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Research Paper

Comparing the Phytochemical Composition of Some Plant Parts Commonly Used in the Treatment of Malaria

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Abstract: *Malaria is endemic to Africa in which Nigeria is the largest country. Medicinal plants are harvested widely in parts or as a whole due to the curative effects derived from their phytochemicals. It is common knowledge that just about 10% of the total plants have their phytochemicals known hence the desire to investigate the various phytochemicals present in the plant parts. Variation in the phytochemicals present in the leaf, root and stem bark of some frequently used Nigerian medicinal plants - Adansonia digitata, Alstonia boonei, Anacardium occidentalis, Azadirachta indica, Carica papaya, Chromolaena odorata, Chrysophyllum albidum and Citrus aurantifolia in the treatment of malaria were therefore investigated. Quantitative phytochemical analysis revealed the presence of tannins, alkaloids, flavonoids, terpenoids, saponins and phenolic acids in various concentrations as there were significant differences ($p<0.05$) between these phytochemicals and their plant parts. The presence of varied active ingredients in the different parts of these plants explains their diverse use in the treatment of different disease conditions. The research into the isolation and identification of these active ingredients in the different plant parts will help to authenticate the diverse claims of herbal practitioners on the use of plants and their parts in the cure of several diseases.*

Keywords: Malaria, Plant parts, Phytochemicals, Medicinal plants.

Introduction

The plant kingdom represents a vast emporium of untapped medical potentialities and this has led to a resurgence of interest in ethnomedicine, ethnobotany and ethnopharmacology (Fadeyi *et al*., 1989). Malaria is a tropical disease caused by the parasite, *Plasmodium* which is transmitted by infected female anopheles mosquitoes commonly found throughout the world especially in sub-saharan Africa. It is characterized by bouts of shivering, sudden rise in temperature and general aching of the body as well as profuse sweating at regular intervals (Ehiagbonare, 2007). Globally, death associated with malaria is highest in Africa and most vulnerable group in the endemic areas are the 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 vector mosquito which is resistant to insecticides (Coker *et al.*, 2000; Masaba *et al*., 2000). Hence, there is an urgent need to find out antimalarial 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 nor have access to modern health facilities (Coker *et al*., 2000).

The medicinal values of plants and their component phytochemicals such as alkaloids, tannins, flavonoids, phenolics and other compounds have been found to produce a definite physiological action on human body (Hill, 1952). A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in nutraceutical and drug research. The presence of active phytochemicals in some herbs used in curing many diseases affecting people in African communities has been documented (Odetola *et al*., 1986). More particularly, the phytochemical properties of some plants used generally in folk medicine in Nigeria have been investigated (WHO, 1978). Examples of such plants are *Morinda morindoides* (Onabanjo, 1983), *Morinda lucida* (Makinde *et al.*, 1985), *Azadirachta indica* (Ekanem, 1978; Obih *et al*., 1985), *Enantia chlorantha, Ocimum gratissimum* (Agomo *et al*., 1992), *Adansonia digitata, Alstonia congeensis, Khaya senegalensis* (Coker *et al*., 2000) and *Tithonia diversifolia* (Oyewole *et al*., 2008).

Although various studies have been carried out on phytochemical constituents in many medicinal plants, not much attention has been paid to the variation in the phytochemicals that appear in the different parts of these plants because chemical composition and biological activities of a plant have been found to be affected by seasonal variation, state of maturity and location among other factors. Therefore, the present work has been designed to evaluate the variation of various phytochemical constituents of some plant parts used in the treatment of malaria in southwestern Nigeria.

Materials and Methods

Plant Selection

Plants were selected based on their high frequency of usage indicated in various Ethnobotanical surveys carried out in some southwestern parts of Nigeria, in the treatment of malaria.

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

The tannins, terpenoids and alkaloids determinations were done using Harborne (1973) method while the flavonoid determination was by the method of Boham and Kocipal-Abyaza (1974). Saponin was determined using the method of Obadoni and Ochuko (2001) and the method of Singleton and Rossi (1965) was used to determine phenolic content.

Statistical Analysis

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

Results

Table 1 shows the survey of frequently used medicinal plants with anti-malarial potentials employed in this study. The plants belonged to different families with varied plant parts used in combination of a minimum of two plant parts. Among the combination of plant parts, leaves were represented in all the plant species, followed by barks used in five plants while the roots were in four plants and the fruits had the least in just two plants.

