Original Article
DOI: https://doi.org/10.70749/ijbr.v3i4.954

INDUS JOURNAL OF BIOSCIENCE RESEARCH
https://induspublishers.com/IJBR
ISSN: 2960-2793/ 2960-2807

Evaluating the Antibacterial Efficacy of *Withania somnifera* (Ashwagandha) against Various Bacterial Strains

Waseem Sajjad[1], Fahim Ullah[1], Rizwan Ullah[1], Zia Ullah[1], Mudassir Ahmad[2], Jawad Zahir[1], Shafiq Ur Rahman[1], Kaleem Ullah[1], Qazi Sami Ul haq[1], Yasar Aziz[1], Jawad Khan[1], Sania[1]

[1]Department of Microbiology, Abbottabad University of Science and Technology, Abbottabad.
[2]Poultry Research Institute Rawalpindi (46000), Punjab, Pakistan.

ARTICLE INFO

Keywords
Withania somnifera, Antibacterial Efficacy, Medicinal Properties, Bacterial Strains

Corresponding Author: Mudassir Ahmad,
Poultry Research Institute Rawalpindi (46000), Punjab, Pakistan.
Email: mudassir.ahmad@uvas.edu.pk

Declaration
Authors' Contribution: All authors equally contributed to the study and approved the final manuscript.
Conflict of Interest: No conflict of interest.
Funding: No funding received by the authors.

Article History
Received: 07-01-2025, Revised: 15-03-2025
Accepted:28-03-2025, Published: 08-04-2025

ABSTRACT

The overuse of chemically synthesized antibiotic drugs and the internal defense mechanism of microorganisms make the currently available antibiotics less efficient because the microbes have developed resistance against them... to handle such kind a solution, the necessity to find new antibacterial agents significantly increases the phytochemical substances present in nature have significantly less production cost and are environmentally friendly. The plant contains an inhibitory mechanism against many bacteria and can be the better alternative to chemically synthesized drugs because of their organic-based origin. The main aim of the current study was to analyze and assess the antimicrobial potential of the selected medicinal plants *Withania somnifera* against some pathogen bacteria such as coli Neisseria. The alcoholic extracts of the medicinal plants were prepared using a soxhlet apparatus. The antimicrobial potential of the extract was evaluated using the healthy diffusion method. As well as the disc diffusion method. The inhibition zones against every bacteria were recorded in diameter, taking millimeters as a measuring scale. Among the selected medicinal plant extracts, the ethanol and chloroform extract, *Withania somnifera*, showed the highest activity against all the tested pathogenic organisms. Among the pathogenic bacteria used in the study, salmonella appeared to be most susceptible to the *Withania somnifera* medicinal plant as they showed susceptibility against the selected plant extract. The result of this study was encouraging. Our result indicates that there is the possibility of using this plant extract in the treatment of bacterial infection. Clinical studies also need to check the potential toxic effects and effectiveness against the infection caused by these pathogenic bacteria.

INTRODUCTION

The herb *Withania somnifera*, frequently called Ashwagandha Indian ginseng or winter cherry, serves traditionally in Ayurvedic medication for boosting life expectancy and vitality and functioning as a rasayana health-enhancing agent to strengthen disease resistance (Verma et al., 2022). The evergreen shrub *Withania somnifera* grows upright with a greyish appearance and pubescent texture and displays tuberous roots extending to medium lengths and a short stem that bears tiny star-shaped trichomes on both stem and branches. Each leaf is up to 10 cm long with oval shape and petiole connection and stand opposite other leaves on the stem (Rhoads & Block, 2007). The tiny blossoms appear in yellowish or greenish colors while growing as single plants or tiny bunches that develop from the leaf axils most of the year. A mature fruit of *Withania somnifera* is smooth and globular with meaty flesh, multiple seeds, and an orange-red color that appears beneath a membrane (Baimey et al., 2020). The shrub reaches about 2 feet tall as it grows as a woody plant and originates from Africa, India, and the Mediterranean regions, with cultivation taking place in the United States (Brightly et al., 2020). The different chemical compositions of *W. somnifera* are extensively cultivated for medicinal uses in India (Polumackanycz et al., 2023). Withania contains 26 species worldwide, and two of these species exist in India under the names W. somnifera and W. coagulans Dunal (Macharia, 2023). Nichols (2005) discovered W. obtusifolia (Tackh) through sampling in South India, although research proved this plant to be a plant with a third set of chromosomes within W. somnifera (2n=48). The Indian germplasm resulted in the identification of W. Ashwagandha as a new species by applying multiple analytical approaches, according to Kumar et al. (2022). The metabolic analysis of *W. somnifera* leaf and root extracts revealed 62 primary and secondary metabolites

