Phytochemical Screening, Atomic Absorption Spectroscopy, GC-MS and Antibacterial Activities of Turmeric (*Curcuma longa* L.) Rhizome Extracts

ABSTRACT

Aim: The study evaluates the phytochemical screening, atomic absorption spectroscopy (AAS), Gas chromatography–mass spectrometry (GC-MS) and antibacterial activities of aqueous and methanolic extracts of turmeric (*Curcuma longa*) rhizome against *Escherichia coli* (*E. coli*) *and Staphylococcus aureus* (*S. aureus*).

Place and Duration of Study: The study was carried out for six months in 2020 in Biochemistry Laboratory, Department of Chemical Sciences, College of Basic Sciences, Lagos State University of Science and Technology (LASUSTECH), Ikorodu, Lagos State, Nigeria.

Methodology: The phytochemical screening, GC-MS and AAS were determined using standard methods. Antibacterial activities were evaluated by disc diffusion and agar well diffusion methods. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) were determined using standard procedures.

Results: The aqueous and methanolic extracts of turmeric (*Curcuma longa*) rhizome showed the presence of phytochemicals like tannins, flavonoids, alkaloids, glycosides and saponin. Mineral composition analysis shows that the plant contains Na, Ca, Mg, K and Fe. Nineteen compounds were identified using GC-MS analysis of turmeric with a R-Turmerone being the most abundant with peak area of 50.05%. The results revealed that at 250 and 500 mg/mL for both aqueous and methanolic root extract of *C. longa* were sensitive to both organism, with zone of inhibition of 22.29±2.35, 29.56±2.23, 21.79±1.04 and 29.95±1.83 against *E. coli* and 22.31±1.59, 28.67±1.42, 22.96± 0.96 and 30.13±1.94mm against *S. aureus* respectively. Azithromycin has zone of inhibition values that ranges from 19.35±1.02 to 32.03±1.23 mm for both organisms tested at 250 and 500 mg/mL respectively. *E. coli* and *S. aureus* were susceptible to erythromycin, ciprofloxacin, roceplin, septrin and streptomycin and resistant to chloramphenicol. The MIC of the aqueous and methanolic root extract of *turmeric* on *E. coli* and *S. aureus* were 62.50, 31.25, 31.250 and 15.625 mg/mL while their MBC values were 250.00, 62.500, 62.500 and 31.2500 mg/ml respectively. MBC/MIC values show that *both* extracts had bactericidal effects.

Conclusions: *Curcuma longa* has essential minerals, phytochemicals, antibacterial activity and may prevent pathogenic diseases caused by *Escherichia coli* and *Staphylococcus aureus.*

Keywords: Antibacterial activity; Turmeric (Curcuma longa); AAS and GC-MS analyses.

1. INTRODUCTION

Curcuma longa L. is commonly called turmeric and is a member of the ginger family. Turmeric is a golden spice derived from the rhizome of the *Curcuma longa* plant, which belongs to the Zingiberaceae family [1]. *Curcuma longa* has been used as the principal ingredient of dishes used from Nigeria, India and Bangladesh for its color, flavor, and taste. In West Africa it's mainly used as a dye to color products, such as cotton cloth, tanned leather, palm fibers and thread to a golden yellow. The use of the yellow color of turmeric rhizome and other plant derivatives as dyes is on the increase toward replacing synthetic additives with natural compounds [2]. The yellow color of turmeric is due to the presence of three main curcuminoids in the rhizome namely: curcumin, demethoxycurcumin, and *bis*-demethoxycurcumin. Dry turmeric

contains: 5.1% oils, 6.3% proteins, 69.43% carbohydrates, 3.5% minerals, and other elements [3]. The bioactive chemical constituents in turmeric have been investigated. Approximately 235 compounds, primarily terpenoids and phenolics, have been identified from various species of turmeric, including 22 diarylheptanoids and diarylpentanoids, 8 phenylpropenes as well as other phenolics, 109 sesquiterpenes, 68 monoterpenes, 5 diterpenes, 4 sterols, 3 triterpenoids, 2 alkaloids, and 14 other compounds [4]. Curcuminoids (mostly curcumin) and essential oils (primarily monoterpenes) are the major bioactive constituents showing different bioactivities. Calebin-A, vanillic acid, vanillin, quercetin, and other phenolic compounds have also previously been identified from turmeric [1, 5]. Studies have shown that aqueous extract of turmeric rhizomes exhibited antibacterial activity against *S aureus* ATCC 25923, *Staphylococus epidermis* ATCC 12228, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 10031 [6, 7].

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 [8]. *Staphylococcus aureus* are Gram positive bacteria and they cause wide range of infections in human and animals. They are found on human skin and mucous membranes. However, it can also be found in other areas of human contact including soil, water, and food products [9]. They causes serious infections like bacteremia, septicemia, osteomyelitis, pneumonia, septic arthritis, wound sepsis, endocarditis, bone and joint infections, toxic shock syndrome and food poisoning [10]. The study evaluates the phytochemical screening, Gas chromatography–mass spectrometry (GC-MS), atomic absorption spectroscopy (AAS) and antibacterial activities of aqueous and methanolic extracts of turmeric (*Curcuma longa*) rhizome against *Escherichia coli and Staphylococcus aureus.*

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Extract

The turmeric was purchased from Ikorodu market and was authenticated by Momoh Johnson from Department of Chemical Sciences (Biochemistry unit), Lagos State University of Science and Technology.

2.2 Mineral Analysis of Turmeric

Two grams of turmeric was digested with 10 mL of aqua regia (Trioxonitrate (v) acid and hydrochloric acid in the ratio 1:3) and the total mixture of the plant and the acids were heated in a crucible for some minutes until brown fumes produced in the process disappeared leaving white fumes. It was then later filtered with filter paper into universal bottle. The micro and macro elements present in the turmeric sample were determined using AGILENT 720 ICP-OES Atomic Absorption Spectrophotometer (AAS). The minerals that were analyzed for were; Ca, Fe, K, Na, Mg, Cu, Zn and Pb.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) analysis of Turmeric

GC-MS analysis of the *Curcuma longa* rhizome was carried out on an Agilent technology 7890 GC system equipped with a mass spectrometric detector (MSD) as described by Momoh *et al.* [11].

2.3.1 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.

2.3.2 Preliminary phytochemical analysis

The presence of saponin, tannins, alkaloids, flavonoids, anthraquinones, glycosides and reducing sugars were determined by qualitative methods [12-14]. The simple qualitative analyses of the extract were based on the intensity of the colour change.

2.3.3 Test organisms

To study the antimicrobial activity of aqueous and methanolic root extracts of turmeric (*Curcuma longa*) extract against two bacterial strains (*Escherichia coli* (Gram negative ATCC # 25922) and *Staphylococcus aureus* (Gram positive, clinical isolates ATCC #6538) were

used for the study. The two microorganisms were maintained at 4°C on Nutrient Agar slant in the Department of Chemical Sciences and fresh subcultures were made before use.

2.3.4 Inoculum preparation

A loopful of isolated colonies of the two organisms were inoculated separately into 4 mL of peptone water, 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 [8]. 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) [8].

2.3.5 Antibiotic susceptibility testing

The susceptibility of the organisms to different antibiotics were tested using the disk diffusion method as described [15, 16]. On freshly prepared Mueller Hinton agar and standardized by the method of Famuyide *et al.* [17] and National Committee for Clinical Laboratory Standard (NCCLS), 2000 [18] using some selected antibiotics namely: Roceplin (25µg/disk), chloramphenicol (30µg/disk), streptomycin (30µg/disk), erythromycin (10µg/disk), ciprofloxacin (10µg/disk), and septrin (30µg/disk). For each combination of the antibiotics and the bacterial strains, the experiment was performed in triplicate.

