



Asian Journal of Research in Pharmaceutical Sciences and Biotechnology

Journal home page: www.ajrpsb.com
<https://doi.org/10.36673/AJRPSB.2025.v13.i02.A03>



IN VITRO ANTIBACTERIAL ACTIVITIES OF GARLIC (*ALLIUM SATIVUM*) AND LEMON (*CITRUS LIMON*) EXTRACTS AGAINST CLINICALLY ISOLATED BACTERIA SPECIES

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ABSTRACT

Background: Garlic and lemon are known to possess antibacterial properties. This study investigates antibacterial activities of their extracts. This would provide valuable insights for the scientific community and pharmaceutical companies in the search for natural antibacterial agents to combat infectious diseases and promote alternative therapeutic approaches. **Objective:** To determine the antibacterial activities of garlic and lemon extracts on Gram-positive and Gram-negative clinical isolates of bacterial species. **Methods:** Fresh garlic and lemon were bought from local market in Hawassa city and were subjected to solvent extraction. The fresh juice, aqueous and ethanol extracts of garlic and lemon were tested for susceptibility pattern using agar plate disk diffusion method against isolates of bacterial species used in the study. Data were sorted manually, computed for descriptive statistics, summarized and presented in tables, frequencies, percentage, mean and standard deviation. **Results:** Of 27 clinical strains, 48.1% were Gram-positive and 51.9% were Gram-negative. Fresh garlic juice demonstrated a high sensitivity of 96.3% across isolates, with Gram-positive bacteria showing up to 100% efficacy. The inhibition zones (IZ) ranged from 9.7±0.6mm for *E. faecalis* to 35.3±0.6mm for MRSA and from 10.7±0.6mm for *P. aeruginosa* to 33.7±0.6mm for *E. coli* in Gram-negative isolates. Aqueous extracts were less effective than fresh juice. Ethanolic extracts of garlic showed resistance at certain concentrations, while fresh lemon juice was effective against all isolates (with IZ ranging from 8.3±0.58mm to 23.7±0.58mm). The results suggest that garlic and lemon can serve as sources of potential antibacterial agents.

KEYWORDS

Garlic, Lemon, Zone of Inhibition, Antibacterial activity, Disk Diffusion Method Hawassa and Ethiopia.

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INTRODUCTION

Plants as a sources of medicinal compounds have continued to show an overriding role in the safeguarding of human health since primordial eras (Verlekar and Chandak, 2018)¹. It is considered that biological/pharmacological activities of medicinal plants are known to be due to the presence of

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phytochemicals, commonly secondary metabolites (Verlekar and Chandak, 2018)¹.

Plants are exceptional foundations of phytochemicals as natural antimicrobials and antioxidants, which can act as an imminent drug in present biomedicine (Gobeziea et al, 2020)². Ethiopia is one of the six hubs of biodiversity in the world with numerous different landscapes, climatic circumstances and different folkloric cultures. The vast majority of population of Ethiopia still depend on traditional medicine and its practitioners (Gobeziea et al, 2020)². World Health Organization (WHO) approximates about 80% of the world's population and 90% of livestock population (Tamene et al, 2020)³ is reliant on herbal medicines. Herbal medicine is a key component in all indigenous peoples, and a mutual element in traditional medicine system in the World. It is estimated that about 25,000 to 75,000 species of higher plants are being used in traditional medicine globally. Medicinal plants still provide substantial value in the health care system of the unindustrialized world (Temam and Dillo, 2016)⁴. This is due to the least or no side effect and easily accessible affordably. Occasionally, herbal medicines from traditional healers are the only available and easily accessible sources of health care to poor community in the third world countries that lack organized health care facilities with availed medicines and skilled man power like Ethiopia (Derbe et al, 2014)⁵.

Ethiopia has a rich tradition of using medicinal plants to treat human diseases, supported by diverse agro-ecological zones and cultural knowledge that foster a wide range of cultivated and wild species. Scholars estimate the country hosts around 6,500 higher plant species, with 12% endemic. However, rising antimicrobial resistance poses a growing challenge both locally and globally, highlighting the urgent need for alternative therapeutic solutions (Tamene et al, 2020)³.

In history, garlic has used for centuries universally by several societies to battle infectious disease. Garlic is among the eldest agricultural harvests, which dating back to 800 B.C and currently used

globally as foodstuff and traditional medicine (Tegegne and Mengiste, 2016)⁶. For instance, like garlic juice, garlic powder and garlic volatile oil are widely used for their antimicrobial actions. According to records in the Bible and other literature garlic has widely known and used for over 5000 years (Zhanga et al, 2020)⁷. In Chinese, Egyptians, Greeks, Indians, Israelis, and Babylonians, garlic has been used for healing a wide variety of disorders in ancient times, including leprosy, diarrhea, constipation, asthma, fever, and infection (Zhanga et al, 2020)⁷.

Garlic has shown a broad antibiotic effect on both gram positive and gram negative bacterial species (Fufa, 2019⁸, Hamza, 2015⁹). This garlic effect is revealed to be due to its most powerful and active constituent Sulphur compounds such as allicin. This is a chemical compound produced enzymatically when the garlic is chopped, bruised or chewed (Chuku, 2015¹⁰, Deresse, 2011¹¹).

The lemon, *Citrus limon*, is a species of small evergreen tree in the flowering plant that belongs to the family Rutaceae, native to South Asia, primarily North eastern India and northern Burma or China although the origin of the lemon is said to be unknown (Klimek-Szczykutowicz et al, 2020)¹². A genomic study of the lemon indicated it was a hybrid between bitter orange (sour orange) and citron.

Traditionally it is used to soothe sore throats, indigestion, relieve intestinal gas and bloating, resolve phlegm and as an additive for flavoring to our foods (Shakya et al, 2019)¹³. Citrus flavonoids have an abundant biological activity such as antibacterial, antifungal, antidiabetic, anticancer and antiviral activities. Flavonoids function as direct antioxidants and free radical scavengers. They have ability to modulate enzymatic actions and prevent cell proliferation. In plants; they appear to play a defensive role against invading pathogens including bacteria, Fungi and Viruses (Shinkafi and Ndanusa, 2013)¹⁴. Therefore, this study aims to establish and evaluate the effectiveness of extracts of garlic (*Allium sativum* L.) and lemon (*Citrus limon*) with the modern antibiotic's drugs against a

number of gram positive and negative isolates from clinical specimen.

MATERIAL AND METHODS

The study was conducted in Hawassa city, the capital of Sidama National Regional state. It is located 275km from Addis Ababa, capital of Ethiopia. The city lies between 07° 05' latitude north and 38° 29' longitude east, with an altitude of 1,697m asl. It covers a total area of 157.2km². It has a mean annual rainfall and temperature of 997.6mm and 20.9°C, respectively. Hawassa city has an estimated population of 351,469 with annual population growth rate of just over 4% and is home to more than 46 ethnic group (CSA, 2015). The study was conducted from July 01/2021 to December 30 2021 at the Regional Reference Laboratory that is located in Hawassa city. It is Accredited Laboratory on limited scope by Ethiopian National Accreditation Office (ENAO). The laboratory provides public health confirmatory diagnostic services for emergency prone diseases and clinical diagnostic services for the regional population as well as in areas/regions nearby Hawassa.

Study Design and Sampling Technique

An *in vitro* cross-sectional study was conducted on 27 clinically isolated bacteria from 217 clinical samples collected at South Nations and Nationalities People Region Public Health Institutes (SNNPR PHI) Microbiology Laboratory by convenience sampling technique during from 01/2021 to December 30 2021.

Microorganisms used in the study

Thirteen gram-positive (seven *Staphylococcus aureus*, five *Enterococcus faecalis* and one *Streptococcus pneumonia*) and fourteen gram-negative (seven *Escherichia coli*, two *Pseudomonas aeruginosa*, four *Klebsiella Pneumonia* and one *Proteus mirabilis*) clinically isolated bacteria from 217 clinical samples, collected at SNNPR PHIL Microbiology Unit, were considered as the subjects for the study.

