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RESEARCH ARTICLE

Phytominerals and Phytochemical Studies of *Azadiracthta indica*, *Leea guineensis* and *Parkia biglobosa* Leaves

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ABSTRACT

Medicinal plants have great importance in African medicine and are also used as precursors in drug discovery. The medicinal value of plants lie in their bioactive constituents which usually allow them to fight against several diseases. Plant-based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds. The mineral and phytochemical analysis of the leaves of *Azadirachta indica*, *Parkia biglobos*a and *Leea guineensis* were investigated. The leaves were collected from the Herbal Garden, Forestry Research Institute of Nigeria, Jericho hills, Ibadan, Nigeria and identified at Forest Herbarium Ibadan. Oyo State. The leaves were air dried and milled to powder using a medium-sized milling machine and stored in an air-tight container until needed for analysis. Phytochemical screening/analysis and mineral analysis were determined using standard analytical methods. Phytochemicals like Alkaloids, Flavonoids, Tannins, Saponins, Anthraquinone, Cardiac glycoside were determined. while Minerals, such as Potassium, Sodium determined using flame photometer; Calcium, Phosphorus, Magnesium, Iron, Zinc, Manganese, and Copper were determined using Atomic Absorption Spectrophotometer. Results indicates that the bioactive contents of the leaves varied significantly ($p<0.05$) among the medicinal plants. The phytochemical analysis showed that the three medicinal plants contained alkaloid, saponins, tannins, flavonoids, polyphenols, cardiac glycoside and anthraquinone in varying quantities with the trend: *A indica* > *P. biglobosa* > *L. guineensis*; the macro elements showed similar trend as the phytochemicals while the micro elements followed the trend: *P. biglobosa* > *L. guineensis* > *A indica*. These results conceivably indicate that the three medicinal plant are good source of essential minerals and phyto-nutrients which possess strong pharmacological activities and provide scientific credence for its therapeutic usage.

Keywords: Phytochemicals, Minerals, *Azadiracthta indica*, *Leea guineensis*, *Parkia biglobosa*

1 Introduction

Medicinal plants have constituted the source of health care all over the world since time immemorial and has thus remain the mainstay of drug discovery [1], [2]; this is believed to be as a result of the presence of certain plant chemicals (phytochemicals). Phytochemicals which may also be referred to as phytonutrients are present in diverse kinds of plants which are consumed as essential components of both human and animal diet where they have marked physiological effects [3] [4]. It is generally believed that the medicinal value of plants is due to some plant secondary metabolites that produce certain physiological action in human body. The bioactive substances of note in plants are alkaloids, flavonoids, tannins, saponins and phenolic compounds [5]; these active substances, alongside the vitamins and minerals have been widely reported to contribute to their various physiologic and protective effects [4], [6] [7]. Other important classes of phytonutrients present in plants are the minerals. Minerals are considered as elements that possesses specific metabolic functions, some of which are present in diverse range of medicinal plants.

Azadirachta indica, Parkia biglobosa and *Leea guineensis* are three medicinal plants of great medicinal values, these plants have found limited

use in conventional medicine; they are known to be used worldwide in pharmaceuticals, food, cosmetics and perfume industries. *Azadirachta indica* (Neem) is a tree that belongs to family Meliaceae which is found in abundance in tropical and subtropical regions like India, Pakistan, Bangladesh and Nepal and now widely distributed all over the world. Its most important active constituent is "azadirachtin"; neem reportedly shows therapeutic properties due to the rich source of antioxidants and other valuable active compounds. [8], [9]. The leaves and roots of neem are known to have antibacterial and antifungal properties [10], [11], [12].

Parkia biglobosa belongs to (Fabaceae - Mimosoideae) commonly called African locust bean tree. It is a native, perennial, deciduous, leguminous tree which grows principally in the savannah region of West Africa Countries [13]. Fermentation process of African locust beans (*P. biglobosa*) is initiated by *Bacillus* species to produce spices called "Iru" or "Dawadawa" in Nigeria and had been described by Bridget *et al*, [14]. The seeds of *P. biglobosa* have been reported to contain up to 29% crude protein and 60% saccharose, high oil content and presence of significant concentration of vitamin C [15]. *P. biglobosa* is one of the ingredients used in the treatment of leprosy and hypertension [16]. Aqueous and methanolic extracts of the fermented seeds of *P. biglobosa* was reported to have antidiabetic and anti-arteriogenic properties of the [17].

