



Research Article

Antibacterial Activity of *Allium Sativum*, *Zingiber Officinale* and Their Synergistic Effect Against *Staphylococcus Aureus* and *Escherichia Coli* Isolated from Milk Samples of a Dairy Farm in Jimma Town Southwestern Ethiopia

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Abstract

The development of antibiotic resistance has recently increased research attention in exploring novel antimicrobial agents sourced from medicinal plants. In Ethiopia, *Allium sativum* (garlic) and *Zingiber officinale* (ginger) are the most valued medicinal plants. This study investigates the antibacterial properties of extracts obtained from the bulbs and roots of *Allium Sativum*, *Zingiber Officinale* and their synergistic effects against *Staphylococcus Aureus* and *Escherichia Coli* strains isolated from milk samples. A 50g powdered bulbs of *A. sativum* and roots of *Z. officinale* were separately macerated with 500 mL of distilled water and 95% ethanol in sterilized flasks. The antibacterial effects of crude aqueous and hydro-ethanol extracts of the both plants and their synergistic effects with 95% ethanol extracts were assessed using disc diffusion method, with concentrations of 50, 75 and 100 mg/mL for susceptibility testing. The 95% ethanol extracts of both plants had lowest yield percentage as compared to aqueous extracts. ANOVA was used for statistical analysis, with a significance level of $P < 0.05$. Both 95% ethanol as well as distilled water extracts and their synergistic effects with 95% ethanol extracts exhibited antibacterial activity against *S. Aureus* and *E. Coli* using *ciprofloxacin* discs as positive and blank discs as a negative control. Among the extracts, the lowest susceptibility was observed for aqueous extracts with inhibition zone of *Z. officinale* at 50 mg/mL against both bacteria, while *E. coli* showed a notable susceptibility to *Z. officinale* at 100 mg/mL. The 95% ethanol extract of *A. sativum* and its combination showed smaller inhibition zone against both bacteria at 50 mg/mL while, larger inhibition zone was seen with *A. sativum* against *E. coli* (27.67 ± 0.58 mm) but *Z. officinale* showed larger inhibitory zone against *S. aureus* (19.33 ± 1.15 mm) at concentration of 100 mg/mL compared to water extract. In both aqueous and 95% ethanol extracts, there was statistically a significant difference ($P \leq 0.000$) in the susceptibility of all tested bacteria. This study indicate that the extracts obtained from of the bulbs of *A. sativum* and the roots of *Z. officinale* have promising antibacterial properties, validating their traditional medicinal use for treating infections.

Keywords

Antibacterial Activity, *Allium Sativum*, *Escherichia Coli*, *Staphylococcus Aureus*, Synergistic Effect, *Zingiber Officinale*

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1. Introduction

Throughout history, plants and their various parts had a significant impact on maintaining the health of humans and animals. Use of plants as medicine can be traced back to ancient times, specifically era of the middle paleolithic, approximately 60,000 years in past, based on fossil records. According to a report by WHO, about 80% of global population predominantly depends on traditional medicine, which extensively uses of plant extracts and their active components [1, 2].

Traditional medicines and medicinal plants are essential components of health-care systems of many developing countries in Asia and Africa, including Ethiopia. In Ethiopia, around 80% of the human population and 90% of livestock dependent on traditional medicine [3, 4]. Modern scientific studies have also shown the effectiveness of certain plants when used in accordance with traditional or folkloric practices [5]. Considering the significance of these resources, which meet the of Ethiopia's populations medical's needs, both for humans and cattle, the possibility that farmers generate income from medicinal plants [6]. Medicinal plants are utilized in various countries, are a significant supplier of new medicines, as well potentially serve as alternatives to conventional drugs. Although traditional medicine and medicinal plants are crucial components of primary healthcare, limited research has been conducted worldwide, resulting in much of the knowledge being retained by traditional healers [7, 8].

Flora of Ethiopia is believed to encompass approximately 6,500–7,000 species with 12–19% of them are being endemic to the country. Ethiopia's diverse geography creates various habitats and vegetation zones, with the highest concentration of medicinal plants located in south and southwest regions due to their abundant biological and cultural diversity [9-11].

The most important medicinal plant species used in Ethiopia are antimicrobial, anthelmintic, and antifungal agents, including *Allium sativum* (garlic), *Allium cepa* (onion), *Zingiber officinale* (ginger), *Emblica officinalis* (amla), *Benincasa hispida* (petha), *Calpurnia aurea* Benth, *Croton macrostachyus*, *Nicotiana tabacum*, *Datura stramonium*, *Malva verticillata*, *Hagenia abyssinica*, *Glinus lotoides*, *Ricinus communis* and *Vernonia amygdalina* [12-14]. According to Abera [15], Gabriel and Guji [16], 39 medicinal plants and 80 medicinal plants have been recognized for treating different diseases in some districts of the Jimma zone of the Oromia Regional State, Southwestern Ethiopia.

Allium sativum and *Zingiber officinale* are frequently utilized in cooking and traditional medicine for their flavoring properties and potential health benefits for human and animal health [17]. Garlic, is a large annual plant that grows in Africa and Ethiopia and is used as traditional medicine for infectious diseases [18, 19]. it has antimicrobial properties against various bacteria [20].

