World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

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GC-MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF AQUEOUS LEAVES EXTRACT OF *PIPER GUINEENSE, GONGRONEMA LATIFOLIUM,* AND *VERNONIA AMYGDALINA*

Osuagwu Olachi Lilian [1,*], Onyinyechi Chijioke Njoku [1], Mohammed Abdulganiyu Galadima [1], Nwabueze Chinonso Queen [2], Nwabueze Obinna Stephen [1], Agidi Onesimus [3] and Itor Moses Oko [4]

[1] Department of Biochemistry, School of Biological Sciences, Federal University of Technology Owerri, Imo State, Nigeria.
[2] Department of Microbiology, Federal University of Technology Owerri, Imo State, Nigeria.
[3] Department of Science Laboratory Technology, Federal University of Technology Owerri, Imo State, Nigeria.
[4] Department of Microbiology and Biotechnology, Dominion University Ibadan, Oyo State, Nigeria.
* Corresponding Author

World Journal of Biology Pharmacy and Health Sciences, 2026, 28(02), 167–177

Publication history: Received on 09 October 2026; revised on 18 November 2026; accepted on 20 November 2026

Article DOI: https://doi.org/10.30574/wjbphs.2026.28.2.0545

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ABSTRACT

Bacterial infection remains a major public health problem causing high number of deaths worldwide. Medicinal plants and their chemical constituents have significant therapeutic properties. This study aimed at evaluating the chemical constituents and antibacterial activity of aqueous leaves extract of *Piper guineense, Gongronema latifolium,* and *Vernonia amygdalina* on *Streptococcus pmeumonia, Klebsiella pneumonia, Escherichia coli,* and *Proteus mirabilis* isolates. The chemical constituents of the plants extract were analyzed using GC-MS technique. Antibacterial effect of the plants extract was evaluated using agar diffusion method. A total of 33, 20, and 29 putative compounds were identified form the *Gongronema latifolium, Piper guineense,* and *Vernonia amygdalina* extract, respectively. The *Gongronema latifolium* extract contained high percentage of n-hexadecanoic acid (23.43 %) and aspidospermidin-17-ol, 1-acetyl 19,21-epoxy-15,16-dimethoxy- (23.43 %). A higher proportion of methyl dihydroisosteviol (11.19 %), 10-methylnonadecane (9.87 %), and 1-octadecene (9.76) were detected in the *Piper guineense* extract. Cyclohexane 2-(2-propenyl)- (5.70 %), 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (5.42 %), and Spiro [2.3] hexan-4-one, 5,5-diethyl (5.40 %) were the highly detected in *Vernonia amygdalina* extract. The percentage prevalence of *Streptococcus pmeumonia, Klebsiella pneumonia, Escherichia coli,* and *Proteus mirabilis* detected in the sputum samples were 46.16 %, 23.08 %, 15.78 %, *and* 15.38 %, respectively. All the three plants extract (50 mg/mL, 100 mg/mL, 150 mg/mL) inhibited the growth of the bacterial isolates with *Vernonia amygdalina* extract showed the highest inhibition against the isolates. At 150 mg/mL, *Vernonia amygdalina* extract showed higher zone of inhibition (27.04 ± 1.79 and 27.00 ± 1.84) against the *Proteus mirabilis* and *Klebsiella pneumonia* comparable to that demonstrated by the reference drug amoxicillin (32.00 ± 1.57 and 29.33 ± 1.41), respectively. The aqueous leaves extract of *Piper guineense, Gongronema latifolium,* and *Vernonia amygdalina* demonstrated inhibitory effect against *Streptococcus pmeumonia, Klebsiella pneumonia, Escherichia coli,* and *Proteus mirabilis* isolates. The inhibitory effect of the plants extract against the bacterial isolates might be attributed to the various compounds identified in the plant extract.

Keywords: Antibacterial activity, Bacterial infection, *Gongronema latifolium*, *Piper guineense, Vernonia amygdalina*

1 INTRODUCTION

Bacterial infection remains a public health issue associated with morbidity and mortality worldwide. Several bacterial species are resistant to many antibiotics and the population of drug-resistance bacteria is predicted to increase by 2030 [1]. Synthetic drugs particularly antibiotics produce serious side effects and effective antibiotics are cost to afford by many local communities. Thus, an investigation is necessary to find effective and safer antimicrobial agent for treatment of bacterial infections. Plants and herbs are effective, have few side effects, and easy to afford than conventional antibiotics. Plants and herbs demonstrate medicinal properties and pharmacological activities as the results they are used for treatment of diseases [2, 3]. Several years ago medicinal plants have been used locally for remedies. Studies showed that plants and herbs demonstrated antioxidants activity [4], antimicrobial activity [5], antiulcer activity [2, 3, 6], antitrypanosomal activity [7], antidiabetic activity [8], and analgesic activity [9]. Research revealed that plants extracts significantly inhibited the growth of bacterial species and modulated microbial antibiotics resistance [5, 10, 11].

Vernonia amygdalina commonly called 'bitter leaf' is a perennial tree belonging to the family Asteraceae and the genus Vernonia [12]. *Vernonia amygdalina* is originated from Africa distributed in sub-Saharan African countries [13]. The plant has been used as chewing stick for promoting oral hygiene, bittering agent, and antimicrobial agent in food preparation and processing. Different parts of *Vernonia amygdalina* have been used in treatment of several diseases such as bacterial infections, fever, malaria, dyspepsia, pains, diabetes, gout, dysentery, tonsillitis, constipation, and ulcers [14, 15, 16]. *Vernonia amygdalina* exhibited many pharmacological activities including anti-sickling activity, plaque development inhibitory effect, and regulate hemostasis activities [17]. In Nigeria, *Vernonia amygdalina* is widely available in various local communities and is locally called 'Onugbu' (Igbo), 'Shuwaka' (Hausa), and 'Ewuro' (Yoruba). *Vernonia amygdalina* leaves are used in many local areas in the country for preparation of bitter leaf soup. Local communities in the country consume aqueous extracts of *Vernonia amygdalina* as tonics for the treatment of diseases.

