

Antimicrobial activity of *Zornia apiculata* and *Ipomoea kituiensis* against WHO-priority drug-resistant clinical isolates

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ABSTRACT

Background. The rise of antibiotic resistance has become a critical medical concern, prompting the World Health Organization (WHO) to publish a list of priority drug-resistant clinical pathogens. Medicinal plants are considered a potential source for developing novel antimicrobial agents. However, studies on the antimicrobial properties of many such plants remain limited, despite their extensive traditional use.

Objective. To evaluate the antimicrobial activity of *Zornia apiculata* and *Ipomoea kituiensis* leaf extracts against multidrug-resistant (MDR) bacteria and yeast to support their traditional medicinal use.

Methods. An in vitro study assessed the antimicrobial potential of leaf extracts from *Z. apiculata* and *I. kituiensis*, sourced from the Omo Valley. Extracts were prepared using selected organic solvents and tested against WHO-prioritized MDR bacterial and fungal isolates.

Results. The overall activity indices were 88.3% for *Z. apiculata* and 83.3% for *I. kituiensis*. The methanol extract of *Z. apiculata* showed an activity index of 100%, with inhibition zones of 21.1 ± 1.3 – 21.8 ± 1.5 mm against Gram-positive and 13.8 ± 1.8 – 20.8 ± 1.5 mm against Gram-negative bacteria. It also exhibited moderate anti-*Candida* activity (12.5 ± 0.5 mm). Against MDR isolates, this extract showed inhibition zones of 18.3 ± 1.2 – 18.5 ± 0.7 mm, with minimum inhibitory concentration (MIC) values of 2.5–10 mg/mL and total antimicrobial activity (TAA) ranging from 9.6–73.8 mL/g. A minimum bactericidal concentration (MBC)/MIC ratio of 0.5 indicated bactericidal action. Both extracts had MIC and MFC values of 5 mg/mL and 10 mg/mL against *Candida albicans*, respectively (MIC/MFC = 0.5).

Conclusion. These findings support the traditional use of *Z. apiculata* and *I. kituiensis* for treating infectious diseases in South Omo communities.

Keywords: incision wound model, fibroblasts, polymorphonuclear cells, granulation tissue, silver sulfadiazine, rat

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INTRODUCTION

A vast majority of the rural population has limited access to healthcare services due to a shortage of professionals and the inability to afford market-based medicines. As a result, they rely heavily on traditional medicines [1]. The World Health Organization (WHO) Traditional Medicines Strategy 2014–2023 provided a framework to support the use of traditional and complementary medicine, thereby aiming to reduce morbidity in low-income nations [2].

Plants inherently produce a variety of bioactive molecules with diverse functions [3]. Medicinal plants are key sources of drugs, and several top-selling plant-based medicines are widely used in the pharmaceutical market [4]. Researchers focused on natural products continue to explore phytochemicals that can be used to develop treatments for infectious diseases. Notably, of the 141 antibacterial drugs approved between 1981 and 2014, more than 58% were classified as “natural products”. Approximately 50% of all drugs approved in the past three decades are linked to natural sources, with 11% derived from native flowering plant species [4], underscoring the significance of plants in drug discovery.

In Ethiopia, traditional healers promote the use of plants and plant-based remedies, which serve over 70% of the human population and 90% of livestock [5,6]. Whole plants and extracts from specific parts account for 95% of such traditional medicines. About 60% of Ethiopia’s plant species are indigenous, and 12% are endemic [7]. These have long been used to treat various ailments – ranging from mild to life-threatening – before the advent of antibiotics and modern drugs. Several Ethiopian plants have demonstrated antimicrobial, anti-inflammatory, acetylcholinesterase inhibitory, and antioxidant properties [8]. However, scientific studies assessing the *in vitro* antimicrobial activity of the country’s vast plant resources remain limited [9]. Strengthening the evidence base for medicinal plants can promote their wider, safer, and more affordable use.

Antibiotic resistance has become an escalating global threat, and Ethiopia is no exception [10]. This challenge has infiltrated healthcare systems worldwide, making bacterial infections increasingly difficult to manage [11]. Moreover, newly developed antibiotics are often costly and inaccessible in developing countries, exacerbating healthcare inequalities.

