

ISSN 1996-3351

Asian Journal of
Biological
Sciences

The Effects of Aqueous *Ocimum gratissimum* Leaf Extract on Some Biochemical and Hematological Parameters in Male Mice

¹A.W. Obianime, ¹J.S. Aprioku and ²C. Esomonu

¹Department of Pharmacology,

²Department of Human Anatomy, Faculty of Basic Medical Sciences,
College of Health Sciences, University of Port Harcourt, Port Harcourt, Nigeria

Corresponding Author: J.S. Aprioku, Department of Pharmacology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Port Harcourt, Nigeria Tel: +234 (0) 8035082379

ABSTRACT

The present study is intended to investigate the dose-and time-dependent effects of the aqueous crude extract of *O. gratissimum* Linn. leaf (OG) on some biochemical and hematological parameters in the male mice. Animals were grouped into 3 (each containing 4 groups, n = 5) and orally administered with OG (11-88 mg kg⁻¹) and observed for 1, 2 and 4 weeks, respectively. A fourth group was used as the control and given only distilled water for 4 weeks. While the serum levels of alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, creatinine and total cholesterol were not significantly (p<0.05) different in experimental animals compared to control animals, 1 week administration of OG significantly decreased the serum levels of urea and total protein in a dose-dependent manner. Furthermore, uric acid, total acid and prostatic acid phosphatases were increased during the fourth week of administration in OG-treated animals, compared to control animals. In addition, the first and second weeks of OG administrations resulted in dose-dependent reductions in PCV, Hb and neutrophils, with increase in WBC and lymphocyte counts. However, administration of OG for 4 weeks had no significant effects on the hematological parameters. The study shows that OG will cause anemia and may affect prostatic/testicular function in the mouse, depending on the duration of exposure to the animal.

Key words: Anemia, hematopoietic, oxidative, phosphatase enzymes, WBC

INTRODUCTION

Ocimum gratissimum Linn. is a perennial herb belonging to the family Lamiaceae. The plant which is believed to originate from Asia and Africa (Sulistiarini, 1999) is also widely distributed in other regions, occurring as different species. The leaves of the plant contain essential oils (Leal *et al.*, 2006) and it is cultivated for various purposes. In Nigeria and most parts of West Africa, it is used as a spice and condiment in dishes, because of its high pungent flavor of clove. The whole plant and the essential oils of the plant have many applications in folk medicine. The crude extracts have been used in the treatment of respiratory tract infections (Lasisi and Ajuwon, 2002); diarrhea (Obuekwe and Obuekwe, 2002); rheumatism and hemorrhoids (Sulistiarini, 1999; Sofowora, 2000); dermatological conditions (Orafidiya *et al.*, 2001, 2002); microbial infections (Akinyemi *et al.*, 2005); epilepsy, high fever and mental illness (Oliver-Bever, 1980; Osifo, 1989).

Phytochemical screening of the leaf extract of *O. gratissimum* (OG) had shown the plant to contain alkaloids, saponins, tannins, alkaloids, anthraquinone, flavonoids, steroids, terpenoids and cardiac glycosides (Holets *et al.*, 2003; Akinyemi *et al.*, 2005; Akinmoladun *et al.*, 2007). In addition, the leaves of the plant contains essential oils made up of eugenol, 1, 8-cineole and linalool, thymol, citral, linalool, ethyl cinnamate and geraniol (Leal *et al.*, 2006) and the quality and quantity of the composition of these essential oils is dependent on the geographical location where the plant is grown, the specie and method of extraction (Leal *et al.*, 2006). Furthermore, OG had been shown to possess diverse pharmacological properties which may be attributed to its usefulness in folk medicine. These properties include antioxidant (Odukoya *et al.*, 2005; Akinmoladun *et al.*, 2007; Aprioku and Obianime, 2008); chemotherapeutic (Dubey *et al.*, 2000); antimutagenic (Obaseki-Ebor *et al.*, 1993); antidiarrhoeal (Offiah and Chikwendu, 1999; Orafidiya *et al.*, 2000; Adebolu and Salau, 2005); antinociceptive (Rabelo *et al.*, 2003, insecticidal (Eze *et al.*, 2006) hypotensive (Lahlou *et al.*, 2004) and antihelmintic (Fakae *et al.*, 2000; Pessoa *et al.*, 2002). This may account for the plants wide use in folk medicine as a remedy for several ailments especially in Africa and India. Although, some commonly used medicinal plants may have little adverse effects on body processes, such as the hematopoietic system (Orhue *et al.*, 2008), it is necessary to investigate the effects of *O. gratissimum* on normal biological processes (qualitatively and quantitatively) in view of its wide medicinal use.

