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# Phytochemical Analysis and Antimicrobial Activity of Roots of Withania somnifera (L.) Dunal

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**Abstract :** *Withania somnifera* (L.) Dunal commonly known as 'Ashwagandha' is a widely used herb in Ayurvedic medicine.In the present study, root extracts of *Withania somnifera* were analysed for phytochemical constituents, and antimicrobial property. Antibacterial activity of root extract was tested by agar-well diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella paratyphi* B. The study also investigated the effect of temperature on its antibacterial activity. Qualitative phytochemical analysis revealed the presence of carbohydrates, reducing sugars, alkaloids, phytosterol, fixed oils, proteins, phenolic compounds and flavonoids. All the bacterial strains were found to be sensitive to acetone, ethyl acetate and ethanol extracts. Acetone and ethanol extracts were more effective against *Klebsiella pneumoniae*, whereas ethyl acetate extract was more effective against *Pseudomonas aeruginosa* and *Salmonella paratyphi* B. The effect of temperature on the antimicrobial potential of the root extracts of the plant remained reasonably unaffected. The present screening demonstrated that *Withania somnifera* root extract has potent antibacterial activity and a potential source of new class of antimicrobial compounds that could be useful for infectious disease chemotherapy and control.

**Keywords :** *Withania somnifera*, Root extract, Antibacterial activity, Thermal stability.

## Introduction

The development of multiple drug resistance bacterial pathogens has diverted the attention of researchers to find out new compounds from natural sources with good antimicrobial potential[1]. *Withania somnifera* Dunal (*Solanaceae*), commonly known as Ashwagandha, Indian ginseng or winter cherry is a one of the most valuable medicinal herb in traditional Indian medicine. Ashwagandha is used for treatment of wounds, cough, asthma, diabetes, tumors, hemiplegia, dyspepsia, diarrhoea, rheumatoid arthritis, lumbago, stress, insomnia, sexual debility, menstrual problems, leucoderma, scabies and leucorrhoea[2-4]. The plant is also used as a dietary supplement as it contains a variety of nutrients and phytochemicals. A decoction of Ashwagandha



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roots is used as a nutrient and health restorative by pregnant women and the elderly. It thickens and increases the nutritive value of the milk when given to nursing mothers[5]. Clinical trials and animal research support the use of the plant for anxiety, Parkinson's disease, attention deficit hyperactivity disorder (ADHD), Alzheimer's disorder and cerebral ischemia[6,7]. Several authors have investigated the antimicrobial potential of *Withania somnifera*[8-10]. However, few reports are available regarding the antimicrobial activities of root extracts of the plant. In the continuation of the strategy of new drug discovery, the present study investigates phytochemical constituents and antibacterial properties of root extracts of *Withania somnifera* L.

## Materials and Methods

### Collection and processing of plant material

The plant *Withania somnifera* L., free from disease was collected from Kolli hills, Eastern Ghats, India. The plant was authenticated by Dr.A.Balasubramaninan, ABS botanical conservation, research and training centre, Salem. The roots were washed under running tap water, air dried in the shade and ground to a fine powder and stored in the airtight bottle.

### Preparation of root extracts

Root powder (50 g) of the Ashwagandha were soaked separately in 200 mL of acetone, ethyl acetate and ethanol in a conical flask and kept at room temperature in laboratory shaker with a shaking speed of 120 rpm for 72 hours. The extracts so obtained were filtered through Whatman No.1 filter paper. The filtrates were concentrated on a rotary evaporator and then stored at 4°C till further use[11].

### Phytochemical screening

The crude root extracts of *Withania somnifera* were tested for the presence of phytochemicals using standard phytochemical methods[12-14].

### Bacterial strains

*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella paratyphi* B used in the present study were from the collection of microbiology laboratory of Vysya College, Salem, India.