Over the past decade, our research team has conducted numerous *in vitro* studies investigating the antimicrobial properties of plants indigenous to different regions of Ethiopia. We have previously reported promising results from species found in the Arba Minch and Chencha regions [9,12]. In this study, we turn our attention to the Omo Valley [13], known for its rich floral diversity and active use of medicinal plants by local healers. Specifically, we examine the antimicrobial properties of two previously unstudied medicinal plants: *Zornia apiculata* and *Ipomoea kituiensis*.

The objective of this study was to scientifically evaluate the antimicrobial activity of *Zornia apiculata* and *Ipomoea kituiensis* leaf extracts against standard reference strains and WHO-priority multidrug-resistant (MDR) clinical isolates. By employing well diffusion assays, MIC/MBC analysis, and total antimicrobial activity (TAA) calculations, we aimed to validate the traditional use of these plants and explore their potential as sources of novel antimicrobial agents.

MATERIALS AND METHODS

Study area

This study was conducted on two medicinal plants collected from the Aari and Benatsemay districts, located in the South Omo Zone of the southern region of Ethiopia. The topography of these districts includes both lowland and highland areas, with an average temperature range of 10.5–35.5° C and mean annual rainfall between 400 and 1600 mm. These areas are known for their rich cultural and ethnic diversity, with traditional medicinal practices still widely in use. Previous ethnobotanical surveys have identified 91 medicinal plant species in this region, spanning 57 genera and 33 families [14,15].

Study design, period, and plant specimens

An *in vitro* experimental study was conducted between October 2021 and June 2022 to evaluate the antimicrobial potency of two medicinal plants (*Zornia apiculata* and *Ipomoea kituiensis*) against type culture and multidrug-resistant (MDR) clinical isolates. The plants were selected based on their reported ethnobotanical use in treating infectious diseases by local traditional healers (Table 1).

Plant samples were collected using sterile tools, including a local digging implement and an industrial pruning shear (Outils Wolf). Samples were transported

TABLE 1. Ethnobotanical usage of *Z. apiculata* and *I. kituiensis*

Scientific name and family	Vernacular name	Ethnobotanical usage	Voucher no.
<i>I. kituiensis</i>	Latata	Malaria Wound infections	AK-01
<i>Z. apiculata</i>	Medanet	Diarrhea Abdominal discomfort	AK-02

in zip-locked polyethylene and isothermal bags under sterile conditions. *I. kituiensis* is a vining species native to regions between Ethiopia and subtropical Africa, belonging to the family *Convolvulaceae*, while *Z. apiculata* is a native species found in Ethiopia, Kenya, and Tanzania [16,17]. Taxonomic identification was confirmed by the National Herbarium at Addis Ababa University.

Preparation of extracts

Tender leaves of both plant species were collected with the assistance of traditional healers and botanists. The leaves were shade-dried and pulverized using a mortar and pestle. For extraction, 1 g of powdered material was combined with 10 mL of methanol (MeOH), ethyl acetate (EtOAc), or chloroform (CHCl₃), and placed on a shaker (Labtech RS-12 R) at 161 g for 10 minutes. The mixtures were filtered through Whatman No. 1 filter paper. This process was repeated three times, and the combined filtrates were concentrated using a rotary evaporator. The final residues were reconstituted in dimethyl sulfoxide (DMSO) to a final concentration of 1 mg/mL and stored at 4° C until further analysis [9].

Preparation of inocula

Type culture strains from the American Type Culture Collection (ATCC), including *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella Typhimurium* (ATCC 13311), *Enterococcus faecalis* (ATCC 29906), and the yeast *Candida albicans*, were obtained from the Ethiopian Public Health Institute.

Multidrug-resistant clinical isolates prioritized by WHO, including methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), and extended-spectrum β-lactamase (ESβL)-producing strains of *Klebsiella pneumoniae*, *E. coli*, *Proteus vulgaris*, *Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter aerogenes*, were obtained from previous clinical isolates stored in our laboratory [9].

