Infection and Drug Resistance

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ORIGINAL RESEARCH

Antibacterial Activity Against Multidrug-Resistant Clinical Isolates of Nine Plants from Chencha, Southern Ethiopia

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Background: The diminishing efficacy of antibiotics currently in use and the emergence of multidrug-resistant bacteria pose a grave threat to public health worldwide. Hence, new classes of antimicrobials are urgently required, and the search is continuing.

Methods: Nine plants were chosen for the current work, which are collected from the highlands of Chencha, Ethiopia. Plant extracts containing secondary metabolites in various organic solvents were checked for antibacterial activity against type culture bacterial pathogens and MDR clinical isolates. The broth dilution technique was used to evaluate the minimum inhibitory and minimum bactericidal concentrations of highly active plant extracts, and time-kill kinetic and cytotoxic assays were performed using the most active plant extract.

Results: Two plants (*C. asiatica* and *S. marianum*) were highly active against ATCC isolates. The EtOAc extract of *C. asiatica* produced the highest zone of inhibition ranging between 18.2±0.8–20.7±0.7 and 16.1±0.4–19.2±1.4 mm against Gram-positive and Gram-negative bacteria, respectively. The EtOH extract of *S. marianum* displayed zones of inhibition in the range of 19.9±1.4–20.5 ±0.7 mm against the type culture bacteria. The EtOAc extract of *C. asiatica* effectively curbed the growth of six MDR clinical isolates. The MIC values of *C. asiatica* against the Gram-negative bacteria tested were 2.5 mg/mL, whereas the corresponding MBC values were 5 mg/mL in each case. The MIC and MBC values were the lowest in the case of Gram-positive bacteria, ie, 0.65 and 1.25 mg/mL, respectively. A time-kill assay showed the inhibition of MRSA at 4 × MIC and 8 × MIC within 2 hours of incubation. The 24 h LD_{50} values of *C. asiatica* and *S. marianum* corresponding to *Artemia salina* were 3.05 and 2.75 mg/mL, respectively.

Conclusion: Overall results substantiate the inclusion of *C. asiatica* and *S. marianum* as antibacterial agents in traditional medicines.

Keywords: plant extract, antimicrobial, cytotoxicity, Chencha, secondary metabolites, drug resistance

Introduction

Plant-derived substances are the primary ingredients and essential components of drugs used in ayurveda, homoeopathy, naturopathy, and traditional medicines of the Native American Indian community.[1] A report by the World Health Organization (WHO) indicates that of the 119 plant-derived drugs, 74% are part of modern medicine and are consistent with their traditional use.[2] Major pharmaceutical corporations constantly do research on plant parts gathered from rainforests and diverse geographic zones to evaluate their potential medicinal values.[3] In addition, a quarter of the drugs prescribed worldwide are of plant origin, consisting of a total number of 121 active compounds.[4] There were 13 drugs approved in the United States between 2005 and 2007, and more than 100 natural product-based drugs are currently undergoing clinical trials.[5] Among the 252 essential medicines on the WHO list, 11% are derived solely from plants.[4]

Received: 6 March 2023
Accepted: 21 April 2023
Published: 27 April 2023

Several plants have therapeutic properties that can heal various diseases and infections naturally and have been in use for centuries because of their antibacterial, antifungal, and antiviral activities.[6] Nowadays, a great quantum of attention has been given to new targets that can be formulated into drugs for treating infectious bacterial and viral diseases and cancer.[7] Bacteria generally have an inherent genetic capability of acquiring and transmitting resistance against synthetic drugs utilized as therapeutic agents. The indiscriminate usage of antibiotics resulted in many bacterial pathogens rapidly becoming resistant to several initially discovered drugs.[8] The efficacy of newly discovered drugs must be adequate to prevent the spread of multidrug-resistant bacteria.[9] Some of the components in plant extracts can play an essential role in conventional as well as modern medicines.[10] A continuous search for new curing agents from medicinal plants results in alternate sources of antibacterial drugs. Plant-based drugs serve as prototypes of more effective and less toxic medicines and drug formulations involving several medications in the upcoming years.[7]

Traditional drugs, mainly derived from medicinal plants, are widely used in Africa because of some cultural connections and economic reasons.[11] As in the case of several countries in the continent, many infectious diseases are treated in Ethiopia's rural areas through traditional medicines.[12] The WHO reports that 90% of the population in the country uses these medicines for their primary health care.[13] Plants account for the origin or source of 95% of traditional medicinal preparations in Ethiopia.[14] The country's flora has a lot of diversity and endemism, making it the fifth-largest in the continent.[15] Many of these plants are believed to contain therapeutic bioactives that could be exploited to treat a variety of diseases ranging from HIV/AIDS to frivolous sore throat,[11] and also many of them appear in traditional medicines and are not scientifically or systematically assessed.[8] Therefore, it is the need of the hour that such plants in use be investigated carefully to validate their efficacy. A data bank describing the effectiveness of herbal plants will encourage the usage of plant-based medicines, which are usually cheaper and harmless.[8] In addition, abundant opportunities exist for isolating specific bioactive molecules from the crude extract of various plant parts and making them even more effective by easy derivatisation.

Our research group has been actively engaged for a decade in the study of antimicrobial properties of plants distributed in different parts of Ethiopia. For instance, recently, we have reported the antimicrobial activities of plants from the suburbs of Arba Minch, Omo Valley, and Nekemte, some of which are even endemic.[8,16–20] Now we are focusing our research on plants in the Chencha district, which is home to diverse species of flora that remain unexplored to a greater extent. More than 135 species of plants from the highlands of Chencha have been documented. Many are part of traditional medicines used to treat wound infections, gastrointestinal infections, and tonsillitis.[21,22] Currently, only limited reports exist on the antibacterial properties of plants from this highland area. Since these plants have long been used in medicines locally, it is worth doing an extensive screening to obtain a data bank, especially by evaluating their antimicrobial properties. The present study involves screening nine selected medicinal plants collected from the Chencha highland against several bacterial pathogens.

Materials and Methods

Study Area

The plant specimens were collected from various sites spread over the Chencha highland, Arba Minch, southern Ethiopia. Chencha is located in the Gamo zone; according to the meteorological report obtained from the Agricultural and Rural Development Office of the District, the annual mean temperature and precipitation are 22.5°C and 1201–1600 mm, respectively.