Bacterial Isolation

All 27 clinical isolates of bacteria were obtained from 217 clinical samples, except blood and CSF, at SNNPR PHIL Microbiology unit were subjected to sub culturing and re-verification in appropriate basic and selective media. Gram positive isolates, and *Enterococcus faecalis* sub-cultured into Blood agar plate (BAP), Trypticase soya agar (TSA) and Mannitol salt agar (MSA) and incubated at 37⁰C for 24 hours. Colony of *Staphylococcus aureus* were confirmed on MSA and tested, from BAP, for its Gram's reaction, catalase teste and tube coagulase test positivity as a confirmatory test. *Staphylococcus aureus* isolates those positive for catalase test, coagulase test and fermented MSA were isolated as pure isolate on TSB. Colony of *Enterococcus faecalis* were confirmed on MSA and tested for biochemical identification on Bile Esculin agar. *Streptococcus pneumonia* was sub-cultured on BAP with optochin disk and incubated at 5% CO₂ condition in a candle jar at 37⁰C for 24 hours. Colonies were checked for alpha hemolysis, susceptibility for optochin disk and Gram's reaction.

Gram negative isolates (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella Pneumonia*, and *Proteus mirabilis*) were sub cultured on MacConkey agar and Trypticase soya agar (TSA) and incubated at 37⁰C for 24 hours (CLSI, 2020)¹⁵. The growth then was subject to further isolation on the basis of colony morphology, biochemical characteristics with the aid of Biochemical media (Triple Sugar Iron (TSI) Agar, Sulfide Indole Motility (SIM) medium, Citrate, Urea, Lysine Iron Agar (LIA), etc.), oxidase strip test, and gram's reaction. Colonies of both Gram positive and negative bacteria were sub cultured into Trypticase soya broth (TSB) and stored at -20⁰C until used for tests.

Plant material collection

Three kilograms of fresh garlic and two kilograms of fresh lemon were bought from the local market in Hawassa city, Ethiopia, after complete isolation and storage of gram positive and gram-negative bacteria of study interest.

Plant material extraction

Preparation of fresh garlic (pure garlic juice)

Fresh garlic bulbs were peeled and decontaminated and cleaned with 70% alcohol and then rinsed with sterile distilled water. These decontaminated bulbs were homogenized using sterile blender. Then, this was filtered through a cheese cloth thoroughly, centrifuged at 12,000rpm for 10 min and filtered twice through a 0.22µm filter to obtain raw fresh garlic extract. This was represented as 100% concentration that was diluted with sterile distilled water to get diluted concentrations of 75% (v/v), 50% (v/v) and 25% (v/v), and tested freshly. Each of the garlic extract was inoculated on nutrient agar media and incubated at 37°C overnight for sterility confirmation (Magrys *et al.*, 2021)¹⁶. The effects of cooking on garlic antibiotic effect under at 100°C, 80°C, 60°C and 40°C for 20 minutes were tested for potency after heating. Moreover, this fresh garlic juice was refrigerated at 4°C for 72 hours and rechecked for the perseverance of antibacterial effect (Annex 11.1).

Preparation of aqueous garlic extract (AGE)

The peeled garlic bulbs were decontaminated and cleaned with 70% alcohol and then rinsed with sterile distilled water and then 100g of cleaned bulbs/cloves was weighed and grounded thoroughly by sterile blender to obtain fine garlic juice. It was homogenized in 1000 ml of distilled water for 15 min. The homogenized mixture was placed at shaker adjusted at 100 rpm 72 hours for shaking and filtered through muslin cloth about three to four times to obtain homogenous aqueous garlic juice. This aqueous extract of garlic was tested immediately, within 72 hours, after extraction process and evaporating the aqueous solvent with the help of rotary evaporator apparatus at 40°C. Each time, the 100% garlic extract was inoculated on nutrient agar media and incubated at 37 °C for 24 hours to check the sterility (Magrys *et al.*, 2021)¹⁶. Bacterial growth was observed to check if there is the contamination. No growth in the plates indicated that the extract was sterile. (Annex 11.2).

Preparation of ethanolic garlic extract

For preparation of ethanolic extract, 150gm of crashed and homogenized fresh garlic was mixed in 1500mL of absolute (96% v/v) ethanol solution in a 2000ml flask and was sealed with an aluminum foil. The content of the flask was mixed thoroughly. The flask was placed at shaker adjusted at 100rpm 72 hours for shaking and then it was filtered by muslin cloth. Centrifugation was performed at 2000rpm for 5 min and supernatant was decanted. The pellet was discarded and supernatant filtrate was placed (Mekonnen and Desta, 2021)¹⁷ and the supernatant was connected to rotary evaporator at 40°C to separate the ethanol solvent from the final concentrated crude garlic. This concentrated crude extract to sterilized, distilled water in a 1:1 (v/v) ratio diluted (Mekonnen and Desta, 2021)¹⁷ (Annex 11.3)., This ethanolic extract of garlic was tested immediately, within 72 hours and the leftover concentrated crude amount was let to stand for above 72 hours at room temperature to convert into dry powder form. Used to prepare 500mg/ml, 200mg/ml and 100mg/ml of garlic extract solution by diluting the dried powder of garlic extract with sterilized distilled water. Each time, the garlic extract was inoculated on nutrient agar media and incubated at 37°C 24 hrs for sterility confirmation (Magrys *et al.*, 2021)¹⁶.

Preparation of fresh lemon extract

The fresh fruits lemon was washed in running tap water in laboratory and then the surface was sterilized with 70% alcohol for 15 minutes and rinsed with sterile distilled water. The lemon was cut open with a sterile surgical blade and the juice of it was pressed out into a sterile beaker container separately and then filtered into another sterile flask to remove the seeds and other tissues and this juice was used freshly as crude without refrigeration or delay (Owhe-Ureghe *et al.*, 2010)¹⁸. This was represented as 100% concentration that was again diluted with sterile distilled water to get diluted concentrations of 75%, 50%, and 25%, and then subsequently used. Each time, the 100% fresh lemon extract was inoculated on nutrient agar

media and incubated at 37 °C overnight for sterility confirmation (Magrys *et al*, 2021)¹⁶. (Annex 11.4).

Preparation of Ethanol Lemon Peel Extract

For preparation of ethanolic extract, 50gm of lemon peel scraping was mixed in 300mL of absolute ethanol solution in a 500ml flask and was sealed with an aluminum foil. The content of the flask was mixed thoroughly. The flask was placed at shaker adjusted at 100rpm for 72 hours for shaking and mixing and then it was filtered by muslin cloth. Centrifugation was performed at 2000rpm for 5 min and supernatant was decanted. The pellet was discarded and supernatant filtrate was connected to rotary evaporator to separate the ethanol solvent from the needed concentrated crude extract of lemon peel. This crude extract was then be diluted with sterilized, distilled water in a 1:1 (v/v) ratio; the extract was stored in sterile falcon tube at 4°C in refrigerator, aseptically to avoid microbial contamination, till used (Annex 11.5). More over the concentrated crude residue obtained after solvent separation by rotary evaporator was allowed to dry at room temperature for 2 weeks and used to prepare 200mg/ml and 100mg/ml of lemon peel extract solution by diluting the dried powder of lemon peel extract with sterilized distilled water. Each time, the 100% lemon peel extract was inoculated on nutrient agar media and incubated at 37°C overnight for sterility confirmation.

Qualitative Phytochemical Analysis

The samples of crude extract of garlic and lemon were transported, by maintaining cold chain properly, to Hawassa University Main Campus Chemistry Laboratory for the required species level analysis and for qualitative determination of phytochemicals (Okigbo *et al*, 2020)¹⁹.

