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Short Communication

Assessment of growth inhibitory activity of *Withania somnifera* L., Indian Ginseng against *Escherichia coli*

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The current investigation is focused on the antibacterial potential of *Withania somnifera* Dunal roots. Ashwagandha root powder contains many bioactive components like alkaloids, tannins, flavonoids, and phenolic compounds. These substances have a variety of recognised medicinal, antimicrobial, and antibacterial properties. This study has investigated the antibacterial activity of *W. somnifera* root ethanol extract (*Ws*-REE). Three concentrations, 0.5, 1, and 2%, were employed for the treatment. The concentration of *E. Coli* cell culture was determined by using a spectrophotometer. The control group exhibited the highest measured cell density of 1.3 X 10^9 cells/mL. In the measured concentration, the dose-dependent impact was noted. For 0.5, 1, and 2% of the sample, the bacterial cell density was 4.24 X 10^8, 1.2 X 10^8, and 8.0 X 10^6, respectively. The growth inhibitory activities of *W. somnifera* extract against *E. coli* may be due to specific chemical compounds or synergistic effects of the compounds present in the extract.

Keywords: *Escherichia coli*, Growth inhibitory, *Withania somnifera*, *Ws*-REE

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Introduction

In the current era of increasing antibiotic resistance, alternative approaches to combat microbial infections are of paramount importance. *Withania somnifera* (Solanaceae), commonly known as Ashwagandha or Indian Ginseng, is native to India and found primarily in Pakistan, Sri Lanka, and Bangladesh. It is distributed across various regions within India, including parts of the Himalayas, central India, and southern India[1]. *W. somnifera* is an evergreen, xerophytic, woody, short perineal shrub[2].

In India, it is grown as a medicinal plant, mainly for its roots, which contain large amounts of bioactive compounds with several pharmacological implications[3,4,5]. A myriad of phytochemicals have been isolated from *W. somnifera* using many separation techniques. Some of the major phytoconstituents present in the plant include withaferin A[6], viscosalactone B[7], 27-deoxywithaferin A[8], pubesenolide[9], jaborosalactone D[10], 4b,27-dihydroxy-L-oxo-22R-witha-2,5,24-trienolide[11], 2,3 dihydrowithaferin A-3b-O-sulfate[6], withanolide A[12], and 27- hydroxywithanolide B[13] (Fig. 1).

Withania somnifera is a medicinal herb that has been used for centuries in Ayurvedic medicine for its various health benefits[14]. It possesses various medicinal properties such as antibacterial, antiviral, antifungal[15], anti-inflammatory[16], antioxidant[17], immuno-modulatory[18], and neuroprotective[19]. *W. somnifera* extract also acts as an anti-stress agent (reducing cortisol levels) and is used as an adaptogenic[20].

Escherichia coli is a gram-negative, rod-shaped bacterium belonging to the family Enterobacteriaceae. It is one of the well-studied microorganisms and serves as a model organism in microbiology and molecular biology. *E. coli* is commonly found in the intestines of warm-blooded animals, including humans, where it plays a role in the digestive process. It is also present in the environment, such as soil, water, and food[21]. While most strains of *E. coli* are harmless and beneficial to humans, some pathogenic strains can cause serious

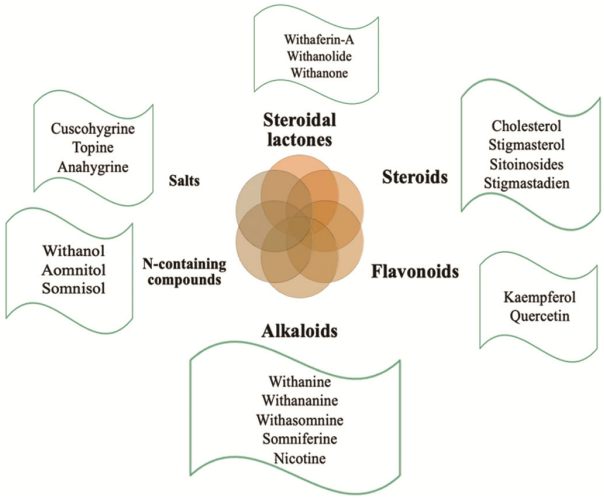

Fig. 1 — Phytoconstituents present in *Withania somnifera* extract.

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illnesses, including food poisoning, urinary tract infections, and gastrointestinal infections[22].

Certain strains of *E. coli* have acquired virulence factors that enable them to cause diseases in humans. Pathogenic *E. coli* strains are classified into various pathotypes based on their virulence mechanisms and clinical manifestations. Examples include enterotoxigenic *Escherichia coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and uropathogenic *E. coli* (UPEC)[23]. *E. coli* is a significant public health concern due to its role in foodborne illnesses and other infections. Outbreaks of *E. coli* infections, often associated with contaminated food or water, can lead to severe illness and even death, particularly in vulnerable populations, such as young children, the elderly, and immuno-compromised individuals[24]. The emergence of antibiotic-resistant *E. coli* strains poses challenges for treating infections. Resistance to multiple antibiotics, including commonly used antibiotics such as fluoroquinolones and third-generation cephalosporins, has become increasingly prevalent among *E. coli* clinical isolates[25].

E. coli is a model organism in antimicrobial studies due to its ease of culturing, rapid growth rate, homology to pathogenic bacteria and well-characterised genome. This study investigated the antibacterial potential of *W. somnifera* extract against *E. coli* DH5α.

