International Journal of Biochemistry Research & Review
10(4): 1-9, 2016, Article no.IJBCRR.24341
ISSN: 2231-086X, NLM ID: 101654445

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antiplasmodial/Antimalarial Effect of Ethanol Extracts of Leaves of *Vernonia amygdalina* and *Gongronema latifolium* on the Activity of Catalase in *Plasmodium berghei*-Parasitized Mice

Ekpo Daniel Emmanuel[1*] and Ekanemesang Udoudo Moses[2]

[1]Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Department of Biochemistry, Obong University, Obong Ntak, P.O.Box 25, Abak, Akwa Ibom State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author EUM designed the study, wrote the protocol and supervised the work. Authors EDE and EUM carried out all laboratories work and performed the statistical analysis. Author EDE managed the analyses of the study. Author EDE wrote the first draft of the manuscript. Author EDE managed the literature searches and edited the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2016/24341
Editor(s):
(1) Richard A. Manderville, Departments of Chemistry and Toxicology, University of Guelph, Canada.
Reviewers:
(1) Umit Tursen, Mersin University, Turkey.
(2) Daniel Arrieta-Baez, National Polytechnic Institute, Mexico.
Complete Peer review History: http://sciencedomain.org/review-history/13411

Original Research Article

Received 15th January 2016
Accepted 6th February 2016
Published 24th February 2016

ABSTRACT

Aim: The study was designed to examine the effect of ethanollic leaf extracts of *Vernonia amygdalina* (VA) and *Gongronema latifolium* (GL) on serum catalase activity in mice exposed to malaria parasite (*Plasmodium berghei*) infection.
Study Design: Forty (40) adult mice weighing 26-30 g were divided into eight (8) Groups of five (5) mice each. Group 1 and 2 served as the normal control (NC) and parasitized control (PC) respectively, both of which received placebo treatment. All the other groups were parasitized and

**Corresponding author: E-mail: ekpodaniele@gmail.com;*

treated with the extracts via oro-gastric intubation for seven (7) days. Group 3 and 4 were treated with 100 mg/kg and 200 mg/kg of VA respectively. Group 5 and 6 were treated with 100 mg/kg and 200 mg/kg of GL respectively. Group 7 and 8 were treated with combination of VA and GL at 100 mg/kg of VA+100 mg/kg of GL and 150 mg/kg of VA+50 mg/kg of GL respectively. Serum catalase activity was determined from blood samples obtained on the 8th day after fasting overnight.

Results: The result showed that the administration of the extracts significantly ($p<0.05$) lowered catalase activity when compared with Group 2 except that of group 4 (200 mg/kg of VA) and 5 (100 mg/kg of GL). There was a significantly lower concentration of catalase in the groups treated with the combination therapy of VA and GL when compared to the untreated Group 2.

Conclusion: The present study revealed that both extracts of *Vernonia amygdalina* and *Gongronema latifolium* had a positive effect on serum catalase activity on parasitized mice. However, combination of both extracts gave a much positive effect. Hence we tend to draw conclusion that combination of both extract may serve as a useful intervention in the management and treatment of malaria parasite infection and would contribute to the development of potential antimalarial drug.

Keywords: Vernonia amygdalina; Gongronema latifolium; malaria parasite; catalase; oxidative stress.

1. INTRODUCTION

Malaria is an infectious disease caused by a parasite known as plasmodium which infects red blood cells. It is characterized by cycles of chills, fever, pain and sweating [1]. Historical records suggest that malaria has infected human since the beginning of mankind. Currently, about 2 million deaths per year worldwide are due to plasmodium infections. The majority occur in children under 5 years of age in sub Saharan African countries. There are about 400 million new cases per year worldwide. Most people diagnosed in the U.S. obtained their infection outside the country usually, while living or travelling through an area where malaria is endemic [1]. Of the four common species of plasmodium that causes malaria, the most serious types is *Plasmodium falciparium* which is of life threatening, however the three other common species (*Plasmodium vivax, Plasmodium ovale and Plasmodium malariae*) are generally less serious, with another relatively new specie (*Plasmodium knowlesi*) that naturally infects only long-tailed and pigtail macaque monkeys and has been recognized to be the cause of zoonotic malaria in humans [2]. It is possible to be infected with more than one species of plasmodium at the same time [1].