Plant Specimens

Depending on their availability, healthy and fresh specimens of plants were garnered from specific spots in the study area (ie, natural forests, home gardens, agricultural landscapes, and sacred sites). These specimens were chosen for their medicinal use based on previous ethnobotanical reports and also in consultations with traditional healers. The plants included in the present study and their current ethnobotanical uses are listed in Table 1. The collected samples were transported to the laboratory in zip-locked polythene and isothermal bags. Taxonomic identification was made with the aid of an eminent botanist Dr Sileshi Nemomissa, and voucher specimens and photographs were retained at the National

Table 1 Ethnobotanical Data of Medicinal Plants Selected for Antibacterial Screening

Scientific Name	Parts Used	Ethno-Botanical Usage	Voucher No.	Reference
Centella asiatica	Leaves	Wound healing Anti-septic	AM01	[67]
Silybum marianum	Latex, rhizome	Hepatitis Stomach aches	AM02	[68]
Pycnostachys abyssinica	Leaves, stem	Hypertension	AM04	[69]
Fuerstia africana	Leaves	Abdominal infection	AM05	[70]
Ranunculus multifidus	Leaves	Respiratory tract infection, gastrointestinal infections, and toothache	AM06	[71]
Plantago lanceolata	Leaves	Asthma	AM07	[72]
Rumex nepalensis	Leaves	Tonsillitis and gastrointestinal infections	AM08	[73]
Nuxia congesta	Leaves	Throat infections and headache	AM09	[74]
Matricaria recutita	Leaves	Menstruation disorders	AM10	[75]

Herbarium of Addis Ababa University. Before extraction, fresh leaves were washed to eliminate debris, minced into small pieces, and kept at room temperature.[8]

Preparation of Extracts

A procedure for extracting crude bioactives described by Manilal et al was followed.[21] Thus, 5 g of respective plant specimens were separately pulverised in a mortar and pestle using organic solvents of increasing polarity, such as chloroform (TCM), ethyl acetate (EtOAc), ethanol (EtOH), and methanol (MeOH); aqueous extracts were also prepared. The resulting extracts were subsequently filtered, and the residues were extracted twice more in the same way. The total extracts were obtained in each case by combining the filtrates from all three consecutive extractions. They were dried in a water bath at 50 $^{\circ}$C, and the concentrated extracts (about 50 mL) were collected in vials and refrigerated. Aliquots (2 mg/mL) of each extract were then prepared with the respective solvent and were tested for their antimicrobial activity against human pathogens.

Assay Bacteria

All the plant extracts were tested against a battery of ATCC and MDR bacterial pathogens (Table 2 and Table 3). Clinical isolates established as MDR were sourced from our laboratory; ATCC strains were obtained from Ethiopian Public Health Institute.

In vitro Antibacterial Screening

The antibacterial activity of each plant extract was detected using the agar well diffusion method described by Manilal et al,[19] followed by the determination of the minimum inhibitory concentration (MIC) of chosen plants. Activity

Table 2 Panel of Pathogens Used for the Primary Screening of Plant Extracts

No.	ATCC Strains no.	Species
1	ATCC 25923	*Staphylococcus aureus* (SA)
2	ATCC 29212	*Enterococcus faecalis* (EF)
3	ATCC 25922	*Escherichia coli* (EC)
4	ATCC 27853	*Klebsiella pneumoniae* (KP)

Table 3 Panel of Pathogens Used for Extended Screening of Highly Active Plant Extracts

Clinical Isolates	Species
Gram-positive	Methicillin-resistant *S. aureus* (MRSA)
	Vancomycin-resistant *Enterococci* (VRE)
Gram-negative	ESBL-*E. coli* (ESBLEC) ESBL-*K. pneumoniae* (ESBLKP) Piperacillin resistant *Pseudomonas aeruginosa* (PRPA) Carbapenem resistant *E. coli* (CRE)

evaluations were done against ATCC bacterial and clinical isolates, including WHO-prioritised drug-resistant strains. A preliminary screening thus revealed the most suitable solvent for extraction. The plant extract with the highest antibacterial activity against ATCC isolates in the preliminary agar diffusion assay was evaluated further against a panel of MDR clinical isolates (extended screening).

The plant extracts which showed the broadest and highest range of antibacterial activity against all the isolates (ATCC & clinical MDR isolates) in both assays were taken up for the final antimicrobial analyses (ie, MIC and time-kill kinetic assays).

Primary Antibacterial Screening

The primary antibacterial screening of extracts against four isolates of ATCC was performed in vitro by the agar diffusion method in Mueller Hinton agar (Himedia, India)[19] (Table 2). A fresh overnight culture of test bacteria, equivalent to 0.5 McFarland standard, was prepared and inoculated onto the agar plates. Afterward, wells were made on agar plates and filled with 120µL of the appropriate plant extract, dissolved in dimethyl sulfoxide or distilled water to minimize the toxicity of solvents. A well filled with the respective solvent served as a negative control.

On the other hand, broad-spectrum antibiotics corresponding to the respective bacteria are taken as the positive control. All the experiments were performed in triplicate (three independent experiments). The diameter of zones of inhibition was measured using a Vernier calliper after an incubation period of 24 h at 37°C. The activity indices of all the solvent extracts of each plant and the overall activity index were calculated using the expressions given elsewhere.[8] The antibiotic, ciprofloxacin, represents the positive control, whereas the solvents are the negative control. This ensures that the solvents used for extraction and dissolution have no influence on the growth of bacteria.[8]

Extended Screening Against MDR Clinical Isolates

On the basis of the results derived from the preliminary agar well diffusion assays, only the plant extract that displayed the highest zone of inhibition and the broadest spectrum of activity against all the ATCC microbial isolates was considered and tested further for antibacterial activity against an extended battery of six MDR clinical isolates (Table 2). The methodology was similar to preliminary screening (agar diffusion assay). The results of this extended screening facilitated the selection of a potent extract for further analysis.[8]

MIC and MBC

The broth dilution method was used to inspect the MIC of crude extracts with the widest and highest range of activities.[19,20] Test solutions consisted of aliquots of dried plant extracts in phosphate-buffered saline (pH 7.2). The dose level of extracts was calculated by a factor of 2 (antilog 0.3) to achieve a final dose in the range of 0.31–40 mg/mL in Mueller Hinton broth (speculated concentrations). Each tube is subsequently inoculated with 100 µL of an overnight culture (exponential phase colonies) of the corresponding clinical isolate and incubated at the ambient temperature for 24 h. The lowest concentration of each plant extract that inhibited visible growth, as indicated by a lack of turbidity, was recorded as MIC. The MIC cultures were inoculated (10 µL) onto Mueller Hinton agar and incubated at 37°C for 24 h; minimum bactericidal concentration (MBC) was considered as the concentration showing zero colony growth compared to the inoculum of the initial culture of the same isolate. The mechanism of

antibiosis (bactericidal or bacteriostatic) shown by the plant extracts was elucidated using the ratio of MBC/MIC or MIC_{index}, as mentioned previously.[19,20]

Time-Kill Kinetics Assay

The time-kill kinetics of the most active plant, *C. asiatica*, was performed as described elsewhere.[23] The extracts were prepared at concentrations equivalent to MIC, twofold of MIC and fourfold of MIC, and the log-phase colonies of *S. aureus* (1.0×10^6 CFU/mL) were seeded and incubated at 37°C. A test tube without the extract serves as a negative control; 1.0 mL aliquots of samples were taken from respective test tubes at specific time intervals (0, 2, 4, 6, 8, 12, and 24 h) and were aseptically inoculated onto tryptic soya agar and incubated at 37°C for 24 h. The number of bacteria was enumerated, and a graph was plotted by taking log CFU/mL versus time.

