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In-vitro Antibacterial and Synergistic Activities of Extracts of Allium cepa and Allium sativum with Selected Antibiotics on Escherichia coli and Staphylococcus aureus

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NCI and IEK designed the study, performed the statistical analysis and wrote the protocol. Author AUK wrote the first draft of the manuscript. Authors AOC and UK managed the analyses of the study. 'Author IEK managed the literature searches. Author NCI wrote the final manuscript. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aim: This study was aimed at investigating the antibacterial activities of extracts of *Allium cepa* and *Allium sativum*, as well as their synergistic activities with some selected antibiotics against the bacterial pathogens *Escherichia coli* and *Staphylococcus aureus*.

Study Design: Onions (*Allium cepa*), garlic (*Allium sativum*) were used in this study. The study assessed how extracts of the plants alone or in combination with some commonly used antibiotics inhibited the growth of some bacterial pathogens using agar well diffusion method.

Place and Duration of Study: The research was carried out in the Department of Microbiology, University of Nigeria, Nsukka over a period of 6 months.

Methodology: Extracts of *Allium cepa* and *Allium sativum* were obtained using ethanol, methanol and water (aqueous). Antimicrobial susceptibility tests were carried out by agar well diffusion technique.

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Results: All extracts obtained showed evidence of antimicrobial property measured as the Inhibition Zone Diameter (IZD) on the test microorganisms. Values of these IZDs showed remarkable increases when used synergistically with antibiotics. For instance, aqueous extract of *Allium sativum* increased from a mean value of 16.5mm at 1.0mg/ml to values of 30.0mm and 38.0mm when used with tetracycline and ampicloxacillin respectively against *Escherichia coli*. The MIC and MBC values also dropped progressively, indicating that fewer synergistic mixtures were required to exert the same effects on the tested organisms. The phytochemical analysis strongly indicated the presence of flavonoids, glycosides and alkaloids, among other plant metabolites. **Conclusion:** This result indicates that *Allium cepa* and *Allium sativum* showed strong antibacterial activity against the bacterial pathogens *E. coli* and *S. aureus*; and that synergism of the extracts with some antibiotics improved those activities. The potential of developing antimicrobials from these plants appears promising.

Keywords: Antimicrobials, Allium cepa, Allium sativum, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Phytochemicals, Synergism.

1. INTRODUCTION

The search for alternatives to replace our failing antibiotics is one of the prime goals of science today [1]. This is because of the unusually high rate of antibiotics resistance currently prevalent worldwide [2]. The World Health Organization put it fittingly when it said that the increasing rate of antibiotic-resisting microorganisms threaten the achievements of modern medicine [3]. The organization pointed out that antibiotic resistance can affect anyone irrespective of age or country, and that it is a threat not just to global health, but also food security and general development today [4]. To make matters worse, it appears that the present era is one in which microorganisms tend to develop resistance faster than new antibiotics are developed [4]. Mankind's indiscriminate and careless use of antibiotics is considered a large contributor to this menace [2]. The role of plants and herbs (phytomedicines) as alternative and complementary medicines (CAM) in health care systems is presently well recognized, as many plants are associated with important medicinal applications [5, 6]. Herbal plants are among the most commonly used antimicrobial agents. They have traditionally been used for thousands of years to control various health complications including infectious diseases [7]. It is therefore not surprising that they are helping in the fight against antimicrobial resistance.

Among the many spices and food condiments used all over the world, only very few enjoy the global reach and acceptance as Allium cepa (onions) and Allium sativum (garlic). Both spices belong to the same family of plants Alliaceae, which is why they share the same generic name [1, 6, 8]. They are also known for their sharp tastes and odors which easily give them away. Both spices also share faint similarities in appearance as they are rounded bulbs. They have been used as medicines in many ancient cultures dating back to many thousands of years [1]. Many pathogenic microorganisms among which the Gram-positive bacteria are Staphylococcus aureus and Streptococcus pneumoniae and their Gram-negative counterparts Escherichia coli, Salmonella typhi and *Klebsiella pneumoniae* have well established history of pathogenicity and have variously been implicated in different human infections with varving degrees of severity. Although antibiotics have been developed against most of these organisms, the problem of antibiotic resistance coupled with the toxicity and many side effects of many antibiotics have made the continuous search for newer antibiotics against these pathogens a necessity [9].

Across the different parts of the world, but especially in the developing countries of Africa and South-East Asia where the health infrastructures are rudimentary, many cases of morbidity and mortality being witnessed are due to infections caused by antibiotic resistant bacteria [10]. In addition, the treatment regime for many hitherto common and innocuous bacterial infections often involves prolonged exposure to broad spectrum antibiotics, leading in several cases to increased toxicity to patients. All of these problems have created an everincreasing need for less toxic, cheap, easily sourced and effective alternative antimicrobial agents that can treat the diseases caused by bacteria and other infectious agents [11]. Many plants, spices and herbs are good alternative options because not only are they usually less toxic than antibiotics and posing fewer side

effects, they are mostly inexpensive and readily available for low socioeconomic population; they also show better patient tolerance [12]. The *Allium* species which are used in the present work present good options in the important search for alternatives to our failing antibiotics [13].