Gongronema latifolium is a tropical rain forest plant that belongs to the family Asclepiadaceae [18]. Gongrenema latifolium is characterizes by a bitter-sweet taste and is abundantly available in West Africa countries [19]. The plant has significant nutritional value and demonstrated various medicinal properties [19]. Gongrenema latifolium exhibited several pharmacologic activities including analgesic, antimicrobial, anti-bacterial, anti-plasmodial, antioxidant, anti-ulcer, anti-sickling, anti-asthmatic, antipyretic, hepatoprotective, hypoglycemic and antiinflammatory properties [20, 21]. In Nigeria, *Gongronema latifolium* is locally called Utazi in Igbo, Madumaro in Hausa, and Arokeke in Yoruba. Different communities in the country have been using the plant as a spice for preparation of soups and stews and for local treatment of many diseases.

Piper guineense (black pepper) is a perennial plant belonging to the Piperaceae family [22]. The plant was originated from West Africa region and is widely distributed in West and Central African countries and used for traditional treatment of different diseases [23]. *Piper guineense* has been used as cosmetic, therapeutic, and insecticidal agent and exhibited antioxidant, anticonvulsant, anti-inflammatory, and neuroprotective activities [24]. In Nigeria, *Piper guineense* is locally known as Uziza in Igbo, Masoro in Hausa, and Iyere in Yoruba. The plant is widely used in Nigerian ethnomedicine for treatment of respiratory infections, gastrointestinal disorders, fever, and reproductive issues [25]. This study aimed at evaluating the chemical constituents and inhibitory effect of aqueous leaves extract of *Piper guineense, Gongronema latifolium,* and *Vernonia amygdalina* on *Streptococcus pmeumonia, Klebsiella pneumonia, Escherichia coli,* and *Proteus mirabilis* isolates from sputum samples of patients attending Federal Medical Centre in Owerri, Imo State.

2 MATERIALS AND METHODS

2.1 Chemicals and Reagents

All the chemicals used in this study were of analytical grade and manufactured by Cormart Nigeria Limited (Lagos, Nigeria) and Maoming Xiongda Chemical Co., Limited (Guangdang, China).

2.2 Plant Materials

The fresh plant leaves of *Piper guineense, Gongronema latifolium,* and *Vernonia amygdalina* were collected at Relief market located in Owerri Municipal Local Government Area, Imo state. The plants samples were identified and assigned voucher numbers by a plant taxonomist, Mr. Francis Iwueze, from the Department of Forestry and Wildlife at the Federal University of Technology Owerri, Imo State. The voucher number of the plants samples *Piper guineense* (FUTO/FWT/ERB/2022/88), *Gongronema latifolium* (FUTO/FWT/ERB/2022/89), and *Vernonia amygdalina* (FUTO/FWT/ERB/2022/90), respectively.

2.3 Extract Preparation

The plants leaves were washed with tap water and subsequently air-dried for two weeks. The samples were ground into fine powder using grinding machine. Five hundred grams of the powdered leaves were soaked in 2.5 L of distilled water for three days with constant shaking at three hours interval. The extract was filtered using bacterial membrane filter and the filtrate was concentrated to dryness. The weight and percentage yield of the extract *Piper guineense* (35.42 g and 7.08 %), *Gongronema latifolium* (32.28 g and 6.46 %), and *Vernonia amygdalina* (44.62 g and 8.92 %), respectively.

2.4 Gas Chromatography- Mass Spectometry Analysis

The chemical constituents of the plants extract were analyzed using GC-MS technique. The GC (Agilent 6890N) and MS (5975B MSD) were equipped with DB-5ms capillary column (30 m × 0.25 mm; film thickness 0.25 µm). The plants extract (2 µL) was injected into the GC column. The initial temperature was set at 40 ^{o}C but increased to 150 ^{o}C at the rate of 10 ^{o}C/min. The temperature was further increased to 230 ^{o}C at the rate of 5 ^{o}C/min and up to 280 ^{o}C at the rate of 20 ^{o}C/min and was then held for 8 minutes. The injector port temperature remained constant at 280 ^{o}C and detector temperature was 250 ^{o}C. Helium was used as the carrier gas with a flow rate of 1 mL/min. Split ratio and ionization voltage were 110.1 and 70eV, respectively. The individual mass spectral peak values of unknown components were compared with the database of National Institute of Science and Technology 2014 (NIST) [26]. Then the phytochemicals were identified after comparing the unknown peak value and chromatogram from the GC-MS against the known chromatogram, peak value from the NIST library database.

2.5 Sputum Samples

A total of twenty-two fresh sputum samples were collected from consenting patients attending Federal Medical Centre in Owerri, Imo State. The samples were obtained using aseptic containers to ensure sterility. The samples were streaked onto nutrient agar and subsequently incubated for 24 hours at 37 ^{o}C. In order to generate uncontaminated cultures of distinct strains, the cultures were subcultivated in blood agar.

2.6 Bacterial Isolates

The sputum samples were streaked on Mannitol salt agar and incubated at 37 ^{o}C for 24 hours. The bacterial colonies were picked and sub-cultured on nutrient agar plates to produce pure cultures. The cultured isolates were maintained on the plates at 4 ^{o}C and then subjected for test. The pure bacterial isolates were identified using microscopic technique and biochemical tests. The bacterial isolates were sub-cultured in nutrient broth at 37 ^{o}C for 6 h prior to antibacterial analysis [27].

2.7 Antibacterial Activity Test

Antibacterial activity of the plants extract was evaluated using agar diffusion method of Kirby-Bauer [28]. The loopful colonies of the bacterial isolates were streaked on nutrient agar plates. The plates were incubated at 37 °C for 24 hours and then the turbidity developed was equally adjusted to Mac Farland standard (0.5) value. The agar discs of diameter 6 mm were aseptically dipped in 2 mL of 50 mg/mL, 100 mg/mL, and 150 mg/mL of the plants extract for 1 minute and then placed over the separate nutrient agar plates. The plates were incubated at 37 °C 24 hours and then observed for development of zone of inhibition measured in millimetre using meter rule. Amoxicillin (30 µg) [29] was used as reference standard.

