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Study of Antibacterial Activity of Withania Somnifera Plant Extract against Some Human Pathogenic Bacteria

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Abstract: ***Withania somnifera Linn ordinarily known as ashwagandha, against human pathogenic microbes. Plants were taken from wild territory with medicines. Antimicrobial action was finished with various solvents acetone, methanol, and chloroform concentrates of the plant (Root and stem) were found to have solid antimicrobial movement. The diverse dissolvable concentrates of withania somnifera indicated huge antibacterial movement against Bacillus subtilis, Escherichia coli, Pseudomonas, Staphylococcus aureus and so on. Withania somnifera root and stem separate demonstrated most elevated antibacterial movement against B. subtilis. Root and stem concentrate of Withania somnifera recorded noteworthy action against all the test microorganisms.***

Keywords: Withania somnifera, Antibactrial, Human Pathogenic Bactria, Disk diffusion method

1. Introduction

Plant items have been a wellspring of therapeudic and restorative specialists since days of yore. The use of plants as medication goes back to ancient period. India has been a customarily wealthy in different kinds of therapeutic plants. Since antiquated occasions, individuals have been utilizing home grown prescriptions to fix infections. Around 20,000 types of plants are being utilized as restorative plants world over. The plant items comprise about 25% of the recommended meds in world (Farnsworth and Bingel 1977 & Principe 1989). The most regularly utilized medications of current prescriptions, for example, anti-inflamatory medicine, quinine, diosgenin, cortisone, and soon have been begun from the therapeutic plant sources (Saifi *et al*., 1971, Mukherjee *et al.*, 1972, Coimbra *et al.*, 1992, Kar *et al.*, 1999, Jafri *et al*., 2000).

Withania somnifera is an evergreen, erect, fanning, tomentose bush, 30-150 cm in stature. Leaves are straightforward, applaud, glabrous, and up to 10 cm long. *W. somnifera* is generally dispersed far and wide from Southern Mediterranean districts to the Canary Island and from South to East Africa; from Palestine to North India covering Israel, Jordan, Egypt, Sudan, Iran, Afghanistan, Baluchistan and Pakistan. In India the plant can be seen developing wild in the North Western areas stretching out to the sloping district of Punjab, Himachal Pradesh and Jammu up to an elevation of 1,500 m (Singh and Kumar, 1998).

The entire plant just as explicit parts (roots, stems, leaves) of plant concentrate and its dynamic constituents have been utilized for the treatment of bigger number of human infirmities. The primary constituents of ashwagandha are alkaloids and steroidal lactones. Withanine, somniferine, somnine, somniferinine, withananine, pseudo-withanine tropane, pseudo-tropine, choline, anaferine, anahydrine, isopelletierine are synthetic constituents present in it. The leaves contain steroidal lactone, which are regularly called as "Withanolides".

W. somnifera has tremendous remedial potential and is known for its immuno-modulatory (Malik *et al.*, 2009; Rasool and Varalakshmi, 2006), hostile to stretch (Archana and Namasivayan, 1998), cardioprotective (Mohanty *et al.*, 2004), against maturing (Singh *et al.*, 2008), cancer prevention agent, calming (Mishra *et al.*, 2000), hostile to tumor (Widodo *et al.*, 2007 and Wadhwa *et al.*, 2013), neuroprotective and hostile to malignant growth exercises (Kataria et al., 2011, 2012, 2013). The therapeutic properties of *W. somnifera* are ascribed to the nearness of a wide exhibit of auxiliary metabolites, including alkaloids like tropine, psudotropine, hygrine, 3-trigloyloxytropine, cushohygrine, choline, dl-isopelletierine, anaferine, anahygrine and withanosomine (Schwarting *et al.*, 1963 and Schroter *et al.*, 1966).

2. Material and method

Collection of the Plant Sample

Withania somnifera plant were collected from Centre of Excellence on MAPs (Medicinal and Aromatic Plants) and NTFP (Non Timber Forest products), Indira Gandhi Krishi Vishwavidyalaya, Raipur – 492012 (C.G.) (21.2382^{o} N, 81.7048^{o} E) and was identified by Dr. P.K. Joshi, Principal Scientist and Team Leader, Centre of Excellence on MAPs and NTFP, Indira Gandhi Krishi Vishwavidyalaya, Raipur – 492012 (C.G.).

