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In vitro antibacterial activity of leaf extracts of *Zehneria scabra* and *Ricinus communis* against *Escherichia coli* and methicillin resistance *Staphylococcus aureus*

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PEER REVIEW

Peer reviewer

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Comments

This paper is very interesting and has wide application in biotechnology where many researchers can follow the screening and extraction of natural products for the discovery of noble compounds. Therefore, this study is helpful to initiate other researchers in the area of interest.
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ABSTRACT

Objective: To evaluate the antibacterial activities of the crude leaves extracts of *Zehneria scabra* (*Z. scabra*) and *Ricinus communis* (*R. communis*) against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and methicillin resistance *S. aureus*.
Methods: The crude powdered leaves of *Z. scabra* and *R. communis* were extracted successively by organic solvents in increasing polarity [benzene, chloroform:acetone (1:1), 70% alcohol and distilled water]. The antibacterial susceptibility of the crude leaves extracts of were tested against standard strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 2923) and clinical isolates of *E. coli*, *S. aureus* and methicillin resistance *S. aureus* using agar well diffusion method.
Results: In *Z. scabra* and *R. communis* leaf extracts, the most sensitive standard strain was *S. aureus* with an inhibition zone of (14.00±1.20) mm and (15.90±2.13) mm, respectively. The minimum inhibitory concentration (MIC) values of *Z. scabra* extracts against test organisms ranged from 1.95 mg/mL for extract 3 in clinical and standard strains of *S. aureus* to 250 mg/mL for extract 1 and 4 in clinical and standard strains of *E. coli*. The MIC values of *R. communis* extracts against test organisms ranged from 1.95 mg/mL for extract 2 and 3 standard strains of *S. aureus* to 250 mg/mL for extract 1 in clinical isolate of *E. coli*. Most of the minimum bactericidal concentration and MIC values of plant extracts were almost similar particularly in *R. communis*, or minimum bactericidal concentration equal to one dilution factor less than MIC value of the extracts mainly in *Z. scabra*.
Conclusions: The potency of plant extracts against test organisms were depend on different organic solvents used. Clinical isolate of bacterial pathogens showed less zones of diameter compared to the standard strains. Gram-positive had wide inhibition zones than Gram-negative bacteria. Further studies should be carried out to isolate the pure compounds and standardization of the methods of plant extracts for an *in vitro* testing.

KEYWORDS
Antibacterial, Extracts, Inhibition zone, MBC, MIC

1. Introduction

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants[1]. This interest in drugs of plant origin is limited due to several reasons, namely, conventional medicine can be inefficient (*e.g.* side effects and ineffective therapy), abusive and/or incorrect use of synthetic drugs results in side effects and other problems[2]. Large percentage of the world's population does not have access to conventional pharmacological treatment, and folk medicine and ecological awareness suggests that natural products are harmless. However, the use of these substances is not always authorized by legal authorities dealing with efficacy and safety procedures, and many published papers explained the lack of quality

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in the production, trade and prescription of phytomedicinal products. So, formulation of plants for standardization and regulation of phytomedicinal products is the most alternative way[3].

In Ethiopia, medicinal plants have been used as traditional medicine to treat number of human and animal ailments by the local people from time immemorial. About 80% of the population in Ethiopia uses traditional medicine, mainly herbal plants[4]. *Zehneria scabra* (*Z. scabra*) and *Ricinus communis* (*R. communis*) are some of the traditionally used medicinal plants. *Z. scabra*, vernacular name is called "Hareg Resa" in Amharic, is a climbing or trailing herb belongs to the Cucurbitaceae that can go up to 10 m. Old stems become woody with corky-ridged bark. It inhabits forest and on forest margins, riverine fringes and exotic plantations across 900-2 100 m above sea level with a widespread in tropical Africa, South Africa, Arabia, India, Java and the Philippines[5]. In Ethiopia, *Z. scabra* is one of the traditional medical plants commonly used for the treatment of alopecia, wound and eczema, burn remedy and skin rashes as part of a poly-herbal preparation, the ash and wash prepared from pounded leaves[6]. *R. communis* popularly called castor bean in English and *Gulo* in Amharic. It is widely spread as a wild plant through East and North Africa. In Ethiopia, castor plant is important to treat tooth ache, cold, dysentery and itachy, fetal membrane retention and rabies in different preparation[7-9].

