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Research Article

COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTIVITY OF ROOT AND LEAF EXTRACTS OF WITHANIA SOMNIFERA *(ASHWAGANDHA)* AND ASPARAGUS RACEMOSUS *(SHATAVARI)*

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ABSTRACT

Withania somnifera (*Ashwagandha*) and *Asparagus racemosus* (*Shatavari*) are well-known Ayurvedic herbs traditionally used for immune modulation and rejuvenation. This study aims to evaluate and compare the antimicrobial efficacy of their root and leaf extracts against selected pathogenic microorganisms. **Methods**: Hydroalcoholic extracts of the root and leaf of *W. somnifera* and *A. racemosus* were prepared using Soxhlet extraction. The antimicrobial activity was tested against certain Gram-positive and Gram-negative bacterial strains, along with selected fungal strain, using the agar well diffusion method. A vehicle control was included to rule out solvent effects. Gentamycin and clotrimazole were used as positive control. **Results:** The leaf extract of *W. somnifera* exhibited significant antimicrobial activity, showing measurable zones of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa* comparable to standard positive controls. The root extract of *W. somnifera* showed moderate activity. The vehicle control showed no activity, confirming the role of active phyto-constituents in microbial inhibition. No antimicrobial activity was observed for the root and leaf extracts of *A. racemosus* against the tested strains. Several strains also showed resistance, suggesting a degree of specificity in the antimicrobial action of *W. somnifera*. **Conclusion**: The findings establish that *W. somnifera*, particularly its leaf extract, possesses selective and effective antimicrobial properties against both Gram-positive and Gram-negative pathogens. This supports its traditional use and highlights its potential as a source for plant-based antimicrobial agents. Conversely, *A. racemosus* did not show any antimicrobial activity under the tested conditions, indicating limited direct antimicrobial potential.

INTRODUCTION

Medicinal plants have long been recognized as rich sources of bioactive compounds with significant therapeutic potential. In recent years, the plant-based therapeutics have gained considerable attention in antimicrobial research for the treatment of infectious diseases, primarily due to the rising resistance to conventional antibiotics and their associated systemic side effects. *Asparagus racemosus* (*Shatavari*) and *Withania somnifera* (*Ashwagandha*) are considered *Rasayana* in Ayurveda- a category of rejuvenating and longevity-promoting agents that enhance strength, vitality, and immunity.[1-4] Both plants are commonly used in their root form, yet their leaves are also reported to possess significant pharmacological activities, including antimicrobial effects.[5.6]

Asparagus racemosus, belonging to the family Asparagaceae, is known for its rich content of steroidal saponins (notably shatavarins), flavonoids, and polyphenols, particularly in its roots. These phytochemicals have shown promising antibacterial and antifungal properties in various in vitro studies. Similarly, *Withania somnifera*, from the family Solanaceae, contains withanolides, alkaloids, and flavonoids, which are found in both roots and leaves.

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These compounds have demonstrated significant biological activity against a range of microbial species.[7-10]

While both species have been individually studied for their antimicrobial potential, the comprehensive evaluations that include both the root and leaf parts under standardized conditions are scarce. Moreover, limited studies are available on the antimicrobial efficacy of hydro-alcoholic extracts of these herbs.

This study aims to bridge this gap by systematically comparing the antimicrobial effects of root and leaf extracts of *A. racemosus* and *W. somnifera.* Using standardized extraction and testing methods, the study will assess their activity against selected bacterial and fungal strains, thereby identifying the most effective plant part and species for antimicrobial formulation. Such comparative analysis not only deepens the scientific understanding of these traditional Ayurvedic herbs but also supports the evidence-based application of their plant extracts in the prevention and management of infectious diseases.

