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Original article

HPLC Profiling of Phenolic Compounds and Flavonoids and Comparative *In Vitro* Antibacterial Activity of *Vachellia karroo* Leaf Extract from Northeastern Libya

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Keywords.
Vachellia Karroo, Antibacterial Activity, HPLC, Phenolic Compounds, Flavonoids.

ABSTRACT

Vachellia karroo is a medicinal plant traditionally used in Africa, but its antibacterial potential and phytochemical constituents in northeastern Libya remain insufficiently investigated. The study aimed to evaluate the antibacterial activity of an 80% ethanolic leaf extract of *V. karroo*, compare its effects with conventional antibiotics, and characterize its phenolic and flavonoid constituents using high-performance liquid chromatography (HPLC). Leaves collected from Shahat, northeastern Libya, were extracted using a cooled maceration method. Antibacterial activity against Gram-positive and Gram-negative bacterial strains, including reference strains and clinical isolates, was assessed using agar well diffusion and minimum inhibitory concentration (MIC) assays. We compared the extract with amoxicillin-clavulanate, cephalexin, imipenem, and vancomycin. HPLC identified eight phenolic compounds and seven flavonoids. Catechol (13.32 µg/ml) and syringic acid (11.45 µg/ml) were the predominant phenolics, while naringin (15.45 µg/ml) and rutin (8.60 µg/ml) were the major flavonoids. The extract showed variable antibacterial activity, with the largest inhibition zone against *Staphylococcus aureus* (23 ± 3.0 mm), followed by clinical *Pseudomonas aeruginosa* (20 ± 7.0 mm) and *Escherichia fergusonii* (18 ± 6.0 mm). The smallest zone was observed against *Acinetobacter baumannii* (13 ± 2.0 mm). Activity exceeded that of selected antibiotics against certain isolates. The results report that the extract demonstrated variable *in vitro* antibacterial activity and diverse phytochemical constituents, warranting further investigation to identify active compounds and clarify its antibacterial potential.

Introduction

Medicinal plants continue to offer promising scaffolds for the development of new drugs due to their diverse phytochemical profiles and broad spectrum of biological activities [1]. Among the ethnobotanically significant species is *Vachellia karroo (Hayne) Banfi & Galasso (*Previously named *Acacia karroo)*. Recent advances in molecular phylogenetics and plant systematics have significantly changed the classification of Acacia sensu lato, and researchers have identified several separate taxa. Most of the African "Acacia" genus is now grouped into Vachellia and Senegalia. Some are still grouped into Acacia, Acaciella, and Mariosousa. This is because of international naming rules, and it is now largely accepted in systematic botany [2,3,4]. People sometimes call the Vachellia genus "thorn trees." This genus is diverse and occurs in Africa, Asia, and Australia [5].

Vachellia karroo is a member of the Fabaceae family and stands out for its extensive use in traditional medicine across Africa. It is widely distributed in sub-Saharan and North African regions, including Libya. This tree is traditionally employed to treat wounds, diarrhea, respiratory infections, and inflammatory conditions [6], and also for decoration.

On the other hand, the escalating global crisis of antimicrobial resistance (AMR) has underscored the urgent need for the discovery and development of novel antibacterial agents. Resistant bacterial pathogens, including methicillin-resistant *Staphylococcus aureus (*MRSA*), Enterobacterales*, extended-spectrum beta-lactamase (ESBL)-producing bacteria, and carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, are increasingly prevalent in clinical settings, rendering many conventional antibiotics ineffective [7,8]. This growing therapeutic challenge has redirected scientific interest toward natural products, particularly plant-derived compounds, which have historically served as a rich source of antimicrobial agents. Antimicrobial resistance (AMR) is a major global health concern that has increased interest in identifying new sources of antibacterial agents. Medicinal plants contain diverse secondary metabolites, including phenolic acids and flavonoids, that may contribute to antibacterial activity. Investigating the antibacterial potential of plant extracts against clinically relevant bacterial isolates and reference strains may provide a basis for further natural-product research. However, the activity of a plant extract should be distinguished from the antibiotic susceptibility profile of the tested bacteria.

