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Full Length Research Paper

Antioxidative Efficacy of Combined Leaves Extracts of *Gongronema Latifolium* and *Ocimum Gratissimum* on Streptozotocin-Induced Diabetic Rat Models

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Abstract

This study investigated the effect of oral administration of combined 80% ethanolic leaves extracts of *Gongronema latifolium* (GL) and *Ocimum gratissimum* (OG) on some oxidative stress indices in diabetic rats. Thirty-six male albino rats were divided into 6 groups with 6 rats in each. Groups A and F received placebo treatment and served as diabetic and normal controls respectively. B and C received extracts of GL and OG at 200mg/kg b.w respectively. D received combined extracts of GL and OG at 100mg/kg b.w each and E received 5 IU/kg b.w insulin subcutaneously. Treatment lasted for 28 days after which animals were sacrificed and serum and liver collected for analysis of antioxidant parameters. From the results, the single and combined extracts treatment significantly ($P<0.05$) decreased hepatic MDA formation, suggesting the role of the extracts in protection against pro-oxidant induced membrane damage. The extracts also significantly increased the hepatic activities of SOD, GPX, CAT and serum levels of ß-carotene, Vitamins A and C. The combined extracts group displayed a more pronounced effect when compared to single extract and insulin groups. The antioxidant effectiveness of the combined extracts might be connected with the synergistic interactions of several bioactive principles present in these leaves.

Keywords: Malondialdehyde, antioxidant enzymes, streptozotocin, diabetes, rats, *Gongronema latifolium, Ocimum gratissimum.*

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by persistent hyperglycemia, glycosuria, increased thirst (polydipsia), polyuria, polyphagia, and weight loss due to absolute or relative lack of insulin (Aguwa, 1996). Free radicals have been described as any chemical species not capable of independent existence and which contains one or more unpaired electrons. Having unpaired electron (s), they are unstable and highly reactive as the attempt is to satisfy their outer electron octet. By so doing, they abstract electrons from neighboring proteins, lipids, carbohydrates and nucleic acids setting in place a chain of reactions that lead to the cell membrane, organelles and DNA damage. Free radicals, including the superoxide radical ($O_2^{\cdot-}$), hydroxyl radical (OH), hydrogen peroxide (H_2O_2) and lipid peroxide radicals have been implicated in a number of disease processes, including diabetes, asthma, cancer, obesity, cardiovascular, gastrointestinal and liver diseases, cataract, macular degeneration, periodontal disease, and other inflammatory processes. These reactive oxygen species (ROS) are normal consequence of biochemical processes formed in the mitochondria and endoplasmic reticulum of aerobic organisms as oxygen is being reduced along the electron transport chain in the body, and also as a result of increased exposure to environmental and/or dietary xenobiotics (Cook and Samman, 1996; Allan and Miller, 1996; Kumpulainen and Salonen, 1999). ROS are increased in various tissues in diabetic condition and are involved in the development of diabetic complications (Brownlee, 2001). Several hypotheses put forth to explain the genesis of free radicals in diabetes include auto-oxidation processes of glucose, the non-enzymatic and

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progressive glycation of proteins with the consequently increased formation of glucose-derived advanced glycosylation end products (AGEs), and enhanced glucose flux through the polyol pathway (Tiwari and Rao, 2002). The increase in ROS themselves has strongly been correlated with chronic hyperglycemia of diabetes (Kaneto *et al.*, 2005). Hence, therapy using free-radical scavengers (antioxidants) has potential to prevent, delay or ameliorate many of these disorders (Delanty and Dichter, 2000). The antioxidant defense system represents a complex network of interactions, synergy and specific tasks for a given antioxidant (Polidori *et al.,* 2001). The efficiency of this defense mechanism is altered in diabetes and, therefore, the ineffective scavenging of free radicals may play a crucial role in determining tissue damage (Wohaieb and Godin, 1987).

