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# Antibacterial Activity and Identification of Bioactive Compounds in Extracts of Garlic cloves and Lemon juice by GC-MS Analysis

JUMARE, F. M[1*] YAKUBU, S. E [1] AMEH, J. B [2]
1*Department of Integrated Science, Federal University of Education, Zaria, Kaduna, Nigeria.
1, 2. Department of Microbiology, Ahmadu Bello University Zaria, Kaduna, Nigeria.
*Email of the corresponding author: fatimajumare@gmail.com

**Abstract**

The search for alternative regimens to treatment of infectious diseases as well as the increase in antibiotic resistance by pathogens has prompted continuous efforts towards discovery of new drugs. In this study, crude aqueous and acetone extracts of garlic cloves and lemon juice were obtained. The extracts were tested against clinical isolates of *Staphylococcus aureus*. The antibacterial activity was determined by agar well diffusion method on Mueller-Hinton Agar plates. All extracts showed inhibitory action against the bacterium with acetone extracts having higher activity than the aqueous extracts. The acetone extracts were further fractionated using solvent – solvent extraction and retested on the bacterium. The bioactive compounds that lead to the antibacterial activity of the fractions of the extracts were identified by Gas Chromatography-Mass Spectrometry analysis where 2,5-Furandione and dihydro-3-methylene were identified in garlic cloves fraction as main compounds with other 8 compounds in trace quantity while 11-Octadecenoic acid methyl ester, Oleic acid and n-Hexadecanoic acid were identified in lemon juice fraction as main compounds with 6 other compounds in trace quantity. These findings claim capacity and future use of these compounds for new drug development. The future work will be to determine toxicity, side effects and pharmaco-kinetic properties of the compounds.
**Keywords:** Antibacterial activity, Garlic cloves, Lemon juice, GC-MS analysis, bioactive compounds.

## 1. Introduction

Medicinal plants play a key role in human health care due to the development of adverse effects and microbial resistance to the chemically synthesized drugs [(17)]. Currently, about 80% of the world population relies on the use of traditional medicine and in many cases; the people claim the good benefit of certain herbal products [(1)]. According to the World Health Organization (WHO), nearly 20,000 medicinal plants exist in 91 countries including 12 mega biodiversity countries. The premier steps to utilize the biologically active compound from plant resources are extraction, pharmacological screening, isolation and characterization of bioactive compound, toxicological evaluation and clinical evaluation [(13)].

Garlic (*Allium sativum*) as a medicinal plant comes from the Celtic word for burning or smarting [(10)]. It has been investigated extensively for health benefits, resulting in more than 1000 publications over the last decade alone, and it is considered one of the best disease-preventive foods [(12)], based on its potent and varied effects [(15)]. While lemon (*Citrus limon*) is probably the best of all antiscorbutics, being almost a specific in scurvy [(11)(9)]. It has long been used as an astringent, diaphoretic, diuretic, gargle, lotion, and tonic. It has also been used externally for acne, fungus (ringworm and athlete's foot), sunburn, and warts [(5)].

Identification of particular bioactive compound of plants has become easier due to the development of modern analytical tools such as gas chromatography–mass spectrometry (GCMS). In this study we tested the antibacterial activity of crude aqueous and acetone extracts of garlic cloves and lemon juice against clinical isolates of *Staphylococcus aureus* and the active extracts were fractionated, retested and analysed by GC/MS machine for bioactive compounds.

## 2. Experimental

### 2.1. Plant material

Fresh garlic and lemon were purchased at Tudun-wada market, Zaria, Kaduna state. These were subsequently authenticated at the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University Zaria.

### 2.2. Extraction

The fresh garlic bulbs were peeled, weighed in to a beaker and cleaned. Cleaned cloves were surface-sterilized with 70% sodium hypochlorite for 2 minutes, rinsed twice with sterile distilled water and crushed using sterile mortar and pestle. Aqueous and acetone extracts of the garlic were prepared by soaking resultant garlic paste in the solvents contained in sterile Erlenmeyer flasks. The flasks were covered with cotton wool plug and then wrapped with aluminium foil. Homogenization of the mixtures and saturation of the solvents were achieved by shaking mechanically for 24hrs on a shaker (lab-line orbit, Melrose Park, ILL) at 100rpm. The mixtures were filtered using muslin cloth and then Whatman no. 1 filter paper in to sterile Erlenmeyer flasks. The filtrates were

condensed in water bath and used as stock. The extracts were stored in the refrigerator at 4°C for subsequent use [4].

The fresh lemon balls were weighed, washed in running tap water in the laboratory, surface sterilized with 70% sodium hypochlorite for 2 minutes, rinsed twice with sterile distilled water and cut open with a sterile knife and the juice was asceptically squeezed into a sterile universal container and then filtered into another sterile Erlenmeyer flasks to remove the seeds and other tissues. Aqueous and acetone extract of the lemon was prepared by soaking resultant lemon juice in the solvents contained in sterile Erlenmeyer flasks. The flasks were covered with cotton wool plug and then wrapped with aluminium foil. Homogenization of the mixtures and saturation of the solvents was achieved by shaking mechanically for 24hrs on a shaker (lab-line orbit, Melrose Park, ILL) at 100rpm. The mixtures were filtered using Whatman no. 1 filter paper in to sterile Erlenmeyer flasks. The filtrates were condensed in water bath and used as stock. The extracts were stored in the refrigerator at 4°C for subsequent use [2].

### 2.3. Collection and Identification of Test organism

Clinical isolates of *S. aureus* were obtained from Hajiya Gambo Sawaba General Hospital, Kofan Gayan, Zaria City, Kaduna State and Saint Lukes Anglican Hospital Wusasa, Zaria, Kaduna State. Ethical Clearance was sought from the Scientific Ethical Committee (SEC), Kaduna State Ministry of Health as well as the hospitals. The isolates were identified on the basis of morphological, biochemical and physiological characteristics.

### 2.4. Preparation of Extract Concentration

This was carried out as described by Srinivasan *et al*., [18]. Stock solution of the plant extracts were prepared by adding 1g of each crude plant extract in 10ml of 10% dimethylsulphuroxide (DMSO) as reconstituting solvent to make 100mg/ml stock solution. From the stock solution, 50mg/ml, 25mg/ml, and 12.5mg/ml concentrations were prepared using Two-fold serial dilution method. These concentrations were labelled and kept in bijou bottles for subsequent use.

