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DPPH free radical scavenging activity of some medicinal plants used in the treatment of malaria in south-western Nigeria

Güneybatı Nijerya'da sıtma tedavisinde kullanılan bazı tıbbi bitkilerin DPPH üzerinden serbest radikal süpürücü etkileri

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SUMMARY
AIM: The Antioxidant activities of the leaf, root and stem bark of 16 selected Nigerian medicinal plants frequently used in the treatment of malaria in South-Western Nigeria were investigated.
METHOD: The radical scavenging activities of the plant extracts against 2,2 Diphenyl -1-picryl hydrazyl, were determined by UV spectrophotometry at 570nm.
RESULTS: Quantitative analysis showed that the different plant parts demonstrated antioxidant activity in various degrees. However, DPPH radical scavenging activity was significantly highest ($P<0.05$) in the leaves of the different plants analyzed. Of the various plants analyzed, *Rauwolfia vomitoria* leaf had the highest radical scavenging activity with percentage inhibition of 2.2 diphenyl -1-picrylhydrazyl (DPPH) radical of 82.8±0.2% followed by that of *Vernonia amygdalina* having 82.6±0.2% inhibition. The stem bark of *Vernonia amygdalina* demonstrated the least antioxidant activity with only 2.9±0.1% inhibition of the DPPH radical.
CONCLUSION: The present work revealed that extracts of these plant parts possess antioxidant activity demonstrated in their various percentage inhibitions of the DPPH radical. However, the high antioxidant activity observed in the plant leaves confirms their frequency of usage in traditional medicine for the treatment of malaria.
Keywords: Antioxidant, medicinal plants, South-Western Nigeria, DPPH free radical.

ÖZET
AMAÇ: Güneybatı Nijerya'da sıklıkla sıtma tedavisinde kullanılan 16 bitkinin yaprak, kök ve kabuklarının antioksidan aktiviteleri incelendi.
YÖNTEM: Bitki ekstraktlarının 2,2 Diphenyl -1-picryl hydrazyl (DPPH) inhibisyonu yöntemiyle radikal süpürücü aktiviteleri UV spektrofotometrede 570 nm'de ölçüldü.
BULGULAR: Kantitatif analizler, farklı bitki parçalarının değişik derecelerde antioksidan aktivite gösterdiğini ortaya koydu. Radikal süpürücü aktivite bitki yapraklarında ($P < 0.05$) anlamlı derecede daha yüksekti. %82.8 ± 0.2 inhibisyona sahip olan *Rauwolfia vomitorium* yaprağı en yüksek radikal süpürücü etkiye sahipken, *Vernonia amygdalina* %82.6 ± 0.2 ile ikinci sıradaydı. *Vernonia amygdalina* kabuğu %2.9 ± 0.1 ile en düşük antioksidan aktiviteye sahipti.
SONUÇ: Bu çalışma, bitki parçalarının ekstrelerinin farklı derecelerde antioksidan aktiviteye sahip olduğunu DPPH inhibisyonu üzerinden ortaya koydu. Yüksek antioksidan aktivitesi olan yaprakların neden sıtma tedavisinde kullanıldıkları hakkında bir fikir verdi.
Anahtar kelimeler: Antioksidan aktivite, şifalı bitkiler, sıtma, Güneybatı Nijerya.

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INTRODUCTION

The medicinal value of plants have assumed a more important dimension in the past few decades owing largely to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potential. Additionally, current research in medicinal plants is beginning to lend credence to their efficacy and potency and in most instances over and above existing conventional and chemotherapeutic options [1]. Nearly all cultures and civilizations from ancient times to the present day have depended fully or partially on herbal medicine because of their effectiveness, affordability, availability, low toxicity and acceptability. The use of herbs for the treatment of malaria is a folk medical procedure in Nigeria. Several researchers have reported the effectiveness of herbs for the treatment of malaria but the herbs used and the quantity vary from one region to another. The important contribution traditional knowledge and practice have given to our modern medicine can be attested from the fact that more than 40% of commonly prescribed medicines throughout the world found their origin directly or indirectly from plants or animals [2]. According to WHO estimate, approximately 80% of the people in developing countries rely chiefly on traditional medicines for their primary health care, of which a major portion involve the use of plant extracts or active principles originating from plant parts. The main drugs developed for malaria and used up till now (quinine alkaloids derived drugs and artemislnin) were discovered based on traditional use and ethnomedical data [3, 4]. Ethnobotanical surveys carried out by several researchers have shown the use of a variety of plant species used singly or in combination for the treatment of malaria. Some of the mostly used plants according to ethnobotanical surveys carried out in the South-Western part of Nigeria incudes; *Azadirachta indica* (Dogoyaro), *Khaya senegalensis* (Oganwo), *Alstonia boonei* (Ahun), *Mangifera indica* (Mangoro), *Morinda lucida (*Oruwo), *Carica papaya (*ewe Ibepe), *Citrus species* (Osan wewe, *Osan gerepu*), *Anacardium occidentalis* (kasu), *Enanthia chlorantha* (Awopa), *Chlomolaena odorata* (Ewe Akintola), *Psidium guajava* (Gova) and *Tithonia diversifolia (Sepeleba*).