The present study is carried out to investigate the dose-and-time-dependent effects of the aqueous crude extract of *O. gratissimum* leaf on some biochemical and hematological indices in the male mice.

MATERIALS AND METHODS

Extraction of plant material: Fresh leaves of *O. gratissimum* L. were collected in the month of June, 2008 from a local garden within the premises of the University of Port Harcourt, Nigeria. The plant was identified and authenticated by Dr. Goodie Uzo Obute- a senior botanist in the botanic garden of the University of Port Harcourt, Nigeria and a voucher specimen was deposited accordingly at the herbarium of the Department of Plant Science, University of Port Harcourt, Port Harcourt, Nigeria.

Extraction: The fresh leaves of the plant were air-dried, pulverized and extracted exhaustively in distilled water. The filtrate was concentrated and evaporated to dryness in vacuo at 40°C, using rotary evaporator. The yield was calculated and the dry extract was stored in a refrigerator at -4°C until use for the experiments.

Animals: A total number of 65 male mice weighing between 30-35 g were used in this study. The animals were obtained from the animal house of the Department of Pharmacology, University of Port Harcourt, Nigeria. The animals were randomly distributed into cages and allowed to acclimatize for 10 days in a well ventilated room at a room temperature of 28.0±2.0°C under natural lighting condition. The animals were allowed free access to standard mouse chow (Topfeeds Ltd., Sapele, Nigeria) and tap water *ad libitum*. All animals used in this study were handled in accordance with the international, national and institutional guidelines for Care and Use of Laboratory Animals as promulgated by the Canadian Council of Animal Care (2009).

Experimental protocol: Animals were divided into four groups- A, B, C and D, representing week 1, 2, 4 and control, respectively. Each group except the control was further subdivided into

four groups of 5 animals each. Group A was given single daily doses of 11, 22, 44 and 88 mg kg⁻¹ of OG for 1 week. Group B received single daily doses of 11, 22, 44 and 88 mg kg⁻¹ of OG for 2 weeks. Group C was given single daily doses of 11, 22, 44 and 88 mg kg⁻¹ of OG for 4 weeks. The control group (group D), containing five animals, was given only distilled water daily for 4 weeks. OG was administered orally using a calibrated 1 mL syringe with attached polythene cannula. At the end of each treatment period, the animals were sacrificed by decapitation under pentobarbital anesthesia (50 mg kg⁻¹, i.p.).

Biochemical assays: Blood was collected into lithium heparinized bottle, centrifuged for 15 min at 3,000 rpm and serum was separated and assayed for alkaline phosphatase using the phenolphthalein method (Babson *et al.*, 1966), total and prostatic acid phosphatases, using colorimetric method (Fishman and Davidson, 2006), uric acid using enzymatic colorimetric method (Fossati *et al.*, 1980), urea using urease-Berthelot method (Weatherbum, 1967), total cholesterol by enzymatic endpoint method (Roeschlau *et al.*, 1974) and creatinine assay was done by alkaline picrate method (Tietz *et al.*, 1986). Serum was also analyzed for ALT and AST.

Hematological assays: Whole blood was collected from the animals into EDTA bottle and assayed for PCV, hemoglobin, WBC and differential cell counts using standard techniques.

