

OPEN ACCESS



Journal of  
**Medicinal Plants Research**

25 November 2018  
ISSN 1996-0875  
DOI: 10.5897/JMPR  
[www.academicjournals.org](http://www.academicjournals.org)



**ACADEMIC  
JOURNALS**  
expand your knowledge

# ABOUT JMPR

**The Journal of Medicinal Plant Research** is published twice monthly (one volume per year) by Academic Journals.

**The Journal of Medicinal Plants Research (JMPR)** is an open access journal that provides rapid publication (twice monthly) of articles in all areas of Medicinal Plants research, Ethnopharmacology, Fitoterapia, Phytomedicine etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JMPR are peer reviewed. Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

## Contact Us

Editorial Office: [jmpr@academicjournals.org](mailto:jmpr@academicjournals.org)

Help Desk: [helpdesk@academicjournals.org](mailto:helpdesk@academicjournals.org)

Website: <http://www.academicjournals.org/journal/JMPR>

Submit manuscript online <http://ms.academicjournals.me/>

## Editors

### **Prof. Akah Peter Achunike**

*Editor-in-chief  
Department of Pharmacology & Toxicology  
University of Nigeria, Nsukka  
Nigeria*

### **Associate Editors**

#### **Dr. Ugur Cakilcioglu**

*Elazig Directorate of National Education  
Turkey.*

#### **Dr. Jianxin Chen**

*Information Center,  
Beijing University of Chinese Medicine,  
Beijing, China  
100029,  
China.*

#### **Dr. Hassan Sher**

*Department of Botany and Microbiology,  
College of Science,  
King Saud University, Riyadh  
Kingdom of Saudi Arabia.*

#### **Dr. Jin Tao**

*Professor and Dong-Wu Scholar,  
Department of Neurobiology,  
Medical College of Soochow University,  
199 Ren-Ai Road, Dushu Lake Campus,  
Suzhou Industrial Park,  
Suzhou 215123,  
P.R.China.*

#### **Dr. Pongsak Rattanachaikunsopon**

*Department of Biological Science,  
Faculty of Science,  
Ubon Ratchathani University,  
Ubon Ratchathani 34190,  
Thailand.*

### **Prof. Parveen Bansal**

*Department of Biochemistry  
Postgraduate Institute of Medical Education and  
Research  
Chandigarh  
India.*

#### **Dr. Ravichandran Veerasamy**

*AIMST University  
Faculty of Pharmacy, AIMST University, Semeling -  
08100,  
Kedah, Malaysia.*

#### **Dr. Sayeed Ahmad**

*Herbal Medicine Laboratory, Department of  
Pharmacognosy and Phytochemistry,  
Faculty of Pharmacy, Jamia Hamdard (Hamdard  
University), Hamdard Nagar, New Delhi, 110062,  
India.*

#### **Dr. Cheng Tan**

*Department of Dermatology, first Affiliated Hospital  
of Nanjing Univeristy of  
Traditional Chinese Medicine.  
155 Hanzhong Road, Nanjing, Jiangsu Province,  
China. 210029*

#### **Dr. Naseem Ahmad**

*Young Scientist (DST, FAST TRACK Scheme)  
Plant Biotechnology Laboratory  
Department of Botany  
Aligarh Muslim University  
Aligarh- 202 002,(UP)  
India.*

#### **Dr. Isiaka A. Ogunwande**

*Dept. Of Chemistry,  
Lagos State University, Ojo, Lagos,  
Nigeria.*

# Editorial Board

**Prof Hatil Hashim EL-Kamali**

*Omdurman Islamic University, Botany Department,  
Sudan.*

**Prof. Dr. Muradiye Nacak**

*Department of Pharmacology, Faculty of Medicine,  
Gaziantep University,  
Turkey.*

**Dr. Sadiq Azam**

*Department of Biotechnology,  
Abdul Wali Khan University Mardan,  
Pakistan.*

**Kongyun Wu**

*Department of Biology and Environment Engineering,  
Guiyang College,  
China.*

**Prof Swati Sen Mandi**

*Division of plant Biology,  
Bose Institute  
India.*

**Dr. Ujjwal Kumar De**

*Indian Veterinary Research Institute,  
Izatnagar, Bareilly, UP-243122  
Veterinary Medicine,  
India.*

**Dr. Arash Kheradmand**

*Lorestan University,  
Iran.*

**Prof Dr Cemşit Karakurt**

*Pediatrics and Pediatric Cardiology  
Inonu University Faculty of Medicine,  
Turkey.*

**Samuel Adelani Babarinde**

*Department of Crop and Environmental Protection,  
Ladoke Akintola University of Technology,  
Ogbomoso  
Nigeria.*

**Dr.Wafaa Ibrahim Rasheed**

*Professor of Medical Biochemistry National Research Center  
Cairo  
Egypt.*

# Journal of Medicinal Plants Research

Table of Contents: Volume 12 Number 28 25 November, 2018

## ARTICLES

- In vivo efficacy of Dialium guineense fruit pulp on hemeoxygenase-1 and angiotensin converting enzyme in experimental diabetes** 483  
Etah E. Nkanu, Gabriel Ujong, Victoria Okon and Iya Eze Bassey
- Evaluation of the phytochemical composition, antimicrobial and anti-radical activities of *Mitracarpus scaber* (Rubiaceae)** 493  
Ouadja B., Anani K., Djeri B., Ameyapoh Y.O. and Karou D. S.
- Bio-guided anti-cariogenic and phytochemical valorization of *Guiera senegalensis* and *Pseudocedrela kotschy* stem extracts** 500  
Guessan Bi Goblé Landry, Kadja Amani Brice, Cottet Kevin, Lecouvey Marc, Mamyrbékova-Békro Janat Akhanovna and Békro Yves-Alain
- Evaluation of herbal cocktail used in the treatment of malaria on liver tissue of adult Wistar rats** 508  
Moronkeji Akinpelu, Eze Ikechi Gerald, Bejide Ronald Ayodele, Anwara Ogbonnaya Akpu and Igunbor Michael Chuks
- Effect of aqueous extract of *Ocimum gratissimum* on acetaminophen induced renal toxicity in male wistar rats** 522  
Okerulu Linda, Celestine Ani, Choice Nworgu, Igwe Uzoma, Ugwuishi Emeka and Nwachukwu Daniel

*Full Length Research Paper*

## ***In vivo* efficacy of *Dialium guineense* fruit pulp on hemeoxygenase-1 and angiotensin converting enzyme in experimental diabetes**

**Etah E. Nkanu\*, Gabriel Ujong, Victoria Okon and Iya Eze Bassey**

Department of Physiology, Faculty of Basic Medical Sciences, Cross River University of Technology, Calabar, Okuku Campus, Yala, Nigeria.

Received 1 September, 2018; Accepted 17 September, 2018

The study aims to investigate the effect of aqueous fruit pulp of *Dialium guineense* on hemeoxygenase, and insulin release, inhibition of angiotensin converting enzyme (ACE) and possible hypoglycemia in streptozotocin-induced diabetic rats. Twenty four male Wistar rats were grouped into control, diabetic (Dm), diabetic + 300 mg/kg body weight *Dialium guineense* (Dm+ DG) and diabetic + 100 mg/kg metformin (Dm +MET). Apart from the control, other rats were made diabetic by a single dose of 50 mg/kg streptozotocin injected intraperitoneally. *Dialium* and metformin were administered orally three days after induction of diabetes. Result showed significant ( $p<0.01$ ) increase in serum ACE, blood glucose, and a decrease ( $p<0.01$ ) in HO-1 and insulin in the Dm group. There was also an increase ( $p<0.01$ ) in TC, TG and LDL-c. Tissue peroxidation (Heart and Kidney) was high in the diabetic untreated rats, superoxide dismutase and catalase activity was attenuated. Administration of aqueous fruit pulp and metformin significantly ( $p<0.001$ ) increased HO-1 and insulin secretion, decreased ACE and blood sugar level ( $p<0.001$ ) as well as the TC, TG and LDL-c. Antioxidant activities in the kidney and liver were potentiated. In conclusion, this study showed that *Dialium guineense* fruit pulp enhances HO-1 and insulin release and inhibits ACE activity. It is hypoglycemic, hypolipidemic and evokes antioxidant activity.

**Key words:** Hemeoxygenase-1 (HO-1), angiotensin converting enzyme (ACE), insulin, diabetes, *Dialium guineense*, metformin.

### **INTRODUCTION**

One major disease that affects man in an alarming rate amongst others is diabetic mellitus. It comes with marked metabolic disorder resulting from different environmental and varied hereditary factors. Commonly associated complications of diabetes mellitus include high toll of

morbidity and mortality abnormal insulin secretion or insulin receptor inactivity, hyperglycemia, hypercholesterolemia, liver and kidney dysfunction as well as derangement of pancreatic  $\beta$ -cell. It is also associated with profound changes in serum lipid, diabetic

\*Corresponding author. E-mail: nkanuee@yahoo.com; nkanuetah@gmail.com. Tel: +2347030299991; 08086127404.

ketoacidosis and culminates in chronic renal failure, neuropathy and coronary heart disease (Arora, 2010). In recognition of all these life threatening factors, concerted efforts to ameliorate the upsurge of the disease have been on course and drugs that can manage diabetes more effectively sorted with vigour.

Research has shown that angiotensin converting enzyme involved in the control of blood pressure plays an essential role in the conversion of inactive angiotensin I to an active angiotensin II which causes vasoconstriction. Studies have also shown that randomized trials using angiotensin converting enzyme inhibitors (ACEI) example, natural ACE inhibitors like polyphenols, flavonoids, xanthines, terpenoids derived from herbs (Kang et al., 2003; Loizzo, et al., 2007) and AT2 receptor blockers significantly decreased the risk of DM (Andraws and Brown, 2007; Abuissa et al.2005), improved insulin sensitivity and glucose metabolism, and reduced plasma glucose in both experimental conditions and in humans with DM (Scheen, 2004).

Similarly, HO-I has been reported to be a key antioxidant enzyme that prevents the development of diabetes by abating oxidative stress via suppression of macrophages and acting as an important component in anti-atherogenic activity (Orozco et al., 2007); it also plays a vital role in evoking insulin release (Ndisang et al., 2010; Mosen et al., 2005).

Substantive evidence indicates that factors such as hyperglycemia, free-fatty acids and adipokines that increase oxidative stress contribute to insulin resistance (Evans et al., 2003) even though the exact mechanism by which this occurs is not fully understood. However, some available information has implicated oxidative stress in the development of different forms of insulin resistance (Evans et al., 2003; Vinayagamoorthi et al., 2008).

It is therefore generally believed that elevated oxidative stress may lead to the cascade of events that impairs insulin-signalling (Vinayagamoorthi et al., 2008), and as such strategies that cause reduction in oxidative stress as well as glucose/insulin intolerance may improve glucose metabolism. Today, attention is redirected to the use of medicinal plants to treat most chronic diseases such as diabetes and hypertension (Liu et al., 2003; Ullah et al., 2015) because of its recognized nutritional and medicinal properties.

*Dialium guineense*, otherwise referred to as black velvet tamarind is an indigenous tropical forest fruit tree that belongs to the family leguminosae. The plant is found in many countries in West Africa and is identified by different names. In countries like Sierra Leone, Senegal, and Guinea Bissau, it is called 'Veludo.' In Nigeria, *Dialium* is called by different names depending on the ethnic group. The Igbos call it Icheku, while the Yorubas call it Awin. In Hausa, it is called Tsamiyarkurm (Orwa et al., 2009). The Yakurr ethnic group in Cross River State, Nigeria calls it Okana gben gbenwen. *D. guineense* has been convincingly used in the

management of various disease conditions such as severe cough, bronchitis, stomach aches, malaria fever, jaundice, antiulcer, hypertension and hemorrhoids (Lawal et al., 2010).

Phytochemical components identified in the sticky pulp of *D. guineense* include gums, hemicelluloses, mucilage, pectin and tannins. It also contains some level of ascorbic acid, minerals (copper, potassium, calcium, iron, selenium, zinc and magnesium), vitamins like vitamin-A, folic acid, riboflavin, niacin and vitamin C, tartaric acid (an anti-oxidant), carbohydrates in the form of soluble sugars, cellulose, iron and lipids (Nahar et al.,1990; Herzog et al.,1994; Gideon et al., 2012), tannins, alkaloids, saponins, flavonoid, steroids and cardiac glycosides and some phenolic compounds (David et al., 2011; Ezeja et al., 2011). This study was therefore aimed at finding out the possible effect of *Dialium giuneense* fruit pulp consumption on heme oxygenase, insulin and ACE activity in streptozotocin-induced diabetic Wistar rats since the fruit is being consumed locally as a socially and an alternative to Vitamin C.

## MATERIALS AND METHODS

### Experimental design

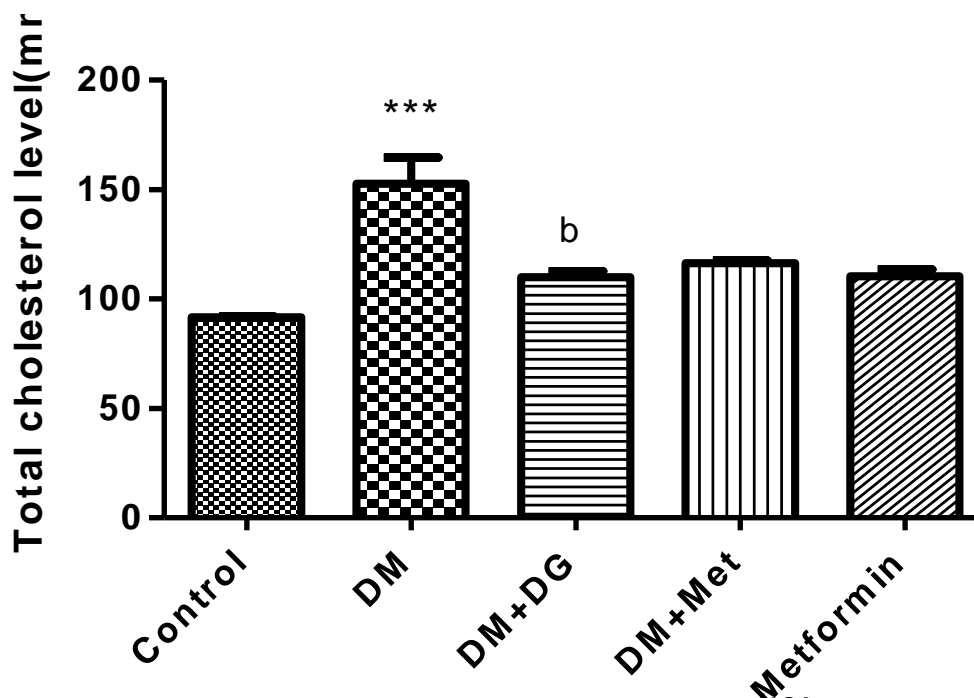
Twenty four male Wistar rats weighing between 170-230 g were used for this study. The animals were randomly selected into four groups of six rats each. Group 1 was the control and received tap water. Group 2, 3 and 4 were injected with 50 mg/Kg body weight of streptozotocin intraperitoneally to render them diabetic. Groups 3 and 4 were then administered 300 mg/kg of *D. guineense* fruit pulp and 100 mg/kg body weight of metformin respectively. Administration of drugs lasted for three weeks. Blood samples were collected by cardiac puncture for biochemical analysis and the tissues removed and used for histological studies and tissue peroxidation.

### Preparation of *Dialium* fruit pulp extract

The fruit pulp of *D. guineense* was purchased from Okuku Market, Yala Local Government Area of Cross River State. *D. guineense* fruits were collected and the dark coloured hard coats broken to expose the soft pink pulp of the fruit. The pulp was peeled from the water proof- like coat and then dried at room temperature by hot air oven (Amstel Hearson Oven, England) to evaporate its water content to a thick orange paste. Dried pulp was blended to powder and used when necessary. Animals received 300 mg/kg body weight of the suspension.

### Induction of diabetes mellitus

The rats except the control group were rendered diabetic on a 12 h fast by a single intraperitoneal injection of 50 mg/kg body weight streptozotocin (SantaCruz Biotechnology, USA) dissolved in 0.01M citrate buffer, at a pH 4.5. All experiments on animals were carried out in absolute compliance with ethical guideline for research, care, and use of laboratory animals. After 3 days of streptozotocin injection, blood glucose concentrations were determined via AccuChek glucometer to confirm diabetes. Blood glucose levels



**Figure 1.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on lipid profile in streptozotocin-induced diabetic rats. n=5; \*\*\*=p<0.01 vs Control; b=p<0.01 vs Dm.

below 130 mg/dl were not considered.

#### Blood collection and analysis

Blood samples were collected from the animals through cardiac puncture. The blood samples were centrifuged at 3000 (rpm) revolutions for 10 min to obtain serum for lipid profile, insulin, angiotensin converting enzyme and hemeoxygenase-1 analysis. Tissues were homogenized, centrifuged and supernatant used to measure malondialdehyde concentration and antioxidant activity.

#### Catalase

The method of Sinha (1972) was used to estimate catalase activity. The major principle involves reduction of dichromate in acetic acid to chromic acetate when heated in the presence of hydrogen peroxide ( $H_2O_2$ ). The perchromic acid formed is an unstable intermediate. The chromic acetate finally produced is measured using the colorimeter.

#### Superoxide dismutase

The activity of SOD was assayed using the method of Mishra et al. (1972). The ability of superoxide dismutase to inhibit the auto-oxidation of epinephrine at pH 10.2 has been used as the basis of a convenient and sensitive assay for this enzyme.

#### Malondialdehyde

Plasma MDA was estimated by method of Jean et al. (1983). After the reaction of thiobarbituric acid with malondialdehyde, the reaction product was extracted in butanol and was measured.

#### Determination of ACE

ACE activity was determined using the method of Hooper et al. (1987) with modifications. In brief, a pre-incubation mixture contained 100 mM Tris-HCl buffer with 300 mM NaCl and 10  $\mu$ M  $ZnCl_2$ , pH 8.3/positive control/test sample of various concentrations and 2 mU of ACE enzyme. The reaction mixture was mixed and pre-incubated at 37°C for 10 min. Following pre-incubation, substrate (N-HippuryL-histidine-L-leucine tetrahydrate) was added to a final concentration of 5 mM. The reaction mixture was mixed and incubated at 37°C for 30 min. The reaction was heated up in boiling in water bath for 4 min. A control reaction was also carried out without the test samples. The reaction mixture was centrifuged at 15,000 rpm for 10 min at 25°C. The supernatant was transferred to HPLC vials and subjected to HPLC analysis.

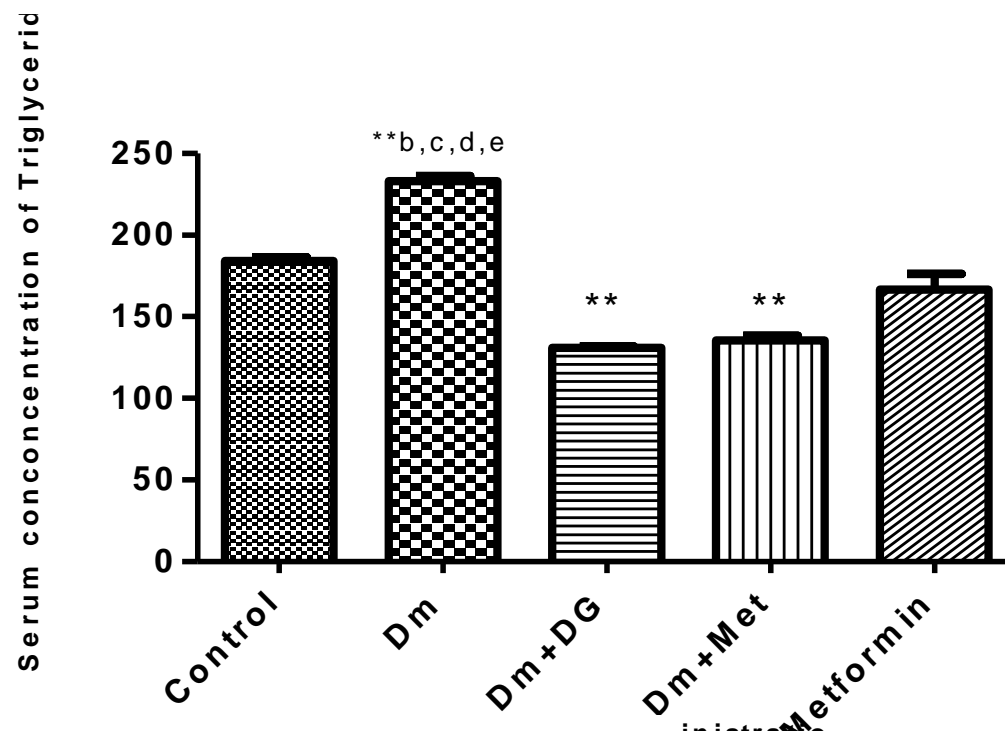
#### Histological studies

Heart and kidney were removed, dissected and washed immediately on ice cold saline. A portion of these tissues was fixed in 10% neutral formal-saline fixative solution for histological studies. After fixation, tissues were embedded in paraffin. Solid sections were cut at 5  $\mu$ m and stained with hematoxylin and eosins as described by Strate et al. (2005). The slides were viewed at magnification of X 400 and photomicrographs were taken.

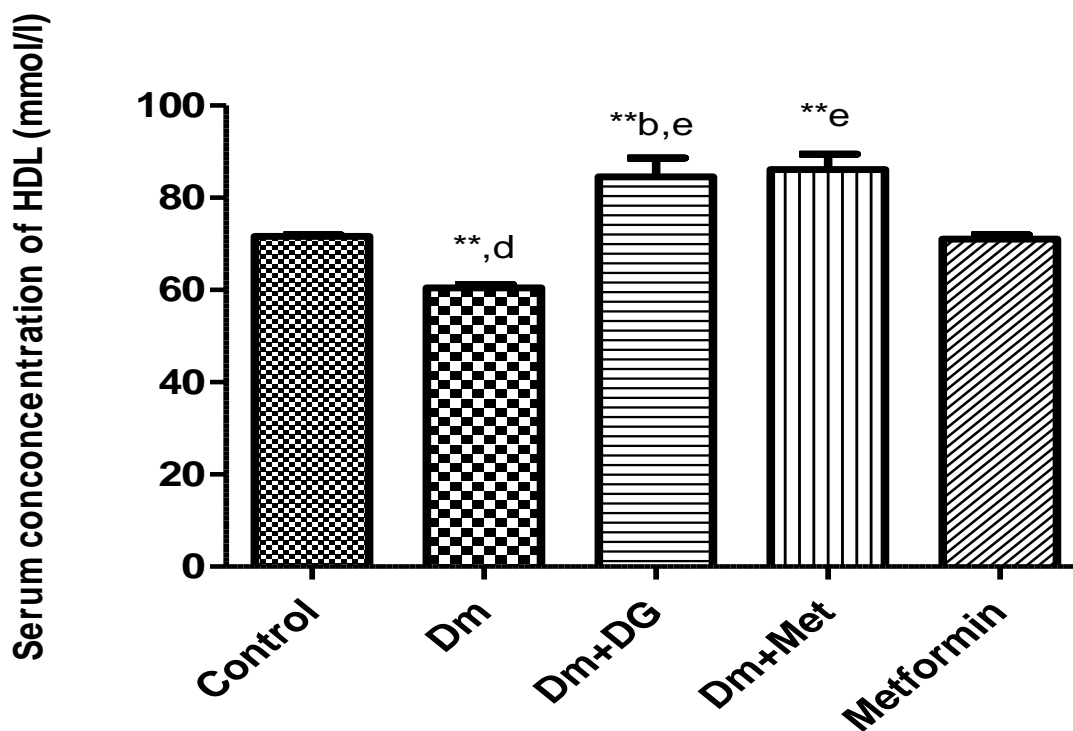
#### Statistical analysis

All data obtained in this study were expressed as mean  $\pm$  standard error of mean. Collected data were analyzed using ANOVA (analysis of variance) followed by Bonferroni's multiple comparison post hoc tests to compare the level of significance between control

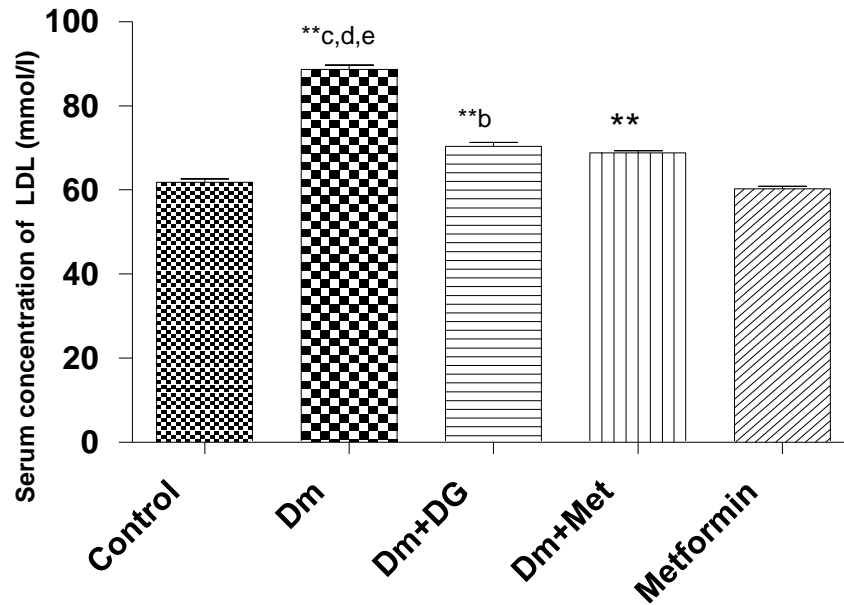




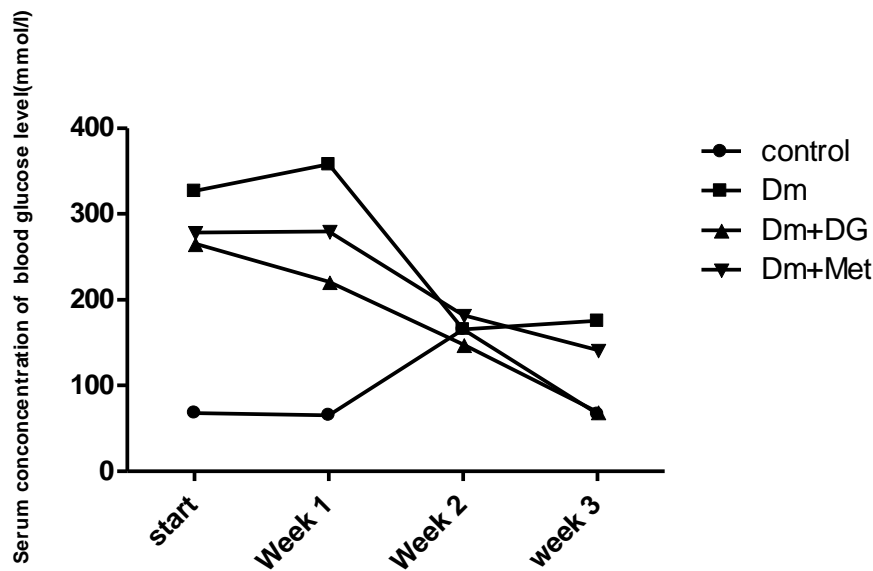
**Figure 2.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on triglyceride concentration in streptozotocin-induced diabetic rats. n=5; \*\*=p<0.01 vs Control; b=p<0.01 vs Dm; c=p<0.01 vs Dm+DG;d=p<0.01 vs Dm Vs Met; e=p<0.01 vs Metformin.