2.8 Molecular Identification of the Bacterial Isolates

The DNA was extracted using Jena Bioscience Kit™ method through lysis, precipitation, and hybridization as described by Bunyan et al. [30]. The polymerase chain reaction was conducted using the method of Abayasekara et al. [31]. The 16SrRNA gene of the bacteria was amplified using the primer pairs 27F-5′ AGAGTTTGATCCTGGCTCAG-3’ and 1492R 5' GGTTACCTTGTTACGACTT-3'. The thermal cycling was run in thermal cycler for an initial denaturation of 95 °C for 15 minutes, followed by 35 amplification cycles of 30 seconds at 95 °C, 1 minute at 61 °C, and 1 minute and 30 seconds at 72 °C. The final extension was run for 10 minutes at 72 °C. The amplification product was separated on a 1.5 % agarose gel, and electrophoresis was carried out at 80 V for 1 hour and 30 minutes. The DNA bands were visualized by ethidium bromide staining. A 100-bp DNA ladder was used as a DNA molecular weight marker. The polymerase chain reaction products were purified and then subjected to DNA Sanger sequencing. The data were analyzed using sequencing analysis software and then searched using the BLAST GenBank.

2.9 Statistical Analysis

The experiments were conducted in triplicate. The results were statistically analyzed using Statistical Package for Social Sciences (SPSS) version 22 software. Significant differences between the mean values were computed at 95 % confidence level by One-way analysis of variance (ANOVA). Significance was considered by two-tailed ($p < 0.05$) values.

3 RESULTS

3.1 Phytoconstituents of the Plant Extract

Figure 1 shows the GC-MS chromatogram of aqueous leaves extract of *Gongronema latifolium*. The chromatogram showed various peaks representing different chemical constituents of the plant.

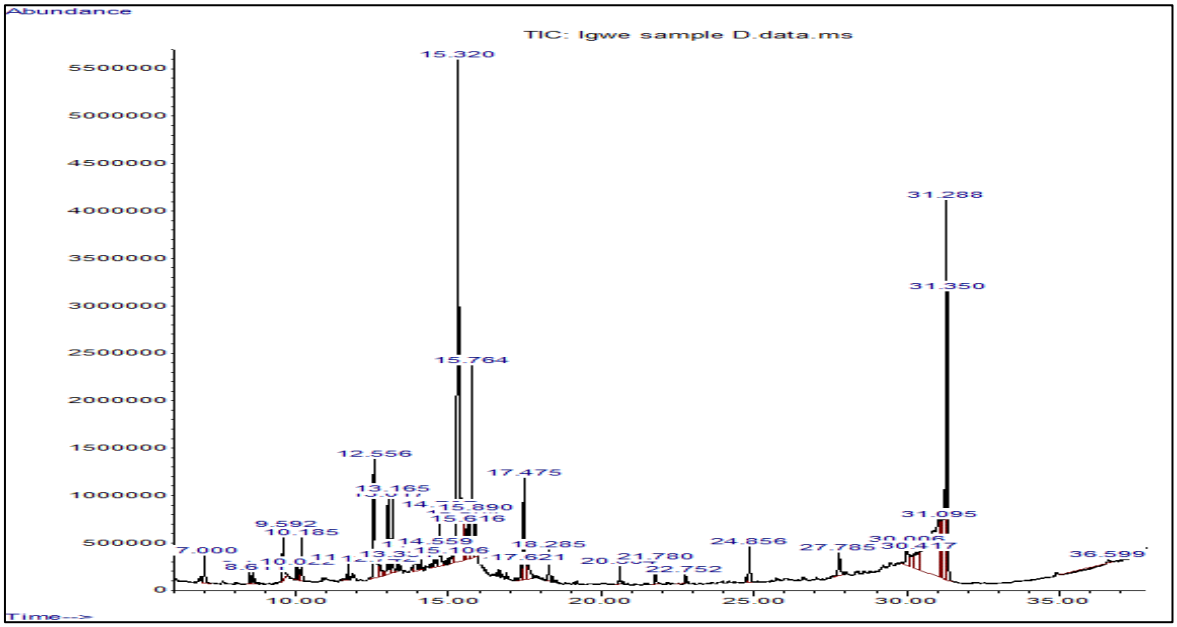


Figure 1: GC-MS Chromatogram of Aqueous Extract of *Gongronema latifolium*

The GC-MS chromatogram of aqueous leaves extract of *Piper guineense* is shown in Figure 2. Many peaks each represents different the plant components were observed on the chromatogram.

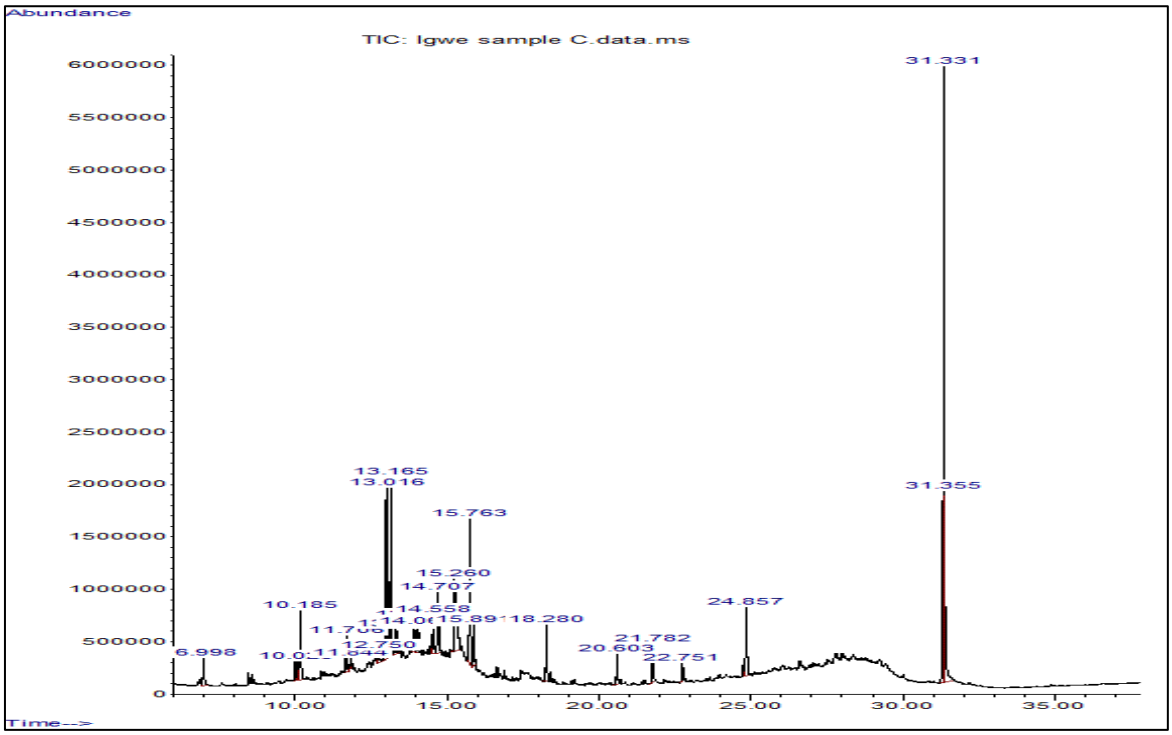


Figure 2: GC-MS Chromatogram of Aqueous Extract of *Piper guineense*

Figure 3 displays the GC-MS chromatogram of aqueous leaves extract of *Veronia amygdalina*. The chromatogram displayed several peaks indicating different constituents present in the plant extract.

