

Original Article

Comparative Antioxidants Status of Leaves Extracts of Some Common Antimalarial Plants in West Africa

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ABSTRACT

Objective: Malaria chemotherapy remains relevance and gives way to the re-evaluation of medicinal plants that has already gain approval in the traditional treatments of Malaria. This work evaluated and compared the antioxidant status of methanol leaves extracts of *Azadirachta Indica* (MAI), *Vernonia Amygdalina* (MVA) and *Carica Papaya* (MCP).

Methods: Phenols, Flavonoids, percentage inhibition of lipid peroxidation and radical scavenging activities using 1,1-diphenyl-2-picryl hydrazyl (DPPH) and Hydroxyl radicals were determined spectrophotometrically based on international standardized methods.

Results: Total Phenolic content in garlic acid equivalence (GAE) were expressed maximally at 700 μ g/ml by 0.015 \pm 0.002, 0.019 \pm 0.017, 0.013 \pm 0.006 mg/g and flavonoids contents at 350 μ g/ml by 0.063 \pm 0.004, 0.020 \pm 0.031, 0.049 \pm 0.002 Mg/g quercetin equivalence (QE) for MAI, MVA and MCP respectively. DPPH scavenging activities of 78.60, 55.55 and 54.96 % were obtained at 350 μ g/ml by MAI, MVA and MCP respectively in the order MAI>MVA>MCP. At 300 μ g/ml, the extracts scavenged hydroxyl radicals significantly ($p \leq 0.05$) by 72.00, 77.80 and 53.15 % in the order MVA>MAI>MCP. Intriguingly, extracts also converted significantly ($p \leq 0.05$), 50% cell protection as they inhibited lipid peroxidation by 50.00, 66.20 and 64.30% in the order MVA>MCP>MAI at 350 μ g/ml, respectively.

Conclusion: Antioxidative properties exhibited by extracts may be correlated with their antimalarial functions, bioactive contents and suggestive of MVA as more potent antimalarial of the evaluated

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plants which may serve as template for malaria drugs and its local usage encouraged in poverty- stricken malarial-endemic areas of West Africa.

Keywords: Azadirachta Indica, Antimalarial, Antioxidants, Carica Papaya, Malaria, Vernonia Amygdalina.

INTRODUCTION

Malaria is a great burden, an infection in many countries of the world responsible for about 200 million infections with almost 90% of these reported in Sub – Sahara Africa each year, and more than 500 thousand deaths annually with children as mostly affected^{1,2}. Although the majority of fatal cases are caused by *Plasmodium falciparum*, other protozoa involved in malaria infection includes, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium vivax*³. Poor tropical and sub tropical areas of the world offers suitable climate for the development of the parasites while the discovery of the disease and various researches on it has span several decades in various countries ranging from Egypt, Greece, Italy France and India with its major devastating effects highly comparable with HIV/AIDS and tuberculosis⁴⁻⁶.

Interestingly, as a complex disease it varies widely in epidemiology and clinical manifestation in different parts of the world but its treatment remains a major challenge due to discovery of resistance, parasites species, vectors control, poverty and fake drugs^{7,8}. Other factors includes current trends in drugs or drugs combinations so as to improve on the old existing drugs⁹, and the exploitation of plants derived phytochemicals as rich sources of anti malarial drugs¹⁰. In many tropical countries herbal remedies mainly from plants are sought for due to lack of funds and scarcity of some known antimalarial drugs¹¹.

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine¹². Traditional plants play an important role in medical system in West African and plant materials remain an important resource to combat serious diseases in the world¹³. However, normal investigations of traditional plants and herbs has become necessary and must be continuous since agents such as quinine and artemisinin have been discovered from them⁹, and the efficacy of these herbs have been shown for decades in Africa¹⁴. Also in Southwest Nigeria ,about fifty medicinal plants have been isolated to have antimalarial properties with remedies made from some of these plants; *Vernonia amygdalina* (bitter leaf), *Carica papaya* (pawpaw leaf) and *Azadirachta indica* (leaf) as local concoctions for malaria therapy^{15,16}.

