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VARIATION IN THE CAROTENOID CONTENT OF SOME FREQUENTLY USED MEDICINAL PLANTS FOR THE TREATMENT OF MALARIA IN SOUTH-WESTERN NIGERIA

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ABSTRACT

Malaria, an infection caused by *Plasmodium*, is a disease transmitted to humans by the female anopheles mosquito. It remains a major cause of death throughout the world especially in sub-Saharan Africa. The use of herbs for the treatment of malaria is a folk medicinal procedure in Nigeria but the herbs used and the quantity vary from one region to another. It has also been revealed that of all the plant parts used in the treatment of malaria, the leaf has the highest frequency of usage. This study was designed to investigate the variation in the carotenoid content of frequently used medicinal plants in the treatment of malaria in South-Western Nigeria. Quantitative phytochemical analysis showed that carotenoids were present in the different plant parts in various concentrations. However, the carotenoid content was significantly higher ($P<0.05$) in the leaves of the different plants. Of all the plants analyzed, carotenoid content was significantly higher ($P<0.05$) in *Morinda lucida* leaf (207.7±2.5mg/kg GAE) and almost absent in the roots of *Chromolaena odorata* and *Chrysophyllum albidum* (having 1.0±0.0 mg/kg GAE, respectively). These results show that carotenoids occur in the different plant parts in varied concentrations. However, the high composition of carotenoids observed in the leaves of the various plants analyzed is suggestive of their high frequency of usage in traditional medicine for the treatment of malaria.

KEYWORDS: Medicinal plants, Malaria, Carotenoid contents, South-Western Nigeria.

INTRODUCTION

A medicinal plant is any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Odugbemi, 2008). The medicinal values 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 (Akinmoladun *et al.*, 2007). 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 (Atangwho *et al.*, 2009). The treatment and control of diseases by the use of the available medicinal plants in a locality will continue to play significant roles in medical health care implementation in the developing countries of the world. Nearly all cultures and civilizations from ancient times to the present-day have depended fully or partially on herbal medicines because of their effectiveness, affordability, availability, low toxicity and acceptability (Atangwho *et al.*, 2009).

Malaria, an infection caused by *Plasmodium*, is a disease transmitted to humans by the female anopheles mosquito. According to WHO, malaria is endemic in 91 countries predominantly in Africa, Asia and Latin America, with about 40% of the world's population at risk. Malaria is distributed widely mainly due to multi-drug resistance developed by *Plasmodium falciparum*. It remains the leading cause of death due to parasitic diseases with approximately 360 million clinical cases annually resulting in an estimated 2,000 000 deaths primarily in children (WHO, 1996; WHO, 1998; Mohammed *et al.*, 2007). According to WHO in 2008,

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there was an estimated 247 million cases of malaria and nearly one million deaths – mostly among children living in Africa. In Africa, a child dies every 45 seconds of Malaria disease and that accounts for 20% of all childhood deaths (WHO, 2010).

Most vulnerable group in the endemic areas constitutes people in the rural environment who often have little or no access to modern medicine. This situation has been complicated further by the emergence of multi-drug resistant strains of *Plasmodium falciparum* and rapid spread of the vector which slows resistance to insecticides (Coker *et al.*, 2000; Masaba *et al.*, 2000). Hence, there is an urgent need to find alternative therapies that are not only effective against resistant malaria but are also available and affordable to this vulnerable group who are not economically buoyant to afford expensive orthodox medicine or have no access to modern health facilities (Coker *et al.*, 2000).

The use of herbs for the treatment of malaria is a folk medicinal practice 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 (Farnsworth *et al.*, 1985). According to WHO estimate, approximately 80% of the people in developing countries rely chiefly on traditional medicines for their primary health care; majority employ 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 artemisinin) were discovered based on traditional use and ethnomedical data (Sofowora, 1984; Muller *et al.*, 2005). In addition, a study by Idowu *et al.* (2010) reported that of all the plant parts (leaf, stem, roots, fruits, seeds and underground stems) used in the treatment of malaria, the leaf had the highest frequency of usage.

