FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 8 No. 6, December, 2024, pp 346 - 354
DOI: https://doi.org/10.33003/fjs-2024-0806-2995

GC-MS PROFILING AND *In vitro* ANTIBACTERIAL EFFICACY OF AQUEOUS LEAF EXTRACTS OF *Ocimum gratissimum* Linn. AND *Vernonia amygdalina* Del

Itah, A. Y. and *Akinjogunla, O. J.

Department of Microbiology, Faculty of Biological Sciences, University of Uyo, P.M.B.1017, Uyo, Akwa Ibom State, Nigeria

*Corresponding authors' email: papajyde2000@yahoo.com Phone: +2348064069404
ORCID iD: https://orcid.org/0000-0001-7105-6771

ABSTRACT

The increasing spread, persistence, and prevalence of multidrug-resistant bacterial strains has become a significant global public health challenge, necessitating the need for novel, potent, and alternative antimicrobial agents, particularly from medicinal plants. The study determined the bioactive phytochemical constituents and antibacterial activities of the aqueous leaf extracts of *Vernonia amygdalina* (ALEVA) and *Ocimum gratissimum* (ALEOG). In ALEVA using the agar-well diffusion method. The ALEVA contained 35 identified compounds, with cis-thujopsene (20.99%), acetic acid (20.77%), and butanoic acid, 2-methyl (12.87%) being the most abundant. Ethosuximide and cyclohexane, 1, 3-butadienylidene were detected in small amounts. The ALEOG had 44 compounds, with butanoic acid, 3-methyl (13.89%), thymol (12.43%), and butanoic acid, 2-methyl (7.22%) in the highest concentrations, while 1H-cyclopenta[a] pentalen-7-ol and 9-octadecynoic acid methyl ester were present in small amounts. The ALEOG and ALEVA showed significant ($p < 0.05$) inhibition at 40 mg/mL against *Helicobacter pylori* and other bacterial isolates, with a highest mean ($\bar{x} \pm$ S.D.) inhibition zone diameter of 17.5 ± 0.5 mm and 18.2 ± 1.1 mm, respectively. The minimum inhibitory concentrations (MIC) values of ALEVA and ALEOG for the isolates ranged between 5 µg/mL and 40 µg/mL; the minimum bactericidal concentrations (MBC) values of ALEVA and ALEOG for the isolates ranged between 5 µg/mL and 80 µg/mL, with an MBC/MIC of ≤ 4. The study has demonstrated that aqueous leaf extracts from *O. gratissimum* and *V. amygdalina* possess significant antibacterial activity, supporting their traditional medicinal uses.

Keywords: Antibacterial, Extracts, Inhibition, *Ocimum gratissimum*, *Vernonia amygdalina*

INTRODUCTION

The increasing spread, persistence, and prevalence of multidrug-resistant bacterial strains has emerged as a significant global public health concern (Akinjogunla *et al*., 2014; Ajayi *et al*., 2024).The overuse of antibiotics has worsened resistance, leading to treatment failures, increased healthcare costs, and higher mortality, particularly in developing countries (Akinjogunla and Divine-Anthony, 2013). This has prompted an intensified global scientific search for novel, potent, and alternative antimicrobial agents, particularly from medicinal plants. Medicinal plants produce secondary metabolites with antimicrobial properties that disrupt bacterial membranes, inhibit protein synthesis, and affect nucleic acid replication (Alozie *et al*., 2024).

Ocimum gratissimum, a member of the Lamiaceae family, commonly known as African basil or clove basil, is a medicinal plant with a long history of traditional use in Africa and Asia. In Nigeria, this plant is known by the Yorubas as 'Efirin'; by the Igbos as 'Nchuanwu'; by the Hausas as 'Daidoya'; and by the Efiks as Nton' (Akinmoladun *et al*., 2007). *Ocimum gratissimum* has been traditionally used for treatment of respiratory infections, diarrhoea, and skin diseases. Its medicinal properties are attributed to the presence of essential oils and bioactive compounds which have antimicrobial (Nwinyi *et al*., 2009), and anti-inflammatory effects (Nguefack *et al*., 2004). This plant is also valued as a culinary herb due to its aromatic qualities and flavour-enhancing properties in food preparation (Orafidiya *et al*., 2007).

Vernonia amygdalina, native to Africa, commonly known as bitter leaf, belongs to the Asteraceae family and is used traditionally in Africa for various ailments (Omoregie and Osagie, 2011). In Nigeria, It is called 'Ewuro' in Yoruba, 'Onugbu' in Igbo, 'Shuwaka' in Hausa and 'Etidot' in Efik (Egedigwe, 2010). It demonstrated antimicrobial properties against *S. typhi*, *K. pneumoniae*, and *P. mirabilis* (Ijeh and Ejike, 2006). Its bioactive compounds, such as saponins, glycosides, flavonoids, and sesquiterpene lactones inhibit microbial enzymes, interfere with DNA replication, and disrupt bacterial cell wall synthesis (Ogunleye and Ibitoye, 2003).

The need for novel and potent alternative antimicrobial agents, particularly from medicinal plants, necessitated this study on the GC-MS profiling and *in vitro* antibacterial efficacy of aqueous leaf extracts of *O. gratissimum* and *V. amygdalina* on gastrointestinal tract bacteria.

MATERIALS AND METHODS

Sources of Medicinal plants

Fresh leaves of *Vernonia amygdalina and Ocimum gratissimum* (Figs. 1 and 2) were collected from their natural habitats in Uyo, Akwa Ibom State. The leaves were transported in zip-lock bags and authenticated by a taxonomist at the Department of Botany and Ecological Studies, University of Uyo. Subsequently, the leaves were taken to the Pharmacognosy and Natural Medicine Laboratory at the University of Uyo for processing. The *O. gratissimum* and *V. amygdalina* leaves were thoroughly washed three times under running water and rinsed with distilled water to remove any extraneous matter, following the methods of Onoruvwe and Olorunfemi (1998); Akinjogunla and Oluyege (2016). The leaves were then chopped into small pieces, air-dried for two weeks in the shade at 28 ± 2 °C, pulverized using a mortar and pestle, and stored in airtight polyethylene bags before extraction.

