DMU

Advanced Journal of Interdisciplinary Studies 9(2025) 2357-2376

AJIDS

Journal homepage: www.ajids.com.et

Volume 9(1), June 2025

Antibacterial activity of *Datura stramonium L.* and *phytolacca dodecandra* L. against some pathogenic bacteria.

Kelemu Tsigea*, Asmamaw Habtamu, Agumas Lemlemu

Department of Biology, College of Natural and computational sciences, Debre Markos University, P.O. Box: 269, Debre Markos, Ethiopia

*Corresponding author's Email: lemlemu13@gmail.com

Abstract

The emergence of antimicrobial-resistant pathogens, coupled with the adverse effects associated with conventional drugs, traditional therapies has made bacterial infections even more of a global public health concern. Traditional medicinal plants, such as *Datura stramonium* L. and *Phytolacca dodecandra* L', have gate attention for their potential antibacterial properties. This investigation sought to assess the in vitro antimicrobial efficacy of these botanical specimens against designated pathogenic bacterial strains. A control experimental laboratory-based research was carried out to undertaken to assess "the antimicrobial effects" of *D. stramonium* and *P. dodecandra*. Samples of *D. stramonium* and *P. dodecandra* were collected from Dembecha Woreda and Debre Markos Town, respectively. The seeds and leaves were air-dried, ground, and soaked in 97% ethanol, 95% hexane, and distilled water. The plant mixtures were agitated in a shaker for duration of 72 hours at ambient temperature, followed by concentration using a rotary evaporator The Ethiopian Public Health Institute (EPHI) provided the bacterial strains Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus used in this study. The antibacterial properties derived from the leaves of Datura stramonium and seed bioactive extracts were evaluated Through the use of two standard microbiological techniques: the disc diffusion procedure along with the agar dilution technique. The disc diffusion test measured the zone of inhibition, while the minimum inhibitory concentration (MIC) was established using the agar dilution technique." The results exhibited considerable bacteria-inhibiting properties showed Impact on strains of *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa.* The area of inhibition zones ranged from 14±0.58 mm to 18±0.58 mm at 400 mg/ml, showing similar efficacy to the positive control, gentamicin. In contrast, the extracts of *P. dodecandra* demonstrated no antibacterial Efficacy against any of the bacterial isolates studied. The ethanol and Extracts obtained using hexane of *D. stramonium* leaves showed the lowest MIC of 100 mg/ml against *P. aeruginosa*, demonstrating an MBC value of 100 mg/ml was also observed for *P. aeruginosa*. The work undertaken confirms the species *Datura stramonium* exhibits promising antimicrobial effect against Selected pathogenic bacteria affecting humans. However, the selection of gentamicin as a control, which has limited

efficacy against Gram-positive species including *S. aureus*,may not be appropriate for comprehensive evaluation. Future studies should consider using antibiotics with broader activity Exhibits inhibitory effects on a broad spectrum of bacteria, including those with Gram-negative and Gram-positive cell walls. to better assess The ability of plant extracts to inhibit bacterial growth.

Keywords: *Datura stramonium*, *P.dodecandra*, disc diffusion, microdilution, *minimum inhibitory concentration*.

1. INTRODUCTION

Reduced effectiveness of antimicrobial therapies (AMR) has become Among the foremost urgent health concerns on a global scale Recent research suggests that antimicrobial resistance is likely to, by 2050, could account for Nearly 10 million annual deaths, potentially exceeding the global mortality burden of cancer. If left unaddressed, this silent pandemic could reverse decades of medical progress, rendering common infections untreatable and routine surgeries high-risk. (Lancet AMR 2024,). The high levels of inappropriate Utilization of antibiotics in both medical practices together with livestock production has fueled the rapid evolution of drug-resistant bacteria. This trend has severely compromised the efficacy of standard antimicrobial therapies, leaving once-manageable infections increasingly difficult to treat (Lancet AMR 2024,). In Ethiopia, the frequency of antimicrobial resistance stands at alarmingly increase. A systematic review encompassing 48,021 study participants revealed that Multidrug-resistant (MDR) bacteria, including Enterobacteriaceae that produce ESBL enzymes bacterial strains have become increasingly widespread Detected within clinical institutions and the broader community, with resistance values spanning 62.9% to 87.4% (Berhe et al., 2021) Notably, *Staphylococcus aureus* strains resistant to methicillin (MRSA) and *Enterococcus* species resistant to vancomycin (VRE) Have appeared as clinically significant nosocomial pathogens, in 5.3% to 32.5% and 5.5% to 41.7% of samples, respectively (Woldu, 2024).

Despite the growing threat of multidrug-resistant bacteria, coupled with the adverse side effects of these treatments, remains a persistent problem, there is a significant gap in the formation of new antibacterial agents (Berhe *et al.,* 2021) lower pace of antibiotic discovery, coupled with the economic challenges associated with developing new drugs, has hindered progress (WHO, 2021). Furthermore, many potentially therapeutic plants remain underexplored, particularly those indigenous to regions like Ethiopia.

For centuries, medicinal plants have served as for therapeutic properties. In Ethiopia, approximately Traditional medicine is used by approximately 80% of the population often utilizing plant-based remedies. Commonly identified as *Datura stramonium* are jimsonweed has been utilized in Ethiopian traditional practices, particularly among students and lay priests (debtrawoch), to "open the mind," fostering receptiveness to learning and enhancing

creativity. This use is rooted in the plant's psychoactive properties, which have been employed in various cultures for spiritual and cognitive purposes. D. stramonium and P. dodecandra are used in Ethiopian traditional medicine for infections (Alemu and Assefa, 2023). Phytolacca dodecandra, known locally as "endod" or "shibti," is a trailing shrub native to Ethiopia and Eritrea. The plant's berries and leaves are traditionally used as a natural detergent for washing clothes, producing a rich lather when mixed with water. Beyond its domestic applications, Phytolacca dodecandra has been recognized for its molluscicidal properties, particularly effective against snails that host the parasite causing schistosomiasis (Karunamoorthi et al. 2008).

