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Determination of Bioactive Compounds and Antimicrobial Capabilities of Purified *Allium sativum* Extract to Multidrug Resistant Enteric Bacteria

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Authors' contributions

This work was carried out in collaboration between both authors. Author OJA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OJA and STA managed the analyses of the study. Author STA managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

There is much concern on spread of pathogenic enteric bacteria and there resistance to commercially available antibiotics. There is a need for development of a new reliable drug for the treatment of diseases caused by these bacteria. This study determines the antimicrobial capabilities of purified *Allim sativum* extract to multidrug resistant enteric bacteria. *In-vitro* antibacterial properties of the extract was carried out using agar well diffusion technique against antibiotics resistant enteric bacteria. The results revealed that Seven fractions of partially purified water extract of *Allium sativum* (fraction 5-11) had significant zone of inhibition to the test enteric bacteria. The Fourier Transform Infrared Spectrophotometer (FTIR) spectra revealed the presence of 24 functional groups in functional groups in water extract of *A. sativum*. Also, Gas Chromatography-Mass Spectrophotometry revealed the presence of different compounds in water extract of *Allium sativum*.

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Keywords: Enteric bacteria; phytochemical; bioactive compound; antimicrobial; Allium sativum.

1. INTRODUCTION

Diseases caused by pathogenic enteric bacteria are major cause of morbidity and mortality [1,2]. It is estimated globally that approximately 1.8 million people die from diarrheal diseases annually, many of which have been linked to diseases acquired from the consumption of contaminated waters and seafood [3].

Gastrointestinal infections are the most common diseases caused by enteric bacteria. Some examples are salmonellosis (*Salmonella* sp.), cholera (*Vibrio cholerae*), dysentery (*Shigella* sp.) and other infections caused by *Campylobacter jejuni*, *Yersinia* sp. and *Escherichia coli* O157:H7 and many other strains. *E. coli* O157:H7 successfully causes infections because of its low infectious dose (ID), which can be as few as ten cells [4]. Antimicrobial resistance (AMR) has emerged as one of the principal public health challenges of the 21st century that threatens the effective prevention and treatment of -increasing range of infections caused by bacteria, parasites, viruses and fungi [5]. The problem of AMR is especially urgent regarding antibiotic resistance in bacteria. Over several decades, to varying degrees, bacteria causing common or severe infections have developed resistance to each new antibiotic. Faced with this reality, the need for action to avert a developing global crisis in health care is imperative. The World Health Organization (WHO) has long recognised the need for an improved and coordinated global effort to contain AMR. In 2001, the WHO Global Strategy for Containment of Antimicrobial Resistance has provided a framework of interventions to slow the emergence and reduce the spread of antimicrobial-resistant microorganisms [5].

In 2012, WHO published The Evolving Threat of Antimicrobial Resistance – Options for Action [6], proposing a combination of interventions that include strengthening health systems and surveillance; improving use of antimicrobials in hospitals and in community; infection prevention and control; encouraging the development of appropriate new drugs and vaccines; and political commitment [5].

Enteric bacteria responsible for common or severe Gastrointestinal infections that have developed resistance to antibiotics remain a major concern to public health.

Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources [7].

Garlic (*Allium sativum*) has been used therapeutically against asthma, candidiasis, colds, diabetes and has been reported to exhibit antibacterial effect against foodborne pathogens such as *Salmonella*, *Shigella* and *Staphylococcus aureus* [8]. There is, therefore, the need to determine the phytochemicals, bioactive compounds and antibacterial efficacy of *Allium sativum* against resistant enteric bacteria.

2. MATERIALS AND METHODOLOGIES

2.1 Materials Used in the Study

Fresh garlic (*Allium sativum*), conical flask, spatula, beakers, stirring rods, measuring cylinder, test tubes, test tube racks, hand gloves, cotton wool, human volunteers, paper tape, muslin clothes, ethanol, aluminum foil, Chloroform, syringe, micropipette, ice pack, nitrogen gas, ethanol, distilled water and n-hexane.