**Figure 3.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on high densitylipoprotein (HDL) concentration in n=5; \*\*=p<0.01 vs Control; b=p<0.01 vs Dm; c=p<0.01 vs Dm+DG;d=p<0.01 vs Dm Vs Met; e=p<0.01 vs Metformin.



**Figure 4.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on high density lipoprotein (HDL) concentration in n=5; \*\*=p<0.01 vs Control; b=p<0.01 vs Dm; c=p<0.01 vs Dm+DG.



**Figure 5.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on blood glucose concentration in streptozotocin-induced diabetic rats. Values are expressed in Mean ± SEM n=5; \*\*=p<0.01 vs Control.

and experimental groups. A value of p<0.05 was considered significant. All analysis was performed using the graph pad version 5 statistical software program.

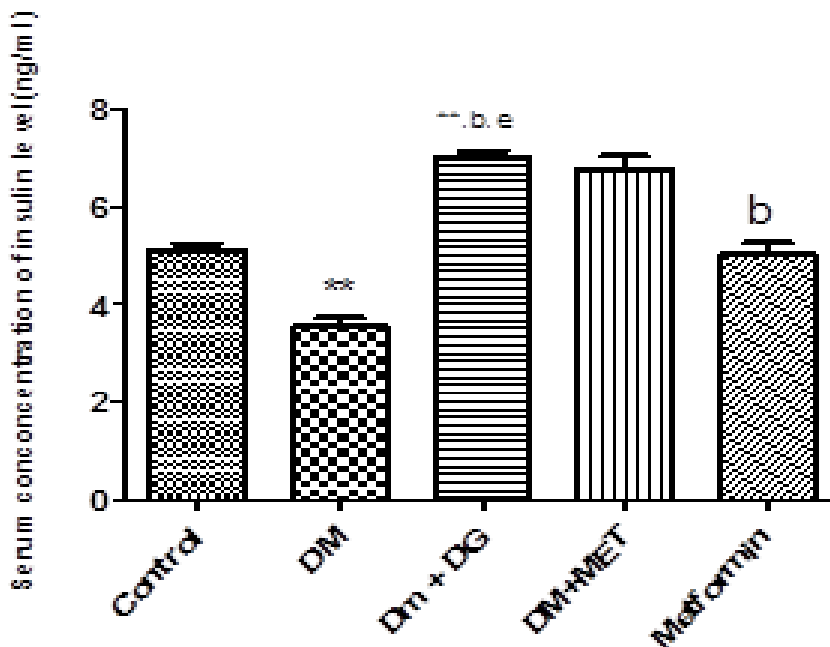
**RESULTS**

The results of this study are presented in Figures 1-9 and

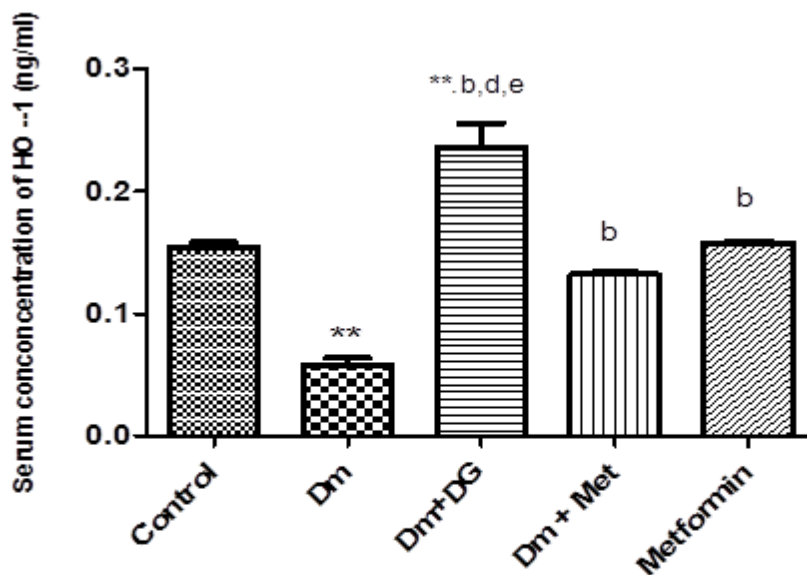
Plates 1 and 2.

**DISCUSSION**

World over, research on ways to ameliorate developing cases of diabetes mellitus has been intensified. This diseases which is life threatening is said to be associated



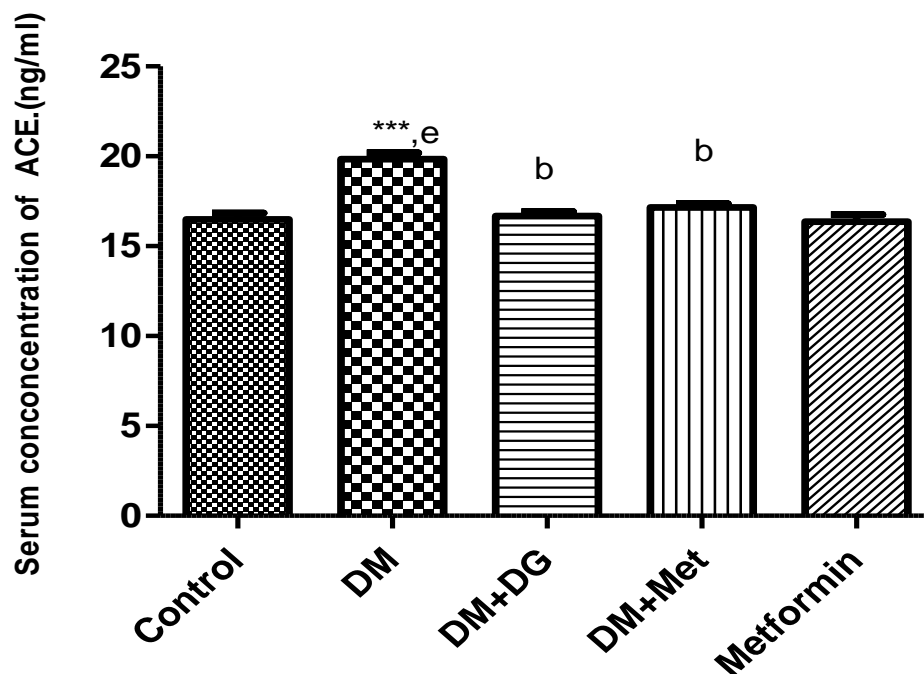
**Figure 6.** Showing effect of daily oral administration of aqueous *Dialium guineense* fruit pulp on insulin concentration in streptozotocin-induced diabetic rats. Values are expressed in Mean ± SEM n=5; \*\*=p<0.01 vs Control; b=p< 0.01 vs Dm, e=p<0.01 vs Met.



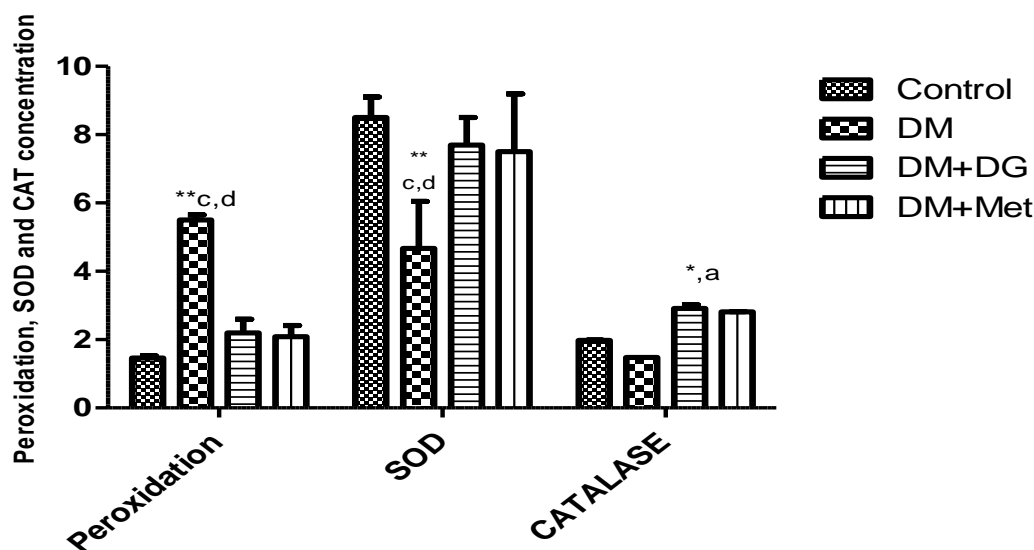
**Figure 7.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on insulin concentration in streptozotocin-induced diabetic rats. Values are expressed in Mean ± SEM n=5; \*\*=p<0.01 vs Control; b=p< 0.01 vs Dm, d=p<0.01 vs Dm+ Met e=p<0.01 vs Met.

with increased oxidative stress, hypercholesterolemia and inflammatory activity with a resultant increase in high incidence of liver and kidney damage. The aim of this

study therefore, was to investigate the effect of aqueous fruit pulp of *Dialium guineense* on hemoxygenase-1, insulin and angiotensin converting enzyme in



**Figure 8.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on insulin concentration in streptozotocin-induced diabetic rats. Values are expressed in Mean ± SEM n=5; \*\*=p<0.01 vs Control; b=p< 0.01 vs Dm, e=p<0.01 vs Met.

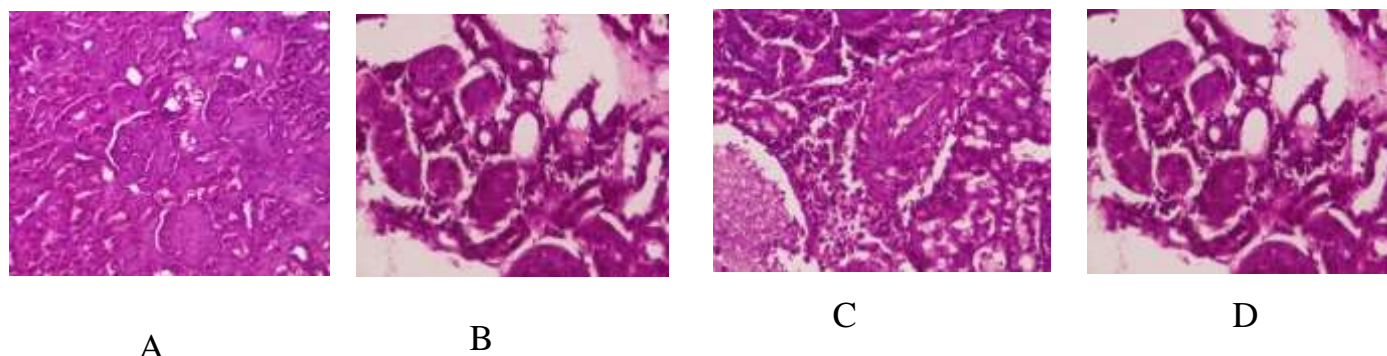


**Figure 9.** Showing effect of daily oral administration of aqueous *D. guineense* fruit pulp on lipid peroxidation, superoxide dismutase and catalase activity in the Kidney in streptozotocin-induced diabetic rats. Values are expressed in Mean ± SEM n=5; \*\*=p<0.01 vs Control; c=p< 0.01 vs DM+DG, d=p<0.01 vs DM+ Met.

streptozotocin-induced diabetic rats, all of which are pointers to the etiology of cardiovascular and coronary heart disease. Streptozotocin used in this study has been

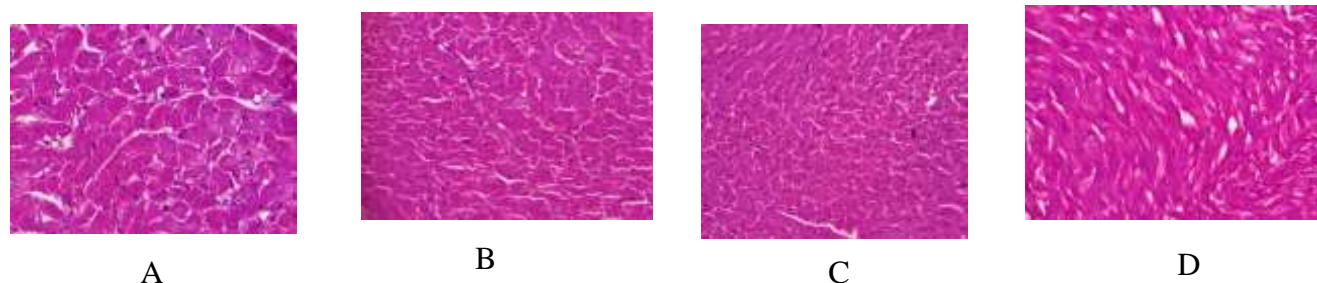
reported to be specific in cytotoxicity, lipotoxicity, generation of hydroxyl free radicals, hyperglycemia and inflammation that may cause lipid peroxidation in

## KIDNEY HISTOLOGY



**Plate 1.** Photomicrographs of renal tissues in (A) **control** showing normal glomeruli, bowman capsules and tubules. No significant lesion seen. (B) **Diabetic untreated** showing moderate perivascular inflammation and peritubular inflammation (C) **Diabetic treated with Dialium guineense** showing normal glomeruli, bowman capsule and tubules. No significant lesion seen. (D) **Diabetic treated with Metformin:** showing moderate perivascular inflammation and mild peritubular inflammation. H&E X400.

## HEART



**Plate 2.** Photomicrographs of cardiac tissue showing no inflammation of myocardium and infiltration of adipocytes in all groups. H & E X400.

pancreatic  $\beta$ -cells resulting in decreased endogenous insulin release (Donath et al., 2009).

In our study, results obtained showed an appreciable decrease in the level of insulin and hemoxygenase-I (HO-1) but with a concomitant increase in blood glucose, TC, LDL-c and angiotensin converting enzyme (ACE) levels in the diabetic untreated group. Similar results have been reported by Erman et al. (1998) and Ustundag et al. (1998). Usually, with hyperglycemia, the pancreatic  $\beta$ -cells become easily destroyed by redox imbalance between free radical production and scavenging processes causing lipid peroxidation,  $\beta$ -cells dysfunction (Lenzen 2008b; Donath, 1999; Djordjevic et al., 2004) and decreased insulin secretion (Robertson et al., 2003).

Treatment of the diabetic group with aqueous fruit pulp of *D. guineense* and a standard antidiabetic drug, metformin, respectively, resulted in a significantly ( $p < 0.01$ ) decreased blood glucose level and ACE but interestingly, increased serum hemoxygenase-1 (HO-1) and insulin concentration. HO-1 and its derivative like carbon monoxide, ferritin and biliverdin have been

reported to be key antioxidant enzymes that prevent the development of diabetes by abating oxidative stress via suppression of macrophages and acting as an important component in antiatherogenic activity (Orozco et al., 2007); they play a vital role in evoking pancreatic beta-cell insulin release and improve glucose metabolism thus reducing hyperglycemia (Ndisang et al., 2010; Mosen et al., 2005; Ndisang et al., 2014).

The mechanism by which *D. guineense* exerts hypoglycemia and induces HO-1 release may not have been unconnected with the reported presence of such components as flavonoid, vitamin C and tanins (Lever et al., 1979; Arogba et al., 2006). There are numerous natural HMOX1 inducers originating from plants, including polyphenols that exert positive effect on diabetic subjects (Bonifaz et al., 2009) Flavonoids is reported to contain quercetin and rutin both of which prevent oxidative stress by scavenging free radicals (Larocca et al., 1995., Cox et al., 2000). Previous studies have shown that quercetin and rutin particularly lower blood glucose level in rats (Vessal et al., 2003), preserve pancreatic

beta cell integrity (Coskun et al., 2005), increase insulin secretion and prevent liver injury (Kobori et al., 2009).

Tanins and phenolic compounds on the other hand, available in several plant derivatives including *D. guineense* are reported to be anti-ACE and act as specific inhibitors of the enzyme (Liu et al., 2006). ACE activity in both experimental animals and humans is usually characteristically high and is required for the conversion of inactive angiotensin-I(AT-I) to the potent and pro-oxidative angiotensin II(AT2). ACE is associated with increased superoxide production and impaired endothelium function that may lead to cardiovascular problems. Our study has shown that hyperglycemia predisposes to hypercholesterolemia and elevated triglyceride and LDL-cholesterol as earlier demonstrated by Arora et al. (2010). These factors are associated with enhanced AT receptor expression (Andraws and Brown, 2007; Abuissa et al., 2005) and are often remote cause of cardiovascular disease and atherosclerosis. The study of these risk factors is nevertheless imperative because they are intervening with the management of diabetes mellitus.

The registered decrease in ACE level due to oral administration of *Dialium* translates to a decrease in serum AT<sub>2</sub> level since ACE is needed to convert inactive AT<sub>1</sub> to the potent and active AT<sub>2</sub> whose effect is to cause systemic vasoconstriction and raise blood pressure. This decrease in ACE concentration is suggestive of a possible ACE inhibition by the fruit pulp. Studies have shown that randomized trials using angiotensin converting enzyme inhibitors (ACEI) and AT<sub>2</sub> receptor blockers significantly decrease the risk of DM (Andraws and Brown, 2007; Abuissa et al., 2005), improve insulin sensitivity and glucose metabolism, and reduce plasma glucose in both experimental conditions and in humans with DM (Scheen, 2004).

Our results therefore strongly indicate that *Dialium guineense* fruit pulp contains agents that promote ACE inhibition. Indeed, some reports have shown that flavonoid, one of the components present in the fruit pulp of *Dialium guineense* presents an anti-atherogenic effect due to its inhibition of ACE *in vitro* (Loizzo et al., 2007). The reparative effect of the fruit pulp and maintenance of tissue integrity was further observed in the improved heart and kidney morphology. The antioxidant effect of *Dialium guineense* was further demonstrated by its ability to significantly ( $p < 0.01$ ) reduce lipid peroxidation in the liver and kidney by promoting superoxide dismutase and catalase enzyme activity. Many studies have shown that oxidative stress becomes apparent in diabetic subjects (Ceriello, 2000; Waggiallah and Alzohairy, 2011).

Consistent with this view, our data provide further evidence that there is presence of oxidative stress with an alteration in antioxidant enzyme activities and increased lipid peroxidation (MDA levels) in diabetic condition. The reduction in serum SOD activity is thought to be as a result of excessive autoxidation and

progressive glycation of enzymatic proteins. The reversal effect of *Dialium* fruit pulp on this unpleasant activity makes it convincing that it holds to an extent promising therapeutic properties.

## Conclusion

The results of this study clearly demonstrated that *Dialium guineense* fruit pulp has a good antioxidant potential and decreases tissue lipid peroxidation, blood glucose level, induces hemeoxygenase and insulin release and acts as ACE inhibitor.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGMENT

The authors of this research study wish to appreciate the technical assistance of Dr. Mrs Iya Eze of the Department of Medical Laboratory Science, University of Calabar and Dr. Sikirulai Olatunde Jeje Department of Physiology, Federal University of Technology, P.M.B 704, Akure, Nigeria.

## REFERENCES

- Abuissa H, Jones PG, Marso SP, O'Keefe JH (2005). Angiotensin converting enzyme inhibitors or angiotensin receptor blockers for prevention of type 2 diabetes: Meta-analysis of randomized clinical trials. *Journal of the American College of Cardiology* 46(5):821-826.
- Andraws R, Brown DL (2007). Effect of inhibition of the renin angiotensin system on development of type 2 diabetes mellitus (meta analysis of randomized trials). *The American Journal of Cardiology* 99(7):1006-1012.
- Arogba SS, Ajoboro AA, Odukwe IJ (2006). A physico-chemical study of Nigeria velvet tamarind (*dialium guineense*) fruit. *Journal of the Science of Food and Agriculture* 66:533-534.
- Arora R, Vig AP, Arora S (2013). Lipid peroxidation: A possible marker for diabetes. *Journal of Diabetes and Metabolism* 11:1-6.
- Bonifaz V, Shan Y, Lambrecht RW, Donohue SE, Moschenross D, Bonkovsky HL (2009). Effects of silymarin on hepatitis C virus and haem oxygenase-1 gene expression in human hepatoma cells. *Liver International* 29(3):366-373.
- Ceriello A (1997). Acute hyperglycaemia and oxidative stress generation. *Diabetic Medicine* 14(S3):S45-S49.
- Coskun O, Kanter M, Korkmaz A, Oter S (2005). Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and  $\beta$ -cell damage in rat pancreas. *Pharmacology Research* 51(2):117-23.
- Cox D, Whichelow MJ, Prevost TA (2000). Antioxidant effects of flavonoids. *Public Health Nutrition* 3:19-29.
- David AA, Olaniyi AT, Mayowa AO, Olayinka AA, Anthony OI (2011). Anti-vibro and preliminary phytochemical characteristics of crude methanolic extracts of the leaves of *Dialium guineense* (Willd). *Journal of Medicinal Plants Research* 5(11):2398-2404.
- Djordjevic A, Spasic S, Jovanovic-Galovic A (2004). Oxidative stress in diabetic pregnancy: SOD, CAT and GSH-Px activity and lipid peroxidation products. *The Journal of Maternal-Fetal and Neonatal Medicine* 16(6):367-372.
- Donath MY, Boni-Schnetzler M, Ellingsgaard H, Ehses JA (2009). Islet

- inflammation impairs the pancreatic B-cell in type 2 diabetes. *Physiology* 24(6):325-331.
- Donath MY, Gross DJ, Cerasi E, Kaiser N (1999). Hyperglycemia-induced b-cell apoptosis in pancreatic islets of *Psammomys obesus* during development of diabetes. *Diabetes* 48(4):738-744.
- Erman A, Van Dyk DJ, Chen-gal B, Iskera D, Giler S, Rosenfeld JB, Boner G (1993). Angiotensin converting enzyme activity in the serum, lung and kidney of diabetic rats. *European Journal of Clinical Investigation* 23(10):615-620.
- Evans JL, Goldfine ID, Maddux BA, Grodsky GM (2003). Are oxidative Stress-activated signaling pathways mediators of insulin resistance and  $\beta$ -cell dysfunction. *Diabetes* 52(1):1-8.
- Ezeja MI, Omeh YS, Ezeigbo II, Ekechukwu AJ (2011). Evaluation of the analgesic activity of the methanolic stem bark extract of *Dialium guineense* (Wild). *Annals of Medical and Health Sciences Research* 1(1):55-62.
- Gideon I.O, Raphael A (2012). Phytochemical analysis and in vivo anti-diarrhoeal potentials of *Dialium guineense*(wild)stem bark extract. *Journal of Intercultural Ethnopharmacology* 1(2):105-110.
- Herzog F, Farah Z, Amado R (1994). Composition and consumption of gathered fruits in the V-Babule, Cote D'Ivoire. *Ecology of Food and Nutrition* 32(3-4):181-196.
- Jakus V (2000). The role of free radicals, oxidative stress and -\*+antioxidant systems in diabetic vascular disease. *Bratislavské lekárske listy* 101(10):541-551.
- Jean CD, Maryse T, Marie JF (1983). Plasma malondialdehyde levels during myocardial infarction. *Clinica Chimica Acta* 129(3):319-322.
- Kang DG, Kim YC, Sohn EJ, Lee YM, Lee AS, Yin MH, Lee HS (2003). Hypotensive effect of butein via inhibition of angiotensin converting enzyme. *Biological and Pharmaceutical Bulletin* 26(9):1345-1347.
- Kobori M, Masumoto S, Akimoto Y, and Takahashi Y (2009). Dietary quercetin alleviates diabetic symptoms and reduces streptozotocin-induced disturbance of hepatic gene expression in mice. *Molecular Nutrition and Food Research* 53(7):859-868.
- Larocca LM, Teofili L, Sica S, Pierelli L, Menichella G (1995). Quercetin inhibits the growth of leukemic progenitors and induces the expression of transforming growth factor-B1 in these cells. *Blood* 85(12):3654-3661
- Lawal IO, Nzokwe NE, Igboanugo ABI, Adio AF, Awosan EA, Nwogwugwu JO, Faleye B, Olatunji BP, Adesoga AA (2010). Ethnomedicinal information on collation and identification of some medicinal plants in research institutes of South-West Nigeria. *African Journal of Pharmacy and Pharmacology* 4(1):001-007.
- Lenzen S (2008b). Oxidative stress: vulnerable beta cells. *Biochemical society Transactions* 36(3):343-347
- Lever M, Vandon-berghe DA, Merilend F, Vlietinck A, Lammense E (1979). Screening of higher plants biological activity/antimicrobial activity. *Plants Medica* 36:311-321.
- Liu JC, Hsu FL, Tsai JC, Chan P, Liu JY, Thomas GN, Tomlinson B, Lo MY, Lin JY (2003). Anti hypertensive effect of tannin isolated from traditional Chinese herbs as non-specific inhibitors of angiotensin converting enzyme. *Life Sciences* 73(12):1543-1555.
- Liu J, Pang Y, Chang T, Bounelis P, Chatham JC, Marchase RB (2006). Increased hexosamine biosynthesis and protein O-GlcNAc levels associated with myocardial protection against calcium paradox and ischemia. *Journal of Molecular and Cellular Cardiology* 40(2):303-312.
- Loizzo MR, Said A, Tundis R, Rashed K, Statti GA, Hufner A, and Menichini F (2007). Inhibition of angiotensin converting enzyme (ACE) by flavonoids isolated from *Ailanthus excelsa* (Roxb) (Simaroubaceae). *Phytotherapy research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 21(1):32-36.
- Mishra HP, Fridovich I (1972). The role of superoxide anion in the auto-oxidation of epinephrine and simple assay for superoxide dismutase. *Journal of Biological chemistry* 247(10):3170-3175.
- Mosen H, salehi A, Ail P (2005). Defective glucose-stimulated insulin release in diabetic Goto-Kakizaka (rat coincides with reduced activity of the islets carbon monoxide signaling pathway. *Endocrinology* 146(3):1553-1558.
- Nahar N, Rahman S, Mosiuhuzzaman M (1990). Analysis of carbohydrates in seven edible fruit of Bangladesh. *Journal of Science of Food and Agriculture* 51:185-192.
- Ndisang JF, Jadhav A (2010). The heme oxygenase system attenuates pancreatic lesions and improves insulin sensitivity and glucose metabolism in deoxycorticosterone acetate hypertension. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 298(1):R211-R223.
- Ndisang JF, Tiwari S (2014). The heme oxygenase system and type-1 diabetes. *Current Pharmaceutical Design* 20(9):1328-1337.
- Orozco JD, Kapturczak MU, Banajas B, Wang X, Weinstein MM, Wong J, Dehane J, Bolissety S, Shaposhnik Z, Shih DM, Agarwal A, Lulis AJ, Araujo JA (2007). Hemeoxygenase -1 expression in macrophages plays a beneficial role in atherosclerosis. *Circulation Research* 100(12).
- Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S (2009). Agro forest tree database: atree reference and selection guide version 4.0. World Agroforestry Centre ICRAF, Nairobi, KE (<http://www.worldagroforestry.org/sites/treedatabases.asp>).
- Rang HP, Dale MM, Ritters JM (1991). The endocrine pancreas and the control of blood glucose. *Pharmacology* 4 p.
- Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H (2003). Glucose toxicity in beta cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes* 52(3):581-587.
- Scheen AJ (2004). Orevention of type 2 diabetes mellitus through inhibition of the renin-angiotensin system. *Drugs* 64(22):2537-2565.
- Sinha KA (1972). Colorimetric assay of catalase. *Analytical biochemistry* 47(2):389-394.
- Strate T O, Mann H, Kleighans S, Rusani C, Schneider E, Yekebas MS, Freitag T, Bloechle C, Izbicki JR (2005). Micro circulatory function and tissue damage is improved after the therapeutic injection of bovine hemoglobin in server acute rodent pancreatitis. *Pancreas* 30(3):254-259.
- Ullah MF, Bhat SH, Abu-Duhier F (2015). Antidiabetic potential of hydro-alcoholic extract of moringapergrina leaves; implication as functional food for prophylactic intervention in prediabetic stage. *Journal of Food Biochemistry* 39(4):360-367.
- Üstundag B, Çay M, Özercan İH (1998). Angiotensin-converting enzyme activity in the serum, lung, liver and kidney in streptozotocin-induced diabetic rats and diabetic nephropathy. *Turkish Journal of Medical Sciences* 28(3):231-238.
- Vessal M, Hemmati M, Vasei M (2003). Antidiabetic effects of quercetin in streptozocin induced diabetic rats. Antidiabetic effects of quercetin in streptozocin induced diabetic rats. *Comparative Biochemistry and Physiology Part C*.
- Vinayagamoorthi R, Bobby Z, Sridhar MG (2008). Antioxidants preserve redox balance and inhibit c-Jun-N-terminal kinase pathway while improving insulin signaling in fat-fed rats: evidence for the role of oxidative stress on IRS-1 serine phosphorylation and insulin resistance. *Journal of endocrinology* 197(2):287-296.
- Waggiallah H, Alzohairy M (2011). The effect of oxidative stress on human red cells glutathione peroxidase, glutathione reductase level, and prevalence of anemia among diabetics. *North American Journal of Medical Sciences* 3(7):344.