Phytochemicals have proven to be beneficial in many ways. They may serve as antioxidants in a bodily system when required (Dhakarey *et al.*, 2005; Singh *et al.*, 2008; Singh *et al.*, 2009). They may also enhance immune response and cell-to-cell communication allowing for the body's built-in defenses to work more efficiently. Phytochemicals may even alter estrogen metabolism, cause cancer cells to die (apoptosis), repair DNA damage caused by smoking and other toxic exposure and detoxify carcinogens by working with bodily enzymes. Some of the common classes of phytochemicals include carotenoids, alkaloids, glycosides, terpenes and polyphenols. Fruits and vegetables that are bright in colour usually have the most phytochemicals and nutrients. It is believed that phytochemicals may be effective in combating or preventing diseases due to their antioxidant effect (Halliwell and Gutteridge, 1992; Farombi *et al.*, 1998). A synergistic relationship amongst phytochemicals is believed to be responsible for the overall beneficial effect derivable from plants (Liu, 2004; Akinmoladun *et al.*, 2007).

Studies have shown that natural compounds including carotenoids, vitamin E and vitamin C may contribute to the radical scavenging activity of plants (Chin *et al.*, 2008). Carotenoids are pigments which play a major role in the protection of plants against photo-oxidative processes. Beta-carotene and other carotenoids are believed to provide antioxidant protection to lipid-rich tissues and it may work synergistically with vitamin E (Percival, 1998). Evidence suggests that cataract progression might be slowed with regular consumption of carotenoids (Jacques, 1994). Carotenoids have also been linked with enhancement of the immune system and decreased risk of degenerative diseases such as cancer, cardiovascular disease, age-related muscular degeneration and cataract formation (Rodriguez-Amaya, 1997). It has also been shown that lycopene and â-carotene are effective in inhibiting the cell growth of various human cancer cell lines (Khachik *et al.*, 2002). Antioxidant activities of carotenoids have also been reported to counteract the effects of free radicals generated in the presence of malaria (Onyesom *et al.*, 2010).

It has been reported that some plants reveal the presence of bioactive properties in their parts (leaves, stem bark and roots) in various degrees or concentrations which also reflects in their therapeutic efficacy (Akharaiyi and Boboye, 2010). Since different parts of some plants have different therapeutic applications, it is important to establish the quantity of the plant raw materials for its constituent plant parts (Sunita and Abishek, 2008). Consequently, for extraction of valuable phytochemicals with great quantity and therapeutic effectiveness, it is important to know the part of the plant with the highest composition.

The aim of this study was to determine variation in the carotenoid content of the different plant parts used in the treatment of malaria in South-Western Nigeria

MATERIALS AND METHODS

Plant Selection/Collection/Identification

Plants were selected based on their high frequency of usage in the treatment of malaria indicated by various ethnobotanical surveys carried out in parts of South-Western Nigeria. All plant materials were collected around Ibadan, Oyo State in South-Western Nigeria and identified at the University of Ibadan Herbarium (UIH), Oyo State, Nigeria.

Processing of Plant Materials

The different parts of the selected plant materials (leaves, stem bark, roots) were air - dried. The dried samples were chopped into small 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.

Phytochemical Screening

Determination of carotenoids using Harborne (1973) method:- One gram each of the samples was measured and extracted with 20 mls acetone and left for 1hour. In a separating funnel, 5 ml each of water and petroleum ether were added by pouring it along the sides of the separating funnel. The petroleum ether layer (containing the carotenoid pigment) was collected and the absorbance (optical density) measured in a spectrophotometer (Spectrum lab 23A) at 440 nm using petroleum ether as a blank. The amount of total carotenoids was calculated in mg per kg.

Statistical Analysis

The results were analyzed by two-way Analysis of Variance (ANOVA), using the General Linear Model (GLM) procedure (Proc GLM) of SAS (Statistical Analysis System). The data were expressed as mean ± standard deviation (mean of 3 determinations) and difference between groups considered significant at $p < 0.05$.

RESULTS

The scientific names, families, local names, common names and parts of plants frequently used in the treatment of malaria are shown in Table 1.

