Phytochemistry of Ethanolic Leaf Extracts of *Ocimumgratissimum* and *Gongronemalatifolium and* their Combined Effects on Red Blood Cell and Platelets Indices of Streptozotocin-Induced Diabetic Rats

Usoh IF[1], *Ekaidem IS[2]

ABSTRACT

The phytochemical screening of Ocimumgratissimum(OG) and Gongronemalatifolium(GL) leaves and their effects on blood parameters of streptozotocin (65mg/kg b.w,)-induced diabetic rats of Wistar strain were studied. Thirty-six male albino Wistarrats were randomised into 6 groups. Each group comprised of 6 rats. Groups A and F were treated with distilled water to respectively serve as diabetic and normal controls, while the diabetic-induced rats in Groups B, C, D and E were respectively treated orally with extracts of GL (200mg/kg body weight), OG (200mg/kg body weight), combined extracts of OG and GL (OGGL:100mg/kg body weight each) and subcutaneous 5 IU/kg body weight of insulin. The animals were treated for 28 days after which they were sacrificed forwhole bloodcollection that was used for haematological assays. The results of phytochemical analysis of the two leaf-extracts demonstratedthe presence of various levels of alkaloids, tannins, phlobatannins, flavonoids, polyphenols, saponins, and reducing sugars. Haematological analysis of the rats demonstrated significant ($p<0.05$) decreases in of red blood cell count (RBC), hemoglobin (HB), mean cell volume (MCV), mean corpuscular haemolobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet count (PLT) in diabeticcontrol rats that were neither treated with the extracts nor insulin compared to the normal control and diabetics rats treated with the extracts or insulin. Total white blood cell counts, percentage lymphocytes and mean platelet volume were on the other hand significantly increased in this group of rats. The combined extracts treated rats showed significant decrease in haematological parameters when compared to single extracts treatments, hence revealing a negative synergistic interaction of the two leaves extracts.

Keywords: Combined extracts, Gongronemalatifolium, Ocimumgratissimum, Red cell indices, Platelet indices.

INTRODUCTION

Diabetes mellitus is a condition associated with many organ complications and is responsible for at least 10% of total expenditure that is related to health care in many countries[2,3]. Absolute or relative deficiency of insulin and/or reduced insulin function is a common underlying cause of this disease. It is characterized by sustained hyperglycemia due to disorders affecting carbohydrate, protein, and fat metabolism. Organ damage, dysfunction, and failure especially those involving the eyes, kidneys, nerves, heart, and blood vessels are associated with diabetes mellitus[4]. Effective therapeutic approach should be multimodal and able to ameliorate the effects of the disease on the various organs. In view of this, several traditional medicinal herbs have been used given the spectrum of active ingredients present in herbs[5]. Over the years, medicinal plants extracts have been known to be effective for treatments of diabetes; and the world's population are currently relying on medicinal plants for their primary health care is about 80%[6,7].

*Gongronemalatifolium (*family: Ascepiadaceae) is a tropical rainforest plant[8]. It is a shrub, with milky or less often, clears latex. The plant, locally called "Utazi" in the Eastern and Southern States of Nigeria is used primarily as a staple vegetable/ spice[9, 10]. The crude leaf-extract of this plant has been shown to maintain a healthy blood sugar levels[11]. Experimental evidences have supported the glucose and lipid lowering potentials as well as antioxidant effects of the aqueous and ethanol extracts of *G.latifolium*leaves[9,10,12,13].