Resistance patterns were confirmed using standard antibiotics (Oxoid, UK) and the Kirby-Bauer disk diffusion method, according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Bacterial suspensions were standardized to 0.5 McFarland and inoculated onto Mueller-Hinton agar (Hi-Media).

Antimicrobial activity testing

Antimicrobial activity of the crude extracts was assessed using the agar well diffusion method [9]. Extracts were tested against both type culture strains and WHO-priority MDR clinical isolates. Commercial antibiotic discs – ceftriaxone (10 µg), piperacillin (100 µg), and ciprofloxacin (5 µg) – were used as positive controls, while extraction solvents served as negative controls.

Anti-yeast activity against *C. albicans* was evaluated on Sabouraud dextrose agar using the same method [18]. All tests were performed in triplicate. Antimicrobial activity indices (MI) were calculated based on the ratio of inhibition zones of extracts to those of positive controls, using a modified formula from previous studies [9,13].

Minimum inhibitory concentration (MIC), minimum bactericidal/fungicidal concentrations (MBC/MFC), and total antimicrobial activity (TAA)

The broth dilution method was used to determine MIC, MBC, MFC, and the MBC/MIC ratio (MIC index) of the most active extracts. The total antimicrobial activity (TAA) was calculated using the method described by Eloff [19].

Quality assurance

All reagents and culture media were checked for expiry, sterility, and performance. Standard ATCC strains were used as controls. Commercial antibiotic discs served as positive controls, and solvents used for extraction served as negative controls. All chemicals and solvents were of analytical reagent grade and procured from Fisher Scientific Co.

Statistical analysis

Each assay was performed in triplicate. Data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was conducted using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA), to compare the effects of different solvents and microbial susceptibility.

Ethical considerations

The study protocol was reviewed and approved by the Institutional Review Board (IRB) of the College of Medicine and Health Sciences, Arba Minch University.

RESULTS

Overall activity indices

Primary antimicrobial screening of leaf extracts indicated that both plant species exhibited activity against at least one of the tested type culture strains. The overall activity indices revealed that *Zornia apiculata* had a higher index (88.8%) compared to *Ipomoea kituiensis* (83.3%). The methanol (MeOH) leaf extract of *Z. apiculata* showed the maximum possible activity index of 100% (Table 2).

TABLE 2. Activity indices of plant extracts

Plant species	Activity indices			OAI
	MeOH	EtOAc	ClFr	
<i>Z. apiculata</i>	100	83.3	83.3	88.8
<i>I. kituiensis</i>	83.3	100	83	83.3

Zones of inhibition ≥ 8 mm were considered indicative of antimicrobial activity. OAI – Overall Activity Index of the respective plant species

Antimicrobial activity of *Z. apiculata* and *I. kituiensis* against type culture isolates

The most effective solvent for extracting antibacterial compounds from *Z. apiculata* leaves was methanol. The MeOH extract produced inhibition zones ranging from 21.1 ± 1.3 to 21.8 ± 1.5 mm against Gram-positive type cultures and from 13.8 ± 1.8 to 20.8 ± 1.5 mm against Gram-negative strains. The highest activity was observed against *S. aureus* (21.8 ± 1.5 mm), *E. faecalis* (21.1 ± 1.3 mm), *P. aeruginosa* (20.8 ± 1.5 mm), and *E. coli* (17.2 ± 1.2 mm).

The next most active extract was from ethyl acetate (EtOAc), which showed inhibition zones of 10.6 ± 1.7 to 17.5 ± 0.8 mm, with the highest activity against *E. faecalis* (17.5 ± 0.8 mm). In contrast, the chloroform (CHCl_3) extract exhibited only moderate activity, with inhibition zones ranging from 8.5 ± 1.3 to 13.9 ± 1.6 mm. Anti-candidal activity was observed only in the MeOH extract (12.5 ± 0.5 mm) (Table 3).

For *I. kituiensis*, EtOAc was the most effective solvent, achieving an activity index of 100% against all type culture isolates tested. However, the inhibition zones were moderate, ranging from 11.1 ± 1.5 mm (*S. Typhimurium*) to 15.5 ± 1.6 mm (*S. aureus*). Anti-candidal activity was shown only by the EtOAc extract (9.9 ± 1.0 mm).