Although anti malarial and anti plasmodial activities of some preparations of these plants were reported, their mechanisms were not understood and not proven scientifically, while there are suspicions that most bioactive components of these plants have antioxidants properties, the correlation between the local anti malarial effects and the possible antioxidants properties of these plants forms the rationale for this study which is worth investigating hence this work was aimed at evaluating and compare the antioxidants status of the extracts of these common anti malarial plants of West Africa; *Vernonia*

amygdalina (bitter leaf), *Carica papaya* (pawpaw leaf) and *Azadirachta indica* (leaf).

MATERIALS AND METHODS

Reagents

All chemicals and reagents were of highest quality and grade and were obtained from Sigma Chemical Company USA, and includes, methanol, follin ciocalteu, gallic acid, quercetin, sodium carbonate, thiobarbituric acid, trichloroacetic acid, 1-1-diphenyl-2-picrylhydrazyl radical (DPPH), distilled water, hydrogen peroxide, sodium acetate, ferrous sulphate *e.t.c* .

Plant materials

Fresh healthy leaves of *Vernonia Amygdalina*, *Carica Papaya* and *Azadirachta Indica* were obtained from the School Teaching and Research Farm, authenticated by the Botany Unit, with the following Herbarium Number, LHO283, LHO412 and LHO214 deposited.

Plant extract preparation

Fresh healthy leaves of these plants were plucked from its stem and air-dried at room temperature inside the Biochemistry laboratory for two weeks, after which it was powdered. The fine powdered leaves were kept in a properly labelled transparent container and ready for use. Methanol extracts of the leaves were prepared by using 200g of each of the powdered leaves samples soaked in 1.5 litres of methanol in the cold for 72hours after which the decanted filtrate was concentrated to dryness at 45⁰C.

Phytochemical study

Determination of the total phenolic contents of the extracts was based on the reduction of Folin-ciocalteu reagent (Phosphomolybdate and Phosphotungstate) by the¹⁷ method. The reduced folin-

ciocalteu reagent is blue and the absorbance was read at 750nm, while results were obtained in garlic acid equivalence using the various concentrations of garlic acid as the standard. The total flavonoids content were estimated using Aluminium Chloride colourimetric method of¹⁸, based on the principle that flavonoids form complexes with Aluminium Chloride. This was determined as quercetin equivalence from a standard curve obtained at different concentrations of quercetin.

Antioxidants Activity

Antioxidants activities studied includes, the free radical scavenging activities determined using the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) by the photometric method of¹⁹, with ascorbic acid as standard and the hydroxyl radicals scavenging activity performed as described by²⁰, using iron/EDTA/H₂O₂ complex. The percentage inhibition of lipid peroxidation were determined in-vitro using ferrous sulphate in a lipid- rich media as described by the method of²¹.

Statistical Analysis

Statistical analysis were based on the Duncan's experimental analysis with mean standard deviation of sample analysed using the student T test²².

RESULTS AND DISCUSSION

Development of novel antimalarial drugs as a tool for combating Malaria Drug Resistance is the reigning trend in Malaria Chemotherapy even as medicinal plants are still widely relevant to health care both in the past and presently²³. Plants that are well-known in folk medicine are currently evaluated to detect their potentials and modulatory effects on malaria parasite.

The malaria parasite when transmitted into the Human Body through

the female Anopheles mosquito ingests the host cytoplasm and breaks down the haemoglobin into amino acids producing hemozoin, a free radical leading to generations of more free radicals and ultimately the chain process of Lipid peroxidation²⁴. Furthermore, Xanthine Oxidase generates free radical superoxide (O_2^-) and a subsequent burst of other oxidative products²⁵, while recent studies have also implicated the parasites in free radical production leading to oxidative stress associated with malaria^{26,27}, hence the need to investigate the antioxidants status and potential of many antimalarial plants with their possible roles.