Ocimumgratissimum (Labiatae) is thought to originate from Africa and Asia, though can now be found in other parts of the World[14]. Different local names such as Daidoya (Housa),

Department of Biochemistry[1], Faculty of Basic Medical Sciences, University of Uyo, Department of Chemical Pathology[2], Faculty of Clinical Sciences, University of Uyo

*Corresponding author: seityjen1@yahoo.com

Nchunwu (Igbo), Efnrin (Yoruba), Nton (Ibibio)[15] is used to describe *O. gratissimum,* but it is popularly known as "scent leaf" in most parts of Nigeria. The plant is commonly used as condiment and spice for preparation of different dishes. The leaf-extract of O. *gratissmium* containssome bioactive substances[16]. Phytochemical screening of the extracts of O. *gratissmium* had shown the plant to contain terpenoids, saponins, alkaloids, tannins, anthraquinone, flavonoids steroids and cardiac glycosides[16-18]. These phytochemicals are known to possess antibacterial[18], antifungal[19], antinoceptive[20], antihypertensive[21], antidiabetic[22], antidiarrheal[23], antioxidant[24], insecticidal and antihelmintic properties which justify its high medicinal use in folk medicine[25].

Polyherbal therapy is a practice that benefits from the enhanced therapeutic effects of more than one plant with minimum side effects[26]. It is thought to derive it enhanced efficacies from phytochemicals present in the plants and they present exciting opportunities for the discovery of new drugs for the treatment of diabetes mellitus.

The constituents of these plant products, though have been shown to demonstrate high level of value in traditional medicine, may sometimes exhibit deleterious effects on some vital body organs and systems including blood and its products. Blood is unique in composition many functions[27]. Its unique nature and functions exposes it to a wide range of abnormal conditions arising from changes in metabolism. These abnormal alterations in blood parameters are results of changes in cellular integrity and exposure to toxic chemicals. Some xenobiotics in blood are converted to reactive oxygen intermediates which react covalently with macromolecules in blood provoking different type of toxicity and poisoning.

The effects of ethanol extracts of *O. gratissimumand G. latifolium*leaves have been reported differently using experimental models, however information is still scanty in the body of literature on their combined effect on hematological parameters of rats with STZ diabetogenesis. In view of this, the phytochemical constituents of ethanolic leaf extracts of *Gongronemalatifolium and Ocimumgratissimum* and their combined effect on some hematological parameters of STZ-induced diabetic rats were studied.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh but matured leaves of *Gongronemalatifolium* and *Ocimumgratissimum* were harvested from Atimbo, Akpabuyo L.G.A in Cross River State. They were both identified and authenticated in the Department of Botany, University of Calabar, Calabar. Each of *Gongronemalatifolium* and *Ocimumgratissimum* leaves were washed with tap water and the debris removed. The plant materials were separately sliced with a knife and 1000g each of *Gongronemalatifolium* and *Ocimumgratissimum* leaves were homogenized with an electric blender in 2.25L and 1.95 L of 80% (v/v) ethanol, respectively. The mixtures were allowed in a refrigerator (4°C)for 48 hours extraction. To obtain a homogenous filtrate, the mixtures were filtered with cheesecloth and later with What man No. 1 filter paper. The filtrates were concentrated *in vacuo* at low temperature (37 to 40°C) to about one tenth of the original volume using a rotary evaporator. The concentrates were further allowed open in a water bath (40°C) for complete dryness, yielding 49.54g and 35.41g of *Gongronemalatifolium* and *Ocimumgratissimum* extracts. The extracts were then refrigerated at 2 to 8°C for animal experiments.

Phytochemical screening

A measured proportion (20gm) of the ground powder sample was mixed with 100mls of distilled water and shook vigorously; and another 20gm in 100mls of ethanol, for 1hour. The extracts were refluxed in a flask two times for 30 minutes. They were filtered with What man No. 1 filter paper and concentrated to 50mls. Qualitative determination of phytochemical constituents of the extracts were performed as described by Harborne[28] and Trease and Evans[29].

Standard laboratory techniques were used for the phytochemical screening. Alkaloids, glycosides (Salkowski test) and saponins (Frothing test) were identified following the method of Sofowora[30]. The presence of phlobatanins, anthraquinones, flavonoids and tannins were tested using the method of Trease and Evans[29].

