

Antimicrobial activity and phytochemical screening of ethanol extracts of six traditionally used medicinal plants in East Amhara, Ethiopia

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Abstract

Background: Antimicrobial resistance (AMR) poses a significant challenge to global health, necessitating the exploration of alternative treatments, particularly from natural sources. This study thus aimed to evaluate the antimicrobial activity and phytochemical profiles of six traditional medicinal plants from East Amhara in Ethiopia against *Staphylococcus aureus* and *Salmonella typhi*.

Methods: Ethanol crude extracts of *Myrtus communis*, *Ziziphus spina-christi*, *Euclea racemosa*, *Syzygium guineense*, *Carissa spinarum*, and *Senna singueanawere* prepared using a 1:10 (w/v) ratio cold maceration extraction technique. Antibacterial activity was assessed via the agar disk diffusion assay method, whereas Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were carried out by broth dilution method. Furthermore, phytochemical screenings of the extracts were conducted to identify secondary metabolites according to the respective protocols and operational manuals.

Results: The findings indicated a dose-dependent antibacterial effect across all the tested plant species. *M. communis* exhibited the strongest antibacterial activity against *S. aureus* (22.33 ± 1.2 mm inhibition zone) followed by *C. spinarum* (18.61 ± 0.33 mm). Regarding the antibacterial effects against *S. typhi*, *M. communis*, and *C. spinarum* again produced the first and second highest activity; 17.01 ± 0.58 mm and 16.62 ± 0.33 mm zone of inhibition respectively. The lowest antibacterial activity against *S. aureus* was recorded by the extract of *S. singueana* (14.61 ± 0.33 mm), whereas, the extract of *E. racemosa* (13.30 ± 0.33 mm) produced the weakest activity against *S. typhi*. The lowest MIC values (6.25 mg/ml) were also noted for *M. communis* against both bacterial strains, indicating potent antibacterial properties. Furthermore, phytochemical screening revealed the presence of multiple bioactive compounds, such as alkaloids, flavonoids, tannins, phenolics, terpenoids, saponins, steroids, glycosides, and anthraquinones among the tested extracts.

Conclusion: The study validates the antimicrobial potential of the selected medicinal plants, supported by their diverse phytochemical profiles. *M. communis* and *C. spinarum* were found to cause potent antibacterial activity. The present study thus suggests that extracts of the leaves of these plant species may serve as valuable sources for developing new antimicrobial agents. Further investigation into their active compounds is also warranted to enhance therapeutic applications.

Introduction

The rise of AMR presents a significant global health challenge, rendering many conventional antibiotics ineffective. Two pathogens of particular concern, *S. aureus*, and *S. typhi*, contribute to severe infections and increasing antibiotic resistance [1,2,3,4]. *S. aureus*, a Gram-positive bacterium, causes infections ranging from minor skin conditions to life-threatening diseases like pneumonia, endocarditis, and sepsis. Methicillin-resistant *S. aureus* (MRSA) is notorious for its resistance to multiple antibiotics, complicating treatment, and increasing morbidity [4,5,6]. Similarly, *S. typhi*, a Gram-negative bacterium, causes typhoid fever, characterized by high fever, abdominal pain, and gastrointestinal disturbances. The emergence of multidrug-resistant (MDR) strains of *S. typhi* has intensified public health burdens, particularly in developing countries where typhoid fever is endemic [7,8,9]. This growing resistance to antibiotics underscores the urgent need for alternative treatments.

Traditional medicinal plants offer a promising source for discovering new antimicrobial agents. For millennia, these plants have been integral to healthcare, especially in regions with limited access to modern medical facilities. They provide a reservoir of bioactive compounds used to treat various ailments [10,11,12]. The use of medicinal plants is deeply rooted in cultural and historical contexts, offering insights into ancient and Indigenous pharmacopeias. In many developing countries, including Ethiopia, a significant portion of the population relies on traditional medicine due to its accessibility and affordability [13,14,15,16].

The East Amhara region in Ethiopia, encompassing Wag Himra, North Wollo, and South Wollo, is noted for its biodiversity and tradition of medicinal plant use. Indigenous knowledge systems have preserved extensive information about plant-based remedies. However, despite the prevalence of traditional medicine and healers in the area, scientific documentation and validation of these plants' pharmacological potential remain limited [17,18,19]. This study focuses on six medicinal plants: *M. communis*, *Z. spina-christi*, *E. racemosa*, *S. guineense*, *C. spinarum*, and *S. singueana*, which are selected for their antibacterial efficacy tests and phytochemical analysis.