From the results obtained in this study, as shown in figure 1 and figure 2, the total phenolic and flavonoids contents of the methanol extract of *Azadirachta indica*, *Vernonia amygdalina* and *Carica papaya* leaves (Denoted MAI, MVA and MCP respectively) were expressed in a concentration dependent manner. Total phenolic contents in these plants at 700 μ g/ml were 0.015mg/g, 0.019mg/g and 0.013mg/g garlic acid equivalence (GAE) for MAI, MVA and MCP while Total flavonoids contents at 350 μ g/ml were 0.063mg/g, 0.0204mg/g and 0.049mg/g Quercetin equivalence (QE) for MAI, MVA and MCP respectively. The apparent presence of these compounds in these plants are indication that these bioactive compounds which are known powerful antioxidants and free radical scavengers²⁸, may be responsible for their local antimalarial effects or may be able to minimize oxidative stress associated with malaria.

A widely used method for testing the ability of a compound to act as a free radical scavenger and to elevate antioxidant activities in biology molecules is the use of DPPH²⁹. This compound is a well known stable Nitrogen synthetic radical and

a scavenger or mother radicals used to evaluate the antioxidant capacity of medicinal herbal products. It has also been used as a screening tool for detecting free radical activities of antioxidants^{30,31}. Results in figure 3, shows the percentage scavenging activity of DPPH which were expressed in a dose- dependent manner. At 350 μ g /ml, MAI, MVA and MCP scavenged DPPH significantly ($p < 0.05$) by 78.60%, 55.55% and 54.96% and in the order MAI>MVA>MCP respectively.

Hydroxyl radicals have been found to very reactive and when generated in the body, attacks membrane structures³², and are definitely involved in the etiology of malaria via the actions and reactions of the malaria parasite and the immune system respectively. In this study, the hydroxyl (OH) radical scavenging activities of the anti malarial plant extracts shows that they scavenged the radical in a concentration dependent manner (Figure, 4) and maximally at 300 μ g/ml by 72.00%, 77.80% and 53.15% for MAI, MVA, and MCP and in the order MVA>MAI>MCP respectively.

The most important biochemical parameter used in the evaluation of chemicals and natural herbal products that can be harnessed as an anti malarial is the ability of such compound to hijack or arrest the process of lipid peroxidation. Lipid peroxidation as an index of oxidative stress is closely associated with malaria²⁴. In figure 5, all the plant extracts showed 50 % cell protection as they inhibited lipid peroxidation in-vitro by 50.00%, 66.19% and 64.29% and in the order MVA>MCP>MAI respectively at 350 μ g/ml concentrations.

Interestingly, from this study, the properties exhibited by these extracts comparatively shows that the extracts of *Vernonia amygdalina* (MVA), exhibited the largest phenolic contents, highest hydroxyl radical scavenging effects and inhibition of

lipid peroxidation which are relatively followed by those of *Azadirachta indica* (MAI) and *Carica papaya* (MCP) respectively.

CONCLUSION

In conclusion, results obtained from various biochemical indices in this study further establishes the antioxidant status of these plants, a correlation for their local anti-malarial properties and usage and an indication of the presence of certain bioactive compounds which may be useful as templates in continuous discovery of potent anti malarial drugs that are cheap and affordable for endemic poor populace of west Africa.

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Authors Disclosure Statement

“No conflict of interests exist”

