Nutritional, phytochemicals, GC-MS and antibacterial activities of aqueous red Onion (*Allium cepa*) extract against *Staphylococcus aureus* and *Escherichia coli*

ABSTRACT

Background of study: Onion (*Allium cepa*) plant has been used for multiple purposes both for modern and traditional medicine. The study evaluates the atomic absorption spectroscopy (AAS), Gas chromatography–mass spectrometry (GC-MS) and antibacterial activities of aqueous red onion (*Allium cepa*) and azithromycin solution against *Staphylococcus aureus* and *Escherichia coli.*

Methodology: The AAS, GC-MS and phytochemical screening of the aqueous red onion (*Allium cepa*) extract were determined using standard procedures. Antibacterial activities were determined by agar well diffusion method. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations were determined using standard procedure.

Results: Mineral analysis shows that the minerals that were found in the red onion are: Na, Mg, Fe, Ca, K, Al and Cu. Aqueous extract of *Allium cepa* shows the presence of secondary metabolites like: saponin, tannins, alkaloids, flavonoids,, phenolic, reducing sugar, steroids etc. 56 compounds were identified using GC-MS analysis with 9, 12-octadecadienoic acid (Z,Z)- being the most abundant with peak area of 50.50% and retention time of 16.563. The results revealed that aqueous *A. cepa* extract with concentration of 250 mg/ml showed strong response against *Staphylococcus aureus* and *Escherichia coli* with zone of inhibition of 22.67±1.585 and 28.18±1.689 respectively. At 100 mg/ml, the onion extract exhibited weak and moderate response against both organisms. The azithromycin solution has zone of inhibition values that ranges from 9.25 ± 0.73 to 20.03 ± 1.16 mm for both organisms tested at 5.00 and 20.00 mg/mL respectively. The MIC values of the azithromycin solution and aqueous red onion extract for *S. aureus* and *E. coli* were 31.25, 31.25, 500 and 250 mg/mL while their MBC values were 62.50, 62.50, 1000.00 and 500.00 mg/ml respectively. MBC/MIC values indicate that azithromycin solution and aqueous red onion extract had bactericidal effects on both organisms tested. The red onion has potential as natural therapeutic agents and may prevent pathogenic diseases caused by *Staphylococcus aureus* and *Escherichia coli.*

Keywords: AAS, Antibacterial activity, GC-MS, red onions, *Escherichia coli* and *Staphylococcus aureus*

1.0 INTRODUCTION

Onion is the most routinely used ingredient in Nigeria for cooking and is also one of the commonly cultivated and consumed vegetables globally. *Allium cepa* commonly called onion belongs to the family of Alliaceae. It is commonly found in the temperate regions [1] and is the second most cultivated vegetable crop in the world. It is called Ayim (Ibibio), Ayo (Ibo), Alubasa (Yoruba) and Albasa (Hausa) [1] in Nigeria. It is an evergreen bulb that grows up to 0.6 meters in height. The bulbs are used as spice in the preparation of virtually all meals in Nigeria [1]. Red onions is also known as purple onions in some European countries with cultivars of the onion (*Allium cepa*), and have purplish-red skin and white flesh tinged with red. They are most commonly used in cooking, but the skin has also been used as a dye. Red onions tend to be medium to large in size and have a sharp flavor and eye-watering qualities. They are often consumed raw, grilled, or lightly cooked with other foods. Natural antimicrobials plant like onion extract is effective against many different bacterial species. According to Sharma et al. [2], the quecertin chemical found in red onions is primarily responsible for the red onion extract's ability to suppress the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus cereus*. In Addition, Kabrah et al. study shows that different concentrations of red onion extract displayed different antibacterial effects on *Pseudomonas aeruginosa* and *Klebsiella Spp* [3].

Researchers are using metabolomics to learn about the chemical make-up of biological materials. Currently, the complex plant metabolite is primarily resolved using gas chromatography mass spectrometry (GC/MS) and ultra-performance liquid chromatography-mass spectrometry (UPLC/MS). GC/MS is most suited for the analysis of volatiles, which determine a plant's scent, whereas UPLC/MS favour's the analysis of non-volatile polar or semi-polar metabolites. As volatiles release results from the breakdown of the non-volatile precursors, i.e. glycosides exclusively detectable with UPLC/MS, GC/MS does in fact provide complementary data to UPLC/MS analysis for identifying Allium aroma [4]. Some of our previous study have shown the GC/MS analysis of some different medicinal plants with their compounds, molecular formulae, molecular weights, retention time and peak area (%) [5-13].

Staphylococcus aureus is a gram positive bacterium and they are found on human mucous membranes and skin. They are also found in other areas of human contact including; food products, water and soil [13]. They cause wide range of infections in animals and human. They causes serious of infections like osteomyelitis, endocarditis, bacteremia, septicemia, pneumonia,

wound sepsis, septic arthritis, bone and joint infections, food poisoning and toxic shock syndrome [13]. "*Escherichia coli* is a gram negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded animals. *E. coli* causes severe infectious diseases associated with high rates of mortality and morbidity" [13]. The aim of the present study is to evaluate the nutritional, phytochemical, GC-MS and antibacterial activities of aqueous red Onion (*Allium cepa*) extract against *Staphylococcus aureus* and *Escherichia coli*.

2. METHODOLOGY

2.1 Collection and Identification of Plant Extract

The red onion was purchased from Ikorodu market and was authenticated by Dr. Johnson O. Momoh from Department of Chemical Sciences (Biochemistry Unit), Lagos State University of Science and Technology.

2.2 Mineral analysis of the red onion (*Allium cepa)*

Mineral analysis of the red onion was determined using method described by Momoh et al. [13]. Two gram of the red onion was digested with 10 ml of aqua regia (HNO_3 and HCl in the ratio 1:3) and the mixture was heated on porcelain crucible until the brown fumes disappeared leaving white fumes. It was later filtered with Whatman filter paper into universal bottle. The mineral elements in the sample were determined by Atomic Absorption Spectrophotometer (Model AGILENT 720 ICP-OES). The minerals that were analyzed are: Mg, Fe, Ca, K, Al, Cu, Pb, Ba, As, Co and Ti.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the red onion (*Allium cepa*)

GC-MS analysis of the red onion was carried out on an Agilent technology 7890 GC system equipped with a mass spectrometric detector (MSD) as described by Momoh et al. [12].

