# Phytochemical Profiling and Antimicrobial Activity of Selected Ethiopian Medicinal Plants: Comparative Evaluation of Solvent Extracts Against Bacterial and Fungal Pathogens

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## Abstract

# Objective

This study evaluated the phytochemical composition and antimicrobial activity of selected Ethiopian medicinal plants traditionally used for the treatment of infectious diseases. Leaves of *Rhamnus prinoides*, *Croton macrostachyus*, and *Vernonia amygdalina* were extracted using methanol, ethanol, and acetone solvents.

# Methods

Preliminary phytochemical screening revealed the presence of bioactive compounds including alkaloids, terpenoids, flavonoids, tannins, saponins, steroids, and phenolics. Antimicrobial activity was assessed against selected bacterial (*S. aureus, S. pneumoniae, S. pyogenes, E. coli, P. aeruginosa*) and fungal (*C. albicans, A. flavus, A. niger Trychophyton spp., Cryptococcus spp.*) strains using the standard agar disc diffusion method, followed by determination of minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) using standard microdilution techniques.

# Results

Among the tested extracts, acetone extracts of *V. amygdalina* exhibited the highest antibacterial activity, with significant inhibition zones against *S. aureus*. MIC values ranged from 500 to 5000 µ/ml, and 500 to 6000 µ/ml indicating moderate to strong antibacterial and antifungal potency, respectively.

# Conclusion

The findings support the ethnopharmacology usage of these plants and suggest their potential as sources of bioactive compounds for the development of alternative antimicrobial agents. Further studies on compound isolation, toxicity, and mechanism of action are recommended.

## 1. Introduction

Despite significant advancements in modern medicine, microbial diseases persist as substantial global threats (Cos et al., 2006). Herbal medicines are the basis of health care worldwide since the earliest days of mankind, and are still widely used (Devi et al., 2010). The WHO emphasizes herbal medicine as a primary healthcare modality in many developing countries. Globally, around 80% of the population depends on traditional medicinal plants for disease treatment, with a more prevalence in African countries (WHO, 2001). Medicinal plants discovered by traditional societies are proving to be a source of potential therapeutic drugs (Devi et al., 2010).

Now days, infectious diseases remain a major global health challenge, exacerbated by the rapid emergence of antimicrobial resistance (AMR). The increasing inefficacy of conventional antibiotics has prompted renewed interest in medicinal plants as alternative sources of antimicrobial agents (Alemu et al., 2024; Lekhak & Sharma, 2009). Ethnopharmacological knowledge plays a crucial role in guiding the selection of plants with therapeutic potential, particularly in developing countries where traditional medicine remains widely practiced.

Medicinal plants are rich in bioactive compounds such as alkaloids, flavonoids, tannins, and phenolic compounds, many of which exhibit antimicrobial activity, biodegradable, safe and have fewer side effects (Ramya et al., 2008). However, less than 10% of recorded floras have been explored phytochemically and evaluated clinically (Reddy, 2010). Numerous studies have demonstrated that plant-derived extracts can inhibit the growth of pathogenic microorganisms through mechanisms including disruption of cell membranes, inhibition of enzyme activity, and interference with nucleic acid synthesis (Bhaskarwar et al., 2008). Standardized antimicrobial assays, such as agar disc diffusion and broth microdilution methods recommended by the Clinical and Laboratory Standards Institute (CLSI), are widely used to evaluate such activities (Kokoska et al., 2002).

Demand for medicinal plants is increasing as the population grows (El-kamali, 2009). The majority of Ethiopian society is not an exception to these traditional healing practices, especially in rural areas. For this study, plants (*Croton macrostachyus, Vernonia amygdalina*, and *Rhamnus prinoides*) were selected based on traditional usage suggestive of medicinal activity against different infectious diseases. On the other hand, test bacteria (*Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa*) and fungi (*Candida albicans, Aspergillus flavus, Aspergillus niger Trychophyton spp., Cryptococcus spp.*) were chosen based on their clinical importance and public health relevance.

Ethiopia has a long history of a traditional health care system, and possesses a rich diversity of medicinal plants that are traditionally used to treat a wide range of infectious diseases (Parvez & Yadav, 2010). Previous studies have reported antimicrobial activities of several Ethiopian plant species. For example, around 80% of the population and 90% of livestock depend on traditional medicinal plants in treating various ailments, bruised ulcer, malaria, diabetes, cancer, and infectious diseases (Lekhak & Sharma, 2009; Tuasha et al., 2018). However, in Ethiopia, studies conducted on traditional medicinal plants are limited in view of the multiethnic, cultural, and flora diversity (Mesfin et al., 2009). Also, studies on comparative analyses across different extraction solvents and microbial strains remain limited. Solvent polarity significantly influences the extraction efficiency of bioactive compounds, thereby affecting antimicrobial activity.

Therefore, the present study aimed to (i) evaluate the phytochemical constituents of selected medicinal plants (*Croton macrostachyus, Vernonia amygdalina*, and *Rhamnus prinoides*), (ii) assess their antibacterial and antifungal activities using standardized methods, and (iii) determine their MIC and

MBC/MFC values. This study provides comparative insights into solvent-dependent antimicrobial efficacy and contributes to the scientific validation of traditional medicinal practices.

## 2. Materials and methods

# 2.1. Description of the study area

Arba Minch, which is the administrative center of Gamo Zone is located in the Southern Region, Ethiopia. The global position of Arba Minch is located at latitude 6°4′ N and longitude 36°27′ E. The specific site lies at an elevation of 1,235 meters above sea level. The mean annual rainfall characterized by a bimodal type is 521–2105 mm including 2 wet seasons (first from the end of March to mid-June, second from mid-September to late November) and 2 dry seasons (first from December to mid-March, second from the end of June to mid-September). The mean annual temperature varies between 14.5°C. Hence, from the combined effect of altitude, temperature and rainfall, one can conclude that the town is classified as dry upper kola eco-climate zone. The study plants were collected from Arba Minch Zuriya Woreda, which is one of the woredas in Gamo Zone, and bordered on the south by the Dirashe special woreda, on the west by Bonke, on the north by Dita and Chencha, on the northeast by Mirab Abaya, on the east by the Oromia Region, and on the southeast by the Amaro special woreda (Fig. 1).

# 2.2. Study design and setting

Laboratory based comparative study was employed to evaluate the antimicrobial activity of the three traditional medicinal plants used in this study. The experiment was laid out with three replications. The investigation was done in Microbiology laboratory of Abaya Campus, Arba Minch University, Ethiopia.