Figure 1: *Vernonia amygdalina* Leaf

Figure 2: *Ocimum gratissimum* Leaf

Preparation of the extracts

Aqueous extract

The pulverized leaves of *V. amygdalina and O. gratissimum* were weighed using a Digital Electronic Laboratory Weighing Balance Scale (*Mettler Toledo Model ME240E*). The aqueous leaf extract of *V. amygdalina* (ALEVA) and *O. gratissimum* (ALEOG) was prepared by soaking 1 kg of the pulverized leaves in 2 L of sterile distilled water for 24 h with occasional shaking at room temperature. The aqueous extract was then filtered using Whatman No. 1 filter paper, and the filtrate was dried on a BIOBASE steam water bath at 50°C for 48 h. The dried extract was weighed, preserved in stoppered sample vials, and stored in a refrigerator at 4°C (Akinjogunla and Oluyege, 2016). Graded concentrations of 10, 20, 40, and 80 mg/mL of the extracts were prepared using 10% DMSO, and the mixtures were shaken vigorously to obtain a homogeneous solution (Alozie *et al*., 2022).

Gas Chromatography-Mass Spectrometry Analysis of V. amygdalina and O. gratissimum

The GC-MS analysis of bioactive compounds in ALEVA and ALEOG was conducted using a Hewlett-Packard (HP) 7890A system with a UV detector and an HP-5 capillary column. Injector temperature (230°C), detector temperature (280°C) and an electron ionization system with an ionization voltage of 70 eV was used (Velmurugan and Anand, 2017). Helium gas (99.9% purity) served as the carrier gas at a constant flow rate of 1 mL/min with an injection volume of 1 µL. The column oven temperature was initially set at 40°C (isothermal for 2 min), increased to 200°C at 5°C/min, and finally to 280°C at 5°C/min. One microliter of the diluted ALEVA and ALEOG (1/100, v/v) was injected manually into the GC-MS using a Hamilton syringe in split mode (ratio 20:1). The relative quantity of each bioactive compound in the extracts was expressed as a percentage based on the peak area in the chromatogram. The mass spectra were interpreted using the National Institute of Standards and Technology (NIST) library database.

Source of Bacterial Isolates

Sixteen (16) bacteria comprising *E. coli* (n = 2), *S. typhi* (n = 2), *H. pylori* (n = 3), *P. aeruginosa* (n = 2), *Enterobacter* spp. (n = 2), *S. aureus* (n = 2), *S. marcescens* (n = 2), *K. pneumoniae* (n = 2), and *P. mirabilis* (n = 2) were obtained from the blood, urine, and stool samples of patients attending both private and public health facilities in Uyo. The bacterial strains were identified using conventional biochemical tests.

Antibacterial Activity of *Vernonia amygdalina and Ocimum gratissimum*

The *in vitro* antibacterial activity of ALEVA and ALEOG was evaluated using the agar well diffusion method (Daoud *et al*., 2019). Sixteen bacterial strains were inoculated onto nutrient agar plates and incubated for 24 h at 37°C. Each bacterial suspension, adjusted to a 0.5 McFarland turbidity standard, was streaked onto Mueller-Hinton Agar plates using a sterile cotton swab. The plates were allowed to dry for 5 minutes. Five wells (6 mm diameter) were punched in each plate using a sterilized cork borer. Three wells were filled with 100 µL of ALEVA and ALEOG at concentrations of 10, 20, and 40 mg/mL, respectively. A fourth well received 10 µL of 1% DMSO (negative control), and a fifth well received 10 µL of 5 mg/mL Augmentin (positive control). The plates were incubated for 18 hours at 35 ± 2°C. All experiments were performed in triplicate.

Determination of Minimum Inhibitory Concentration (MIC) of ALEVA and ALEOG

The MIC of ALEVA and ALEOG against *H. pylori* and other enteric bacteria was determined by the macro-broth dilution technique (CLSI, 2018). To prepare the dilutions, 100 microliters of an 80 mg/mL stock solution of ALEVA were serially diluted with nutrient broth in test tubes to achieve concentrations of 40, 20, 10, 5, and 2.5 mg/mL. Then, 100 µL of each ALEVA concentration was added to 9.9 mL of nutrient broth to obtain final concentrations of 80, 40, 20, 10, 5, and 2.5 µg/mL. Then, 100 µL of each bacterial suspension containing approximately 10^6 CFU/mL was added into each test tube. Two control tubes were included: one with nutrient broth and bacteria (positive control) and one with nutrient broth and ALEVA (negative control). All test tubes were incubated at 37ºC for 24 h and examined for bacterial growth. The same procedure was followed for ALEOG. The MIC value was taken as the lowest concentration of ALEVA and ALEOG that visibly inhibited the growth of the test bacteria after 24 h of incubation at 37 °C (Akinjogunla *et al*., 2021).

Determination of minimum bactericidal concentration (MBC) of ALEVA and ALEOG

A loopful from each MIC broth tube without visible bacterial growth was streak-inoculated onto freshly prepared nutrient agar plates. The inoculated plates were incubated at 37°C for 24 h, then examined for bacterial growth (CLS1, 2018; Akinjogunla *et al*., 2021). The MBC value was taken as the lowest concentration that killed 99.9% of the test bacteria after 24 h of incubation at 37°C.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS, Version 25.0) was used for data analysis. Results were expressed as mean ± standard deviation (SD), and significant differences were determined using Duncan's Multiple Range Test at 95% levels of significance.

RESULTS AND DISCUSSION

The retention time, peak area, molecular weight, and chemical compound names of bioactive compounds in the ALEVA are presented in Table 1 and Fig. 3. The results identified a total of 35 bioactive phytochemical compounds in ALEVA. The mass spectra of these compounds are shown in Fig. 3. The compounds present in the highest concentrations were Cis-Thujopsene (20.99%), acetic acid (20.77%), Butanoic acid, 2-methyl (12.87%), 1-Heptatriacotanol (10.50%), and Aromadendrene oxide (9.70%), with retention times (minutes) of 20.9, 4.756, 4.886, 21.067, and 9.7, respectively (Table 1). The bioactive compounds present in very small amounts in ALEVA were geranyl-α-terpinene, Ethosuximide, Androstan-17-one, 3-ethyl-3-hydroxy-, (5α), 2, 9-Heptadecadiene-4, 6-diyn-8-ol, Cyclopropaneoctanoic acid, 2-[[2-[(2- ethylcycl)], and Cyclohexane, 1, 3-butadienylidene (Table 1).