Table 1: Frequently used antimalarial plants in Ibadan, Southwestern Nigeria

S/No	SCIENTIFIC NAME	FAMILY NAME	LOCAL NAME (Yoruba)	COMMON NAME	PARTS USED
1	*Adansonia digitata* L	Bombacaceae	Ose	Baobab	Leaf, stem, roots
2	*Alstonia boonei* De Wild	Apocynaceae	Ahun	Stool wood	Root, bark, lef
3	*Anacardium occidentalis* L	Anacardiaceae	Kasu	Cashew-nut tree	Bark, leaf
4	*Azadirachta indica* A.Juss	Meliaceae	Dongonyaro	Neem	Bark, leaf
5	*Carica papaya* L	Caricaceae	Ibepe	Pawpaw	Leaf, fruit
6	*Chromolaena odorata* (L) King&robinson	Asteraceae	Ewe -Akintola, Ewe – Awolowo	Siam weed	Root, leaf
7	*Chrysophyllum albidum*	Sapotaceae	Agbalumo	African star apple	Bark, leaf
8	*Citrus aurantifolia* L	Rutaceae	Osan-wewe	Lime	Root, bark, leaf, fruit
9	*Citrus paradisi*	Rutaceae	Osan-gerepu	Grape	Fruit, leaf, root, stem
10	*Khaya senegalensis* Desr	Meliaceae	Oganwo	Mahogany	Bark
11	*Mangifera indica* L	Anacardiaceae	Mangoro	Mango	Bark, leaf
12	*Morinda lucida* Benth	Rubiaceae	Oruwo	Brimestone tree	Bark, leaf
13	*Psidium guajava* L.	Myrtaceae	Gilofa	Guava	Bark, leaf
14	*Rauwolfia vomitoria* Afzel	Apocynaceae	Asofeyeje	Swizzle stick	Root, bark, leaf
15	*Tithonia diversifolia* A.Grey	Asteraceae	Jogbo, Agbale	Tree marigold	Leaf, stem, twigs
16	*Vernonia amygdalina* Del.cent	Asteraceae	Ewuro	Bitterleaf	Leaf

The present study carried out on the carotenoid content of some antimalarial plants commonly used in Ibadan, South-Western Nigeria, revealed the presence of carotenoids in various quantities in the different plant parts (leaf, root and stem bark) analyzed quantitatively. The quantitative estimation of the carotenoid content is summarized in Table 2.

Table 2: Carotenoid content of the different plant parts analyzed

S/No	Plants	Parts	Carotenoids (mg/kg GAE)
1	*Adansonia digitata*	Leaf	181.0±1.0a
		Root	10.0±0.0c
		Stem bark	64.0±2.0b
2	*Alstonia boonei*	Leaf	180.0±2.0a
		Root	5.0±0.0c
		Stem bark	30.0±0.0b
3	*Anacardium occidentalis*	Leaf	193.7±3.2a
		Root	29.0±1.0b
		Stem bark	5.0±0.0c
4	*Azadirachta indica*	Leaf	190.0±5.0a
		Root	98.0±2.0b
		Stem bark	30.0±2.0c
5	*Carica papaya*	Leaf	190.7±4.5a
		Root	14.0±1.0c
		Stem bark	22.7±1.5b
6	*Chromolaena odorata*	Leaf	196.0±4.0a
		Root	1.0±0.0c*
		Stem bark	21.0±1.0b
7	*Chrysophyllum albidum*	Leaf	190.0±2.0a
		Root	1.0±0.0c*
		Stem bark	23.0±2.0b
8	*Citrus aurantifolia*	Leaf	186.0±2.0a
		Root	87.0±3.0b
		Stem bark	30.0±0.0c
9	*Citrus paradisi*	Leaf	189.0±1.0a
		Root	179.0±1.0b
		Stem bark	137.0±2.0c
10	*Khaya senegalensis*	Leaf	189.0±1.0a
		Root	11.0±1.0c
		Stem bark	20.0±0.0b
11	*Mangifera indica*	Leaf	189.7±1.5a
		Root	17.0±3.0b
		Stem bark	5.0±0.0c
12	*Morinda lucida*	Leaf	207.7±2.5a
		Root	6.0±0.0c
		Stem bark	75.0±0.0b
13	*Psidium guajava*	Leaf	191.0±2.0a
		Root	59.0±1.0b
		Stem bark	24.0±1.0c
14	*Rauwolfia vomitoria*	Leaf	191.0±1.0a
		Root	11.0±0.0c
		Stem bark	28.0±2.0b
15	*Tithonia diversifolia*	Leaf	162.3±2.5a
		Root	5.0±0.0c
		Stem bark	57.3±2.5b
16	*Vernonia amygdalina*	Leaf	190.0±5.0a
		Root	5.0±0.0c
		Stem bark	37.0±3.0b

Figures are expressed as mean ±SD
Figures bearing different alphabets differ significantly ($P<0.05$)
*not significantly different ($P>0.05$)

There was significant difference ($P<0.05$) in the carotenoid content of the different plant parts studied in all plants except the roots of *Chromolaena adorata* and *Chrysophyllum albidum* (Table 2). From the results obtained in this study, the carotenoid content 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. Of all the plants analyzed, carotenoid content was significantly higher ($P<0.05$) in *Morinda lucida* leaf (207.7±2.5mg/kg GAE) followed by *Chromolaena odorata* leaf (196.0±4.0mg/kg GAE) but absent in the roots of *Chromolaena odorata* and *Chrysophyllum albidum,* both of which 1.0±0.0mg/kg GAE (Table 2).

