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Assessment of chemical compositions of three antimalarial plants from Akure, Southwestern Nigeria: A preliminary study

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Malaria has been a menace to the health conditions of both rural and urban populations in Nigeria. Ethnobotanical survey revealed the use of *Anthocleista djalensis* A. Chev, *Lophira alata* Banks ex C.F. Gaertn. and *Olax subscorpioidea* Oliv. in the treatment of malaria in Akure, Southwestern Nigeria. The powdered plant samples were screened for phytochemical constituents, proximate composition and mineral elements according to standard protocols. There was no significant difference in the alkaloids, cardiac glycosides, saponins and tannins of the three samples. Anthraquinones and flavonoids were altogether absent. Available carbohydrate was highest in *A. djalensis* (66.48%) and least in *O. subscorpioidea* (66.40%) whereas crude fibre was highest in *O. subscorpioidea* (19.93%) and least in *L. alata* (15.84%). The crude protein in *A. djalensis* and *L. alata* almost tied with the least recorded for *O. subscorpioidea* (2.39%). The fat content in the three samples was generally low. Calcium was highest in *L. alata* (11767.83±29.17 mg/kg) and least in *A. djalensis* (7413.67±17.16 mg/kg); whereas, magnesium was highest in *A. djalensis* (1582.33±26.10 mg/kg) and least in *O. subscorpioidea* (1180.33±33.38 mg/kg). However, *L. alata* was found to contain 333.63±1.74 mg/kg of iron while *A. djalensis* and *O. subscorpioidea* had 301.33±4.04 and 249.68±4.72 mg/kg, respectively. Similarly, phosphorus content was highest in *L. alata* (934.58±0.51 mg/kg) and least in *O. subscorpioidea* (552.95±2.38 mg/kg). The zinc content was highest in *A. djalensis* (80.67±2.08 mg/kg). Manganese was found to be 67.71±4.19 mg/kg in *L. alata*, 50.71±1.58 mg/kg in *A. djalensis*, and 30.94±2.13 mg/kg in *O. subscorpioidea*. Lead tested negative in all the three samples. The plant samples contained major mineral elements and nutritive compounds. They may help to prevent opportunistic infections associated with malaria, as well as help to manage metabolic diseases. Anti-nutritive compounds and heavy metal composition in the samples are negligible and as such make the plants safe for consumption.

Key words: Malaria, *Anthocleista djalensis*, *Lophira alata*, *Olax subscorpioidea*, phytochemicals, minerals, Akure, Nigeria.

INTRODUCTION

Malaria, an infectious disease caused by *Plasmodium* species, has been a menace to the health conditions of both rural and urban populations in Nigeria (NGA, 2005). Although it is a global epidemic, the incidence and severity are higher in the tropics especially in the sub-

Saharan Africa, where pregnant women and children are the most susceptible (Nmorsi et al., 2007; Nguta et al., 2010). Worldwide, malaria afflicts about 40% amounting to over 300 million people annually (WHO, 2000) affecting more than 100 countries in virtually all the

continents of the world (Rowe, 2006). In Nigeria, reports indicated that pregnant women are most vulnerable and hence malaria causes 10% of all deaths in pregnant women (Ayoola et al., 2008). The cost of management or eradication of malaria with conventional approach and its attendant effect on the standard of living and the economy of a nation calls for an urgent review of natural products to combat the ancient scourge.

Admittedly, plants have been used in prehistoric times and are considered effective in the management of malaria. However, and more recently, the plant world has been revisited in the ongoing fight against the disease primarily because of the deficiencies of western drugs in terms of cost, access, and drug-resistance by the malaria parasite, or more importantly the diversity of plant life, relative cheapness, acclaimed potency, and cultural relevance.

Anthocleista djalensis A. Chev – Gentianaceae is a large tree which grows up to 20 feet; bole up to 4 cm in diameter, stilt-rooted, twig sometimes erect, spines above the leaf axils and with white flowers that are scented (Jensen and Schripsema, 2002). Traditionally, the plant is used to treat wound, malaria, constipation, dysentery, diarrhoea, hepatitis, skin infection, and inflammation (Okoli and Iroegbu, 2004; Aiyelaja and Bello, 2006).