Traditional healers in these areas possess vast knowledge about the medicinal uses of local plants, passed down through generations. This ethnobotanical knowledge is invaluable for identifying plants with therapeutic potential [16,17]. For instance, *M. communis*, or common myrtle, also known as “Ades” in its Amharic vernacular name, is valued for its aromatic leaves and berries, traditionally used for respiratory and digestive disorders, skin infections, and inflammation. Its essential oils have documented antimicrobial properties. In many parts of the country, the leaves of the plant are used for cosmetic purposes as well [20,21]. *Z. spina*, commonly known as Christ's thorn jujube or “Qurkura” in Amharic vernacular, is utilized for analgesic, anti-inflammatory, and antimicrobial properties, traditionally treating wounds and gastrointestinal issues. Its fruits, leaves, and roots are rich in bioactive compounds [16,22,23,24]. *E. racemosa* also known as “Dedebo” in the Amharic dialect, treats skin conditions and infections, often applied topically for wounds and burns, with roots containing naphthoquinones known for antimicrobial activity [16,25].

S. guineense, or waterberry, also known as “Dokma” in Amharic, is used for its antifungal, antibacterial, antimalarial, and antioxidant properties, traditionally treating diarrhea and infections. Its leaves and bark are abundant in polyphenols and tannins [26,27]. *C. spinarum*, or the conkerberry or bush plum also known as “Agam” in the Amharic language, treats fever and gastrointestinal disorders, showing notable antimicrobial and anti-inflammatory activities [16,28,29]. Likewise, *S. singueana* is locally known as “Gufa” in Amharic, recognized for its laxative properties and use in treating skin diseases and respiratory infections, with documented antimicrobial activity. In addition, it has been used for the treatment of malaria, typhoid, gonorrhea, bilharzia, cancer, epilepsy, and ulcers. People also used it for smoke baths [30,31].

Given the traditional uses of the above-mentioned medicinal plants for treating infections, this study thus aimed to scientifically validate their antimicrobial activity against common causes of bacterial infection in the community (*S. aureus* and *S. typhi*), and performing phytochemical screening to identify the bioactive compounds.

Materials and Methods

Description of plant collection area

Plant materials were collected from three distinct locations within the East Amhara region of Ethiopia. *M. communis* and *Z. spina-christi* were collected from Woldiya town in the North Wollo Zone, *E. racemosa* and *S. guineense* from Amdework town in the Wag Himra Zone, and *C. spinarum* and *S. singueana* were from Kombolcha town in the South Wollo Zone. These areas are generally characterized by diverse geographical features, including highlands, plateaus, and valleys, which contribute to their unique climatic and ecological conditions. The areas experience a bimodal rainfall pattern, with annual precipitation ranging from 600 to 1,200 mm, primarily between June and September. Temperature variations are significant, with highland areas averaging between 10°C and 20°C, while lowland regions can exceed 30°C [32,33]. The fertile volcanic soils in this region enhance agricultural productivity, particularly in the highlands where subsistence farming is prevalent. Local farmers grow staple crops such as teff, barley, and wheat, and engage in livestock rearing, forming the backbone of their livelihoods. Additionally, many communities participate in trade and local crafts, bolstering economic resilience. The vegetation ranges from dense forests in the highlands to shrubland and grassland in lower elevations, supporting rich biodiversity and medicinal plants. The interplay of these ecological and socioeconomic factors makes the East Amhara region in Ethiopia a significant area for biodiversity in general and herbal medicine in particular, offering valuable plant resources for both local use and scientific research [34,35,36].

Plant collection and authentication procedures

Plant collection: The leaves of *M. communis*, *Z. spina-christi*, *E. racemosa*, *S. guineense*, *C. spinarum*, and *S. singueana* were collected during their peak growing season to ensure optimal maturity and enhance the potency of their bioactive compounds. Collection sites were selected based on accessibility, the abundance of target plants, and minimal human disturbance to preserve the specimens' integrity. Healthy, mature leaves were carefully harvested by hand, ensuring the plants' overall structure remained unharmed. The collected materials were placed in separate, clean, breathable cloth bags to prevent microbial growth and degradation [37]. They were then transported to a temporary, clean, and shady mobile laboratory on the same day to maintain their freshness and chemical integrity. After drying well in the shade, the materials were transported to the Microbiology Laboratory at Debre Markos University for further analysis.

