Phytochemical (qualitative, quantitative), proximate analysis, toxicological implication and characterisation of aqueous *Ocimum Gratissmum* leaf extract

[1]**Joseph O. Soola,** [1]**Boone I. Kukoyi,** [2*]**Wasiu E. Olooto,** [2]**Hameed A. Adenusi and** [2]**Monisola A. Ayodele**

[1]Department of Physiology, Olabisi Onabanjo University, Ago-Iwoye, Nigeria
[2]Department of Chemical Pathology and Immunology, Olabisi Onabanjo University, Ago-Iwoye, Nigeria

ABSTRACT

Background: Plant materials has been in use globally for medical reasons with little or no knowledge of phytochemicals therein present and often times no scientific basis.

Methods: The determination of the types, quantity of some phytochemicals present in aqueous *Ocimum gratissimum* leaf extract and its toxicological implications were performed using different doses of the extract administered orally to Albino rats for 60 days. Blood was collected, centrifuged to obtain the plasma which was used to determine plasma alanine transaminase (ALT) and aspartate transaminase (AST) activities; total protein; albumin; bilirubin; urea; and creatinine concentrations.

Results: Phytochemistry showed that *Ocimum gratissimum* contain flavonoids, alkaloids, terpenoids, anthraquinone, tannin, phlobotannin, phenol, and saponin but lack cardiac glycoside and cardenolide. Proximate analysis revealed that it contain 46.1% carbohydrate, 15% crude fibre, 15.4% crude protein, 5% crude fat, 9.2% moisture, and 9.1% ash. The LD_{50} for *Ocimum gratissimum* is ≥5000mg/kg while markers of hepatorenal toxicity revealed an increase in ALT and AST activities; albumin; and urea; and a decrease in total bilirubin, conjugated bilirubin, total protein and creatinine concentrations on chronic administration. The result of GC-MS showed the presence of various bioactive compounds in aqueous *Ocimum gratissimum* leaf extract.

Conclusion: This study revealed that *Ocimum gratissimum* leaf contains different phytochemicals and compounds of potential medicinal importance in appreciable quantities and its ingestion has no toxicological implication.

Keywords: *Ocimum gratissimum*, phytochemicals, proximate analysis, toxicity.

1. INTRODUCTION

Basically, drugs are chemical entities used in the treatment, cure, prevention, or diagnosis of disease or otherwise used for the enhancement of physical or mental state. It is obtained either through chemical synthesis or isolated from natural products [1]. Natural products are obtainable from plants, microorganisms, fungi and bacteria. A number of different drug therapies (singly or combined) had been incorporated into the management of diseases Worldwide with some showing little or no significant improvement in the prognosis of such diseases. Also, lifelong drug use for treatment of chronic ailments has overall economic implications which have increasingly drawn the attention of researchers to the need to discover naturally existing, readily available, economic friendly and non-toxic phytochemicals present in plant materials for the management of ailments in Africa and the World as a whole. *Ocimum gratissimum*, as an example of plant with medicinal potentials, is a herbaceous plant that is indigenous to tropical areas especially India and Nigeria, where it is found in the Savannah and coastal areas. In Nigeria it is called various names by different tribes such as "effinrin-nla" by the Yorubas, "Ahuji" by the Igbos, and "Daidoya" by the Hausas [2]. Traditionally, various parts of *Ocimum gratissimum* (leaves, roots, the whole plant) in different forms (decoctions, concoctions, infusions) has been used extensively in traditional medicine for the treatment of various diseases. Notable ones include epilepsy, high fever, diarrhoea, mental illness, septic wound, infections, cold, catarrh, stomach upset and haemorrhoids [2, 3, 4]. These claims mostly have no scientific basis but are generally based on "testimonies" and other anecdotal reports from individuals who have used such remedies. The scientific community thus need much more proof to know the truth about the characteristics, safety and efficacy of such remedies and possible complications from their use. This research thus focused on

* Corresponding author: Email: olotoenny@yahoo.com, Phone: +2348053131571

determining the types and quantity of active compounds present in aqueous *Ocimum gratissimum* leaf extract and its toxicological implication.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1 Reagents

Sulfuric acid, sodium hydroxide, ferric chloride, petroleum ether, methanol, hydrochloric acid, Mayer's reagent, albumin, total protein, bilirubin (total and direct), alanine transaminase, aspartate transaminase, urea and creatinine laboratory kit reagents (Randox Laboratories, Crumlin, England).