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Phytochemical investigations of many plants belonged to Fabaceae family have revealed that it contains a wide array of bioactive secondary metabolites, such as tannins, flavonoids, alkaloids, saponins, and phenolic acids, all of which are associated with antimicrobial, antioxidant, and immunomodulatory properties [9,10,11]. Despite the documented traditional applications and preliminary phytochemical data, there remains a paucity of rigorous scientific evaluation after the reclassification, particularly regarding the antibacterial efficacy of Libyan ecotypes of *V. karroo* against clinically relevant bacterial isolates. Furthermore, no previous studies have correlated the antimicrobial activity of this Libyan species with detailed phytochemical profiling using high-performance liquid chromatography (HPLC), a critical step in linking biological effects to specific bioactive constituents [12].

Recent trends in ethnopharmacology emphasize the importance of validating traditional remedies through standardized methodologies, including *in vitro* antimicrobial assays and chemical fingerprinting, to ensure reproducibility and translational potential [13]. In this context, this study evaluates the antibacterial activity of *V. karroo* leaf extract collected from north-eastern Libya against a panel of Gram-positive and Gram-negative bacteria, including reference strains and clinical isolates. Concurrently, HPLC analysis was employed to identify and quantify key phenolic and flavonoid constituents, providing a chemical basis for the observed bioactivity. The findings contribute to the growing body of evidence supporting the pharmacological value of *V. karroo* and highlight its potential as a source of lead compounds in the fight against drug-resistant pathogens.

Although *Vachellia karroo* has been investigated for its phytochemical composition and antibacterial properties, evidence concerning leaf extracts collected from northeastern Libya remains limited. Specifically, the extract's HPLC-based phenolic and flavonoid profile, as well as its antibacterial efficacy against a wide panel of Gram-positive and Gram-negative reference strains and clinical isolates, have not been sufficiently described. Thus, the purpose of this study was to assess the *in vitro* antibacterial activity of an 80% ethanolic leaf extract of *V. karroo*, compare its inhibitory effects with those of specific reference antibiotics, ascertain its minimum inhibitory concentrations (MICs), and use HPLC to identify its main phenolic and flavonoid constituents.

Materials and Methods

Chemicals and Reagents

Solvents used for the HPLC experiment were of chromatographic grade (Merck, South Korea). Phenolic acid standards (including Catechol, syringic acid, cinnamic acid, caffeic acid, salicylic acid, pyrogallol, ferulic acid, and chlorogenic acid) and flavonoid standards (including Naringin, rutin, luteolin, catechin, quercetin, apigenin, and kaempferol) were purchased from Sigma Chemical Co. (USA). Antibiotic discs including amoxicillin-clavulanate (AMC, 30 µg), cephalexin (30 µg), imipenem (IPM, 10 µg), and vancomycin (VA, 10 µg) were obtained from Al-Akeed Laboratory, Benghazi, Libya. Nutrient agar and Mueller-Hinton agar (MHA) used for bacterial culture, 0.5 McFarland standard used for calibration and inoculum preparation, and ethanol (80%) used as the extraction solvent are materials sourced from the Department of Biomedical Sciences, Faculty of Pharmacy, Omar Al-Mukhtar University, Libya.

Plant Collection and Identification

Leaves of *Vachellia karroo* were collected in June 2024 from the suburban areas of Shahat City, northeastern Libya. The plant was authenticated and registered by the taxonomist at the Department of Plant Sciences, Faculty of Science, Omar Al-Mukhtar University, and voucher specimens were deposited at the Silphium Herbarium at the Faculty of Science of the same university. The collected leaves were washed thoroughly with distilled water, air-dried at room temperature away from direct sunlight, and ground into a fine powder using an electric blender. The resulting powder was stored in airtight glass containers at 4°C, ready for the extraction process.

Extraction Procedure

The crude extract was prepared using the cold maceration method. Briefly, 50 g of dried leaf powder was macerated in 500 mL of 80% ethanol (v/v) with intermittent shaking for 72 hours. The mixture was filtered through cotton wool followed by Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator (Büchi R-210) to obtain a concentrated crude extract.