in the leaves, while the roots contained 48 metabolites. A total of 29 metabolites were detected in both leaf and root tissues of *W. somnifera* by Kumar et al. (2022b), who reported fatty acids, organic acids, amino acids, sugars, flavones, and sterol derivatives. Metabolic concentrations in these metabolites showed quantitative differences between leaf and root structures (Figure 1). Antineoplastic activity describes cancer, which represents an excessive growth of abnormal cells that can replicate actively and spread to other tissues.

Nations et al. (2020) calculated that the global cancer case numbers in 2008 amounted to 28.8 million. Mortality rates increase mainly from colorectal cancer, joined by breast cancer, followed by gastric cancer and prostate cancer, with pulmonary cancer as the fourth contributor to elevated mortality rates. Distinct therapeutic approaches are applied singularly or jointly to treat different tumor types. The pharmacological properties physicians want ineffective tumor treatments for involve high selectivity, tumor cell growth limitation, cell homeostasis restoring capabilities, and minimal side effects (Dua et al., 2021). Multiple treatment failures result from cancer cells' various genetics together with complex tumor relationships, which create conditions for drug-resistant cells to evolve into treatment-resistant clones. Scientists have shown interest in natural medicinal compounds because they can prevent or reduce treatment resistance by targeting various cancer hallmarks, including cell proliferation together with resistance to apoptosis, replicative immortality and invasion and metastasis and tumor-promoting inflammation (Roy, 2024). Metastases that appear during cancer treatment play a vital role in determining the survival odds of cancer patients. The research conducted by Egbuna and Hassan (2021) presented *W. somnifera* as a potential sustainable therapeutic option for halting cancer cell growth conditions. The research examined how the root extract samples reacted against the protein vimentin, which drives metastatic behavior. Tests were conducted with numerous *W. Somnifera* extracts to measure their ability to inhibit cell motion inside breast cancer tumors. The data indicated that the dosages of rats at lower *Withania somnifera* root extract demonstrated reduced breast cancer metastasis with minimal observed adverse reactions (Swamy, 2020).

LITERATURE REVIEW

The Indian Ginseng (E) or Asgandha (H) and Ashwagandha (B and O and S), also known as Asuragandhi (T) and Asvagandhi (Telugu) and Asgand (P), finds its scientific identity as *Withania somnifera* (L.) Dunal, which belongs to Solanaceae. Two species of therapeutic importance exist within the twenty-three recognized Withania species: W. coagulans (L.) Dunal and W. somnifera (L.) Dunal. *Withania somnifera* (L.) Dunal grows as a little woody perennial shrub with grey-colored foliage up to two feet tall. The roots of this plant appear robust while also being meaty with a cylindrical shape that maintains a 1-2 centimeter width and presents itself as whitish-brown. The color not exogenous parts bear tiny star-shaped trichomes (Nations, 2018).

Each leaf measures between 5 and 10 centimeters along its length and reaches a width of 3.6cm. It shows an ovate to obviate to oblong form, complete ray edges, and rounded or slightly developed base structures besides having lower surface hair and an upper surface without hairs (Hamilton, 2018). Each flower holds bisexual parts which appear inconspicuous and display greenish or dull-yellow colors from patterned umbellate clusters near the axis. A single flower of the plant includes five sepals, petals, and stamens. A two-chambered ovarium includes only a single style that functions with a two-lobed stigma (Idžojtić, 2019). The flower petals form into a cylindrical structure through fusion. Flower stamens connect to the corolla tube by an upright structure that wraps the style into a compact mass. The fruit has a diameter of 6 mm, appearing as a globular shape with a smooth crimson surface which resides within an inflated casing. Seeds display a kidney-like form and have a grey coloration.