2.1.2 Equipment

Electric blender, porcelain crucible, desiccator, soxlet reflux flask, rotary evaporator, glass wares, thermometer, weighing scale, and Perkin Elmer gas chromatograph interfaced to a Mass Spectrometer (GCMS),

2.1.3 Biological materials

Female Wistar rats, *Ocimum gratissimum* leaf

2.2. Methods

2.2.1 Preparation of aqueous leaf extract of ocimum gratissimum

Fresh leaves of *Ocimum gratissimum* were washed, air-dried at room temperature for two weeks and milled to powder with an electric blender. About 150g of powdered *Ocimum gratissimum* was added to 450mL of water in an air-tight container. The mixture was frequently shaken for a period of one week to allow the soluble matters present in the plant materials dissolve in water. At the end of one week, the mixture was decanted and filtered. The filtrate was concentrated using rotary evaporator and the final obtained suspension was further dried using water bath to obtain the aqueous residue. The stock solution of the extract was prepared by dissolving 100g of extract in 10mL of water to give a concentration of 10g/mL. The stock solution was labelled appropriately and refrigerated at 4^{o}C until required for use.

2.2.2 Experimental animal

The study was carried out using female adult healthy Wistar strain Albino rats weighing 200-250g which were obtained from Veterinary Medicine Department, University of Ibadan. The rats were acclimatised for 2 weeks before being exposed to the experimental procedures. The rats were kept and maintained under conventional laboratory condition of temperature; humidity and light in accordance with the US Public Health Service Guidelines [5].

2.2.3 Phytochemical qualitative and quantitative analysis of Ocimum Gratissmum

Phytochemical screening of aqueous *Ocimum gratissimum* leaf extract was performed using standard procedures [6, 7]. This included test for anthraquinones, terpenoids, flavonoids, saponins, tannins, alkaloids, cardenolide, phenols and cardiac glycosides. Proximate analysis was done by analysing the leaves for crude protein, crude fat, crude fibre, ash, and moisture, and carbohydrate was calculated by difference. Qualitative analysis of the aqueous extract was done by determining the concentrations of alkaloids, tannin, saponins, phlobatannins, phenolics, oxalate, and phytate present using standard methods [8-16].

2.2.4 Acute toxicity study

Acute toxicity study was carried out using thirty female Albino rats using standard methods [17, 18]. Female rats were used because literature surveys of conventional LD_{50} tests show that whenever differences are observed in sensitivity between the sexes, females are generally slightly more sensitive than male [19]. The acute oral median dose (LD_{50}) was also estimated and calculated using the method of maximum likelihood [20, 21]. In the night preceding the test, food was withdrawn but not water. In the morning of the test, the rats were weighed and the administered doses (300, 500, 1000, 2000 and 5000mg/kg body weight) of *Ocimum gratissimum* calculated. The *Ocimum gratissimum* powder was dissolved in 1mL water and administered as a single dose, using oral cannula. Food was further withheld for about 4 hours. The rats were group-caged according to the dose and observed individually for signs of toxicity every fifteen minutes during the first 30 minutes, hourly during the first six hours, two-hourly during the first 24 hours, and daily for a total of 14 days. Toxicity signs considered include changes in skin, fur and eyes; respiratory efforts; tremors; convulsions; behavioral pattern; salivation; diarrhea; sleep and moribund condition. The control group was given 1mL water instead of aqueous *Ocimum gratissimum* extract.

