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THE PRIMITIVE IN-VITRO PHARMACOLOGICAL STUDIES OF THE EXTRACTS FROM *WITHANIA SOMNIFERA*

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ABSTRACT

From ancient times, Medicinal plants were used for the treatment of various deadly diseases. Especially Asian countries like India, china etc., were used medicinal plants as a potential curing agent for various diseases and used in their different medicinal system named ayurvedic, siddha etc.,. Withania somnifera is an indigenous herb belonging to family Solanacae, commercially known as Ashwagandha. It is extensively used in most of the Indian herbal pharmaceuticals and nutraceuticals, It was spread all over the India and in some other Asian countries. It has phytochemicals named alkaloid, steroidal lactones, flavonoids, saponins and tannins; it has various pharmacological activities whereas in the present study we clearly investigate their antibacterial, antifungal and antioxidant activities in their leaves and root extracts. Among the various solvent used our research reveals that the Aqueous, chloroform and methanol extracts shows maximum inhibitory effect against the tested bacteria and fungi. In addition to that we proved that these extracts also have antioxidant activity. Furthermore we concluded that the aqueous leaf extract and methanolic root extract has virulent activities when compared to other.

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INTRODUCTION

Withania somnifera belonging to the family *Solanaceae* as herb and is spread all over the world especially in Asian countries. It's cultivated more over all parts of India. From ancient times it was used for a variety of aliments. It contains the phytochemicals such as alkaloids, tannins, flavonoids, and terpenes, including choline, tropanol, pseudotopanol, cuscokygrene, 3- tigioyloxytropana, isopelletierine and several other steroidal lactories. Because of that, it exhibits pharmacological activities such as antioxidant, and anti-microbial and in ancient traditional medicine it was used to treating rheumatism, nervous exhaustion, brain-fag, low of memory, loss of muscular energy and spermatorrhoea. In addition to that, It increases body energy. Furthermore, flavonoids like apigenin, kaempferol, astragalin were isolated to investigate the pharmacological activities such as antioxidant, antimicrobial (F Aquil et al., 2006). In the pharmaceutical industry drug development is depend on the natural products especially the plants. Even though the origin of all modern drugs were natural product but mechanistic study of action of natural products against the disease were very low. So our research mainly focused on to find the mechanism of the action of the drug compounds present in the *Withania somnifera* against various diseases. For that we initially plan to do the antioxidant, and anti-microbial studies of *Withania somnifera*.

MATERIAL AND METHODS

Plant collection:

The leaves and roots used were selected from the healthy, mature and disease free *Withania somnifera* plant, Collected from the Botanical Garden, Bon Secours College For Women, Thanjavur, Tamilnadu, India. All the chemicals were analytical grade from hi-media and all the glass wares used were completely sterilized and every process was done at completely sterilized condition.

Extraction:

The leaves and roots of *Withania somnifera* were washed with distilled water and 100 gm of fresh leaves and roots were crushed using mortor and pestal separately with distilled water and the extracts were filtered through Whatman No. 1 filter paper and centrifuged up to the complete removal of debris. The whole process was repeated three times and finally, the concentrated extracts were collected separately in closed containers and kept in a refrigerator at 4 °C. All the steps mentioned above were done separately for roots and leaves. The same process was done for methanol and chloroform extracts.

Bacterial strains:

Gram Positive

Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, and Staphylococcus epidermis.

Gram negative

Pseudomonas aeruginosa , Salmonella typhi, E.coli, were the bacterial strains used to check the antibacterial activity of *Withania somnifera.*

Antibacterial test:

The final solution contains 0.8mg/50 µl concentration, was considered as the test samples for each solvents. For the preparation of sample disc, paper discs of 5 mm diameter were made from Whattman filter paper by punch machine and then autoclaved at 121°C for 15 min. 50 µl of extract was applied to the paper discs under aseptic conditions. Blank discs were also prepared using solvents only. Chloramphenicol discs (30 µg/disc) were used as standards.