HPLC Analysis of Phenolic Compounds and Flavonoids

An Agilent Series 1100 HPLC system (Agilent Technologies, USA), equipped with an autosampler, solvent degasser, dual LC pumps, ChemStation software, and a UV/Vis detector, was used for phytochemical profiling. Separation was achieved on a reversed-phase C18 column (125 mm × 4.60 mm, 5 µm particle size) maintained at 25°C. A gradient elution program was employed for phenolic acids using solvent A (methanol) and solvent B (aqueous acetic acid, 1:25 v/v). Detection was set at 250 nm, and the gradient profile was as follows: 0–3 min, 100% B; 3–8 min, linear increase to 50% A; 8–10 min, increase to 80% A; 10–15 min, return to 50% A. For flavonoids, isocratic elution was conducted with acetonitrile (A) and 0.2% (v/v) aqueous formic acid (B) at a ratio of 70:30. The flow rate was 1.0 mL/min, injection volume was 25 µL, and detection was performed at 360 nm. Identification of compounds was based on retention times and peak areas compared

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to reference standards. Quantification was carried out using external calibration curves, and results were expressed as µg per mL of extract.

Antibacterial Assay

Bacterial Strains and Culture Conditions

The following bacterial isolates were included in this study: *Staphylococcus aureus* (clinical), *Staphylococcus argenteus* DSM 28299, *Escherichia fergusonii* ATCC 3569, *Klebsiella pneumoniae* (clinical). *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* (clinical isolate), and *Acinetobacter baumannii* (clinical). All strains were cultured on nutrient agar slant and maintained at 4°C. Fresh suspensions were prepared in sterile saline 0.9% and adjusted to the 0.5 McFarland standard (~1.5×10^8 CFU/mL) before testing.

Agar Well Diffusion Method

The antibacterial activity of the crude extract (100 mg/ml) was evaluated using the agar well diffusion method as described by Mukhtar and Ghori [14]. Briefly, 100 µL of standardized bacterial inoculum was spread onto Mueller-Hinton agar plates. Wells (7 mm diameter) were created using a sterile cork borer and filled with 100 µL of the test extract. Plates were pre-diffused for 5 minutes and incubated aerobically at 37°C for 24 hours. The diameter of inhibition zones (IZD) was measured in millimeters (mm) using digital calipers. Each assay was performed in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined via the agar well diffusion method according to Andrews [15]. Six two-fold serial dilutions of the extract (50, 25, 12.5, 6.25, 3.125, and 1.56 µg/mL) were prepared in sterile saline. Each dilution (100 µl) was introduced into pre-punched wells on MHA plates seeded with the respective bacterial suspension. After incubation at 37°C for 24 h, inhibition zones were measured. The lowest concentration showing visible inhibition was recorded as the MIC.

Evaluation of Standard Antibiotics

The agar well diffusion method was used to evaluate the antibacterial activity of the standard antibiotics. The discs were placed on MHA plates inoculated with each bacterial strain. Plates were incubated at 37°C for 24 h, and inhibition zones were measured. Interpretation of susceptibility was based on CLSI (2023) guidelines: <9 mm = weak, 10–12 mm = moderate, ≥13 mm = strong inhibition.

Statistical Analysis

Data are expressed as mean ± standard deviation (SD) of three independent replicates. One-way ANOVA followed by Tukey's post-hoc test was used to compare inhibition zones among treatments. Differences were considered statistically significant at $P < 0.05$. Statistical analyses were performed using SPSS version 26 (IBM Corp., USA).

Results

Phenolic and flavonoid Compounds of V. karroo Leaf Ethanol Extract

V. karroo leaf ethanol extract was analyzed by HPLC for the identification and quantification of eight different phenolic acids viz., Catechol, syringic acid, cinnamic acid, caffeic acid, salicylic acid, pyrogallol, ferulic acid, and chlorogenic acid, and seven flavonoid compounds viz., Naringin, rutin, luteolin, catechin, quercetin, apigenin, and kaempferol. The related representative HPLC chromatograms of phenolic acids and flavonoids are clearly revealed in (Figure 1). The phytochemical analysis revealed that the *V. karroo* extract's most abundant phenolic acid in µg/mL was catechol, with a concentration of 13.32, followed by syringic acid (11.45) and cinnamic acid (7.61). At the same time, naringin was present with the highest concentration (15.40 µg/mL) in flavonoid compounds, followed by rutin (8.60), and luteolin (7.48) (Table 1).

