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 700µg/ml by 0.015±0.002, 0.019±0.017, 0.013±0.006 mg/g and flavonoids contents at 350µg/ml by 0.063±0.004, 0.020±0.031, 0.049±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 350µg/ml by MAI, MVA and MCP respectively in the order MAI>MVA>MCP. At 300µg/ml, the extracts scavenged hydroxyl radicals significantly($p \leq 0.05$) by72.00, 77.80 and 53.15 % in the order MVA>MAI>MCP. Intriguingly, extracts also convered significantly ($p \leq 0.05$), 50% cell protection as they inhibited lipid peroxidation by50.00, 66.20 and 64.30% in the order MVA>MCP>MAI at 350µg/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

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

Keywords: AzadirachtaIndica, Antimalarial, Antioxidants, CaricaPapaya, 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 tuberculosis[4-6] .

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 drugs[9], and the exploitation of plants derived phytochemicals as rich sources of anti malarial drugs[10] . In many tropical countries herbal remedies mainly from plants are sought for due to lack of funds and scarcity of some known antimalarial drugs[11].

Medicinal plants, since times immemorial, have been used in virtually all cultures as a source of medicine[12]. 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 world[13]. 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 them [9] , and the efficacy of these herbs have been shown for decades in Africa[14] .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 45^0C.

Phytochemical study

Determination of the total phenolic contents of the extracts was based on the reduction of Folin-ciocalteau reagent (Phosphomolybdate and Phosphotungstate) by the[17] method. The reduced folin-ciocalteau 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 metod of[18], 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 of[19] , with ascorbic acid as standard and the hydroxyl radicals scavenging activity performed as described by[20], using iron/EDTA/H_2O_2complex. The percentage inhibition of lipid peroxidation were determined in-vitro using ferrous sulphate in a lipid- rich media as described by the method of[21].

Statistical Analysis

Statistical analysis were based on the Duncan`s experimental analysis with mean standard deviation of sample analysed using the student T test[22].

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 presently[23]. 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 peroxidation[24]. Furthermore, Xanthine Oxidase generates free radical superoxide (O_2^-) and a subsequent burst of other oxidative products[25], 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 figure1 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 700µg/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 350µg/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 scavengers[28], 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 DPPH[29]. 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 350µg /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 structures[32], 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 300µg/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 malaria[24] . 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 350µg/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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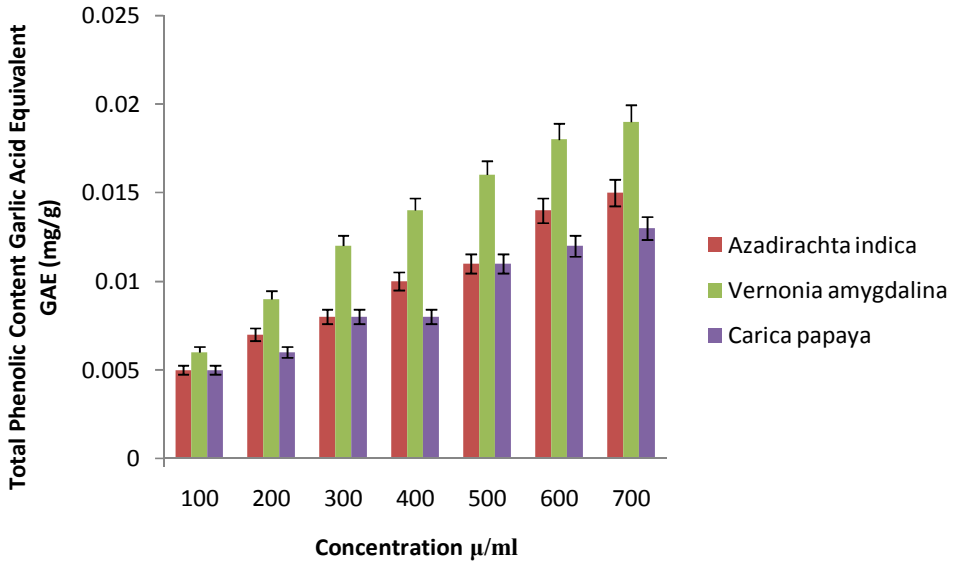

Figure 1: Comparative assessment of the Total Phenolic contents of methanol extracts of the anti-malarial plants (Garlic acid equivalent (GAE:mg/g)). (Data obtained from three determinations with $p<0.05$ as level of significance)