Experimental Animals

Thirty-six (36) male albino rats of Wistar strain weighing between 164-258g were obtained from the animal house of the College of Medical

Sciences, University of Calabar. The animals were allowed to acclimatize for two weeks in the Department of Biochemistry animal house facility, University of Calabar, where the experiment was carried out. The animals were housed in well ventilated cages (wooden bottom and wire mesh top) where bedding was replaced every two days, and kept under controlled environmental conditions (room temperature of about 27°C and 12 hour light/dark cycle). The animals were fed with grower's marsh and tap water *ad libitum*

Induction of Experimental Diabetes

Prior to diabetes induction, the rats were subjected to 12 hour fast and then diabetes was induced by intraperitoneal injection of 65mg/kg b.wstreptozotocin (STZ)[30] (Sigma St. Louis, MO, USA) reconstituted in 0.1M Na citrate buffer (pH 4.5). Seven days after, diabetes was confirmed in STZ treated rats with a fasting blood sugar concentration =200mg/dl. The diabetic rats were then divided randomly into the different groups.

Experimental design and treatment of animals

Thirty-six (36) male albino *Wistar* rats were divided into 6 groups of 6 rats each as shown in table 1. The plant extracts reconstituted in distilled water (vehicle) were administered via oral gastric intubation at a dose of 200mg/kg body weight daily for single extract treatment and 100mg/kg body weight each in combined extract treatment twice per day (7.00am and 7.00pm). Insulin (5IU/kg body weight) was administered subcutaneously (S.C) once daily post prandial. The dosages of plant extracts and insulin used were according to the methods of Ebong *et al.*[26] and the treatment lasted for 28 days.

Table 1: Experimental Design

Group	No. of animals	Treatment
A	6	Placebo (Diabetic Control)
B	6	GL extract (200mg/kg bw)
C	6	OG) extract (200mg/kg bw)
D	6	GL (100mg/kg) + OG (100mg/kg)
E	6	Insulin (5 IU/kg bw)
F	6	Placebo (normal control)

Collection of samples for haematological analysis

At the end of the 28 days, food was withdrawn from the rats and they were fasted overnight but had free access to water. They were euthanized under chloroform vapour and sacrificed. Whole blood was collected into EDTA bottlesvia cardiac puncture using sterile syringes and needles for full blood count. Full blood counts were estimated using the Sysmsex® Automated Haematology Analyzer KX-21N, Sysmex Corporation, Kobe-Japan. The pre-diluted (PD) sample method was used where blood was diluted manually, and then fed into the transducers of the analyzer.

Statistical analysis

Data were presented as Mean ± standard deviation. Differences between means were compared employing student's t-test and ANOVA post hoc, a probability of $p < 0.05$ was considered significant.

RESULTS

The results of qualitative phytochemical screening of GL and OG are presented in table 2. Alkaloids and saponins were present in both plant extract in higher concentration than tannins, phlobatannins and reducing sugar. Although the content of polyphenols in GL was higher than in OG, flavonoid content of OG was two times higher than in GL. Anthraquinons and cardiac glycosides were not present in both plant extracts. Red cell indices namely, total red cell counts, haemoglobin concentration (HB), mean cell volume (MCV), mean cell haemoglobin

(MCH) and mean corpusularhaemoglobin concentration (MCHC), in normal control rats, diabetic control (untreated) rats and diabetic rats treated with GL and OG extracts are shown in table 3. Significantly($p< 0.05$) lower red cell indices were observed in diabetic control rats when compared with normal control group. Treatment of the diabetic rats with separate extracts of GL and OG showed significant improvement in red cell indices to values compared to those receiving insulin treatment. However, treatment with a combination of the two extracts was not as beneficial as those treated with separate extracts. The untreated diabetic rats control rats showed significant($p< 0.05$) increase in total white blood cell counts and percentage lymphocytes as compared with normal control rats (table 4). Similarly, treatment with extracts of both leaves separately and in combination reduced these parameters significantly($p< 0.05$) to values comparable with insulin treatment. Platelet counts of diabetic untreated rats were significantly($p< 0.05$) lower than those of normal control while the mean platelet volume (MPV) values were higher in diabetic control rats than normal control. These changes were reversed significantly by treatment with both plant extracts either in combination or separately, and insulin.