2.3.6 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 and methanolic root extracts of turmeric *(Curcuma longa)* extract. Eighteen hours of broth culture of the two microorganisms 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 about 25 mL of an autoclaved nutrient agar on sterile plates and left to solidify. Then, the surface of each plate was drilled using a sterile cork borer (6 mm) and 3 wells were punched out on each plate. A total of 100 µL of a standardized culture (adjusted to 0.5 McFarland) of the two organisms were added into the different agar plates followed by loading of 100 µL of the aqueous and methanolic root extracts of turmeric 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 (*Staphylococcus aureus* and *Escherichia coli*) to aqueous and methanolic extracts of turmeric were assayed using standard method [8]. The experiment was repeated thrice, for each replicate, the readings were taken in three different fixed directions and the average values were recorded [8]. 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 [19].

2.3.7 Minimum inhibitory concentration (MIC) of aqueous and methanolic root extracts of turmeric (*Curcuma longa*)

Minimum inhibition concentration is the lowest extract concentration that inhibited the growth of the test organisms as indicated by the absence of visible turbidity in the tube compared with the control tubes. The MIC of the aqueous and methanolic root extracts of turmeric rhizome extracts were determined according to standard method [8]. The MIC of the aqueous and methanolic root extracts of turmeric extract were assayed using serial dilution method. Briefly, a total of 1 mL of Mueller-Hinton broth was poured to a set of different test tubes and autoclaved. Subsequently, 1 ml of 100% aqueous and methanolic root extracts of turmeric (2g/mL) were poured to the first separate test tubes to make a concentration of 50%, and two-fold serial dilutions were made by transferring 1 mL from one tube to another to get the following series: 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%, 0.39% etc. Then, an overnight broth culture of the different test organisms were adjusted to McFarland turbidity standard and 100 µL of the different cell suspensions were added to each of the separate tubes. The 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 and methanolic root extracts of turmeric extract. The lowest concentration of the dilution without bacterial

growth was considered as the minimum inhibition concentration.

2.3.8 Minimum Bactericidal Concentration (MBC) of the aqueous and methanolic root extracts of turmeric extract

The MBC of the aqueous and methanolic root extracts of Turmeric extract were carried out by standard method [8]. In the procedure, 0.1 mL aliquots of test samples taken from the non-turbid tubes of the minimum inhibition concentration assay test 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 and methanolic root extracts at which no colonies of *Escherichia coli* and *Staphylococcus aureus* were taken as the minimum bactericidal concentration. The results were compared with that of control tube using sterilized distilled water. The experiment was performed in triplicate. The MBC was taken as the concentration of the aqueous and methanolic root extracts of Turmeric 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 either bactericidal or bacteriostatic.

2.4 Statistical Analysis

All analyses were carried out in triplicate determination and results were expressed as mean ± SD. Students -test was used for comparison. The data analysis was done using one way analysis of variance (ANOVA) Post Hoc Turkey Graph Pad prism computer software version 5.01. *P*-value < 0.05 was considered significant.

3. RESULTS

Table 1. Mineral composition of *Curcuma longa*

Elements	Conc. in mg/L	%RSD
Na	1.3638 ±0.002	NIA
Mg	0.8025±0.001	NIA
Ca	0.7973 ±0.001	1.14
K	0.0018 ±0.000	0.53
Fe	1.0109 ± 0.002	0.4
Zn	0.0484 ± 0.000	30.3
Ag	0.0019 ± 0.000	23.6
As	0.0097 ± 0.000	50.0
Cd	0.0059 ±0.001	80.0
Co	0.0086 ± 0.000	140.5
Cu	0.0061 ± 0.000	307.1
Ni	0.0016 ±0.000	12.1
Pb	-0.0028 ± 0.000	363.8

NIA indicate not available. Values are mean ± standard deviation for triplicate determinates

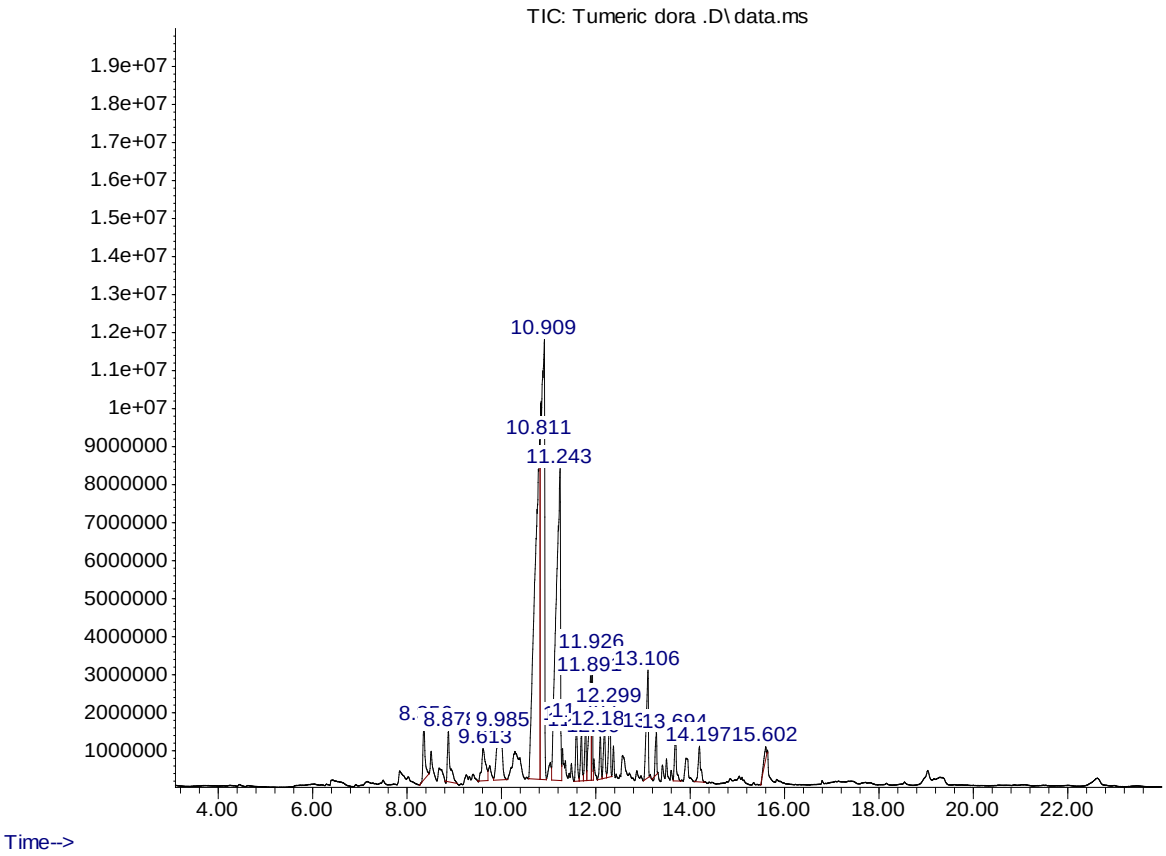

Fig. 1. Gas-Chromatography–Mass Spectrometry chromatogram of turmeric (*Curcuma longa*)

Table 2. Compounds found in the turmeric analyzed using Gas Chromatography–Mass Spectrometry

