Kabarak Journal of Research & Innovation

https://journals.kabarak.ac.ke/index.php/kjri/

RESEARCH ARTICLE

Phytochemical Profiling and Antimicrobial Efficacy of *Withania somnifera* and *Allium vineale* against *Escherichia coli* and *Staphylococcus aureus*

Carolyne Chepkirui*[1], Micah Lagat[2], & Jackline Kirui[2]

1. Department of Preclinical & Pharmacognosy, Kabarak University
2. Department of Physical & Biological sciences, Kabarak University

Correspondence: carochep2005@gmail.com

Submitted 24th March, 2025, Accepted 14th May, 2025 and Published 15th October 2025

ABSTRACT

The emergence of antimicrobial resistance necessitates the exploration of plant-derived alternatives with therapeutic potential. This study evaluates the antimicrobial efficacy and phytochemical composition of *Withania somnifera* (Ashwagandha) and *Allium vineale* (Wild Onion), two ethnomedicinal plants traditionally used for infectious disease management. Methanolic extracts of both plants were tested against *Escherichia coli* and *Staphylococcus aureus* using disc diffusion and minimum inhibitory concentration (MIC) assays. Phytochemical profiling was conducted via standardized qualitative methods to identify bioactive constituents. Ashwagandha exhibited broad-spectrum activity, with inhibition zones of 9.52 ± 0.04 mm (*E. coli*) and 10.84 ± 0.04 mm (*S. aureus*) at 1g/ml, while Wild Onion showed preferential potency against *S. aureus* (MIC: 0.25g/ml vs. 0.5g/ml for *E. coli*). Phytochemical analysis revealed high concentrations of tannins, flavonoids, and alkaloids in Ashwagandha, correlating with its robust antimicrobial effects. Wild Onion, rich in sulfur compounds and flavonoids, demonstrated targeted efficacy against Gram-positive pathogens, likely due to membrane-disruptive mechanisms. Sterile water controls (5.0 mm inhibition) confirmed solvent neutrality, and positive controls (Erythromycin: 24.16 mm; Gentamicin: 25.34 mm) validated assay reliability. These findings underscore the potential of Ashwagandha and Wild Onion as sustainable alternatives to synthetic antibiotics, particularly in combating multidrug-resistant *S. aureus*. The study bridges traditional knowledge and modern pharmacology by linking phytochemical diversity to antimicrobial function. Future research should focus on isolating active compounds, optimizing extraction protocols, and evaluating in vivo efficacy to advance plant-based therapeutics in clinical settings.

Keywords: Antimicrobial resistance, *Withania somnifera*, *Allium vineale*, Phytochemicals, Gram-positive Pathogens, Natural Therapeutics

INTRODUCTION

The rise of antimicrobial resistance (AMR) presents a critical challenge to global health, as it reduces the effectiveness of existing treatments and complicates infection management (Aljeldah,

2022; Salam et al., 2023; Tang et al., 2023). This crisis is particularly concerning due to the high mortality associated with resistant bacterial infections (Talebi Bezmin Abadi et al., 2019). Two pathogens, *Escherichia coli* and *Staphylococcus aureus*, significantly contribute to this burden (Gagliotti et al., 2011). Globally, *E. coli* is responsible for an estimated 2.8 million infections and 1.3 million deaths annually, while *S. aureus*, including methicillin-resistant strains (MRSA), contributes to approximately 1.5 million infections and 500,000 deaths each year (Murray et al., 2022; OECD, 2018). In Africa, the impact of these pathogens is also severe, with *E. coli* and *S. aureus* being leading causes of morbidity and mortality particular in young children and infants (Bagamian et al., 2020; Mduma et al., 2022). *E. coli* infections alone result in about 1.1 million deaths annually across the continent (Croxen et al., 2013) (WHO, 2021). *Staphylococcus aureus* infections, including MRSA, contribute to an estimated 200,000 deaths per year in Africa (Irek et al., 2018). Kenya faces a significant health burden from these pathogens, as *E. coli* is a major cause of both hospital-acquired and community-acquired infections, while S. aureus remains a key concern in both medical and community settings (Berkley et al., 2005; Omwenga et al., 2022). Therefore, there is an urgent need to discover and develop new antimicrobial agents.