The therapeutic effects of several plants and vegetables which are used in traditional medicine have also been attributed to their antioxidant compounds. Antioxidants include a large group of chemicals that acts to fight and directly oppose oxygen charged molecules that are out to damage our cells. They act as a defense system against oxidative damage in our bodies and may be helpful in avoiding chronic diseases and effects of ageing. Some examples of antioxidants are vitamins A, C, and E and beta-carotene. Antioxidants protect other molecules (in vivo) from oxidation when they are exposed to free radicals and reactive oxygen species which have been implicated in the aetiology of many diseases and in food deterioration and spoilage [5-8]. They block the action of free radicals [9-11] by acting as a reducing agent reversing the oxidation by donating electrons and hydrogen ions [12]. Antioxidants are also used to preserve food quality mainly because they arrest oxidative deterioration of lipids.

Plant-based antioxidants are now preferred to the synthetic ones because of safety concerns [13, 14]. Natural antioxidants are also in high demand for application as nutraceuticals/functional food/bio-pharmaceuticals because of consumer preferences [15].These factors have inspired the widespread screening of plants for possible medicinal and antioxidant properties, the isolation and characterization of diverse phytochemicals and utilization of antioxidants of natural origin [16, 17]. It is therefore evident that a profile of the chemical composition of a plant together with knowledge of its antioxidant activity will give a fair estimate of its therapeutic potential [18]. The aim of this study is to determine the antioxidant activities of the different plant parts used in the treatment of malaria in South-Western Nigeria.

MATERIALS AND METHOD

Procedures

Plant selection, collection and processing: Plants were selected based on their high frequency of usage indicated in various Ethnobotanical surveys carried out in the South-Western part of Nigeria, on plants used in the treatment of malaria.

All plant materials shown in table 1 were collected between the months of January to March, 2010 around Ibadan, Oyo State in the South-western part of Nigeria and Identified at the Department of Botany Herbarium, University of Ibadan, Oyo state, Nigeria.

The different parts of the plants (leaves, stem bark, roots) were shade dried. The dried samples were chopped into smaller pieces and ground separately to powder using an electronic mill (commercial / generic disc attrition mill). The powdered samples were then stored in small plastic airtight containers.

Antioxidant Activity

Antioxidant activity was measured by a slightly modified method previously described by [19, 20].

Extraction Procedure

Two grams of each sample was extracted with 20mls acetone (80%) and 0.2% formic acid (20%) for 2mins. The extract was then filtered through Whatman filter paper 1. The supernatant was used for the analysis.

The radical scavenging activities of the plant extracts against 2,2 Diphenyl -1-picryl hydrazyl radical(Sigma - Aldrich) were determined by UV spectrophotometry at 570nm.

Determination of Antioxidant Activity using 2, 2Diphenyl-1-picryl hydrazyl (DPPH) radical

2mls of the extract was measured into a test tube and 3mls of methanol was added, followed by 0.5ml of 1mM DPPH in methanol. This was allowed to stand for 2mins.A blank solution was prepared containing the same amount of methanol and DPPH, The absorbance was measured on a spectrophotometer (Spectrum lab 23A) at 517nm and the radical scavenging activity was converted to percentage inhibition using the formula:

$$\% IH = \frac{A_a - A_b}{A_b} \times \frac{100}{1}$$

Where % IH = percentage inhibition

A_a = Absorbance of extract at 517nm

A_b = Absorbance of blank at 517nm

Statistical analysis

The results were analyzed by two-way Analysis of variance (Anova), using GLM procedure (Proc GLM) of SAS (statistical analysis system). All data is expressed as mean ± standard deviation (mean of 3 determinations) and difference between groups considered significant at $p < 0.05$.