Plant authentication: Preliminary identification of the plants was conducted in the field using morphological characteristics such as leaf shape, flower structure, and root features, guided by standard botanical keys and local floras. The collected specimens were then taken to the Herbarium at Debre Markos University for authentication. Plant taxonomists verified the identity of the specimens by comparing them with authenticated herbarium samples and consulting botanical literature [37]. Finally, voucher specimens of the plants were prepared and deposited at the Debre Markos University Herbarium with their specimen collection IDs (Table 1).

Preparation of crude extracts

The crude extracts of the leaves of the collected plants were prepared using maceration techniques with 80% ethanol. The leaves were thoroughly washed with distilled water to remove dirt and impurities. The leaves were then air-dried at room temperature in a shaded, well-ventilated area to prevent the degradation of sensitive compounds. Once dried, they were ground into a fine powder using an electric grinder. Then, a precise electronic balance was used to measure 100 grams of the powdered leaf for each extraction process. One hundred grams of the powdered leaf was placed in a clean, dry container, and 1000 ml of 80% ethanol was

added to the container, ensuring that the powder was fully submerged in the solvent. The mixture was then sealed and allowed to macerate at room temperature for 72 hours. During this period, the mixture was occasionally shaken to enhance the extraction process. After the maceration period, the mixtures were filtered using Whatman number 1 filter paper to separate the liquid extracts from the residues. The filtrates were collected in clean, labeled containers. The solvent from the filtrates was removed using a rotary evaporator under reduced pressure at temperatures not exceeding 40°C to avoid thermal degradation of the extracts. This process yielded concentrated crude extracts. The concentrated crude extracts were then transferred to pre-weighed, labeled amber glass bottles to protect them from light and stored in a refrigerator at 4°C until use [38].

Experimental pathogens

The study utilized two bacterial strains: one Gram-positive and one Gram-negative. *Staphylococcus aureus* (ATCC 25923) was the representative for the Gram-positive category, while *Salmonella typhi* (ATCC 19430) served as the Gram-negative representative. These strains were obtained from the Clinical Bacteriology Laboratory at the Amhara Public Health Institute (APHI). Upon arrival, the bacterial strains were reactivated by sub-culturing them in nutrient broth. Each strain was inoculated into separate sterile tubes filled with nutrient broth and incubated at 37°C for 24 hours. After reactivation, the bacterial cultures were streaked onto nutrient agar plates to isolate colonies. The plates were then incubated at 37°C for 24 hours, and the isolated colonies were examined to confirm the purity and identity of the bacterial strains. The confirmed strains were subsequently preserved on nutrient agar slants, which were also incubated at 37°C for 24 hours to promote adequate growth. Finally, the cultures were stored at 4°C to maintain their viability and integrity for future antibacterial activity assessments [39].

Study design: It is a controlled experimental design, and all the antibacterial assay tests were carried out three times.

Preparation of discs for antimicrobial susceptibility testing

To prepare the discs for antimicrobial susceptibility testing, paper disks measuring approximately 6 mm in diameter were individually punched from Whatman number 1 filter paper using a sterilized two-hole punch. Care was taken to avoid overlapping holes to ensure uniform size. The punched discs were then placed on aluminum foil inside a beaker, which was covered and autoclaved at 121°C for 15 minutes to achieve sterility. After autoclaving, four serial dilutions (200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml) of each plant extract were prepared using a 10% DMSO (Dimethyl sulfoxide) stock solution. These concentrations were made on a weight-by-volume (w/v) basis, reflecting the weight of the plant extract per volume of the 10% DMSO solution. Each dilution was mixed thoroughly to ensure consistency. Using sterile forceps each cooled and autoclaved paper disc was dipped into one of the prepared plant extract dilutions, ensuring complete submersion for uniform impregnation. The excess solution was removed by gently touching the edge of each disc against the beaker's side. The impregnated discs were then carefully placed onto a clean, sterile Petri dish and allowed to air-dry at room temperature. Finally, the discs were stored in sterile containers, sealed with parafilm to prevent contamination, until they were needed for use [40].