Funding Source Declaration

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REFERENCES

- Vogel G. Infectious disease - New map illustrates risk from the ‘other’ malaria. *Science* 2010; 329: 618–618.
- World Health Organization (WHO). World Malaria Report 2011; World Health Organization Press: Geneva, Switzerland.
- Nogueira CR, Lopes LMX. Antiplasmodial natural products. *Molecules* 2011; 16: 2146-2190
- Greenwood BM, Fidock DA, Kyle DE, Kappe SHI, Alonso PL, Collins FH, Duffy PE. Malaria: progress, perils and prospects for eradication. *J Clin. Invest* 2008; 118: 1266-1276.
- Gilles HM. Clinical Features of Malaria in Pregnancy and Children. 2010;
- Victor-Silva S, Reyes-Lecca RC, Pinheiro TRA and Lacerda MVG. Malaria is associated with poor school performance in an endemic area of the Brazilian Amazon. *Malaria Journal* 2009; 8: 230doi:10.1186/1475-2875-8-230.
- Foster SD. Pricing, distribution, and use of antimalarial drugs. *Bulletin of the World Health Organization* 1991; 69: 349–363.
- Sendagire H, Kaddumukasa M, Ndagire D, Aguttu Nassejje CM, Pettersson M, Gote Swedberg G and Kironde F. Rapid increase in resistance of *Plasmodium falciparum* to chloroquine-Fansidar in Uganda and the potential of amodiaquine-Fansidar as a better alternative. *Acta Tropica* 2005; 95: (3), 172-182.
- Phillipson JD, Wright CW. Antiprotozoal agents from plant sources. *Planta Medica* 1991; 57: (1), 53-59.
- Basco LK, Mitaku S, Skaltsounis AL, Ravelomanantsoa N, Tillequin F, Koch M, Le Bras J. *In vitro* activities of acridone alkaloids against *Plasmodium falciparum*. *Antimicrobial Agents and Chemotherapy* 1994; 5: 1169-1171.
- Zirih GN, Mambu L, Guede-Guina F, Bodo B, Grellier P. *In vitro* antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. *Journal of Ethnopharmacology* 2005; 98: 281-285.
- Hoareau L, and Dasilva EJ. Medicinal plants: a reemerging health aid. *Electronic Journal of Biotechnology* 1999; 2(2): 56-69.
- Anselem A. Herbs for healing pax herbals Edo State Nigeria. 2004;
- Elujoba T. Book Review “Traditional Medicinal Plants and Malaria.” *Afr. J. Tradit. Complement. Alternative Medicine* 2005; 2(2): 206-207.
- Abosi AO, and Raseroka BH. *In vivo* antimalarial activity of *Vernonia amygdalina*. *British Journal of Biomedical Science* 2003; 60(20): 89-91.

16. Udoh P, Essien I, Udoh F. Effect of Carica papaya (paw paw) seeds extract on the morphology of pituitary-gonadal axis of male Wistar rats. *Phytother Res* 2005; 19: 1065–8.
17. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chem.* 2001; 73: 73.
18. Chang CC, Yang MH, Wen HM, and Chern JC. Estimation of total Flavonoid content of propolis by two complimentary colorimetric methods. *J. Food drug analysis* 2002; 10: 117- 182.
19. Mensor LL, Menezes FS, Leitao GG, Reis AS, dos Santos TC, Coube CS, and Leitao SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *phytother. Res.* 2001; 15: 127-130.
20. Halliwell B. Free radicals and metal ions in health and disease. *Proc. Nutr. Soc.*1987; 46: 13-26.
21. Ruberto G, Baratta MT, Deans SG, and Dorman HJD. Analysis of chemical composition and bioactive property evaluation. *Asian Pac J.* 2000; 31: 3-15.
22. Bliss CI. Statistics in biology. Statistical methods for research in the Natural sciences, (1) Mc Graw Hill Book company. N.Y. 1967; 558.
23. Ajaiyeoba EO, Abiodun OO, Falade MO, Ogbole NO, Ashidi JS, Happi, CT, Akinboye DO. *In vitro* cytotoxicity studies of 20 plants used in *Nigerian antimalarial ethnomedicine*. *Phytomedicine* 2006; 13: 295-298.
24. Cabrales P, Zanini GM, Meays D, Frangos JA, Carvalho LJM. Nitric Oxide protection against murine cerebral malaria is associated with improved cerebral microcirculatory physiology. *J. Infect. Dis,* 2011; 203: 1454–1463.
25. Dockrell HM, Playfair JH. Killing of Plasmodium yoelli by enzyme-induced products of the oxidative burst. *Infect. Immun.* 1984; 43: 451–456.
26. Potter SM, Mitchell AJ, Cowden WB, Sanni LA, Dinauer M, Haan JB, Hunt NH. Phagocyte-derived reactive oxygen species do not influence the progression of murine blood-stage malaria infections. *Infect. Immun,* 2005; 73: 4941–4947.
27. Keller CC, Kremsner PG, Hittner JB, Misukonis MA, Weinberg JB, Perkins DJ. Elevated nitric oxide production in children with malarial anemia: Hemozoin-induced nitric oxide synthase type 2 transcripts and nitric oxide in blood mononuclear cells. *Infect. Immun.* 2004; 72: 4868–4873.
28. Ghasemzadeh A, Jaafar HZE, Rahmat A. Antioxidant activities, total Phenolics and flavonoidscontent in two varieties of Malaysia Young Ginger (*Zingiber officinale* Roscoe). *Molecules* 2010; 15: 4324-4333.
29. Braca A, Tomasi ND, Bari LD, Pissa C, Polliti M, Morelli I, Antioxidant principles from *Bauhinia terapotensis*. *J.Nat.prod.* 2001; 64: 892-895.
30. Karki PD, Youngblood D, Sarma G, Wood Z, Karplus P, Poole L. Analysis of the link between enzymatic activity and oligomeric state in Ahp C, a bacterial peroxiredoxin. *Biochemistry* 2005; 44: (31), 10583-92.
31. Werber Jay, Wang Y, John Milligan, Micheal Li, Xiaohua Ji, Junyan A. Analysis of 2,2- azobis (2-amidinopropane) dehydrochloride degradation and hydrolysis in aqueous solutions. *Journal of Phamaceutical Sciences* 2011; 100: (8) 3307-15.
32. Candeias LP, Patel KB, Stratford MRL, Wardman P. Free hydroxyl radicals are formed as reaction between the neutrophil-derived species superoxide and hypochlorous acid. *FEBS Lett:* 1993; 333: 151-153.