Many medicinal plants including *Gongronema latifolium* and *Ocimum gratissimum* have been reported to possess free radical scavenging (antioxidant) properties (Ugochukwu *et al*., 2003; Ogundipe *et al.*, 2003, Trevisan *et al.,* 2006, Akinmoladun *et al*., 2007). They contain several different pharmacologically active compounds that may act individually, additively or synergistically to improve health (Azarzeh *et al*., 2003). *Gongronema latifolium* Bent Hook, is a herbaceous shrub, with yellow flowers and the stem that yields characteristics milky exudates when cut. It is locally called “utasi” by the Efiks, Ibibios and Quas; ‘’utazi” by the Igbos and “arokeke” by the Yorubas in Nigeria. The Efiks and the Quas in Calabar use *G. latifolium* crude leaf extract in the treatment of malaria, diabetes, hypertension, and as laxative. The use of crude leaf extract of this shrub in maintaining a healthy blood sugar levels has been reported (Okafor and Ejiofo, 1996). Scientific studies have established the hypoglycaemic, hypolipidaemic and antioxidative effects of aqueous and ethanol extracts of *G. latifolium* leaf (Ugochukwu *et al*., 2003; Ogundipe *et al.*, 2003). Morebise *et al*. (2002) showed that the leaf extract has anti-inflammatory activity with its potential nutritional and food processing properties. Some phytochemicals such as B-sistosterol, lupenyl esters, pregnane ester, glycosides, essential oils and saponins are associated with parts of this herb (Schneider *et al*., 1993; Morebise *et al*., 2002). It is plausible that one or more of these phytochemicals that are found in *G. latifolium* is likely to influence cellular proteins with enzymatic activity.

Ocimum gratissimum (Labiatae) is a native of Africa and Asia but is now distributed to other parts of the World including the United States of America (Sulistiarini, 1999). In Nigeria, *O. gratissimum* is described by different local names: Daidoya (Hausa), Nchunwu (Igbo), Efnrin (Yoruba), Nton (Ibibio) (Owulade, 2004) but it is popularly known as “scent leaf” in most parts of the country. The plant is used as a condiment and spice in most parts of the World including Nigeria for preparation of different dishes. It is also used widely in folk medicine for the treatment of several ailments including fever, cough and respiratory disorders (Correa, 1932; Oliver, 1980), sore throat, kidney stones, epilepsy and dermatitis (Sofowora, 1993), headache, stress and mental diseases (Osifo,1989). Studies have shown that the leaf extract of O. *gratissimum* contains potent bioactive components (essential oils) made up of eugenol, citral, linalool, charvicol, thymol, gerianol, triterpenoids, saponins, alkaloids, etc. (Sulistiarini, 1999; Leal *et al*., 2006; Matasyoh *et al*., 2007). These phytochemicals posses antibacterial (Akinyemi *et al*., 2005), antifungal (Dubey *et al*., 2000) antinoceptive (Rabelo *et al*., 2003), antihypertensive (Interaminense *et al.,* 2005, 2007), antidiabetic (Mohammed *et al.,* 2007), antidiarrheal (llori *et al*., 1996; Adebolu and Salau, 2005), antioxidant (Odukoya *et al*., 2005), insecticidal (Eze *et al*., 2006) and antihelmintic properties which justify its high medicinal use in folk medicine (Pessoa *et al*., 2002).

Polyherbal therapy allows for combination of secondary metabolites which would not only exert a combined effective action in various mechanistic targets but also potentiate maximum therapeutic efficacy with minimum side effects (Tiwari and Rao, 2002; Ebong *et al*., 2008). From the foregoing, this study is aimed at investigating the combined antioxidant action of the ethanolic leaves extracts of *Gongronema latifolium and Ocimum gratissimum* in streptozotocin-induced diabetic Wistar rats.

MATERIALS AND METHODS

Collection and preparation of plant materials

Fresh but matured leaves of *Gongronema latifolium* and *Ocimum gratissimum* were collected from Atimbo, Akpabuyo Local Government Area of Cross River State. They were both identified and authenticated in the Department of Botany, University of Calabar, Calabar. 500g each of *Gongronema latifolium* and *Ocimum gratissimum* were thoroughly washed with clean tap water to remove dust particles**,** and debris and shade dried. The dried plant materials were separately ground into powder with KENWOOD electric blender (KENWOOD LTD. ENGLAND). The powdered samples were each soaked in 80% ethanol and kept in a glass container with a plastic screwed cap and kept in a refrigerator for 48 hours at 4°C. They were filtered using a cheese material and afterwards Whatman No.1 filter paper. The filtrates were separately concentrated *in vacuo* at 37-40°C using a rotary evaporator. The concentrates were allowed open in a water bath (40°C) for complete ethanol removal. The dried extracts were refrigerated at 2-8°C until required for use. The concentration of the extract was determined by drying a known volume and measuring the dry weight.