*Full Length Research Paper*

# **Evaluation of the phytochemical composition, antimicrobial and anti-radical activities of *Mitracarpus scaber* (Rubiaceae)**

**Ouada B., Anani K., Djeri B.\*, Ameyapoh Y.O. and Karou D. S.**

Laboratoire de Microbiologie et de Contrôle de Qualité des Denrées Alimentaires (LAMICODA),  
Université de Lomé, Togo.

Received 20 June, 2018; Accepted 13 July, 2018

***Mitracarpus scaber* is a medicinal plant used in traditional practices for the treatment of dermatoses and liver diseases. The objective of this study was to quantify the content of phenolic compounds and to evaluate the anti-radical and antimicrobial activities of four types of its total extracts on ten microbial strains. The Folin-Ciocalteu method was used to determine total phenol content, condensed tannin content by the Butanol-HCl method and anti-radical activity by reduction of phosphomolybdate. The micro-dilution technique coupled with spreading in an agar medium made it possible to evaluate the antimicrobial activity. The results obtained showed that the total phenol content varies according to the nature of the extracts and ranges from  $36.75 \pm 1.62$  mg / g to  $14.63 \pm 0.44$  mg / g of extract. The contents of condensed tannins ranged from  $41.83\% \pm 0.03$  mg CE / g to  $0.39\% \pm 0.14$  mg CE/g. The anti-free radical activity was between  $0.48 \pm 0.06$  mg AAE / g and  $0.21 \pm 0.00$  mg AAE/g. The antimicrobial activity gave MIC of 6.25 to 50 mg / ml. The hydroalcoholic extract showed lower MIC and would therefore be best suited for the treatment of microbial diseases.**

**Key words:** *Mitracarpus scaber*, antimicrobial activity, antiradical activity, phenols.

## **INTRODUCTION**

Plants in their diversity are a gift of nature to man. They contain a lot of important molecules that justifies their therapeutic use in traditional medicine. The number of plant species was estimated to be about 400,000 to 500,000 (Karou et al., 2006). The use of plants in therapeutic care was known to all peoples. Even today, they continue to prove themselves especially in countries where low-income indigenous populations do not have access to modern medical care. Thus, in African traditional medicine, *Mitracarpus scaber*, an annual tropical plant of Rubiaceae family about 10 to 50 cm

high (Nathalie, 2002) with rough leaves (Olorode et al., 1984) was used. At maturity, this plant makes white flowers at the level of each armpit of the leaves. *M. scaber* grows on degraded soils in Africa and Asia (Moussa et al., 2015). It was also found in Latin America (Yaméogo, 1982). In Togo, *M. scaber* can be harvested from June to November. In Togolese traditional medicine, the plant was used to treat infected wounds, skin (Magbefon et al., 2009). It was also used orally in combination with sesame to treat liver problems. Similarly, in several other countries in Africa, it was known and

\*Corresponding author. E-mail: [djerifr2002@gmail.com](mailto:djerifr2002@gmail.com).



used to treat dermatoses, headaches, toothache, amenorrhea, dyspepsia, venereal diseases (Kerharo and Adam, 1974). As a result, these traditional practices have prompted researchers to conduct scientific study to verify many therapeutic effects that *M. scaber* is said to have. For this reason, several extracts of the plant have been studied in order to test its antimicrobial, antifungal activities and even its hepato-protective effect. At the same time, other researchers have been interested in the qualitative chemical composition of plant extracts. This study was undertaken to contribute to the evaluation of its phytochemical constituents and also, to explore the anti-radical and antimicrobial potentials of its various extracts.

## MATERIALS AND METHODS

### Plant material

The whole plant of *M. scaber* was harvested.

### Solvents

The solvents used include the following: Chloroform, petroleum ether and methanol.

### Culture media and reagents

To carry out extractions and phytochemical tests, the following reagents were used: 95° ethanol, methanol, Merck's Folin-Ciocalteu reagent, butanol, hydrochloric acid, gallic acid, PROLABO ascorbic acid, Sodium carbonate, ammoniacal iron sulfate, sulfuric acid, sodium phosphate and ammonium molybdate from PROLABO. Muller Hinton agar from MAST House and Liofichem nutritious as well as Muller Hinton Broth from MAST House were used for antimicrobial testing.

### Microbial strains

Antimicrobial tests were performed with reference strains: *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Salmonella* OMB; wild strains of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* ATCC 27853, *Citrobacter diversus* and on *Candida albicans* provided by Laboratory of Microbiology and Quality Control of Foodstuffs (LA-MI-CO-DA).

### Sample collection

Fresh whole plant of *M. scaber* was collected between June and November 2015 in Kozah at 400 km north of Lomé (Togo). The plants was identified and confirmed at the Herbarium of Department of Plant Biology, Faculty of Sciences, University of Lomé (FDS-UL).

### Preparation of extracts

The plant materials (fresh leaves) was dried in the laboratory (LASEE-UK) at room temperature (25°C) and pulverized into a fine powder for extraction by a Moulinex brand of Binatone. Several

extracts were prepared from the powder obtained. The procedure was carried out using water, ethanol-water (70:30 v/v) methanol and organic solvent such as chloroform.

### Hydroalcoholic extraction

100 g of the powdered plant materials was extracted using percolation process in 500 ml distilled water- ethanol (30:70).

### Aqueous extraction

For aqueous extraction, 100 g of powder was mixed in 500 ml of distilled water.

### Chloroform extraction

Chloroform extract was prepared adding 100 g of powder to 500 ml of chloroform.

### Methanol extraction

For methanol extract, 100 g of powder was diluted with petroleum ether before adding to 500 ml of methanol using percolation process overnight. Each mixture was subjected to continuous stirring by an orbital stirrer for 48 h. At the end of the stirring, the mixture was decanted and filtered on Wattman paper. The filtrate was evaporated to dryness to obtain residue in vacuole using a Heidolph Laborata 4000 rotavapor at 60°C.

### Evaluation of total polyphenol content

The total polyphenols content was determined according to the Folin-Ciocalteu (FCR) method described by Karou et al. (2006). For this test, the standard curve was prepared using gallic acid; linear-dose-response regressing curve was generated at absorbance of 760 nm with a UNICO model 12 spectrophotometer against a negative control consisting of a mixture of 0.5 ml of FCR, 0.5 ml of sodium carbonate, distilled water and a positive control consisting of extract and distilled water.

### Estimation of proanthocyanidol content

The proanthocyanidol content was evaluated by the method of Butanol-HCl, developed by Porter et al. (1986). The test consisted of mixing 0.2 ml of each extract with 0.2 ml of ammoniacal iron sulfate (20 g/L) and 7 ml of a solution of butanol / hydrochloric acid (95/5 ml) in the tubes. After 40 min incubation in a water bath at 95°C, the tubes were cooled to room temperature and their absorbance read at 540 nm. The concentration of proanthocyanidin extracts was obtained by the following relationship developed by Aboh et al. (2014):

$$X = (\text{Absorbance} \times 1 \text{ CE/g}) / 0.280$$

Absorbance = optical density of extract measured at 540 nm;  
CE = equivalent catechin).

Evaluation of the anti-radical activity of the extracts by the phosphomolybdate reduction method. The reduction of the phosphomolybdate was carried out according to the method described by Prieto et al. (1999) and Karou et al. (2006). 1 ml of each extract was added to 9 ml of reagent (phosphomolybdate)

**Table 1.** Content of total phenols, proanthocyanidols and antiradical compounds.

Components extracts	Total polyphenols (mg AGE/g)	Proanthocyanidols (mg CE/g)	Antiradical component (mg AAE/g)
EM1	31.58±2.42	1.83%±0.03	0.48 ±0.06
EM2	25.74±0.09	1.59%±0.03	0.44 ±0.04
EC	14.63±0.44	0.39%±0.14	0.21±0.00
EH	19.13±1.32	0.61%±0.01	0.29±0.01
EA	36.75±1.62	1.01%±0.15	0.28±0.00

EM1 non-delipidated methanol extract; EM2 delipidated methanol extract; EC chloroform extract; EH hydroalcoholic extract; EA aqueous extract; Mg AGE/g milligram gallic acid equivalent per gram of extract; Mg CE/g milligram catechin equivalent per gram of extract; Mg AAE/g milligram equivalent ascorbic acid per gram of extract.

and the whole was heated at 95°C for 90 min in a water bath after which the mixture obtained was cooled to room temperature. Ascorbic acid was used as a standard antioxidant under the same experimental conditions. The results were expressed in milligrams of equivalent of ascorbic acid per gram of crude extract.

#### Antibiogram of the germs studied

A bacterial suspension was prepared in sterile distilled water from pure culture of 24 h from nutrient agar. This suspension was compared to the standard of the Mc Farland 0.5 solution which corresponds to 108 CFU/ml. The suspensions thus obtained were seeded by swabbing on Mueller Hinton agar. The thickness of the (Comité de l'Antibiogramme de la Société Française de Microbiologie, 2015). The antibiotic discs were placed on Petri dishes. After 10 to 15 min, the plates were incubated at 37°C for 24 h. The diameters of the zones of inhibition were measured and compared with sensitive one.

#### Microbicidal activity of extracts

Minimal Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined using the microdilution technique in 96-well plates with Muller Hinton broth (MHB) (Clinical and Laboratory Standards Institute, 2012). To this end, stock solutions of the total extracts sterilized by millipore membrane filtration of 0.45 µm in diameter were prepared at 100 mg/ml in an ethanol / water mixture (10/90). One hundred microliters (100 µl) of MHB were deposited in the wells of the microplate and successive dilutions of 100 to 12.5 mg/ml of extract were made with BMH. Microbial turbidity suspensions corresponding to Mac Farland 0.5 were made with microorganisms from a 24-h culture at 37°C after a control Gram. Then, 100 µl of this microbial suspension was brought into contact with the extracts and their dilutions in the wells. Positive control wells (microbial suspension without extract) and negative controls (MHB + extract) were also formed. Gentamicin was used as a reference antibiotic. Finally, the plates were stirred and incubated at 37°C for 24 h. The MBC was determined by taking 100 µl of suspension in the wells without visible growth and by seeding on the nutrient agar. Incubation was carried out at 37°C for 24 to 48 h at the end of which the colonies were counted.

#### Bactericidal kinetics

For this test, the ten strains studied were tested by a single

concentration of MIC of the hydroalcoholic extract. Thus, 100 µl of each microbial suspension (108 CFU/ml) of MHB was brought into contact with 100 µl of extract (100 mg/ml) at initial time (t = 0). Samples of 100 µl were plated on nutrient agar at t = 0 and after incubation times of 15, 30, 45 min and 24 to 48 h for certain germs. The dishes were incubated at 37°C and the colonies were counted in 24 h. Control microbial suspensions without extract were made.

#### Statistical analysis

The statistical analyses were carried out using Epi-info version 6.04 dfr. The parametric analyses were performed by the ANOVA (Variance Analysis) test. The difference between the averages is considered statistically significant at the 5% threshold (P <0.05).

## RESULTS AND DISCUSSION

### Content of total polyphenols, proanthocyanidols and antiradical activity of extracts of *M. scaber*

Quantitative chemical analyses were carried out on the total extracts of the powder of the plant studied. The results indicated that the aqueous extract was richer in total phenols (36.75 ± 1.62 mg / g of extract). The chloroform extract had the lowest phenol value (14.63 ± 0.44 mg / g extract); while the methanolic extracts were rich in condensed proanthocyanidols or tannins (1.86% CE/g extract for the non-dilapidated methanolic extract followed by the dilapidated methanol extract, 1.59% CE/g). The results are recorded in Table 1.

Studies were carried out on *M. scaber*. There are limited quantitative studies on the phenolic compounds of the total extracts of the plant in Nigeria. Aboh et al. (2014) studied the phenolic qualitative composition of *M. scaber* using diethyl ether and tannins with ethyl acetate. It appears from their study that the said plant had a content of 9% of phenols and 1.4% of tannic compounds. Methodological, soil and climatic differences could explain the differences between the results obtained.

Koudoro (2015), on several plant extracts, also showed that the aqueous extracts had the highest total phenol contents than the other solvents. The differential solubility

**Table 2.** Sensitivity of microbial strains studied to antibiotics.

Germ	Antibiotics					
	Lincomycine	Penicilline G	Norfloxacin	Nétilmicine	Tobramycine	Ceftriaxone
<i>E. coli</i>	R	R	R	S	S	S
<i>E. coli</i> ATCC 25922	R	R	S	S	S	S
<i>S. aureus</i>	S	I	I	S	S	S
<i>S. aureus</i> ATCC 29213	R	I	I	S	S	S
<i>S. aureus</i> ATCC 25922	S	I	I	S	S	S
<i>P. aeruginosa</i> ATCC 27853	R	R	I	S	S	S
<i>Salmonella</i> OMB	R	R	I	S	S	S
<i>K. pneumoniae</i>	R	R	S	S	S	S
<i>C. diversus</i>	R	I	S	S	S	S
<i>C. albicans</i>	S	I	I	S	S	S

S: Sensitive; R: Resistant; I: Intermediate.

of total phenols in solvents was explained by the PH and the polarity of these molecules and solvents. Other studies have shown that phenolic compounds possess antioxidant activities (Karou et al., 2006; Koudoro, 2015).

The present study evaluated the anti-radical activity of *M. scaber* and the reduction of molybdate VI to molybdate V in hot and acid medium. The results of this quantitative test show that there was a good correlation between the contents of the proanthocyanidol and the measured values of the antiradical activity ( $r^2 = 0.92$ ). The high value was obtained with methanol extract (0.48 mg AA/g extract) and the low value was obtained with the chloroform extract (0.21 mg/g extract). In this context, the antiradical activity of methanol extract was evaluated with the DPPH method. At the end, 50% effective concentration (EC<sub>50</sub> of  $41.64 \pm 1.5 \mu\text{g/ml}$ ) was obtained (Germano et al., 2000). Anti-free radicals were substances that could neutralize or reduce the damage caused by free radicals in the body. Thus, the use of *M. scaber* in cosmetic products and in liver therapy could therefore be justified by the above results. The results of the statistical analyses (total phenols = 0.000501, P proanthocyanidols = 0.001311, antiradical activity = 0.020491) showed that the content of total phenol extracts, proanthocyanidins and antiradical compounds depends on the solvents nature ( $P < 0.05$ ).

### Test of sensitivity of strains studied to conventional antibiotics

The results of this test are shown in Table 2. All of the Gram-negative microbial strains tested were resistant to Lincomycin including the reference strain *S. aureus* ATCC 29213. Similarly, all enterobacteria resisted the action of Penicillin G. *E. coli* strain was sensitive to three antibiotics tested (Tobramycin, Netilmicin and Ceftriaxone). While the *E. coli* ATCC strain 25922 in addition to these three was sensitive to Norfloxacin. Any antibiotics inhibited in vitro all of organisms tested growth.

The resistance of the tested microbial strains to antibiotics would be linked to several factors. Indeed, to be active, an antibiotic must first enter the bacterial cytoplasm, without being modified.

Other antibiotics act either by inhibiting nucleic acid synthesis of the bacteria or by disrupting the cytoplasmic membrane or by disrupting bacterial proteins or by acting on membrane permeability or by acting on the intermediate metabolism (Marjorie, 2007). Despite these various mechanisms of action, microorganisms were also endowed with mechanisms of resistance that were natural or acquired. For example, Gram-negative bacilli (*E. coli*, *Salmonella* sp., *P. aeruginosa* etc.) were naturally resistant to hydrophobic antibiotics. The bacterium can modify the point of attachment of the antibiotic and thus become resistant. Some antibiotics pass through the outer membrane of the wall of these bacteria with difficulty because of the presence of lipids. Lincomycin belongs to lincosamides and Penicillin of first-generation  $\beta$  lactams. These two molecules were comparable in spectrum of action. They were ineffective on Enterobacteriaceae and on genus of *Pseudomonas* (Anne, 2014).

*E. coli* strain was resistant to Norfloxacin which was a fluoroquinolone and targeted at bacterial DNA. This result could be explained by a resistance acquired by *E. coli* strain by transfer of plasmid or chromosomal mutation. Ceftriaxone belongs to the third-generation of cephalosporins. The susceptibility of the strains was due to the lack of production of cephalosporinases of the strains or to an ineffective production of this enzyme in the face of the antibiotic. Similarly, the microbial strains studied were sensitive to Tobramycin and Netilmicin, which were water-soluble and positively charged aminosides (Anne, 2014).

### Evaluation of antimicrobial activity of total extracts of *Mi. scaber*

The antimicrobial tests carried out with the five extracts

Table 3. Antibacterial activity of total extrats of *M. scaber*.

Extracts/ Concentrations (mg/ml)	Microbial strains									
	<i>Candida albicans</i>	<i>S. a</i>	<i>S. a</i>	<i>S. a</i>	<i>E. c</i>	<i>E. c</i>	<i>P. a</i>	<i>S. OMB</i>	<i>K. p</i>	<i>C. d</i>
			ATCC	ATCC		ATCC	ATCC			
			25923	29213		25922	27853			
EH, MIC	12.5	25	25	25	25			25	25	6.25
MBC	12.5	25	50	50	50	25		50	50	50
CMB/CMI	1	1	2	2	2	50		2	2	8
						2				
EM1, CMI	50	25	25	25	50			50	50	50
CMB	>50	50	50	50	>50	50		50	50	>50
CMB/CMI	-	2	2	2	-	>50		1	1	
EM2, CMI	>50	50	50	50	50			>50	>50	>50
CMB	>50	50	50	50	50	50	-	>50	>50	>50
CMB/CMI		1	1	1	1	50				
						1				
EC, CMI	50	50	>50	>50	>50			>50	>50	>50
CMB	50	50	>50	>50	>50	>50		>50	>50	>50
CMB/CMI	1	1				>50				
EA,CMI	50	50	50	50	>50			>50	>50	>50
CMB	>50	50	50	50	>50	>50		>50	>50	>50
CMB/CMI		1	1	1		>50		-	-	-
Gentamycine 20 mg/ml	S	S	S	S	S			S	S	S

*C. a*; *S. a*: *Staphylococcus aureus*; *E. c*: *Escherichia coli*; *P. a*: *Pseudomonas aeruginosa*; *K. p*: *Klebsiella pneumoniae*; *C. d*: *Citrobacter diversus*; EH: hydroalcoholic Extract; EM1: non delipided methanolic extract; EM2: delipided methanolic extract; EC: chloroformic extract; EA: aqueuse extract; S: Sensitive; - : non given antibiotic capacity.

made it possible to determine the Minimum Inhibitory Concentrations (MIC), the Minimum Bactericidal Concentrations (MBC) and the antibiotic potency (MBC/MIC) of each extract. The antibiotic potency was considered bactericidal if  $MBC / MIC < 1$ ; it was bacteriostatic if  $1 < MBC / MIC$ . The results are shown in Table 3. The analysis results showed that the hydroalcoholic extract was the most active on all of the microorganisms studied while the chloroform and

aqueous extracts were the least active. The hydroalcoholic extract exerts a bactericidal effect on *Candida albicans* with  $MIC = 12.5$  mg/ml and on *Citrobacter diversus* with  $MIC = 6.25$  mg / ml. The effect on the other microbial strains tested was bacteriostatic. The extract EM1 inhibited the growth of all the germs tested. There was a bactericidal effect at 50 mg / ml on salmonella OMB and *Klebsiella pneumonia* but bacteriostatic effect was observed on *S. aureus*. The EM2

extract inhibited *S. aureus* and *E. coli* growth at 50 mg/ml. Similarly, at 50 mg/ml EC and EA extract inhibited the growth of *S. aureus* and *C. albicans*. All strains tested were sensitive to gentamycin, the reference antibiotic (20 mg / ml) was used as control. The results obtained therefore indicate that the antimicrobial activity of *M. scaber* extracts could be attributed to phenolic and alkaloids compounds plant extract. However, the aqueous extract richest in phenolic compounds was the

**Table 4.** Viability of microorganisms as a function of time.

Number of colony/strains	Time					
	0 min	15 min	30 min	45 min	24 h	48 h
<i>E. coli</i>	108	460±40	9±1	1±1	-	-
<i>E. coli</i> ATCC 25922	108	0±0	0±0	0±0	-	-
<i>S. aureus</i>	108	108	0±0	0±0	-	-
<i>S. aureus</i> ATCC 25923	108	108	0±0	0±0	-	-
<i>S. aureus</i> ATCC 29213	108	108	108	27±2	-	-
<i>P. aeruginosa</i> ATCC 27853	108	108	3±1	1±1	-	-
<i>Salmonella</i> OMB	108	1973±428	9±6	2±1	-	-
<i>K. pneumoniae</i>	108	57±1	1±1	0±0	1112±171	1310±105
<i>C. diversus</i>	108	1600±401	1380±80	1287±173	-	-
<i>C. albicans</i>	108	108	24±6	2±1	-	-

least active on the microbial strains tested while the hydroalcoholic extract less concentrated in these compounds was the most active on all the microorganisms studied.

Bgaguidi et al. (2005) showed that the alkaloid Azaanthraquinone benzo (g) isoquinoline isolated from an alcohol extract of *M. scaber* was responsible for this antibacterial activity. MIC was 19 µg/ml on *S. aureus* ATCC 25923, 150 µg/ml on *E. coli* ATCC 25922 and >10 mg / ml on *P. aeruginosa* ATCC 27853. Alkaloids were organic substances with complex alkaline molecular structures with high pharmacological activity at low concentration (Bruneton, 1999). Among these pharmacological properties were the antimicrobial properties (Karou et al., 2006).

On the other hand, the antimicrobial activity of seven polyphenolic compounds isolated from methanol extract was examined by Bisignano et al. (2000). They showed that gallic acid and 3, 4, 5-trimethoxybenzoic acid isolated from the said plant inhibited the standard and clinical strains of *S. aureus* (MIC 3.90 and 0.97 µg/ml), and that 4-methoxyacetophenone and 3, 4, 5-trimethoxyacetophenone also inhibited the reference and clinical strains of *C. albicans* (MIC 1.95 and 0.97 µg / ml). Thus, the antimicrobial activity of *M. scaber* was due to the synergistic action of the phenols and alkaloids of this plant.

In addition, Bisignano et al. (2000) also tested total methanol extracts and found MICs of 31, 25 µg / ml on *S. aureus* ATCC 25923 and 62.50 µg / ml on *C. albicans*. Drying and storage of the extracts would influence the quality of the extracts. The fresh plant of *M. scaber* contains harounoside, a molecule with antimicrobial activity whereas it was absent in the dry plant (Harouna et al., 1995).

Similarly, Karou et al. (2015) showed that the conservation of extracts had a negative impact on their pharmacological quality. Indeed, the author has demonstrated that the phenols in contact with oxygen

undergo an auto-oxidation and give insoluble polymers of high molecular weights that penetrate the bacterial wall. To better understand the action of this plant, the hydroalcoholic extract was selected for its interesting activity to conduct a kinetic on the microbial strains studied. The results of this test are recorded in Table 4. The hydroalcoholic extract of *M. scaber* exerted a bactericidal effect at 100 mg/ml on all of germs tested. After 45 min of contact, growth of *E. coli* ATCC 25922, *S. aureus* 25922, *K. pneumoniae* was completely inhibited. This bactericidal action was observed from the 15th min for *E. coli* strain, 30th min for *C. albicans* and 45th min for *S. aureus* ATCC 29213. For *Citrobacter diversus* strain, there was an escape phenomenon. Indeed, the effect exerted by the extract on this strain would be of bacteriostatic type 15 min after contacting the germs.