### 2.5. Preparation of Turbidity Standard

McFarland standards are used as a reference to adjust the turbidity of microbial suspension so that number of bacteria will be within a given range. Firstly, (1% w/v) $BaCl_2$ and (1% v/v) $H_2SO_4$ were prepared by dissolving 1g of $BaCl_2$ in 100ml of sterile distilled water and 1ml of concentrated $H_2SO_4$ in 99ml of sterile distilled water respectively to serve as stock solutions for the preparation of the McFarland standard. From the stock solutions, 0.5 McFarland scale was prepared by adding 9.95ml of (1% v/v) $H_2SO_4$ to 0.05ml of (1% w/v) $BaCl_2$ with constant stirring to maintain a suspension of (1% w/v) $BaSO_4$ whose density is equivalent to $1.5 \times 10^8$ CFU /ml or 150million/ml approximate cell density of bacteria. The barium sulphate suspension in 4- to 6-ml aliquots were transferred in to screw-cap tubes, tightly sealed, and stored in the dark at room temperature to prevent loss by evaporation. This was subsequently used for comparison with the turbidity of the bacterial inoculum [6].

### 2.6. Standardization of Bacterial Inoculum

For inoculum standardization, density of isolated cultures was adjusted equal to that of 0.5 McFarland standards ($1.5 \times 10^8$CFU/ml) by suspending some quantity of the bacterial culture in to 2ml of sterile physiological saline as suspending medium. The physiological saline was prepared by dissolving 8.5g of $NaCl_2$ in 1L of distilled water and sterilised. To aid comparison, the test organisms and standard were compared against a white background with contrasting black lines [6].

### 2.7. Susceptibility of S. aureus Isolates to plant Extracts

The antibacterial activity of crude aqueous and acetone extracts of garlic cloves and lemon juice against the test organism was evaluated by using agar well diffusion method of sensitivity test described by Srinivasan *et al*., [18]. Mueller Hinton agar plates were inoculated with 100$\mu$l of standardized inoculum of the bacterium (in triplicates) using a micropipette of 100$\mu$l size and spread uniformly with sterile swab sticks. Wells of 8 mm size were made with sterile cork borer into the agar plates containing the bacterial inoculum. Using the micropipette, 100$\mu$l volume of the various concentrations; 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml each of the extracts were poured into wells of inoculated plates. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extracts into the agar and then incubated for 18 to 24 hrs at $37^0$C. The diameter of inhibition zone (DIZ) was measured and expressed in millimetres. The mean values of the diameter of inhibition zones were calculated to the nearest whole number [18]. In order to check the activity of the extracts, the reconstituting solvent (DMSO) was used as negative control. Commercially available standard antibiotics; Erythromycin (10µg), Ciprofloxacin (10g), Ampicillin (30g) and Gentamycin (10g) were used as positive control parallel with the extracts. For these antibiotics, inhibition zones were interpreted in accordance with the CLSI (Clinical Laboratory Standards Institute) interpretation guideline [7].

### 2.8. Fractionation of Crude Plant Extracts

The active crude extracts of garlic cloves and lemon juice were fractionated in accordance with the procedures of Venskutonis *et al.*[19] The extraction solvents were; petroleum ether, ethyl acetate, n- butanol and water. The procedure was carried out in a separating funnel in which fractions obtained were evaporated to dryness on a water bath to remove the solvent. Detail of the procedure steps for the fractionation is represented in figure 1 as a schematic diagram.

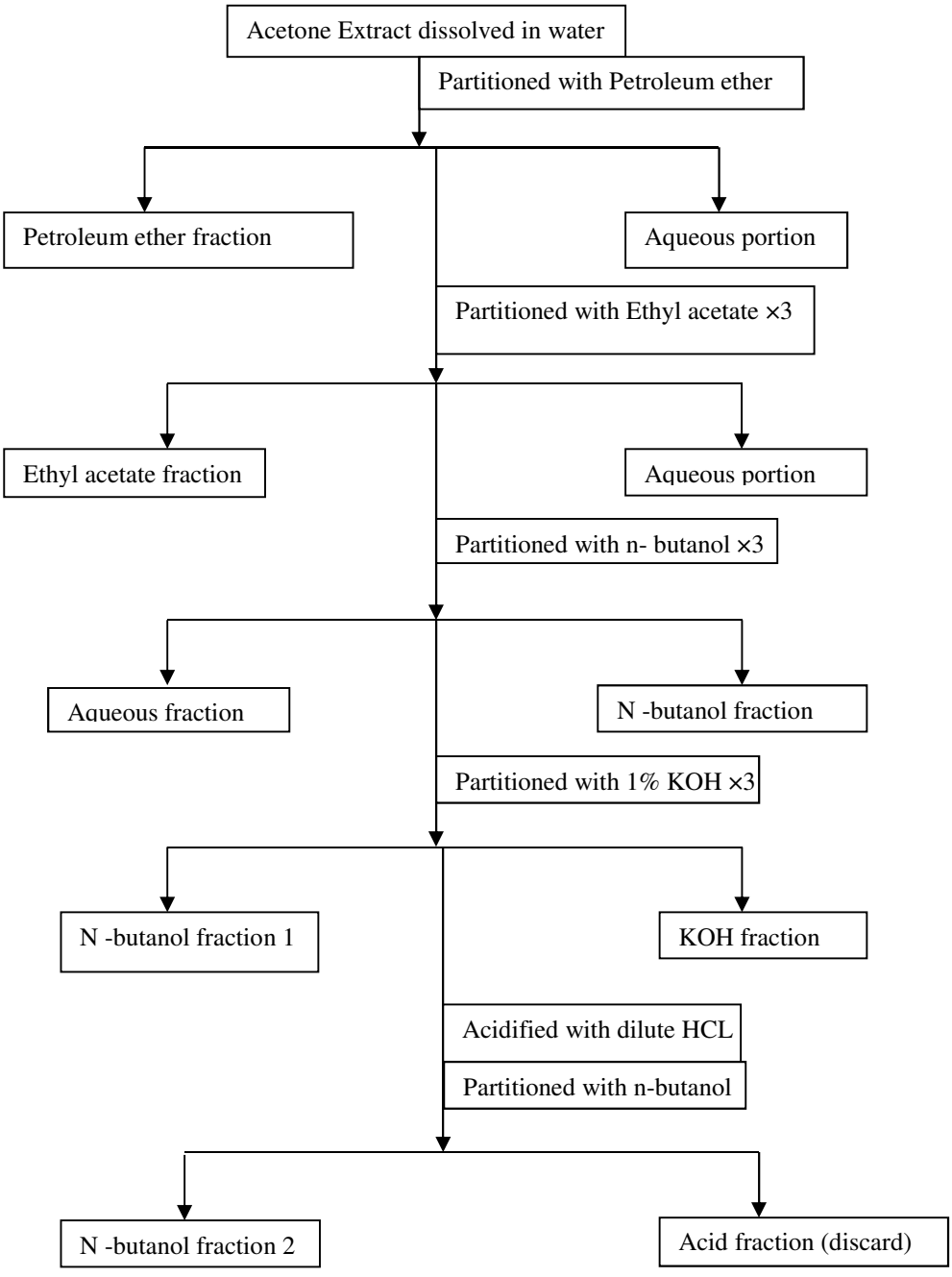



Figure 1: Schematic diagram of procedure steps for fractionation of crude plant extracts.