Pk#	RT	Name of the compound	Molecular Formulae	Molecular Weight (g/mol)	Peak Area (%)	Ref#	CAS#
1	8.358	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	$C_{15}H_{22}$	202.3352	1.67	66865	000644-30-4
2	8.877	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	$C_{15}H_{24}$	204.3511	1.88	68734	020307-83-9
3	9.611	Benzene, 1-ethyl-3,5-dimethyl-	$C_{10}H_{14}$	134.2182	1.85	15214	000934-74-7
4	9.987	Benzene, 1-(1,5-dimethylhexyl)-4-methyl-	$C_{15}H_{24}$	204.3511	5.83	68654	001461-02-5
5	10.811	aR-Turmerone	$C_{15}H_{20}O$	216.3187	50.05	79922	000532-65-0
6	11.244	2-Methyl-6-(4-methylenecyclohex-2-en-1-yl)hept-2-en-4-one	$C_{15}H_{22}O$	218.335	20.03	81679	082508-14-3
7	11.592	3-Methyl-6-(6-methylhept 5-en-2-yl)- cyclohex-2-enone	$C_{15}H_{24}O$	220.3505	1.42	83600	066964-98-5
8	11.696	Gamma -Terpinene	$C_{10}H_{16}$	136.2340	1.12	16078	000099-85-4
9	11.787	Binapacryl	$C_{15}H_{18}N_2O_6$	322.317	1.26	180130	000485-31-4
10	11.892	Benzonitrile, 3-hydroxy	C_7H_5NO	119.1207	1.30	9294	000873-62-1 43
11	11.925	(E)-Atlantone	$C_{15}H_{22}O$	218.3346	2.14	81630	108645-54-1
12	12.096	Cumenyl angelate, o	$C_{14}H_{18}O_2$	218.29	0.98	81511	1000383-67-2 38
13	12.187	3,5-Dimethylanisole	$C_9H_{12}O$	136.1910	1.29	16778	000874-63-5
14	12.296	Prop-2-ynyl (E)-2-methylbut-2-enoate	$C_8H_{10}O_2$	138.16	1.99	17804	1000373-72-5 22
15	13.106	Diglycolic acid, nonyl 3-phenylpropyl ester	$C_{22}H_{34}O_5$	378.5	3.35	241428	1000382-18-0 35
16	13.287	(S)-3-Methyl-6-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-enone	$C_{15}H_{22}O_2$	234.33398	0.88	96682	949081-10-1
17	13.696	But-2-enamide, N-ethyl-N-(3-methyl phenyl)-3-methyl-	$C_{14}H_{19}NO$	217.31	1.30	80637	1000308-23-6 38
18	14.196	Cyclohexanecarboxylic acid, 4-nitrophenyl ester	$C_{13}H_{15}NO_4$	249.2625	1.26	110342	1000307-70-8
19	15.601	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4455	0.40	140138	000060-33-3

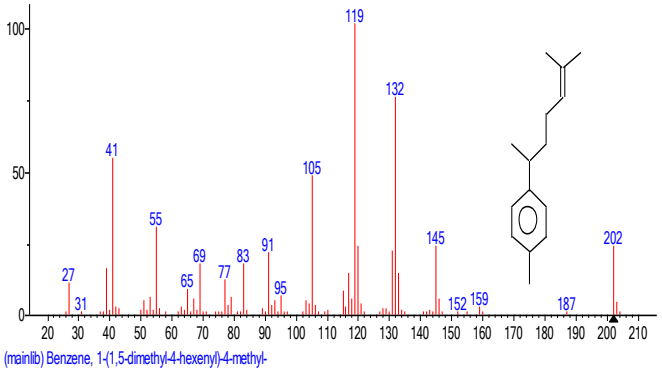

Fig.2a. Mass spectrum of Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- structure.

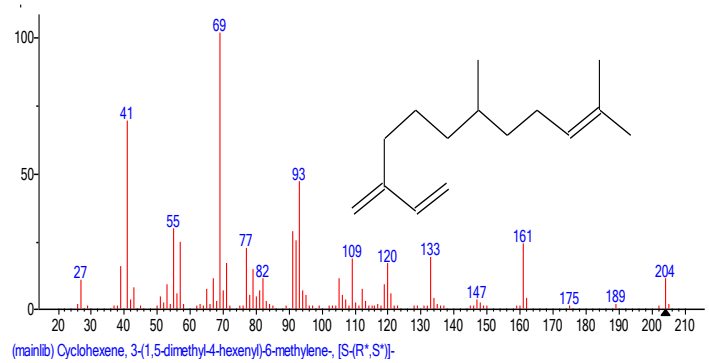

Fig.2b. Mass spectrum of Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]- structure

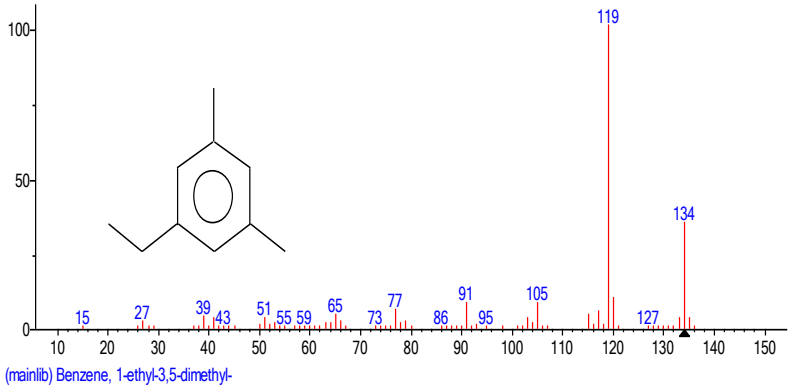

Fig. 2c. Mass spectrum of Benzene, 1-ethyl-3,5-dimethyl- structure

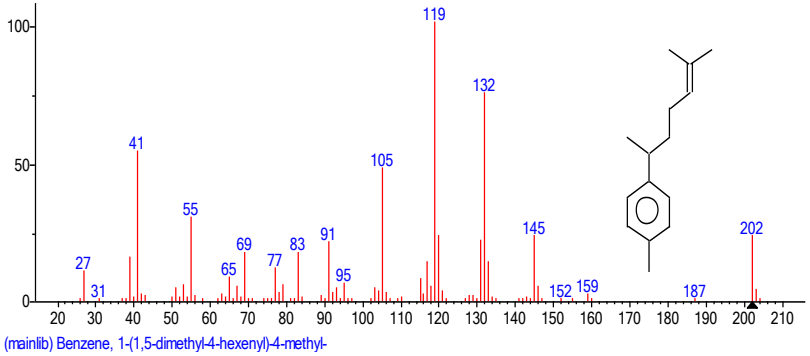

Fig. 2d. Mass spectrum of Benzene, 1-(1,5-dimethylhexyl)-4-methyl- structure

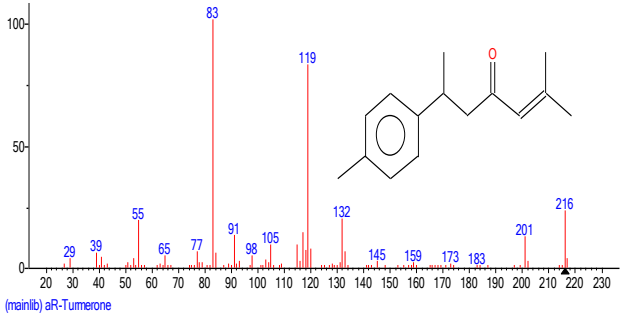

Fig. 2e. Mass spectrum of aR-Turmerone structure

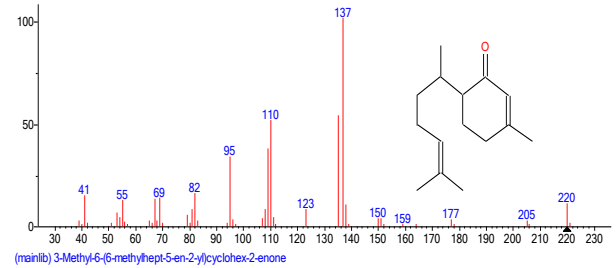

Fig. 2g. Mass spectrum of 3-Methyl-6-(6-methylhept 5-en-2-yl)-cyclohex-2-enone structure.

