SCIENTIFIC CULTURE
www.sci-cult.com

SCIENTIFIC CULTURE, Vol. 12, No. 4, (2026), pp. 5035-5050
Open Access. Online & Print

DOI: 10.5281/zenodo.12426548

QUANTITATIVE ANTIBACTERIAL PROFILING OF LEAF, BARK, AND ROOT EXTRACTS OF *SEARSIA RHEMANNIANA* USING STANDARDIZED MIC AND IC_{50} DETERMINATION

Rampana D.E.[1]*, Makhoahle P.M[2]

[1]Department of Health Sciences, Faculty of Health and Environmental Sciences
[2]Central University of Technology Free State, Bloemfontein, SOUTH AFRICA, Centre for Quality of Health and Living

Received: **28/12/2025**
Accepted: **02/03/2026**

Corresponding Author: Rampana D.E. (drampana@cut.ac.za)

Abstract

Antimicrobial resistance (AMR) is a major global public health concern that threatens the effective management of infectious diseases. The increasing resistance of clinically important pathogens such as Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae has intensified the search for alternative antimicrobial agents, particularly from medicinal plants with diverse bioactive compounds. This study evaluated the antibacterial activity of aqueous, dichloromethane (DCM), and methanolic (MeOH) extracts derived from leaf, bark, and root tissues of Searsia rhemanniana. Antibacterial activity was assessed using a broth microdilution assay with extract concentrations ranging from 7.8 to 1000 µg/mL. Minimum inhibitory concentration (MIC) and half-maximal inhibitory concentration (IC_{50}) values were determined against S.aureus ATCC 25923, K.pneumoniae ATCC 700603, and E. coli ATCC 25922. All experiments were performed in triplicate across three independent biological replicates. Polymyxin B sulfate, doxycycline, and kanamycin served as positive controls to validate assay performance. Results demonstrated a clear solvent- and strain-dependent activity profile. Methanolic extracts exhibited the greatest antibacterial activity, particularly against S. aureus ATCC 25923, with bark and root extracts showing IC_{50} values of approximately 60–125 µg/mL. Aqueous extracts displayed moderate inhibition, while DCM extracts were largely inactive. K. pneumoniae showed intermediate susceptibility, whereas E. coli exhibited pronounced resistance across all extract types. None of the extracts achieved bactericidal MIC thresholds within the tested concentration range, indicating predominantly bacteriostatic effects. These findings highlight the antibacterial potential of polar extracts of S.rhemanniana and support further bioassay-guided fractionation and phytochemical investigation for antimicrobial drug discovery.

KEYWORDS: Antimicrobial resistance; Searsia rhemanniana; medicinal plants; antibacterial activity; minimum inhibitory concentration (MIC); Gram-positive bacteria; Gram-negative bacteria.

1 INTRODUCTION

One of the major international health topics of concern, which has become one of the most severe global health issues, is antibacterial resistance (AMR) posing a threat to the successful prevention and treatment of infectious diseases (1). AMR is a major morbidity and mortality causative agent in all parts of the world, especially in low- and middle-income countries where the infectious diseases are still widespread (2). Toxic bacteria like *Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae) and Pseudomonas aeruginosa (P. aeruginosa)* have become resistant to the most prescribed antibiotics (3) leading to prolonged disease, high cost of healthcare, and deaths (4). The declining efficiency of currently available antibiotics has only heightened the hunt on other antimicrobial agents, including the ones that are natural (5). The traditional healthcare system is increasingly exploring medicinal plants as sources of new antibacterial compounds, given that medicinal plants have been used in traditional healthcare systems over centuries (6). The most promising antimicrobials are those derived in the plant kingdom, due to their chemical diversity, availability, and ability to counteract bacterial resistance mechanisms due to multi-target mechanisms (7).

Medicinal Plants and Antibacterial Activity

Medical plants produce a diverse array of secondary metabolites including flavonoids, phenolic acids, tannins, alkaloids and terpenoids many of which have antibacterial properties (8). Such compounds can have an antibacterial effect by destabilizing bacterial cell membranes, preventing protein synthesis, disrupting the replication of nucleic acids, or disrupting important metabolic pathways (9). Research has shown that crude extracts of plants or isolated plant phytochemicals can exert a high degree of inhibitory activity against Gram-positive and Gram-negative bacteria including the multidrug-resistant strains (10). There are also instances where plant extracts have exhibited synergistic activity with conventional antibiotics to increase their effectiveness as well as to lower the dosages administered (11). The results underline the recent popularity of medicinal plants as new sources of new antibacterial agents. Southern Africa is an especially biodiverse region as far as plant biodiversity and ethnomedicinal knowledge is concerned, though, several native species have not been studied scientifically yet (12). These plants should therefore conduct systematic antibacterial screening to validate traditional claims and come up with promising drug development candidates.

Minimum Inhibitory Concentration (MIC) as a Measure of Antibacterial Potency

Measuring the MIC is an element of antimicrobial pharmacology (13). It gives a reproducible and quantitative measure of the lowest concentration which a test substance prevents the growth of visible bacterial growth which allows researchers to classify extracts as active, moderately active, or inactive by predetermined concentrations (14). It is also a method of making comparisons across studies and can be used to create a database of MIC values of different plant extracts, which is a valuable resource in prioritizing those who will proceed to further pharmacological development (15). With the scourge of emerging drug resistance, recognition of plant extracts that have demonstrated high antibacterial activity may spur more research on their mechanisms of action, possible synergism with antibiotics, and future possibilities of its translation to therapeutic products (16). The conventional therapies are still at risk due to antibiotics resistance, and thus new alternative sources of antimicrobials like phytochemicals could provide alternative solutions to effective and accessible treatment (17).

The phytochemical studies on various species of *Searsia* have shown that they possess considerable amounts of flavonoids, phenolic acids, tannins, and other secondary metabolites that have been strongly related to the antibacterial and antioxidant effects (18). As an illustration, the plants, *Searsia leptodictya* and *Searsia undulata*, have been reported to contain gallic acid and protocatechuic acid that have been recorded to have antimicrobial activity in vitro (19). Generally, the genus *Searsia* can be described as having a multifaceted phytochemical profile that is likely to be useful in a variety of bioactivities, including those of antibacterial activity, so it can serve as an attractive target in future research (20). Published studies on *Searsia rhemanniana* are lacking. This gap indicates that to ascertain whether *S. rhemanniana* extracts show similar or better inhibitory action on common and drug-resistant strains of bacteria, systematic evaluation of MIC values of *S. rhemannia* extracts against selected bacterial strains is both timely and scientifically sound. This study would help to broaden the pharmacological research of the *Searsia* genus, confirm or refute possible traditional applications, and find new plant-based antibacterial leads. Moreover, setting up MIC values offers a platform of upcoming investigations to focus on bioassay guided fractionation, mechanism of action investigation, as well as prospective therapeutic progression.

2 MATERIALS AND METHODS

Plant material

The identification of the plant material was done by botanists at the botanical garden in KwaZulu-Natal, South Africa as *S. rhemanniana*. After the verification, the plant material was carefully washed using distilled water to get rid of soil and debris. The plant was then divided into its major parts of anatomy, which were roots, bulbs, and leaves. All the parts of the plants were dried in an oven with ventilation at a temperature between 30 and 60 °C to allow a steady dehydration and an inactivation of phytochemicals. After complete drying, the material was crushed coarsely in a hammer mill and kept at room temperature in airtight containers until they were extracted.

Extract preparation

Fine powder plant material was obtained through an IKA grinder (IKA Labortechnik, Germany) at the laboratory of the Central University of Technology. Then powdered was now taken to the bioassaix (in vitro screening of drugs) lab to continue processing. At bioassay (in vitro drug screening) lab powdered plant material the extraction of compounds in methanol (MeOH), dichloromethane (DCM), and water (H_2O) in a ratio of about 1:4 (w/v) was also done. The maceration was put on a shaker (Labcon, Lab Design Engineering, Maraisburg, South Africa) and allowed to run 72 hours. After extraction, the mixture was filtered in vacuum filtration system, using Whatman No. 1 filter paper (Merck Chemicals (Pty) Ltd, Wadeville, South Africa). This was done until the filtrate became clear. Under low pressure, the organic solvents (MeOH and DCM) were eliminated using a BÜCHI rotovapor (Labotec (Pty) Ltd, Halfway House, South Africa) and the extracts (remained as organic) were dried at room temperature under a fume hood and stored at 4 °C. Aqueous extract was then frozen at -80 C and freeze-dried to a powder, and then kept in 4 °C.