## Conclusion

*M. scaber* was an herbaceous plant well known to populations of several African countries. Renowned for its antifungal and antimicrobial activities, its aerial parts are mostly used for the treatment of dermatoses and mycoses by indigenous populations. These popular practices have attracted the attention of researchers who had carried out several studies in different research laboratories to confirm the traditional uses and understand the active principles responsible for the activities of the plant. Thus, in vitro tests of the antifungal and anti-microbial activity of *M. scaber* extracts carried out on several germs had proved to be active and thus confirmed the traditional practices. In addition, the molecules responsible for its activities have been isolated from extracts and even from the essential oil of the leaves of the plant. The results obtained can benefit some pharmaceutical companies who make ointments and cosmetic soaps. From all this work, *M. scaber* has an international reputation and, as a result, work on this

rubiaceae must continue on other aspects, especially ash analysis, other insect-destroying agricultural products and the ability of the plant to potentiate the action of the antimicrobial agents that certain bacteria defy etc. in order to optimize its use for the benefit the indigenous populations.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

- Aboh MI, Olayinka BO, Adeshina GO, Oladosu P (2014). Antifungal activities of phyto compounds from *Mitracarpus villosus* (Sw.) DC from Abuja, Nigeria. *Journal of Microbiology Research* 4(2):86-91.
- Kokou A, Adjarah Y, Ameyapoh Y, Karou SD, Agbonon A, de Souza C, Gbeassor M (2015). Effects of hydroethanolic extracts of *Balanites aegyptiaca* (L.) *Delile* (Balanitaceae) on some resistant pathogens bacteria isolated from wounds. *Journal of Ethnopharmacology* 164:16-21.
- Anne D (2014). Antibiotiques classification, 13p <http://anne.decoester.free.fr/atb/atb.htm>.
- Bisignano G, Sanogo R, Marino A, Aquino R, D'Angelo V, Germano MP, De Pasquale R, Pizza C (2000). Anti-microbial activity of *Mitracarpus scaber* extract and isolated constituents. *Letters in Applied Microbiology* 30(2):105-108
- Bruneton J (1999). Pharmacognosie, phytochimie, plantes medicinale. Lavoisier Technique et Documentation. Paris 418-419
- Comité de l'Antibiogramme de la Société Française de Microbiologie (2015). Recommandations 2015 ([www.sfm-microbiologie.org](http://www.sfm-microbiologie.org)).
- Gbaguidi F, Giulio M, Georges A, Mansour M, Quetin-Leclercq J (2005). Densitometric HPLC Quantification of 2-Azaanthraquinone isolated from *Mitracarpus scaber* and antibacterial activity against *Dermatophilus congolensis*. *Journal of Planar Chromatography-Modern TLC* 18(105):377-379.
- Germano B, Sanogo R, Marino A, Aquino R, D'Angelo V, Germano MP, R De Pasquale, Pizza C (2000). Antimicrobial activity of *Mitracarpus scaber* extract and isolated constituents. *Letters in Applied Microbiology* 30(2):105-108.
- Harouna H, Faure R, Elias R, Debrauwer L, Saadou M, Balansard G, Boudon G (1995). Harounoside a pentalongin hydroquinone diglycoside from *Mitracarpus scaber*. *Phytochemistry* 39(6):1483-1484.
- Karou D, Savadogo A, Canini A, Yameogo S, Montesano C, Simpore J, Traore AS (2006). Antibacterial activity of alkaloids from *Sida acuta*. *African Journal of Biotechnology* 5(2):195-200.
- Kerharo J, Adams JJ (1974). La pharmacopée sénégalaise traditionnelle. Plantes médicinales et toxiques.
- Koudoro YA, Oronce DL, Mahudro Y, Pascal AD, Paul TF, Alain AG, Koko SD (2014). Chemical characterization, antiradical and antibacterial activities of extracts of the root bark of *Cochlospermum planchonii* of Benin. *International Journal of Innovation and Applied Studies* 7(4):1582
- Magbefon T, Eklou-Kadegbeku K, Aklirikou AK, Agbonon A, de Souza C, Gbeassor M (2009). Infected wound healing and antimicrobial effects of *Chenopodium ambrosioides* and *Mitracarpus scaber*. *International Journal of Biological and Chemical Sciences* 3(3).
- Marjorie EE (2007). Etude de la phytochimie et des activités antibactériennes et antifongiques de cinq plantes médicinales utilisées dans le traitement traditionnel des dermatoses au Mali. Thèse de Doctorat en pharmacie, 175p ([www.keneya.net/fmpos/theses/2007/pharma/pdf/07P17.pdf](http://www.keneya.net/fmpos/theses/2007/pharma/pdf/07P17.pdf)).
- Moussa MB, Rabiou H, Inoussa M, Bakasso Y, Mahamane A (2015). Bio-indicateurs de la fertilité des sols et la perception paysanne à l'ouest du Niger. *Journal of Applied Biosciences* 89(1) :8281-8290.
- Nathalie BR (2002). Contribution de l'acide ursolique dans l'activité antifongique de *Mitracarpus scaber* ZUCC (RUBIACEAE). Thèse de doctorat, Université de Ouagadougou, 98p ([www.beep.ird.fr/collect/uouaga/index/assoc/M08552.dir/M08552.pdf](http://www.beep.ird.fr/collect/uouaga/index/assoc/M08552.dir/M08552.pdf))
- Clinical and Laboratory Standards institute (2012). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standards-M-07-A9, 9th edition volume 32. [www.clsi.org](http://www.clsi.org)
- Olorode O (1984). Taxonomy of West African flowering plants. Published in London and New York. pp 64 & 65.
- Porter LJ, Hritsch LN, Chan B (1986). The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* 25(1):223-230.
- Prieto P, Pineda M, Aguilar M (1999). Spectrophotometric quantification of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application of vitamin E. *Analytical Biochemistry* 269(2):337-341.
- Yaméogo AM (1982). Plantes médicinales africaines IX. Contribution à l'étude d'une plante spontanée *Mitracarpus scaber* Zucc. (Rubiaceae). *Revue Médecine Afrique Noire* 29:519-21.

Full Length Research Paper

# Bio-guided anti-cariogenic and phytochemical valorization of *Guiera senegalensis* and *Pseudocedrela kotschy* stem extracts

Guessan Bi Goblé Landry<sup>1, 2</sup>, Kadja Amani Brice<sup>1\*</sup>, Cottet Kevin<sup>2</sup>, Lecouvey Marc<sup>2</sup>, Mamyrbékova-Békro Janat Akhanovna<sup>1</sup> and Békro Yves-Alain<sup>1</sup>

<sup>1</sup>Laboratoire de Chimie Bio-Organique et de Substances Naturelles/UFR-SFA/ Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire.

<sup>2</sup>Université Paris 13, Sorbonne Paris Cité, Laboratoire CSPBAT, CNRS UMR 7244, F-93017 Bobigny Cedex, France.

Received 25 September, 2018; Accepted 12 October, 2018

The objective of the present study is to carry out bio-guided phytochemical investigation of *Guiera senegalensis* and *Pseudocedrela kotschy* stem extracts. The two plants are used as toothpicks for oral hygiene. The inhibition test revealed a bacteriostatic effect of hexane extract PK1 of *P. kotschy* against *Streptococcus mutans* ATCC 25175 and *Streptococcus salivarius* ATCC 20560, two bacterial cariogenic strains, with a decrease in the number of bacterial colonies of 1 Log/control. The aqueous extract GS5 obtained from the stems of *G. senegalensis* is bactericidal, with total inhibition of *S. salivarius* ATCC 20560. The antimicrobial effect of the stem extracts from the two plants studied varies according to the plant species and the type of bacterial strain. The phytochemicals 8-Hydroxy-6,7-dimethoxy-3-methylisochroman-4-one, 1-(4-hydroxy-3-methoxyphenyl) propane-1,2-dione and (4E, 15E)-Nonadeca-4,15-dien-10-one were isolated, respectively from GS5A extracts of *G. senegalensis* and PK1 of *P. kotschy* by normal column chromatography.

**Key words:** *Guiera senegalensis*, *Pseudocedrela kotschy*, stem extracts, *Streptococcus mutans*, *Streptococcus salivarius*, spectroscopic characterization.

## INTRODUCTION

Plants have always been an indispensable source for human beings as regards health and food, especially in sub-Saharan Africa. In general, plant stems are used in oral care. Such plant stems include *Guiera senegalensis* (Combretaceae) and *Pseudocedrela kotschy* (Meliaceae), which are commonly used as toothbrush by the populations. *G. senegalensis* and *P. kotschy* are often

used in sub-Saharan regions to cure various diseases (Kerharo et al., 1948; Faye et al., 1980; Sanogo et al., 1998; Ancolio et al., 2002; Adamu et al., 2005; Alex et al., 2005; Hadissa and Deschamps, 2006; Ahua et al., 2007; Dieye et al., 2008; Somboro et al., 2011; Ohemu et al., 2014; Traore et al., 2014; Diarra et al., 2015; Sonibare et al., 2015; Kantati et al., 2016; Kpodar et al., 2016).

\*Corresponding author. E-mail: [kadjamanib@yahoo.fr](mailto:kadjamanib@yahoo.fr). Tel:(+225) 07 32 80 41 / 07 90 89 61.

The African continent is endowed with an impressive floristic biodiversity, with a large variety of plants for food and therapeutic needs. This natural floristic richness is only slightly valued chemically and pharmacologically. The bacterial resistance towards synthetic antibiotics is one of the major concerns of the medical research today (Ouelhadj et al., 2017). Yet medicinal plants are interesting alternatives to explore alongside synthetic drugs. That is why, the objective of this study consists of a phytochemical investigation of *G. senegalensis* and *P. kotschyi* stem extracts by a bio-guided anti-cariogenic way to find out more new and effective antibacterials.

## MATERIALS AND METHODS

### Plant

The plant parts (leaves, stems) were harvested in July 2016 from Longorola in Sikasso region (11° 11' 59" North, 7° 05' 49" West) in Southern Mali. The leaves of each plant were identified at Abidjan National Floral Center (CNF) according to herbariums N<sup>os</sup> 7569 and 8664, respectively for *G. senegalensis* and *P. kotschyi*. The *G. senegalensis* (GS) and *P. kotschyi* (PK) stems used for phytochemical and antibacterial analyses were dried under permanent air conditioning (16°C) for 10 days. Then, they were reduced to powders with an electric grinder to obtain the analysis extracts.

### Pathogenic bacteria

Two international cariogenic bacterial strains from the American type culture collection, namely *Streptococcus mutans* (*S. mutans* ATCC 25175) and *Streptococcus salivarius* (*S. salivarius* ATCC 20560) were used for antibacterial tests.

### Preparation of extracts

The powder maceration of each plant (200 g) in hexane (600 mL) under agitation at ambient temperature during 48 h, gave dry hexanic extracts from *G. senegalensis* (GS1) and *P. kotschyi* (PK1) after filtration and solvent elimination, using a rotary evaporator (Büchi R-210 Rotavapor TM).

The marc (10 g) from GS and PK after delipidation with hexane, were brought into contact in different solvents at room temperature for 48 h to provide the dry extracts: GS2, PK2 (acetone, 50 mL); GS3, PK3 (dichloromethane-methanol, 50:50 mL); GS4, PK4 (methanol-water, 70:30 mL); GS5, PK5 (water, 50 mL) after filtration and concentration with the rotary evaporator.

### Antibacterial power evaluation

The antibacterial potency of the different extracts GS1-GS5 and PK1-PK5 were evaluated according to Yew (2015). The bacterial strains to be tested were cultured for 24 h in culture media specific for streptococci. The initial absorbance (650 nm) was first measured for each tube. Then, 300 µL of the overnight culture was added to each tube, and the mixture was adjusted to an absorbance of 1.0 ± 0.05 at the initial wavelength. After 24 h of culture, the absorbance was determined for each tube to estimate bacterial growth. A series of dilutions of 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup>, and 10<sup>-6</sup> for the

seeded tubes were performed. An aliquot of 50 µL of the dilutions obtained were subsequently spread on the specific agar media. Colony counting was performed between 24 and 72 h after seeding, at which time the averages of the triplicates of each series were calculated. The results obtained were converted into Colony Forming Units per mL of medium (CFU/mL) and expressed in logarithmic decimals (CFU Log/mL).

### Aglyconic extracts preparation

The aglyconic extracts were obtained from plant extracts according to Alilou et al. (2014) method, and it showed a better antibacterial profile.

GS5 and PK5 (15 g) were put in touch with HCl (328 mL, 2N) in a flask. The reactional mass was refluxed for 150 min. After filtration and cooling at room temperature, the hydrolyzate was treated with diethyl ether (3 × 100 mL). After decantation, the organic phase was washed with water (3 × 100 mL), then recovered and dried on anhydrous MgSO<sub>4</sub> for 60 min. After filtration on Whatman paper and elimination of the solvent with a rotary evaporator to dryness (40°C), GS5A and PK5A aglyconic extracts were provided.

### Secondary phytoconstituants fractionation, purification and separation

PK1, GS5A and PK5A extracts were retained for chromatographic fractionation and purification with regard to their better antibacterial profile. Isolation and purification were achieved through an elution series on silica gel in the normal phase (Figures 1 and 2).

### Isolated phytoconstituants spectroscopic characterization

Structural elucidation of secondary metabolites has been performed on purified native phytoconstituants. The <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained on BRÜKER Avance 400 MHz. IR spectra were recorded on Perkin Elmer FT-IR 2000 between 4000 and 500 cm<sup>-1</sup>.

## RESULTS AND DISCUSSION

### Extraction yields

The extraction yield of each plant extract is shown in Table 1.

In Table 1, maceration in water supplied the best extracts yields; which seems to accredit the recurring use of this extraction process in endogenous phytotherapy. However, the latter also shows that the yield may differ from a botanical species in another one. The aglyconic extracts yields from *G. senegalensis* and *P. kotschyi* are 0.78 and 0.67%, respectively.

### Plant extracts antibacterial profile

The antibacterial profile of ten raw extracts (GS1-GS5, PK1-PK5) has been estimated towards the two pathogenic bacterial strains directly involved in tooth decay. The results are shown in Tables 2 and 3.

Extracts effect was appreciated according to the



**Table 1.** Extraction yields.

Extract	<i>G. senegalensis</i>					<i>P. kotschy</i>				
	GS1	GS2	GS3	GS4	GS5	PK1	PK2	PK3	PK4	PK5
Yield (%)	1.34	1.79	1.58	0.96	<b>3.89</b>	0.53	3.1	6.72	4.4	<b>8.21</b>

GS1, PK1: Hexanic extracts; GS2, PK2: acetone extracts; GS3, PK3: dichloromethane-methanol extracts; GS4, PK4: hydromethanolic extracts; GS5, PK5: aqueous extracts.

**Table 2.** Antibacterial profile of plant extracts (in CFU Log/mL) by inhibition of *S. mutans* ATCC 25175 growth.

Extract	GS1	GS2	GS3	GS4	GS5	PK1	PK2	PK3	PK4	PK5
Average triplicate (Log CFU/mL) (0.1 g/L)	8.56	8.60	8.43	8.75	8.74	8.22	8.73	8.69	8.71	8.56
Average triplicate (Log CFU/mL) (0.2 g/L)	8.51	8.62	8.45	8.85	8.62	7.92	8.48	8.56	8.85	8.38
Average triplicate (Log CFU/mL) (0.5 g/L)	8.38	8.41	8.48	9.03	-	7.64	8.26	8.46	8.58	8.58
Witnesses: Average triplicate (CFU Log/mL)	8.79	8.62	8.33	8.64	8.71	8.80	8.76	8.40	8.86	8.58

**Table 3.** Antibacterial profile of plant extracts (in log CFU/mL) by inhibition of *S. salivarius* ATCC 20560 growth.

Extract	GS1	GS2	GS3	GS4	GS5	PK1	PK2	PK3	PK4	PK5
Average triplicate (Log CFU/mL) (0.1 g/L)	6.87	7.52	6.66	7.14	5.78	6.67	7.04	7.30	6.86	6.38
Average triplicate (Log CFU/mL) (0.2 g/L)	6.90	7.35	6.79	6.91	0	6.28	6.83	7.42	6.73	6.97
Average triplicate (Log CFU/mL) (0.5 g/L)	6.20	7.06	7.06	6.64	-	6.05	6.07	6.81	6.34	6.41
Witnesses: Average triplicate (CFU Log/mL)	6.99	7.74	6.3	7.22	7.15	6.81	6.92	6.9	6.9	6.26

classification of Yew (2015). Indeed, an extract is bacteriostatic with regard to a decrease in the number of 1 Log/control colonies. On the other hand, it is bactericidal if the total inhibition of bacterial culture is observed.

The results of the antibacterial tests reveal that, GS1, GS2, GS3, GS4, PK2, PK3, PK4 and PK5 do not exhibit any significant inhibition effect on the growth of *S. mutans* and *S. salivarius* at the tested concentrations. PK1 is bacteriostatic towards *S. mutans* (Table 2). Indeed, this extract inhibits the growth of this bacterial strain by stopping the number of colonies of more than 1 Log/control at concentrations of 0.2 and 0.5 g/L. GS5 inhibits the growth of *S. salivarius* because no bacterial colony is found on the agar in its presence. GS5 is therefore bactericidal at 0.2 g/L (Table 3).

On the other hand, the latter showed no inhibitive activity against *S. mutans* (Table 2). GS1 and PK1 extracts caused a decrease in the number of *S. salivarius* colonies from about 0.75 Log/control to 0.5 g/L. This value close to 1 Log/control could be improved either by increasing the concentration of these extracts, or by isolating the active phytochemicals which they contain.

Phytochemical screening of *G. senegalensis* and *P. kotschy* stems has already been performed (Kadja, 2014). The author reports that these organs contain phytochemicals (coumarins, flavonoids, etc.) and terpenes among other identified active second principles. Besides,

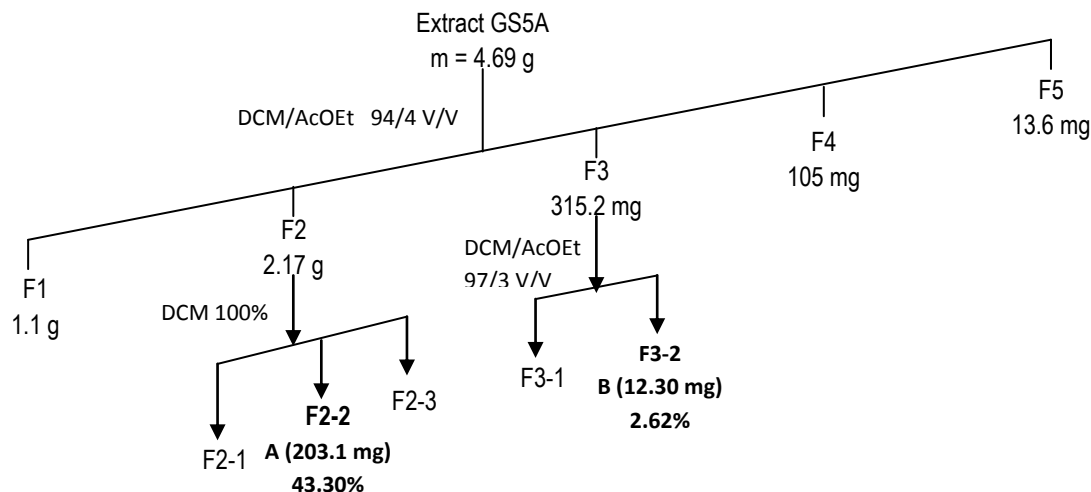
the beneficial effects of terpenes and polyphenols on oral health are known (Bitty, 1982; Kadja et al., 2011; Atsain et al., 2016). Thus, the antibacterial activities exhibited by hexanic extracts GS1 and PK1 suggest a synergic action due to the existence of terpenic phytoconstituents. As for the aqueous extracts of GS5 and PK5, their manifest antibacterial profile seems to have a correlation with the aforementioned extracts of water-soluble secondary metabolites with antibacterial potential.

### Isolated phytochemical structural elucidation

PK1, GS5A and PK5A extracts were chromatographically fractionated on a normal phase silica gel column. Fractionation and purification of GS5A led to the isolation of phytoconstituents A and B, with yields of 43.30 and 2.62%, respectively, with regard to the mass fractions (Figure 1). As for PK1, its fractionation and purification allowed the isolation of phytochemical C, with a yield of 4.92% (Figure 2). The interpretation of IR spectra was done according to the method of Brown et al. (1992) and Robert et al. (2005).

### Structure of isolated phytochemical A from GS5A extract

Analysis of <sup>13</sup>C NMR, JMOD and DEPT 135 spectra of



**Figure 1.** Fractionation and purification of GS5A. P: Petroleum ether, DCM: dichloromethane; AcOEt: Ethyl acetate. Column: length 45 cm, diameter 4 cm, height of the layer of normal silica gel 15 cm.

**Table 4.** NMR spectral data of compound A.

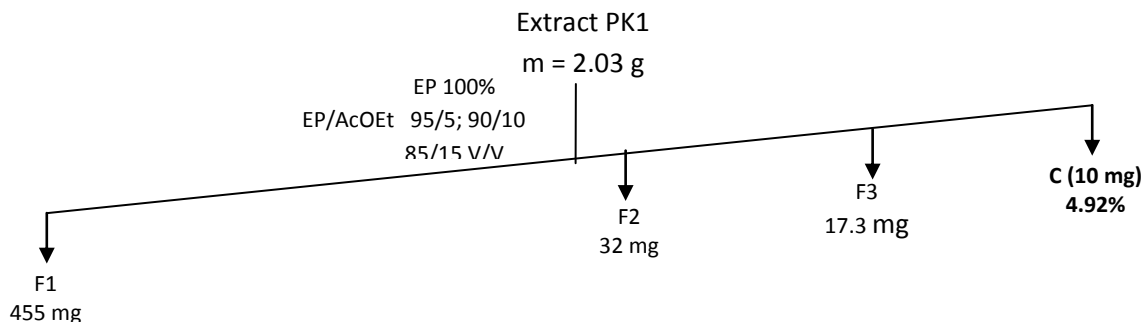
N <sup>o</sup> C	Type	<sup>13</sup> C δ (ppm)	HSQC δ (m, J Hz)	HMBC δ (ppm)	COSY δ (m, J Hz)
1	C	190.59	-	-	-
2	C	201.54	-	-	-
3	CH <sub>3</sub>	26.98	2.58 (s)	190.59; 201.54	-
1'	CH	127.19	7.60 (dd; 8.3; 1.9)	152.57	6.98 (d; 8.3)
2'	CH	111.35	7.58 (d; 1.9)	127.19; 190.59; 147.25; 152.59	-
3'	C	147.25	-	-	-
4'	C	152.57	-	-	-
5'	CH	114.69	6.98 (d; 8.3)	124.68; 147.25; 152.57	7.60 (dd; 8.3; 1.9)
6'	C	124.68	-	-	-
7'	CH <sub>3</sub>	56.40	3.96 (s)	147.25	-

compound A (Table 4) reveals the presence of 5 C quaternary  $sp^2$  hybridization, of which 2 C=O at 190.59 and 201.54 ppm; 2 C primary at 2.98 and 56.48 ppm; C3 secondary ethylenic 111.35, 114.49 and 127.19 ppm. Thus, 10 C is the carbon skeleton of the compound. The <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>) shows the presence of 3 H (CH<sub>3</sub>O-) at 3.96 ppm; 3 H aromatics at 6.97, 7.57 and 7.61 ppm; 1 H at 6.23 ppm; and 3 H (CH 3) at δ 2.51 ppm. The 2D spectrum, COSY, shows the correlation of proton signals resonating at 7.61 and 6.97 ppm; confirmed by the HSQC and HMBC spectra in the sense that these 2H are carried by adjacent carbons whose signals resonate at 114.69 and 127.19 ppm. IR spectrum analysis shows 3408 cm<sup>-1</sup> absorption bands (C4'-OH, valence vibration); 1658 cm<sup>-1</sup> (C1=O, deformation vibration), 1710 cm<sup>-1</sup> (C2=O, valence vibration), 3080 cm<sup>-1</sup> (aromatic C-H, valence vibration), 1294 cm<sup>-1</sup> (aromatic C-H, deformation vibration), 1588 and 1513 cm<sup>-1</sup> (cyclic C=C, valence vibrations), 1267 cm<sup>-1</sup> (asymmetric C-O-C,

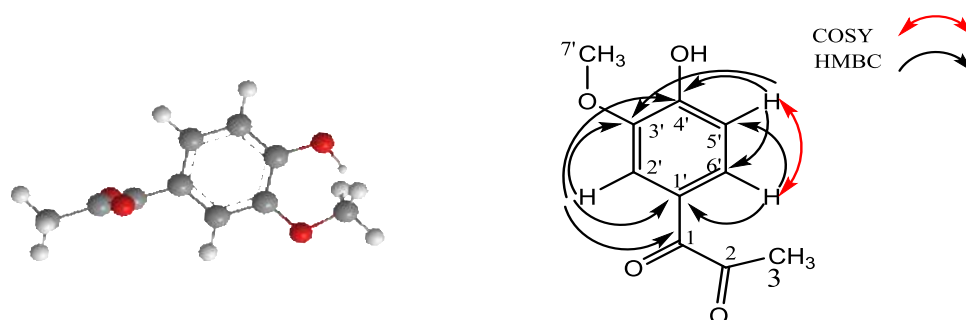
valence vibration), 2940 and 2840 cm<sup>-1</sup> (aliphatic C-H). All the spectral data allowed identification of the phytocompound A (Figure 3).