Antibiotic Susceptibility Test (AST)

AST for Modern Drugs (Conventional Medicine)

All of clinically isolates, obtained from clinical samples at SNNPR PHIL Microbiology Unit, were tested for their drug sensitivity and resistance pattern with the disk diffusion method on Mueller-Hinton agar (MHA) at 37°C for 16-18 hours of incubation and for *Streptococcus pneumonia* MHA with 7% de-fibrinated sheep blood used at 5% CO₂

at 37°C (CLSI, 2020)¹⁵. An agar plate of antibiotic disk diffusion method was used by selecting 3-5 colonies of isolate and was emulsified in 1ml sterile physiological saline solution (the colony suspension) to 0.5 McFarland turbidity standards.

Drugs of choice, for each study isolates, were selected, tested and results were interpreted according Clinical and Laboratory Standards Institute (CLSI) drug profile guide (CLSI, 2020)¹⁵. The zone of inhibition was measured with a ruler and the mean value and standard deviation of triplicates was used (Annex 11.8).

AST for Herbal Extracts (Garlic and Lemon)

Loop full growths from bacterial pure isolate were inoculated into nutrient broth and incubated at 37°C for 18 hours. The bacterial suspension was then diluted with normal saline until the turbidity was adjusted and compared with standard tube (McFarland number 0.5) to yield a uniform suspension containing 1.5×10^8 CFU/ml (CLSI, 2020). Cotton swab was dipped into it and streaked on the Mueller Hinton agar (for all tested bacteria) surface on the plates and the plates were left for 5-15 minutes at room temperature to dry (for *Streptococcus pneumonia* MHA with 7% defibrinated sheep blood used at 5% CO₂ at 37°C) {CLSI, 2020 #62}. An agar plate disk diffusion method was used by preparing a Wattman № 1 filter paper that was punched exactly at 6mm diameter by using paper puncher that punches at 6 mm diameter and this 6mm disks were saturated by soaking within particular extracts of the garlic and lemon extracts solutions (The plates were performed in triplicates) (Liu *et al*, 2021)²⁰, Shinkafi and Ndanusa, 2013¹⁴). The average saturation amount for this 6mm diameter disk was $35.1 \pm 5 \mu\text{l}$. All plates of the tested organisms were incubated at 37°C for overnight. After 24 hrs of incubation, each extract was noted for zone of inhibition for against every isolate of the study. The diameter of the zone of inhibitions was measured by a ruler in millimeter (mm) and expressed as Means \pm SD (mm) of triplicate tests (Liu *et al*, 2021)²⁰.

Quality Control

The performance of each batch of every culture media, which were required for culturing and isolation, were controlled by testing with control strains like *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus pneumoniae* (ATCC 49619) and *Enterococcus faecalis* (ATCC 29212) to check the quality of the media. And the sterility of every culture media and biochemical test media used for the study purpose were checked by incubating 5% of prepared media at 37 °C for 24 hours (Teka et al, 2022)²¹. Moreover, ATS were controlled for the antimicrobials disk efficacy and determined for quality performance of zone of inhibition was within expected range or not with reference ATCC strains. Sterility of all the extracts was examined on nutrient agar. About 1ml of each extract was inoculated in nutrient agar plates and incubated at 37°C for 24 hours. Bacterial growth was observed to check the contamination and no growth in the plates was used as indicator that the extracts were sterile.

Data processing and analysis

Data was computed for descriptive statistics such as frequencies, percentages, mean and standard deviations were used to analyze and characterize the findings of the study.

RESULTS AND DISCUSSION

Drug resistance status of isolates

The 217 different samples were collected from urine (80, 37%), stool (53, 24.4%), ear discharge (21, 9.4%), pus/wound (17, 7.9%), nasopharyngeal swab (16, 7.1%), vaginal/cervical discharge (12, 5.5%), throat swab (8, 3.9%), urethral discharge (5, 2.4%) and sputum (5, 2.4%). About 27(12.4%) different clinically isolated bacteria obtained from the study laboratory, 13(48.1%) were gram-positive bacteria and the remaining 14 (51.9%) isolates were gram-negative bacteria (Figure No.2). The activity tests revealed that of the total 27 study isolates, 24(88.9%) were resistant to at least one antibacterial drug among three or more antibacterial

drug groups and said to be MDR developed isolates. Among gram-positive bacteria, 11(84.6%, n=13) were MDR developed, and 13 (92.8%, n=14) gram-negative bacteria were also found to be MDR bacteria. Additionally, 24 (88.9%) isolates were resisted for about four (4) and more antibiotic drug disks, of different class/groups, tested (Figure No.1).

Staphylococcus aureus (S. aureus)

Seven *Staphylococcus aureus* isolates were tested against 11 antibiotics. Of these, six (85.7%) were resistant to Cefoxitin, identifying them as methicillin-resistant *S. aureus* (MRSA), while one was methicillin-susceptible (MSSA). All isolates showed susceptibility to clindamycin but resistance to both ampicillin and penicillin. Additionally, six isolates (85.7%) were classified as multidrug-resistant (MDR) strains.

Escherichia coli (E. coli)

All the 7(100%) *Escherichia coli* isolates were showed resistant to Penicillin, Ampicillin, Amoxycillin-clavulanic acid and Tetracycline. Additionally, 6(85.7%) isolates of *Escherichia coli* were resistant to Trimethoprim-sulfamethoxazole and Azithromycin. From the 7 *Escherichia coli* isolates 6(85.7%) and 5(71.4%) isolates showed susceptibility for Gentamycin and Nitrofurantoin respectively. All (100%) of *Escherichia coli* isolates were developed MDR.

Enterococcus faecalis

Five *Enterococcus faecalis* isolates were tested against eight antibiotics. All isolates (100%) showed resistance to Ampicillin and Tetracycline. Additionally, resistance was observed in 4 isolates each for Ciprofloxacin, Erythromycin, and Gentamycin; 3 for Vancomycin; and 2 for Chloramphenicol. Nitrofurantoin was effective against three isolates, while Erythromycin and Gentamycin showed the least susceptibility, each effective against only one. Overall, 80% were multidrug-resistant (MDR) and 60% were vancomycin-resistant strains.

Klebsiella pneumonia

All four *Klebsiella pneumoniae* isolates were tested against 11 antibiotics. Each showed resistance to

Trimethoprim-sulfamethoxazole, Penicillin, Nitrofurantoin and Cefuroxime. Three isolates were also resistant to Ceftriaxone, Azithromycin, Ampicillin, Amoxicillin-clavulanic acid, and Tetracycline. Notably, one wound-derived isolate was resistant to all 11 antibiotics. Among the 27 total study isolates, only one *Klebsiella pneumoniae* strain showed susceptibility to Ampicillin. All were classified as multidrug-resistant (MDR).

Pseudomonas Aeruginosa

Both *Pseudomonas aeruginosa* isolates exhibited resistance to Trimethoprim-sulfamethoxazole, Penicillin, Ceftriaxone, Ampicillin, and Imipenem. However, they remained susceptible to Azithromycin and Gentamycin. Notably, both isolates were identified as multidrug-resistant (MDR) strains.

Proteus mirabilis

The *Proteus mirabilis* isolate was tested for antimicrobial susceptibility using eight antibiotics: Azithromycin, Ciprofloxacin, Nitrofurantoin, Trimethoprim-sulfamethoxazole, Ampicillin, Penicillin, Amoxicillin-clavulanic acid and Gentamycin. It showed susceptibility to Azithromycin, Ciprofloxacin, Amoxicillin-clavulanic acid, and Gentamycin, while exhibiting resistance to Nitrofurantoin, Ampicillin and Penicillin.

Streptococcus pneumonia

Streptococcus pneumoniae was tested for antimicrobial susceptibility against 13 antibiotics, including Ampicillin, Clindamycin, Ciprofloxacin, Trimethoprim-sulfamethoxazole, Oxacillin, Penicillin, Tetracycline, Erythromycin, Azithromycin, Ceftriaxone, Vancomycin, Rifampicin, and Chloramphenicol. It showed susceptibility to Clindamycin, Penicillin, Azithromycin, Vancomycin, and Rifampicin, while demonstrating resistance to the remaining eight antibiotics. Notably, it was the only isolate among the 27 tested that was susceptible to Penicillin.