Current studies have further demonstrating antimicrobial Capacity of medicinal plants. For instance, Fungal endophytes obtained from *Rosmarinus officinalis* (rosemary) confirmed significant Inhibitory effects on methicillin-resistant S. aureus (MRSA) and extended-spectrum β-lactamase (ESBL)-positive Escherichia coli strain (Almuhayawi et al., 2021). Additionally, rosemary essential oils exhibited synergistic effects with antibiotics, particularly against Pathogenic Gram-negative bacteria (Bouyahya et al., 2022). Such findings align with broader research emphasizing plant-derived compounds as promising alternatives to combat antimicrobial resistance (Saxena et al., 2023).

These findings suggest that underutilized plants may offer novel compounds to combat resistant pathogens. This experimental analysis evaluates the antimicrobial efficacy of leaf and seed extracts from selected medicinal plants indigenous to Ethiopia. By focusing on plants that have not been extensively studied, this research seeks to fill existing knowledge gaps and contribute to the development of alternative antimicrobial agents.

2. Materials and Methodology

2.1 Specimen Gathering

The experiment utilized botanical specimens collected from distinct ecological zones in northwestern Ethiopia. *Phytolacca dodecandra* L. specimens were obtained from Debre Markos, located at 10°20′N latitude and 37°43′E longitude a highland area situated 300 km northwest of Addis Ababa at 2,446 meters elevation. This location experiences temperate climatic conditions characterized by mean annual precipitation of 1,380 mm and stable temperatures averaging 18°C (Debre Markos University Meteorological Data, 2018).

In contrast, *Datura stramonium* L. samples were collected from Dembecha district (10°20'N, 37°40'E), located 346 km from the national capital at a moderately high elevation of 2,075 meters. This region exhibits distinct seasonal variations with 780 mm yearly rainfall and temperature fluctuations between 18-24°C (Dembecha Agricultural Meteorological Report, 2018).

All laboratory analyses were conducted at Debre Markos University's Department of Biology, utilizing standard phytochemical research facilities.

2.2 Research Design and Study Period

A controlled experimental The research was carried out in the Microbiology Laboratory at Debre Markos University; the experiment employed a randomized block design incorporating triplicate measurements to enhance statistical validity and experimental consistency. The study period spanned from December 2019 to June 2020, during which the antibacterial efficacy of leaf and seed extracts from two selected medicinal plants was evaluated against specific pathogenic bacteria. This timeframe allowed for comprehensive testing and analysis of the plant extracts' antimicrobial properties.

2.3 Collection plant materials and authentication

The study specimens (Phytolacca dodecandra L. leaves and Datura stramonium L. seeds/leaves) were harvested during peak flowering season (October–December 2019) to ensure optimal phytochemical content. Taxonomic authentication was performed by Dr. Haimanot Reta (Senior Botanist, Debre Markos University Herbarium) using voucher specimens deposited at the university's plant science department. The plant samples were washed 3 times with Piped water and air Air-dried in a shaded area at ambient temperature (25–30°C). until the sample parts was completely dry According to Thippeswamy et al. (2011), 1kg of *P. dodecandra L.* Leaf and *D. stramonium L*. Seed and leaf part air dried in shade for two weeks. The plants were finely ground by using electrical grinding and powders of those plant parts were macerates in water, hexane and ethanol at the rate of 50g of each powder per 500ml of ethanol and hexane solvent and 50g per 1000ml in aqueous solvent and then shaken for 72 hours (Jembere, 2002). And filter with the extracts were filtered using Whatman No. 1 filter paper and subsequently concentrated with a rotary evaporator then the filtrates were dried in bottle.

2.4 Sampling method and sample Size

The numbers of treatments were composed from combination of two types of plants those were *P.dodecandra* and *D.stramonium* extracts with four bacterial species obtained from Ethiopian public health institute. The experimental test was repeated 3 times.

2.5 Preparation of aqueous extract

Approximately 50 grams of seeds and leaves from each plant were soaked in 1000 mL of sterile distilled water in conical flasks and agitated on a shaker for 72 hours, following the method described by Subbarayan et al. (2010). The resulting macerates were initially filtered through double-layered muslin cloth. The filtrate was then passed through Whatman No. 1 filter paper to remove finer particles. The combined extracts were concentrated using a water bath, after which the concentrated extracts were stored in brown bottles at 4°C until further use.

2.6 Preparation of solvent extracts

Precisely 50g of both plants' extract powder was the precipitates were re-soaked individually using the same solvents and under comparable conditions after being exposed to 500 mL batch soaking conical flacks and kept placed on a mechanical shaker at 120 revolutions per minute (rpm) for 72 hours “at room temperature, as

reported by Mohana et al. (2016). After filtration was complete, they were concentrated at 60 rpm and 45°C. At the conclusion of the procedure, each extract's % yield was stored for later use at 4ºC.

2.7 Standard drug

The antibacterial assay were incorporated quality controls with 10 µg gentamicin discs (positive control) and 10% Tween 80 solution (negative control) to validate the disc diffusion methodology

2.8 Bacterial Strains

The study utilized bacterial strains acquired from the Ethiopian Public Health Institute (EPHI) for in vitro antimicrobial susceptibility testing. The tested bacterial strains comprised Gram-negative species including *Escherichia coli* (ATCC 29522), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603), and the Gram-positive species *Staphylococcus aureus* (ATCC 29523).”

2.9 Solvent type and their purification

During the experiment there were solvent used for extracting the test plant part which were listed below in the table.