Ginger, a plant with an underground stem known as a rhizome, is commonly used in cooking and found in different

colors ranging from white to brown. Color depends on whether the outer layer is removed and how it is initially processed. In East Africa, particularly in southwest Ethiopia, ginger is used as both a spice and an herb [21] and has microbial properties against infectious bacteria and fungi [22-24], the potential antimicrobial effects of ginger might be attributed to phenolic compounds. Historically, ginger has been utilized in the treatment of intestinal infections, especially those linked with digestive well-being [26]. Certain phytochemicals or essential oils along with a variety of other chemical constituents like tannins, saponins, flavonoids, glycosides, steroids, alkaloids, carotenoid phenols, terpenoids, polyphenols, anthraquinones, oxalates and phytates, are responsible antimicrobial activity of garlic and ginger species are a result of distinct [27-29].

The type and composition of the spice, its quantity and the type of bacteria, the food's composition, the pH level and temperature are the main factors of that affect antibacterial activity [30].

The growing number of resistant strains causing infections has led to a decrease in the effectiveness of the currently available antibiotics. This can be linked to factors such as overuse of antimicrobial drugs, incorrect dosing of antimicrobial and uncontrolled access to drugs [31]. The majority of bacteria can generate resistance illnesses which has led to mortality and morbidity. This emphasizes the urgent need for solutions to counteract bacterial resistance [32]. The risk of transferring a resistant bacteria or resistance genes, from food animals to humans exists when such bacteria are present in the food animals [33, 34].

Furthermore, most rural residents in developing countries including Ethiopia, do not have no access to antibacterial drugs for treating livestock illness or primary healthcare.

A little evidence available about the effects of crude extracts of garlic and ginger on *Staphylococcus aureus* and *Escherichia coli* despite some research on the antibacterial efficacy against certain bacteria. Hence, the purpose of this study was to evaluate in vitro antibacterial activity of aqueous and 95% ethanol crude extracts of garlic bulbs and ginger roots as well as their synergistic effects against *Staphylococcus aureus* and *Escherichia coli*.

2. Methods and Materials

Geographical Location of the Plants

This research was carried out in Jimma Town, located in the Oromia, southwestern Ethiopia. The Jimma situated at 7°40' N latitude and 36°50' E longitudes, has an altitude of 1780 meters above sea level and receives 1200 to 2000 mm of rainfall annually. The area experiences yearly lowest and maximum temperatures of 6 and 31 degree Celsius respectively, with average temperature of approximately 18.5 degree Celsius. It is well known for farming cattle, producing coffee, and

cultivation of crop [35]. An estimated population of 2,212,962 cattle head, 866,561 sheep, 457,311 goats, 96,782 horses, 17,644 mules, 77,767 donkeys, 546,722 beehives and

1,951,129 poultry in the zone [36]. The study area's map is displayed in (Figure 1).

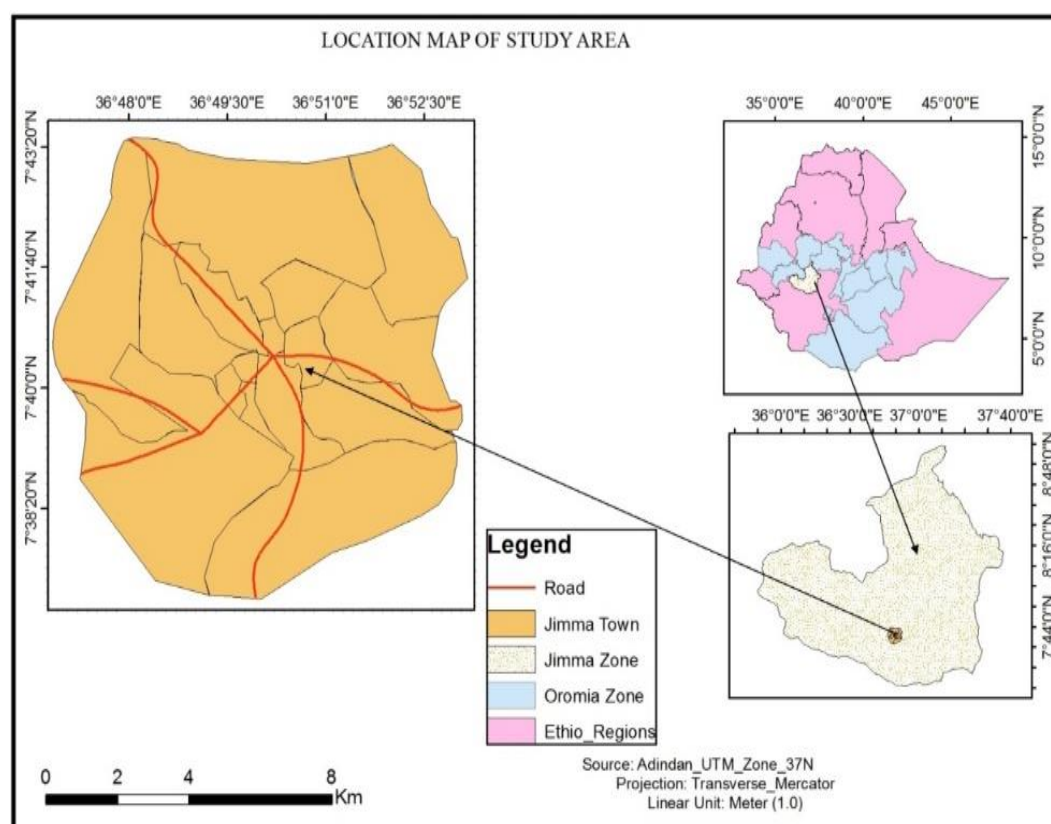


Figure 1. Jimma town administrative boundary (source: Ethio GIS, 2014).