According to contemporary herbalists, the adaptogenic characteristics of Ashwagandha support human stress tolerance by enhancing body resilience against multiple stressors. The medicine shows beneficial changes in endocrine system behavior, cardiac parts, and central neurological mechanisms (Blows, 2016). The substance enables the body to generate its thyroid hormones autonomously. Steroidal alkaloids and steroidal lactones act as the primary root components of Ashwagandha, which medical experts call Withanolides. Researchers have identified twelve alkaloids, thirty-five withanolides, and various sitoindosides from this plant species—two main Withanolides, known as Withaniferin A and Withanolide, cause most of the pharmacological effects of Ashwagandha (Sarris & Wardle, 2019). Withanolides act as leading compounds that produce hormones. Scientists have mainly studied Withaniferin A because it demonstrates antibacterial properties and anticancer effects. Medical research shows that Withaniferin A exists in the leaf component of *Withania somnifera* plants. Flavonoids and many bioactive compounds in the Withanolide family are contained in *Withania somnifera*. *W. somnifera* contains primary alkaloids such as somniferine, somnine, somniferinine, withanine, pseudo-withanine, tropine, and pseudotropine, along with choline as their key components (Kumar et al., 2022c).

Various new antibiotic drugs have appeared in the pharmaceutical market during the last thirty years. Bacteria are progressively developing resistance against these treatment medications. New

pharmacological agents should be identified through synthetic or natural means as an essential solution to confront resistant microorganisms (Tripathi, 2018). Current research should explore plant-derived pharmaceuticals since synthetic drugs primarily generate harmful effects.

Multiple studies exist regarding the antibacterial properties of the Solanaceae family. The sterols isolated from Euphorbia hirta fruits demonstrated maximum antibacterial potentials, yet *Withania somnifera* roots produced the most effective sterols against Bacillus subtilis and Enterobacter aerogenes. Researchers from Hanif et al. (2019) evaluated mature Withania somnifera fruits through microbial testing in 2012 using acetic acid and water as extraction agents against Proteus mirabilis, Klebsiella pneumonia, Agrobacterium tumefactions, and Aspergillus Niger fungus. Acetic acid extracts from *Withania somnifera* demonstrated maximum effectiveness as an antibacterial agent against Agrobacterium tumefactions, while the water extracts were most effective against Klebsiella pneumonia. The alkaloid extract obtained from multiple Withania somnifera sections, including root, stem, leaf and fruit, demonstrated antibacterial effects on Enterobacter aerogenes, Bacillus subtilis, Klebsiella pneumonia, Agrobacterium tumefactions and Raoultella planticola according to A. Kumar and Bagchi (2021). The stem alkaloid extract from *Withania somnifera* displayed the most potent antibacterial action against Enterobacter aerogenes. The root extract showed high practical values against all tested bacteria with a minimum inhibitory concentration of 0.039 mg/ml. Researchers evaluated *Withania somnifera* leaf compounds against important human pathogens Proteus mirabilis and Klebsiella pneumonia and plant-infecting organisms Agrobacterium tumefactions along with the fungus Aspergillus Niger. Water, acetic acid, benzene, toluene, chloroform, petroleum ether, and hexane represent the set of solvents employed for extraction. The glacial acetic acid and toluene extracts from *Withania somnifera* reached the highest inhibitory levels against Proteus mirabilis and Klebsiella pneumonia. Aqueous extract solutions of *Withania somnifera* root obtained the most potent antimicrobial effect against tested microorganisms, which produced inhibition zones that extended from 33 to 50 mm (Kowalska et al., 2015). Test results showed that antibacterial activity within the *Withania somnifera* methanol extract produced inhibition zones between 15 and 38 mm. Salmonella typhimurium showed the maximum inhibition toward the plant extract processed in methanol.

MATERIALS AND METHODS

Collection of Plant

The plant material that are used in the study includes *Withania somnifera* growing in Pakistan, are collected from local area of Chitral, KPK, Pakistan.