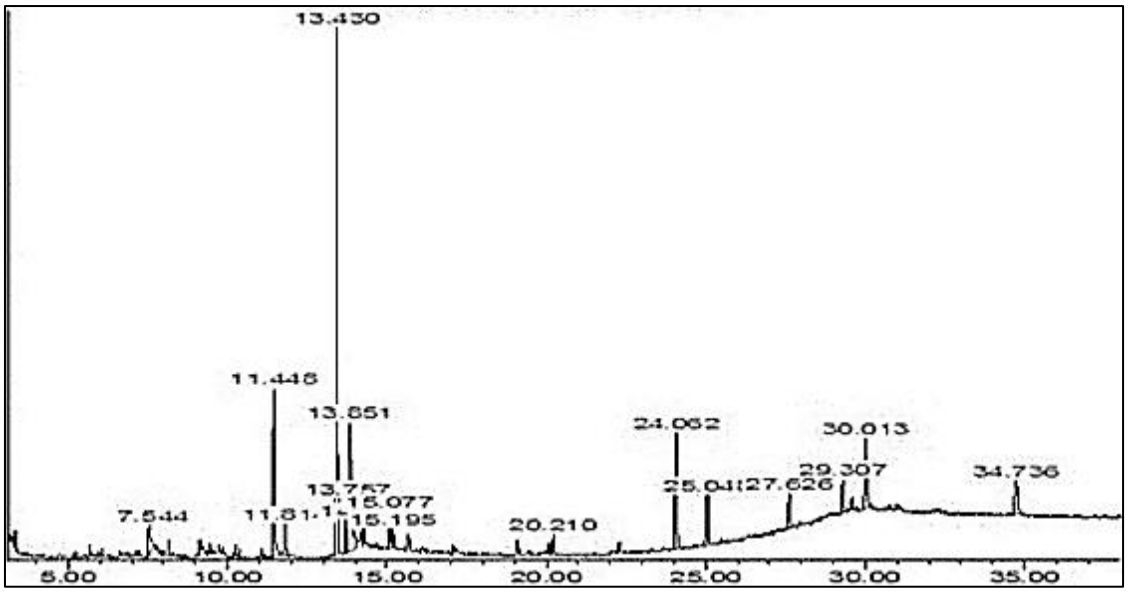


Figure 3: GC-MS Chromatogram of Aqueous Extract of *Veronia amygdalina*

Table 1 shows the bioactive compounds present in aqueous leaves extract of *Gongronema latifolium*. A total of thirty-three compounds were identified form *Gongronema latifolium*. The extract contained high percentage of n-hexadecanoic acid (23.43 %) and aspidospermidin-17-ol, 1-acetyl 19,21-epoxy-15,16-dimethoxy- (23.43 %) than the other components.

Table 1: Bioactive Compounds Present in Aqueous Leaves Extract of *Gongronema latifolium*

CLASS OF COMPOUND	RT	PEAK AREA (%)	BIOACTIVE COMPOUND
Alkane	7.00	0.88	Tetradecane
	10.19	1.11	Hexadecane
	11.71	0.50	Heptadecane
	13.17 31.05 13.92	2.18 13.73 0.94	Octadecane Cyclotrisiloxane, hexamethyl- 9-Heptadecanone
Phenol	8.48	0.46	2,4-Di-tert-butylphenol
	8.61	0.35	Pentadecane
	30.42	2.27	Thymol, TMS derivative
Carboxylic Acids	12.56	5.07	Tetradecanoic acid
Fatty Acid	13.02	2.24	9-Eicosene, (E)-
	15.32	23.43	n-Hexadecanoic acid
	9.59	1.87	Dodecanoic acid
	17.48	4.15	cis-Vaccenic acid
Alkyl	14.06	0.65	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione
Ketone	14.56	0.74	Nonadecane
	31.35	5.59	3-(3,4-Dimethoxycinnamoyl)-4-hydroxy-6-methyl-2H-pyran-2-one
Alkene	15.76 22.75	5.64 0.16	1-Docosene 17-Pentatriacontene

Glycoside	17.37	0.61	9,12-Octadecadienoic acid (Z, Z)-
Ester	21.78 14.71 20.04 31.10	0.72 2.03 0.47 1.82	Diisooctyl phthalate Dibutyl phthalate Pentadecafluorooctanoic acid, octadecyl Silicic acid, diethyl bis(trimethylsilyl) ester
Steroid	24.86	1.13	Squalene
	27.79	1.22	Ergosterol
	31.29	11.30	16-Pregnenolone
Alkaloid	30.01 15.11	1.52 23.43	N-Acetoacetyl-deacetylcolchicine Aspidospermidin-17-ol, 1-acetyl 19,21-epoxy-15,16-dimethoxy-
Isothiazolinone	36.60	1.04	1,2-Benzisothiazol-3-amine, TBDMS derivative
Sulfides	15. 62 13.33	1.70 0.49	Tert-Hexadecanethiols 1-Octadecanesulphonyl chloride

The bioactive compounds identified in aqueous leaves extract of *Piper guineense* was presented in Table 2. A total of twenty putative compounds were identified in the extract. A higher proportion of methyl dihydroisosteviol (11.19 %), 10-methylnonadecane (9.87 %), and 1-octadecene (9.76) were detected in the extract compared with the other identified compounds.