Antibacterial Activity of V. karroo Leaf Ethanol Extract

The examined bacteria showed differing degrees of antibacterial activity. At 23mm ± 3.0, *Staphylococcus aureus* had the largest mean inhibition zone, followed by the clinical isolate of *Pseudomonas aeruginosa* (20mm ± 7.0), *Escherichia fergusonii* ATCC 3569 (18mm ± 6.0), and *K. pneumoniae* with a 17mm ± 1.1 inhibition zone. On the other hand, *Staphylococcus argentous* DSM 28299 and the standard *P. aeruginosa* strain showed lower mean inhibition zones of 14mm ± 0.6, while *Acinetobacter baumannii* showed the lowest mean inhibition zone of 13mm ± 2.0 (Table 3). The studied bacterial isolates revealed different MIC values for *Vachellia karroo* extract. *Escherichia fergusonii, Acinetobacter baumannii*, and *Klebsiella pneumoniae* demonstrated the lowest inhibition at 25 mg, while *Staphylococcus aureus* showed the lowest inhibition at 6.25 mg. *Staphylococcus argentous DSM (28299)* was inhibited at an MIC of 6 µg, while a concentration of 100 µg was the MIC of both standard and clinical *Pseudomonas aeruginosa* isolates.

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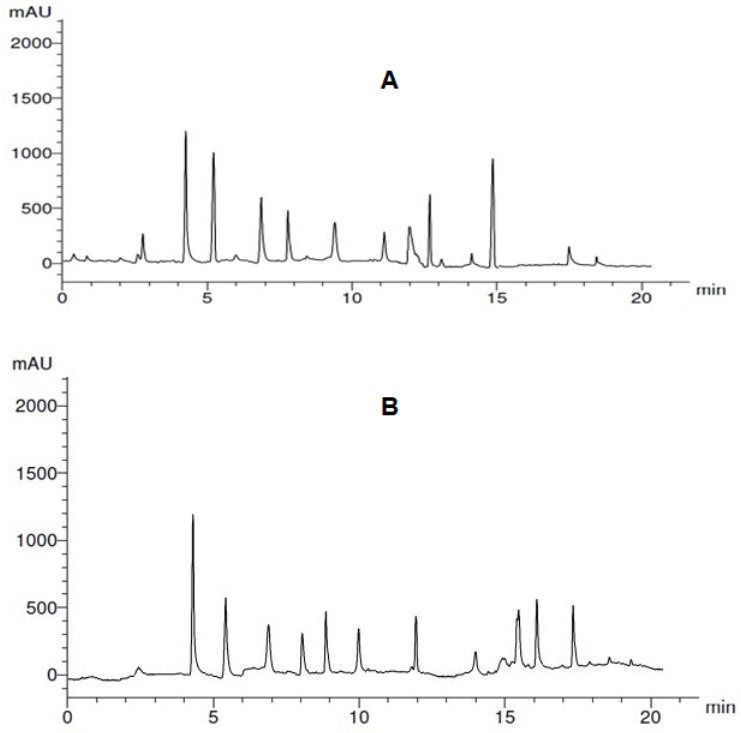

Figure 1. HPLC chromatograms of A: phenolic acids and B: Flavonoid compounds identified in the V. karroo leaf etanol extract.

Table 1. Concentrations (µg/mL) of phenolic and flavonoid compounds in the V. karroo leaf ethanol extract quantified using HPLC

Phenolic acids			Flavonoid compounds		
RT	**Compound**	**Concentration**	**RT**	**Compound**	**Concentration**
2.9	Chlorogenic	1.40	4.6	Naringin	15.45
4.0	Catechol	13.32	5.2	Rutin	8.60
5.0	Syringenic	11.45	7.0	Quercetin	5.49
7.0	Cinnamic	7.61	8.1	Kampferol	4.66
8.0	Caffeic	5.48	9.0	Luteolin	7.48
9.4	Pyrogallol	4.21	10.0	Apegenin	5.49
11.0	Ferulic	2.35	12.01	Catechin	6.78
12.0	Salicylic	4.72			

Table 3. Antibacterial Activity of the 80% Ethanol Leaf Extract of Vachellia karroo Against the Tested Bacterial Isolates.

Tested Isolates	Number of replicates	Mean ± SD
Staphylococcus argentous DSM (28299)	3	14±0.6
Staphylococcus aureus	3	23±3.0
Escherichia fergusonii (ATCC 3569)	3	18±6.0
Acinetobacter baumannii	3	13±2.0
Pseudomonas aeruginosa (standard)	3	14±0.6
Pseudomonas aeruginosa (clinical)	3	20±7
Klebsiella pneumonia	3	17±1.1

Antibacterial Activity of Extract versus Antibiotics: References

The antibacterial activity of the 80% ethanolic *V. karroo* leaf extract (100mg/ml) was compared with the inhibition zones produced by selected reference antibiotics against the tested bacterial isolates and reference strains. The revealed inhibition zones varied significantly (P = 0.00) among bacterial species and between the extract and individual antibiotics.