Lophira alata Banks ex C.F. Gaertn. – Ochnaceae is usually straight, without buttress roots, but sometimes with a swollen base, and is usually clear of branches up to about 30 m with glabrous twigs and found in the subtropical and tropical moist lowland forests of Cameroun, the republic of Congo, Ivory Coast, Equatorial Guinea, Gabon, Ghana and Nigeria (Burkill, 1985). Traditionally, the bark is used in treatment of inflammation, toothache and as analgesic. In South-western Nigeria, Kayode (2006) reported the use of the leaves, stem bark, root, and seed in the treatment of malaria.

Olax subscorpioidea Oliv. – Olacaceae is a tree or sometimes a many-stemmed shrub up to 10m high of deciduous forest (Burkill, 1985). The leaf, bark, and root are used in the treatment of venereal diseases, arthritis, and rheumatism and as febrifuge (Oni, 2010).

This study was carried out to investigate the chemical constituents of three antimalarial plants used in Akure, South-western Nigeria with a view to evaluating the nutritive potentials of the plants.

MATERIALS AND METHODS

Study area

Akure is a popular metropolis in Ondo State. Akure South Local Government supports a population of over 400,000 people (NBS, 2006). The mean annual rainfall is about 1350 mm with bimodal

distribution spanning between March and November; the relative humidity averaged 80% with temperature range between 23 and 30°C which is suitable for agricultural production (Folayan, 2013). Civil servants are the major inhabitants of the city which is the centre of administration of the Ondo State Government. However, farming and trading are other occupation of the residents who majored in food crops and livestock production (Folayan, 2013).

Collection and identification of plant materials

Fresh stem barks of *Anthocleista djalensis*, *Lophira alata* and root of *Olax subscorpioidea* were collected, and identified at the University of Ibadan Herbarium (UIH) and thereafter air-dried.

Preparation of plant samples

The dried plant samples were pulverized to coarse powder using a laboratory mill (Model 4 Arthur Thomas, USA).

Chemical analysis

The powdered plant samples were screened for phytochemical constituents, proximate composition and mineral elements according to standard protocols reported by Walsh (1971), Harbone (1973), AOAC (1990), Evans (2002), and Sofowora (2008).

Alkaloids

The powdered plant sample (500 mg) was weighed and extracted with 10 ml of hydrochloric acid (HCl). The HCl extract was then filtered with Whatman filter paper (No. 1). The filtrate of about 2.5 ml was treated with few drops of Dragendoff's reagent. A precipitate indicated the presence of alkaloids.

Antraquinones

The powdered plant sample (500 mg) was shaken with 10 ml of benzene. The solution was filtered and 5 ml of 10% ammonium hydroxide (NH₄OH) solution was added to the filtrate. A violet colour was observed in the lower phase. It indicated presence of anthraquinones.

Cardiac glycosides

One gram (1 g) of sample was extracted with 40 ml of distilled water; the extract was placed in the oven at 100°C for 15 min. 1 ml of the preparation was added to 5ml distilled water and 2 ml Glacial Acetic Acid, and a drop of FeCl₃. Thereafter, 1ml of concentrated H₂SO₄ was introduced from the side of the test tube. A brown ring (with violet or green ring) signifies the presence of cardiac glycosides.

Flavonoids

A few drops of concentrated hydrochloric acid (HCl) were added to

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a small amount of an extract (0.5 g) of the plant material; development of red colour was taken as an indication of the presence of flavonoids.

Saponins

The sample (200 mg) was shaken with 5ml of distilled water and then heated to boil. Persistent frothing showed the presence of saponins.

Tannins

The sample (500 mg) was mixed with 10ml of distilled water and heated on a water bath. The mixture was filtered and ferric chloride (FeCl_3) was added to the filtrate. Appearance of blue black colouration showed the presence of tannins.