Table 2: Bioactive Compounds Present in Aqueous Leaves Extract of *Piper guineense*

Class of compound	Rt	Area (%)	Bioactive compound
Alkane	7.00	1.811	Tetradecane
	10.19	3.82	Hexadecane
	11.71	1.86	Heptadecane
	10.03	1.14	5-Tetradecene, (E)-
	13.16	9.87	10-Methylnonadecane
	13.38	2.12	1-Chloroeicosane
	13.93	2.95	9-Heptadecanone
	14. 56	2.27	Tetratetracontane
Steroid	24.86	4.73	Squalene
	15.26	7.64	n-Hexadecanoic acid
Sesquisterpernoid	11.84	0.55	Dodecane, 2,6,10-trimethyl-
Alcohol	12.75	0.80	1-Decanol, 2-hexyl-
Alkene	14.06	1.62	Cetene
	13.02	9.76	1-Octadecene
	15.90	1.96	Octadecane
	18.28	2.96	1-Docosene
Ester	14.71 10.03	4.62 1.14	Didodecyl phthalate Pentadecafluorooctanoic acid, octadecyl ester
	21.78	2.27	Bis(2-ethylhexyl) phthalate
Steviosides	31.36	11.19	Methyl dihydroisosteviol

Table 3 indicates the bioactive compounds identified in aqueous leaves extract of *Vernonia amygdalina*. A total of twenty-nine compounds were identified in the extract. Cyclohexane 2-(2-propenyl)- (5.70 %), 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (5.42 %), and Spiro [2.3] hexan-4-one, 5,5-diethyl (5.40 %) were the highly detected compounds in the extract.

Table 3: Bioactive Compounds Present in Aqueous Leaves Extract of Vernonia amygdalina

CLASS OF COMPOUNDS	RT	AREA (%)	BIOACTIVE COMPUND
Aldehyde	6.04	2.63	13-Octadecenal, (Z)-
	7.06	2.42	Tridecanal
	7.51	0.65	9-Octadecenal, (Z)-
Fatty acid	29.30	1.05	Oleic Acid
	34.95	0.67	Dodecanoic acid, ethenyl ester
	6.11	2.12	1-Tricosene
Alkane	6.43	2.31	6-Nitroundec-5-ene
	36.99	5.70	Cyclohexane 2-(2-propenyl)-
	36.31	2.00	Cyclodecano l-aminomethyl
	35.90	2.91	9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester
	35.74	4.81	Cyclohexane, 1,1'-dodecylidenebis 4-methyl-
	32.23	0.34	Dodecanoic acid, 1,2,3-propanetriyl ester
	34.03	5.40	Spiro [2.3] hexan-4-one, 5,5-diethyl
Fatty Triazole	16.87	4.46	Fluconazole
Ester	37.73	2.11	9-Octadecanoic acid 2 -(octadecyoxyl) ethyl ester
	34.78	3.19	9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl) oxy] propyl ester
	36.65	4.68	Oleic acid, 3-hydroxypropyl ester
	33.61	0.40	Vinyl lauryl ether
Alcohol	37.18	3. 69	2-(4, 5-Dicholoroimidazole 1-yl) ethanol
Acyl-glycerol	35.19	4.55	3'H-Cycloprop (1,2)-5-cholest-1-en- 3-one, 1'-carboethoxy-1'cyano-1,2-dihydro-
	34.63	5.42	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester
Azole	33.04	3.43	1-Ethyl-2-hydroxymethylimidazole

3.2 Prevalence of the Bacterial Isolates

Figure 4 shows the percentage prevalence of the bacterial isolates from the sputum samples of the patients. The percentage prevalence of *Streptococcus pmeumonia, Klebsiella pneumonia, Escherichia coli,* and *Proteus mirabilis* detected in the sputum samples were 46.16 %, 23.08 %, 15.78 %, *and* 15.38 %, respectively.

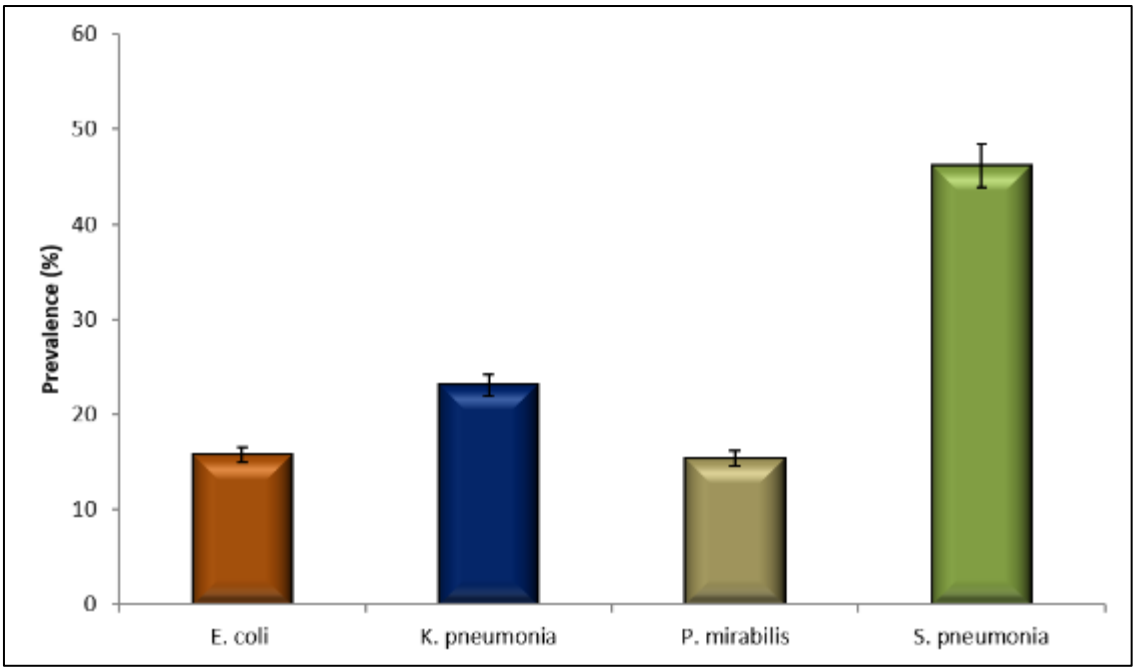


Figure 4: Prevalence of the Bacterial Isolates

3.3 Antibacterial Activity

Table 4 shows the inhibitory effect of aqueous leaves extract of *Piper guineense, Gongronema latifolium,* and *Vernonia amygdalina* on *Streptococcus pmeumonia, Klebsiella pneumonia, Escherichia coli,* and *Proteus mirabilis* isolate. The plants extract showed inhibitory effect against the isolates in dose dependent manner. At all the concentrations (50 mg/mL, 100 mg/mL, 150 mg/mL) *Vernonia amygdalina* extract displayed the highest inhibition against the isolates than other plants extract. At 150 mg/mL, *Vernonia amygdalina* extract showed higher zone of inhibition (27.04 ± 1.79 and 27.00 ± 1.84) against the *Proteus mirabilis* and *Klebsiella pneumonia* comparable to that demonstrated by the reference drug amoxicillin (32.00 ± 1.57 and 29.33 ± 1.41), respectively.

