqueous, *n*-hexane and chloroform extract were found totally inactive against *Salamonella*. Highest zone of inhibition against *B. atropeousi* 11 mm was showed by ethyl acetate fraction. Similarly Highest zone of inhibition against *S. aureuses* 11 mm was showed *n*-Hexane fraction While all other extracts found active against all seven tested pathogen. It is recommended that *Withania somnifera* is an important plant from medicinal point of view and can be a potential candidate for further bio-assays which would lead to synthesis of safe herbal drugs of global interests.

CONCLUSION

Medicinal plants maintain the health & vitality of individuals & also cure disease, without causing toxicity. As *Withania somnifera* possess good antimicrobials and medicinally important chemicals, such as Withaferins, sitoindosides and various alkaloids, they protect the cells from oxidative damage and diseases. Thus consume a good diet will provide health- protective effects. In conclusion, this article provides the therapeutic knowledge about *Withania somnif*era, which is used by the people all over the world. Also, it is of significance to exploit novel medicines from *Withania somnifera*. Although, the results from this review are quite promising for the use of this plant as a multi-purpose medicinal agent.

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