Sample preparation

The extractions were done in the solvents of aqueous, dichloromethane (DCM) and methanol (MeOH). To obtain the necessary level of solubility, all the lyophilized extracts were resolubilized in dimethyl sulfoxide (DMSO) to a standardized working concentration of 70 mg/mL and sonicated in 30 min before use. The first positive control to screen MIC was polymyxin B sulfate, whereas doxycycline and kanamycin were added as extended positive controls to ensure the quality of the assay and validate its performance.

Bacterial strain and culture conditions

Activity against three bacteria clinically relevant as reference strains (*K. pneumoniae* ATCC 700603, a β-lactamase producing bacterium with the SHV-18 gene; *S. aureus* ATCC 25923, a well-characterized, methicillin sensitive quality control bacterium commonly used as a standard in antimicrobial susceptibility testing and measurement of bacterial growth; and Escherichia coli ATCC 25922, a drug-s Stocks of all strains of bacteria were transferred to tryptic soy agar (TSA; Sigma-Aldrich) and left to incubate overnight at 37 °C. One, well isolated colony per plate was incubated in 20 mL of Mueller Hinton broth (MHB; Sigma-Aldrich) and left to incubate for 18 h at 37 °C with vigorous agitation (250 rpm). The next day, subcultures were prepared, inoculating fresh MHB at a 1:99 dilution and incubating further at 37 °C to get actively growing cultures. Bacterial suspensions were then standardized to a 0.5 McFarland standard of turbidity, and it was approximated that 1 × 108CFU/mL. The entire bacterial manipulation was performed under the containment of biosafety level 2 (BSL-2).

Minimal inhibitory concentration (MIC) assay

To determine the minimum inhibitory concentrations (MICs) of the plant extracts, a two-fold serial dilution series was prepared in Mueller–Hinton broth (Sigma-Aldrich) using 96-well round-bottom microtiter plates (Greiner Bio-One), yielding final extract concentrations ranging from 7.8 to 1000 µg/mL. Each diluted extract was inoculated with a standardized bacterial suspension to a final assay volume of 100 µL per well. All samples were tested in triplicates within each plate, and the entire experiment was conducted across three independent biological replicates. Microtiter plates were incubated at 37 °C for 18 h under static conditions. Appropriate experimental controls as shown below were incorporated to verify assay validity. To minimize evaporative effects and maintain consistent incubation conditions, all plates were placed in a humidified chamber containing distilled water and incubated at 37 °C for a total of 24 h.

Table 1: Types of controls, contents and purpose

Code	Control Type	Contents	Purpose / Relevance
PC	Positive Control	Known antibiotic + bacteria	Validates assay performance; reference for inhibition
GC	Growth Control	Bacteria only	Defines 100% growth baseline
SC	Sterility Control	Medium only (no bacteria)	Checks sterility; ensures no contamination
VC	Vehicle Control	Solvent + bacteria	Ensure solvent has no inhibitory effect
PC+	Extended Positive Control	Duplicate/alternative antibiotic	Monitors consistency and reproducibility

Microtiter experimental plates readout and analysis

The bacterial growth measured in terms of culture turbidity was measured using a Tecan Infinite 500 microplate reader. The optical density values at 620 nm bandwidth of 10 nm with an integration setting of 10 flashes per well were measured. Microsoft Excel was used to process raw data. The values of minimum inhibitory concentration (MIC) and half-maximal inhibitory concentration (IC50) of each sample were estimated using measures of percent viability and percent inhibition. Percentage viability obtained was:

%Viability = 100 × (Extract − mean sterile control) / (mean growth control − mean sterile control), and percentage inhibition was calculated as:

%Inhibition = 100 − %Viability.

All graphical analyses and data visualizations were generated using GraphPad Prism and Microsoft Excel.

3 RESULTS

Assay performance validation

The MIC assay is controlled in a highly discriminatory, biologically coherent, and consistent way across all the bacterial strains tested thus supporting the strength of the assay (Figure 1). Growth controls produced the most uniformly generated metabolic signals, which were confirmation of optimal culture conditions and a good normalization reference, whereas vehicle controls were like growth controls in each strain, which indicated that no significant solvent-related antibacterial or cytotoxic effects existed. Strain dependent slight decreases in vehicle control signals were present but quite minor, reproducible, and biologically insignificant compared to the intense inhibition of positive controls. The difference in the extent of overlap between growth and vehicle controls inter-strain variability showed the presence of inherent physiological differences as opposed to artifacts of the assay making it important to assess the control as strain specific. The use of Doxycycline and Kanamycin always resulted in significant growth inhibition of all strains, easily separating itself by both negative control and positive activity against the assay sensitivity and range, and the selective inhibition of both antibiotics in and among strains conformed to the established mechanisms of action and activity spectrum. All three components preserved hierarchy of control (growth control > vehicle control ≫ positive controls), low background interference and reproducible strain-appropriate responses indicate high assay discrimination capacity and technical stability to provide confidence that any inhibitory effect was due to test compounds as well as to compare antimicrobial screening between strains of interest.

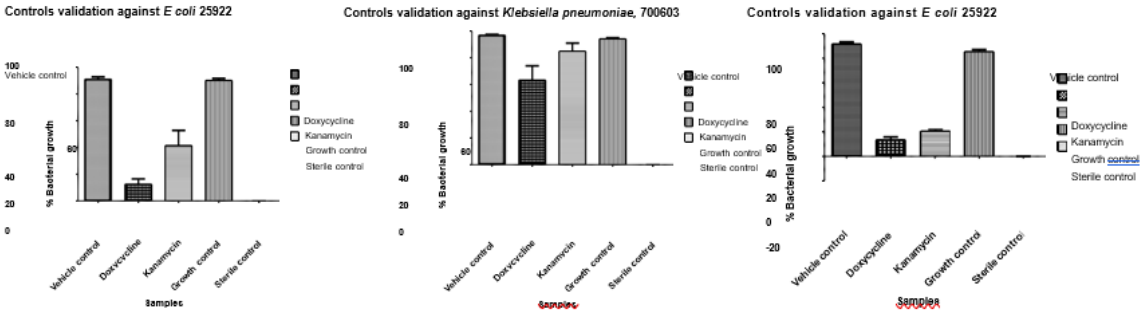

Figure 1: Control performance in the CUTMIC assay across bacterial strains.

Growth controls showed maximal metabolic activity, while vehicle controls closely matched growth with minor strain-dependent deviations. Doxycycline and kanamycin produced clear, strain-specific growth inhibition. The consistent separation between negative

MIC/IC50 determination

The subsequent tables and figures proved a comprehensive analysis of the MIC and IC50 estimated values for the various plant extracts (Aqueous, DCM, and MeOH) against the three bacterial strains: *E coli,* 25922, *klebsiella pneumoniae, 700603, and Staphylococcus aureus,* 25923.

Table 2: MIC/IC50 assessment of the aqueous extract vs E. coli ATCC 25922

Sample ID	Organism	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	MIC	IC50
Leaf (Aqueous)	*E. coli*, 25922	3	Absorbance	1000	83.19	16.81	> 1000	> 1000
		3	Absorbance	500	79.69	20.31		
		3	Absorbance	250	81.58	18.42		
		3	Absorbance	125	85.01	14.99		

		3	Absorbance	62.5	91.24	8.76		
		3	Absorbance	31.25	95.85	4.15		
		3	Absorbance	15.625	98.59	1.41		
		3	Absorbance	7.8125	100.00	0.00		
Bark (Aqueous)	*E. coli*, 25922	3	Absorbance	1000	83.96	16.04	> 1000	1000
		3	Absorbance	500	82.06	17.94		
		3	Absorbance	250	82.07	17.93		
		3	Absorbance	125	82.41	17.59		
		3	Absorbance	62.5	86.52	13.48		
		3	Absorbance	31.25	93.36	6.64		
		3	Absorbance	15.625	97.20	2.80		
		3	Absorbance	7.8125	97.48	2.52		
Root (Aqueous)	*E. coli*, 25922	3	Absorbance	1000	85.59	14.41	> 1000	> 1000
		3	Absorbance	500	82.52	17.48		
		3	Absorbance	250	82.58	17.42		
		3	Absorbance	125	83.51	16.49		
		3	Absorbance	62.5	87.76	12.24		
		3	Absorbance	31.25	93.91	6.09		
		3	Absorbance	15.625	97.03	2.97		
		3	Absorbance	7.8125	98.97	1.03		
Polymyxin B Sulfate	*E. coli*, 25922	3	Absorbance	1000	6.81	93.19	< 1000	<1000
		3	Absorbance	500	8.30	91.70		
		3	Absorbance	250	8.02	91.98		
		3	Absorbance	125	6.70	93.30		
		3	Absorbance	62.5	5.34	94.66		
		3	Absorbance	31.25	4.49	95.51		
		3	Absorbance	15.625	48.51	51.49		
		3	Absorbance	7.8125	95.43	4.57		