Antimicrobial Activity of Fresh Garlic (*Allium sativum*) Juice on Isolated Bacteria

Fresh garlic juice demonstrated antibacterial activity against 26 out of 27 clinical isolates

(96.3%), with inhibition zones ranging from $9.7 \pm 0.6\text{mm}$ to $35.3 \pm 0.6\text{mm}$. The only resistant strain was *Pseudomonas aeruginosa*, showing no inhibition. Among Gram-positive bacteria, inhibition ranged from $9.7 \pm 0.6\text{mm}$ (*Enterococcus faecalis*) to $35.3 \pm 0.6\text{mm}$ (MRSA), while Gram-negative strains ranged from $10.7 \pm 0.6\text{mm}$ (*Pseudomonas aeruginosa*) to $33.7 \pm 0.6\text{mm}$ (*Escherichia coli*) (Table No.2).

Among 24 highly drug-resistant isolates (88.9% of 27), 23 (95.8%) were sensitive to fresh garlic juice. When heated in a water bath at 40°C , 60°C , 80°C and 100°C for 20 minutes, garlic's antimicrobial activity declined. For MRSA, the inhibition zone dropped from $35.0 \pm 1.0\text{mm}$ (fresh) to $32.67 \pm 0.58\text{mm}$, $31.67 \pm 0.58\text{mm}$, $29.33 \pm 0.58\text{mm}$ and $6.00 \pm 0.00\text{mm}$ respectively. *Enterococcus faecalis*, with the lowest fresh garlic inhibition ($9.7 \pm 0.6\text{mm}$), showed no inhibition at any temperature. Overall, 7(25.9%), 8(29.6%), 8(29.6%) and all 27(100%) isolates showed no inhibition at 40°C , 60°C , 80°C and 100°C respectively. All *Enterococcus faecalis* and *Pseudomonas aeruginosa* isolates were completely resistant after heating.

Fresh garlic juice stored at $2-8^\circ\text{C}$ for 72 hours exhibited nearly the same antibacterial activity as unrefrigerated juice, with only slight reductions in inhibition zones ($0-2.3\text{mm}$) for most isolates. For example, *Escherichia coli* dropped from $24.3 \pm 0.6\text{mm}$ to $22.00 \pm 1.00\text{mm}$, MRSA from $35.0 \pm 1.0\text{mm}$ to $33.00 \pm 1.00\text{mm}$, *Klebsiella pneumoniae* from $28.7 \pm 0.6\text{mm}$ to $27.00 \pm 1.00\text{mm}$ and *Proteus mirabilis* from $21.0 \pm 1.0\text{mm}$ to $19.67 \pm 0.58\text{mm}$. One MRSA isolate maintained a consistent zone of $29.0 \pm 1.0\text{mm}$.

Among the Gram-positive bacteria, MRSA exhibited the highest inhibition zone of $35.33 \pm 0.58\text{mm}$ with fresh garlic juice, which decreased to $14.33 \pm 0.58\text{mm}$, $11.67 \pm 0.58\text{mm}$, and $6.00 \pm 0.00\text{mm}$ at 75%, 50%, and 25% concentrations, respectively. *Enterococcus faecalis*, initially showing $9.7 \pm 0.6\text{mm}$, displayed no inhibition at any diluted concentration. Remarkably, MSSA showed a strong $33.33 \pm 0.58\text{mm}$ inhibition

with fresh garlic but no inhibition at all when diluted to 75%, 50%, or 25%.

Among the Gram-negative bacteria, *Escherichia coli* showed the highest inhibition zone of 33.7 ± 0.58 mm with fresh garlic juice, which decreased to 16.33 ± 0.58 mm, 14.67 ± 0.58 mm and 6.00 ± 0.00 mm at 75%, 50% and 25% concentrations, respectively. *Pseudomonas aeruginosa*, with an initial zone of 10.7 ± 0.58 mm, exhibited no inhibition at any diluted concentration. Overall, out of 27 isolates, 23(85.2%) showed no inhibition at 25% concentration, 15(55.5%) at 50% and 4(14.8%) at 75%. Detailed inhibition data are presented in Table No.3.

Aqueous extract was showed lower antibacterial effect than fresh garlic juice. The overall antibacterial activity of aqueous garlic extract for all study isolates is displayed below on Table No.4. The results of the antibacterial examinations by means of the disk diffusion method for ethanolic extract of garlic are given below in Table No.5

Antimicrobial Activity of Fresh Lemon (*Citrus lemon*) Juice on Isolates

Fresh lemon juice was shown antibacterial effect to all 27(100%) of isolates with the zone of inhibition ranging from 8.3 ± 0.58 mm to 23.7 ± 0.58 mm which was lesser than that of fresh garlic juice. The highest zone of inhibition revealed for gram positive bacteria with this fresh lemon juice was 23.7 ± 0.58 mm to *Enterococcus faecalis* and *Streptococcus pneumoniae*. The lower zone of inhibition revealed for gram positive bacteria with this fresh lemon juice was 8.3 ± 0.58 mm to MRSA isolate. In addition, the highest and lowest zone of inhibition revealed for gram negative bacteria were 20.7 ± 0.58 mm to *Pseudomonas aeruginosa* and *Escherichia coli* and 11.7 ± 0.58 mm to *Escherichia coli* isolates respectively. Additionally, this fresh juice was considered as 100% and then further diluted by sterile distilled water to provide concentrations of 75%, 50% and 25% fresh lemon juice. The results of the antibacterial examinations by means of the disk diffusion method for fresh lemon juice and its decreasing concentration are given below on Table No.6.

Lemon (*Citrus lemon*) Peel Ethanolic Extracts was shown no zone of inhibition for all the 27 isolates of gram positive and gram-negative bacteria. The zone inhibitions recorded below (Table No.7) as 6.0 ± 0.0 mm were the size of the diameter of Watman's No.1 filter paper disk that was prepared to saturate the extract and to test disk diffusion test on MHA.

Discussion

Natural antibacterial agents are being more cost-effective with minimum toxicity, side effects and AMR have extended the consideration of contemporary world. Researchers from different parts of the world have confirmed the antibacterial, antioxidant, antiviral, anticancer and antifungal properties of herbals (Liu *et al*, 2021)²⁰. So, studies so far have revealed that garlic have broad spectrum antibacterial effect against both gram positive and gram negative bacteria. Although few studies have been conduct on antibacterial activity of lemon, its effect against gram positive and negative bacteria have been discovered. For long periods of time, garlic has stayed as a seasoning agent with essential therapeutic features (Martins *et al*, 2016). Several writers (Anggraini *et al*, 2020²², Batiha *et al*, 2020²³, Bin *et al*, 2020²⁴, Deresse, 2011¹¹, Fufa, 2019⁸, Hailu *et al*, 2020²⁵) proposed the use of garlic because of its antibacterial usefulness against gram positive and gram negative bacteria, besides to its bioactive constituents that present others essential nutritional value.