Ethno-botanical studies revealed that *Z. scabra* and *R. communis* are being used in the treatment of pathogenic organisms in the traditional health care system in Ethiopia. However, very little work has been done to evaluate their efficacy in scientific way[10]. Therefore, this study was aimed to evaluate the *in vitro* antibacterial activity of crude leaf extractions of *Z. scabra* and *R. communis* against standard and clinical isolates of *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) including methicillin resistance *S. aureus* (MRSA).

2. Materials and methods

2.1. Study area

The study was conducted at University of Gondar, Gondar town. It is located in the North West Ethiopia of Amhara region. The town has an elevation of 2 080 m above sea level with a mid-altitude climate and an average annual maximum temperature of 30 °C and minimum temperature of 16 °C[11].

2.2. Plant material

2.2.1. Sampling, sample collection and identification

Through random sampling, healthy and disease free of the leaves of a wild growing *Z. scabra* and *R. communis* were collected from gardens in Gondar town by scissors and samples were kept in plastic bags. The plants were identified and confirmed using standard manuals by plant taxonomist.

2.2.2. Preparation of crude extract

The leaves of *Z. scabra* and *R. communis* were transported to Microbiology Laboratory, Department of Biology, Faculty of Natural and Computational Sciences, University of Gondar. The plant materials were washed using distilled water and air dried at room temperature under shade for 10 d and powdered using wooden-made pestle and mortar. The powdered materials were sieved and stored in air tight container until use.

A 100 g of air-dried plant powdered leaves were measured by electronic balance (CY510) and then placed in a 2000 mL clean round bottomed flask at room temperature caped with aluminum foil. After that, 1000 mL benzene was added to the flask which contained powdered leaves material [1 part test material and 10 parts of solvent (w/v)]. The mixture was kept on a rotary shaker at 200 r/min for 24 h. The macerate was first filtered through double layer muslin cloth then through Whatman No. 1 filter paper and it was assigned as extract 1. The residue was taken again and mixed with chloroform/acetone with ratio 1:1 in a volume of 1000 mL, and the filtrate was assigned as extract 2. The same procedure was followed using 70% alcohol and distilled water to obtain extract 3 and extract 4, respectively. These successive cold maceration methods were done with increasing order of their polarity[13]. The filtrates were allowed to concentrate under rotary vapor (RE 2000) at 40 °C, weighed and stored in sterilized air tight container at 4 °C for further analysis.

2.3. Test organisms

Standard strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 2923) were collected from Biomedical and Laboratory Sciences Research Center, University of Gondar while clinical isolates of *E. coli*, MRSA and *S. aureus* were collected from Ethiopian Health and Nutrition Research Institute, Addis Ababa. The microorganisms were transported to Microbiology Laboratory by using nutrient agar slant and preserved at 4 °C for further use.

2.4. Standard antibiotics

Ciprofloxacin (in the form of powder) having a broad spectrum property was used as a positive control (5 μg/mL).

2.5. Inocula preparation

The inocula preparation was carried out by growth methods[14,15]. The test organisms from nutrient agar slant were transferred into a nutrient agar medium to get a pure colony at 37 °C for 24 h. Three to five pure colonies were selected and transferred into a sterile test tube containing 5 mL of sterile nutrient broth and the solutions were mixed by a vortex mixer. The broth culture was incubated at 37 °C until it reached the turbidity of (10^5-10^6 CFU/mL) which was equivalent to 0.5 McFarland standards (usually about 8 h) and turbidity was adjusted visually by comparing the test.

2.6. Agar well diffusion method

Susceptibility tests were performed by agar well diffusion method using Muller-Hinton agar[16,17]. A sterile cotton swabs were dipped into the adjusted suspension by pressing and rotating the swabs firmly against the inside of the tube above the fluid level. The swab was then evenly streaked over the entire surface of the Muller-Hinton agar plate repeatedly by rotating the plate approximately 600 each time and the rim of the agar was swabbed to obtain uniform inoculums. On each plate, six equidistant wells (one in the center and five wells at the corner) were made with a 6 mm

diameter sterilized cork borer, 2 mm from the edge of the plate[15].

Five of the holes were aseptically filled with 50 μL of different concentrations of each plant extracts (*i.e.* 100 mg/mL, 200 mg/mL, 300 mg/mL, 400 mg/mL and 500 mg/mL) sequentially, and at the center, ciprofloxacin was added as a positive control. The amount of control was the same as the tested sample on the wells. Petri plates were placed at 4 °C for 2 h to allow diffusion of the extract into the agar and then incubated at (37±0.1) °C for 24 h. At the end the inhibition zones formed were measured to the nearest millimeters and the experiment was performed in duplicate. Experiments that gave contradicting results were done for the third time for an easy decision.

2.7. Determination of minimum inhibitory concentration (MIC)

MIC of crude extracts of the *Z. scabra* and *R. communis* were performed using two fold broth dilution methods[18]. The extract solution (500 mg/mL) was serially diluted with nutrient broth as 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256 to bring 250 mg/mL, 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.63 mg/mL, 7.81 mg/mL, 3.95 mg/mL and 1.95 mg/mL concentrations, respectively and 20 μL of a standard suspension of the test organism was added to each concentration of the extract. Two test tubes containing nutrient broth without antimicrobial agent were added in each test. One of these tubes was inoculated with the test organism; the other was left uninoculated and served as a control for media sterility. The broth plates were incubated at 37 °C for 24 h. The lowest concentration, at which there was no turbidity, was regarded as MIC value of the extract.

2.8. Determination of minimum bactericidal concentration (MBC)

MBC is the lowest concentration of an antibiotic required to kill a microorganism[18]. The MBC were determined by sub-culturing 20 μL of the test dilutions from MIC tubes on to fresh nutrient agar plates incubating at 37 °C for 24 h. The lowest concentration that killed the entire bacterial colony on the plates was recorded as MBC.

2.9. Statistical analysis

One-way analysis of variance (ANOVA) was used for determination of the antimicrobial susceptibility test of phyto-chemical extracted by different solvents in the specific concentration. Values were expressed as mean ±SEM by using SPSS version 16 software and presented as tables. *P* values less than 0.05 were taken as statistically significant[19].

3. Results

3.1. Agar well diffusion assay of the crude leaf extracts of Z. scabra and R. communis

Among the standard test strains, *S. aureus* (ATCC 2923) was the most susceptible bacteria [(14.00±2.00) mm], followed by *E. coli* (ATCC 25922) [(6.85±0.91) mm] to the extracts of *Z. scabra*. Among clinical isolates most susceptible strains for extracts of *Z. scabra* was *S. aureus* [(9.95±1.79) mm] followed by MRSA [(7.80±1.24) mm] and *E. coli* [(3.50±0.97) mm]. Similarly, the most susceptible standard strains for extracts of *R. communis* was *S. aureus* (ATCC 2923) [(15.90±2.13) mm], followed by *E. coli* (ATCC 25922) [(15.20±1.53) mm] and clinical isolates of *S. aureus* [(9.90±1.98) mm], MRSA [(9.50±2.12) mm] and *E. coli* [(7.75±0.91) mm] (Table 1).

Table 1

Zone of inhibition against test organisms of leaf extract fractions of *Z. scabra* and *R. communis*.

leave type	Organisms	Diameter of zone of inhibition (mm)	
		IZE	Ciprofloxacin
Z. scabra	*E. coli**	3.50±0.97	26.75±0.33
	*E. coli***	6.85±0.91	31.25±0.19
	MRSA	7.80±1.24	21.00±0.32
	*S. aureus**	9.95±1.79	22.00±0.22
	*S. aureus***	14.00±2.00	28.00±0.49
R. communis	*E. coli**	7.75±0.91	28.00±0.16
	*E. coli***	15.20±1.53	33.75±0.25
	MRSA*	9.50±2.12	21.25±0.33
	*S. aureus**	9.90±1.98	24.00±0.49
	*S. aureus***	15.90±2.13	28.25±0.50

Data are expressed as mean±SEM. *: Clinical isolates; **: Standard strains; IZE: Inhibition zone of extract; MRSA: Methicillin resistant *S. aureus*.

3.2. The MIC values of Z. scabra and R. communis extracts against test organisms

The MIC value of plant extracts of *Z. scabra* against the test bacteria ranged from 1.95 mg/mL (extract 3 for both clinical and standard stains of *S. aureus*) to 250.00 mg/mL (extract 1 and extract 4 for both clinical and standard strains of *E. coli*), respectively, (Table 2). The MIC values of extract 3 ranged from 1.95 mg/mL to 62.5 mg/mL with the least MIC values compared to other crude leaf extract fraction. Extract 4 had MIC values ranged from 15.62 mg/mL (on standard strains of *S. aureus*) to 250.00 mg/mL (on standard strains of *E. coli*) where as MIC values were negligible to other strains (particularly on clinical isolates). The overall trend showed that the MIC values of Gram-positive bacteria were lower than Gram-negative bacteria (Table 2).

Table 2

The MIC values of *Z. scabra* leaf extract fractions against test organisms using two fold broth dilution methods.

Organisms	Plant extracts (mg/mL)			
	Extract 1	Extract 2	Extract 3	Extract 4
*E. coli**	250.00	62.50	62.50	–
*E. coli***	125.00	31.25	31.25	250.00
MRSA*	62.50	15.62	7.81	–
*S. aureus**	15.62	3.91	1.95	31.25
*S. aureus***	3.91	1.95	1.95	15.62

*: Clinical isolates; **: Standard strains; –: No MIC value.

As indicted from Table 3, *R. communis* leaf extract showed MIC value ranging from 1.95 mg/mL to 250.00 mg/mL. The most effective extract that inhibited the growth of bacteria was extract 3 with value ranged from 1.95 mg/mL to 62.5 mg/mL. Extract 4 inhibited all standard strains in low MIC values ranged from 3.91 mg/mL (*S. aureus* (ATCC 2923) to 7.81 mg/mL (*E. coli* (ATCC 25922). Standard strain of *S. aureus* (ATCC 2923) was the most sensitive with MIC value of 1.95 mg/mL to

extracts 2 and 3 followed by standard strain of *E. coli* (ATCC 25922) and clinical isolate of *S. aureus* (1.95 mg/mL) to extract 3. The most resistant bacteria were clinical isolate of *E. coli* (62.5 mg/mL) at extract 2 and 3 followed by clinical isolate of *E. coli* (250.00 mg/mL) at extract 1 (Table 3).

Table 3

The MIC values of *R. communis* leaf extract fractions against test organisms using two fold dilution methods.

Organisms	Plant extracts (mg/mL)			
	Extract 1	Extract 2	Extract 3	Extract 4
*E. coli**	250.00	62.50	62.50	–
*E. coli***	7.81	3.91	1.95	7.81
MRSA*	62.50	15.62	7.81	–
*S. aureus**	62.50	3.91	1.95	31.25
*S. aureus***	3.91	1.95	1.95	3.91

*: Clinical isolates; **: Standard strains; –: No MIC value.

3.3. The MBC values of *Z. scabra* and *R. communis* extracts against test organisms

MBC value of fraction 3 from leaf extract of *Z. scabra* was ranged from 1.95 mg/mL against clinical and standard strains of *S. aureus* to 62.5 mg/mL against clinical isolate of *E. coli*. The second important extract in *Z. scabra* leaf was extract 2 ranged from 3.91 mg/mL against standard strain *S. aureus* to 62.5 mg/mL against clinical isolate of *E. coli* followed by extract 1 that ranged from 7.81 mg/mL against standard strain *S. aureus* to 500.00 mg/mL against clinical isolate of *E. coli* (Table 4).

Table 4

The MBC of *Z. scabra* leaf extract fractions against test organisms.

Organisms	Plant extracts (mg/mL)			
	Extract 1	Extract 2	Extract 3	Extract 4
*E. coli**	500.00	62.50	62.50	–
*E. coli***	250.00	31.25	31.25	250.00
MRSA*	62.50	15.62	7.81	–
*S. aureus**	15.62	7.81	1.95	31.25
*S. aureus***	7.81	3.91	1.95	15.63

*: Clinical isolates; **: Standard strains; –: No MBC values.

R. communis leaf extract fraction showed greater effects in inhibiting bacterial growth in this study, with MBC values ranged from 1.91 mg/mL of extract 3 against standard and clinical isolates of *S. aureus* to 250 mg/mL of extract 1 against clinical isolate strains of *E. coli*. Standard strain of *E. coli* was the most sensitive strains next to *S. aureus* (3.91 mg/mL from extract 3) (Table 5).

Table 5

The MBC of *R. communis* leaf extract fraction against test organisms.

Organisms	Plant extracts (mg/mL)			
	Extract 1	Extract 2	Extract 3	Extract 4
*E. coli**	250.00	125.00	62.50	–
*E. coli***	15.62	7.81	3.91	15.62
MRSA*	62.50	15.62	7.81	–
*S. aureus**	62.50	7.81	1.95	125.00
*S. aureus**	31.25	1.95	1.95	62.50

*: Clinical isolates; **: Standard strains; –: No MBC values.