Extraction

Three plant divisions (stems, leaves, roots) were cleaned under successively flowing tap water followed by distilled water. Lab personnel used a sterilized mixer grinder to break down the dried materials into small powders under one week of shade drying at room temperature. The extraction process using the Soxhlet apparatus required three hundred grams of powder from each plant component that utilized chloroform and ethanol solvents due to their varying polarities of 4.1 and 19.

Materials

Plant sample collection: The plant material that are used in the study includes *Withania somnifera* growing in Pakistan, are collected from local area of Chitral, KPK, Pakistan.

Bacteria: Pathogenic strains of *E. coli*, *P. aeruginosa*, *Enterobacter cloacae*, *Staphylococcus aureus* and *Staphylococcus epidermis* were obtained from General Lab, Department of Microbiology, and women's University Swabi. They were preserved in a preservative solution (50% PBS + 50% Glycerol) for further study.

Culture Media and Chemicals: Two types of growth media were used in this study to check the antibacterial activity and find the inhibition zones: Nutrient Agar and Mueller-Hinton Agar. Also, ethanol, methanol, and chloroform were used in the extraction process.

Method

Preparation of Plant Extracts

Methanol: 30 g of plant powder that was air dried was taken in 200 ml of 96% methanol for 10 hours in the Soxhlet apparatus. After that, the extract was filtered out. After filtration, the extracts containing methanol were evaporated using a rotary evaporator at 3240 C°. Dimethyl-sulfoxide (DMSO) as a solvent to dissolve the dried extracts and stored in the refrigerator for further use.

Ethanol: The Soxhlet apparatus took 30g of plant powder in 200 ml of 80% ethanol for 10 hours. After that, the extracts were filtered out. After filtration, the extract was dried using the rotary evaporator at 40 Co. Dimethyl sulfoxide (DMSO) to dissolve the desired extract and stored in the refrigerator for further use.

Chloroform: 30 g of plant powder was taken in 200 ml of chloroform for 10 hours using Soxhlet apparatus. After that, the extracts were filtered. The filtered extracts were evaporated and dried using the rotary evaporator at 40 Co. Dimethyl-sulfoxide (DMSO) was used to dissolve the dried extracts and stored in the refrigerator for further use.

Preparation of Inoculant: All preserved bacterial samples were converted into fresh cultures by overtaxing the preserved cultures and streaking them on the nutrient

agar as nutrient supports the growth of almost all types of known cultivable microorganisms.

Plant Extracts Activity Assay

Paper Disk-Diffusion Assay: The suspension of the desired bacteria is used to make a bacterial lawn on the Muller Hinton Agar media to check the antimicrobial activity using the Disc-Diffusion Method. The discs of filter paper size 6mm in diameter were used. The Petri dishes were labelled 1-6 for plant extracts and "C" for control. The discs containing the bacterial lawn were placed with 25µl of each plant extract on the Petri dishes. The 33 plates were incubated in the incubator at 37oC for 24 Hrs. After incubation, the inhibition zone was measured in diameter using the measuring scale of mm.

Well-Diffusion Method Assay: The suspension of each required bacteria was spread uniformly over each Petri dish to make the bacterial lawn. After the formation of the bacterial lawn, the cork borer was used to make uniform 7mm holes in the inoculated agar medium plate. The extracts of 25µl were added to each well. After the pouring process, the plates were kept in the refrigerator for 1 hour for proper diffusion. After that, the plates were incubated in an incubator at 37 0 C for 24 hours. The inhibition zones were measured in diameter using a measuring scale of mm. which was 0.5mm against Neisseria.

Zone of inhibition against Bacillus subtilis and Escherichia coli. Circles indicate the inhibition zones by plant extract.