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Among Gram-positive isolates, for *S. argenteus* DSM 28299, the extract produced lower inhibition zone than those of amoxicillin–clavulanate (31 ± 2.8 mm), cephalexin (16 ± 0.5 mm), imipenem (35 ± 0.0 mm), and vancomycin (39 ± 1.2 mm), while against *S. aureus*, the extract exhibited larger inhibition zone compared with that of amoxicillin–clavulanate (13 ± 1.5 mm) and cephalexin (0 ± 0.0 mm), while its inhibition zone was lower than imipenem (35 ± 0.0 mm) and vancomycin (40 ± 0.0 mm) as presented in (Figure 2).

Table 4. Antibacterial Activity (Diameter of inhibition zones in mm) of V. karroo leaf extract versus antibiotic references against tested bacterial isolates.

Tested Isolates	Extract 100mg/ml	AMC 30µg	Cephalexin 30 µg	IPM 10 µg	VA 10 µg
S. argentous DSM (28299)	14±0.6^{c}	31±2.8^{b}	16±0.5^{d}	35±0.0^{b}	39±1.2^{a}
S. aureus	23±3.0^{c}	13±1.5^{d}	0±0.0^{e}	35±0.0^{b}	40±0.0^{a}
E. fergusonii (ATCC 3569)	18±6.0^{b}	11±0.0^{b}	0±0.0^{c}	30±0.0^{a}	Not done
A. baumannii	13±2.0^{c}	34±0.6^{a}	32±2.5^{ab}	30±0.0^{b}	Not done
Ps. aeruginosa (standard)	14±0.6^{b}	35±0.0^{a}	35±0^{a}	37±2.5^{a}	Not done
Ps. aeruginosa (clinical)	20±7.0^{b}	12±1.5^{b}	0±00^{c}	30±0.0^{a}	Not done
K. pneumonia	17±1.1^{b}	0±0.0^{d}	37±0.6^{a}	13±2.6^{c}	Not done
P value	< 0.00				

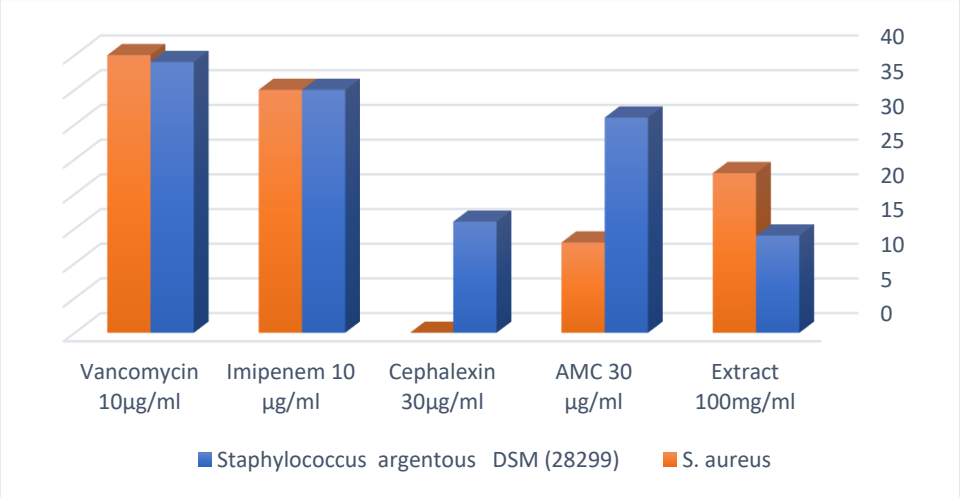

Figure 2. Inhibition zones (mm) of tested extract versus Antibiotic references against Gram-positive isolates