Materials and Methods

Plant sample collection and extraction

W. somnifera root powder was procured with a reputed brand name from the market. Exactly 40 g of *W. somnifera* powder was used to extract 400 mL ethanol with a Soxhlet extraction apparatus at 45°C for 72 h. The extract was then concentrated using a rotary vacuum evaporator at 45°C. The concentrated extract was collected in a glass bottle and stored at 4°C. The yield of the extract was calculated as

Percentage of the yield

The percentage of the yield was calculated by following the formula

$$\text{Percentage yield of extract} = \frac{\text{Weight of extract x } 100}{\text{Weight of } W.somnifera \text{ powder used for extraction}}$$

Antibacterial activity

A culture of *E. coli* (DH5α) was prepared in Luria-Bertani (LB) broth. In a laminar airflow chamber, 20 µL of inoculum was added to 20 mL of autoclaved LB broth in sterilised borosil test tubes. The mouth of the test tubes was sealed with sterile cotton (non-absorbant) plugs and kept overnight in an orbital shaker at 37°C and 110 RPM.

For the antibacterial bioefficacy of *W. somnifera* root ethanol extract (*Ws*-REE), it was diluted using DMSO. In the treatments, different concentrations of the plant extract were added to make the final concentrations of the tests as 2, 1, and 0.5%. In control, DMSO was added in place of the extract. Both control and test experiment test tubes were kept in the shaker for bacterial growth. After 24 h, bacterial growth in each treatment and control was observed by measuring the optical density (OD) of each sample using a UV-Vis spectrophotometer (Cary UV-Vis, Agilent Technologies). The OD values were converted to microbial density with the help of Agilent Genomics: Tool-Bio Calculators[26] (https://www.chem.agilent.com/store/biocalculators/calcODBacterial.jsp). All the experiments were replicated three times. The results are presented as Mean±SE.

Results and Discussion

A total of 3.6 g *Ws*-REE was obtained by the extraction method used in the present study, which constituted the plant extract yield of 9%.

The results on the bioefficacy of *Ws*-REE on *E. coli* indicate that the extract had significant growth inhibitory activity. Significant bacterial growth was observed in the control after 24 h. of incubation; the cell density in the control was 1.3 X 10^9. While in the treatments with *Ws*-REE, a dose-dependent growth inhibition of *E. coli* was observed. At 2% concentration of the extract, negligible absorbance was recorded, indicating that the growth of *E. coli* was inhibited with 1.02 X 10^7 cell density. Significant inhibition was also observed at 1% of *W. somnifera* extract; the cell density was 1.19 X 10^8. At 0.5% concentration, the impact on growth was the least, out of the three doses, and the cell density was 4.22X 10^8 (Fig. 2).

The qualitative study of the phytocomponents of *W. somnifera* reported the presence of alkaloids, carbohydrates, saponins, glycosides, proteins, phytosterols, phenolic compounds, flavonoids, terpenoids, tannins, anthraquinones, and emodins[27]. Similarly, eleven compounds were reported in *W. somnifera*, including oleic acid, phytol, and n-hexadecenoic acid. The extracts of this plant were found to be effective (showing larger zones of

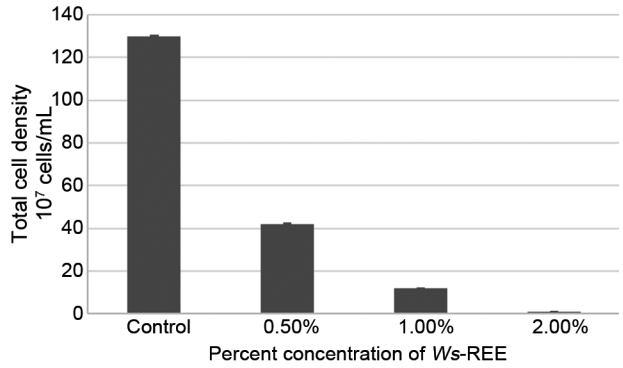

Fig. 2 — Growth inhibitory activity of *Ws*-REE against *E. coli*.

inhibition) against *Escherichia coli* and *Klebsiella pneumoniae*[28].

A phytochemical study of the roots of *W. somnifera* showed the presence of flavonoids, alkaloids, and steroids and reported an antibacterial effect against *E. coli*[29]. The pharmacological activities of roots and leaves of *W. somnifera* were studied on different bacterial and fungal test organisms, and the antibacterial and antifungal activities were reported[30]. The phytochemicals possessing antibacterial activities were identified in leaves of *W. somnifera*, including cyclotrisiloxane and hexamethyl, with high abundance. In the roots, 2,2-dimethoxybutane has been reported to have high abundance and antimicrobial properties[31].

Conclusion

A literature survey of GC-MS analysis of *W. somnifera* ethanol extract shows the presence of the various classes of phytochemicals that have been reported with antimicrobial activity; for example, N-Hexadecenoic acid, Alkaloids, Saponin, Phenolic compounds, Tannins, Anthraquinone, Phlorotannin's, and Coumarins. The growth inhibitory activities of *W. somnifera* extract against *E. coli* may be due to specific chemical compounds or synergistic effects of the compounds present in the extract. The present study suggests that *W. somnifera* can be potentially used to explore pharmaceutically important phytocompounds that may be useful for various bacterial diseases.

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Conflicts of interest

The author/s declares no conflicts of interest regarding the publication of this paper.

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