Table 2 and Fig. 4 show the identification of 44 compounds in ALEOG. The main bioactive compounds, identified based on the relative contents, were Butanoic acid, 3-methyl (13.89%), Thymol (12.43%), Butanoic acid, 2-methyl (7.22%), cis-Thujopsene (7.10%), 1-Heptatriacotanol (4.79%), 9-Octadecenamide (4.18%), Ethanone, 1-(6-methyl-7-oxabicyclo [4.1.0] hept (3.73%), 4-dihydroxy-p-menth-2-ene (3.43%), Acexamic acid (3.43%), 1S,2S,5R-1,4,4-Trimethyltricyclo [6.3.1.0(2,5)] (3.33%). The bioactive compounds present in very small amounts in ALEOG were 1H-Cyclopenta[a]pentalen-7-ol, decahydro-3, 3; Nerolidol isobutyrate, 3-Pentanol, 2,4-dimethyl, 1,7-Octadien-3-ol, 9-Octadecynoic acid, methyl ester, and 1-Heptatriacotanol (Table 2).

The results of the susceptibility of *H. pylori* and other bacterial isolates to ALEOG are detailed in Table 4. The maximum inhibitory zone diameter (IZD) observed was 17.5 ± 0.5 mm, while the minimum IZD was 8.5 ± 0.0 mm, with activity indices (A.I.) ranging from 0.51 to 1.19. Discs containing 40 mg/mL ALEOG exhibited strong inhibitory effects on *H. pylori* HPS2 and HPS13, *S. typhi* STS17, and *K. pneumoniae* KPS5 (IZD: ≥ 15 mm). Discs with 20 mg/mL ALEOG showed moderate inhibitory effects on 75% of the bacterial isolates (IZD: 10 to 14 mm) and mild inhibitory effects on 12.5% of the isolates (IZD: < 10 mm). Of the 16 bacterial isolates tested, 18.8% showed resistance to the growth inhibitory effects of 10 mg/mL ALEOG (Table 4).

Helicobacter pylori and other bacterial isolates susceptibilities to ALEVA are presented in Table 4. The IZDs ranged from 18.2 ± 1.1 mm to 8.8 ± 0.2 mm, with activity indice (A.I.) between 0.54 and 1.21. All the *H. pylori* and bacterial isolates exhibited sensitivity to ALEVA at a concentration of 40 mg/mL; 14 isolates (87.5%) were sensitive to a 20 mg/mL concentration of ALEVA, while 31.2% (n = 5) of the bacterial isolates (*E. coli* ECS9, *S. typhi* STS17, *Enterobacter* spp. EBS11, *S. aureus* SAS11, and *P. mirabilis* PMS7) showed resistance to 10 mg/mL ALEVA (Table 4).

The MIC and MBC values of ALEOG and ALEVA for *H. pylori* and other enteric bacteria are presented in Tables 5 and 6. Of the 16 isolates tested, the MIC value of ALEOG for nine isolates was 5 µg/mL, while the MIC value of ALEOG for *S. typhi* STS17 was 40 µg/mL. The MBC value for ALEOG was 80 µg/mL for two isolates (*S. typhi* STS17 and *S. aureus* SAB11); the MBC value of ALEOG for *H. pylori* HPS13 was 5 µg/mL; and an MBC/MIC of ≤ 4 was obtained (Table 5). The MIC values of ALEVA for the isolates ranged between 5 µg/mL and 40 µg/mL, while the MBC values of ALEVA ranged between 10 µg/mL and 80 µg/mL, with an MBC/MIC of ≤ 4 (Table 6).

Table 1: Bioactive Compounds in Aqueous Leaf Extracts of *V. amygdalina* using GC- MS

Peak	RT (min.)	Peak Area (%)	Names of Chemical Compounds	Mol. Weight (g/mol)
1	4.756	20.77	Acetic acid	60.052
2	4.886	12.87	Butanoic acid, 2-methyl-	*102.13*
3	5.241	1.15	2-Pentanamine	87.16
4	5.751	0.67	3-Pentanol, 2,4-dimethyl-	116.20
5	8.095	3.87	2-Piperidinone	99.13
6	8.517	0.82	Catechol	110.10
7	8.767	0.42	Alpha.-(Aminomethylene) glutaconic anhydride	*139.11*
8	8.906	0.24	Ethosuximide	141.16
9	10.689	1.31	5,6-Dihydro-5-methyluracil	128.12
10	12.841	1.00	Diethyl Phthalate	222.24
11	14.103	0.31	Benzene, 1,3,5-triethyl-	162.27
12	14.147	0.24	Cyclohexane, 1,3-butadienylidene-	*134.22*
13	14.226	0.51	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5	*210.31*
14	14.372	0.33	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetr	*380.50*
15	14.653	0.51	Acetic acid, 2-(2, 2, 6-trimethyl-7-oxa-bicyclo[4.	*238.32*
16	14.775	0.37	Indoleacetic acid	175.18
17	15.240	1.45	1(2H)-Naphthalenone, 3,4,5,6,7,8-hexahydro-	*150.22*
18	15.623	0.90	2,3a-Dimethylhexahydrobenzofuran-7a-ol	*152.23*
19	15.782	0.68	Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-	*152.14*
20	16.223	0.29	2,9-Heptadecadiene-4,6-diyn-8-ol, (Z,E)-	*244.37*
21	16.316	0.65	Cyclopentanol, 1,2-dimethyl-3-(1-methylethen	*154.24*
22	18.129	0.78	Phytol	296.53
23	18.385	0.29	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha)	*318.50*

24	18.420	0.63	Hexadecanamide	255.25
25	18.968	1.57	1-Heptatriacotanol	*452.50*
26	19.131	0.35	Lycopodane, 4,5-didehydro-15-methyl-, (15R)-	*231.38*
27	19.820	0.27	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcycl)	282.50
28	19.884	3.24	9-Octadecenamide, (Z)-	281.47
29	19.966	0.32	1,2,3,4,6,7,8,8a-Octahydronaphthalene-6,7-dio	*136.23*
30	20.267	0.53	6-epi-shyobunol	*222.37*
31	20.655	0.29	geranyl-.alpha.-terpinene	*272.46*
32	20.789	9.70	Aromadendrene oxide-(2)	*220.35*
33	20.900	20.99	cis-Thujopsene	204.35
34	21.067	10.50	1-Heptatriacotanol	*452.50*
35	21.208	1.18	Caryophyllene oxide	220.35