2.2 Source of Microorganism

Enteric bacteria used in this study were stock cultures from our previous work. They were isolates from Ogbese river water and stored at microbiology department microorganism bank of Federal university of Technology, Akure.

2.3 Collection of *Allium sativum*

Fresh garlic (*Allium sativum*) bulbs were purchased from Oba's market, Akure, Ondo State, Nigeria. The plant was identified and authenticated by experts at the Crop, Soil and Pest Department, Federal University of Technology, Akure, Nigeria. Garlic bulbs were separated into a sterile containers, washed with running clean tap water and dried at room temperature. The garlic bulb was milled to paste, and stored in an airtight container at 4^{o}C temperature in a refrigerator until when required.

2.4 Preparation of Extracts from *A. sativum*

A. sativum was extracted with water using the method described by [9]. *A. sativum* extracts were prepared by chopping the edible portion of garlic using pestle and mortar and put in sterile plastic containers. 200g of chopped garlic was homogenized in 1litre of sterile distilled water. The homogenate was kept in a covered sterile container for three days. Sterile muslin cloth was used to remove the large particles from the homogenate and then filtered using Whatman No. 1 filter paper. The Extract obtained was then concentrated in vacuum using rotary evaporator to remove the solvent [10].

2.5 Storage of Stock Concentration of *A. sativum* Extracts

The 100% stock concentration extracts *A. sativum* extract was obtained and stored at 4°C in a well corked universal bottle. It was reconstituted with DMSO to a required concentration at each use.

2.6 Partial Purification of Water Extract of *A. sativum*

Partial purification was carried out using column chromatography as described by [11]. Twenty grams of water extract of *A. sativum* was subjected to column chromatography on silica gel (100 – 200 mesh – Merck) packed and eluted with mixture of n-Hexane, chloroform, ethyl acetate, ethanol, methanol and water of increasing polarity to obtain fractions respectively.

2.7 Determination of the Retention Factor (Rf) of Water Extract of *A. sativum*

The retention factor of water extract of *A. Sativum* was determined by developing Thin Layer Chromatography; 30 g silica gel G (with $CaSO_4$ as binder) was placed in a beaker and shook vigorously with 60-65ml of distilled water for about 1min, transferred to the applicator and spread uniformly on the plate 20x20 cm. The thickness of the layer was 0.25 mm. Plates were allowed to dry for 5-10 min in dust free conditions. Gel was activated prior to use for 5 min at 110^{o}C in a hot oven. Gel was divided into a number of lanes by drawing lines with a lead. Different known volume (5, 10 µl) of the sample extracts were spotted in various lanes carefully with a ball pen or micro syringe on the line 2.5 cm away from each other. The plate was developed in a solvent system in a chromatographic tank for about 50 min and Rf value was calculated:

Rf Value =

Distance moved by the molecule ÷ Distance moved by the mobile phase

(Location by the spot) (Solvent Front)

2.8 Determination of the Chemical Properties and Functional Groups of Water Extract of *Allium sativum*

The chemical properties and functional groups of water extract of *A. sativum* was determined using Fourier Transform Infrared Spectroscopy analysis (FTIR) as described by [12]. A FT-IR spectrometer (Infrared spectrometer Varian 660 MidIR Dual MCT/DTGS Bundle with ATR) was used to confirm the chemical structure of all samples. Before analysis, the samples were dried in an auto- desiccator for 24 hours. Samples were directly applied to a diamante crystal of ATR and resulting spectra of them were corrected for background air absorbance. Potassium bromide (KBr) disks were prepared from powdered samples mixed with dry KBr in the ratio of 1:100. The spectra were recorded in a transmittance mode from 4000 to 500/400 cm^{-1} at a resolution of 4 cm. Infrared spectrum was Fourier transformed and recorded in the absorption mode. The refractogram obtained from FT-IR spectroscopy between wave number and absorption is tabulated below. IR solution software is employed for getting the spectrum. The region of IR radiation helps to identify the functional groups of the active components present in extract based on the peaks values of the FTIR spectrum. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio.