DISCUSSION

The phytochemical composition of the leaf, root and stem bark of the antimalarial plants employed in this study revealed the presence of carotenoids in variable quantities as there were significant differences between the plant part compositions of these phytochemicals. From the results obtained, it was also revealed that the carotenoid content was significantly highest in the leaves of the different parts of 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. Consequently, this result may suggest the frequent use of plant leaves in the treatment of malaria as indicated in a study by Idowu *et al.* (2010).

This variation in carotenoid composition in the plant parts could be as a result of seasonal variation among other reasons. Singh *et al.* (2010) reported that the presence of phytochemicals in plant parts is greatly influenced by environmental conditions and degree of development. It has also been reported that some plants reveal the presence of bioactive properties in their parts (leaves, stem bark and roots) in various degrees or concentration which also reflects in their therapeutic efficacy (Musyimi *et al.*, 2008; Akharaiyi and Boboye, 2010). In most cases, maximum accumulation of chemical constituents occur at the time of flowering which then decline at the beginning of the fruiting stage (Mendonca – Fillio, 2006). This was also observed by Makinde *et al.* (1994) in their report on *Morinda lucida* leaf extract against *Plasmodium berghei* in mice. This may indicate that at a certain period of the year, the bioactive components present in some plant specimen could have reduced to minimum levels such that when these plants are tested again, contrary results may be obtained. This could explain why certain plants are used within certain periods of the year for effective cure of diseases in ethnomedicine (Adomi and Umukoro, 2010). State of maturity is another factor responsible for the observed variation in plant part composition of these plants. It was reported that younger leaves of tropical rainforest plants contained secondary metabolites that were either present in very little quantities or totally absent in matured leaves, and the extract from these younger leaves showed better biological activity when tested for anticancer activity and against *Bacillus subtilis* and *Artemia salina* (Brime shrimp) (Kursar *et al.*, 1999). This is, however, in contrast to the report given by Singh *et al.* (2010) on variation of some phytochemicals in methi and saunf plants at different stages of development. From their report, it was observed that phytochemicals showed significant increase in mature plants in comparison to early stage plants and their parts in both methi and saunf, suggesting that these phytochemicals increase with maturity.

In many areas endemic to malaria, high rates of malnutrition and micronutrient deficiencies are observed. Several of these micronutrients, including vitamins A and E, carotenoids and zinc, play essential roles in immune function (Semba, 1998; Hughes, 1999; Meydani and Beharka, 1998; Shankar and Prasad, 1998) and are implicated in resistance to many infectious diseases (Shankar and Prasad, 1998; Black, 1998). Increased importance has been given to the role that nutrition plays in malaria, and vitamin deficiencies have been associated with morbidity and mortality due to malaria. Individuals with malaria have lower plasma concentrations of several micronutrients compared to controls (Adelekan *et al.*, 1997; Das *et al.*, 1996; Hautvast *et al.*, 1998). It had been reported that supplementation of vitamin A in children reduces morbidity due to malaria by 30% (Shanker *et al.*, 1999), and supplementation of zinc resulted in 38% reduction in attendance in a *Plasmodium falciparum* health centre by preschool children (Shankar *et al.*, 2000). It has been demonstrated in a study by Akpotuzor *et al.*(2007) that betacarotene levels, along with levels of other antioxidants in children with malaria infection are depressed. A similar observation was also made in other reports (Cooper *et al.*, 2002; Galloway *et al.*, 2000; Adelekan *et al.*, 1997).The low circulating beta-carotene is attributed in part to increased consumption in the face of enhanced free radical activity (Galloway *et al.*, 2000). Biochemical findings have demonstrated the presence of carotenoid biosynthesis in the intraerythro-cytic stages of the apicomplexan parasite *P. falciparum* (Tonhosolo *et al.*, 2009). Antioxidant activities of carotenoids have also been reported to counteract the effects of free radicals generated in the presence of malaria (Onyesom *et al.*, 2010).

The reduction of these antioxidants in the face of malaria infection may, however, pre-dispose children to free radical attack. To avert this consequence, it is recommended that antioxidant agent (particularly Vitamin C) and leafy vegetables rich in carotenoids be made one of the component drug regimens/nutritional supplements for the treatment of malaria infection.

CONCLUSION

Results from the present investigation show that these plants are rich in carotenoids which occur in various quantities in the different plant parts analyzed. However, the high composition of carotenoids observed in the leaves of the plants is indicated in the frequency of usage of this part in traditional medicine for the treatment of malaria.

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