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Research Article

# *In-vitro* Antimicrobial Activity of *Withania somnifera* Leaf and Root Extracts grown in Heavy Metal toxic soils

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**ABSTRACT**

The experiment was conducted to evaluate the antimicrobial activity of leaf and root extracts of *Withania somnifera* L. Dunal (ashwagandha), against human pathogenic bacteria and plant pathogenic fungi. Plants were grown in pots in semi black soil with three treatments, Treatment No I (without any additions to the soil), Treatment No II (Cadmium 10ppm, Chromium 20ppm, Nickel 16ppm), Treatment No III (1 % of Calcium hydroxide along with heavy metals). Antimicrobial activity was done with plants, harvested 6 months after date of sowing. The different solvents viz., acetone, methanol, petroleum ether, chloroform and ethyl alcoholic extracts of the plant (leaves) were found to possess strong antimicrobial activity. The present study revealed that acetone extract demonstrated highest antibacterial activity, and petroleum ether extract showed no antibacterial activity against all the four test human pathogens viz., *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.* The crude extract exhibited moderate activity against the bacterial pathogens *S. aureus, B. subtilis* and *E. coli* whereas; no activity was shown against *P. aeruginosa*. Control plant (Treatment No I) leaf extract showed maximum inhibition to solvents acetone, methanol, petroleum ether, chloroform and ethyl alcohol extracts as compared to other two treatments. Five solvent extracts from roots and leaves of *Withania somnifera* in three treatments screened against plant pathogenic fungi *Fusarium oxysporum* and *Colletotrichum capsici* showed antifungal activity.

**Key words**: - *Withania somnifera*, heavy metal treated soil, antimicrobial activity, human pathogens.

## INTRODUCTION

There has been continuous increase all over the world in the resistance of pathogens to drugs. Consequently, new infections can occur in hospitals resulting in high mortality. Therefore, several medicinal plants have been tried against pathogenic microorganisms[1, 2]. Using herbs as antimicrobials played an important role in nearly every culture on earth, including Asia, Africa, Europe and America[3]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by microbial pathogens has led to the screening of medicinal plants for their potential antimicrobial activity[4]. Almost all traditional medicine involved the use of plant extracts crude or sometimes pure active compounds[5]. *Withania somnifera* (Solanaceae), commonly known as ashwagandha, Indian ginseng, and winter cherry[6], has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years. Several studies on this plant indicated that it possesses immunomodulatory, hemopoetic and rejuvenating properties besides positively influencing the endocrine, cardiopulmonary and central nervous systems[7], and antiserotogenic, arthritis[8, 9, 10].

Roots and leaves of this species have traditionally been used, among its many other applications, as an anti-infective remedy against a number of health disorders such as ulcers, rashes, painful swellings, gonorrhoea, syphilis, conjunctivitis, stomach-ache, colds or tuberculosis[11, 12]. *W. somnifera* used for its aphrodisiac, liver tonic, astringent, emaciation, insomnia, senile dementia[13], analgesic effect[14],

memory-improving effects[15], exhibit antibacterial, and anti-fungal[16].

The plant has been found useful in the treatment of burns, wounds, and dermatological disorders, and gastrointestinal diseases, dysfunctions of the respiratory system, asthma, bronchitis, anti-cancer and geriatric problems[17, 18, 19], and including inhibitory effects on enzymes, modulatory effect on some cell types, protection against allergies, antiviral, anti-malarial, antioxidant, anti inflammatory[20, 21].

In the present study, we evaluated antimicrobial activity of *Withania somnifera* leaf and root crude extracts using different solvents. The wide usage of the plant is the reason for conducting the present research and further to investigate the antimicrobial activity of the leaf and root extracts, which adds another advantage to the plant users.

## MATERIALS AND METHODS

### 1. Plant material source

*Withania somnifera* seed materials were obtained from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Hyderabad, Telangana State, India.