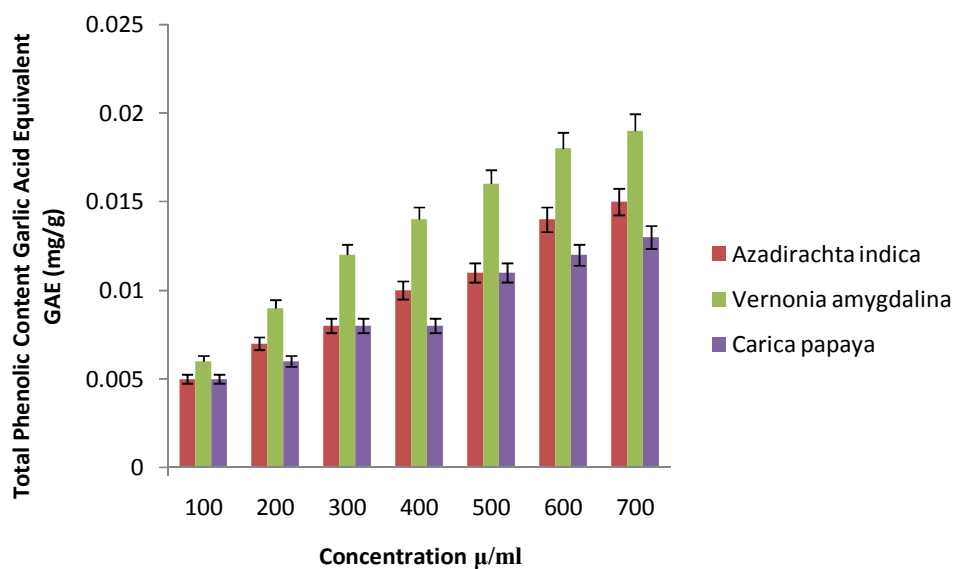


Figure 1: Comparative assessment of the Total Phenolic contents of methanol extracts of the anti-malarial plants (Garlic acid equivalent (GAE:mg/g)). (Data obtained from three determinations with $p < 0.05$ as level of significance)