2.2.5 Chronic toxicity study

This was carried out using forty female Albino rats administered with different doses (100 and 200mg/kg body weight) of *Ocimum gratissimum* for a period of 60 days. On the 60th day, blood was collected from the rats by cardiac puncture under diethylether anaesthesia into heparinised bottle, centrifuged at 5000 rpm to obtain the

plasma which was stored at -20ºC till analysed. Plasma urea, creatinine, aspartate transaminase (AST) and alanine transaminase (ALT) concentrations were determined [22-24].

2.2.6 Gas chromatography-mass spectrophotometry

Gas chromatographic-Mass spectrometric (GC-MS) analysis of aqueous *Ocimum gratissimum* leaf extract was performed using a Perkin Elmer GC Claurus 500 system and GC-MS equipped with Elite-1 fused silica capillary column (30m x 1µMdf composed of 100% Dimethyl polysiloxane). In the Mass Spectrometry (MS) step of GC-MS, compounds leaving the GC column are fragmented by electron impact. The charged fragments were detected, and the subsequent spectra obtained were used to identify the molecule. The time taken by the compounds to pass through the column to a detector (retention time) was used for identification when compared to a reference. For detection, an electron ionisation energy system with ionisation energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1ml/min and an injection volume of 2µl was employed (split ratio of 10:1). Injector temperature was 250°C; and ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min. to 280°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass. Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

2.3 Data Analysis

The statistical analysis was done using SPSS software version 21. Descriptive statistics and graphical representations were used to describe and represent variables. Independent t-test was used to compare differences in mean between two groups while one way ANOVA was used to compare differences in mean between more than two groups. The level of statistical difference was set at $p < 0.05$

3.0 RESULTS

3.1 Phytochemical screening

The result of phytochemical screening and phytoquantitative analysis of aqueous *Ocimum gratissimum* leaf extract shows the presence of flavonoids, alkaloids, terpenoids, anthraquinone, tannin, phlobotannin, phenol, and saponin and absence of cardiac glycoside and cardenolide (Tables 1 and 2).

Table 1: Phytochemical screening and phytoquantitative analysis aqueous *Ocimum gratissimum* leaf extract

Phytochemicals	**Present/absent**
Alkaloids	Present
Saponin	Present
Phenol	Present
Phlobatanin	Present
Anthraquinones	Present
Tannin	Present
Cardiac glycosides	Present
Flavonoids	Present
Steroids	Present
Cardenolide	Absent
Terpenes	Present

3.2 Proximate analysis

The result of proximate analysis revealed that *Ocimum gratissimum* leaf extract contain 46.1% carbohydrate, 15% crude fibre, 15.4% crude protein, 5% crude fat, 9.2% moisture, and 9.1% ash (Table 3).

Table 2: Phytoquantitative analysis of aqueous *Ocimum gratissimum* leaf extract

Parameters	Composition (mg/100g)
Alkaloid	0.290
Saponin	0.220
Phenol	0.029
Phlobatannin	0.008
Phytate	0.130
Oxalate	0.120

3.3 Acute toxicological studies

Results of acute toxicity study showed no single death of rat recorded in all the administered doses, even at the highest dose of 5000mg/kg. Thus, the LD_{50} for *Ocimum gratissimum* leaf extract is ≥ 5000 (Table 4).

3.4 Chronic toxicological studies

The result of chronic toxicity study revealed an increase in plasma AST and ALT activities; albumin; and urea; and a decrease in total bilirubin, conjugated bilirubin, total protein and creatinine concentrations on chronic administration of aqueous *Ocimum gratissimum* leaf extract when compared to the controls (Table 5).