2.1. Collection and Preparation of Plant Samples

From November 2019 to January 2021, medicinal plants used for treating livestock diseases were collected in Jimma Town, “Merkato” (Table 1 and Figure 2), based on traditional

knowledge and literature reviews. Garlic and ginger were purchased from the local market, and the plants were sent to the Ethiopian Herbarium for classification. Identification was confirmed by Dr. Ermias Luleka from Addis Ababa University. Fresh bulbs and roots were washed, cut into smaller pieces, and air-dried for three weeks. After drying, the samples were ground into a fine powder, sifted, weighed, and stored in sealed polythene bags in a refrigerator at 4 °C for future use.

Table 1. Descriptions of the plant material.

Scientific name	Common Name	Local Name	Parts Used
<i>Allium sativum</i>	Garlic	Qullubii adii (A. O) Nechi shunkurt (A)	Bulbs
<i>Zingiber officinale roscoe</i>	Ginger	Jinjibilla (A.O) Zinjibil(A)	Roots

A.O= Afaan Oromoo; A=Amharic

2.1.1. Preparations of Crude Extracts

Crude extraction was carried out by weighing approximately 50g of each test plant [37] using a delicate balance and transferring it to a flask containing solvents with 500 mL distilled water and 95% ethanol. The solvents were added separately and mixed vigorously for 30 minutes to ensure the proper blending and maceration of the plant components. After that, mixture was given a 72 hr incubation period at room temperature. Following this period, the extract's suspension was filtered through gauze plug followed by filter paper (Whatman no. 1 filter paper, Whatman Ltd, England) to gain

a solid-free solution and discarding any remaining plant residues. The solvent was then removed at 50 °C using a vacuum rotary evaporator [38, 39]. Yield of each extraction was measured independently by measuring weight of each extracts and calculating the yield percentage as extract weight divided by dry powder weight×100 according to Pandey and Chawdhry [40]. The concentrated extracts obtained from each plant material were then transferred to bijou bottles that fit tightly together by aluminum foil and labeled with the name of the corresponding plant (Figure 3). They were stored refrigerator at 4 °C until they were prepared for the antimicrobial activity test. We used the following formula to find percentage yield of each plant extract:

$$\text{Percentage extract yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of dried powder}} \times 100$$

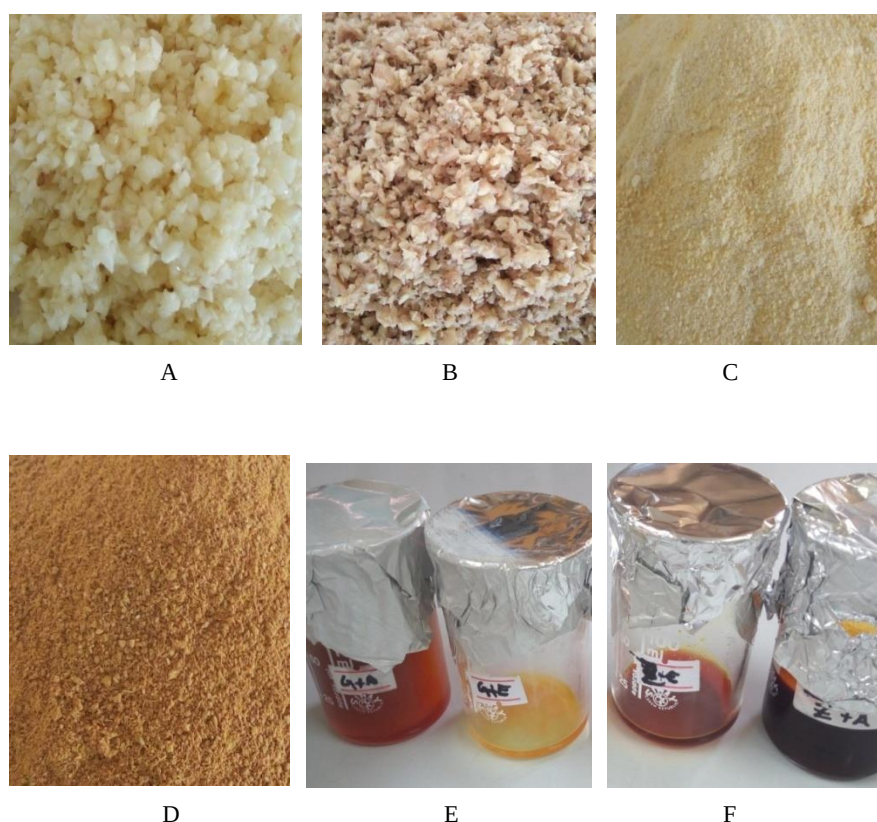


Figure 2. Chopped roots of *Allium sativum* (garlic) and *Zingiber officinale* (ginger) (A and B); fine powder (C and D); and crude filtered extract (E and F); the left pictures shows the roots of garlic and the right picture shows bulbs of ginger.