Detection of components

Analysis of mass spectrum GC-MS was conducted by the database of the National Institute Standard and Technique (NIST) which contained more than 62,000 patterns. The spectrum of the unidentified compound was compared with the spectrum of the identified compounds stored in

the National Institute Standard and Technique library. The names, molecular weight, structure of the compounds in the test material were ascertained.

2.4 Preparation of aqueous red onion (*Allium cepa)* extract

The aqueous red onion extract was prepared according to the method described by Momoh et al. [14]. The red onion bulbs were cleaned with water to remove any adhering soil on their outer surfaces. 100 g of the red onion were taken after removal of their outer skin surfaces and cut into small pieces by sterile scalpel. The small pieces were blended with 200 ml sterile distilled water using sterile blender for 5 min at medium speed. The homogenized mixture was filtered through white cloth, centrifuged at 2000 × g for 10 minutes and the clear supernatant was used for the experiment. The filtered extract was used for the study within three hours of preparation.

2.5 Qualitative phytochemical analysis of aqueous red onion (*Allium cepa)* extract

Phytochemical analysis for secondary metabolites constituents were carried out on the aqueous red onion extract using standard phytochemical procedures [13, 15, 16].

2.6 Test organisms

To study the antimicrobial activity of aqueous *Allium cepa* extract, two bacterial strains (*Staphylococcus aureus* a gram positive bacterium, clinical isolates ATCC #6538 and *Escherichia coli* a gram negative bacterium ATCC #25922) were used for the study. The two microorganisms were maintained at 4°C on nutrient agar slant in the Department of Chemical Sciences (Biochemistry Laboratory) and fresh sub cultures were made before use.

2.7 Inoculum preparation

A loopful of isolated colonies of the two different organisms were inoculated into separate tubes containing 4 ml of peptone water and later incubated at 37°C for 4 hours. These actively growing bacterial suspensions were then adjusted with peptone water to obtain turbidity visually comparable to that of 0.5 McFarland standards using standard procedure [13]. The 0.5 McFarland standard was prepared by mixing 0.5ml of 1.75% (w/v) barium chloride dehydrate ($BaCl_2$. $2H_2O$) with 99.5 ml of 1% (v/v) H_2SO_4. This turbidity was equivalent to approximately 1 x 10^8 colony forming units per ml (CFU/ml) [13].

2.8 Determination of diameter of zone of inhibition using agar well diffusion method

Agar well-diffusion method was employed to determine the antimicrobial activity of aqueous red onion (*Allium cepa)*. Eighteen hours of broth culture of the two different microorganisms (*Staphylococcus aureus* and *Escherichia coli*) were suspended into the sterile nutrient broth. It was standardized by gradually adding 9% normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1 x 10^8 colony forming units per ml. Petri-dishes were prepared by loading 25 ml of an autoclaved nutrient agar on sterile plates and left to solidify after one hour. Then, the surface of each plate was drilled using a sterile cork borer (6 mm) and 3 wells were punched out on each plate. 0.1 ml of a standardized culture (adjusted to 0.5 McFarland) of the two organisms were added into the different agar plates followed by loading of 0.1 ml of the aqueous red *Allium cepa* extract in the wells and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 37ºC for 18-24 hours for bacterial pathogens. The diameters of the inhibition zone (mm) were measured. The susceptibility of the two different organisms to aqueous red onion extract was assayed using standard method [13]. The experiment was repeated thrice, for each replicate, the readings were taken in three different fixed directions and the average values were recorded. The inhibitory responses were classified as potent response, ++++, zone diameter >30 mm; strong response, +++, zone diameter between 21-30 mm; moderate response, ++, zone diameter between 16-20 mm; weak response, +, zone diameter between 10-15 mm; and little or no response, zone diameter <10 mm [13].

2.9 Minimum inhibitory concentration (MIC) of aqueous red onion (*Allium cepa)*

Minimum inhibition concentration is the lowest aqueous red onion extract concentration that inhibited the growth of the test organism as indicated by the absence of visible turbidity in the tube compared with the control tubes. The MIC of aqueous red onion extract was determined according to standard method using procedure described by Momoh et al. [13]. The MIC of the aqueous red onion extract was assayed using serial dilution method. A total of 1 ml of Mueller-Hinton broth agar was poured to a set of different test tubes and autoclaved. Subsequently, 1 ml of 100% aqueous red onion extract (2g/ml) was poured to the first test tube to make a concentration of 50%, and two-fold serial dilutions were made by transferring 1 ml from one tube to another. Then, an overnight broth culture of the different test organisms were adjusted to McFarland turbidity standard and 0.1 ml of the different cell suspensions were added to each of the separate test tubes. The test tubes were incubated aerobically at 37°C for 18 hours. Negative

control tube was made by pouring 1ml of normal saline instead of the aqueous red onion extract. The lowest concentration of the dilution without bacterial growth was considered as the minimum inhibition concentration.

2.10 Minimum Bactericidal Concentration (MBC) of aqueous red onion (*Allium cepa)*

The MBC of the aqueous red onion (*Allium cepa)* extract was carried out by standard method described by Momoh et al. [13]. In the procedure, 0.1 ml aliquots of test samples taken from the non-turbid tubes of the minimum inhibition concentration assay tubes were sub-cultured onto nutrient agar plates. The resulting plates were then incubated aerobically at 37°C for 24 hours. The lowest concentration of the aqueous red onion extracts at which there were no colonies of *Staphylococcus aureus* and *Escherichia coli* were taken as the minimum bactericidal concentrations. The results were compared with that of control tube using sterilized distilled water. The MBC was taken as the concentration of the aqueous red onion that did not show any growth on a new set of agar plates. The lowest MIC value that revealed no visible growth was regarded as the minimum bactericidal concentration. The MBC/MIC value was also calculated as bactericidal or bacteriostatic.