### 2. Experimental material and design of the experiment

Seeds of *Withania somnifera* were grown in earthen pots at green house of Botanical Garden, Department of Botany, Osmania University and Hyderabad. Sandy loam semi black soil collected was passed through a 2mm sieve and air dried for one week, and was filled into 15 pots for individual exposure. Treatment No I (without any addition to the soil),Treatment No II (Cadmium 10ppm, Chromium 20ppm, Nickel 16ppm were introduced into the soil),and Treatment No III (1 % of Calcium hydroxide was also added along with heavy metals to soil). Plants were grown up to the productivity levels.

### 3. Collection of the plant materials

*Withania somnifera* (L) leaves and roots were collected from our experimental pots in the Botanical Garden, Department of Botany, Osmania University, Hyderabad, Telangana State, India. The plant material was washed under running tap water, shade dried and powdered using mechanical grinder. The powdered form of plant material was stored in air tight glass bottles protected from sunlight until required for analysis.

### 4. Preparation of extracts from root and leaves

The different solvents like methanol, chloroform, petroleum ether, ethanol and acetone were used for extractions. Ten grams of leaves and root powder was dissolved in 100ml of different solvents in a conical flask and kept at room temperature in a rotary shaker for 48 hours. Then, it was filtered through Whatman No1 filter paper, allowed to evaporate and stored at room temperature until use.

### 5. Micro-organisms collection and maintenance

The bacterial pathogens *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus* and fungal pathogens *Fusarium oxysporum* and *Colletotrichum capsici* were obtained from stock culture in the Department of Botany, Osmania University, Hyderabad. The organisms were cultured on nutrient agar (bacteria) and PDA (fungi) slants, stored at $4^{o}$C until use.

### 6. Disc diffusion assay

Antibacterial tests were carried out by the disc diffusion method[22] using 100µL of suspension, containing $10^{8}$ colony forming units (CFU) $mL^{-1}$ of bacteria spread on nutrient agar medium. The discs (6mm in diameter) impregnated with leaf extractions of *Withania somnifera* (L) were placed on the pathogen inoculated agar plates. The plates were incubated at room temperature for 24 hours. Antimicrobial activity was evaluated by measuring the zone of inhibition (mm) against the test organisms. Three replicates were maintained and each assay was repeated 3 times.

### 7. Agar well diffusion method:

The minimum inhibitory concentrations (MIC) of antimicrobial extracts of *Withania somnifera* (methanol, ethyl alcohol, acetone, chloroform and petroleum ether) was determined against two fungal pathogens (*Fusarium oxysporum* and *Colletotrichum capsici*) by well diffusion method [23]. 100µl (volume) concentrations of different extracts were added to wells and measure the zone of inhibition after incubation.

## RESULT AND DISCUSION

### 1. Leaf extraction against bacteria:

The results of antibacterial activity of crude leaf extract of *Withania somnifera* against four human pathogens likes *Staphylococcus aureus, Bacillus subtilis and Escherichia coli* are presented (Table 1 and Fig1). The leaf extract of treatments in all the solvents (except petroleum ether) showed antibacterial activity on *S. aureus* (except methanol extract of Treatment II), acetone and chloroform solvent extract in Treatment I showed highest anti bacterial activity recording zone of inhibition 3mm, followed by ethyl alcohol extract 2mm and least (1mm) by methanol extract. In Treatment II and III the zone of inhibition against *S. aureus* was recorded

to be only 1mm in all the four solvent extracts (except acetone i.e. 2mm).

In Treatment II ethyl alcohol extract showed antibacterial activity against the three pathogens tested. Acetone and chloroform extracts showed least activity against *S. aureus and E. coli* whereas; no activity was shown against *B. subtilis*. In treatment II methanol extract showed antibacterial activity against *E. coli* while no activity was exhibited against *S. aureus* and *B. subtilis.* Methanol, ethyl alcohol, acetone and chloroform extracts of Treatment III showed antibacterial activity against the three tested pathogen. However petroleum ether extracts of all the three different treatments showed no anti bacterial activity against all the three tested pathogen.

2. **Root extraction against bacteria:**

Root extraction of three treatments in all the five different solvents showed no antibacterial activity against all the three tested pathogen bacteria (Table 2).