2.1.2. Test Microorganisms

Bacteria preparation for the experiment

The study involved isolating and identifying from milk samples collected from cows infected with mastitis including *Staphylococcus aureus* and *Escherichia coli*. The laboratory work was carried out at the Jimma University Veterinary Microbiology Laboratory, following standard protocols for media preparation, sample collection, culturing, isolation, and

identification in accordance with clinical veterinary microbiology guidelines [25, 41].

S. aureus isolation and identification from Cows milk samples

S. aureus was identified from milk samples obtained aseptically as the guidelines of the National Mastitis Council at Jimma University's Kito Furdisa Dairy Farms Enterprise. The milk samples that showed positive results in the CMT test were carefully centrifuged and streaked onto blood agar media.

Subsequently, a single bacterial colony was sub-cultured on nutrient agar media and then cultured on Mannitol Salt Agar (MSA) media [42, 43]. The identification of bacteria as *S. aureus* was based on their bacterial morphology observed in Gram staining, colony characteristics on MSA described by

Jahan et al [4] (Figure 3C), beta hemolytic patterns on blood agar containing 7% (v/v) sheep blood (Figure 3B), as well as positive results in catalase, and tube coagulase tests (Figure 3A).

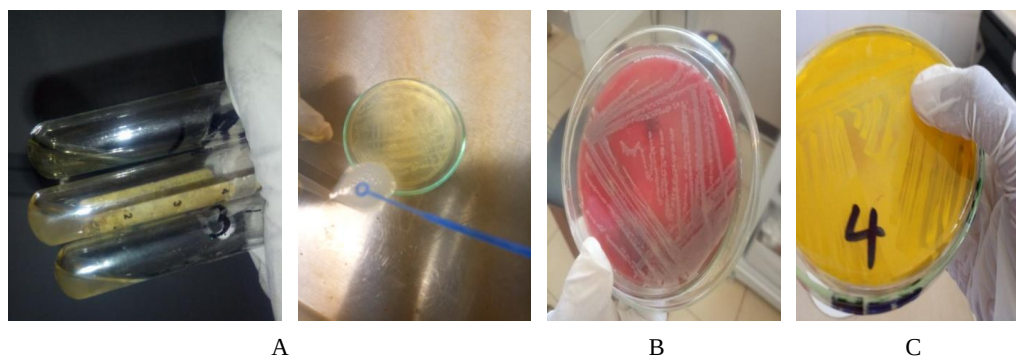


Figure 3. Tube coagulase positive *S. aureus* (A); beta hemolytic *S. aureus* colony on 7% sheep blood agar (B); and golden yellow *S. aureus* colony on mannitol salt agar (C).

Escherichia Coli isolation and identification from milk samples

Escherichia coli was obtained by streaking milk samples that tested positive for CMT from Jimma University Kito Furdisa Dairy Farms Enterprise onto blood agar, followed by sub-culturing onto MacConkey agar. The resulting pure colonies underwent Gram staining and were cultured on MacConkey agar to distinguish lactose fermenting and non-fermenting gram-negative bacteria [44], *E. coli* was identified as the Lactose positive colonies [41, 45-47]. These colonies were then further cultured on xylose lysine deoxycholate agar for Enterobacteriaceae identification [48]. Additionally, *E. coli* colonies exhibiting a distinct metallic sheen [41] cultural response on eosin methylene blue agar following a 24 hour of incubation period at 37 °C, the motility test, the triple sugar iron agar test (Figure 4B), and the citrate utilization test [42] were performed.

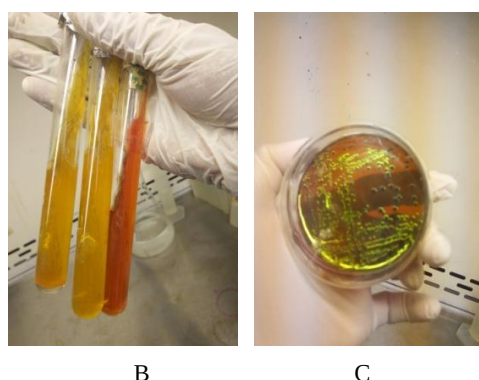


Figure 4. The yellow slant and butt with splitting of the agar by gas formation in the TSI tube (B), and blue black metallic sheen colonies of *E. coli* growing on eosin methylene blue agar (C).