2.11 Statistical Analysis

Experiments were performed in triplicate and results were expressed as mean ± SD. The data analysis was done using one way analysis of variance (ANOVA) Post Hoc Turkey Graph Pad prism computer software version 5.01. *P* values < 0.05 were considered significant.

3.0 RESULT

3.1 Mineral analysis of red onion

The minerals that were found in the red onion are: Na, Mg, Fe, Ca, K, Al, Cu, Cd, Pb, Ba, Cr, Ag, As, Co and Ti.

Table 1. **Mineral composition of the red onion.**

Elements	Conc. in mg/L	%RSD
Na	0.00201±0.0001	1.06
Mg	2.1655±0.001	0.29
Al	0.00064±0.00001	12.50
Ca	0.05687±0.0001	2.41
K	0.8525±0.001	0.57
Cu	0.0060±0.00001	0.32
Fe	0.88812±0.0001	0.55

Cr	0.00557±0.00001	1.96
Ag	ND	0.90
Pb	ND	0.33
Cd	ND	0.71
Ba	ND	1.11
As	ND	2.73
Co	ND	0.61
Ti	ND	0.19

ND mean not detected

3.2 Result for Gas-Chromatography–Mass Spectrometry of the red onion

The Gas-Chromatography–Mass Spectrometry chromatogram and the compounds found in the red onion are shown in Figure 1 and Table 1 below.