The degree of antibacterial activity expressed by many plants has been found to correlate very well with the extraction solvents used [14, 15]. Water, ethyl acetate and ethanol are more frequently used compared to other solvents such as acetone, butanol and chloroform [16-18]. This study was aimed at investigating the antibacterial activity of these two important and strategic Allium plants as additional proofs of their medicinal values. To do so three extracts of each of them: water (aqueous), ethanol and hexane were used against some selected bacterial strains.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh samples of onions (*Allium cepa*) and garlic (*Allium sativum*), used in this study were bought from the Ogige Market, located along University Market Road in Nsukka, Enugu State, Nigeria. They were then taken for proper identification and authentication by Mr Chijoke Onyeukwu, a botanist and curator of the Herbarium of the Department of Plant Science and Biotechnology (PSB), University of Nigeria, Nsukka. Voucher numbers were given to the samples as follows: UNH 313 (*Allium cepa*) and UNH 214 (*Allium sativum*)

2.2 Bacterial Strains

strains of Escherichia coli and Clinical Staphylococcus aureus were obtained from the National Agency for Food and Drug Administration and Control (NAFDAC), Agulu, Anambra State. The strains were collected as nutrient agar slants and immediately transported in an icebox to one of the research labs of Microbiology Department, University of Nigeria, Nsukka and stored at -20°C. Thereafter, strains were maintained on both nutrient agar slants and broths just before use.

2.3 Preparation of Extracts

Aqueous, ethanol and hexane extracts of onions and garlic respectively, were prepared separately. The fresh cloves of onions and garlic were first peeled, sliced and sun dried for twelve days. After drying, the slices of each of the spices or nuts were ground to fine powder separately using a manual blender. The powders obtained were thereafter, macerated with the different solvents in 1000 mL conical flasks for 48 hours in a powder to solvent ratio (g/L) of 1:5 (typically, 100 g of each powder in 500 mL of solvent) according to the method described by Nnamchi et al., [1]. Afterwards, the flasks were incubated at room temperature for 48 hours with shaking at 120 rpm. Filtrates of the extracts (ethanol and methanol), were evaporated at while the aqueous extracts 50°C were evaporated at 80°C in a rotary evaporator.

2.4 Standardization of Inoculum

Freshly prepared nutrient broth cultures of the two bacteria were used as inocula by diluting with sterile saline solution. Each inoculum was thereafter prepared according to the CLSI approved guideline for bacteria and yeasts [19]. This was done as follows: 10mL of the sterile saline solution was transferred to separate test tubes and left to cool. Thereafter, 0.1ml of each of overnight broth cultures of the bacterial strains were individually dispensed into the separate test tubes containing the solutions. The turbidity of each bacterial broth suspension was then adjusted to match the appearance of 0.5 McFarland"s standard using sterile normal saline [19]. This served as the standard inocula for the antibacterial activity testing and for the of determination minimum inhibitory concentration of the extracts.

2.5 Phytochemical Screening of Plant Extracts

The extracts of *Allium sativum* and *Allium cepa* were screened for the presence or otherwise of phytochemical compounds such as saponins, tannins, flavonoids, alkaloids, phlobatannins, and anthraquinones according to the method described by Khan et al. [20], Auwal et al. [21] and Gul et al. [22].

2.6 Antimicrobial Assay using Disc Diffusion Method

The antimicrobial assay of spices was performed by well diffusion method on Mueller Hinton agar plates using the Kirby-Bauer disc diffusion method as described by Gashe et al. [23]. All the experiments were performed under sterile conditions. Aseptically prepared Mueller Hinton agar plates were inoculated separately with 1.5 x 108 CFU/mL of each test bacterial culture and then spread evenly on the plate. Wells were then bored (10mm) on the plates aseptically as previously described with modifications [24] and filled with 50 µl of each of the extracts. Controls were also placed in each of the plates. For synergism tests, 0.025mls of extract was combined with 0.025mls of standardized antibiotic. The plates were left at ambient temperature for about 15 minutes and then incubated at 37°C for 24 hours and observed for zone of inhibition (IZD) was measured (in millimeters) using a meter rule and recorded. The end or edge of inhibition zone is where the bacterial growth starts. Antimicrobial assay was performed in triplicate for the extracts alone and in duplicate for the synergism of extract and antibiotic with each bacterial strain.

2.7 Determination of Minimum Inhibitory Concentration (MIC) by Agar Well Diffusion Method

The MIC of the different garlic and onions extracts was determined by the method described by Natta et al. [25] with minor modifications. The extracts were diluted ranging from 100 mg/ml to 0.01 mg/ml and checked for MIC against bacterial strains. Sterile pipettes were used to measure out 50 µl of the different dilutions of aqueous, ethanol and methanol extracts of garlic and onions and filled into 10mm holes bored on Mueller Hinton agar plates seeded with 1.5 × 108 CFU/ml of each bacterial cultures separately. For synergism tests, 0.025mls of extract was combined with 0.025mls of standardized antibiotic to give a 1:1 ratio. Plates were placed at 37°C for 24 hours. The zone of inhibition (IZD) in each case was measured as the diameter of the clearing zones and results were recorded. Each experiment was performed in triplicate for extracts alone and duplicates for synergism of extract and antibiotic.