Antimicrobial Susceptibility Test

Disc Diffusion Assay

Mueller–Hinton agar (MHA, Oxoid, Ltd, England) was utilized for the antimicrobial sensitivity test. 38 g of powder were dissolved in 1,000 mL of distilled water, thoroughly mixed, were boiled, and then autoclaved for 15 minutes at 121 °C. The medium was then transferred into sterilized 90 mm agar plates and allowed to solidify. Plates were then incubated for 4 hours at 37 °C to ensure sterility. After a 24-hour period, if there was no visible growth, plates were deemed sterile and prepared for testing for antimicrobial sensitivity. 30 µL of plant extracts was soaked for 30 minutes on sterile filter paper discs Whatman no. 1 (6 mm in diameter). The Mueller–Hinton agar plates that had been previously inoculated were covered with filter paper discs that had soaked in the extract. The plates were then allowed to stand for 30 minutes at room temperature to allow the extract to diffuse properly [49]. The top 4–5 well-isolated colonies with the appearance were then picked from the nutrient agar using a wire loop, mixed with sterile normal saline, and agitated by a vortex mixer. Using sterile swabs, the bacterial suspension was spread over MHA media after its turbidity was adjusted by comparing it to 0.5 McFarland turbidity standards which is equivalent to 1.5×10^8 CFU/mL. 0.05 mL of 1.175% aqueous solution of barium chloride (0.048NBCL₂H₂O) and 9.95 mL of 1% sulfuric acid (0.036NH₂SO₄) were used prepare the McFarland turbidity standard. The test suspensions were measured in 10mL sized tubes that were matched to the standard by contrasting them visually with a white background that had black lines. Depending on level of turbidity of *S. aureus* and *E. coli* saline or colonies were added to modify the turbidity. On the agar plates, discs impregnated with crude extract and regular antibiotics were positioned at 24 mm from the center and 15 mm from

the edge. After being inverted, the plates were labeled and incubated for 24 hours at 37 °C. The zone diameter was measured in millimeters by using a caliper after the incubations. Three duplicates of the experiments were conducted [52].

Determination of in vitro Minimum Inhibitory Concentration (MIC)

Established protocols were followed in order to determine the MIC of the aqueous and ethanolic crude extracts [50]. The stock solution was diluted in a 2-fold manner to create working concentration 100, 75, and 50 mg/mL. To prepare the stock solution of each extract, 1,000 mg was added to a beaker with 5 mL of dimethyl sulfoxide (DMSO) and thoroughly mixed by vortexing. To get 100mg/mL, the preliminary of 200 mg/mL concentration of the plant extracts was diluted twice by transferring 1 mL of the stock solution to 1 mL of DMSO. Then, to get 75mg/mL, 1mL of the 100mg/mL solution was moved to another test tube containing 1mL of DMSO. After that, 1mL was transferred to a test tube containing 1mL of DMSO to get 50mg/mL of concentration. The filter paper discs with sterile discs were then placed at each concentration and allowed them to absorb the solution before being analyzed. Every paper disc had the capacity to absorb 0.01 mL [51].

Inhibitory Potential of Plant Extract

Sterile 6 mm Whatman filter paper no.1 discs were placed on MHA inoculated with test microorganisms. Using sterilized forceps, different concentrations of 100 µL aqueous and

ethanol extracts of garlic, ginger, and a combination of both were applied to the discs. Negative controls included blank discs with sterile distilled water, while 5 µg of ciprofloxacin served as the positive controls.

2.2. Statistical Analysis

Results of the laboratory test including the inhibition zone from individual plant extract, the MIC, and bacteriocidal potential against the both bacteria were all documented in a Microsoft Excel spreadsheet. This data was then transferred to SPSS version 20 for further analysis. The comparison of the average inhibition zones of each extract at varying concentrations with those of the controls against the test microorganisms was conducted using ANOVA, and a statistical significance level at $P < 0.05$.

3. Results

Plants and Their Extract Yields

The study showed that 95% ethanol extracts of garlic and ginger yielded the lower quantities of extract 2.87g and 3.92 g respectively while the aqueous extraction resulted in higher yields of 14.2 g for garlic and 10.67g for ginger, as in Table 2.

Table 2. Percentage yield of the crude extracts obtained from *Allium sativum*(garlic) and *Zingiber officinale* (ginger).

Extraction solvents	Raw plant powder (grams)	Extracted plant (grams)	Percentage Yield (%)
Aqueous extracts of garlic	50	14.2	28.4
95% extracts of garlic ethanol	50	2.87	5.74
Aqueous extracts of ginger	50	10.67	21.34
95% ethanol extracts of ginger	50	3.92	7.84

Physical characteristics of the semisolid extracts of the crude 95% ethanol and aqueous water extracts from both *Allium sativum* and *Zingiber officinale* were observed. The extract obtained from *A. sativum* was light yellow with sticky consistency and emitted an allacin scent, while the extract recovered from *Z. officinale* was brown with a sticky texture and a strong smell (Figure 6). The appearance of the extracts closely similarly with the description provided by Yusha'u et al [53].

Antibacterial Activity of *Allium Sativum* and *Zingiber officinale*

The bulbs and roots extracts of *Allium sativum* and *Zingiber officinale* have shown antibacterial activity against *S. aureus* and *E. coli*. At 50 mg/mL, the aqueous extracts from both plants showed the smallest inhibition zones against *S. aureus*,

while at 100 mg/mL, exhibited highest inhibition concentration against *E. coli*. Conversely the ethanol-based extract from both plants showed the smaller inhibition zones (9.33 ± 0.58 and 9.67 ± 0.58) at 50 mg/mL against *E. coli* but larger inhibitory zones against *E. coli* (27.67 ± 0.58) and *S. aureus* (19.33 ± 1.15) at a concentration of 100 mg/mL. The combination of the two plant extracts through ethanol showed the synergistic effects against both bacteria *S. aureus* (16.00 ± 1.00) and *E. coli* (14.67 ± 0.58) compared to their individual aqueous extracts (Table 4 and Figure 7). ANOVA analysis showed significant difference in the antibacterial activities of garlic, ginger and ciprofloxacin against both microorganisms (F-value=6.37; $P < 0.000$) against *E. coli* (F-value=11.25; $P < 0.000$) when tested against *S. aureus*. The least square differ-