2.9 Separation of the Bioactive Compound of Water Extract of *Allium sativum*

The bioactive compounds of water extract of *Allium sativum* were separated using Gas Chromatography - mass spectrometry (GC – MS) as described by [13]. Analysis was done using a Varian 3800 gas chromatograph equipped with a Agilent MS capillary column (30 m × 0.25 mm i.d.) connected to a Varian 4000 mass spectrometer operating in the EI mode (70 eV; m/z 1 – 1000; source temperature 230°C and a quadruple temperature 150°C). The column temperature was initially maintained at 200°C for

2 min, increased to 300°C at 4°C/min, and maintained for 20 min at 300°C. The carrier gas was Nitrogen at a flow rate of 1.0 mL/min. The inlet temperature was maintained at 300°C with a split ratio of 50:1. A sample volume of 1µL in chloroform was injected using a split mode, with the split ratio of 50:1. The mass spectrometer was set to scan in the range of m/z 1-1000 with electron impact (EI) mode of ionization, runtime were 40 minutes. Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC – MS compounds present in the samples were identified. Samples of water extract of *allium sativum* and replicates were continuously injected as one batch in random order to discriminate technical from biological variations. Additionally, the prepared pooled samples were used as quality controls (QCs), which were injected at regular intervals throughout the analytical run to provide a set of data from which the repeatability can be assessed.

2.10 Sensitivity Pattern of Bacterial Isolates to Partially Purified Extracts of *A. sativum*

Each of the fractions obtained from partial purification of the plants extracts were screened for antimicrobial activity on antibiotics resistant enteric bacteria by performing disc diffusion method as described by [9]. The plants extracts fractions were being reconstituted using 30% v/v Dimethyl sulfoxide and sterilized (by filtration) using sterile injection filters of 0.22 µm pore size. The various plants extracts fractions to be screened were reconstituted to concentration of 50 mg/ml respectively. A disc shaped sterile paper was soaked overnight inside the various plants extracts fractions respectively. The discs were placed on the agar wells in each of the test bacterial seeded agar aseptically. The negative control for the experiment was 30% aqueous DMSO while ciprofloxacin (0.63 mg/ml) was used as the positive control. All the plates were incubated at 37°C for 24 hours after which the zones of inhibitions were measured.

3. RESULTS

3.1 Partial Purification of Water Extracts of *Allium sativum*

After the partial purification of water extracts of *Allium sativum*, water extract of *Allium sativum* has 11 fractions.

3.2 Determination of the Retention Factor (Rf) of Water Extract of *A. sativum*

The Thin layer Chromatogram of water extracts of *Allium sativum* is represented in Plate 1. Retention factor of the extract is calculated by;

R.F (Retention factor) = distance moved by solute/distance moved by solvent
Distance moved by solvent of the extract is = 15.1.
Distance moved by solute (*Allium sativum*) is = 12.7
R.F of Water Extracts of *Allium sativum* is =12.7/15.1=0.84.

Plate 1. Chromatogram of water extract of *Allium sativum*

3.3 Determination of Chemical Properties and Functional Groups of Water Extract of *Allium sativum*

The peaks and the functional groups of Fourier Transform Infrared Spectroscopy analysis of water extract of *Allium sativum* after partial purification is represented in Table 1 and Fig. 1 respectively. Purified water extract of *Allium sativum* had some functional groups such as Amine salt, Aliphatic primary amine, isothiocynate, α,β-unsaturated ketone, Aromatic ester, Vinyl ether, Aliphatic ether and Fluoro compound.