#### Structure of isolated phytocompound B from GS5A extract

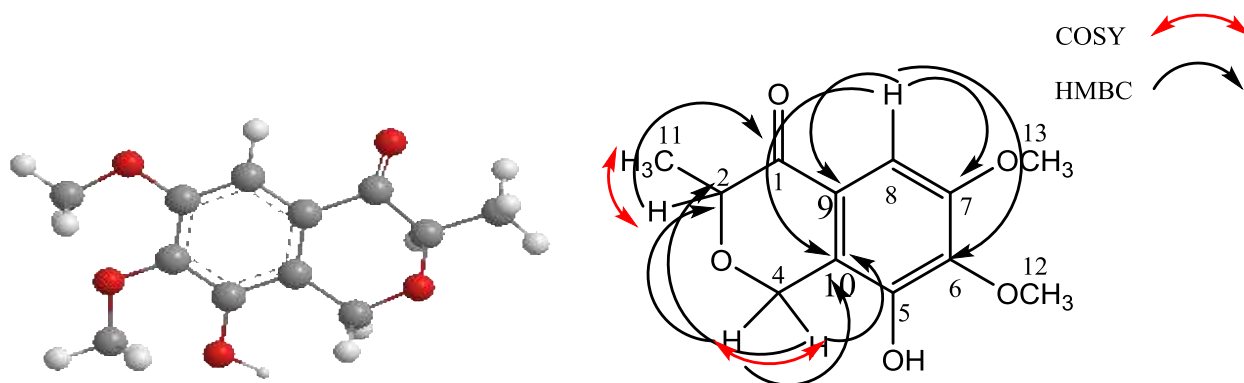
The <sup>1</sup>H NMR spectrum of compound B (CDCl<sub>3</sub>) shows the presence of 14 H including 1 H aromatic at 7.34 ppm; 6 H of 2 (CH<sub>3</sub>O) at 3.91 and 3.94 ppm; 3 H (CH<sub>3</sub>) at 1.50 ppm; 2 H appearing at 4.77 and 5.06 ppm as doublets each; 1 H at 4.20 ppm as a quartet; 1 H (OH) at 6.20 ppm wide signal. <sup>13</sup>C NMR, DEPT 135 and JMOD show the presence of 12 C including 6 C quaternary (C1; C2; C3; C4; C5; C6), 1 aromatic CH (C7) resonant at 103.96 ppm; 2 CH<sub>3</sub> methoxy (C10; C11) appearing at 56.78 and 60.89 ppm; 1 CH<sub>3</sub> at 16.11 ppm; 1 C secondary (C9) at 62.48 ppm. The HSQC, HMBC and COSY data is shown



**Figure 2.** Fractionation and purification of PK1. EP: Petroleum ether, DCM: dichloromethane, AcOEt: ethyl acetate. Column: length 30 cm, diameter 3 cm, height of the layer of normal silica gel 15 cm.



**Figure 3.** 1-(4'-hydroxy-3'-methoxyphenyl) propane-1, 2-dione with different COSY and HMBC correlations



**Figure 4.** 5-Hydroxy-6,7-dimethoxy-2-methylisochroman-1-one with different COSY and HMBC correlations.

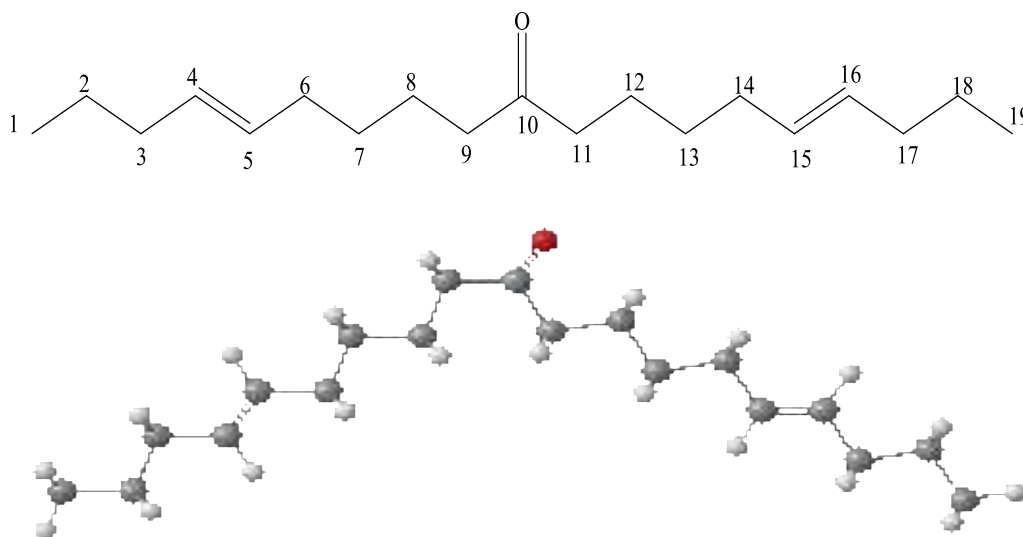
in Table 5. The IR spectrum reveals the presence of an absorption band at  $3504\text{ cm}^{-1}$  (C5-OH, valence vibration in dilute solution);  $3054\text{ cm}^{-1}$  (aromatic C-H, valence vibration);  $1683\text{ cm}^{-1}$  (C1=O, valence vibration);  $1016\text{ cm}^{-1}$  (C2-O-C4, valence vibration); and  $2986.74\text{ cm}^{-1}$  (aromatic C-H, deformation vibration). All the spectral data allowed identification of the phytocompound B (Figure 4).

#### Structure of isolated phytocompound C from PK1 extract

NMR analysis shows that compound C has molecular symmetry. The  $^{13}\text{C}$  NMR and HMBC correlation spectra revealed the presence of 2 primary C which resound in 14.08 ppm; 16 secondary C among which 4 C equivalent type C=C (C4=C5, C15=C16) appearing at 128.07 and

**Table 5.** NMR spectral data of compound B.

N°C	Type	<sup>13</sup> C δ (ppm)	HSQC δ (m, J Hz)	HMBC δ (ppm)	COSY δ (ppm)
1	C	195.21	-	-	-
2	C	78.06	4.20 (q; 6.7)	195.21	1.50
4	CH <sub>2</sub>	62.85	5.06 (d; 15.7) 4.77 (d; 14.4)	78.05; 121.64; 141.73; 195.21 78.05; 121.63; 195.21	4.77 5.06
5	C	144.10	-	-	-
6	C	141.73	-	-	-
7	C	147.54	-	-	-
8	CH	103.96	7.30(s)	121.63; 144.10; 147.54; 195.21	-
9	C	130.51	-	-	-
10	C	121.63	-	-	-
11	CH <sub>3</sub>	16.11	1.50 (s)	78.05; 195.21	4.20
12	CH <sub>3</sub>	56.78	3.94 (s)	147.54	-
13	CH <sub>3</sub>	60.89	3.91 (s)	141.73	-

**Figure 5.** (E, E)-Nonadeca-4,15-dien-10-one.

130.03 ppm, and 12 C (CH<sub>2</sub>) equivalents (C2-C18, C3-C17; C6-C11, C7-C13, C8-C12 and C9-C11), resounding at 29.71, respectively; 27.19, 22, 70, 25.63, 24.67, and 34.06 ppm; 1 quaternary C in 180 ppm. <sup>1</sup>H NMR spectra, HSQC direct correlations and COSY correlations allowed to assign the different values of protons and carbons (Table 6). Analysis of the IR spectrum showed an absorption band at 3054 cm<sup>-1</sup> (C-H asymmetric, valence vibration in CH=CH); 1680 cm<sup>-1</sup> (C=C, E configuration, valence vibration); 1709 cm<sup>-1</sup> (C=O, valence vibration); and 738 cm<sup>-1</sup> (C-H, deformation vibration). The spectral data set confirm the molecular structure of phytocompound C (Figure 5).

## Conclusion

In this work, valorization of the extracts of stems for *G. senegalensis* and *P. kotschy* has been undertaken, for two plant species used by the populations in sub-Saharan Africa used like toothbrush for the maintenance of oral health. Biologically, the stem extracts of these plants have exhibited inhibitory effects on both tested cariogenic bacterial strains. These antibacterial effects were owed to the synergic combination of the active secondary metabolites, which they contain. On the one hand, these results support the first intention, the utility of popular use of both plants as toothpicks; on the other

**Table 6.** NMR spectral data of compound C.

N°C	Type	<sup>13</sup> C δ (ppm)	HSQC δ (m, J Hz)	HMBC δ (ppm)	COSY δ (m, J Hz)
1=19	CH <sub>3</sub>	14.13	0.84 (m)	22.61; 31.90	1.25
2=18	CH <sub>2</sub>	29.71	1.25 (t, 7.1 Hz)	-	-
3=17	CH <sub>2</sub>	27.19	2,00 (d, 4.6 Hz)	127.89; 128.07; 130.01, 130.19	2.73 (t, 6.5 Hz)
4=16	C	130.03	5.30 (s)	25.60	-
5=15	C	128.07	5.30 (s)	25.60	-
6=14	CH <sub>2</sub>	22.70	1.25 (t, 7.1 Hz)	-	-
7=13	CH <sub>2</sub>	25.63	2.73 (t, 6.5 Hz)	127.89; 128.07; 130.01, 130.19	2,00 (d, 4.6 Hz)
8=12	CH <sub>2</sub>	24.67	1.59 (dd, 14; 6.9 Hz)	29.14; 34.06; 180.01	2.30 (t, 7.5 Hz)
9=11	CH <sub>2</sub>	34.06	2.30 (t, 7.5 Hz)	24.66 ; 29.14 ; 34.06; 180.01	1.59 (dd, 14; 6.9 Hz)
10	C	180.01	-	-	-

hand, it recommends the possibility of the use of these plants in the prevention of dental caries. In the phytochemical plan, three isolated phytoconstituants have been characterized. These are 1-(4'-Hydroxy-3'-methoxyphenyl) propane-1, 2-dione, 5-hydroxy-6, 7-dimethoxy-2-methylisochroman-1-one and (E, E)-nonadeca-4, 15-dien-10-one. The study of their anticariogenic potential is in progress.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

- Adamu HM, Abayeh OJ, Agho MO, Abdullahi AL, Uba A, Dukku UH, Wufem BM (2005). An ethnobotanical survey of Bauchi State herbal plants and their antimicrobial activity. *Journal of Ethnopharmacology* 99 (1):1-4.
- Ahua KM, Ioset JR, Loset KN, Diallo D, Muel J, Hostettmann K (2007). Antileishmanial activities associated with plants used in the Malian traditional medicine. *Journal of Ethnopharmacology* 110(1):99-104.
- Alex A, Oteng-Yeboah AA, Odamtten GT, Simmonds MS (2005). Ethnobotanical study of some Ghanaian anti-malarial plants. *Journal of Ethnopharmacology* 99(2):273-279.
- Alilou H, Bencharki B, Mina L, Hassani I (2014). Screening phytochimique et identification spectroscopique des flavonoïdes d'*Asteriscus graveolens* sub sp. odorus. *Afrique Science: Revue Internationale des Sciences et Technologie* 10(3).
- Ancolio C, Azas N, Mahiou V, Ollivier E, Di Giorgio C, Keita A, Timon-David P, Balansard G (2002). Antimalarial activity of extracts and alkaloids isolated from six plants used in traditional medicine in Mali and Sao Tome. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 16(7):646-649.
- Atsain AMR, Kadja AB, Guessennd NK, N'guessan JK, Mamyrbékova-Békro JA, Békro YA (2016). Etude phytochimique bio-guidée, activités antibactérienne et analgésique de décoctions éthanoliques d'une Mimosaceae de Côte d'Ivoire. *International Journal of Innovation and Applied Studies* 18(2):569-577.
- Bitty MJ (1982). Study of some plants used as stick friction tooth Ivory Coast, thesis for the state diploma of doctor of dental surgery in Ivory Coast, Thèse de doctorat, Université de Clermont I pp. 99
- Brown DW, Floyd AJ, Sainsbury M (1992). *Organic spectroscopy* (traduit du russe). Edition Mir, Moscou, 300 p. Côte d'Ivoire P 125.
- Diarra N, Togola A, Diallo D, Willcox M, Jong JD (2015). Ethnobotanical study of plants used against malaria in Sélingué subdistrict, Mali. *Journal of Ethnopharmacology* 166:352-360.
- Dieye AM, Sarr A, Diop SN, Ndiaye M, Sy GY, Diarra M, Rajraji-Gaffary I, Ndiaye-Sy A, Faye B (2008). Medicinal plants and the treatment of diabetes in Senegal: Survey with patients. *Fundamental and Clinical Pharmacology* 22(2):211-216.
- Faye O, Olschwang D, Giono-Barber H, Pousset JL (1980). African medical plants II. Antitussive action of lyophilized *Guiera senegalensis* (Combretaceae). *Dakar Medical* 25(4):285-292.
- Hadissa T, Deschamps JP (2006). Use of medicinal plants for the treatment of oral diseases in Burkina Faso. *Journal of Ethnopharmacology* 104(8):68-78.
- Kadja AB (2014). Sept plantes africaines utilisées comme cure-dents: compositions minérale, phénolique et activités biologiques. Thèse de doctorat unique, Université Nangui Abrogoua,
- Kadja AB, Mamyrbékova-Békro JA, Benie A, Boua BB, N'gaman KC, Békro YA (2011). *Erythrophleum africanum Afzel* (Caesalpinaceae), an African toothpick: Phytochemical screening, total flavonoid content and antioxidant activity. *Journal of Medicinal Plants Research* 5(27):6273-6277.
- Kantati YT, Kodjo KM, Dogbeavouoffi S, Vaudry D, Leprince J, Gbeassor M (2016) Ethnopharmacological survey of plant species used in folk medicine against central nervous system disorders in Togo. *Journal of Ethnopharmacology* 181:214-220
- Kerharo J, Bouquet A, Heintz R (1948). Le Wilinwiga des Mossi (*Guiera senegalensis*, Lam.), ses usages thérapeutiques indigènes et son application au traitement des diarrhées cholériques. *Acta Tropica* 5(4):345-348.
- Kpodar MS, Karou SD, Katawa G, Anani K, Gbékley HE, Adjrah Y, Tchacondo T, Batawila K, Simpore J (2016). An ethnobotanical study of plants used to treat liver diseases in the Maritime region of Togo. *Journal of Ethnopharmacology* 181:263-273.
- Ohemu TL, Agunu A, Olotu PN, Ajima U, Dafam OG, Azila JJ (2014). Ethnobotanical survey of medicinal plants used in the traditional treatment of viral infections in Jos, Plateau state, Nigeria. *International Journal of Medicinal and Aromatic Plants* 4(2):74-81.
- Ouelhadj A, Ait Salem L, Djenane D (2017). Activité antibactérienne de l'huile essentielle de *Pelargonium x asperum* et son potentiel synergique avec la nisine. *Phytothérapie* pp. 1-9.
- Robert MS, Francis XW, David JK (2005). *Spectrometric identification of organic compounds*. John Wiley and sons.
- Sanogo R, Crisafi G, Germanò MP, De Pasquale R, Bisignano G (1998). Evaluation of Malian traditional medicines: screening for antimicrobial activity. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 12(S1):S154-S156.
- Somboro AA, Patel K, Diallo D, Sidibe L, Chalchat JC, Figueredo G, Ducki S, Troin Y, Chalard P (2011). An ethnobotanical and phytochemical study of the African medicinal plant *Guiera senegalensis*, J. F. Gmel. *Journal of Medicinal Plants Research*

- 5(9):1639-1651.
- Sonibare MA, Okorie PN, Aremu TO, Adegoke A (2015). Ethnomedicines for mosquito transmitted diseases from Southwestern Nigeria. *Journal of Natural Remedies* 15(1):33-42.
- Traoré MS, Diane S, Diallo MST, Balde ES, Balde MA, Camara A, Diallo A, Keita A, Cos P, Maes L, Pieters L, Balde AM (2014). *In vitro* antiprotozoal and cytotoxic activity of ethnopharmacologically selected Guinean plants. *Planta medica: Natural products and medicinal plant research*. Stuttgart 80(15):1340-1344.
- Yew JY (2015). Etude de l'effet de quatre composés contenant du miel sur deux bactéries cariogènes: *Streptococcus mutans* et *Lactobacillus rhamnosus*. Diplôme d'Etat en chirurgie, Université de Bordeaux, France 60 p.

*Full Length Research Paper*

## **Evaluation of herbal cocktail used in the treatment of malaria on liver tissue of adult Wistar rats**

**Moronkeji Akinpelu<sup>1</sup>, Eze Ikechi Gerald<sup>2</sup>, Bejide Ronald Ayodele<sup>3</sup>, Anwara Ogbonnaya Akpu<sup>4\*</sup> and Igunbor Michael Chuks<sup>5</sup>**

<sup>1</sup>Department of Clinical Laboratory, Baptist Medical Centre, Saki, Oyo State, Nigeria.

<sup>2</sup>Department of Anatomy, University of Benin, Benin, Nigeria.

<sup>3</sup>Department of Morbid Anatomy and Forensic medicine, Obafemi Awolowo University, Ile-ife, Nigeria.

<sup>4</sup>Federal Teaching Hospital, Ido-Ekiti, Ekiti State, Nigeria.

<sup>5</sup>Department of Medical Laboratory Science, University of Benin, Benin City, Nigeria.

Received 17 August, 2018; Accepted 17 September, 2018

Interest is renewed in herbal medicine since it is believed it has less side effects and is safer. In addition, there has been continued demand to obtain more drugs from plant sources to alleviate various ailments of mankind. This study is aimed at investigating the antimalarial activity in an herbal cocktail and individual plant extracts contained on *Plasmodium berghei* in infected Wistar rats. Thirty five Wistar rats randomly assigned into seven groups of five were used. The cocktail and individual aqueous extracts of *Azadirachta indica*, *Mangifera indica*, *Carica papaya*, and *Citrus limon* were orally administered to the infected Wistar rats weighing an average of 200 g at standard doses of 100 mg/kg/day for seven days, with the exception of aqueous leaf extract of *A. indica* which was administered at a dose of 10 mg/kg/day. The therapeutic effects of the cocktail and the individual extracts against *P. berghei* were investigated and the effects on the liver were histologically assessed. Biochemical assays for liver markers aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP) and albumin (ALB) were also assessed. The results showed that the cocktail and individual extracts possess antimalarial activity, by reducing the degree of parasitaemia, inducing recovery of hepatic cells and reduction of malaria associated liver pathology. Administration of the extracts did not significantly alter the level of albumin and total protein, no increase was observed in AST activity ( $P > 0.05$ ). Significant increase was observed in the ALT activities among the rats administered with the cocktail or that which contained extract ( $P < 0.0001$ ). In conclusion, the cocktail and the individual extracts possess antimalarial activity, thus justifying their usage in traditional medical practice. Extensive studies for validation of various medicinal plants used in treating malaria should be further conducted

**Key words:** *Plasmodium berghei*, cocktail, aspartate aminotransferase, alanine aminotransferase, total protein, albumin.

### **INTRODUCTION**

Phytotherapy has been a backbone of medicine since way back in which various herbs and their extracts containing active ingredients of therapeutic significance is used (Tiwari et al., 2014). Natural plant extracts contain a variety of phenolic compounds which are assigned

various biological activities (Makni et al., 2018). Malaria remains a public health problem worldwide and a leading cause of death and disease in many developing countries especially the tropical and subtropical regions of the world where young children and pregnant women are

mostly affected (Vineet and Bagai, 2014; Iyama and Idu, 2015).

Malaria is one of the most common vector-borne diseases recognized as a crucial parasitic disease of humans, it is a life-threatening blood disease caused by parasites transmitted to human through female anopheles mosquitoes bite (Arzoo and Kumari, 2017). Until now, malaria is still a health problem worldwide, especially in tropical countries like Africa and Asia and about 3.3 billion people worldwide are at risk of malaria (Taek et al., 2018). Variety of Nigerian medicinal plants has demonstrated antimalarial potential and could serve as possible sources of antiplasmodial compound (Ibrahim et al., 2012). The World Health Organization defines herbal medicines to include herbs, herbal materials, herbal preparations and finished herbal products; containing active ingredients plants parts, or other plant materials, or combinations of both (WHO, 2005). In Nigeria, malaria remains a major public health problem and the high cost of the effective antimalarial drugs, poor quality drugs and increased emergence of Plasmodial resistance necessitates the need for alternative source of medicine in malaria treatment and prevention (Okere et al., 2014). Malaria is responsible for approximately 60% of outpatient visits and 30% of admissions in Nigeria and it is also believed to contribute up to 11% of maternal mortality, 25% of infant mortality, and 30% of under-5 mortality (FMOH, 2015). It is estimated that about 110 million clinically diagnosed cases of malaria and nearly 300,000 malaria-related childhood deaths occur each year (FMOH, 2015). Children under age five and pregnant women are mostly affected and Africa still bears over 80% of the global malaria burden which Nigeria accounts for about 29% of this burden. Moreover, in combination with the Democratic Republic of Congo, Nigeria contributes up to 40% of the global burden (WHO, 2014).

Malaria infection has been reported to induce acute injuries to vital organs and the most pronounced changes inflicted due to this disease involve the blood, spleen, liver and kidney of the infected host (Vineet and Bagai, 2014). The greatest impact of the disease is on the poor people of the world and most of these populations are found in the rural settings, especially in African communities where the people have poor nutritional status and also lack access to good health facilities (Ajala et al., 2011). Thus, the rural dwellers depend more on herbs and other forms of traditional medicines for cure (Idowu et al., 2010). Various medicinal properties have been attributed to natural herbs (Vaghasiya et al., 2011) and plants constitute the main source of new pharmaceuticals and healthcare products (Ivanova et al.,

2005).

WHO (2016), indicated malaria to be endemic in 91 countries as at the beginning of 2016 and a total funding for malaria control and elimination in 2015 was estimated at US\$2.9 billion. About 212 million cases of malaria occurred worldwide, where 429,000 deaths were recorded globally and 303,000 malaria deaths are estimated to have occurred in children aged under 5, which is equivalent of to 70% of the global total (WHO, 2016).

Plants provide many advantages such as ornament, oxygen, food, beverages, clothing, perfumes and building materials and they are source of an enormous number of compounds, known as 'secondary metabolites' (Laudicina et al., 2013). In Nigeria, various plants are used for the management of malaria and these vary from one locality to another (Aiyeloja and Bello, 2006; Odugbemi et al., 2007). Within the context of traditional practice, malaria is commonly treated with decoctions or infusions from bitter plants (Randrianarivelosia et al., 2003), and the popularity of herbs in traditional medicine has been linked to their higher likelihood of containing pharmacologically active compounds compared to woody plant forms (Thomas et al., 2009).

In the last decade, there has been resurgence in search for new lead compounds from plants to treat malaria (Noronha et al., 2018). A number of traditional herbs have been tested and used in the prevention and treatment of malaria including, leaves of *Carica papaya*, *Azadirachta indica* popularly called Dongoyaro in Nigeria, *Mangifera indica* and *Citrus limon* (Idowu et al., 2010; Gbolahan et al., 2014). Chloroform extract of *C. papaya* have been found to be active against the malarial parasites (Abass et al., 2017).

Etuk et al. (2010) and Ene and Atawodi (2012) reported the use of *M. indica* leaf decoctions in the treatment of malaria. Various parts of *M. indica* tree have been used in traditional medicine for the treatment of different ailments and a number of bioactive phytochemical constituents such as polyphenols, terpenes, sterols, carotenoids, vitamins, and amino acids, and so forth have been reported with several studies proving the pharmacological potential of different parts of mango trees such as leaves, bark, fruit peel and flesh, roots, and flowers as anticancer, anti-inflammatory, anti-diabetic, antioxidant, antibacterial, antifungal, anthelmintic, gastroprotective, hepatoprotective, immunomodulatory, antiplasmodial, and antihyperlipemic (Ediriweera et al., 2017).

A large variety of chemical compounds have been reported in *M. indica* which includes mangiferin, gallic

\*Corresponding author. E-mail: theosbright@yahoo.com.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)



acid, catechins, quercetin, kaempferol, protocatechuic acid, ellagic acids, propyl and methyl gallate, rhamnetin, and anthocyanins as the major polyphenolic compounds found in *M. indica* (Nayan et al., 2017). *C. papaya* belongs to the family of Caricaceae. It is commonly called paw-paw and it is known for its food and nutritional values worldwide (Melariri et al., 2012).

The properties of papaya fruit and other parts of the plant are also well known in traditional system of medicine and the medicinal application of papaya makes it a valuable nutraceutical fruit plant. The different parts of the *C. papaya* plant includes leaves, seeds, latex and fruit exhibited to have medicinal value and have been used in the treatment of various ailments. Young leaves are rich in flavonoids (kaempferol and myricetin), alkaloids (carpaine, pseudocarpaine, dehydrocarpaine I and II), phenolic compounds (ferulic acid, caffeic acid, chlorogenic acid), and cynogenetic compounds (benzyl glucosinolate). Both the leaf and fruit of *C. papaya* Linn. possess carotenoids namely  $\beta$ -carotene, lycopene, anthraquinones glycoside, as compared to matured leaves; hence, possess medicinal properties like anti-inflammatory hypoglycaemic, anti-fertility, abortifacient, hepatoprotective, wound healing. Recently, its antihypertensive and antitumor activities have also been established (Anjum et al., 2013).

The latex from unripe papaya fruit contains enzymes papain and chymopapain, while vitamin C and E are also constituents of the leaves (Yogiraj et al., 2014). Variety of substances are present in citrus fruits and they include carbohydrates, fibre, vitamin C, potassium, folate, calcium, thiamine, niacin, vitamin B6, vitamin A, phosphorus, magnesium, copper, riboflavin, pantothenic acid and a variety of phytochemicals which are necessary for proper functioning of the body, although some confer additional protection against chronic disease over basic nutrition. In addition, phytochemicals including essential oils, alkaloids, flavonoids, coumarins, psoralens and carotenoids are also present with previous pharmacological studies reporting the antimicrobial, anthelmintic, insect repellent, antioxidant, anticancer, cardiovascular, central nervous, anti-inflammatory, analgesic, antidiabetic, reproductive, gastrointestinal, immunological, respiratory and many other pharmacological effects of the *C. limon* (Al-Snaf, 2016).

Titanji et al. (2008) reported the use of *C. papaya*, *M. indica* as part of the medicinal plants used in the treatment on malaria in Cameroon in the form of decoction, dried powder or ground material prepared from plants for consumption as teas, steam bath or enema which are sold as herbal remedies. Water extracts of pawpaw and mango leaves have been confirmed to show potencies against malaria parasites and they compare favorably with an established long acting orthodox anti-malarial drug, sulphadoxine/pyrimethamine (Gbolahan et al., 2014). Indigenous dwellers also claimed that the usage of selected forest plants leaves and parts

are effective in the management of malaria. This study evaluates the efficacy of a herbal cocktail which contains fruit extract of *C. limon*, aqueous leaf extract of *C. papaya*, *A. indica* and *M. indica* commonly used in the treatment of malaria in some communities in Nigeria, as well as to determine the biochemical or histomorphological changes that could occur in the liver following treatment with the cocktail or extracts contained therein.

## MATERIALS AND METHOD

### Experimental animals

This experiment was conducted in strict compliance with the humane animal care standards of the University of Benin, Benin City, Nigeria. Six weeks old inbred Wistar rats, weighing an average of 200 g obtained from the Anatomy Department of the University of Benin animal house were used as experimental animals for this study. All animals purchased were kept in ventilated cages in the Animal House at the Anatomy Department, University of Benin, Benin city, Nigeria. They were fed with growers mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo State, Nigeria throughout the duration of this study.