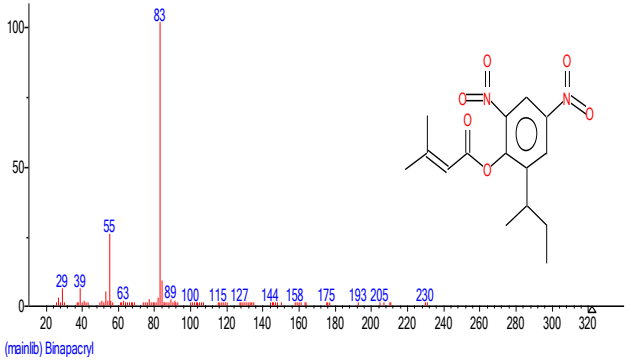

Fig. 2i. Mass spectrum of Binapacryl structure

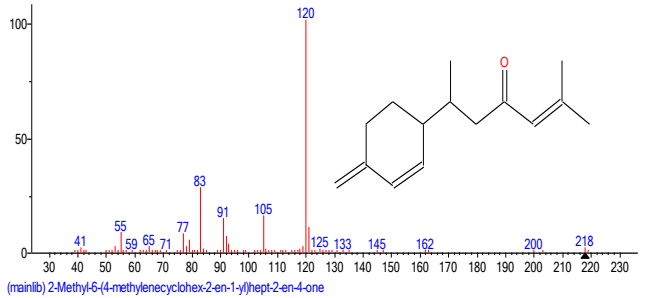

Fig. 2f. Mass spectrum of 2-Methyl-6-(4-methylenecyclohex-2- en-1-yl)hept-2-en-4-one structure

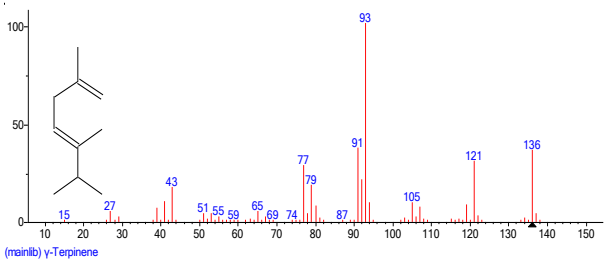

Fig. 2h. Mass spectrum of gamma.-Terpinene structure

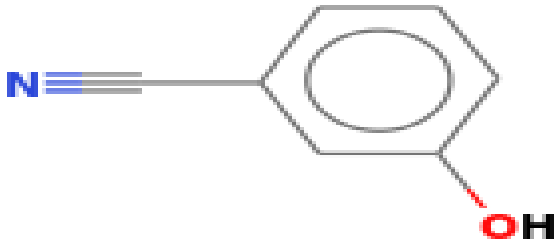

Fig. 2j. Structure of Benzonitrile, 3-hydroxy

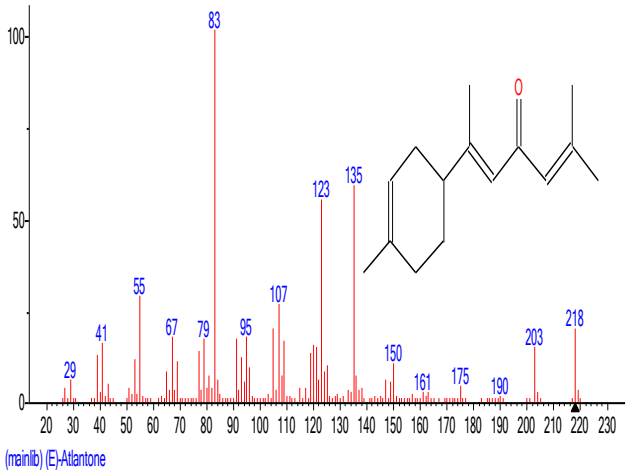

Fig. 2k. Mass spectrum of (E)-Atlantone structure

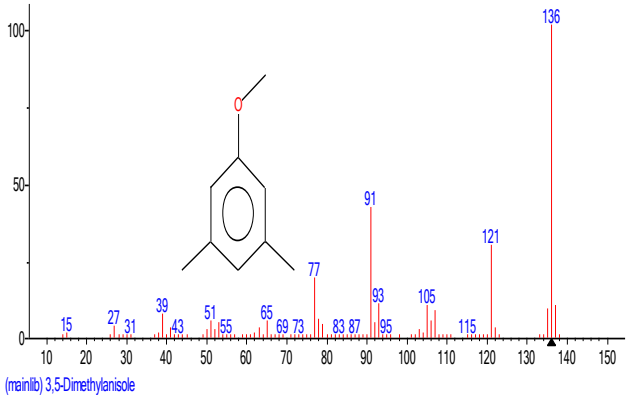

Fig. 2m. Mass spectrum of 3,5-Dimethylanisole Structure

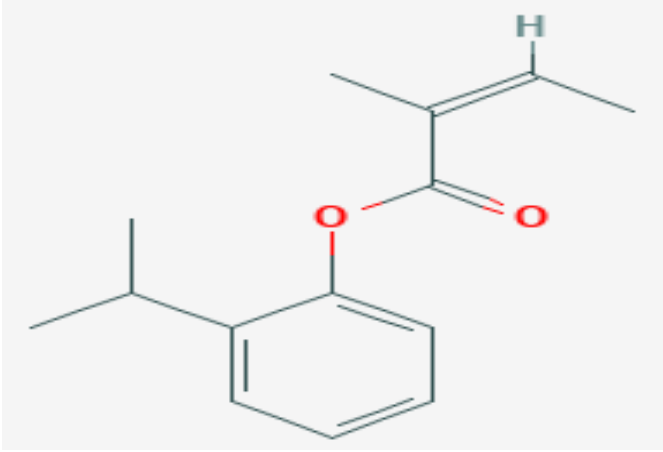

Fig. 2l. Structure of Cumenyl angelate, o-

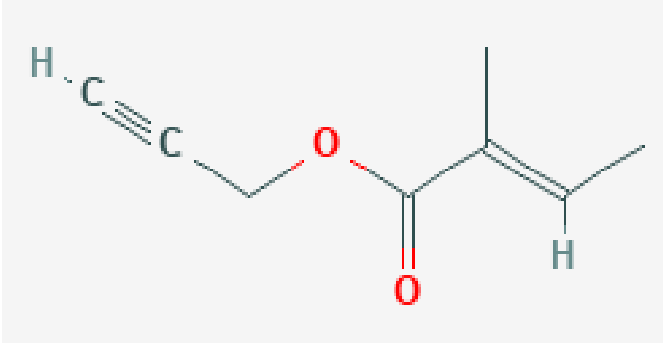

Fig. 2n. Structure of Prop-2-ynyl (E)-2-methylbut-2-enoate

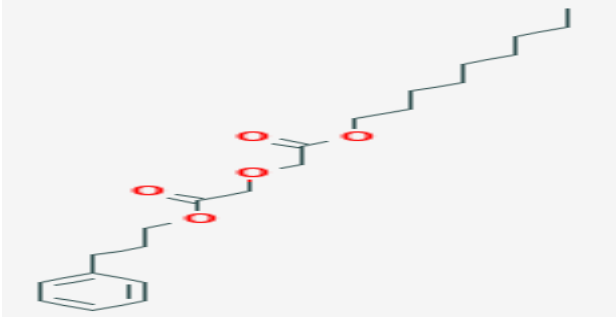

Fig. 2o. Diglycolic acid, nonyl 3-phenylpropyl ester

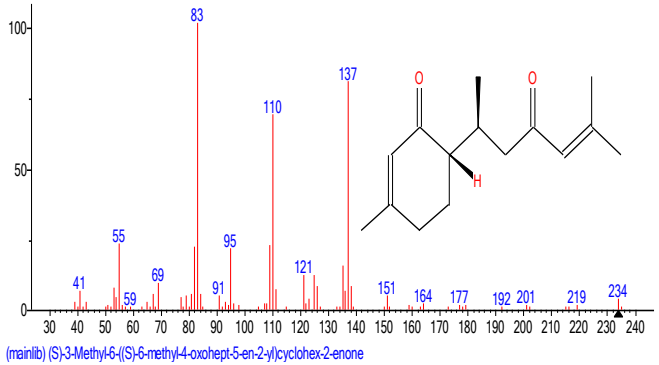

Fig. 2p. Mass spectrum of (S)-3-Methyl-6-((S)-6-methyl-4-oxohept-5-en-2-yl)cyclohex-2-enone structure