ence of the two-way comparison showed that there were differences between garlic aqueous and garlic ethanol, in which garlic ethanol extract showed high antibacterial activity (P -value 0.000); garlic ethanol and ciprofloxacin, in which garlic ethanol extract showed higher activity ($P=0.002$); garlic ethanol extract and ginger aqueous extract ($P=0.000$); ginger ethanol extract ($P=0.022$); and garlic ethanol extract and their combination ethanol extract ($P=0.019$) where garlic ethanol extract showed higher antibacterial activity. However, the antibacterial activity of aqueous ginger was significantly different from that of the ginger ethanol extract and the synergistic effect of garlic and ginger ethanol extract, where the latter two had more activity than the former ($P=0.007$, 0.008 respectively). In terms of concentration, 100 mg/mL of garlic extract exhibited higher antibacterial activity against *E. coli* compared to 75 and 50 mg/mL concentrations for both aqueous and ethanol extracts.

Ethanol extract of ginger significantly inhibited *S. aureus* compared to the aqueous garlic extract positive control, and all other extracts, except for the synergistic effects of garlic and ginger. The effect of the concentration 100 mg/mL of aqueous extract of garlic was more potent against *E. coli* than that of the other concentrations tested, which was also observed for the ginger extracts.

Positive control ciprofloxacin, showed varied effectiveness against the two bacteria strains. The smallest zone of inhibition (10.78 ± 0.50) against *E. coli*. However, it showed the largest zone diameter (11.22 ± 0.50) against *S. aureus* when compared to 50 mg/mL concentration of both extracted plants, their combination, and aqueous extract of garlic and ginger (50, 75, and 100 mg/mL, except garlic extract against *E. coli* at 100 mg/mL concentrations). Distilled water did not exhibit any activity (Table 3 and Figure 7).

Table 3. Antibacterial activity of *Allium sativum* and *Zinger officinale* extract against *staphylococcus aureus* and *Escherichia coli*.

Extracts solvents	Concentration (mg/mL)	Garlic (<i>Allium sativum</i>)		Ginger (<i>Zinger officinale</i>)	
		Zone of inhibition (mm)		Zone of inhibition (mm)	
		<i>S.aureus</i>	<i>E. coli</i>	<i>S.aureus</i>	<i>E. coli</i>
Aqueous	50	6.33 ± 1.15^a	7.67 ± 0.58^a	6.00 ± 1.00^a	6.00 ± 0.58^a
	75	7.67 ± 0.58^a	8.67 ± 0.58^a	7.67 ± 0.58^a	7.67 ± 0.58^a
	100	9.00 ± 1.00^a	11.67 ± 0.58^a	10.00 ± 1.00^a	9.00 ± 1.00^a
95% Ethanol	50	10.00 ± 1.00^a	9.33 ± 0.58^a	10.33 ± 0.58^a	9.67 ± 0.58^a
	75	11.33 ± 0.58^a	12.67 ± 0.58^a	14.67 ± 0.58^a	12.67 ± 0.58^a
	100	15.67 ± 0.58^a	27.67 ± 0.58^a	19.33 ± 1.15^a	15.33 ± 0.58^a
Positive control		11.22 ± 0.50^b	10.78 ± 0.50^b	11.22 ± 0.50^b	10.78 ± 2.50^b
Negative control		0.00 ± 0.00^c	0.00 ± 0.00^c	0.00 ± 0.00^c	0.00 ± 0.00^c

Notes: The table presented as the mean $\pm$ SEM: ^a against 100, 75, and 50 mg/mL of plant extract by ethanol, aqueous, and their synergistic effect, ^b against positive control, ^c against negative control.

Table 4. Synergetic effects of both plants extract by 95% Ethanol against *S.aureus* and *E.coli*.

Extracts solvents	Concentration (mg/mL)	Zone of inhibition (mm)	
		<i>S. aureus</i>	<i>E. coli</i>
95% Ethanol	50	9.67 ± 0.58^a	10.00 ± 1.00^a
	75	12.33 ± 0.58^a	12.67 ± 0.58^a
	100	16.00 ± 1.00^a	14.67 ± 0.58^a
Positive control		11.22 ± 0.44^b	10.78 ± 0.44^b
Negative control		0.00 ± 0.00^c	0.00 ± 0.00^c

Notes: The table presented as the mean $\pm$ SEM: ^a against 100, 75, and 50 mg/mL of plant extract by ethanol, aqueous, and their synergistic effect, ^b against positive control, ^c against negative control.