### Parasite and infection

Chloroquine-sensitive *Plasmodium berghei* (NK 65 strain) obtained from the Institute of Advance Medical Research and Training (IMRAT), University of Ibadan, Nigeria was used for this study. Thirty-five (35) adult Wistar rats weighing an average of 200 g were divided into seven groups labeled I, II, III, IV, V, VI and VII. Each of the groups consisted of 5 rats, which were allowed to attain the requisite weights before commencement of the experiment.

Group I: Non-infected control group (Negative control) (Distilled water was administered only).

Group II: Infected untreated group (*P. berghei* + Distilled water was administered).

Group III: *P. berghei* + Herbal cocktail was administered.

Group IV: *P. berghei* + *A. indica* extract was administered.

Group V: *P. berghei* + *M. indica* extract was administered.

Group VI: *P. berghei* + *C. papaya* extract was administered.

Group VII: *P. berghei* + *C. limon* extract was administered.

### Inoculation procedure

Three donor mice with rising parasitemia of 25% were sacrificed and blood was collected into ethylene diaminetetraacetic (EDTA) bottle and diluted with phosphate buffer saline to  $10^8$  parasitized erythrocytes/mL. Healthy Wistar rats were inoculated intraperitoneally with preparation of the infected blood (Peter and Anatoli, 1998).

### Blood cytology evaluation

A small drop of blood from the tail of each infected rats was collected on clean grease free slide. Thin blood films were stained with Giemsa and were allowed to air-dry and viewed with oil immersion objectives (Akin-Osaniye et al., 2013). The percentage parasitaemia evaluation was determined (total number of pRBC/total number of RBC  $\times$  100) for each infected rat (Innocent et al., 2017). Rats with parasitaemia, at least equal to 25%, were

either treated with the cocktail or each constituent extract on the seventh post-infection day.

## Plant

### Cocktail and individual extract

The plant samples collected were identified by a plant Taxonomist from Plant Biology and Biotechnology Department of the University of Benin, Benin City, Nigeria as *A. indica* (family Meliaceae), *M. indica* (family Anacardiaceae), *C. papaya* (family Caricaceae), and *C. limon* (family Rutaceae) (Arzoo and Parina Kumari, 2017). The cocktail, *C. limon* juice extract and aqueous leaf extracts of *M. indica* and *C. papaya* were administered to respective groups at a dose of 100 mg/kg/body weight, while aqueous leaf extract of *A. indica* was administered at 10 mg/kg/body. The chemotherapeutic effect of the extract against *P. berghei* was investigated after seven days of administration. Phytochemical studies were carried out at the Pharmacognosy Department of the University of Benin, Benin City, Nigeria to determine the presence of carbohydrate, alkaloid, flavonoid, tannins and saponin in aqueous leaf extract of *A. indica*, *M. indica*, *C. papaya* and fruit extract of *C. limon*, using standard procedures proposed by Sofowora (1982) and Evans (2002).

### Histopathology studies

The liver was excised and immediately transferred into 10% neutral buffered formalin and processed for light microscopic study, using an automatic tissue processor machine (Shandon 2000, Leica, Frankfurt, Germany). Tissues were dehydrated in various grades of alcohol then cleared in two changes of xylene, infiltrated in two changes of wax bath and finally embedded in paraffin wax. Five microns thick paraffin sections were obtained, which were finally stained using the Hematoxylin and Eosin staining procedure and the sections mounted with DPX and examined microscopically by means of x10 and x40 objective lenses (Avwioro, 2014).

### Biochemical studies

Biochemical markers associated with liver functions were determined using the plasma obtained from the Wistar rats upon necropsy after seven days of treatment. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein and albumin were analyzed using commercial diagnostic kits from Randox laboratory, United Kingdom. The kits employed the procedure of Reitman and Frankel (1957) for the analysis of AST and ALT, while total protein was estimated using the procedure of Tiez (1995). Albumin was estimated using the method of Grant (1987).

### Statistical analysis

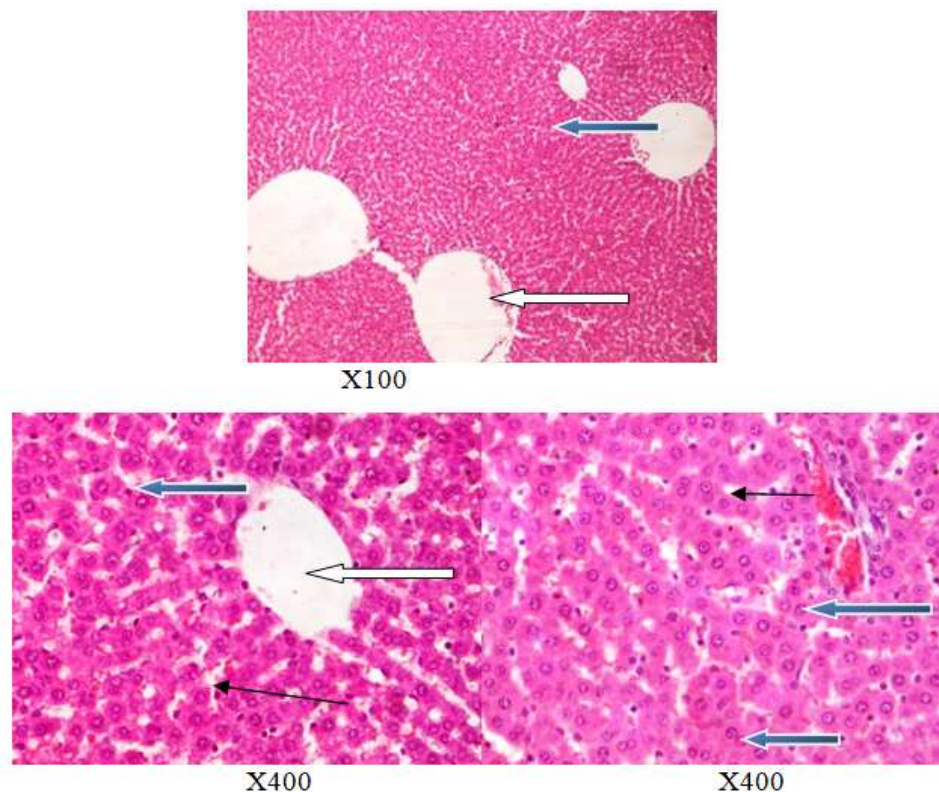
Data analysis was performed using statistical package for social sciences (SPSS) version 20.0. Data were expressed as mean  $\pm$  standard deviation (SD). Test of significance was calculated using paired Student's t-test.  $p < 0.05$  was considered to be significant.

## RESULTS

### Histopathological findings

Histopathological findings indicated that extracts of *C.*

*papaya* and *C. limon* were the most efficacious as there was significant reduction in the malaria associated liver pathology; while the groups administered with aqueous leaf extract of *M. indica* was observed to be least potent. Sections of uninfected rats administered with distilled water only showed normal histological features composed only of hepatocytes, portal vein and sinusoids, the morphology of the hepatocytes appeared normal and the sinusoids were not infiltrated; and no pathological lesion observed (Figure 1). Liver sections of the infected untreated group showed very poor architecture. There is severe portal triaditis and the portal tracts showed periportal infiltration of inflammatory cells with mild congestion of the portal vein, some of the hepatocytes showed foamy cytoplasm and some showed cellular debris engulfed by Kupffer cells. Heavy infiltrates of inflammatory cells and mild vascular congestion was observed. Parasites were engulfed in Kupffer cells and malaria pigment (haemozoin) was also present (Figure 2). Liver sections of infected rats administered with the cocktail showed sinusoids mildly packed with parasite-laden Kupffer cells and malaria pigment, mild inflammatory cellular infiltrates were also observed to be present around the portal zone, the hepatic portal vein was not congested (Figure 3). Liver sections of infected rats treated with aqueous leaf extract of *A. indica* showed decreased inflammatory cellular infiltrates indicating resolving malaria infection; however, the sinusoids were mildly packed with Kupffer cells which have engulfed haemozoin pigment and cellular debris and mild congestion was observed within the portal vein (Figure 4). Comparison with the untreated infected group administered with distilled water only showed no observable congestion in the blood vessels and there was significant reduction in the polymorphonuclear cellular infiltrates (Figure 4). Liver sections of infected rats treated with the aqueous extract of *M. indica* showed sinusoids moderately packed with parasite-laden Kupffer cells and cellular debris (Figure 5). There was a mild observable congestion in the portal vein with severe periportal infiltration by inflammatory cells, and various regions of the liver were packed with haemozoin. The morphology of the hepatocytes showed micro vesicular steatosis and the cytoplasm are vacuolated and infiltrated with fat. Few cells with engulfed parasites are also seen (Figure 5). Infected group administered with aqueous leaf extract of *C. papaya* showed normal central venules without congestion. There is no observable congestion of the hepatic portal vein and the hepatocytes appeared normal. Mild sinusoidal Kupffer cell activation is present; however, the central venules is not congested and the sinusoids appeared normal and not infiltrated, no pathological lesion seen (Figure 6) Liver sections of infected group treated with fruit extract of *C. limon* showed normal morphology of most of the hepatocytes, however, with a few engulfed parasites. There was no portal vein congestion, liver sinusoids were mildly packed



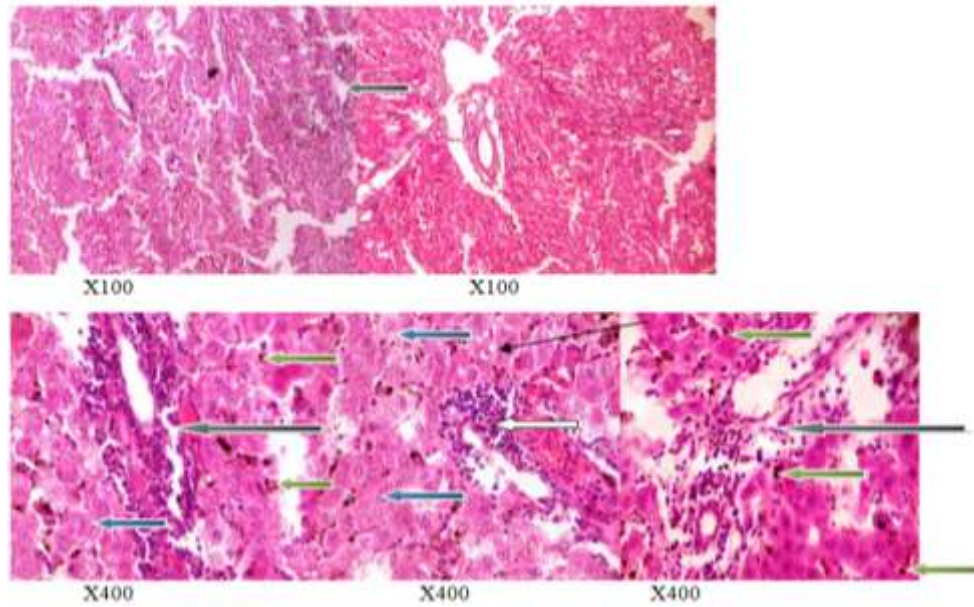
**Figure 1.** Photomicrograph of a liver section of uninfected rats administered with distilled water only showing normal central venules without congestion (white arrow), the morphology of the hepatocytes appear normal (blue arrow), the sinusoids appear normal and not infiltrated (slender arrow), no pathological lesion seen.

with Kupffer cells and haemozoin pigment was seen in various regions (Figure 7).

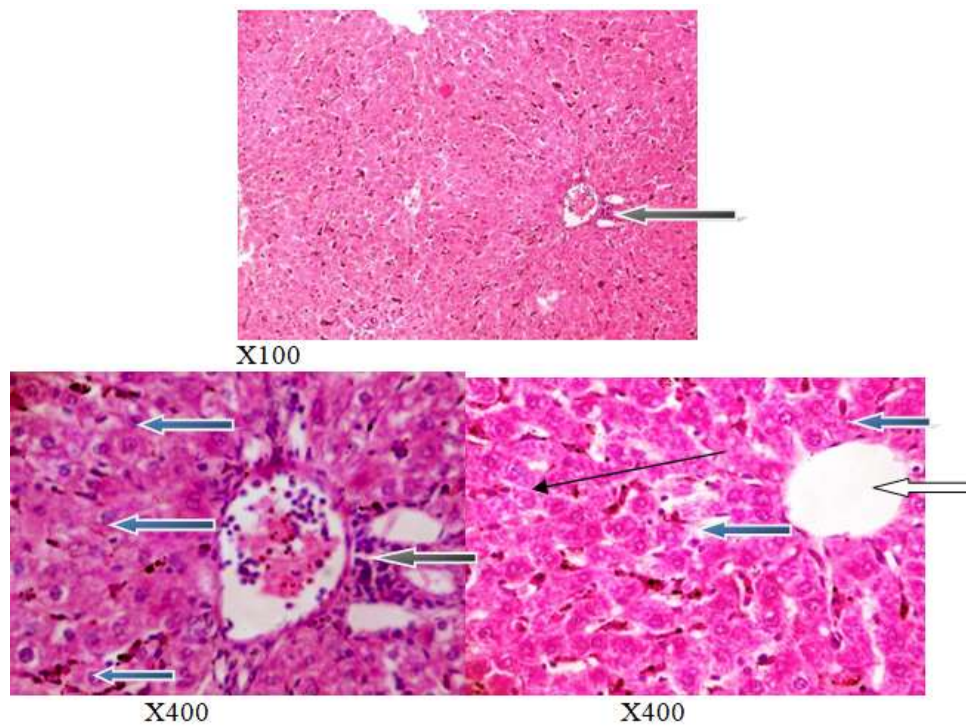
### Biochemical studies

Table 1 shows the mean and standard deviation of liver enzymes, albumin and total protein obtained in all groups. Table 2 shows the comparative analysis of liver enzymes, albumin and total protein obtained in all groups administered with the cocktail or either extract contained therein. There were statistical significant variations in ALT activity ( $P < 0.0001$ ) when all groups administered with the cocktail or either of the constituent extract were compared with the non-infected control group administered with distilled water only. AST activities and albumin values were observed to be normal among all groups ( $P > 0.05$ ), while rats administered with the cocktail showed increase in total protein values ( $P < 0.04$ ). Table 3 shows the comparative analysis of liver enzymes, albumin and total protein between the infected untreated groups and groups administered with the cocktail or either extracts showed no increase in albumin values and AST activity was observed to be

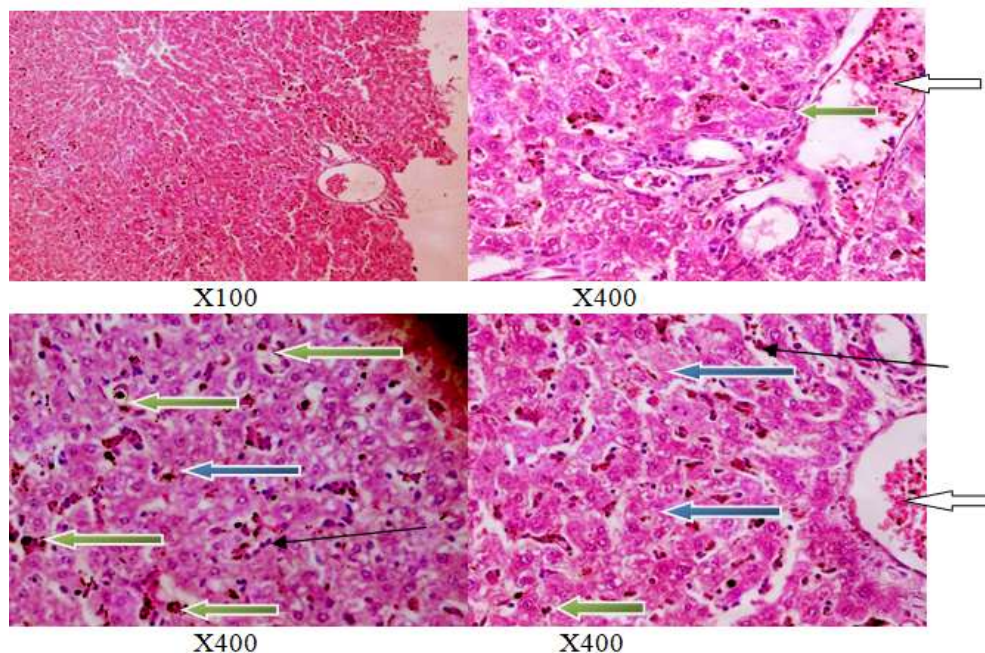
normal ( $P > 0.05$ ). However, there was a significant increase in ALT activities in rats administered with either of the cocktail ( $P < 0.0003$ ), *A. indica* ( $P < 0.0008$ ) or *C. papaya* ( $P < 0.01$ ). Significant increase was observed in the total protein values among rats administered with the cocktail ( $P < 0.04$ ) when compared with the infected untreated group administered with distilled water only. Comparative analysis of liver enzymes, albumin and total protein between the cocktail group and groups administered with various individual plant extract showed no significant increase in AST activity ( $P > 0.05$ ). However, significant increase in ALT activity ( $P < 0.0001$ ) was observed in other groups. Significant increase was observed in the groups administered with *A. indica* ( $P < 0.03$ ) and in the total protein values of groups administered with aqueous leaf extract of *C. papaya* ( $P < 0.001$ ) shown in Table 4. In addition, comparative analysis of liver enzymes, albumin and total protein between groups administered with individual extracts showed no statistical significant increase in the albumin values and AST activities; however, there was variations in the ALT activities ( $P < 0.00001$ ) with the exception of rats administered with aqueous leaf extract of *M. indica* or *C. limon* ( $P > 0.05$ ) while increase in total protein



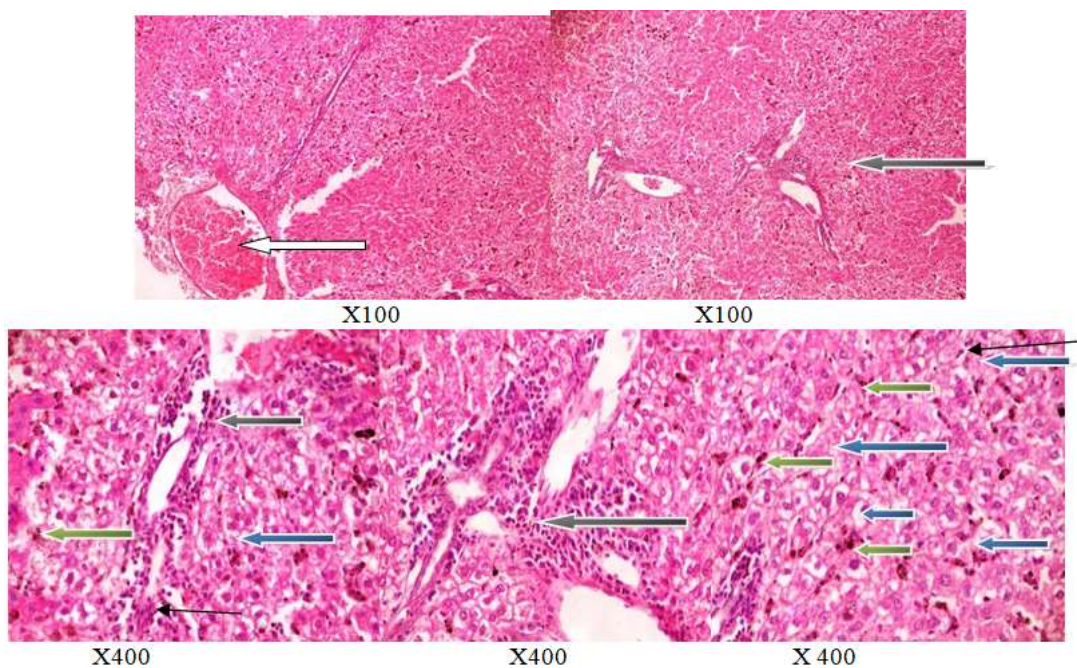
**Figure 2.** Photomicrograph of a liver section of Rat induced with *Plasmodium berghei* showing very poor architecture. Sinusoids are densely packed with malaria pigments (haemozoin) and cellular debris engulfed by Kupffer cells, there is severe portal triaditis; the portal tracts show periportal infiltration of inflammatory cells with mild congestion of the portal vein (white arrow), some of the hepatocytes show foamy cytoplasm (blue arrow) and some show engulfed parasites (green arrow). The sinusoids appear normal and not infiltrated (slender arrow).



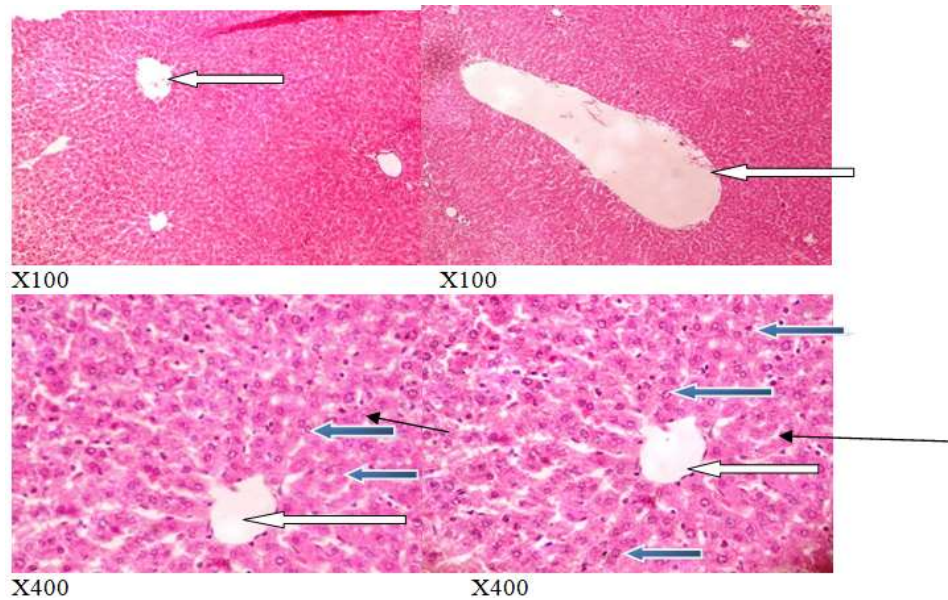
**Figure 3.** Photomicrograph of a rat liver infected with *P. berghei* and treated with the herbal mixture (Cocktail) showing sinusoids mildly packed with parasite laden Kupffer cells, normal central venules without congestion (white arrow), however, the portal tract show mild infiltration of inflammatory cells-mild portal triaditis (black arrow), the morphology of the hepatocytes appear normal (blue arrow), the sinusoids appear normal and not infiltrated (slender arrow).



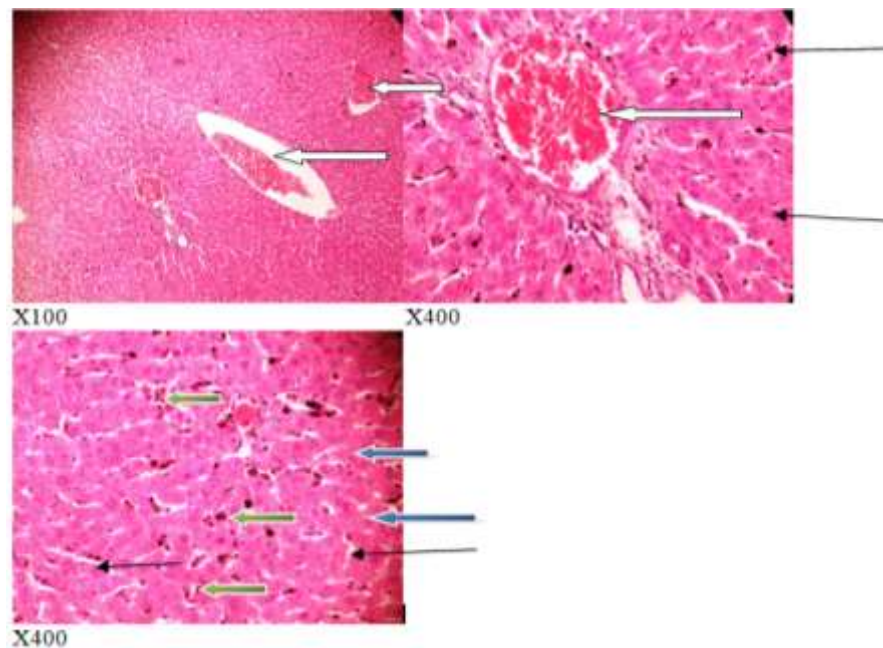
**Figure 4.** Liver section of Rat induced with *P. berghei* and treated with 10 mg/kg of aqueous leaf extract of *A. indica* showing sinusoids mildly packed with Kupffer cells which have engulfed haemozoin pigments and cellular debris. There is mild congestion within the portal vein (white arrow), the morphology of the hepatocytes show vacuolated and spongy-like cytoplasm (blue arrow) as well as engulfed parasites, the sinusoids are mildly infiltrated (slender arrow).



**Figure 5.** Rat liver infected with *P. berghei* and treated with *M. indica* showing sinusoids moderately packed with Kupffer cells laden parasites and cellular debris, mild congestion within the portal vein (white arrow) severe periportal infiltration by inflammatory cells (black arrow), the morphology of the hepatocytes show microvesicular steatosis; the cytoplasm is vacuolated and infiltrated by fat (blue arrow). Few cells with engulfed parasites are seen (green arrow).



**Figure 6.** Rat liver infected with *P. berghei* and treated with *C. papaya* showing mild sinusoidal Kupffer cell activation. The central venules is not congested (white arrow), the morphology of the hepatocytes appear normal (blue arrow), the sinusoids appear normal and not infiltrated (slender arrow), no pathological lesion seen.



**Figure 7.** Rat liver infected with *P. berghei* and treated *C. limon* showing, mildly packed Kupffer cells, the portal vein is mildly congested (white arrow), the morphology of most of the hepatocytes appear normal (blue arrow) while other few show engulfed parasites (green arrow), the sinusoids appear normal (slender arrow).

values was observed in rats administered with either *M. indica* or *C. papaya*  $<0.002$  and groups administered with *C. papaya* or *C. limon* ( $P < 0.02$ ) (Table 5).