Polyphenols

One gram (1 g) of sample was added to 25 ml of water. The preparation was put in oven at 100°C for 15 min. The presence or absence of polyphenols was determined by adding a few drops of 1% (w/v) solution of ferric chloride followed by 1% (w/v) gelatin in sodium chloride of the same concentration. The formation of a precipitate indicated the presence of polyphenols.

Proximate composition - carbohydrate

Carbohydrate content was estimated by difference using the formula: % Available carbohydrate = $100 - (\% \text{ protein} + \% \text{ moisture} + \% \text{ ash} + \% \text{ fibre} + \% \text{ fat})$

Crude fibre

Two grams (2 g) of each sample was digested with 20% H_2SO_4 and NaOH solutions.

Crude protein

Half a gram (0.5 g) of each sample was weighed into a filter paper and put into a Kjeldahl flask; 10 cm^3 of concentrated H_2SO_4 was added and then digested in a fume cupboard until the solution became colourless. Distillation was carried out with 10 cm^3 of 40% NaOH. The distillate was received with 5 cm^3 of 4% boric acid in a mixed indicator till the boric acid solution turned green. Titration was done in the receiver flask with 0.01 M HCl until the solution turned red.

Fat (ether extract)

Two grams (2 g) of each sample was extracted with petroleum ether for 5 h in a Soxhlet extractor.

Moisture content

Two grams (2 g) of each sample was put into the crucible and dried in an oven at 105°C overnight. The dried samples were cooled in a dessicator for 30 min and weighed to a constant weight. The percentage loss in weight was taken as the moisture content.

Total ash

Two grams (2 g) of each sample was placed in a crucible and ashed at 600°C for 3 h. The hot crucibles were cooled in a dessicator and weighed. The percentage residual weight was taken for ash content.

Mineral analysis

After wet digestion, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), and lead (Pb) were analyzed using Atomic Absorption Spectrophotometer (FC 210/211 VGP Bauusch Scientific AAS); phosphorus was determined using Vanadomolybdate (Yellow method). Percentage transmittance was determined at 400 nm using Spectronic 20 (Bausch and Lomb) Colorimeter.

Data analysis

Data were statistically analysed and expressed as mean \pm SD. Differences in means were assessed for significance by Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

RESULTS AND DISCUSSION

Ethnobotanical investigation revealed the use of a recipe comprising the stem barks of *Anthocleista djalonenis*, *Lophira alata* and the root of *Olax subscorpioidea* in the treatment of malaria in Akure, southwestern Nigeria. Table 1 shows the plant profile of the medicinal plants. Alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, tannins and polyphenols are the anti-nutritional factors assessed, and are in fairly low concentrations (Table 2). There was no significant difference in the alkaloids of the three samples. The amount of cardiac glycosides, saponins tannins and polyphenols were very close. Anthraquinones and flavonoids were altogether absent. Ayandele and Adebisi (2007) reported the presence of tannins, glycosides, and saponins in the water extract of the stem of *O. subscorpioidea*; the absence of flavonoids was in line with the result obtained in this study. However, the authors reported flavonoids in the ethanol extract. This could be due to the extraction solvent as this is important for the determination of complete dissolution of bioactive compounds and the improvement of the kinetics of metabolites (Kratchnova et al., 2010). Phytochemical screening of the methanol, petroleum ether and hot-water leaf extracts of *A. djalonenis* showed the presence of tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides (Akinoyemi and Ogundare, 2014). Furthermore, Onocha et al. (2003) reported the isolation of phtalide, xanthenes, monoterpene-diol, djalonenol as well as iridoid glucoside, djalonenoside from *A. djalonenis*. The isolation of lophirosides and related groups from *L. alata* has been reported by Tih et al. (1994). Phytochemical analysis of *L. alata* by Haliru et al. (2013) indicated the presence of alkaloids, tannins, saponins, anthocyanosides and

Table 1. Specimen profile of three medicinal plants used in the management of malaria in Akure, Southwestern Nigeria.