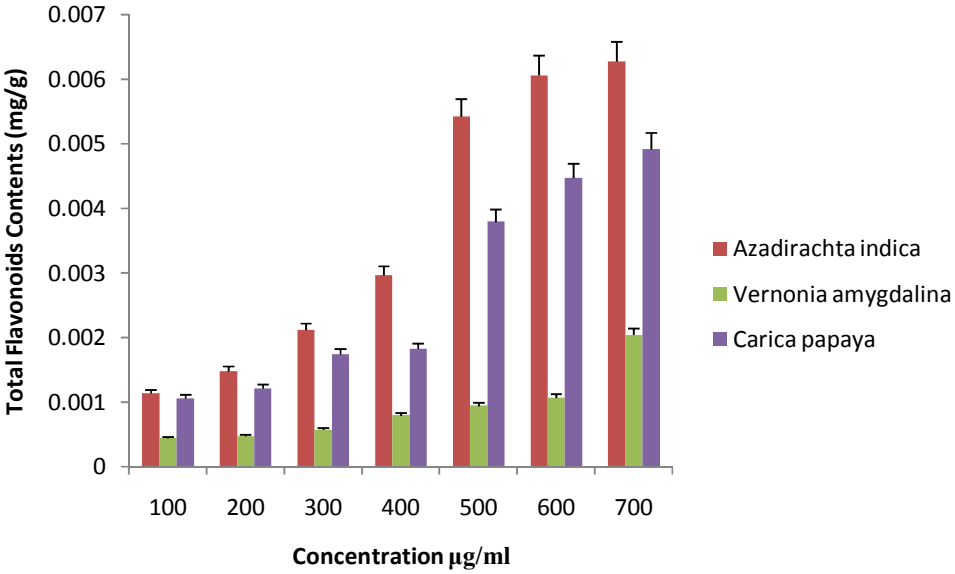

Figure 2: Comparative assessment of Total Flavonoids contents of the methanol extracts of the anti-malarial plants (Quercetin equivalent (QE:mg/g)). (Data obtained from three determinations with $p<0.05$ as level of significance)

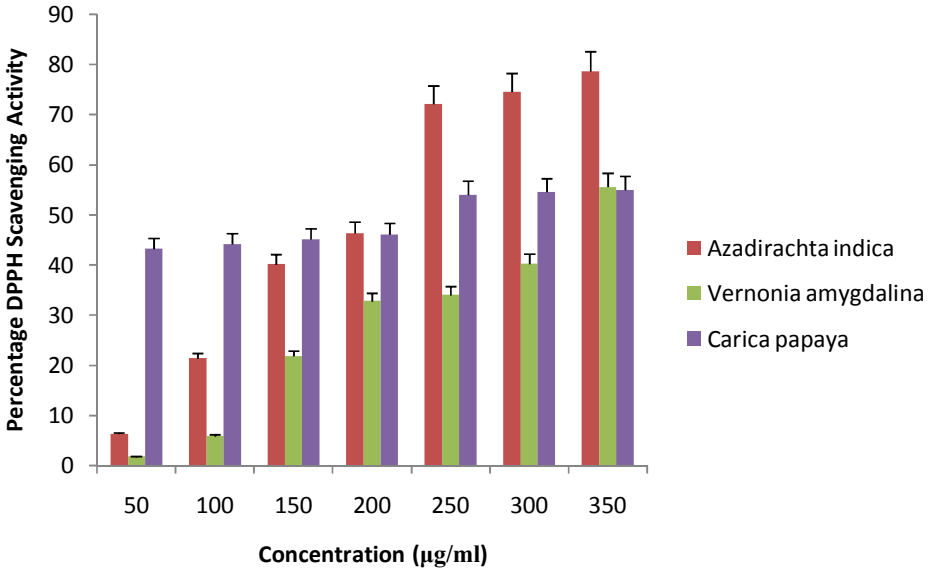

Figure 3: The Percentage DPPH Scavenging activity by various concentrations of methanol extracts of the anti-malarial plants. (Data obtained from three determinations with $p<0.05$ as level of significance)