Across the tested concentration range (7.81–1000 µg/mL) as shown in Table 2 and Figure 2, the maximum inhibition observed was ~20.3% at 500 µg/mL. No concentration achieved near-complete growth inhibition (commonly ≥90% inhibition or absence of visible growth). MIC was therefore *not reached* within the tested range and is reported as: MIC > 1000 µg/mL. Additionally, A 50% inhibition threshold was not achieved at any tested concentration. Even at the highest concentration (1000 µg/mL), inhibition remained below 25%. Curve fitting would require substantial extrapolation beyond the experimental data and would not be scientifically robust. The IC_{50} was therefore reported as: IC_{50} > 1000 µg/mL.

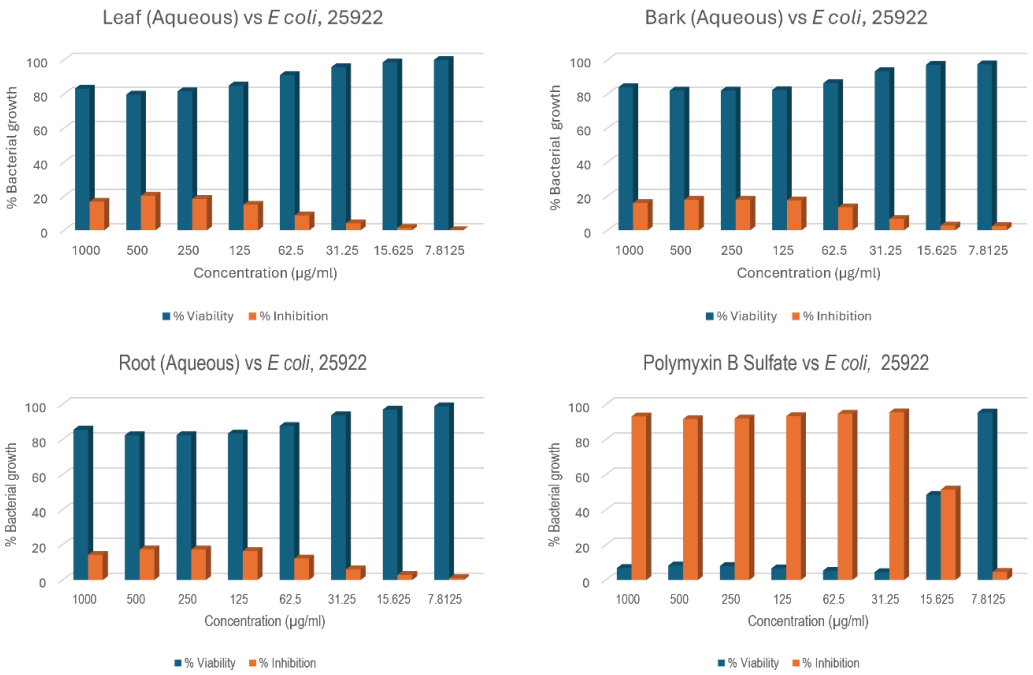

Figure 2: % viability and % inhibition assessment of the aqueous extract vs E. coli ATCC 25922

Table 3: MIC/IC50 assessment of the DCM extract vs E. coli ATCC 25922

Sample ID	Organism	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	MIC	IC50
Leaf (DCM)	*E. coli, 25922*	3	Absorbance	1000	100.00	0.00	N/A	N/A
		3	Absorbance	500	91.55	8.45		
		3	Absorbance	250	91.12	8.88		
		3	Absorbance	125	90.93	9.07		
		3	Absorbance	62.5	88.36	11.64		
		3	Absorbance	31.25	86.47	13.53		
		3	Absorbance	15.625	85.31	14.69		
		3	Absorbance	7.8125	84.32	15.68		
Bark (DCM)	*E. coli, 25922*	3	Absorbance	1000	100.00	0.00	N/A	N/A
		3	Absorbance	500	99.63	0.37		
		3	Absorbance	250	96.61	3.39		
		3	Absorbance	125	91.90	8.10		
		3	Absorbance	62.5	94.43	5.57		
		3	Absorbance	31.25	96.68	3.32		
		3	Absorbance	15.625	93.90	6.10		
		3	Absorbance	7.8125	93.10	6.90		
		3	Absorbance	1000	99.73	0.27		
		3	Absorbance	500	98.81	1.19		
		3	Absorbance	250	94.77	5.23		
Root (DCM)	*E. coli, 25922*	3	Absorbance	125	96.04		3.96	N/A
		3	Absorbance	62.5	97.35		2.65	
		3	Absorbance	31.25	96.22		3.78	
		3	Absorbance	15.625	93.64		6.36	
		3	Absorbance	7.8125	93.34		6.66	
Polymyxin B Sulfate	*E. coli, 25922*	3	Absorbance	1000	6.03		93.97	< 1000
		3	Absorbance	500	7.73		92.27	
		3	Absorbance	250	7.13		92.87	
		3	Absorbance	125	6.76		93.24	
		3	Absorbance	62.5	5.59		94.41	
		3	Absorbance	31.25	4.44		95.56	
		3	Absorbance	15.625	25.57		74.43	
		3	Absorbance	7.8125	94.20		5.80	

Table 3 and Figure 3 demonstrated that the DCM leaf, bark, and root extracts had low antibacterial activity against E. coli ATCC 25922 over the whole concentration range (7.8125-1000 µg/mL). No ≥50% growth inhibition of any concentration was obtained in none of the extracts and the highest values of inhibition were relatively low; about 15.7% in the leaf extract, ≤8.1% in the bark extract and ≤6.7% in the root extract. Therefore, no values of MIC or IC_{50} were obtained (N/A) in all three DCM extracts, which implies that the inhibitory strength was not meaningfully significant in the conditions of the assay. Conversely, the positive control of polymyxin B sulfate showed good antibacterial activity, with >90 percent being inhibited at most concentrations; though, according to the data sets criteria, its MIC and IC 50 Could well be reported as >1000 µg/mL, which is the reporting cutoff used in the current analysis.

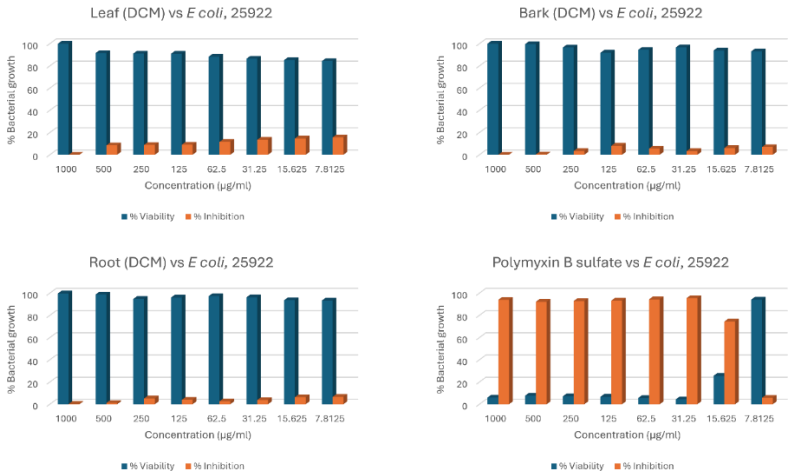

Figure 3: % viability and % inhibition assessment of the DCM extract vs E. coli ATCC 25922

Table 4: MIC/IC50 assessment of the Methanol (MeOH) extract vs E. coli ATCC 25922