Table1: Frequently used medicinal plants with anti-malarial potentials

S/No	Plants	Family	Local Name	Common Name	Parts Used
1.	*Azadirachta indica* A.Juss	Meliaceae	Dongoyaro	Neem	Bark, leaves
2.	*Chrysophyllum albidum*	Sapotaceae	Agbalumo	African star apple	Bark, leaves
3.	*Anacardium occidentalis* L	Anacardiaceae	Kaju	Cashew-nut	Bark, leaves
4.	*Alstonia boonei* De Wild	Apocynaceae	Ahun	Stool wood	Root, bark leaves
5.	*Carica papaya* L	Caricaceae	Ibepe	Pawpaw	Leaves, fruit
6	*Chromolaena odorata* (L) King & Robinson	Asteraceae	Ewe - Akintola, Ewe - Awolowo	Siam weed	Root, leaves
7	*Citrus aurantifolia* L	Rutaceae	Osan-wewe	Lime	Root, bark, leaves, fruit
8	*Adansonia digitata* L	Bombacaceae	Ose	Baobab	Leaves; stem, roots

The present study carried out on variation in the phytochemical constituents of some plant parts frequently used in the treatment of malaria in southwestern Nigeria, revealed the presence of medicinally active compositions in various quantities in the different plant parts. The quantitative estimation of the crude phytochemical constituents in the plant parts studied is summarized in Table 2.

Table 2: Quantitative phytochemical composition of the potential anti-malarial plant parts analyzed

Plants	Parts	Tannins (mg/kg GAE)	Alkaloids (mg/kg GAE)	Flavonoids (mg/kg GAE)	Terpenoids (mg/kg GAE)	Saponins (mg/kg GAE)	Phenolic acids (mg/kg GAE)
Adansonia digitata	Leaf	166.0±1.0A	4200.3±1.5A	16289.7±7.5A	162.0±0.0A	750.0±0.0B	11.0±0.0B
	Root	37.7±1.5B	1456.3±7.2B	3419.7±4.5B	113.7±1.5B	170.0±2.0C	8.0±0.0C
	Stem bark	19.3±1.5C	970.0±0.0C	175.3±0.6C	58.7±1.5C	1400.0±5.0A	78.0±2.0A

Alstonia boonei	Leaf Root Stem bark	147.0±1.0A 7.0±1.0C 11.0±0.0B	3399.7±2.5A 1610.3±3.5B 710.3±1.5C	74.7±2.5C 495.0±5.0B 2530.0±2.0A	165.0±1.0A 14.0±2.0C 32.0±1.0B	370.0±5.0B 410.0±3.0A 50.0±0.0C	81.0±1.0A 45.0±2.0B 47.0±2.0B
Anacardium occidentalis	Leaf Root Stem bark	137.0±1.0A 55.0±2.0C 105.0±1.0B	1000.3±3.5 C 2030.3±2.5 B 3940.0±1.0A	2080.0±0.0C 5050.3±2.5A 4300.3±3.5B	127.0±2.0A 72.3±2.5B 38.0±2.0C	120.3±4.5C 3880.3±4.5A 3290.3±0.6B	36.0±1.0C 54.0±1.0B 122.3±2.5A
Azadirachta indica	Leaf Root Stem bark	104.0±2.0A 22.3±0.6B 102.7±1.5A	2860.0±0.0B 2149.7±2.5 C 4706.7±5.6A	2850.0±2.0A 2435.0±5.0B 344.0±4.0C	159.7±1.5A 113.0±2.0C 128.0±3.6B	300.0±0.0C 990.0±10.0A 920.0±5.0B	42.0±2.0A 21.0±1.0C 27.7±2.5B
Carica papaya	Leaf Root Stem bark	160.7±2.5A 5.3±0.6B 6.0±1.0B	2480.3±3.5B 3160.3±1.5A 1138..7±1.5C	2839.7±3.5B 6590.0±5.0A 174.7±0.6C	163.0±3.0A 52.7±2.5C 65.3±1.5A	1150.0±5.0B 380.0±5.0C 2006.7±15.3A	50.0±2.0A 11.0±0.0B 5.7±0.6C
Chromolaena odorata	Leaf Root Stem bark	166.0±2.0A 5.7±0.6C 21.0±1.0B	2369.7±3.5B 1730.3±1.5C 2690.3±2.5A	3855.0.±3.0A 655.0±0.0C 2453.0±9.8B	107.0±2.0A 46.3±1.5C 70.0±0.0B	1330.0±2.0A 250.0±4.0B 20.0±0.0C	77.7±2.5A 8.0±0.0B 10.0±0.0B
Chrysophyllum albidum	Leaf Root stem bark	149.3±3.5A 36.3±0.6B 24.0±1.0C	3237.0±7.9A 2349.7±0.6B 1540.3±4.5C	960.0±5.0C 2190.3±3.5B 2314.7±2.5A	162.3±2.5A 10.0±0.0C 70.7±4.5B	3950.0±15.0A 2460.0±5.0C 3099.7±5.5B	35.0±0.0C 45.0±3.0B 84.0±2.0A
Citrus aurantifolia	Leaf Root Stem bark	105.0±2.0A 10.7±0.6B 11.0±1.0B	2599.7±3.5B 2790.3±3.5A 1100.3±0.6C	840.3±2.5B 22470.3±4.5A 684.7±2.5C	65.0±3.0A 19.0±1.0C 33.0±0.0B	320.0±5.0B 3720.0±5.0A 30.0±0.0C	54.0±1.0B 71.0±1.0A 20.0±0.0C

