

Original Research Article

Effect of Ethanolic Leaf Extracts of *Vernonia amygdalina* and *Gongronema latifolium* on Serum Catalase Activity in Plasmodium Berghei-Infected Mice

ABSTRACT

Aim: The study was designed to examine the effect of ethanolic 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 26-30g were divided into 8 groups of five mice each. Group 1 and 2 served as the normal control (NC) and parasitized control (PC) respectively that received placebo treatment. All the other groups were parasitized and treated with the extracts via oro-gastric intubation orally for 7 days. Group 3 and 4 were treated with 100 and 200mg/kg of VA respectively. Group 5 and 6 were treated with 100 and 200mg/kg of GL respectively. Group 7 and 8 were treated with combination of VA and GL at 100mg/kg of VA+100mg/kg of GL and 150mg/kg of VA+50mg/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 (200mg/kg of VA) and 5 (100mg/kg of GL). There was a significantly lower concentration of catalase in the mice 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 falciparum* 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 parasite (protozoan) and it infects mammals 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 direct practical to man or his domestic animals but rather it is of interest for practical model organisms in the laboratory for experiment 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 erythrocytes. The multiplication of the parasite in the blood causes pathology such as anaemia and damage of essential organs such as lungs, liver and spleen. *Plasmodium berghei* infection may also affect the brain and cause cerebral complications in laboratory mice.

During malaria infection, oxidative stress is said to be of high increase in human system as oxidative stress is isolated in infected red blood cells (IRBC). H_2O_2 (hydrogen peroxide) production is reported in *Plasmodium berghei*-IRBC, O_2 -production in *Plasmodium falciparum*-IRBC. They have been demonstrated to increase levels of lipid peroxidation [3]. 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 [4]. During malaria infection, massively recruited and activated monocytes and neutrophils produce increasing levels of ROS and therefore ROS plays a role in the pathology of malaria and excessive oxidation stress and particularly at unwanted places (vascular lining, blood brain barrier) will damage the defence system. When there is excessive oxidation stress, antioxidant enzymes like catalase tends to increase in the blood. Other antioxidant enzymes include superoxide dismutase (SOD), glutathione etc. Catalase is an important intracellular antioxidant enzyme which is present in high concentration in the liver cells and red blood cells. It is also prominent in other body organs such as brain, heart and kidney. Catalase reactivity is important when H_2O_2 concentration 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. Serum catalase catalyses the decomposition of hydrogen peroxide to water and oxygen.

Medicinal plants have formed the basis of health care throughout the world and therefore 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 the third world countries [5]. 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 treatment of malaria.

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 a characteristics odour and taste [6]. It is known as "Ewuro" in Yoruba, "Etidot" in Ibibio, "Onugbu" in Igbo and "Chusa" in Hausa tribes of Nigeria [7]. 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 part of Nigeria. It contains wide array of phytochemicals like oxalates, phytates and tannin [8], high amount of flavonoid [9]. Also contains major minerals like calcium, potassium, magnesium [10] and vitamins A, C and E, as well as niacin, riboflavin, thiamine [11].

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 [12]. It contains phytochemicals such as flavonoid, tannins, alkaloid, glycosides etc. [13], vitamins A, C, E, niacin, riboflavin and thiamine [11]. It is medicinally used as antibacterial [14], antioxidant [15] and as well hypoglycaemic, hypotensive and hepato-preventive properties [16].

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. 1.40kg of *Gongronema latifolium* and 1.83kg of *Vernonia amygdalina* was homogenized using an electric homogenizer (QL4-18L40) and separately agitated in 1.95 and 2.25 litres of 80% (v/v) ethanol respectively. The mixtures were allowed for 48hours in the refrigerator at 4°C 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°C) under pressure to about one-tenth the original volume using a rotary evaporator. The concentrates were allowed open in a water bath (40°C) for complete dryness yielding 46.6g (4.054%) and 36.4g (3.471%) of greenish brown and brown oily substances for *Gongronema Latifolium* and *Vernonia amygdalina* respectively. The extracts were then refrigerated at 2-8°C prior to administration.

2.2.2 Experimental Animals

Forty (40) adult mice weighing between 26-30g were obtained from the animal house of faculty of Basic Medical Sciences of the University of Uyo, 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 and 12 hour light/dark cycle prior to experimentation. They were fed with growers mash and water ad libitum. All animals were carefully monitored and all the experimental protocol with the animals was in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols were duly approved by the Institutional Ethical Committee of NITR.