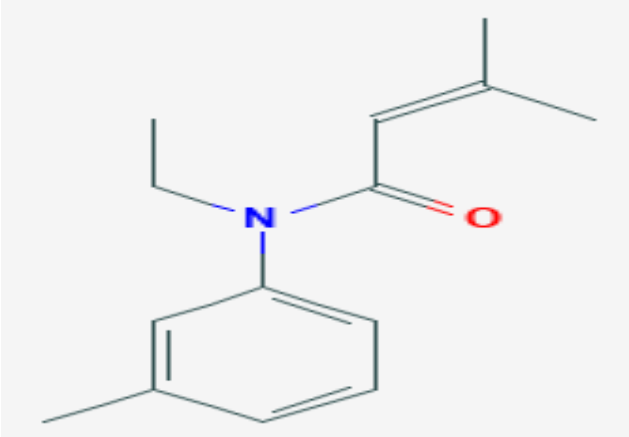

Fig. 2q. Structure of But-2-enamide, N-ethyl-N-(3-methyl phenyl)-3-methyl-

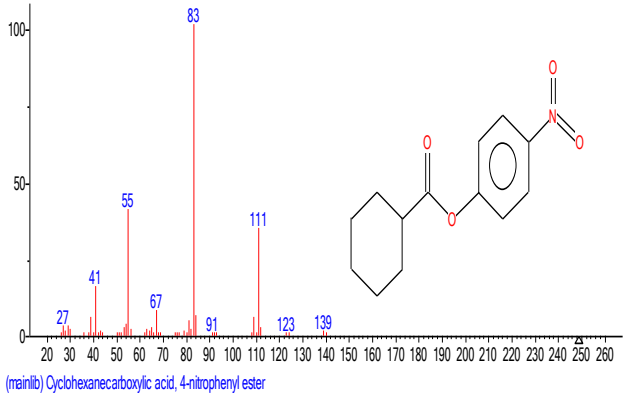

Fig. 2r. Mass spectrum of Cyclohexanecarboxylic acid, 4-nitrophenyl ester structure

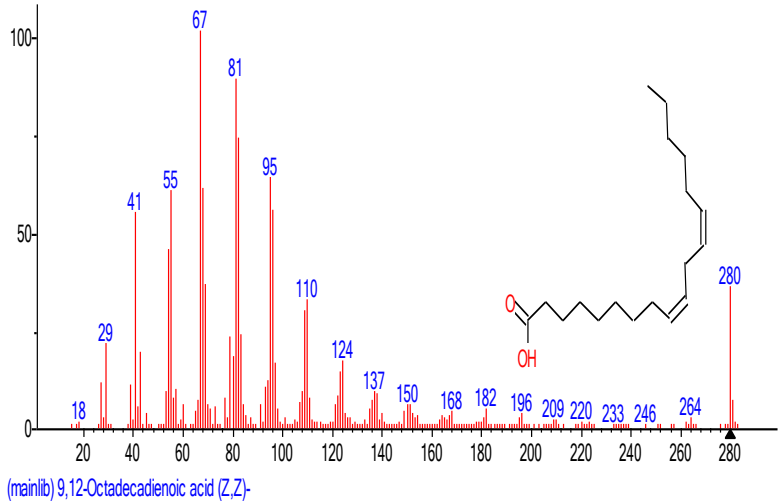

Fig. 2s. Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)- structure

Fig. 2. Mass spectrum and structure of 19 different compounds obtained during GC-MS analysis of *Curcuma longa*

Table 3. Phytochemistry of aqueous and methanolic extracts of tumeric (*Curcuma longa*)

Phytochemical constituent	Test performed	Water	Methanol
Alkaloids	Mayer`s test	+	+
Tannins	Ferric chloride test	+	+
Saponins	Froth test	+	+
Flavonoids	Lead Acetate test	+	+
Simple phenolics	Ferric Chloride test	+	+
Steroid		-	+
Protein	Biuret test	+	+
Test for reducing sugar	Fehling`s solution test	+	+
Carbohydrate	Molisch`s test	+	+

Notes: (+) indicates absent, (-) indicates present

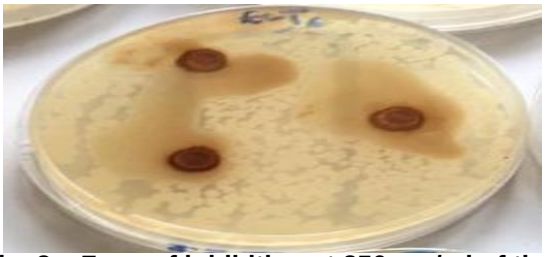

Fig. 3a. Zone of inhibition at 250 mg/ml of the aqueous extract of *Curcuma longa* against *Escherichia coli*

Fig. 3b. Zone of inhibition at 250 mg/ml of the methanolic extract of *Curcuma longa* against *Escherichia coli*

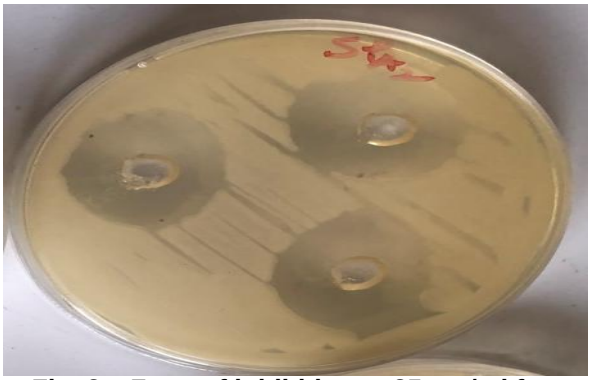

Fig. 3c. Zone of inhibition at 25 mg/ml for azithromycin solution against *Staphylococcus aureus*

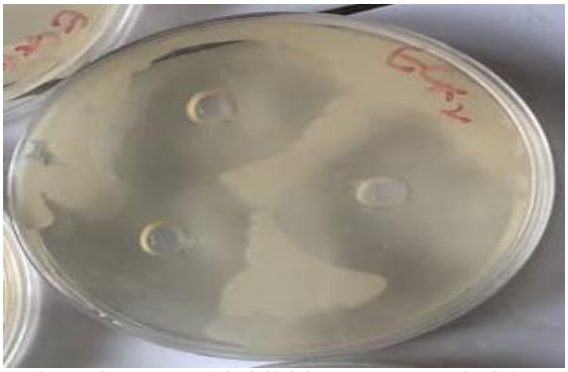

Fig. 3d. Zone of inhibition at 25 mg/ml for azithromycin solution against *Escherichia coli*