# 2.3. Plant material collection

Fresh leaves of *Croton macrostachyus* (Amharic name: bisana), *Vernonia amygdalina* (Amharic name: grawa) and *Rhamnus prinoides* (Amharic name: gesho) were collected from Arba Minch Zuriya Woreda located 6°00'38"N 37°34'05"E 1,194 m in May 2020, Ethiopia. The plants were authenticated by a botanist Mr. Fekede Endale, and voucher specimens, BWL005/2019, were given and deposited at the herbarium of Addis Ababa University for future reference. These plants are selected based on botanical diversity and ethinobotanical medicinal use by the local communities (Table 2). The plant species in this study *Croton macrostachyus*, *Vernonia amygdalina*, and *Rhamnus prinoides* are not listed as threatened or endangered on the IUCN Red List, nor are they included in the appendices of the CITES of Wild Fauna and Flora. Plant materials of *C. macrostachyus*, *V. amygdalina*, and *R. prinoides* were collected in accordance with local and national regulations, and prior informed consent was obtained from relevant local authorities and/or landowners before sample collection.

# 2.4. Preparation of the plant extracts

Collected plant materials were washed with distilled water and air-dried at room temperature under shade for two weeks. The dried samples were ground into fine powder using a mechanical grinder. Plant

extraction was done as described by (Mostafa et al., 2018) with minor modification. Three batches of 100 g of powdered plant material were extracted separately using methanol, ethanol, and acetone through maceration for 72 hours with intermittent shaking. The extracts were filtered using Whatman No. 1 filter paper and concentrated using a rotary evaporator under reduced pressure. The crude extracts were stored at 4°C until further use. The extract yield percentages were calculated using the following formula:

$$\%\ \text{Yield of plant extact} = \frac{(\text{Weight of dried extract}) \times\ 100}{\text{Weight of the dried plant sample}}$$

# 2.5. Phytochemical screening

Preliminary phytochemical analysis was conducted to detect the presence of major secondary metabolites, including alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, phenolics, and glycosides, using standard qualitative methods (Amabye, 2015; Agidew, 2022; Nigussie et al., 2025; Alemu et al., 2024).

# 2.6. Test microorganisms and inoculum preparations

The antibacterial activity of each plant extract was evaluated against selected pathogenic microorganisms including: bacteria *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Escherichia coli*, and *Pseudomonas aeruginosa*, and fungi *Candida albicans, Aspergillus flavus, Aspergillus niger Trychophyton spp. and Cryptococcus spp*. All microbial strains were obtained from the Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. For this study, the inoculum preparation followed the same procedure as described by Mostafa et al. (Mostafa et al., 2018). Microbial suspensions were prepared in sterile saline and adjusted to match 0.5 McFarland turbidity standard (~ 1 × $10^8$ CFU/mL for bacteria), following standard protocols.

# 2.7. Antibacterial activity

The antimicrobial activity of the extracts was evaluated using the agar disc diffusion method in accordance with CLSI guidelines. Sterile Mueller-Hinton agar (Difco) for bacteria and Sabouraud dextrose agar (for fungi) plates were inoculated with standardized microbial suspensions. Sterile filter paper discs (6 mm diameter) were impregnated with known concentrations (100 µl) of plant extracts and placed on the inoculated agar surface (Terreaux et al., 2002). The plates were incubated at 37°C for 24 hours (bacteria), and 28°C for 48–72 hours (fungi). A parallel comparative assay was performed using standard commercial antimicrobial agents, including Gentamicin (10 µg), Amoxicillin (10 µg), Vancomycin (30 µg), Tetracycline (30 µg), and Streptomycin (10 µg), to compare their antimicrobial efficacy with that of the plant extracts. Ketoconazole was used as the positive control for antifungal activity, whereas 5% dimethyl sulfoxide (DMSO, 0.1 mL) served as the negative control. Zones of inhibition were measured in millimeters (mm) by Vernier caliper. Each experiment was performed in triplicate, and results were expressed as mean ± standard deviation.

# 2.8. Determination of MIC

The minimum inhibitory concentration (MIC) was determined using the broth microdilution method. Serial dilutions of the extracts were prepared in sterile broth media. In dilution technique, two-fold serial dilutions of the extracts were prepared in concentrations ranging from 500 to 6000 µg/ml. Each tube was inoculated with standardized microbial suspension and incubated under appropriate conditions. MIC was the lowest concentration of extract that resulted in no visible growth on the surface of the agar.

## 2.9. Determination of MBC/MFC

The determination of MBC was conducted following established protocols assembled by (Eloff, 1998) and (Alemu et al., 2024). To determine minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC), aliquots from MIC tubes showing no growth were subcultured onto fresh agar plates. The plates were incubated according to growth requirement of each organism. The absence of turbidity in the recovery medium was evidence of bactericidal activity.

## 2.10. Statistical analysis

The quantitative data were subjected to ANOVA using SPSS version 27 software, and Least Significant Difference (LSD) was applied for mean comparison. Significance was accepted at $P < 0.05$. The data of all the parameters was statistically analyzed and expressed as mean. Python 3 software was used to analyze the effectiveness of plant extracts against bacterial and fungal species provide insight clustered bar graphs for comparison of MIC.

# 3. Results

## 3.1. Percentage yield of plant extracts

The percentage yield represents the amount of extract obtained from the leaves of each plant species using different solvents. *Croton macrostachyus* has the highest percentage (33.33%) yield in water extract, while *Vernonia amygdalina* has the highest (33.03%) in methanol extract, while the lowest (17.78%) yields were collected from acetone extracts. The choice of solvent significantly influences the yield, with methanol generally providing higher yields compared to acetone and water. Ethnobotanical data, such as local names and family names, can be useful for understanding the cultural and traditional significance of these plants. This information is valuable for researchers in the field of natural products and ethnobotany, providing insights into the extractive potential of these plants for further pharmacological and medicinal studies (Table 1).

Table 1
Percentage yield of leaf extracts of *Rhamnus prinoides, Croton macrostachyus* and *Vernonia amygdalina* and their ethnobotanical data (n = 3).

| Plant species name | Family name | Local name | Plant part extracted | Percentage yield (w/w) (mean ± SEM) | | |
|---|---|---|---|---|---|---|
| | | | | Solvents used | | |
| Acetone | | | | Methanol | Water | |
| *R. prinoides* | Rhamnaceae | *Gesho* | Leaves | 14.21 ± 2.04 | 26.84 ± 2.83 | 25.76 ± 3.09 |
| *C. macrostachyus* | Euphorbiaceae | *Bisana* | Leaves | 14.92 ± 3.48 | 23.38 ± 2.50 | 33.33 ± 2.71 |
| *V. amygdalina* | Asteraceae | *Grawa* | Leaves | 17.78 ± 2.75 | 33.03 ± 3.04 | 24.91 ± 2.61 |

Table 2
Traditional uses of plant extracts by local community

| Plant species | Traditional uses |
|---|---|
| *Croton macrostachyus* | Treat malaria, fever, diarrhea, stomach aches, ringworm, pneumonia, fungal skin infections, sexually transmitted infections, typhoid, blood clotting. |
| *Vernonia amygdalina* | antiparasitic, used as appetizer, to treat diarrhea, stomach aches, to treat fever, coughs, headaches, skin infections. |
| *Rhamnus prinoides* | Used to treat stomach ache, sexually transmitted diseases, pneumonia, skin infections and wounds, back pain, chest pain, intestinal parasites |

# 3.2. Phytochemical composition

Phytochemical screening revealed the presence of several bioactive compounds, including alkaloids, flavonoids, tannins, steroids, glycosides, saponins, and phenolic compounds across all plant extracts (*R. prinoides*, *C. macrostachyus*, and *V. amygdalina*). However, variations were observed depending on the extraction solvent, with acetone and methanol extracts generally showing a broader range of phytochemicals (Table 3).