Extract Preparation

The stem and root were extracted from the plants, washed with running faucet water and dried in conceal for hardly any days. Dried stem and root was coarsely powdered utilizing mechanical processor and oppressed for extraction. The concentrates were set up by utilizing Soxhlet extraction technique. It was expendable extraction utilizing solvents viz. acetone, methanol and choloroform for the time of around 48 hour or 22 cycles or till the dissolvable in the siphon container of an extractor become shading less. Arranged concentrates were sifted with the assistance of

channel paper and thought utilizing water shower at 40-50oC and dried in sight-seeing oven at 40oC. The dried powders were put away in sterile compartments and kept in fridge at 4oC for additional examination (Okeke *et al.*, 2001).

Test organisms used for the analysis

MTCC bacterial isolates *viz*., *Enterococcus* (MTCC-439), *Bacillus fusiformis* (MTCC-1286), *Pseudomonas luminescens* (MTCC-130), *Enterobacter* (MTCC-509), *Bacillus cereus* (MTCC-6909), *Bacillus subtilis* (MTCC-1789), *Staphylococcus aureus* (MTCC-7443), *Escherichia coli* (MTCC-3221), *Klebsiella pneumoniae* (MTCC-9544) and *Pseudomonas* (MTCC-3163)

Antimicrobial Screening of extract-

Agar disc diffusion method was utilized to assess antibacterial action of Withania somnifera root and stem as portrayed by (Bauer et al., 1966). The assess antibacterial activity by agar disc diffusion method sterile petri plates were set up with 20 ml of Muller Hinton Agar. The standard inoculums of bacterial suspension acclimated to 0.5 McFarland turbidity standard which is comparable to 1 x 108 CFU/ml of microorganisms were cleaned on the cemented media and permitted to dry for 10 min. what's more, with recently arranged WS root and stem and at centralizations of 50 mg/ml, 75 mg/ml, 100 mg/ml, 6 mm cleaned channel papers circles (Whatmann No. 1) were immersed. The impregnated circles were then positioned onto the outside of the hardened agar medium. Petri plates were then brooded for 24 h at 35±1°C. At last the zone of restraint shaped by WS root and stem was recorded after 24 h hatching at 35±1°C. The impacts were contrasted and that of standard, streptomycin (positive control). Each concentrate was tried in triplicate alongside streptomycin (1mg/circle). The plates were kept at 4°C for 1h for dispersion of concentrate, from that point were hatched at 37±2°C for 24 h. Zone of hindrance (IZ) or discouraged development of microorganisms was estimated and the 'Activity Index' (AI) for each concentrate was determined.

$$\text{Activity index (AI)} = \frac{\text{Inhibition Zone of the sample}}{\text{Inhibition Zone of the standard}}$$

3. Result

Antibacterial activity using Disk Diffusion Method

The extracts obtained above were screened for their antibacterial activity in comparison with standard antibiotic Streptomycine (30 µg/mL) *in-vitro* by disc diffusion method using *S. aureus*, *B. cereus*, *E. coli*, *Pseudomonas*, *Klebsiella*, *P.luminescens*, *Enterobacter*, *B. subtilis*, *Enterococcus*, *B. fusiformis* as test organisms. Each extract was individually loaded on the 6 mm sterile disc at the concentration of 50µg/mL, 75µg/mL and 100µg/mL and subjected to antibacterial activity. The results were recorded by measuring the zone of growth inhibition surrounding the disc. The experiments were done in triplicate.

Table 1: Antibacterial activity (zone of inhibition) of Methanol, Acetone and Chloroform crude extracts (Soxhlet Extraction unit) of Withania somnifera(L.) stem against different types of pathogenic bacteria

Antimicrobial activity *W.somnifera* stem extract																
	Microorganism	E.coli			Pseudomonas			Klebsiella			P. Luminescens			Enterobacter		
	Conc. (µg/ml)	50	75	100	50	75	100	50	75	100	50	75	100	50	75	100
Withania sonmifera L. Stem Extract	Acetone	08.33 ±0.23	09.66 ±0.68	12.00 ±0.29	10.33 ±1.62	10.66 ±1.63	11.00 ±1.36	08.33 ±0.35	09.00 ±0.67	10.00 ±0.72	10.33 ±0.56	10.66 ±0.23	11.00 ±0.62	11.33 ±1.21	12.00 ±1.70	12.66 ±1.67
	Methanol	13.33 ±0.25	12.00 ±0.26	14.66 ±1.21	NI	NI	NI	NI	09.66 ±0.67	10.33 ±0.54	09.66 ±0.90	10.00 ±0.92	10.66 ±0.26	10.33 ±1.29	11.00 ±1.28	12.33 ±1.27
	Chloroform	11.66 ±0.77	09.66 ±0.65	13.00 ±1.54	08.00 ±1.25	09.00 ±1.36	10.33 ±1.73	NI	NI	08.33 ±0.52	09.33 ±0.26	10.33 ±0.48	10.66 ±0.23	09.33 ±1.42	11.00 ±1.35	12.00 ±1.38
Negative control		No Inhibition			No Inhibition			No Inhibition			No Inhibition			No Inhibition		
Positive control		19.00 ± 0.21			20.66±0.58			20.33±1.34			20.66±0.30			19.66±0.87		
	Microorganism	Bacillus subtilis			Staphylococcus aureus			B. cereus			Enterococcus			Bacillus fusiformis		
	Conc. (µg/ml)	50	75	100	50	75	100	50	75	100	50	75	100	50	75	100
Withania sonmifera L. Stem Extract	Acetone	08.66 ±0.26	10.00 ±0.25	10.66 ±1.29	09.00 ±1.35	10.00 ±0.67	10.66 ±1.72	10.33 ±0.41	11.33 ±0.70	12.00 ±0.97	09.33 ±0.23	09.66 ±0.68	10.00 ±0.29	10.33 ±0.21	11.33 ±0.58	12.00 ±0.93
	Methanol	NI	NI	NI	NI	09.66 ±0.67	10.33 ±1.54	08.33 ±0.24	09.66 ±0.28	10.66 ±0.27	11.33 ±0.25	11.66 ±0.36	12.00 ±0.61	10.66 ±0.87	11.33 ±0.65	12.33 ±0.92
	Chloroform	10.00 ±0.48	11.33 ±1.23	11.66 ±1.43	NI	NI	08.33 ±0.52	10.33 ±0.95	11.33 ±0.46	11.66 ±0.70	10.66 ±0.77	11.33 ±0.65	12.00 ±0.54	09.66 ±0.62	10.33 ±0.63	11.33 ±0.32
Negative control		No Inhibition			No Inhibition			No Inhibition			No Inhibition			No Inhibition		
Positive control		21.66±0.67			19.66±0.89			21.00±1.11			19.33±0.98			18.66±0.22		

Table 2: Antibacterial activity (zone of inhibition) of Methanol, Acetone and Chloroform crude extracts (Soxhlet Extraction unit) of Withania somnifera(L.) root against different types of pathogenic bacteria.