3.4 Determination of Bioactive Compound of Water Extract of *Allium sativum*

The gas chromatography-mass spectrophotometry of partially purified water extract of *Allium sativum* reveals that it possess antimicrobial bioactive compounds Table 2 and Fig. 2. Partially Purified water extract of *Allium sativum* had nineteen peaks and nineteen bioactive compounds (1,2-Benzenediol, 3-Methylpyridazine, Benzoic acid,4-ethoxy ethyl ester, Eicosane, 2,6,10,14,18-pentamethyl, Octadecanoic acid, 2-hydroxy-1- hydroxymethyl) ethyl ester, n-Hexadecanoic acid, Hexadecanoic acid, methyl ester, 1-Heptacosanol, Ethanone, 1-(3-hydroxyphenyl)-, Hexadecanoic acid, 15-methyl-, methyl ester, Phytol, n-Hexadecanoic acid, Benzofuran, 2,3-dihydro-, 3-Buten-2-one, 4-(2-furanyl)-, 9,12, Octadecadienoic acid (ZZ)-, 2,4-Di-tert-butylphenol, and 2-Pentanone and 4-hydroxy-4-methyl-, Tetracosane).

3.5 Sensitivity Pattern of Bacterial Isolates to the Fractions of Partially Purified Water Extracts of *Allium sativum*

The antimicrobial susceptibility patterns of the fractions of partially purified water extract of *Allium sativum* Fig. 3. Eleven fractions of partially purified water extract of *Allium sativum* were tested for antimicrobial susceptibility. Seven fractions of partially purified water extract of *Allium sativum* (fraction 5-11) had significant zone of inhibition while fraction 1-4 does not have significant zone of inhibition. Fraction eight had the highest zone of inhibition 24.33 ± 0.33 mm while fraction five had the least zone of inhibition 1.66 ± 1.66 mm.

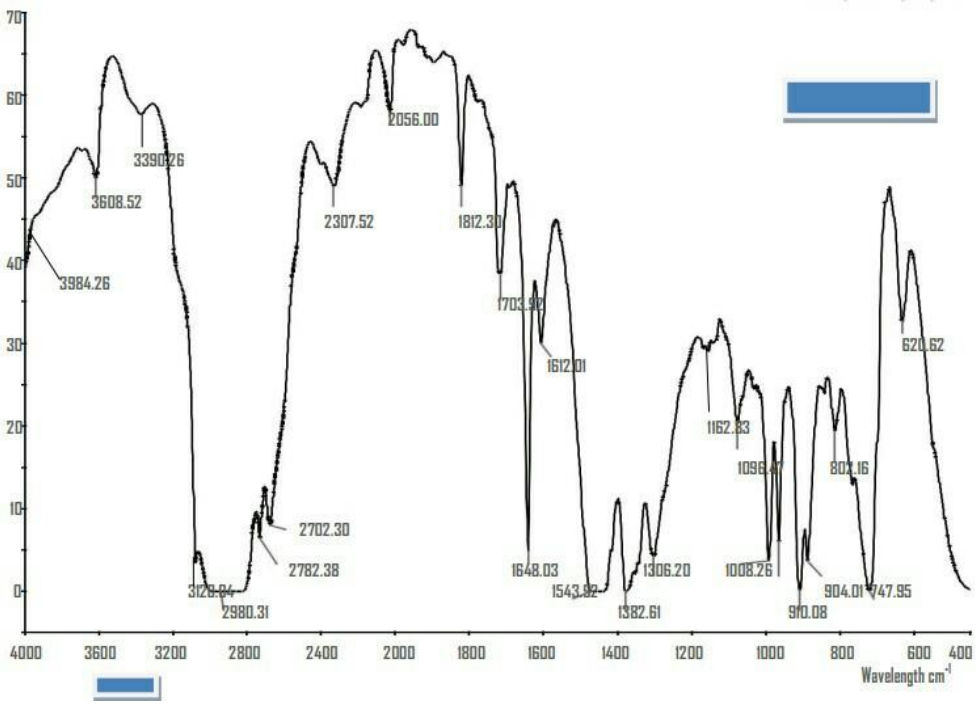
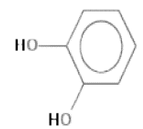
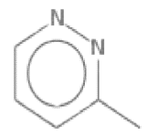
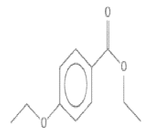
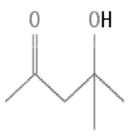
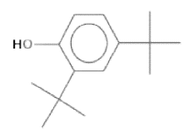