Table 3: Proximate analysis of aqueous *Ocimum gratissimum* leaf extract

Parameters	% Composition
Ash	9.1
Moisture	9.2
Carbohydrate	46.1
Crude protein	15.4
Crude fat	5.0
Crude fibre	15.0

Table 4: Acute toxicity study of aqueous *Ocimum gratissimum* leaf extract in rat

Dose	Control (Water)	300 mg/kg	500 mg/kg	1000 mg/kg	2000 mg/kg	5000 mg/kg
Dose difference	0	300	200	500	1000	3000
Number of rats	5	5	5	5	5	5
Number of death	0	0	0	0	0	0
Mean Death	0	0	0	0	0	0

Calculation of $LD_{50,}$

LD_{50} = Maximum dose – Y/ Number of rats per group

Y= Sum of mean death; LD_{50} = 5000 – 0/5 = 5000

Table 5: Chronic toxicity study of aqueous *Ocimum gratissimum* leaf extract in rat

Dose/ Parameters	100mg/kg	200mg/kg	Control	F value	P value
AST(IU/L)	36.7±4.9	54.0±2.41*	43.5±1.74	1.28	0.29
ALT(IU/L)	33.4±2.04	24.5±3.20	32.8±3.7	1.79	0.36
Total bilirubin (mg/dL)	0.49±0.33	0.46±0.34	0.59±0.17	3.69	0.06
C. bilirubin (mg/dL)	0.36±0.08	0.36±0.07	0.48±0.23	53.141	0.48
Albumin (mg/dL)	3.13±0.32	3.46±0.15	3.83±0.09	28.95	0.00
Total protein (mg/dL)	5.63±0.09	5.61±0.97	5.95±1.04	6.26	0.00
Urea (mg/dL)	48.75±10.88	52.1±2.8*	45.2±2.6	1.39	0.33
Creatinine (mg/dL)	0.7±0.03	0.8±0.02	0.8±0.21	0.18	0.94

Values are Mean ± Standard deviation (SD) where AST = Aspartate aminotransaminase, ALT = Alanine aminotransaminase, and C. bilirubin = Conjugated. Bilirubin. * = Statistically significant ($p<0.05$) when compared with the control.

3.5 GC-MS studies

The result of GC-MS showed the presence of various bioactive compounds in aqueous *Ocimum gratissimum* leaf extract (Table 6).