Table 3
Phytochemical screening of acetone, methanol, and aqueous leaf extracts of *Rhamnus prinoides*, *Croton macrostachyus*, and *Vernonia amygdalina*.

| Phytochemicals | Tests | Rp | | | Cm | | | Va | | | Observed color |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | W | M | A | W | M | A | W | M | A | |
| Flavonoids | Alkaline reagent | - | + | + | + | + | + | + | + | + | Yellow |
| Tannins | $FeCl_3$ test | - | + | + | + | + | + | - | - | - | Black-green |
| Terpenoids | Salkowski test | - | + | + | + | + | + | + | + | + | Red-brown |
| Saponins | Foam test | - | + | + | + | + | + | + | + | + | Foam |
| Steroids | Salkowski test | - | + | + | + | + | + | + | + | + | Blue-green |
| Alkaloids | Chloroform + $H_2SO_4$ | - | + | + | + | + | + | + | + | + | Brownish-red |
| Phenolic | Iodine test | - | + | + | + | + | + | + | + | + | Bluish-black |
| Glycosides | Keller-Killiani test | - | - | - | + | + | + | + | + | + | Green-blue |

+: present; - : absent; W: water; M: methanol; A: acetone; Rp: *Rhamnus prinoides*; Cm: *Croton macrostachyus*; Va: *Vernonia amygdalina*

# 3.3. Antibacterial activities of plant extracts

All plant extracts exhibited varying degrees of antibacterial activity against the tested bacterial strains. Among the tested plants, *Vernonia amygdalina* extracts demonstrated the highest antibacterial activity, particularly against *Staphylococcus aureus*, with the largest zones of inhibition (16.0 ± 0.6 mm) observed in acetone extracts. Moderate activity was observed against *Escherichia coli* and *Salmonella typhi*, although inhibition zones were generally smaller compared to Gram-positive bacteria. The antibacterial activity varied significantly depending on the solvent used, indicating that solvent polarity influences the extraction of active compounds. No inhibitory effect was observed for *P. aeruginosa, C. macrostachyus* (acetone extract), and *R. prinoides* (methanol extract), with inhibition zones measuring 0.0 ± 0.0 mm. The positive controls showed larger inhibition zones, including Gentamicin (23.0 ± 0.6 mm against *S. pyogene*), Amoxicillin (23.0 ± 0.6 mm against *S. aureus* and 22.0 ± 0.6 mm against *P. aeruginosa*), Vancomycin (19.0 ± 0.6 mm against *S. pneumoniae* and *E. coli*), Tetracycline (19.0 ± 0.6 mm against *P. aeruginosa*), and Streptomycin (20.0 ± 0.6 mm against *S. pneumoniae*), while the negative control (DMSO) showed no activity (0.0 ± 0.0 mm) against all tested organisms (Table 4).

Table 4
Antibacterial activity of plant extracts expressed as mean inhibition zone diameter (n = 3) against selected bacterial pathogens.

| Plants and solvents used | Mean diameter of inhibition zone in (mm) ± SEM of pathogens | | | | |
|---|---|---|---|---|---|
| | Sa | Spy | Spn | Ec | Pa |
| Rp (Acetone) | 8.3 ± 0.3 | 10.7 ± 0.3 | 14.0 ± 0.6 | 8.3 ± 0.3 | 8.3 ± 0.3 |
| Rp (Methanol) | 10.3 ± 0.3 | 9.0 ± 0.6 | 13.3 ± 0.3 | 10.0 ± 0.6 | 0.0 ± 0.0 |
| Rp (Water) | 9.0 ± 0.6 | 11.0 ± 0.6 | 10.3 ± 0.3 | 8.3 ± 0.3 | 8.3 ± 0.3 |
| Cm (Acetone) | 15.0 ± 0.6 | 9.0 ± 0.6 | 0.0 ± 0.0 | 14.0 ± 0.6 | 14.0 ± 0.6 |
| Cm (Methanol) | 14.0 ± 0.6 | 9.0 ± 0.6 | 0.0 ± 0.0 | 11.0 ± 0.6 | 11.0 ± 0.6 |
| Cm (Water) | 12.0 ± 0.6 | 7.3 ± 0.3 | 0.0 ± 0.0 | 10.0 ± 0.6 | 8.3 ± 0.3 |
| Va (Acetone) | 16.0 ± 0.6 | 16.0 ± 0.6 | 13.0 ± 0.6 | 0.0 ± 0.0 | 12.0 ± 0.6 |
| Va (Methanol) | 14.0 ± 0.6 | 17.0 ± 0.6 | 9.0 ± 0.6 | 0.0 ± 0.0 | 14.0 ± 0.6 |
| Va (Water) | 10.0 ± 0.6 | 11.0 ± 0.6 | 9.0 ± 0.6 | 0.0 ± 0.0 | 10.0 ± 0.6 |
| G (10 µg) | 10.0 ± 0.6 | 23.0 ± 0.6 | 20.0 ± 0.6 | 19.0 ± 0.6 | 9.0 ± 0.6 |
| A (10 µg) | 23.0 ± 0.6 | 17.0 ± 0.6 | 17.0 ± 0.6 | 16.0 ± 0.6 | 22.0 ± 0.6 |
| V (30 µg) | 18.0 ± 0.6 | 18.0 ± 0.6 | 19.0 ± 0.6 | 19.0 ± 0.6 | 11.0 ± 0.6 |
| T (30 µg) | 12.0 ± 0.6 | 14.0 ± 0.6 | 14.0 ± 0.6 | 11.0 ± 0.6 | 19.0 ± 0.6 |
| S (10 µg) | 12.0 ± 0.6 | 19.0 ± 0.6 | 20.0 ± 0.6 | 14.0 ± 0.6 | 12.0 ± 0.6 |
| Nc (DMSO) | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |

Within each column, different superscript letters indicate significant differences according to one-way ANOVA followed by Tukey HSD ($p < 0.05$). Rp: *Rhamnus prinoides*, Cm: *Croton macrostachyus*, Va: *Vernonia amygdalina*, Sa: S. aureus, Spy: *S. pyogenes*, Spn: *S. pneumoniae*, Ec: *E. coli*, Pa: *P. aeruginosa*, G: Gentamicin, A: Amoxicillin, V: Vancomycin, T: Tetracycline, S: Streptomycin, DMSO: Dimethyl sulfoxide, Nc: Negative control