Medicinal plants, with their diverse array of bioactive compounds; flavonoids, alkaloids and terpenes represent a promising source for novel antimicrobials with membrane disruption being their most common mechanism (Álvarez-Martínez et al., 2021; Radulovic et al., 2013). *Allium vineale*, a perennial herbaceous plant belonging to the Amaryllidaceae family, is renowned for its slender, grass-like. In traditional medicine, *Allium* species have been utilized for its remarkable antimicrobial, anti-inflammatory, antioxidant, and digestive properties (Alam et al., 2023; Forma et al., 2021; Štajner et al., 2006). The primary antimicrobial activity is attributed to compounds such as allicin, thiosulfinates, and their transformation products, which impede microbial growth by reacting with sulfhydryl groups found in cellular proteins (Kyung KyuHang, 2012). The methanolic extracts of *A. vineale* displayed antibacterial activity against various gram-positive and gram-negative bacteria (Durmaz et al., 2006). *Withania somnifera* (Ashwagandha), is a prominent herb in Ayurvedic medicine (Mukherjee et al., 2021), renowned for its diverse pharmacological properties including anti-inflammatory(Chandra et al., 2012; Srivastav & Das, 2014), antioxidant (Srivastav & Das, 2014) and anticancer (Mazurkiewicz et al., 2024; Mehta et al., 2021) properties particularly due to the presence of withanolides like withaferin-A and withanolide-D.

METHODOLOGY

Collection and Identification of the Plant

Wild onion leaves were collected from Nakuru county, Rongai sub-county and taken to Kabarak University Pharmacognosy Laboratory where identification and authentication by a taxonomist was made and the voucher specimen was archived at the pharmacognosy lab, Kabarak University.

Extraction Process

The collected wild onion plant samples were dried at normal room temperature in the laboratory and thereafter ground using an electric mill. Methanolic extraction was carried out according to

methods described by Nimbeshaho et al. (2020), with modifications. Using an electric analytical beam balance, 1000 grams of powdered dried plant part will be weighed.

At each time, 42.25 g was placed in soxhlet apparatus wrapped in a filter paper and distilled with absolute methanol. The extracted samples were mixed in a conical flask then placed in a rotary vacuum evaporator in a water bath at 40^{o}C to recover the solvent (methanol) and concentrate the crude extract. The semi-solid extract was placed in sterile beaker and left in the laminar flow hood for 24 hours for complete evaporation of the solvent. The total yield of the solid crude extract was weighed and put in tightly screwed capped glass containers and stored in the refrigerator at 40^{o}C prior to use for biological assays.

Methodology for Phytochemical Assays

The phytochemical screening of *Withania somnifera* (Ashwagandha) and *Allium vineale* (Wild Onion) methanolic extracts was conducted using standardized qualitative protocols to identify bioactive constituents. Dried leaves of both plants were ground to a fine powder and macerated in 70% methanol (1:4 w/v) for 72 hours at room temperature with periodic agitation. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator. The dried extracts were stored at 4°C until analysis.

Phytochemical tests were performed to detect major classes of compounds, including saponins, tannins, phenolics, flavonoids, carbohydrates, proteins, alkaloids, cardiac glycosides, anthraquinones, sterols, and triterpenoids. Established methods were employed: the Froth Test for saponins (Lawal et al., 2019), Ferric Chloride and Lead Acetate tests for tannins (Abayomi, 1993), and Shinoda, Sulphuric Acid, Alkaline, and Lead Acetate tests for flavonoids. Carbohydrates were identified via Fehling's and Benedict's tests, while proteins were assessed using the Biuret reaction. Alkaloids were detected using Mayer's, Wagner's, Dragendorff's, and Hager's reagents. Cardiac glycosides were evaluated with the Keller-Killiani and Kedde tests, and sterols/triterpenoids with Lieberman-Burchard and Salkowski tests (Lawal et al., 2019).

Positive controls such as quercetin for flavonoids, atropine for alkaloids and negative controls (distilled water) were included to validate assay specificity. Reactions were scored based on intensity (+++, ++, +) or absence (-) of observable changes, with triplicate measurements ensuring consistency. All procedures adhered to published guidelines to ensure reproducibility and minimize experimental bias.