MATERIALS AND METHODS

1. **Plant Material Authentication and Processing**
   Fresh leaves of *Asparagus racemosus* and *Withania somnifera* were collected from the herbal garden of the All India Institute of Ayurveda and their roots were indented from the pharmacy of same institute. The plant materials were authenticated in the pharmacognosy laboratory of the institute by a qualified taxonomist. Samples were washed with distilled water, tray-dried at 35–40°C, and pulverized into coarse powder and stored in sealed polyethylene bags within airtight containers at room temperature until extraction.
2. **Microbial strains and culture conditions**
   Bacterial and fungal strains were procured from the Institute of Microbial Technology (MTCC–IMTECH), Chandigarh, India. The strains used were *Staphylococcus aureus* (MTCC 737), *Escherichia coli* (MTCC 1687), *Pseudomonas aeruginosa* (MTCC 1688), *Klebsiella pneumoniae* (MTCC 9544), *Salmonella enterica Typhi murium* (MTCC 1251), and *Candida albicans* (MTCC 183). All microbial strains were periodically sub-cultured on their respective media to maintain the viability.
3. **Preparation of plant extracts and stock solution**
   Hydro-alcoholic extracts were prepared using methanol and distilled water (50:50, v/v) by Soxhlet extraction at a drug-to-solvent ratio of 1:10 (w/v) for 6–8 h, until the siphon solvent becomes colorless. The hydro-alcoholic solvent system was selected due to its ability to dissolve a broad spectrum of phytoconstituents, including alkaloids, flavonoids, and terpenoids, thereby ensuring maximal recovery of both polar and moderately non-polar compounds. The extracts were subsequently concentrated at ≤50°C in a hot air oven to prevent degradation of thermolabile constituents. The dried extracts were weighed, labelled, and stored in amber-coloured glass containers at −20°C until further analysis. The extracts were screened for any microbial contamination in the microbiology laboratory and were confirmed to be sterile before use.
   The stock solution of each extract was prepared at the concentration of 100mg/ml using their respective extraction solvent and stored at 4°C until use in antimicrobial testing.
4. **Microbial Culture and Inoculum Preparation**
   Lyophilized cultures were revived aseptically in a sterile nutrient broth (for bacteria) or a Sabouraud dextrose broth (for fungi) and incubated at 37°C for 18–24 h (bacteria) and 24–48 h (fungi).
   Revived cultures were streaked onto selective and differential media specific for each organism: MacConkey agar for *E. coli*, Cetrimide agar for *P. aeruginosa*, Hichrome UTI agar for *K. pneumoniae*, Blood agar for *S. aureus,* Salmonella- Shigella (SS) agar for *Salmonella,* and Sabouraud Dextrose Agar (SDA) for *C. albicans*. Plates were incubated at 37°C for 24–48 hours in a BOD incubator. A loopful of fresh colonies was suspended in 1ml of sterile normal saline and incubated at 37°C for 2–4 hours until visible turbidity developed, to obtain a standardized inoculum.
5. **Antimicrobial Activity Assessment**
   The antimicrobial activity of plant extracts was evaluated using the agar well diffusion method. Mueller–Hinton Agar (MHA) plates were prepared according to the manufacturer's instructions, and were uniformly inoculated with the standardized microbial suspension using a sterile cotton swab to obtain a confluent lawn culture. Wells of 6mm diameter were aseptically punched into the agar using sterile pipette tips, and the plates were appropriately labelled. Each well was loaded with 50µL of the respective plant extract (100mg/mL). Gentamicin (10µg/disc) was used as a positive control for bacterial strains, and clotrimazole (10 µg/disc) served as a positive control for the fungal strain. The extraction solvent (hydro-alcohol; 50:50) was used as a negative vehicle control. All plates were incubated at 37°C for 18–24 hours for bacteria and 24–48 hours for fungi. After incubation, the antimicrobial activity was assessed by measuring the diameter of the clear zone of inhibition around each well in millimetres (mm). All experiments were performed in triplicate, and

results were expressed as mean ± standard deviation.

RESULTS

The anti-microbial activity of root and leaf extracts of *Withania somnifera* and *Asparagus racemosus* was evaluated using the agar well diffusion method. The activity was assessed by measuring the diameter of the zone of inhibition (mm). The results are expressed as mean ± standard deviation (SD) at a 95% confidence level and are presented in Table 1.

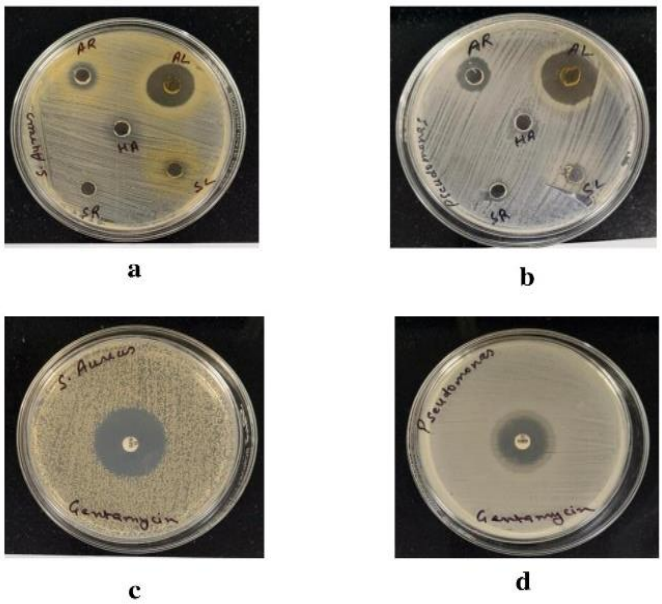
Both root and leaf extracts of *Withania somnifera* exhibited antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with the leaf extract showing significantly stronger inhibition than the root extract ($p<0.05$). No zone of inhibition was observed for the negative control, confirming that the activity of *W. somnifera* extracts was statistically significant ($p<0.05$). The remaining bacterial and the fungal strains were resistant under the tested conditions.