Table 1 Solvent type and their purification

No	Solvent type	Alcoholic proportion in %	Made in	Manufacturing date	Expired date
1	Ethanol	97%	Ethiopia	April,2018	April,2020
2	Hexane	95%	India	May, 2018	May,2020
3	Distilled water	0	D/R/H	-	-

2.10 Antibacterial Test Determination of Crude Extracts by Disc Diffusion method

The antimicrobial activity of the plant extracts was assessed using the disc diffusion technique, following the procedure described by Heatley (1944). Cultures of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* were grown on nutrient agar plates and incubated at 37°C for 24 hours. Subsequently, the bacterial samples were inoculated using a sterilized inoculation loop into a test tube containing nutrient broth to facilitate growth overnight in an incubator maintained at 37 degrees Celsius. Following a 24-hour incubation period, the bacterial culture from the nutrient broth was streaked onto nutrient agar in accordance with the manufacturer's specifications. A bacterial suspension was prepared with a standardized cell density of $1.5x10^7$ cells $ml^{-1}$from bacterial colony grown on nutrient agar for 24 hours in incubator at 37^0 C The turbidity is typically measured at 600 nm wavelength (OD600 a standard corresponds Suspensions

of bacteria were adjusted to match the 0.5 McFarland standard, which is estimated to contain 1.5 × 10^8 CFU/mL." These standards are used to adjust bacterial suspensions to match the desired turbidity, ensuring accurate and reproducible testing conditions. Using a sterile swab, the bacteria were applied to Mueller Hinton Agar (MHA) plates. 2 mg/disc, 1mg/disc and 0.5 mg/disc crude extracts of water, hexane and ethanol of the leaf and seed parts of those plants were loaded on the separate filter paper (Whatman No.1filter) which is 6 mm in diameter. The disc which was loaded with extracts was allowed drying so that the solvent evaporated. The extract loaded discs were carefully put on bacteria swab Mueller Hinton Agar with sterilized forceps. Similarly, the standard antibiotics which were gentamycin (10µg/disk), as a positive control and 10% tween 80 as a negative control were carefully put on bacterial swabbed Mueller Hinton Agar in Petri dish. Then the bacteria in experimental groups, negative control and positive control Petri dish were incubating for 24 hours at 37C^o. Each experiment was conducted in triplicate. Following incubation, the zones of bacterial inhibition around each disc were measured using a ruler, as described by Shahwar and Raza (2009).

2.11 Evaluation of Extracts' Minimum Inhibitory Concentration

Antimicrobial effect was evaluated via the standardized agar dilution technique following established protocols (Andrews, 2001). swabs, ensuring even distribution of the inoculum swabs, ensuring even distribution of the inoculum.

For each test extract (aqueous, hexane, and ethanol), a homogeneous mixture was prepared by combining 19 mL of heated Mueller-Hinton agar with One milliliter of the respective Concentrated plant extract at 200 mg/mL. This mixture was carefully the medium was aseptically poured into sterile Petri dishes and left to solidify. under laminar airflow to prevent condensation.

Bacterial inocula were prepared by suspending test organisms Using 0.85% solution ofphysiologicaly Suspended in saline to match the turbidity of the 0.5 McFarland standard (1-2 × 10^8 CFU/mL). A standardized volume of 150 µL ((1-2 × 10^8 CFU/mL) the volume for each bacterial suspension was uniformly inoculated in the agar-extract using sterile plate (ESCMID, 2000) and was swabbed on 19 ml and 1 ml of extracts of seed and leaf from each plant at concentration of water extract (200 mg/ mL). for all bacteria type, ethanol extract of leaf and seed 400,200,200 and 200,100 and At concentrations of 200 mg/mL, the extracts were tested against *S. aureus*, *P. aeruginosa*, and *E. coli*, respectively. while hexane extract of the leaf 400,100,200 and 200mg/ml of seed against *S. aureus*, *P. aeruginosa*, and *E. coli*) together Incubation took place at 37^o C for one full day and observed for any visible growth within minimum concentration. The minimum inhibitory concentration (MIC) was assessed by visually inspecting bacterial growth

2.12. Evaluation of Extracts' Minimum Bactericidal Concentration

To determine the lowest bactericidal concentration (MBC), a small amount were taken from the wells of the MIC test plate and transferred onto nutrient agar by streaking across the surface. The samples that showed no visible bacterial growth were then re-inoculated spread across Mueller-Hinton agar plates. Following incubation After incubation at 37°C for 24 hours, the MBC was determined. At the least concentration at which no bacterial colonies appeared on the Mueller-Hinton agar.

2.13. Data Analysis

The results were entered into SPSS version 20 for analysis. Data are presented as mean values ± standard error of the mean (SEM). Statistical significance was evaluated at a 95% confidence level, with P-values less than 0.05 considered significant. Differences in antibacterial susceptibility were analyzed using one-way ANOVA.

3. RESULT

3.1 Percentage yield of plant extract

The ethanol extract of Datura stramonium seeds produced the highest percentage yield, while the lowest yield was recorded from the aqueous extract of Phytolacca dodecandra seeds, as shown in Table 2.

Table 2. Percentage yield of plant *D. stramoniumL.*and *P.dodecandra Ex*tract

Plant name	Part of plant	Amount of powder(gm)	Solvent type(1:10)	Yield of extraction (%)
D. stramoniumL.	Leaves	100	Ethanol	10.34
	Leaves	100	water(1:20)	9.14
	Leaves	100	Hexane	10.53
	seed	100	Ethanol	10.74
	seed	100	water(1:20)	8.3
	seed	100	Hexane	8.14
P.dodecandra L.	Leaves	100	Ethanol	10.12
	Leaves	100	water(1:20)	9.21
	Leaves	100	Hexane	8.35
	seed	100	Ethanol	8.11
	seed	100	water(1:20)	7. 9
	seed	100	Hexane	8.41

3.2 Evaluation of Antimicrobial Activity of Datura stramonium Leaf and Seed Extract

Antibacterial properties of the plant's were evaluated using disc diffusion method. Leaf and seed extracts obtained from *Datura stramonium* demonstrated Showed considerable antimicrobial properties against *S. aureus*, *E. coli*, and *P. aeruginosa* at concentrations of 400, 200, and 100 mg/mL. However, not all concentrations were

consistently effective, and none showed activity against *Klebsiella pneumoniae*. The antibacterial efficacy of water, ethanol, and hexane extracts from *D. stramonium* leaves and seeds against the four bacterial strains is summarized in Tables 3, 4, and 5.

At a measured concentration of 400 mg/mL, the water-based extract of *D. stramonium* leaves produced inhibition zones measuring 14 ± 0.78 mm for *P. aeruginosa*, 11.33 ± 1.2 mm for *S. aureus*, and 12 ± 0.58 mm for *E. coli*. Similarly, the seed extract in water showed an inhibition zone of 15 ± 0.58 mm against *P. aeruginosa* at the same concentration. However, this seed extract exhibited no inhibitory effect against *S. aureus* and *E. coli* at any tested concentration.