3. **Leaf extraction against fungi:**

The results of antifungal activity of leaf extracts in different treatments of *Withania somnifera* in different solvents are presented (Table 3 and Fig 2). In treatment I, methanol and acetone extractions showed no anti fungal activity against *F. oxysporum.* However, showed minimum activity against *C. capsici* with zone of inhibition 1 and 2mm respectively. Petroleum ether showed minimum antifungal activity against *F. oxysporum* and *C. capsici* with zone of inhibition 2mm each. Ethyl alcohol and chloroform extract showed highest antifungal activity recording maximum zone of inhibition 6 and 5mm against *F. oxysporum* and *C. capsici* respectively. However, showed moderate activity with zone of inhibition 3mm against *C. capsici* and least inhibition of only 1mm against *F. oxysporum* respectively.

In Treatment II, methanol extraction showed maximum antifungal against *F. oxysporum* and *C. capsici* with zone of inhibition 6 and 5mm respectively. Petroleum ether extraction showed zone of inhibition 4mm against *C. capsici* while minimum activity with zone of inhibition 1mm against *F. oxysporum*. Ethyl alcohol extracts showed moderate antifungal activity with zone of inhibition 3mm against *F. oxysporum*, Whereas, no activity was recorded against *C. capsici*. However, acetone and chloroform extracts showed no antifungal activity against both the fungal pathogens tested. Treatment III ethyl alcohol extracts showed maximum antifungal activity with zone of inhibition 5and 8mm against *F. oxysporum* and *C. capsici* respectively. However, acetone and methanol have showed no antifungal activity against both the fungal pathogen tested except methanol which showed minimum activity against *F. oxysporum* with zone of inhibition 1mm. Petroleum ether and chloroform showed least antifungal activity against *F. oxysporum* and *C. capsici* with zone of inhibition 1mm each respectively.

4. **Root extraction against fungi:**

The results of antifungal activity of root extracts from three different treatments of *Withania somnifera* in different solvents are presented (Table 4 and Fig 3). In Treatment I, the extracts in all the solvents showed antifungal activity (ethyl alcohol showed no activity against *C. capsici*) ranging from 3-6mm and 1-8mm against *F. oxysporum* and *C. capsici* respectively. Methanol extract showed maximum antifungal activity with zone of inhibition 6 and 8mm against *F. oxysporum* and *C. capsici* respectively. Acetone, petroleum ether showed zone of inhibition 6 and 4mm and 5 and 6mm against *F. oxysporum* and *C. capsici* respectively. Chloroform extract showed good antifungal activity against *F. oxysporum* showed zone of inhibition 4mm. However, showed least activity (1mm) against *C. capsici.* Ethyl alcohol extracts showed antifungal activity against *F. oxysporum* (3mm), whereas no activity was shown against *C. capsici.*

In Treatment II, the extract of all the solvents exhibited no antifungal activity against the test pathogens. Except ethyl alcohol and petroleum ether extract showed antifungal activity against *C. capsici* with zone of inhibition 2 and 6mm respectively. Petroleum ether extract showed highest antifungal activity against *C. capsici* recording zone of inhibition 6mm. In Treatment III, root extract in acetone showed antifungal activity against *F. oxysporum* and *C. capsici* with zone of inhibition 2 and 3mm respectively, whereas ethyl and chloroform showed antifungal activity against *C. capsici* with zone of inhibition 2mm. However, no activity was exhibited by methanol and petroleum ether extractions against *F. oxysporum* and *C. capsici*. Ethyl alcohol and chloroform showed no antifungal activity against *F. oxysporum*.

Antibacterial activity of *W. somnifera* leaf acetone extract, which correspond with results of previously published papers reporting similar effects of various plant-derived products (extracts or compounds) against intestinal and pathogenic bacteria[24,25,26,27] . Many reports are available on the antiviral, antibacterial, antifungal, antihelminthes, antimolluscal and anti-inflammatory properties of plants[28, 29, 30, 31, 32, 33, 34]. The presence of bioactive compounds in plants has been reported to confer resistance against microbial pathogens and therefore

explains the demonstration of antibacterial activity by the plant extracts used in this study[35]. The study is a preliminary evaluation of antimicrobial activity of Carica papaya and *W. somnifera*[36]. The earlier worked done by[37] in *Withania somnifera* revealed that the fungal species composition was almost similar in Treatment I and Treatment II soils, where as in Treatment III soil, the fungal species has decreased in number.