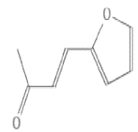
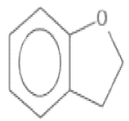
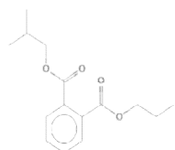
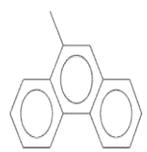
Fig. 1. Fourier transform INFRARED spectrophotometer (FTIR) spectra of partially purified water extract of *A. sativum*

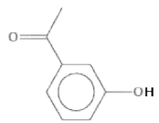
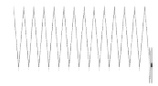
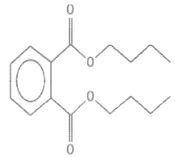
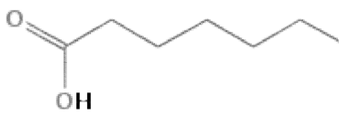
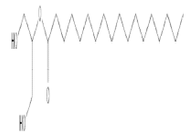
Table 1. FTIR spectral peak values and functional groups obtained from partially purified water extract of *A. sativum*

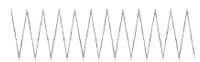
Run #	Peak (cm^{-1})	Functional group	Interpretation
1	3904.26	O-H Stretching	Alcohol
2	3606.52	O-H (bonded), N-H stretching	Unidentified
3	3390	C–H stretch	Alkene
4	3120.04	C≡N stretching	Unidentified
5	2980.31	O-H (bonded),C-H, N-H stretching	Amine salt
6	2782.36	N-H stretching	Aliphatic primary amine
7	2702.30	C-H stretching	Aldehyde
8	2307.52	O=C=O stretching	Unidentified
9	2056.00	N=C=S stretching	Isothiocynate
10	1812.30	C-O stretching	Acid halide
11	1703.92	C=O Stretching	Conjugated aldehyde
12	1648.03	C=O stretching	Unidentified
13	1612.01	C=C stretching	α,β-unsaturated ketone
14	1543.92	N-O stretching	Nitro compound
15	1382.61	C-F, N-Ostretching	Unidentified
16	1306.20	C–O stretching	Aromatic ester
17	1162.83	C-O, C-F stretching	Vinyl ether
18	1096.47	C-O Stretching	Aliphatic ether
19	1008.26	C-F Stretching	Fluoro compound
20	910.08	C=C Bending	Alkene
21	904.01	S=O, C-O Stretching	Unidentified
22	802.16	para directing benzene ring	Unidentified
23	747.95	C=C, C-H Bending	Unidentified
24	620.42	C-Br stretching	Halo compound

Table 2. Gas chromatography-mass spectrophotometry compounds present in the partially purified water extract of *A. sativum*

Peak #	Retention Time	Compound Detected	Mol. Formula.	Mol. Wt.	Peak Area (%)	% Composition	MS Fragment ions	Structures
1	5.46	1,2-Benzenediol	$C_6H_6O_2$	110	1.66	1.09	27, 64, 110	
2	5.81	3-Methylpyridazine	$C_5H_6N_2$	94	2.21	2.96	39, 65, 94	
3	6.04	Benzoic acid,4-ethoxy ethyl ester	$C_{11}H_{14}O_3$	194	4.98	4.21	65, 121, 194	
4	6.21	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	116	9.41	9.81	43, 59, 116	
5	6.47	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206	3.87	3.03	57, 191, 206	