These inhibitions were significantly higher than the negative control, 10 % tween 80. at 400mg/ mL concentration the leaves and seed of *D.stramonium* had similar significant value with positive control of Gentamycin. The variations identified between the Statistical analysis indicated significant differences in mean values ($P \leq 0.05$). The size of the inhibition zones was influenced by the concentration of the water extracts from both the leaves and seeds—indicating that as the concentration increased, the diameter of the inhibition zones also increased, as presented in Table 3.

Table 3. Evaluation of the antibacterial Impact of *Datura stramonium* leaf and seed extracts aqueous extracts on pathogenic bacteria using the disc diffusion technique.

Extract /solvent	Concentration in mg/ml	Mea ± SEM in mm			
		E.coli	*S.aureus*	*P.aeruginosa*	*K.pneumoneae*
Leaf of *D.stramonium l*	400mg/ Ml	≠b 12±0.58*a	≠b11.33±1.2*a	14±0.78*a	-
	200mg/ Ml	≠b 8.33±0.33*a	≠b 8±0.58*a	≠b 10.66±0.33*a	-
	100mg/ Ml	-	≠b 7±0.0*a	≠b 8.33±0.66*a	-
Seed of *D.stramonium l*	400mg/ mL	-	-	15±0.58*a	-
	200mg/ mL	-	-	≠b 11.66±0.23*a	-
	100mg/ mL	-	-	≠b 8.33±0.3*a	-
TWEEN 80	10%TWEEN 80	- L	- L	- L	- L
		-S	- S	- S	- S
Positive control	Gentamycin10µg/disk	20.33±0.3L*a	22.13±1.4L*a	20±0.11 L*a	20±57 S*a
		22.11±0.21S*a	21.21±1.45S*a	20±57 S*a	20±58 S*a

Key: - *: significantly higher than; ≠ = significantly lower than a = inhibition of 10% tween 80; b= inhibition zone Gentamycin = no zone of inhibition, L= leaf, S= seed.

The ethanol extract of *leaves* of *D. stramonium* had zone of inhibition of 18±0.38mm, 10±0.58mm and 8.66±0.23mmagainst *Pseudomonas aeruginosa*

and *Staphylococcus aureus* and *E. coli*, respectively at 400mg/ mL concentration. The ethanol extract of seed of *D. stramonium* had zone of inhibition of 14±0.58mm, against *P. aeruginosa*. *and* 12±0.58mm *S.aureus* at 400mg/ mL concentration. But there was no inhibition zone on *E. coli* in ethanol extract of *D.stramonium* seed at all concentration. These inhibitions were significantly higher than the inhibition zone of negative control, 10 % tween 80. In this test the ethanol extract of *leaves* of *D. stramonium which had* zone of inhibition (18±0.38mm), (15±0.58mm) and (14±0.58mm) against *P. aeruginosa*. were significantly similar from the inhibition of positive control of Gentamycin but not the remaining. the variations observed among the statistical analysis revealed significant differences in mean values ($p \leq 0.05$)." The inhibition zone size for the ethanol extracts of both leaves and seeds was concentration-dependent, similar to the pattern observed with the water extracts, as illustrated in Table 4.

Table 4. Assessment of antimicrobial effects of ethanol extracts from *Datura stramonium* leaves against pathogenic bacteria using the disc diffusion method.

No	**Extract**	**Concentration in mg/ml**	**Mea ± SEM in mm**			
			E.coli	*S.aureus*	*P. aeruginosa.*	*K.pneumonia*
1	Leaf of*D.stramonium l*	400mg/ mL	$^{\neq b}$8.66±0.23*^{a}	b≠10±0.58*^{a}	18±0.38*^{a}	-
		200mg/ mL	$^{\neq b}$7±0.33*^{a}	-	15±0.58*^{a}	-
		100mg/ mL	-	-	-	-
2	Seed of*D.stramonium l*	400mg/ mL	-	$^{\neq b}$12±0.58*^{a}	14±0.58*^{a}	-
		200mg/ mL	-	$^{\neq b}$8±0.58*^{a}	$^{\neq b}$11.33±0.33*^{a}	-
		100mg/ mL	-	-	$^{\neq b}$8.33±0.33*^{a}	-
	Tween 80	10%TWEEN 80	-L	-L	-L	-
			-S	-S	-S	-
	Positive control	Gentamycin10 µg/disk	20.66±0.66L*^{a}	21.33±0.88 L*^{a}	20.33±0.56L*^{a}	-
			21.0±0.23 S*^{a}	20.33±0.18 S*^{a}	20.13±0.18S*^{a}	-

Key: - *: significantly higher than: $^{\neq}$ = significantly lower than; a = inhibition of 10% tween 80; b= inhibition zone Gentamycin - = no zone of inhibition, L= leaf, S= seed.

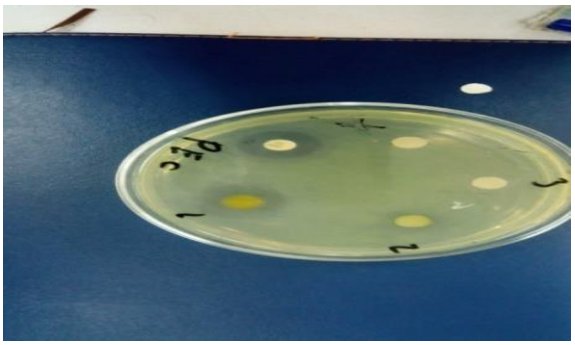

Figure 1 inhibition zone of ethanol extracts of d.stramonium leaf against *P. aeruginosa*.