Table 1:

Plant Materials and Phytochemical Analysis

Phytochemicals	**Test procedure**	**Expected observation**
Alkaloids	2 ml + Mayer's reagent	Yellow colored precipitate
Carbohydrates	2 ml + Naphthol + Sulphuric acid	Violet color

Glycosides	5 ml extract + 5 ml water shake	Foam produced
Phytosterols	2 ml extract + 2 ml $CHCl_3$+2 ml H_2S0_4	Golden yellow color
Flavonoids	2 ml extract + a few drops of NaOH	Yellow color that clears on adding dilute HCL
Phenol and Tannins	Extract + 4 drops of $FeCl_3$	Blue-black coloration

RESULTS

Antimicrobial Assay

The antimicrobial activity of *Withania somnifera* (Ashwagandha) and *Allium vineale* (Wild Onion) extracts was evaluated using the disc diffusion method against *Escherichia coli* and *Staphylococcus aureus*. The results were analyzed using two-way ANOVA to assess the effects of treatment and concentration on the zone of inhibition.

Table 2:

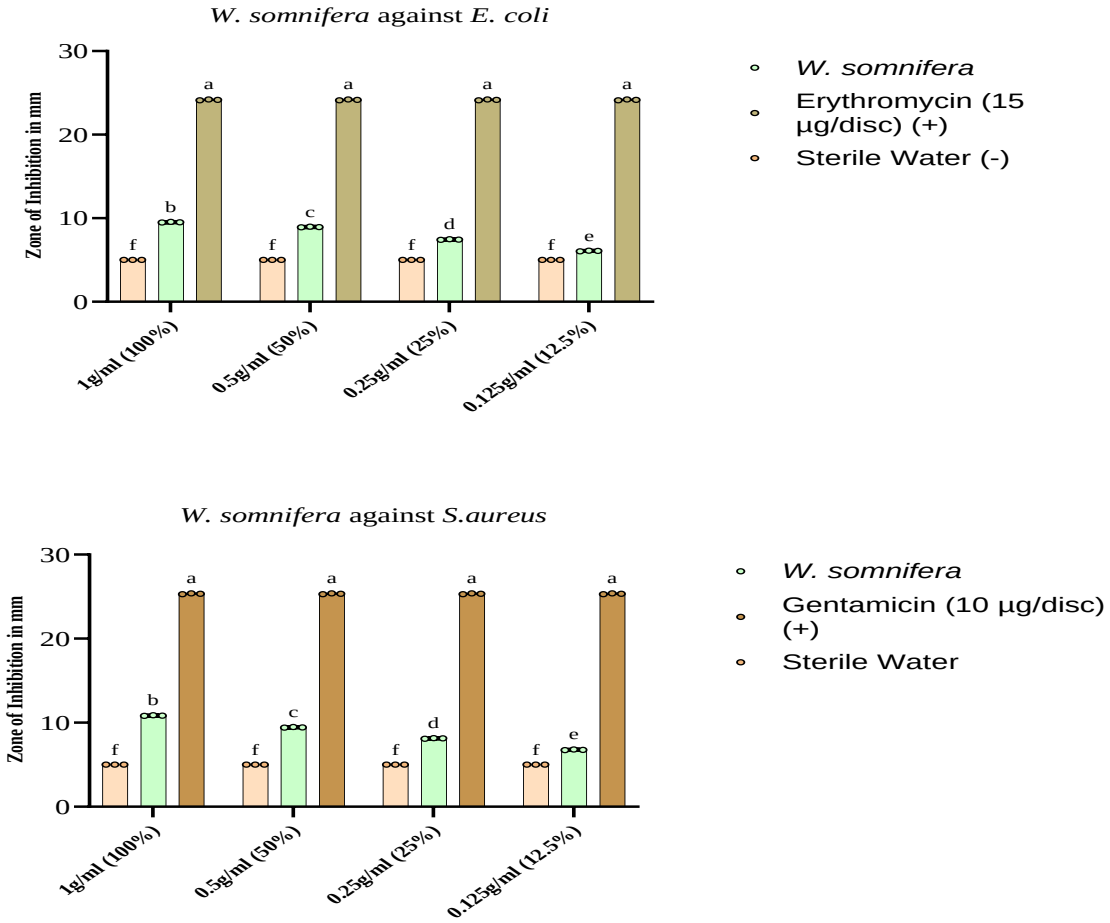
Ashwagandha Leaf-extract Against E. coli

Extract Concentration	Zone of Inhibition (mm) Replicate 1	Zone of Inhibition (mm) Replicate 2	Zone of Inhibition (mm) Replicate 3
1g/ml (100%)	9.52	9.48	9.55
0.5g/ml (50%)	8.95	8.91	8.98
0.25g/ml (25%)	7.44	7.40	7.49
0.125g/ml (12.5%)	6.09	6.05	6.12