Leea guineensis commonly called Red tree vine is an evergreen shrub or small tree that belongs to the family Leeaceae. It is locally called alugbokita and usually propagated by seed or stem cutting; the seed germinates in 14-21 days at 70° F and can grow up to 20ft high; the plant is widely distributed in moist, intermediate temperate zones in tropical Africa including Cote d'Ivoire, Liberia, Sierra Leone, Ghana, Cameroon and Nigeria [18], [19]. The plant is reported to exhibits potential in-vivo anti-tumour and antioxidant activity [20]; while it's anti-inflammatory activity was also demonstrated in experimental animals. [21]. These medicinal plants represent different classes of medicinal plants found in the herbal garden of Forestry Research Institute of Nigeria; therefore, the present study aims at investigating the comparative potential of *Azadirachta indica, Parkia biglobosa and Leea guineensis* leaves as source of nutritional (minerals) and therapeutic (phytochemical) purposes.

2 Materials and Methods

2.1 The Plant

Fresh leaves of the plants (*A. indica, P. biglobosa, L. guineensis*) were collected in May 2017 at the herbal garden of Forestry Research Institute Nigeria. It is located on Latitude $7^0$23′48.287.5N and Longitude $3^0$51′48.96.0E. The plant samples were identified by a taxonomist at the taxonomy unit of the Institute. Leave samples were air-dried on a cabinet drier, milled using a milling machine and then kept in an air tight jar ready for further analysis.

2.2 Phytochemical Assay and Mineral Analysis

2.2.1 Qualitative and Quantitative Assay

Qualitative phytochemical screening of the leaves was determined using the standard methods described by Boye *et al.,* [22] and Omoruyi *et al.,* [23] while the quantitative phytochemical analysis estimating the quantity of saponins, alkaloids, flavonoids, anthraquinone, cardiac glycosides, phenolics and tannins were determined using the standard methods described by Mbaebie *et al.,* [24].

2.2.2 Mineral Analysis

The mineral analysis was done according to the method Uddin *et al*., [25], 1g of leave samples were wet digested using 9ml of freshly prepared aqua regia (a mixture of 65% nitric acid and 37% hydrochloric acid in a ratio of 1:3), the mixture boiled gently over a water bath at 95 ^{0}C for 5h until sample is completely dissolved; filtered and made up to standard volume with deionized water. The respective minerals were analysed using Atomic Absorption Spectrophotometer.

2.3 Statistical analysis

Quantitative data were expressed as Mean ±SD of triplicate measurement; analysis of variance (ANOVA) was used to detect significant

difference between mean of measured parameters of different species, while specific differences were identified using Least Significant Difference (LSD) statistical test at 5% level of probability. SPSS version 20 was used for the statistical analysis.

3 Results

Table 1 presents the results of the qualitative phytochemical screening of leaves of the medicinal plants. The qualitative phytochemical screening of the leaves of *Azadirachta indica, Parkia biglobosa* and *Leea guineensis* revealed the presence of a variety of plant secondary metabolites such as alkaloids, tannins, flavonoids, cardiac glycosides, saponins and anthraquinone.

Table 1: *Qualitative phytochemical screening of the leaves*

Phytochemicals	***A. indica***	***P. biglobosa***	***L. guineensis***
Alkaloids	+	+	+
Cardiac Glycosides	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Phenolic	+	+	+
Tannins	+	+	+
Anthraquinone	+	+	+

* + Present, - Absent

Table 2 presents the results obtained for the quantitative phytochemical analysis of the three medicinal plants. All the medicinal plants had the presence of some secondary metabolites in varying quantities.

Table 2: *Phytochemical analysis (Quantitative) (mg/100g) of the leaves*

Phytochemicals	***A. indica***	***P. biglobosa***	***L. guineensis***
Alkaloids	21.19±1.25a	12.81±0.57b	11.69±0.52b
Cardiac Glycosides	2.02±0.85a	4.92±0.08b	4.55±0.91c
Saponins	15.35±0.52a	10.47±0.80b	7.53±0.30c
Flavonoids	4.46±0.23a	9.42±0.83b	7.36±0.17c
Polyphenol	3.17±0.22a	1.01±0.59b	1.30±0.01c
Tannins	8.33±0.09a	7.23±0.02b	3.95±0.01c
Anthraquinone	3.03±0.02a	1.03±0.02b	1.02±0.01b

*Values are expressed as mean and SD of triplicate measurements; means with same alphabets in the same row are not significantly different at p<0.05

A. indica had significantly higher ($p<0.05$) concentration of alkaloids, saponins, polyphenol, tannin and anthraquinone compared to the other two species, while *P. biglobosa* had significant higher ($p<0.05$) concentration of cardiac glycosides and flavonoid compared with *A. indica* and *L. guineensis.*

Table 3 presents the results obtained for the mineral analysis of the three medicinal plants. For the macro elements, *A. indica* had significantly higher ($p<0.05$) concentration of calcium, magnesium, potassium and phosphorus compared with the other two species; for the micro elements, *P. biglobosa* had a significantly higher concentration ($p<0.05$) of iron, copper and manganese compared to *A. indica* and *L. guineensis.*

Table 3: *Result of mineral analysis of three medicinal plants*

Minerals (%)	***Azadirachta indica***	***Parkia biglobosa***	***Leea guineensis***
Macro Elements			
Calcium(Ca)	5.26±0.59a	3.27±0.01b	2.75±0.01b
Magnesium(Mg)	4.84±0.09a	2.2 7±0.11b	2.85±0.04c
Potassium (K)	5.12±0.03a	1.14±0.02b	1.17±0.03b
Sodium(Na)	0.06±0.02a	0.25±0.01b	0.16±0.12c
Phosphorus(P)	0.99±0.33a	0.24±0.10b	0.15±0.10b
Micro Elements			
Zinc(Zn)	0.012±0.002a	0.016±0.002a	0.014±0.002a
Iron (Fe)	0.04±0.01a	0.07±0.012b	0.04±0.02a
Copper(Cu)	0.002±0.001a	0.008±0.002b	0.003±0.001c
Manganese (Mn)	0.003±0.001a	0.02±0.002b	0.004±0.002a

Values are expressed as mean and SD of triplicate measurements; means with same alphabets in the same row are not significantly different at p<0.05Values are expressed as mean and SD of triplicate measurements.

4 Discussion

Plants used in the treatment of diseases contain bioactive principles with biological activity some of which are responsible for the characteristic odour, pungencies and colour of plant, while others give the particular plant its culinary, medicinal or poisonous virtue [26]. The result obtained in this study reveals the presence of some secondary metabolites in the three medicinal plants, which is in consonance with those reported for other plants [27], [28], [29]. Comparable result is similarly reported by Borokini, and Omotayo, [30] for *L. guineensis,* and

Ajaiyeoba [16] for *P. biblobosa*. The high content of Alkaloid in *A. indica* may be partly responsible for the bitter taste of neem plant. *P. biglobosa* showed an increased level of alkaloids, saponins and flavonoids, unlike cardiac glycosides, tannins, polyphenols and anthraquinone. In this study, *L. guineensis*, also shows an abundance of alkaloids, saponin and flavonoids compared to cardiac glycosides, tannins, polyphenols and anthraquinone; these phytochemicals are known to exhibit various pharmacological and biochemical actions when ingested.

A. indica contained significantly higher concentration ($p<0.05$) of Alkaloids, Saponin, polyphenols, tannin and anthraquinones than other medicinal plants used in this study. These values are on the lower side compared to that reported by Ndukwe *et al.,* [31] and Harry-Asobara [32] where higher levels of alkaloid, phenols, saponins and tannin in *A. indica* from Abia state Nigeria were observed which may reflect the effect of provenance. There are increased levels of cardiac glycosides and saponins in *P. biglobosa* which is the same with that reported by Ajaiyeoba [16], unlike flavonoids, alkaloids, tannins and anthraquinone. Tannins is reported to serve as antidotes for many poisons [33], hence, the presence of tannin in all the medicinal plants makes them a useful source of antidotes for poisons. Alkaloids are beneficial chemicals to plants, serving as repellent to predators and parasites. Alkaloids have been noted to possess antimicrobial, antihypertensive, antifungal, anti-inflammatory, anti-fibrogenic effect as well as anti-diarrheal effect which is probably due to their effects on small intestine [34]. However, when ingested by animals, they affect glucagon, thyroid stimulating hormones and inhibit certain enzymatic activities [35]. Flavonoids generally serve as flavouring ingredients in plants. Besides their role as flavouring agents they are also expressed in plants in response to microbial infection suggesting their antimicrobial activity [36]. Flavonoids have also been implicated as antioxidants both in physiological and diseased states [37]. For instance, tea flavonoids have been reported to reduce the oxidation of low-density lipoprotein, lower the blood level of cholesterol and triglycerides [38].

Intakes of elements such as macronutrients and micronutrients are essential for physiological and metabolic processes. The macro-elements are required in amounts greater than 100 mg/dl and the micro-elements are required in amounts less than 100 mg/dl [39]. The result reported in this study for minerals shows a significantly higher level of macro elements in the leaves of *A. indica,* while *L. guineensis* leaves had d lowest levels of both macro and micro elements which is also lower than the result reported by Ajiboye *et al.*, [18], in their study on the chemical composition of the seed of *L. guineensis*. Adequate phosphorus availability stimulates early growth and hastens maturity in plants [40]. Higher concentration of P (0.99%) concentration was observed in the leaves of *A. indica* compared with *P. biglobosa* (0.24%) and *L. guineensis* (0.15%) which are all significantly different from each other ($P< 0.05$), this is within the range of 0.15-0.68% generally reported for an array of medicinal plants such as *Cybopogon* sp, *Anacardium* sp, *Psidium guajava, Carica papaya* [31], *Melanthera scandens, L. guineensis, Ocimum gratissimum* [41] and *Cruscentia cujete* leaves [42]; however, this range is lower than the range of 1.33-1.62 % reported by Chavan *et al.,* [43] for Phosphorus in the leaves of *Artemisia* nilagirica, *Cythocline purpurea* and *Sphaeranthus indicus*. Calcium helps in building and maintaining bone mass and strength, thus, is an essential component of diet [43]. The leaves of *A indica* had the highest concentration of Ca (5.26%) followed by *P. biglobosa* (3.27%) and *L. guineensis* (2.75%). This range is relatively higher than 0.85-1.5% range in *Blumea eriantha, Artemisia* nilagirica, *Cythocline purpurea* and *Sphaeranthus indicus* as reported by Chavan *et al.,* [43], 0.15% reported for *A. indica* by Ndukwe *et al.,* [31], 0.51%, 0.39%, 0.36% reported for *O. gratissimum*, *M. scandens* and *L. guineensis* respectively [41] and 0.23% in *Abrus precatorious* [45].

The leaves of *A. indica, P. biglobosa and L. guineensis* also shows an abundance of magnesium at varying degree. The range observed in this study is higher than the levels reported in the leaves of *Cruscentia cujete* [42], *Diospyros mespiliformis* [46], *Mucuna poggei* leaves [47] and *Abrus precatorious*

[45]. Dietary Potassium (K) lowers blood pressure in a human, which can reduce the risk of stroke; however, some of the protective effect of K appears to extend beyond its ability to lower blood pressure. The Potassium content of the leaves of the medicinal plants varied significantly. *A. indica* has a significantly higher amount of K (5.12%) compared to *P. biglobosa* (1.14%) and *L. guineensis* (1.17%). This is higher compared to that reported by Ndukwe *et al.*, [31], for *Psidium guajava* (0.23%) and *Vernonia amygdalina* (0.26%), Paul *et al.,* [45] for *Abrus precatorious* and Fagbohun *et al.*, [41] for *O. gratissimum*, M. *scandens* and *L. guineensis.* Sodium is a mineral element that plays an important role in the human body. When sodium level in the blood is too low is dangerous and can cause seizures and coma. Very high sodium levels can lead to seizures and death [48]. The importance of sodium in cellular homeostasis and physiological function cannot be overemphasized. Excess dietary intake of sodium may aggravate the condition of high blood pressure and may also have an adverse effect on target organs [49]. The Sodium concentration of the three medicinal are varies significantly from each other with *P. biglobosa* having the highest concentration of 0.25%. The values reported in this study falls within the range generally reported for common tree species and tropical plants [31]; [43]. It is noteworthy that the value of macro elements reported in this study is also higher than those of Ndukwe *et al.*, [31] and Harry-Asobara and Samson [32] both results obtained from *A. indica* leave samples obtained from Abia State Nigeria; this may also emphasize the effect of provenance. Microelements are used in relatively small amounts and is made up of less than 0.1% of dry plant tissue; they perform a variety of functions as well as being component of enzymes, activation of enzymes and certain redox reaction in plant metabolism [50], the iron concentration was higher (minor concentration) in all the medicinal plants compared to other micro elements which all occurred in trace amount i.e. Zn, Cu, Mn which are also lower than those reported for other plants.

5 Conclusion

The leaves of the three plants used in this study were found to contain important phytochemical constituents needed to combat various kinds of infection in human and minerals, which are useful for a wide range of metabolic functions. The following trend was observed for phytochemicals and macro element composition: *A indica* > *P. biglobosa* > *L. guineensis*; while for micro element, the trend *P. biglobosa* > *L. guineensis* > *A indica* were observed, thus, the ethno-medicinal importance of these plants as regards its folkoric information on the treatments of arrays of ailments, diseases and conditions could be justified.

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