Statistical analysis: Data were expressed as Mean±SE of mean. Comparisons between control values and values of treated groups of guinea-pigs were performed with one-way Analysis of Variance (ANOVA). Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Biochemical parameters: Administration of *O. gratissimum* (11-88 mg kg⁻¹) for 1 week significantly ($p < 0.05$) decreased the serum levels of urea and total protein in treated animals in a dose-dependent manner, compared to the control animals group. The serum urea and total protein levels in animals given 88 mg kg⁻¹ of OG were 2.07±0.39 and 42.0±1.5 g L⁻¹, compared to 4.43±0.46 and 53.0±0.91 g L⁻¹, respectively in the control animals (Table 1). Furthermore, while basal serum levels of total acid phosphatase (ACP), prostatic acid phosphatase (ACPP) and uric acid in control animals were not significantly different from serum levels obtained in OG-treated animals in the first and second weeks, there were significant dose-dependent increases in the serum levels of these parameters in experimental animals during the fourth week of OG administration (Table 1). However, OG caused no significant effects on the serum levels of the hepatic enzymes: alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and the other biochemical parameters investigated (Table 1).

Hematological parameters results: One week administration of OG (11-88 mg kg⁻¹) caused significant ($p < 0.05$) dose-dependent decreases in PCV and Hb levels, without significant effects during the second and fourth weeks of administration. Maximal responses were obtained at 88 mg kg⁻¹ of OG during the 1st week of administration in treated animals, with PCV of 19.3±1.3% and Hb, 6.57±0.47 g dL⁻¹, respectively, compared to 34.0±1.5% and 10.0±0.43 g dL⁻¹, respectively in control animals (Fig. 1A, B). Furthermore, WBC count (9.13±2.4 ×10⁹ mL⁻¹) in animal groups given 88 mg kg⁻¹ for 1 week were higher, compared to the WBC count (4.45±0.92 ×10⁹ mL⁻¹) obtained in control animals group (Table 2). In addition, 2 weeks administration of OG resulted in a dose-dependent decrease in neutrophil counts and an increase in lymphocyte counts (Table 2).

Table 1: The effects of 4 weeks oral administration of aqueous *Ocimum gratissimum* leaf extract (11-88 mg kg⁻¹) on the biochemical parameters of male mice

Dose (mg kg ⁻¹)	Week 1		Week 2		Week 4	
	ALP	ACP	ALP	ACP	ALP	ACP
Control	31.0±0.7	6.68±1.3	31.00±0.7	6.68±1.3	31.00±0.7	6.68±1.3
11	30.0±1.2	7.30±1.1	31.67±0.88	10.37±1	28.67±1.5	12.20±2.1*
22	25.0±1.0*	8.33±1.6	28.30±7.0	8.80±1.1	32.00±1.2	13.33±1.5**
44	32.3±1.5	8.87±2.0	25.00±0.3*	7.89±0.77	30.00±1.0	13.87±1.0**
88	31.7±1.2	7.20±0.68	29.30±1.2	7.80±0.88	27.00±0.5*	17.47±1.2***

Dose (mg kg ⁻¹)	Week 1		Week 2		Week 4	
	ACPP (IU L ⁻¹)	Uric acid (mmol L ⁻¹)	ACPP (IU L ⁻¹)	Uric acid (mmol L ⁻¹)	ACPP (IU L ⁻¹)	Uric acid (mmol L ⁻¹)
Control	1.98±0.45	352.75±13.28	1.98±0.45	352.75±13.28	1.98±0.45	352.75±13.28
11	2.43±0.87	402.30±28.3	3.87±0.71	358.30±32.0	4.83±1.5*	388.00±15.5*
22	2.53±1	378.00±41.1	3.80±0.3	409.00±8.5*	5.27±0.75**	378.33±13.2*
44	3.00±0.82	385.70±16.4	4.50±0.50*	389.00±19.3	7.13±0.55***	394.67±8**
88	2.47±0.20	380.70±19.5	4.13±1.2*	360.70±25.6	7.43±0.92***	411.33±6.4**

Dose (mg kg ⁻¹)	Week 1		Week 2		Week 4	
	Urea	Total protein	Urea	Total protein	Urea	Total protein
Control	4.43±0.46	53.00±0.91	4.43±0.46	53.00±0.91	4.43±0.46	53.00±0.91
11	4.03±0.43	49.30±1.2*	3.70±0.46	49.30±1	5.80±1.4	53.33±2.7
22	3.60±0.35*	50.40±1.0	3.17±0.54	49.67±0.88	6.80±2.2*	46.67±1.2
44	2.83±0.2**	46.30±0.88**	4.73±0.69	49.00±1.7	7.50±0.58*	48.33±0.88
88	2.07±0.39***	42.00±1.5**	3.40±0.35	44.67±5.0*	8.47±0.86**	47.67±3.8