In vitro Cytotoxic Assay

A brine shrimp test utilising *Artemia salina* larvae is beneficial for monitoring the cytotoxicity of natural products, including plant extracts. It was evaluated on newly hatched nauplii of *A. salina* (Sanders Great Salt Lake, Brine Shrimp Company L.C., USA).[24] The assay was done with 2 mL of saline (35 ppt) containing a chosen concentration of the plant extract in cavity blocks (embryo cups). Afterward, 20 numbers of nauplii were added to the experimental, positive, and negative control wells. Pure organic solvent served as a positive control, while saline water served as a negative control. A probit scale was used to calculate the LD_{50} value based on the percent mortality.[25]

Data Analysis

All experiments were conducted in triplicate to ensure statistical validity. The data are represented as the mean standard deviation (SD); SPSS 25 was used to conduct a one-way analysis of variance (ANOVA) of the differences among mean values (Statistical Package for Social Services, Chicago, IL, USA).

Ethical Considerations

All the clinical isolates used in this study are collected from the stock culture deposited at the Medical Microbiology and Parasitology Laboratory, Department of Medical Laboratory Science. No ethics approval was required for this study because it did not involve any human or animal subjects.

Results

Overall Antibacterial Screening Against ATCC Bacterial Isolates

Plant specimens were extracted separately using TCM, EtOAc, EtOH, MeOH and water. Water is a common and convenient solvent for most of the traditional healers due to its availability. However, it cannot extract antibacterial compounds that are non-polar or medium-polar in nature. Therefore, in this work, we included other solvents to extract compounds of varying degrees of polarity. Antibacterial screening revealed that crude extracts of all nine plant species showed in vitro antibacterial activity against at least one of the tested ATCC bacterial isolates. The activity indices of plant extracts are tabulated in Table 4. Further, it was found that the activity of plant extracts differed significantly according to the type of solvent used for extraction.

Results of the overall activity index of plants indicated that *C. asiatica* has the highest spectrum (93.7%) of antibacterial activity, followed by *S. marianum* (75%). EtOAc, EtOH, and MeOH, extracts of *C. asiatica*, showed the highest rank of activity indices (100%), followed by TCM (75%). In the case of *S. marianum*, both EtOAc and EtOH extracts showed activity indices of 100% (Table 4). The mean difference was significant at the 0.05 level. On comparing the activity indices of all organic extracts, it was found that EtOAc and EtOH were the most suitable solvents for extracting antibacterial components. Aqueous extracts of all the plants showed no bactericidal activity (data not shown). Also, none of the negative controls showed any inhibitory effect. However, the zone of inhibition of the positive control, ie, ciprofloxacin, was significantly higher than that of the extracts.

Table 4 Antibacterial Activity Indices of Plant Extracts (%)

Plant Species	TCM	EtOAc	EtOH	MeOH	OAI
C. asiatica	75	100	100	100	93.7
S. marianum	50	100	100	50	75
P. lanceolata	50	100	25	25	50
P. abyssinica	50	100	50	50	62.5
F. africana	0	0	50	100	37.5
R. multifidus	0	50	25	0	18.75
R. nepalensis	25	75	100	50	62.5
N. congesta	25	50	100	25	62.5
M. recutita	25	75	75	50	56.25

Notes: Activity index was expressed as the relative antimicrobial activity of the respective solvent extract of the plant species against five ATCC bacteria. Overall activity was expressed as the relative antimicrobial activity of all the solvent extracts of a particular plant species against four ATCC bacteria. Zone of Inhibition ≥ 10 mm were considered active.
Abbreviations: TCM, chloroform; EtOAc, ethyl acetate; EtOH, ethanol; MeOH, methanol; OAI, overall activity index of respective plant species.

Antibacterial Activity of Different Plant Extracts Against ATCC Bacterial Isolates

Centella asiatica

The overall activity index of *C. asiatica* extract was 93.7%. Three solvents, such as EtOAc, EtOH, and MeOH, performed well in terms of the extent of extraction of antibacterial metabolites. These extracts efficiently inhibited the growth of 100% of ATCC isolates. The crude EtOAc extract of *C. asiatica* exhibited a zone of inhibition in the range of 16.1±0.4–19.2±1.4 mm against Gram-negative bacteria and 18.2±0.8–20.7±0.7 mm against Gram-positive bacteria. High levels of inhibitory activity were observed, particularly against Gram-positive bacteria. The EtOAc extract showed impressive activity against *E. faecalis, S. aureus*, and *E. coli*, corresponding to inhibitory zones, 20.7±0.7, 18.2±0.8, and 19.2±1.4 mm, respectively. However, only a lower level of activity was detected against *K. pneumoniae* (16.1±0.4 mm). The second most active was the EtOH extract. It produced a zone of inhibition ranging from 14.7±0.6 to 20.4±0.8 mm and also showed the highest activity against *E. coli* (20.4±0.8 mm). The MeOH extract controlled the growth of bacteria by producing inhibitory zones in the range of 11.6±0.6–13.5±0.8 mm (Table 5).

Table 5 Inhibition Zones of Different Plant Extracts in Primary Antibacterial Screening Against ATCC Isolates

Plant	Solvents	SA	EF	EC	KP
C. asiatica	TCM	12.7 ± 0.8^{fg}	13.2 ± 0.8^{ef}	11.8 ± 0.8^{fg}	9.1 ± 0.9^{hi}
	EtOAc	20.7 ± 0.7^{a}	18.2 ± 0.8^{b}	19.2 ± 1.4^{c}	16.1 ± 0.4^{c}
	EtOH	14.7 ± 0.6^{d}	15.4 ± 0.7^{c}	20.4 ± 0.8^{b}	17.5 ± 0.9^{b}
	MeOH	11.8 ± 0.6^{hi}	13.5 ± 0.9^{de}	11.6 ± 0.6^{fg}	12.6 ± 0.3^{d}

(*Continued*)

Table 5 (Continued).

Plant	Solvents	SA	EF	EC	KP
S. marianum	TCM	7.5±0.5^{k}	9.1±0.7^{ij}	14.6±0.9^{e}	12.5±0.5^{de}
	EtOAc	18.8±0.3^{b}	21±1.7^{a}	21.7±0.9^{a}	20.5±1.6^{a}
	EtOH	20.5±0.7^{a}	19.9±1.4^{ab}	21.4±1.0^{ab}	21.8±2.4^{a}
	MeOH	16.9±0.6^{c}	9.1±0.8^{ij}	12.6±1.0^{f}	7.6±0.7^{ij}
P. lanceolata	TCM	12.7±0.7^{fg}	8.2±1.1^{k}	12.2±1.0^{fg}	9.0±0.9^{hi}
	EtOAc	16.8±0.6^{c}	19.6±1.5^{ab}	17.9±0.5^{d}	14.8±0.7^{c}
	EtOH	12.9±0.8^{ef}	9.0±0.6^{ij}	7.8±0.4^{j}	7.8±0.2^{ij}
	MeOH	12.3±0.5^{gh}	8.7±0.5^{jk}	8.4±0.6^{j}	8.2±1.4^{ij}
P. abyssinica	TCM	8.2±0.7^{ijk}	12.3±1^{ef}	12.5±0.5^{f}	8.5±0.8^{ij}
	EtOAc	16.6±0.4^{c}	16.4±1.1^{c}	15.5±0.9^{e}	16.0±1.3^{c}
	EtOH	17±0.8^{c}	15.9±1.4^{c}	8.6±0.5^{j}	8.9±0.4^{hi}
	MeOH	17.3±1.4^{c}	15.2±2.3^{cd}	8.2±0.2^{j}	8.3±0.5^{ij}
F. africana	TCM	8.4±0.7^{ijk}	5.3±0.5^{m}	0.0	0
	EtOAc	6.0±1^{L}	0	0.0	5.4±0.5^{k}
	EtOH	13.9±0.8^{de}	8.7±0.6^{kl}	12.5±0.5^{f}	5.3±0.2^{k}
	MeOH	15.1±1.1^{d}	15.3±.9^{cd}	11.6±0.5^{fg}	10.4±0.5^{gh}
R. multifidus	TCM	8.4±0.6^{ijk}	8.4±0.5^{k}	7.5±0.2^{j}	5.3±0.4^{k}
	EtOAc	13.2±0.8^{ef}	8.9±0.5^{jk}	11.4±0.4^{fg}	5.2±0.1^{k}
	EtOH	7.5±0.4^{k}	12.0±0.8^{ef}	5.4±0.4^{k}	5.6±0.4^{k}
	MeOH	9.0±0.2^{j}	9.1±0.7^{ij}	5.2±0.3^{k}	5.2±0.3^{k}
R. nepalensis	TCM	11.5±0.4^{i}	8.7±0.5^{jk}	8.3±0.3^{j}	7.1±0.2^{j}
	EtOAc	9.2±0.5^{j}	10.9±0.5^{gh}	11.8±0.1^{fg}	10.7±1^{fg}
	EtOH	11.6±0.4^{hi}	12.6±1.2^{ef}	11.9±0.5^{fg}	11.2±0.7^{de}
	MeOH	8.5±0.8^{ijk}	11.4±0.8^{fg}	10.6±0.3^{i}	7.9±0.7^{ij}
N. congesta	TCM	9.2±0.7^{j}	10.6±4^{hi}	11.4±0.3^{fg}	11±0.7^{ef}
	EtOAc	13.9±0.2^{de}	11.6±0.7^{ef}	8.6±0.3^{j}	9.0±0.4^{hi}
	EtOH	13.8±0.8^{de}	12.7±1.4^{ef}	10.8±0.7^{hi}	12.2±1.2^{de}
	MeOH	8.5±0.4^{ijk}	12.0±0.6^{ef}	8.5±0.5^{j}	8.9±0.7^{hi}
M. recutita	TCM	9.1±0.6^{j}	11.1±0.3^{gh}	8.1±0.1^{j}	8.9±0.8^{hi}
	EtOAc	9.1±0.3^{j}	13.5±1.2^{de}	11.2±0.2^{gh}	11.3±0.7^{de}
	EtOH	14.1±0.8^{de}	11.3±0.9^{fg}	12.3±0.3^{fg}	7.1±0.3^{j}
	MeOH	8.8±0.6^{ijk}	13.1±1.6^{ef}	8.4±0.05^{j}	10.9±0.4^{ef}
Ciprofloxacin (5µg)		34±1.6	34±1.2	25±1.5	32±1.1

Notes: Values are mean ± standard deviations. Means followed by different letters in the same column differ significantly at $P = 0.05$ according to Duncan's new multiple range tests.

Silybum marianum

The overall activity index of *S. marianum* was 75%; EtOAc and EtOH were found to be the most effective in extracting antibacterial metabolites from this plant. Both produced activity indices of 100% against the ATCC isolates. The former extract showed a higher level of activity against three bacteria, such as *S. aureus* (20.5±0.7 mm), *K. pneumoniae* (21.8 ±2.4 mm), and *E. coli* (21.4±1 mm). Only a slightly lower level of activity was shown against *E. faecalis* (19.9±1.4 mm). The second most effective solvent is EtOAc, and this extract showed higher levels of antibacterial activity against *E. faecalis* (21±1.7 mm), *K. pneumoniae* (20.5±1.6 mm), and *E. coli* (21.7±0.9 mm). The highest activity against *E. coli* was shown by chloroform extract (14.6±0.9 mm) (Table 5), whereas it was the least effective against *K. pneumoniae*.

Plantago lanceolata

The overall activity index of the extract of *P. lanceolata* was 50%; EtOAc was found to be the best extraction solvent in this case, producing an activity index of 100%. This extract showed very effective inhibition against *E. faecalis* (19.6 ±1.5 mm), *E. coli* (17.9±0.5 mm), and *S. aureus* (16.8±0.6 mm). However, a relatively lower rank of activity is only detected against *K. pneumoniae* (14.8±0.7 mm); chloroform extract showed only a moderate activity against *S. aureus* (12.7±0.7 mm) and *E. coli* (12.2±1 mm). On the other hand, the EtOH extract showed a comparatively lower range of activity (9.0±0.6 to 12.9±0.8 mm) (Table 5).

Pycnostachys abyssinica

The overall activity index of the extract of *P. abyssinica* was 50%. It is to be noted that the EtOAc extract of this plant displayed an activity index of 100%. The crude EtOAc extract produced the highest zone of inhibition, ranging between 15.5±0.9 and 16.6±0.4 mm, against ATCC bacterial pathogens. Among the Gram-negative bacteria, *K. pneumoniae* is the most susceptible isolate, corresponding to an inhibitory zone of 16.0±1.3 mm. In the case of its Gram-positive counterpart, *S. aureus*, which is the most susceptible, an inhibitory zone of 16.6±0.4 mm was observed. Besides, inhibitory zones were produced by the EtOH extract of this plant against *S. aureus* (17±0.8 mm) and *E. faecalis* (15.9±1.4 mm).

Fuerstia africana

The overall activity index of *F. africana* extract was only 37.5%. It was found that MeOH was the best solvent for the extraction of this plant, producing an activity index of 100%. This extract showed the highest inhibition against *S. aureus* (15.1±1.1) and *E. faecalis* (15.3±.9 mm). The EtOAc extract showed only moderate levels of activity against *S. aureus* (13.9±0.8 mm) and *E. coli* (12.5±0.4 mm). However, TCM and EtOAc extracts showed no activity (Table 5).

Rumex nepalensis

The overall activity index of *R. nepalensis* extract was 62.5%; EtOH was the most effective solvent for the extraction of antibacterials from this plant, producing an activity index of 100%. This extract displayed the greatest level of inhibition against *E. faecalis* (12.6±1.2 mm), *E. coli* (11.2±0.7 mm), and *S. aureus* (11.6±0.4 mm). However, the EtOAc extract showed only moderate levels of activity against *S. aureus* (9.2±0.5 mm) and *E. faecalis* (10.9±0.5 mm), whereas chloroform and MeOH extracts showed a comparatively lower range of activities (Table 5).