Antibacterial sensitivity test

The agar disk diffusion method was utilized in this study to assess the antibacterial activity of crude leaf extracts from the selected plants on nutrient agar. Stock cultures of the bacterial strains were preserved on nutrient agar slants at 4°C. Bacterial suspensions were created by inoculating nutrient broth with pure colonies, adjusting the turbidity to match the McFarland 0.5 standard, which corresponds to a concentration of 1.5×10^8 Colony Forming Units (CFU)/ml. The prepared discs were carefully placed on the surface of Mueller Hinton agar plates that had been inoculated with bacterial suspensions. Each plate contained six discs: one disc with 5 µg of Ciprofloxacin as a positive control to verify assay performance, one disc with 10% DMSO as a negative control to evaluate any solvent effects, and four discs containing varying concentrations of plant extracts (200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml). To ensure proper contact and diffusion of the extracts, the impregnated discs were gently pressed onto the agar surface using sterile forceps. The plates were then inverted and incubated at 37°C for 24 hours to allow bacterial growth and the diffusion of bioactive compounds from the plant extracts into the agar medium. After incubation, the plates were examined for clear zones of inhibition around each disc, indicating the antibacterial activity of the plant extracts against the respective bacterial strains. The diameter of each zone was measured with a calibrated ruler to quantitatively evaluate the degree of bacterial growth inhibition [39].

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of crude extracts from the leaves of the tested plants against bacterial strains was assessed using the broth dilution method in 96-well microplates. A series of two-fold dilutions, ranging from 256 mg/ml to 2 mg/ml, was prepared for each plant extract in nutrient broth; with 10% DMSO serving as the solvent for organic extracts to ensure solubility and consistency. Each well of sterile 96-well microplates was filled with 100 µl of nutrient broth, followed by the addition of 10 µl of each dilution of the plant extract. Positive controls with antibiotics and negative controls containing only broth and 10% DMSO were included to validate the assay. Subsequently, 10 µl of a standardized bacterial inoculum, adjusted to the McFarland 0.5 standard, was added to each well containing the plant extract dilutions and broth. Careful mixing ensured an even distribution of bacteria throughout the wells. The microplates were sealed with sterile lids to prevent evaporation and contamination and then incubated at 37°C for 24 hours to facilitate bacterial growth and interaction with the plant extract dilutions. After the incubation period, the microplates were visually examined for turbidity, indicating bacterial growth. The MIC was defined as the lowest concentration of the plant extract at which no visible bacterial growth was observed compared to the growth control wells. The MIC values were recorded as the endpoint for each tested bacterial strain, providing quantitative data on the antimicrobial potency of the extracts [39].

Determination of Minimum Bactericidal Concentration (MBC)

The MBC of crude extracts against bacterial strains was assessed using the broth dilution method in 96-well microplates. After incubation, the microplates were examined for signs of bacterial growth, and wells exhibiting no visible growth were selected based on the MIC results. To determine the MBC, 10 µl of the contents from each selected well (where no visible growth was detected) was transferred onto fresh nutrient agar plates. These plates were then incubated at 37°C for an additional 24 hours. The MBC was defined as the lowest concentration of the plant extract that completely prevented visible bacterial growth on the agar plates following the subculture. The MBC values were recorded and analyzed, providing further insights into the bactericidal activity of the extracts against the tested bacterial strains [41].

Phytochemical screening of extracts

The qualitative phytochemical profile of ethanol crude extracts of plants was conducted following the protocols described by Harborne (1998), Edeoga et al., (2005), and Zhang and Wang(2013). These protocols encompass tests for alkaloids, flavonoids, tannins, phenolic content, terpenoids, saponins, steroids, glycosides, and anthraquinones, allowing for a comprehensive analysis of the phytochemical profile of the studied plants [42,43,44].

Data analysis

Experimental data were carefully recorded and entered into Microsoft Excel to ensure consistency and accuracy. The data were then transferred to Statistical Software for Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA) for analysis. Analysis of variance (ANOVA) was utilized to assess significant differences in zones of inhibition, as well as MIC and MBC values across various concentrations, and bacterial strains. Qualitative data regarding secondary metabolites were analyzed descriptively, with the occurrence of each compound type calculated based on positive detection in the extracts. A statistical significance level of $p < 0.05$ was considered.

Results

Extract yields

The ethanol crude extract yields from the plant materials are summarized in (Table 1). Using the cold maceration extraction method with a 1:10 w/v ratio, the extraction results showed varying yields across the six medicinal plants. *C. spinarum* had the highest yield (23%), followed by *E. racemosa* (21%), while *S. singueana* produced the lowest yield (12.5%). These differences in yield reflect the variations in phytochemical content and solubility of compounds among the species.