Botanical Name	Description	Voucher
<i>Anthocleista djalonenis</i> A. Chev	Large tree, 20 feet, stilt-rooted, spiny, flowers white, scented, sapwood; fruits green, slash brownish, granular fruits round.	UIH 12668, UIH 12618
*Sapo; Cabbage tree		UIH 21055
<i>Lophira alata</i> Banks ex Gaertn f.	Big tree; used to treat inflammation, toothache and cancer	UIH 14207, UIH 15627
*Ponhan; Red ironwood		
<i>Olox subscorpioidea</i> Oliv.	Woody shrub with leafy branchlets, flowers whitish, fruits round and bright yellow when ripe, 12mm in diameter; and has a green calyx.	UIH 16044
*Ifon; -		

a= Voucher specimen numbers of representative taxa. Source: University of Ibadan Herbarium (UIH). *Vernacular name (Yoruba); Common name.

Table 2. Phytochemical constituents of three antimalarial plants used in Akure, Southwestern Nigeria.

Parameter	Composition (%)		
	<i>A. djalonenis</i>	<i>L. alata</i>	<i>O. subscorpioidea</i>
Alkaloids	0.68 ^a ± 0.01	0.73 ^a ± 0.01	0.61 ^a ± 0.00
Anthraquinones	0.68 ^a ± 0.01	0.00 ^a ± 0.00	0.01 ^a ± 0.00
Cardiac Glycosides	0.21 ^a ± 0.00	0.25 ^a ± 0.00	0.24 ^a ± 0.00
Flavonoids	0.00 ^a ± 0.00	0.00 ^a ± 0.00	0.00 ^a ± 0.00
Saponins	0.35 ^a ± 0.00	0.31 ^a ± 0.00	0.27 ^a ± 0.00
Tannins	0.03 ^a ± 0.00	0.02 ^a ± 0.00	0.03 ^a ± 0.00
Polyphenols	0.10 ^a ± 0.00	0.13 ^a ± 0.00	0.14 ^a ± 0.01

Values are mean ± SD of triplicate determinations. Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test (DMRT) at p<0.05.

reducing compounds; cardiac glycosides and anthraquinones were absent. Although, secondary metabolites function as defence against herbivores, microbes and competing plants, and as signal compounds in pollination process (Wink, 1988), they have proved to be pharmacologically important and are used as analgesics and narcotics, central nervous system stimulant, mydriatic, miotics and antihypertensive (Evans, 2002). The phytochemicals assessed in the three antimalarial plants were present in very low concentrations; this seems to question the acclaimed therapeutic activity of the plants. However, the proximate and mineral composition can be deduced to complement the bioactivity of the plants in traditional medicine.

Table 3 shows the proximate composition of the three antimalarial plants. Available carbohydrate was highest in *A. djalonenis* (66.48%) and least in *O. subscorpioidea* (66.40%) whereas crude fibre was highest in *O. subscorpioidea* (19.93%) and least in *L. alata* (15.84%). The crude protein in *A. djalonenis* and *L. alata* was

almost the same with the least recorded for *O. subscorpioidea* (2.39%). The fat content in the three samples was generally low with 1.64% in *A. djalonenis* and 1.50% in *O. subscorpioidea*. A relatively high fat content was recorded for *L. alata* (3.48%). The moisture content was lowest in *L. alata* (8.47%) and highest in *O. subscorpioidea* (9.35%). In other words, the dry matter (DM) was highest in *L. alata* and lowest in *O. subscorpioidea*. The highest ash content was found to be in *O. subscorpioidea* (3.37%) and the least recorded in *A. djalonenis* (2.48%). The non-nutrient compositions (anti-nutrient factors) of the seed of *O. subscorpioidea* have been reported by Otori and Mann (2014). The moisture content of the plant samples was very low; this is an indication that the plants could withstand long storage. Carbohydrate was high in the samples; carbohydrates provide the energy required for normal physiological functions; they help to power cells and tissues in the body. Crude fibre, made up of cellulose with little quantity of lignin, is indicative of the level of non-digestible

Table 3. Proximate composition of three antimalarial plants used in Akure, Southwestern Nigeria.