Table 2: Qualitative phytochemical screening of GL and OG leaf extracts

S/N	Phytochemicals	GL	OG
1	Alkaloids	++	++
2	Tannins	+	+
3	Phlobatannins	+	+
4	Flavonoids	+	++
5	Polyphenols	++	+
6	Anthraquinons	-	-
7	Saponins	++	++
8	Cardiac Glycoside	-	-
9	Reducing sugars	+	+

Key: + = Present, ++ = Present in excess, - = Absent

Table 3: Red blood cell indices of treated and untreated diabetic rats.

Group/ Treatment	RBC (10^6/µL)	HB (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
DC	6.50± 0.01*^{c}	10.04± 0.02*^{c}	42.10± 0.01*^{c}	12.02± 0.01*^{c}	21.60± 0.01*^{c}
D_{GL}	10.55± 0.00*^{,a,c}	13.97± 0.01*^{,a,b,c}	61.20± 0.01*^{,a}	17.06± 0.01*^{,a,c}	29.70± 0.01*^{,a,c}
D_{OG}	10.54± 0.01*^{,a,c}	15.34± 0.01*^{,a,b,c}	61.15± 0.01*^{,a}	16.78± 0.01*^{,a,b,c}	29.72± 0.01*^{,a,c}
D_{GLOG}	8.79± 1.76*^{,a}	13.31± 2.66*^{,a}	51.01± 1.20*^{,a,}	14.26± 2.85*^{,a}	24.79± 4.95*^{,a}
D_I	10.43± 0.01*^{,a,b}	12.76± 0.02*^{,a,b}	60.10± 0.02*^{,a,}	16.13± 0.03*^{,a,b}	25.64± 0.02*^{,a,b}
NC	10.89 ±0.01^{a}	16.52 ±0.01^{a}	68.27 ±1.62^{a}	20.50 ±0.01^{a}	30.58 ±0.10^{a}

*$p<0.05$ vs NC; a = $p<0.05$ vs DC; b = $p<0.05$ vs D_{GLOG}; c = $p<0.05$ vs D_I
Values are expressed as mean ± SEM, n = 6.

Table 4: Platelets and white blood cells changes in diabetic treated and untreated rats

Group/ Treatment	PLT (10^3/µL)	MPV(fL)	WBC (10^3/µL)	Lym%
DC	259.50 ± 7.21*	8.09 ± 0.02*	16.50 ± 0.03*	58.29 ± 0.02*
D_{GL}	467.17 ± 1.62*	6.11 ± 0.02*,b	13.60 ± 0.02*,a,b,c	48.20 ± 0.01*,a,c
D_{OG}	460.83 ± 0.54*	6.12 ± 0.01*,b	13.25 ± 0.04*,a,b,c	50.18± 0.01*,a,c
D_{GLOG}	352.70 ± 8.26*,a	6.82 ± 1.16*,a	15.91 ± 2.78*,a	53.33 ± 8.87*
D_I	384.17 ± 2.43*	6.07 ± 0.02*,b	12.08 ± 0.1*,a,b	44.80 ± 0.01*,a,b
NC	579.50 ± 1.78[a]	5.62 ± 0.05[a]	9.70 ±0.02[a]	32.90 ± 0.01[a]

*p<0.05 vs NC; a = p<0.05 vs DC; b = p<0.05 vs D_{GLOG}; c = p<0.05 vs D_I
Values are expressed as mean ± SEM, n = 6.