Table 1: Percentage yield of ethanol crude extracts from the leaves of the plants studied

Plant species	Voucher specimen ID	Plant part	Ratio (w/v)	Yield %
<i>Z. spina</i>	BG001	Leaf	1:10	20
<i>M. communis</i>	BG003	Leaf	1:10	16
<i>E. racemosa</i>	AG001	Leaf	1:10	21
<i>S. guineense</i>	AG002	Leaf	1:10	15
<i>S. singueana</i>	TM001	Leaf	1:10	12.5
<i>C. spinarum</i>	TM002	Leaf	1:10	23

Antibacterial sensitivity

The antibacterial activity of ethanol extracts from the leaves of the six plant species was evaluated against two pathogenic bacteria, *S. aureus*, and *S. typhi*, at concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml). The results revealed a dose-dependent antibacterial effect across all the plant species tested. Notably,

M. communis extracts displayed the strongest antibacterial activity against *S. aureus* with an inhibition zone of 22.33 ± 1.2 mm, followed by *C. spinarum* extracts, which achieved an 18.61 ± 0.33 mm inhibition zone at the highest concentration (200 mg/ml). In contrast, *S. singueana* extracts demonstrated the weakest antibacterial effect, with an inhibition zone of 14.61 ± 0.33 mm at the same concentration (Table 2).

Table 2: Antibacterial sensitivity of ethanol crude extracts from the leaves of six plant species against *S. aureus*.

Dose (mg/ml)	<i>M. communis</i>	<i>Z. spina</i>	<i>E. racemosa</i>	<i>S. guineense</i>	<i>C. spinarum</i>	<i>S. singueana</i>
200	22.33 ± 1.2	17.83 ± 0.44	15.31 ± 0.33	17.33 ± 0.33	18.61 ± 0.33	14.61 ± 0.33
100	19.66 ± 0.88	16.53 ± 0.28	13.31 ± 0.33	14.72 ± 0.44	16.07 ± 0.58	13.61 ± 0.33
50	15.33 ± 1.24	15.33 ± 0.61	12.50 ± 0.29	13.01 ± 0.58	15.11 ± 0.05	11.10 ± 0.51
25	13.66 ± 0.88	13.16 ± 0.72	10.72 ± 0.33	11.33 ± 0.33	13.61 ± 0.33	10.54 ± 0.28
5µg Ciprofloxacin	24.11 ± 0.12	22.16 ± 0.51	22.33 ± 0.67	22.02 ± 1.00	23.33 ± 0.33	23.31 ± 0.33
10% DMSO	-	-	-	-	-	-

When tested against *S. typhi*, the extracts of *M. communis* and *C. spinarum* once again demonstrated the highest activity, resulting in inhibition zones of 17.01 ± 0.58 mm and 16.62 ± 0.33 mm, respectively. In contrast, *E. racemosa* extracts exhibited the weakest inhibition, measuring 13.30 ± 0.33 mm (Table 3). Additionally, the extracts showed better efficacy against *S. aureus* than *S. typhi* across all tested plant species and concentrations.

Table 3: Antibacterial sensitivity of ethanol crude extracts from the leaves of six plant species against *S. typhi*

Dose (mg/ml)	<i>M. communis</i>	<i>Z. spina</i>	<i>E. racemosa</i>	<i>S. guineense</i>	<i>C. spinarum</i>	<i>S. singueana</i>
200	17.01 ± 0.58	15.11 ± 0.29	13.30 ± 0.33	15.01 ± 0.58	16.62 ± 0.33	14.61 ± 0.33
100	11.66 ± 0.33	13.52 ± 0.29	11.50 ± 0.29	12.71 ± 0.33	12.31 ± 0.33	10.01 ± 0.57
50	11.32 ± 0.21	11.81 ± 0.29	8.82 ± 0.44	9.03 ± 0.58	10.33 ± 0.33	9.36 ± 0.33
25	10.46 ± 1.01	9.83 ± 0.44	7.72 ± 0.33	7.35 ± 0.33	9.34 ± 0.33	8.05 ± 0.03
5µg Ciprofloxacin	21.32 ± 0.01	21.51 ± 0.52	22.31 ± 0.33	22.72 ± 0.33	22.61 ± 0.33	22.65 ± 0.33
10% DMSO	-	-				