Ashwagandha extract demonstrated significant antimicrobial activity, with inhibition zones ranging from 9.52 ± 0.04 mm at 1g/ml (100%) to 6.09 ± 0.04 mm at 0.125g/ml (12.5%) (Table 2). Two-way ANOVA revealed a significant effect of concentration ($p < 0.05$), with higher concentrations yielding larger inhibition zones. The positive control, Erythromycin (15 µg/disc), showed a mean inhibition zone of 24.16 ± 0.06 mm, while the negative control (sterile water) showed no activity (5.0 mm) (Figure 1).

Figure 1:

Two-way ANOVA Analysis of Ashwagandha (W. somnifera) Extract Against E. coli and S. aureus. Treatments Include Ashwagandha Extract, Erythromycin (Positive Control), and Sterile Water (Negative Control)



The extract exhibited stronger activity against *S. aureus*, with inhibition zones ranging from 10.84 ± 0.04 mm at 1g/ml to 6.77 ± 0.04 mm at 0.125g/ml (Table 3). Two-way ANOVA confirmed a significant concentration-dependent effect ($p < 0.05$). The positive control, Gentamicin (10 µg/disc), produced a mean inhibition zone of 25.34 ± 0.06 mm, while the negative control showed no activity (5.0 mm) (Figure 1).

Table 3:

Ashwagandha Leaf-extract Against S. aureus

Extract Concentration	Zone of Inhibition (mm) Replicate 1	Zone of Inhibition (mm) Replicate 2	Zone of Inhibition (mm) Replicate 3

1g/ml (100%)	10.84	10.80	10.88
0.5g/ml (50%)	9.45	9.41	9.49
0.25g/ml (25%)	8.13	8.10	8.17
0.125g/ml (12.5%)	6.77	6.73	6.80

Wild Onion (*A. vineale*) Leaf Extract

Wild onion extract showed moderate antimicrobial activity, with inhibition zones ranging from 7.69 ± 0.04 mm at 1g/ml to 5.25 ± 0.04 mm at 0.125g/ml (Table 4). Two-way ANOVA indicated a significant effect of concentration ($p < 0.05$). The positive control, Erythromycin (15 µg/disc), yielded a mean inhibition zone of 24.16 ± 0.06 mm, while the negative control showed no activity (5.0 mm) (Figure 2).

Table 4*:*

Wild Onion Extract on E. coli

Extract Concentration	Zone of Inhibition (mm) Replicate 1	Zone of Inhibition (mm) Replicate 2	Zone of Inhibition (mm) Replicate 3
1g/ml (100%)	7.69	7.65	7.72
0.5g/ml (50%)	7.18	7.15	7.21
0.25g/ml (25%)	6.56	6.52	6.59
0.125g/ml (12.5%)	5.25	5.21	5.28

Enhanced activity was observed against *S. aureus*, with inhibition zones ranging from 9.58 ± 0.04 mm at 1g/ml to 5.34 ± 0.04 mm at 0.125g/ml (Table 5). Two-way ANOVA confirmed a significant concentration-dependent effect ($p < 0.05$). The positive control, Gentamicin (10 µg/disc), produced a mean inhibition zone of 25.34 ± 0.06 mm, while the negative control showed no activity (5.0 mm) (Figure 2).

Table 5:

Wild Onion Extract on S. aureus

Extract Concentration	Zone of Inhibition (mm) Replicate 1	Zone of Inhibition (mm) Replicate 2	Zone of Inhibition (mm) Replicate 3
1g/ml (100%)	9.58	9.55	9.62

0.5g/ml (50%)	8.32	8.29	8.35
0.25g/ml (25%)	7.10	7.06	7.13
0.125g/ml (12.5%)	5.34	5.30	5.37

Figure 2:

Two-way ANOVA Analysis of Wild Onion (A. vineale) Extract Against E. coli and S. aureus. Treatments Include Wild Onion Extract, Gentamicin (Positive Control), and Sterile Water (Negative Control)