Sample ID	Organism	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	MIC	IC50
Leaf (MeOH)	*E. coli, 25922*	3	Absorbance	1000	93.82	6.18	> 1000	> 1000
		3	Absorbance	500	81.77	18.23		
		3	Absorbance	250	80.49	19.51		
		3	Absorbance	125	85.94	14.06		
		3	Absorbance	62.5	94.14	5.86		
		3	Absorbance	31.25	100.00	0.00		
		3	Absorbance	15.625	99.78	0.22		
		3	Absorbance	7.8125	97.51	2.49		
Bark (MeOH)	*E. coli, 25922*	3	Absorbance	1000	82.38	17.62	> 1000	> 1000
		3	Absorbance	500	81.49	18.51		
		3	Absorbance	250	81.26	18.74		
		3	Absorbance	125	82.40	17.60		
		3	Absorbance	62.5	84.31	15.69		
		3	Absorbance	31.25	94.08	5.92		
		3	Absorbance	15.625	98.76	1.24		
		3	Absorbance	7.8125	99.77	0.23		
Root (MeOH)	*E. coli, 25922*	3	Absorbance	1000	95.84	4.16	> 1000	> 1000
		3	Absorbance	500	86.20	13.80		
		3	Absorbance	250	84.86	15.14		
		3	Absorbance	125	85.76	14.24		
		3	Absorbance	62.5	88.78	11.22		
		3	Absorbance	31.25	96.31	3.69		
		3	Absorbance	15.625	99.32	0.68		
		3	Absorbance	7.8125	99.88	0.12		
Polymyxin B Sulfate	*E. coli, 25922*	3	Absorbance	1000	6.48	93.52	<1000	<1000
		3	Absorbance	500	7.47	92.53		
		3	Absorbance	250	7.01	92.99		
		3	Absorbance	125	6.63	93.37		
		3	Absorbance	62.5	5.47	94.53		
		3	Absorbance	31.25	4.41	95.59		
		3	Absorbance	15.625	34.29	65.71		
		3	Absorbance	7.8125	94.16	5.84		

The MeOH extracts had low antibacterial activity to E. coli ATCC 25922s over the entire concentration range studied (7.81-1000 µg/mL), which is comparable to the aqueous leaf extract; the highest inhibition was approximately 19.51 percent at 250 µg/mL (Table 4 and Figure 4). No concentration was able to achieve near-complete growth inhibition (typically 90% or more growth inhibition, or no growth observed at all). The indicator of MIC is MIC > 1000 µg/mL because it was not achieved in the measured range. Moreover, the inhibition threshold was not achieved at any concentration tested (50%). At the highest concentration (1000 µg/mL), it was not inhibited more than 20%. Curving over would not be a scientifically good idea and would require a lot of extrapolations of the confines of the experiments. Due to this, the IC50 had been reported as IC50 > 1000 µg/mL.

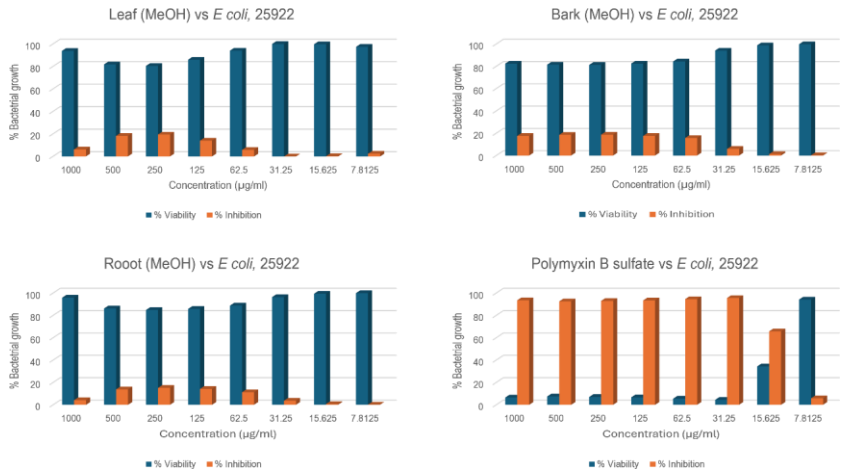

Figure 4: % viability and % inhibition assessment of the MeOH extract vs E. coli ATCC 25922

Table 5: MIC and IC_{50} of Aqueous Extracts vs K. pneumoniae 700603

Sample ID	*Organism*	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	*MIC*	*IC50*
Leaf (Aqueous)	*K. pneumoniae, 700603*	3	Absorbance	1000	53.50	46.50	>1000	>1000
		3	Absorbance	500	54.52	45.48		
		3	Absorbance	250	53.42	46.58		
		3	Absorbance	125	67.23	32.77		
		3	Absorbance	62.5	75.61	24.39		
		3	Absorbance	31.25	85.93	14.07		
		3	Absorbance	15.625	90.98	9.02		
		3	Absorbance	7.8125	91.79	8.21		
Bark (Aqueous)	*K. pneumoniae, 700603*	3	Absorbance	1000	51.92	48.08	>1000	~500
		3	Absorbance	500	48.70	51.30		
		3	Absorbance	250	52.97	47.03		
		3	Absorbance	125	62.49	37.51		
		3	Absorbance	62.5	72.93	27.07		
		3	Absorbance	31.25	81.76	18.24		
		3	Absorbance	15.625	86.62	13.38		
		3	Absorbance	7.8125	89.95	10.05		
		3	Absorbance	1000	52.52	47.48		
		3	Absorbance	500	46.50	53.50		
		3	Absorbance	250	51.11	48.89		
Root (Aqueous)	*K. pneumoniae, 700603*	3	Absorbance	125	61.66	38.34	>1000	~500
		3	Absorbance	62.5	71.66	28.34		
		3	Absorbance	31.25	82.28	17.72		
		3	Absorbance	15.625	87.67	12.33		
		3	Absorbance	7.8125	91.07	8.93		
Polymyxin B Sulfate	*K. pneumoniae, 700603*	3	Absorbance	1000	8.14	91.86	<1000	<1000
		3	Absorbance	500	5.97	94.03		
		3	Absorbance	250	5.14	94.86		
		3	Absorbance	125	5.47	94.53		
		3	Absorbance	62.5	5.29	94.71		
		3	Absorbance	31.25	4.74	95.26		
		3	Absorbance	15.625	4.65	95.35		
		3	Absorbance	7.8125	78.05	21.95		

None of the aqueous plant extracts achieved ≥90% growth inhibition across the tested concentration range (1000–7.81 µg/mL); therefore, no MIC was reached for leaf, bark, or root extracts (Table 5 and Figure 5). In contrast, the positive control (polymyxin B sulfate) demonstrated clear bactericidal activity with an MIC ≤1000 µg/mL. Leaf aqueous extract reached Maximum inhibition of ~46–47% at 1000–250 µg/mL Inhibition decreased progressively with dilution; MIC was not reached with IC_{50} not reached (IC_{50} >1000 µg/mL) Bark aqueous extract Inhibition exceeded 50% at 500 µg/mL (~51.3%). Lower concentrations showed a clear dose-dependent decline, MIC was not reached with IC_{50}: ~500 µg/mL. Root aqueous extract showed comparable profile to bark, with ~53.5% inhibition at 500 µg/mL.Sub-50% inhibition at ≤250 µg/mL, MIC was not reached with IC_{50}: ~500 µg/mL.