### Determination of antimicrobial activity

The antimicrobial activities of *Withania somnifera* root extracts were determined using the agar-well diffusion method following a published procedure with slight modification[15].The test bacterial strain was inoculated in to Mueller-Hinton broth and incubated at 37°C for 24 hours. The cultures were later diluted with sterile Mueller-Hinton broth, adjusted to 0.5 McFarland turbidity standard and inoculated on Mueller-Hinton agar plates by spreading the culture over the surface of the medium using a sterile cotton swab. Wells of 6 mm diameter were punched in the medium using a sterile cork borer and filled with 100 µL of crude rootextracts (5 mg/mL) dissolved in dimethylsulfoxide (4.0%, v/v). Negative and positive controls were also run in parallel on the same plate with dimethylsulfoxide and ciprofloxacin (50µg/mL), respectively. The plates were incubated in an upright position at 37°C for overnight in an incubator. At the end of incubation period, antibacterial activities were assessed by measuring the diameters of the zone of inhibition around each well, excluding the diameter of well in mm

### Determination of thermal stability of root extracts

Thermal stability of *Withania somnifera* root extract was tested using the method described by Simlai and Roy[16]. Dried acetone, ethyl acetate and ethanol extract of the plant material was kept at 60°C and 100°C for 30 minutes. Then the samples were cooled at room temperature and stored in a refrigerator at 4°C till further use. Afterwards, residual antimicrobial activity of plant extract was tested by agar-well diffusion method as described above.

## Results and Discussion

Acetone, ethyl acetate and ethanol extract of roots of *Withania somnifera* showed the presence of carbohydrates, reducing sugars, alkaloids, phytosterol, fixed oils, proteins, phenolic compounds and flavonoids (Table 1). Phytochemicals such as alkaloids, flavonoids, tannins and several other aromatic compounds are secondary metabolites of plants that serve as defence mechanism against plant pathogens[17, 18]. This may therefore explain the demonstration of antimicrobial property of Ashwagandha.

**Table 1: Phytochemical screening of *Withania somnifera*(L.) root extracts**

| **Phytochemicals** | **Test** | **Acetone extract** | **Ethyl acetate extract** | **Ethanol extract** |
|---|---|---|---|---|
| Carbohydrates | Molisch's test | + | + | + |
| Reducing sugars | Fehling's test | - | - | - |
| | Benedict's test | - | - | - |
| | Barford's test | + | + | + |
| Alkaloids | Mayer's test | - | - | + |
| | Dragendorff's test | + | + | + |
| | Hager's test | + | + | + |
| | Wagner's test | + | + | + |
| Phytosterols | Salkowski test | + | + | + |
| Fixed oil | Spot test | + | + | + |
| Saponins | Froth test | + | + | + |
| Proteins and free aminoacids | Biuret test | + | + | + |
| | Ninhydrin test | - | - | - |
| | Xanthoprotein test | + | + | + |
| | Millon's test | - | - | - |
| Phenolic compounds | Ferric chloride test | + | + | + |
| Flavonoids | Alkaline reagent test | + | + | + |

+: Positive, - : Negative

Acetone, ethyl acetate and ethanol extract of roots of *Withania somnifera* showed good antibacterial activity against all the tested microorganisms. Acetone extract displayed highest antibacterial activity against *Klebsiella pneumoniae* and the lowest activity against *Escherichia coli*. Maximum antimicrobial activity was recorded for ethyl acetate extract against *Pseudomonas aeruginosa* and *Salmonella paratyphi* B. The largest zone of inhibition for ethanol extract was against*Klebsiella pneumoniae,*and the smallest zone of inhibition was against*Staphylococcus aureus*.Gentamicin showed inhibition zone ranging from 26.33±0.47- 36.33±0.47 mm.