Dose (mg kg ⁻¹)	Week 1		Week 2		Week 4	
	Total cholesterol (mmol L ⁻¹)	Creatinine (µmol L ⁻¹)	Total cholesterol (mmol L ⁻¹)	Creatinine (µmol L ⁻¹)	Total cholesterol (mmol L ⁻¹)	Creatinine (µmol L ⁻¹)
Control	3.83±0.11	50.25±1.7	3.83±0.11	50.25±1.7	3.83±0.11	50.25±1.7
11	3.93±0.23	50.00±1.5	3.30±0.06	45.67±2.8	4.03±0.23	59.67±12.7
22	3.97±0.35	50.00±7.6	3.67±0.32	50.67±2.2	3.90±0.17	70.00±5*
44	3.57±0.2	56.30±4.4	3.57±0.15	62.33±11.1*	3.67±0.2	51.67±2.2
88	3.30±0.06*	47.70±0.88	3.13±0.35*	46.67±3.5	3.70±0.21	66.33±10.4

Dose (mg kg ⁻¹)	Week 1		Week 2		Week 4	
	AST	ALT	AST	ALT	AST	ALT
Control	17.00±2.8	9.25±0.48	17.00±2.8	9.25±0.48	17.00±2.8	9.25±0.48
11	15.00±1.7	8.70±0.89	12.30±1.5	7.67±1.9	12.67±1.2	6.33±0.88
22	17.00±1.5	11.30±0.88	18.67±1.8	12.67±1.8	13.33±2.4	7.67±2.2
44	13.30±2.4	8.67±1.8	14.33±2.3	9.00±2.6	18.33±3.3	11.33±1.9
88	16.30±1.3	10.70±0.88	12.30±1.5	7.30±1.3	16.00±3.1	8.33±1.2

ALP: Alkaline phosphatase; ACP: Acid phosphatase; ACPP: Prostatic acid phosphatase; AST: Aspartate aminotransferase and ALT: Alanine aminotransferase. Data given as Mean±SEM. *Indicates a significant difference (p<0.05) from control. **Indicates a significant difference (p<0.01) from control. ***Indicates a significant difference (p<0.001) from control

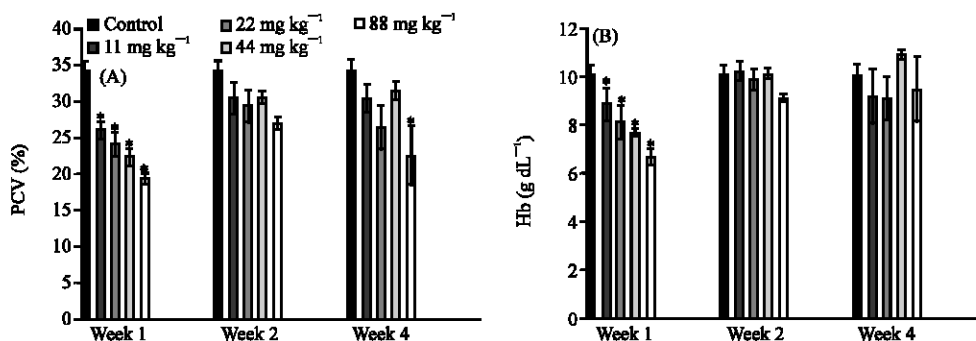


Fig. 1: The effects of 4 weeks oral administration of aqueous *Ocimum gratissimum* leaf extract (11-88 mg kg⁻¹) on (A): PCV and (B): Hb in male mice. Data given as Mean±SEM. *Indicates a significant difference (p<0.05) from control

Table 2: The effects of 4 weeks oral administration of aqueous *Ocimum gratissimum* leaf extract (11-88 mg kg⁻¹) on some hematological parameters in male mice