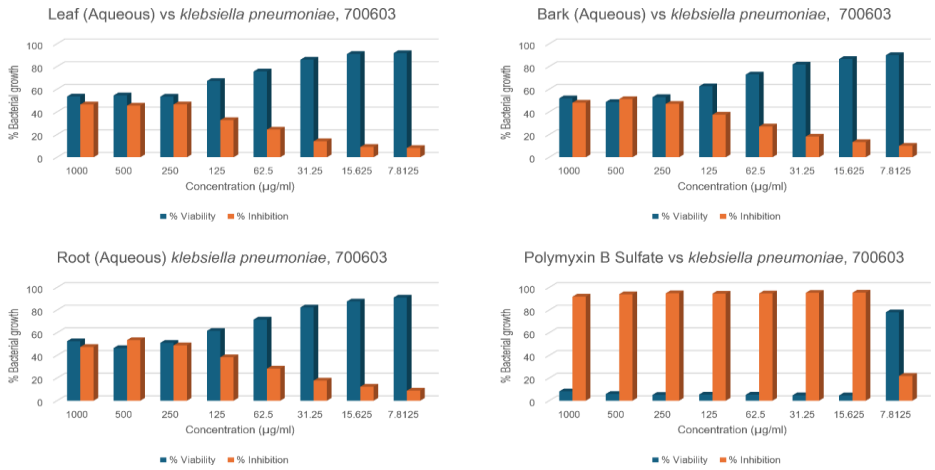

Figure 5: % viability and % inhibition of Aqueous Extracts vs K. pneumoniae 700603

Table 6: MIC and IC_{50} of DCM Extracts vs K. pneumoniae 700603

Sample ID	*Organism*	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	*MIC*	*IC50*
Leaf (DCM)	*K. pneumoniae*, 700603	3	Absorbance	1000	89.56	10.44	N/A	N/A
		3	Absorbance	500	90.88	9.12		
		3	Absorbance	250	93.17	6.83		
		3	Absorbance	125	95.94	4.06		
		3	Absorbance	62.5	95.68	4.32		
		3	Absorbance	31.25	95.29	4.71		
		3	Absorbance	15.625	95.24	4.76		
		3	Absorbance	7.8125	96.70	3.30		
Bark (DCM)	*K. pneumoniae, 700603*	3	Absorbance	1000	97.49	2.51	N/A	N/A
		3	Absorbance	500	96.13	3.87		
		3	Absorbance	250	92.46	7.54		
		3	Absorbance	125	87.36	12.64		
		3	Absorbance	62.5	92.37	7.63		
		3	Absorbance	31.25	93.52	6.48		
		3	Absorbance	15.625	93.66	6.34		
		3	Absorbance	7.8125	94.17	5.83		
		3	Absorbance	1000	87.41	12.59		
		3	Absorbance	500	88.99	11.01		
		3	Absorbance	250	89.14	10.86		
Root (DCM)	*K. pneumoniae, 700603*	3	Absorbance	125	88.94	11.06	N/A	N/A
		3	Absorbance	62.5	92.89	7.11		
		3	Absorbance	31.25	93.05	6.95		
		3	Absorbance	15.625	91.09	8.91		
		3	Absorbance	7.8125	96.57	3.43		
Polymyxin B Sulfate	*K. pneumoniae, 700603*	3	Absorbance	1000	9.80	90.20	<1000	<1000
		3	Absorbance	500	6.92	93.08		
		3	Absorbance	250	6.21	93.79		
		3	Absorbance	125	6.51	93.49		
		3	Absorbance	62.5	6.03	93.97		
		3	Absorbance	31.25	4.88	95.12		
		3	Absorbance	15.625	4.84	95.16		
		3	Absorbance	7.8125	80.98	19.02		

DCM extracts (leaf, bark, and root) demonstrated weak antibacterial activity against *K. pneumoniae* 700603 across the full tested concentration range (1000–7.81 µg/mL), (Table 6 and Figure 6). None of the extracts achieved ≥50% inhibition at any concentration, with maximal inhibition remaining low (≤12.6%). Consequently, MIC values could not be determined for any of the DCM extracts, and IC_{50} values were not reached, indicating that bacterial viability remained high (>85–95%) even at the highest concentrations tested. Among the extracts, bark and root DCM extracts showed marginally higher inhibition at 1000–125 µg/mL, but these effects were not concentration-dependent and remained biologically insignificant.

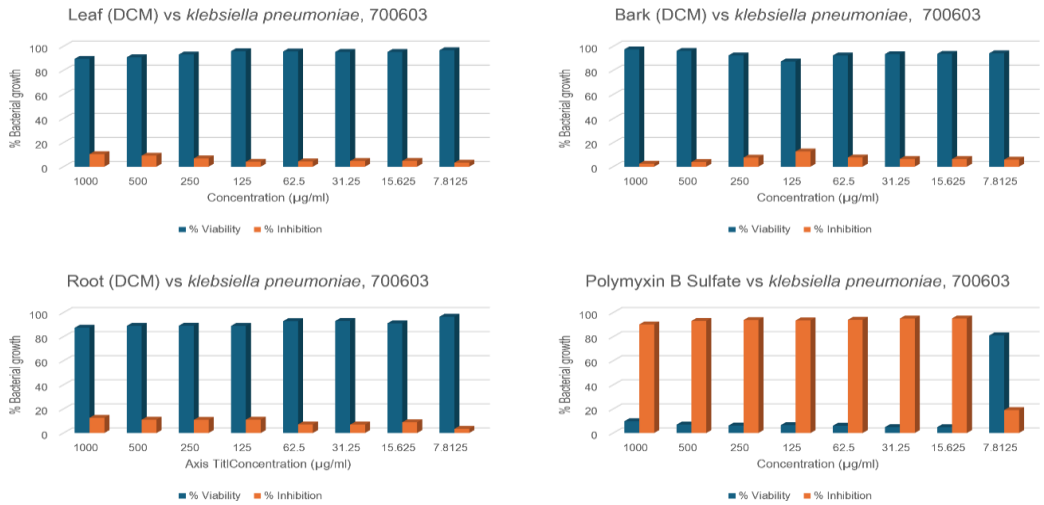

Figure 6: % viability and % inhibition of DCM Extracts vs K. pneumoniae 700603

Table 7: MIC and IC_{50} of MeOH Extracts vs K. pneumoniae 700603

Sample ID	Organism	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	MIC	IC50
		3	Absorbance	1000	61.93	38.07		
Leaf (MeOH)	*K. pneumoniae, 700603*	3	Absorbance	500	47.45	52.55	>1000	≈300–400
		3	Absorbance	250	54.12	45.88		
		3	Absorbance	125	69.63	30.37		
		3	Absorbance	62.5	81.52	18.48		
		3	Absorbance	31.25	87.90	12.10		
		3	Absorbance	15.625	90.33	9.67		
		3	Absorbance	7.8125	94.28	5.72		
Bark (MeOH)	*K. pneumoniae, 700603*	3	Absorbance	1000	51.23	48.77	>1000	≈ 450–550
		3	Absorbance	500	36.39	63.61		
		3	Absorbance	250	43.16	56.84		
		3	Absorbance	125	57.49	42.51		
		3	Absorbance	62.5	69.46	30.54		
		3	Absorbance	31.25	81.65	18.35		
		3	Absorbance	15.625	86.71	13.29		
		3	Absorbance	7.8125	91.55	8.45		
Root (MeOH)	*K. pneumoniae, 700603*	3	Absorbance	1000	61.89	38.11	>1000	≈ 400–500
		3	Absorbance	500	44.28	55.72		
		3	Absorbance	250	49.86	50.14		
		3	Absorbance	125	63.87	36.13		
		3	Absorbance	62.5	74.11	25.89		
		3	Absorbance	31.25	83.65	16.35		
		3	Absorbance	15.625	87.42	12.58		
		3	Absorbance	7.8125	91.90	8.10		
Polymyxin B Sulfate	*K. pneumoniae, 700603*	3	Absorbance	1000	11.25	88.75	≤500	≤500
		3	Absorbance	500	6.48	93.52		
		3	Absorbance	250	5.71	94.29		
		3	Absorbance	125	6.31	93.69		
		3	Absorbance	62.5	5.79	94.21		
		3	Absorbance	31.25	4.63	95.37		
		3	Absorbance	15.625	22.55	77.45		
		3	Absorbance	7.8125	74.24	25.76		

MeOH extracts from leaf, bark, and root tissues exhibited moderate, concentration dependent on antibacterial activity against *K. pneumoniae* 700603 (Table 7 and Figure 7). Across all three extracts, MIC values were not reached within the tested concentration range (≤1000 µg/mL), as none achieved ≥90% growth inhibition. Bark MeOH was the most active plant extract, reaching a maximum inhibition of ~63.6% at 500 µg/mL, with an estimated IC_{50} ≈ 300–400 µg/mL. Leaf MeOH showed moderate inhibition, peaking at ~52.6% at 500 µg/mL, corresponding to an IC_{50} ≈ 450–550 µg/mL. Root MeOH displayed comparable but slightly weaker activity, with ~55.7% inhibition at 500 µg/mL and an IC_{50} ≈ 400–500 µg/mL.

In contrast, the positive control polymyxin B sulfate demonstrated potent antibacterial activity, achieving >90% inhibition at ≤500 µg/mL and a clear MIC ≤500 µg/mL, validating assay sensitivity and confirming the comparatively lower potency of the MeOH extracts.