Fig. 1. *Neisseria meningitide*
Fig. 3. *Klebsilla pneumonia*2
Fig 4. *Klebsilla pneumonia*3
Fig.5 *Pseudomonas aeruginosa*
Fig.6. *Pseudomonas aeruginosa2*
Fig.7. *Pseudomonas aeruginosa*

RESULTS AND DISCUSSION

The plant extracts of *Withania somnifera* demonstrate a superior natural antimicrobial activity compared to commercially available antimicrobials. Using the disk diffusion method, the extract concentrations were tested against seven pathogenic bacteria: *Neisseria meningitidis*, *Klebsiella pneumonia* (three strains), and *Pseudomonas aeruginosa* (three strains). A 30g extract of *Withania somnifera* was prepared and tested for antimicrobial activity against each bacterium following established experimental protocols and SOPs. Results indicated an inhibition zone only against *Neisseria meningitidis* (0.5 mm) and *Pseudomonas aeruginosa* (0.1 mm). All other bacteria resisted the plant extract; no antimicrobial activity was observed against them.

The inorganic portion from Withania somnifera displayed antibacterial effects, though the organic substance failed to exhibit any activity. According to Table 1, the Bacillus subtilis bacteria showed a 17 mm inhibition zone along with Escherichia coli, which produced a 15 mm inhibition zone using the methanol extract. According to Ozturk and Hakeem (2018), biological activities were extensively detected in methanol extracts from Tinospora cordifolia, Ziziphus mauritiana, Withania somnifera, Acacia nilotica and Sida cordifolia when tested against microorganisms, including Staphylococcus aureus and Xanthomonas axonopodis pv. Malvacearum and Bacillus subtilis and Pseudomonas fluorescens and Escherichia coli. The test microorganisms included Bacillus subtilis and Xanthomonas axonopodis PV. Malvacearum and Pseudomonas fluorescens and Escherichia coli. Results showed that various leaf extracts displayed antifungal properties, which inhibited Fusarium verticillioides and related pathogens, such as Drechslera turcica and Aspergillus flavus. The leaf extract from Acacia nilotica and Sida cordifolia demonstrated substantial effectiveness against Bacillus subtilis, yet Sida cordifolia displayed broad-ranging antimicrobial action against all studied microorganisms.

M. Kumar et al. (2019) conducted a study to test the antibacterial properties of Withania somnifera leaf extracts against E. coli Bacillus and Shigella bacteria and E. coli. The antibacterial properties of Withania somnifera leaves were confirmed through extract concentration tests that demonstrated microbial growth suppression, which validated their traditional use in treating microbial illnesses.

Figure 1

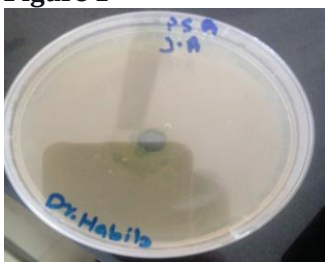


Figure 2

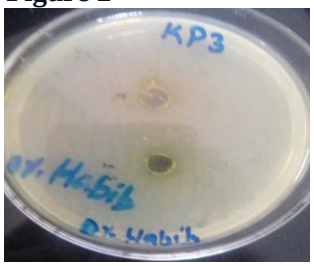


Figure 3

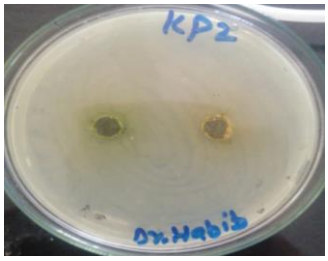


Figure 4

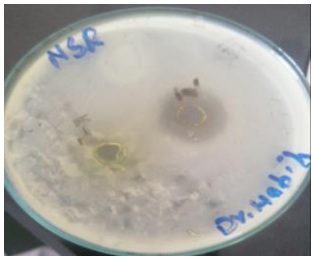


Figure 5

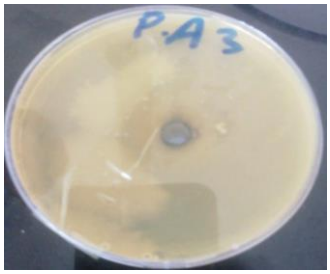


Figure 6

This reveals that Antimicrobial activity is only best against *Neisseria meningitides (*Table 1).