Plasmodium berghei is a unicellular protozoan parasite that causes malaria in mammals (rodents) other than humans. It is one of the four plasmodium species that have been described in African murine rodents, others been *Plasmodium chaubausi*, *Plasmodium vinckei* and *Plasmodium yoeli*. These parasites are not directly practical to man or his domestic animals. Rather, it is of interest for practical model organisms in the laboratory for experimental study of human malaria. Like all malaria parasite of mammals, *Plasmodium berghei* is transmitted by anopheles mosquitoes and infects the liver after been injected into the blood stream by a bite of an infected female mosquito. After a short period (few days) of development and multiplication, these parasites leave the liver and invade the erythrocytes. The multiplication of the parasite in the blood results in pathological cases such as anaemia, and damage to essential organs such as lungs, liver and spleen. *Plasmodium berghei* infection may also affect the brain and cause cerebral complications in laboratory mice. These symptoms are to an extent comparable to the symptoms of cerebral malaria in patients infected with *Plasmodium falciparum* which causes malaria parasite in humans [3].

During malaria infection, oxidative stress is said to be of high increase in the human system as oxidative stress is isolated in infected red blood cells (IRBC). Hydrogen peroxide production is reported in *Plasmodium berghei*-IRBC and O_2^- production in *Plasmodium falciparium*-IRBC. They have been demonstrated to increase levels of lipid peroxidation [4]. Oxidative stress is the result of a disturbance in the balance of naturally generated oxidants and antioxidants. This can be caused by an increase in the production of reactive oxygen species (ROS) and/or a decrease in the activity of the antioxidant system [5]. During malaria infection, massively recruited and activated monocytes and neutrophils produce increasing levels of ROS. Hence ROS plays a role in the pathology of malaria and excessive oxidation stress, particularly at

unwanted places (vascular lining, blood brain barrier) will damage the defence system. When there is excessive oxidative stress, the concentration of antioxidant enzymes such as catalase, superoxide dismutase (SOD) and glutathione peroxidase increases in the blood.

Catalase is an important intracellular antioxidant enzyme which is present in high concentration in the liver and red blood cells. It is also prominent in other body organs such as brain, heart and kidney. Its reactivity is important when the concentration of hydrogen peroxide is raised. The transformation of O_2^- and H_2O_2 into very reactive hydroxyl radicals is catalysed by transition metal ions such as copper and iron. This hydroxyl radical rapidly reacts at first encounter with macromolecules such as lipids, proteins or DNA often causing molecular or cellular damage. Catalase catalyses the decomposition of hydrogen peroxide to water and oxygen.

Medicinal plants have formed the basis of healthcare worldwide. Hence, the use of plant derived natural products as part of herbal preparations for alternative sources of medicine continues to play a major role in chemotherapy especially in third world countries [6]. Plants by means of secondary metabolism contain a variety of herbal and non-herbal ingredients that can ameliorate a disease condition by acting on a variety of targets in host organism. *Vernonia amygdalina* and *Gongronema latifolium* have been known and identified by many researchers to possess herbal ingredients for the treatment of malaria parasite infection and have also been studied for their effect on hematology (blood chemistry) [7,8].