Dose(mg kg ⁻¹)	Week 1		Week 2		Week 4	
	WBC (×10 ⁹ mL ⁻¹)	Neutrophils (%)	WBC (×10 ⁹ mL ⁻¹)	Neutrophils (%)	WBC (10 ⁹ mL ⁻¹)	Neutrophils (%)
Control	4.45±0.92	46.25±0.92	4.45±0.92	46.25±0.92	4.45±0.92	46.25±0.92
11	5.47±0.41*	58.00±0.8	3.73±0.46	41.67±3.4*	2.93±0.75	46.67±0.88
22	6.20±1.5*	47.67±5.9	4.27±0.41	37.33±2.0**	2.53±0.27	46.00±2.1
44	7.65±1.2**	56.00±4.2	5.80±1.9	31.67±1.2***	4.47±0.52	46.00±0.58
88	9.13±2.4***	47.67±3.3	3.50±0.5	28.33±1.7***	4.03±1.6	46.67±2.6

Dose(mg kg ⁻¹)	Week 1		Week 2		Week 4	
	Lymphocyte	Monocyte	Lymphocyte	Monocyte	Lymphocyte	Monocyte
Control	53.0±2.0	0.75±0.48	53.00±2.0	0.75±0.48	53.00±2.0	0.75±0.48
11	42.0±0.8	0.00±0	58.33±1.2*	1.33±0.88	53.00±1.2	0.33±0.33
22	52.3±5.9	0.00±0	62.67±2.3**	1.00±0.58	53.00±2.5	1.00±0.58
44	42.67±5.2	0.00±0	68.30±0.7***	0.00±0	53.33±0.88	0.67±0.67
88	49.67±4.7	2.67±1.3*	70.0±1.5***	0.33±0.33	53.33±2.6	0.00±0

Data given as Mean±SEM. *Indicates a significant difference (p<0.05) from control. **Indicates a significant difference (p<0.01) from control. ***Indicates a significant difference (p<0.001) from control

The present study was carried out to investigate the effects of 4 weeks daily oral administration of different doses of the aqueous leaf extract of *O. gratissimum* (OG) on some biochemical and hematological parameters of male mice. The biochemical results show that the effects of OG on the biochemical parameters investigated were dependent on the dose and duration of administration. Urea and total protein were significantly (p<0.05) decreased dose-dependently during the first week of OG (11-88 mg kg⁻¹) administration, while total acid phosphatase, prostatic acid phosphatase, urea and uric acid were significantly increased dose-dependently when OG was administered for a period of 4 weeks. Total and prostatic acid phosphatase enzymes are produced by the liver and prostate gland and elevation of their serum levels is indication of toxicity to these organs (Yam, 1974; Lin *et al.*, 1980; Chu and Lin, 1998). Additionally, elevation of serum uric acid level, which is a purine metabolite is also a marker of toxicity, mediated through oxidative stress (Hooper *et al.*,

2000; Knapp *et al.*, 2004). Furthermore, increase in the serum levels of total protein and urea are indications of renal impairment mediated via oxidative stress (Traynor *et al.*, 2006). However, the serum levels of the hepatic enzymes: ALP, AST and ALT (which are usually elevated during damage to liver cells and tissues) were not significantly altered. This suggests that hepatic function in the mouse is not adversely affected by OG, which is consistent with the previous works of Al-Sobayil *et al.* (2008). Thus, the biochemical result suggests that OG may have a reduced oxidative effect during short period of administration, which is consistent with our previous study (Aprioku and Obianime, 2008), while it may cause increased oxidative stress particularly on the prostate/testes during prolonged administration, with little (protective) or no effects on hepatic cells.

Furthermore, the hematology result showed that administration of OG (11-88 mg kg⁻¹) in the 1st week decreased PCV and Hb levels, while WBC and lymphocyte counts were increased during the first and second weeks of OG administration, respectively. These results were dose-dependent and consistent with the reports of Ephraim *et al.* (2000). However, the effects were not time-dependent and the serum levels of the hematological parameters were reversed to control values (i.e., refractoriness) in the second and fourth weeks of administration. This is however contrary to the results of Ephraim *et al.* (2000), who reported that hematopoietic parameters time-dependently decreased over a period of 1 month. These differences in time may be due to specie differences and differences in the experimental protocols. The extract was administered twice weekly in the other studies, using rabbits.