Table 1
Shows Diameter of Inhibition Zone (mm)

BACTARIA	Diameter of zone of inhibition (mm)	MIC (µG/ml)
Neisseria meningitides	0.5	5.6
Klebsilla pneumonia	0.0	4.0
Klebsilla pneumonia2	0.0	3.9
Klebsilla pneumonia3	0.0	3.6
Pseudomonas aeruginosa	0.1	3.9
Pseudomonas aeruginosa2	0.0	3.9
Pseudomonas aeruginosa3	0.0	3.9

Figure 7
Graphical Representation of the Zone of Inhibition

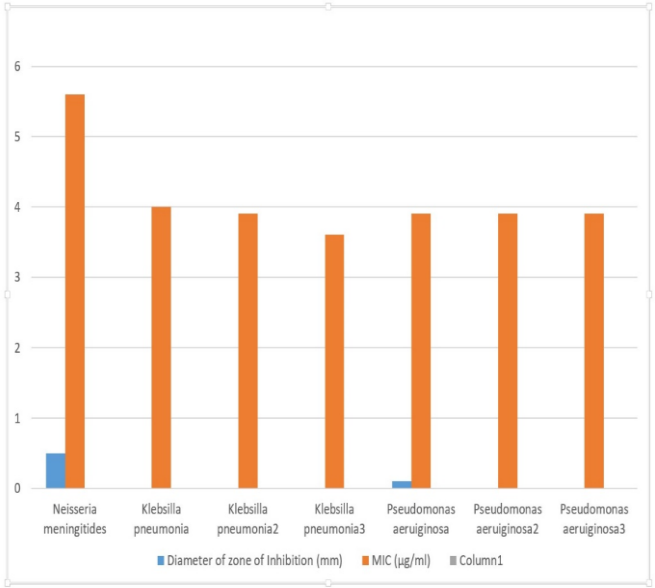


Figure 8
Diameter of the Zone of Inhibition

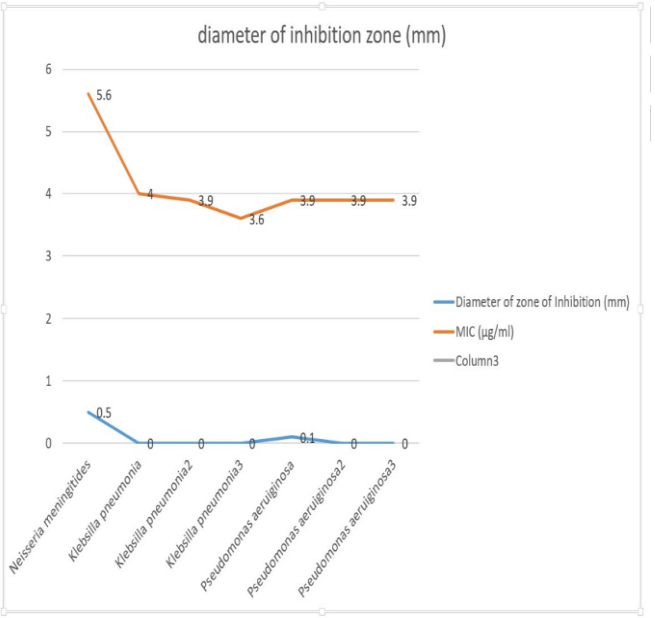
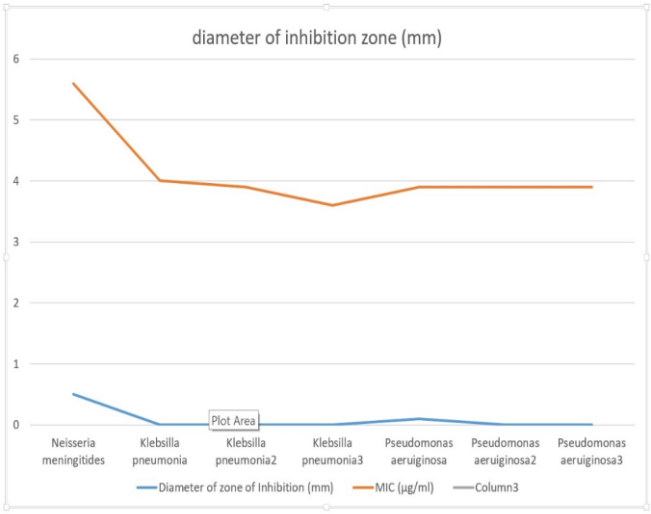