# 3.4. MIC/MBC of extracts

The MIC and MBC assays revealed that solvent type and plant species strongly influenced antibacterial activity. Methanol and acetone extracts of *Croton macrostachyus* and *Vernonia amygdalina* consistently exhibited the lowest MIC and MBC values (500–750 µg/ml), indicating potent activity against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Escherichia coli*. In contrast, aqueous extracts generally required higher concentrations (≥ 1500 µg/ml MIC; up to 3000 µg/ml MBC), particularly for *Rhamnus prinoides*. Among the tested organisms, *Pseudomonas aeruginosa* was the most resistant, with MIC and MBC values reaching 3000–6000 µg/ml for aqueous extracts, while *S. pneumoniae* was the most sensitive, showing inhibition at 500 µg/ml with methanol extracts. Overall, methanol and acetone

extracts, especially from *C. macrostachyus* and *V. amygdalina*, demonstrated superior antibacterial efficacy compared to water extracts, with *P. aeruginosa* emerging as the least susceptible pathogen (Table 5, Fig. 3).

Table 5
MIC and MBC of selected plant extracts against five bacterial pathogens

Rp: *Rhamnus prinoides*, Cm: *Croton macrostachyus*, Va: *Vernonia amygdalina*, Sa: S. aureus, Spy: *S. pyogenes*, Spn: *S. pneumoniae*, Ec: *E. coli*, Pa: *P. aeruginosa*

| Plants and solvents | µg/ml | Sa | Spy | Spn | Ec | Pa |
|---|---|---|---|---|---|---|
| Rp (Acetone) | MIC | 1250 ± 29 | 1250 ± 29 | 625 ± 15 | 625 ± 15 | 2500 ± 58 |
| | MBC | 1250 ± 29 | 2500 ± 58 | 625 ± 15 | 625 ± 15 | 5000 ± 115 |
| Rp (Methanol) | MIC | 1000 ± 29 | 1000 ± 29 | 500 ± 12 | 1000 ± 29 | 2000 ± 58 |
| | MBC | 1000 ± 29 | 2000 ± 58 | 500 ± 12 | 1000 ± 29 | 4000 ± 115 |
| Rp (Water) | MIC | 1500 ± 29 | 1500 ± 29 | 750 ± 12 | 1500 ± 29 | 3000 ± 58 |
| | MBC | 1500 ± 29 | 3000 ± 58 | 750 ± 12 | 1500 ± 29 | 6000 ± 115 |
| Cm (Acetone) | MIC | 625 ± 15 | 625 ± 15 | 625 ± 15 | 1250 ± 29 | 1250 ± 29 |
| | MBC | 625 ± 15 | 1250 ± 29 | 625 ± 15 | 2500 ± 58 | 1250 ± 29 |
| Cm (Methanol) | MIC | 1000 ± 29 | 1000 ± 29 | 500 ± 12 | 1000 ± 29 | 1000 ± 29 |
| | MBC | 1000 ± 29 | 2000 ± 58 | 500 ± 12 | 2000 ± 58 | 1000 ± 29 |
| Cm (Water) | MIC | 1500 ± 29 | 1500 ± 29 | 750 ± 12 | 1500 ± 29 | 1500 ± 29 |
| | MBC | 1500 ± 29 | 3000 ± 58 | 750 ± 12 | 3000 ± 58 | 1500 ± 29 |
| Va (Acetone) | MIC | 625 ± 15 | 1250 ± 29 | 625 ± 15 | 1250 ± 29 | 625 ± 15 |
| | MBC | 625 ± 15 | 2500 ± 58 | 625 ± 15 | 2500 ± 58 | 625 ± 15 |
| Va (Methanol) | MIC | 1000 ± 29 | 1000 ± 29 | 500 ± 12 | 1000 ± 29 | 1000 ± 29 |
| | MBC | 1000 ± 29 | 2000 ± 58 | 500 ± 12 | 2000 ± 58 | 1000 ± 29 |
| Va (Water) | MIC | 1500 ± 29 | 1500 ± 29 | 750 ± 12 | 1500 ± 29 | 1500 ± 29 |

Rp: *Rhamnus prinoides*, Cm: *Croton macrostachyus*, Va: *Vernonia amygdalina*, Sa: S. aureus, Spy: *S. pyogenes*, Spn: *S. pneumoniae*, Ec: *E. coli*, Pa: *P. aeruginosa*

| | | | | | | |
|---|---|---|---|---|---|---|
| | **MBC** | **1500 ± 29** | **3000 ± 58** | **750 ± 12** | **3000 ± 58** | **1500 ± 29** |

Rp: *Rhamnus prinoides*, Cm: *Croton macrostachyus*, Va: *Vernonia amygdalina*, Sa: S. aureus, Spy: *S. pyogenes*, Spn: *S. pneumoniae*, Ec: *E. coli*, Pa: *P. aeruginosa*

Rp: *Rhamnus prinoides*, Cm: *Croton macrostachyus*, Va: *Vernonia amygdalina*

# 3.5. Antifungal activities of plant extracts

The comparative analysis of inhibition zones demonstrates that both plant species and solvent type exert a decisive influence on antifungal efficacy. Methanol extracts of *C. macrostachyus* and *V. amygdalina* consistently produced inhibition zones that were statistically indistinguishable from the positive control (Ketoconazole), underscoring their strong antifungal potential across all tested pathogens. Acetone extracts exhibited moderate activity, particularly in *V. amygdalina*, but remained significantly less effective than methanol counterparts. In contrast, aqueous extracts failed to inhibit fungal growth, highlighting the poor extraction efficiency of water for bioactive compounds. *R. prinoides* displayed limited antifungal activity, with negligible inhibition against *Trychophyton* and *Cryptococcus*, suggesting a weaker phytochemical profile. Collectively, these findings indicate that methanol is the most effective solvent for isolating antifungal metabolites, and that *C. macrostachyus* and *V. amygdalina* represent promising candidates for the development of plant-derived antifungal agents, while aqueous extracts and *R. prinoides* are unlikely to provide therapeutic benefit (Table 6).