2.8 Determination of Minimum Inhibitory Concentration (MIC) by Broth Dilution Method

The broth dilution method as described by National Committee for Clinical Laboratory Standard [19] was used in the determination of MIC. Varying concentrations of the extracts (0.05mg/ml to 10.0mg/ml) were prepared. A portion (0.1ml) of each concentration was added to each 9ml of nutrient broth containing 0.1ml of standardized test organism of bacterial cells. The tubes were incubated at $37^{\circ}C$ for 24h. The tube

with the least concentration of the extracts without growth (turbidity) after incubation was taken and recorded as the minimum inhibitory concentration (MIC).

2.9 Determination of Minimum Bactericidal concentration (MBC)

The contents of the MIC tubes that show no growth was sub cultured onto Antibiotic-free liquid medium, incubated at 37° C for 24h and examined for bacterial growth. The tubes that show no growth at this stage were further sub-cultured onto nutrient agar plate and incubated at 37° c for 24h. The lowest concentration of extract that showed no growth on the plate after 24hours was taken as the Minimum Bactericidal Concentration (MBC).

2.10 Statistical Analysis

All assays done in the course of this work were in triplicates. Values of inhibition zone diameters were calculated as mean \pm SE (standard error) of triplicate measurements obtained for all extracts. This applied to both the single and synergistic assays. The statistical package used was SPSS (Statistical Package for Service Solutions) version 23.

3. RESULTS AND DISCUSSION

The use of onions and garlic as natural supplements is a healthy choice for the treatment of cardiovascular diseases [26, 27], hypertension [28], diabetes [29], Alzheimer's disease [30] inflammation, thrombosis [31] and cancer [32]. Synergisms of onions with allopathic therapeutics have been reported in the treatment and management of obesity [33], cold, cough, bronchitis and influenza [34]. Garlic has equally been used to enhance the antifungal activity of amphotericin B used in the treatment of fungal infections of Candida albicans and Aspergillus fumigatus but did not enhance its cytotoxic effects [35]. The combination of garlic and omeprazole showed synergistic effect against Helicobacter pylori strains resistant to clarithromycin and metranizadole [36] and the combination of ajoene, a garlic compound (50mg/kg) and chloroquine (4.5mg/kg) given as a single dose on a dav completely prevented subsequent development of parasitaemia in treated mice [37].

Results of the present study showed that the three extracts obtained from *Allium cepa* and *Allium sativum* (onions and garlic) inhibited the growth of the bacterial species they were challenged with, with the extent of inhibition depending on the concentration used. This agrees with Gull *et al.* [38] who established in their study that spices reduce and inhibit the growth of food pathogens, and therefore surmised that the use of spices decreases the chances of foods.

The percentage yield obtained from the extraction of each of the four dried powders of the different spices with the three solvents: water (aqueous), ethanol and methanol, is shown in Table 1. The Table shows that the highest yield was obtained when onions was extracted with methanol (OME, 26.48%) followed by onions extracted with ethanol (OEE, 12.73%) while the least was obtained with garlic extracted with

ethanol (GaEE, 4.05%). The yields are rated thus: OME> OEE> GaME> GaAE> OAE> GaEE.

Table 2 shows the phytochemical composition of the different extracts. In total. seven phytochemicals viz., alkaloids, flavonoids, glycosides, saponins, steroids, tannins and terpenoids were tested. The results indicate that alkaloids, flavonoids and glycosides are present in all the six extracts, while saponins, steroids, tannins and terpenoids were not present in one or more of the plant extracts. For instance, ethanol extracts of onions (Allium cepa) and garlic (Allium sativum) had six of the seven phytochemicals, and lacked only one of them. The same six phytochemicals was also contained in the methanolic extracts of Allium cepa. The table thus represents another impact of how the different extractants affect the phytochemical contents of the different plant materials. These results corroborate with the results reported by Roy et al. [39].

Table 1. Dried extract yields and percentage yield

	Aqueous	Methanol	Ethanol
Garlic	8.64%	9.72%	4.05%
Onions	4.24%	26.48%	12.73%

	Extract	Allium cepa	Allium sativum
	Aqueous	+	+
Alkaloids	Ethanolic	+	+
	Methanolic	+	+
	Aqueous	+	+
Flavonoids	Ethanolic	+	+
	Methanolic	+	+
	Aqueous	+	+
Glycosides	Ethanolic	+	+
	Methanolic	+	+
	Aqueous	-	+
Saponins	Ethanolic	+	+
	Methanolic	+	+
	Aqueous	+	+
Steroids	Ethanolic	-	+
	Methanolic	-	+
	Aqueous	-	-
Tannins	Ethanolic	+	-
	Methanolic	+	-
	Aqueous	+	+
Terpenoids	Ethanolic	+	+
	Methanolic	+	-