Peak #	Retention Time	Compound Detected	Mol. Formula.	Mol. Wt.	Peak Area (%)	% Composition	MS Fragment ions	Structures
6	6.51	9, 12, Octadecadienoic acid (ZZ)-	$C_{18}H_{32}O_2$	280	5.01	6.83	67, 81, 280	
7	7.15	3-Buten-2-one, 4-(2-furanyl)-	$C_8H_8O_2$	136	6.09	5.92	65, 121, 136	
8	7.63	Benzofuran, 2,3-dihydro-	C_8H_8O	120	8.20	8.46	65, 91, 120	
9	8.62	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.86	8.99	43, 60, 256	
10	10.26	1,2-Benzene dicarboxylic acid, bis [2-methyl propyl] ester	$C_5H_{22}O_4$	278	3.88	4.73	57, 149, 278	
11	10.52	Phytol	$C_{20}H_{40}O$	296	3.90	2.82	96, 165, 192	

Peak #	Retention Time	Compound Detected	Mol. Formula.	Mol. Wt.	Peak Area (%)	% Composition	MS Fragment ions	Structures
12	10.62	Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284	15.05	13.73	74, 87, 284	
13	11.22	Ethanone, 1-(3-hydroxyphenyl)-	$C_8H_8O_2$	136	3.32	3.25	93, 121,	
14	13.19	1-Heptacosanol	$C_{27}H_{56}O$	396	3.87	3.33	83, 97, 396	
15	13.24	Hexadecanoic acid, methyl ester	$C_{16}H_{12}O_4$	278	3.32	3.42	149, 205, 278	
16	14.32	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	11.17	9.45	43, 73, 256	
17	14.92	Octadecanoic acid, 2-hydroxy-1-hydroxymethyl) ethyl ester	$C_{21}H_{42}O_4$	358	1.61	2.55	43, 98, 358	

Peak #	Retention Time	Compound Detected	Mol. Formula.	Mol. Wt.	Peak Area (%)	% Composition	MS Fragment ions	Structures
18	16.10	Eicosane, 2,6,10,14,18-pentamethyl	$C_{25}H_{52}$	352	1.66	1.45	57, 99, 352	
19	16.21	Tetracosane	$C_{24}H_{50}$	338	1.11	1.15	57, 71, 338	

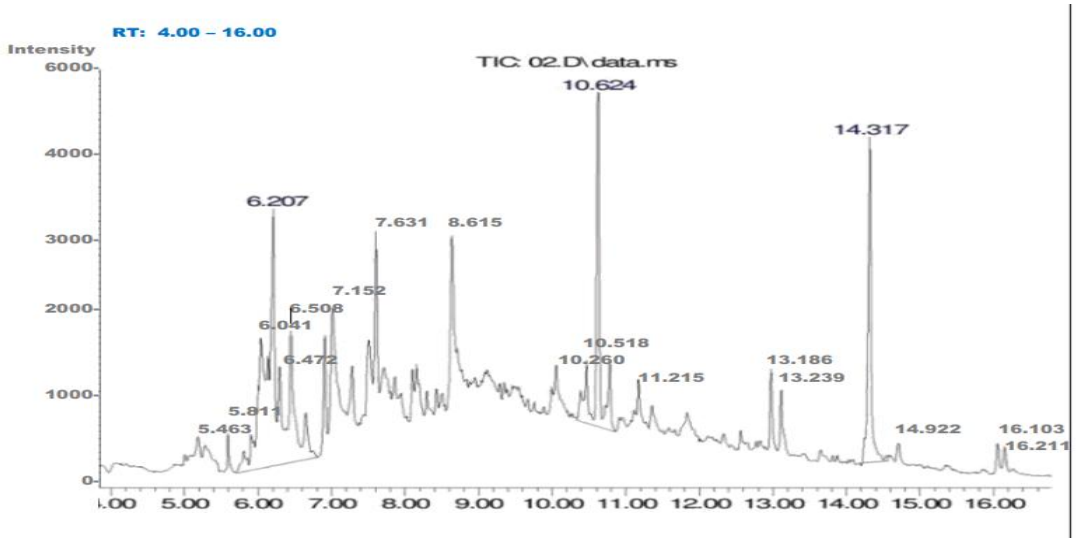


Fig. 2. Gas chromatography-mass spectrophotometry spectra of partially purified water extract of *A. sativum*