Figures are expressed as mean ±SD

Figures bearing different alphabets differ significantly ($P > 0.05$)

Figures bearing the same alphabets are not significantly different ($P < 0.05$)

There was no significant difference ($P<0.05$) between the tannin contents of the leaf and the stem bark of *Azadirachta indica*, the root and stem bark of *Carica papaya* and the root and stem bark of *Citrus aurantifolia*. There was also, no significant difference ($P<0.05$) in the phenolic acid contents of the root and the stem bark of *Alstonia boonei* and the root and the stem bark of *Chromolaena odorata*.

Discussion

The results (Table 2) of the investigation into the phytochemical composition of leaf, root and stem bark of the various antimalarial plants employed in this study have revealed that there is varied quantities of phytochemicals, with significant differences ($p<0.05$) between the plant parts. The different plant parts showed that leaves of *Adansonia digitata* had the highest composition of tannins, alkaloids, flavonoids and terpenoids while the stem bark had the highest composition of saponins and phenolic acids. However, *Adansonia digitata* stem bark had the least composition of tannins, alkaloids, flavonoids and terpeniods while the root had the least composition of saponins and phenolic acids. Masola *et al. (*2009) reported the presence of tannins, phlobatannins, terpenoids, cardiac glycosides and saponins in the stem bark extract of *Adansonia digitata*. The presence of phenols in its root bark was also reported. They concluded that stem and root barks of *A. digitata* contained bioactive constituents responsible for the antimicrobial activity. This could possibly explain the scientific basis for the use of the crude stem and root bark extracts of *A. digitata* in the traditional system of medicine for the treatment of diseases such as white bacillary diarrhoea (pullorum disease) and diarrhoea symptom of Newcastle disease ((Gueye, 1999; Wynn and Fougere, 2006). The report of Tanko *et al.* (2008) also revealed the presence of tannins, carbohydrate, terpenes, saponins, flavonoids and alkaloids and that flavonoids and terpenes present in *A. digitata* may also be responsible for their antidiabetic activity. In support of these current findings, *A. digitata* was one of the plants earlier documented as being active in relieving fever caused by malaria in humans (Gruenwald and Galizia, 2005).

In the case of *Alstonia boonei* (Table 2) the leaves gave the highest composition of tannins, alkaloids, terpenoids and phenolic acids, but the least composition of flavonoids as compared to *A boonei* stem bark which also gave the highest composition of flavonoids and the least composition of alkaloids and saponins while the roots gave the highest composition of saponins and the least composition of tannins, terpenoids and phenolic acids. The presence of these phytochemicals had been reported in the leaves (Akinmoladun *et al.,* 2010) and stem bark (Fasola and Egunyomi, 2005; Akinmoladun *et al.,* 2007). The variation observed in the phytochemicals of *A. boonei* plant parts was evident in the research findings of Adomi (2006) who reported that aqueous extract of the stem bark of *A.boonei* was active against two gram-positve and gram-negative bacteria tested whereas there was lack of response of tested bacteria on the root bark extract of *A.boonei.* This was attributed to a reduced level of bio-active the components as illustrated by Adomi and Umukoro (2010).