DISCUSSION

The preliminary phytochemical analyses of the two leaves extracts showed the presence of alkaloids, tannins, phlobatannins, flavonoids, polyphenols, saponins, and reducing sugars. Alkaloidsplay some metabolic role and also to control development in the living system[31]. The presence of saponins in the leaves may be the basis for the use of the leaves in lowering plasma cholesterol level and therefore could serve to control atherosclerosis and other related disease conditions[5]. Flavonoids have been already known to possess some antioxidants properties and could promote healthy endothelial function. Tannins are known bioactive compounds with notable antiviral and anticacer activities[32].

Significant reduction in red blood cell indices in diabetic (untreated) control rats were observed in this study. This observation indicates that diabetes is associated microcytic, hypochromic anaemia which may be similar to those observed in iron deficiency anaemia. Diabetic condition has been known to be associated with increased oxidative stress usually causing depletion of intracellular reduced glutathione and other cytosolic antioxidants in erythrocytes. This oxidant status in the red cells predisposes to intravascular lysis leading to anaemia. Diabetes mellitus also present a chronic inflammatory state probably resulting from glucose toxicity on endothelial cells producing increased levels of pro - inflammatory cytokines namely: interleukin 1 (IL - 1), interleukin 6 (IL - 6) and tumor necrosis factor α (TNF - α). These cytokines can induce hepatic synthesis of hepcidin, a negative controller of ferroportin function, thereby reducing iron absorption from the gastrointestinal tract[36], this may be responsible for the microcytic form of anaemia observed in the streptozotcin induced diabetes in rats.

Exposure of rats to STZ and treatment with GL, OG, GLOG (combined) and insulin produced significant changes in some haematological indices. Administration of some medicinal compounds or drugs had been associated with different levels of alteration in haematological parameters from the normal range. Inthis study, there was significant increase in erythrocytes (RBC) counts, mean cell volume (MCV) and mean corpuscular haemoglobin concentration (MCHC), of diabetic single and combined GL and OG extracts treated groups compared to diabetic control. These findings suggest that the plants extracts may impact some regulatory role on the production of hematopoietic chemical mediators such as colony-stimulating factors and erythropoietin by stromal cells and macrophages in the bone marrow[33]. This provides the local environment

for haematopoiesis. The extracts of the leaves have been shown to contain high level of polyphenols/flavonoids which are known antioxidant compounds and alkaloids with notable anti - inflammatory activities[34]. The effects of these components of the plants extracts on protecting the erythrocytes and other cells from oxidative damage and inflammatory reactions may also be responsible for their anti anaemia properties as observed in this study.

From the values of WBC in diabetic treated groups compared to diabetic control (DC), it may be inferred that the elevation in the WBC count is an inflammatory reaction of animal to chronic cellular dysfunctions caused by diabetes mellitus. Leucocytosis observed here in diabetic untreated rats may suggest a stimulation of the immune system aimed at protecting the rats against infections and necrotizing host cells. The level of leukocytosis has been said to be directly related to the severity of the inducing stress condition, and may be the result of increased leucocytes mobilization[35]. Significant increase in the percentage lymphocytes in diabetic untreated control groups compared to normal control and extracts treated rats reflects possible immune modulatory effects of the extracts.

The platelet counts of diabetic untreated rats were significantly lower than those of normal control and diabetic groups treated with extracts and insulin. Chronic hyperglycemia can contribute to alterations resulting in endothelial dysfunction and vascular injuries of diabetic complications[36]. Vascular lesions of diabetes mellitus arising from disturbances in polyol pathways and activation of protein kinase C may increase platelet activation and consumption. Platelet activation may involve change of shape, adherence to sub-endothelial surfaces, secretion of intracellular organelles contents and aggregation to form a thrombus. Thus, platelet activation may play a critical rolein the pathogenesis of advanced atherosclerosis in diabetes[39,40].