RESULTS

Table 1 shows frequently used medicinal plants in the treatment of malaria employed in this study. The present study carried out on the antioxidant activities of some antimalarial plants commonly used in South-Western Nigeria, revealed a variation in the percentage inhibition of DPPH radical in the different plant parts (Leaf, root and stem bark) analyzed quantitatively. The result from this study is summarized in table 2.

Table 1. Frequently used medicinal plants in the treatment of malaria.

S/NO	SCIENTIFIC NAME	FAMILY NAME	LOCAL NAME	COMMON NAME	PARTS USED	ETHNOMEDICINAL USES	REFERENCE
1.	*Azadirachta indica* A.Juss	Meliaceae	Dongoyaro	Neem	Bark, leaves, Fruits.	Decoction of leaves and stem bark is used for the treatment of malaria. -Young twigs are chewed to keep the teeth and gums healthy -Fruits are recommended for piles -Oil from the seeds is used for skin diseases e.g scabies, ringworm, eczema -Decoction of fruit is used as an insecticide in farms	[21-29].
2.	*Morinda lucida* Benth	Rubiaceae	Oruwo	Brimestone tree	Bark, leaves	The bark, root and leaf are bitter and used in infusion or decoction for treatment of yellow fever and other forms of fever. -Twigs are used as chewing stick -A preparation from this plant is given for any febrile condition in childbirth -The very bitter leaf decoction is applied to the breast of women at weaning of their infants	[21, 26].

Table 1. Resume (Frequently used medicinal plants in the treatment of malaria

S/NO	SCIENTIFIC NAME	FAMILY NAME	LOCAL NAME	COMMON NAME	PARTS USED	ETHNOMEDICINAL USES	REFERENCE
3.	*Psidium guajava* L.	Myrtaceae	Gilofa	Guava	Bark, leaves	It is boiled with other leaves to cure fever -Decoction of fresh leaves is used as a remedy for stomach ache and diarrhoea -Leaves are chewed to relieve toothache -Leaves are boiled with lemon grass and the decoction taken for cough -Ripe fruit is mildly laxative, unripe fruit is astringent and anti-diarrhoetic -Leaves are used in diabetic preparations -Infusion of leaves is used to promote fertility in women	[21, 23, 25, 26].
4.	*Chrysophyllum albidum*	Sapotaceae	Agbalumo	African star apple	Bark, leaves	The bark is used for treatment of malaria, sleeping sickness and yellow fever. -.Leaves is used as emollients and for treatment of skin eruption.	[23].
5.	*Anacardium occidentalis* L	Anacardiaceae	Kasu	Cashew-nut tree	Bark, leaves	. In the south eastern part of Nigeria, the leaf extracts is ents. An infusion of the stem bark and leaves of the plant is used as a remedy for toothache and sore gums while the astringent bark is given for severe diarrhoea and thrush. -It is also used for the treatment of pile and scurvy prevention. Vinegar is obtained from it by fermentation	[23, 25, 27].
6.	*Mangifera indica* L	Anacardiaceae	Mangoro	Mango	Bark, leaves	The stem bark is used as blood booster and antibiotic -The leaves are used for malaria -The seeds are used as worm expellant -The fruits is a rich source of vitamin C -The skin of the fruit is used to treat uterine haemorrhage -The leaves are chewed to tone up the gums -The bark is astringent and is used as a remedy for skin lessons, sore gums, diarrhoea, dysentery and high blood pressure	[22-29].
7.	*Alstonia boonei* De Wild	Apocynaceae	Ahun	Stool wood	Root, bark leaves	The latex is also used as anaesthesia for pain relief -The root bark is chewed frequently to induce maturity and development of breast	[21, 23, 26].

Table 1. Resume (Frequently used medicinal plants in the treatment of malaria

S/NO	SCIENTIFIC NAME	FAMILY NAME	LOCAL NAME	COMMON NAME	PARTS USED	ETHNOMEDICINAL USES	REFERENCE
8.	*Carica papaya* L	Caricaceae	Ibepe	Pawpaw	Leaves, fruit	*Carica papaya* L is cultivated for its fruits, it is favoured by the people of the tropics as breakfast and as ingredients in jellies, preserved or cooked in various ways. The juice makes a popular beverage; young leaves, shoot and fruits are cooked as vegetables. . The seed is used for intestinal worm, unripe fruit as remedy for ulcer and impotence. The tea prepared with green leaves promotes digestion and aid in the treatment of ailments such as chronic indigestion, overweight and Obesity, anteriosclerosis, high blood pressure and weakening of the heart . -Fruit is applied on ringworm. It is a source of vitamin A and has some vitamin C. -Leaves are taken for malaria. Leaves mixed with lemon grass and guava leaves are also used in treatment of hypertension.	[23-28].
9.	*Vernonia amygdalina* Del.cent	Compositae	Ewuro	Bitterleaf	Leaves	leaf decoction is taken as laxative, digestive, tonic and for fever -infusion of bark and roots is used for rheumatism, as wash for itching, parasitic infection, ringworm, small pox, chicken pox and measles. -The twigs are chewing sticks for gastrointestinal troubles -root infusions is also used as an aphrodisiac -leaves are used in the treatment of diabetes -the decoction of the root with others is given for cough, cold and general weakness of the body. -fruit is recommended for piles, dysentery, colic, emetie and menstrual disorder.	[22-27].
10.	*Chromolaena odorata* (L) King&robinson	Compositae	Ewe - Akintola, Ewe – Awolowo	Siam weed	Root, leaves	Despite the negative sides to the plant which includes suppressing young plantation and being poisonous to livestock, it still has patronage from practitioners of traditional medicine. In the southern part of Nigeria, the leaves are used for wound dressing, skin infection and to stop bleeding -leaf infusion is used to treat fever and diabetes, crushed leaves are used to treat skin rashes, leaves are squeezed on fresh cuts to sterilize them. Leaves are also used to alleviate headache and toothache when chewed.	[23, 26].

Table 1. Resume (Frequently used medicinal plants in the treatment of malaria

S/NO	SCIENTIFIC NAME	FAMILY NAME	LOCAL NAME	COMMON NAME	PARTS USED	ETHNOMEDICINAL USES	REFERENCE
11.	*Rauwolfia vomitoria* Afzel	Apocynaceae	Asofeyeje	Swizzle stick	Root, bark leaves	The use of its root, root bark and stem bark are extensive particularly for their aphrodisiac, antihelminthic, dysenteric, diarrhoetic, cardiotonic, emetic, febrifugic, insecticidal and abortive properties reports that *Rauwolfia vomitoria* is good for the treatment of hypertension, insomnia, nervous disorder, Jaundice, fever, diarrhoea, dysentery, scabies, mental disorder, anti-helminthic and malaria. -A decoction of the root can be used as a sedative and the root bark for treatment of convulsion in children -root and leaf are used for urethral discharge -the leaves and berries are powerfully emetic -leaves, seeds and roots are used in treatment of mental related ailments -Itching is treated with crushed leaves while hot infusion of pulverized rootbark treats insomnia	[23, 26].
12.	*Khaya senegalensis* Desr	Meliaceae	Oganwo	Mahogany	Bark	The bark extract is used for treating jaundice, dermatoses, hookworm infection and malaria. The seeds and leaves are used to treat fever and headache and the roots are used to treat mental illness, syphilis and leprosy The stembark and leaves of *Khaya senegalensis* have been used in Adamawa State in Northern Nigeria in forms of decoction and concoctions to cure mucous diarrhoea, syphilils, pyrexia, and malaria fever.	[24, 27].
13.	*Citrus aurantifolia* L	Rutaceae	Osan-wewe	Lime	Root, bark, leaves, fruit	*Citrus aurantifolia* is one plant that has been in use for ailments such as common cold, depressive illness and alcoholism and has been acclaimed to possess anti-inflammatory antirheumatic, anti-scorbatic, anti-coagulant, anti-spasmodic and anti-infective properties.	[23].
14.	*Citrus paradise*	Rutaceae	Osan-gerepu	Grape	Fruit, leaves, root, stem	building up resistance to common colds and wound infections	[23].
15.	*Tithonia diversifolia* A.Grey	Compositae	Jogbo, Agbale	Tree marigold	Leaves, stem, twigs	Ethnobotanical surveys have shown that extracts from this plant exhibited antimalarial, antidiarrhoeic, anti-inflammatory, antibacterial, and anti proliferation properties. It is also commonly used in decoction for convulsions and fever or as an external application. It has also been known to relieve rheumatism and the flowers are beneficial in treatment of eye diseases	[23].

Table 1. Resume (Frequently used medicinal plants in the treatment of malaria

S/NO	SCIENTIFIC NAME	FAMILY NAME	LOCAL NAME	COMMON NAME	PARTS USED	ETHNOMEDICINAL USES	REFERENCE
16.	*Adansonia digitata* L	Bombacaceae	Ose	Baobab	Leaves; stem, roots	*Adansonia digitata* is a plant traditionally employed in several African regions as food stuffs and for medicinal purposes.The native African population commonly used baobab fruit as famine food, to prepare decoctions, sauces and natural refreshing drinks due to its nutritional properties The pulp is therapeutically employed as analgesic anti-diarrhoea and for treatment of small pox and measles.The fruit pulp of *A. digitata* is traditionally used for the treatment of fever, diarrhoea, dysentery, haemolysis and small pox in humans Leaf infusions are used as treatment for diarrhoea, fever, inflammation, kidney and bladder diseases, blood clearing and asthma in humans.The bark is used for treatment of fever especially that caused by malaria As far as ethnovetrinary medicine is concerned, reports indicates that bark of *Adansonia digitata* is used for treatment of diarrhea in poultry and fruits are used for treatment of New castle diseases in poultry.	[27].

From the results obtained from this study, radical scavenging activity was significantly highest ($P<0.05$) in the leaves of the different plants analyzed. This may be due to the fact that the leaf tends to possess more phytochemicals as a result of their brightness in colour. The results also showed that the leaves of *Rauwolfia vomitoria* and *Vernonia amygdalina* demonstrated high radical scavenging activity with percentage inhibition of 82.8±0.2% and 82.6±0.2% respectively. However antioxidant activity was lowest in the stem bark of *Vernonia amygdalina* having only 2.9±0.1% inhibition of the DPPH radical. (Fig.1)

DISCUSSION

The different plants analyzed demonstrated antioxidant activities in various percentages. However, Antioxidant activity was highest in the leaves of *Rauwolfia vomitoria*, followed by the leaves of *Vernonia amygdalina* while *Vernonia amygdalina* stem bark demonstrated the least antioxidant activity (Fig 1). Antioxidants have been reported to offer protection against the oxidative stress induced by malaria infection [30, 31]. The antioxidant activity of some phytochemicals such as carotenoids has also been reported to counteract the effects of free radicals generated in the presence of malaria [31].

Increase in reactive oxygen species (ROS) has already been described in *Plasmodium vivax* malaria [32]. As a result of the increased metabolic rate of the rapidly growing and multiplying parasite, large quantities of toxic redox-active byproducts are generated. Furthermore, a reduction in antioxidant enzymes such as glutathione peroxidase, catalase and superoxide dismutase has been observed in plasma of malaria-infected individuals [33-35]. These changes in oxidants and anti-oxidants have been associated with severe malaria in children [36]. Oxidative stress (OS) in malaria can be caused by two main mechanisms. Firstly, by the parasite which reproduces in the erythrocytes, changing the structure and affecting parameters such as stiffness, viscosity and volume. Central to the generation of OS is the degradation of host haemoglobin by the parasite. Secondly, the OS mechanisms involve the host immune response, which initiates a cascade of defense mechanisms culminating with the release of free radicals by activated macrophages, to tackle the parasite [37- 38]. Reactive hydroxyl radicals (•OH) generated via mitochondrial OS, have been shown to play an important role in the liver apoptosis in a murine model of malarial infection [39-40].

Table 2. Antioxidant activity of the different plant parts analyzed.

Plants	Parts	Antioxidant activity (%inhibition)
Adansonia digitata	Leaf	80.5±0.0a
	Root	12.5±0.3c
	Stembark	28.6±0.2b
Alstonia boonei	Leaf	71.6±0.1a
	Root	12.7±0.2b
	Stembark	11.3±0.2c
Anacardium occidentalis	Leaf	71.1±0.2a
	Root	40.5±0.2c
	Stembark	59.9±0.1b
Azadirachta indica	Leaf	67.7±0.2a
	Root	19.7±0.4c
	Stembark	61.4±0.2b
Carica papaya	Leaf	72.3±0.2a
	Root	36.7±0.2b
	Stembark	6.0±0.0c
Chromolaena odorata	Leaf	81.2±0.3a
	Root	10.8±0.2b
	Stembark	11.1±0.1b
Chrysophyllum albidum	Leaf	79.7±0.3a
	Root	6.3±0.0c
	Stembark	45.4±0.5b
Citrus aurantifolia	Leaf	58.3±0.2a
	Root	7.0±0.0c
	Stembark	19.1±0.5b
Citrus paradise	Leaf	64.5±0.3b
	Root	13.8±0.2c
	Stembark	69.1±0.1a
Khaya senegalensis	Leaf	38.2±0.3c
	Root	44.2±0.2a
	Stembark	43.2±0.6b
Mangifera indica	Leaf	81.7±0.3a
	Root	31.4±0.2c
	Stembark	50.4±0.1b
Morinda lucida	Leaf	78.9±0.3a
	Root	8.3±0.1c
	Stembark	28.6±0.1b
Psidium guajava	Leaf	67.6±0.1a
	Root	44.5±0.3b
	Stembark	41.8±0.3c
Rauwolfia vomitoria	Leaf	82.8±0.2a
	Root	9.6±0.4b
	Stembark	7.8±0.3c
Tithonia diversifolia	Leaf	58.7±0.5a
	Root	7.0±0.0C
	Stembark	9.3±0.2b
Vernonia amygdalina	Leaf	82.6±0.2a
	Root	17.5±0.3b
	Stembark	2.9±0.1c

Figures are expressed as mean ±SD
Figures bearing different alphabets differ significantly ($P<0.05$)

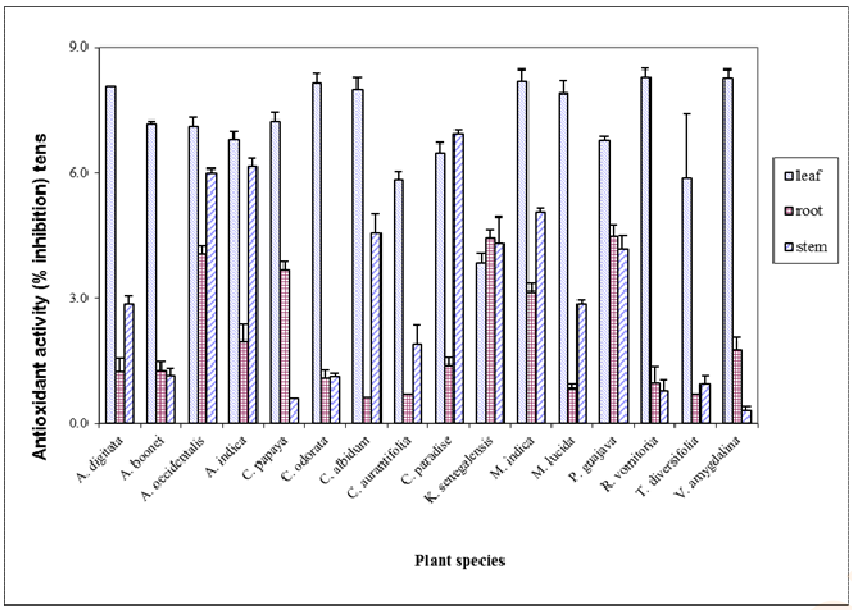

Figure 1. Antioxidant activity of the different plant parts analyzed

Several reports in the literature suggest that drugs used to treat malaria, such as chloroquine and primaquine lead to oxidative stress, particularly in erythrocytes [41-43]. In addition total antioxidant level varies with the severity of malaria. Patients with severe malaria have been shown to have significantly lower total antioxidant levels than those with mild/moderate malaria. Antioxidants are used up to counteract the effects of free radicals generated in the course of malaria infection. This explains why reduction in antioxidant level is dependent on the severity of malaria.

Vitamins A, C, and beta-carotene, which are contributors to antioxidants activities, have been measured in a study [30]. From their observations, these antioxidants are reduced in malaria infection and reduction of these antioxidants in turn caused the reduction of the total antioxidant levels of malaria-infected children. Furthermore, lowered levels of antioxidants, especially of vitamin C in malaria infection also suggest lowered immunity of host which may be responsible for some of the complications of malaria infection. The reduction of these antioxidants in the face of malaria infection may pre-dispose the children to free radical attack.

To reduce morbidity due to *Plasmodium falciparum*, dietary modifications, including foods rich in antioxidants (such as vegetables, fruits etc.) should be encouraged [31]. The antioxidant activity may result from the neutralization of free radical initiating oxidation processes or from the termination of radical chain reactions [44]. The relatively low antioxidant activity of the stem bark of *Vernonia amygdalina* may however, not imply a low medicinal value [18].

CONCLUSION AND RECOMMENDATION

The present work revealed that extracts of these plant parts possess antioxidant activity demonstrated in their various percentage inhibitions of the DPPH radical. However, the high antioxidant activity observed in the plant leaves confirms their frequency of usage in traditional medicine for the treatment of malaria.

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