ANTIOXIDANT, INHIBITION OF ADVANCED GLYCATION END-PRODUCT FORMATION AND ANTIMICROBIAL ACTIVITIES OF *OCIMUM GRATISSIMUM* (SCENT LEAF) METHANOLIC LEAF EXTRACT AGAINST *Escherichia coli* AND *Bacillus SPP*

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ABSTRACT

The study investigates the antioxidant, inhibition of advanced glycation end-product formation and antimicrobial activities of methanolic leaf extract of *Ocimum gratissimum*. GC-MS, qualitative, antioxidant scavenging and antiglycation activities of the extract of *Ocimum gratissimum* were determined using standard procedures. The antimicrobial activity was evaluated by agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using standard methods. The GC-MS analysis of *Ocimum gratissimum* leaf revealed the presence of 49 compounds with P –cymene been the most abundant. The phytochemical screening of the extract shows the presence of alkaloids, tannins, phenolic, flavonoids, saponins etc. The *in-vitro* antioxidant assay of the extract was found to have β-carotene, lycopene, total phenolic compounds, total flavonoid compounds, reducing power and DPPH scavenging activities. The extract showed significant inhibitory effects on the formation of compounds containing two carbonyl groups and Advanced Glycated End products formation with IC_{50} of 75.85 ±4.87 µg/mL. Agar well diffusion assay was characterized by inhibition zones of 21.0 ± 0.30, 19.66 ± 0.20, 33.78± 0.30 and 33.25 ± 0.40 mm for *Escherichia coli* and *Bacillus spp* respectively at 250 mg/ml for *Ocimum gratissimum* and 25 mg/ml for tetracycline solution. The MIC values for *E.coli* and *Bacillus spp* were 33.33, 66.67, 0.78 and 0.78 for both extract and tetracycline while their MBC values were 66.67, 133.00, 1.56 and 1.56 respectively. MBC/ MIC values showed that *Ocimum gratissimum* extract and tetracycline had bactericidal effects. These results indicated that *Ocimum gratissimum* possesses antioxidant, prevents glycation and has antimicrobial activities.

Keywords: Antioxidant, GC-MS, *Ocimum gratissimum*, antiglycation properties and antimicrobial activities.

INTRODUCTION

The use of synthetic drugs in the treatment of various viral and bacterial diseases has leads to complication due to some of the chemical composition of these drugs can be cytotoxic and carcinogenic when administered in large doses [1] The use of natural or ethnomedicinal herbs as an alternative for orthodox drugs is very important in order to prevent the negative side effects and toxicity of synthetic drugs. Herbal medicines are used in place of synthetic drugs and

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may look primitive when compared to orthodox drugs, which are believed to be better than those made from herbal plants. In developing countries, 75- 80% of their population depend on herbal medicines as their primary health care. *Ocimun Gratissimum* also called scent leaf is widely distributed in the tropics of Asia and Africa. It belongs to the family Labiatae and *Ocimum Gratissimum* is the most abundant of the genus Oscimum. In Nigeria, scent leaf is called *Nchonwu* by Igbo, *effinrin-nia* by the people of the Yoruba´s speaking tribe, while in the northern region of Nigeria; the Hausas call it *Daidoya*. *Ocimum gratissimum* is well known for its antioxidant property and its medicinal value may be due to its rich polyphenolic compounds [2]. It is commonly used by traditional medicine practitioners and has also been reported to show certain biological activities such as: antimicrobial, phytotherapeutic properties, as well as insect repellant and antioxidant activities [3-6]. *Ocimum gratissimum* has been used locally for the treatment of dysentery, diarrhea, candidiasis and gastrointestinal disorders caused by various gastrointestinal organisms.

Glycation process occurs when carbonyl group of reducing carbohydrates reacts with amino group of proteins, nucleic acids and other molecules non-enzymatically to initiate the process glycation (fructosamine products or amadori) [7]. The Amadori products formed will then undergo numerous irreversible reactions to form highly reactive carbonyl species like 3-deoxy-glucosone, glyoxal and methylglyoxal. These reactive carbonyls species react with sulfhydryl, amino and functional groups found in guanidine of extracellular and intracellular proteins to produce stable advanced glycation end products (AGEs). The AGE products can cross-link with proteins like lens crystallins, collagen, low density lipoprotein and hemoglobin which lead to the alteration of the structures and functions of these proteins *in vivo*. The formation of protein glycation is a key molecular basis of diabetic complications which results from chronic increase in blood sugar. Synthetic AGEs inhibitor drug like aminoguanidine (AG), plant extracts and their purified constituents have been shown to possess antiglycation properties and suppress AGE formation. Antiglycation properties of plant extracts have been attributed to the present of substance containing phenolic compounds and correlated to their free radical scavenging properties. Therefore, glycation is an essential important target for the treatment of complications arising from diabetes. Despite the popular use of this medicinal plant, there are few data available on their antiglycation and antimicrobial potentials. For these reasons, this study aims to determine the antioxidant, antiglycation and antimicrobial activities of methanolic leaf extract of *Ocimum gratissimum*.