Results of MIC and MBC of the extracts

The MIC and MBC were evaluated to better understand the effectiveness of the plant extracts against the bacterial pathogens tested. The extract from *M. communis* exhibited the lowest MIC value (6.25 mg/ml) for

both bacteria, indicating a strong inhibitory effect even at lower concentrations. However, the *S. singueana* extract showed the highest MIC value (25 mg/ml), indicating relatively weaker antibacterial activity. For bactericidal effects, the *S. guineense* extract had the lowest MBC (12.5 mg/ml) against *S. typhi*, while the *S. singueana* extract required the highest concentration (100 mg/ml) to achieve a bactericidal effect against *S. aureus*. These results indicate that extracts from *M. communis* and *S. guineense* have significant antibacterial potential, whereas the extract from *S. singueana* is less potent (Figure 1).

Phytochemical screening

Phytochemical analysis of the ethanol crude extracts revealed the presence of several bioactive compounds across the six plant species. Alkaloids were detected in all six plant extracts, indicating their widespread presence. Flavonoids, known for their antioxidant and antimicrobial properties, were present in all extracts except for the extract of *S. guineense*. Tannins, Phenols, Terpenoids, Saponins, Steroids, Glycosides, and Anthraquinones were variably distributed among the plants’ extract. Notably, extracts of *C. spinarum* and *E. racemosa* exhibited a full spectrum of these phytochemicals, suggesting a rich chemical composition that could contribute to their observed antibacterial activities. On the other hand, the extract of *S. singueana* lacked Terpenoids and Steroids, which might explain its lower antibacterial efficacy. The consistent presence of Alkaloids, Tannins, and Phenols across all extracts highlights their potential role in the antibacterial effects observed in this study, although the specific contributions of these compounds were not further dissected (Table 5).

Table 5: Secondary metabolites of ethanol crude extracts from the leaves of six plant species

Phytochemicals	Test	Results					
		<i>M. communis</i>	<i>Z. spina</i>	<i>E. racemosa</i>	<i>S. guineense</i>	<i>C. spinarum</i>	<i>S. singueana</i>
Alkaloids	Wagner	+	+	+	+	+	+
Flavonoid	Alkaline	+	+	+	-	+	+
Tannins	Braymer’s Test	+	+	+	+	+	+
Phenol	Ferric chloride	+	+	+	+	+	+
Terpenoid	Salkowski	+	+	+	+	+	-
Saponins	Foam	+	-	+	+	+	+
Steroids	Salkowski	+	+	+	+	+	-
Glycosides	Bortanger	+	+	+	+	+	+
Anthraquinones	NaOH	+	+	+	-	+	+

Key: +: present; -: absent

Discussion

The current study evaluated the antimicrobial efficacy and phytochemical profile analysis of six medicinal plants commonly used in East Amhara, Ethiopia, with a particular focus on their activity against *S. aureus* and *S. typhi*. The findings revealed substantial differences in the antimicrobial potency of the ethanol extracts of the tested plant species. Notably, *M. communis* exhibited the highest antibacterial activity against both bacterial strains. These findings are consistent with previous reports that have documented the potent antimicrobial properties of *M. communis* [20,45]. Its abundant phytochemicals, such as essential oils, flavonoids, and tannins could be implicated in the high antibacterial activity of the plant extract [21]. Likewise, the strong inhibitory effects of *M. communis* against both Gram-positive and Gram-negative bacteria suggest its potential utility in managing infections caused by various bacterial infections. Previous studies have similarly demonstrated the broad-spectrum antimicrobial activity of *M. communis* [20,21,45,46,47]. Moreover, the presence of bioactive compounds such as 1,8-cineole and α -pinene, known for their antimicrobial properties, further supports the therapeutic potential of *M. communis* [21,48,49,50].

Similarly, the notable antibacterial activity exhibited by *C. spinarum* validates its longstanding use in traditional medicine for the treatment of various infections [28,29]. The antimicrobial efficacy of the plant is likely attributed to the presence of bioactive compounds such as saponins and flavonoids. These phytochemicals are known for their ability to disrupt bacterial cell membranes and interfere with microbial metabolic processes, thereby inhibiting bacterial growth and survival [51,52]. Previous reports also demonstrated that plant extracts constituting similar bioactive compounds produced potent antibacterial activities [53,54,55].