4. Discussion

In this study, leaf extracts of *Z. scabra* showed maximum zone of inhibition (14.00±2.00) mm against standard strain of *S. aureus* (ATCC 2923) followed by (9.95±1.79) mm against clinical isolate of *S. aureus*. The least diameter of zone of inhibition recorded was (3.50±0.97) mm against clinical isolate of *E. coli*. Similarly, other study conducted by Anand *et al.* indicated that ethanol, methanol, ethyl acetate and aqueous extracts of the shoot of *Z. scabra* from the standard bacterial pathogen of *S. aureus* showed maximum zone of inhibition (50.00±0.11) mm[20], while in a study conducted by Bruck[21], methanol leaves extracts of *Z. scabra* had no effect on the tested microbes except at higher concentration low activity against standard strain of *S. aureus* (6 mm). These variations in zone of inhibitions may be due to the difference in concentration of active pricnciple; type of solvent used for extraction and type of bacterial strains tested[20].

Crude leaves extracts of *R. communis* demonstrated maximum zone of inhibition against standard strains of *S. aureus* (ATCC 2923) was (15.90±2.13) mm, followed by *E. coli* (ATCC 25922) [(15.20±1.53) mm], clinical isolates of *S. aureus* [(9.90±1.98) mm] and MRSA [(9.50±2.12) mm]. The least inhibition zone was recorded from clinical isolate of *E. coli* (7.75±0.91) mm. A similar study conducted by Kensa and Yasmin showed that the most sensitive strain was *E. coli* (11.3 mm)[22]. While other reports by Chukwuka *et al.* showed that *R. communis* leaf extract did not inhibit *E. coli*[23]. The difference might be related to many factors such as age of the plant, plant part used, solvent concentration, tested strains used and extraction procedures followed[21].

The results of the present study showed that standard bacterial strain of *S. aureus* was the most susceptible bacterium, which may be attributed to the absence of outer membrane of the organism that makes it more accessible to permeation by active principles of the leaves extracts of *Z. scabra* and *R. communis*[18]. Promising inhibition zone was also obtained against MRSA which shows that herbal preparations are important alternatives for drug resistant bacteria pathogens. Crude leaves extracts of *Z. scabra* and *R. communis* have shown an interesting profile of antibacterial activity against standard bacterial strains more than clinical isolates and Gram-positive strains are more sensitive to the extracts. Therefore, further study is needed to isolate the pure compounds from these crude extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

In developing countries, about 80% of the population uses medicinal plants as traditional medicine to treat infectious

diseases. *Z. scabra* and *R. communis* are being used for treatment of pathogenic organisms in the traditional health care system. This study aimed to evaluate antibacterial activity of crude extract from *Z. scabra* and *R. communis* against clinical and standard strains.

Research frontiers

In this study, leaf extracts of *Z. scabra* and *R. communis* showed maximum zone of inhibition against standard strain of *S. aureus*. Promising inhibition zone were also obtained against MRSA which shows that medicinal plant extracts are important alternatives for drug resistant bacterial pathogens. Crude leaves extracts of *Z. scabra* and *R. communis* have shown an interesting profile of antibacterial activity against standard bacterial strains more than clinical isolates. Gram-positive were more sensitive than Gram-negative strains.

Related reports

The antimicrobial effect of plant extracts against test organisms were depend on different solvents used. Similar study reported by Anand *et al*., 2012 indicated that ethanol, methanol, ethyl acetate and aqueous extracts of the shoot of *Z. scabra* showed maximum zone of inhibition for standard strains of *S. aureus*.

Innovations and breakthroughs

Promising inhibition zone were obtained against *S. aureus* and MRSA which shows that medicinal plant extracts from *Z. scabra* and *R. communis* are important alternatives for drug resistant bacterial pathogens.

Applications

In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. *Z. scabra* and *R. communis* have antimicrobial components, therefore looking for its formulation and components of the extract may lead to the production of important antibiotic alternative for the treatment of infectious diseases.

Peer review

This paper is very interesting and has wide application in biotechnology where many researchers can follow the screening and extraction of natural products for the discovery of noble compounds. Therefore, this study is helpful to initiate other researchers in the area of interest.

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