MATERIALS AND METHODS.

Collection and Identification of Ocimum gratissimum

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The leaf of *Ocimum gratissimum* was obtained from Lagos State University of Science and Technology Ikorodu in Lagos State, Nigeria. The leaves were identified using PictureThis-Plant identifier.

Preparation of Ocimum gratissimum methanolic leaf extract.

The leaves of *Ocimum gratissimum* were washed, air dried under shade in chemistry Laboratory in Ikorodu, it was later pulverised to coarse power using blender. The Extraction process was carried out by dispersing 200 grams of the grounded scent leaves into 1 liter of 80% methanol and shaked for 72 hour using GFL shaker. The solution was filtered and concentrated using rotary evaporator at a temperature not exceeding 40°C. The concentrated extract was dried to complete dryness in an aerated oven at 40°C for 48 hours. The *Ocimum gratissimum* methanolic leaf extract was later stored in a refrigerator at 4°C.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Ocimum gratissimum

GC-MS analysis was performed using 8860 gas chromatographs coupled to 5977C inert mass spectrometer with triple axis detector and electron impact source using Agilent Technologies. The stationary phase of separation of the compounds was carried out on HP-5 capillary column coated with 5% of Phenyl Methyl Siloxane (30 m length × 0.32 mm diameter × 0.25 μm film thickness). The carrier gas used was helium at a constant flow rate of 1.573 ml/min, an initial nominal pressure of 1.9514 psi and at an average velocity of 46 cm/s. One microliter of the *Ocimum gratissimum* sample was injected in split less mode at an injection temperature of 260°C. Purge flow was 21.5 ml/min at 0.50 min with a total gas flow rate of 23.355ml/min and gas saver mode was switched on. The oven was initially programmed at 60°C (1 min), then ramped at 4°C/min to 110°C (3 min), followed by temperature program rates of 8°C/min to 260°C (5 min) and 10°C/min to 300°C (12 min). The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230°C, quadrupole temperature of 150°C and transfer line temperature of 280°C. Scanning of possible compounds was from m/z 30 to 550 amu at 2.62s/scan rate and was identified by comparing measured mass spectral data with those in NIST 14 Mass Spectral Library.

Detection of components

The interpretation of mass spectrum gas chromatography-mass spectrometry was conducted by the database of the National Institute Standard and Technique (NIST) which contain more than sixty-two thousand patterns. The spectrum of the unidentified compound was compared with the spectrum of the identified compounds stored in the NIST library. The names, molecular weight, structure of the compounds in the test material were ascertained.

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Qualitative phytochemical analysis of Ocimum gratissimum methanolic leaf extract

Phytochemical analyses for secondary metabolite constituents were carried out on the *Ocimum gratissimum* methanolic leaf extract using standard phytochemical procedures [8,9].

In-vitro plant antioxidant assay

Determination of β-Carotene and lycopene determination

β-carotene and lycopene were determined in the dried plant of *Ocimum gratissimum* by the method described by Barros et al., (2007) [10]. The contents of β-carotene and lycopene were calculated according to the following equations:

β-carotene (mg/100 mL) = 0.216 (A663) − 0.304 (A505) + 0.452 (A453);

lycopene (mg/100 mL) = −0.0458 (A663) + 0.372 (A505) − 0.0806 (A453). The assays were carried out in triplicates, the results were mean ± SD and expressed as µg of carotenoid/g of *Ocimum gratissimum* extract.

Total phenolic contents determination

The methanolic leaf extract of *Ocimum gratissimum* was used for the determination of total phenolic content (TPC) using Folin-Ciocalteu reagent with standard procedure [11].

Determination of total flavonoid content and ferric reducing antioxidant power assay (FRAP) of methanolic leaf extract of Ocimum gratissimum

Total flavonoids content was determined in the extract by using the protocol of Sakanaka et al., (2005) [12]. The ferric reducing power capacities of the methanolic leaf extract of *Ocimum gratissimum* was determined using method described by Wang et al., (2008) [13], with ascorbic acid being used as the positive controls. The experiments were carried out in triplicate.