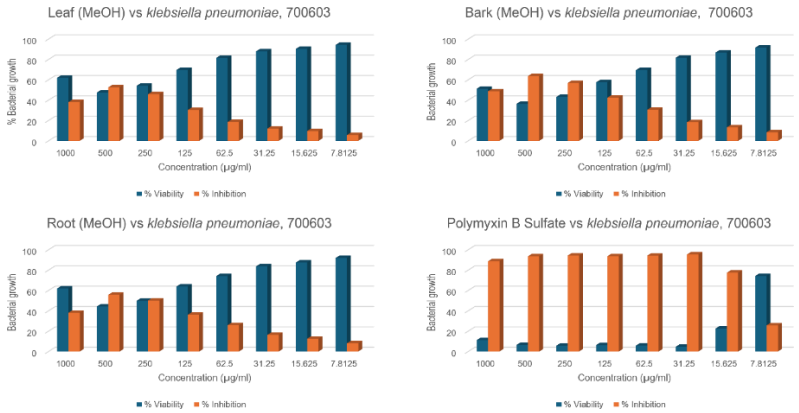

Figure 7: % viability and % inhibition of MeOH Extracts vs K. pneumoniae 700603

Table 8: MIC and IC_{50} Summary of Aqueous extracts vs Staphylococcus aureus, 25923

Sample ID	*Organism*	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	*MIC*	*IC50*
Leaf (Aqueous)	*S. aureus, 25923*	3	Absorbance	1000	57.93	42.07	>1000	~250 – 500
		3	Absorbance	500	49.84	50.16		
		3	Absorbance	250	46.45	53.55		
		3	Absorbance	125	60.25	39.75		
		3	Absorbance	62.5	71.00	29.00		
		3	Absorbance	31.25	87.02	12.98		
		3	Absorbance	15.625	99.58	0.42		
		3	Absorbance	7.8125	99.99	0.01		
Bark (Aqueous)	*S. aureus, 25923*	3	Absorbance	1000	40.15	59.85	>1000	~125 – 250
		3	Absorbance	500	48.08	51.92		
		3	Absorbance	250	48.28	51.72		
		3	Absorbance	125	44.61	55.39		
		3	Absorbance	62.5	48.94	51.06		
		3	Absorbance	31.25	61.38	38.62		
		3	Absorbance	15.625	83.45	16.55		
		3	Absorbance	7.8125	100.00	0.00		
Root (Aqueous)	*S. aureus, 25923*	3	Absorbance	1000	36.90	63.10	>1000	~125 – 250
		3	Absorbance	500	37.55	62.45		
		3	Absorbance	250	39.55	60.45		
		3	Absorbance	125	45.08	54.92		
		3	Absorbance	62.5	52.09	47.91		
		3	Absorbance	31.25	60.61	39.39		
		3	Absorbance	15.625	78.47	21.53		
		3	Absorbance	7.8125	100.00	0.00		
Polymyxin B Sulfate	*S. aureus, 25923*	3	Absorbance	1000	4.58	95.42	<500	<500
		3	Absorbance	500	5.98	94.02		
		3	Absorbance	250	26.83	73.17		
		3	Absorbance	125	38.21	61.79		
		3	Absorbance	62.5	68.98	31.02		
		3	Absorbance	31.25	96.82	3.18		
		3	Absorbance	15.625	98.15	1.85		
		3	Absorbance	7.8125	95.13	4.87		

None of the aqueous plant extracts achieved ≥90% growth inhibition within the tested concentration range (7.8–1000 µg/mL), (Table 8 and Figure 8). Consequently, no definitive MIC was reached for any aqueous extract, and MIC values are interpreted as >1000 µg/mL. In contrast, concentration-dependent inhibition trends allowed estimation of IC_{50} values. The leaf aqueous extract produced moderate inhibition, reaching ~54% inhibition at 250 µg/mL and ~50% at 500 µg/mL, with reduced activity at lower concentrations. The IC_{50} is therefore estimated between 250 and 500 µg/mL, while the MIC was not reached (>1000 µg/mL). The bark aqueous extract showed slightly stronger activity than the leaf extract, exceeding 50% inhibition at 125–250 µg/mL. Accordingly, the IC_{50} is estimated between 125 and 250 µg/mL, with no MIC observed within the tested range (>1000 µg/mL). The root aqueous extract displayed the highest inhibitory effect among the aqueous extracts, consistently producing >54% inhibition from 125 to 1000 µg/mL. The IC_{50} is estimated between 125 and 250 µg/mL, while the MIC remained >1000 µg/mL.

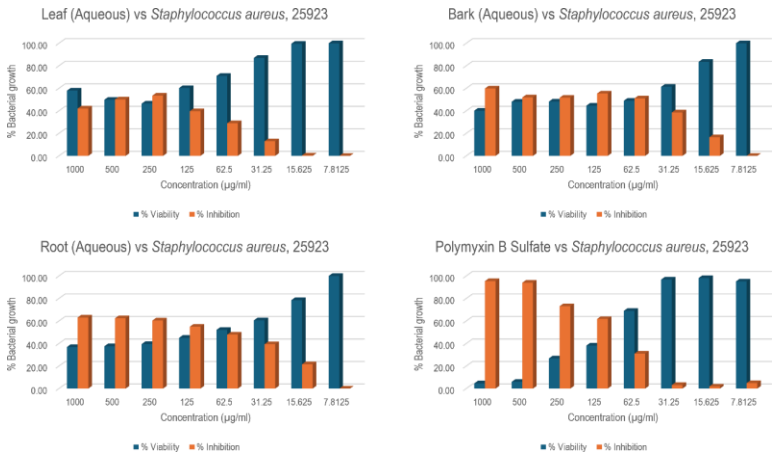

Figure 8: % viability and % inhibition Summary of Aqueous extracts vs Staphylococcus aureus, 25923

Table 9: MIC and IC_{50} of Dichloromethane (DCM) Extracts vs S. aureus, 25923

Sample ID	*Organism*	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	*MIC*	*IC50*
Root (DCM)	*S. aureus, 25923*	3	Absorbance	62.5	90.71	9.29	N/A	N/A
		3	Absorbance	31.25	89.98	10.02		
		3	Absorbance	15.625	83.03	16.97		
		3	Absorbance	7.8125	73.29	26.71		
Polymyxin B Sulfate	*S. aureus, 25923*	3	Absorbance	1000	3.46	96.54	< 250	< 250
		3	Absorbance	500	3.60	96.40		
		3	Absorbance	250	19.83	80.17		
		3	Absorbance	125	35.24	64.76		
		3	Absorbance	62.5	87.84	12.16		
		3	Absorbance	31.25	94.98	5.02		
		3	Absorbance	15.625	100.00	0.00		
		3	Absorbance	7.8125	95.42	4.58		

Across all DCM extracts (leaf, bark, and root), no minimum inhibitory concentration (MIC) or half-maximal inhibitory concentration (IC_{50}) could be determined within the tested concentration range (7.8–1000 µg/mL), (Table 9 and Figure 9). The leaf DCM extract demonstrated an unprecedented highest activity among the plant-derived samples, with a maximum inhibition of ~68.6% observed only at the highest concentration (1000 µg/mL), while inhibition at lower concentrations remained ≤20%. Bark and root DCM extracts exhibited consistently weak antibacterial effects, generally producing ≤27% inhibition even at the lowest tested concentrations, and no clear dose–response sufficient to define MIC or IC_{50} values.