Fig. 3. Zone of inhibition of azithromycin solution, aqueous and methanolic extracts of *Curcuma longa* rhizome against *Staphylococcus aureus and Escherichia coli* at 25 and 250 mg/mL

Table 4. Antimicrobial susceptibility pattern of standard antibiotics agent against *Escherichia coli*

Antibiotic sensitive disc	Concentration (µg)	Diameter of zone of inhibition (mm)	Interpretation
Chloramphenicol (CH)	30	14.80 ± 0.37	+
Ciprofloxacin (CPX)	10	19.16± 1.250	++
Streptomycin (S)	30	21.37± 1.32	+++
Roceplin (R)	25	23.00± 1.95	+++
Septrin (SXT)	30	18.07 ± 0.64	++
Erythromycin (E)	10	20.00± 1.66	++

Table 5. Antimicrobial susceptibility pattern of standard antibiotics agent against *Staphylococcus aureus*

Antibiotic sensitive disc	Concentration (µg)	Diameter of zone of inhibition (mm)	Interpretation
Chloramphenicol (CH)	30	15.13 ± 0.60	+
Ciprofloxacin (CPX)	10	18.55± 0.27	++
Streptomycin (S)	30	18.21 ± 0.17	++
Roceplin (R)	25	18.00 ± 0.58	++
Septrin (SXT)	30	16.83± 0.24	++
Erythromycin (E)	10	16.64± 0.39	++

Table 6. Zone of inhibition of *Curcuma longa* aqueous and methanolic extract against *Escherichia coli* and *Staphylococcus aureus*

Test organisms	Aqueous extract of Tumeric concentration (mg/ml)	Zone of inhibition for aqueous extract of Tumeric (mm)	Methanolic extract of Tumeric concentration (mg/mL)	Zone of inhibition of methanolic extract of Tumeric (mm)	Concentration of azithromycin solution used (mg/mL)	Zone of inhibition of azithromycin solution (mm)
Escherichia coli	250	22.29 ± 2.35^{b}	250	21.79 ± 1.04^{b}	12.50	21.67 ± 1.04^{c}
Staphylococcus aureus	250	22.31 ± 1.59^{b}	250	22.96 ± 0.96^{b}	12.50	19.35 ± 1.0^{c}
Escherichia coli	500	29.56 ± 2.231^{a}	500	29.95 ± 1.83^{a}	25	$32..03\pm1.23^{a}$
Staphylococcus aureus	500	28.67 ± 1.42^{a}	500	30.13 ± 1.94^{a}	25	28.76 ± 0.92^{b}

Comparisons across the column was done using One way ANOVA Post Hoc Turkey test. The superscript a has the highest value followed by b and c has the lowest value. A. P<0.05 was considered statistically significant

Table 7. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *Curcuma longa* extracts against *Escherichia coli* and *Staphylococcus aureus*

ORGANISMS	*Escherichia coli*	*Staphylococcus aureus*
MIC for aqueous extract of Tumeric (mg/mL)	62.500	31.250
MIC for methanolic extract of Tumeric (mg/mL)	31.250	15.6250
MBC for aqueous extract of Tumeric (mg/mL)	250.00	62.500
MBC for methanolic extract of Tumeric (mg/mL)	62.500	31.250
MBC/MIC for aqueous extract of tumeric	4.00	2.00
MBC/MIC for aqueous extract of tumeric	2.00	2.00

4. DISCUSSION

The result of this study shows that sodium (1.3638 ±0.000) was the most abundant element present in the turmeric followed by iron (1.0109 ± 0.0041), magnesium (0.8025±0.000) and calcium (0.7973 ±0.000). Other elements like: K, Zn, Ag, As, Cd, Co, Cu, Ni and Pb were found to be present in very small quantities that are not significant (Table 1). Enemor *et al.* [20] study shows that *Curcuma longa* rhizomes had higher contents of calcium, magnesium, potassium and sodium in parts per million (ppm) at 38.68 ± 0.114, 19.75 ± 0.001, 9.20 ± 0.002 and 7.06 ± 0.014 respectively. Their result is similar to the result obtained in our study. Ogidi *et al.* [21] study indicates that sodium element helps in the treatment of heart diseases. In a research work carried out by Hartwig [22], magnesium plays fundamental roles in genomic stability and DNA repair processes. Other studies show that 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 [23, 24]. Okwu, [25] study shows that Magnesium and calcium are used for 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. Calcium ions help in the activation of numerous enzymes activities in the body. Iron is an important element that is used in the formation of red blood cells.

Fig. 1 shows the Gas-Chromatography–Mass Spectrometry chromatogram of *Curcuma longa* (turmeric). A total of 19 compounds were identified consisting of 2 prominent compounds and 17 minor compounds (Table 2). The two major compounds and their percentage abundance are: aR-Turmerone (RT=10.811 and peak area = 50.05%) and 2-Methyl-6-(4-methylenecyclohex-2-en-1-yl)hept-2-en-4-one (RT=11.244 and peak area = 20.03%). aR-Turmerone (peak area = 50.05%), is the most abundant compound followed by 2-Methyl-6-(4-methylenecyclohex-2-en-1-yl)hept-2-en-4-one (peak area = 20.03%). Ar-turmerone, the major volatile component in the rhizome, showed potent α-amylase (IC_{50} of 24.5 µg) and α-glucosidase (IC_{50} of 0.28 µg) inhibition [26]. Hoi-Seon, [27] study shows that at 2 and 1 mg/disk, ar-turmerone strongly inhibited the growth of *C. perfringens* and moderately inhibited the growth of *E. coli* without any adverse effects on the growth of four lactic acid-bacteria (*B. adolescentis, B. bífídum, B. longum,* and *L. casei*) at 2 mg/disk. Marliyana et al. [28] study shows that ar-turmerone was not active against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923*, Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 13883 using disc diffusion method. Hucklenbroich et al [29] experimental findings show that in-vitro and in-vivo study of aromatic (ar-) turmerone induces neural stem cells (NSC) proliferation. Ar-turmerone support regeneration in neurologic disease. Studies have shown that antitumor properties, exerted via the induction of apoptosis [30] and inhibition of tumor cell invasion have been attributed to ar-turmerone [31]. Park *et al.* [31] study shows that ar-turmerone also possesses anti-inflammatory properties resulting from the blockade of key signaling pathways in microglia. Microglia activation is a hallmark of neuroinflammation and is associated with various neurologic disorders, including neurodegenerative diseases [32, 33] and stroke [34, 35].

The preliminary qualitative analysis of the different secondary metabolites present in both extracts of *Curcuma longa* was investigated. The aqueous and methanolic root extract of turmeric showed that they contain alkaloids, flavonoids, tannins, saponins, simple phenolics, steroids (steroids was absent in the aqueous root extract), protein, reducing sugar and carbohydrate. Flavonoids are generally more soluble in water or polar solvents because they bonds with hydroxyl groups. Glycosides are compounds that contain sugar and non-sugar components. Saponins are generally in the form of glycosides so they tend to be polar. Saponins are surface active compounds that produce foam if shaken in water. This happens because saponins have polar and non-polar groups that will form micelles. When the micelle is formed the polar group will face out while the non-polar groups face inside so it looks like foams. Tannins which are phenolic compounds tend to dissolve in water and tend to be polar. Terpenoids are fat soluble. One of the terpenoids which has the potential as an antimicrobial is triterpenoid, while steroids are fat groups and are part of the triterpenoid.