Vernonia amygdalina is commonly known as bitter leaf which is a shrub or small tree of 2-5m belonging to the family; “Asteraceae”. The leaves are green with characteristic odour and a bitter taste [9]. The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides [10]. *Vernonia amygdalina* produces no seeds. Hence, they are distributed through cutting [11]. It is known as “Ewuro” in Yoruba, “Etidot” in Ibibio, “Onugbu” in Igbo and “Chusa”in Hausa tribes of Nigeria [12]. The leaves are mostly used as vegetables for preparation of soup to stimulate the digestive system and also used for treatment of fever in South Southern and South Eastern Nigeria. It contains a wide variety of phytochemicals such as oxalates, phytates and tannins [13], as well as high amount of flavonoid [14]. It also contains major minerals like calcium, potassium, magnesium [15], and vitamins A, C and E, as well as niacin, riboflavin, thiamine [16]. It grows under a range of ecological zones in Africa and produces large mass of foliage and is tolerant to drought [17].

Gongronema latifolium is atypical rainforest plant belonging to “Ascepidaceae” family with simple, occasional whorled leaf. It is widely used in West African sub-region for a number of medicinal and nutritional purposes. In South-Eastern and South-Southern States of Nigeria, it is known as “Utazi” and used primarily as a staple vegetable/spice [18]. It contains phytochemicals such as flavonoid, tannins, alkaloid, glycosides etc. [19], vitamins A, C, E, niacin, riboflavin and thiamine [16]. It is medicinally used as antibacterial [20], antioxidant [21] and as well hypoglycaemic, hypotensive and hepato-preventive properties [22].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection of plant materials

Fresh but matured leaves of *Vernonia amygdalina* (Del.) and *Gongronema latifolium* (Benth) were respectively purchased from a local farm in Uyo local Government area of Akwa Ibom state in August 2014. They were authenticated by Dr. (Mrs) Margaret E. Bassey, a Plant Taxonomist, Department of Botany, University of Uyo, Uyo, by comparing the plant specimen with Voucher specimen deposited in a herbarium in the Department of Botany (Voucher Nos. UUH 2083 and UUH 2084, Uyo respectively. The leaves were rinsed severally with clean tap water to remove dust particles and debris and thereafter allowed to completely drain.

2.2 Methods

2.2.1 Preparation of plant extracts

The drained leaves were separately chopped into bits with a knife on a chopping board. 1.40 kg of *Gongronema latifolium* and 1.83 kg of *Vernonia amygdalina* was homogenized using an electric homogenizer (QL4-18L40) and separately agitated in 1.95 L and 2.25 L of 80% (v/v) ethanol respectively. The mixtures were allowed for 48 hours in the refrigerator at 4℃ for thorough extraction of the plants active components. These were then filtered with cheesecloth and

later with Whatman No. 1 filter paper to obtain a homogenous filtrate. These filtrates were then separately concentrated at low temperature (37-40℃) under pressure to about One-tenth the original volume using a rotary evaporator. The concentrates were allowed open in a water bath (40℃) for complete dryness yielding 46.6 g (4.054%) and 36.4 g (3.471%) of greenish brown and brown oily substances for *Gongronema latifolium* and *Vernonia amygdalina* respectively. The extracts were then refrigerated at 2-8℃ prior to administration.

2.2.2 Experimental animals

Forty (40) adult mice weighing between 26-30 g were obtained from the Animal House of the Faculty of Basic Medical Sciences, University of Uyo. They were allowed to acclimatize to the laboratory condition for 2 weeks in properly ventilated cages, where bedding was replaced every two days at room temperature with a 12 hour light/dark cycle maintained prior to experimentation. They were fed with standard growers mash feeds and water ad libitum. All animals were carefully monitored and handled in accordance with internationally accepted principles for care and use of laboratory animals.

2.2.3 Induction of experimental plasmodium

The mice were infected with the *Plasmodium berghei* parasites by obtaining parasitized blood from cut-tip of the tail of infected mice (3 to 4 drops) and diluted in 0.9 ml of phosphate buffer (pH 7.4). The mice were inoculated intra-peritoneally with 0.1 ml (106) *Plasmodium berghei* parasitized red blood cell (pRBC) maintained by passage and were considered to have cerebral malaria (CM) if they display neurological symptoms such as paralysis, deviation of the head, ataxia, convulsions or coma upon infection. The percentage of parasitaemia was then determined by blood film (smears) made by collecting blood from cut-tip of the tail of the mice, stained with Geimisa stain and calculated as the number of pRBC per 100 RBC.