In contrast, neither root nor leaf extracts of *Asparagus racemosus* exhibited antimicrobial activity against any of the tested bacterial or fungal strains. Representative zones of inhibition were observed for *Withania somnifera* extracts against susceptible strains as shown in Figure 1.

Table 1: Antimicrobial activity of root and leaf extracts of *Withania somnifera* and *Asparagus racemosus* against selected pathogenic strains (Zone of inhibition, mm; mean ± SD, n = 3)

	Zone of inhibition (mm)					
S.No	***S.aureus***	***E. Coli***	***Pseudomonas***	***Klebsella***	***Candida***	***Salmonella***
AR	10.67 ±0.65	R	14.33±0.65	R	R	R
AL	20.33 ±0.65	R	21.67±0.65	R	R	R
SR	R	R	R	R	R	R
SL	R	R	R	R	R	R
HA	R	R	R	R	R	R
GM	24.67±0.65	19.33 ±1.31	20 ±0.00	18.67 ±1.31	NA	21.33±1.31
CC	NA	NA	NA	NA	43 ±1.96	NA

Note: AR- *Ashwaghandha* root, AL- *Ashwaghandha* leaf, SR- *Shatavari* root, SL -*Shatavari* leaf
HA- Hydro-alcohol (vehicle negative control), GM- Gentamycin, CC-Clotrimazole (positive control)



a b c d

FIGURE 1: **Representative zones of inhibition demonstrating notable antimicrobial activity of *Withania somnifera* root and leaf extracts against susceptible strains**

(a) Antimicrobial activity of *Withania somnifera* extracts against *Staphylococcus aureus*
(b) Antimicrobial activity of *Withania somnifera* extracts against *Pseudomonas aeruginosa*
(c) Antimicrobial activity of Positive control (Gentamycin) against *Staphylococcus aureus*
(d) Antimicrobial activity of Positive control (Gentamycin) against *Pseudomonas aeruginosa*

DISCUSSION

The present study demonstrated that both leaf and the root extracts of *Withania somnifera* exhibited antimicrobial activity, specifically against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, with the leaf extract showing a stronger inhibitory effect. Notably, the zone of inhibition produced by the leaf extract was comparable to that of gentamicin, the standard positive control, indicating a potent antibacterial potential. In contrast, the vehicle control showed no activity, confirming that the observed antimicrobial effects were attributable to the active phytoconstituents of the extracts rather than the solvent.

The greater efficacy of the leaf extract compared to the root extract may be attributed to a rich profile of withanolides, alkaloids, flavonoids, and phenolic compounds, which have been previously associated with antimicrobial and anti-inflammatory activities.[11-12] These secondary metabolites may exert their effects through mechanisms such as disrupting bacterial cell membranes or interfering with essential metabolic enzymes.[13]

The observation that only *Pseudomonas aeruginosa* and *Staphylococcus aureus* were susceptible to the *Withania somnifera* extracts indicates a selective antimicrobial spectrum. *P. aeruginosa* is a Gram-negative bacterium with intrinsic resistance mechanisms, including efflux pumps and a low-permeability outer membrane, making it challenging to inhibit with conventional agents.[14] The ability of *W. somnifera* extracts to inhibit its growth suggests that the specific phytoconstituents may target pathways or structures unique to these bacteria. Similarly, *S. aureus*, a Gram-positive pathogen commonly associated with skin and soft tissue infections, is highly adaptable and has developed various resistance mechanisms due to the widespread use of antibiotics,[15] was found to be susceptible to the extracts in the study. The selective sensitivity may be attributed to the differences in cell wall composition, membrane permeability, and the presence of target molecules that interact effectively with the bioactive compounds in the extracts.