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)

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 Health Sciences, University of Calabar. The animals were allowed to acclimatize for two weeks in the Biochemistry departmental animal house facility, University of Calabar where 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 water from tap *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.w (Ugochukwu and Babady, 2003) streptozotocin (STZ) (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.

Experimental design and treatment of animals

The diabetic rats (n = 30) were divided randomly into five 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 postprandia**l**. The dosages of plant extracts and insulin used were according to the methods of Ebong *et al.,* (2006) and Sonia and Scrinvasan, (1999). The normal control group, F (n=6) was fed with grower's marsh and water without treatment. Treatment lasted for 28 days.

Preparation of serum and microsomal fractions of liver homogenate

After 28 days of treatment, the animals were anaesthetized with chloroform vapor, quickly brought out of the jar and sacrificed. Whole blood was collected by cardiac puncture from each animal using sterile needle to pierce through the heart. It was emptied into sterile test tubes containing no anticoagulant and allowed to stand for about 15 minutes to clot and further spun in a westerfuge centrifuge (Model 1384) at 10,000g at 4^{o}C for 10 mins. Serum was separated from the clot with Pasteur pipette into sterile sample tubes for the determination of serum β –Carotene and vitamins A and B. The liver was immediately removed, washed in ice cold 1.15% KCl solution, blotted, weighed and homogenized in 4 volumes of the homogenizing buffer (pH7.4) using a potterelvegin homogenizer. The resulting liver homogenate was centrifuged at 105,000g for 1 hour in a BECKMAN L5 - 50B ultracentrifuge with a type 35 fixed angle rotors. Pellet microsomes were suspended in 0.25M sucrose solutions and this was stored in a frozen condition. These procedures were carried out at temperature between 0^{o}C and 4^{o}C so as to retain enzyme activity. The liver microsome was used to determine lipid peroxidation (LP), glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT).

Determination of lipid peroxidation

Lipid peroxidation in microsomes prepared from liver was estimated by thiobarbituric acid-reactive substances (TBARS) as described by the procedure of Varshney and Kale, (1990). It was determined by quantifying MDA concentrations, which was spectrophotometrically measured by the absorbance of a red-colored product with thiobarbituric acid.

Determination of liver superoxide dismutase (SOD) activity

SOD activity in hepatocytes was measured using assay kits (Randox laboratories, Crumlin, Ireland). The method

for SOD employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazonium chloride (INT) to form a red formation dye (Woolliams *et al.,* 1983). The SOD activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT. The absorbance was spectrophotometrically measured.

Determination of liver glutathione peroxidase (GPx) activity

GPx activity in hepatocytes was measured using assay kits (Randox laboratories, Crumlin, Ireland). The method is based on that of Paglia and Valentine (1967). GPX catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione (GSSG) is immediately converted to the reduced form (GSH) with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance was spectrophotometrically measured.

Determination of catalase (CAT) activity

Catalase (CAT) activity in hepatocytes was spectrophotometrically determined using assay kits from Bioxytech ® catalase-520 ^{TM}, based on Aebi (1984). The principle involves dismutation of hydrogen peroxide (H_2O_2) to water and molecular oxygen whose rate is directly proportional to the concentration of catalase. The sample containing catalase of unknown activity is incubated in the presence of a known concentration of H_2O_2, for exactly one minute, after which the reaction is stopped / quenched with sodium azide. The amount of H_2O_2 remaining in the reaction mixture is then quantified by the oxidative coupling reaction of 4-aminophenazone (4-aminoantipyrene,) and 3,5-dichloro-2-hydroxy benzenesulphonic acid (DHBS) catalyzed by a versatile enzyme, horseradish peroxidase (HRP). The color intensity of the resulting product (measured at 520nm) is directly proportional to amount of H_2O_2 remaining in reaction mixture but inversely proportional to catalase activity in the sample.

Determination of serum β–carotene and vitamins A and C

Serum β-carotene and vitamin A were assayed according to the method of Suzuki and Katoh (1990) as described by Kokcam and Naziroglu (1999). Serum vitamin C was determined chemically according to the procedure described by Erel *et al.* (1997) using dinitrophenylhydrazine (DNPH).