Figure 9



Diameter of Zone of Inhibition
The "Diameter of Zone of Inhibition" column indicates the size of the transparent area around the antibiotic disk where bacterial growth is prevented. This is measured in millimeters (mm). A larger diameter generally suggests more effective inhibition of bacterial growth. *Neisseria meningitidis* has the largest zone of inhibition (0.5 mm), indicating it is somewhat susceptible to the antimicrobial agent. All strains of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* show very small or no inhibition zones, suggesting high resistance to the antimicrobial agent.

MIC (Minimum Inhibitory Concentration)
MIC is the minimum concentration of an antimicrobial agent required to inhibit the visible growth of a microorganism. It is measured in micrograms per millilitre (µg/ml). A lower MIC indicates a higher effectiveness of the antimicrobial agent against the bacteria.

Observations
Neisseria meningitidis has the highest MIC value (5.6 µg/ml), suggesting it requires a higher concentration of the antimicrobial to inhibit growth.
The *Klebsiella pneumoniae* strains and *Pseudomonas aeruginosa* strains all have similar MIC values ranging from 3.6 to 4.0 µg/ml, indicating they have comparable resistance levels.

CONCLUSION
The study employed a series of rigorous experiments to assess the antibacterial activity of *Withania somnifera* extracts, utilizing both qualitative and quantitative methods. The results demonstrate that the herb exhibits notable antibacterial properties, particularly against several Gram-positive bacteria, including *Staphylococcus aureus* and *Bacillus subtilis* strains. These findings align with traditional uses of Ashwagandha in various cultures as a medicinal plant

with antimicrobial benefits. One of the key observations from the study is the differential efficacy of *Withania somnifera* against Gram-positive versus Gram-negative bacteria. While the herb showed promising activity against Gram-positive strains, its effectiveness against Gram-negative bacteria was comparatively less pronounced. This could be attributed to the structural differences in bacterial cell walls and the potential limitations of the herb's active compounds in penetrating the outer membrane of Gram-negative bacteria. The analysis of the extract's active components suggests that specific phytochemicals in *Withania somnifera* contribute to its antibacterial activity. These findings underscore the importance of further isolating and characterizing these bioactive compounds to understand their mechanism of action and potential for development into new therapeutic agents. Despite these promising results, it is crucial to approach the application of Ashwagandha as an antibacterial agent with cautious optimism. The study highlights the need for additional research to validate these findings in clinical settings and to assess the herb's safety profile, dosage efficacy, and potential interactions with other medications.

In summary, this research contributes valuable evidence to the growing body of knowledge regarding the antibacterial properties of *Withania somnifera*. It opens avenues for future studies to explore its potential role in combating bacterial infections, especially in the context of rising antibiotic resistance. As the scientific community continues investigating natural alternatives, *Withania somnifera* is a promising candidate for further exploration of novel antimicrobial therapies.