Nuxia congesta

The overall activity index of the extract of *N. congesta* was 62.5%; EtOH was the most suitable solvent to extract antibacterial metabolites from *N. congesta*. This extract produced an activity index of 100% against ATCC isolates, and the highest level of activity was exhibited against three bacteria, such as *S. aureus* (13.8±0.8 mm), *E. faecalis* (12.7 ±1.4 mm), and *K. pneumoniae* (12.2±1.2 mm). However, only a slightly lower activity level was shown against *E. coli* (10.8±0.7 mm). The second most effective solvent is EtOAc, and the extract showed higher levels of antibacterial activity against *S. aureus* (13.9±0.2 mm) and *E. faecalis* (11.6±0.7 mm); MeOH extract showed the highest activity against *E. faecalis* (12.0±0.6) (Table 5).

Matricaria recutita

The overall activity index of *M. recutita* extract was 56.25%. It was found that EtOH and EtOAc were the best solvents for extraction, both producing an activity index of 75%. These extracts showed the highest inhibition against *S. aureus* (14.1 ±0.8 mm), *E. coli* (12.3±1 mm), and *E. faecalis* (11.3±0.9 mm). The EtOAc extract of this plant showed only a moderate activity against *S. aureus* (9.1±0.3 mm). However, it showed a higher range of activities against all other isolates (Table 5).

Antibacterial Activity of Different Plant Extracts Against MDR Clinical Isolates

Based on the results of the overall activity indices with respect to ATCC isolates, EtOAc and EtOH extracts of *C. asiatica, S. marianum*, and *P. lanceolata*, EtOAc extract of *P. abyssinica, R. nepalensis*, and *N. congesta*, MeOH extract of *F. africana* and EtOAc extract of *M. recutita* were further subjected to a comprehensive antibacterial screening against a panel of six drug-resistant bacterial isolates sourced from clinical samples. The above-mentioned plant extracts efficiently prevented the growth of MDR isolates to varying degrees. On the other hand, CRE was observed to be the most resistant MDR clinical isolates against most of the plant extracts tested.

C. asiatica

Ethyl acetate extract of this plant showed the highest inhibition zones against penicillin-resistant *P. aeruginosa* (24.2 ±1.3 mm) and MRSA (22.6±0.8 mm), followed by VRE (19.8±0.67 mm) and ESBLKP (19.5±0.6 mm). The antibacterial activity of the EtOH extract of *C. asiatica* was found to be moderate against MDR clinical isolates, and the activity was in the range of 12.5±1.2–20.4±1.1 mm (Table 6). However, the MeOH extract proved only less effective against four MDR isolates, and the inhibition zones are in the range of 10.3±0.3–14.8±0.3 mm.

S. marianum

The extent of antibacterial activity of the EtOAc extract of *S. marianum* against MDR isolates was considerably great. Inhibitory zones of 19.7±1.1, 17.5±0.9, and 17.4±0.8 mm were shown against three clinical isolates such as ESBLEC,

Table 6 Inhibition Zones of Different Plant Extracts in Extended Antibacterial Screening Against MDR Isolates

Plant	Solvents	MRSA	VRE	PRPA	ESBLKP	ESBLEC	CRE
C. asiatica	EtOAc	22.6±0.8	19.8±0.67	24.2±1.3	19.5±0.6	17.7±1.3	18.3±2.8
	EtOH	12.5±1.2	12.7±0.8	20.4±1.1	19.6±1.3	13.9±0.7	15.7±1.3
	MeOH	11.0±0.4	11.2±0.9	8.4±0.5	10.3±0.3	14.8±0.3	5.5±0.5
S. marianum	EtOAc	15.5±0.6	17.5±0.9	17.4±0.8	15.7±0.8	19.7±1.1	10.7±0.6
	EtOH	15.8±1.4	17±1.2	16.8±0.9	14.9±0.4	17.4±0.8	11.3±1.4
P. lanceolata	EtOAc	12±2.2	10.8±0.7	15.5±1.2	11.2±0.7	12.8±0.7	6±0.9
P. abyssinica	EtOAc	13.8±1.1	14.4±1	12.1±0.4	10.9±0.8	12.9±0.2	6.9±1.3
R. multifidus	EtOAc	8.2±1.1	11.7±0.8	7.7±0.5	0	0	0
F. africana	MeOH	12.9±2.2	12.6±1	8.5±0.7	9±0.2	6.9±0.8	8.1±1
R. nepalensis	EtOH	6.1±1	7.4±1	7.1±0.6	0	0	0
N. congesta	EtOH	11.8±1.5	12±0.8	13±1.1	11.8±0.5	10.6±0.5	7.8±1.2
M. recutita	EtOAc	8.1±0.8	9.8±0.9	7.3±0.6	8±0.9	8.8±0.7	5.5±0.5
	EtOH	8.9±0.4	11.7±1.1	6.7±0.4	5.5±0.4	7.8±0.1	5.9±0.3
Ciprofloxacin (5µg)		35±1.45	20.7±2.50	22±1.4	32±1.50	24±2.3	19.2±1.7

VRE, and PRPA, respectively. Only a lower rank of activity was shown against CRE (10.7±0.6 mm). The second most active solvent is EtOH, which showed higher levels of antibacterial activity against VRE (17±1.2 mm) and ESBLEC (17.4±0.8 mm) (Table 6).

P. abyssinica

The antibacterial activity of the EtOAc extract of *P. abyssinica* against MDR isolates was respectably high. Inhibitory zones of 14.4±1, 13.8±1.1, and 12.9±0.2 mm were shown against three clinical isolates, VRE, MRSA, and ESBLEC, respectively. A lower degree of activity was only shown against ESBLKP (10.9±0.8 mm) (Table 6).

P. lanceolata

The EtOAc extract of this plant showed a high extent of inhibition against PRPA (15.5±1.2 mm), followed by MRSA (12 ±2.2 mm) and ESBLEC (12.8±0.7 mm). However, no activity was exhibited against CRE.

Antibacterial Activity of Other Plant Extracts

The EtOH extract of *N. congesta* was active against five MDR isolates, and the activity in terms of inhibition zones ranged from 10.6± 0.5 to 13±1.1 mm; MeOH extract of *F. africana* inhibited the growth of MRSA (12.9±2.2) and VRE (12.6±1 mm); EtOH extract of *M. recutita* displayed activity against only one of the MDR clinical isolates, ie, VRE (11.7 ±1.1 mm). Likewise, the EtOAc extract of *R. multifidus* showed activity against only VRE (11.7±0.8). The extract of *R. nepalensis* showed no activity against MDR clinical isolates.