Statistical analysis

The results were reported as means ± SD from six repeated determinations and evaluated with the analysis of student's t-test. Differences were considered to be statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Malondialdehyde (MDA) levels and liver SOD, GPx and CAT activities

The results in table 2 revealed a significant significant decrease in the activities of SOD, GPx and CAT and significant increase in the concentration of MDA in hepatocytes of STZ-induced diabetic rats (DC) compared to normal control (NC) rats ($p<0.05$). Treatment with GL and OG extracts significantly brought down the level of MDA and increased the activities of the antioxidant enzymes when compared to the DC ($p<0.05$). Insulin treated group (D_I) showed a significant decrease in the concentration of MDA and the activities of antioxidant enzymes when compared to extract treated groups. The combined extracts treated group (D_{GLOG}) exhibited a significant decrease in the concentration of MDA and increase in the activities of SOD, GPx and CAT when compared to single extract treated groups.

Serum β-carotene, vitamins A and C levels

Serum levels of β-carotene, vitamins A and C significantly decreased in DC group and increased ($p<0.05$) in the combined extracts treated group (D_{GLOG}) when compared to normal control (table 3). These levels also increased significantly in extracts and insulin (D_I) treated groups when compared to DC. The single extract treated groups displayed significant decrease ($p<0.05$) in the levels of these parameters as compared to combined extract treated groups.

Streptozotocin used in the induction of diabetes is a known pathogenic factor of free radicals generation or increased lipid peroxidation (Tatsuki *et al*., 1997; Szkudelski, 2001; Goycheva *et al*., 2006) and the resultant radicals do induce the antioxidant defense enzymes particularly in the liver, the tissue with the most abundant antioxidant defense enzymes (Tatsuki *et al.,* 1997), causing an initial increase. But if this process continues and become chronic, the enzyme systems may become overwhelmed, and the induction system also exhausted or worn out hence leading to usually observed decrease in these antioxidant enzymes. The decrease is in line with the report of Ugochukwu *et al.* (2003) who suggested it to be as a result of the radicals inactivating the enzymes or glycation of the enzyme protein themselves. Other researchers also reported a

Table 2. Some oxidative stress indices in liver of treated and untreated diabetic rats

Group/ treatment	MDA (µmol/g protein)	SOD (U/g protein)	GPx (U/g protein)	CAT (U/g protein)
DC	30.25 ±0.04*	9.52 ±0.03*	8.40 ±0.03*	40.17 ±0.06*
D_{GL}	20.03 ±0.01*,a,b,c	15.10 ±0.01*,a,b,c	12.59 ±0.04*,a,b,c	40.49 ±0.03*,a,b,c
D_{OG}	19.72 ±0.01*,a,b,c	15.20 ±0.05*,a,b,c	12.65 ±0.01*,a,b,c	45.25 ±0.02a,b,c
D_{GLOG}	16.50 ±0.02*,a	17.25 ±0.02*,a	14.00 ±0.02*,a	47.67 ±0.13*,a
D_I	15.50 ±0.05*,a,b	10.62 ±0.01*,a,b	9.60 ±0.02*,a,b	42.10 ±0.01*,a,b
NC	15.72 ±0 .01	19.70 ±0.03	16.20 ±0.03	50.24 ±0.02

Values are expressed as mean ± SEM, n = 6.
*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
DC= Diabetic control;
D_{GL}= Diabetic treated with *Gongronemalatifolium extract*
D_{OG} =Diabetic treated with *Ocimumgratissimum* extract
D_{GLOG} =Diabetic treated with combined extracts of *Gongronemalatifolium* and *Ocimum gratissimum*
D_I= Diabetic treated with insulin NC= Normal control

Table 3. Serum β-carotene and vitamins A and C levels of treated and untreated diabetic rats

Group/ treatment	Vitamin A (µmol/l)	Vitamin C (mg/100ml)	ß-carotene (µg/100ml)
DC	0.50 ±0.11*	0.33 ±0.01*	12.00 ±0.10*
D_{GL}	1.30 ±0.10*,a,b,c	0.50 ±0.06*,a,b,c	16.00 ±1.22*,a,b,c
D_{OG}	1.31 ±0.09*,a,b,c	0.52 ±0.02*,a,b,c	16.06 ±0.50*,a,b,c
D_{GLOG}	2.90 ±0.20*,a	0.99 ±0.08*,a	21.30 ±0.20*,a
D_I	1.50 ±0.05*,a,b	0.58 ±0.01*,a,b	16.60 ±0.02*,a,b
NC	1.80 ±0.10	0.62 ±0.05	18.10 ±0.50