DPPH Radical scavenging activity assay of methanolic leaf extract of Ocimum gratissimum

1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was measured in the plant according to the procedure described by Gyamfi et al., (1999) [14]. The DPPH radical scavenging activity was calculated by using the following equation:

$$\text{Radical scavening activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

The IC_{50} was defined as the concentration (in μg/mL) of the *Ocimum gratissimum* leaf extract required to deplete the amount of DPPH radical by 50%. Ascorbic acid (AA) was used as the standard.

***In vitro* antiglycation inhibition assay**

In vitro antiglycation activity of the methanolic leaf extract of *Ocimum gratissimum* was examined by bovine serum albumin (BSA)-glucose model. Antiglycation activity of the plant extract was determined using standard reported method [15], with slight modification. In different tubes assays, each test tube contain 60 µL reaction mixtures containing 20 µL of BSA,

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20µL glucose anhydrous and 20 µL of methanolic leaf extract of *Ocimum gratissimum* sample of different concentration. Glycated control test tube contain 20 µL BSA, 20 µL glucose, and 20 µL sodium tetraoxophosphate (vi) buffer (pH 7.4, 67mM), while blank control test tube contains 20 µL of BSA and 40 µL sodium tetraoxophosphate (vi) buffer. The reaction mixture was incubated at 37ºC for 7 days. After incubation, 6 µL (100%) of trichloroacetic acid (TCA) was added to each test tube and centrifuge at 1500 rpm for 20 minutes at 4ºC. After centrifugation, the pellets were rewashed with 60 µL (10%) of TCA. The supernatant containing glucose inhibitor and interfering substance was removed and pellet contains AGE-BSA were dissolved in 60 µL sodium tetraoxophosphate (vi) buffer (PBS). The assessment of fluorescence spectrum (ex. 370 nm), and change in fluorescence intensity (ex. 370 nm to 440 nm) based on AGEs were monitored by using a spectrofluorimeter. Percentage (%) inhibition was calculated using the following formula:

% inhibition = [1 – [fluorescence of sample/fluorescence of glycated)] × 100.

Test organisms

To study the antimicrobial activity of *Ocimum gratissimum* methanolic leaf extract, we use two bacterial strains (*Escherichia coli* and *Bacillus spp)* obtained from Chemical Sciences Department (Biochemistry unit), Lagos State University of Science and Technology, Lagos, Nigeria. 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.

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

The procedure of agar well-diffusion method was employed to determine the antimicrobial activity of *Ocimum gratissimum*. The susceptibility of the two different organisms (*Escherichia coli* and *Bacillus spp)* to *Ocimum gratissimum* methanolic leaf extract were assayed using standard method [16]. The experiment was repeated thrice, for each replicate, the readings were taken in three different fixed directions and the average values were recorded [16].

Minimum inhibitory concentration (MIC) of Ocimum gratissimum extract

Minimum inhibition concentration is the lowest extract concentration that inhibited the growth of the test organism as indicated by the absence of visible turbidity in the tube compared with the control tubes. The MIC of *Ocimum gratissimum* methanolic leaf extract was determined according to standard method [16]. The MIC of the *Ocimum gratissimum* extract was assayed using serial dilution method. The lowest concentration of the dilution of the methanolic leaf extract in mg/ml without bacterial growth was taken as the minimum inhibition concentration.

Minimum Bactericidal Concentration (MBC) of Ocimum gratissimum extract

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The minimum bactericidal concentration of the *Ocimum gratissimum* methanolic leaf extract was carried out by standard method [16]. The lowest concentration of the *Ocimum gratissimum* extracts at which no colonies of *Escherichia coli* and *Bacillus spp* were taken as the minimum bactericidal concentration. The MBC/MIC values were calculated as either bactericidal or bacteriostatic.

Statistical Analysis

The *in vitro* BSA-AGE assay for the *Ocimum gratissimum* methanolic leaf extracts and IC_{50} assays for the extract were represented as mean ± standard deviation. The data analysis was done using Graph Pad prism computer software version 5.01. Unpaired t-test suggested significant differences at $p < 0.05$.

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RESULTS AND DISCUSSION.