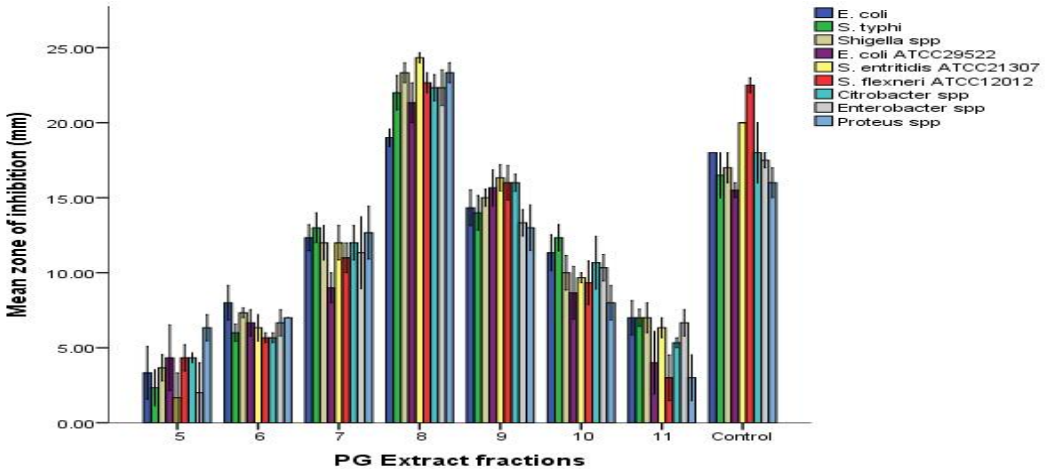


Fig. 3. Sensitivity pattern of bacterial isolates to the fractions of partially purified water extracts of *Allium sativum*

4. DISCUSSION

Partial purification of water extracts of *Allium sativum* reveals water extract of *Allium sativum* had 11 fractions. Thin layer chromatogram of water extracts of *Allium sativum* shows distance moved by solvent for the extract was 15.1 cm, while the distance moved by water extracts of *Allium sativum* was 12.7cm. The retention factor for water extracts of *Allium sativum* was calculated at 0.84.

Purified water extract of *Allium sativum* had 24 peaks and functional groups such as Amine salt, Aliphatic primary amine, isothiocynate, α,β-unsaturated ketone, Aromatic ester, Vinyl ether, Aliphatic ether and Fluoro compound.

The gas chromatography-mass spectrophotometry of partially purified water extract of *Allium sativum* reveals that water extract of *Allium sativum* had nineteen peaks and nineteen bioactive compounds (1,2-Benzenediol, 3-Methylpyridazine, Benzoic acid,4-ethoxy ethyl ester, Eicosane, 2,6,10,14,18-pentamethyl, Octadecanoic acid, 2-hydroxy-1- hydroxymethyl) ethyl ester, n-Hexadecanoic acid, Hexadecanoic acid, methyl ester, 1-Heptacosanol, Ethanone, 1-(3-

hydroxyphenyl)-, Hexadecanoic acid, 15-methyl-, methyl ester, Phytol, n-Hexadecanoic acid, Benzofuran, 2,3-dihydro-, 3-Buten-2-one, 4-(2-furanyl)-, 9,12, Octadecadienoic acid (ZZ)-, 2,4-Di-tert-butylphenol, and 2-Pentanone and 4-hydroxy-4-methyl-, Tetracosane), this is related to the findings of [14] in "GC/MS analysis, antimicrobial and Antioxidant Effect of Ethanol Garlic Extract" in which they reported similar bioactive compounds which can be used in production of irresistible antimicrobials and other medicinal products.