Our study revealed that MDR developed isolates were 88.9% (n=27). This is comparable with (81.7%) of Sidama, Ethiopia (Alemayehu, 2021)²⁶, (72.2%) of Gondar, North Ethiopia (Assefa *et al*, 2022)²⁷, (73.4%) of Addis Ababa, Ethiopia (Woreta *et al*, 2022)²⁸, (76%) of Bahir dar, Ethiopia (Temesgen *et al*, 2019)²⁹ and (72.6%) of Egypt (El-Sokkary *et al*, 2018)³⁰. However, lower rate of MDR reported with the studies done at (60.65%) of Gondar, Ethiopia (Biset *et al*, 2020)³¹, (65.5%) of Addis Ababa, Ethiopia (Asres *et al*, 2017)³², (64.3%) of Saudi Arabia (Al Hamdan *et al*, 2022)³³ and (40.2%) of Czech Republic (Samal *et al*, 2022)³⁴. This relatively lower MDR rate than our

study may be due to the study method difference, the sampled community may use properly antimicrobial drug, proper prescription of antibacterial, minimum discontinuation of prescribed antibacterial and majorly may be due to our study site was regional reference laboratory and majority of samples were requested after frequent treatment failure of patients so that the rate of MDR rate could probably be higher in our case. Among 24(88.9%, n=27), 23(95.8%, n=24) were sensitive for fresh garlic juice and 100% of MDR developed isolates were effectively inhibited with lemon juice. The antibacterial effect of fresh garlic (*Allium sativum*) juice performed in our study showed that 100% effectiveness for gram positive and 92.8% for gram negative isolates which is similar with the studies done in Hawassa, Ethiopia (Deresse, 2011)¹¹, (Abiy and Berhe, 2016)³⁵, Nigeria (Okigbo et al, 2020)¹⁹, Indonesia (Anggraini et al, 2020)²², Bosnia (Strika et al, 2017)³⁶, Romania (Rotaru et al, 2020)³⁷ and Poland (Magrys et al, 2021)¹⁶.

This study showed that fresh garlic juice has no (6.0±0.0mm) or least antibacterial effect against *P. aeruginosa* isolate uniquely which is in line with the studies of China (Li et al, 2015)³⁸ and Poland (Magrys et al, 2021)¹⁶. However, similar studies showed, in another way, sensitivity in (21.9mm) Romania (Rotaru et al, 2020)³⁷, China (Li et al, 2015)³⁸ and Saudi Arabia (Saad and Mona, 2013)³⁹. This may be due to the different species of garlic that could have higher antibacterial potential and it may be also the bacterial genetic difference and biological factors associated with environment.

In our study, fresh raw garlic juice revealed the highest antibacterial effect against MRSA isolates (ranging from 29.0±1.0 to 35.3±0.6mm). This is similar to the studies done in Nigeria (Abdulrahman et al, 2017)⁴⁰, China (Li et al, 2015)³⁸, Bosnia (Strika et al, 2017)³⁶.

In our study the antimicrobial effect of fresh garlic juice at room temperature and its part refrigerated at 4 °C for 72 hours showed the similar antibacterial effectiveness, however; the fresh garlic shows a little greater effectiveness. This finding lines with

similar studies done in Hawassa, Ethiopia (Deresse, 2011)¹¹, Nigeria (Ikoni et al, 2017)⁴¹.

In this study the heat treatment of garlic showed that sensitive for 85.7% of gram negative and 61.5% for gram positive isolates at 40°C, 85.7% for gram negative and 53.8% for gram positive isolates at 60°C and 85.7% for gram negative and 53.8% for gram positive at 80°C. However, when the temperature of the study becomes 100°C the result was 0% sensitive and 100% resistant that is no antibacterial effect seen among all (100%) study isolates. This finding lines with the studies done at Hawassa, Ethiopia (Deresse, 2011)¹¹, at Harar, Ethiopia (Rasheed and Thajuddin, 2011)⁴², Israel (Gorinstein et al, 2009) and (Locatelli et al, 2015). With increasing temperature the zone of inhibition decreases and eventual at 100°C for 20 minutes all its antibacterial effect stops.

This is may be due to the effect of temperature (heating garlic) can gradually affect the antibacterial active compound of the garlic. This could be also due to high instability nature of the allicin. Studies proved that heating has a damaging effect on valuable possessions of garlic and loses allicin potential. Probably the assertion that sulphoxides and thiosulfonates are thermally unstable and may decompose into several sulfur compounds at high temperature that corresponding with reports of (Ismail et al, 2020)⁴³. However, our study showed no agreement with study done at Oman (Sah et al, 2016)⁴⁴ that revealed antibacterial effect of garlic treated at 100°C for 30 minutes.

This difference may be due to the *Allium sativum* species differences that have somewhat strong bioactive compounds in nature, the more susceptible genetic variation of the bacterial strain and also could be tested after the log phase of bacterial growth time.

Our study revealed the concentration of garlic affects the antibacterial effect of garlic. In our case, the higher the concentration (100%) of garlic revealed the larger the zone of inhibition with 13(100%) of gram positive and 13(92.8%) of gram negative study isolates. Also, the lower the concentration fresh garlic juice (25%) revealed

antibacterial effectiveness with 4(14.8%, n=27) gram negative isolates and have no antibacterial effect with 13(100%) gram positive and 10(71.4%) gram negative isolates (Table No.5). This is similar with the study conducted at Hawassa, Ethiopia (Abiy and Berhe, 2016)³⁵ and Injibara, Ethiopia (Fufa, 2019)⁸. This prosperity of garlic may help to understand that garlic (*Allium sativum*) has both bacteriostatic and bactericidal potential. However, some studies done Iran (Houshmand et al, 2013)⁴⁵ showed similar sensitivity for different concentration. This could be happened due to the genetic variation of bacteria at different environment and some methodical difference.

In our study 13(100%) gram positive isolates were sensitive to aqueous extracted garlic. However, 13(92.8%) gram negative were sensitive to aqueous extracted garlic. This is similar with the studies conducted in Ambo, Ethiopia (Bekele and Gebisa, 2022)⁴⁶, Abia State, Nigeria (Olugbue et al, 2017)⁴⁷, Aden, Yemen (Ismail et al, 2020)⁴³ and Maharashtra, India (Vasant et al, 2020)⁴⁸. As our study, the concentrated crude and dried (for above 72 hours) garlic ethanolic extract was shown no antibacterial effect on 100% gram positive and negative isolates. This study is similar with studies conducted at Chain (MRSA, *Escherichia coli*, *Pseudomonas Aeruginosa*, *Klebsiella pneumonia*) (Liu et al, 2021)²⁰ and Nigeria (Chinedu, 2019)⁴⁹. This is may be the bioactive compound in the garlic called allicin and other phytochemical that helpful as antibacterial were decomposed or lost its antibacterial potential. Since the difference in solubility and reactivity of bioactive compounds be affected with the extraction solvent. However, the ethanolic extract of garlic tested immediately within 72 hours of extraction was shown antibacterial effect on 100% of gram positive and negative isolates that lines with studies at Tamil Nadu, India (Karuppiah and Rajaram, 2012) and China (Bin et al, 2020)²⁴. The antibacterial effect of this extract was lesser than that of fresh garlic juice.

In our study, juice extract of lemon was found to be more effective against the 100% gram-positive and negative bacteria. This is similar with the studies

done at Nepal (Shakya et al, 2019)¹³, Nigeria. (Okigbo et al, 2020)¹⁹, Nigeria (Okeke et al, 2015)⁵⁰ and Bangladesh (Liya and Siddique, 2018)⁵¹. This effect of lemon juice may be primarily due to the essential oils (EO) known to penetrate the cell membrane and mitochondrial membranes, thereby increasing permeability of the organelles, which lead to ion leakage, growth inhibition and death of bacteria.

The likely mechanisms wherein EOs interferes with bacterial proliferation may include the following:

The disintegration of the bacterial outer membrane or phospholipid bilayer,

Alteration of the fatty acid composition,

Increase in membrane fluidity resulting in leakage of potassium ions and protons;

Interference with glucose uptake, and

Inhibition of enzyme activity or cell lysis (Angane et al, 2022)⁵².

Our study revealed the decreasing of fresh lemon juice concentrations (100% (v/v) to 25% (v/v)) directly proportional to the zone of inhibition. This is similar with study done at Indonesia (Ekawati and Darmanto, 2019)⁵³, Egypt (Eldin et al, 2020)⁵⁴ and Indonesia (Hendersona et al, 2018)⁵⁵. From overall 27 different isolates 21(77.8%), 10(37%) and 4(14.8%) isolates showed no zone of inhibition with 25%, 50% and 75% of fresh lemon juice respectively. From the total, 6 (22.2%, n=27) isolates were showed zone of inhibition (sensitivity) for all 75%, 50% and 25% diluted of fresh lemon juice (Table No.8) even though the zone of inhibition varies.