Values are expressed as mean ± SEM, n = 6.
*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
DC= Diabetic control
D_{GL}= Diabetic treated with *Gongronemalatifolium extract*
D_{OG} = Diabetic treated with *Ocimumgratissimum* extract;
D_{GLOG} = Diabetic treated with combined extracts of *Gongronemalatifolium* and *Ocimum gratissimum*
D_I= Diabetic treated with insulin NC= Normal control

decrease in the activity of these antioxidant enzymes in the liver of diabetic rats (Anuradha and Selvam, 1993; Stanelly *et al.,* 2001). In this study, the significant increase in the level of MDA (an index of lipid peroxidation) and decrease in the activities of SOD, GPx and CAT in diabetic control group as compared to

normal control and extract treated groups might be linked to the production of ROS by STZ-induced hyperglycemia in rats. Ozmen *et al.* (2000) indicated in their report, an inverse relationship between ROS concentration and SOD, CAT and GPx activities in liver, heart and kidneys of diabetic animals. Lipid peroxidation is one of the characteristic features of chronic diabetes. The increased free radicals produced may react with polyunsaturated fatty acids (PUFA) in cell membranes leading to lipid peroxidation. The mechanism is that hydroxyl radical ($OH^{\cdot}$) attacks polyunsaturated fatty acid (PUFA), forming a carbon-centred lipid radical. The radical rearrange to form a conjugate dienyl radical. This radical reacts with ambient oxygen (O_2), forming a hydroperoxyl radical, which then abstracts hydrogen from a neighboring lipid, forming lipid peroxide and starting a chain reaction. This reaction continues until the supply of PUFA is exhausted, unless a termination reaction occurs. GL and OG extracts (singly and in combination) may suppress lipid peroxidation through different chemical mechanisms, including free radical quenching, electron transfer, radical addition, or radical recombination (Liangli Yu *et al*., 2002). In this study insulin was more effective in reducing the MDA concentration than the extract while the extract was more effective in increasing the activity of antioxidant enzymes when compared to insulin group. This might be because the standard drug promoted the uptake of glucose into cells and therefore prevented hyperglycemic induced membrane peroxidation.

The significant ($p<0.05$) decrease in diabetic control rats (as compared to normal control) and increase in treatment groups (as compared to diabetic control) of serum levels of β-carotene, vitamins A and C could be linked to increased production of ROS and to the ability of the extracts to mitigate ROS generated by STZ diabetes respectively. Beta-carotene is the precursor of vitamin A, and its ability to act as an antioxidant is due to the stabilization of organic peroxide free radicals within its conjugated alkyl structure. Since β-carotene occurs at low oxygen concentration, it compliments antioxidant properties of vitamin E which is effective at higher oxygen concentrations. Vitamin C is known to act as an effective antioxidant on its own and it also shows excellent synergistic activity with vitamin E in the inhibition of oxygen radical-induced lipid peroxidation *in vitro* (Stocker, *et al.,* 1986). Vitamin C within the body is maintained in the reduced form by shuttling the dehydroascorbate across the erythrocyte membrane for reconversion to ascorbate (Orroinger and Roear, 1979). Vitamin C exists as the enolate anion at physiological pH which spontaneously reduces superoxide, organic ($R^{\cdot}$) and vitamin E radicals, forming dehydroascorbyl radical ($AS^{\cdot}$). This radical undergoes a second reduction reaction to form dehydroascorbate which is recycled to ascorbate by dehydroascorbate reductase, a GSH-dependent enzyme present in all cells. Plants often contain substantial amounts of antioxidants including flavonoids, polyphenols, minerals (Se, Cu, Zn and Cr) carotenoids, β-carotene and vitamins such as tocopherol (vitamin E) and ascorbic acid (vitamin C), (Larson, 1988: Battel *et al*., 1999: Gorman, 1992). Vitamin E is the major chain terminating antioxidant in membranes; it reduces both conjugated dienyl and hydroperoxyl radicals, quenching the chain or cycle or lipid peroxidation reactions. This might have contributed to the enhanced activity of antioxidant enzymes displayed by the extract treated rats and reduced concentration of MDA, an index of lipid peroxidation.