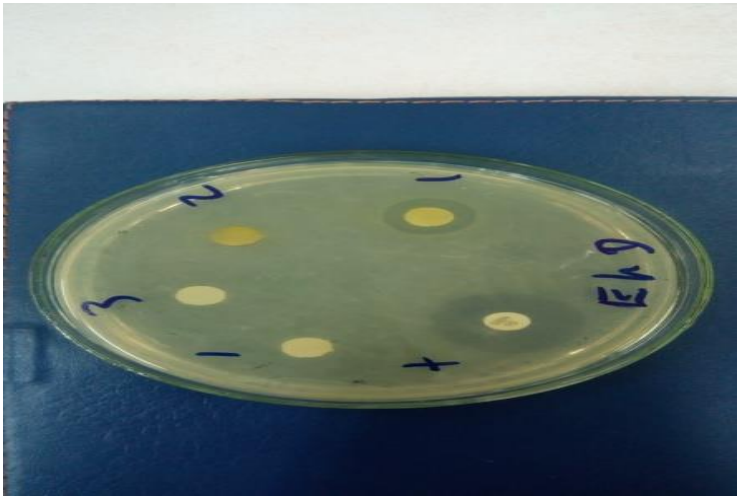

Figure 2. Inhibition zone produced by hexane Bioactive extracts obtained from *Datura stramonium* seeds against *Escherichia coli*. The hexane leaf extract of *D. stramonium* exhibited.

The greatest inhibition diameter was 14.33 ± 0.27 mm against *Pseudomonas aeruginosa* At 400 mg/mL concentration, as shown in Table 5. At the same concentration, the hexane extract of the leaves showed inhibition zones measuring Inhibition zones measured 14.33 ± 0.27 mm, 9.33 ± 0.58 mm, and 10.66 ± 0.33 mm against *Pseudomonas aeruginosa, Staphylococcus aureus*, and *E. coli*, respectively. Meanwhile, the aqueous seed extract of *D. stramonium* produced inhibition zones of 14.33 ± 0.33 mm, 14 ± 0.58 mm, and 13.66 ± 0.33 mm Tested on *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* at 400 mg/mL concentration.These inhibitions were significantly higher than the inhibition zone of negative control, 10 % tween 80. The observed differences among the mean was statistically significant which was ($P \leq 0.05$) except the concentration of at 100mg/ mL on the leaf and seed extracts on all the test bacteria's which had not zone of inhibition.

Table 5. Antimicrobial activity of plant extracts which were hexane extracts against pathogenic bacteria by disc diffusion method.

No	Extract	Concentration in mg/ mL	Mea ± SEM in mm			
			E.coli	*S.aureus*	*P.aregenasa*	*K.pneumonia*
	Leafof *D.stramonium l*	400mg/ mL	≠b 10.66±0.33*a	≠b 9.33±0.58*a	14.33±0.27*a	-
		200mg/ mL	≠b 8.33±0.67*a	≠b 8±1.0*a	≠b 9.66±0.33*a	-
		100mg/ mL	-	-	-	-
2	Seed of *D.stramonium l*	400mg/ mL	≠b 13.66±0.33*a	≠b 14±0.58*a	14.33±0.33*a	-
		200mg/ mL	≠b 8±0.58*a	≠b 8±0.88*a	≠b 10±0.58*a	-
		100 mg/mL	-	-	-	-
	tween 80	10%tween 80	L-	L-	L-	L-
			S-	S-	S-	S-
	Positive control	Gentamycin10µg/disk	22.66±0.88L*a	22±1.15L*a	17±1.15L*a	-
			21±0.58S*a	23.7±0.88S*a	18.8±1.12S*a	-

Key: - *: significantly higher than: ≠ = significantly lower than; a = inhibition of 10% tween 80; b= inhibition zone Gentamycin - = no zone of inhibition, L= leaf, S= seed.

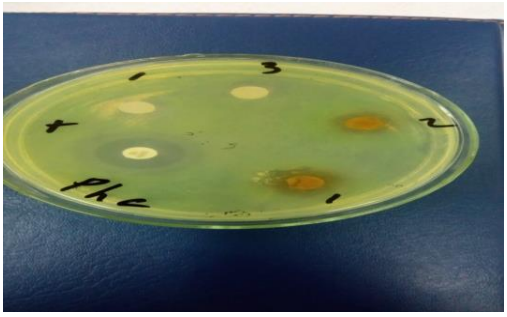

Figure 2 inhibition zone of *hexane* extracts on D.stramonium L. seed

Against P. aeruginosa

3.3 Evaluation of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Crude Extracts from *Datura stramonium* Leaves and Seeds

3.3.1 Assessment of the Minimum Inhibitory Concentration (MIC) for Crude Extracts Derived from *Datura stramonium* Leaves and Seeds

The aqueous extract from the leaves showed a minimum inhibitory concentration (MIC) of 200 mg/mL against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. In contrast, the seed extract did not demonstrate any MIC against the bacteria tested in this study. Ethanol extracts of the leaves exhibited MIC values of 400 mg/mL for *S. aureus* and 200 mg/mL for both *E. coli* and *P. aeruginosa*. Meanwhile, the ethanol seed extracts displayed MICs of 100 mg/mL for all three bacterial strains, as detailed in Table 6.

Table 6. Minimum Inhibitory Concentrations (MIC) of Aqueous, Ethanol, and Hexane Extracts of *Datura stramonium* L.

Bacteria spp.	Extract	Concentration in mg/mL for MIC					
		12.5	25	50	100	200	400
E.coli	*D.stramonium* ^{La}	+	+	+	+	*	-
	D.stramonium^{Lb}	+	+	+	+	*	-
	D.stramonium^{Lc}	+	+	+	+	*	-
	D.stramonium^{Sb}	+	+	+	+	*	-
	D.stramonium^{Sc}	+	+	+	+	*	-
S. aureus	*D.stramonium* ^{La}	+	+	+	+	*	-
	D.stramonium^{Lb}	+	+	+	+	+	*
	D.stramonium^{Lc}	+	+	+	+	+	*
	D.stramonium^{Sb}	+	+	+	+	*	-
	D.stramonium^{Sc}	+	+	+	+	*	-
P.aeruginosa	*D.stramonium* ^{La}	+	+	+	+	*	-
	D.stramonium^{Lb}	+	+	+	+	*	-
	D.stramonium^{Lc}	+	+	+	*	-	-
	D.stramonium^{Sb}	+	+	+	*	-	-
	D.stramonium^{Sc}	+	+	+	+	*	-

Key: a = aqueous extract, b = ethanol extract, c = hexane extract; L = leaf, S = seed; + indicates bacterial growth; − indicates no bacterial growth; * denotes the minimum inhibitory concentration (MIC).