Table 6

Inhibition zone of acetone, methanol and aqueous plant extracts against *C. albicans, A. flavus, A. niger, Trychophyton spp.* and *Cryptococcus* spp.

| Plant species | Solvents used | Mean mycelia diameter (mm) ± SEM | | | | |
|---|---|---|---|---|---|---|
| | | ***Ca*** | ***Af*** | ***An*** | ***Tr*** | ***Cr*** |
| Rp | Acetone | 5.99 ± 1.23$^{b}$ | 7.70 ± 2.12$^{b}$ | 6.50 ± 2.23$^{b}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{c}$ |
| | Methanol | 7.38 ± 2.40$^{b}$ | 5.60 ± 1.04$^{b}$ | 5.59 ± 2.40$^{b}$ | 0.00 ± 0.00$^{c}$ | 1.97 ± 1.00$^{c}$ |
| | Water | 4.45 ± 1.45$^{b}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{c}$ |
| Cm | Acetone | 8.08 ± 1.32$^{b}$ | 5.15 ± 2.33$^{b}$ | 0.00±.00$^{c}$ | 2.42 ± 1.21$^{b}$ | 3.25 ± 2.32$^{b}$ |
| | Methanol | 10.35 ± 2.51$^{a}$ | 7.80 ± 2.14$^{a}$ | 9.83 ± 3.52$^{a}$ | 10.93 ± 2.45$^{a}$ | 9.43 ± 2.45$^{a}$ |
| | Water | 0.00 ± 0.00$^{c}$ | 2.95 ± 1.05$^{b}$ | 3.86 ± 1.56$^{b}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{c}$ |
| Va | Acetone | 9.53 ± 4.91$^{ab}$ | 8.72 ± 3.15$^{a}$ | 6.23 ± 3.45$^{b}$ | 7.96 ± 3.56$^{ab}$ | 8.10 ± 3.25$^{ab}$ |
| | Methanol | 9.65 ± 1.70$^{a}$ | 9.93 ± 2.32$^{a}$ | 9.91 ± 2.54$^{a}$ | 9.90 ± 2.51$^{a}$ | 9.31 ± 1.52$^{a}$ |
| | Water | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{c}$ |
| Control | Keto. | 11.65 ± 4.12$^{a}$ | 10.42 ± 2.45$^{a}$ | 10.32 ± 3.48$^{a}$ | 12.25 ± 2.11$^{a}$ | 10.12 ± 2.14$^{a}$ |
| C- | 5% DMSO | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{c}$ |

Letters indicating significant differences (ANOVA + Tukey HSD, $p < 0.05$), values sharing the same letter are not significantly different, while different letters indicate significant differences in the same raw against the positive control. Data reported are the mean diameters expressed in mm. Values are presented as mean ± SEM. The experiments were conducted with triplicates samples (n = 3). Keto: ketoconazole; C-: negative control. Rp: *Rhamnus prinoides*, Cm: *Croton macrostachyus*, Va: *Vernonia amygdalina*, Ca: *C. albicans*, Af: *A. flavus*, An: *A. niger*, Tr: *Trichophyton* spp., Cr: *Cryptococcus* spp.

# 3.6. MIC/MFC of extracts

The MIC and MFC assays revealed clear differences in antifungal efficacy depending on plant species and solvent type. Methanol and acetone extract of *Croton macrostachyus* and *Vernonia amygdalina* consistently exhibited the lowest MIC values (500–750 µg/ml) and correspondingly low MFC values (1000–1500 µg/ml), indicating strong inhibitory and fungicidal activity against *Candida albicans*, *Aspergillus niger*, and *Trichophyton* spp. In contrast, aqueous extracts generally required higher concentrations to achieve inhibition, with MIC values exceeding 1000 µg/ml and MFC values up to 3000 µg/ml, particularly for *Rhamnus prinoides*. Among the tested pathogens, *Cryptococcus* spp. was the least susceptible, requiring the highest MIC and MFC values across all extracts. Overall, these findings demonstrate that methanol and acetone are superior solvents for extracting antifungal metabolites, and that *C. macrostachyus* and *V. amygdalina* represent promising candidates for further development of plant-derived antifungal agents, while aqueous extracts and *R. prinoides* showed limited efficacy.

Table 7
Minimal inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of plant extracts on five fungal pathogens.

| Plants vs solvents | µg/ml | Ca | Af | An | Tr | Cr |
|---|---|---|---|---|---|---|
| *Rp* (Acetone) | MIC | 750 ± 32 | 1000 ± 41 | 625 ± 29 | 875 ± 35 | 1250 ± 44 |
| | MFC | 1500 ± 48 | 2000 ± 66 | 1250 ± 37 | 1750 ± 54 | 2500 ± 71 |
| *Rp* (Methanol) | MIC | 625 ± 28 | 875 ± 36 | 500 ± 24 | 750 ± 33 | 1000 ± 39 |
| | MFC | 1250 ± 39 | 1750 ± 58 | 1000 ± 31 | 1500 ± 47 | 2000 ± 63 |
| *Rp* (Water) | MIC | 1000 ± 41 | 1250 ± 48 | 875 ± 36 | 1125 ± 44 | 1500 ± 52 |
| | MFC | 2000 ± 69 | 2500 ± 78 | 1750 ± 55 | 2250 ± 72 | 3000 ± 84 |
| *Cm* (Acetone) | MIC | 500 ± 26 | 750 ± 33 | 625 ± 29 | 875 ± 35 | 1000 ± 38 |
| | MFC | 1000 ± 34 | 1500 ± 49 | 1250 ± 41 | 1750 ± 56 | 2000 ± 62 |
| *Cm* (Methanol) | MIC | 625 ± 28 | 875 ± 36 | 500 ± 23 | 750 ± 31 | 875 ± 35 |
| | MFC | 1250 ± 39 | 1750 ± 57 | 1000 ± 32 | 1500 ± 48 | 1750 ± 55 |
| *Cm* (Water) | MIC | 1000 ± 42 | 1250 ± 49 | 875 ± 36 | 1125 ± 45 | 1250 ± 47 |
| | MFC | 2000 ± 68 | 2500 ± 79 | 1750 ± 56 | 2250 ± 71 | 2500 ± 77 |
| *Va* (Acetone) | MIC | 500 ± 25 | 875 ± 37 | 625 ± 28 | 750 ± 32 | 875 ± 34 |
| | MFC | 1000 ± 33 | 1750 ± 59 | 1250 ± 40 | 1500 ± 50 | 1750 ± 56 |
| *Va* (Methanol) | MIC | 625 ± 27: | 750 ± 34 | 500 ± 22 | 750 ± 31 | 1000 ± 39 |
| | MFC | 1250 ± 38 | 1500 ± 49 | 1000 ± 30 | 1500 ± 47 | 2000 ± 61 |
| *Va* (Water) | MIC | 875 ± 35 | 1125 ± 44 | 750 ± 31 | 1000 ± 39 | 1250 ± 46 |
| | MFC | 1750 ± 56 | 2250 ± 71 | 1500 ± 48 | 2000 ± 64 | 2500 ± 78 |

Rp: *Rhamnus prinoides*, Cm: *Croton macrostachyus*, Va: *Vernonia amygdalina*, Ca: *C. albicans*, Af: *A. flavus*, An: *A. niger*, Tr: *Trichophyton* spp., Cr: *Cryptococcus* spp.