In our study the effect of lemon peel ethanolic extract has shown no zone of inhibition or resistant against all 27(100% of both gram positive and negative) of isolates. However, this finding showed no agreements with studies that revealed antibacterial effect of lemon peel ethanolic extract, Wollo, Ethiopia (Wolela, 2020)⁵⁶, Iraq (Hindi and Chabuck, 2013)⁵⁷, Indonesia (Hendersona et al, 2018)⁵⁵, India (Gupta et al, 2017)⁵⁸. This difference could possibly be due to our method of extraction that allowed concentrated crude of lemon peel, to dry to powder form at room temperature for above

72 hours. Thus this stay could also decompose the essential bioactive compounds in lemon peel. Another possible reason for our study’s resistance of isolates towards lemon peel could be the way of lemon fruits presented at direct sun light at market place. And the chain of supply until purchasing could also affect and/or decompose antibacterial active compounds in the peel.

Pandey *et al*, (2011) stated that the peel of *Citrus limon* comprises of flavonoides, tannins, reducing sugars but lacks saponinis and phlobatannins. The lemon juice extract composed of all these phytochemicals therefore the lemon juice extract revealed supreme zone of inhibition than lemon peel (Shakya *et al*, 2019)¹³. This idea is similar with our study finding that the phytochemical compounds presented (Table No.3) in limon juice and peel were almost similar.

Qualitative Phytochemical Garlic (*Allium sativum*) and Lemon (*Citrus lemon*)
Table No.1: Qualitative test results for secondary metabolite of garlic and lemon extracts

S.No	Secondary Metabolites Tested	Raw Garlic	Aqueous Extract of Garlic	Garlic at 100°C	Ethanollic Extract of Garlic	Lemon Juice	Lemon peel Ethanolic Extract
1	Phenol	+	+	-	-	+	+
2	Flavonoids	+	+	+	-	+	+
3	Glycoside	+	+	+	+	+	+
4	Antra Quinones	+	+	+	+	-	-
5	Terpenoids	+	+	+	+	-	+
6	Steroids	-	-	-	-	+	+
7	Saponinis	+	+	-	+	+	-
8	Tannis	-	-	-	-	+	+
9	Sulphur/allicin	+	+	-	+	NA	NA

Key: - +=Present, - = absent, NA= not applicable

Table No.2: Antimicrobial effect of fresh garlic (*Allium sativum*) juice on isolates bacteria and effect of temperature on garlic juice bioactivity

S.No	Name of Isolated Organism	Fresh Raw Garlic Juice Zone of Inhibition (mm)	Garlic at 40°C for 20 min Zone of Inhibition	Garlic at 60°C for 20 min Zone of Inhibition	Garlic at 80°C for 20 min Zone of Inhibition millimeter	Garlic at 100°C for 20 min Zone of Inhibition millimeter	Garlic at 4°C for 72 hours Zone of Inhibition millimeter
1	<i>Pseudomonas aeruginosa</i>	10.7±0.6	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	10.33±0.58
2	<i>Pseudomonas aeruginosa</i>	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
3	<i>Escherichia coli</i>	33.7±0.6	29.67±0.58	26.00±0.00	24.00±0.00	6.00±0.00	32.67±0.58
4	<i>Escherichia coli</i>	11.0±1.0	9.67±0.58	9.33±0.58	8.67±0.58	6.00±0.00	10.67±0.58
5	<i>Escherichia coli</i>	24.3±0.6	21.33±0.58	19.67±0.58	17.00±1.00	6.00±0.00	22.00±1.00
6	<i>Escherichia coli</i>	24.0±0.0	22.67±0.58	20.00±0.00	17.67±0.58	6.00±0.00	23.33±0.58
7	<i>Escherichia coli</i>	25.7±0.6	23.67±0.58	21.67±0.58	19.67±0.58	6.00±0.00	24.33±0.58
8	<i>Escherichia coli</i>	22.3±0.6	20.33±0.58	18.67±0.58	15.67±0.58	6.00±0.00	21.67±0.58
9	<i>Escherichia coli</i>	23.0±0.0	20.67±0.58	18.00±1.00	14.67±0.58	6.00±0.00	22.67±0.58
10	<i>Klebsiella pneumonia</i>	16.7±1.5	16.00±1.00	14.67±0.58	11.67±0.58	6.00±0.00	15.00±1.00
11	<i>Klebsiella pneumonia</i>	13.7±0.6	11.67±0.58	11.00±0.00	10.00±0.00	6.00±0.00	13.67±0.58
12	<i>Klebsiella pneumonia</i>	28.7±0.6	25.67±0.58	22.67±0.58	19.67±0.58	6.00±0.00	27.00±1.00
13	<i>Klebsiella pneumonia</i>	15.0±1.0	13.33±0.58	11.67±0.58	9.67±0.58	6.00±0.00	15.00±1.00
14	<i>P. mirabilis</i>	21.0±1.0	16.33±0.58	15.33±0.58	14.00±0.00	6.00±0.00	19.67±0.58
15	<i>Staphylococcus aureus (MRSA)</i>	34.7±0.6	29.00±1.00	22.33±0.58	19.67±0.58	6.00±0.00	34.00±0.00
16	<i>Staphylococcus aureus (MRSA)</i>	20.7±1.2	10.67±0.58	6.00±0.00	6.00±0.00	6.00±0.00	20.33±0.58
17	<i>Staphylococcus aureus (MRSA)</i>	35.3±0.6	32.67±0.58	28.33±0.58	25.67±0.58	6.00±0.00	34.00±1.00
18	<i>Staphylococcus aureus (MRSA)</i>	35.0±1.0	32.67±0.58	31.67±0.58	29.33±0.58	6.00±0.00	34.33±0.58
19	<i>Staphylococcus aureus (MRSA)</i>	35.0±1.0	32.00±1.00	31.00±0.00	29.00±1.00	6.00±0.00	33.00±1.00
20	<i>Staphylococcus aureus (MSSA)</i>	33.3±0.6	27.67±0.58	23.33±0.58	19.67±0.58	6.00±0.00	32.33±0.58
21	<i>Staphylococcus aureus (MRSA)</i>	29.0±1.0	18.33±0.58	16.67±0.58	15.33±0.58	6.00±0.00	29.00±1.00
22	<i>Enterococcus faecalis</i>	15.3±0.6	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	15.00±0.00
23	<i>Enterococcus faecalis</i>	9.7±0.6	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	9.33±0.58
24	<i>Enterococcus faecalis</i>	17.0±1.0	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	16.33±0.58
25	<i>Enterococcus faecalis</i>	15.0±1.0	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	15.67±0.58
26	<i>Enterococcus faecalis</i>	14.3±0.6	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00	14.67±0.58
27	<i>Streptococcus pneumonia</i>	29.0±1.0	17.67±0.58	16.33±0.58	14.33±0.58	6.00±0.00	28.33±0.58

Table No.3: Effect of different concentration of fresh garlic juice on 27 clinically isolated bacteria