Staphylococcus aureus and *Escherichia coli* were selected for the study and tested against some selected antibiotics, aqueous and methanolic extracts of turmeric. Roceplin and streptomycin antibiotics showed strong response with zone diameter between 21-30 mm against *Escherichia coli*, ciprofloxacin, septrin and

erythromycin showed moderate response with zone diameter between 16-20 mm while chloramphenicol showed weak response with zone diameter less than 16 mm (Table 4). Ciprofloxacin, streptomycin, roceplin, septrin and erythromycin antibiotics showed moderate response to *Staphylococcus aureus* with zone diameter between 16-20 mm, while chloramphenicol showed weak response with zone diameter less than 16 mm (Table 5). In the present study, the aqueous and methanolic extracts of turmeric extract exhibited strong response antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* with zone of inhibition ranging from 21.79±1.04 to 30.13±1.94 at concentration of 250 and 500 mg/dl respectively. The study shows that 500 mg/ml aqueous and methanolic rhizome extracts of tumeric were sensitive to the tested organisms and were significantly (P< 0.004) different from the 250 mg/mLof the different extracts used in the study. *Staphylococcus aureus* was more sensitive to the two different extracts used in the study (Table 6).

Azithromycin solution at 25 mg/ml showed potent response against *Escherichia coli* with zone diameter greater than 30 mm and strong response against *Staphylococcus aureus* with zone diameter less than 30 mm. At 12.50 mg/ml, azithromycin exhibited strong response against *Escherichia coli* and moderate response against *Staphylococcus aureus* (Table 6).

Kim *et al.* [36] and Chandrana *et al.* [37] studies reported that turmeric extract was effective against *Bacillus subtilis, Escherichia coli*, and *Staphylococcus aureus* which may be due to the presence of curcuminoid which is a phenolic compound. Negi *et al.* [38] research work reported that curlone and turmerone components of turmeric possessed better antibacterial activity against a wide range of microbes including: *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Bacillus coagulans, Psuedomonas aeruginosa* and *Escherichia coli.* The antimicrobial activity of turmeric is reported to be due to the presence of curcuminoids, turmerol, curcumins, veleric acid, essential oil and turmeric oil [39-41].

The antibacterial activity of aqueous and methanolic extracts of turmeric against *S. aureus* and *E. coli* pathogens were investigated for their MIC and MBC values. MIC or MBC values are the lowest concentration of an antimicrobial agent necessary to inhibit bacterial growth or kill bacteria respectively [8]. Aderele *et al.* [8] study shows that MIC test is important in the laboratory to confirm the resistance of microorganisms to an antimicrobial agent and also used it to monitor the activity of new antimicrobial agents. The aqueous and methanolic rhizome extracts of turmeric have MIC values of 62.500 and 31.250 mg/ml for *E. coli,* 31.250 and 15.6250 mg/ml for *S. aureus* respectively. The two extracts also have MBC values of 250.00 and 62.500 for *E. coli,* 62.500 and 31.250 mg/ml for *S. aureus* respectively (Table 7). The result of this study showed that the gram-negative bacterium (*Escherichia coli*) was less susceptible to the two rhizome extracts when compared to the gram-positive bacterium (*Staphylococcus aureus*).

Study has shown that curcumin, the active constituent of turmeric exhibited inhibitory activity on methicillin-resistant *S. aureus* strains (MRSA) with MIC values ranging from 125–250 µg/mL [38]. This compound also displayed good antibacterial activity with MIC values ranging from 5 to 50 µg/mL against 65 clinical isolates of *Helicobacter pylori* [42].

Different studies show that aqueous extract of turmeric rhizomes exhibited antibacterial effects against *S aureus* ATCC 25923, *Staphylococus epidermis* ATCC 12228, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 10031 with MIC values ranging from 4–16 µg/mL [6, 7]. The methanolic extract of *C. longa* has inhibitory effects against *S. aureus* (MIC value 128 µg/mL) and *Bacillus subtilis* (MIC value of 16 µg/mL) [43]. In another study carried out by Lawhavinit et al. [44], the methanol and hexane extracts of turmeric also showed antibacterial effect against an array of bacteria including, *Vibrio vulnificus, Vibrio harveyi, Vibrio parahaemolyticus, Vibrio alginolyticus, Vibrio cholerae, Bacillus cereus, B. subtilis, Aeromonas hydrophila, S. aureus, Streptococcus agalactiae, Staphylococcus. epidermidis, Edwardsiella tarda and Staphylococcus intermedius* with MIC values ranging from 125–1000 µg/mL [44]. All the above studies support the result obtained in our research concerning the antibacterial activity of turmeric against *Staphylococcus aureus* and *Escherichia coli.* Aderele et al., [8] 2020 study has shown that calculated MBC/MIC ratio is bactericidal if the values of MBC/MIC ratio are less than or equal to 4 and bacteriostatic if the MBC/MIC ratio is > 4. The aqueous and methanolic extracts of turmeric rhizome have bactericidal effects on *Escherichia coli* and *Staphylococcus aureus* respectively.

5. CONCLUSION

Curcuma longa has essential minerals, phytochemicals and other natural therapeutic agents that possess antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* and may prevent pathogenic diseases caused by these organisms.

ACKNOWLEDGEMENTS

The authors are grateful to Onayade Omobolanle Funmilola, Iwuji Jovita Ogornna and Adeniran Nofisat Anuoluwapo for the assistance render when carrying out the laboratory research work. Our sincere gratitude goes to Mr. Oluwasegun Michael Adekunle for his patience, commitment and assistance when carrying out the phytochemical and antimicrobial assays in the laboratory.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gupta SC, Sung B, Kim JH, Prasad S, Li S, Aggarwal BB. Multitargeting by turmeric, the golden spice: From kitchen to clinic. Molecular Nutrition and Food Research. 2013;57(9):1510–1528.
2. Ravindran PN. Turmeric: the Genus Curcuma. CRC Press, London; 2007.
3. Islam F, Karim M, Shahjahan M, Hoque M, Alam MR, Hossain MA. Study on the effect of plant spacing on the production of turmeric at farmer's field. Asian Journal of Plant Sciences. 2002; 1(6): 616-617.
4. Li S, Yuan W, Deng G, Wang P, Yang P, Aggarwal B. Chemical composition and product quality control of turmeric (*Curcuma longa* L.). Pharmaceutical Crops. 2011; 2:28–54.
5. Miean KH, Mohamed S. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. Journal of Agricultural and Food Chemistry. 2001;49(6):3106–3112.
6. Moghadamtousi SZ, Kadir HA, Hassandarvish P, Tajik H, Abubakar S, Zandi K. A review on antibacterial, antiviral, and antifungal activity of curcumin. Biomed. Res. 2014; Int. 186864.
7. Niamsa N, Sittiwet, C. Antimicrobial activity of *Curcuma longa* aqueous extract. J. Pharmacol. Toxicol. 2009;4(4): 173–177.
8. Aderele OR, Rasaq AK and Momoh JO. Phytochemical Screening, Mathematical Analysis and Antimicrobial Activity of Methanolic Seed Extract of *Hunteria umbellata*. European Journal of Medicinal Plants. 31(16): 1-17, 2020*;* Article no.EJMP.61248 ISSN: 2231-0894, NLM ID: 101583475.
9. Chambers HF. The changing epidemiology of *Staphylococcus aureus* Emerg Infect Dis. 2001;6:178-182.
10. Ajayi DO, Onasanya AA, Daramola GO. Evaluation of Different Genotypes and Genetic Virulence in *Staphylococcus aureus* Isolates from Clinical Samples. JAMB. 2021; 21(12): 135-149. Article no.JAMB.80358.
DOI: 10.9734/JAMB/2021/v21i1230422.
11. Momoh JO, Damazio OA, Ajetunmobi AO, Babalola AO, Adekunle OM, Busari NO, Musa AA. Phytochemical analysis and antiplasmodial (curative) activities of methanolic leaf extract of *Morinda lucida* (Ewe Oruwo) in male Swiss mice infected with *Plasmodium berghei NK65.* IJTDH. 2019;37(1):1-13. Article no.IJTDH.47956.
DOI: 10.9734/IJTDH/2019/v37i130156.
12. Trease GE, Evans WC. *Pharmacognsy*. 11th edition, London. Brailliar Tiridel Can Macmillian Publishers. 1986;60-75.
13. Sofowora A. *Medicinal plants and traditional medicines in Africa.* Spectrum Book Ltd. Ibadan, Nigeria. 1993; 289.
14. Momoh JO, Aderele OR, Rasaq AK. Sub-acute and protective effect of *Cymbopogon citratus* against carbon tetrachloride-induced liver damage, Afr. J. Biochem. 2020;14(4):112-124.
DOI: 10.5897/AJBR2019.1064.
15. Momoh J, Oluremi NO, Odetunde SK Antimicrobial and Antioxidant Properties of Aqueous Garlic (*Allium sativum*) Extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* British Microbiol Res J. 2016;14(1):1-11 Article no.BMRJ.24095 ISSN: 2231-0886, NLM ID: 101608140.
16. Cheesbrough M. *District Laboratory practice in Tropical Countries* Part 2: Cambridge University Press. UK. 2002; 136-142.
17. Famuyide IM, Aro AO, Fasina FO, Eloff JN, McGaw LJ. Antibacterial activity and mode of action of acetone crude leaf extracts of under investigated *Syzygium* and *Eugenia* (Myrtaceae) species on multidrug resistant porcine diarrhoeagenic