2.2.3 Induction of Experimental Plasmodium

The mice were infected with parasites (*Plasmodium berghei*) by obtaining parasitized blood from cut-tip of the tail of infected mice (3 to 4 drops) and diluted in 0.9ml phosphate buffer (PH 7.4). The mice were inoculated intra-peritoneally with 0.1ml (10⁶) *Plasmodium berghei* parasitized red blood cell (pRBC) maintained by passage. The mice 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 100mg/kg body weight |
| Group 4 | Parasitized + VA 200mg/kg body weight |

- 127 Group 5 Parasitized + GL 100mg/kg body weight
 128 Group 6 Parasitized + GL 200mg/kg body weight
 129 Group 7 Parasitized + 100mgVA + 100mg/kg GL body weight³
 130 Group 8 Parasitized + 150mgVA + 150mg/kg GL body weight

131

132 **2.2.5 Collection of Blood Sample and Preparation**

133 At the end of the animal experiment, feed was withdrawn from the mice and they were fasted overnight
 134 prior to sacrifice. The mice were euthanized under chloroform vapour and were immediately dissected
 135 medico-ventrally to expose the heart. Blood samples were collected from the cardiac puncture with the
 136 aid of sterile syringe and needle into labelled plain sample tubes (with no anticoagulant inside) and
 137 allowed to clot before been subjected to centrifugation in order to obtain the serum. The serum was used
 138 for catalase analysis.

139

140 **2.2.6 Determination of Serum Catalase Concentration**

141 Serum catalase activity was determined by the method of Aebi (1985) [17]. The concentration in the
 142 sample was calculated using quadratic formulae. Thus the equation for calculating catalase concentration
 143 is given as follows:

144

145

$$146 \text{ Catalase (U/mL)} = \frac{8.39 \times 10^{-3} \sqrt{(-8.39 \times 10^{-5})^2 - 4 \times 2.38 \times 10^{-5} (1.18 - \text{Absorbance})}}{4.76 \times 10^{-5}}$$

147

148

149 **2.3 Statistical Analysis**

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

154 **3. RESULTS**

155 The effect of repeated oral administration of *Vernonia amygdalina* and *Gongronema latifolium* in
 156 plasmodium berghei-infected mice is shown in Table 1. Induction of plasmodium in mice resulted in a
 157 significantly higher catalase activity in group 2 (48.07 $\pm$ 0.25) when compared to group 1 (43.71 $\pm$ 0.10).
 158 Serum catalase activity was observed to be significantly ($P < 0.05$) lower in the treated groups (3-7) when
 159 compared with the parasitized control group 2 mice, with better reductions observed in the group that
 160 received combined treatment of VA and GL. Although there was reduction in catalase concentration in
 161 group 4 and 5, the reduction was statistically non-significant ($p > 0.05$) when compared with the parasitic
 162 control group 2 mice.

163 Table 1: The effect of *Vernonia amygdalina* and *Gongronema latifolium* on Serum Catalase Concentration
 164 of Plasmodium berghei-Infected Mice.

Groups	Treatment Doses	Catalase (U/mL)	P-value
Group 1	Normal Control (Normal Saline)	43.71 $\pm$ 0.10	>0.05
Group 2	Parasitized Control (Normal Saline)	48.07 $\pm$ 0.25	<0.05
Group 3	VA 100mg/kg b.wt	46.20 $\pm$ 0.18**	<0.05
Group 4	VA 200mg/kg b.wt	47.30 $\pm$ 1.54*	<0.05
Group 5	GL 100mg/kg b.wt	47.50 $\pm$ 1.20*	<0.05
Group 6	GL 200mg/kg b.wt	46.70 $\pm$ 1.21**	<0.05

Group 7	VA 100mg/kg + GL 100mg/kg b.wt	45.70±0.16**	<0.05
Group 8	VA 150mg/kg + GL 150mg/kg b.wt	45.50±0.50**	<0.05

Values are means $\pm$ 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.

The data reported in table 1 above is presented graphically on the bar charts given below.