Table 5. ANOVA table for the significance tests of garlic and ginger extracts and different concentration against *E. coli* and *S. aureus*.

zone of inhibition Result					
	Sum of Squares	df	Mean Square	F	Significance
ANOVA table type of extract against <i>E. coli</i>					
Between Groups	452.611	5	90.52	6.37	0.000
Within Groups	682.222	48	14.21		
Total	1134.833	53			
ANOVA table effect of concentrations against <i>E. coli</i>					
Between groups	1118.167	17	65.78	142.07	0.000
Within groups	16.667	36	.463		
Total	1134.833	53			
ANOVA table effect of type of extracts on <i>S. aureus</i>					
Between groups	351.778	5	70.36	11.25	0.000
Within groups	300.222	48	6.26		
Total	652.000	53			
ANOVA table effect of concentrations of extracts on <i>S. aureus</i>					
Between groups	632.000	17	37.18	66.92	0.000
Within groups	20.000	36	.56		
Total	652.000	53			

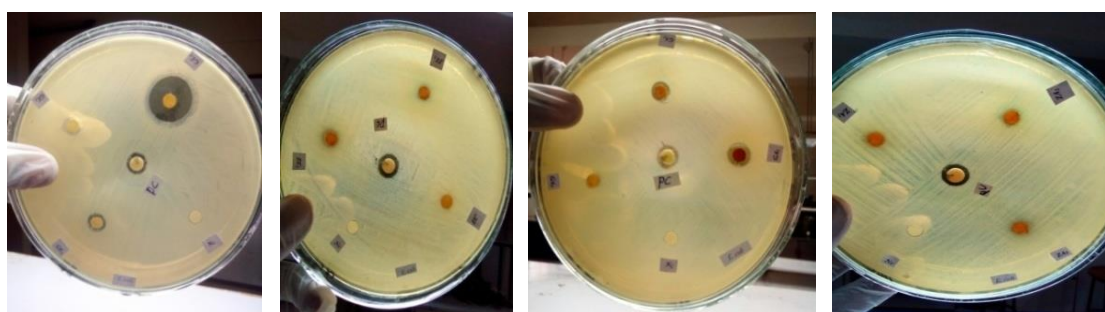


Figure 5. Antibacterial effects of study plants at different concentration.

GE₁, 100%; GE₂, 75%; GE₃, 50% (concentration of garlic ethanol extract); ZE₁: 100%; ZE₂, 75%; and ZE₃, 50% (concentration of ginger ethanol extract); GA₁: 100%; GA₂, 75%; GA₃: 50% (concentration of garlic aqueous extract); ZA₁: 100%; ZA₂, 75%; ZA₃, 50% (concentration of ginger aqueous extract); Nc, negative control; Pc, positive control.

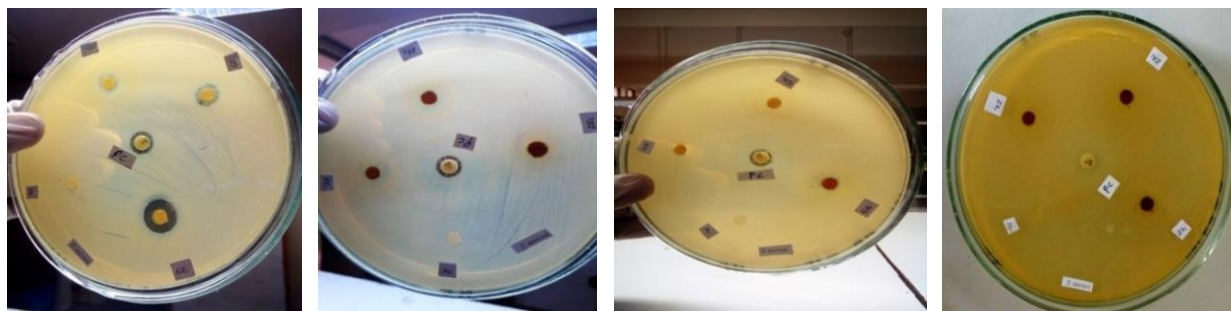


Figure 6. Antibacterial effects of study plants at different concentration for identifying good efficacy.

GE₁: 100%; GE₂: 75%; GE₃: 50% (concentration of garlic ethanol extract); ZE₁, 100%; ZE₂, 75%; and ZE₃: 50% (concentration of ginger ethanol extract); GA₁: 100%; GA₂, 75%; GA₃, 50% (concentration of garlic aqueous extract); ZA₁: 100%; ZA₂, 75%; ZA₃, 50% (concentration of ginger aqueous extract); Nc, negative control; Pc, positive control.

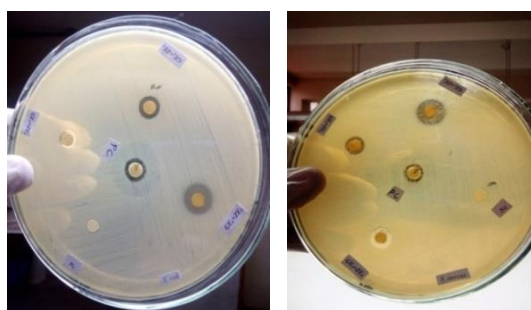


Figure 7. synergistic effects of antibacterial of study plants.

GE₁+GE₁= 100% of garlic mixed with 100% of ginger; GE₂+GE₂= 75% of garlic mixed with 75% ginger; GE₃+GE₃ = 50% of garlic mixed 50% ginger (their synergistic effects) of 95% ethanol extracts of plants; Nc= negative control; Pc= positive control.