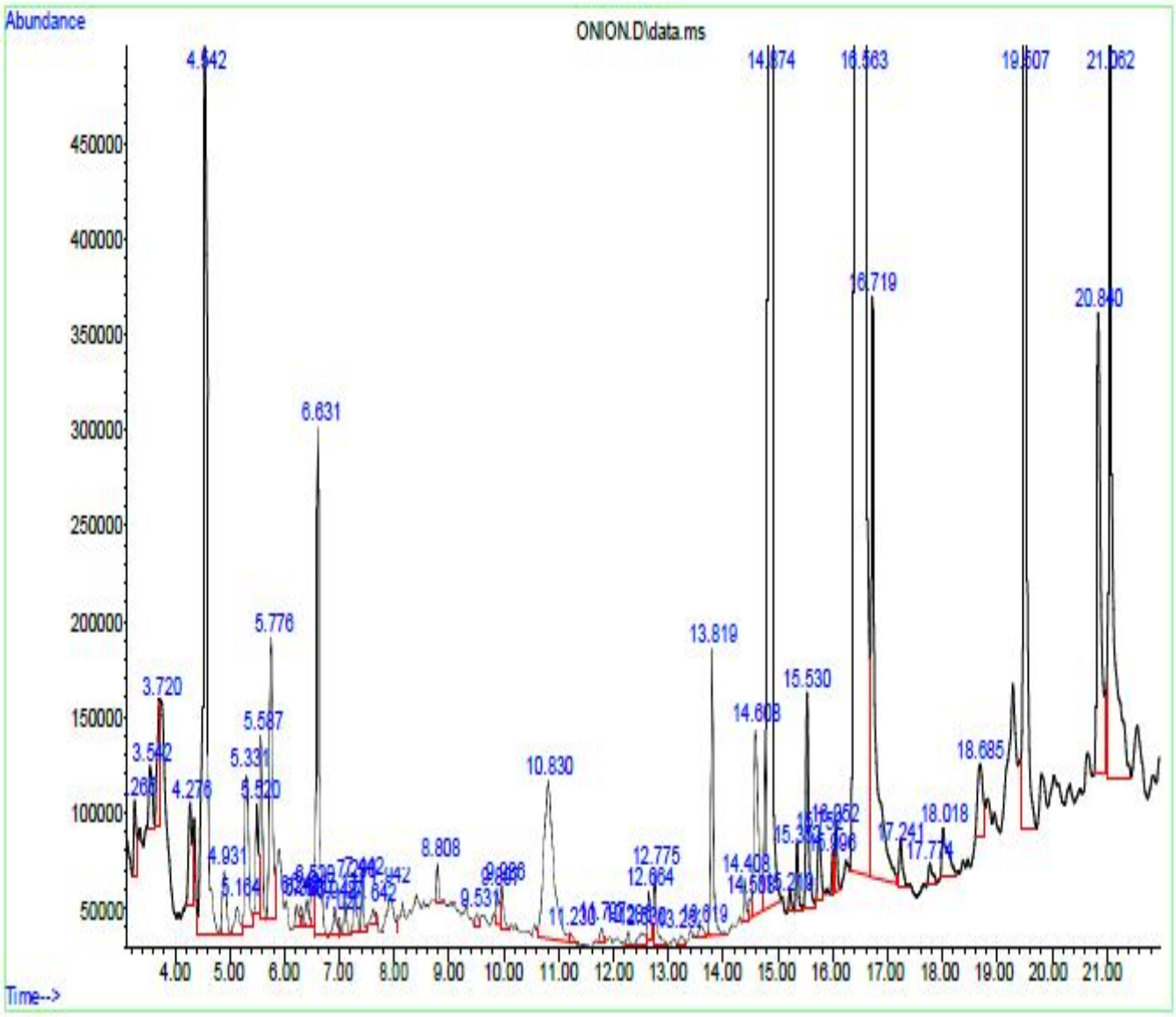


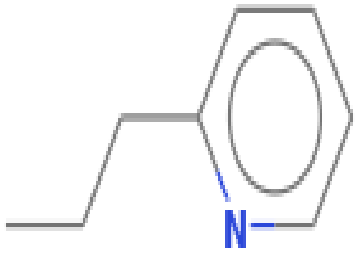
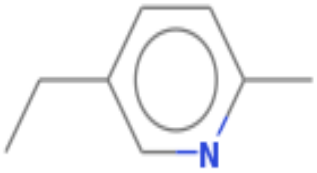
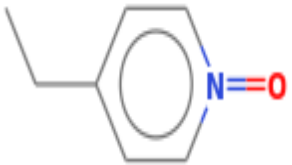
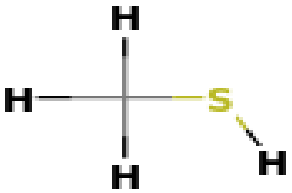
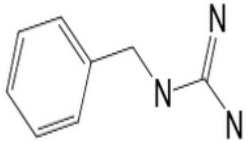
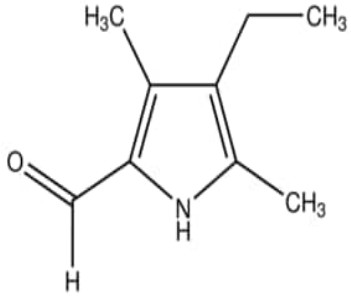
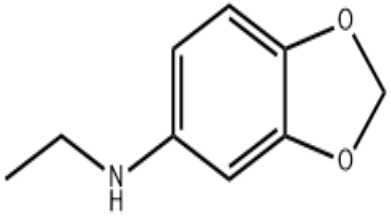
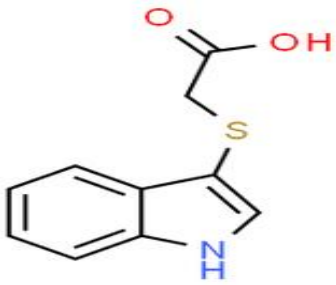
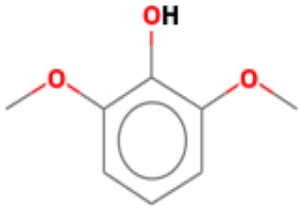
Figure 1: **Gas-Chromatography–Mass Spectrometry chromatogram of the red onion.**

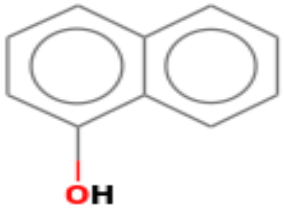
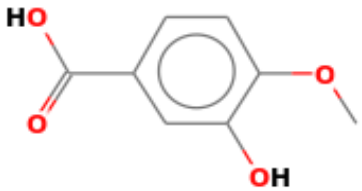
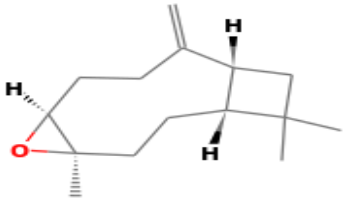
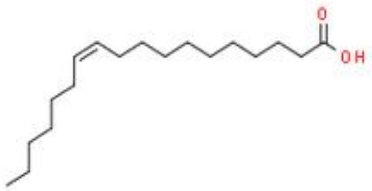
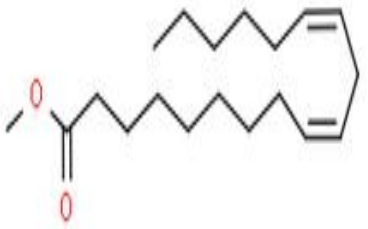
Table 2: Compounds found in the red onion analyzed using Gas-Chromatography–Mass Spectrometry

Pk#	RT	Name of the compound	Molecular Formulae	Molecular Weight (g/mol)	Peak Area (%)	Ref#	CAS#	Qual
1	3.265	Pyridine, 2-propyl-	$C_8H_{11}N$	121.1796	0.27	9751	000622-39-9	70
2	3.542	1H-Pyrrole-2-acetonitrile, 1-methyl-	$C_7H_8N_2$	120.15	0.30	9526	024437-41-0	30
3	3.720	Pyridine, 5-ethyl-2-methyl-	$C_8H_{11}N$	121.1796	0.55	9811	000104-90-5	38
4	4.276	Pyridine, 4-ethyl-, 1-oxide	C_7H_9NO	123.1525	0.47	10387	014906-55-9	50
5	4.542	Methanethiol	CH_4S	48.107	6.11	10419	069687-78-1	87
6	4.931	Phenol, 3-amino-4-methyl-	C_7H_9NO	123.15	0.36	10368	002836-00-2	52
7	5.164	2-(3-Pentyl)pyridine	$C_{10}H_{15}N$	149.23	0.17	23990	007399-50-0	38
8	5.331	1H-Pyrrole, 3,4-diethyl-2-methyl-	$C_9H_{15}N$	137.22	0.80	17220	034874-30-1	86
9	5.520	Naphthalene	$C_{10}H_8$	128.174	0.29	12196	000091-20-3	80
10	5.587	1H-Pyrrole, 2-ethyl-3,4,5-trimethyl-	$C_9H_{15}N$	137.22	0.83	17223	069687-79-2	90
11	5.776	Propanethiol	C_3H_8S	76.16	1.33	17222	000520-69-46	91
12	6.242	Guanidine, (phenylmethyl)-	$C_8H_{11}N_3$	149.095297	0.09	24162	002211-57-6	46
13	6.320	Phenol, 4-methylamino, ethyl(ether	$C_9H_{13}NO$	151.21	0.08	25744	003154-18-5	38
14	6.431	cyclopropane, 1-(1'-propenyl)-2-hydroxymethyl-	$C_7H_{12}O$	112.17	0.12	6738	1000197-30-5	42
15	6.520	1-Nitro-3-n-hexylbenzene	$C_{12}H_{17}NO_2$	207.27	0.15	71433	127118-85-8	49
16	6.631	4-Ethyl-3,5-dimethyl-1H-pyrrole-2-carboxaldehyde	$C_9H_{13}NO$	151.21	2.08	25769	006250-80-2	72
17	6.942	Benzene, 1-(1,1-dimethylethoxy)-4-methyl-	$C_{11}H_{16}O$	164.24	0.13	34967	015359-98-5	55
18	7.020	Butanoic acid, 3-[(1-phenylethyl-2-propynyl)oxy]	$C_{15}H_{18}O_3$	246.30	0.05	107857	1000196-79-9	38
19	7.131	3-Ethylphenyl isocyanate	C_9H_9NO	147.17	0.11	23169	023138-58-1	42
20	7.298	Phenol, 4-amino-3,5-diethyl	$C_{10}H_{15}NO$	165.23	0.21	35681	108451-25-8	89