The leaves of *Anacardium occidentalis* gave the highest amount of tannins, and terpenoids but least composition for alkaloids, flavonoids, saponins and phenolic acids. On the other hand, the plant stem bark gave the highest composition of alkaloids and phenolic acids as well as the least composition of terpenoids while the root gave the highest composition of flavonoids and saponins but least composition of tannins. The presence of these phytochemicals has also been reported in the leaves (Goncalves *et al*., 2005; Agedah *et al.,* 2010), stem bark (Ayepola and Ishola, 2009) and nuts (Rajesh-kannan *et al.,* 2009) of *A.occidentalis*. The obtained result is also in accordance with the reports of Tedong *et al. (*2006) whose phytochemical analysis of *A. occidentalis* revealed the presence of alkaloids, polyphenols and saponins. The fungicidal activities of *A. occidentalis* extracts have been reported and the strong antifungal properties of the plant extracts were demonstrated based on the presence of secondary metabolites (Geyid *et al.,* 2005). Again, this variation in the phytochemical composition of *A. occidetanlis* was evident in the report of Rajesh-Kannan *et al, (*2009). They reported that the variation in the antifungal activity of *A. occidentalis* was based on differences in the concentration of bioactive components in the plant parts or synergistic reaction of various phytochemicals in the extracts and that the broad and narrow spectrum activities of the plant extracts on the fungus were directly related to the nature and potential of the chemical compounds. The antimicrobial properties of *A. occidentalis* have also been attributed to its polyphenol contents (Agedah *et al.,* 2010). Furthermore, the antibacterial property of *A. occidentalis* reported by Ayopola and Ishola (2009) has also supported the folkloric usage in treatment of diarrhoea, dysentery, pile, toothache, sore gums and oral thrush.

Azadirachta indica leaves gave the highest composition of tannins, flavonoids, terpenoids, and phenolic acids but least composition of saponins. The stem bark gave the highest composition of alkaloids but least composition of flavonoids. The root gave the highest composition of saponins but least composition of tannins, alkaloids, terpenoids and phenolic acids (Table 2). This tallies with the results of phytochemical analysis reported on the leaves (Suresh *et al.,* 2008; Atangwho *et al.,* 2009), stem bark (Fasola and Egunyomi, 2005; Ndunagu *et al.,* 2008) and the root bark (Ndunagu *et al.,* 2008) of *A. indica*. Atangwho *et al.* (2009) reported that *A. indica* owes its antidiabetic properties to its selective chemical composition and that proper knowledge of the proximate, phytochemical and micronutrient composition is fundamental to understanding the mode of antidiabetic action of this medicinal plant.

The leaves of *Carica papaya* gave the highest composition of tannins, terpenoids and phenolic acids. The stem bark gave the highest compositon of saponins and the least composition of alkaloids, flavonoids and phenolic acids while the root gave the highest composition of alkaloids and flavonoids but the least composition of tannins terp,enoids and saponins. The report of Ayoola and Adeyeye (2010) on the phytochemical and nutrient evaluation of *Carica papaya* leaves revealed the presence of saponins, cardiac glycosides, alkaloids but absence of tannins. The absence of tannin is in contrast to the obtained result in which the highest tannin content was found in the leaves. This variation could be attributed to seasonal variation among other reasons such as the state of maturity as well as environmental conditions affecting the plant. The research findings of Oloyede (2005) revealed the presence of saponins in the unripe fruit pulp of *C. papaya*. The presence of these phytochemicals, vitamins and minerals in *C. papaya* partly supports the use of this plant in herbal medicine and that it is a rich and potential source of food and drug (Ayoola and Adeyeye, 2010).

Chromolaena odorata leaves gave the highest composition of tannins, flavonoids, terpenoids, saponins and phenolic acids. The stem bark gave the highest composition of alkaloids but least composition of saponins while *C. odorata* roots gave the least composition of tannins, alkaloids, flavonoids, terpenoids and phenolic acids. The obtained result is in accordance with the report of Akinmoladun *et al.* (2007) on the phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata.* They revealed the presence of tannins, terpenoids, and flavonoids in the leaf. These phytochemicals have been documented to possess medicinal properties and health promoting effects (Salah *et al.,* 1995; Del-Rio *et al.,* 1997; Okwu, 2004; Liu, 2004). However in contrast to these results, Akinmoladun *et al.,* (2007) reported low phenolic content in the leaf extract of *C. odorata* in their study. They concluded that low phenolic levels (and any other phytochemical) and low antioxidant indices in plants do not translate to poor medicinal properties. The present investigation has claimed that *C. odorata*, though described as a plant of low economic value, is not worthless after all as its use in traditional medicine attests to this.