2.2.4 Animal treatment

Following induction of experimental plasmodium, the animals were randomly distributed into eight (8) groups of five mice each and received treatment as follows:

Group 1 Normal control + Normal saline
Group 2 Parasitized control + Normal saline
Group 3 Parasitized + VA 100 mg/kg body weight
Group 4 Parasitized + VA 200 mg/kg body weight
Group 5 Parasitized + GL 100 mg/kg body weight
Group 6 Parasitized + GL 200 mg/kg body weight
Group 7 Parasitized + 100 mgVA + 100 mg/kg GL body weight
Group 8 Parasitized + 150 mgVA + 150 mg/kg GL body weight

2.2.5 Collection of blood sample and preparation

At the end of the seventh (7^{th}) day period of treatment with VA and GL, feed was withdrawn from the mice and they were fasted overnight prior to sacrifice. The mice were euthanized under chloroform vapour and were immediately dissected medico-ventrally to expose the heart. Freshly pumped blood samples were collected from heart via cardiac puncture with the aid of sterile syringe and needle, and subsequently emptied into neatly labelled plain sample tubes (without anticoagulant inside). The blood samples were allowed to clot before been subjected to centrifugation at 3000 rpm for 10 minutes to obtain the serum which was subsequently assayed for catalase activity.

2.2.6 Determination of serum catalase concentration

Serum catalase activity was determined by the method of Aebi, [23]. The concentration in the sample was calculated using quadratic formulae. Thus the equation for calculating catalase concentration is given as follows:

$$\text{Catalase (U/mL)} = \frac{8.39\times10^{-3}\sqrt{-(-8.39\times10^{-5})^2 - 4\times2.38\times10^{-5}\,(1.18 - \text{Absorbance})}}{4.76\times10^{-5}}$$

2.3 Statistical Analysis

The data were analysed using IBM Statistical Product and Service Solutions (SPSS) version 21 and the results were expressed as mean ± standard error of mean (SEM). Significant differences were established by the one-way analysis of variance (ANOVA). Mean values with $p<0.05$ were considered statistically significant.

3. RESULTS

The effect of repeated oral administration of *Vernonia amygdalina* and *Gongronema latifolium* in *Plasmodium berghei*-infected mice is shown in Table 1. Induction of plasmodium in mice resulted in a significantly higher catalase activity in Group 2 (48.07±0.25) when compared to Group 1 (43.71±0.10). Serum catalase activity was observed to be significantly ($P<0.05$) lower in the treated Groups (3 to 7) when compared with the parasitized control group, with greater reductions observed in the groups that received combined treatment with VA and GL. Although there was a decrease in catalase concentration in Groups 4 and 5, this was however found to be non-significant ($p>0.05$) when statistically compared to the parasitized control Group 2 mice.

The data reported in Table 1 is presented graphically on the bar chart (Fig. 1).

4. DISCUSSION

The reason for assessing the effect of *Vernonia amygdalina* and *Gongronema latifolium* on serum catalase activity during malaria parasite infection in mice was to ascertain the possible usefulness of such intervention in the treatment and management of malaria parasite infection.

Vernonia amygdalina and *Gongronema latifolium* are known for several medicinal uses, and have been studied for different pharmacological properties. Phytochemical analysis of these plants revealed the presence of various secondary metabolites such as flavonoids, terpenoids, steroids, tannins, alkaloids, saponins and glycosides that possess antiplasmodial and antioxidant activities. This accounts for the antiplasmodial and antioxidant potentials of both plant extracts.