Earlier studies had shown that the aqueous leaf extract of *O. gratissimum* has different pharmacological actions including antioxidative properties (Rabelo *et al.*, 2003; Odukoya *et al.*, 2005; Leal *et al.*, 2006; Aprioku and Obianime, 2008). In addition, the crude leaf extract of OG had been shown to block and reduce cadmium-induced elevations of the serum levels of some biochemical parameters in the male guinea-pig (Aprioku *et al.*, 2009), while administration of OG over 24 h, reduced basal serum levels of acid and prostatic acid phosphatases (Aprioku and Obianime, 2008). This is consistent with the biochemical data of the present study which shows that specific oxidative parameters (total protein and urea) were reduced during the first week of OG administration, while (ACP, ACP, urea and uric acid) increased during the 4th week of OG administration. Furthermore, the results of this study show that the hematopoietic system is more sensitive to OG than the biochemical parameters, causing anemia and increase in leucocytes during acute administration (1 week). These effects may be due to oxidative compounds like saponnins and triterpenes which are contained in the crude extract of *O. gratissimum* which are deleterious to blood cells (Watt and Breyer-Brandwijk, 1962). The reduced hematological effects of OG after the first week of administration in the present study may be due to adaptation through stimulation of adaptive mechanisms by the body against OG-induced toxicity on the blood cells. It thus suggests that OG may have dual responses (i.e., oxidative or antioxidative), depending on the tissue/organ system under investigation or the duration of administration. This may be explained by the complex amount or nature of phytochemical compounds contained in the crude extract of *O. gratissimum* which have both oxidative (e.g., saponnins, triterpenes, alkaloids) and antioxidative (e.g., eugenol, flavonoids, citral, linalool) properties.

In conclusion, the aqueous crude leaf extract of *O. gratissimum* cause microcytic anemia and proliferation of WBC and lymphocytes during short periods of administration in the mouse, which normalize during prolonged administration. It also causes decrease in urea and total protein during short periods of administration (i.e., antioxidative) and increases acid and prostatic acid phosphatases, urea and uric acid during prolonged periods of administration (i.e., oxidative).

ACKNOWLEDGMENT

Authors thank the technical staff of the Departments of Pharmacology and Clinical Chemistry of the University of Port Harcourt, Port Harcourt, Nigeria for their technical assistance.

REFERENCES

- Adebolu, T.T. and A.O. Salau, 2005. Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in Southwestern Nigeria. Afr. J. Biotechnol., 4: 682-684.
- Akinmoladun, A.C., E.O. Ibukun, E. Afor, E.M. Obuotor and E.O. Farombi, 2007. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. Scientific Res. Essay, 2: 163-166.
- Akinyemi, K.O., O. Oladapo, C.E. Okwara, C.C. Ibe and K.A. Fasure, 2005. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC Complement. Altern. Med., 5: 6-6.
- Al-Sobayil, K.A., M.M. Zeitoun, M.H. Khalil and A.M. Abdel-Salam, 2008. Effect of oral administration of a functional synbiotic syrup on libido, semen characteristics, serum testosterone and liver and kidney function of goat's bucks. Asian J. Biol. Sci., 1: 11-18.
- Aprioku, J.S. and A.W. Obianime, 2008. Antioxidant activity of the aqueous crude extract of *Ocimum gratissimum* Linn. leaf on basal and cadmium-induced serum levels of phosphatases in male guinea-pigs. JASEM., 12: 33-39.
- Aprioku, J.S., A.W. Obianime and B.C. Didia, 2009. *Ocimum gratissimum* Linn. reverses cadmium-induced toxicity of spermatoc parameters of the male guinea-pig. J. Expt. Clin. Anal., 8: 1-7.
- Babson, L.A., S.J. Greeley, C.M. Coleman and G.D. Phillips, 1966. Phenolphthalein monophosphate as a substrate for serum alkaline phosphatase. Clin. Chem., 12: 482-490.
- Canadian Council On Animal Care, 2009. The Care and Use of Farm Animals in Research, Teaching and Testing. CCAC., Ottawa, ON., pp: 12-15.
- Chu, T.M. and M.F. Lin, 1998. PSA and acid phosphatase in the diagnosis of prostate cancer. J. Clin. Ligand Assay, 21: 24-34.
- Dubey, N.K., T.N. Tiwari, D. Mandin, H. Andriamboavonjy J.P. and Chaumont, 2000. Antifungal properties of *Ocimum gratissimum* essential oil (ethyl cinnamate chemotype). Fitoterapia, 7: 567-569.
- Ephraim, K.D., H.A. Salami and T.S. Osewa, 2000. The effect of aqueous leaf extract of *Ocimum gratissimum* on haematological and biochemical parameters in rabbits. Afr. J. Biomed. Res., 3: 175-179.
- Eze, S.C., J.E. Asiegbu, B.N. Mbah, G.C. Orkwor and R. Asiedu, 2006. The effects of four agrobotanical extracts and three types of bags on the control of insect pests and moulds of stored yam chips. Agro-Science, 5: 8-12.
- Fakae, B.B., A.M. Campbell, J. Barrett, I.M. Scott, P.H. Teesdale-Spittle, E. Liebau and P.M. Brophy, 2000. Inhibition of glutathione-S-transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. Phytoter. Res., 1148: 630-634.
- Fishman, H.W. and M.H. Davidson, 2006. Determination of serum acid phosphatases method. Biochem. Anal., 4: 257-284.
- Fossati, P., L. Prencipe and G. Berti, 1980. Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin. Chem., 26: 227-231.

- Holets, F.B., T. Ueda-Nakamura, B.P.D. Filho, D.A.G. Cortez, J.A. Morgado-Diaz and C.V. Nakamura, 2003. Effect of essential oil of *Ocimum gratissimum* on the trypanosomatid *Herpetomonas samuelpessoai*. Acta Protozoologica, 42: 269-276.
- Hooper, D.C., G.S. Scott, A. Zborek, T. Mikheeva, R.B. Kean, H. Koprowski and S.V. Spitsin, 2000. Uric acid, A peroxy nitrite scavenger, inhibits CNS inflammation, blood-CNS barrier permeability changes and tissue damage in a mouse model of MS. FASEB., 14: 691-698.
- Knapp, C.M., C.S. Constantinescu, J.H. Tan, R. McLean, G.R. Cherryman and I. Gottlob, 2004. Serum uric acid levels in optic neuritis. Mult. Scler., 10: 278-280.
- Lahlou, S., L.F.L. Interaminense, J.H. Leal-Cardoso, S.M. Morais and G.P. Duarte, 2004. Cardiovascular effects of the essential oil of *Ocimum gratissimum* leaves in rats: Role of the autonomic nervous system. Clin. Exp. Pharmacol. Physiol., 31: 219-225.
- Lasisi, A.O. and A.J. Ajuwon, 2002. Beliefs and perceptions of ear, nose and throat-related conditions among residents of a traditional community in Ibadan, Nigeria. Afr. J. Med. Med. Sci., 31: 45-48.
- Leal, P.F., F. Chaves, M. Celio, L.C. Ming, A.J. Petenate and M.M.A. Angela, 2006. Global yields, chemical compositions and antioxidant activities of Clove basil (*Ocimum gratissimum* L.) extracts obtained by supercritical fluid extraction. J. Food Process Eng., 29: 547-559.
- Lin, M.F., C.L. Lee, J.W. Wojcieszyn, M.C. Wang, L.A. Valenzuela, G.P. Murphy and T.M. Chu, 1980. Fundamental biochemical and immunological aspects of prostatic acid phosphatase. Prostate, 1: 415-425.
- Obaseki-Ebor, E.E., K. Odukora, H. Telikepalli, L.A. Mtscher and D.M. Shankel, 1993. Antimutagenic activity of extracts of leaves of four common edible vegetable plants in Nigeria (West Africa). Mutat. Res., 302: 109-117.
- Obuekwe, I.F. and I.C. Obuekwe, 2002. Indigenous methods used for the management of diarrhoea in an urban community in Edo State, Nigeria. J. Med. Biomed. Res., 1: 7-14.
- Odukoya, O.A., O.O. Ilori, M.O. Sofidiya, O.A. Aniunoh, B.M. Lawal and I.O. Tade, 2005. Antioxidant activity of Nigerian dietary spices. Elect. J. Environ. Agric. Food Chem., 4: 1086-1093.
- Offiah, V.N. and U.A. Chikwendu, 1999. Anti-diarrhoeal effects of *Ocimum gratissimum* leaf extract in experimental animals. J. Ethnopharmacol., 68: 327-330.
- Oliver-Bever, O., 1980. Medicinal Plants in Nigeria. 1st Edn., Nigerian college of Arts, Science and Technology, Ibadan, pp: 90-94.
- Orafidiya, O.O., A.A. Elujoba, F.O. Iwalewa and I.N. Okeke, 2000. Evaluation of antidiarrhea properties of *Ocimum gratissimum* volatile oil and its activity against enteroaggregative *Escherichia coli*. Pharm. Pharmacol. Lett., 10: 9-12.
- Orafidiya, L.O., A.O. Oyedele, O.A. Shittu and A.A. Eluioba, 2001. The formulation of an effective topical antibacterial product containing *Ocimum gratissimum* leaf essential oil. Int. J. Pharm., 224: 177-183.
- Orafidiya, L.O., E.O. Agbani, A.O. Oyedele, O.O. Babalola and O. Onayemi, 2002. Preliminary clinical tests on topical preparations of *Ocimum gratissimum* Linn. leaf essential oil for the treatment of acne vulgaris. Clin. Drug Investig., 22: 313-319.
- Orhue, E.S., M. Idu, J.E. Ataman and L.E. Ebite, 2008. Haematological and histopathological studies of *Jatropha tanjorensis* (J.L. Ellis and Soroja) leaves in rabbits. Asian J. Biol. Sci., 1: 84-89.