MIC and MBC of *C. asiatica* Extract Against ATCC Isolates

The MIC and MBC values of *C. asiatica* against the ATCC bacteria ranged between 0.65–2.5 mg/mL and 1.25–5 mg/mL, respectively. The lowest MIC and MBC values were observed against *S. aureus*, ie, 0.65 and 1.25 mg/mL, respectively. The *E. coli* and *K. pneumoniae* corresponded to MIC and MBC values of 2.5 and 5 mg/mL, respectively (Table 7).

MIC and MBC of *C. asiatica* Extract Against MDR Clinical Isolates

The determined MIC values of this extract against MDR clinical isolates ranged between 2.5 and 10 mg/mL. The lowest MIC value of 2.5 mg/mL with a corresponding MBC of 5 mg/mL was recorded against MRSA. However, the MIC and MBC values corresponding to other MDR isolates were slightly higher (5 and 10 mg/mL) (Table 8).

MIC and MBC of *S. marianum* Extract Against ATCC Isolates

The MIC and MBC values of *S. marianum* against ATCC isolates ranged between 2.5–5 mg/mL and 5–10 mg/mL, respectively. The lowest values of MIC and MBC were observed against *S. aureus*, and they are 2.5 and 5 mg/mL, respectively. Gram-negative counterparts, *E. coli* and *K. pneumoniae*, corresponded to an equal level of MIC and MBC values, ie, 5 and 10 mg/mL, respectively (Table 9).

Table 7 MIC and MBC of EtOAc Extracts of *C. asiatica*

Test Organisms	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
E. coli	2.5	5	2
K. pneumoniae	2.5	5	2
E. faecalis	1.25	2.5	2
S. aureus	0.65	1.25	2

Table 8 MIC and MBC Values of EtOAc Extract of *C. asiatica* Against MDR Clinical Isolates

Test organisms	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
ESBL-*E. coli*	5	10	2
ESBL-*K. pneumoniae*	5	10	2
PR-*P. aeruginosa*	5	10	2
MRSA	2.5	5	2

Table 9 MIC and MBC of EtOAc Extract of *S. marianum*

Test Organisms	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
E. coli	5	10	2
K. pneumoniae	5	10	2
E. faecalis	5	10	2
S. aureus	2.5	5	2

Table 10 MIC and MBC Values of EtOAc Extract of *S. marianum* Against MDR Clinical Isolates

Test Organisms	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
ESBL-*E. coli*	10	20	2
ESBL-*K. pneumoniae*	10	20	2
PR-*P. aeruginosa*	5	20	2
MRSA	5	10	2

MIC and MBC of *S. marianum* Extract Against MDR Clinical Isolates

The MIC values obtained in the case of MDR clinical isolates ranged between 5 and 10 mg/mL. The lowest value of MIC, ie, 5 mg/mL with a corresponding MBC of 10 mg/mL, was recorded against MRSA. However, the MIC and MBC values of other MDRs were slightly higher (10 and 20 mg/mL, respectively) (Table 10).

Time Kill Kinetic Assay

According to our study, the MIC against MRSA was 2.5 mg/mL. The killing rate of *C. asiatica* enhanced with an increase in the extent of MIC. At 1× MIC of extract, the growth of MRSA is fully inhibited at 24 h. On the other hand, at 4 and 8× MIC, bactericidal effects were observed within just 4 hours of incubation (Figure 1). The time-kill kinetics showed a dose-dependent bactericidal effect on MRSA, indicating an impressive antibacterial property of the extract, directly in proportion to the rate of death of *S. aureus*.

Brine Shrimp Cytotoxicity

The toxicity of extracts of *C. asiatica* and *S. marianum* to *A. salina* nauplii were pronounced at higher concentrations, and the 24 h LD_{50} values were 3.05 and 2.75 mg/mL, respectively. The *C. asiatica* and *S. marianum* extracts at concentrations less than one mg/mL were not toxic to nauplii, whereas it was found to be toxic at a concentration above

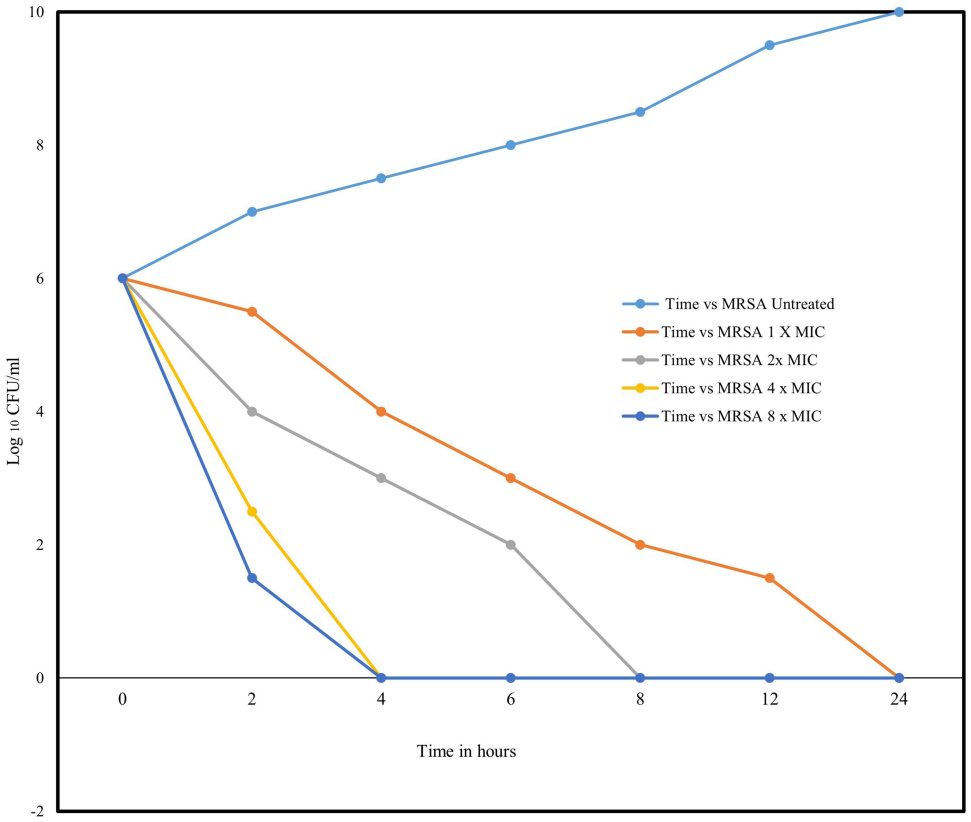

Figure 1 Time kill kinetic assay showing the inhibition of the MRSA by the *C. asiatica* at 1× MIC, 2× MIC, 4× MIC and 8× MIC. At 1× MIC, the complete inhibition was achieved within 24 hours of incubation, and when MIC was increased to 4× and 8×, the bactericidal effect was noticed within 4 hours of incubation.

8 mg/mL. It is to be mentioned that the toxicity of ethyl acetate, a vehicle control used for solubilising the extracts, was negligible. At higher concentrations, ie, >8 mg/mL, the nauplii treated with both extracts showed hyperactive movements and paralysis.