# 3. Discussion

The present study demonstrated that extracts of *Croton macrostachyus, Rhamnus prinoides*, and *Vernonia amygdalina* possess notable antimicrobial activity against selected bacterial and fungal pathogens. The observed activity can be attributed to the presence of bioactive phytochemicals such as flavonoids, glycosides, saponins, tannins, alkaloids, steroids and phenolic compounds, which are known to exhibit antimicrobial properties through multiple mechanisms. Phytochemical screening revealed that methanol and acetone extracts of all three plant species contained a broad spectrum of secondary metabolites. In contrast, several water-based extracts, particularly those from *Rhamnus prinoides*, were deficient in these constituents, which is consistent with earlier reports indicating that aqueous extraction is less effective for recovering tannins, polyphenols, alkaloids, and flavonoids (Molla et al., 2016). Comparable findings have been reported for *Croton macrostachyus* seeds and roots, where crude extracts contained alkaloids, phenolics, tannins, terpenoids, saponins, and flavonoids (Tedla & Kibret, 2024), and for *R. prinoides*, and *Vernonia amygdalina* in studies conducted in Ethiopia (Amabye, 2015; Degu et al., 2024). The presence of these phytochemicals is particularly relevant given their established antimicrobial and antioxidant properties, with flavonoids and phenolics frequently associated with microbial growth inhibition. Thus, the comparatively weaker activity of aqueous extracts, especially from *R. prinoides*, may be attributed to the absence of these bioactive compounds. Overall, the findings emphasize the importance of solvent polarity in determining both extraction yield and phytochemical composition, which in turn likely influences the antimicrobial efficacy of the extracts.

Extraction yields varied considerably depending on both the plant species and the solvent employed, underscoring the critical role of solvent polarity in phytochemical recovery. For instance, aqueous extraction of *C. macrostachyus* produced the highest yield (33.33%), while methanol extraction of *V. amygdalina* yielded 33.03%. In contrast, acetone consistently produced the lowest yields (~ 17.78%), reflecting its reduced efficiency in solubilizing polar metabolites. These findings corroborate previous reports that polar solvents, particularly methanol, are more effective in extracting flavonoids, phenolics, and other secondary metabolites than less polar solvents such as acetone or water (Assefa et al., 2024; Beshir et al., 2025; Nigussie et al., 2025). The relatively poor performance of aqueous extracts, as observed in *R. prinoides*, may be attributed to the absence of several phytochemical classes, thereby limiting extraction efficiency. Collectively, the data reinforce the conclusion that solvent choice is a decisive factor influencing extraction yield and, by extension, the potential biological activity of plant-derived preparations.

The antibacterial assays confirmed that all three plant extracts possessed inhibitory activity, though the magnitude varied with plant species, solvent type, and bacterial strain. Acetone and methanol extracts of *Croton macrostachyus* and *Vernonia amygdalina* consistently produced larger inhibition zones, such as 15.0 ± 0.6 mm against *Staphylococcus aureus* and 17.0 ± 0.6 mm against *Streptococcus pyogenes*,

respectively, whereas aqueous extracts were generally less effective. Positive control antibiotics exhibited substantially greater inhibition zones, validating assay sensitivity, while the negative control (DMSO) showed no activity, confirming that observed effects were attributable to the extracts. MIC and MBC values ranged from 500 to 5000 µg/ml, with methanol and acetone extracts demonstrating stronger activity than aqueous extracts. The lowest MIC/MBC values (500–625 µg/ml) were observed against *S. pneumoniae* and *S. aureus*, while *Pseudomonas aeruginosa* was the most resistant, requiring up to 6000 µg/ml. These findings are consistent with previous reports highlighting the broad-spectrum antimicrobial potential of *C. macrostachyus* and *V. amygdalina* (Effah-yeboah & Agyapong, 2021; Tedla & Kibret, 2024; Beshir et al., 2025). The reduced susceptibility of Gram-negative bacteria such as *E. coli* and *P. aeruginosa* supports the established notion that their outer membrane limits penetration of plant-derived phytochemicals (Kimani et al., 2025; Molla et al., 2016). Similarly, antifungal assays revealed MIC and MFC values between 500 and 6000 µg/ml, with methanol and acetone extracts particularly from *C. macrostachyus* and *V. amygdalina* exhibiting the strongest activity against *Candida albicans* (MIC 500 µg/ml). In contrast, aqueous extracts consistently required higher concentrations, especially for *R. prinoides*, while *Cryptococcus* spp. was the most resistant. Collectively, these results underscore the importance of solvent polarity in determining phytochemical yield and antimicrobial efficacy, and they highlight *C. macrostachyus* and *V. amygdalina* as promising candidates for further development of plant-derived antibacterial and antifungal agents.

These findings provide foundational support for the bioprospecting of novel antibacterial and antifungal agents from these plants. Furthermore, the results underscore the importance of integrating traditional knowledge with modern scientific research. Traditional health practitioners accumulate knowledge and skills on medicinal plants and their application. These findings support traditional Ethiopian medicinal practices, emphasizing that traditional healers recognize tissue-specific phytochemical distributions (Adefa et al., 2025). Integrating traditional knowledge with modern research can guide the discovery of plant-based antibacterial agents and inform public health strategies.

The present study demonstrates several important strengths that enhance its scientific and practical relevance. First, the experimental design is robust, incorporating multiple medicinal plant species and a range of extraction solvents, which allows for a comprehensive comparative evaluation of solvent-dependent bioactivity. Such an approach is essential, as solvent polarity significantly influences the extraction efficiency of phytochemicals and, consequently, their antimicrobial potential (Mehmood et al., 2022; Panda et al., 2016). Furthermore, the inclusion of quantitative antimicrobial parameters, including MIC, MBC, and MFC, provides a more rigorous assessment of antimicrobial efficacy than qualitative screening alone, which is often lacking in similar studies (Kebede et al., 2021).

In addition, the study is strongly grounded in ethnomedicinal knowledge, contributing to the scientific validation of traditional Ethiopian medicinal practices and supporting the exploration of plant-based alternatives in the context of increasing antimicrobial resistance. Finally, the use of a broad panel of microbial strains, including both Gram-positive and Gram-negative bacteria as well as fungal pathogens,

enhances the scope and applicability of the findings, enabling a more comprehensive evaluation of the antimicrobial spectrum of the investigated plant extracts.

### Limitations of the study

Despite these promising results, this study has several limitations. The crude extracts used in this study contain complex mixtures of compounds, and the specific active constituents responsible for the observed antimicrobial activity were not identified. In addition, toxicity and in vivo efficacy were not evaluated. Future studies should focus on the isolation and characterization of active compounds, as well as assessment of their safety and pharmacological properties.