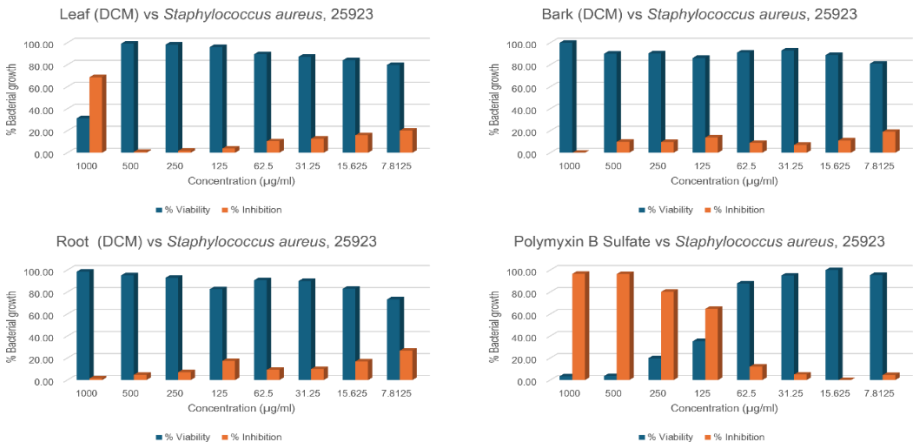

Figure 9: % viability and % inhibition Summary of DCM extracts vs Staphylococcus aureus, 25923

Table 10: MIC and IC_{50} Summary of MeOH extracts vs Staphylococcus aureus ATCC 25923

Sample ID	Organism	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	MIC	IC50
Leaf (DCM)	*S. aureus, 25923*	3	Absorbance	1000	31.36	68.64	N/A	N/A
		3	Absorbance	500	99.16	0.84		
		3	Absorbance	250	98.24	1.76		
		3	Absorbance	125	96.10	3.90		
		3	Absorbance	62.5	89.55	10.45		
		3	Absorbance	31.25	87.20	12.80		
		3	Absorbance	15.625	84.07	15.93		
		3	Absorbance	7.8125	79.85	20.15		
Bark (DCM)	*S. aureus, 25923*	3	Absorbance	1000	100.00	0.00	N/A	N/A
		3	Absorbance	500	90.04	9.96		
		3	Absorbance	250	90.30	9.70		
		3	Absorbance	125	86.14	13.86		
		3	Absorbance	62.5	91.02	8.98		
		3	Absorbance	31.25	92.88	7.12		
		3	Absorbance	15.625	88.87	11.13		
		3	Absorbance	7.8125	81.00	19.00		
		3	Absorbance	1000	98.54	1.46		
		3	Absorbance	500	95.16	4.84		
		3	Absorbance	250	92.91	7.09		
		3	Absorbance	125	82.57	17.43		

Sample ID	Organism	Replicates	Readout	Concentration (µg/ml)	% Viability	% Inhibition	MIC	IC50
		3	Absorbance	1000	41.51	58.49		
		3	Absorbance	500	44.17	55.83		
		3	Absorbance	250	45.09	54.91		
		3	Absorbance	125	49.82	50.18		
Leaf (MeOH)	*S. aureus, 25923*	3	Absorbance	62.5	77.21	22.79	>1000	~125
		3	Absorbance	31.25	96.28	3.72		
		3	Absorbance	15.625	100.00	0.00		
		3	Absorbance	7.8125	98.08	1.92		
		3	Absorbance	1000	36.21	63.79		
		3	Absorbance	500	28.53	71.47		
		3	Absorbance	250	31.11	68.89		
		3	Absorbance	125	35.51	64.49		
Bark (MeOH)	*S. aureus, 25923*	3	Absorbance	62.5	41.81	58.19	>1000	~60–125
		3	Absorbance	31.25	57.47	42.53		
		3	Absorbance	15.625	83.50	16.50		
		3	Absorbance	7.8125	100.00	0.00		
		3	Absorbance	1000	45.67	54.33		
		3	Absorbance	500	26.93	73.07		
		3	Absorbance	250	35.53	64.47		
		3	Absorbance	125	39.02	60.98		
Root (MeOH)	*S. aureus, 25923*	3	Absorbance	62.5	45.65	54.35	>1000	~60–125
		3	Absorbance	31.25	61.37	38.63		
		3	Absorbance	15.625	87.95	12.05		
		3	Absorbance	7.8125	100.00	0.00		
		3	Absorbance	1000	3.91	96.09		
		3	Absorbance	500	5.72	94.28		
		3	Absorbance	250	20.04	79.96		
		3	Absorbance	125	30.55	69.45		
Polymyxin B Sulfate	*S. aureus, 25923*	3	Absorbance	62.5	86.12	13.88	<250	<250
		3	Absorbance	31.25	98.65	1.35		
		3	Absorbance	15.625	100.00	0.00		
		3	Absorbance	7.8125	96.35	3.65		

As reported in table 10 and Figure 10, none of the MeOH plant extracts achieved ≥90% growth inhibition within the tested concentration range (7.8–1000 µg/mL), indicating MIC values >1000 µg/mL for all three extracts. The leaf MeOH extract showed moderate activity, reaching ~50% inhibition at 125 µg/mL, corresponding to an IC_{50} ≈125 µg/mL. The bark MeOH extract exhibited stronger inhibition at higher concentrations (≈64–71% at 125–500 µg/mL), with an estimated IC_{50} between ~60–125 µg/mL. The root MeOH extract demonstrated comparable moderate potency, achieving ~55–73% inhibition at 62.5–500 µg/mL, yielding an IC_{50} in the ~60–125 µg/mL range. As expected, the positive control polymyxin B sulfate displayed potent activity with ≥90% inhibition at low concentrations (MIC within the tested range), validating assay performance.

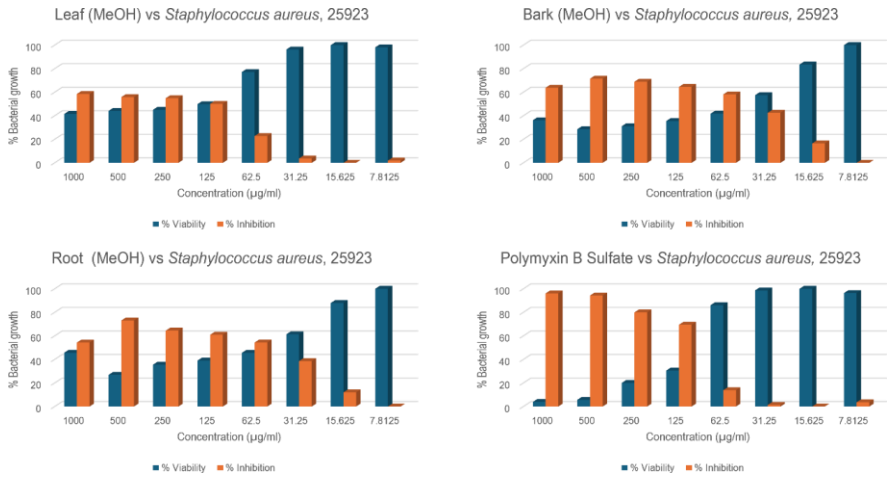

Figure 10: % viability and % inhibition Summary of MeOH extracts vs Staphylococcus aureus, 25923

Across the antibacterial screening figures 2-10, clear solvent- and strain dependent susceptibility patterns were observed among *S. aureus*, *E. coli*, and *K. pneumoniae*. Overall, MeOH extracts exhibited the

strongest antibacterial activity, with *S. aureus* remaining the most susceptible strain, showing the highest levels of growth inhibition. Notably, *K. pneumoniae* displayed a more pronounced inhibitory response to the solvent extracts than *E. coli*, particularly for the MeOH extracts, indicating greater sensitivity of *K. pneumoniae* to the extracted bioactive constituents. Aqueous extracts demonstrated moderate antibacterial activity, again with *S. aureus* as the most susceptible strain, while *K. pneumoniae* showed consistently greater inhibition than *E. coli*, albeit at lower levels than those observed for MeOH extracts. In contrast, DCM extracts exhibited the weakest antibacterial effects overall, with low inhibition across all strains; however, even under these conditions, *K. pneumoniae* tended to be slightly more susceptible than *E. coli*, reinforcing the relative resistance of *E. coli* across solvent systems. Importantly, polymyxin, included as a positive control, consistently induced near-complete inhibition across all tested strains, validating assay robustness and providing a reliable benchmark for extract performance. Collectively, these results highlight the strong influence of extraction solvents on antibacterial efficacy and demonstrate that, across solvent systems, *K. pneumoniae*. is more susceptible than *E. coli*, while MeOH extracts show the greatest overall antibacterial potential, particularly against *S. aureus*.