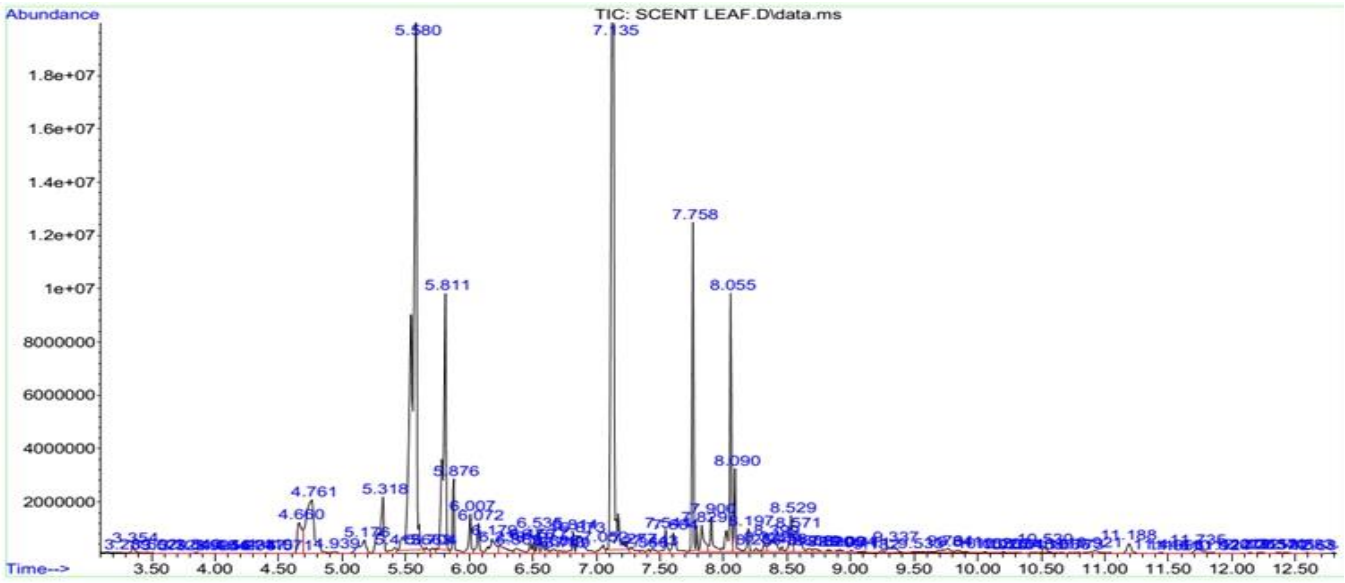

Figure 1: GC-MS analysis of *Ocimum gratissimum* leaf

Table 1: Phytocomponents identified in the leaf of *Ocimum gratissimum using* GC-MS analysis

S/N	RT	Compounds	Molecular formula	Molecular weight	Peak Area %
1	3.354	Butyric acid hydrazide	$C_4H_{10}NO_2$	102.1350	0.25
2	3.502	Methylene chloride	CH_2Cl_2	84.93	0.07
3	3.846	3-methoxy-2,2-dimethyloxirane	$C_5H_{10}O_2$	102.133	0.08
4	4.161	Methylene chloride	CH_2Cl_2	84.93	0.03
5	4.410	Methylene chloride	CH_2Cl_2	84.93	0.03

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6	4.761	Bicyclo[3.1.0]hex-2-ene,4-methyl-1-(1-methylethyl)-	$C_{10}H_{16}$	136.23	6.78
7	4.939	Camphene	$C_{10}H_{16}$	136.24	0.08
8	5.176	Bicyclo[3.1.0]hexane,4 methylene -1-(1-methylethyl)-	$C_{10}H_{16}$	136.23	0.99
9	5.318	Beta-myrcene	$C_{10}H_{16}$	136.23	2.65
10	5.413	Alpha.-Phellandrene	$C_{10}H_{16}$	136.23	0.10
11	5.580	P –cymene	$C_{10}H_{14}$	134.21	25.30
12	5.811	Gamma.-terpinene	$C_{10}H_{16}$	136.23	7.23
13	5.882	Cyclohexanol, 1-methyl-4-(1-methyletheyl)-,cis-	$C_{10}H_{20}$	156.26	1.48
14	6.007	O –Isopropenyltoluene	$C_{10}H_{12}$	130.20	1.39
15	6.179	6-Azabicyclo[3.2.1]octane	$C_7H_{13}N$	111.18	1.61
16	6.369	4H-Pyran-4-one,2,3,-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144.12	0.36
17	6.535	3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-,(R)-	$C_{10}H_{18}O$	154.25	0.60
18	6.666	2-pyridine acetic acid, hexahydro-	$C_7H_{13}NO_2$	145.15	0.10
19	6.814	Benzene,2-methoxy-4-methyl-1-(1-methylethyl)-	$C_{12}H_{20}O_2$	196.29	1.14
20	7.034	o-cymene	$C_{10}H_{14}$	134.22	0.27
21	7.135	Thymol	$C_{10}H_{14}O$	150.22	24.78