Table 2. Phytochemical components of extracts

KEY: + = Present; - = Absent

The differences shown in the class or type of phytochemicals extracted by the different solvents could be explained on the basis of differences in their nature as extractants. It is known for example that the extraction principle depends on like dissolving and extracting like, which is the same as saying that only the metabolites that can dissolve in each solvent can be extracted by it from the overall components of the plant material used. This process which is also known as selective solubility is a basic chemistry concept today [40, 41]. These differences obviously have great impact on the phytochemical properties of the different extracts as non-polar solvents typically solubilize mostly lipophilic compounds such as fatty acids, alkanes, waxes, pigments, alkaloids etc., while polar solvents solubilize those like flavonoid glycosides, tannins and some alkaloids among others while those in between like ethanol in this case extract compounds of intermediate polarity which include some alkaloids and flavonoids [41probably explains 441. This whv the phytochemical analysis of the extracts (Table 2). show that each extract comprise different phytochemical components such as alkaloids, flavonoids, glycosides, saponins, steroids and tannins with none of them containing all the components. Since the nature and scope of the present work is essentially preliminary it is concerned only with the qualitative assessment of the presence or otherwise of the different phytochemical compounds.

Table 3 shows the inhibition zone diameters of the extracts on *Escherichia coli*. All extracts of *Allium cepa* showed no activity only at 0.01mg/ml. The aqueous extract of *Allium cepa*

equally showed no activity only at 0.1mg/ml. However, the aqueous extract of *Allium sativum* showed appreciable activity at all concentrations while the ethanol and methanol extracts showed activity only at 10mg/ml and 100mg/ml concentrations.

The aqueous and methanol extracts of *Allium cepa* showed no activity on *Staphylococcus aureus* only at 0.01mg/ml and 0.1mg/ml concentrations. Equally, the ethanol (at 0.01mg/ml) and methanol (at both 0.01mg/ml) and 0.1mg/ml) extracts of *Allium sativum* showed no activity. The ethanol extract of *Allium cepa* and aqueous extract of *Allium sativum* showed increasing activity with increasing concentrations (Table 4).

Tables 5 and 6 both show the synergistic activities of the plant extracts with tetracycline and ampicloxacillin separately against *Escherichia coli* and *Staphylococcus aureus* at 0.01mg/ml, 0.1mg/ml, 1mg/ml. 10mg/ml and 100mg/ml concentrations. The results indicated that all concentrations were active. Analysis showed that the activities were significantly different from each other.

The minimum inhibitory concentrations (MICs) of the extracts of *Allium cepa* and *Allium sativum* on *E. coli* and *S. aureus* are respectively shown in figures 1 and 2. The highest value (0.1mg/ml) was seen for; all extracts of *Allium cepa* used alone on *E. coli*. Aqueous extract of *Allium cepa* used alone on *S. aureus,* ethanol and methanol extracts of *Allium sativum* used alone on *E. coli* and ethanol and methanol extracts of *Allium sativum* used alone on *S. aureus*.

Table 3 Antibacterial activity of different solvent extracts of test plants against <i>Escherichia coli</i>
at various concentrations

Test Test extract			Concentration (mg/ml)					
plant			0.01	0.1	1.0	10	100	
	Aqueous		-	-	15.5±3.54	16.5±2.12	19.0±1.00	
Allium	Ethanolic		-	18.0±0	16.67±2.89	21.0±1.73	22.07±2.51	
сера	Methanolic		-	14.0±0	16.0±2.83	16.67±2.08	21.67±1.53	
	Aqueous		15.0±0	16.0±0	16.5±2.12	17.33±2.52	20.0±0.58	
Allium	Ethanolic		-	-	-	14.67±1.15	18.33±2.89	
sativum	Methanolic		-	-	-	18.0±0	19.33±1.15	
Positive	Tetracycline					50±0		
control	Ampicloxacillin					63±0		
	Aqueous	-						
Negative	Ethanol	-						
control	Methanol	-						

Values are diameter of zone of inhibition in mm (Mean±SD); (-) No activity. Values in the same column differ significantly (p<0.05)

Test Test extract Concen			centration (m	entration (mg/ml)			
plant			0.01	0.1	1.0	10	100
	Aqueous		-	-	14.0±0	15.67±0.58	19.67±0.58
Allium	Ethanolic		15.0±0	15.33±0.58	15.67±1.15	17.67±0.57	19.33±1.15
сера	Methanolic		-	-	19.0±1.41	19.0±3.61	22.67±2.52
	Aqueous		16.0±0	16.0±1.0	16.67±1.53	18.33±1.53	24.0±1.0
Allium	Ethanolic		-	14.5±0.71	17.0±0	17.0±0	18.5±0.71
sativum	Methanolic		-	-	19.0±0.41	19.0±3.61	22.67±1.15
Positive	Tetracycline					40±0	
control	Ampicloxacillin					48±0	
	Aqueous	-					
Negative	Ethanol	-					
control	Methanol	-					