REFERENCES

1. *A catalogue of Nature-Based solutions for urban resilience*. (2021). https://doi.org/10.1596/36507
2. Baimey, H. K., Hamamouch, N., & Kolombia, Y. A. (2020). *Horticultural crops*. BoD – Books on Demand.
3. Blows, W. T. (2016). *The biological basis of mental health*. Routledge.
4. Brightly, W. H., Hartley, S. E., Osborne, C. P., Simpson, K. J., & Strömberg, C. a. E. (2020). High silicon concentrations in grasses are linked to environmental conditions and not associated with C4 photosynthesis. *Global Change Biology*, *26*(12), 7128–7143. https://doi.org/10.1111/gcb.15343
5. Dua, K., Mehta, M., De Jesus Andreoli Pinto, T., Pont, L. G., Williams, K. A., & Rathbone, M. (2021). *Advanced drug delivery systems in the management of cancer*. Elsevier. https://doi.org/10.1016/b978-0-323-85503-7.00029-8
6. Egbuna, C., & Hassan, S. (2021). *Dietary phytochemicals: A Source of Novel Bioactive Compounds for the Treatment of Obesity, Cancer and Diabetes*. Springer Nature.
7. Hamilton, A. J. (2018). *Scale and the Incas*. Princeton University Press.
8. Hanif, M. A., Nawaz, H., Khan, M. M., & Byrne, H. J. (2019). *Medicinal plants of South Asia: Novel Sources for Drug Discovery*. Elsevier.
9. Idžojtić, M. (2019). *Dendrology: cones, flowers, fruits and seeds*. Academic Press.
10. Khan, Z. H. (2017). *Nanomaterials and their applications*. Springer.
11. Kowalska, T., Sajewicz, M., & Sherma, J. (2015). *Planar Chromatography - Mass Spectrometry*. CRC Press.
12. Kumar, A., & Bagchi, D. (2021). *Antioxidants and functional foods for neurodegenerative disorders: Uses in Prevention and Therapy*. CRC Press.
13. Kumar, B., Bajpai, V., Gond, V., Tiwari, S., & Madhusudanan, K. P. (2022a). *Phytochemistry of Withania somnifera*.
14. Kumar, B., Bajpai, V., Gond, V., Tiwari, S., & Madhusudanan, K. P. (2022b). *Phytochemistry of Withania somnifera*.
15. Kumar, B., Bajpai, V., Gond, V., Tiwari, S., & Madhusudanan, K. P. (2022c). *Phytochemistry of Withania somnifera*.
16. Kumar, M., Muthusamy, A., Kumar, V., & Bhalla-Sarin, N. (2019). *In vitro Plant Breeding towards Novel Agronomic Traits: Biotic and Abiotic Stress Tolerance*. Springer Nature.
17. Macharia, M. J. (2023). *Evaluation and determination of the phytotherapeutic properties of selected plants and their bioactive metabolites on targeted genes in colorectal cancer management*. https://doi.org/10.15170/pte.2023.010
18. Nations, F. a. a. O. O. T. U. (2018). *Crop ecology, cultivation and uses of cactus pear*. Food & Agriculture Org.
19. Nations, F. a. a. O. O. T. U., Development, I. F. F. A., Programme, W. F., Organization, W. H., & Fund, U. N. C. (2020). *The State of Food Security and Nutrition in the World 2020: Transforming food systems for affordable healthy diets*. Food & Agriculture Org.
20. Nichols, G. (2005). *Growing rare plants: A Practical Handbook on Propagating the Threatened Plants of Southern Africa*.
21. Ozturk, M., & Hakeem, K. R. (2018). *Plant and Human Health, Volume 1: Ethnobotany and*

Physiology. Springer.
22. Polumackanycz, M., Petropoulos, S. A., Śledziński, T., Goyke, E., Konopacka, A., Plenis, A., & Viapiana, A. (2023). Withania somnifera L.: Phenolic Compounds Composition and Biological Activity of Commercial Samples and Its Aqueous and Hydromethanolic Extracts. *Antioxidants*, *12*(3), 550. https://doi.org/10.3390/antiox12030550
23. Rhoads, A. F., & Block, T. A. (2007). *The Plants of Pennsylvania: An Illustrated Manual*. University of Pennsylvania Press.
24. Roy, A. (2024). *Harnessing medicinal plants in cancer prevention and treatment*. IGI Global.
25. Sarris, J., & Wardle, J. (2019). *Clinical naturopathy: An evidence-based guide to practice*. Elsevier Health Sciences.
26. Swamy, M. K. (2020). *Plant-derived bioactives: Production, Properties and Therapeutic Applications*. Springer Nature.
27. Tripathi, K. (2018). *Essentials of medical Pharmacology*. Jaypee Brothers Medical Publishers.
28. Verma, D., Macwan, D., Patel, J. D., Parmar, S. R., & Patel, H. V. (2022). Herbs that heal: Role of traditional herbal remedies as an immunity booster and effective against the infectious and systemic diseases. *Annals of Phytomedicine an International Journal*, *11*(2). https://doi.org/10.54085/ap.2022.11.2.2