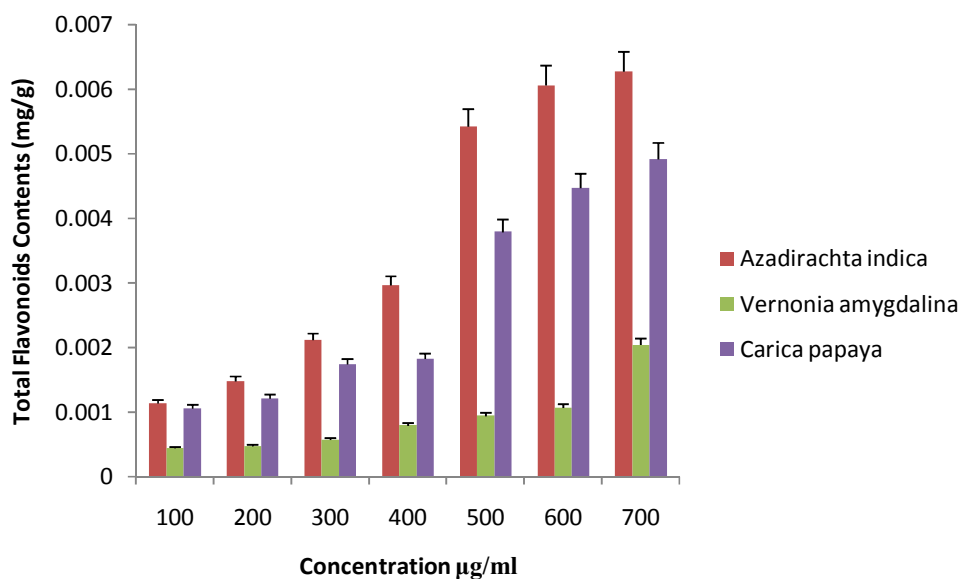


Figure 2: Comparative assessment of Total Flavonoids contents of the methanol extracts of the anti-malarial plants (Quercetin equivalent (QE:mg/g)). (Data obtained from three determinations with $p < 0.05$ as level of significance)

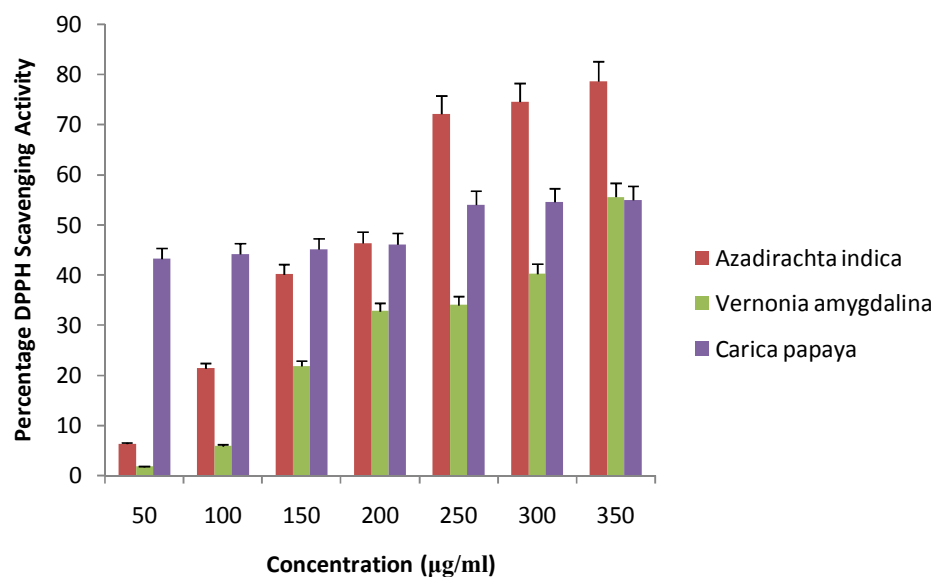


Figure 3: The Percentage DPPH Scavenging activity by various concentrations of methanol extracts of the anti-malarial plants. (Data obtained from three determinations with $p < 0.05$ as level of significance)