### 2.9. Antibacterial Activity Assay of Extracts Fractions

The antibacterial activity of each fraction of garlic cloves and lemon juice crude extracts against the test organism was evaluated by using agar well diffusion method of sensitivity test described by Srinivasan *et al*.,[18] . Mueller Hinton agar plates were inoculated with 100$\mu$l of standardized inoculum of each bacterium using a micropipette of 100$\mu$l size and spread uniformly with sterile swab sticks. Wells of 4 mm size were made with sterile cork borer into the agar plates containing the bacterial inoculum. Using the micropipette, 100$\mu$l volume of the various fractions; petroleum ether, ethylacetate, n-butanol 1, n-butanol 2 and aqueous fraction were poured into wells of inoculated plates. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extracts into the agar and then incubated for 18 to 24 hrs at $37^0$C. The diameter of inhibition zone (DIZ) was measured and expressed in millimetres [18].

### 2.10. Statistical Analysis

The data generated are presented in tables and charts and were analysed statistically using the S.P.S.S (Statistical Package for Social Sciences) package- SPSS 18. ANOVA was used to compare means of the plant extracts at different concentrations and the positive control antibiotics if there is any statistically significant difference in the diameter of zones of inhibition and P values < 0.05 is considered significant. Subsequently, these were further ranked by the Duncan's multiple range tests.

### 2.11. GC-MS Analysis

To identify the bioactive compounds in the extracts, it was further analysed by GC/MS machine after insertion into the GC-MS inlet port in a GC-MS vial. The results were printed out from the computer system connected to the GC/MS machine [14]. The GC-MS is composed of two major building blocks; the gas chromatograph and the mass spectrometer. GC analysis separates all of the components in a sample and provides a representative spectral output. The technician injects the sample into the injection port of the GC device. The GC instrument vaporizes the sample and then separates and analyzes the various components. Each component ideally produces a specific spectral peak that may be recorded on a paper chart or electronically. The time elapsed between injection and elution is called the "retention time." The retention time can help to differentiate between some compounds. The size of the peaks is proportional to the quantity of the corresponding substances in the specimen analyzed. The peak is measured from the baseline to the tip of the peak. MS identifies substances by electrically charging the specimen molecules, accelerating them through a magnetic field, breaking the molecules into charged fragments and detecting the different charges. A spectral plot displays the mass of each fragment. A technician can use a compound's mass spectrum for qualitative identification [3].

## 3. Results

The plant materials, garlic and lemon used for this study were identified at the herbarium section, department of Biological Sciences ABU Zaria. Garlic was identified as *Allium sativum L* (voucher no. 990) belonging to the familiy Liliaceae while lemon was identified as *Citrus limon L* (voucher no. 2196) of the Rutaceae family.

The clinical isolates of *Staphylococcus aureus* obtained from Hajiya Gambo Sawaba General Hospital, Kofan Gayan, Zaria City, Kaduna State (HGSH) and Saint Lukes Anglican Hospital Wusasa, Zaria, Kaduna State (SLAH) are presented in Figure 2. This shows that a total of 51 isolates were obtained as presumptive isolates of *Staphylococcus aureus* among which 30 were from High Vaginal Swab samples (HVS) where 35.3% were from HGSH and 23.5% were from SLAH, 17 were from Wound Swab samples (WS) where 23.5% were from HGSH and 7.8% were from SLAH and 4 were sourced from Urine specimens where 3.9 % were from HGSH and 5.9% were from SLAH.

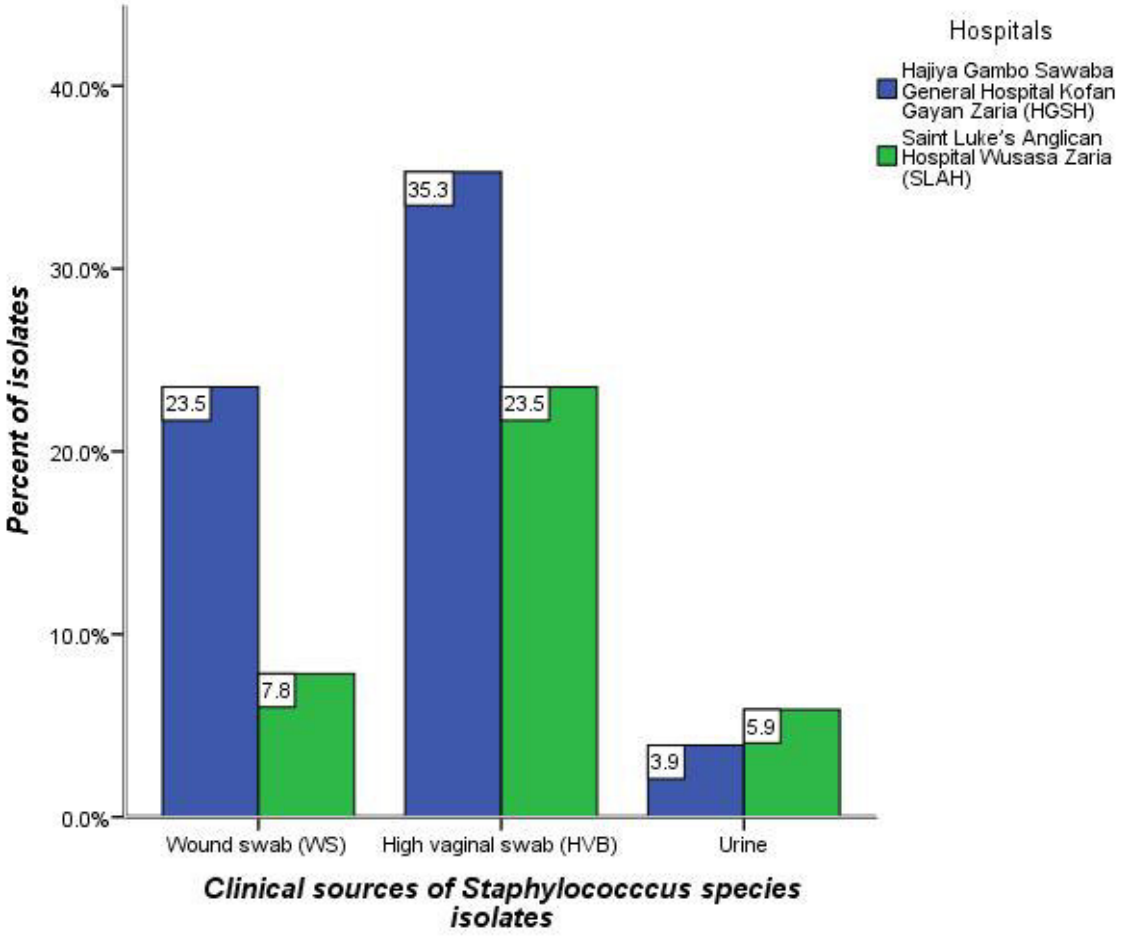



**Figure 2: Distribution of Clinical isolates of *Staphylococcus aureus* obtained from hospitals and their various sources**