Discussions

This study is part of an ongoing screening program aimed at the discovery of Ethiopian medicinal plants with antibacterial activities.[8,16–20] This is the first study demonstrating the in vitro antibacterial and cytotoxic potentials of plants, which are widely used in conventional medicines by traditional healers in Chencha. The selection of plants based on ethnobotanical and ethnomedicinal information can probably yield good floral candidates having peculiar potential as the source of antibacterials compared to a random selection.[26] Plants included in the present study were selected based on ethnomedicinal data collected from the highlands of Chencha. There are many opportunities to discover the antibacterial activity of ignored plants with medicinal value. In addition, antibacterial screening of crude plant extracts is more practical and quicker than looking for the activity of selected isolated pure compounds of plant origin.[27]

The antibacterial activity varies greatly among plant species, and also, in the case of each plant type, it may fluctuate depending on the type of solvent used for extraction and the genus of bacteria tested.[19] Ipso facto, Gram-negative bacteria are more virulent than their Gram-positive counterparts due to their typical morphology and some innate traits.[28] As a result, antimicrobial activity screening should be continued in a broader sense in order to discover newer and more effective compounds, particularly for dealing with the former, many of which are known as MDR strains. The plant extracts examined in this study displayed remarkable antibacterial activity, particularly against Gram-positive bacteria. Alterations in the antibacterial activities of plant species may be correlated to variations in the harvesting time,[29] developmental stage, method of extraction employed, virulence factors of bacteria tested, and even the specific plant part used.[20] A fascinating aspect of our study is that many of the plant extracts exhibited impressive activity against

WHO-prioritised pathogens such as MRSA, VRE, ESBL, and CRE. However, not much extensive research was done on the antibacterial potentials of these plants against MDR isolates.

Antimicrobial Activities of Plants

The present study showed that several plants used by traditional healers in Chencha possess promising antimicrobial activities. The antibacterial activity of a plant extract is attributed to a wide variety of phytochemical compounds contained in them. However, secondary metabolites and their functionalities believed to contribute to the antibacterial activities of the listed plants in this study are not elucidated. In the current study, the EtOAc extract of *C. asiatica* demonstrated the highest and most comprehensive spectrum of activities against ATCC and MDR isolates, making it the most valuable plant. Furthermore, it effectively curbed the growth of all bacteria tested. The second most effective was the EtOH extract of the same plant; MeOH extract, however, showed moderate to low activity only. This was in contrast with the results of a study done in South Africa, which reported that MeOH and acetone extracts of *C. asiatica* were more active compared to EtOH and aqueous extracts.[30] The significant activity displayed by EtOAc and EtOH extracts could be attributed to some soluble and active polar compounds. Earlier published work from South Africa,[31] Malaysia,[32] and India[33] indicated the antibacterial activity of *C. asiatica* extract in various solvents as well as that of essential oils. However, there is no published report on the antimicrobial activity of *C. asiatica* from Ethiopia so far.

According to our findings, EtOH extract showed significant antibacterial activity against ESBL-*E. coli*, CR-*K. pneumoniae*, and CR-*P. aeruginosa*.[34] Similarly, another couple of studies done in Malaysia reported that EtOH extract was considerably effective against *B. cereus, E. coli* O157:H7, *E. coli, S. enterica* serovar Typhimurium, and *S. aureus*.[32,35] The second most active plant identified in our study was *S. marianum*. EtOAc and EtOH extracts of this plant effectively inhibited the growth of ATCC and MDR isolates. A literature scan indicates that the antibacterial activity of *S. marianum* from Ethiopia has not yet been reported. Several studies conducted in other parts of the world reported the antibacterial activity of *S. marianum* against various types of bacteria. For instance, different concentrations of the aqueous and MeOH extracts of *S. marianum* demonstrated antibacterial activity against *S. aureus, E. coli, K. pneumoniae, P. aeruginosa, Enterobacter aerogenes*, and *C. albicans*.[36] In addition, a study done in India reported that the EtOH extract of this plant was effective against all four ATCC bacterial strains, such as *S. aureus, E. faecalis, E. coli*, and *P. aeruginosa*.[37] Likewise, a few studies done in Pakistan revealed the antibacterial activity of aqueous and EtOH extracts of the seeds of *S. marianum* against *S. aureus, Bacillus subtilis, E. coli, P. vulgaris, K. pneumoniae*, S. Typhimurium, and *P. aeruginosa*.[38,39] A couple of publications from Iran reported that *S. marianum* has significant antimicrobial activity only against Gram-positive bacteria.[40,41] According to a study done in Brazil, two compounds such as silymarin and silibinin, isolated from *S. marianum*, showed remarkable activity against MDR.[42] On the basis of the overall activity index obtained, we found that the next most active plant was *P. abyssinica*. The EtOAc extract of this plant was active against both ATCC and MDR isolates. A previous study done in Ethiopia reported that petroleum ether extract of the roots of *P. abyssinica* inhibited the growth of *S. aureus* and *P. aeruginosa*.[43] Another study from Ethiopia showed that acetone extract of *P. abyssinica* had antimicrobial activities against *S. aureus, E. coli*, S. Typhi, and *B. cereus*.[44] Another active plant found in our study was *R. nepalensis*. The EtOH extract of this plant entirely prevented the growth of ATCC, and the second most effective was the EtOAc extract. However, the former extract could not produce a consistent result against the MDR isolates. A series of studies conducted in various parts of Ethiopia revealed that *R. nepalensis* has some antimicrobial properties; for instance, *R. nepalensis* sourced from the Amhara region of Ethiopia displayed activity against *B. cereus* and *S. epidermidis*.[45] Likewise, *R. nepalensis* collected from Jimma town exhibited activity against S. Typhimurium and *P. aeruginosa*.[46]

Nuxia congesta was another plant that demonstrated impressive anti-ATCC activity. EtOH extract of this plant actively inhibited the growth of all four reference strains. Furthermore, it showed activities against MDR clinical isolates like MRSA, VRE, PRPA, ESBLKP, and ESBLEC. A study done in Gondar, Ethiopia, also reported that EtOH and MeOH extracts of this plant possessed antibacterial activity against *E. coli, E. faecalis, S. aureus, P. mirabilis, K. pneumoniae*, and *P. aeruginosa*.[47] In addition, another study conducted in Ethiopia reported that the MeOH extract and a fraction of the aqueous extract of *N. congesta* have anti-malarial activities.[48]

Plantago lanceolata was another plant included in our study, and the results revealed that the EtOAc extract of this plant fully subjugated the growth of all four ATCC isolates tested. At the same time, it prevented the growth of only four MDR clinical isolates. An earlier study in Gondar, Ethiopia, reported that crude extracts of *P. lanceolata* showed activity against four bacterial species (Gram-positive bacteria: *S. aureus* and *S. agalactiae* and Gram-negative bacteria: *E. coli* and S. Typhi).[49] Also, a previous study done in Ethiopia showed that this plant demonstrated antibacterial activities against *Listeria monocytogenes, Streptococcus, S. aureus, Salmonella*, and *E. coli*.[50] In addition, *P. lanceolata* leaf extract in various solvents also exhibited antibacterial activity against *S. pneumoniae*, MRSA, *S. aureus, S. boydii, E. coli*, and *K. pneumoniae*.[51]