21	7.442	N-Ethyl-3,4-(methylenedioxy)aniline	$C_9H_{11}NO_2$	165.19	0.18	36068	032953-14-3	68
22	7.642	2-(1H-indol-3-ylthio) acetic acid	$C_{10}H_9NO_2S$	207.249	0.06	71224	054466-88-5	46
23	7.942	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	154.1632	0.33	28298	000091-10-1	38
24	8.808	Quinaldic acid, 1,2,3,4-tetrahydro-8-hydroxy-4-oxo-, L-	$C_{10}H_9NO_4$	207.18	0.12	71263	004886-42-4	49
25	9.531	1H-Indole, 5-methyl-2-phenyl-	$C_{15}H_{13}N$	207.27	0.05	71661	013228-36-9	43
26	9.897	1-Naphthalenol	$C_{10}H_8O$	144.1699	0.14	20929	000090-15-3	43
27	9.986	3-Hydroxy-4-methoxybenzoic acid, methyl ester	$C_9H_{10}O_4$	182.1733	0.18	50230	006702-50-7	55
28	10.830	3-Hydroxy-4-methoxybenzoic acid	$C_8H_8O_4$	168.146	2.16	38787	000645-08-9	87
29	11.230	1H-Isoindole-1,3(2H)-dione, 2-buty l-4,5,6,7-tetrahydro-	$C_{12}H_{17}NO_2$	207.27	0.06	71546	054934-85-9	30
30	11.797	5-Acetamido-4,7-dioxo-4,7 dihydrobenzo furazan	$C_8H_5N_3O_4$	207.14	0.06	71759	153136-27-7	51
31	12.286	Aromadendrene oxide-(1)	$C_{15}H_{24}O$	220.3505	0.06	83545	1000151-98-4	43
32	12.530	2,5,5,6,8a-Pentamethyl-trans-4a,5, 6,7,8,8a-hexahydro-gamma-chromene	$C_{14}H_{24}O$	208.34	0.10	72665	1000215-77-8	44
33	12.664	3H-3a,7-Methanoazulene, 2,4,5,6,7,8-hexahydro-1,4,9,9-tetramethyl-, [3aR-(3a.alpha.,4.beta.,7.alpha.)]	$C_{15}H_{24}$	204.3511	0.16	68862	002387-78-2	70
34	12.775	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.370	0.32	91415	000544-63-8	95
35	13.252	Caryophyllene oxide	$C_{15}H_{24}O$	220.3505	0.06	83536	001139-30-6	38
36	13.619	Pyrido[2,3-d]pyrimidine, 4-phenyl-	$C_{13}H_9N_3$	207.23	0.08	71621	028732-75-4	30
37	13.819	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.3975	1.08	104282	001002-84-2	97
38	14.408	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	0.15	130820	000112-39-0	86
39	14.508	1,2,5-Oxadiazol-3-amine, 4-(3-methoxy phenoxy)-	$C_9H_9N_3O_3$	207.19	0.07	71817	1000351-72-8	49
40	14.608	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282. 46136	1.12	142073	000506-17-2	90
41	14.874	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4241	10.06	117419	000057-10-3	99
42	15.219	1,E-8,Z-10-Hexadecatriene	$C_{16}H_{28}$	220.39	0.07	83741	080625-33-8	53
43	15.352	11,13-Dimethyl-12-tetradecen-1-olacetate	$C_{18}H_{34}O_2$	282.4614	0.24	142133	1000130-81-0	64
44	15.530	2(1H)-Naphthalenone, octahydro-4a-methyl-	$C_{14}H_{24}O$	208.34	0.84	72729	022089-89-0	93

		7-(1-methylethyl)-, (4a.alpha., 7.beta., 8a.beta.)-						
45	15.752	2-Myristynoyl-glycinamide	$C_{16}H_{28}N_2O_2$	280.41	0.33	139899	1000111-57-7	35
46	15.996	Methyl octadecadienoate	$C_{19}H_{34}O_2$	294.4721	0.12	153842	1000336-43-1	98
47	16.052	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.4721	0.22	153892	000112-63-0	95
48	16.563	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4455	50.50	140139	000060-33-3	99
49	16.719	Thiopropanal S-Oxide	C_3H_6OS	90.144	3.43	144272	000057-11-4	99
50	17.241	Alpha.-Santonin	$C_{15}H_{18}O_3$	246.30	0.22	108109	121732-53-4	62
51	17.774	2-Myristynoyl-glycinamide	$C_{16}H_{28}N_2O_2$	280.41	0.11	139899	1000111-57-7	58
52	18.018	5-Acetamido-4,7-dioxo-4,7-dihydrobenzo furazan	$C_8H_5N_3O_4$	207.14	0.40	71759	153136-27-7	38
53	18.685	3-methyl-N-(5-methyl-4,5-dihydro-1,3-thiazol-2-yl)11yridine-2-amine	$C_{10}H_{13}N_3S$	207.30	0.56	71191	1000362-45-9	42
54	19.507	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330.5026	4.54	188252	023470-00-0	49
55	20.840	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	354.5	2.55	208883	002277-28-3	95
56	21.062	Octadecanoic acid	$C_{18}H_{36}O_2$	284.4772	4.76	144268	000057-11-4	45

 Structure of pyridine, 2-propyl-	 Structure of pyridine, 5-ethyl-2-methyl-	 Structure of pyridine, 4-ethyl-, 1-oxide
 Structure of methanethiol	 Structure of guanidine, (phenylmethyl)-	 Structure of 4-Ethyl-3,5-dimethyl-1H-pyrrole-2- carboxaldehyde
 Structure of N-Ethyl-3,4-(methylenedioxy) aniline	 Structure of 2-(1H-indol-3-ylthio) acetic acid	 Structure of phenol, 2,6-dimethoxy-