Due to the broadest and highest range of activity, the MeOH extract of *Z. apiculata* and the EtOAc extract of *I. kituiensis* were selected for further testing against WHO-priority multidrug-resistant (MDR) clinical isolates.

Antibacterial activity against

WHO-priority drug-resistant clinical isolates

The MeOH extract of *Z. apiculata* demonstrated high antibacterial activity against the MDR isolates: VRE (18.5 ± 0.7 mm), MRSA (18.1 ± 0.7 mm), and ES β L-producing *P. aeruginosa* (18.3 ± 1.2 mm). A slightly lower activity was seen against ES β L-producing *E. coli* (13.9 ± 1.2 mm). The highest microbial indices were against VRE (3.08), ES β L-producing *E. aerogenes* (2.9), and ES β L-producing *K. pneumoniae* (2.2) (Table 4).

The EtOAc extract of *I. kituiensis* also showed considerable activity against all WHO-priority MDR isolates. The largest inhibition zones were recorded for ES β L-producing *E. aerogenes* (15.4 ± 1.5 mm), *A. baumannii* (15.06 ± 1.3 mm), and MRSA (14.3 ± 0.7 mm).

TABLE 3. Inhibition zones of leaf extracts against ATCC strains and *C. albicans*

Plant species	Solvents	ATCC					
		<i>S. aureus</i>	<i>E. faecalis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. Typhimurium</i>	<i>C. albicans</i>
<i>Z. apiculata</i>	MeOH	21.8 ± 1.5^a	21.1 ± 1.3^a	20.8 ± 1.5^a	17.2 ± 1.2^a	$13.8 \pm 1.8^{a,b}$	12.5 ± 0.5^a
	EtOAc	10.6 ± 1.7^c	17.5 ± 0.8^b	16 ± 1.4^b	14.7 ± 1^b	15.7 ± 1.5^a	7.4 ± 0.3^d
	ClFr	10.8 ± 1^c	13.9 ± 1.6^c	13.3 ± 1.04^c	9.5 ± 0.8^c	8.5 ± 1.3^c	$7.5 \pm 1.3^{c,d}$
<i>I. kituiensis</i>	MeOH	14.6 ± 1.8^b	9.9 ± 1.2^d	9.9 ± 0.5^d	10.4 ± 0.8^c	9.3 ± 1.5^c	$7.2 \pm 0.4^{c,d}$
	EtOAc	15.5 ± 1.6^b	14.5 ± 1.3^c	14.5 ± 1.3^c	11.2 ± 1.7^c	11.1 ± 1.5^b	9.9 ± 1^b
	ClFr	8.5 ± 0.5^c	9.9 ± 1.3^d	7.8 ± 2.02^e	11.06 ± 1.4^c	8.4 ± 2.2^c	$6.9 \pm 1.05^{c,d}$

Note: Values are presented as mean $\pm$ standard deviation. Means followed by different letters within the same column differ significantly at $p < 0.05$ according to Duncan's New Multiple Range Test.

TABLE 4. Antibacterial activity of *Z. apiculata* and *I. kituiensis* against WHO-prioritised MDR clinical bacterial isolates

Plant extracts	ATCC							
	MRSA	VRE	ESBL-KP	ESβL-PA	ESβL-PV	ESβL-EC	ESβL-AB	
<i>Z. apiculata</i>	18.1 ± 0.7	18.5 ± 0.7	15.8 ± 1.0	18.3 ± 1.2	15.5 ± 1.1	13.9 ± 1.2	16.4 ± 2.0	17.7 ± 1.5
MI	0.86	3.08	2.2	0.76	0.96	1.9	1.2	2.9
<i>I. kituiensis</i>	14.3 ± 0.7	13.0 ± 2.1	12.3 ± 1.7	12.8 ± 1.8	11.5 ± 1.8	13.2 ± 1.1	15.06 ± 1.3	15.4 ± 1.5
MI	0.68	2.1	1.7	0.53	0.71	1.8	1.15	2.5
Ceftriaxone	–	6	7	–	10	7	9	6
Ciprofloxacin	21	–	–	–	–	–	–	–
Piperacillin	–	–	–	10	–	–	–	–

Note: The microbial index (MI) was calculated as the relative zone of inhibition (mm) of the clinical isolate compared to that of the respective antibiotic.