The leaves of *Chrysophyllum albidum* had the highest composition of tannins, alkaloids, terpenoids and saponins while the flavonoids and phenolic acids were the least. On the other hand, the stem bark had the highest composition of flavonoids and phenolic acids but least composition of tannins and alkaloids. The root of *C. albidum* had the least composition of terpenoids and saponins (Table 2). This result is in accordance with the findings of Okoli and Okere (2010) on the antimicrobial activity of the phytochemical constituents of *Chrysophyllum albidum* plant that revealed the presence of alkaloids, tannins, phenols and flavonoids in the seed, root, leaves and stem slash. However, the absence of tannins in the leaves of their findings is in contrast to the obtained results. Likewise, the highest saponin composition found in the roots is also in contrast to the obtained result in which the least saponin composition was present in the root. The high tannin content of this plant may be responsible for the ethno-medicinal usage as reported by Adewusi (1997) and this includes its anti-inflammatory effects. The high levels of flavonoids in the stem slash reported by Okoli and Okere (2010) is in accordance with the obtained result. Results of several researches on the antimicrobial activities of phytochemicals are further evidenced by their active role in plant disease resistance (Amadioha and Obi, 1998; Ijeh & Omodamiro, 2006; Okwu & Morah, 2007) and antioxidant activity. Results from Okoli and Okere (2010) indicate that the extracts of the seeds and roots of *Chrysophyllum albidum* have good potentials as anti-inflammatory, anti-diarrhoeal and anti-haemorrhoidal compound and further provide a rationale for the use of the seed and root extracts of this plant in the traditional medicine practice in Nigeria.

Citrus aurantifolia leaf gave the highest composition of tannins and terpenoids. The root gave the highest composition of alkaloids, flavonoids, saponins and phenolic acids but least composition of tannins and terpenoids while the stem bark of *C. aurantifolia* gave the least composition of alkaloids, flavonoids, saponins and phenolic acids (Table 2). The presence of alkaloids, saponins, flavonoids, phenols and tannins had also been reported in the leaf and peels of *Citrus aurantifolia* (Okwu *et al.,* 2007). The low alkaloid content and high tannin content found in the leaf is also in accordance with the obtained results. Okwu *et al.* (2007) also reported that the alkaloid and phenolic content were highest in the peel than the leaf. The reason may be that during fruiting, more alkaloids and phenolic constituents are produced in the peels in order to protect and preserve the seeds from microbial attack (Okwu and Emenike, 2006) thus, giving them antimicrobial activities. The presence of tannins may be the reason for the bitter and astringent taste of unripe *Citrus aurantifolia* fruits. The presence of saponins may also be responsible for bitter taste and antifungal properties of this plant. The result of a study by Taiwo *et al.* (2007) on the *in vitro* antimicrobial activity of crude extracts of *Citrus aurantifolia* and *Tithonia diversifolia* on clinical bacterial isolates revealed that *C. aurantifolia* fruit juice had broad spectrum antibacterial effects but more on gram negative bacteria. These findings also agree with that of Onyeagba *et al.* (2004) and others (Oboh *et al.*, 1992; Oboh and Abulu, 1997) which showed that lime juice contained active ingredients that justified their use in traditional African medicinal practice.

The variation in phytochemical composition of the plant parts analysed could be as a result of seasonal variation among other reasons. Singh *et al.* (2010) reported that the presence of phytochemicals in plants and its parts is greatly influenced by environmental conditions and degree of development. It has also been reported that some plant parts (leaves, stem bark and roots) had bioactive properties to 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 – filho, 2006). This was also observed by Makinde *et al.* (1994) in their reports 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 might have reduced to minimum levels such that when these plants are tested again, contrary results may be obtained. In explanation, this may propably be 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. 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 as anticancer 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 this report they observed that phytochemicals showed significant increase in mature plants in comparison to early stage plants and their parts in both methi and saunf therefore suggesting that these phytochemicals increase with maturity.

As different parts of some plants have different clinical indications or therapeutic applications, it is important to establish the quantity of plant materials for its constituent plant parts composition as reported by Sunita and Abhishek (2008).

Conclusion

In the phytochemical composition obtained in this study, the leaves of the different plants analysed had more tannins, alkaloids, terpenoids and phenolic acids as compared to the root and stem bark while the stem bark gave the highest composition of flavonoids and saponins as compared to the root and the leaves. However, the high composition of phytochemicals observed in the plant leaves confirms their frequency of usage in traditional medicine for the treatment of malaria as reported by Idowu *et al.* (2010). The presence of varied active ingredients in the different parts of these plants could also explain their diverse use in the treatment of different disease conditions, so the need for research into the isolation and identification of these active ingredients in the different plant parts will help to authenticate the diverse claims of herbal practitioners on several disease conditions across Nigeria.

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