 Structure of 1-Naphthalenol	 Structure of 3-Hydroxy-4-methoxybenzoic acid	 Structure of 3H-3a,7-Methanoazulene, 2,4,5,6,7,8-hexahydro-1,4,9,9-tetramethyl-, [3aR-(3a.alpha.,4.beta.,7.alpha.)]
 Structure of tetradecanoic acid	 Structure of caryophyllene oxide	 Structure of pentadecanoic acid
 Structure of cis-Vaccenic acid	 Structure of n-hexadecanoic acid	 Structure of methyl octadecadienoate

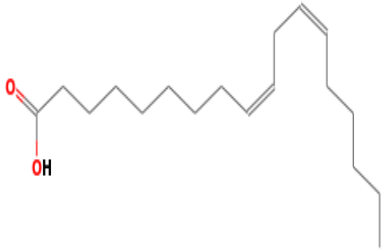
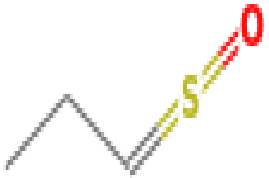
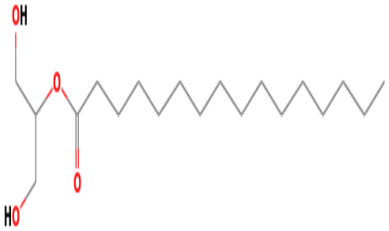
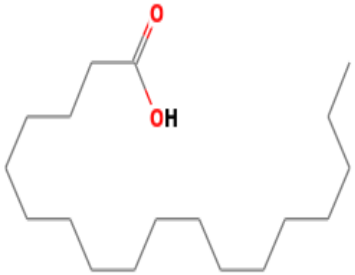
 Structure of 9,12-Octadecadienoic acid (Z,Z)-, methyl ester	 Structure of 9,12-Octadecadienoic acid (Z,Z)-	 Structure of thiopropanal S-Oxide
 Structure of hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	 Structure of octadecanoic acid	

Figure 2. Structure of some different compounds obtained during GC-MS analysis of the red onion.

Table 3: Qualitative phytochemical constituents of aqueous red onion extract

The results of the phytochemical screening showed that; flavonoids**,** reducing sugar, carbohydrate, protein, cardiac glycosides, steroids, saponins, tannins, phenolic compounds, polyphenol and alkaloid were present.

Table 3: Qualitative phytochemical constituents of aqueous red onion extract

Phytochemical constituent	Inference
Alkaloids	+
Tannins	+
Saponins	+
Flavonoids	+
Polyphenol	+
phenolic compounds	+
Anthranoid	-
Antraquinone	-
Protein	+
Reducing sugar	+
Carbohydrates	+
Cardiac glycosides	+
Steroids	+

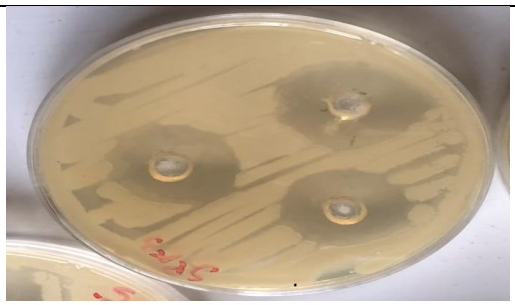


Fig. 3a. Zone of inhibition at 20 mg/ml concentration for Azithromycin solution against *Staphylococcus aureus*

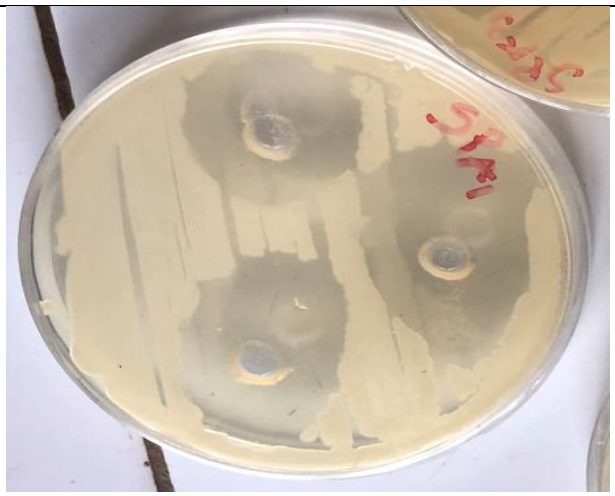


Fig. 3b. Zone of inhibition at 20 mg/ml concentration for Azithromycin solution against *Staphylococcus aureus*

Fig. 3c. Zone of inhibition at 100 mg/ml concentration for aqueous red onion extract against *Escherichia coli*

Fig. 3d. Zone of inhibition at 100 mg/ml concentration for aqueous red onion extract against *Staphylococcus aureus*

Figure 3. Zone of inhibition of azithromycin solution and aqueous red onion extract against *Staphylococcus aureus* and *Escherichia coli* was determined using agar well diffusion method.