3.3.2 The MBC of *D. stramonium L*. leaves and seed of crude extracts

The highest minimum bactericidal concentration (MBC) observed for the aqueous leaf extract of *Datura stramonium* was 400 mg/mL against *Staphylococcus aureus*, while for *Escherichia coli* and *Pseudomonas aeruginosa*, it was 200 mg/mL. Meanwhile, the ethanol leaf extract exhibited MBC values of 400 mg/mL for both *E. coli* and *S. aureus*, with a lower value of 200 mg/mL against *P. aeruginosa*. For the hexane leaf extract, the minimum inhibitory concentration (MIC) was 400 mg/mL against *E. coli* and *S. aureus*, and 100 mg/mL for *P. aeruginosa*, as summarized in Table 7.

Table 6.The MBC of water, ethanol and hexane extract of the D.stramonium L.

Bacteria spp.	Extract	Concentration in mg/mL for MBC		
		400	200	100
E.coli	*D.stramonium* ^{La}	#	-	-
	D.stramonium^{Lb}	#	-	-
	D.stramonium^{Lc}	#	-	-
	D.stramonium^{Sb}	+	#	-
	D.stramonium^{Sc}	#	-	-
S. aureus	*D.stramonium* ^{La}	+	#	-
	D.stramonium^{Lb}	#	-	-
	D.stramonium^{Lc}	#	-	-
	D.stramonium^{Sb}	#	-	-
	D.stramonium^{Sc}	#	-	-
P. aeruginosa.	*D.stramonium* ^{La}	+	#	-
	D.stramonium^{Lb}	+	#	-
	D.stramonium^{Lc}	+	+	#
	D.stramonium^{Sb}	+	#	-
	D.stramonium^{Sc}	+	#	-

Key: a= water, b = ethanol, c=hexane, L=leaf, S=seed, - = no growth of bacteria; += higher bacteria growth, #= Minimum bacterial concentration.

3.4 Antimicrobial Activity of *P.dodecandra L.* leaves and seed of crude extracts

In this research, none of the water, ethanol, or hexane extracts from the leaves and seeds of *Phytolacca dodecandra* exhibited antimicrobial effects against any of the tested bacterial strains at any concentration.

4. DISCUSSION

In the present study, water extracts of leaf of *D.stramonium* showed inhibition zone of 14±0.58 mm against *P. aeruginosa*. at the concentration of 400 mm/mL and followed by E. coli (12±0.58). Consistent with these results, Ali (2017) reported that the aqueous leaf extract exhibited antibacterial activity against *E. coli*, producing a 10 mm inhibition zone. Conversely, The smallest inhibition zone, measuring 11.33 ± 1.2 mm, was recorded against *Staphylococcus aureus*. In this research, the aqueous extract from the leaves of *Datura stramonium* produced notably larger inhibition zones against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* at concentrations of 400 mg/mL and 200 mg/mL compared to the negative control (Tween 80). However, these zones did not significantly exceed those caused by the positive control, Gentamycin. Lower extract concentrations showed no antibacterial effect against *E. coli*.

The strong antibacterial activity observed may be linked to the presence of various bioactive secondary compounds such as tannins, saponins, steroids, flavonoids, alkaloids, glycosides, and phenolic compounds (Shagal et al., 2012). This is consistent with findings by Ali (2017), who reported that both aqueous and ethanol extracts of *Datura stramonium* contain these metabolites, which contribute to antimicrobial properties against *S. aureus*, *E. coli*, and *P. aeruginosa*. The diverse phytochemical content highlights the plant's potential for pharmaceutical development.

Conversely, the aqueous seed extract lacked antibacterial effects on most bacteria tested, except *P. aeruginosa*, which was sensitive at all tested concentrations for both leaf and seed extracts. Ali (2017) also investigated sequential extracts from whole plants using different organic solvents against *E. coli*, *S. aureus*, and *P. aeruginosa*. The lack of activity in the seed extract may be due to water's limited capacity to extract essential secondary metabolites.

At 400 mg/mL, the aqueous extracts of both leaves and seeds inhibited *P. aeruginosa* with zones measuring 14 ± 0.78 mm and 15 ± 0.58 mm, respectively, comparable to Gentamycin's inhibition. Ethanol extracts of *Datura stramonium* leaves at the same concentration demonstrated antibacterial activity with inhibition zones of 18 ± 0.58 mm for *P. aeruginosa*, 10 ± 0.58 mm for *S. aureus*, and 8.66 ± 0.33 mm for *E. coli*. These findings agree with Solomon Girmay (2014), who reported a zone of inhibition of 8.74 ± 0.22 mm against *E. coli* at 20 µg/mL.

In contrast, Gachande and Khillare (2013) observed greater antibacterial activity from ethanol leaf extracts of *Datura stramonium*, with zones of inhibition measuring 18 mm against *E. coli* and 24 mm against *S. aureus*. However, the ethanol extract did not inhibit *Klebsiella pneumoniae*. This study also found that *K. pneumoniae* was resistant to

the extracts, though the mechanisms behind this resistance were not investigated. These contributing to this resistance, such as efflux pump activity and biofilm formation. *K. pneumoniae* is known to possess multiple multidrug resistance (MDR) efflux pumps, including the AcrAB-TolC system, which actively expels a wide range of antibiotics, thereby reducing drug levels within cells and their role in promoting resistance. This finding was agreed with (Reddy, 2009*)*. Consistent with the current results, Priyanka et al. (2012) observed that ethanol Datura stramonium extracts leaves shows significant antimicrobial effects on*Staphylococcus aureus* and *Escherichia coli*. Consistent with Reddy (2009) investigated that the ethanol extract of *D.stramonium* leaves revealed inhibition zone (8.0+0.50 mm) on *S.aureus* .at concentration of 40mg/ml which was similar to this study that recorded (10±0.58mm) When prepared at 400 mg/mL, the ethanol extracts of *Datura stramoniu* leaves produced significantly larger Clear zones indicating antibacterial activity toward *E. coli* and *P. aeruginosa* at both 400 and 200 mg/mL compared to the negative control, which was 10% Tween 80.

In this finding the leaf ethanol extract of *Datura stramonium* with a concentration of 400mg/mL had higher zone of inhibition (18±0.38mm)(15±0.58) and seed of *D. stramonium*(14±0.58mm) against to the bacteria *P. aeruginosa*. which had similar significantly value to the inhibition of positive control of Gentamycin.