For each of the test organisms, the pre-culture was taken from stock cultures and was grown in nutrient broth at 37°C for 24 h. using sterile forceps, all the sample and blanks discs were placed on the marked positions on the seeded petri dishes maintaining an aseptic condition. The standard discs were placed separately onto another set of seeded Petri dishes. The plates were kept at 4°C for 24 h to allow sufficient time for the test material to diffuse to a considerable area of the medium. Afterthat, they were incubated at 37°C for 24 h. The resulting clear zones were measured by a transparent scale (Arora S *et al.,* 2004)

Fungal strains:

Aspergillus flavus, Colletotrichum corchori, and Fusarium equiseti were the fungal strains used to check the antifungal activity of *Withania somnifera*

Antifungal test:

The anti-fungal screening was done by disc diffusion method. The final solution contains 0.8 mg/50 µl concentrations, was considered as the test samples for each solvents. For each of test fungi, separate plate was prepared. Similarly, another set of plates were prepared using a standard antibiotic clotrimazole at a concentration of 80 µg/ml. A set of control plates were also prepared using PDA plates alone. All of the plates were incubated at 24°C for 4 days after which the inhibition of fungal colony was measured with a transparent scale in mm and the percentage of inhibition of mycelial growth was calculated. Finally the antibiotic clotrimazole was employed as a standard for comparison (P Singariya et al., 2014).

Antioxidant Test:

The Radical Scavenging Activity (RSA) of extracts of *W. somnifera* was done by using DPPH assay. Different concentrations (200, 400, 600, 800) of these extracts mixed with methanol and adjusted to nearly 8.5 ml and .5 ml of 0.1 mM methanolic solution of DPPH was added to each tubes and vortexed. Then the tubes were kept undisturbed 20 min at room temperature. Absorbance was measured at 517nm using uv-visible spectrophotometer. RSA was expressed in inhibition percentage and calculated by a standard formula (Dhanani D et al., 2013).

RESULTS AND DISCUSSION

Antibacterial activity:

The leaves and roots extracts of *Withania somnifera* were tested for antibacterial activities. All the samples were tested for antibacterial activities against seven pathogenic bacteria including gram-positive and gram-negative using disc diffusion method and the results were discussed in Table 1 & 2. The zone less than 7mm was considered as resistant. All the studied pathogens were found to be moderately susceptible to AE extract of leaves and ME extract of root with a zone of inhibition ranging from 7 to 14 mm. The highest activity of methanol root extract was found against *Bacillus subtilis* and *E.coli* (with a zone of inhibition 10 mm). The aqueous leaf extract was also found to have potential antibacterial activity against all the bacteria studied. The highest activity was found against *Staphylococcus aureus, Pseudomonas aeruginosa, E.coli* (with a zone of inhibition of 10, 11,13 mm respectively). The above result proved that methanol root extract and aqueous leaf extract had a stronger antibacterial activity. However, the standard antibiotic chloramphenicol showed very strong inhibition against almost all of the tested bacteria.

Table -1: Antibacterial activity of *Withania somnifera* root extract.

Zone of inhibition				
Gram Positive root				
Name of Bacteria	**Aqueous extract**	**Methanol extract**	**Chloroform Extract**	**Chloramphenicol**
Bacillus cereus	7	9	-	20
Bacillus subtilis	-	10	-	15
Staphylococcus aureus	7	8	7	10
Staphylococcus epidermis	-	7	-	25
Gram Negative root				
Name of Bacteria	Aqueous extract	Methanol extract	Chloroform Extract	Chloramphenicol
Pseudomonas aeruginosa	7	9	-	25
Salmonella typhi	-	-	-	20
E.coli	9	10	7	16

Table -2: Antibacterial activity of *Withania somnifera* leaf extract.