Abbreviations: MRSA – methicillin-resistant *S. aureus*; VRE – vancomycin-resistant Enterococci; ESBL-KP – extended-spectrum β-lactamase-producing *K. pneumoniae*; ESBL-PA – extended-spectrum β-lactamase-producing *P. aeruginosa*; ESBL-PV – extended-spectrum β-lactamase-producing *P. vulgaris*; ESBL-EC – extended-spectrum β-lactamase-producing *E. coli*; ESBL-AB – extended-spectrum β-lactamase-producing *A. baumannii*; ESBL-EA – extended-spectrum β-lactamase-producing *E. aerogenes*; MI – microbial index.

mm). Moderate activity was observed against VRE (13 ± 2.1 mm), ESβL-producing *E. coli* (13.2 ± 1.1 mm), and *P. aeruginosa* (12.8 ± 1.8 mm). The highest microbial indices were against VRE (2.5), ESβL-producing *E. aerogenes* (2.1), and *K. pneumoniae* (1.7) (Table 4).

Minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC), and total antimicrobial activity (TAA)

The MIC values of *Z. apiculata* leaf extracts against type cultures ranged from 0.65 to 5 mg/mL. The lowest MIC was observed against *S. aureus* (0.65 mg/

mL), with a corresponding MBC of 1.25 mg/mL. MIC values for *E. coli* and *P. aeruginosa* were 1.25 mg/mL, with MBC values of 2.5 mg/mL each.

For *I. kituiensis*, the MIC values of the EtOAc extract ranged from 1.25 to 10 mg/mL. The lowest MIC and MBC values (1.25 and 2.5 mg/mL, respectively) were recorded against *S. aureus*, *E. coli*, and *P. aeruginosa* (Table 5).

Both plant extracts exhibited notable MIC and MBC values against all tested WHO-priority MDR clinical isolates. For *Z. apiculata*, MIC and MBC values ranged from 2.5–10 mg/mL and 5–10 mg/mL, respectively. The

TABLE 5. IC, MBC, MFC, and TAA of methanol extract of *Z. apiculata* and ethyl acetate extract of *I. kituiensis* against type culture strains and WHO-prioritised MDR bacterial isolates

Bacterial isolates	<i>Z. apiculata</i>				<i>I. kituiensis</i>			
	MIC	MBC	MIC/MBC	TAA	MIC	MBC	MIC/MBC	TAA
	mg/mL			mL/g	mg/mL			mL/g
<i>P. aeruginosa</i>	1.25	2.5	0.5	38.4	1.25	2.5	0.5	36
<i>E. coli</i>	1.25	2.5	0.5	38.4	1.25	2.5	0.5	36
<i>S. aureus</i>	0.65	1.25	0.5	73.8	1.25	2.5	0.5	36
Yeast	MIC	MFC	MIC/MFC	TAA	MIC	MFC	MIC/MFC	TAA
<i>C. albicans</i>	5	10	0.5	9.6	5	10	0.5	9
WHO prioritised clinical isolates								
<i>P. aeruginosa</i>	2.5	5	0.5	19.2	5	10	0.5	9
<i>E. coli</i>	2.5	5	0.5	19.2	2.5	5	0.5	18
<i>S. aureus</i>	2.5	5	0.5	19.2	5	10	0.5	9
<i>E. faecalis</i>	2.5	5	0.5	19.2	5	10	0.5	9
<i>P. vulgaris</i>	5	10	0.5	9.6	5	10	0.5	9
<i>K. pneumoniae</i>	2.5	5	0.5	19.2	2.5	5	0.5	18
<i>A. baumannii</i>	2.5	5	0.5	19.2	2.5	5	0.5	18
<i>E. aerogenes</i>	2.5	5	0.5	19.2	2.5	5	0.5	18

Note: Yield of MeOH extract = 4.8%; Yield of EtOAc extract = 4.5%. **Abbreviation:** TAA – total antimicrobial activity.

same concentration range was observed for *I. kituiensis*. TAA values for *Z. apiculata* were higher (9.6 to 73.8 mL/g) compared to those for *I. kituiensis* (9 to 36 mL/g).