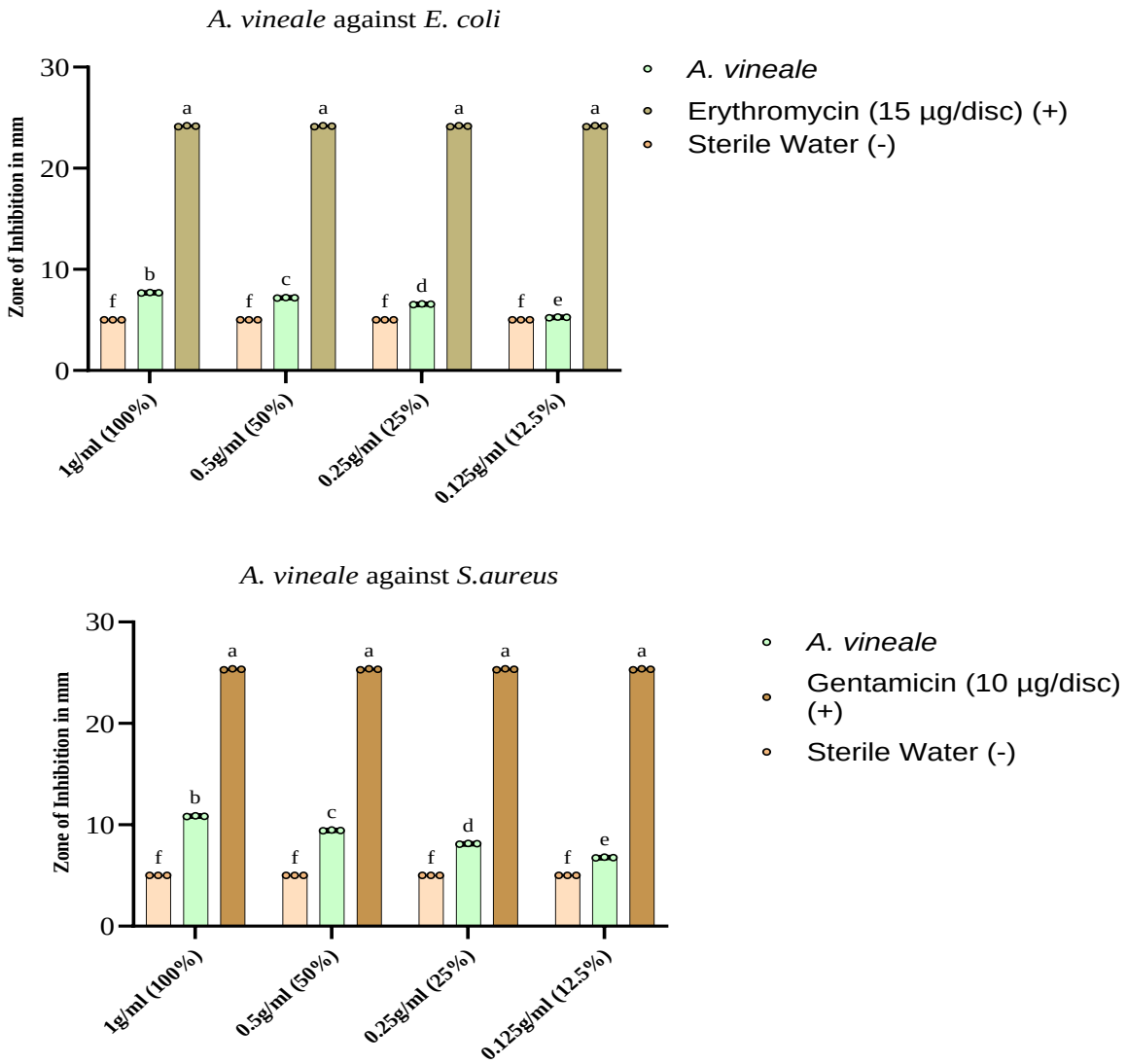


Table 6:

Negative Control with Replicates

Organism	Control	Zone of Inhibition (mm) Replicate 1	Zone of Inhibition (mm) Replicate 2	Zone of Inhibition (mm) Replicate 3
E. coli	Sterile Water	5.0	5.0	5.0
S. aureus	Sterile Water	5.0	5.0	5.0

Table 7:

Positive Control with Replicates

Organism	Antibiotic	Concentration	Zone of Inhibition (mm) Replicate 1	Zone of Inhibition (mm) Replicate 2	Zone of Inhibition (mm) Replicate 3
E. coli	Erythromycin	15 µg/disc	24.16	24.10	24.22
S. aureus	Gentamicin	10 µg/disc	25.34	25.28	25.40

Minimum Inhibitory Concentration (MIC)

Wild Onion Extract:

For *E. coli*: MIC = 0.5g/ml (50%).