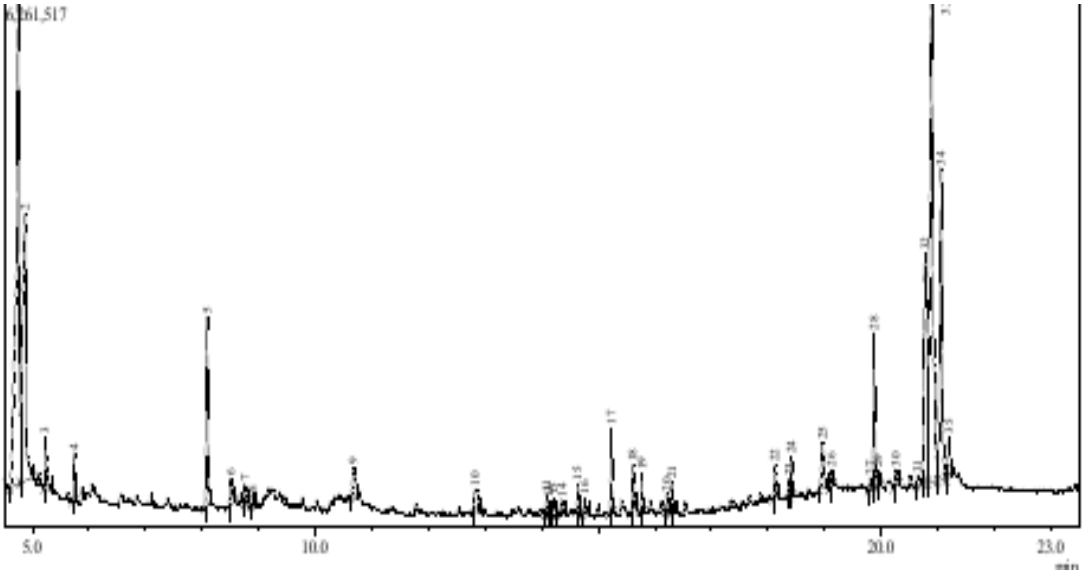

Figure 3: Gas Chromatography-Mass Spectroscopy Analysis of Aqueous Leaf Extract of *V. amygdalina*

Table 2: Bioactive Compounds in Aqueous Leaf Extracts of *O. gratissimum* using GC-MS

Peak	RT (min.)	Peak Area (%)	Names of Chemical Compounds	Mol. Weight (g/mol)
1	4.559	1.19	1,3-Propanediol	76.09
2	4.781	13.89	Butanoic acid, 3-methyl-	102.13
3	4.914	7.22	Butanoic acid, 2-methyl-	102.13
4	5.179	0.64	Butyrolactone	86.08
5	5.792	0.45	3-Pentanol, 2,4-dimethyl-	*116.20*
6	6.178	0.50	But-3-enyl (E)-2-methylbut-2-enoate	*182.26*
7	6.871	0.65	2-Pyrrolidinone	85.11
8	7.091	0.88	But-3-enyl (E)-2-methylbut-2-enoate	*182.26*
9	8.117	1.80	2-Piperidinone	99.133
10	8.299	0.31	1,7-Octadien-3-ol	*126.20*
11	8.507	6.28	Catechol	110.10
12	8.710	0.81	Benzofuran, 2,3-dihydro-	*120.15*
13	8.915	0.58	alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]ox	*170.25*
14	9.246	0.68	Epoxy-.alpha.-terpenyl acetate	196.29
15	9.376	3.43	1,4-dihydroxy-p-menth-2-ene	*170.25*
16	9.550	12.43	Thymol	150.22
17	9.880	3.73	Ethanone, 1-(6-methyl-7-oxabicyclo[4.1.0] hept	*154.21*
18	9.943	3.43	Acexamic acid	195.19
19	10.455	2.92	1,3-Benzenediol, 4-ethyl-	*138.16*
20	10.635	0.53	5,7-Octadien-3-ol, 2,4,4,7-tetramethyl-, (E)-	*182.31*
21	10.699	0.42	Benzenemethanol, .alpha.,.alpha.,4-trimethyl-	150.21
22	11.269	3.02	Benzenemethanol, 4-(methoxymethyl)-.alpha.-	*166.21*
23	12.409	2.36	Phenol, 3-methoxy-2,4,5-trimethyl-	*166.22*
24	12.532	1.06	1,6-Octadiene, 3-ethoxy-3,7-dimethyl-	*182.30*
25	13.798	0.41	2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1	*199.35*

26	13.990	0.80	Cyclopropane, 1,1-dimethyl-2-(1-methylethoxy	*288.40*
27	14.149	0.46	Bicyclo[6.3.0]undeca-1,7-dien-3-one, 5,5-dime	190.28
28	14.230	0.61	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5	*210.31*
29	14.671	0.96	Acetic acid, 2-(2, 2, 6-trimethyl-7-oxa-bicyclo [4.	*278.39*
30	15.029	0.96	2,3-Bis(1-methylallyl)pyrrolidine	*179.30*
31	15.247	2.26	1(2H)-Naphthalenone, 3,4,5,6,7,8-hexahydro-	150.21
32	15.635	1.18	2,3a-Dimethylhexahydrobenzofuran-7a-ol	170.25
33	15.789	0.58	Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-	*298.40*
34	16.559	0.33	Nerolidol isobutyrate	*292.45*
35	18.421	0.59	Hexadecanamide	*255.44*
36	18.737	0.46	1,8-Diazacyclotetradecane-2,7-dione	*226.32*
37	18.976	0.61	1-Heptatriacotanol	*537.00*
38	19.693	0.34	1H-Cyclopenta[a]pentalen-7-ol, decahydro-3,3,	*264.40*
39	19.821	0.40	9-Octadecynoic acid, methyl ester	*294.47*
40	19.887	4.18	9-Octadecenamide, (Z)-	281.47
41	20.792	3.33	1S,2S,5R-1,4,4-Trimethyltricyclo[6.3.1.0(2,5)]	*204.35*
42	20.885	7.10	cis-Thujopsene	*204.36*
43	21.068	4.79	1-Heptatriacotanol	*537.00*
44	21.206	0.42	1-Heptatriacotanol	*537.00*