In contrast to *M. communis* and *C. spinarum*, *S. singueana* exhibited relatively lower antibacterial activity in this study, particularly against Gram-negative bacteria (*S. typhi*). A study reported by Nyalo et al., (2022) also revealed that the plant extract has more activity against Gram-positive than Gram-negative bacteria, further supporting the present finding [56]. The weak antibacterial activity of *S. singueana* in this study raises concerns about its effectiveness as a standalone treatment for bacterial infections, despite its traditional use for various ailments in Ethiopian folk medicine [30,31]. While *S. singueana* is traditionally valued for its medicinal properties, its modest antibacterial activity might be attributed to the lower concentration of bioactive compounds in the tested extracts. However, the presence of anthraquinones in *S. singueana*, compounds known for their therapeutic properties, suggests that this plant may still hold potential in different therapeutic contexts, such as in combination therapies where it could enhance the efficacy of other antimicrobial agents [57,58]. Reports by Nyalo et al., (2022) also demonstrated the same finding with herein study [56].

The determination of MIC and MBC provided further insights into the antimicrobial efficacy of the plant extracts studied. *M. communis* was particularly noteworthy, exhibiting the lowest MIC value against both *S. aureus* and *S. typhi*. This low MIC indicates a strong inhibitory effect, even at minimal concentrations, highlighting *M. communis* as a potent candidate for developing herbal formulations. In addition to *M. communis*, *S. guineense* emerged as another plant species with significant antimicrobial potential, particularly against *S. typhi*. The MIC and MBC results for *S. guineense* suggest that this plant possesses notable bactericidal properties, warranting further investigation.

Phytochemical analysis of the studied plants revealed a diverse array of secondary metabolites, including alkaloids, flavonoids, tannins, phenolic compounds, saponins, terpenoids, and glycosides. These bioactive compounds are often closely linked to the antimicrobial activities observed in medicinal plants, as evidenced

by the significant antimicrobial effects found in *M. communis*, *C. spinarum*, and *S. guineense*. Flavonoids, for example, are known for their ability to disrupt bacterial cell walls and membranes, leading to increased permeability and subsequent cell lysis [51]. The strong inhibitory effects of *M. communis*, identified as the most potent antibacterial agent in this study, can be attributed to its rich flavonoid content and other phytochemicals. Tannins further enhance antimicrobial activity by precipitating proteins and inhibiting essential enzymatic processes within microbial cells [59,60], supporting the observed effectiveness of these plants against *S. aureus* and *S. typhi*.

The presence of other secondary metabolites, such as saponins and terpenoids, also contributes to the overall antimicrobial efficacy of these plants. The significant antibacterial activity of *C. spinarum* against *S. aureus* reinforces its traditional use in folk medicine, likely due to its saponin and flavonoid content, which disrupt bacterial membranes and hinder microbial metabolism [51,52,61]. On the other hand, the limited antimicrobial activity observed in *S. singueana* against *S. typhi* raises questions about its effectiveness as a standalone treatment, suggesting that its therapeutic potential may lie in synergistic effects with other plants' extracts or compounds.

The findings of this study underscore the hypothesis that medicinal plants are valuable reservoirs of bioactive compounds with significant therapeutic potential, highlighting their relevance in developing new antimicrobial agents to combat the rising challenge of antibiotic resistance. The rich biodiversity of the East Amhara region in Ethiopia provides a wealth of medicinal resources that have been utilized by indigenous populations for centuries. The validation of traditional medicine through scientific research not only enhances our understanding of these plants but also fosters a greater appreciation for the cultural practices surrounding their use.

Conclusion

The present study demonstrates the significant antimicrobial potential of the six medicinal plants tested from East Amhara, Ethiopia, particularly highlighting extracts of *M. communis* as the most potent antibacterial agent against *S. aureus* and *S. typhi*. The low MIC values indicate its promise as a natural alternative to conventional antibiotics. Notably, *C. spinarum* and *S. guineense* also exhibited substantial antibacterial activity, supporting their traditional use in treating infections. The diverse array of bioactive compounds, including flavonoids, saponins, alkaloids, and others suggests these plants are valuable reservoirs for developing effective herbal formulations. Future research should focus on the isolation and characterization of bioactive compounds, which may lead to the discovery of novel antibiotics.

Abbreviations

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, AMR: Antimicrobial resistance, ATCC: American type culture collection, LC: Lethal concentration, DMSO: Dimethyl-sulfoxide, CFU: Colony forming unit, SPSS: Statistical software for social sciences, ANOVA: Analysis of variance, PC: Positive control, NC: Negative control

Declarations

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Authors' contribution: BG, TM, and AG conceived the study and conducted the laboratory investigations. SY, AH, and AL performed the statistical analysis. All authors contributed to interpreting the results, drafting the manuscript, and approving the final version for submission and publication.