Table 6: Active compounds present in aqueous *Ocimum gratissimum* leaf extract

S/N	Retention time	% Peak Area	Name of the compound	Reference
1	8.751	0.81	Semicarbazide, 1-(1-[5-methyl-1-(4-amino-furazan-3-yl)-1H-[1,2,3] triazol-4-yl]-ethylidene-	114942
			Silane, dimethyl [(methylsilyl)]methyl]	8646
			5-Hexyn-1-ol	3142
2	8.990	1.32	Pyrazine, Methoxy-,1-oxide	11036
			1-(phenylsulfinyl)-2-methylenecyclopropane	43657
			Carbamothioic acid, o-butyl ester	14634
3	9.147	1.02	[1,2,4] Triazolo [1,5-a] pyrimidine-6-carboxylic acid, 7-amino- ethyl ester	67118
			Cyclohexanecarboxamide, N-furfuryl	66823
			1-Heptyne	2828
4	12.599	0.87	Acetic acid, 2-(5-amino-9H-furazano [3,4-b] 1,2,4-triazolo [4,3-d]pyrazin-8-yl)-2,2-dinitro-, ethyl este	186056
			1H-pyrazole-3-carboxylic acid, 2,5 –dinitro-5-oxo-	11994
			Spiro[2.5]octane-1,1-dicarbonitril	30712
5	13.106	1.08	1,2- Benzenediol, 3-[[(3,5-dichlorophenyl) imino]methyl]-	128086
			6-Chloro-3-ethyl-2-methyl-4-phenylquinoline	128402
			9H-Imidazole [1,2-a] benzimidazole, 2-(4-chlorophenyl)-9-methyl-	128282
6	13.194	4.91	2-Anilino-6-methyl-6,7,8,9-tetrahydro-5H-1,2,4-triazolo[1,5-c] 1,3-diazepin-5-ylidenecyanamide	128107
			7H-Dibenzo [b,g] carbazole, 7-methyl	128504
			4-[N'-2-Oxo-1,2-dihydro-indol-3-ylidene]-hydrazinol]-benzoic acid	128193
7	13.469	0.86	Benzofurazan, 4,5,6,7-tetrahydro-4-nitro-	37649
			4,6-Bis (diethylamino)-1,3,5-triazine-2-carbonylhydrazide	127972
			4-Oxo-7,7-dinitro-4,5,6,7-tetrahydro(2H) benzotriazole	83679
8	13.557	0.76	2-Pyrimidinamine, N-(4,5-dihydro-5-methyl-2-thiozolyl)-3-methyl-	66594
			2,4-Dichlorobenzaldehyde 1-methyl-	216769
			1-(2,4,6-trinitrophenyl)hydrazone	66600
			N-(3-Chlorophenyl)maleimide	
9	14.270	0.90	[1,3,5]Triazino [1,2-a] [1,3] benzimidazol-2-amine, 4-(2-fluorophenyl)-1,4-dihydro-	128202
			Propane, 1,1,2,3, 3-pentachloro-	72916
			Methylphosphonic acid	2936
10	15.921	0.90	Dithioacetic acid .alpha.-[p-nitrophenyl] 3-carbamylpyridinium	171277
			1,5-Hexadiyne	1022
			1,4-Cyclohexadiene -1,2-dicarboxylic anhydride	24378
11	17.379	1.38	Quinoline, 2-chloro-6-methoxy-4-methyl-	66667
			Cyclopentene-1-carboxylic acid, 4- [2-(diphenylmethyl)-2-propen-1-yl]-, methyl ester	171148
			Aresenous acid, tris (trimethylsilyl) ester	178799
12	17.954	1.05	Propane, 1-(4-nitrophenyl)-3-Phenylamino	117120
			6H-Indolo [2,3-b] quinoxaline, 9-chloro-6-methyl-	116367
			2-(Trimethylsilyl) amino-4-methylphenol trimethylsilyl ether	116252
13	18.573	0.98	Benzaldehyde, 2-nitroso-	15448
			phenol, 4-(2-amino-5-nitrophenyliminomethyl)-2-methoxy-	133411
			Ethanamine, 2-phenoxy-	16723
14	18.986	0.92	Phenol, 2-ethyl-	9915
			Phenol, 2,4-dimethyl	9936
			Phenol, 3,5-dimethyl	9940
15	19.168	0.84	2-pyridinamine, 5-bromo-6-methyl-	50538
			4-pyridinamine, N-methyl-N, 3-dinitro-	60009