- Osifo, N.G., 1989. A System of Traditional Health Care. Vol. 2, Ethiope Publishing Corporation, Benin City, Nigeria, ISBN-13: 9781230541, pp: 106.
- Pessoa, L.M., S.M. Morais, C.M.L. Bevilaqua and J.H.S. Luciano, 2002. Antihelminthic activity of essential oil of *Ocimum gratissimum* Linn. and eugenol against *Haemonchus contortus*. *Vet. Parasitol.*, 9: 59-63.
- Rabelo, M., E.P. Souza, P.M.G. Soares, A.V. Miranda, F.J.A. Matos and D.N. Criddle, 2003. Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae) in mice. *Braz. J. Med. Biol. Res.*, 36: 521-524.
- Roeschlau, P., P. Bernt and W. Gruber, 1974. Enzymatic determination of total cholesterol in serum. *Z. Klin. Chem. Klin. Biochem.*, 12: 226-226.
- Sofowora, A., 2000. Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons Canada, Ltd., Ibadan, Nigeria, ISBN-13: 9780471902447.
- Sulistiarini, D.L., 1999. *Ocimum gratissimum* L. In: Plant Resources of South-East Asia. No. 19: Essential oils Plants. Oyen, P.A. and X.D. Nguyen (Eds.). Prosea Foundation, Bogor, Indonesia, pp: 140-142.
- Tietz, N.W., E.L. Pruden and O. Siggard-Anderson, 1986. Electrolytes, Blood Gasses and Acid Base Balance. In: Textbook of Clinical Chemistry, Tietz (Ed.). Saunders Publishers, Philadelphia, ISBN-13: 9780721688862, pp: 1188-1196.
- Traynor, J., R. Mactier, C.C. Geddes and J.G. Fox, 2006. How to measure renal function in clinical practice? Clinical review. *BMJ.*, 333: 733-737.
- Watt, J.M. and M.J. Breyer-Brandwijk, 1962. The Medicinal and Poisonous Plants of Sourthern and Eastern Africa. Elsevier Health Sciences, Edinburgh, ISBN-13: 9780443005121, pp: 1425.
- Weatherbum, M.W., 1967. Phenylhypochlorite reaction for determination of ammonia. *Analytica Chemica*, 39: 971-971.
- Yam, L.T., 1974. Clinical significance of the human acid phosphatases: A review. *Am. J. Med.*, 56: 604-616.