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22	7.230	Phenol,p-tert-butyl-	$C_{11}H_{15}NO_2$	193.24	0.45
23	7.443	2-methyl-7-endo-vinylbicyclo[4.2.0]oct-1(2)-ene	$C_{11}H_{16}$	148.24	0.21
24	7.544	Alpha –copaene	$C_{15}H_{24}$	204.35	1.06
25	7.758	Caryophyllene	$C_{15}H_{24}$	204.35	5.41
26	7.900	Humulene	$C_{15}H_{24}$	204.35	1.96
27	8.055	Naphthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-[4aR-(4a.alpa., 7.alpha.,8a.beta)]	$C_{15}H_{24}$	204.35	6.10
28	8.197	(2s, 4aR, 8aR)-4a, 8-dimethyl-2-(prop-1-en-2-yl)-1, 2, 3, 4, 4a, 5,6,8a-octa hydronaphthalene	$C_{15}H_{24}$	204.35	0.68
29	8.399	9-12-octadecadienoic acid(z,z)-methylester	$C_{19}H_{34}O_2$	294.5	1.23
30	8.529	Caryophyllene oxide	$C_{15}H_{24}O$	220.35	2.58
31	8.672	Spiro[4.4]nonan-2-one	$C_9H_{14}O$	138.21	0.46
32	8.909	Isoaromadendrene epoxide	$C_{15}H_{24}O$	220.35	0.21
33	9.111	Indane,2-methoxy-3-(2-methyl-1-propenyl-1)-	$C_{14}H_{18}O$	202.29	0.08
34	9.337	Methyl stearate	$C_{19}H_{38}O_2$	298.5	0.67
35	9.533	Cyclopropane octanal,2-octyl	$C_{19}H_{36}O$	280.5	0.16
36	9.764	2-methyl-z,z-3,13 0ctadecadienol	$C_{19}H_{36}O$	280.5	0.78
37	10.055	Heptadecanoic acid,16-methyl-,methylester	$C_{19}H_{38}O_2$	298.5	0.07
38	10.203	1,2,-dioctylcyclopropene	$C_{19}H_{36}$	264.5	0.14

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39	10.530	Dibutylphthalate	$C_{16}H_{22}O_4$	278.34	0.39
40	10.773	Naphthalene,1,2,3,4,4a,5,6,7,-octahydro-4a-methyl	$C_{23}H_{26}$	302.5	0.05
41	10.921	7-hexadecenoic acid, methylester,(z),	$C_{17}H_{32}O_2$	268.4	0.27
42	11.188	Hexadecanoic acid ,methylester	$C_{17}H_{34}O_2$	270.4507	0.45
43	11.444	9-octadecenal,(z),	$C_{18}H_{34}O$	266.5	0.06
44	11.556	1-propyl 9-tetradecenoate	$C_{17}H_{32}O_2$	268.4	0.03
45	11.735	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	0.52
46	12.079	Oleic acid	$C_{18}H_{34}O_2$	282.5	0.07
47	12.215	Cis-1-chloro-9-octadecene	$C_{18}H_{35}Cl$	286.9	0.13
48	12.441	3,4,diethylphenol	$C_{10}H_{14}O$	150.22	0.13
49	12.583	1-[4-acetyl-1-(2 amino-4,5-dimethyl-phenyl)-2,5-dimethyl-1H-pyrrol-3-yl]-ethanone	$C_{18}H_{22}N_2O_2$	298.4	0.05

Table 2: The qualitative phytochemical constituents of *Ocimum gratissimum* methanolic leaf extract

Phytochemical constituent	Inference
Saponins	+
Cardiac glycoside	+
Alkaloids	+
Phenolic	+
Terpenoids	+
Tannins	+
Phlobatanins	+
Reducing sugar	-
Flavonoids	+
Steroids	-

+ mean present and – means absent

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Table 3: Estimation of antioxidant and free radical scavenging activities of methanolic leaf extract of *Ocimum gratissimum*