			4-pyridinamine, N-methyl-3-nitro-	26306
16	19.255	5.57	Cyclopentasiloxane, decamethyl-	196316
			Cyclopentasiloxane, decamethyl-	196318
			Cyclopentasiloxane, decamethyl-	196317
17	22.483	1.31	2-[5-(2-Isobutoxyphenyl)-4H-1,2,4-triazol-3-ylthio] acetic acid	150179 48424
			Cyclopropane, 1-chloro-2,2-dimethyl-1-3-(3,3-dimethyl-1-butynyl)-	128488
			Cyclopentanecarboxamide, 3-ethenyl-2-(3-pentenylidene)-N-phenyl-, [1.alpha.,2Z (E), 3. Alpha.]-	
18	24.341	0.83	Acetamide, N-isoxazole [5,4-b]pyridine-3-yl-	43477
			Boronic acid, phenyl-	9804
			4-Methyl -2-mecarptopyridine-1-oxid	18904
19	25.266	7.85	Cyclohexasiloxane, dodecamethyl-	225656
			1,3,5,7,9-Pentaethylcyclopentasiloxane	196319
			Tetradecanoic acid, tripropylsilyl ester	204643
20	25.754	0.76	1-Phthalazinecarboxamide, N-(bicycle[2.2.1] hept-2-ylmethyl)-3,4-dihydro-4-oxo-	141904 32086
			Pyridine, 3-(1-methyl-2-pyrrolodinyl)-, (S)-	32079
			Pyridine, 2-(1-methyl-2-pyrrolidinyl)-	
21	25.948	0.98	1-Hexane, 3-chloro-	8792
			Phosphine, methylbis (1-methylethyl) –	14483
			Benzenesulfonamide, 2-methyl-	39303
22	26.305	0.93	Hexestrol, di-TMS	217253
			Propanoic acid , 2-[(5,7-dimethyl[1,2,4]triazolo[1,5-a] pyrimidine-2-yl)thiol]-, ethyl ester	126978 67840
			pyrazolo[1,5-a]pyridine, 3-methyl-2-phenyl-	
23	27.312	0.86	(9-Oxo-9, 10-dihydroacrin-4-yl) acetic acid	104732
			propanenitrile, 3-[2-(4-pyridyl)-1-indolyl)-	99843
			1H-Isoindole-1-3(2H)-dione, 2-buty 1-4, 5,6,7-tetrahydro-	66908
24	28.025	0.92	Silicic acid, diethyl bis (trimethylsilyl) ester	140472
			1-Dimethyl (phenyl) silyloxypentane	79127
			1,2,4-Benzenetricarboxylic acid,-dodecyl dimethyl ester	214443
25	28.776	0.76	Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl]-	66795
			4-Quinolinecarboxylic acid, 2-chloro-	66606
			Cyclohexa-2,5-diene-1, 4-dione, 2-methyl-5-(4-morpholinyl)-	66749
26	30.684	8.36	Cycloheptasiloxane, tetradecamethy 1-	236968
			Octasiloxane, 1,1,3,3,5,5,7,7,9,9,	240341
			11,11,13,13,15,15, -hexadecamethyl-	236969
			Cycloheptasiloxane, tetradecamethy 1-	
27	31.372	0.83	2,6-lutidine 3,5-dichloro-4-dodecylthio-	199835
			Terephthalic acid, di(2-methoxyethyl) ester	128845
			Chlorodibromoacetic acid, methyl ester	114302
28	32.085	0.93	5-Bromopyrazolo [3,4-d]-s-triazin-4 (3H)-one	73566
			2-(5-Bromo-3-pyridyl)-5-[(5-chlorosalicylidene)amino]benzoxazole	221277 48996
			1,3-Bis(2-chloroethyl) urea	
29	33.211	0.86	4,6-Bis (diethylamino)-1, 3,5 triazine-2-carbonylhydrazide	127972
			Phenol, 4-(2-amino-5-nitrophenyliminomethyl1)-2-methoxy-	133411
			Benzoic acid, 2,5-dichloro-3-hydroxy-6-methoxy-	91077
30	34.562	0.78	Diethylmalonic acid, 2-fluoro-3-trifluoromethylphenyl nonyl ester	226730 209410
			Glutaric acid, 4-fluorobenzyl undecyl ester	
			Isobutyl isothiocyanate	
31	35.600	0.76	S- [N-[2-Napthalenemethyl] thiosulfuric acid	152387
			2-Norbornanone, 1-(epithioethyl)-7, 7-dimethyl-, S,S-dioxide	83929
			Propanoic acid, 2-chloro-	5221

32	35.675	4.32	Silane, [[4-[1,2-bis[(trimethylsilyl)oxy]ethyl]-1,2-phenylene]bis(oxy)bis[trimethyl- Silane, [[4-[1,2 bis[(trimethylsilyl)oxy]ethyl]-1,2-phenylene] bis(oxy)]bis[trimethyl- Silane, [[4-[1-2-bis[(trimethylsily)oxy]ethyl]-1,2-phenylene]bis (oxy)]bis[trimethyl-	228734 228736 228735
33	36.438	0.83	N-Benzyl-1H-benzimidazole (2Z) -3- Amino-3-[(2-hydroxyethyl)sulfanyl]-2-propene-1,1,2-tricarbonitrile N,N'-Dicyclohexylformamidine	67811 68232 67790
34	36.589	0.94	2-1-Phenyl ethylidiene-hyrazono-3-methyl-2, 3-dihydrobenzothiazole Rhodanine	128311 14568 14567
35	37.245	9.93	Fumaric acid, 2-hexyl undecyl este cis-2, 4, 5-Trimethoxy-.beta.-methyl-.beta.-nitrostyrene	186655 104529
36	37.683	2.20	Benzophennone-2,4', 5-tricarboxylic acid, trimethyl ester Cyclohexanecarboxamide, N-[5-(3,4-methylenedioxyphenyl)-1,3,4-thiadiazol-2-yl]- Hexasiloxane, tetradecamethyl-	187734 169775 228692
37	38.190	4.79	6-(2-Aminophenyl)-1,2,4-triazine -3, 5(2H, 4H)-dione tritms 2-(4-Iodo-phenyl)-6-pentyl-5,6,7,8-tetrahydro-quinoline Silane, dimethyl(1-phenylpropoxy) hexadecyloxy-	219094 214089 223346
38	42.206	25.13	Ethisterone 2-Propenoic acid, 3-[(phenylmethyl) thiol]-, (E)- Norgestrel	155012 55694 155010

4.0 DISCUSSION

The roles played by phytochemicals differ from one to another. Tannins act on proteins to form protective layer on mucus membranes; flavonoids stabilise membranes, inhibits lipid peroxidation, and affects intermediary metabolism; phenols have antioxidant properties and protects cells either by preventing the production of free radicals or by scavenging free radicals produced in the body [25-27]. Result of the phytochemical screening revealed that aqueous *Ocimum gratissimum* leaf extract is abundantly rich in flavonoids, alkaloids and terpenoids; appreciably rich in anthraquinone, tannin, phlobotannin and phenol; rich in saponin but lack cardiac glycoside and cardenolide (Table 1). This showed no major differences in the phytochemical constituents from the results of other previous investigations [28-30]. Phytoquantitative analysis indicates that *Ocimum gratissimum* leaf is rich in alkaloids and saponin and also contain appreciable amount of phytates (Table 2). This corroborates the earlier findings reported by Talabi and Makanjuola (2017) but contradict the findings of Akinmoladun *et al.* (2007) who reported absence of alkaloid in aqueous leaf extract [4, 31]. The presence of phytates allows it to form complexes with metals or proteins and therefore reduces their bioavailability in the gastrointestinal tract. Proximate analysis result showed that *Ocimum gratissimum* leaf contain 46.1% carbohydrate, 15% crude fibre, 15.4% crude protein, 5% crude fat, 9.2% moisture, and 9.1% ash (Table 3). This is similar to earlier findings by Talabi and Makanjuola (2017) who reported 44.36% carbohydrate, 17.37% crude fibre, 15.08% crude protein, 5.10% crude fat, 8.32% moisture, and 9.79% ash content in *Ocimum gratissimum* leaf [31]. The high carbohydrate content indicates the important nutritional benefits of *Ocimum gratissimum* and its roles in metabolism and energy generation for cellular activities. The high crude fiber and protein contents indicate its anti-hyperglycemic potentials and importance in cellular repairs respectively. Toxicological studies are done using crude plant materials, aqueous, polar or non-polar extracts of such materials. This toxicity may be inherent of the phytochemicals therein present or consequent of the method of extraction [32]. Investigation of acute toxicity of unknown substance is done using animal models and the results obtained from such studies must be carefully inferred in man as the result provides only an approximate estimate of lethality in human` [33]. The index of acute toxicity is LD_{50} which is interpreted as follows: very toxic $\leq$ 5mg/kg; toxic > 5 $\leq$ 50mg/kg; harmful, > 50 $\leq$ 500mg/kg and no label, > 500 $\leq$ 2000mg/kg[17]. Any LD_{50} values greater than 5000mg/kg are usually considered to be of no practical interest [20]. From this study, the LD_{50} value for aqueous *Ocimum gratissimum* leaf extract was found to be above 5000mg/kg (Table 4), thus nontoxic when administered by the oral route and is considered safe for animal consumption. This finding is similar to the work of Ojo *et al.* (2013) who reported LD_{50} value for aqueous *Ocimum gratissimum* leaf extract to be 4242.64mg/kg [29]. Chronic oral toxicity of aqueous *Ocimum gratissimum* leaf extract was assessed by determining plasma aspartate transaminase (AST) and alanine transaminase (ALT) activities; and plasma urea and creatinine concentrations. It was observed that there was a significant increase ($p < 0.05$) in plasma AST activities, at a dose of 200mg/kg) and a non-significant difference in plasma ALT activities among the extract treated groups when compared to the controls, though all values were