Antimicrobial activity of *W. somnifera* root extract

	Microorganism	E.coli			Pseudomonas			Klebsiella			P. Luminescens			Enterobacter		
	Conc. (µg/ml)	50	75	100	50	75	100	50	75	100	50	75	100	50	75	100
Withania sonmifera L. Root Extract	Acetone	12.66 ± 1.28	14.00 ±1.56	14.66 ± 1.41	07.00 ±1.28	08.33 ±1.62	09.33 ±1.26	09.00 ± 1.76	09.33 ± 0.25	10.33 ± 1.92	11.33 ± 1.62	11.66 ± 1.54	12.00 ± 1.90	10.00 ±1.21	11.66 ±1.19	12.00 ±1.67
	Methanol	11.33 ± 0.74	12.33 ± 1.87	13.66 ± 1.89	07.66 ±1.87	8.33± 1.63	09.66 ±1.84	09.66 ± 1.23	10.66 ± 0.76	11.00 ± 1.93	10.00 ± 1.76	11.33 ± 1.93	11.66 ± 1.35	11.66 ±0.54	12.00 ±0.35	12.33 ±0.20
	Chloroform	11.33 ± 0.26	11.33 ± 1.23	12.66 ± 1.43	NI	07.33 ±1.27	08.66 ±1.87	07.66 ± 0.87	06.33 ± 1.28	08.66 ± 1.23	08.66 ± 1.35	08.66 ± 1.72	09.00 ± 1.23	08.00 ±1.66	08.33 ±1.23	09.33 ±1.70
Negative control (In highest Conc.)		No Inhibition			No Inhibition			No Inhibition			No Inhibition			No Inhibition		
Positive control (In highest Conc.)		19.00 ± 0.21			20.66±0.58			20.33±1.34			20.66±0.30			19.66±0.87		
	Microorganism	Bacillus subtilis			Staphylococcus aureus			B. cereus			Enterococcus			Bacillus fusiformis		
	Conc. (µg/ml)	50	75	100	50	75	100	50	75	100	50	75	100	50	75	100
Withania sonmifera L. Root Extract	Acetone	09.66 ±0.77	10.33 ±0.53	11.33 ±1.91	09.00 ±1.76	10.00 ±0.25	11.66 ±1.92	12.33 ±0.31	12.33 ±0.19	12.66 ±0.67	12.66 ±0.32	13.00 ±0.56	13.33 ±0.41	10.33 ±0.33	10.66 ±0.36	11.33 ±0.60
	Methanol	09.33 ±1.59	09.66 ±1.87	10.33 ±1.89	10.66 ±1.23	11.66 ±0.76	11.66 ±1.93	11.00 ±0.64	11.33 ±0.35	12.33 ±0.20	12.00 ±0.33	12.33 ±0.87	12.66 ±1.89	10.00 ±0.95	11.66 ±0.27	12.00 ±0.61
	Chloroform	08.00 ±0.59	08.66 ±1.26	09.66 ±1.21	07.66 ±0.87	08.00 ±1.28	09.66 ±1.23	10.00 ±0.38	11.33 ±0.84	11.66 ±0.70	11.33 ±0.26	12.33 ±0.23	12.66 ±0.43	09.66 ±0.32	10.33 ±0.62	10.66 ±0.40
Negative control		No Inhibition			No Inhibition			No Inhibition			No Inhibition			No Inhibition		
Positive control		21.66±0.67			19.66±0.89			21.00±1.11			19.33±0.98			18.66±0.22		

- Negative Control = Solvent and Positive control = Streptomycin 30 µg/ml.
- standard error (SEM); n=3 in each group.± Values expressed as Mean
- Inhibition zones were measured in mm and disc diameter was included.

4. Discussion

W. somnifera root and stem removal demonstrated great restraint of Enterococcus, E.coli, and Enterobacter which were safe against routinely utilized first-line antibiotics. As in the past investigation, (Mathur et al., 2007) methanolic root concentrates of W. somnifera uncovered 4 mm inhibitory zone against Escherichia coli and 10 mm inhibitory zone at 10 µg/ml against Enterococcus, which is exceptionally not as much as that saw in our investigation. This distinction might be because of an alternate part of the plant extract being utilized. Owais et al., 2005 watched 22 mm zone of inhibition against S. aureus at a concentration of 20 mg/ml of W. somnifera leaf extract. In another investigation, (Mahesh et al., 2008) 15 mm zone of inhibition at a grouping of 100 µg/ml against S. aureus and E. coli by W. somnifera leaf extract was accounted for. In present investigation great inhibitory zone against E.coli, Enterobacter, and Enterococcus at the grouping of 100 µg/ml was watched. Distinction in the zone of inhibition may be ascribed to the difference in bacterial strains utilized, technique utilized, and diverse land territory from where the plant was obtained.

Reference standard institutes like Clinical Laboratory Standard Institute or European Committee on Antibiotic Susceptibility have rules for the interpretation of disk diffusion and minimum inhibitory fixation for antibiotics, yet there is no such standard zone outline or rule accessible for W. somnifera separate. In the current examination, persuading zone regarding hindrance was found by W. somnifera root and stem remove in pathogenic Gram-positive and Gram-negative isolates which were impervious to routinely utilized first line anti-infection agents (with no zone of inhibition). In any case, the test size of the current examination was low; henceforth, this requires a substantially more comprehensive in vitro and in vivo investigation including distinguishing proof and seclusion of explicit W. somnifera mixes acting against a safe strain.

5. Conclusion

This finding supports the use of root and stem extract of *W. somnifera* in the treatment of bacterial pathogens by alternative systems of medicine. Clinical trials with *Withania somnifera* for its activity against bacterial infections should be conducted.

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