Parameters	Concentration obtained
β-Carotene (mg)	0.136 ± 0.01
Lycopene (mg/g)	0.035±0.03
Total phenolic compounds as mg gallic acid equivalent (GAE mg/g extract)	317.95 ± 8.5
Total flavonoids compounds as rutin equivalent (mg/g dry extract)	18.46 ± 2.18
Reducing power for ascorbic acid	0.924±0.012
Reducing power for *Ocimum gratissimum* extract	0.873±0.05
IC_{50} of DPPH scavenging activity of ascorbic acid	7.16 ±0.85 μg/mL
IC_{50} of DPPH scavenging activity of *Ocimum gratissimum* extract	74.09 ± 4.17 μg/mL

Values are represented as mean ± SD of triplicate determination

Table 4: Result showing the percentage inhibition of glycation by methanolic leaf extract of *Ocimum gratissimum*

Plant/standard	Concentration (µg/ml)	Antiglycation activity (% Inhibition)
Ocimum gratissimum	500	65.3
Ocimum gratissimum	1000	74.8
Ocimum gratissimum	1500	94.1
Ascorbic acid	500	58.8
Ascorbic acid	1000	64.4
Ascorbic acid	1500	90.7

Values are expressed as mean ± SD

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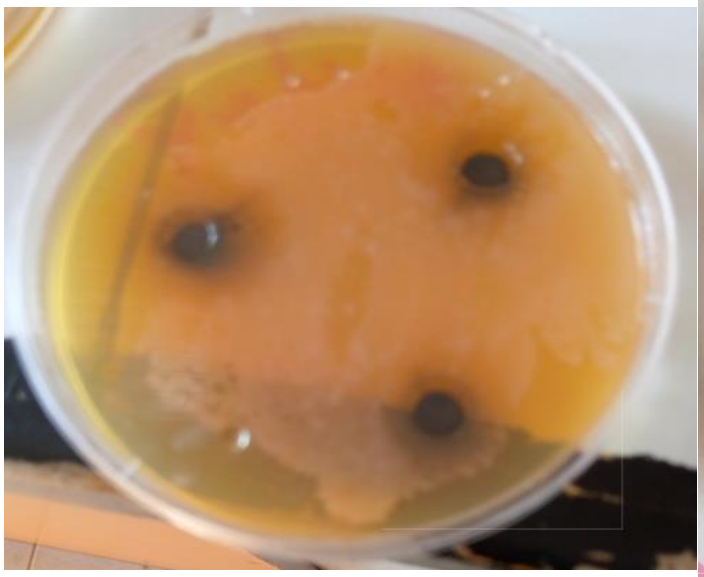

Figure 2: Zone of inhibition at 250 mg/ml concentration of the *Ocimum gratissimum* extract against *E.coli*

Figure 3: Zone of inhibition at 250 mg/ml concentration of *the Ocimum gratissimum* extract against *Bacillus spp*.

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Figure 4: Zone of inhibition at 12.5 mg/ml concentration of tetracycline against *E.col*	Figure 5: Zone of inhibition at 25 mg/ml concentration of tetracycline against *E.coli*
 Figure 6: Zone of inhibition at 12.5 mg/ml concentration of tetracycline against *Bacillu spp*	 Figure 7: Zone of inhibition at 25 mg/ml Concentration of tetracycline against *Bacillus spp*

Figure 2-7. Zone of inhibition of tetracycline solution and *Ocimum gratissimum* methanolic leaf extract against *E. coli and Bacillus spp* at 12.5, 25 and 250 mg/ml.

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Table 5: Zone of inhibition of *Ocimum gratissimum* methanol leaf extract and tetracycline solution against *Escherichia coli* and *Bacillus spp*

Test organisms	Concentration of methanolic leaf extract of *Ocimum gratissimum* (mg/ml)	Zone of inhibition for methanolic leaf extract of *Ocimum gratissimum* (mm)	Concentration of tetracycline solution (mg/ml)	Zone of inhibition for tetracycline solution (mm)	Interpretation
Escherichia coli	50	Nil	12.5	30.75± 0.20	Only tetracycline was sensitive
Bacillus spp	50	Nil	12.5	29.13 ± 0.3	Only tetracycline was sensitive
Escherichia coli	250	21.0 ± 0.30	25	33.78± 0.30	Both are sensitive
Bacillus spp	250	19.66 ± 0.20	25	33.25 ± 0.40	Both are sensitive

Table 6: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) for *Ocimum gratissimum* methanolic leaf extract and tetracycline solution against *Escherichia coli* and *Bacillus spp*