**Table 1**
**Zone of inhibition (mm) antibacterial activity of crude extracts in different solvents from *Withania somnifera* leaves of plants grown in three different treatments.**

| Treatments | Zone of inhibition (mm) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Methanol | | | Ethyl alcohol | | | Acetone | | | Chloroform | | | Petroleum ether | | |
| | Sa | Bs | Ec | Sa | Bs | Ec | Sa | Bs | Ec | Sa | Bs | Ec | Sa | Bs | Ec |
| Treatment I | 1 | - | 1 | 2 | - | 1 | 3 | - | - | 3 | + | 1 | - | - | - |
| Treatment II | - | - | + | 1 | 1 | + | 2 | - | + | 1 | - | + | - | - | - |
| Treatment III | 1 | + | 1 | 1 | 1 | + | 1 | + | + | 1 | 1 | 1 | - | - | - |

Bs= *Bacillus subtilis*, Ec= *Escherichia coli*, Sa= *Staphylococcus aureus*, - = absent, + = present.

**Table 2**
**Zone of inhibition (mm) antibacterial activity of crude extracts in different solvents from *Withania somnifera* root of plants grown in three different treatments.**

| Treatments | Zone of inhibition (mm) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Methanol | | | Ethyl alcohol | | | Acetone | | | Chloroform | | | Petroleum ether | | |
| | Sa | Bs | Ec | Sa | Bs | Ec | Sa | Bs | Ec | Sa | Bs | Ec | Sa | Bs | Ec |
| Treatment I | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Treatment II | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Treatment III | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

Bs= *Bacillus subtilis*, Ec= *Escherichia coli*, Sa= *Staphylococcus aureus*, - = absent, + = present.

**Table 3**
**Zone of inhibition (mm) antifungal activity of crude extracts in different solvents from *Withania somnifera* leaf of plants grown in three different treatments.**

| Treatments | Zone of inhibition (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Methanol | | Ethyl alcohol | | Acetone | | Chloroform | | Petroleum ether | |
| | Fo | Cc | Fo | Cc | Fo | Cc | Fo | Cc | Fo | Cc |
| Treatment I | - | 1 | 6 | 3 | - | 2 | 1 | 5 | 2 | 2 |
| Treatment II | 6 | 5 | 3 | - | - | - | - | - | 1 | 4 |
| Treatment III | 1 | - | 5 | 8 | - | - | 1 | 1 | 1 | 1 |

Fo- *F. oxysporum*, and Cc- *C. capsici*

**Table 4**
**Zone of inhibition (mm) antifungal activity of crude extracts in different solvents from *Withania somnifera* roots of plants grown in three different treatments.**

| Treatments | Zone of inhibition (mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Methanol | | Ethyl alcohol | | Acetone | | Chloroform | | Petroleum ether | |
| | Fo | Cc | Fo | Cc | Fo | Cc | Fo | Cc | Fo | Cc |
| Treatment I | 6 | 8 | 3 | - | 6 | 5 | 4 | 1 | 4 | 6 |
| Treatment II | - | - | - | 2 | - | - | - | - | - | 6 |
| Treatment III | - | - | - | 2 | 2 | 3 | - | 2 | - | - |