Among Gram-negative isolates, for *E. fergusonii* ATCC 3569, the extract produced an inhibition zone exceeding that of AMC (11 ± 0.0 mm) and cephalexin (0 ± 0.0 mm), but was lower than that of imipenem (30 ± 0.0 mm). Against *A. baumannii*, the extract was lower than AMC (34 ± 0.6 mm), cephalexin (32 ± 2.5 mm), and imipenem (30 ± 0.0 mm). However, against standard *P. aeruginosa*, the extract was less active compared with 35 ± 0.0 mm for AMC, 35 ± 0.0 mm for cephalexin, and 37 ± 2.5 mm for imipenem, and was higher than AMC (12 ± 1.5 mm) and cephalexin (0 ± 0.0 mm), and lower than imipenem (30 ± 0.0 mm) against clinical *P. aeruginosa*. Higher activity shown by the extract against *K. pneumoniae* compared with AMC (0 ± 0.0 mm) and imipenem (13 ± 2.6 mm), but it sowed lower inhibition zone than cephalexin 37 ± 0.6 mm (Figure 3).

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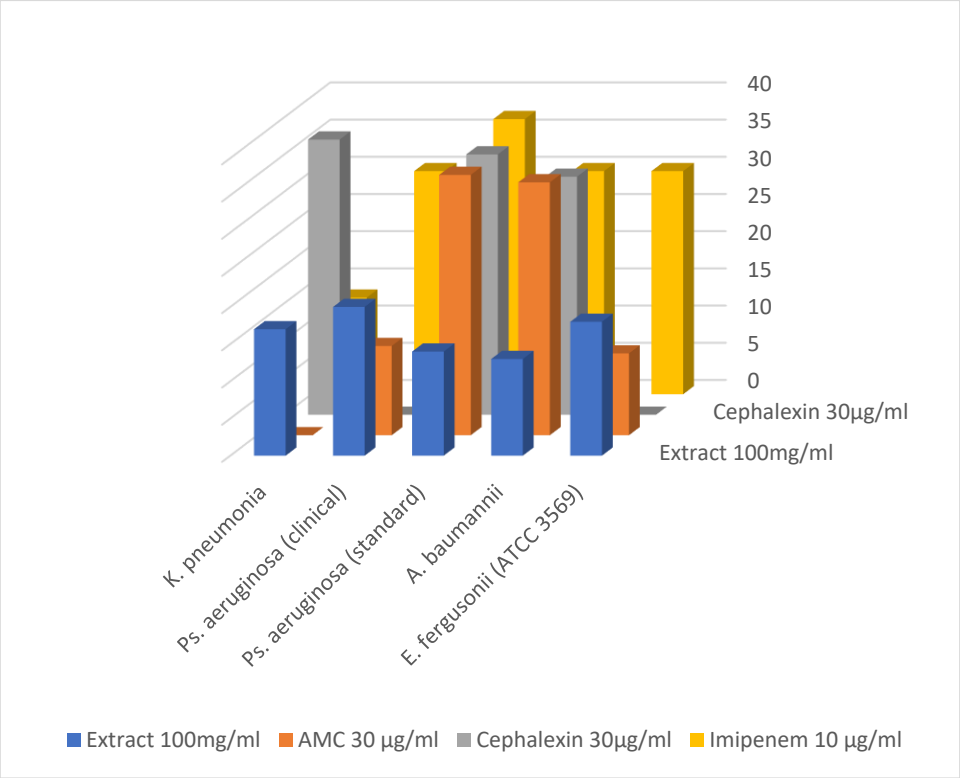

Figure 3. Inhibition zones (mm) of tested extract versus Antibiotic references against Gram-negative isolates

Discussion

The global population reached approximately 8.2 billion in 2025, continuing the steady growth observed in recent decades, with an annual growth rate of around 0.85–0.9%, according to demographic estimates and population tracking systems [16]. Approximately 80% of the global population relies on traditional herbal medicine, named medicinal plants, for healthcare, particularly in China, India, and Africa, due to cultural traditions and limited access to conventional healthcare in some regions [17]. This guided the researchers' attention to studying medicinal plants, especially as they are well known as rich sources of healthy compounds that promote health and well-being [18], and this has guided this study to focus on this plant as one of many medicinal plants, notably because it has recently become a widely naturalized plant established in Libya and because it has not been addressed in any previous Libyan research since its reclassification and separation from the genus "Acacia".