Organisms	*Escherichia coli*	*Bacillus spp*
MIC for *Ocimum gratissimum* methanolic leaf extract (mg/ml)	33.33	66.67
MIC for tetracycline solution (mg/ml)	0.78	0.78
MBC for *Ocimum gratissimum* methanolic leaf extract (mg/ml)	66.67	133.00
MBC for tetracycline solution (mg/ml)	1.56	1.56
MBC/MIC for *Ocimum gratissimum*	2.00	1.99
MBC/MIC for tetracycline solution	2.00	2.00

Discussion

Medicinal plants are now consumed in many cultures and countries of the world for therapeutic purposes against wide variety of diseases due to their easy accessibility and little or no side effects. In the case of microbial diseases, the emergence of high rate of resistance of microorganisms such as, *E.coli, Salmonella, Bacillus, Klebsella* etc to antibiotics, have led researchers to explore the use of herbal

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plants as an antimicrobial agent. Figure 1 shows the Gas-Chromatography–Mass Spectrometry chromatogram of *Ocimum gratissimum*. A total of 49 compounds were identified consisting of five prominent compounds and 44 minor compounds (Table 1). The two major compounds and their percentage abundance are: P–cymene (RT=5.580, 25.30%) and Thymol (RT=7.135, 24.78%). P–cymene has insecticidal property, antifungal, antinociceptive, herbicidal and anti-inflammatory activities [17-18], while Thymol possess antimicrobial activity [19-20]. Gamma.-terpinene (RT=5.811, 7.63%) compound found in the plant possesses antioxidant and antimicrobial activities [21,22].

The preliminary qualitative analysis of the active components (secondary metabolites) of *Ocimum gratissimum* leaf using methanolic extraction showed the presence of different constituents such as alkaloids, flavonoids, terpenoids, phenolic, cardiac glycosides, tannins which have been reported to have curative effects on pathogens by serving as defense mechanism against micro-organisms, in addition to their various biological activities such as, ant diabetes, anti-inflammatory, antimicrobial and anticancer [23].

The values obtained for lycopene and β-Carotene were 0.035±0.03 µg/g and 0.136 ± 0.01 µg respectively. The reducing power activity assay shows that the *Ocimum gratissimum* extracts exhibited reducing capacity (0.873±0.05 OD at 700 nm) less than ascorbic acid (0.924±0.012 OD at 700 nm). The total flavonoids and total phenolic compounds obtained (Table 3) shows that *Ocimum gratissimum* may serve as good scavenger of free radicals. The free radicals produce by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) are normally used for the screening of medicinal plants to investigate their antioxidant properties. The principle of this antioxidant assay is the ability of 1, 1-diphenyl-2-picrylhydrazyl, a stable free radical, to diminish the deep purple color in the presence of an antioxidant. The color of 1, 1-diphenyl-2-picrylhydrazyl radical is as a result of the presence of an odd electron found in it. When an antioxidant compound donates an electron to 1, 1-diphenyl-2-picrylhydrazyl, the DPPH is decolorized; this can easily be quantified by noting the change in absorbance at 517 nm. *Ocimum gratissimum* extracts was assessed for their antioxidant activity using 1, 1-diphenyl-2-picrylhydrazyl radical method and expressed as percent DPPH inhibition as shown in Table 3 above. The strong antioxidant activities of *Ocimum gratissimum* extracts are possible as a result of flavonoids and phenolics which have been shown to have high antioxidant properties [24,25].

There are few studies reported on the antiglycation activity of *Ocimum gratissimum*, however, it is well known that active ingredients in this plant extracts are responsible for this activity [26,27]. The mechanism of glycation process is also well understood, as oxygen free radicals such as, ROS (reactive oxygen species) plays a crucial role in protein glycation via lipid peroxidation during AGEs formation. Incorporation of these free radicals into the glycation process leads to the formation of AGEs [28], hence, it is possible that the reduction of these

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free radical formation contribute to AGEs inhibition. Several plants with secondary metabolites that act as antioxidants such as, polyphenols and flavonoids with free radicals scavenging and reducing capacity [29,30] have been reported for their high inhibitory activity against non-enzymatic glycation process [25,31]. The antiglycation activity of *Ocimum gratissimum* methanolic leaf extracts was evaluated for the inhibition of advanced glycation endproducts formation based on the BSA/glucose system. The results showed that *Ocimum gratissimum* exhibited potential antiglycation activity (> 50% inhibition) in a dose dependent manner more than ascorbic acid used in the study (Table 4). The study revealed a positive correlation between flavonoid and phenolic contents of *Ocimum gratissimum* with antiglycation activities (data not shown). Different studies have suggested that flavonoid and phenolic compounds in plants extracts are responsible for the antiglycation activities of plants [24,32,33]. The results indicated that the methanolic leaf extracts of *Ocimum gratissimum* had significantly higher inhibitory activity-against AGE formation induced by BSA-glucose model than ascorbic acid (IC_{50} = 75.85 ± 4.87 µg/mL) (p<0.05). This may be due to the phenolic and flavonoid compound found in the plant [34,35]. A study reported the antiglycation activity of saponin, which may be another contributing factor as suggested by Devendran and Balasubramanian [36]. This directly justified the antiglycation potential of *Ocimum gratissimum* methanolic extract in our result, because of the presence of saponin and other active components responsible for this activity [26,27].