CONCLUSION

The extracts of GL and OG showed a significant synergistic effect on STZ-induced diabetic rats. This implies that combined extract treatment may be more beneficial and useful relative to individual extracts in diabetes management as antioxidant defense system. Further studies are warranted to determine the exact components in GL and OG responsible for the observed synergistic effect and such components may be candidates for use as prophylactic agents against free radicals generated by STZ diabetes.

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REFERENCES

Adebolu TT, Salau AO (2005). Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhea causing bacteria in Southwestern Nigeria. *African Journal of Biotechnology*, Vol.4, No. 7, pp. 682-684.

Aebi H (1984). Catalase *in vitro.* In: SP. Colowick, N.O. Kaplanes (eds*). Method in Enzymology.* Vol. 105, pp. 121-126.

Aguwa CN (1996). Diabetes mellitus. In: Therapeutic Basis of clinical pharmacy in the tropics.Optimal Publishers Enugu, Nigeria, pp. 1-453.

Akinmoladun AC, Ibukun EO, Emmanuel A, Obuotor EM, Farombi EO (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Science Research Essay,* Vol. 2, pp. 163-6.

Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA (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 Alternative Medicine,* Vol. 5, pp.6-10.

Allan L, Miller ND (1996). Antioxidant Flavonoids: structures, functions and clinical usage. *Alternative Medicine Review,* Vol. 1, pp. 103-111.

Anuradha CV, Selvam R (1993). Effect of oral methionine on tissue lipid peroxidation and antioxidants in alloxan induced diabetic rats. *Journal of Nutritional Biochemistry*, Vol. 4, pp. 212–217.

Azaiszeh H, Fulder S, Khalil K, Said O (2003). Ethnobotanical knowledge of local Arabs practitioners in the middle Eastern region.