Table 4. Zone of inhibition for aqueous red onion extract and Azithromycin solution against *Staphylococcus aureus* and *Escherichia coli*

Test organisms	Concentration of aqueous red onion extract (mg/ml)	Zone of inhibition for aqueous red onion extract (mm)	Interpretation	Concentration of azithromycin solution (mg/ml)	Zone of inhibition for azithromycin solution (mm)	Interpretation
Staphylococcus aureus	100	14.12 ± 0.64^{d}	+	5	9.25 ± 0.73^{b}	little or no response
Staphylococcus aureus	250	22.67 ± 1.59^{b}	+++	20	20.03 ± 1.16^{a}	++
Escherichia coli	100	16.16 ± 0.86^{c}	++	5	9.37 ± 0.53^{b}	little or no response
Escherichia coli	250	28.18 ± 1.69^{a}	+++	20	19.93 ± 0.93^{a}	++

Data represent means ± SD (n=3). a=highest, b= medium, d=lowest. Those alphabets that have different letters are statistically significant ($P<0.05$), while those alphabets that have the same letters are statistically not significant ($P>0.05$). The inhibitory responses were classified as strong response, +++, zone diameter between 21-30 mm; moderate response, ++, zone diameter between 16-20 mm; weak response, +, zone diameter between 10-15 mm; and little or no response, zone diameter <10

Table 5: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) for aqueous red onion extract and azithromycin solution against *Staphylococcus aureus* and *Escherichia coli*

Organisms	*Staphylococcus aureus*	*Escherichia coli*
MIC for aqueous red onion extract (mg/ml)	500	250
MIC for azithromycin solution (mg/ml)	31.25	31.25
MBC for aqueous red onion extract (mg/ml)	1000	500
MBC for azithromycin solution (mg/ml)	62.50	62.50
MBC/MIC for aqueous red onion extract	2.00	2.00
MBC/MIC for azithromycin solution	2.00	2.00

4.0 DISCUSSION

Allium cepa has been used for centuries either as raw vegetables for culinary purposes or as ingredients in traditional medicine worldwide for treatment of different diseases and infections. The present study was designed to investigate the possible antibacterial effects of aqueous red onion (*Allium cepa*) extracts on pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli*. Atomic absorption spectrometry is an analytical method used for the qualitative and quantitative determination of different chemical elements found in the red onion. The result of this study shows that magnesium (2.1655±0.001) was the most abundant element present in the red onion followed by iron (0.88812±0.0001), potassium (0.8525±0.001) and calcium (0.05687±0.0001). Study has shown that magnesium plays major roles in genomic stability and DNA repair processes. Magnesium activates over 300 different enzymes and thus participates in many metabolic processes, which makes it an important micronutrient, and also helps in electrolyte transport across cell membranes [13]. Iron is important in the formation of erythrocytes cells in the body. Calcium ion helps in the activation of numerous enzymes activities in the body [13]. Calcium and Magnesium helps in the formation of strong bone and teeth. The presence of Calcium ions help to convert prothrombin to thrombin during blood coagulation and are also used in milk clotting. Other elements like: Al, Cu, and Cr were found to be present in very small quantities that are not significant (Table 1). These elements increase the nutritional component of the red onion. Other elements like: Ag, Pb, Cd, Ba, As, Co and Ti were not detected. The extract of onion was shown to contain minerals like: calcium (222 mg/kg), magnesium (211.3 mg/kg), potassium (159 mg/kg), phosphorus (35 mg/kg), iron (0.31 mg/kg), zinc (0.21 mg/kg), manganese (180.1 mg/kg) and sodium (3.2 mg/kg) [17].

The GC-MS involves the separation of components of the sample and later using various detectors to identity different component present. The separation of the component takes place based on the difference in the affinity of various components present in the sample toward the stationary phase. The bioactive composition of the red onion was accessed using GC-MS. Figure 1 shows the Gas-Chromatography–Mass Spectrometry chromatogram of the red onion. A total of 56 compounds were identified consisting of 2 prominent compounds and 54 minor compounds (Table 2) using GC-MS analysis with 9, 12-octadecadienoic acid (Z,Z)- (also called alpha-linoleic acid) being the most abundant with peak area of 50.50% and retention time of 16.563.

The high concentration of (Z,Z)-9,12-octadecadienoic acid found in the aqueous extract of red onion may be responsible for the antibacterial activities of the plant because different research have shown that the compound have inhibitory action against some bacterial species [18, 19]. Z, Z-9, 12-Octadecadienoic acid was found to be the most abundant component, both in the light petroleum and aerial parts and dichlomethane extracts of *Helleborus bocconei Ten.* subsp. and the study shows that the compound (Z, Z-9,12-Octadecadienoic acid) may be responsible for its antibacterial activities of the extracts [20]. Rossellia et al. [20] study also indicates that the second most abundant compound in the extracts was n-Hexadecanoic acid and maybe responsible for its antibacterial activities. This is a clear positive indication that that the antibacterial activities of aqueous red onion against *Staphylococcus aureus* and *Escherichia coli* may be due to the presence of Z, Z-9,12-Octadecadienoic acid and n-Hexadecanoic acid since they constitute 60.56% of the constituent of the extract of red onion. Momoh et al. [5] study shows that 9, 12-octadecadienoic acid (Z,Z)- possess hepatoprotective, hypocholesterolemic, antiarthritic, anti-inflammatory, anti-histaminic and nematicidal activities. n-Hexadecanoic acid ($C_{16}H_{32}O_2$) is the second most abundant compound found in the red onion with peak area of 10.06% and retention time of 14.874. The compound has antibacterial and antifungal activities [21]. Momoh et al. [5] study shows that n-Hexadecanoic acid has antibacterial, antifungal, and anti-inflammatory activities. Powered red onion extracted with six different organic solvents (methanol, acetone, chloroform, benzene, petroleum ether and ethyl acetate) were analyze for their chemical constituent using GC-MS. A total of 43 compounds were identified by GC-MS analysis and out of them dodecanoic acid was found common in all the extracts [22]. Yadav et al. [22] study also shows that the extracts contained 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, Hexadecanoic acid and Hexadecanoic acid, methyl ester which were found in our study and the extracts poses antibacterial activities. Machová et al. [23] study shows that 19 organosulfur compounds were detected with a different course of release. The most abundant sulfur-containing substance for the onion analyze using HS-SPME/GC-MS was thiopropanal S-oxide, followed by methylprop(en)yldisulfide and prop(en)yltrisulfide respectively. "GC-MS analysis of essential onion oil shows that it contain 22 compounds with dipropyl disulfide and dipropyl trisulfide were the most representative compounds found in the essential oils of onion" [24].