Since the MBC/MIC ratio was ≤ 2 , both extracts were considered bactericidal.

The MIC of both extracts against *C. albicans* was 5 mg/mL, and the minimum fungicidal concentration (MFC) was 10 mg/mL. The MIC/MFC ratio was 0.5 for both extracts. TAA values were 9.6 mL/g for *Z. apiculata* and 9 mL/g for *I. kituiensis*.

DISCUSSION

Natural antimicrobial agents may offer a cost-effective means of treating various diseases, particularly for people in rural areas worldwide. Plant-derived medicines are often preferred because they tend to have fewer adverse effects than their synthetic counterparts [18]. In many cultures, traditional plant-based remedies are an integral part of indigenous healthcare systems and complement the formal public health infrastructure [9]. Antimicrobial screening is a critical step in validating the efficacy of plant extracts or drug formulations against pathogenic microorganisms.

Although many medicinal plants are used traditionally, a large proportion of them have not been systematically investigated for antimicrobial properties. Evaluating antimicrobial activity is thus an essential method for assessing the potential of plants to produce bioactive compounds. In the present study, the tender leaves of *Zornia apiculata* and *Ipomoea kituiensis* were extracted using three solvents of varying polarity and screened for antimicrobial activity. These solvents – methanol (MeOH), ethyl acetate (EtOAc), and chloroform (CHCl₃) – are known for their efficacy in extracting phytochemicals of diverse structures and polarities.

Our findings revealed significant differences in antimicrobial activity depending on the plant species, solvent used, and microbial strain tested. Such variation may result from differences in the types and concentrations of bioactive compounds extracted and the virulence factors of the target bacteria [20]. Notably, MeOH and EtOAc (both medium-polar solvents) were more effective than CHCl₃, underscoring the importance of solvent polarity. This agrees with prior studies indicating that antimicrobial metabolites in plants are more soluble in medium-polar solvents [12,21–24]. The

low activity of CHCl₃ extracts suggests they contain fewer active antimicrobial constituents. Furthermore, the negative control (solvent only) did not show any microbial inhibition, confirming the genuine activity of the plant extracts.

Antimicrobial efficacy also depends on multiple factors, including plant species, geographic location, harvesting time, plant part used, extraction process, and the microbial strains tested [25, 26]. In this study, the MeOH and EtOAc extracts of *Z. apiculata* demonstrated moderate to high antibacterial activity, particularly against Gram-positive type cultures such as *S. aureus* and *E. faecalis*. This may be attributed to the solubility and abundance of medium-polar antibiotic constituents in the leaf extracts. These findings align with prior studies on *Zornia diphylla*, which showed moderate activity against *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *Bacillus subtilis* [27]. We also observed that only the MeOH extract of *Z. apiculata* leaves exhibited activity against *Candida albicans*, which corroborates previous findings of lower antifungal activity in related species [27]. This suggests *Z. apiculata* leaves may contain anti-candidal agents.

Traditionally, chopped roots of *Z. apiculata* are used to treat abdominal pain and vomiting in children, while *Z. glochidiata* is used to treat malaria after boiling with coffee leaves [14]. While other *Zornia* species like *Z. latifolia* and *Z. diphylla* have recognized medicinal properties, *Z. apiculata* remains largely unstudied [27]. Other species such as *Z. milneana* and *Z. brasiliensis* have demonstrated antimicrobial activity against pathogens like *Aeromonas hydrophila*, *Fusarium spp.*, and various fungi and bacteria [28–30].

For *I. kituiensis*, EtOAc was the most effective solvent. It exhibited appreciable activity against *S. aureus*, *E. faecalis*, and *P. aeruginosa* type culture strains. Similar findings have been reported for other *Ipomoea* species. For instance, *I. carnea* acetone extract showed activity against *P. vulgaris* and *S. Typhimurium*, while its ethanol extract inhibited *P. aeruginosa* [31]. *I. mauritiana* also demonstrated notable activity against *Bacillus cereus* [32], and *I. batatas* showed broad-spectrum activity against several pathogens [33]. In Pakistan, the MeOH extract of *I. hederacea* displayed strong antimicrobial effects against a wide range of bacteria and fungi [34], and *I. staphylinia* showed activity against *E. coli* in India [35]. These variations in results may stem from differences in extraction methods, phytochemical profiles, or microbial

resistance.