Platelet hyper-reactivity in patients with diabetes has been said to be multifactorial[41,42]. It may be related to some biochemical factors namely hyperglycemia and hyperlipidemia, insulin resistance, inflammatory and oxidant state. It is also associated with increased expression of glycoprotein receptors and growth factors[40,41]. Insulin regulates platelet function through a functional insulin receptor (IR) found on platelets[45] and had been shown to inhibit platelet interaction with collagen and attenuates the platelet aggregation effect of some coagulation agonists[41]. In chronic inflammatory state such as seen in diabetic condition, superoxide increases intra-platelet release of calcium following activation thus enhancing platelet reactivity[42]. Superoxides have also been shown to limit the physiological action of nitric oxide (NO). Platelets from patients with diabetes have been reported to show increased expression of surface P-selectin and glycoprotein (GP) IIb/IIIa receptors and were more sensitive to aggregation compared to platelets from patients without diabetes[42].

The mean platelet volumes(MPV) of rats in the diabetic (untreated) control group were significantly higher than those of normal control and diabetic rats treated with single or combined extracts or insulin. MPV is an index of the average size and activity of platelets. Larger platelets are younger, more reactive and readily form aggregates. They contain denser granules, secrete more serotonin and β-thromboglobulin, and produce more thromboxane A2 than smaller platelets[43]. All these conditions represent pro-coagulant effects and predisposes to thrombotic vascular complications. Our findings of increased MPV in untreated diabetes suggest a relationship between the platelet function especially MPV and diabetic vascular complications[40,43]. The ability of the extracts to reduce the MPV of diabetic rats may be related to the anti-inflammatory and anti-oxidant properties of the phytochemicals present in the leaves. In this study, the amelioration of platelet activities in terms of platelet counts and MPV was better with insulin treatment and when the extracts were administered separately than in the combined extract administration.

The reason for the antagonistic effects of the two extracts (GL and OG) on red blood cells and platelets indices of diabetic rats is not fully understood. Phytochemical studies as presented above showed that total polyphenol concentration is higher in GL while Flavonoid components of the polyphenol were greater in OG. The interaction between other forms of polyphenol and flavonoids aimed at reducing the antioxidant and anti - inflammatory effects which characterize the flavonoid compounds may be a possible explanation.

In conclusion, the leaf - extracts of GL and OG singly and in combination at the

specified doses for the period of the experiment have been observed to significantly improve the haematological parameters of experimental diabetic animals. However, the GL and OG in combination interacted in such a way that the blood parameters of rats treated with single extracts performed better than those with the combined treatment.