S.No	Name of Isolated Organism	Fresh Raw Garlic Zone of Inhibition millimeter	Garlic fresh 75% Zone of Inhibition millimeter	Garlic fresh 50% Zone of Inhibition millimeter	Garlic fresh 25% Zone of Inhibition millimeter
1	<i>Pseudomonas aeruginosa</i>	10.7±0.58	6.00±0.00	6.00±0.00	6.00±0.00
2	<i>Pseudomonas aeruginosa</i>	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
3	<i>Escherichia coli</i>	33.7±0.58	16.33±0.58	14.67±0.58	6.00±0.00
4	<i>Escherichia coli</i>	11.0±1.00	6.00±0.00	6.00±0.00	6.00±0.00
5	<i>Escherichia coli</i>	24.33±0.58	16.67±0.58	14.33±0.58	6.00±0.00
6	<i>Escherichia coli</i>	24.00±0.00	15.33±0.58	6.00±0.00	6.00±0.00
7	<i>Escherichia coli</i>	22.33±0.58	16.33±0.58	13.33±0.58	10.00±0.00
8	<i>Escherichia coli</i>	25.67±0.58	16.00±1.00	12.33±0.58	6.00±0.00
9	<i>Escherichia coli</i>	23.00±0.00	16.33±0.58	13.67±0.58	10.67±0.58
10	<i>K. pneumonia</i>	16.67±1.53	14.33±0.58	13.33±0.58	11.00±0.00
11	<i>K. pneumonia</i>	13.67±0.58	10.33±0.58	6.00±0.00	6.00±0.00
12	<i>K. pneumonia</i>	28.67±0.58	18.33±0.58	16.67±0.58	11.67±0.58
13	<i>K. pneumonia</i>	15.00±1.00	11.67±1.15	6.00±0.00	6.00±0.00
14	<i>P. mirabilis</i>	21.00±1.00	14.67±0.58	6.00±0.00	6.00±0.00
15	<i>Staphylococcus aureus (MRSA)</i>	34.67±0.58	14.33±0.58	6.00±0.00	6.00±0.00
16	<i>Staphylococcus aureus (MRSA)</i>	20.67±1.15	13.67±0.58	6.00±0.00	6.00±0.00
17	<i>Staphylococcus aureus (MRSA)</i>	35.33±0.58	14.33±0.58	11.67±0.58	6.00±0.00
18	<i>Staphylococcus aureus (MRSA)</i>	35.00±1.00	17.33±0.58	15.33±0.58	6.00±0.00
19	<i>Staphylococcus aureus (MRSA)</i>	35.00±1.00	16.67±0.58	14.33±0.58	6.00±0.00
20	<i>Staphylococcus aureus (MSSA)</i>	33.33±0.58	6.00±0.00	6.00±0.00	6.00±0.00
21	<i>Staphylococcus aureus(MRSA)</i>	29.00±1.00	18.33±0.58	6.00±0.00	6.00±0.00
22	<i>Enterococcus faecalis</i>	15.3±0.6	15.67±0.58	12.67±0.58	6.00±0.00
23	<i>Enterococcus faecalis</i>	9.7±0.6	6.00±0.00	6.00±0.00	6.00±0.00
24	<i>Enterococcus faecalis</i>	17.0±1.0	12.33±0.58	6.00±0.00	6.00±0.00
25	<i>Enterococcus faecalis</i>	15.0±1.0	10.33±0.58	6.00±0.00	6.00±0.00
26	<i>Enterococcus faecalis</i>	14.3±0.6	12.33±0.58	6.00±0.00	6.00±0.00
27	<i>Streptococcus pneumonia</i>	29.00±1.00	17.67±0.58	13.33±0.58	6.00±0.00

Table No.4: Antibacterial effect of aqueous extract of garlic on 27 clinically isolated bacteria

S.No	Name of Isolated Organism	Garlic aqueous extract
		Zone of Inhibition (mm)
1	<i>Pseudomonas aeruginosa</i>	11.7±0.58
2	<i>Pseudomonas aeruginosa</i>	6.00±0.00
3	<i>Escherichia coli</i>	19.7±0.58
4	<i>Escherichia coli</i>	11.7±0.58
5	<i>Escherichia coli</i>	19.7±0.58
6	<i>Escherichia coli</i>	17.3±0.58
7	<i>Escherichia coli</i>	18.7±0.58
8	<i>Escherichia coli</i>	19.3±0.58
9	<i>Escherichia coli</i>	19.7±0.58
10	<i>K. pneumonia</i>	18.0±1.00
11	<i>K. pneumonia</i>	12.0±0.00
12	<i>K. pneumonia</i>	20.0±1.00
13	<i>K. pneumonia</i>	14.7±1.53
14	<i>P. mirabilis</i>	19.3±0.58
15	<i>Staphylococcus aureus (MRSA)</i>	19.3±1.53
16	<i>Staphylococcus aureus (MRSA)</i>	18.7±1.53
17	<i>Staphylococcus aureus (MRSA)</i>	18.3±1.53
18	<i>Staphylococcus aureus (MRSA)</i>	19.7±0.58
19	<i>Staphylococcus aureus (MRSA)</i>	20.7±0.58
20	<i>Staphylococcus aureus (MSSA)</i>	13.3±1.53
21	<i>Staphylococcus aureus (MRSA)</i>	17.0±1.00
22	<i>Enterococcus faecalis</i>	20.3±0.58
23	<i>Enterococcus faecalis</i>	16.7±0.58
24	<i>Enterococcus faecalis</i>	16.0±1.00
25	<i>Enterococcus faecalis</i>	14.7±0.58
26	<i>Enterococcus faecalis</i>	19.7±1.53
27	<i>Streptococcus pneumonia</i>	21.0±1.00

Table No.5: Antibacterial effect ethanolic extract of garlic on 27 clinically isolated bacteria during 72 hours of extraction and above 72 hours of extraction

S.No	Name of Isolated Organism	Garlic Ethanol Extract (72 hours)	Garlic Ethanol Extract (>72 hrs) at room T°	Garlic Ethanol Extract AST (above 72 hrs) at room T°		
		Zone of Inhibition (mm)	Zone of Inhibition (mm)	Zone of Inhibition (mm)		
				500mg/ml	200mg/ml	100mg/ml
1	<i>Pseudomonas aeruginosa</i>	12.7±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
2	<i>Pseudomonas aeruginosa</i>	18.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
3	<i>Escherichia coli</i>	18.7±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
4	<i>Escherichia coli</i>	10.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
5	<i>Escherichia coli</i>	16.7±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
6	<i>Escherichia coli</i>	15.7±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
7	<i>Escherichia coli</i>	15.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
8	<i>Escherichia coli</i>	15.0±0.00	6.0±0.0	6±0.0	6±0.0	6±0.0
9	<i>Klebsiella pneumonia</i>	15.0±0.00	6.0±0.0	6±0.0	6±0.0	6±0.0
10	<i>Klebsiella pneumonia</i>	11.7±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
11	<i>Klebsiella pneumonia</i>	18.7±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
12	<i>Klebsiella pneumonia</i>	11.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
13	<i>P. mirabilis</i>	16.7±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
14	<i>Staphylococcus aureus (MRSA)</i>	16.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
15	<i>Staphylococcus aureus (MRSA)</i>	18.0±0.00	6.0±0.0	6±0.0	6±0.0	6±0.0
16	<i>Staphylococcus aureus (MRSA)</i>	18.7±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
17	<i>Staphylococcus aureus (MRSA)</i>	20.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
18	<i>Staphylococcus aureus (MRSA)</i>	18.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
19	<i>Staphylococcus aureus (MSSA)</i>	15.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
20	<i>Staphylococcus aureus (MRSA)</i>	16.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
21	<i>Enterococcus faecalis</i>	17.7±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
22	<i>Enterococcus faecalis</i>	13.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
23	<i>Enterococcus faecalis</i>	15.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
24	<i>Enterococcus faecalis</i>	12.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
25	<i>Enterococcus faecalis</i>	13.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0
26	<i>Streptococcus pneumonia</i>	20.3±0.58	6.0±0.0	6±0.0	6±0.0	6±0.0

Table No.6: Antimicrobial Activity of Fresh Lemon (*Citrus lemon*) Juice on clinically isolate with also at certain level of concentration