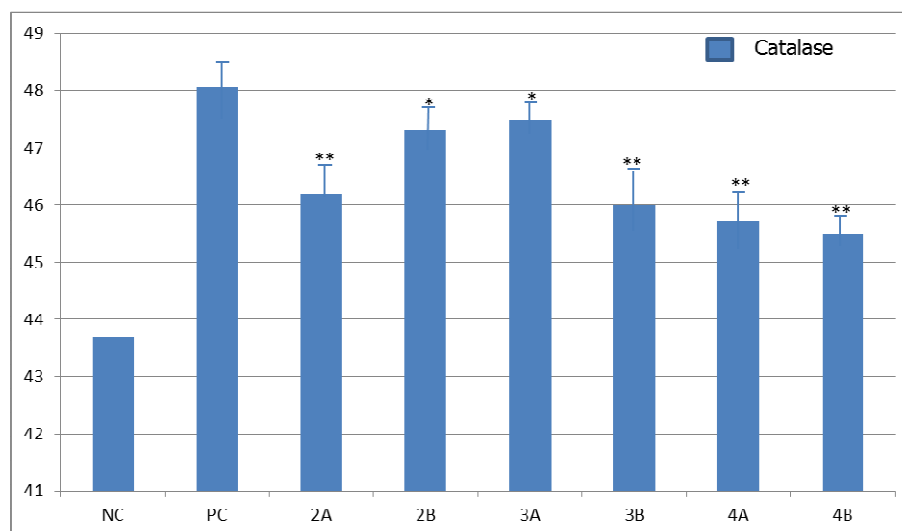


Fig. 1. Effect of different doses of ethanolic extracts of VA and GL on Plasmodium berghei-Infected Mice.

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 $\pm$ Standard error of mean (SEM).

4. DISCUSSION

The reason for assessing the effects of *Vernonia amygdalina* (VA) and *Gongronema latifolium* (GL), in serum catalase activity during malaria parasite infection in mice was to ascertain the possible usefulness of such intervention in management and treatment of malaria parasite infection. The significant increase in catalase concentration of group 2 when compared to group 1 may have resulted from the induction of plasmodium berghei which develops and multiplies after a period of time. The parasites leave the liver and invade the red blood cells giving rise to this condition. However, repeated daily administration of the extracts (VA and GL) resulted in decrease catalase activity. This reduction may be due to the presence of antioxidants in the leaves as indicated by many studies. Flavonoids have been for their antioxidant properties and it's a major secondary metabolite that acts as an antioxidant [18]. The presence of flavonoid in VA and GL has indicated the significant reduction in catalase activity. The presence of other phytochemicals in VA and GL like tannin and alkaloids may also have contributed to the reduction in catalase activity of infected mice. The combination effect of these phytochemicals along with flavonoid has been associated with antioxidant activity [19]. It may also be hypothesized that the catalase reduction observed in mice group that received VA and GL intervention may be as a result of 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 another possible reason may be due to the increased presence of antioxidant vitamins (A, C and E) in the leaves of VA and GL. They must have exert its effect by neutralizing the ROS been generated during malaria infection by donating an electron to these toxic radicals so as to stabilize them.

A striking observation in these intervention regimen is the significant ($P < 0.05$) decrease in serum catalase activity in groups that received combined treatment with VA and GL when compared to parasitized

control. Superoxide dismutase (SOD) is the key defence enzyme against oxidative stress because it has the ability to convert superoxide anion to hydrogen peroxide [20]. However, catalase protects the cell from effects 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 H₂O₂ formed by SOD and other processes thereby protecting the cells from harmful effects of H₂O₂ [21]. With this observation, it would be hypothesized that fewer superoxide radicals are produced and used by SOD to generate H₂O₂ for catalase to decompose in the groups that received VG and GL. In group 8, the dosage of VG is more than that of GL but yet produced almost the same reduction value in catalase activity with that of group 7 that has same dosage of VG and GL. *Vernonia amygdalina* contains significant amount of flavonoid such as luteolin which may account for its increased antioxidant activity [22]. The values obtained in oxidative stress indicator "catalase" in groups 4 and 5 when compared to parasitized non-treated control was statistically non-significant. This may indicate treatment resistance in the mice at high dose of VA and low treatment effect of lower dose of GL in oxidative stress treatment.

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 VG and GL 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 University of Uyo Teaching Hospital Health, Research and Ethical Committee Uyo, Akwa Ibom State Nigeria. 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.