Fo- *Fusarium oxysporum*, and Cc- *Colletotrichum capsici*

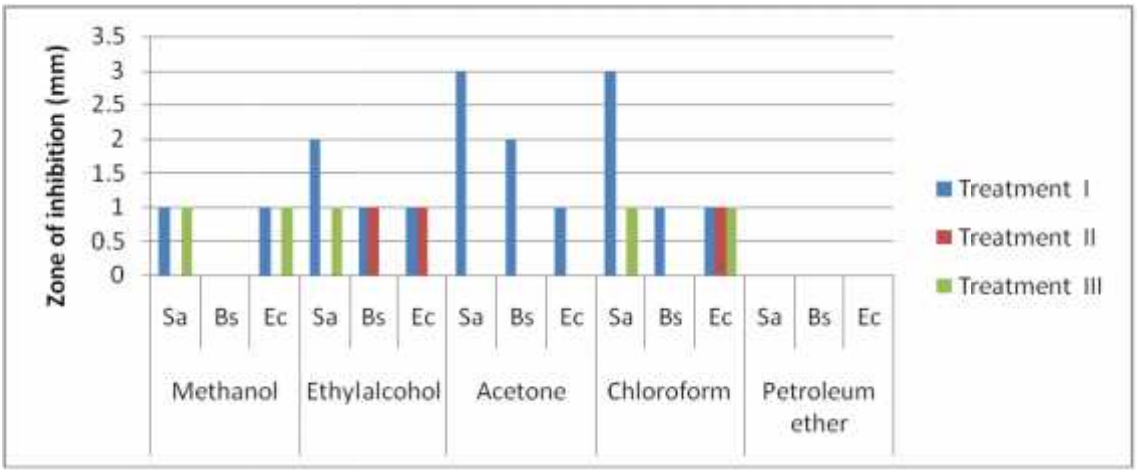


**Figure 1**
**Antibacterial activity of crude extracts in different solvents of *Withania somnifera* leaf of plants grown in three treatments.**

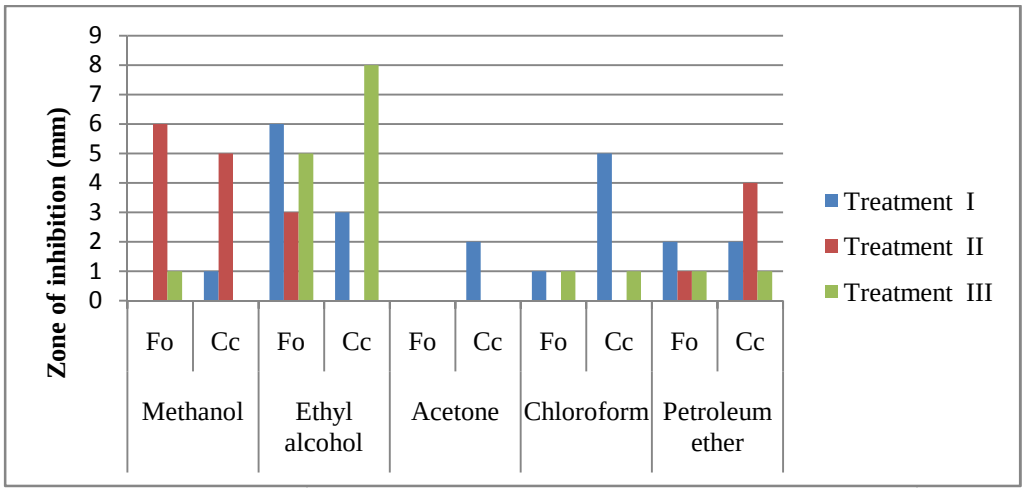


**Figure 2**
**Antifungal activity of crude extracts in different solvents of *Withania somnifera* leaf of plants grown in three different treatments.**

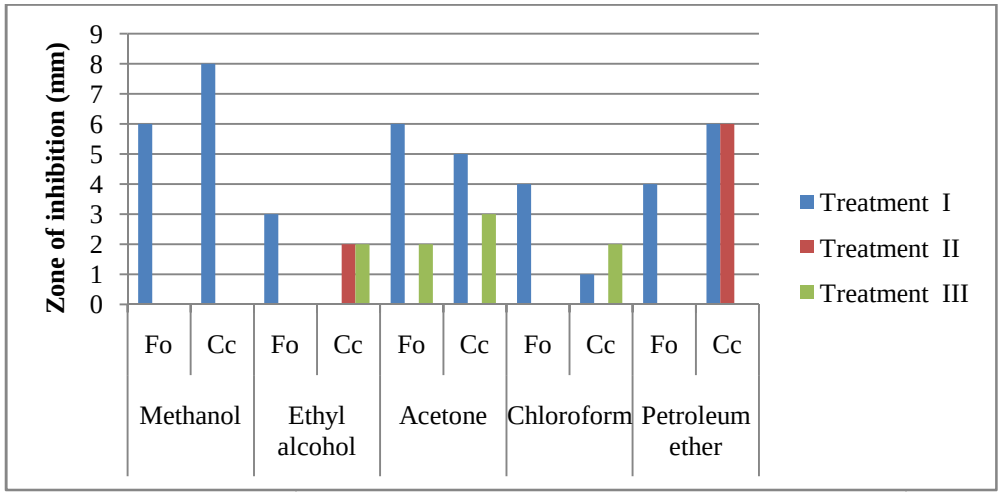


**Figure 3**
**Antifungal activity of crude extracts in different solvents of *Withania somnifera* root of plants grown in three different treatments.**

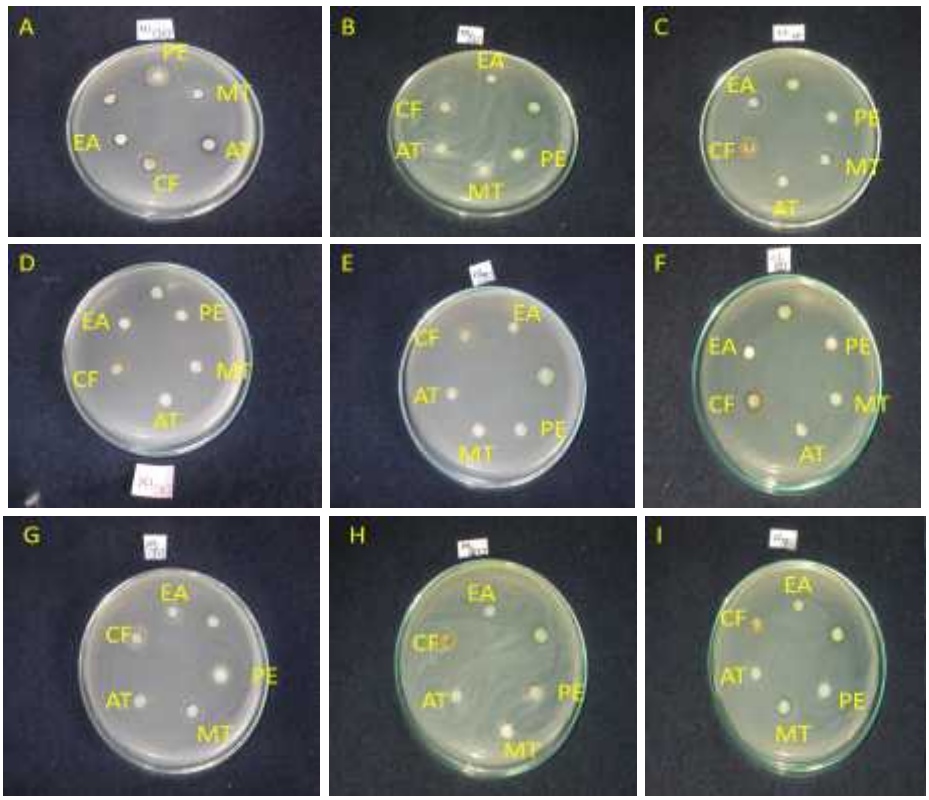


**Fig 4**
**Antibacterial activity of *Withania somnifera* leaf extracts against *S. aureus* (A- Treatment I, B- Treatment II and C- Treatment III ), against *B. subtilis* (D- Treatment I, E- Treatment II and F- Treatment III) and against *E. coli* (G- Treatment I, H- Treatment II and I- Treatment III).**

## CONCLUSION

A detailed antibacterial and antifungal activity study was carried out on *Withania somnifera* leaf extracts in different solvents from three treatments. The crude extract exhibited moderate activity against the three human pathogens *B. subtilis, E. coli* and *S. aureus*, and no activity was shown against *P. aeruginosa*. The acetone extract showed highest antibacterial activity whereas, petroleum ether extract showed no activity against all the three pathogenic bacteria. Roots and leaves extracts of *Withania somnifera* not only have antibacterial activity but also antifungal activity against pathogenic fungi. It may be useful for biocontrol activity.

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