### Phytochemical studies

The results obtained from the phytochemical analysis of

**Table 1.** Mean and standard deviation of liver enzymes, albumin and total protein obtained in all groups.

Parameter	Non-Infected control	Infected untreated group	Cocktail	<i>A. indica</i>	<i>M. indica</i>	<i>C. Papaya</i>	<i>C. limon</i>
AST (U/L)	83±6.8	92±9.0	91±8.0	83±21.0	93±15.0	90±16	96±23.0
ALT (U/L)	28±0.8	29±1.4	47±1.9	51±0.5	33±2.7	39±2.6	33±1.5
ALB (g/dl)	4.1±0.1	4.0±0.2	4.0±0.1	4.2±0.1	4.1±0.1	4.1±0.2	4.1±0.1
TP (g/dl)	6.3±0.3	6.3±0.2	6.5±0.1	6.4±0.3	6.5±0.1	6.3±0.1	6.4±0.1

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALB: albumin; TP: total protein.

**Table 2.** Comparative analysis of the liver enzymes, albumin and total protein obtained in all groups administered with the cocktail or various individual extracts.

Parameter		Non-Infected control vs. Infected untreated group	Non-Infected control vs. Cocktail	Non-Infected control vs. <i>A. indica</i>	Non-Infected control vs. <i>M. indica</i>	Non-Infected control vs. <i>C. papaya</i>	Non-Infected control vs. <i>Citrus limon</i>
AST (U/L)	"t"	0.10	-1.77	0.04	-1.37	-0.87	-1.18
	"p"	0.17	0.06	0.48	0.11	0.21	0.14
ALT (U/L)	"t"	-0.43	20.68	-54.20	-3.79	-9.04	-7.09
	"p"	0.34	0.0001***	0.0001***	0.003**	0.0001***	0.0005***
ALB (g/dl)	"t"	1.12	1.58	-1.0	0.41	0.00	1.0
	"p"	0.15	0.08	0.17	0.35	0.5	0.17
TP (g/dl)	"t"	-0.14	-1.92	-0.69	1.50	0.36	-0.77
	"p"	0.45	0.04*	0.26	0.09	0.36	0.18

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALB: albumin; TP: total protein. \*Values are considered statistically significant. (\*p<0.05) relative to the non-infected control group. t= (student's t-test).

*M. indica*, *C. papaya*, *A. indica* and *C. limon* extract showed the presence of some secondary metabolite like carbohydrate, alkaloid, flavonoid, tannins and saponin. The presence of these secondary metabolites in this extract may be responsible for the antiplasmodial activity of the various extract (Table 6).

## DISCUSSION

This study indicated that the herbal cocktail and individual extract contained therein has antiplasmodial effect on *P. berghei*. Histological studies of the infected rats showed recovery of the hepatocytes from congested black pigmentation (haemozoin pigment) as induced by these plant extracts; thus highlighting their importance in traditional treatment of malaria. Examination of the liver section clearly indicate that the administration of aqueous leaf extract of *C. papaya* and fruit extract of *C. limon* significantly reduced malaria associated with liver pathology when compared with the infected untreated group administered with distilled water only. Rats

administered with the cocktail and plants extracts of *A. indica* also showed ameliorative effect on malaria associated liver pathology; while the group administered with aqueous leaf extract of *M. indica* was observed to be least potent. Photomicrographs of the liver of the uninfected control group administered with distilled water appeared normal as no endemic inflammation of hepatocytes was observed. Liver changes in severe malaria reported by Whitten et al. (2011) were observed in the untreated infected group administered with distilled water only. Sinusoids was observed to be densely packed with malaria pigments (haemozoin) and cellular debris engulfed by Kupffer cells. Heavy infiltrates of inflammatory cells was also confirmed to be present as observed by Olayode et al. (2015), mild vascular congestion of the hepatic portal vein was observed. The sinusoids of the infected rats treated with the cocktail was observed to be mildly packed with parasite laden Kupffer cells and haemozoin, a mild inflammation was also observed around the portal zone. The hepatic portal vein was not congested. Infected rats treated with aqueous leaf extract of *A. indica* showed decreased inflammatory

**Table 3.** Comparative analysis of liver enzymes, albumin and total protein between the infected untreated group and groups administered with the cocktail and individual extracts.

Parameter		Infected untreated vs. Cocktail	Infected untreated vs. <i>A. indica</i>	Infected untreated vs. <i>Mangifera indica</i>	Infected untreated vs. <i>C. papaya</i>	Infected untreated vs. <i>Citrus limon</i>
AST (U/L)	"t"	-1.18	0.74	-0.07	0.22	-0.27
	"p"	0.14	0.24	0.47	0.42	0.40
ALT (U/L)	"t"	-5.36	-6.64	-0.98	-2.79	-1.21
	"p"	0.0003***	0.0008***	0.18	0.01*	0.13
ALB (g/dl)	"t"	0.00	-1.63	-0.87	-0.87	-0.69
	"p"	0.5	0.07	0.20	0.20	0.26
TP (g/dl)	"t"	-1.93	-0.60	-1.47	0.6	0.88
	"p"	0.04*	0.28	0.09	0.28	0.20

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALB: albumin; TP: total protein. \*Values are considered statistically significant. (\*p<0.05) relative to the infected untreated group. t= (student's t-test).

**Table 4.** Comparative analysis of liver enzymes, albumin and total protein between the cocktail group and groups administered with various individual plant extract.

Parameter		Cocktail vs. <i>A. indica</i>	Cocktail vs. <i>M. indica</i>	Cocktail vs. <i>C. papaya</i>	Cocktail vs. <i>C. limon</i>
AST (U/L)	"t"	0.86	-0.22	0.21	-0.41
	"p"	0.21	0.42	0.42	0.35
ALT (U/L)	"t"	-4.08	9.84	5.82	12.89
	"p"	0.002**	0.0001***	0.0002***	0.0001*
ALB (g/dl)	"t"	-2.14	-1.21	-1.05	-1.0
	"p"	0.03*	0.13	0.16	0.17
TP (g/dl)	"t"	0.81	0.77	4.32	1.43
	"p"	0.22	0.23	0.001*	0.09

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALB: albumin; TP: total protein. \*Values are considered statistically significant. (\*p<0.05) relative to the infected untreated group. t= (student's t-test).

cellular infiltrates in the liver sections; indicating that infection is resolving, which is in line with the observation of Anyaechie (2009) who documented that an active ingredient Irocin A isolated from Neem leaves is toxic to causative strains of malaria. Comparison with the infected untreated group administered with distilled water only showed no observable congestion in the blood vessels, and there was significant reduction in the polymorphonuclear cellular infiltrates.

The sinusoids of infected rats administered with aqueous extract of *M. indica* were moderately packed with parasite-laden Kupffer cells and cellular debris; mild observable congestion in the portal vein and various

regions of the liver were packed with haemozoin. The infected group treated with aqueous leaf extract of *C. papaya* had mild sinusoidal Kupffer cell activation and haemozoin pigments were observed in various regions of the liver section. Relatively few cellular infiltrates was observed to be present, indicating a resolving plasmodial infection. There was no observable congestion of the hepatic portal vein. This observation is in line with Longdet and Adoga (2017) who observed that extract of *C. papaya* leaf confers a dose dependent decrease on the level of parasitaemia when compared to the non-infected control group. This study corroborates Arise et al. (2012) who reported that extract of *C. papaya* gave



**Table 5.** Comparative analysis of liver enzymes, albumin and total protein between groups administered with individual extracts.

Parameter		<i>A. indica</i> vs. <i>M. indica</i>	<i>A. indica</i> vs. <i>C. papaya</i>	<i>A. indica</i> vs. <i>C. limon</i>	<i>M. indica</i> vs. <i>C. papaya</i>	<i>M. indica</i> vs. <i>C. limon</i>	<i>C. Papaya</i> vs. <i>C. limon</i>
AST (U/L)	"t"	-0.89	-0.60	-0.94	0.34	-0.23	-0.49
	"p"	0.20	0.28	0.19	0.37	0.41	0.32
ALT (U/L)	"t"	14.86	10.22	25.40	-3.71	-0.43	4.20
	"p"	0.0001**	0.00001*	0.0001*	0.003*	0.34	0.002*
ALB (g/dl)	"t"	1.26	0.45	1.77	-0.23	0.45	0.49
	"p"	0.12	0.27	0.68	0.41	0.33	0.32
TP	"t"	-0.42	1.16	0.00	4.02	0.79	-2.53
	"p"	0.34	0.14	0.5	0.002*	0.23	0.02*

AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALB: albumin; TP: total protein. \*Values are considered statistically significant. (\* $p < 0.05$ ) relative to the infected untreated group. t= (student's t-test).

**Table 6.** The phytochemical constituents of the aqueous leaf extract of *M. indica*, *C. papaya*, *A. indica* and *C. limon*.

Phytocompound	<i>M. indica</i>	<i>C. papaya</i>	<i>A. indica</i>	<i>C. limon</i>
Carbohydrate	++	+	++	+
Alkaloid	+	++	++	+
Flavonoid	+	+	++	+
Tannins	++	+	+	++
Saponin	+	+	+	+

+: Present in low concentration; ++: present in moderate concentration.

significant suppression ( $P < 0.05$ ) of parasitemia, following five days administration in established infection. Fatmawaty et al. (2017) further buttressed the antiplasmodial activity of *C. papaya* in *P. berghei* infected mice and histological examination of liver tissues of treated and untreated mice; which further supports the potential antimalaria of this plant, hence validating its traditional use in the treatment of malaria. Thomas et al. (2004), Fatmawaty (2013) and Okpe et al. (2016) also reported the antilarvicidal and antimalarial potential of fruit and leaf extract of *C. papaya*, both *in vitro* and *in vivo*. Liver sections of the group treated with *C. limon* showed reduced inflammatory cellular infiltrates, and the sinusoids were mildly packed with Kupffer cells and haemozoin pigment was seen in various regions. There was no portal vein congestion observed.

Observation in this study further corroborates Saganuwan et al. (2014) who indicated that many Nigerian plants can be used for the treatment of malaria as the groups administered with the cocktail or either extracts of *A. indica*, *M. indica*, *C. papaya* or *C. limon* and they showed reduction in the liver pathology associated with malaria infection. The leaves used in this study (*C. papaya*, *A. indica*, *M. indica*) showed the presence of

phytochemical constituents, including flavonoid, alkaloid and tannin which compounds are potentially used for various treatments such as herbal remedies (Longdet and Adoga, 2017). This study corroborates the observation of some studies (Dhar et al., 1996; Mulla and Su, 1999; Nathan et al., 2005) that showed that azadirachtin and other limonoids available in neem extracts are active on malaria vectors. Furthermore, observation in these study are in line with Osanaiya et al. (2013) who confirmed that antimalarial activity of *A. indica* leaf extract in *P. berghei* infected albino mice.

Biochemical studies indicated that the cocktail and individual extracts do not alter the AST activity in the infected rats administered with the cocktail or that of the constituent extracts contained therein when compared with the non-infected group administered with distilled water only. However, there was an observable increase in the activity of ALT in the rats treated with the cocktail or that of the extract. Rats administered with aqueous leaf extract of *M. indica* showed a reduction in ALT activity compared to those administered with the cocktail or either of the extracts of *A. indica*, *C. papaya* or *C. limon*. Thus, the result obtained in this study agrees with the findings of Olayode et al. (2017) who observed that

*M. indica* has an ameliorative effect against hepatocellular injury. There was variation in the AST among the rats in the different group but no significant increase was observed in the enzyme activity ( $p > 0.05$ ). Also, there was no significant change in albumin levels  $P > 0.05$  and total protein values among groups administered with *A. indica*, *M. indica*, *C. papaya* and *C. limon* were observed to be normal, while rats administered with the cocktail showed a significantly raised albumin level. AST activity in the infected groups administered with cocktail or that of the extracts remained normal when compared with the infected group administered with distilled water only; there was also no significant difference in the ALT activity among rats in the groups treated with *M. indica* or *C. limon* when compared with the infected untreated group administered with distilled water.

Additionally, no difference in the total protein and albumin values were observed in test groups compared to the untreated infected group administered with distilled water; however, the group administered with the cocktail showed an elevated total protein concentration. Comparison of *A. indica*, *M. indica*, *C. papaya* and *C. limon* extracts with cocktail group showed no significant difference in the AST activity. Notwithstanding, an increase in ALT activity in various groups administered with either of the extract when compared to the group administered with the cocktail was observed. No observable difference in the albumin level between the infected rats administered with the cocktail and groups administered with either of the plant extracts, with the exception of the groups administered with the aqueous leaf extract of *A. indica*, showed an increased level of albumin when compared to the group administered with cocktail.

Comparative analysis of total protein values among infected rats in the group administered with cocktail and infected rats administered with either extracts of *A. indica*, *M. indica*, *C. papaya* or *C. limon* showed no significant difference, with the exception of the rats in the group treated with aqueous leaf extract of *C. papaya* that showed an increase in total protein value when compared with the group administered with the cocktail. No observable difference in the AST activity between various individual extracts when statistically compared with one another. Significant elevation of ALT activity induced by individual extracts was observed.

In addition, increased activity of ALT enzyme in the groups administered with *M. indica*, *C. papaya* and *C. limon* when compared with the groups treated with aqueous leaf extract of *A. indica* was also observed. Furthermore, there was increase in ALT activity in groups treated with *C. papaya* upon comparison with the groups administered with *M. indica*; while the activity in the group treated with *C. limon* was observed to be insignificant ( $P > 0.05$ ). Increase in ALT activity occurred in groups administered with *C. limon* fruit extract when compared with the group administered with aqueous leaf extract of

*C. papaya*; while albumin levels among the various groups treated with various extracts showed no significance. There was increase in the total protein concentration in the group treated with *C. papaya* compared to the group treated with *M. indica*. Increase in total protein values in the groups treated with *C. limon* when compared with the groups administered with aqueous leaf extract of *C. papaya* was as well observed.

## Conclusion

This study indicated that the cocktail and individual plant extracts contained therein possess antiplasmodial effect by inducing recovery of hepatic cells from congested black pigmentation (haemozoin pigment) and reduction in malaria associated liver pathology. Thus, observation in this study gave credence to the traditional use of the plants for the treatment of malaria.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## REFERENCES

- Abass AY, Muhammad I, Bilbis LS, Saidu Y, Onu A (2017). *In vitro* antimalarial activity of some Nigerian medicinal plants. *Journal of Pharmacognosy and Phytochemistry* 6(6):885-888
- Aiyelaja AA, Bello OA (2006). Ethnobotanical potentials of common herbs in Nigeria: A case study of Enugu state. *Education Research and Reviews* 1(1):16-22.
- Ajala TO, Igwilo CI, Oreagba IA, Oluwatoyin, Odeku OA (2011). The antiplasmodial effect of the extracts and formulated capsules of *Phyllanthus amarus* on *Plasmodium yoelii* infection in mice. *Asian Pacific Journal of Tropical Medicine* 4(4):283-287.
- Akin-Osaniye BC, NokAJ, Ibrahim S, Inuwa HM, Onyike E, AmLabu E, Haruna E (2013). "Antimalarial effect of Neem leaf and Neem stem bark extracts on *Plasmodium berghei* infected in the pathology and treatment of malaria". *International Journal of Research in Biochemistry and Biophysics* 3(1):7-14.
- Al-Snafi AE (2016). Nutritional value and pharmacological importance of citrus species grown in Iraq. *IOSR Journal of Pharmacy* 6(8):76-108
- Anjum V, Ansari SH, Naquvi KJ, Arora P, Ahmad A (2013). Development of quality standards of *Carica papaya* Linn. Leaves. *Scholars Research Library* 5(2):370-376.
- Anyaehe UB (2009). Medicinal properties of fractionated acetone/water neem (*A. indica*) leaf extract from Nigeria: A review. *Nigerian Journal of Physiological Sciences* 24:157-159.
- Arise RO, Malomo SO, Lawal MM (2012). Comparative antimalarial and toxicological effects of artemisinin with methanolic extract of *C. papaya* leaves and bark of *Alstonia broonai* in animal models. *Advances in Natural and Applied Sciences* 6(2):116-123.
- Arzoo and Parina K (2017). A review on plants having antimalarial activity. *International Journal of Universal Pharmacy and Bio Sciences* 6(3):142-158.
- Avwioro OG (2014). *Histochemistry and tissue pathology; Principles and Techniques*. 3<sup>rd</sup> Edition. Society for Cellular Pathology Scientist of Nigeria pp. 97-98.
- Dhar RH, Dawar S, Garg S, Basir F, Talwar GP (1996). "Effect of volatiles from neem and other natural products on gonotrophic cycle and oviposition of *anopheles stephensi* and *An. Culicifacies* (Diptera: Culicidae)". *Journal of Medical Entomology* 33(2):195-201.
- Ediririweera MK, Tennekoon KH, Samarakoon SR (2017). A review on ethnopharmacological applications, pharmacological activities, and bioactive compounds of *Mangifera indica* (Mango) Evidence-Based

- Complementary and Alternative Medicine pp. 1-24.
- Ene AC, Atawodi SE (2012). Ethnomedicinal survey of plants used by the Kanuris of North-eastern Nigeria. *Indian Journal of Traditional Knowledge* 11(4): 640-645.
- Etuk EU, Bello SO, Isezuo SA, Mohammed BJ (2010). Ethnobotanical survey of medicinal plants used for the treatment of Diabetes mellitus in the north western region of Nigeria. *Asian Journal of Experimental Biological Sciences* 1(1):55-59.
- Evans WC (2002). *Trease and Evans Pharmacognosy*, 15<sup>th</sup> Edition. London: W.B. Sanders. pp. 183-393.
- Fatmawaty F, Astuti H (2013). Antimalarial activity of *Delonix regia* on mice with *Plasmodium berghei*. *Journal of Natural Products* 6: 61-66.
- Fatmawaty F, Rosmalena R, Amalia A, Syafitri I, Prasasty VD (2017). Antimalarial effect of flamboyant (*Delonix regia*) bark and papaya (*C. papaya* L) leaf ethanolic extracts against *Plasmodium berghei* in mice. *Biomedical and Pharmacology Journal* 10(3):1081-1089.
- Federal Ministry of Health (2015). National Malaria Elimination Programme, FMOH. Federal republic of Nigeria. Abuja, Nigeria.
- Gbolahan WA, Titus AOI, Isiaka AA, Gbolahan-Ayoade EE (2014). Composition of some traditional malaria remedies and their antiplasmodial effects on (*Plasmodium berghei*). *International Journal of Scientific Research Publications* 4(3):1-8.
- Grant GH (1987). *Amino Acids and Proteins*. In: *Fundamentals of Clinical Chemistry*, Tietz NW, 3<sup>rd</sup> edition, Philadelphia, USA: WB Saunders Company pp. 328-329.
- Ibrahim HA, Imam IA, Bello AM, Umar U, Muhammad S, Abdullahi SA (2012). The potential of Nigerian medicinal plant as antimalarial agent: A review. *International Journal of Science and Technology* 2(8):600-605.
- Idowu OA, Soniran OT, Ajana O, Aworinde DO (2010). Ethnobotanical survey of antimalarial plants used in Ogun State, Southwest Nigeria. *African Journal of Pharmacy and Pharmacology* 4(2):55-60.
- Innocent AE, Aniekan IP, Aquaisi NA (2017). Histopathological effect of *Nauclea latifolia* ethanolic leaf extract and Artemether/Lumefantrine on the hippocampus of *P. berghei*-infected mice. *International Journal of Brain and Cognitive Sciences* 6(1):9-16.
- Ivanova D, Genova D, Chervenkov T, Yankova T (2005). Polyphenols and antioxidant capacity of Bulgarian medicinal plants. *Journal of Ethnopharmacology* 96(1-2):145-150.
- Iyamah PC, Idu M (2015). Ethnomedicinal survey of plants used in the treatment of malaria in Southern Nigeria. *Journal of Ethnopharmacology* 173:287-302.
- Laudicina VA, Palazzolo E, Germanò MA (2013). Current and Potential Use of Citrus Essential Oils. *Current Organic Chemistry* 17:3042-3049
- Longdet I, Emmanuel AA (2017). Effect of Methanolic leaf extract of *C. papaya* on *Plasmodium berghei* infection in Albino Mice. *European Journal of Medicinal Plants* 20(1): 1-7.
- Makni M, Jemai R, Kriaa W, Chtourou Y, Fetoui H (2018). *Citrus limon* from Tunisia: Phytochemical and physicochemical properties and biological activities. *BioMed Research International* pp. 1-8.
- Melariiri P, Campbell W, Etusim P, Smith P (2012). *In vitro* antiplasmodial activities of extracts from five plants used singly and in combination against *Plasmodium falciparum* parasites. *Journal of Medicinal Plants Research* 6(47):5770-5779.
- Mulla MS, Su T (1999). Activity and biological effects of Neem products against arthropods of medical and veterinary importance. *Journal of the American Mosquito Control Association* 15 (2):133-152.
- Nathan SS, Kalaivani K, Muruga K (2005). Effects of Neem limonoids on the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Acta Tropica* 96(1):47-55.
- Nayan V, Onteru SK, Singh D (2017). *Mangifera indica* flower extract mediated biogenic green gold nanoparticles: Efficient nanocatalyst for reduction of 4-nitrophenol. *Environmental Progress and Sustainable Energy* 37(1):283-294.
- Noronha M, Guleria S, Jani D, George LB, Highland H, Subramanian RB (2018). Ethnobotanical database based screening and identification of potential plant species with antiplasmodial activity against chloroquine-sensitive (3D7) strain of *Plasmodium falciparum*. *Asian Pacific Journal of Tropical Biomedicine* 8(2):92-97.
- Odugbemi TO, Akinsulire OR, Aibinu IE, Fabeku PO (2007). Medicinal plants useful for malarial therapy in Okeigbo, Ondo State, Southwest Nigeria. *African Journal of Traditional, Complementary and Alternative Medicine* 4(2):191-198.
- Okere SO, Janet OS, Eunice O, Moses DA, Mercy OS (2014). Antiplasmodial activity of aqueous leaf extract of *Cymbopogon citratus* against *Plasmodium falciparum* infected rats. *American Journal of Biomedical and Life Sciences* 2(3):60-64 available at [www.sciencepublishinggroup.com/j/ajbls](http://www.sciencepublishinggroup.com/j/ajbls)
- Okpe O, Nathan H, Joseph I, Vicent AU, Stanley IR, Okoduwa, Omiagocho TI (2016). Antimalarial potential of *C. papaya* and *Vernonia amygdalina* in mice infected with *Plasmodium berghei*. *Journal of Tropical Medicine* pp. 1-6.
- Olayode AA, Ofusori DA, Ogunniyi TA, Olusola SS, Bejide RA, Adelodun ST (2017). Modulatory role of aqueous leave extracts of *M. indica* (Linn) on liver function test in plasmodium - infected albino mice. *Anatomy Physiology and Biochemistry International Journal* 2(2): 001-005. <http://dx.doi.org/10.19080/APBIJ.2017.02.555584>
- Olayode AA, Ofusori DA, Ogunniyi TA, Olusola SS (2015). Histomorphological studies of the liver of Plasmodium-infected albino mice after administration of aqueous leaf extract of *M. indica* (Linn.). *Anatomy* 9(3).
- Peter IT, Anatoli VK (1998). The current global malaria situation. *Malaria parasite biology, pathogenesis and protection*. Washington D.C, USA: ASM Press pp. 11-12.
- Randrianarivelosia M, Rasidimanana VT, Rabarison H, Cheplogoi PK, Ratsimbason M, Mulholland DA, Mauclère P (2003). Plants traditionally prescribed to treat tazo (malaria) in the eastern region of Madagascar. *Malaria Journal* 2:25.
- Reitman S, Frankel S (1957). A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. *American Journal of Clinical Pathology* 28:56-58.
- Saganuwan SA, Onyeyili PA, Etuk EU (2104). Immunomodulatory potentials and histopathological effects of aqueous leaf extracts of *Abrus precatorius* leaf in mus musculus. *Journal of Haematology Research* 1(2):54-62.
- Sofowora A (1982). *Medicinal plants and traditional medicine in Africa*. John Wiley and sons Ltd.
- Taek MM, Bambang PEW, Mangestuti A (2018). Ethnomedicinal plants used for the treatment of malaria in Malaka, West Timor. *Journal of Young Pharmacists* 10(2).
- Thomas EI, Vandebroek SS, Van Damme P (2009). Cultural significance of medicinal plant families and species among Quechua farmers in Apillapampa, Bolivia. *Journal of Ethnopharmacology* 122:60-67.
- Tietz NW (1995). *Clinical guide to laboratory tests*. 3<sup>rd</sup> Edition. Philadelphia USA: WB Saunders Company pp. 518-519.
- Titanji IV PK, Zofou D, Ngemenya MN (2008). The antimalarial potential of medicinal plants used for the treatment of malaria in cameroonian folk medicine. *African Journal of Traditional Complementary and Alternative Medicine* 5(3):302-321.
- Tiwari R, Amit KV, Sandip C, Kuldeep D, Shoor VS (2014). *Neem (Azadirachta indica)* and its potential for safeguarding health of animals and humans: A Review. *Journal of Biological Sciences* 14(2):110-123.
- Vaghasiya Y, Dave R, Chanda S (2011). Phytochemical analysis of some medicinal plants from western Region of India. *Research Journal of Medicinal Plant* 5(5):567-576.
- Vineet K, Upma B (2014). Structural changes in spleen architecture upon *Plasmodium berghei* (NK-65) infection in BALB/c mice. *Journal of Pharmacy and Biological Sciences* 9(4):16-20.
- Whitten R, Milner DAJr, Yeh MM, Kamiza S, Molyneux ME, Taylor TE (2011). Liver pathology in Malawian children with fatal encephalopathy. *Human Pathology* 42:1230-1239.
- World Health Organization (2005). *World Malarial Report*. Geneva. Malaria prevention and control. ISBN 92 4 159319 9 Available at: <http://rbm.who.int/wmr>
- World Health Organization (2014). *World Malaria Report*. Geneva. ISBN 978 92 4 156483 0. Available at [http://www.who.int/malaria/publications/world\\_malaria\\_report\\_2014/en/](http://www.who.int/malaria/publications/world_malaria_report_2014/en/)
- World Health Organization. (2016). *World Malaria Report*. Geneva. ISBN 978-92-4-151171-1 available at <https://creativecommons.org/licenses/by-nc-sa/3.0/igo>

Yogiraj V, Goyal PK, Chauhan CS, Goyal A, Vyas B (2014). *Carica papaya* Linn: an overview. *International Journal of Herbal Medicine* 2(5): 01-08.

*Full Length Research Paper*

# **Effect of aqueous extract of *Ocimum gratissimum* on acetaminophen induced renal toxicity in male wistar rats**

**Okerulu Linda<sup>1</sup>, Celestine Ani<sup>2\*</sup>, Choice Nworgu<sup>1</sup>, Igwe Uzoma<sup>3</sup>, Ugwuishi Emeka<sup>2</sup> and Nwachukwu Daniel<sup>1</sup>**

<sup>1</sup>Department of Physiology, College of Medicine, University of Nigeria, Enugu Campus, Nigeria.