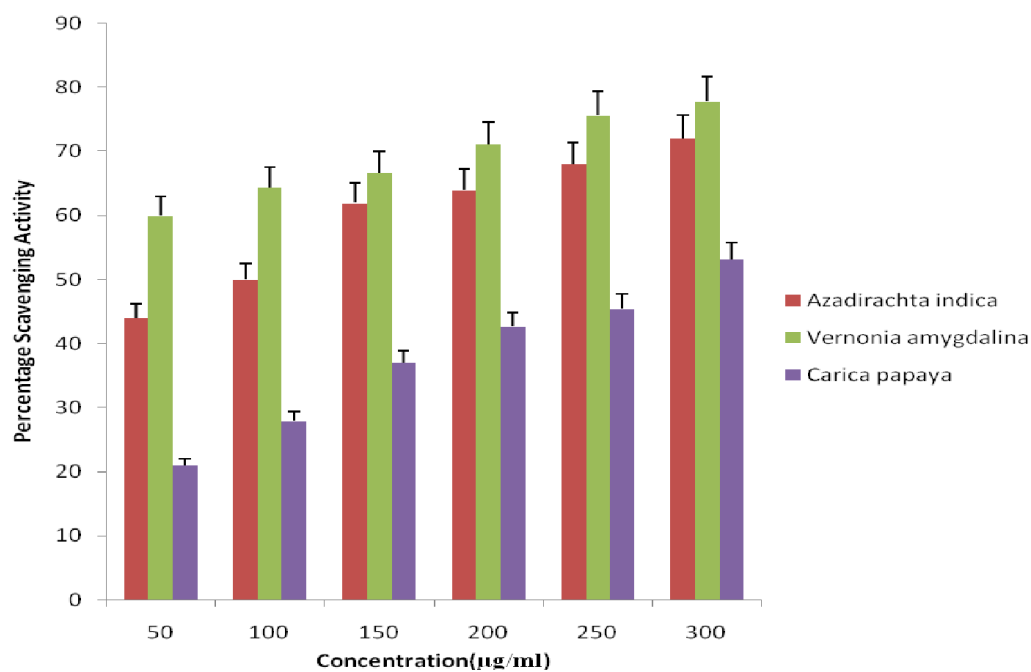


Figure 4: The percentage hydroxyl radical Scavenging activity by various concentrations of methanol extracts of the anti-malarial plants. (Data obtained from three determinations with $p < 0.05$ as level of significance)

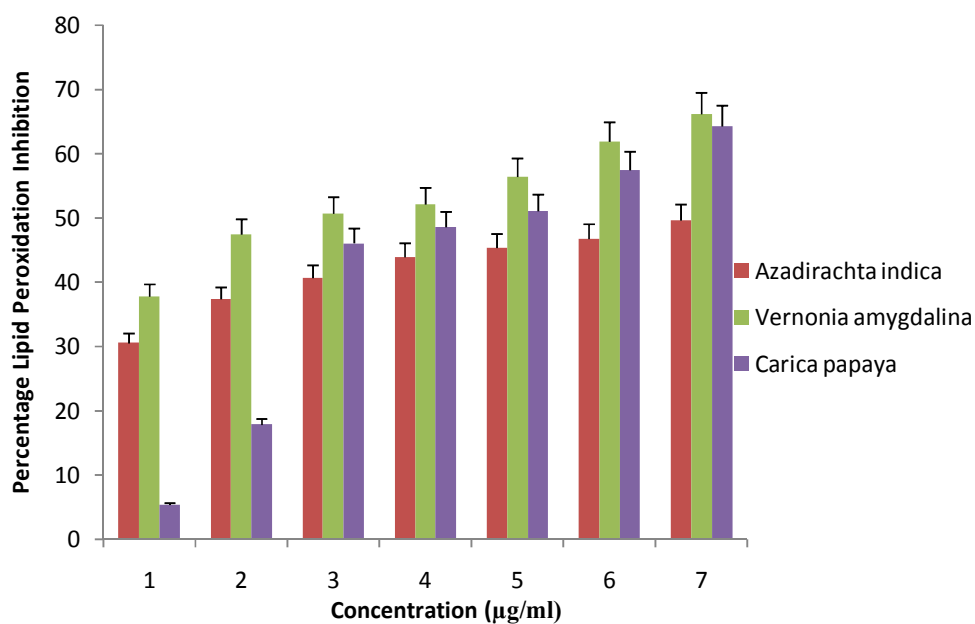


Figure 5: The percentage inhibition of lipid peroxidation by various concentrations of methanol extracts of the anti-malarial plants. (Data obtained from three determinations with $p < 0.05$ as level of significance)