Table 4: Inhibitory Effect of Aqueous Leaves Extract of the Plants on Bacterial Isolate

Extract	Bacterial Isolate			
	S. pmeumonia	*E. coli*	*P. mirabilis*	*K. pneumonia*
Amoxicillin (30 µg)	28.33 ± 1.32	28.50 ± 1.50	32.00 ± 1.57	29.33 ± 1.41
P. guineense (50 mg/mL)	2.33 ± 0.47*	5.50 ± 0.45*	5.04 ± 0.48*	5.66 ± 0.49*
P. guineense (100 mg/mL)	4.31 ± 0.86*	7.00 ± 0.87*	9.50 ± 0.88*	9.33 ± 0.85*
P. guineense (150 mg/mL)	6.30 ± 1.95*	13.03 ± 1.02*	12.00 ± 0.97*	12.00 ± 0.97*
G. latifolium (50 mg/mL)	1.33 ± 0.32*	3.50 ± 0.35*	5.26 ± 0.51*	5.33 ± 0.51*
G. latifolium (100 mg/mL)	1.83 ± 0.83*	4.00 ± 0.44*	5.50 ± 0.53*	7.00 ± 0.66*
G. latifolium (150 mg/mL)	4.43 ± 0.74*	11.50 ± 0.80*	10.24 ± 1.00*	9.42 ± 0.87*
V. amgdalina (50 mg/mL)	1.16 ± 0.14*	8.00 ± 0.71*	14.00 ± 1.12*	14.65 ± 1.22*
V. amgdalina (100 mg/mL)	4.33 ± 0.97*	11.23 ± 0.83*	17.50 ± 1.50*	19.66 ± 1.76*
V. amgdalina (150 mg/mL)	7.83 ± 1.49*	14.11 ± 1.02*	27.04 ± 1.79	27.00 ± 1.84

Values are expressed as mean ± SEM (n = 3)

3.4 Molecular Identity of the Bacteria Isolates

The identity of the bacterial isolates is shown in Table 5. As revealed by BLAST analysis, isolates number 2 and 4 showed maximum identities of 100 % to *Escherichia coli* and *Streptococcus pneumoniae* strain. Isolate number 1 displayed identity of 99 % to *Klebsiella pneumoniae*, while isolate number 3 exhibited sequence homology level of 98 % with *Proteus mirabilis*.

Table 5: Molecular Identity of the Bacteria Isolates

Isolate No	Bacterial Specie	Strain	Percentage identity	Ascension Number	GenBank
1	*Klebsiella pneumoniae*	KP169	99 %	5_23-C_B09_01	CP041023
2	*Escherichia coli*	B16_27	100 %	5_27-F_B09_06	MH734837
3	*Proteus mirabilis*	P1	98 %	5_21D_B09_03	MK430917
4	*Streptococcus pneumoniae*	VN_O27	100 %	5_20D_B09_04	AY525794.1

4 DISCUSSION

Microbial infections have been posing threat to society and are associated with high mortality worldwide. In this study, the aqueous leaves extract of *Vernonia amygdalina*, *Gongronema latifolium,* and *Piper guineense* significantly inhibited the growth of *Streptococcus pmeumonia, Klebsiella pneumonia, Escherichia coli,* and *Proteus mirabilis* isolate. This finding is in line with the result of similar study which showed that methanol leaves extract of *Vernonia amygdalina* significantly inhibited the growth of *Staphylococcus aureus, Streptococcus agalactiae,* and *Lactobacillus rhamnosus* isolates [32]. Relevant research showed that ethanol leaf extract of *Vernonia amygdalina* exhibited inhibitory effect against *S. aureus* and *Shigella* [33]. Similar study by Zubairu et al. [34] revealed that *Vernonia amygdalina* leaf extract exhibited anti-bacterial effect against *E. coli, S. aureus* and *S. typhi*. The result of this study is in line with the finding of similar research which indicated that 300 mg/mL of ethanolic extract of *Gongronema latifolium* significantly inhibited the growth of *Staphylococcus aureus* isolates from skin of human subjects up to 85.71 % [35]. The present finding agrees with the finding of Ilodibia et al. [36] who reported that ethanol leaf and stem extracts of *Gongronema latifolium* exhibited significant inhibitory effect against the bacterial isolates. Similar study by Nwinyi et al. [37] revealed that ethanolic extract of *G. latifolium* showed higher inhibitory effect against the bacterial isolates. Similar research by George and Bill [38] hexanolic and aqueous extracts of *Piper guineense* exhibited significant inhibitory effects against Staphylococcus aureus, *Escherichia coli*, *Klebsiella pneumonia*e, Proteus vulgaris, Pseudomonas aeruginosa, and Candida albicans. Ogunmefun et al. [39] reported that ethanolic and aqueous leaf extracts of *P. guineense* demonstrated antimicrobial activity against some selected bacteria. Relevant studies displayed that *Piper guineense* displayed antimicrobial activities [40, 41]. In this study, significant percentage of *Streptococcus pmeumonia, Klebsiella pneumonia, Escherichia coli,* and *Proteus mirabilis* was detected in the sputum samples of the patients. The BLAST analysis finding revealed that the bacterial isolates displayed similarity with *Escherichia coli*, *Streptococcus pneumonia, Klebsiella pneumoniae*, and *Proteus mirabilis* strain. In the present study, a total of 33, 20, and 29 putative compounds were identified form the *Gongronema latifolium, Piper guineense,* and *Vernonia amygdalina* extract, respectively. The chemical constituents in plants inhibited the growth of certain microorganisms including *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella sp*. [42]. The inhibitory effect of the plants extract against the bacterial isolates might be attributed to the various compounds identified in the plant extract.