## Conclusion

This study revealed that the selected Ethiopian medicinal plants *Rhamnus prinoides, Croton macrostachyus*, and *Vernonia amygdalina* possess significant antimicrobial potential against a range of bacterial and fungal pathogens. The findings confirm that these plants contain diverse bioactive phytochemicals, including alkaloids, flavonoids, tannins, and phenolic compounds, which likely contribute to their observed antimicrobial effects. The use of multiple extraction solvents revealed that solvent polarity plays a crucial role in determining the efficacy of extracted compounds, with acetone extracts generally exhibiting superior antimicrobial activity. Importantly, the determination of MIC, MBC, and MFC provided quantitative evidence of both bacteriostatic and bactericidal properties of the extracts, thereby strengthening the reliability of the findings. Such quantitative assessments are essential for validating antimicrobial efficacy and are often emphasized in high-quality studies of medicinal plants. Furthermore, the study provides scientific support for the traditional use of these plants in Ethiopian ethnomedicine, highlighting their potential as promising sources of novel antimicrobial agents in the context of rising antimicrobial resistance. The broad-spectrum activity observed against both Gram-positive and Gram-negative bacteria, as well as fungal pathogens, underscores their potential applicability in the development of alternative therapeutic options.

## Declarations

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### Author contributions

BW was conceived and designed the study; carried out and analysed and interpreted the sample data. BW drafted the manuscript. BW and AC revised the manuscript, read and corrected the final copy of the manuscript.

We declare that this study is our original work representing one of the first reported on the phytochemical and antimicrobial activity of these plants within this study area.

**Declaration of conflicting interests**

The authors declared no conflicts of interest with respect to the research, authorship, and publication of this article.

**Ethical approval**

The ethical approval for this study was obtained from the Ethical Review Board of the Arba Minch University, Ethiopia, with the clearance number (IRB/ 1504/2018).

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**Consent for publication**

The various authors consent for publication were sorted while the journal option was discussed and agreed upon. However, there are no individual authors data/report included in the study

**Animal welfare**

Guidelines for human animal treatment did not apply to the present study because it is an in vitro study and tests/experiments were not done on live animals.

**Availability of data and materials**

All data, machines, experiments, and analysis sources were appropriately acknowledged as necessary while writing the manuscript. Any other information needed are available on request.

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# Figures

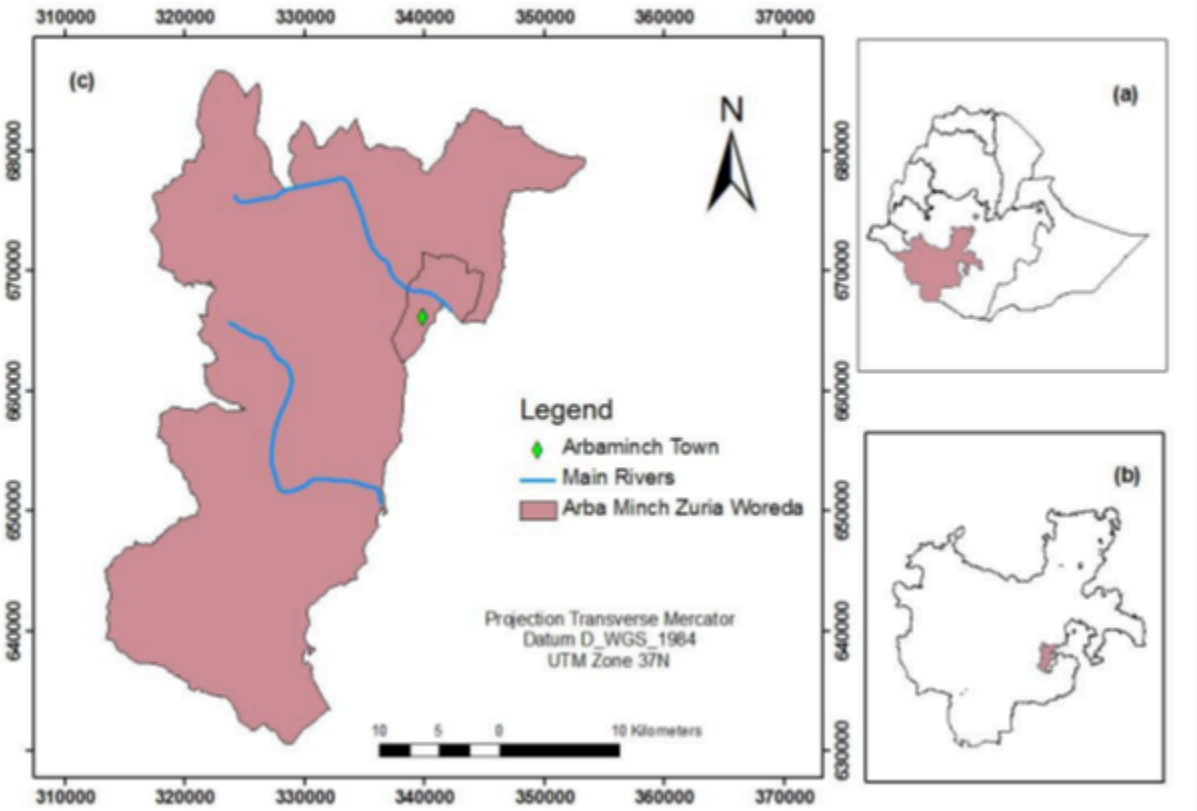
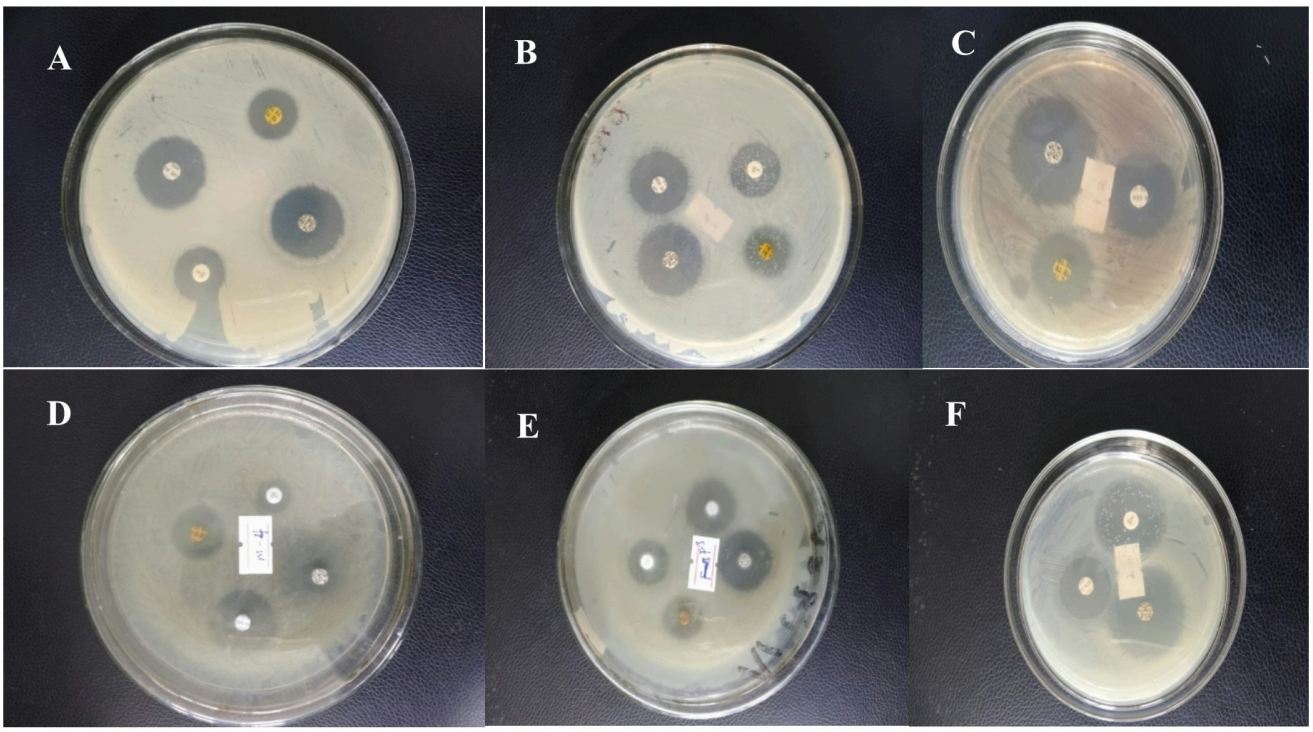