The cultural and physiologic properties of clinical isolates of *Staphylococcus aureus* as presented in Table 1 and plates I shows that out of 51(100%) clinical isolates of *Staphylococcus aureus* obtained from the hospitals, 20 (39.2%) appeared as golden yellow, slightly raised colonies on mannitol salt agar, gram positive, cocci and clustered under the microscope, catalase positive, coagulase positive, ferment mannitol, positive for latex agglutination and thus, identified as *Staphylococcus aureus.* Table 2 shows the results of biochemical characterization and identification of clinical isolates of *S. aureus* using Microgen* *S. aureus* Identification Kit.

**Table 1: Cultural and Physiologic Properties of Clinical Isolates of *Staphylococcus aureus***

| Isolates | Colonial morphology | Gram reaction | Cell Morphology | Cell arrangement | Catalase | Coagulase | Mannitol | Latex agglutination | Identification |
|---|---|---|---|---|---|---|---|---|---|
| A8 | Golden yellow, slightly raised | Positive | Cocci | Clusters | + | + | + | + | *S. aureus* |
| A16 | Golden yellow, slightly raised | Positive | Cocci | Clusters | + | + | + | + | *S. aureus* |
| A25 | Golden yellow, slightly raised | Positive | Cocci | Clusters | + | + | + | + | *S. aureus* |
| A32 | Golden yellow, slightly raised | Positive | Cocci | Clusters | + | + | + | + | *S. aureus* |
| A49 | Golden yellow, slightly raised | Positive | Cocci | Clusters | + | + | + | + | *S. aureus* |
| A26 | Golden yellow, slightly raised | Positive | Cocci | Clusters | + | + | + | + | *S. aureus* |
| A30 | Golden yellow, slightly raised | Positive | Cocci | Clusters | + | + | + | + | *S. aureus* |
| A13 | Golden yellow, slightly raised | Positive | Cocci | Clusters | + | + | + | + | *S. aureus* |
| A3 | Golden yellow, slightly raised | Positive | Cocci | Clusters | + | + | + | + | *S. aureus* |
| A36 | Golden yellow, slightly raised | Positive | Cocci | Clusters | + | + | + | + | *S. aureus* |

**Key: A- isolate code, + = positive.**

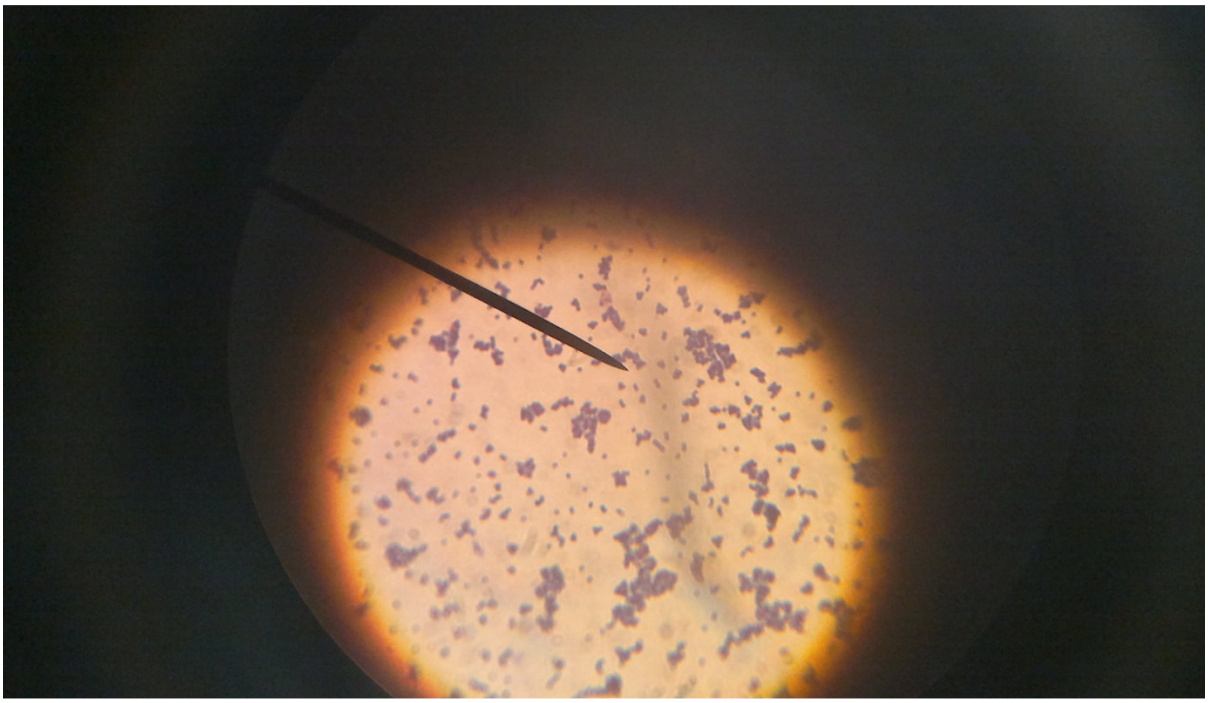


**Plate I: photomicrograph of *Staphylococcus aureus***

**Table 2: Biochemical Characterization and Identification of Clinical Isolates of *S. aureus* using Microgen* *S. aureus* Identification Kit**

| IS | L | C | N | S | T | M | N | M | T | P | B | B | U | A | PYR | % probability | Profile No | Final identification |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A8 | + | + | + | + | + | + | + | + | + | + | - | + | + | + | - | 96.55% | 77756 | *S. aureus subsp aureus* |
| A16 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | 100% | 77776 | *S. aureus subsp aureus* |
| A25 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | 100% | 77776 | *S. aureus subsp aureus* |
| A32 | + | + | + | + | + | + | + | + | + | + | + | - | + | - | - | 100% | 77764 | *S. aureus subsp aureus* |
| A36 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | 100% | 77776 | *S. aureus subsp aureus* |
| A49 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | 100% | 77776 | *S. aureus subsp aureus* |
| A26 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | 100% | 77776 | *S. aureus subsp aureus* |
| A30 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | 100% | 77776 | *S. aureus subsp aureus* |
| A13 | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | 98.57% | 77767 | *S. aureus subsp aureus* |
| A3 | + | + | + | + | + | + | + | + | - | + | + | + | + | + | - | 76.21% | 77676 | *S. aureus subsp aureus* |