Zone of inhibition				
Gram Positive leaf				
Name of Bacteria	**Aqueous extract**	**Methanol extract**	**Chloroform Extract**	**Chloramphenicol**
Bacillus cereus	9	-	7	20
Bacillus subtilis	8	7	8	15
Staphylococcus aureus	10	7	9	10
Staphylococcus epidermis	7	-	-	25
Gram Negative leaf				
Name of Bacteria	Aqueous extract	Methanol extract	Chloroform Extract	Chloramphenicol
Pseudomonas aeruginosa	11	8	9	25
Salmonella typhi	7	-	-	20
E.coli	13	9	10	16

Antifungal activity:

The leaves and roots extracts of *Withania somnifera* were tested for antifungal activities. All the samples were tested for antifungal activities against three fungi using disc diffusion method and the results were discussed in Table 3 & 4. The zone less than 7mm was considered as resistant. All the studied pathogens were found to be moderately susceptible to AE extract of leaves and ME extract of root with a zone of inhibition ranging from 7 to 14 mm. The highest activity of methanol root extract was found against *Fusarium equiseti* (with a zone of inhibition 22 mm). The aqueous leaf extract was also found to have potential antifungal activity against all the fungi studied and The highest activity was found against *Aspergillus flavus* (with a zone of inhibition of 18 mm respectively). The above result proved that methanol root extract and aqueous leaf extract had a stronger antifungal activity. However, the standard antibiotic clotrimazole showed very strong inhibition against almost all of the tested fungi.

Table – 3: Antifungal activity of *Withania somnifera* root extract.

Root extract				
Name of fungi	**Aqueous extract**	**Methanol extract**	**Chloroform Extract**	**Clotrimazole**
Aspergillus flavus	9	20	8	35
Colletotrichum corchori	7	16	7	20
Fusarium equiseti	-	22	-	25

Table – 4: Antifungal activity of *Withania somnifera* leaf extract.

Leaf Extract				
Name of fungi	**Aqueous extract**	**Methanol extract**	**Chloroform Extract**	**Clotrimazole**
Aspergillus flavus	18	11	13	35
Colletotrichum corchori	12	-	-	20
Fusarium equiseti	10	-	7	25

Anti-oxidant activity:

All the extracts and ascorbic acid showed a dose-dependent activity but the methanolic root extract and aqueous leaf extract shows strong activity. Among the four different concentrations (100, 200, 400 and 800 μg/ml) *Withania somnifera* methanolic root extract, showed scavenging activity 56.40, 80.40, 85.20and 86.40 percentages respectively and aqueous leaf extract showed scavenging activity 50.20, 75.25, 80.30, 82.40 percentages respectively. Among the above mentioned four different concentrations the highest scavenging activity of *Withania somnifera* methanolic root extract 800 μg/ml was the highest one as well as aqueous leaf extract 800 μg/ml was the highest one. The DPPH free radical scavenging activity of the *Withania somnifera* methanolic root extract, aqueous leaf extract and ascorbic acid is tabulated in Table 5.

Table: 5 Antioxidant activity of Ascorbic acid *and Withania somnifera.*

Test material	Concentration	Scavenging activity
Ascorbic acid	100	84.60
	200	87.70
	400	89.40
	800	92.20
Withania somnifera Root extract	100	56.40
	200	80.40
	400	85.20
	800	86.40
Withania somnifera Leaf extract	100	50.20
	200	75.25
	400	80.30
	800	82.40

CONCLUSION AND FUTURE PERSPECTIVES

Finally we concluded that the results of the study reveal that the aqueous leaf and methanolic root extracts of *Withania somnifera* exhibits a very potential antimicrobial activity. In addition to that aqueous leaf extract and methanol root extract of *Withania somnifera* shows strong antioxidant activity. These results can be the strong scientific evidence for the use of this plant as a useful source of antioxidant, antibacterial and antifungal references. Furthermore, all our studies were in-vitro to observe other activities of *Withania somnifera*, we need to study the in vivo activities of it. so our future studies will be in vivo especially on cancer studies in mice.

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