In the present study, the mice were infected with *Plasmodium berghei* parasite via intra-peritoneal inoculation with 0.1 ml *Plasmodium berghei* parasitized red blood cells (pRBC) maintained by passage, and the effect on serum catalase activity following oral administration of the extracts to the mice for seven days was assayed. There was a significant ($p<0.05$) increase in catalase activity observed in Group 2 when compared to Group 1, and this is as a result of the increased oxidative stress associated with induction of the *Plasmodium berghei* parasite. The parasites then develop and multiply after a period of time. They leave the liver and invade the red blood cells giving rise to this condition.

Repeated daily administration of *Vernonia amygdalina* and *Gongronema latifolium* resulted in decrease in catalase activity. This reduction is indicative of the anti-plasmodia/anti-malarial activity of *Vernonia amygdalina* [24,25] and *Gongronema latifolium* [26]. There was a significant decrease in catalase activity of Group 3 mice when compared to those of Group 2. The presence of phytochemicals such as tannins, alkaloids and flavonoids which possesses antioxidant activity in the leaves of *Vernonia amygdalina* and *Gongronema latifolium* could be attributed to this as well as a plethora of

Table 1. Effect of *Vernonia amygdalina* and *Gongronema latifolium* on serum catalase concentration of *Plasmodium berghei*-infected mice

Groups	Treatment doses	Catalase (U/mL)	*P*-value
Group 1	Normal control (Normal saline)	43.71±0.10	>0.05
Group 2	Parasitized control (Normal saline)	48.07±0.25	<0.05
Group 3	VA 100 mg/kg b.wt	46.20±0.18**	<0.05
Group 4	VA 200 mg/kg b.wt	47.30±1.54*	<0.05
Group 5	GL 100 mg/kg b.wt	47.50±1.20*	<0.05
Group 6	GL 200 mg/kg b.wt	46.70±1.21**	<0.05
Group 7	VA 100 mg/kg + GL 100 mg/kg b.wt	45.70±0.16**	<0.05
Group 8	VA 150 mg/kg + GL 150 mg/kg b.wt	45.50±0.50**	<0.05

*Values are mean ± standard error of means of duplicate determination. Significant values are at P<0.05. VA: Vernonia amygdalina, GL: Gongronema latifolium, b.wt: body weight *: not significant when compared to group 2, **: Significant when compared to group 2*

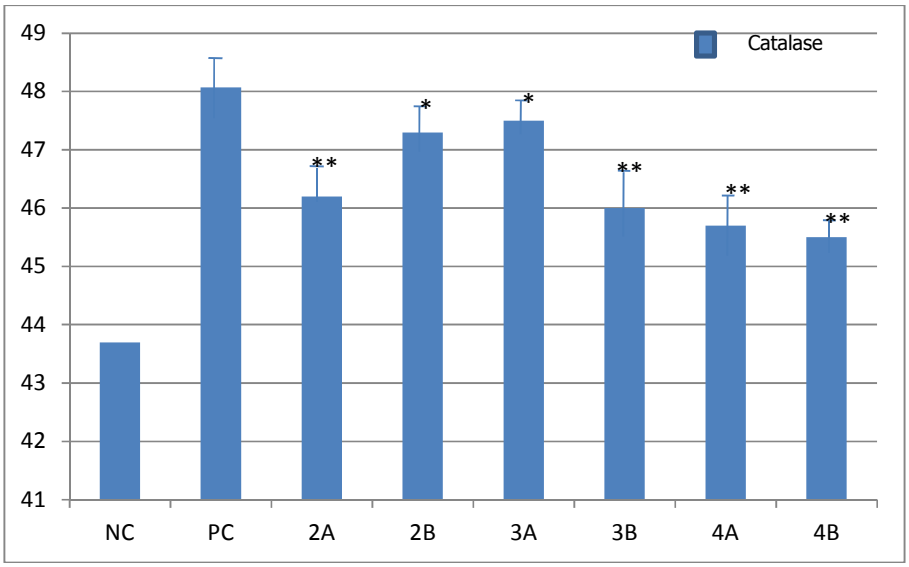

Fig. 1. Effect of different doses of ethanolic extracts of VA and GL on *Plasmodium berghei*-infected mice

*NC: Normal control, PC: Parasitized control, 2A: Group 3; 2B: Group 4, 3A: Group 5, 3B: Group 6, 4A: Group 7, 4B: Group 8. Significant from NC and PC: ** P < 0.05; Values are Mean ± Standard error of mean (SEM)*

biological activities possessed by both plants [27-29]. These active ingredients may act singly, additively or synergistically to produce the results for which the medicinal values of these plants was investigated.