Table 4. Antibacterial activity of different solvent extracts of test plants against Staphylococcus aureus at various concentrations

Values are diameter of zone of inhibition in mm (Mean±SD); (-) No activity. Values in the same column differ significantly (p<0.05)

Table 5. Synergistic antibacterial activity of different solvent extracts of test plants with Tetracycline and ampicloxacillin against *Escherichia coli* at various concentrations

Test plant and	Test extract	t Concentration (mg/ml)				
antibiotic		0.01	0.1	1.0	10	100
	Aqueous	30.5±0.71	30.0±2.83	32.5±3.54	34.5±0.71	35.5±0.71
Allium cepa and	Ethanolic	35.0±0	34.5±0.71	32.0±1.41	30.0±0	33.5±3.54
Tetracycline	Methanolic	29.5±0.91	32.5±3.54	35.5±3.54	36.5±2.12	38.5±0.71
Allium sativum	Aqueous	26.5±2.12	37.5±0.71	30.0±0	30.5±0.71	32.5±0.71
and	Ethanolic	30.0±0	33.0±0	30.0±0	32.5±2.12	35.0±0
Tetracycline	Methanolic	36.0±1.41	35.5±0.771	31.5±2.12	33.5±2.12	37.5±0.71
	Aqueous	29.5±0.71	32.5±0.71	33.5±2.12	36.5±0.71	39.5±0.71
Allium cepa and	Ethanolic	34.0±8.49	35.0±0	30.5±0.91	35.5±6.36	35.0±7.07
Ampicloxacillin	Methanolic	38.5±0.71	30.0±0	30.5±0.71	33.0±0	34.0±1.41
Allium sativum	Aqueous	33.5±0.71	35.5±0.71	38.0±0	38.5±0.71	40.5±0.71
and	Ethanolic	32.5±0.61	22.5±3.54	30.5±9.19	34.0±5.66	32.5±2.54
Ampicloxacillin	Methanolic	37.5±3.54	40.0±0	40.0±0	42.0±1.41	40.0±0

Values are diameter of zone of inhibition in mm (Mean±SD); (-) No activity. Values in the same column differ significantly (p<0.05)

Table 6. Synergistic antibacterial activity of different solvent extracts of test plants with Tetracycline and ampicloxacillin against *Staphylococcus aureus* at various concentrations

Test plant and	Test extract	Concentration (mg/ml)				
antibiotic		0.01	0.1	1.0	10	100
Allium cepa and	Aqueous	30.0±0	35.0±1.41	35.0±0	33.0±2.83	37.0±0
Tetracycline	Ethanolic	24.0±1.41	23.5±2.12	31.5±4.95	32.5±3.54	36.5±2.12
	Methanolic	28.5±2.12	29.0±1.41	30.5±0.71	33.0±0	37.5±3.54
Allium sativum	Aqueous	25.0±0	30.0±0	29.0±1.41	31.5±2.12	30.0±0
and	Ethanolic	31.5±2.12	32.5±3.54	30.5±0.71	32.0±2.83	37.5±3.54
Tetracycline	Methanolic	28.0±4.24	30.5±4.95	29.5±0	30.41±1.41	33.0±4.24
Allium cepa and	Aqueous	30.0±0	33.5±4.95	34.5±0.71	35.0±0	37.5±0.71
Ampicloxacillin	Ethanolic	37.0±0	38.5±2.12	38.5±3.54	37.5±3.54	37.0±1.41
	Methanolic	40.0±0	40.0±0	36.5±4.95	35.0±7.07	37.5±3.54
Allium sativum	Aqueous	32.5±0.71	36.5±2.12	40.0±0	39.5±0.71	41.0±0
and	Ethanolic	34.5±3.54	32.5±0.71	34.5±0.71	32.5±0.71	33.5±2.12
Ampicloxacillin	Methanolic	40.0±0	40.0±0	36.5±4.95	35.0±7.07	37.5±3.54

Values are diameter of zone of inhibition in mm (Mean±SD); (-) No activity. Values in the same column differ significantly (p<0.05)

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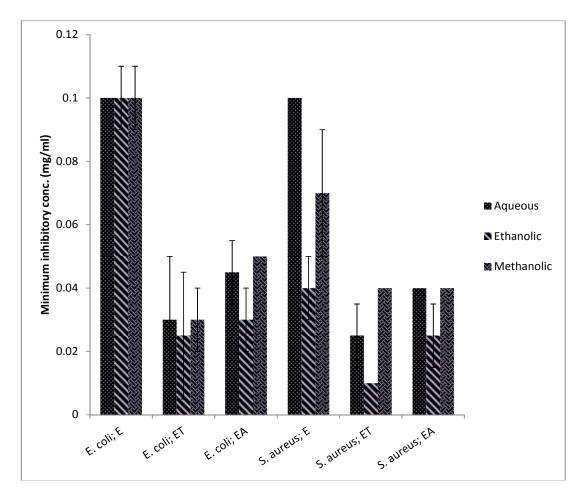