Fitoter., Vol. 74, pp.98-108
Battell ML, Rodrigues B, Yuen VG, McNeill JH (1999). Treatment and pharmacological interventions in streptozotocin diabetes. In J. H. McNeill's (Ed.) Experimental Models of Diabetes (pp. 195-216). U.S.A.: CRC Press LLC
Brownlee M (2001).Biochemistry and molecular cell biology of diabetic complications. *Nature.* Vol. 414, No. 6865, pp. 813-820.
Cook MC, Samman S (1996). Flavonoids-chemistry, metabolism, card is protective effects and dietary sources. *Nutritional Biochemistry*, Vol.7, pp. 66-76.
Correa MP (1932). Dictionary of plant utilized in Brazil, Ministry of Agriculture, Rio de Janeir pp.63.
Delanty N, Dichter M (2000). Antioxidant therapy in Neurologic diseases. *Archives of Neurology*. Vol.57, pp. 1265-1270.
Dubey NK, Tiwari TN, Mandin D, Andriamboavonjy H, Chaumont JP (2000). Antifungal properties of *Ocimum gratissimum* essential oil (ethyl cinnamate Chemotype. *Fitoterepia,* Vol. 7, No. 15, pp. 567-569.
Ebong PE, Atangwho IJ, Eyong EU, Egbung GE (2008). The antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta* indica (A.Juss) (Neem) and *Vernonia amyglalina* (Del.) (African bitter leaf). *American Journal of Biochemistry and Biotechnology.* Vol. 4, No.3, pp. 239-244.
Ebong PE, Atangwho IJ, Eyong EU, Ukere C, Obi AU (2006). Pancreatic beta cell regeneration. A probable parallel mechanism of hypoglycaemic action of Vernomia amygdalina and *Azadirachta indica*. Proceedings of International Neem Conferencce, Kuming, China. Nov.11-12.
Erel O, Kocyigit A, Avci S, Aktepe N, Bulut V (1997). Oxidative stress and antioxidative statue of plasma and erythrocytes in patients with malaria. *Clin. Biochem.*, Vol. 30, pp. 631-639.
Eze SC, Asiegbu JE, Mbah BN, Orkwor GC, Asiedu R (2006). Biocontrol of strorage insect and use as fungicide were explored. *Agro-Science*, Vol.5, No.1, pp. 8-12.
Gorman LS (1992). *Lipids and Lipoproteins*. In: Bishop, ML., Duben-Engelkirk, J.L. and Fody, E.P. (Eds.), Clinical Chemistry.Principles,Procedures, Correlations. 2nd edition. Philadelphia: J. B Lippincott Company. pp. 354-357.
Goycheva P, Gadjeva V, Popov B (2006). Oxidative Stress and its complications in diabetes mellitus. *Trakia Journal of Sciences*. Vol.4, No.1, pp. 1-8.
Ilori M, Sheteolu AO, Omonigbehin EA, Adeneye AA (1996). Antidiarrhoeal activities of *Ocimum gratissimum* (Lamiaceae*). Journal of Diarrhoea Disease Research,* Vol.14, No.4, pp. 283-285.
Interaminense LF, Jucá DM, Magalhães PJ, Leal-Cardoso JH, Duarte GP, Lahlou S (2007). 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 Pharmacology.* Vol. 21, pp.497-506.
Interaminense LF, Leal-Cardoso JH, Magalhães JC, Duarte GP, Lahlou S (2005). Enhanced hypotensive effects of the essential oil of *Ocimum gratissimum* leaves and its main constituent, eugenol, in DOCA-salt hypertensive conscious rats. *Planta Medical.* Vol.71, pp.376
Kaneto H, Matsuoka T, Nakatam Y, Kavirnori D, Matsuhisa M, Yamasaki O (2005). Oxidative stress and the JN pathway in Diabetes. *Current Diabetes Reviews.* Vol.1, pp,65-72.
Kokcam I, Naziroglu M (1999). Antioxidant and lipid peroxidation status in blood of patients with ascariasis. *Clin. Chim. Act.,* Vol. 289, pp.23-31
Kumpulainen JT, Salonen JT (1999). Natural Antioxidant and Anticarcinogens in Nutrition, Health and Disease. The Royal Society of Chemistry UK, pp. 178-187.
Larson RA (1988). The antioxidants of higher plants. *Phytochemistry.* Vol. 27, pp. 969-978.
Leal PF, Chaves F, Celio M, Ming LC, Petenate AJ, Meireles M, Angela A (2006). Global yeilds, chemical composotions and antioxidant activities of Clove basil (Ocimum *gratissimum* L.). *Journal of Food Processing Engineer.* Vol. 29, No. 5, pp. 547-559.
Liangli Y, Haley S, Perret J, Harris M (2002). Antioxidant properties of hard winter wheat extracts. *Food Chem*., 78: 457-461.
Matasyoh LG, Matasyoh JC, Wachira FN, Kinyua MG, Muigai TAW, Mukiama TK (2007). Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya. African Journal of Biotechnology, Vol. 6, No. 6, pp. 760-765
Mohammed A, Tanko Y, Okasha MA, Magaji RA, Yaro AH (2007). Effects of aqueous leaves extract of *Ocimum gtatissimum* on blood glucose level of steptozocin induced diabetic Wistar rats. *African Journal of Biotechnology,* Vol 6, pp. 2087-90.
Morebise O, Fafunso MA, Makinde JM, Olayide OA, Awe E (2002). Anti-inflammatory Property of *Gongronema latifolium*. *Phytotherapy Research,* Vol. 16, pp. 575-577.
Odukoya AO, Ilori OO, Sofidiya MO, Aniunoh OA, Lawal BM, Tade IO (2005). Antioxidant activity of Nigerian dietary spices. *EJEAF Chemistry,* Vol. 4, No. 6, pp. 1086- 1093.
Ogundipe OO, Moody JO, Akinyemi TO, Raman A (2003). Hypoglycaemic potentials of methanolic extracts of selected plant foods in alloxanized mice. *Plant Foods Human Nutrition*, Vol. 58, pp. 1-7.
Okafor JC, Ejiofor MAU (1996). Strategies for enhancement of utilization of potential edible woody forest species of southeastern Nigeria. The biodiversity of African plant. Kluwe. The Netherlands, pp. 684-695.
Oliver B (1980). Medicinal plants in Nigeria. Nigerian College of Arts, Science and Technology: Ibadan, p. 90.
Orroinger EP, Roear ME (1979). An ascorbate-Mediated trans membrane reducing system of the human erythrocyte. *J. Clin. Invest.,* Vol. 63, pp. 53-58.
Osifo NG (1989). A System of Traditional Health Care .Ethiope Publishing Corporation, Benin city, Nigeria. Vol. 2, p. 106.
Owulade MO (2004). Handbook African medicinal plants. CRC press, Boca Raton, Florida, USA, pp. 214-215.
Ozmen D, Ozmen B, Esin E, Guner I, Sara H, Bayindir O (2000). Lens peroxide dimutase and catalase activities in diabetic cataract. *Turkish Journal of Endocrinology and Metabolism,* Vol.1, pp. 1-4.
Paglia DE, Valentine WN (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine*. Vol. 70, pp.158-169 .
Pessoa LM, Morais SM, Bevilaqua CML, Luciano JHS (2002). Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn. and eugenol against *Haemonchus contortus Veterinary Parasitology*. Vol. 109, pp. 59-63.
Polidori MC, Stahl W, Eichler O, Niestroj I, Sies H (2001). Profiles of antioxidants in human plasma. Free Radical Biological Medicine, Vol.30, pp. 456–462.
Rabelo M, Souza EP, Soares PMG, Miranda AV, Matos FJA, Criddle DN (2003). Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae) in mice. *British Journal of Medical Biology Research,* Vol.36, pp. 521- 524.
Schneider C, Rotscheidt K, Breitmaimeri E (1993). Four new pregnane glycoside from *Gongronema latifolium (*Asclpidaeceae*)*. *Annalen Der Chemie* 10: 1057-1062.
Sofowora LA (1993). *Medicinal plants and traditional medicine in Africa*. Spectrum Books Ltd, Ibadan, Nigeria. Nigeria. p. 55.
Sonia B, Scrinivasan BP (1999). Investigations into the anti-diabetic activity of *Azadirachia* indica*. Indian Journal of Pharmacology,* Vol.31, pp. 38 -141.
Stanely P, Prince M, Menon VP (2001). Antioxidant action of *Tinospora cordifolia* root extract in alloxan diabetic rats. *Phytotherapy Research,* Vol. 15, pp. 213–218.
Stocker R, Weidemann MJ, Hunt NH (1986). Possible mechanism responsible for the increased ascorbic acid content of *plasmodium vinckei* infected mouse erythrocytes.*Biochemica and Biophysica Acta*. Vol. 881, pp. 391-397
Sulistiarini DL (1999). *Ocimum gratissimum* L. In: Oyen PA, Nguyen ED (eds) Plant Resources of South-East Asia. No. 19: Essential oils Plants (Prosea Foundation, Bogor, Indonesia) p. 140
Suzuki J, Kattoh M (1990). A simple and cheap method for measuring serum vitamin A in cattle using only spectrophotometer. *Jpn. J. Vet. SCP*. Vol. 124, pp. 83-88.
Szkudelski T (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*. Vol. 50, pp. 536- 546.
Taksuki R, Satoh K, Yamamoto A, Hoshi K, Ichihara, K (1997). Lipids Peroxidation in the Pancreas and Other Organs in Streptozotocin Diabetic Rats. Japan *Journal of Pharmacology*. Vol. 75, pp. 27-273.