**KEY**

IS=Isolate No.
L= Latex agglutination test
C=Colony pigmentation
N=Nitrate
S=Sucrose
T=Trehalose
M=Mannitol
PYR=Pyrrolidonyl arylamidase
M=Mannose
T=Turanose
P=Alkaline Phosphatase
β= β- Glucosidase
β= β- Glucuronidase
U=Urease
A=Arginine N=N-Acetyl Glucosamine

The susceptibility of *Staphylococcus aureus* to different concentrations of aqueous and acetone extracts of garlic cloves and lemon juice as well as the antibiotics where means with different superscripted alphabets along a column are significantly different at $P < 0.05$ is presented in Table 3, figure 3 and Plate II. The result indicates that the isolates of *S. aureus* exhibited varying degrees of resistance and susceptibility to different concentrations of aqueous and acetone extracts of garlic cloves and lemon juice as well as the antibiotics used as positive control in the susceptibility analysis (Ciprofloxacin (10µg), Erythromycin (10µg), Ampicillin(30µg) and Gentamicin(10µg)). Thus, Different concentrations of aqueous and acetone extracts of garlic cloves yielded low inhibitory activity against *S. aureus* isolates with mean inhibition zone diameter ranging from 0.0 ± 0.00 to 5.4 ± 1.34mm while different concentration of aqueous and acetone extracts of lemon juice yielded high inhibitory activity against *S. aureus* isolates with mean inhibition zone diameter ranging from 2.8 ± 1.07 to 21.9 ±

6.46mm which is higher than the inhibitory activity of some of the antibiotics used as positive control such as Ampicillin and Gentamicin that has mean inhibition zone diameter of 4.5 ± 1.06 and 5.8 ±1.15 respectively. However, the other two antibiotics Ciprofloxacin and Erythromycin yielded the highest inhibitory activity compared to the extracts and the aforementioned antibiotics with mean inhibition zone diameter of 32.7 ± 1.44 and 32.1 ± 0.86 respectively.

**Table 3: Susceptibility of *S. aureus* Clinical isolates to Different Concentrations of Aqueous and Acetone Extracts of Garlic cloves and Lemon juices**

| Extracts | Dosages (mg/ml) | Mean Inhibition Zone Diameters (mm) ± SEM |
|---|---|---|
| **AQG** | 100.0 | $0.0\pm0.00^{i}$ |
| **AQG** | 50.0 | $0.0\pm0.00^{i}$ |
| **AQG** | 25.0 | $4.9\pm1.28^{fg}$ |
| **AQG** | 12.5 | $4.5\pm1.04^{fg}$ |
| **AQL** | 100.0 | $20.4\pm0.36^{b}$ |
| **AQL** | 50.0 | $16.0\pm0.51^{cd}$ |
| **AQL** | 25.0 | $13.6\pm0.44^{d}$ |
| **AQL** | 12.5 | $2.8\pm1.07^{gh}$ |
| **ACG** | 100.0 | $0.0\pm0.0^{i}$ |
| **ACG** | 50.0 | $0.9\pm0.65^{hi}$ |
| **ACG** | 25.0 | $5.4\pm1.34^{fg}$ |
| **ACG** | 12.5 | $5.3\pm1.04^{fg}$ |
| **ACL** | 100.0 | $21.9\pm0.46^{b}$ |
| **ACL** | 50.0 | $17.5\pm0.46^{c}$ |
| **ACL** | 25.0 | $14.0\pm0.33^{d}$ |
| **ACL** | 12.5 | $9.9\pm1.13^{e}$ |
| **Ciprofloxacin** | 10µg | $32.7\pm1.44^{a}$ |
| **Erythromycin** | 10µg | $32.1\pm0.86^{a}$ |
| **Ampicillin** | 30µg | $4.5\pm1.06^{fg}$ |
| **Gentamycin** | 10µg | $5.8\pm1.15^{f}$ |

**Key: Means with the different superscripted alphabets along a column are significantly different at P < 0.05. AQG=Aqueous garlic extract, AQL=Aqueous lemon extract, ACG=Acetone garlic extract, ACL=Acetone lemon extract.**