Fig. 1. Minimum inhibitory concentrations (MIC) of different *Allium cepa* extracts on tested bacterial strains. (Key: E= extract; ET= extract and tetracycline and EA= extract and ampicloxacillin

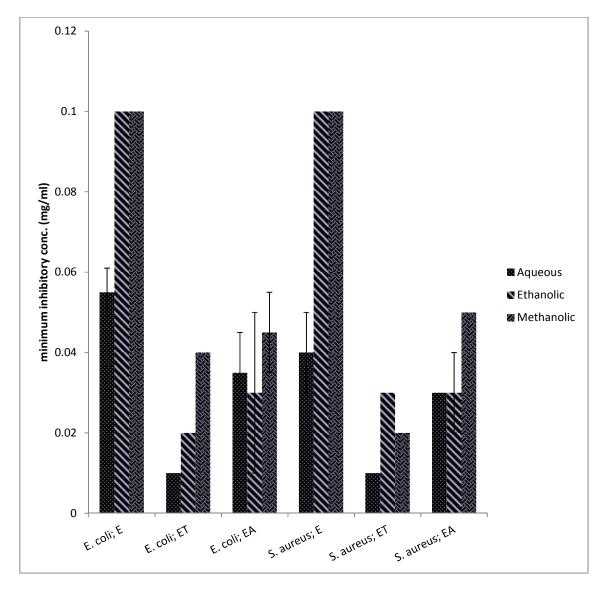


Fig. 2. Minimum inhibitory concentrations (MIC) of different *Allium sativum* extracts on tested bacterial strains. (Key: E= extract; ET= extract and tetracycline and EA= extract and ampicloxacillin)

Table 7. Minimum bactericidal concentrations (MBC) of different Allium cepa extracts on the
tested bacterial strains

	Test extract	Escherichia coli	Staphylococcus aureus
	Aqueous	10mg/ml	10mg/ml
Extract	Ethanolic	10mg/ml	1mg/ml
	Methanolic	10mg/ml	1mg/ml
	Aqueous	1mg/ml	1mg/ml
Extract and	Ethanolic	1mg/ml	1mg/ml
tetracycline	Methanolic	1mg/ml	1mg/ml
	Aqueous	1mg/ml	1mg/ml
Extract and	Ethanolic	1mg/ml	1mg/ml
ampicloxacillin	Methanolic	1mg/ml	1mg/ml

	Test extract	Escherichia coli	Staphylococcus aureus
	Aqueous	1mg/ml	1mg/ml
Extract	Ethanolic	10mg/ml	1mg/ml
	Methanolic	10mg/ml	1mg/ml
	Aqueous	1mg/ml	1mg/ml
Extract and	Ethanolic	1mg/ml	1mg/ml
tetracycline	Methanolic	1mg/ml	1mg/ml
	Aqueous	1mg/ml	1mg/ml
Extract and	Ethanolic	1mg/ml	1mg/ml
ampicloxacillin	Methanolic	1mg/ml	1mg/ml

Table 8. Minimum bactericidal concentrations (MBC) of different Allium sativum extracts on the tested bacterial strains

The MIC values showed progressive decline when the extracts were used in synergism with tetracycline and ampicloxacillin. The ethanol extracts of *Allium cepa* used in synergism had the lowest MIC values while the aqueous extracts of *Allium sativum* had the lowest MIC values on the average on both organisms. The methanol extracts of both *Allium cepa* and *Allium sativum* had the highest MIC values even when used in synergism.

Tables 7 and 8 shows the minimum bactericidal concentrations (MBC) of *Allium cepa* and *Allium sativum* extracts respectively. The results indicated that at least 10mg/ml concentrations were required for bactericidal activity of; all extracts of *Allium cepa* on *E. coli*, aqueous extracts of *Allium cepa* on *S. aureus*, ethanol and methanol extracts of *Allium sativum* on *E. coli*. All other extracts and all synergistic mixtures required only 1mg/ml for bactericidal activity.

4. CONCLUSION

This study has shown that extracts of Allium sativum and Allium cepa have strong antibacterial activities against the bacterial strains used. They further reinforced that result with the good synergistic activities they showed with selected antibiotics against the bacterial strains. The phytochemical screening of the extracts revealed that they contain important constituents such as saponins, alkaloids, tannins, steroids, glycosides, terpenoids and flavonoids which are most likely responsible for the antibacterial activities observed. The minimum inhibitory concentrations of extracts obtained in this study showed that the plants have great potentials for use as complementary medicines to antibiotics or also as alternatives. Further