For *S. aureus*: MIC = 0.25g/ml (25%)

Phytochemical Assays

Qualitative phytochemical screening of Ashwagandha and Wild Onion extracts revealed the presence of various bioactive compounds, as summarized in Table 8.

Table 8:

Phytochemical Analysis of Ashwagandha (W. somnifera) and Wild onion (A. vineale) Extracts

Phytochemical Constituents	Chemical Tests	*W. somnifera*	*A. vineale*
Saponins	a) Foam/Froth Test	++	-
Tannins	a) Ferric Chloride Test	+++	+++

	b) Lead Acetate Test	+++	+++
Phenolic Compounds	a) Ferric Chloride Test	+++	+
Flavonoids	a) Shinoda Test	++	++
	b) Sulphuric Acid Test	+++	+++
	c) Alkaline Test	+++	+++
	d) Lead Acetate Test	+++	+++
Carbohydrates	a) Fehling's Test	Traces (green)	+
	b) Benedict's Test	+	+
Proteins	a) Biuret Test	-	-
Alkaloids	a) Mayer's Test	+++	+++
	b) Wagner's Test	+++	++
	c) Dragendorff's Test	+++	+++
	d) Hager's Test	++	++
Cardiac Glycosides	a) Keller-Killiani Test	+++	++
	b) Kedde Test	+	++
Anthraquinone Glycosides	a) Borntrager's Test	-	-
	b) Modified Borntrager's Test	-	-
Sterols and Triterpenoids	a) Lieberman-Burchard Test	+++	++
	b) Salkowski Test	+++	+++

*Key: **[+++]**: Strongly positive; **[++]**: Moderately positive; **[+]**: Weakly positive; **[-]**: Negative; **[Traces (green)]**: Indicates a faint positive result (specific to Fehling's test)*

Ashwagandha (W. somnifera) Extract

The phytochemical profile of Ashwagandha extract was rich in secondary metabolites. Tannins were strongly detected through both Ferric Chloride and Lead Acetate tests, indicated by intense coloration and precipitate formation, respectively. Phenolic compounds were also strongly present, as confirmed by the Ferric Chloride test, which produced a greenish-blue coloration. Flavonoids were identified via multiple tests, including Shinoda (orange/purple coloration), Sulphuric Acid

(yellow/red solutions), Alkaline (intense yellow color), and Lead Acetate (yellow precipitate). Alkaloids were strongly detected using Mayer's (creamy precipitate), Wagner's (reddish-brown color), Dragendorff's (orange-red color), and Hager's (yellow precipitate) tests. Cardiac glycosides were strongly positive in the Keller-Killiani test, showing bluish-green and reddish-brown layers, and weakly positive in the Kedde test (purple coloration). Sterols and triterpenoids were strongly detected using Lieberman-Burchard (brown/green rings) and Salkowski (red/yellow layers) tests. Saponins were moderately present, as indicated by persistent foam formation in the Froth test. Carbohydrates were weakly detected, with a faint green trace in Fehling's test and a positive Benedict's test. Proteins and anthraquinone glycosides were absent in the extract.

Wild Onion (A. vineale) Extract

Wild Onion extract exhibited a distinct phytochemical profile. Tannins were strongly detected, similar to Ashwagandha, through Ferric Chloride and Lead Acetate tests. Phenolic compounds were weakly present, as indicated by a faint greenish-blue coloration in the Ferric Chloride test. Flavonoids were strongly detected across all tests, including Shinoda, Sulphuric Acid, Alkaline, and Lead Acetate. Alkaloids were strongly positive in Mayer's and Dragendorff's tests, moderately positive in Wagner's and Hager's tests, and weakly positive in the Kedde test. Cardiac glycosides were moderately detected in the Keller-Killiani test and weakly in the Kedde test. Sterols and triterpenoids were strongly detected in the Salkowski test and moderately in the Lieberman-Burchard test. Carbohydrates were weakly present, as indicated by a faint green trace in Fehling's test and a positive Benedict's test. Saponins, proteins, and anthraquinone glycosides were absent in the extract.

The phytochemical screening highlighted the presence of tannins, flavonoids, alkaloids, and sterols/triterpenoids as major constituents in both extracts. However, Ashwagandha exhibited a broader spectrum of bioactive compounds, including phenolics and cardiac glycosides, which were either weakly present or absent in Wild Onion. These findings align with the observed antimicrobial activity, suggesting that the bioactive compounds contribute to the extracts' efficacy against *E. coli* and *S. aureus*.