Flavonoids are known to be good antioxidants, and luteolin (a flavonoid found in VA) has been reported to be a strong antioxidant [30]. *Vernonia amygdalina* contains significant amount of flavonoid such as luteolin which may account for its increased antioxidant activity. Igile et al. [31] confirmed that luteolin is a more potent an antioxidant than butylated hydroxytoluene (BHT), and they reported that its glucosides (luteolin 7-O-β- glucuroniside and luteolin 7-O-β-glucoside) also have antioxidant activities. Thus, the decrease in serum catalase activity in the treatment groups can also be attributed to the presence of flavonoids in these plants.

A striking observation in these intervention regimen is the significant ($p<0.05$) decrease in serum catalase activity in Groups 7 and 8 which received combined treatment with VA and GL when compared to parasitized control (Group 2). This is indicative of the additive/synergistic effect of both extract when administered together, and can be attributed to the quenching effects of these hydrogen rich organic acids on the free radicals generated within the parasitized red blood cells, therefore stabilizing the cell membrane from harmful manifestations of the free radicals generated from parasite degradation of host haemoglobin. Also, the increased presence of antioxidant vitamins (A, C and E) in the leaves of VA and GL can exert their effect by neutralizing the toxic reactive oxygen species (ROS) generated during malaria parasite infection by donating an electron to these toxic radicals thus stabilizing them.

In vitro antioxidant and free radical scavenging activity of the extracts (total flavonoid content, reducing power, DPPH radical, total phenol and total antioxidant capacity) revealed both medicinal plants to be useful antioxidant reservoir. This justifies the possible use of these plants in combating free radical related diseases such as malaria often caused by oxidative stress conditions. The DPPH radical scavenging activity of both extract was dependent on the phenols and flavonoids in the extracts which is an indication of the antioxidant potentials of the extracts.

Superoxide dismutase (SOD) is the key defence enzyme against oxidative stress because it has the ability to convert superoxide anion to hydrogen peroxide [32]. However, catalase protects the cells from the effect of hydrogen peroxide by catalysing its decomposition to oxygen and water without the production of free radicals. In this way, catalase functions as a

scavenger of (hydrogen peroxide) H_2O_2 formed by superoxide dismutase and other processes thereby protecting the cells from the harmful effect of hydrogen peroxide [33]. From our observation, the fewer superoxide radicals produced were used up by SOD to generate H_2O_2 for catalase to decompose in the Groups that received VA and GL.

The activity of catalase observed in Groups 7 and 8 was found to be non-significantly ($p>0.05$) lower when compared to the catalase activity in Groups 3 and 6. However, Groups 4 and 5 recorded catalase concentrations which were found to be non-significantly lower when compared to parasitized non-treated Group 2 mice. This may indicate treatment resistance in the mice at higher doses of the extract of *Vernonia amygdalina* and decrease response to treatment with lower dose of *Gongronema latifolium.*

5. CONCLUSION

This study has shown the anti-plasmodial activity of *Vernonia amygdalina* and *Gongronema latifolium* as seen in its ability to suppress increased catalase activity during malaria parasite infection with *Plasmodium berghei*. Therefore, traditional use of these plants to treat malaria in parts of Nigeria may be based on its anti-parasitic activity. However, the combination therapy of *Vernonia amygdalina* and *Gongronema latifolium* might prove to be necessary intervention in the management and treatment of malaria parasite infection as its hyper ability to suppress increased catalase activity was observed. This combination therapy may contribute to the development of potential antimalarial drug from Nigerian ethno botanical diversity.

ETHICAL APPROVAL

The experimental procedures and protocols used in this study were approved by the Institutional Health, Research and Ethical Committee. All efforts were made to minimize animal suffering and reduce the number of animals used. All authors hereby declare that the principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. The animals were used in accordance with NIH Guide for the care and use of laboratory animals.

ACKNOWLEDGEMENTS

Authors are very grateful to Barr. Udofia Akpan Obot and Mr/Mrs Emmanuel George Ekpo for providing the funds necessary to facilitate this research work. We also in a special way thank Innocent Edagha (PhD) and Mr. James Prince Ogaranya for their intellectual contributions to the success of this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. D'Acremont V, Lengeler C, Genton B. Reduction in the proportion of fevers associated with *Plasmodium falciparium* parasitaemia in Africa: A systematic review. Malaria Journal. 2010;9:240-248.
2. Singh BL, Kimsung S, Matusop A. A large focus of naturally acquired *Plasmodium knowlesi* infection in human beings. Lancet. 2004;363(9414):1017-1024.
3. Franke-Fayard B, Fonager J, Braks A, Kahn SM, Janse CJ. Sequestration and tissue accumulation of human malaria parasites: Can we learn anything from rodent models of malaria? PLoS Pathogens. 2010;9(6):e10001032.
4. Narkornchai S, Anantavara S. Oxygen free radicals in malaria. In: Lipid Soluble Antioxidants, Biochemistry and Clinical Applications. 1st Eds, Omg ASH, Paker Publishers, Birkhause Velag, Basel. 1993;355-362.
5. Postma NS, Mommers EC, Eling WM, Zuldema J. Oxidative stress in malaria: Implications for prevention and therapy. Pharmacy World and Science. 1996; 18:121-129.
6. Joy PP, Thomas J, Matthew S, Skaria BP. Medicinal plants. Kerala Agricultural University, Kerala, India. 1998;3-8.
7. Akinnuga AM, Bamidele O, Ekechi P, Adeniyi OS. Effects of an ethanolic Leaf extract of *Gongronema latifolium* on some haematological parameters in rats Afr. J. Biomed. Res. 2011;14:153-156.
8. Oyedeji KO, Bolarinwa AF, Akintola AM. Effect of methanolic extract of *Vernonia amygdalina* on hematological and plasma biochemical parameters in male albino rats. J. Dental Med. Sci. 2013;3(5):64-67.

9. Singha SC. Medicinal plants in Nigeria. National Press Ltd, Lagos, Nigeria. 1996; 49.
10. Ologunde BO, Akinpelu D, Aregheoro EM. Antimicrobial activity of *Vernonia amygdalina* leaves. Fitoterapia J. stud. Med. Plant. 1999;70:432.
11. Tende JA, Ezea ED, Tende YA. Hematological effects of aqueous extract of *Vernonia amygdalina* in wistar rats. Scientific Journal of Biological Sciences. 2013;2(3):62-67.
12. Egedigwe CA. Effect of dietary incorporation of *Vernonia colorata* on blood lipid profile and relative organ weights in albino rats (Thesis). Department of Biochemistry. MOUAU, Nigeria; 2010.
13. Eleyimi AF, Sporns P, Bressler DC. Nutritional composition of *Gongronema latifolium* and *Vernonia amygdalina*. Journal of Ethanopharmacology. 2008; 106:117-120.
14. Igile GO, Oleszek W, Jurzysta M, Burda S, Fanfunso M, Fasanmada AA. Flavonoids from *Vernonia amygdalina* and their antioxidant activities. Journal of Agric & Food Chemistry. 1994;42:2445-2448.
15. Mensah JK, Okoli RI, Ohaji-Obodo JO, Eifedyl K. Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. African Journal of Biotechnology. 2008;7:859-865.
16. Atangwho IJ, Ebong PE, Eyong EU, Williams IO, Eteng MU, Egbung GE. Comparative chemical composition of leaves of antidiabetic medicinal plants: *Azardirachta indica*, *Vernonia amygdalina* and *Gongronema latifolium*. African Journal of Biotechnology. 2009;8:4685-4689.
17. Bonsi GO, Lessen WA, Burda S, Jurszsta M. Nutritional assessment of *Vernonia amygdalina* leaves in growing mice. J. Agric. Food Chem. 1995;43:2162-2166.
18. Ugochukwu NH, Babady NE. Antihyperglycaemic Effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen Metabolism in livers of normal and streptozotocin-induced diabetic rats. Journal of Life Sciences. 2003;1925-1938.
19. Iweala EJ, Obidoa O. Effect of a long term consumption of a diet supplemented with leaves of *Gongronema latifolium* benth on some biochemical and histological parameters in male albino wistar rats. Journal of Biological Sciences. 2009;9: 859-865.
20. Etetim EN, Useh MF, Okokon JE. Pharmacological screening and evaluation of antiplasmodial activity of *Gongronema latifolium* (utazi) against *Plasmodium berghei* infection in mice. Nigerian Journal of Health and Biomedical Science. 2008;7(2):51-55.
21. Odukoya OA, Inya-agha SI, Segun FI, Sofodiya MO, Ilori OO. Antioxidant activity of selected green leafy vegetables. American Journal of Food Technology. 2007;2(3):169-175.
22. Ugochukwu NH, Babady NE, Cobourne M, Gasset SR. The effect of *Gongronema latifolium* leaf extract on serum lipid profile and oxidative stress of hepatocytes of diabetic rats. Journal of Bioscience. 2003; 28:1-5.
23. Aebi H. Catalase *in vitro*: Methods in Enzymology. Enzymology. 1985;105:121-126.
24. Masaba SC. The antimalarial activity of *Vernonia amygdalina* Del. (Compositae). Trans. Roy Soc. Trop. Med. Hyg. 2000; 94:694-695.
25. Abosi AO, Raseroka BH. *In vivo* antimalarial activity of *Vernonia amygdalina*. Br. J. Biomed. Sci. 2003; 60:89-91.
26. Eleyinmi AF. Chemical composition and anti-bacterial activity of *Gongronema latifolium*. J Zhejiang Univer Sci. 2007; 8(5):352-358.
27. Igile GO, Oleszek W, Jurzysta M, Burda S, Fanfunso M, Fasanmade AA. Flavonoids from *Vernonia amygdalina* and their antioxidant activities. J. Agric. Food Chem. 1994;42:2445-2448.
28. Igile GO, Oleszek W, Jierzysta M, Burda S. Nutritional assessment of *Vernonia amygdalina* and *Gongronema latifolium* leaves in growing mice. Journal of Agric and Food Chemistry. 1995;23:22-27.
29. Tona L, Cimanga RK, Mesia K, Musuamba CT, De Bruyne T, Apers S, Hermans N, Van Miret S, Pieters L, Totte J, Vlietink AJ. *In vitro* antiplasmodial activity of extracts and fractions of seven medicinal plants used in the Democratic Republic of Congo. J. Ethnopharmacol. 2004;93:27-32.
30. Torel J, Cillard J, Cillard P. Antioxidant activity of flavonoids and reactivity with peroxy radical. Phytochem. 1986;25:383-385.

31. Ijeh II, Chukwunonso ECCE. Current perspectives on the medicinal potentials of *Vernonia amygdalina* Del. Journal of Medicinal Plants Research. 2011;5(7): 1051-1061.
32. Clark IA, Chaudhri G, Cowden WB. Some roles of free radicals in malaria: Review article. Free Radic Biol Med. 1989;6(3): 315-321.
33. Zamocky M, Koller F. Understanding the structure and function of catalases: Clues from molecular evolution: An *in vitro* mutagenesis. Biophysics & Molecular Biology. 1999;72:19-66.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13411