5 CONCLUSION

The aqueous leaves extract of Piper guineense, Gongronema latifolium, and Vernonia amygdalina exhibited significant inhibitory effect against Streptococcus pmeumonia, Klebsiella pneumonia, Escherichia coli, and Proteus mirabilis isolate. The plants extract showed the presence of various putative compounds that have been documented with different medicinal properties and pharmacological activities. The inhibitory effect of the plants extract against the bacterial isolates might be attributed to the various compounds identified in the plant extract.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The authors declared that there was no conflict of interest.

REFERENCES

[1] Mohammad AG, Amaechi JC, Abubakar I, Ibrahim S, Said SS, Aliyu JD. Phytochemicals screening and anti-bacterial effect of aqueous leaves extract of Cola acuminate. Journal of Advancement in Pharmacognosy. 2025a; 5(2): 10 – 23.

[2] Abubakar I, Muhammad HY, Shuaibu YB, Abubakar MG, Hassan SW. Anti-ulcerogenic activity of the fractions of methanol leaves extract of *Hannoa klaineana* in Wistar rats. International Journal of Pharma Science. 2021; 12(2): 27 – 40. http://dx.doi.org/10.22376/ijpbs.2021.12.2.p27-40

[3] Abubakar I, Muhammad HY, Shuaibu YB, Abubakar MG. Anti-ulcer activity of methanol extract of the leaves of *Hannoa klaineana* in rats. Journal of Phytopharmacology. 2020a; 9(4): 258 – 264. https://doi.org/10.31254/phyto.2020.9408

[4] Ibrahim IB, Abubakar I, Ibrahim S, Adiya ZSG, Buhari HB, Shehu SR. Phytochemicals screening, proximate composition and anti-oxidants analysis of Italian *Citrus paradisi* fruits. Journal of Tropical Pharmacy and Chemistry. 2024; 8(1): 2087 – 7099. https://doi.org/10.25026/jtpc.v8i1.629

[5] Dagne A, Degu S, Abebe A, Bisrat D. Antibacterial activity of a phenylpropanoid from the root extract of *Carduus leptacanthusfresen*. Journal of Tropical Medicine. 2023; 1–6. https://doi.org/10.1155/2023/4983608

[6] Abubakar I, Muhammad HY, Shuaibu YB, Abubakar MG. Gastro-protective effect of ethylacetate fraction of the leaves of *Hannoa klaineana* on aspirin and histamine-induced gastric ulcer in rats. International Journal of Pharmacology and Toxicology. 2020b; 8(1): 70-77. http://dx.doi.org/10.14419/ijpt.v8i1.30534

[7] Goronyo JI, Ibrahim YKE, Tytler BA, Hussaini M. In vivo antitrypanosomal activities of Acacia nilotica stem bark methanol extract in Wistar rats infected with Trypanosoma brucei brucei. AROC in Natural Products Research. 2022; 2: 21 – 27.

[8] Shubham K, Anu M, Dinesh B, Amit M. Herbal medicines for diabetes management and its secondary complications. Current Diabetes Reviews. 2021; 17: 437 – 456.

[9] Abubakar I, Abubakar MG, Aliyu JD, Ibrahim S, Abdullahi Z, Zubairu A, Sahabi AU. Analgesic effect of ethylacetate fraction of the methanol leaves extract of Hannoa klaineana in rats. Journal of BioScience and Biotechnology. 2024; 13(2): 155 – 161. https://doi.org/10.69085/jbb20242155

[10] Gonfa YH, Tessema FB, Gelagle AA, Getnet SD, Tadesse MG, Bachheti A. Chemical compositions of essential oil from aerial parts of Cyclospermum leptophyllum and its application as antibacterial activity against some food spoilage bacteria. Journal of Chemistry. 2022; 22: 1 – 9. https://doi.org/10.1155/2022/5426050

[11] Degu S, Abebe A, Gemeda N, Bitew A. Evaluation of antibacterial and acute oral toxicity of Impatiens tinctoria A. Rich root extracts. Plos one. 2021; 16(8): e0255932. https://doi.org/10.1371/journal.pone.0255932

[12] Ijeh II, Ejike CEC. Current perspectives on the medicinal potentials of *Vernonia amygdalina Del*. Journal of Medicinal plants Research. 2011; 5 (7): 1051 – 1061.

[13] Echem OG, Kabari LG. Heavy metal content in bitter leaf (*Vernonia amygdalina*) grown along heavy traffic routes in Port Harcourt. Agricultural Chemistry. 2013; 201–210. https://doi.org/10.5772/55604

[14] Girma Z, Abdela G, Awas T. Ethnobotanical study of medicinal plant species in Nensebo District, south-eastern Ethiopia. Ethnobotony Research Applied. 2022; 24: 1 – 25. https://doi.org/10.32859/era.24.28.1-25

[15] Mekonnen AB, Mohammed AS, Tefera AK. Ethnobotanical study of traditional medicinal plants used to treat human and animal diseases in sedie muja district, south gondar, Ethiopia. Evidence-based Complementary Alternative Medicine. 2022; 1 – 22. https://doi.org/10.1155/2022/7328613

[16] Habtamu A, Melaku Y. Antibacterial and antioxidant compounds from the flower extracts of *Vernonia amygdalina*. Advanced Pharmacology Science. 2018; 18: 4083736. http://doi.org/10.1155/2018/4083736

[17] Satria D, Harahap U, Dalimunthe A, Septama AW, Hertiani T, Nasri N. Synergistic antibacterial effect of ethyl acetate fraction of Vernoniaa mygdalina delile leaves with tetracycline against clinical isolate methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. Advanced Pharmacology Science. 2023. https://doi.org/10.1155/2023/2259534

[18] Elijah NA, Ndelekwute EK, Afolabi KD. Effect of dietary levels of *Gongronema latifolium* (utazi) on nutrient retention and live weight of broiler chickens. Nigerian Journal of Agriculture Food and Environment. 2022; 18(1): 20 – 25.