Matricaria recutita was another active plant incorporated in our study; EtOAc and EtOH extracts of this plant revealed promising antimicrobial activity against the test ATCC bacteria, while it showed only a lower activity level against MDR isolates. In contrast to our results, a previous study done in Debre Berhan, Ethiopia, reported that *M. recutita* failed to show any antimicrobial activity.[52] Interestingly, some studies done in Djibouti and Iran reported a broad spectrum antibacterial activities of *M. recutita*.[53] In the case of *F. africana*, the MeOH extract showed the highest activity against ATCC bacteria; however, in the extended screening test, it only inhibited the growth of MRSA and VRE. Our findings align with those of a study conducted in Kenya, which reported that *F. africana* is the only plant that showed intense activity against MRSA.[54] This result is also consistent with the findings of another study that reported activity against *S. aureus* and MRSA.[55] The MeOH extract of *F. africana* showed the remarkable activity against *S. aureus, B. subtilis, B. pumilus, K. pneumoniae*, and *E. coli*.[56] Extract of *R. multifidus* exhibited only a diminished level of activity. EtOAc extract of this plant can only inhibit the growth of a couple of isolates of the ATCC and only a single isolate of MDR groups of test bacteria, making it the plant with the lowest antimicrobial activity as per our study. A prior study done in Jimma, Ethiopia, reported the antibacterial activity of the chloroform extract of this plant against *S. aureus, P. aeruginosa*, and *E. coli*.[57] Likewise, another study performed in Adama, Ethiopia, reported that the aerial part of *R. multifidus* displayed impressive activity against *S. aureus* and *S. pyogenes*.[58]

Based upon the results of antibacterial activities, only two plants, such as *C. asiatica* and *S. marianum*, were selected for further advanced assays, such as the mechanism of antibiosis and cytotoxicity. Our findings revealed some differences in the MIC and MBC values corresponding to the ATCC and MDR isolates of bacteria tested. The MBC/MIC ratio was calculated to arrive at the type of activity, ie, bactericidal or bacteriostatic, exhibited by the crude extract of *C. asiatica* and *S. marianum*. The MBC/MIC ratio against four ATCC strains was 2, and from this value, it can be inferred that the mechanism of antibiosis of the respective plant extract is bactericidal. MRSA had the lowest value of MIC among all MDR clinical isolates. It could be envisaged that the mechanism of antibiosis against MDR isolates is also bactericidal because the ratio of MBC/MIC is 2.[20] MIC and MBC values may fluctuate depending on factors such as the crude nature of the extract, the concentration of different active ingredients, cytoplasmic permeability of the metabolites, and virulence factors.[8,20] MICs ranging from 1.25 to >10 mg/mL were observed against Gram-positive and Gram-negative bacteria in the case of the crude EtOAc extract of *C. asiatica*.[30] Another study conducted in South Africa reported lower MIC values between 0.039 and 1.25 mg/mL.[31] A higher MIC value ranging from 26 to 62 mg/mL was observed in a study done on *C. asiatica* in Kenya.[59] The authors reported MBC values ranging from 52 to 125 mg/mL in the same study.

The ratio of MBC/MIC against four ATCC strains was 2, indicating that the mechanism of antibiosis of the plant extract is again bactericidal. Furthermore, the antibiosis mechanism against MDR isolates is also bactericidal, as is shown by the ratio of MBC/MIC, ie, 2. In *C. asiatica*, triterpenes are present, which are assumed to be polar compounds. Thus, it is possible to inhibit bacterial growth by ionisation of molecules combined with polyphenol adsorption onto bacterial membranes. This may effectively disrupt cell membranes, eventually resulting in cell death.[60]

MIC values of petroleum ether and EtOH seed extracts of *S. marianum* have already been reported from India.[61] The MIC values corresponding to a couple of compounds isolated from *S. marianum* against *E. coli* were 512 g/mL (for silymarin) and 64 g/mL (for silibinin).[42] The lowest MIC values in the case of seed extracts of this plant, ranging from 125 to 500 g/mL, were found in a study done in Turkey.[62]

Time-kill kinetics revealed that EtOAc extract of *C. asiatica* applied at different concentrations had a dose and time-dependent effect on the death of *S. aureus*. The growth of *S. aureus* was efficiently arrested at 1, 4, and 8 × MIC within 24 and 2 h of incubation, respectively. Therefore, it is presumed that dose-dependent kinetics can happen when the plant extract reaches the target site at a higher concentration, killing *S. aureus*. On the other hand, time-dependent kinetics occur when the

concentration of the extract exceeds the MIC value, as shown in the case of *S. aureus*.[59] Major bioactive constituents in *C. asiatica* from different locations include triterpenes such as Asiatic acid, madecassoside, and madecassic acid.[63]

Cytotoxicity

The cytotoxic activity demonstrated by *C. asiatica* leaf extract (LD_{50} = 3.055 mg) is probably due to the existence of antitumor metabolites, and the values were more or less similar to the results (LD_{50} = 1.926 mg) of a previous study.[64] The cytotoxic activity of *C. asiatica* extracts with varied values of LD_{50} against *A. salina* has already been reported.[65] The extent of cytotoxic activity of *S. marianum* found in our study was by and large similar to that reported earlier.[66] A slight discrepancy in LD_{50} values reported by various authors may be attributed to the difference in seasonality, the geographical distribution of plants, the age and parts of the plant used, the method of extraction, and the solvent type.

Limitations of the Study

Seasonality variation of plant specimens was not conducted.

Conclusion

The antibacterial activity of the nine medicinal plants studied varied widely according to the species, type of extraction solvents, and bacteria strains tested. Under the current set of experimental conditions, EtOAc and EtOH were effective solvents for extracting antimicrobial components from most of the plant species. Two plant species, *C. asiatica* and *S. marianum*, showed remarkable antibacterial activity against MDR. Among the nine plants screened, the crude EtOAC extract prepared from *C. asiatica* efficiently subjugated the growth of both ATCC and MDR bacterial isolates (WHO prioritised pathogens), indicating that metabolites present in this extract are antibacterial with a broad spectrum of activities. In addition, both extracts produced impressive cytotoxic activity. Based on these overall findings, it could be envisaged that *C. asiatica* is a promising source of novel antibiotics as well as cytotoxic leads. Overall results substantiate the traditional use of *C. asiatica* and *S. marianum* as antibacterial agents, especially against drug-resistant bacteria. More in-depth analysis is required to identify the antimicrobial compounds existing in these plants, as well as their full spectrum of efficacy.

Funding

This research was supported by Research Directorate, College of Medicine and Health Sciences, Arba Minch University [GOV/AMU/TH14/CMHS/MeLs/02/13].

Disclosure

The authors report no conflicts of interest in this work.

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