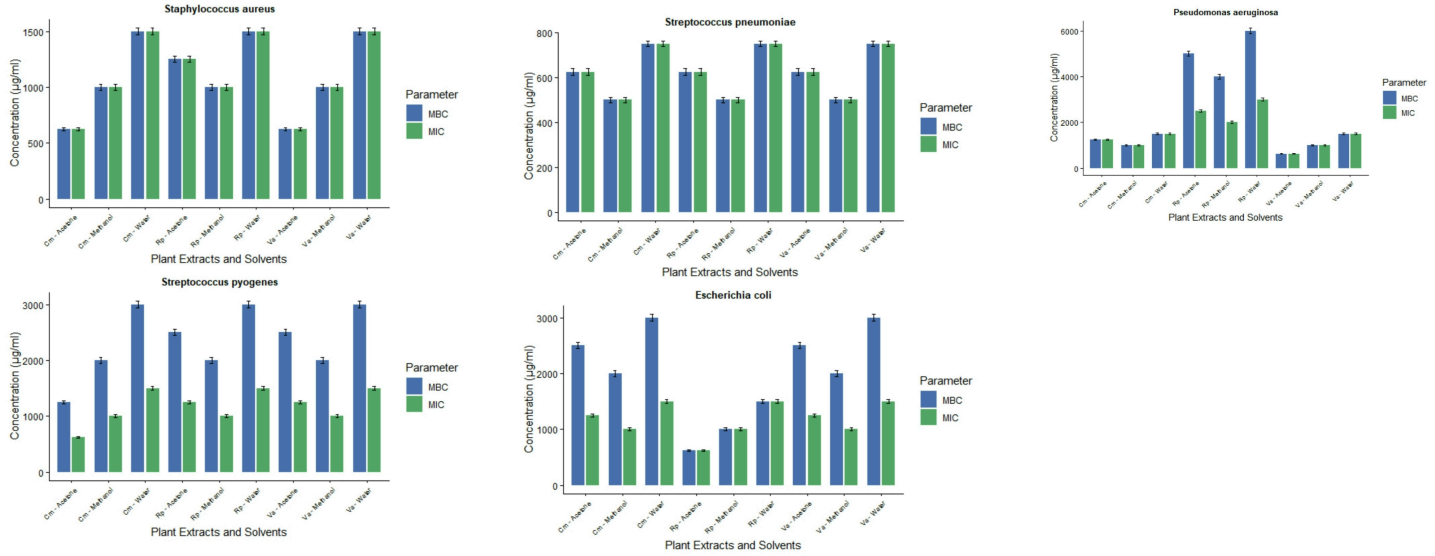

**Figure 1**

Location of the study plant collection area. (a) Southern Region in Ethiopia (b) Arba Minch Zuria Woreda in Southern Region (c) Arba Minch Zuria Woreda



## Figure 2

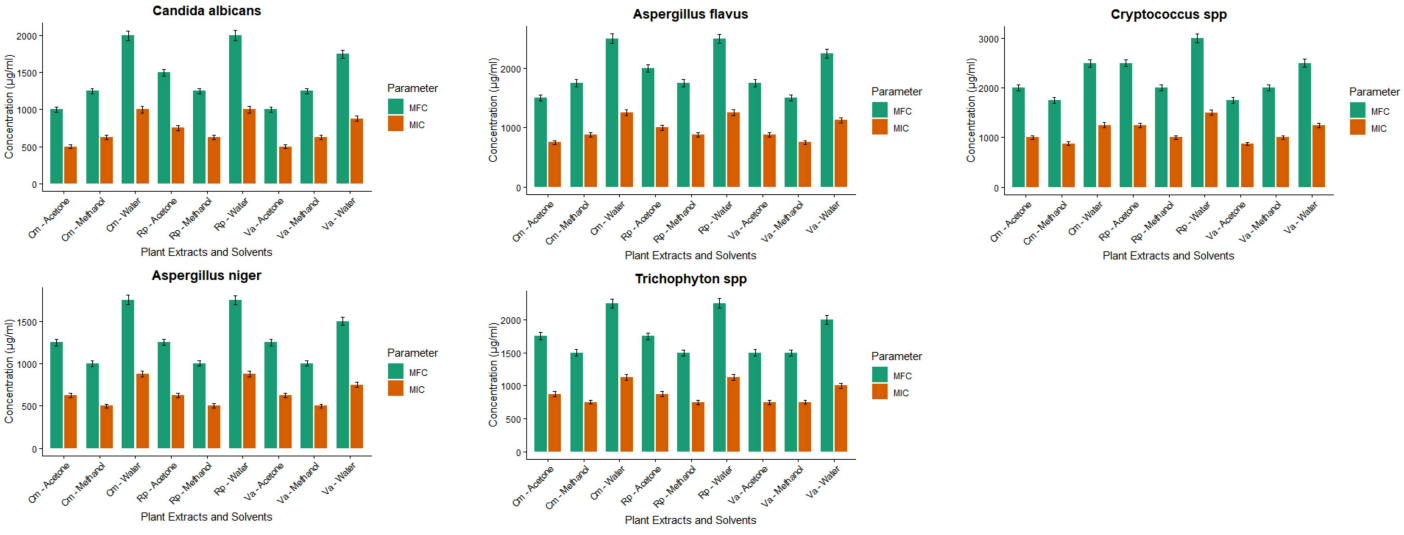
Growth inhibition caused by plant extracts against selected bacterial pathogens A) *E. coli*; B) *S. aureus*; C and F) *S. pneumonia*; D) *P. aeruginosa*; E) *S. pyogenes*




## Figure 3

MIC and MBC of examined plant extracts against five bacterial pathogens.

Rp: *Rhamnus prinoides*, Cm: *Croton macrostachyus*, Va: *Vernonia amygdalina*




## Figure 4

MIC and MFC values of the plant extract against fungal human pathogens