Competing interests: All authors declared that they have no competing interests.

Availability of data: All data analyzed during this study are included in this manuscript.

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Figures

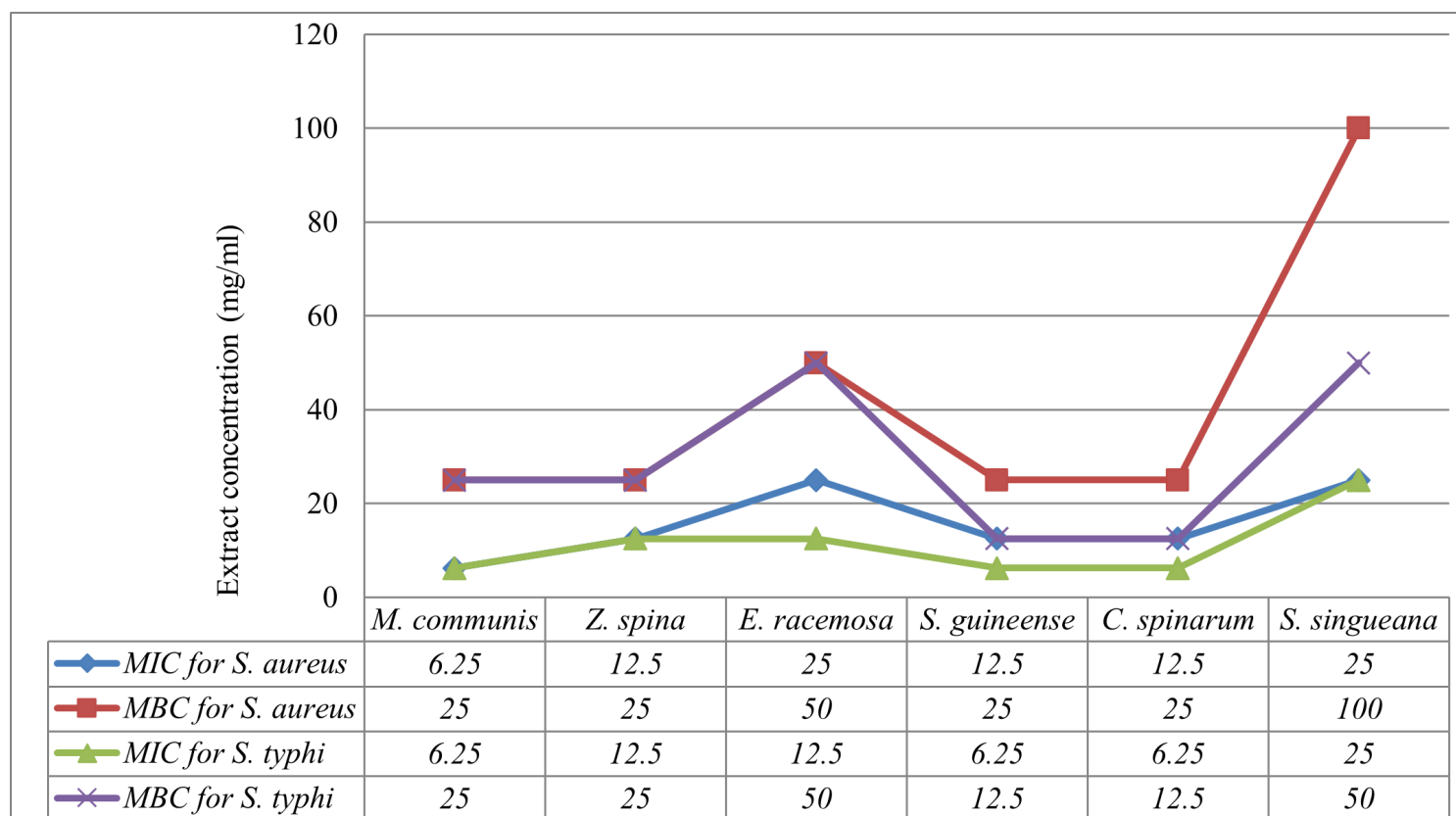


Figure 1

MIC and MBC results of ethanol crude extracts from the leaves of six plant species against *S. aureus* and *S. typhi*