The hexane extract of leaf of *D.stramonium* showed inhibition zone of 14.33±0.27mm against *P. aeruginosa*. at the concentration of 400 mm/mL and followed by *E. coli* (10.66±0.33). In agreement with study hexane extracts of the leaves inhibited *E. coli* (14.00 ± 0.22 mm) and *S.* aureus (18.00 ± 0.27 mm) at 40 µg/mL (Solomon Girmay, 2014). The seed hexane extract of *Datura stramonium* exhibited antibacterial properties. (14.33±0.33mm) against *P. aeruginosa*. 400mg/mL), followed by *S. aurous* (14±0.58mm) and *E. coli* which was (13.66±0.33mm) and they were significantly higher than the negative control of 10%tween80.

In hexane extract of leaves and seed of *D. stramonium*at 400mg/MLhad higher zone of inhibition (14.33±0.27) and (14.33±0.33) respectively against to the bacteria of *P.aregenasa* that had similar significant value to the inhibition of positive control.

The leaf and seed extracts of *Datura stramonium* showed inhibitory effects against *Escherichia coli*, with minimum inhibitory concentration (MIC) values ranging from 100 mg/mL to 200 mg/mL across all three solvents tested.

(water, ethanol, and hexane). But on *P. aeruginosa*. S. *aurous* most of the MIV value was 200mg/ml. In contrast to this finding investigated lower MIC values of ethanol extract of *D.stramonium L*. leaf was 24, 44 and 40mg/ml on *Staphylococcus aureus*, *E. coli* together with *P. aeruginosa*,. Similarly, Mawahibsho et al. (2012) found that the Inhibitory and bactericidal concentrations values of ethanol extracts from *Datura stramonium* leaves ranged between 6.25 and 12.5 mg/mL against these bacterial strains. which was much less concentration than the present and second discovered. "These variations may result

from the solvent's reduced effectiveness in extracting the phytochemical constituents of the test plant, indicating that the solvent's polarity influences the plant's phytochemical profile. (Bissa and Bohra, 2012).

In *P.dodecandra* test the disk of test extract were exposed by bacteria in against way of the target experiment. The leaf and seed extracts of *Phytolacca dodecandra* exhibited no antimicrobial activity against the tested bacterial strains. This lack of efficacy may can be ascribed to the absence of bioactive compounds within the extracts. Phytochemical analyses have identified saponins, alkaloids, and phenolic compounds found in the fruits of *Phytolacca dodecandra*, which are recognized for their antimicrobial properties. However, the presence and concentration of these compounds may differ based on variables like plant part used extraction method, and solvent polarity. (Bissa and Bohra, 2012) the efficacy of medicinal plant depends at the combination of secondary bioactive compounds such as alkaloids, saponins, tannins, steroids, flavonoids, phenolic compounds, and glycosides.

There was totally no antibacterial activity was seen against *K.pneumonia* by the plant part of (leaves and seed) *D.stramonium* this was because of the bacteria was became resistant than the other gram negative bacteria. In a similar manner (Reddy*,* 2009*)* Ethanol extract of the leaf *of D.stramonium L.* did not have antimicrobial activity on *K.pneumonia.* When comparing the bacteria strain in this study, *P.aregenasa* was more sensitive than the remaining bacteria.

In all the extract type of leaves and seed of *D. stramonium* at 400mg/MLhad higher zone of inhibition against to the bacteria of *P. aeruginosa*. which had similar significantly value to the inhibition of positive control of Gentamycin. Due to these reason further study would be fundamental by increasing the concentration type and using different type of polar and non-polar solvent to extract the secondary metabolites of the plant effectively. In this finding most of the observed differences among the mean was statistically The results were significant ($P \leq 0.05$), and the size of the inhibition zones varied according to the concentration Fom the plant extracts tested inhibiting the bacteria. When comparing of the positivity of the plant part within solvent type at 400mg/mL (in water extract the seed (15±0.58mm) leaves (14±0.78mm) and in ethanol leaves (18±0.38mm) seed (14±0.58mm) of the plant highly significant respectively but in hexane the seed(14.33±0.33mm) than leaves (14.33±0.27mm) of the plant did not significant difference between seed and leaves and this difference lined with concentration of the extract.

5. CONCLUSION

Datura stramonium L. demonstrated promising antibacterial effects against the tested bacterial strains, indicating its capability to serve as a source of antimicrobial compounds. Therefore, further research involving isolation and identification of its bioactive compounds is advised for biological investigations. In contrast, *Phytolacca dodecandra* L. Did not exhibit any antimicrobial effects against the tested bacterial strains.

Acknowledgments

Debre Markos University has been fully acknowledged for its contribution to laboratory equipment and identification of plant.

Conflict of Interest

The authors declare that there are no conflicts of interest associated with this study.

Authors 'Contributions

Each authors contributed equally to the study's conception and design, data gathering, analysis, interpretation, and writing of the manuscript. Each author has reviewed and given approval for the final manuscript version.

Grant Support

This research was conducted without external funding.

Reference

Alemu, L., & Assefa, E. (2023). Phytochemical investigation and structural elucidation on seed extracts of *Datura stramonium*. *Journal of Science and Inclusive Development, 5*(1), 38–57. https://doi.org/10.20372/jsid/2023-216

Ali, E. A. (2017). Medical importance of *Datura fastuosa* (*Datura metel*) and *Datura stramonium*. *Journal of Pharmacy, Thiqar University, 7*(2), 43–58.

Almuhayawi, M. S., Ramadan, W. S., Harakeh, S., Aljaouni, S. K., Bharali, D. J., Mousa, S. A., & Almuhayawi, S. M. (2021). Antimicrobial potential of endophytic fungi isolated from *Rosmarinus officinalis* against multidrug-resistant bacterial strains. *Saudi Journal of Biological Sciences, 28*(1), 656–664. https://doi.org/10.1016/j.sjbs.2020.10.058

Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy, 48*(6), 5–16. https://doi.org/10.1093/jac/48.suppl_1.5

Antimicrobial Resistance Collaborators**.** (2024). Global burden of bacterial antimicrobial resistance 1990-2021: A systematic analysis with forecasts to 2050. *The Lancet, 404*(10459), 1199-1226. https://doi.org/10.1016/S0140-6736(24)01867-1

Berhe, D. F., Beyene, G. T., Seyoum, B., Gebre, M., Haile, K., Tsegaye, M., & Abdissa, A. (2021). Prevalence of antimicrobial resistance and its clinical implications in Ethiopia: A systematic review. *Antimicrobial Resistance & Infection Control, 10*, 1–14. https://doi.org/10.1186/s13756-021-00939-2.