[19] Ugochukwu NH Babady EN. Antioxidant effects of *Gongronema latifolium* in hepatocytes of rat models of noninsulin dependent diabetes mellitus. Life Science. 2023; 73(15): 1925 – 1938. https://doi.org/10.1007/BF02970124

[20] Conrad OA, Chinedu OC. Genetic diversity, conservation, and improvement of *Gongronema latifolium* Benth. in South-Eastern Nigeria using the internal transcribed spacer – ITS. IOP Conference Series: Earth and Environmental Science. 2022; 1054(1): 012013. https://doi.org/10.1088/17551315/1054/1/012013

[21] Morebise O. A review on *Gongronema latifolium*, an extremely useful plant with great prospects. European Journal of Medicinal Plants. 2015; 10: 1 – 9. https://doi.org/10.9734/EJMP/2015/19713

[22] Isikhuemen EM, Ogbomwan BO, Efenudu IU. Evaluation of phytochemical and mineral constituents of *Piper guineense* Schum. & Thonn and Piper umbellatum Linn: Implications for ethnomedicine. E ur opean Journal of Medicinal Plants. 2020; 31(1): 84 – 97.

[23] Mgbeahuruike EE, Onwubuya O. An ethnobotanical survey and antifungal activity of *Piper guineense* used for the treatment of fungal infections in West-African traditional medicine. Journal of Ethnopharmacology. 2019.

[24] Christianah BA. In-Vitro Antibacterial Activity of *Piper Guineense* Extracts and their Silver Nanoparticles Against Bacterial from Gastrointestinal Tract. London Journal of Research In Science: Natural and Formal. 2024; 24(4): 23 – 37.

[25] Ogunmefun OT, Akharaiyi FC, Adegunle SJ. Phytochemical and antimicrobial properties of *Piper guineense* (Schumach. & Thonn) on selected human pathogens. Journal of Chemical and Pharmaceutical Research. 2020; 9 (11): 180 – 186.

[26] National Institute of Standard and Technology (NIST). Program handbook details the criteria used for OWM Laboratory Recognition. NIST Hanbook. 2014; 143.

[27] Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press. 2005. https://www.medbox.org/preview/5255d6e105d4-41a9-beb2-02b60e695ecc/doc.pdf.

[28] Kirby-Bauer A. Antimicrobial sensitivity testing by agar diffusion method. Journal of Clinical Pathology. 1996; 44, 493.

[29] Udensi JU, Anyanwu CO, Emedoh AE, Anyanwu EO, Danladi M, Pius T, Ezera A. Therapeutic potential of Chromolaena odorata, Cola acuminata, and Cymbopogon citratus against pathogenic bacteria. Scientific Reports. 2025; 15: 217. https://doi.org/10.1038/s41598-024-84696-3

[30] Bunyan IA, Naji SS, Aljodoa HH. Isolation and identification of some bacterial strains isolated from throat infections in Hilla Province, Iraq. Indian Journal of Public Health Research and Development. 2019; 10(6).

[31] Abayasekara LM, Perera J, Chandrasekharan V, Gnanam VS, Udunuwara NA, Liyanage DS, Ilango J. Detection of bacterial pathogens from clinical specimens using conventional microbial culture and 16S metagenomics: a comparative study. BMC Infectious Diseases. 2017; 17(1): 1 – 11.

[32] Mohammad AG, Amaechi JC, Abubakar I, Ibrahim S, Said SS. Phytochemicals composition and anti-bacterial activity of methanol leaves extract of *Vernonia amygdalina*. *Biology, Medicine, & Natural Product Chemistry*. 2025b; 14(1): 519 – 527. https://doi.org/10.14421/biomedich.2025.141.519-527

[33] Ogundare AO. Antibacterial properties of the leaf extracts of *Vernonia amygdalina*, Ocimum gratissimum, Corchorous olitorius and Manihot palmate. Journal of Microbiology Antimicrobial. 2011; 3 (4): 77 – 86.

[34] Zubairu AY, Mukhtar M, Saidu I, Ibrahim Z, Isah S, Garga MA, Kebbi HS. Antibacterial activity of methanolic extract of bitter leaf (*Vernonia amygdalina*) from various component fractions using column chromatography. GSC Biological and Pharmaceutical Sciences. 2019; 7(2): 16 – 21.

[35] Merenini IF, Upla PU, Ezaka EI, Egbobe JO, Sani BE, Yayra KA, Baffour Gyawu S, Chatterjee A, Nfongeh JF. Antibacterial Effect of *Gongronema latifolium* Leaf Extract on Staphylococcus aureus isolates from skin of human subjects in Lafia metropolis. Lafia Journal of Scientific and Industrial Research. 2025; 3(1): 33 – 39. https://doi.org/10.62050/ljsir2025.v3n1.367

[36] Ilodibia CV, Ezeja IJ, Akachukwu EE, Chukwuma MU, Egboka TP, Emeka AN. Phytochemical screening and antimicrobial effects of aqueous and ethanol leaf and stem extracts of *Gongronema latifolium* Benth. Research Journal of Botany. 2015; 10(2): 50 – 60. https://doi.org/10.17311/rjb.2015.50.60

[37] Nwinyi O, Chinedu SN, Ajani O. Evaluation of antibacterial activity of Psidium guajava and *Gongronema latifolium*. Journal of Medicinal Plant Research. 2008; 2: 189 – 192. http://eprints.covenantuniversity.edu.ng/1936/1/AjaniNwinyi%20et%20al%202008%20JMPR.pdf

[38] George BT, Bill AE. Phytochemical proling, spectroscopic characterization, and antimicrobial assessment of *Piper guineense* seeds. Research Squsre. 2026; 1 – 13. https://doi.org/10.21203/rs.3.rs-8611550/v1

[39] Ogunmefun OT. et al. Phytochemical and antimicrobial properties of *Piper guineense* (Shumach and Thonn) on selected Human pathogens. Journal of Chemistry Pharmacy and Research. 2017.

[40] Madu AN, Iwu IC, Edeh EC, Joseph EE. Extraction and GC-MS characterization of leaf extract of *Piper guineense* . African Journal of Biology and Medical Research. 2023; 6(2): 71 – 83.

[41] Adefegha SA, Oboh G, Ademosun AO, Akinyemi AJ. Comparative phytochemical proling and GC-MS analysis of selected culinary spices including *Piper guineense*. Scientific Reports. 2022; 12: 25204.

[42] Mancuso G, Midiri A, Gerace E, Biondo C. Bacterial antibiotic resistance: The most critical pathogens. Pathogens. 2021; 10(10): 1310. https://doi.org/10.3390/pathogens10101310