DISCUSSION

The antimicrobial and phytochemical properties of *Withania somnifera* (Ashwagandha) and *Allium vineale* (Wild Onion) extracts were investigated in this study. The results demonstrate significant antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*, supported by the presence of diverse bioactive compounds. These findings align with previous studies on medicinal plants and their potential as natural antimicrobial agents.

Antimicrobial Activity

Ashwagandha extract exhibited dose-dependent antimicrobial activity, with higher inhibition zones against *S. aureus* compared to *E. coli*. This is consistent with studies showing that *W. somnifera* possesses broad-spectrum antimicrobial properties, attributed to its alkaloids, flavonoids, and tannins (Ezez et al., 2023). The stronger activity against *S. aureus* may be due to the Gram-positive bacterium's susceptibility to plant-derived compounds that disrupt cell wall synthesis (Nascimento et al., 2000).

Wild Onion extract also showed significant activity, particularly against *S. aureus*, with a lower MIC (0.25g/ml) compared to *E. coli* (0.5g/ml). This aligns with studies reporting the antimicrobial efficacy of Allium species, which is attributed to sulfur-containing compounds like allicin (Bastaki et al., 2021). The observed activity against *S. aureus* is particularly noteworthy, as this pathogen is often resistant to conventional antibiotics (Foster, 2017; Monaco et al., 2017).

The positive controls (Erythromycin and Gentamicin) demonstrated robust activity, validating the assay's reliability. The negative control (sterile water) showed no significant inhibition, confirming that the observed effects were due to the plant extracts.

Phytochemical Composition

The phytochemical screening revealed that both extracts contain bioactive compounds known for their antimicrobial properties. Ashwagandha's strong presence of tannins, flavonoids, and alkaloids aligns with its broad-spectrum activity. Tannins and flavonoids are known to disrupt microbial cell membranes and inhibit enzyme activity, (Donadio et al., 2021; Huang et al., 2024) while alkaloids interfere with DNA replication and protein synthesis (Abookleesh et al., 2022).

Wild Onion's high content of flavonoids and sulfur compounds likely contributes to its antimicrobial efficacy. Flavonoids are known to inhibit bacterial efflux pumps and biofilm formation (Lopes et al., 2017), while sulfur compounds like allicin exhibit potent bactericidal effects (Bhatwalkar et al., 2021). The absence of saponins in Wild Onion contrasts with Ashwagandha, which may explain the latter's broader activity.

The findings are consistent with previous research on medicinal plants. For example, Sebaro et al. (2023) reported that *W. somnifera* extracts inhibit *S. aureus* and *E. coli* through mechanisms involving cell wall disruption and oxidative stress. Similarly, studies on Allium species have highlighted their antimicrobial potential, particularly against Gram-positive bacteria (Feknous et al., 2024). However, the current study provides new insights into the specific phytochemical profiles and MIC values of these extracts, contributing to the growing body of knowledge on natural antimicrobial agents.

Limitations

While the results are promising, this study has limitations. The use of methanolic extracts may not fully capture the plants' bioactive potential, as other solvents could yield different phytochemical

profiles. Additionally, in vitro assays do not account for the complex interactions *in vivo*, necessitating further studies to evaluate efficacy in clinical settings.

CONCLUSION

This study demonstrates that *Withania somnifera* and *Allium vineale* extracts possess significant antimicrobial activity against *E. coli* and *S. aureus*, supported by their rich phytochemical profiles. Ashwagandha exhibited broader-spectrum activity, while Wild Onion showed preferential potency against *S. aureus*. These findings highlight the potential of these plants as natural alternatives to conventional antibiotics, particularly in addressing antibiotic-resistant pathogens.

RECOMMENDATIONS

Further research should investigate the efficacy of these extracts against other clinically relevant pathogens, including multidrug-resistant strains. In addition, exploring the use of different solvents could enhance the extraction of bioactive compounds, thereby improving their potential applications. To establish practical relevance, in vivo studies involving animal and clinical trials are necessary to evaluate both the safety and efficacy of these extracts in real-world settings. Finally, mechanistic studies should be undertaken to elucidate the specific modes of action of the bioactive compounds, particularly in relation to resistant strains.

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