S.No	Isolates	Lemon Juice (100%) Zone of Inhibition (mm)	Lemon Juice (75%) Zone of Inhibition (mm)	Lemon Juice (50%) Zone of Inhibition (mm)	Lemon Juice (25%) Zone of Inhibition (mm)
1	<i>Pseudomonas aeruginosa</i>	20.7±0.58	13.3±0.58	6.0±0.00	6.0±0.00
2	<i>Pseudomonas aeruginosa</i>	19.3±0.58	17.7±0.58	12.7±0.58	10.0±0.00
3	<i>Escherichia coli</i>	17.7±0.58	15.7±0.58	11.3±0.58	6.0±0.00
4	<i>Escherichia coli</i>	11.7±0.58	6.0±0.00	6.0±0.00	6.0±0.00
5	<i>Escherichia coli</i>	15.7±0.58	6.0±0.00	6.0±0.00	6.0±0.00
6	<i>Escherichia coli</i>	17.7±0.58	6.0±0.00	6.0±0.00	6.0±0.00
7	<i>Escherichia coli</i>	20.7±0.58	16.7±0.58	13.7±0.58	10.0±0.00
8	<i>Escherichia coli</i>	17.7±0.58	16.7±0.58	12.3±0.58	6.0±0.00
9	<i>Escherichia coli</i>	19.3±0.58	17.7±0.58	16.0±0.00	10.7±0.58
10	<i>K. pneumonia</i>	18.3±0.58	14.3±0.58	11.3±0.58	6.0±0.00
11	<i>K. pneumonia</i>	15.7±0.58	14.3±0.58	12.3±0.58	6.0±0.00
12	<i>K. pneumonia</i>	18.3±0.58	16.0±0.00	14.3±0.58	6.0±0.00
13	<i>K. pneumonia</i>	12.0±0.00	6.0±0.00	6.0±0.00	6.0±0.00
14	<i>P. mirabilis</i>	16.3±0.58	15.3±0.58	11.7±0.58	6.0±0.00
15	<i>Staphylococcus aureus (MRSA)</i>	17.3±0.58	15.3±0.58	11.7±0.58	6.0±0.00
16	<i>Staphylococcus aureus (MRSA)</i>	16.0±0.00	14.7±1.15	11.0±0.00	6.0±0.00
17	<i>Staphylococcus aureus (MRSA)</i>	19.7±0.58	18.3±0.58	13.7±0.58	10.0±0.00
18	<i>Staphylococcus aureus (MRSA)</i>	8.3±0.58	7.7±0.58	6.0±0.00	6.0±0.00
19	<i>Staphylococcus aureus (MRSA)</i>	16.7±0.58	15.3±0.58	11.7±0.58	6.0±0.00
20	<i>Staphylococcus aureus (MSSA)</i>	19.3±0.58	17.3±0.58	11.3±0.58	6.0±0.00
21	<i>Staphylococcus aureus (MRSA)</i>	17.7±0.58	16.7±0.58	13.3±0.58	6.0±0.00
22	<i>Enterococcus faecalis</i>	19.0±1.00	18.7±0.58	15.7±0.58	12.0±0.00
23	<i>Enterococcus faecalis</i>	23.7±0.58	21.0±0.00	6.0±0.00	6.0±0.00
24	<i>Enterococcus faecalis</i>	20.3±0.58	18.3±0.58	6.0±0.00	6.0±0.00
25	<i>Enterococcus faecalis</i>	21.7±0.58	19.3±0.58	6.0±0.00	6.0±0.00
26	<i>Enterococcus faecalis</i>	21.7±0.58	20.7±0.58	6.0±0.00	6.0±0.00
27	<i>Streptococcus pneumonia</i>	23.7±0.58	22.0±0.00	16.3±0.58	12.3±0.58

Table No.7: Effect of lemon peel ethanolic extract on 27 clinically isolated bacteria

S.No	Name of Isolated Organism	Lemon Peel ethanol extract
		Zone of Inhibition millimeter
1	<i>Pseudomonas aeruginosa (n=2)</i>	6.0±0.0
2	<i>Escherichia coli(n=7)</i>	6.0±0.0
3	<i>Klebsiella pneumonia (n=4)</i>	6.0±0.0
4	<i>P. mirabilis (n=1)</i>	6.0±0.0
5	<i>Staphylococcus aureus (n=7)</i>	6.0±0.0
6	<i>Enterococcus faecalis (n=5)</i>	6.0±0.0
7	<i>Streptococcus pneumonia (n=1)</i>	6.0±0.0

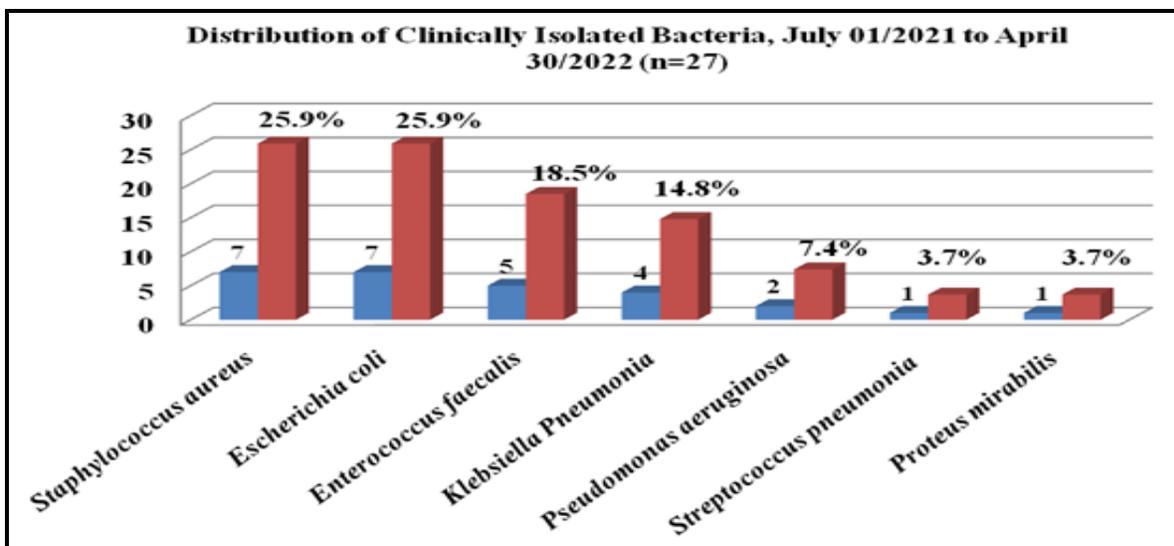


Figure No.1: The distribution of clinically isolated bacteria, July 01/2021 to April 30/2022 (n=27)

CONCLUSION

Garlic (*Allium sativum*) and Lemon (*Citrus limon*) can act as either bacteriostatic or bactericidal agent to inhibit and stop the growth of clinical isolated bacteria. The emergence of antimicrobial resistance can be combated with the use of garlic by having raw garlic on daily meals or by using garlic phytochemicals as a raw material for production of new antimicrobial agents. So, the result of our study proved that the antibacterial value of garlic and lemon juices is in line with the uses of these herbs in traditional medicine.

The authors also recommended that Specific bioactive compounds that particularly exerting antibacterial action should be fractionated (isolated) and characterized from these herbs, *Allium sativum* and *Citrus limon*, and need to be known for next action.

Comprehensive *in vivo* experimental studies need be performed on animal model and/or controlled clinical trials to demonstrate the levels of effectiveness, mechanism of action and side effects in order to discovery of novel antimicrobial agents.

ACKNOWLEDGEMENT

We acknowledge Medical Laboratory Science School, Hawassa University.

AUTHOR CONTRIBUTIONS

MD conducted and analyzed the laboratory research and statistical analysis and wrote the initial manuscript. LA and DD edited the manuscript. All authors have read and approved the final manuscript.

FUNDING

There is no funding to report.

CONFLICTS OF INTEREST

All authors declare that there is no conflict of interest regarding the publication of this paper.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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Please cite this article in press as: Legesse Adane et al. *In vitro* antibacterial activities of garlic (*Allium Sativum*) and lemon (*Citrus Limon*) extracts against clinically isolated bacteria species, *Asian Journal of Research in Pharmaceutical Sciences and Biotechnology*, 13(2), 2025, 24-46.