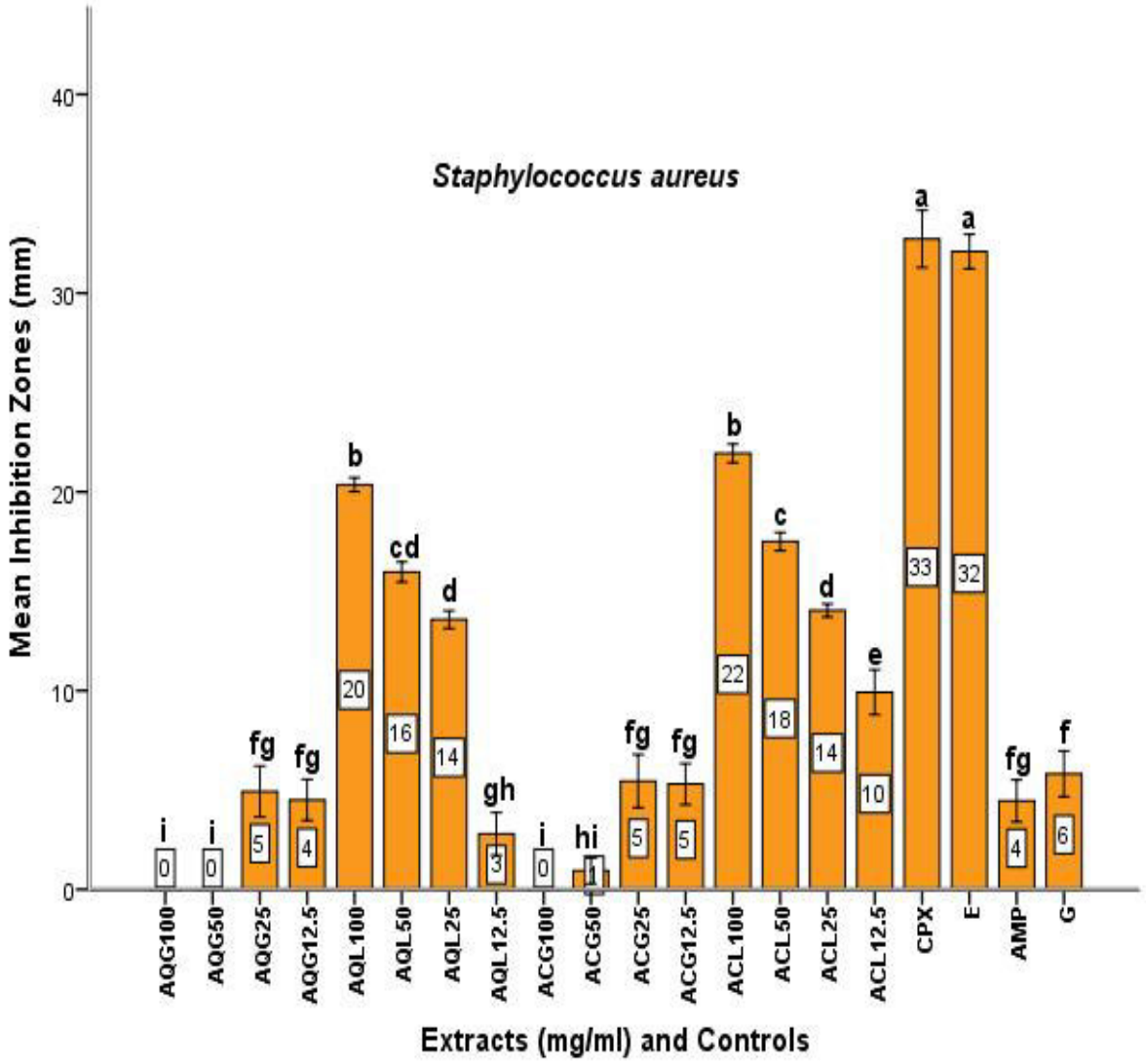



**Figure 3: Susceptibility of Clinical Isolates of *S. aureus* to Different Concentrations of Aqueous and Acetone Extracts of Garlic cloves and Lemon juice**

**Key: AQG=Aqueous garlic extract, AQL=Aqueous lemon extract, ACG=Acetone garlic extract, ACL=Acetone lemon extract, CPX= Ciprofloxacin, E= Erythromycin, AMP= Ampicillin, G= Gentamycin.**

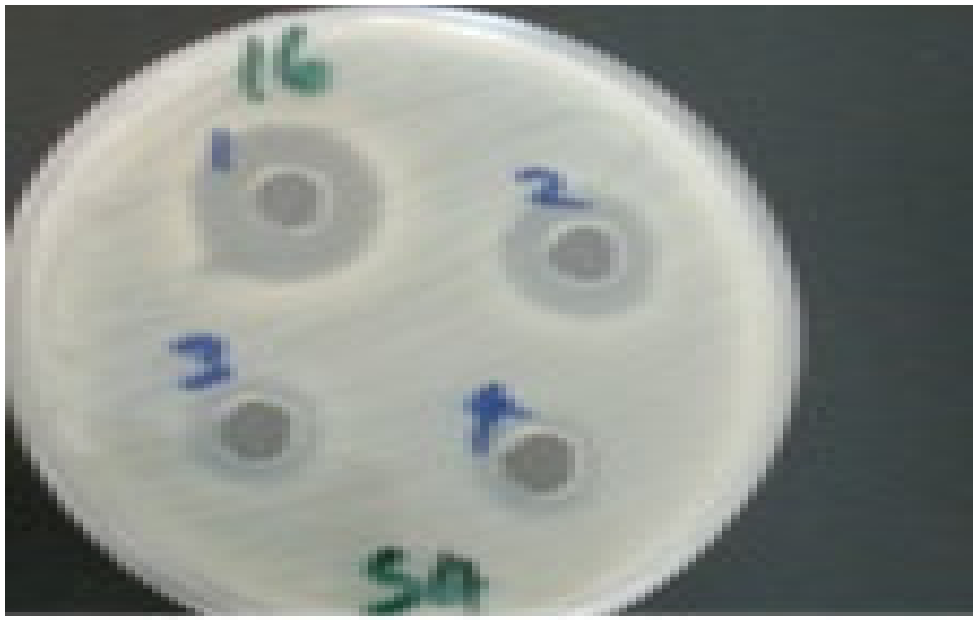



**Plate II: Antibacterial susceptibility plates of *S. aureus* and extracts 18-24hrs after incubation**

The result of antibacterial susceptibility test of various fractions of garlic cloves and lemon juice acetone extracts to *S. aureus* clinical isolate is presented in Table 4. The result shows that n-butanol 1 fraction of lemon juice had susceptibility on the isolate with mean inhibition zone diameter of 16.0 + 0.00mm. The n-Butanol 2 fraction of garlic cloves also had susceptibility on the isolate with mean inhibition zone diameter of 15.0± 0.00mm.

**Table 4: Antibacterial Susceptibility test of various fractions of Acetone Extracts of Garlic cloves and Lemon juice on *S. aureus* Clinical Isolates**

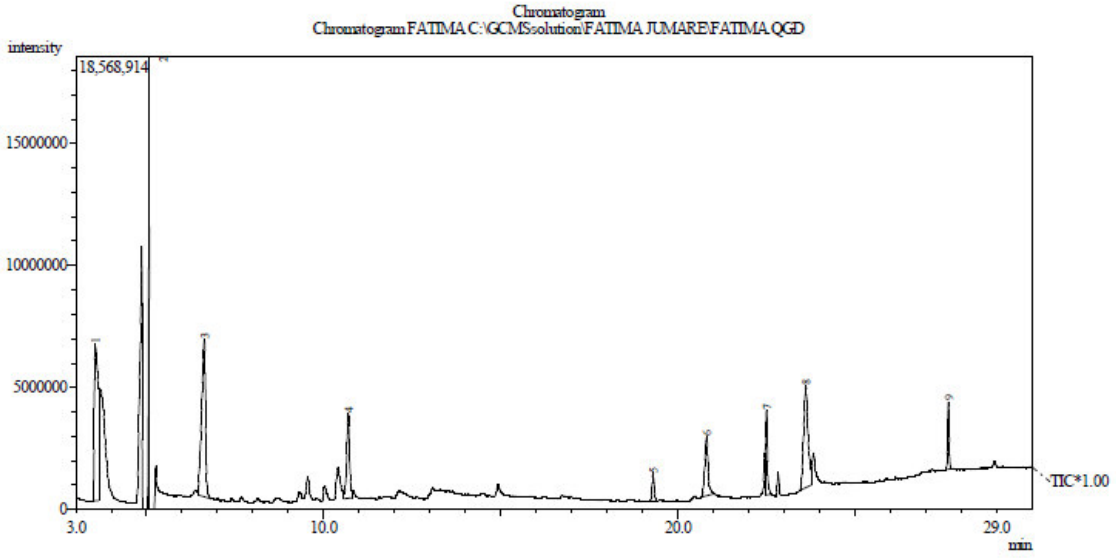
| Garlic and Lemon Fractions | Mean Inhibition Zone Diameter of *S.aureus* Isolate (mm) |
|---|---|
| **EL(1)** | NZ |
| **EG(1)** | NZ |
| **EL(2)** | NZ |
| **PG(2)** | NZ |
| **B1L(3)** | 16. 0±0.00 |
| **B1g(3)** | NZ |
| **B2L(4)** | NZ |
| **B2g(4)** | 15. 0±0.00 |
| **AL(5)** | NZ |
| **AG(5)** | NZ |