Escherichia coli. BMC Vete Res. 2019; 15:162.
18. National Committee for Clinical Laboratory Standards (NCCLS). Performance standard for antimicrobial susceptibility testing, 10th information supplement approved standard. 2000;M100-S10. Wayne P.A.
19. Hoi-Seon L. Antimicrobiai Properties of Turmeric *Curcuma longa* L.) Rhizome—Derived ar-Turmerone and Curcumin. Food Sci. Biotechnol. 2006;15(4):559-563.
20. Enemor VHA, Ogbodo UC, Nworji OF, Ezeigwe OC, Okpala CO, Iheonunekwu GC. Evaluation of the Nutritional Status and Phytomedicinal Properties of Dried Rhizomes of Turmeric (*Curcuma longa*). J. Biosci Medicines.2020;8:163-179. https://doi.org/10.4236/jbm.2020.88015.
21. Ogidi OI, Esie NG, Dike OG. Phytochemical, Proximate and Mineral Compositions of *Bryophyllum pinnatum* (Never Die) Medicinal Plant J. Pharmacognosy Phytochem. 2019;8:629-635.
22. Hartwig A. Role of Magnesium in Genomic Stability. Mutation Research. 2001;475: 113-121. https://doi.org/10.1016/S0027-5107(01)00074-4.
23. Pasternak K, Kocot J, Horecka A. Biochemistry of Magnesium. Journal of Elementology. 2010;15:601-616.
24. Wolf FI. Cittadini A. Chemistry and Biochemistry of Magnesium. Molecular Aspects of Medicine.2003;24: 3-9. https://doi.org/10.1016/S0098-2997(02)00087-0.
25. Okwu DE. Phytochemicals, vitamins and mineral contents of two Nigerian Medicinal Plants. Int. J. Mol. Med. Adv. Sci. 2005; 1: 375-381.
26. Lekshmi PC, Arimboor R, Indulekha PS, Menon AN. Turmeric (*Curcuma longa* L.) volatile oil inhibits key enzymes linked to type 2 diabetes. Int. J. Food Sci. Nutr. 2012;63(7): 832–834.
27. Hoi-Seon L. Antimicrobiai Properties of Turmeric *Curcuma longa* L.) Rhizome—Derived ar-Turmerone and Curcumin. Food Sci. Biotechnol. 2006;(15) 4: 559-563*.*
28. Marliyana SD, Wibowo FR, Wartono1 MW, Munasah G. Evaluation of antibacterial activity of sesquiterpene Ar- Turmerone from *Curcuma soloensis* Val. Rhizomes. IOP Conf. Series: Materials Science and Engineering. 2019;578**:** 012060 IOP Publishing DOI:10.1088/1757-899X/578/1/012060.
29. Hucklenbroich J, Klein R, Neumaier B, Graf R, Fink GR, Schroeter M, Rueger MA. Aromatic-turmerone induces neural stem cell proliferation in vitro and in vivo. Stem Cell Res. Therapy. 2014;5(100): 1-9.
30. Lee Y. Activation of apoptotic protein in U937 cells by a component of turmeric oil. BMB Rep. 2009;42:96–100.
31. Park SY, Jin ML, Kim YH, Kim Y, Lee SJ. Anti-inflammatory effects of aromatic-turmerone through blocking of NF-kappaB, JNK, and p38 MAPK signaling pathways in amyloid beta-stimulated microglia. Int Immunopharmacol. 2012; 14:13–20.
32. Hirsch EC, Hunot S. Neuroinflammation in Parkinson's disease: a target for neuroprotection? Lancet Neurol. 2009;8:382–397.
33. Perrin RJ, Fagan AM, Holtzman DM. Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. Nature. 2009; 461:916–922.
34. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. Nat Med. 2011; 17:796–808.
35. Schroeter M, Dennin MA, Walberer M, Backes H, Neumaier B, Fink GR, Graf R. Neuroinflammation extends brain tissue at risk to vital peri-infarct tissue: a double tracer [(11)C]PK11195- and [(18)F]FDG-PET study. J Cereb Blood Flow Metab. 2009; 29:1216–1225.
36. Kim KJ, Yu HH, Cha JD, Seo SJ, Choi NY, You YO. Antibacterial activity of *Curcuma longa* L. against methicillin resistant *Staphylococcus aureus*. Phytother Res.2005; 19:599–604.
37. Chandrana H, Baluja S, Chanda SV. Comparison of antibacterial activities of selected species of Zingiberaceae family and some synthetic compounds. Turk J Biol.2005;29(29):83–97
38. Negi PS, Jayaprakasha GK, Jagan Mohan Rao L, Sakariah KK. Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture. J Agric Food Chem. 1999;47(10):4297–4300. DOI: 10.1021/jf990308d. PMID: 10552805.
39. Rai D, Singh JK, Roy N, Panda D. Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity. Biochem J.2008;410:147–155.
40. Cikricki S, Mozioglu E, Yylmaz H. Biological activity of curcuminoids isolated from *Curcuma longa*. Rec Nat Prod.2008;12:19–24.

41. Basniwal RK, Butter HS, JainVK, JainN(2011) Curcumin nanoparticles: preparation, characterization, and antimicrobial study. J Agric Food Chem.2011;59:2056–2061.
42. De R, Kundu P, Swarnakar S, Ramamurthy T, Chowdhury A, Nair GB. Antimicrobial activity of curcumin against *Helicobacter pylori* isolates from India and during infections in mice. Antimicrob. Agents Chemother.2009;53(4):1592–1597.
43. Ungphaiboon S, Supavita T, Singchangchai P, Sungkarak S, Rattanasuwan P, Itharat A. Study on antioxidant and antimicrobial activities of turmeric clear liquid soap for wound treatment of HIV patients. Songklanakarin J. Sci. Technol.2005;27(2):269–578.
44. Lawhavinit OA, Kongkathip N, Kongkathip B, 2010. Antimicrobial activity of curcuminoids from *Curcuma longa* L. on pathogenic bacteria of shrimp and chicken. Kasetsart J. Nat. Sci.2010; 44 (3):364–371.