On the other hand, the lack of activity against *Escherichia coli, Klebsiella pneumoniae, Salmonella enterica,* and *Candida albicans* suggests either intrinsic resistance or insufficient concentrations of active phytochemicals in the extracts to inhibit these strains. Gram-negative bacteria, in particular, possess outer membrane barriers that can limit the penetration of many plant-derived compounds.[14] Additionally, resistance in other bacterial and fungal strains may result from variations in cell wall structure, efflux mechanisms, biofilm formation, impermeable membranes, or the presence of resistance genes, rendering them less susceptible to the phytochemicals present in *W. somnifera*. [16,17]

Interestingly, neither leaf nor root extracts of *Asparagus racemosus* exhibited antimicrobial activity against any of the tested organisms under the conditions and concentrations used in this study, indicating limited direct inhibitory or bactericidal effects. Despite its well-documented adaptogenic, immunomodulatory, and anti-inflammatory properties,[18] the antimicrobial potential of *A. racemosus* appears to be strain-specific or indirect. Its therapeutic effects may instead be mediated through modulation of host immune responses, enhancing the body's ability to combat infections rather than directly inhibiting microbial growth.

These findings are consistent with previous reports supporting the antibacterial properties of *W. somnifera*[19-20] and additionally provide comparative evidence of its superior antimicrobial activity relative to *Asparagus racemosus*. Moreover, these observations highlight the need to further study the targeted signalling pathways of *W.Somnifera* in the above susceptible bacterial strains, the resistance mechanism of non-susceptible bacterial strains, biofilm inhibition assays, studies on different assay models that better mimic host-pathogen interactions, ex vivo tissue models, or in vivo studies. Ultimately, such effects would require evaluation in animal or clinical studies to fully assess the interplay between the extract, host immune system, and pathogens.

It is also important to acknowledge the variability in plant-based antimicrobial efficacy due to factors such as plant part used, extraction method, solvent polarity, and microbial strain specificity. In this study, extracts were standardized to a uniform concentration (100mg/ml), but the actual concentration of active constituents may differ between leaf and root preparations. Furthermore, the agar well diffusion method is qualitative and influenced by compound diffusion rates in the medium; therefore, further quantitative evaluations, including minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and mechanistic studies, would be valuable to comprehensively assess their antimicrobial potential.

The antimicrobial activity demonstrated by both root and leaf extracts of *Withania somnifera* aligns with their therapeutic use in classical Ayurvedic literature.[21] In Ayurvedic texts, the root is prominently classified as a rejuvenative (*Rasayana)* and strength-promoting (*Balya*), traditionally valued for enhancing *Ojas,*[1-4] promoting systemic immunity,

and increasing resistance against recurrent infections. These effects reflect host-targeted or systemic modes of action, supporting overall immune modulation rather than direct microbial killing. The root is also described for antimicrobial (*Krimighna*), anti-inflammatory (*Shothahara*), detoxifying (*Vishahara*), and wound-healing (*Vranahara*) properties.[22] These effects may contribute directly through *Krimighna* (antimicrobial) action, and indirectly to infection control by enhancing host defences and promoting tissue repair. In contrast, the leaf, though less commonly utilized internally, is emphasized in classical texts for external applications, particularly in conditions like *Visarpa* which is described as a rapidly spreading skin disorder often compared to cellulitis or erysipelas., where it is prescribed with *Gomutra*.[23] In these applications, the therapeutic effects may include direct antimicrobial activity at the site of application, along with anti-inflammatory and detoxifying actions, complementing host defence mechanisms locally.

The integration of classical Ayurvedic insights with modern experimental evidence strongly supports the rationale for exploring *Withania somnifera* in managing microbial infections through both systemic and topical approaches. The findings validate its traditional use in treating infections and indicate that the leaf may be a more effective source for developing antimicrobial formulations. These results also underscore the need for further phytochemical profiling and bioassay-guided fractionation to isolate and characterize the active constituents responsible for the observed antimicrobial activity. Notably, unlike many previous studies focusing on root extracts, our study highlights the superior potency of its leaf extract, which may provide a more sustainable and readily available source of bioactive compounds compared to the resource-intensive harvesting of roots.

CONCLUSION

The study demonstrates the selective antimicrobial potential of *Withania somnifera*, with its leaf extract showing significant activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, highlighting its therapeutic value as a source of plant-based antimicrobials. In contrast, *Asparagus racemosus* root and leaf extracts showed no detectable activity under the tested conditions, indicating limited or strain-specific efficacy. Further studies are needed to isolate, study the active phytoconstituents, and their respective targeted signalling pathways, to optimize extraction methods, validate clinical potential, and assess antimicrobial activity against a broader spectrum of microbial pathogens.

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