<sup>2</sup>Department of Human Physiology, College of Medicine, Enugu State University of Science and Technology, Parklane, Enugu, Nigeria.

<sup>3</sup>Department of Physiology, Faculty of Basic Medical Sciences, Alex Ekwueme Federal University, Ndufu-Alike, Ebonyi State, Nigeria.

Received 15 September, 2018; Accepted 4 October, 2018

The need for an alternative means of managing renal toxicity has become necessary instead of kidney transplant. This study investigated the effect of aqueous extract of *Ocimum gratissimum* (AEOG) on acetaminophen (ACE) induced renal toxicity. Twenty-five male Wistar rats were assigned into five groups (1, 2, 3, 4, and 5) with five rats in each group. Group 1 served as the normal control and received normal saline as placebo. Group 2 served as the negative control and received 500 mg/kg of ACE orally for 21 days. Groups 3, 4 and 5 served as the treatment groups and received 100, 200 and 400 mg/kg AEOG in addition to 500 mg/kg ACE orally for 21 days. The animals were sacrificed on the 22nd day. Blood was collected for the biochemical analysis (serum creatinine, urea and electrolytes). The kidney was harvested, rinsed in 1% KCl and preserved in 10% formaldehyde solution for the histological examination. The phytochemical analysis indicates the presence of tannins, flavonoids, saponin, alkaloids, phenols, phlobatamin, anthraquinone, terpenoids, steroids, cardiac glycosides and absence of cardenolides and chalcones. Biochemical analysis showed significant increase in creatinine and urea levels in the negative control groups when compared with the normal control while there was no significant decrease in both creatinine and urea in the treatment groups when compared with the negative control. There was no significant difference ( $p > 0.05$ ) in the serum electrolytes ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$ ). The histological examination showed mild and moderate healing of the renal tissues in the treatment groups when compared with the negative control group which showed severely damaged renal tissues. Based on the aforementioned observations, it was concluded that AEOG possessed ameliorative effect in ACE induced renal toxicity.

**Key words:** Renal toxicity, acetaminophen, *Ocimum gratissimum*, rats, electrolytes.

## **INTRODUCTION**

Plants have been an old companion of man providing food, shelter, wealth and have helped in maintaining

\*Corresponding author. E-mail: [anicelestine2006@gmail.com](mailto:anicelestine2006@gmail.com). Tel: +2348034607689 or +2348159416345.

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

relatively good health by its preventive and curative potentials when properly utilized (Kumar et al., 2011). *O. gratissimum* (commonly known as scent leaf) is known as Nchuanwu or Ahuji in Igbo, Effirin in Yoruba, Daidoya in Hausa and Aramogbo in Edo (Ephrain et al., 2000). It is used mainly to flavor food and meat (Okigbo et al., 1977). It is known to contain alkaloids, tannins, phytates, flavonoids and oligosaccharides (Ijeh et al., 2004). The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhea, headache, and diseases of the eye (Adebolu et al., 2005). It has been shown to possess antibacterial activity (Ofokansi et al., 2003), antioxidant properties (Obob et al., 2008), antimicrobial and antihelminthic activities (Sofowora, 2008), hepatoprotective properties (Gbolade et al., 2009). There have been speculations that scent leaves may possess hypoglycemic activity in streptozotocin induced diabetic rats (Egesie et al., 2006).

The kidney is important for the regulation of body fluids and electrolytes. It is also a major excretory organ for waste substances like creatinine and urea (Nwangwu et al., 2015). Creatinine and urea are major catabolic products of carbohydrate and protein metabolism respectively. The reduction in creatinine concentration in the urine is indicative of impaired renal function (Smith et al., 1990). Accumulation of urea and creatinine in the serum is indicative of kidney impairment (Nisha et al., 2017). The excretion of body fluid with reduced amounts of potassium, sodium and water intake restriction, as well as excessive intake of potassium may result in rare conditions of hyperkalemia and hypernatremia, respectively (Kang et al., 2002). In spite of several scientific reports on *O. gratissimum*, there are couple of studies on *O. gratissimum* on its effect on the kidney (Ogundipe et al., 2017). The incidence of kidney failure or chronic kidney failure has doubled over the last 15 years (Arhgothoro et al., 2012). Most of the patients who suffer from kidney disease are not able to afford the cost of kidney transplant. *O. gratissimum* has been reported to possess medicinal properties and it is used in most local dishes/foods especially here in South East, Nigeria; there is need to investigate its effect on the kidney with an aim in establishing its usefulness or otherwise in preventing renal impairment.

Therefore, this work is aimed at investigating the effect of aqueous extract of *O. gratissimum* on acetaminophen induced renal toxicity in male Wistar rats using these renal biomarkers, serum creatinine level, serum urea level, serum electrolytes, and histology of the kidney.

## MATERIALS AND METHODS

### Collection of plant sample, identification and authentication

The fresh leaves of *O. gratissimum* were purchased from a local market in Enugu State. A sample of the leaf of this plant was identified and authenticated at the herbarium section of the Department of Plant Science and Biotechnology, University of

Nigeria Nsukka. A voucher specimen was deposited in the herbarium for further reference with no. (UNH350a).

### Preparation of extract

The aqueous extraction of the leaves of *O. gratissimum* was carried out according to the method of Ojo et al. (2013). The leaves were washed and air dried at room temperature, pulverized using a laboratory mechanical grinder and the fine powder obtained and stored until needed. Seven hundred grams of the powdered sample was extracted with 1 L of distilled water (via maceration) for 48 h. The mixture was decanted and filtered using sterile Whatman filter paper No. 1. The filtrate was concentrated to dryness using a water-bath at a temperature of 50°C giving a dark-green paste with a yield of 8.8%. The extract was later reconstituted such that 1 g of the paste was dissolved in 10 ml of normal saline to make up the concentration of the stock solution which was labeled appropriately and refrigerated at 4°C until required for use.

$$\% \text{ Yield} = \frac{\text{weight of extract}}{\text{weight of sample}} \times 100$$

$$\frac{61.8}{700} \times \frac{100}{1}$$

$$\% \text{ Yield} = 8.8\%$$

### Phytochemical screening

Phytochemical screening was carried out using the method of Ighodalo et al. (2012).

### Acute toxicity study

The modified method of Nirmala et al. (2012) was used to assess the acute oral toxicity of *O. gratissimum* leaves. Healthy Wistar rats weighing (140 to 180 g) were used for this purpose. Four doses of 500, 1000, 2000 and 4000 mg/kg of the extract were given to 4 groups containing 4 animals in each group. Single dose of the extract was administered orally to each animal. The animals were observed individually during the first 30 min and thereafter 24 h for a period of 14 days. Signs of toxicity, body weight, feed and water intake for each group was observed every day for 14 days. The extract was devoid of toxicity even at a dose of 4000 mg/kg by oral route in rats. Hence, 100, 200 and 400 mg/kg doses of the extract were selected for this experiment.

### Experimental design

Twenty five male Wistar rats with weights ranging from 100 to 200 g were obtained from the Animal House of Faculty of Basic Medical Sciences, University of Nigeria, Enugu Campus. The animals were acclimatized for two weeks at 12 h light/dark cycle at a normal room temperature of 27±0.05°C with the standard laboratory conditions. The animals were housed in clean well-ventilated, standard wire mesh cages which were cleaned daily. They were also fed with normal rat chow (Vital Feeds Ltd Jos, Plateau State) and clean tap water *ad libitum* and divided as follows:

- Group 1: Normal control and received normal saline as placebo;
- Group 2: Received 500 mg/kg of ACE orally for 21 days. (Negative control);
- Group 3: Received 100 mg/kg AEOG;
- Group 4: Received 200 mg/kg AEOG;
- Group 5: Received 400 mg/kg AEOG and 500 mg/kg ACE orally for

**Table 1.** Phytochemical analysis of *O. gratissimum* showed that tannins, flavonoids, saponins, alkaloids, phenols and reducing sugar were present while anthraquinones and steroids were absent.

Phytochemicals	Quantitative analysis (mg/100 g)
Tannins	210.07±0.09
Flavonoids	269.86±0.13
Saponin	5.88±0.1
Alkaloids	68.71±0.20
Phenols	0.00±0.00
Reducing Sugar	24.14±0.16
Anthraquinones	0.00±0.00
Steroids	0.00±0.00

**Table 2.** Biomarkers of kidney function.

Kidney biomarkers	Control	500 mg/kg ACE	100 mg/kg <i>O. gratissimum</i> + 500 mg/kg ACE	200 mg/kg <i>O. gratissimum</i> + 500 mg/kg ACE
Creatinine (umol/l)	0.65±0.11	0.91±0.14*	0.65±0.06	0.64±0.04
Urea (mmol/l)	16.92±5.52	20.18±7.71*	17.25±2.17	16.53±1.75

Each value represents the Mean ± Standard deviation. ACE: Acetaminophen. \*P<0.05 shows a significant difference.

21 days.

#### Histology of the kidney

After sacrificing the animal, the kidneys from the rats were quickly removed using a surgical blade to cut the rat open and fixed in 10% formal saline. Afterwards the tissues were processed into slides.

#### Ethical clearance

This study was obtained from the Research Ethics Committee of the College of Medicine, University of Nigeria, Enugu Campus. The protocol number is: 036/12/2017

#### Statistical analysis

All data were expressed as mean±standard deviation. Statistical package for social science (SPSS) version 20 was used for data analysis. One way analysis of variance (ANOVA) was used to determine the difference between the means of various groups. Value of P <0.05 was considered significant.

## RESULTS

### Phytochemical analysis

The phytochemical analysis showed that *O. gratissimum* contains various phytochemical such as tannins, flavonoids, saponins, etc. Elevation of urea and creatinine levels in the serum is taken as the index of

nephrotoxicity (Partwardhan et al., 2005). Aqueous extract of *O. gratissimum* non-significantly decreased serum creatinine and urea level in a dose-dependent manner. The highest dose showed greater improvement in renal function which was also confirmed by the histological analysis where there was mild healing in the group treated with *O. gratissimum* 100 mg/kg, moderate healing in the groups treated with 200 and 400 mg/kg *O. gratissimum* (Table 1). There was a significant increase (p<0.05) in creatinine level in the negative control group when compared with the normal control group. However, there was non-significant decrease in creatinine level in the treated groups when compared with the negative control. There was a significant increase in urea level in the negative control group when compared with the normal control group. However, there is no significant difference (P>0.05) in urea level in the treated groups when compared with the negative control group (Table 2). Phytochemical are known to perform different biological activities. This ameliorative effect of *O. gratissimum* may be due to the presence of flavonoids which exhibits antioxidant activity (Abdullahi et al., 2012). There was no significant change in the serum electrolytes (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup>) which indicates that *O. gratissimum* maintained electrolyte balance (Table 3).

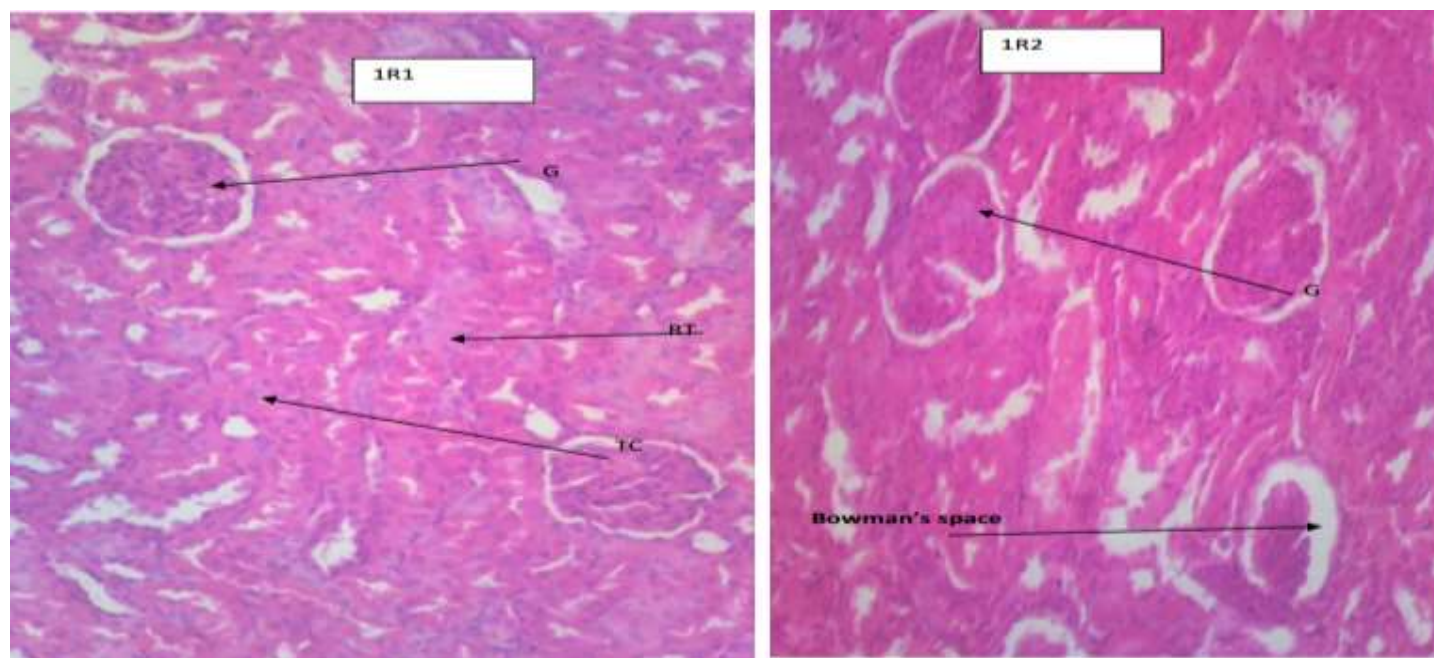
### Histological analysis

However, there are clumping of the renal tubules (CRT) and mild tubular dilation (MTD) and mild fatty changes

**Table 3.** Serum electrolytes.

Serum electrolytes	Control	500 mg/kg ACE	100 mg/kg <i>O. gratissimum</i> + 500 mg/kg ACE	200 mg/kg <i>O. gratissimum</i> + 500 mg/kg ACE
Na <sup>+</sup> (mmol/l)	136.13±2.22	130.53±3.74	134.25±2.05	134.53±1.64
K <sup>+</sup> (mmol/l)	5.76±0.29	5.47±0.64	6.17±0.92	6.17±0.92
Cl <sup>-</sup> (mmol/l)	96.93±1.64	91.58±3.82	93.28±1.13	93.28±1.13
HCO <sub>3</sub> <sup>-</sup> (mmol/l)	32.90±6.38	31.50±1.71	28.23±3.95	28.23±3.95

Values were expressed as Mean± Standard deviation. There was no significant difference ( $P>0.05$ ) in sodium (Na<sup>+</sup>), Potassium (K<sup>+</sup>), Chloride (Cl<sup>-</sup>) and Bicarbonate (HCO<sub>3</sub><sup>-</sup>).



**Figure 1.** Photomicrograph of group 1r1r2 control section of kidney (X400) (H/E) shows normal architecture with glomeruli (G), Bowman space (BS), renal tubules (RT) and tubular cell (TC).

(MFC). The result of histology study confirms an improvement in the histoarchitecture in groups treated with *O. gratissimum* that had moderate regeneration of the glomeruli, moderate regeneration of the tubular cell, mild and moderate healing of renal tissues when compared with the negative control group that had severe coagulative necrosis of glomeruli, tubular cell necrosis and severely damaged renal tissue. The improvement in histology was also dose dependent which may be due to the presence of tannins which protects kidneys from inflammation (Figures 1 to 4) (Just et al., 1998).

## Conclusion

The result of the present study has shown that the aqueous extract of *O. gratissimum* exerted an

ameliorative effect on acetaminophen induced renal toxicity in a dose dependent manner. Thus consumption of *O. gratissimum* may protect the kidney from injury caused by some toxic agents.

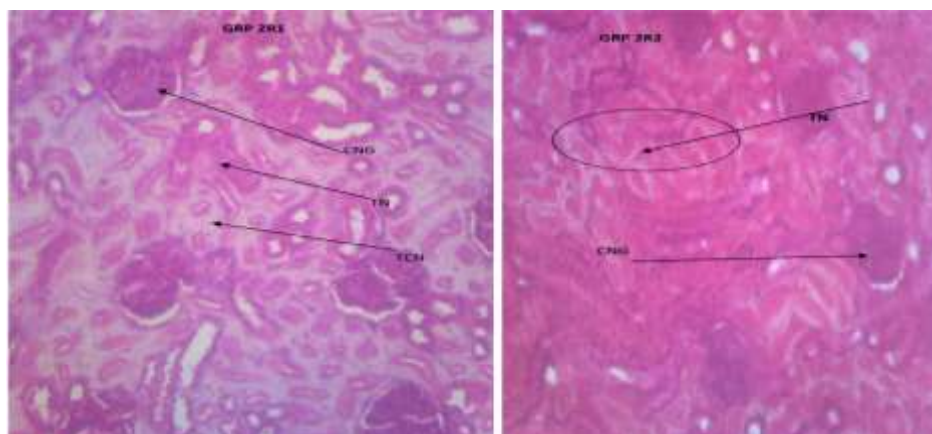
## ACKNOWLEDGEMENT

The authors sincerely acknowledged the effort of Prof. Nwachukwu Daniel who initiated and supervised this research work at the Prestigious University of Nigeria, Enugu Campus.

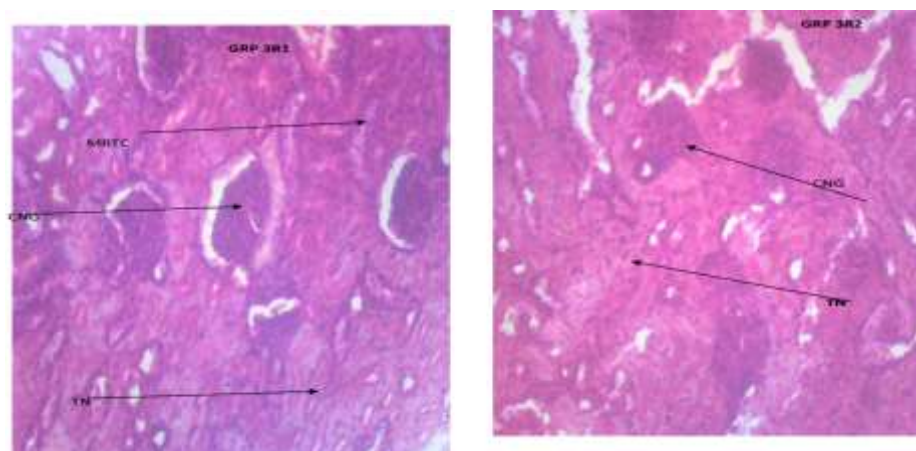
## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

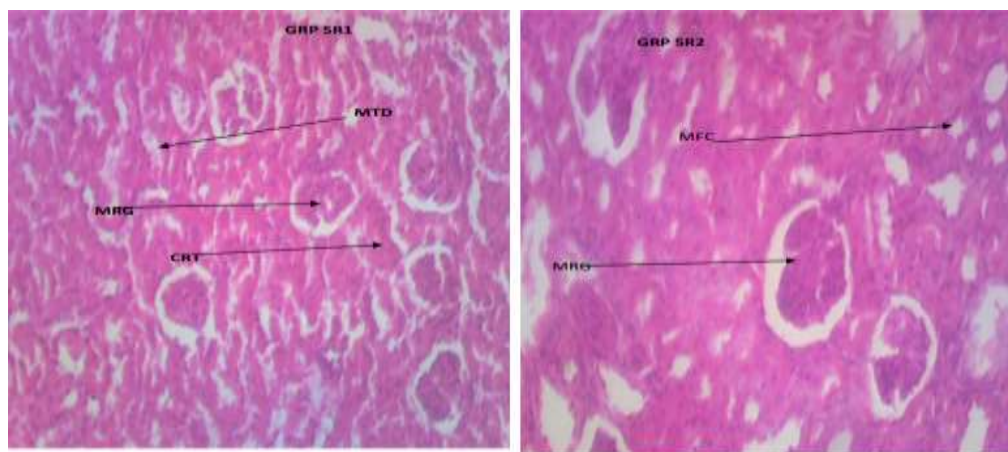




**Figure 2.** Photomicrograph of 3r1r2 section of kidney administered with 100 mg/kg of *Ocimum gratissimum* 1 h before inducing 500 mg/kg of acetaminophen for 21 days (X400) (H/E) shows mild healing with coagulative necrosis of glomeruli (CNG), tubular necrosis (TN) with moderate regeneration of the tubular cell (MRTC).



**Figure 3.** Photomicrograph of 4r1r2 section of kidney administered with 200 mg/kg of *ocimum gratissimum* 1 h before inducing 500 mg/kg of acetaminophen for 21 days (X400) (H/E) shows moderate healing with moderate regeneration of the glomeruli (MRG) and the renal tubules (RT). However there are fatty changes (MFC) and tubular cell necrosis (TCN)



**Figure 4.** Photomicrograph of group 5r1r2 section of kidney administered with 400mg/kg of *Ocimum gratissimum* 1 h before inducing 500 mg/kg of acetaminophen for 21 days (X400) (H/E) shows moderate healing with moderate regeneration of the glomeruli (MRG) and the renal tubules (RT).

## REFERENCES

- Abdullahi M (2012). Phytochemical constituents and antimicrobial and grain protectant activities of cloves basil (*ocimum gratissimum L.*) grown in Nigeria. *International Journal of Plant Research* 2(1):18
- Adebolu TT, Salau AO (2005). Antimicrobial activity of leaf extracts of *ocimum gratissimum* on selected diarrhea causing bacteria in south western Nigeria. *African Journal of Biotechnology* 4(7):682-684.
- Arhgooro EM, Anosike EO, Uwakwe AA (2012). *Ocimum gratissimum* aqueous extract enhances recovery in cisplatin-induced nephrotoxicity in albino Wistar rats. *Indian Journal of Drugs and Diseases* 1(5):129-142.
- Egesie UG, Adelaiye AB, Ibu JO, Egesie OJ (2006). Safety and hypoglycemic properties of aqueous leaf extract of *Ocimum gratissimum* in STZ-induced diabetic animals". *Nigerian Journal of Physiological Science* 21(1-2):31-35.
- Ephrain KD, Salami HA, Osewa TS (2000). Effect of aqueous extract of *Ocimum gratissimum* on biochemical parameters in rabbits. *African Journal Biomedical Research* 3(3):175-179.
- Gbolade A (2009). Inventory of antidiabetic plants in selected districts of Lagos state, Nigeria. *Journal of Ethnopharmacology* 121(1):135-139.
- Ighodalo OM, Agubiade JO, Kuti OA (2012). Evaluation of the chemical, nutritional, antimicrobial and antioxidant-vitamin profiles of *Ptilostigma thonningii* leaves (Nigeria species). *Research Journal of Medicinal Plants* 6 (7):537-543.
- Ijeh II, Njoku OU, Ekenze EC (2004). Medicinal evaluation of *xylopiia ethiopica* and *Occimum gratissimum*". *Journal of Medicinal and Aromatic Plant Science*, 26 (5):19-21.
- Just MJ, Recio MC, Giner RM, Cueller MV, Manez, S, Billa AR, Rios JI (1998). Anti Inflammatory activity of unusual lupine saponins from *Bupleurum fruticosens*. *Planta Medica* 64(05):404-407.
- Kang SK, Kim W, Oh MS (2002). Pathogenesis and treatment of hypernatremia. *Nephron* 92:14-17.
- Kumar VI, Padhy BM (2011). Protective effect of aqueous suspension of dried latex of *Calotropisprocera* against oxidative stress and renal damage in diabetic rats. *Biocell* 35(3):63-69.
- Nirmala M, Giriya K, Lakshman K, Divya T (2012). Hepatoprotective activity of *Musa paradisiaca* on experimental animal models. *African Pacific Journal of Biomedicine* 2(1):11-15.
- Nisha R, Srinivasa KSR, Thanga MK, Jagatha P (2017). Biochemical evaluation of creatinine and urea in patients with renal failure undergoing hemodialysis. *Journal of Clinical Pathology and Laboratory Medicine* 1(2):1-5.
- Nwangwu SCO, Josiah SJ, Offor CE, Ekhyame EC (2015). Evaluation of the effects of aqueous extracts of *Ocimum gratissimum* on some kidney function parameters in normal male rats. *Global Journal of Pharmacology* 9(1):72-76.
- Oboh G (2008). Antioxidant Potential of *ocimum gratissimum* and *Ocimum canum* leaf polyphenols and protective effects on some pro-oxidants induced lipid peroxidation in rat brain. An *in vitro* study. *Anal Journal of Food Technology* 3(5):325-334.
- Ofokansi KC, Adikwu MU, Esimone CO, Nwodo NM (2003). Antibacterial activity of the leaf extract of *Ocimum gratissimum* (famlabiateae). *Journal of Biological Research and Biotechnology* 9(1):35-37.
- Ogundipe DJ, Akomolafe RO, Sanusi AA, Imafidon CE, Olukiran OS, Oladele AA (2017). *Ocimum gratissimum* Ameliorates gentamicin-induced kidney injury but decreases creatinine clearance following sub-chronic administration in rats. *Journal of Evidence Based Complementary Alternative Medicine* 22(4):592-602.
- Ojo OA, Oloyede OI, Olanrewaju OI, Ojo AB, Ajiboye BO, Onikanni SA (2013). Toxicity studies of the crude aqueous leaves extracts of *Ocimum gratissimum* in albino rats. *IOSR Journal of Environmental Science, Toxicology and Food Technology* 6:434-439.
- Ogigbo EN (1977). Neglected plants of horticultural and nutritional importance in traditional farming systems of tropical Africa. *Acta Horticulture* 53:31-150.
- Partwardhan B, Warude D, Pushpangadan P, Bhatt N (2005). Ayurveda and traditional Chinese medicine: A comparative overview. *Evidence Based Complementary and Alternative Medicine* 2(4):465-473.
- Smith CL, Hampton EM (1990). Using estimated creatinine clearance for individualizing drug therapy: A reassessment DICP pp. 1185-1190.
- Sofowora A (2008). Medicinal plants and traditional medicine in Africa. 3<sup>rd</sup> Edn. Spectrum Books Limited Ibadan, Nigeria pp. 199-204
- Sofowora A (2008). Medicinal plants and traditional medicine in Africa. 2<sup>nd</sup> Edn Spectrum Books Ltd Ibadan Nigeria ISBN-13:9789782462190, P 289.

## Related Journals:

