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Phytochemical, nutritional and anti-nutritional properties of leaves, stems bark and roots of trees used in popular medicine for the treatment of malaria in South Eastern Nigeria

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Environmental factors are known to affect genes in various ways. They are affecting the gene products including the production of both primary and secondary metabolites, hence the need to assess the phytochemical and nutritional compositions of *Morinda lucida* Benth and *Alstonia boonei* De Wild growing in Nsukka, Enugu state, south eastern Nigeria. The qualitative analyses of the plant parts showed the presence of all the tested phytochemicals in various levels ranging from trace (+) to very heavily present (++++). The quantitative estimates showed significant variations in their values in leaves, stems bark and roots in some phytochemical components. Results of the proximate also showed the contents of protein were 2.46 - 17.69 and 1.45-2.1; fats 0.49-1.88 and 0.84- 1.4; carbohydrates 68.76- 81.63 and 17.81- 31.38; and ash 2.33-2.9 and 0.78- 2.75 in *M. lucida* and *A. Boonei* plant parts, respectively. Vitamins A, E and K and anti-nutritional factors, phytate and oxalate also varied in the leaves, stems bark and roots. The results showed that these medicinal plants grown in Nsukka, a Derived Savanna Agro-Ecology, are rich in phytochemicals and proximate nutrients. The rich presence of these phytochemicals and other nutritive values supports the use of the different parts of these plants in ethno-medicine and equally creates the possibility for their use in drug formulation.

Key words: *Alstonia boonei,* ethno-medicine, *Morinda lucida,* phytochemicals, Nsukka.

INTRODUCTION

Alstonia boonei De wild belonging to the family Apocynaceae is a tree commonly called pattern wood or stool wood (Aigbokhan, 2014). The local names of this plant in South Eastern Nigeria are Egbu, Egun, or Egbe. This plant species is primarily a timber; however, the natives of South Eastern Nigeria, use virtually all the parts of this plant - leaves, stems bark and roots, for medicinal purposes (Aigbokhan, 2014).

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However, *A. boonei*, is not used only in South Eastern Nigeria, but there are various reports of its use in ethno-medicine across Africa, especially in countries where it is not considered a sacred plant (Terashima, 2003; Betti, 2004; Alshawsh et al., 2009; Tepongning et al., 2011). *A. boonei* is used widely in the prevention and treatment of malaria among the indigenous population of South Eastern Nigeria. Many authors have reported its use in the treatment of diverse ailments like fever, intestinal helminthes, rheumatism, hypertension and diarrhea (Terashima, 2003; Adomi, 2008; Bello et al., 2009). *Morinda lucida* (Benth), on the other hand, belongs to the family of Rubiaceae with common names as Indian mulberry, Brimstone tree and Hog tree apple (Aigbokhan, 2014). This family is known to be widely used in traditional medicine. Its primary use in South Eastern Nigeria is as a medicinal plant. The vernacular names among the Igbos are Ogere, Huka, Ezeogu and Njisi (Aigbokhan, 2014). *M. lucida*, like many other medicinal plants, is a multipurpose medicinal plant. It is reported to be used in the treatment of yellow fever, malaria, hypertension, dysentery, stomach ache, ulcer, gonorrhea and leprosy (Adesida and Adesogan, 1972; Makinde and Obih, 1985; Tona et al., 1999). Medicinal plants and their rich phytochemical components have diverse anti-bacterial and anti-plasmodium activities on humans and other organisms. Reported phytochemicals in plants include alkaloids, tannins, flavoids, phenolics and many others. Phytochemicals associated with *A. boonei* and *M. lucida* include alkaloids, tannins, saponins, glycosides, flavonoids and triterpenoids (Kucero et al., 1972; Ayiku, 1992; Fasola and Egunyomi, 2005; Afolabi et al., 2007). There are several alkaloids of which echitamine and echitamidine compounds are the major ones that have been isolated from *A. boonei* stems bark (Adotey et al., 2012). Medicinal plants have nutritional functions; these nutrients are essential for the physiological activities of the human body. Such nutrients are carbohydrates, proteins, fats, mineral elements, and vitamins and even dietary fiber. All these play important role in satisfying human needs for energy and life processes (Hoffman et al., 1998; Dingman, 2002). Several researchers have worked on the phytochemicals, proximate and/or vitamins and anti nutritional components of medicinal plants. Several studies have shown that plant and plant products under the control of multiple genes are affected by the environment in diverse ways (Kucero et al., 1972; Ayiku, 1992; Fashola and Egunyomi, 2005; Afolabi et al., 2007). Environmental stresses could trigger defense or adaptive mechanisms resulting in the accumulation of a wide range of antioxidants in some environments more than the others (Hare et al., 1999; Hasegawa et al., 2000).

Antioxidants are used by plants as defense mechanism against several forms biotic and abiotic stresses. This implies that changes in a particular environment and/or plants growing in different environments could accumulate various levels of these secondary metabolites.

It has been reported that plants respond to environmental stresses such as high or low temperatures, drought, salts, UVB/light and acclimatize to these conditions in varying degrees (Hare et al., 1999; Hasegawa et al., 2000; Tattini et al., 2004; Myungmin, 2008).Environmental conditions that induce stress on plants affect the concentrations of antioxidants as they are produced to serve as plant defense mechanism. Some researchers have reported that high temperature induces phytochemical accumulation in several plant species as a defense strategy (Lefsrud et al., 2005). So even though several researchers had reported their findings on the chemical properties of *M. lucida* and *A. boonei* in some parts of Nigeria and other countries, there is also need to study the phytochemicals, nutritional and anti-nutritional properties of these multipurpose medicinal plants growing in Nsukka. The aim of this research is therefore, to study the phytochemicals, proximate and anti-nutrient compositions of *M. lucida* and *A. boonei*; and the vitamin composition of *M. lucida*.

MATERIALS AND METHODS

Collection and identification of plant samples

The plant parts (leaves, stems bark and roots) of *Morinda lucida* were collected at Zoological garden, Department of Zoology and Environmental Biology while *A. boonei* plant parts were collected from the Botanical garden, Department of Plant Science and Biotechnology, both at the University of Nigeria, Nsukka (UNN). Nsukka is in a Derived Savanna Agro-Ecology located at latitude 06° 52’ N longitude 07° 24’ E and altitude 442 m above sea level. The plant specimens were identified and authenticated at Department of Plant Science and Biotechnology, UNN and Bioresources Development and Conservative Program (BDCP), Nsukka, Enugu state.

Processing of plant extracts

The plant materials were dried at room temperature. The dried plant parts were ground to powder with a milling machine in the laboratory and the powdered samples were stored in separate air tight containers.

Extraction process

Hot water extract of the sample was used for the analyses. The process was performed by soaking 50 g of the powdered sample in 500 ml of distilled water and boiling for about 10 min, thereafter, it was double filtered with cheese cloth. The filtrate was allowed to cool before drying in hot-air oven.

Chemical analyses of the samples

The phytochemicals, proximate, vitamin and antinutrient analyses were determined using standard methods (AOCC, 2002; Edeoga et al., 2005). The qualitative phytochemical composition was determined before quantitative analysis. The phytochemical analyses were conducted for steroids, alkaloids, terpenoids, tannins, saponins, flavonoids, phenols, hydrogen cyanide and

Table 1. Qualitative phytochemical analyses of the leaves, stems bark and roots of *Morinda lucida* and *Alstonia boonei*

Phytochemical	*Morinda lucida*			*Alstonia boonei*		
Phytochemicals	**Leaves**	**Bark**	**Roots**	**Leaves**	**Bark**	**Roots**
Steroids	++	++	+	++	+	++
Alkaloids	+++	++	+	++	++	+++
Terpenoids	+	++	+	++	+	+++
Tannins	+++	++	+	+	++	+++
Saponins	+	+	+	+	+	++
Flavonoids	+++	++	++	++	++	+++
Phenols	++++	+++	++	++	+	++
HNC	+	+	++	+	+	+
Glycosides	++	+	+	+++	+++	+++

++++ = very heavily present, +++ = heavily present, ++ = present, + = trace.

glycosides in each of the plant samples – leaves, stems bark and roots. The proximate analysis was done for proteins, fats, fiber, moisture, ash and carbohydrates, and for vitamins A, E and K. The anti-nutritional factors determined were phytate and oxalate.

Data analyses

The data collected from the replicated plant samples for the quantitative phytochemicals, proximate, vitamin and antinutrient analyses were used in the analyses of variance using GENSTAT package (Genstat release 7.22 DE) (GENSTAT 2009). The means values were tested for significance and separated using Fisher's least significant difference (F-LSD) only when the F-ratio is significant (Obi, 2002).

RESULTS

The qualitative analyses showed strongly present, present and trace amount of the different phytochemicals in both *M. lucida* and *A. boonei* plant parts (Table 1). The presence of alkaloids, tannins, glycosides, terpenoids, saponins, phenols, hydrogen cyanide, flavoniods and steroids were observed in both *M. lucida* and *A. boonei* plant parts. The quantitative analyses showed significant variations in most of the phytochemical constituents of plant parts at FLSD $P \leq 0.05$ (Table 2). Equally the tannins content in the leaves of *M. lucida* (1.49 mg/100 g) varied significantly from the quantity in the stems bark (1.37 mg/100 g) and roots (0.47 mg/100 g). The tannins contents in *A. boonei* were much lower than the values obtained in *M. lucida*. The leaves, stems bark and roots of *M. lucida* contained higher values in terpenoids, alkaloids, steroids and phenols, while *A. boonei* leaves, stems bark and roots had higher values in hydrogen cyanide, saponins and glycosides. The different parts of these two medicinal plants are rich in phytochemical constituents. The proximate analysis showed that these plant parts contain nutritive components such as fats, proteins and carbohydrates at diverse levels (Tables 3 and 4). The protein contents ranged from 2.46 to 17.69 and 1.45 to 2.81 in *M. lucida* and *A. boonei* plant parts, respectively (Tables 3 and 4). The fiber contents were higher in *A. boonei* roots, stems bark and leaves. It was observed that *M. lucida* which had higher carbohydrate contents also had lower moisture content.The vitamin contents of *M. lucida* showed higher retinol (vitamin A) in the leaves than in the stems bark and roots ranging from 17.56 in the leaves to 1.25 in the roots (Table 5). Equally, vitamin K was higher in the leaves (2.61 to 0.62), while the values of vitamin E did not vary significantly in the leaves and bark; however, both varied significantly from the roots (Table 5). Table 6 shows the anti-nutrient compositions of the plant parts. Different levels of phytate and oxalate were observed in *M. lucida* and *A. boonei* plant parts as shown in the table. Phytate was significantly higher in the leaves of both *M. lucida* and *A. boonei* than in the other parts. The values obtained for oxalate were also significantly higher in the leaves of both plants ranging from 2.43 to 2.11 and 0.33 to 14 in *M. lucida* and *A. boonei*, respectively.

DISCUSSION

The qualitative screening of these medicinal plants from this ecological zone showed rich presence of bio-active phytochemicals while the detailed quantitative analyses revealed the actual values in the different plants parts; thus serving as a reference to their use in ethno-medicine among the people of South Eastern Nigeria. Plant phytochemicals have antibiotic, antiviral, anti-plasmodial and anti-parasitic properties as has been reported in several studies (Oliver-Bever, 1986; Adomi, 2008; Ene et al., 2008; Alshawsh et al., 2009). These results corroborate some previous studies on the phytochemical screening of *Morinda lucida* in other ecological zones, which also revealed the presence of alkaloids and flavonoids (Adeyemi et al., 2014), tannins, alkaloids,

Table 2. Phytochemical quantitative analyses of leaves, stems bark and roots of *Morinda lucida* and *Alstonia boonei*

Medicinal plants	Sample	Tannins (mg/100 g)	HNC (mg/g)	Flavonoids (mg/100 g)	Terpenoids (mg/100 g)	Alkaloids (mg/100 g)	Saponins (mg/g)	Steroids (mg/g)	Phenols (mg/100 g	Glycosides (mg/g)
Morinda lucida	Leaves	1.49^{a} ± 0.003	0.16^{a} ± 0.002	0.28^{a} ± 0.003	2.31^{b} ± 0.009	2.64^{b} ± 0.008	0.06^{a} ± 0.004	1.16^{a} ± 0.003	4.84^{a} ± 0.003	0.43^{a} ± 0.004
	Bark	1.37^{b} ± 0.004	0.15^{b} ± 0.003	0.08^{b} ± 0.006	3.55^{a} ± 0.011	3.01^{a} ± 0.005	0.06^{a} ±0.004	1.16^{a} ± 0.003	4.84^{a} ± 0.003	0.43^{a} ± 0.004
	Roots	0.47^{c} ± 0.003	0.14^{c}± 0.002	0.08^{b} ± 0.003	1.95^{c} ± 0.003	0.25^{c} ± 0.003	0.02^{b} ± 0.006	0.44^{b} ± 0.004	1.05^{b} ± 0.004	0.14^{b} ± 0.004
	LSD (P ≤ 0.05)	0.003	0.002	0.003	0.007	0.005	0.004	0.003	0.003	0.003
Alstonia boonei	Leaves	0.366^{b}±0.004	1.994^{b}±0.002	0.785^{a}±0.004	0.426^{a}±0.003	0.125^{a}±0.003	1.375^{a}±0.005	0.195^{a}±0.004	0.064^{a}±0.0003	1.375^{b} ±0.004
	Bark	0.375^{a}±0.002	1.094^{c} ±0.004	0.075^{b}±0.005	0.227^{b}±0.002	0.026^{c} ±0.0046	1.254^{b}±0.004	0.034^{c} ±0.004	0.041^{b}±0.0006	1.394^{a}±0.002
	Roots	0.036^{c} ±0.002	0.375^{a}±0.002	0.004^{c} ±0.0003	0.075^{c} ±0.004	0.035^{b}±0.0020	0.375^{c}± 0.003	0.035^{b} ±0.005	0.005^{c} ±0.0005	1.076^{c} ±0.002
	LSD (P ≤ 0.05)	0.002	0.0027	0.0029	0.0024	0.0028	0.0035	0.0033	0.0004	0.0022

HNC= Hydrogen cyanide. * Values = mean ± Standard Deviation, Values followed by different letters are significantly different from one another.

Table 3. Range and mean of the proximate composition (%) of the leaves, stems bark and roots of *Morinda lucida.*

Medicinal plant	Plant part	Fats		Proteins		Moisture		Fiber		Ash		Carbohydrates	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Morinda lucida	Leaves	1.825-2.0	1.88^{a}±0.058	17.43-18.2	17.69^{a}±0.26	6.92-7.0	6.93^{a}±0.35	2.3-3.0	2.9^{c}±0.05	2.907-2.94	2.9^{a}±0.002	68.09-69.6	68.76^{b}±0.67
	Bark	0.643-0.70	0.66^{b}±0.19	4.371-4.378	4.38^{b}±0.002	5.3-5.485	5.43^{b}±0.6	5.928-6.0	5.96^{b}±0.13	2.33-2.3388	2.33^{c}±0.002	80.4-81.25	80.9^{a}5±0.28
	Roots	0.41-0.50	0.49^{c}±0.008	2.452-2.459	2.46^{c}±0.002	4.2-5.73	4.9^{b}±0.45	6.77-7.2	6.96^{a}±0.13	2.422-2.56	2.47^{b}±0.05	80.6-82.16	81.63^{a}±0.052
	LSD (P≤ 0.05)	-	0.12	-	0.51	-	0.9	-	0.27	-	0.09	-	1.78

Values = mean ± Standard Deviation, Values followed by different letters are significantly different from one another.

Table 4. Proximate composition (%) of the leaves, stems bark and roots of *Alstonia boonei.*

Medicinal plant	Plant part	Fats	Proteins	Moisture	Fiber	Ash	Carbohydrates
Alstonia boonei	Leaves	0.844^{b} ± 0.0036	2.106^{b} ± 0.005	67.254^{a} ± 0.004	7.153^{c} ± 0.003	2.155^{b} ± 0.002	20.488^{b} ± 0.008
	Bark	0.404^{c} ± 0.004	2.811^{a} ± 0.008	67.055^{b} ± 0.0045	9.164^{b} ± 0.004	2.754^{a} ± 0.004	17.814^{c} ± 0.015
	Roots	1.414^{a} ± 0.0038	1.45^{c} ± 0.005	52.515^{c} ± 0.0032	12.454^{a}±0.004	0.785^{c} ± 0.003	31.387^{a} ± 0.012
	LSD (P≤ 0.05)	0.0031	0.0047	0.0031	0.0028	0.0022	0.0099

Values = mean ± Standard Deviation, Values followed by different letters are significantly different from one another.

Table 5. Vitamin contents of leaves, stems bark and roots of *Morinda lucida.*

Sample	Vitamin A	Vitamin E	Vitamin K
Leaves	17.56^{a} ± 0.002	1.57^{a} ± 0.002	2.61^{a} ± 0.006
Bark	6.64^{b} ± 0.006	1.54^{b} ±0.003	1.57^{b} ± 0.005
Roots	1.25^{c} ± 0.004	0.96^{c} ±0.006	0.62^{c} ± 0.005
LSD (P ≤ 0.05)	0.003	0.004	0.004

Values = mean ± Standard Deviation, Values followed by different letters are significantly different from one another.

Table 6. Anti-nutrient composition of leaves, stems bark and roots of *Morinda lucida* and *Alstonia boonei*

Medicinal Plant	Sample	Phytate (mg/100 g)	Oxalate (%)
Morinda lucida	Leaves	3.65^{a} ± 0.006	2.43^{a} ± 0.003
	Bark	1.89^{b} ± 0.003	2.12^{b} ± 0.003
	Roots	1.87^{b} ± 0.005	2.11^{b} ± 0.004
	LSD(P ≤ 0.05)	0.003	0.004
Alstonia boonei	Leaves	1.345^{a} ± 0.0042	0.334^{a} ± 0.0045
	Bark	0.408^{b} ± 0.0056	0.311^{b} ± 0.005
	Roots	0.157^{c} ± 0.0021	0.144^{c} ± 0.0025
	LSD(P ≤ 0.05)	0.0034	0.003

L.S.D (P ≤ 0.05), Values = mean ± Standard Deviation, Values followed by different letters are significantly different from one another.

flavonoids and glycosides (Ajaiyeoba et al., 2006). Flavonoids, which are present in both medicinal plant parts are effective as free radial scavengers as has been observed in several studies (Del-Rio et al., 1994; Selah et al., 1995; Okwu, 2004). Flavonoids have also anti-allergic, anti-inflammatory, anti-viral, anti-proliferative and anti-carcinogenic properties (Middleton and Kandaswami, 1993), hence, the importance of their presence in these medicinal plants.

These plant parts are rich in phenolic compounds which have been reported as one of the largest and most ubiquitous groups of plant metabolites (Singh et al., 2007). They possess several biological properties, some of which are antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han et al., 2007). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds. Phenolic compounds are generally known to have antioxidant properties. Some of the characteristics of saponins include formation of foams in aqueous solutions, range from haemolytic activity to cholesterol binding properties and bitterness (Sodipo et al., 2000; Okwu, 2004). Plant parts have saponins, thus increasing their efficacy as drugs making them capable of boosting immune system (Okwu, 2004).The presence of steroids in these plant parts also makes them useful against cerebral malaria, thus, confirming their effectiveness as anti- plasmodial agents as reported by David et al. (2004). Several functions of steroids in living organisms range from acting as haemolytic activity, cholesterol binding and anti bacterial properties (Sodipo et al., 2000; Okwu, 2004; Raguel, 2007). The presence of steroids in *Morinda lucida,* however, is in disagreements with the report of Saganuwan et al. (2009) which showed that steroids were absent in this plant. This contradiction may be due to environmental interactions between organisms and their environment. As a result of these interactions, the environment may have influence on cellular productions via their actions on the genes. Thus, the production of any phytochemical, steroids in this case may depend on the combinations of necessary defense mechanism of the plant based on the prevalent environmental stresses. Alkaloids are the most essential of all phytochemicals, it is abundant in all the plant parts. They have a wide range of activities of which anti-parasitic properties are one of them (Louw et al., 2002; Abu et al., 2014). The presence of these phytochemicals supports the use of these two indigenous medicinal plants *A. boonei* and *M. lucida* in the herbal treatment of malaria, and beyond this the rich presence of varied active ingredients in *A. boonei* and *M. lucida* justifies their wide usage in the herbal treatment

of other ailments in South Eastern Nigeria.

Equally, the nutritional components of the plants showed moderate quantities of proximate compounds – carbohydrates, protein, fat, fiber, ash and high moisture content. Malaria patients tend to lose appetite and these herbal drugs in addition to their bioactive phytochemicals against the parasites also serve as sources of energy, protein, mineral elements and vitamins to the patients. All these contribute to the quick recovery of the body cells. The crude fiber contents of most samples were quite high. Fiber helps to absorb excess water in the colon, retain a good amount of moisture in the fecal matter and can offer protection against conditions like hemorrhoids, colon cancer, chronic constipation and rectal fissures (Jimaima et al., 2003). Diabetes affects about 10% of Nigerian adults (Global Nutrition Report, 2014) and diets high in fiber is generally efficient in the management of plasma glucose concentration in diabetic individuals. The low fats and carbohydrates composition of *A. boonei* leaves confirms the fact that vegetables are relatively low in calories and fats (Jayaraj et al., 2008). The observed high protein and carbohydrate values of *M. lucida* leaves could be attributed to its low moisture content which made the nutrients to be more concentrated. The vitamin composition of the samples were appreciable showing that they can alleviate the problem of 'hidden hunger' (micronutrient deficiencies) in the patients by contributing to daily vitamin intake. Micronutrient deficiencies are becoming widespread especially in those countries in the developing part of the globe. Vitamins A and E are powerful antioxidants and could be effective in combating degenerative diseases like atherosclerosis (Nwanjo, 2005). An inherent malaria sign/symptom is the depletion of red blood cells. Boosting the intake and absorption of iron and other nutrients necessary for blood formation is therefore very necessary. Vitamin A improves iron status probably by reducing the levels of infection, improving production and proliferation of red blood cells in the bone marrow, increasing the absorption of iron from food in the intestine and mobilization from body stores (Nnam, 2011). The appreciable ash values of the samples show that they are good sources of minerals and vitamins which are essential for day to day metabolic regulation of the body. This suggests that these plants are not only of high medicinal value but also of high nutritional value and studies have shown that nutritional and medicinal properties of plants are as a result of the interaction between their phytochemical and nutrient constituents (Nnam et al., 2012).

Conclusion

The different secondary metabolites responsible for the enthno-medicinal properties of these plant parts are alkaloids, tannins, saponins, terpenoids etc. *M. lucida* and *A. boonei* in combination with other potential anti-malarial plants may therefore be possible sources for the discovery of new chemotherapeutic agents in targeting of *P. falciparum* and other related parasites causing malaria. The plant parts have additional nutritional values because of the proximate and vitamins contents, hence these plants may also supply needed macro and micro-nutrients to the patients.

Conflict of Interests

The authors have not declared any conflict of interests.

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