REFERENCES

1. Gipsen WH, Biessels GJ. Cognition and synaptic plasticity in diabetes mellitus. Trends Neurosci 2000; 23:542-9.
2. King H, Aubert RE, Herman WH. Global burden of diabetes, 19952025: prevalence, numerical estimates, and projections. Diabetes Care 1998;21:1414-31.
3. Kamtchouing P, Kahpui SM, Djomeni Dzeufiet PD, T'edong L, Asongalem EA, Dimoa T. Anti-diabetic activity of methanol/methylene chloride stem bark extracts of Terminaliasuperba and Canariumschweinfurthii on streptozotocininduced diabetic rats. *J Ethnopharmacol* 2006;104:306-9.
4. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2007;30: S42-S47
5. Atangwho IJ, Ebong PE, Eyong EU, Williams IO, Eteng MU, Egbung GE. Comparative chemical composition of leaves of some antidiabetic medicinal plants: *Azadirachtaindica, Vernoniaamygdalina* and *Gongronemalatifolium.* African Journal of Biotechnology 2009a; 8:4685-89
6. Shinde V, Dhalwal K. Pharmacognosy: The Changing Scenario. *Journal of Pharmacognosy* 2007; 1:1.
7. WHO. (1999). WHO Monographs on Selected Medicinal Plants. Geneva, 1:4.
8. Chattopadhyay RR. A comparative evaluation of some blood sugar lowering agents of plant origin. Journal of Ethnopharmacology 1999;67:367-372.
9. Ugochukwu MH, Babady NE, Cobourne MK. Gasset SR. The effect of *Gongronema Latifolium* extracts on serum lipid profile and oxidation stress on Hepatocyte of Diabetic rats. *Journal of Bioscience* 2003;28:1-5.
10. Morebise O, Fafunso MA, Makinde JM, Olayide OA, Awe EO. Anti-inflammatory Property of *Gongronemalatifolium*. *Phytotherapy Research* 2002;16:575-7.
11. Okafor JC, Ejiofor MAU. Strategies for enhancement of utilization of potential edible woody forest species of southeastern Nigeria. The biodiversity of African plant. Kluwe. The Netherlands, 1996; pp. 684-695.
12. Ogundipe OO, Moody JO, Akinyemi TO, Raman A. Hypoglycaemic potentials of methanolic extracts of selected plant foods in alloxanized mice. *Plant Foods Human Nutrition* 2003;58:1-7.
13. Schneider C, Rotscheidt K, Breitmaimeri E. Four new pregnane glycoside from *Gongronemalatifolium (*Asclpidaeceae*).Annalen Der Chemie* 1993;10:1057-62.
14. Sulistiarini DL. *Ocimumgratissimum*L. In: Oyen PA, Nguyen ED (eds) Plant Resources of South-East Asia. No. 19: Essential oils Plants (Prosea Foundation, Bogor, Indonesia) 1999; p. 140
15. Owulade MO. *Handbook African medicinal plants.* CRC press, Boca Raton, Florida, USA, 2004; pp. 214 -5.
16. Leal PF, Chaves F, Celio M, Ming LC, Petenate AJ, Meireles M, Angela A. Global yeilds, chemical composotions and antioxidant activities of Clove basil (Ocimum*gratissimum* L.). *Journal of Food Processing Engineer*. 2006; 29:547-559.
17. Holetz FB, Ueda Nakamura T, Ficho BPD, Cortez DAG, morgado-Diaz JA, Nakamura CV. Effects of essential oil of Ocimumgratissimum on the Trypanosomatid herpetomonas samud pessoal. Acta Protozool 2003; 42:269-2276.
18. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus*activity. *BMC*

Complement Alternative Medicine, 2005; 5: 6-10.