The increasing global burden of antimicrobial resistance (AMR) has catalyzed renewed interest in plant-derived natural products as potential sources of novel antibacterial agents. In this context, the present study provides compelling evidence that the 80% ethanolic leaf extract of *Vachellia karroo* exhibits significant activity against pathogens [19]. The observed bioactivity is supported by comprehensive phytochemical profiling via HPLC, which revealed the presence of key phenolic and flavonoid compounds known for their antimicrobial and antioxidant properties [20] in North African folk medicine, and also positions this species as a promising candidate for further development in the fight against drug-resistant infections caused by a range of clinically relevant bacterial isolates, particularly Gram-positive and select Gram-negative bacteria (Naidoo et al., [21].

The crude extract demonstrated potent antibacterial effects with the highest inhibition zone (23.0 ± 3.0 mm) recorded against *Staphylococcus aureus*, a major Gram-positive pathogen associated with skin, soft tissue, and systemic infections. This level of inhibition exceeds the threshold for strong activity (≥13 mm) as defined by CLSI (2023), and is comparable to or superior to several standard antibiotics tested in this study, including amoxicillin-clavulanate (AMC) and cephalexin. The observed activity against *E. fergusonii* is particularly noteworthy, as this emerging pathogen (often misidentified as *E. coli*) has been increasingly associated with gastroenteritis and extraintestinal infections, including bacteremia [22]. While data on *E. fergusonii*'s susceptibility to plant extracts are scarce, our results align with recent studies indicating that phenolic-rich botanicals can disrupt the outer membrane of certain Gram-negative Enterobacteriaceae through lipopolysaccharide (LPS) binding and membrane permeabilization [23,24]. However, the complete resistance of both standard and clinical isolates of *P. aeruginosa* to the extract in the MIC assay, despite a visible inhibition zone in the well diffusion test, suggests a complex interaction. This discrepancy may be attributed to the extract's limited ability to penetrate the highly impermeable outer membrane of *P. aeruginosa*, which is fortified with porin restrictions and efflux pump systems (e.g., MexAB-OprM) that actively expel antimicrobial agents [25]. The zone of inhibition observed in the agar diffusion assay may reflect bacteriostatic effects at high local concentrations near the well,

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which are not sustained at lower, systemically relevant concentrations tested in the MIC assay. This phenomenon has been previously reported with plant extracts rich in polyphenols, where high initial concentrations cause localized membrane disruption, but sub-inhibitory levels fail to maintain efficacy [33]. HPLC analysis identified a diverse array of phenolic and flavonoid compounds, with catechol (13.32 µg/ml), syringic acid (11.45 µg/ml), and naringin (15.45 µg/ml) being the most abundant. These compounds are well-documented for their antimicrobial mechanisms. Catechol, a simple phenol, has been shown to inhibit bacterial growth by generating reactive oxygen species (ROS) and disrupting membrane integrity [26]. Naringin, a flavanone glycoside, enhances membrane permeability and inhibits biofilm formation in *S. aureus* [27], which may explain the strong activity observed in this study. The presence of quercetin (5.49 µg/mL) and rutin (8.60 µg/mL) further supports the extract's bioactivity. Quercetin has been reported to inhibit DNA gyrase and topoisomerase IV in Gram-positive bacteria [28], while rutin modulates quorum sensing and reduces virulence factor expression in *P. aeruginosa* [29]. However, despite these favorable constituents, the lack of MIC below 100mg/ml against *P. aeruginosa* suggests that synergistic interactions among compounds were disrupted during dilution. Interestingly, caffeic acid (5.48 µg/mL) and ferulic acid (2.35 µg/mL) were also detected. These compounds are known to inhibit sortase A in *S. aureus*, thereby reducing cell wall anchoring of virulence proteins [30]. Their presence may contribute to the extract's selective efficacy against Gram-positive bacteria, which possess a thick peptidoglycan layer accessible to such inhibitors, unlike the outer membrane barrier in Gram-negatives.