**Key: EL=Ethylacetate lemon juice fraction, EG=Ethylacetate garlic cloves fraction, PL=Petroleum ether lemon juice fraction, PG=Petroleum ether garlic cloves fraction, B1L=n-Butanol 1 lemon juice fraction, B1g= n-Butanol 1 garlic cloves fraction, B2L=n-Butanol 2 lemon juice fraction, B2g= n-Butanol 2 garlic cloves fraction, AL=Aqueous lemon juice fraction, AG=Aqueous garlic cloves fraction, NZ= no zone of inhibition.**

The result of Gas-Chromatography/Mass-Spectrometry (GC-MS) analysis of fractions of acetone extracts of garlic cloves and lemon juice is presented in Figure 4, Figure 5, Table 5 and Table 6. Figure 4 shows the GC-MS peak report nine (9) peaks were obtained in the n- Butanol 2 fraction of acetone extract of garlic clove and Table 5 shows the interpretation based on peak spectra of garlic clove fraction (n-Butanol 2) where each peak has a probable chemical compound name, its formula and structure. Figure 5 shows the GC-MS peak report of lemon juice fraction (Ethylacetate) where nine (9) peaks were also obtained in the Ethylacetate fraction of acetone

extract of lemon juice and Table 6 shows the interpretation based on peak spectra of lemon juice fraction where each peak has a probable chemical compound name, its formula and structure.



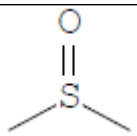
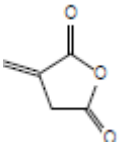
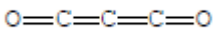
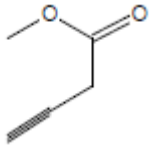
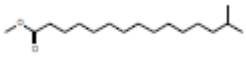
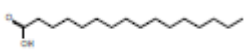
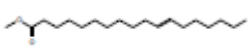
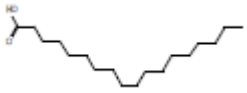
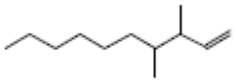

Peak Report TIC

| Peak# | R.Time | I.Time | F.Time | Area | Area% | Height | Height% |
|---|---|---|---|---|---|---|---|
| 1 | 3.562 | 3.492 | 3.658 | 46844566 | 22.10 | 6437514 | 13.15 |
| 2 | 5.066 | 5.033 | 5.083 | 19410457 | 9.16 | 18568914 | 37.93 |
| 3 | 6.635 | 6.450 | 6.825 | 50011845 | 23.59 | 6472985 | 13.22 |
| 4 | 10.700 | 10.575 | 10.817 | 18946474 | 8.94 | 3495867 | 7.14 |
| 5 | 19.308 | 19.225 | 19.408 | 4791079 | 2.26 | 1162340 | 2.37 |
| 6 | 20.821 | 20.675 | 21.025 | 16124814 | 7.61 | 2446057 | 5.00 |
| 7 | 22.512 | 22.475 | 22.642 | 11770291 | 5.55 | 3470177 | 7.09 |
| 8 | 23.621 | 23.450 | 23.775 | 36099104 | 17.03 | 4150917 | 8.48 |
| 9 | 27.648 | 27.583 | 27.725 | 7998126 | 3.77 | 2749703 | 5.62 |
| | | | | 211996756 | 100.00 | 48954474 | 100.00 |

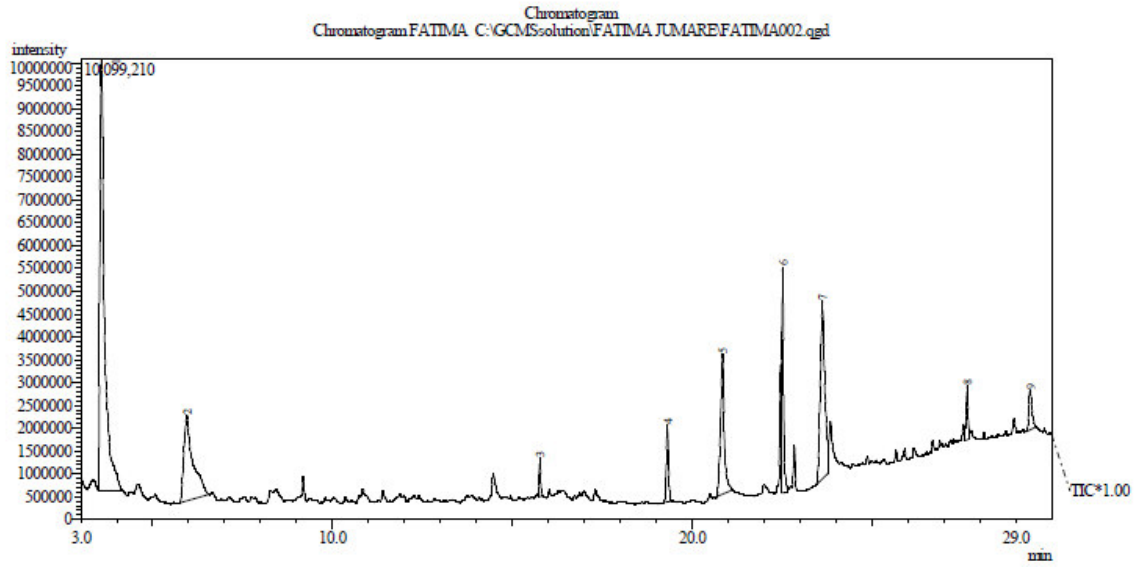
**Figure 4: Gas-Chromatography (GC) Analysis of Fraction of Acetone Extract of Garlic Cloves**

**Table 5: Mass-Spectrometry (MS) of Probable Chemical Compounds in fraction of Acetone Extract of Garlic Cloves**

| Peak | Compound Name | Compound Formula | Compound Structure |
|---|---|---|---|
| 1 | Dimethyl Sulfoxide | $C_2H_6OS$ |  |
| 2 | 2,5-Furandione,dihydro-3-methylene- | $C_5H_4O_3$ |  |
| 3 | 1,2-Propadiene-1,3-dione | $C_3O_2$ |  |
| 4 | Methyl 3-butynoate | $C_5H_6O_2$ |  |
| 5 | Pentadecanoicacid,14-methyl-, methyl ester | $C_{17}H_{34}O_2$ |  |
| 6 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ |  |
| 7 | 11-Octadecenoic acid, methyl ester | $C_{19}H_{36}O_2$ |  |
| 8 | Oleic Acid | $C_{18}H_{34}O_2$ |  |
| 9 | 1-Decene, 3,4-dimethyl- | $C_{12}H_{24}$ |  |