4. Discussion

The effectiveness of medicinal plant extracts against drug resistant strains is thought to be attributed to compounds such as flavonoids, tannins, saponins, phenolic compounds and essential oils. These components exhibit strong antimicrobial properties, particularly against strains that are resistant to traditional antibiotics [54].

In this study, 95% ethanol extract of garlic (*Allium sativum*) showed a lower yield (2.87) grams or (5.74%) while, the aqueous extract gave higher yield of (14.2 g) or (28.4%), which is in close agreement with Wolde et al [55] reported a yield of (1.86 g) and (22.24 g) by using 95% of ethanol and aqueous extract, respectively. Ginger (*Zinger officinale*) 95% ethanol extract showed the lower yield (3.92 g) or (7.84%) while, the aqueous extract gave higher yield of (10.67 g) or (21.34%), slightly higher than a report of (5.26%) Mostafa et al [56] using of ethanol extract and but, lower (32%) using aqueous extract of *Zinger officinale* [57]. The variation in the yield could be due to the polarity of the solvents, amount of extracted

plants, seasonal variation (winter or summer), and the methodology used. Ethanol extracts of the ginger roots were demonstrated greater efficacy against the bacterial strains *S. aureus* and *E. coli* compared to the aqueous extracts. This finding aligns with a previous study on medicinal plants targeting clinical isolates of multidrug-resistant bacterial strains [58]. Similarly comparing the ethanol extracts of garlic bulbs to the aqueous extracts, the former were exhibited high effectiveness against *S. aureus* and *E. coli* which is consistent with study done on human food pathogens [59].

Garlic crude ethanol extracts displayed inhibition zones of range of 15.67 ± 0.58 mm for *S. aureus* and 27.67 ± 0.58 mm for *E. coli*, while ginger crude ethanol extract exhibited inhibition zone of 19.33 ± 1.15 mm and 15.33 ± 0.58 mm for the same strains. The combination extract of both plants resulted in inhibition zones of 16.00 ± 1.00 mm and 14.67 ± 0.58 mm for *S. aureus* and *E. coli*, respectively. The study indicated that the crude ethanol extracts of garlic exhibited better antibacterial effects against *E. coli* at various concentrations compared to *S. aureus*, whereas ginger was more effective against *S. aureus* at different concentrations, (Tables 5, and Figures 6 and 7) supporting previous findings of Aliyu et al [20]. However,

the synergistic effect of the combined garlic and ginger extracts was lower than in the present study.

Aqueous extracts of both plants exhibited lower activity against *S. aureus* and *E. coli*, although ginger aqueous extract showed potent antimicrobial property against *E. coli* at a concentration of 50% mg/mL, similar to Sikrodia *et al* [60] and showed strong antimicrobial activity against different concentration of ginger 100, 50, and 25% the zone of inhibition was 15, 12 and 10 mm for *S. aureus* and 20, 15, and 13 mm for *E. coli*, respectively. While, there was no zone of inhibition of 100, 50, and 25% of garlic extract for *S. aureus* and *E. coli* which was in contrast to the current study which garlic inhibited zone formation. This variation might be due to the time of collection of the plants material, climate, concentration level, temperature, methods of extraction, time of maceration, and varying amounts of active constituents in the plant materials.

The study also found that the synergistic effect of both plant extracts in 95% ethanol were more effective against *S. aureus* and *E. coli* compared to aqueous extracts, but with smaller inhibition zones than those observed with ginger and garlic ethanol separately. This is contrary to previous studies which reported a synergistic effect of ethanol extracts of ginger and garlic against *Bacillus* spp. and *S. aureus* [40, 61], this study found a higher inhibitory activity with ethanol alone. Ethanol is effective in extracting lipophilic and lipophobic bioactive compounds that can penetrate the bacterial cell walls, resulting in high inhibition zones against both against both *S. aureus* and *E. coli*.

Due to variations in cell structure and permeability barriers, medicinal plants tend to suppress the growth of gram-positive bacteria more efficiently than gram-negative bacteria [62, 63]. The presence of various active phyto-constituents in the plants extracts, such as saponins, flavonoids, tannins, glycosides, steroids, alkaloids, carotenoids, polyphenols, phenols, terpenoids, anthraquinones, oxalates, Phytates [29] likely contributes to their inhibitory effects on both gram-negative and gram-positive pathogens in both aqueous and ethanol extraction [64].

5. Conclusion

The recent study revealed that crude extracts from *Allium sativum* and *Zinger officinale* demonstrated potential antibacterial activity against *S. aureus* and *E. coli*. Ethanolic extracts showed greater antibacterial properties compared to the aqueous extracts. This finding suggest that these plant extracts could be utilized in treating of bacterial infections caused by *S. aureus* and *E. coli*, potentially aiding in combating antimicrobial resistance. However, further investigations is necessary to confirm the active ingredients of these plants as well as to assess their safety, efficacy, toxicity, and clinical evaluation.

Abbreviations

CMT California Mastitis Test

ANOVA	Analysis of Variance
DMSO	Dimethyl Sulfoxide
MSA	Mannitol Salt Agar
WHO	World Health Organization
SPSS	Statistical Package for the Social Sciences

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Conflicts of Interest

No competing interests exist in this study, according to the authors.

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