Tiwari AK, Rao JM (2002). Diabetic mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Current Science*, Vol. 8, No. 31, pp. 30-37.

Trevisan MTS, Silva MGV, Pfundstein B, Spiegelhalder B, Owen RW (2006). Characterization of the volatile pattern and antioxidant capacity of essential oil from different species of the genus Ocimum. *Journal of Agric and Food Chemistry.* Vol. 54, pp, 4378-82.

Ugochukwu MH, Babady NE, Cobourne MK, Gasset SR (2003). The effect of *Gongronema Latifolium* Extracts on serum lipid profile and oxidation stress on Hepatocyte of Diabetic rats. *Journal of Bioscience,* Vol. 28, No. 1, pp 1-5.

Ugochukwu NH, Babady NE (2003). Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. *Life Science,* Vol. 73, No. 15, pp. 1925-1938.

Varshney R, Kale RK (1990). Effects of Calmodulin antagonist on radiation induced lipid peroxidation in microsomes. *Int. J. Rad Biol*. Vol. 58, pp. 733-744.

Wohaieb SA, Godin DV (1987). Alterations, in free radical tissue defense mechanism in streptozotocin-induced diabetes in rats: effects of insulin treatment. *Diabetes,* Vol. 36, pp. 1014–1018.

Wooliams JA, Wiener G, Anderson PH, McMurray CH (1983).*Research in verterinary. Science.* Vol. 34, pp.253-56

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