REFERENCES

1. D,Acremont V, Lengeler C, Genton, B. Reduction in the proportion of fevers associated with plasmodium falciparum 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. 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; pp 355-362.
4. 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.
5. Joy PP, Thomas J, Matthew S, Skaria BP. Medicinal Plants, Kerala Agricultural University, Kerala, India, 1998; pp 3-8.
6. Singha SC. Medicinal Plants in Nigeria. National Press Ltd, Lagos, Nigeria, 1996; p.49.
7. Egedigwe CA. Effect of dietary incorporation of vernonia colorata on blood lipid profile and relative organ weights in albino rats (Thesis). Department of Biochemistry, 2010; MOUUAU, Nigeria.
8. Eleyimi AF, Sporns P, Bressler DC. Nutritional composition of gongronema latifolium and vernonia amygdalina. *Journal of Ethanopharmacology*, 2008; 106: 117-120.
9. 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.

- 249 10. Mensah JK, Okoli RI, Ohaji-Obodo JO, Eifedyl K. Phytochemical, nutritional and medical properties of
250 some leafy vegetables consumed by Edo people of Nigeria. *African Journal of Biotechnology*, 2008; 7:
251 859-865.
- 252 11. Atangwho IJ, Ebong PE, Eyong EU, Williams IO, Eteng MU, Egbung GE. Comparative chemical
253 composition of leaves of antidiabetic medicinal plants: *azadirachta indica*, *vernonia amygdalina* and
254 *gongronema latifolium*. *African Journal of Biotechnology*, 2009; 8: 4685-4689.
- 255 12. Ugochukwu NH, Babady NE. Antihyperglycaemic Effect of aqueous and ethanolic extracts of
256 *gongronema latifolium* leaves on glucose and glycogen Metabolism in livers of normal and streptozotocin-
257 induced diabetic rats. *Journal of Life Sciences*, 2003; 1925-1938.
- 258 13. Iweala EJ, Obidoa O. Effect of a long term consumption of a diet supplemented with leaves of
259 *gongronema latifolium* benth on some biochemical and histological parameters in male albino wistar rats.
260 *Journal of biological sciences*, 2009; 9: 859-865.
- 261 14. Etetim EN, Useh MF, Okokon JE. Pharmacological screening and evaluation of antiplasmodial activity
262 of *gongronema latifolium* (utazi) against plasmodium berghei infection in mice. *Nigerian Journal of Health*
263 *and Biomedical Science*, 2008; 7(2): 51-55.
- 264 15. Odukoya OA, Inya-agma SI, Segun FI, Sofodiya MO, Ilori OO. Antioxidant activity of selected green
265 leafy vegetables. *American Journal of Food Technology*, 2007; 2(3): 169-175.
- 266 16. Ugochukwu NH, Babady NE, Cobourne M, Gasset SR. The effect of *gongronema latifolium* leaf
267 extract on serum lipid profile and oxidative stress of hepatocytes of diabetic rats. *Journal of Bioscience*,
268 2003; 28: 1-5.
- 269 17. Aebi H. Catalase Invitro: Methods in Enzymology. *Enzymology*, 1985; 105: 121-126.
- 270 18. Igile GO, Oleszek W, Jierzysta M, Burda S. Nutritional assessment of *vernonia amygdalina* and
271 *gongronema latifolium* leaves growing mice. *Journal of Agric and Food Chemistry*, 1995; 23: 22-27.
- 272 19. Owoyele BY, Olayele SB, Elegba RA. Anti-inflammatory and analgesic activities of leaf extract of
273 *landolphia owariensis*. *African Journal of Biomedical Research*, 2002; 4(3): 131-133.
- 274 20. Clark IA, Chaudhri G, Cowden WB. Some roles of free radicals in malaria: review article. *Free Radical*
275 *Biological Medicine*; 6: 315-321.
- 276 21. Zamocky M, Koller F. Understanding the structure and function of catalases: clues from molecular
277 evolution: an invitro mutagenesis. *Biophysics & Molecular Biology*, 1999; 72: 19-66.
- 278 22. Owolabi MA, Jaja SI, Oyekanmi OO, Olatunji OJ. Evaluation of the antioxidant activity and lipid
279 peroxidation of the leaves of *vernonia amygdalina*. *Journal of Internal Medicine*, 2008; 5:1550-1560.