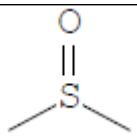
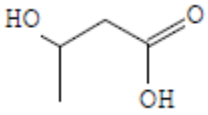
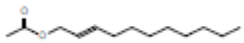
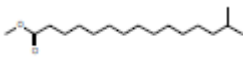
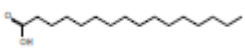
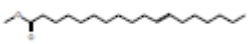
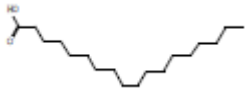
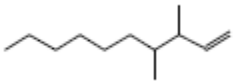
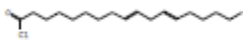

Peak Report TIC

| Peak# | R.Time | I.Time | F.Time | Area | Area% | Height | Height% |
|---|---|---|---|---|---|---|---|
| 1 | 3.583 | 3.500 | 4.167 | 85245837 | 40.07 | 9466556 | 33.98 |
| 2 | 5.955 | 5.758 | 6.558 | 32986110 | 15.51 | 1880263 | 6.75 |
| 3 | 15.770 | 15.725 | 15.858 | 2499355 | 1.17 | 847563 | 3.04 |
| 4 | 19.316 | 19.225 | 19.442 | 7038012 | 3.31 | 1692525 | 6.08 |
| 5 | 20.854 | 20.558 | 21.133 | 22853198 | 10.74 | 3072720 | 11.03 |
| 6 | 22.522 | 22.483 | 22.675 | 17761670 | 8.35 | 4949229 | 17.77 |
| 7 | 23.628 | 23.458 | 23.800 | 34782214 | 16.35 | 3900098 | 14.00 |
| 8 | 27.651 | 27.600 | 27.725 | 4200927 | 1.97 | 1175422 | 4.22 |
| 9 | 29.403 | 29.308 | 29.567 | 5373344 | 2.53 | 871745 | 3.13 |
| | | | | 212740667 | 100.00 | 27856121 | 100.00 |

**Figure 5: Gas-Chromatography (GC) Analysis of Fraction of Acetone Extract of Lemon Juice**

**Table 6: Mass-Spectrometry (MS) of Probable Chemical Compounds in Fraction of Acetone Extract of Lemon juice**

| Peak | Compound Name | Compound Formula | Compound Structure |
|---|---|---|---|
| 1 | **Dimethyl Sulfoxide** | $C_2H_6OS$ |  |
| 2 | **Butanoic acid, 3-hydroxy-** | $C_4H_8O_3$ |  |
| 3 | **Acetic acid, undec-2-enyl ester** | $C_{13}H_{24}O_2$ |  |
| 4 | **Pentadecanoic acid, 14-methyl-, methyl ester** | $C_{17}H_{34}O_2$ |  |
| 5 | **n-Hexadecanoic acid** | $C_{16}H_{32}O_2$ |  |
| 6 | **11-Octadecenoic acid, methyl ester** | $C_{19}H_{36}O_2$ |  |
| 7 | **Oleic Acid** | $C_{18}H_{34}O_2$ |  |
| 8 | **1-Decene, 3,4-dimethyl-** | $C_{12}H_{24}$ |  |
| 9 | **9,12-Octadecadienoyl chloride** | $C_{18}H_{31}C_{lO}$ |  |

## 4. Discussion

The results of the current study reveals the antibacterial activity and bioactive compounds in aqueous and acetone extracts of garlic cloves and lemon juice based on in vitro evaluation of antibacterial activity and identification of bioactive compounds in aqueous and acetone extracts of garlic cloves (*Allium sativum*) and lemon juice (*Citrus limon*) on clinical isolates of *Staphylococcus aureus.* The aqueous and acetone extracts of lemon juice inhibited the *S. aureus* isolates with mean inhibiting zone diameter ranging from 2.8 ± 1.07 to 21.9 ± 6.46mm.This indicates the antibacterial effects of lemon juice as previously studied by Al-ani *et al.* [(2)] on the evaluation of antibacterial activity of citrus fruit juices against *Staphylococcus aureus*, *Proteus vulgaris* and *Pseudomonas aeruginosa* and concluded that the use of different concentrations of Citrus juice extracts had an effective antibacterial activity against the tested organisms.

Similarly, acetone extract of garlic cloves also had antibacterial activity on the isolates with mean inhibition zone diameter ranging from 0.0 ± 0.00 to 5.4 ± 1.34mm which gives support to previous findings of Ankri and Mirelman [(4)] on the report that garlic cloves when crushed yields *allicin*, a thiosulfinate compound that interferes with RNA synthesis. If RNA cannot be produced or produced in fewer amounts then protein synthesis will be greatly affected. It would be stopped at every stage due to the absence of messenger RNA, ribosomal RNA and transfer RNA, if amino acids and proteins cannot be produced then growth and development of the organism will not occur as they are essential for all parts of cell structure.

Gas-Chromatography/Mass-Spectrometry (GC-MS) analysis showed the presence of 2, 5-Furandione, dihydro-3-methylene- as main compound with other 8 compounds in trace quantity (Figure 4 and Table 5) in the fraction of acetone extract of garlic cloves. Similarly, the result for lemon juice fraction showed the presence of 11-Octadecenoic acid methyl ester, Oleic Acid and n-Hexadecanoic acid as main compounds with other 6 compounds in trace quantity (Figure 5 and Table 6). From the results, it can be interpreted that the identified compounds were responsible for the antibacterial activity of garlic cloves and lemon juice on the isolates. The present findings on garlic cloves support previous study by Karwan [8] on antimicrobial activity and GC/MS analysis of different garlic crude extracts including acetone extract where the GC-MS analysis identified the compound, {2-Furancarboxaldehyde, 5- (hydroxymethyl)} as the major compound in all the extracts which is similar 2, 5-Furandione, dihydro-3-methylene- identified in a fraction of the crude extract in this study. Similarly, the study by Naseer *et al.* [13] on evaluation of antibacterial activity of five selected fruits including lemon juice on bacterial wound isolates has determined that the antibacterial action of lemon juice is linked with the presence of complex organic acids (succinic, citric, tartaric, etc.).

The compounds identified in the lemon juice fraction as main compounds, 11-Octadecenoic acid methyl ester, Oleic Acid and n-Hexadecanoic acid are also complex organic acids although not the same as that of the crude extract. Thus, all these findings implies that similar but not the same compounds from crude plant extract are identified in the fractions given that the number of peaks produced in the fractions have been reduced to the minimum far less than the number of peaks produced in the crude extract [3][14][16].

## 5. Conclusion

Conclusively, the results obtained in this study have consistently demonstrated the efficacy of aqueous and acetone extracts of garlic cloves and lemon juice on *S. aureus* pathogenic bacteria of clinical origin. The current study has also revealed probable chemical compounds which are responsible for these antibacterial activities contained in fractions of the plant extracts by GC/MS analysis. Thus, these plants are potential sources for new antibacterial drugs.

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