Furthermore, this study has proceeded with a comparative Efficacy versus standard Antibiotics, and the results revealed that the *V. karroo* extract outperformed cephalexin and AMC against *S. aureus* and *E. fergusonii*. Vancomycin did not tested against Gram-negative bacteria due to its lack of intrinsic activity against Gram-negative bacteria and poor outer membrane penetration [31]. This paradoxical result may be explained by the extract's multi-target mechanism, including membrane disruption, enzyme inhibition, and ROS generation, which collectively overcome resistance mechanisms that typically render single-target antibiotics ineffective. In contrast, imipenem showed superior activity against most Gram-negative isolates, reflecting its stability against extended-spectrum β-lactamases (ESBLs) and efficient porin-mediated uptake. The extract's inferior performance against *Klebsiella pneumoniae* and *Acinetobacter baumannii* compared to carbapenems and β-lactam/β-lactamase inhibitors underscores the challenge of targeting highly resistant Gram-negative pathogens with plant extracts alone. However, the moderate inhibition of *A. baumannii* (13.0 ± 2.0 mm) is encouraging, as this pathogen is frequently pan-drug resistant and difficult to treat [32]. Flavonoids like quercetin and naringin exhibit potent *in vitro* activity, their efficacy is often reduced in Gram-negative bacteria due to efflux and enzymatic degradation [33]. Moreover, the absence of alkaloids or terpenoid compounds known to enhance membrane permeability in detectable amounts in this extract may further limit its spectrum. On the other hand, *S. argenteus* is emerging as a cause of invasive infections in tropical regions [34], and few studies have evaluated plant extracts against this species; our findings suggest that *V. karroo* may possess broad anti-staphylococcal properties, warranting further investigation.

Interestingly, the results show that the extract has a significant ($p = 0.05$) antibacterial activity against both tested Gram-negative and Gram-positive isolates with varied MICs; the lowest was 6.25 mg against *S. aureus*. Similar to this study, More et al., 2026 [11] reported and proved the effectiveness of the ethanol 80% leaf extract of *V. karroo* leaves, but reported lower MICs of 1.25mg/ml and 2.5mg/ml compared to 25mg/ml and 6.25mg/l against *S. aureus*, and Escherichia species, respectively, with the note that they tested different Escherichia species (*E.coli*), while *Escherichia fergusonii* (ATCC 3569) is the one tested in this study. The difference in MICs between the two studies may be attributed to many points, such as different species and strains, in addition to the different collection sites, where the plant material was collected from the campus of the University of Pretoria (UP), located in Hatfield, South Africa, in More's study, and from the suburban areas of Shahat City, northeastern Libya, in this study. Limited studies were found testing the antibacterial potential of 80% ethanol extract of *V. karroo* leaf against isolates included in this study. Two separate studies done by Baloyi and Adeosun and their teams were found to target the effectiveness of the methanol (not ethanol) extract against standard bacterial isolates rather than clinical ones [35, 21]. Both studies agreed with this study in that this plant has antibacterial activities. Even though Adeosun's study agreed with this study on the effectiveness point, it disagreed on the MIC values, where it revealed a lower MIC of 3.12mg/ml against *P. aeruginosa* ATCC 9721 and *S. aureus* ATCC 25923 compared to 100mg/ml and 6.25mg/ml this study reported, respectively. Also, Adeosun's study reported a lower MIC of 6.25mg/l against *E. coli* ATCC 10536 and *K.* pneumoniae ATCC 33495, compared to 25mg/ml revealed against *Escherichia fergusonii* ATCC 3569 and clinical *K.* tested in this study. These findings not only validate the traditional use of *V. karroo* in North African folk medicine but also position this species as a promising candidate for further development in the fight against drug-resistant infections.

Limitations and recommendations

This study is limited by the absence of bioautography or fractionation to identify the exact bioactive compound(s) responsible for the observed effects. Additionally, the lack of time-kill kinetics, biofilm inhibition assays, and in vivo validation restricts the translational potential of the findings. Future studies should explore synergistic interactions between the extract and conventional antibiotics, particularly against MDR Gram-negative pathogens. Moreover, nano

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formulation strategies could enhance the delivery and stability of the active constituents, potentially overcoming penetration barriers in resistant bacteria.

Conclusion

Vachellia karroo leaf ethanol extract showed selective and potent antibacterial activity, especially against Gram-positive pathogens and some Gram-negative species, including *E. fergusonii*. This activity may be attributed to the extract's richness in phenolic compounds and flavonoids, including catechol, naringin, and quercetin. In addition, the extract's potential to outperform standard antibiotics in some cases supports its ethnopharmacological use and merits further investigation into its modes of action, synergistic potential, and therapeutic efficacy. Future research should include extensive mechanistic studies, isolation and purification of active compounds, validation of the activities of pure compounds, and safety assessments.

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