4 CONCLUSION

This paper shows that methanol plant extracts have been the most potent in terms of antibacterial properties, especially against *S. aureus* ATCC 25923 and moderate activity can be observed with *K. pneumoniae* ATCC 700603. As a contrast, *E. coli* ATCC 25922 demonstrated to be highly resistant against all the extracts tested. The screening of *S.rhemaniana* aqueous, DCM and MeOH extracts of leaves, barks and root tissues showed a clear solvent- and strain-dependency profile. Overall, MeOH extraction showed the highest antibacterial activity, aqueous extracts had intermediate activity whereas DCM extracts had very limited activity. *S. aureus* ATCC 25923 was the most sensitive of the tested organisms, followed by *K. pneumoniae* ATCC 700603 which was intermediate, and *E. coli* ATCC 25922 was the most resistant of all solvent systems. The DCM leaf, bark and root extracts in all the three bacterial strains were largely inactive to all three bacterial strains since it was unable to reach either MIC or IC_{50} values in the concentration range of 7.8-1000 mcg/mL. The same trends were observed with aqueous, DCM and MeOH extracts against *E. coli* ATCC 25922 that had weak activity (less than 25 percent) suggesting intrinsic resistance of this strain and no significant antibacterial effect under the test conditions. The best antibacterial activity was recorded with MeOH extracts against *S. aureus* ATCC 25923, especially the MeOH extracts of bark and roots, and they recorded IC_{50} of the range of approximately 60-125 µg/m. Aqueous root and bark extracts were also moderately concentration-effectively inhibiting *S. aureus* and *K. pneumoniae*, with IC_{50} values between 125-500 µg/m. The MeOH extracts, particularly the bark, showed consistent mid-range inhibitory activity against *K. pneumoniae*, making it possible to identify this strain as a second susceptible target, to polar solvent extracts. Excellent sensitivity and performance in the assays were ensured by the addition of polymyxin B sulfate, together with doxycycline and kanamycin. Polymyxin B uniformly provided close to full (>90%) growth inhibition with all strains with minimal concentrations, creating a strong reference standard. It was confirmed that the distinct separation of growth, vehicle and positive controls confirmed the strength of the assays, reproducibility and appropriateness to estimate MIC and IC_{50}. Among the most important findings is that Extraction solvent was a critical factor of antibacterial activity, where MeOH, finally by DCM, was used as the solvent. *S. aureus*, gram-positive organism was the most vulnerable; the highest and continuous inhibition was observed. *K. pneumoniae* was more sensitive than the *E. coli* especially to the MeOH and aqueous extracts. None of the plant extracts reached bactericidal levels of MIC (≥90% inhibition) in the tested range, which presented mostly bacteriostatic or moderate levels of inhibition. The seen patterns of activity were dose-dependent and repeatable, making them biologically relevant as opposed to artefact of assay. Even though the extracts under the current conditions did not achieve conclusive MIC values, several MeOH and aqueous extracts exhibited IC_{50} values in the biologically relevant ranges, which is sufficient to justify additional bioassay-directed fractionation, phytochemical characterization and expanded antimicrobial profiling. In general, the work has a sound, well-managed background.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to the Faculty of Health and Environmental Sciences assistant research office for their support and assistance. We also extend our thanks to the Department of Higher Education and Training (DHET) through the University Capacity Development Programme (UCDP) Grant for providing the funding that made the publication of this study possible.

FUNDING

There was no funding or nonprofit entities of the paper.

DISCLOSURE

None.

AUTHORS CONTRIBUTION

The authors have made significant contributions to the article.

ETHICAL CONSIDERATION

No ethical consideration was required for this study as it does not involve any interventions with humans or animals.

DATA AVAILABILITY

All relevant data supporting the findings of this article are included within the manuscript. No supplementary materials are required.

REFERENCES

Salam, M.A., Al-Amin, M.Y., Salam, M.T., Pawar, J.S., Akhter, N., Rabaan, A.A. and Alqumber, M.A., 2023, July. Antimicrobial resistance: a growing serious threat for global public health. *In Healthcare* (Vol. 11, No. 13, p. 1946). MDPI.

Sulis, G., Sayood, S. and Gandra, S., 2022. Antimicrobial resistance in low-and middle-income countries: current status and future directions. *Expert review of anti-infective therapy*, 20(2), pp.147-160.

Mancuso, G., Midiri, A., Gerace, E. and Biondo, C., 2021. Bacterial antibiotic resistance: the most critical pathogens. *Pathogens*, 10(10), p.1310.

Pal, M., Kerorsa, G.B., Marami, L.M. and Kandi, V., 2020. Epidemiology, pathogenicity, animal infections, antibiotic resistance, public health significance, and economic impact of staphylococcus aureus: a comprehensive review. *American Journal of Public Health Research*, 8(1), pp.14-21.

Ghosh, C., Sarkar, P., Issa, R. and Haldar, J., 2019. Alternatives to conventional antibiotics in the era of antimicrobial resistance. *Trends in microbiology*, 27(4), pp.323-338.

Abdallah, E.M., Alhatlani, B.Y., de Paula Menezes, R. and Martins, C.H.G., 2023. Back to nature: Medicinal plants as promising sources for antibacterial drugs in the post-antibiotic era. *Plants*, 12(17), p.3077.

Begum, S. and Shrivastava, S., 2024. Bioactive Compounds: Characterization And Mechanistic Research Examining the Opportunities and Difficulties in the Development of Antimicrobial and Antiviral Agents based on Plants. *International Journal of Multiphysics*, 18(3).

Hussein, R.A. and El-Anssary, A.A., 2019. Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. *Herbal medicine*, 1(3), pp.11-30.

Donadio, G., Mensitieri, F., Santoro, V., Parisi, V., Bellone, M.L., De Tommasi, N., Izzo, V. and Dal Piaz, F., 2021. Interactions with microbial proteins driving the antibacterial activity of flavonoids. *Pharmaceutics*, 13(5), p.660.

Kebede, T., Gadisa, E. and Tufa, A., 2021. Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PloS one*, 16(3), p.e0249253.

Saquib, S.A., AlQahtani, N.A., Ahmad, I., Kader, M.A., Al Shahrani, S.S. and Asiri, E.A., 2019. Evaluation and comparison of antibacterial efficacy of herbal extracts in combination with antibiotics on periodontal pathobionts: an in vitro microbiological study. *Antibiotics*, 8(3), p.89.

Tiwari, S., 2025. Ethnobotanical Documentation and Antimicrobial Potential of Indigenous Flora.

Wenzler, E., Maximos, M., Asempa, T.E., Biehle, L., Schuetz, A.N. and Hirsch, E.B., 2023. Antimicrobial susceptibility testing: An updated primer for clinicians in the era of antimicrobial resistance: Insights from the Society of Infectious Diseases Pharmacists. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, 43(4), pp.264-278.

Hossain, T.J., 2024. Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. *European Journal of Microbiology and Immunology*, 14(2), pp.97-115.

Porras, G., Chassagne, F., Lyles, J.T., Marquez, L., Dettweiler, M., Salam, A.M., Samarakoon, T., Shabih, S., Farrokhi, D.R. and Quave, C.L., 2020. Ethnobotany and the role of plant natural products in antibiotic drug discovery. *Chemical reviews*, 121(6), pp.3495-3560.

AL-Azzawi, M.K., Hasan, N.A. and Barrak, M.M., 2024. A review of the development of an understanding of antibiotic interactions, from mechanisms of action to novel resistance and the search for natural alternatives. *Journal of Medical Genetics and Clinical Biology*, 1(6), pp.78-102.

Khare, T., Anand, U., Dey, A., Assaraf, Y.G., Chen, Z.S., Liu, Z. and Kumar, V., 2021. Exploring phytochemicals

for combating antibiotic resistance in microbial pathogens. *Frontiers in pharmacology*, 12, p.720726.

Hawu, O., Mokoboki, H.K., Lebopa, C.K., Mkhize, Z. and Ravhuhali, K.E., 2025. The proximate and secondary metabolites of the leaf fodder of Searsia species are affected by seasons in the North West province of South Africa. *Agroforestry Systems*, 99(4), p.79.

Koki, M., Yalo, M., Makhaba, M., Nako, N., Rautenbach, F., Badmus, J.A., Marnewick, J., Hussein, A.A. and Mabusela, W.T., 2022. Phytochemical investigation and biological studies on selected Searsia species. *Plants*, 11(20), p.2793.

Majoang, D., Ogwuegbu, M.C., Mthiyane, D.M., Singh, M. and Onwudiwe, D.C., 2025. Green synthesis and evaluation of the anticancer potency of Ag, Cuo, and Ag/Cuo nanoparticles synthesized using Searsia lancea fruit extract. *Discover Materials*, 5(1), p.149.