clinical evaluation of the effectiveness of *Allium species* in *in vivo* experiment is recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Nnamchi CI, Okefum VE, Amaechi CD, Ugwu K, Nsofor CA. Antibacterial and Synergistic Effects of Extracts of Allium cepa, Allium sativum, Zingiber officinale and Garcinia kola on Selected Bacterial Strains. Tropical Journal of Natural Product Research. 2021; 5(4):763-771
- Nnamchi CI, Nwosa IB. (2013) Preliminary evaluation of the synergistic effects of ethanol extracts of garlic and some conventional antimicrobial agents on bacterial isolates from HVS. Plant Product Research Journal. 16: 54 – 63.
- 3. World Health Organization. Antimicrobial resistance: global report on surveillance [Online]. 2014 [cited 2021 Jan 26]. Available from: https://www.who.int/drugresistance/docum ents/surveillancereport/en/
- 4. World Health Organization, Antimicrobial resistance. [Online]. 2020 [cited 2021 Jan 30]. Available from: https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance
- Nnamchi CI, Mbah OB, Ugwu K. (2020). Preliminary assessment of the antimicrobial susceptibility pattern of some halitosis-causing oral bacterial isolates to extracts of the plant Garcinia kola. South

Asian Research Journal of Natural Products 3(4): 31-40.

- Sherifat OE, Al-Hassan AA, Abdulameen SA, Ramatu A, Mary UO. Antibacterial Activities of the Extracts of Allium sativum (Garlic) and Allium cepa (Onion) Against Selected Pathogenic Bacteria. Tanzania Journal of Science 2020;46(3):914-922
- Kalu AU, Nwankwo EO, Odoh CK. In Vitro Antimicrobial Activity of Polyporus Alveolaris on Clinical Pathogens. Journal of Current Biomedical Research. 2017;1(1):47-54.
- Ponmurugan K and Shyamkumar R. Antibacterial effect of Allium sativum cloves and Zingiber officinale rhizomes against multiple-drug resistant clinical pathogens. Asian Pac. J Trop Biomed. 2012; 2:597-601.
- 9. Price LB, Hungate BA, Koch BJ, Davis GS, Liu CM. Colonizing opportunistic pathogens (COPs): the beast in all of us. PLoS Pathog. 2017; 13 (8): e1006369.
- 10. Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. Clin. Microbiol. Infect. 2016; 22 (5): 416-422.
- Wise R, Blaser M, Carrs O, Cassell G, Fishman N, Guidos R, Levy S, Powers J, Norrby R, Tilottson J, Davies R, Projan S, Dawson M, Monnet D, Keogh-Brown M, Hand K, Garner S, Findlay D, Morel C, Bax R, Burke F, Chopra I, Czaplewski L, Finch R, Livermore D, Piddock LJV, White T. The urgent need for new antibacterial agents. J. Antimicrob. Chemother. 2011; 66(9): 1939-1940.
- Adeshina GO, Jibo S, Agu VE, Ehinmidu JO. Antibacterial activity of fresh juices of Allium cepa and Zingiber officinale against multidrug resistant bacteria. International Journal of Pharma and Bioscience. 2011;2:289–295.
- Rakhi NK, Tuwani R, Mukherjee J, Bagler G. Data-driven analysis of biomedical literature suggests broad-spectrum benefits of culinary herbs and spices. PLoS ONE. 2018; 13:e0198030.
- Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: extraction, isolation and identification of bioactive compounds from plants extracts. Plants (Basel) 2017;6: 42-65.

- Akpi Uchenna Kalu, Okobo Uchenna Jude, Ebeifenadi Uche Jonathan. Antimicrobial activity of whole fruit body of Auricularia auricular on clinical pathogenic bacteria and fungi. European Journal of Biotechnology and Bioscience 2020;(8)4:33-38.
- Gupta R, Thakur B, Singh P, Singh HB, 16. Sharma VD. Katoch VM and Chauhan. SVS. Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant Mvcobacterium tuberculosis isolates. Indian J. Med. Res. 2010:131:809-813.
- 17. Gangadhar M, Shraddha K, Ganesh M. Antimicrobial screening of garlic (Allium sativum) extracts and their effect on glucoamylase activity in-vitro. J. Appl. Pharm. Sci. 2012;2(1):106-108.
- Lekshmi NCJP, Viveka S, Jeeva S, Raja BJ. Efficacy of crude extracts of Allium sativum and Allium cepa against human pathogens. Adv Appl. Sci. Res. 2015a; 6 (1): 72-78.
- National Committee for Clinical Laboratory Standards (now CLSI): Reference method for broth dilution susceptibility testing of bacteria and yeasts: Approved standard. NCCLS document M35-A. 2002, National Committee for Laboratory Clinical Standards, Wayne, PA.
- Khan WS, Shah SZ, Khan MS, Akhtar N, Munir I, Khan H. A preliminary phytochemical screening of medicinal plants: a case study of selected plant species at three phenological stages. Pak. J. Weed Sci. Res. 2016; 22 (2): 329-352.
- Auwal MS, Saka S, Mairiga IA, Sanda KY, Shuaibu A, Ibrahim A. Preliminary phytochemicals and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica. Vet. Res. Forum 2014;5(2):95-100.
- 22. Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan. Sci. World J. 2017:1-7.
- Gashe F, Mulisa E, Mekonnen M, Zeleke G. Antimicrobial resistance profile of different clinical isolates against third-

generation cephalosporins. J Pharm. 2018; 9:5070742.