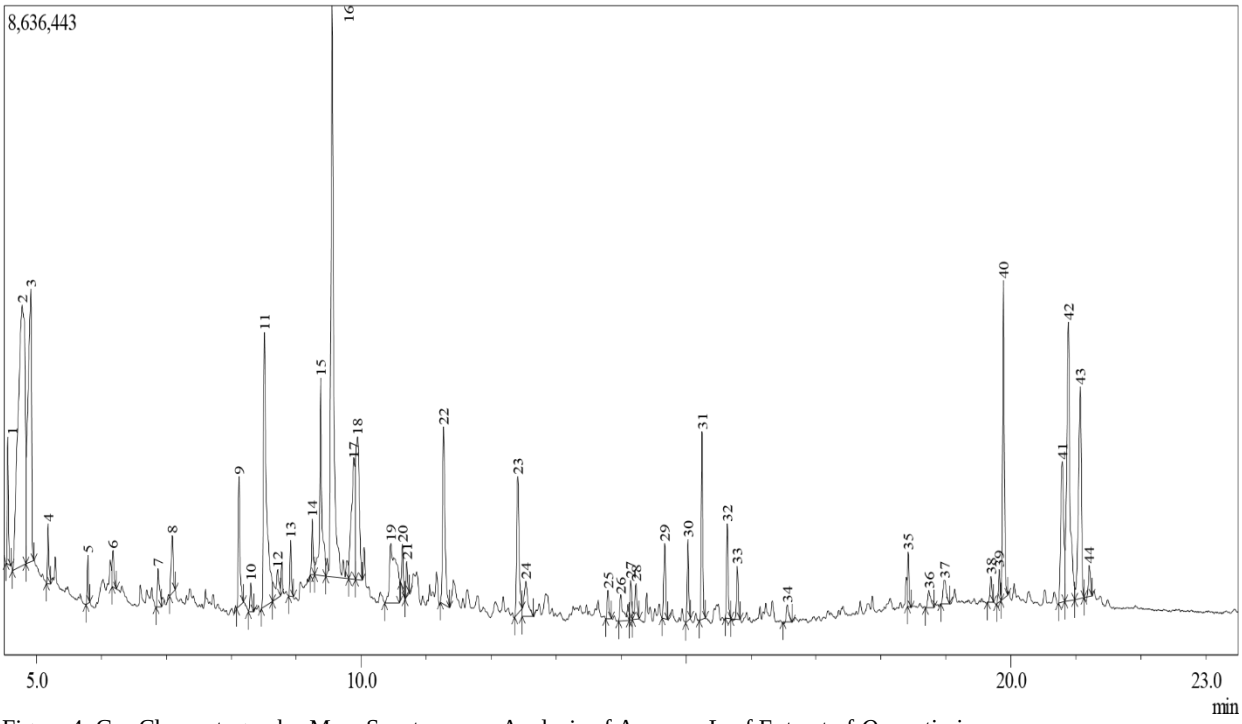

Figure 4: Gas Chromatography-Mass Spectroscopy Analysis of Aqueous Leaf Extract of *O. gratissimum*

Table 3: Antibacterial Efficacies of Aqueous Leaf Extracts of *O. gratissimum*

Bacterial Isolates	Isolates Code	Mean Zone of Inhibition (mm)							
		10 mg/mL	**A.I**	**20 mg/mL**	**A.I**	**40 mg/mL**	**A.I**	**Amoxicillin**	**DMSO**
E. coli	ECS12	11.7 ± 0.3^a	0.67	13.5 ± 1.0^b	0.77	14.8 ± 0.2^b	0.85	17.5 ± 0.5^c	NZ
	ECS9	8.5 ± 0.0^a	0.68	10.6 ± 0.3^a	0.75	12.0 ± 0.0^a	0.85	14.2 ± 0.2^b	NZ
S. typhi	STS17	NZ	0.0	NZ	0.0	9.5 ± 0.5^a	1.19	8.0 ± 0.0^a	NZ
	STS8	12.6 ± 0.2^a	0.61	14.5 ± 0.5^b	0.71	16.0 ± 1.0^b	0.78	20.5 ± 1.0^c	NZ
	HPS13	13.0 ± 1.0^b	0.86	16.5 ± 1.0^b	1.1	17.5 ± 0.5^c	1.17	15.0 ± 0.0^b	NZ
H. pylori	HPS2	11.7 ± 0.2^a	0.75	13.8 ± 0.2^b	0.89	16.5 ± 0.5^b	1.06	15.5 ± 0.5^b	NZ
	HPS19	9.7 ± 0.0^a	0.61	11.5 ± 0.5^a	0.92	12.0 ± 0.0^a	0.96	12.5 ± 0.0^a	NZ
Enterobacter spp.	EBS11	10.4 ± 0.1^a	0.63	12.0 ± 0.0^a	0.73	13.3 ± 0.3^b	0.81	16.5 ± 0.5^b	NZ
S. marcescens	SMS22	9.5 ± 0.0^a	0.59	10.7 ± 0.3^a	0.67	12.0 ± 0.0^a	0.75	16.0 ± 1.0^b	NZ
	SMS10	9.5 ± 0.5^a	0.54	11.0 ± 0.0^a	0.63	13.6 ± 0.2^a	0.78	17.5 ± 0.5^c	NZ
S. aureus	SAS11	NZ	0.0	9.5 ± 0.5^a	0.72	11.5 ± 0.3^a	0.87	13.2 ± 0.2^b	NZ
	SAS16	9.4 ± 0.1^a	0.51	12.0 ± 0.0^a	0.65	14.0 ± 1.0^b	0.76	18.5 ± 1.5^c	NZ
K. pneumoniae	KPS1	NZ	0.0	8.5 ± 0.5^a	0.65	9.8 ± 0.1^a	0.75	13.0 ± 0.0^b	NZ

	KPS5	12.0 ± 1.0[a]	0.80	14.0 ± 1.0[b]	0.93	15.8 ± 1.1[b]	1.05	15.0 ± 0.3[b]	NZ
P. mirabilis	PMS7	11.5 ± 0.5[a]	0.65	12.5 ± 0.5[a]	0.71	13.5 ± 0.5[b]	0.77	17.5 ± 1.0[c]	NZ
	PMS9	10.5 ± 0.0[a]	0.66	12.0 ± 1.0[a]	0.75	13.5 ± 0.0[b]	0.84	16.0 ± 1.0[b]	NZ

Keys: A.I: Activity Index, mm: Millimetre; X: Mean; S.D.: Standard Deviation; NZ: No zone of Inhibition; DMSO: Dimethyl Sulphoxide; Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test ($P < 0.05$).