Escherichia coli (gram negative bacterium) and *Bacillus spp* (gram positive) were used in this present study because of their reported prevalence in infection causing diseases as contained in literatures [37,38]. The leaf extract of methanolic solvent system of *Ocimum gratissimum* sowed inhibitory activities against the tested organisms, *E. coli* and *Bacillus spp* exhibited zone of inhibition at a concentration of 250 mg/ml. *Ocimum gratissimum* showed an inhibition zone of 21.0 ± 0.30 mm as compared to tetracycline (30.75 ± 0.20 mm at 12.5 mg/ml) against *Escherichia coli* and Inhibition of 19.66 ± 0.20 mm compared to tetracycline (29.13 ± 0.30 mm at 12.5 mg/ml) against *Bacillus spp* (gram positive). This may be because *Ocimum gratissimum* is a good source of natural antioxidants, natural drugs and contain secondary metabolites such as, flavonoids, tannins, steroids, saponins, and phenolics with inhibitory effects on these microorganisms. Talabi and Makanjuola [39] study shows that *Ocimum gratissimum* aqueous ethanolic leaf extracts were active against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Bacillus* cereus and the aqueous extract of the leaf was active against *P. aeruginosa* [40-42]. Studies also reported that methanolic leaf extract of *Ocimum gratissimum* showed potential antibacterial activities which support the present findings. The difference observed in the zones of inhibition of the extract suggests the susceptibility of the test organisms to different secondary metabolites present in the plant extracts. The mechanism of action of flavonoids, alkaloids, and glycosides with antimicrobial activity is available in literatures [43-

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46], hence, it is possible that the presence of these secondary metabolites in the methanolic extracts of *Ocimum gratissimum* has revealed by the preliminary phytochemical studies contributes to its antimicrobial activity, although this activity may not be due to one of these active ingredients alone but the mixture of the components. The plant extract did not show any inhibition against the two tested organisms at a low concentration of 50 mg/ml.

The study shows that the MIC values for *E.coli* and *Bacillus spp* were 33.33, 66.67, 0.78, and 0.78 for both *Ocimum gratissimum* extract and tetracycline while their MBC values were 66.67, 133.00, 1.56 and 1.56 respectively. Umar et al., (2019) [1] study shows that methanol and chloroform extracts of *Ocimum gratissimum* have an MIC of 50 mg/mL against S. *typhi* and *E. coli* and the same extracts recorded MBC of 50 mg/mL for *B. subtilis* and *S. typhi*. MBC/ MIC results showed that *Ocimum gratissimum* methanolic leaf extract and tetracycline had bactericidal effects. *Ocimum gratissimum* has strong potency against these microorganisms with *E. coli* being the most susceptible. Aderele et al., (2020) [16] study shows that calculated MBC/MIC ratio has bactericidal effect if the values of the ratio are less than or equal to 4 and bacteriostatic if the ratio is greater than 4. *Ocimum gratissimum* and tetracycline solution showed remarkable bactericidal effects on the two organisms tested.

CONCLUSION

The phytochemical screening of the methanolic leaf extract of *Ocimum Gratissimum* extract shows the presence of alkaloids, tannins, phenolic, flavonoids, saponins etc. A total of 49 compounds were identified using GC-MS with P–cymene been the most abundant. The *in-vitro* antioxidant assay of the extract was found to have β-carotene, lycopene, total phenolic compounds, total flavonoid compounds, reducing power and DPPH radical scavenging activities. *Ocimum gratissimum* has potential as natural therapeutic agents against diabetes as shown from the antiglication activity. The methanolic leaf extract of *Ocimum Gratissimum* is effective on *Escherichia coli* and *Bacillus spp.* This is an indication that the extract has antimicrobial activity. The MIC values for *E.coli* and *Bacillus spp* were 33.33, 66.67 while their MBC values were 66.67, 133.00 respectively. MBC/MIC values showed that *Ocimum gratissimum* extract had bactericidal effects.

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