The antimicrobial susceptibility patterns of the fractions of partially purified water extract of *Allium sativum* shows that fractions of the purified extract were able to inhibit most of the isolates. Fraction eight of the partially purified water extract of *Allium sativum* had the highest zone of inhibition 24.33 ± 0.33 mm. This is in accordance with the findings of [15], in which they reported the highest zone of inhibition of fractions of *Allium sativum* against some Enterobacteriaceae sp isolated from sprouted Mung bean to be higher (24 mm).

5. CONCLUSION

The findings from this study suggest that *Allium sativum* extracts has numerous and bioactive compounds that can be used for production irresistible antimicrobials against pathogenic enteric bacteria and other medicinal products that are highly beneficial to human being and animals. Hence, further research should be carried out using advance techniques to confirm this claim.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Clasen T, Schmidt W, Rabie T, Roberts I, Cairncross S. Interventions to improve water quality for preventing diarrhoea: systematic review and meta-analysis. British Medical Journal. 2007;1-10.
2. WHO (World Health Organization). UN-water global annual assessment of sanitation and drinking-water GLAAS): Targeting resources for better results. Geneva: WHO Press; 2010.
   (Cited April 23, 2010)
   Available:http://www.unwater.org/d
3. WHO. (World Health Organization). 2005. Guidelines for Laboratory and Field Testing of Mosquito Larvicides. WHO communicable disease control, prevention and eradication. WHO pesticide evaluation scheme.
   WHO/CDS/WHOPES/GCDPP/2005.13.
4. Johanna Santamaria, Gary A. Toranzos. Enteric pathogens and soil: a short review. International Microbiology. 2003;6: 5–9.
5. Francesca Prestinaci, Patrizio Pezzotti, Annalisa Pantosti. Antimicrobial resistance: A global multifaceted phenomenon. Review: Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanita, Rome, Italy. Taylor and Francis Group. Pathogens and Global Health. 2015;109(7):309-318.
6. WHO. (World Health Organization). The evolving threat of antimicrobial resistance. Options for action. Geneva: WHO Library Cataloguing-in-Publication Data; 2012.
7. Mukeshwar Pandey, Mousumi Debnath, Shobit Gupta, Surender K. Chikara. Phytomedicine: An ancient approach turning into future potential source of therapeutics. Journal of Pharmacognosy and Phytotherapy. 2011;3(1):113-117.
8. Teferi G, Hahn HJ. Treatment of malaria in Ethiopia folk medicine. Tropical Doctor. 2002;32:206-207.
9. Olukunle OF, Adenola OJ. Comparative antimicrobial activity of lemon grass (*Cymbopogon citratus*) and garlic (*Allium sativum*) extracts on *Salmonella typhi*. Journal of Advances in Medical and Pharmaceutical Sciences. 2019;20(2): 1-9.
10. Oluduro AO. Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. leaf in South Western Nigeria, Malaysian Journal of Microbiologyy. 2012;8(2):59-67.
11. Devika R, Koilpillai J. Column Chromatographic Separation of Bioactive Compounds from *Tagetes erecta* Linn. International Journal of Pharmaceutical Sciences and Research. 2015;6(2):762-66.
12. Nagarajan D, Ramesh Kumar T. Fourier transform infrared spectroscopy analysis of garlic (Allium). International Journal of Zoology Studies. 2017;2(6):11-14.
13. Satyal Prabodh, Craft Jonathan, Dosoky Noura, Setzer William. The chemical compositions of the volatile oils of garlic

(*Allium sativum*) and wild garlic (*Allium vineale*). Foods (Basel, Switzerland). 2017; 6:63.

14. Park Na-Hye, Jang Hye-Ri, Lee Seung-Jin, Ark Seung-Chun. GC/MS analysis, antimicrobial and antioxidant effect of ethanol garlic extract. International Journal of Phytomedicine. 2017;9(2).

15. Fahad A, Alzowahi M, Ahmed Abu-Taleb, Amani As-Suhbani, Kadam TA. The inhibitory effects of garlic extract and its fractions against some Enterobacteriaceae sp isolated from sprouted Mung bean. International Journal of Current Microbiology and Applied Sciences. 2013; 2(7):104-115.

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