Table 4: Antibacterial Efficacies of Aqueous Leaf Extracts of *V. amygdalina*

Bacterial Isolates	Isolates Code	Mean Zone of Inhibition (mm)							
		10 mg/mL	A.I	20 mg/mL	A.I	40 mg/mL	A.I	Amoxicillin	DMSO
E. coli	ECS12	9.5 ± 0.0[a]	0.54	11.5 ± 1.0[a]	0.66	14.0 ± 1.0[b]	0.80	17.5 ± 0.5[c]	NZ
	ECS9	NZ	0.0	NZ	0.0	10.5 ± 0.0[a]	0.74	14.2 ± 0.2[b]	NZ
S. typhi	STS17	NZ	0.0	8.6 ± 0.4[a]	1.08	9.7 ± 0.5[a]	1.21	8.0 ± 0.0[a]	NZ
	STS8	13.5 ± 0.5[b]	0.66	15.0 ± 1.0[b]	0.73	18.2 ± 1.1[c]	0.84	20.5 ± 1.0[c]	NZ
	HPS13	9.3 ± 0.0[a]	0.74	12.0 ± 1.0[a]	0.96	13.8 ± 0.4[b]	1.10	12.5 ± 0.0[a]	NZ
H. pylori	HPS2	12.3 ± 0.3[a]	0.70	14.5 ± 0.5[b]	0.83	16.8 ± 0.2[b]	0.96	17.5 ± 0.5[c]	NZ
	HPS19	8.8 ± 0.2[a]	0.67	10.5 ± 0.0[a]	0.80	13.5 ± 0.5[b]	1.02	13.2 ± 0.2[b]	NZ
Enterobacter spp.	EBS11	NZ	0.63	8.5 ± 0.5[a]	0.52	10.5 ± 0.0[a]	0.64	16.5 ± 0.5[b]	NZ
S. marcescens	SMS22	10.5 ± 0.5[a]	0.66	13.2 ± 0.2[b]	0.67	15.0 ± 1.0[b]	0.75	16.0 ± 1.0[b]	NZ
	SMS10	13.0 ± 1.0[b]	0.75	14.5 ± 1.5[b]	0.83	15.0 ± 0.0[b]	0.97	15.5 ± 0.5[b]	NZ
S. aureus	SAS11	NZ	0.0	NZ	0.0	8.0 ± 0.0[a]	0.62	13.0 ± 0.0[b]	NZ
	SAS16	10.7 ± 0.1[a]	0.58	12.7 ± 0.3[a]	0.67	16.0 ± 1.0[b]	0.86	18.5 ± 1.5[c]	NZ
K. pneumoniae	KPS1	10.0 ± 0.0[a]	0.67	12.4 ± 0.1[a]	0.83	14.0 ± 0.5[b]	0.93	15.0 ± 0.0[b]	NZ
	KPS5	11.9 ± 0.1[a]	0.79	13.5 ± 1.0[b]	0.90	16.0 ± 1.0[b]	1.07	15.0 ± 0.3[b]	NZ
P. mirabilis	PMS7	NZ	0.0	9.0 ± 1.0[a]	0.51	11.5 ± 0.5[a]	0.66	17.5 ± 1.0[c]	NZ
	PMS9	12.5 ± 0.5[a]	0.78	14.7 ± 0.7[b]	0.92	17.5 ± 1.5[b]	1.09	16.0 ± 1.0[b]	NZ

Keys: A.I: Activity Index, mm: Millimetre; X: Mean; S.D.: Standard Deviation; NZ: No zone of Inhibition; DMSO: Dimethyl Sulphoxide; Mean within the column followed by the different superscript letters are significant as determined by Duncan's multiple range test ($P < 0.05$).

Table 5: Minimum Inhibitory and Minimum Bactericidal Concentrations of Aqueous Leaf Extracts of *O. gratissimum*

Bacterial Isolates	Code	Concentrations of ALEOG (µg/mL)						Conc. (µg/mL)		MBC / MIC
		2.5	5	10	20	40	80	MIC	MBC	Ratio
E. coli	ECS12	+	-	-	-	-	-	5	10	2
	ECS9	+	+	-	-	-	-	10	40	4
S. typhi	STS17	+	+	+	+	-	-	40	80	2
	STS8	+	-	-	-	-	-	5	20	4
	HPS13	+	-	-	-	-	-	5	5	1
H. pylori	HPS2	+	-	-	-	-	-	5	20	4
	HPS19	+	+	-	-	-	-	10	40	4
Enterobacter spp.	EBS11	+	-	-	-	-	-	5	40	4
S. marcescens	SMS22	+	+	-	-	-	-	10	20	2
	SMS10	+	+	-	-	-	-	10	10	1
S. aureus	SAB11	+	+	+	-	-	-	20	80	4
	SAS16	+	-	-	-	-	-	5	10	2
K. pneumoniae	KPS1	+	+	-	-	-	-	10	20	2
	KPS5	+	-	-	-	-	-	5	20	4
P. mirabilis	PMS7	+	-	-	-	-	-	5	10	2
	PMS9	+	-	-	-	-	-	5	20	4

Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; - : No growth; +: Growth; ALEOG: Aqueous Leaf Extract of *O. gratissimum*

Table 6: Minimum Inhibitory and Minimum Bactericidal Concentrations of Aqueous Leaf Extracts of *V. amygdalina*

Bacterial Isolates	Code	Concentrations of ALEVA (µg/mL)						Conc. (µg/mL)		MBC / MIC
		2.5	5	10	20	40	80	MIC	MBC	Ratio
E. coli	ECS12	+	+	-	-	-	-	10	20	2
	ECS9	+	+	+	-	-	-	20	80	4
S. typhi	STS17	+	+	+	-	-	-	20	40	2
	STS8	+	-	-	-	-	-	5	10	2
	HPS13	+	-	-	-	-	-	5	20	4
H. pylori	HPS2	+	+	-	-	-	-	10	20	2
	HPS19	+	+	-	-	-	-	10	40	4
Enterobacter spp.	EBS11	+	+	+	-	-	-	20	40	2
S. marcescens	SMS22	+	-	-	-	-	-	5	20	4
	SMS10	+	-	-	-	-	-	5	10	2
S. aureus	SAB11	+	+	+	+	-	-	40	80	2
	SAS16	+	+	-	-	-	-	10	40	4
K. pneumoniae	KPS1	+	+	-	-	-	-	10	40	4
	KPS5	+	-	-	-	-	-	5	10	2
P. mirabilis	PMS7	+	+	+	-	-	-	20	40	2
	PMS9	+	-	-	-	-	-	5	10	2

Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; - : No growth; +: Growth;
ALEVA: Aqueous Leaf Extract of *V. amygdalina*

Discussion

The use of plants as traditional remedies remains central in developing countries, particularly among rural communities, where many plants have proven highly effective in treating various ailments (Omojasola and Awe, 2004). The chemical profile analysis of ALEVA in our study revealed several bioactive compounds. Among the compounds detected in high concentrations were **cis-**Thujopsene**,** acetic acid, butanoic acid, 2-methyl and aromadendrene oxide. The detection of these compounds in ALEVA agrees with the findings of Olufunmilayo *et al*. (2017) on GC-MS analysis of phyto-components in the root, stem bark and leaf of *Vernonia amygdalina***.** The cis-thujopsene and acetic acid have been documented for antimicrobial and insecticidal properties (Bisht *et al*., 2019), suggesting a possible contribution to ALEVA's medicinal efficacy. The presence of butanoic acid, 2-methyl, a short-chain fatty acid, in ALEVA may contribute to enhancement of antimicrobial activities of ALEVA. Additionally, 9-heptadecadiene-4, 6-diyn-8-ol and cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcycl)] were detected in low concentrations in ALEVA. These compounds may enhance the therapeutic effectiveness of the more dominant components in ALEVA by improving overall permeability, bioavailability, and antimicrobial activity, thereby contributing to a synergistic effect within the extract (Wagner and Ulrich-Merzenich, 2009).

This study has reve aled a rich variety of bioactive compounds in ALEOG, with the major components as butanoic acid, 3-methyl (13.89%), thymol (12.43%), butanoic acid, 2-methyl (7.22%), cis-thujopsene (7.10%), and 1-heptatriacotanol (4.79%). The presence of thymol in ALEOG in this study is in conformity the findings of Ogundoju *et al*. (2023) in their study on GC-MS profiling and antibacterial efficacy of *O. gratissimum* against bacteria associated with gastroenteritis. Thymol is a well-established compound in the essential oils of many aromatic plants, including *Ocimum* spp (Koba *et al.*, 2009). It has strong antimicrobial, antioxidant, and anti-inflammatory properties, making it a key contributor to the medicinal value of the extracts. The high concentration of thymol (12.43%) in ALEOG suggests that it could play a central role in its overall bioactivity ((Koba *et al.*, 2009). In this study, Nerolidol isobutyrate, 1, 7-Octadien-3-ol, and 9-Octadecynoic acid, methyl ester were present in small quantities. The presence of these bioactive compounds, although in small quantities, may enhance the synergistic effects of the extract (Koba *et al.*, 2009).

In this study, ALEOG exhibited strong antibacterial activity, with inhibition zone diameters (IZDs) of ≥ 15 mm against *H. pylori*, *S. typhi*, and *K. pneumoniae*. This observation is consistent with the findings of Adebolu and Oladimeji (2005) and Matasyoh *et al.* (2008), which reported significant antibacterial effects of *O. gratissimum*, particularly against *H. pylori* and other gastrointestinal pathogens. Similarly, *H. pylori* and other bacterial isolates were highly susceptible to growth inhibition by ALEVA at concentration of 40mg/mL. The results of the sensitivity of *H. pylori* and other bacterial isolates to ALEVA are in agreement with the findings of Orafidiya (2007), who reported significant antibacterial activity of *O. gratissimum* against various pathogens, including *H. pylori*. However, a small number of bacterial isolates demonstrated resistance to ALEOG and ALEVA, which is consistent with the report by Silva *et al.* (2016). Their study highlighted that some bacterial strains have developed resistance to natural plant extracts, often due to mechanisms such as efflux pumps or biofilm formation (Mirghan *et al*., 2022). Our results showed that both ALEOG and ALEVA exhibited strong antibacterial activity, with MIC values as low as 5 µg/mL, indicating high effectiveness. The MBC/MIC ratio of ≤ 4 was obtained for both extracts, and this confirmed their efficacy as bactericidal agents as reported by Perez *et al*. (1990).

CONCLUSION

The study has shown the antibacterial efficacy of aqueous leaf extracts from *O. gratissimum* and *V. amygdalina*, even at low concentrations, while also validating their traditional medicinal uses. However, further research is necessary to explore their underlying mechanisms of action against gastrointestinal bacteria and to assess their potential clinical applications.

ACKNOWLEDGEMENT

The authors sincerely acknowledge TETFund, Nigeria, for providing financial support for this study through the Institutional-Based Research (IBR) Programme.

REFERENCES

Adebolu, T.T. and Oladimeji, S.A. (2005). Antibacterial activity of *Ocimum gratissimum* on selected diarrhoea-causing bacteria in Southwestern Nigeria. *African Journal of Biotechnology***,** 4(7): 682-684.

Ajayi, A.O., Odeyemi, A.T., Akinjogunla, O.J., Adeyeye, A. B. and Ayo-Ajayi, I. (2024) Review of antibiotic resistant bacteria and antibiotic resistance genes within the one health framework, *Infection Ecology and Epidemiology*, 14:1, 2312953.

Akinjogunla, O. J., Ajayi, A.O. and Ekeh, N.O. (2014): Virulence factors and MDR *Staphylococcus* spp from the anterior nares of undergraduate students in Uyo. *American Journal of Research Communication*, 2 (11): 158-180.

Akinjogunla, O. J. and Divine-Anthony, O. (2013). Asymptomatic bacteriuria among apparently healthy undergraduate students in Uyo, South- South, Nigeria. *Annual Review and Research in Biology*, 3(3): 213-225.

Akinjogunla, O J. and Oluyege, A.O. (2016). Thermostability and *in-vitro antibacterial* activity of aqueous extracts of *T. tetraptera* pods on multidrug resistant clinical isolates. *British Journal of Pharmaceutical Research*, 14(2):1-13.

Akinjogunla, O. J., Umo, A. N., Alozie, M. F., Oshosanya, G.O., and Saturday, G. I. (2021). Antibacterial potentiality and time kill kinetics of amlodipine, thioridazine and promethazine against pathogenic bacterial isolates from clinical samples. *African Journal of Clinical and Experimental Microbiology*, 22 (3): 397- 406.

Akinmoladun, A. C., Ibukun, E. O., Afor, E., Obuotor, E. M. and Farombi, E. O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Scientific Research and Essays*, 2(5), 163-166.