The use of medicinal plant extracts is nowadays essential in the search for new active antibacterial biomolecules agents. *Allium cepa* extract acts through the presence of potentially

bioactive components such as alkaloids, phenol, glycosides, flavonoids, and tannins etc. The results of the phytochemical screening shows that; flavonoids**,** reducing sugar, carbohydrate, protein, cardiac glycosides, phenolic compounds, steroids saponins,, tannins, polyphenol and alkaloid were present. Anthranoid and antraquinone were absent in the aqueous red onion extract (Table 3). Okonkwo and Achilike [25] study shows that the results of the phytochemical screening of onion contain; cardiac glycosides, flavonoid, terpernoids, carbohydrate, polyphenol, saponins, phlobatannins, anthraquinone and alkaloid were present. Ogbonna et al. [17] results revealed that "*Allium cepa* extract contains flavonols like quercetin, pigments like anthocyanin and fructan in higher concentrations while diallylsulfide, thiosulfinate and tannins in moderate concentrations. Lower concentrations of glycosides, alkaloids, saponin, citric acid, myritic acid, ferulic acid, glutamic acid and malic acid were also observed. The red Onion used in this study contains flavonoids and phenolic compounds". Study has shown that "flavonoids are second class of health enhancing chemical compounds active against microorganisms; they have been found in-vitro to be effective antimicrobial substances against a wide range of microorganisms" [26]. "It has been reported that phenolic compounds may incorporate into lipid monolayers of gram-positive microorganism which may led to increase in the membrane fluidity. In addition, phenolic compounds may interrupt the lipid–protein complexes and increase membrane permeability, affecting their physiology and metabolism resulting to cell death" [27]. Studies have suggested that "the antimicrobial components of the plant extracts (phenolic compounds, terpenoid and alkaloid) interact with enzymes and proteins of the microbial cell membrane causing its disruption to disperse flux of protons towards cell exterior which induces cell death or may inhibit enzymes necessary for amino acids biosynthesis" [28, 29]. "Other studies attributed the inhibitory effect of these plant extracts to hydrophobicity characters of these plants extracts which enable them to react with protein of microbial cell membrane and mitochondria which disturb their structures and change their permeability" [30, 31].

Antimicrobial agents from plants are essential to provide new compounds for chemists to improve the bioactivity through continuous investigation of chemical and pharmacological activities of antimicrobial plants usefulness in discovering new drugs to overcome resistance bacteria against antibiotics that are already established. In the present study, the aqueous red onion extract exhibited strong response antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* with zone of inhibition of 22.67 ± 1.59 and 28.18 ± 1.69 at concentration of 250

mg/ml. *Escherichia coli* was more susceptible to the red onion extract compared to the *Staphylococcus aureus.* At concentration of 100 mg/ml, *Staphylococcus aureus* showed weak response while *Escherichia coli* showed moderate response. Azithromycin solution at 20 mg/ml, showed moderate response to both organisms and at 5 mg/ml the standard drugs showed little or no response (Table 4).

Mnayer et al. [24] study shows that "*Staphylococcus aureus* and *Listeria monocytogenes* were highly sensitive (P<0.05) to onion oil with diameters of 15.5 and 15.0 mm, respectively. *Salmonella typhimurium* and *Campylobacter jejuni* were also sensitive with inhibition zones of 12.0 and 9.0 mm, respectively while *Escherichia coli* was the only resistant bacteria to the essential onion oil". "Antibacterial activity of extracts of *Allium cepa* was analyzed against *S. aureus* and *Bacillus subtillis* and the zone of inhibition measured were 9 mm and 8 mm respectively. *Allium cepa* extract in combination with *Zingiber officinale* extract showed the zone of inhibition of 11 mm and 13 mm against *S. aureus* and *Bacillus subtillis* respectively" [32]. This result indicates that synergistic effects increase the potential of the *A. cepa*. Kim et al. [33] studied the effect of *A. cepa* extracts on oral pathogenic bacteria like: *Streptococcus sobrinus, Streptococcus mutans, Prevotella intermedia* and *Porphyromonas gingivalis*. They found that the extracts were active against all of these bacteria. In another study, antimicrobial activity of onion juice tested on 8 microorganisms using well-diffusion and disc-diffusion methods. It was observed that *Pseudomonas aeruginosa, Escherichia coli,* and *Bacillus subtilis* were resistant while the most sensitive was *Candida albicans* [23]. "*Allium cepa* methanol extract shows good antibacterial activity against different bacterial strains and the methanol extract was found most potent against *K. pneumonia* and *S. marcescens* with the zone of inhibition of 26±0.76 mm for both strains" [22].

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent to prevent visible growth of a microorganism. The MIC value was determined by the level of turbidity of the dilution of the onion extract. In this study, the MIC of azithromycin solution and red onion extract was assessed against *S. aureus* and *E. coli*. The result in Table 5 showed that the aqueous red *Allium cepa* extract showed antibacterial activity against *S. aureus* and *E. coli* with MIC and MBC values of 500, 250, 1000 and 500 mg/ml respectively while azithromycin solution has MIC and MBC values of 31.25 and 62.50 mg/ml respectively for both

organisms. Phakawan and Tepsorn [27] study shows that "the MIC values of red onion crude extract ranged from 15.00 to 19.00 % (w/v) and the MBC values ranged from 25.00 to 35.00 % (w/v) against *Salmonella Typhimurium, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* O157:H7 and *Bacillus cereus* respectively". Study has shown that calculated MBC/MIC ratio is bacteriostatic if the MBC/MIC ratio is > 4 and bactericidal if the values of MBC/MIC ratio are less than or equal to 4 [6, 13]. The aqueous red onion extract has bactericidal effects on *Staphylococcus aureus and Escherichia coli* respectively.

5. CONCLUSION

Red onion (*Allium cepa*) contains essential mineral elements and phytochemicals that possess antioxidants and other natural therapeutic agents that have antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* and may prevent pathogenic diseases caused by these bacteria. The results of this study suggest that red aqueous onion contains potential bioactive compounds that have antibacterial activities and the compounds can further be analyzed to determine the main component responsible for its action.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript

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