within the normal ranges (Table 5). Both AST and ALT are markers of liver toxicity with ALT much specific about measure of hepatocellular integrity. This finding corroborates the earlier reported increase in AST and ALT in rats fed with *Ocimum gratissimum* [29]. The observed increase in plasma AST could be from tissues, other than liver, where AST is found. However, increase in plasma ALT activities in rats administered with *Ocimum gratissimum* leaf extract compared to the control group confirmed that damage has been inflicted on the plasma membrane of the liver which might lead to the compromise of its integrity. Plasma albumin and bilirubin concentrations indicate the secretory and synthetic functions of the liver and can be used to ascertain types of liver damage. Bilirubin is the major breakdown product from physiologic destruction of senescent red blood cells. It is removed from the blood by the liver; hence it is a good indicator of liver function. Bilirubin concentration is elevated in the blood either due to increased production or decreased liver uptake. Result from this study showed a significant ($p < 0.05$) decrease in the total and conjugated bilirubin concentrations in rats administered with *Ocimum gratissimum* when compared with the control (Table 5). This observation corroborates the findings of Ojo *et al.* (2013) and suggests that the extract had no adverse effect on the liver [29]. A rise in the concentration of serum bilirubin indicates or suggests liver damage since the liver serves as an excretory unit rather than a distributing unit for bilirubin. Albumin is produced by the liver and is a major protein that circulates in the blood stream. Low serum albumin has been associated with low protein intake. A significant decrease ($p < 0.05$) in plasma albumin and a non-significant decrease ($p > 0.05$) in plasma total protein were observed in rats administered with *Ocimum gratissimum* when compared to the control group (Table 5). This observation corroborates findings from other studies [29]. The observed decrease may be due to presence of anti-nutrient factors in the extract which affects the digestibility of its protein content, synthetic function of the liver and or increases excretion of albumin. Considering nephrotoxicity measures, a significant ($p < 0.05$) increase in plasma urea concentration was observed at 200mg/kg dose (Table 5), though the values are within the normal range [34]. Also, a non-significant ($p > 0.05$) change in the plasma creatinine concentration was observed in the entire doses used (Table 5). Thus, the aqueous *Ocimum gratissimum* leaf extract is not nephrotoxic on chronic use. Characterising aqueous *Ocimum gratissimum* leaf extract using GC-MS revealed the presence of different active compounds (Table 6). These bioactive compounds may be found very useful in the formulation of novel drugs as they possess various pharmacological activities.

5.0. CONCLUSION

The choice of plant materials for therapeutic use in human is dependent on its toxicological indices extrapolated from animal studies. This study had revealed that *Ocimum gratissimum* leaf contain different phytochemicals of potential medicinal importance in appreciable quantities. Also, oral consumption of *Ocimum gratissimum* leaf has no toxicological implications both in short- and long-term use. The identified compounds therein present make *Ocimum gratissimum* leaf a promising plant in drug discovery.

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