19. Dubey NK, Tiwari TN, Mandin D, Andriamboavonjy H, Chaumont JP. Antifungal properties of *Ocimumgratissimum* essential oil (ethyl cinnamatechemotype) *Fitoterepia,* 2000;7:567-9.
20. Rabelo, M, Souza, E.P., Soares, P.M.G., Miranda, A.V., Matos, F.J.A. & Criddle, D.N. Antinociceptive properties of the essential oil of *Ocimumgratissimum* L. (Labiatae) in mice. *British Journal of Medical Biology Research*, 2003;36:521-4.
21. Interaminense LF, Jucá DM, Magalhães PJ, Leal-Cardoso JH, Duarte GP, Lahlou S. Pharmacological evidence of calcium - channel blockade by essential oil of *gratissimum* and its main constituent, eugenol, in isolated aortic rings from DOCA-salt hypertensive rats. *Fundamental Clinical Pharmacol*ogy, 2007;21:497-506.
22. Mohammed A, Tanko Y, Okasha MA, Magaji RA, Yaro AH.. Effects of aqueous leaves extract of *Ocimumgtatissimum* on blood glucose level of steptozocin induced diabetic wistar rats. *African Journal of Biotechnology,* 2007;6:2087-90.
23. Ilori M, Sheteolu AO, Omonigbehin EA, Adeneye AA. Antidiarrhoeal activities of *Ocimumgratissimum* (Lamiaceae*). Journal of Diarrhoea Disease Res*earch, 1996; 14: 283-5.
24. Odukoya AO, Ilori OO, Sofidiya MO, Aniunoh OA, Lawal BM, Tade IO. Antioxidant activity of Nigerian dietary spices. *EJEAF Chemistry,* 2005; 4):1086-93.
25. Pessoa LM, Morais SM, Bevilaqua CML, Luciano JHS. Anthelmintic activity of essential oil of *Ocimumgratissimum* Linn. And eugenol against *Haemonchuscontortus*. *Veterinary Parasitol*ogy 2002;109:59-63.
26. Ebong P E, Atangwho IJ, Eyong E U, Egbung GE. The antidiabetic efficacy of combined extracts from two continental plants, *Azadirachtaindica* and *Vernoniaamygdalina*(Del). American Journal of Biochemistry and Biotechnology, 2008; 4, 239-244
27. Martini H. *Fundamentals of Anatomy and Physiology.* 3rd edn, Prentice Hall, Inc, New Jersy, 1995; pp. 648-998.
28. Harborne JB. Phytochemical methods: A guide to Modern technique of plant analysis. Chapman and Hall, London.
29. Trease GE, Evans WC. Pharmacognosy 1996; 4thedn, WB Saunders pp 234-6.
30. Ugochukwu NH, Babady NE. Antioxidant effects of *Gongronemalatifolium* in hepatocytes of rat models of non-insulin dependent diabetes mellitus. Fitoterapia, 2002; 73,612-8.
31. Edeoga HO, Eriata DO. Alkaloid, tannins and saponin contents of some Nigerian medicinal plants. J. Med. Aromatic Plant Sci.2001;23:344-9
32. Chang HY, Lin CC, Lin TC. Antiherpes simplex virus type 2. Chemistry 2002; 43:2162-66
33. Chang-Gue S, Seung-Hyun H, Jung- Hyo C, Jang- Woo S, Chin- Ho C, Yeon-Weot L, Chong-Kwan C. Induction of hemopoiesis by saenghyulden, a mixture of ginseng radix, paconial radix alba and homnis placenta Extracts. Acta Pharmacologica Sciencia 2003;5: 21-26
34. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and diseases. Oxidative Medicine and Cellular Longevity 2009; 2:270-8.
35. Celik L, Seizek H. The haematological effects of methyl parathion in rats. J. Hazadous Materials 2008; 153:1117-21.
36. Bae SH, Lee J, Roh KH, Kim J. Platelet activation in patients with diabetic retinopathy. Korean J Ophthalmol. 2003;17:1404.
37. Maitra A. The Endocrine System. In: Kumar V, Abbas AK, Fausto N, Aster JC, editors. Robbins and Cotran Pathologic Basis of Disease. 8th ed. New Delhi: Elsevier; 2010. pp. 1097-164.

38. Mitchell RN. Hemodynamic Disorders, Thromboembolic Disease and Shock. In: Kumar V, Abbas AK, Fausto N, Aster JC, editors. Robbins and Cotran Pathologic Basis of Disease. 8th ed. New Delhi: Elsevier; 2010. pp. 111-34
39. Colwell JA, Nesto RW. The platelet in diabetes-focus on prevention of ischemic events. Diabetes Care. 2003;26:21818.
40. Vinik AI, Erbas T, Park TS, Nolan R, Pittenger GL. Platelet dysfunction in type 2 diabetes. Diabetes Care. 2001;24:1476-85.
41. Yngen M, Norhammar A, Hjemdahl P, Wallén NH. Effects of improved metabolic control on platelet reactivity in patients with type 2 diabetes mellitus following coronary angioplasty. DiabVasc Dis Res. 2006;3:52-6.
42. Chang HA, Hwang HS, Park HK, Chun MY, Sung JY. The Role of Mean Platelet Volume as a Predicting Factor of Asymptomatic Coronary Artery Disease. Korean J Fam Med. 2010;31:600-6.
43. Jindal S, Gupta S, Gupta R, Kakkar A, Singh HV, Gupta K, et al. Platelet indices in diabetes mellitus: indicators of diabetic microvascular complications. Hematology. 2011;16:86-89.