Bissa, T., & Bohra, E. (2012). Antibacterial and antifungal studies of marigold (*Tagetes erectus*) leaf extracts. *Journal of Pharmacy Research, 3*(1), 58–64.

Bouyahya, A., Chamkhi, I., Benali, T., Guaouguaou, F.-E., Balahbib, A., El Omari, N., Taha, D., Belmehdi, O., Ghokhan, Z., & El Menyiy, N. (2022). Mechanisms of rosemary essential oil–antibiotic synergy against Gram-negative bacteria. *Phytomedicine, 104*, 154299. https://doi.org/10.1016/j.phymed.2022.154299

Debre Markos University Corporate Communication Directorate. (2018). *Geographic and climatic data of Debre Markos Town*

Dembecha Woreda Agriculture Development Office. (2018). *Agricultural and environmental profile of Dembecha Woreda*

ESCMID (European Society of Clinical Microbiology and Infectious Diseases). (2000). *Guidelines for the determination of minimum inhibitory concentrations (MICs) by agar dilution*.

Egharevba, R., & Ikhatua, M. (2008). Ethno-medical uses of plants in the treatment of various skin diseases in Ovia North East, Edo State, Nigeria. *International Journal of Advanced Research, 4*(1), 58–64.

Gachande, B., & Khillare, E. (2013). In vitro evaluation of *Datura* species for potential antimicrobial activity. *Journal of Pharmacognosy and Phytochemistry, 4*(1), 78–81.

Heatley, N. G. (1944). A method for the assay of penicillin. *Biochemical Journal, 38*(1), 61– 65.

Jembere, B. (2002). Evaluation of the toxicity potential of *Milletia ferruginea* (Hochest) Baker against *Sitophilus zamias* (Motsch.). *International Journal of Pest Management, 48*, 29–32. https://doi.org/10.1080/09670870110087483

Karunamoorthi, K., Bishaw, D., & Mulat, T. (2008). Laboratory evaluation of Ethiopian local plant *Phytolacca dodecandra* extract for its toxicity effectiveness against aquatic macroinvertebrates. *European Review for Medical and Pharmacological Sciences, 12*(6), 381–386.

Mawahib, E., Futooh, Z., Rahman, M., & Sanaa, O. (2012). Callus induction and antimicrobial activities of callus and intact plant extracts of *Datura stramonium* L. *International Journal of Science and Research, 3*(8), 1–5.

Mohan, N., Hanumantha, R., Ranjith, K., & Sivasubramanian, V. (2016). Mass cultivation of *C. turgidus* and *Oscillatoria* sp. and effective harvesting of biomass by low-cost methods. *International Journal of Advanced Research in Biomedical Sciences, 3*(2), 247–260.

Newire, E., Ahmed, S., & House, B. (2013). Detection of new SHV-12, SHV-5, and SHV-2a variants of extended-spectrum beta-lactamase in *Klebsiella pneumoniae* in Egypt. *Pakistan Journal of Biological Sciences, 12*(16), 1120–1125.

Priyanka, R., Srivastava, S., & Shukla, A. (2012). Antimicrobial properties of *Datura stramonium*: A review. *Journal of Ethnopharmacology, 142*(3), 123–130. https://doi.org/.

Reddy, B. U.(2009). Antimicrobial activity of *Datura stramonium* and *Tylophora indica* (Burm.f.) Merr. *Pharmacology Online, 1*, 1293–1300.

Saxena, M., Saxena, J., & Khare, S. (2023). Plant-derived antimicrobials in the fight against antibiotic resistance: Current status and future perspectives. *Frontiers in Microbiology, 14*, 1129823. https://doi.org/10.3389/fmicb.2023.1129823

Selvamohan, T., Ramadas, V., & Kishor, S. (2012). Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. *Journal of Advances in Applied Science Research, 3*(5), 3374–3381.

Shagal, M., Modibbo, U., & Liman, A. (2012). Pharmacological justification for the ethnomedical use of *Datura stramonium* stem-bark extract in treatment of diseases caused by some pathogenic bacteria. *Journal of Tropical Biomedicine, 2*(1), 16–19.

Shahwar, D., & Raza, M. (2009). In vitro antibacterial activity of extracts of *Mimusops elengi* against Gram-positive and Gram-negative bacteria. *African Journal of Microbiology Research, 3*(12), 960–964.

Solomon, G. (2014). Preliminary phytochemical screening and in vitro antimicrobial activity of *Datura stramonium* leaves extracts collected from Eastern Ethiopia. *Journal of Biological Sciences, 4*(1), 1–5.

Subbarayan, P., Sarkar, M., Impellizzeri, S., Raymo, F., Lokeshwar, B., Kumar, P., Agarwal, R., & Ardalan, B. (2010). Anti-proliferative and anti-cancer properties of *Achyranthes aspera*: Specific inhibitory activity against pancreatic cancer cells. *Indian Journal of Experimental Biology, 48*(1), 78–82.

Thippeswamy, S., Praveen, P., Mohana, D., & Manjunath, K. (2011). Antimicrobial evaluation and phytochemical analysis of known medicinal plant *Samanea saman* Merr. against some human and plant pathogenic bacteria and fungi. *Journal of Herbs, Spices & Medicinal Plants, 17*(4), 443–452. https://doi.org/10.1080/10496475.2011.632081

Woldu, M. A. (2024). *Antimicrobial resistance in Ethiopia: Current landscape, challenges, and strategic interventions* [Doctoral dissertation, Addis Ababa University].

World Health Organization. (2021). *Global action plan on antimicrobial resistance*. https://www.who.int/publications/i/item/9789241509763