 Chaudhary HS, Yadav J, Shrivastava AR, Singh S, Singh AK, Gopalan N. Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city of central India). J Adv Pharm Technol Res. 2013;4(2):118-123.

DOI: 10.4103/2231-4040.111528

- Natta L, Orapin K, Krittika N, Pantip PB. Essential oil from Zingi beraceae for antifood borne bacteria. Int Food Res J. 2008;15:337-346.
- 26. Mahmoudi M, Mercer J, Bennett M. DNA damage and repair in atherosclerosis. Cardiovascular research. 2006;71(2):259-68.
- Bordia A, Verma SK, Srivastava KC. Effect of ginger (Zingiber officinale Rosc.) and fenugreek (Trigonella foenumgraecum L.) on blood lipids, blood sugar and platelet aggregation in patients with coronary artery disease. Prostaglandins, leukotrienes and essential fatty acids. 1997;56(5):379-384.
- Benavides GA, Squadrito GL, Mills RW, Patel HD, Isbell TS, Patel RP, Darley-Usmar VM, Doeller JE, Kraus DW 2007 Proc Natl Acad Sci USA 104:17977–17982.
- 29. Banerjee SK, Maulik SK. Effect of garlic on cardiovascular disorders: a review. Nutrition journal. 2002;1(1):1-4.
- Dos Santos-Neto LL, de Vilhena Toledo MA, Medeiros-Souza P, de Souza GA. The use of herbal medicine in Alzheimer's disease-a systematic review. Evid Based Complement Alternat Med. 2006;3(4):441-445.

DOI: 10.1093/ecam/nel071

- Fukao T, Fukuda Y, Kiga K, Sharif J, Hino K, Enomoto Y, Kawamura A, Nakamura K, Takeuchi T, Tanabe M. An evolutionarily conserved mechanism for microRNA-223 expression revealed by microRNA gene profiling. Cell. 2007; 129 (3):617-631.
- Hsing AW, Chokkalingam AP, Gao YT, Madigan MP, Deng J, Gridley G, Fraumeni Jr JF. Allium vegetables and risk of prostate cancer: a population-based study. Journal of the National Cancer Institute. 2002; 94 (21):1648-6451.

- Lee CM, Huxley RR, Wildman RP, Woodward M. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. Journal of Clinical Epidemiology. 2008; 61 (7): 646-653.
- Kumar D, Michaels MG, Morris MI, Green M, Avery RK, Liu C, Danziger-Isakov L, Stosor V, Estabrook M, Gantt S, Marr KA. Outcomes from pandemic influenza A H1N1 infection in recipients of solid-organ transplants: a multicentre cohort study. The Lancet infectious diseases. 2010; 10 (8): 521-526.
- Ogita A, Fujita KI, Taniguchi M, Tanaka T. Enhancement of the fungicidal activity of amphotericin B by allicin, an allyl-sulfur compound from garlic, against the yeast Saccharomyces cerevisiae as a model system. Planta medica. 2006; 72 (13): 1247-1250.
- Jonkers DM, Van den Broek E, Van Dooren I, Thijs C, Dorant E, Hageman G, Stobberingh E. Antibacterial effect of garlic and omeprazole on Helicobacter pylori. Journal of Antimicrobial Chemotherapy. 1999; 43 (6): 837-839.
- Perez HA, De la Rosa M, Apitz R. In vivo activity of ajoene against rodent malaria. Antimicrobial agents and chemotherapy. 1994; 38 (2): 337-339.
- Gull I, Saeed M, Shaukat H, Aslam SM, Samra ZQ, Athar AM. Inhibitory effect of Allium sativum and Zingiber officinale extracts on clinically important drug resistant pathogenic bacteria. Annals of clinical microbiology and antimicrobials. 2012; 11(1):1-6.
- Roy J, Shakleya DM, Callery PS, Thomas JG. Chemical constituents and antimicrobial activity of a traditional herbal medicine containing garlic and black cumin. African Journal of Traditional, Complementary and Alternative Medicines. 2006;3(2):1-7.
- 40. Smith WL. Selective solubility: like dissolves like. J Chem Educ. 1977; 54: 228.
- 41. Amaro HM, Fernandes F, Valentao P, Andrade PB, Sousa-Pinto I, Malcata FX, Guedes AC. Effect of solvent system on extractability of lipidic components of Scenedesmus obliquus (M2-1) and Gloeothece sp. on antioxidant scavenging

Nnamchi et al.; SAJRM, 10(2): 32-44, 2021; Article no.SAJRM.71487

capacity thereof. Mar Drugs. 2015; 13: 6453-6471.

- 42. Seidel V. Initial and bulk extraction of natural products isolation. Mol Biol. 2012; 864:27-41.
- 43. Halim R, Danquah MK, Webley PA. Extraction of oil from microalgae for

biodiesel production: a review. Biotechnol Adv. 2012; 30:709-732.

44. Bucar F, Wube A, Schmid M. Natural product isolation how to get from a biological material to pure compounds. Nat Prod Rep. 2013; 30: 525-545.

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