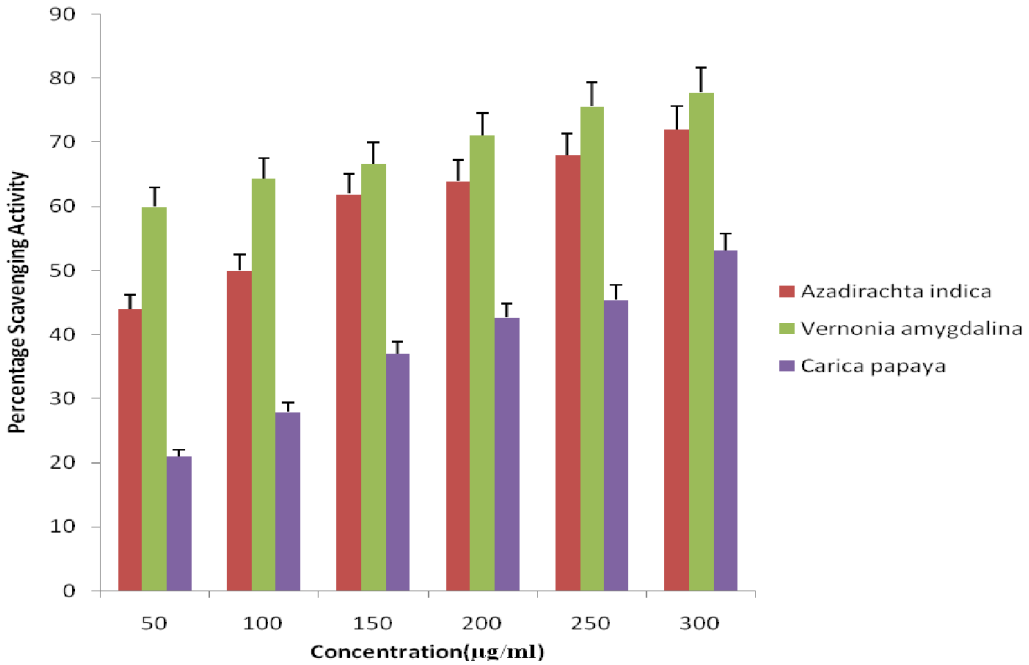

Figure 4: The percentage hydroxyl radical Scavenging activity by various concentrations of methanol extracts of the anti-malarial plants. (Data obtained from three determinations with $p<0.05$ as level of significance)

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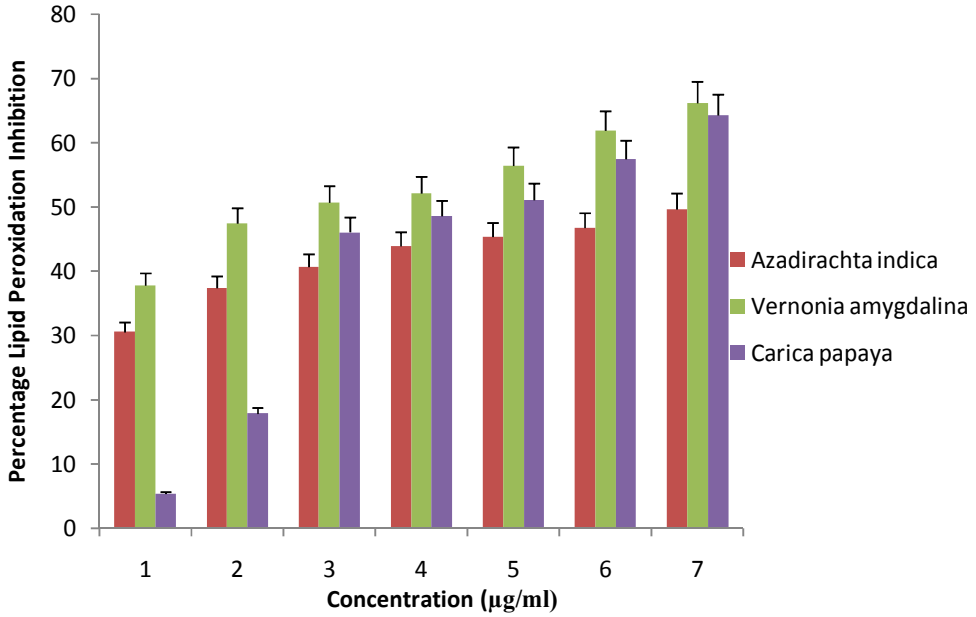

Figure 5: The percentage inhibition of lipid peroxidation by various concentrations of methanol extracts of the anti-malarial plants. (Data obtained from three determinations with $p<0.05$ as level of significance)