Alozie, M.F., Ekong, U.S., Effiong, D.E., Udofa, E.J and Akinjogunla, O.J. (2022). Evaluation of the antibacterial properties of the extract and fractions of *Ipomoea triloba* I. (Convolvulaceae) on selected enteric diarrheagenic bacteria**.** *Journal of Biological Research and Biotechnology* (*Bio-Research*), 20 (1): 1398-1408.

Alozie, M.F. Ekong, U.S., Anwana, U.I., Udofa, E.J. and Akinjogunla, O.J. (2024). Acute toxicity and in vivo antidiarrhoeal activity of ethanol extract and fractions of *Ipomea triloba* by model infection and protection tests in mice against selected clinical isolates. *British Journal International*, 28 (6): 35-40.

Bisht, M., Sharma, S. and Singh, R. (2019). Antimicrobial and insecticidal properties of cis-thujopsene and acetic acid. *Journal of Essential Oils Research*, *31*(4): 234-245.

Clinical and Laboratory Standards Institute. (2018). *Performance Standards for Antimicrobial Susceptibility Testing* (28th ed., CLSI supplement M100S).

Daoud, A., Malika, D., Bakari, S., Hfaiedh, N., Mnafgui, K., Kadri, A. and Gharsallah, N. (2019). Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars. *Arabian Journal of Chemistry*, 12(8): 3075-3086.

Egedigwe, C.A. (2010). Effects of dietary incorporation of *Vernonia amygdalina* and *Vernonia colorata* on blood lipid profile and relative organ weights in Albino Rats", M.Sc Dissertation, Department of Biochemistry, Michael Opara University of Agriculture Umudike, Nigeria.

Ijeh, I. I. and Ejike, C. E. C. C. (2006). Current perspectives on the medicinal potentials of *Vernonia amygdalina* Del. *Journal of Medicinal Plants Research*, 5(7): 1051-1061.

Jodi, S. M., Umar, N., Ungokore, H. Y., and Ngwai, Y. B (2020). Antimicrobial activity of *Acacia nilotica* against some bacterial isolates associated with wound infections. *FUDMA Journal of Sciences*, 4(2): 807 - 811.

Koba, K., Poutouli, P. W., Raynaud, C. and Sanda, K. (2009). Antifungal activity of the essential oils from *Ocimum gratissimum* L. grown in Togo. *Journal of Scientific Research, 1*(1): 164-171.

Matasyoh, L.G., Matasyoh, J.C., Wachira, F.N., Kinyua, M.G., Thairu, A.W. and Mukiama, T.K. (2008). Antimicrobial activity of essential oils of *Ocimum gratissimum* L. from different populations of Kenya. *African Journal of Traditional, Complementary and Alternative Medicines***,** 5(2):187-193.

Mirghani, R., Saba, T., Khaliq, H., Mitchell, J., Do, L., Chambi, L., Diaz, K., Kennedy, T., Alkassab, K., Huynh, T., Elmi, M., Martinez, J., Sawan, S. and Rijal, G. (2022). Biofilms: Formation, drug resistance, and alternatives to conventional approaches. *AIMS Microbiology*, 8(3): 239-271.

Nguefack, J., Somda, I., Mortensen, C. N. and Nkengfack, A. E. (2004). Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. *International Journal of Food Microbiology*, 94(3): 329- 334.

Nwinyi, O. C., Chinedu, N. S., Ajani, O. O., Ikpo, C. O. and Ogunniran, K. O. (2009). Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineense* on *E. coli* and *S. aureus*. *African Journal of Food Science*, 3(3): 77-81.

Ogundoju, R., Oladunmoye, M. and Babatunde, O. (2023). GC-MS profiling and antibacterial efficacy of *Ocimum gratissimum* (Linn.) against bacteria associated with gastroenteritis. *Microbes and Infectious Diseases*. Doi: 10.21608/mid.2023.216316.1538.

Ogunleye, D. S. and Ibitoye, S. F. (2003). Studies of antimicrobial activity and chemical constituents of *Ximenia americana*. *Tropical Journal of Pharmaceutical Research*, 2(2): 239-241.

Olufunmilayo, L. A., Oshiobugie, M. J. and Iyobosa, A. I. (2017). Gas chromatography-mass spectrometry (GC-MS) analysis of phyto-components in the root, stem bark, and leaf of *Vernonia amygdalina*. *World Journal of Pharmaceutical Research*, 6(2): 35-49.

Omojasola, P.F. and Awe, S. (2004) The Antibacterial activity of the leaf extracts of *Anacardium occidentale* and

Gossypium hirsutun against some selected microorganisms. *Bioscience Research Communications*, 60: 25-58.

Omoregie, E. S. and Osagie, A. U. (2011). Effect of *Jatropha tanjorensis* leaves supplement on the activities of some antioxidant enzymes, vitamins, and lipid peroxidation in rats. *Journal of Food Biochemistry*, 35(2): 409-424.

Onoruvwe, O. and Olorunfemi, P. O. (1998). Antibacterial screening and pharmacognostical evaluation of *Dichrostachys cinerea* root. *West African Journal of Biological Sciences*, 7: 91-99.

Orafidiya, L. O., Kaloustian, J., Agbani, E. O., Portugal, H., Abudu, A., Igbeneghu, O. A., Abou, L., Mikail, C., Ivaldi, B. and Adetogun, G. A. (2007). A study on the composition, autoxidation, and antimicrobial activity of the leaf essential oil of *Ocimum gratissimum* Linn. against *Helicobacter pylori* and *Neisseria sicca*. *International Journal of Essential Oil Therapeutics*, 1: 1-7.

Pérez, C., Paul, M. and Bazerque, P. (1990). Antibiotic assay by agar-well diffusion method. *Acta Biologiae et Medicinae Experimentalis*, 15: 113-115.

Silva, M. K. N., Carvalho, V. R. A., & Matias, E. F. F. (2016). Chemical profile of essential oil of *Ocimum gratissimum* L. and evaluation of antibacterial and drug resistance-modifying activity by gaseous contact method. *Pharmacognosy Journal*, 8(1): 4-9.

Velmurugan, G. and Anand, S.P. (2017). GC-MS Analysis of bioactive compounds on ethanolic leaf extract of *Phyllodium pulchellum* L. Desv. *International Journal of Pharmacognosy and Phytochemical Research*, 9(1): 114-118.

Wagner, H. and Ulrich-Merzenich, G. (2009). Synergy research: Approaching a new generation of phytopharmaceuticals. *Phytomedicine, 16*(2-3): 97-110.