In some African regions, the stem of *I. kituiensis* is traditionally used to manage digestive disorders, anthrax, and malaria. Its leaves, however, are primarily employed as an acaricide or pesticide and are toxic if consumed in large quantities. Prior studies have extracted alkaloids, flavonoids, saponins, tannins, and terpenoids from its leaves using a 1:1 methanol-dichloromethane solvent system [36].

The growing prevalence of WHO-priority drug-resistant clinical isolates poses a severe global threat to public, animal, and environmental health. A noteworthy result of this study is the significant activity of *Z. apiculata* MeOH extract against MRSA, ES β L-producing *E. faecalis*, and *P. aeruginosa*, with moderate activity against other resistant strains. Likewise, *I. batatas* and *I. muruoides* have been previously reported to inhibit drug-resistant strains such as *E. aerogenes*, *S. aureus*, and *E. coli* [33,37]. Thus, both *Z. apiculata* and *I. kituiensis* may serve as potential sources of antimicrobial agents against high-priority resistant pathogens.

Our MIC analysis showed variability depending on the bacterial strain. The MIC values against type cultures were generally lower than those for MDR isolates. The lowest MIC values were recorded against *S. aureus* type strains, ranging from 0.65 to 2.5 mg/mL for *Z. apiculata*, and 1.25 to 5 mg/mL for *I. kituiensis*. These were higher than those reported for *Z. diphylla* in India (5.3–10 μ g/mL) [27], but lower than values observed for *I. triloba* (250–1000 mg/mL) and *I. mauritiana* in other studies [32,38]. Differences in MIC values may result from the crude nature of the extracts, variations in constituent concentrations, microbial resistance mechanisms, or differences in cytoplasmic permeability.

The exact mechanism of antimicrobial action of these crude extracts remains unclear. However, the active compounds may disrupt bacterial cytoplasmic membranes, inhibit DNA replication and transcription, or interfere with virulence factors. This study suggests that bioassay-guided fractionation and targeted purification could lead to the discovery of novel antibacterial compounds capable of combating MDR pathogens.

Although the specific bioactive compounds in *Z. apiculata* and *I. kituiensis* were not isolated in this study, literature shows that species from these genera contain diverse chemical constituents. For instance,

antimicrobial activity in *Zornia* species has been linked to monoterpenoids like sabinene and terpinene-4-ol [27], and sesquiterpenes such as trans-nerolidol, germacrene D, trans-caryophyllene, α -humulene, and farnesene have been identified in *Z. brasiliensis* [30]. Isoflavones are also commonly reported in *Zornia* species [39,40]. Likewise, *Ipomoea* species are rich in bioactive compounds, including alkaloids, flavonoids, tannins, saponins, cardiac glycosides, phenols, terpenoids, and steroids [41, 42].

Limitations

This study had several limitations. It did not assess seasonal variations, perform phytochemical profiling, evaluate toxicity, or conduct *in vivo* validations. Future work should incorporate bioassay-guided fractionation, purification, structural elucidation, and toxicity testing to identify and characterise the specific secondary metabolites responsible for the observed antimicrobial effects.

CONCLUSIONS

The present study demonstrated that the methanol extract of *Zornia apiculata* and the ethyl acetate extract of *Ipomoea kituiensis* leaves possess notable and diverse antimicrobial activity against both type culture strains and WHO-priority drug-resistant clinical isolates, as evidenced by well diffusion and MIC assays. To our knowledge, this is the first report on the anti-candidal effects of leaf extracts from *Zornia* and *Ipomoea* species. These findings support the traditional use of both plants by local healers in South Omo for treating infectious diseases. However, further studies are necessary to elucidate the exact molecular mechanisms of action and any potential synergistic effects involved in the therapeutic outcomes. It is also important to investigate the selective toxicity and safety profiles of these extracts.

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Conflict of interest:

The authors declare no conflicts of interest related to this work.

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