Acetone, ethyl acetate and ethanol extract of roots of the plant demonstrated antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Salmonella paratyphi* B to different magnitudes. This finding supports the traditional uses of the *Withania somnifera* for respiratory and gastrointestinal disorders[4].It has been reported that methanolic extract of roots of *Withania somnifera*possess moderate antimicrobial activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [19]. In the present study, acetone, ethyl acetate and ethanol extract of roots of the plant showed strong antimicrobial activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* Ethyl acetate extract of roots of the plant showed antibacterial against *Staphylococcus aureus,Escherichia coli* and *Pseudomonas aeruginosa* which is agreement with the results obtained by Sundaram *et al.*[20].

**Table 2: Antimicrobial activity of *Withania somnifera*(L.) root extracts**

| Bacterial strains | Diameter of zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | Acetone extract | Ethyl acetate extract | Ethanol extract | Gentamicin |
| *Staphylococcus aureus* | 13.66±0.47 | 12.33±0.47 | 12.66±0.47 | 26.33±0.47 |
| *Escherichia coli* | 12.33±0.47 | 13.33±0.47 | 17.33±0.47 | 26.33±0.47 |
| *Klebsiella pneumoniae* | 21.33±0.47 | 16.33±0.47 | 18.33±0.47 | 36.33±0.47 |
| *Proteus mirabilis* | 15.33±0.47 | 12.66±0.47 | 15.66±0.47 | 34.33±0.47 |
| *Pseudomonas aeruginosa* | 16.00±0.81 | 19.33±0.47 | 17.00±0.81 | 33.33±0.47 |
| *Salmonella paratyphi* B | 18.00±0.81 | 19.33±0.47 | 14.33±0.47 | 31.33±0.47 |

Values are means of triplicate determinations ± standard deviation

The effect of temperature on antimicrobial potential of acetone extract of roots of *Withania somnifera*revealed that the antimicrobial activity was not much affected at 60°C and 100°C, suggesting that phytochemicals responsible for antimicrobial activity were relatively heat stable. However, acetone extract of roots of the plant lost its activity against *Salmonella paratyphi* B at 60°C and 100°C. Ethyl acetate and ethanol extract of roots of the plant showed decrease in antimicrobial activity and no antimicrobial activity suggesting that the phytochemicals responsible for antimicrobial activity were heat sensitive. At elevated temperature, the antimicrobial compounds, being protein in nature might be degraded and resultantly, activity is lost[21].It is a well-known fact that the loss of antimicrobial property of plant extracts by heating may be due to volatilization and/or physical changes that take place during heating[22].

**Table 3: Effect of temperature on antimicrobial potential of *Withania somnifera*(L.)root extracts**

| Bacterial strains | Diameter of zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | 60°C | | | 100°C | | |
| | AE | EA | EE | AE | EA | EE |
| *Staphylococcus aureus* | 11.33±0.47 | 10.33±0.47 | 10.33±0.47 | 11.33±0.47 | 0 | 0 |
| *Escherichia coli* | 10.33±0.47 | 11.33±0.47 | 12.33±0.47 | 10.33±0.47 | 0 | 10.33±0.47 |
| *Klebsiella pneumoniae* | 14.33±0.47 | 12.33±0.47 | 10.33±0.47 | 13.33±0.47 | 0 | 0 |
| *Proteus mirabilis* | 13.33±0.47 | 11.33±0.47 | 12.33±0.47 | 12.33±0.47 | 0 | 0 |
| *Pseudomonas aeruginosa* | 16.33±0.47 | 13.66±0.47 | 15.33±0.47 | 14.33±0.47 | 10.33±0.47 | 13.33±0.47 |
| *Salmonella paratyphi* B | 0 | 15.00±0.81 | 11.33±0.47 | 0 | 9.33±0.47 | 0 |

Values are means of triplicate determinations ± standard deviation
AE: Acetone extract, EA: Ethyl acetate extract, EE: Ethanol extract

## Conclusion

The present study concludes that acetone, ethyl acetate and ethanolic extract of roots of *Withania somnifera* possess broad spectrum antibacterial activity and could be used as an alternate to antibiotics for the treatment of infectious diseases.

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