Parameter	Composition (%)		
	<i>A. djalensis</i>	<i>L. alata</i>	<i>O. subscorpioidea</i>
Ash	2.48 ^b ± 0.34	2.57 ^b ± 0.09	3.37 ^a ± 0.14
Carbohydrate	66.48 ^a ± 0.62	66.32 ^a ± 0.94	63.40 ^b ± 0.37
Crude Fibre	19.27 ^a ± 0.27	15.84 ^b ± 0.16	19.93 ^a ± 0.08
Crude Protein	3.38 ^a ± 0.23	3.31 ^a ± 0.21	2.39 ^b ± 0.12
Fat	1.64 ^b ± 0.10	3.48 ^a ± 0.44	1.50 ^b ± 0.12
Moisture	8.50 ^b ± 0.36	8.47 ^b ± 0.47	9.35 ^a ± 0.19

Values are mean ± SD of triplicate determinations. Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test (DMRT) at p<0.05.

Table 4. Mineral element composition of three antimalarial plants used in Akure, Southwestern Nigeria.

Mineral	Composition (mg/kg)		
	<i>A. djalensis</i>	<i>L. alata</i>	<i>O. subscorpioidea</i>
Calcium	7413.67 ^c ± 17.16	11767.83 ^a ± 29.17	11667.67 ^b ± 28.88
Copper	22.67 ^a ± 2.08	8.38 ^b ± 0.58	8.66 ^b ± 0.59
Iron	301.33 ^b ± 4.04	333.63 ^a ± 1.74	249.68 ^c ± 4.72
Lead	0.00 ^a ± 0.00	0.00 ^a ± 0.00	0.00 ^a ± 0.00
Magnesium	1582.33 ^a ± 26.10	1227.36 ^b ± 7.47	1180.33 ^c ± 33.38
Manganese	50.71 ^b ± 1.58	67.71 ^a ± 4.19	30.94 ^c ± 2.13
Phosphorus	715.47 ^b ± 2.75	934.58 ^a ± 0.51	552.95 ^c ± 2.38
Potassium	0.55 ^a ± 0.03	0.18 ^b ± 0.01	0.76 ^a ± 0.01
Sodium	0.61 ^a ± 0.04	0.22 ^b ± 0.02	0.77 ^a ± 0.02
Zinc	80.67 ^a ± 2.08	57.25 ^b ± 0.46	57.44 ^b ± 1.49

Values are mean ± SD of triplicate determinations. Means in the same row followed by the same letter are not significantly different by Duncan's Multiple Range Test (DMRT) at p<0.05.

carbohydrate (Onwuka, 2005; Akpaibo and Ikpe, 2013). Low levels are reported for the samples. This is good as high level could cause indigestion and irritation of the bowels. Lipids are good sources of energy. They act as insulators and protect delicate organs of the body, and are important in many cellular functions; this study, however, reports low values of fat in the samples. Protein and ash are generally low in the samples. Proteins are useful in bakery and confectioneries (Khalil et al., 2012) whereas ash content represents the mineral matter of food samples (Onwuka, 2005).

Calcium, magnesium, phosphorus, and iron were found to be very high in the three samples (Table 4). Calcium was highest in *L. alata* (11767.83±29.17 mg/kg) and lowest in *A. djalensis* (7413.67±17.16 mg/kg) whereas magnesium was highest in *A. djalensis* (1582.33±26.10 mg/kg) and least in *O. subscorpioidea* (1180.33±33.38 mg/kg). However, *L. alata* was found to contain 333.63±1.74 mg/kg of iron while *A. djalensis* and *O. subscorpioidea* had 301.33±4.04 mg/kg and 249.68±4.72mg/kg respectively. Similarly, phosphorus content was highest in *L. alata* (934.58±0.51 mg/kg) and

least in *O. subscorpioidea* (552.95±2.38 mg/kg). Potassium and sodium were found to be very low in the plant samples with highest values recorded in *O. subscorpioidea* and lowest values in *A. djalensis* in each case. The zinc content obtained for *A. djalensis* was 80.67±2.08 mg/kg whereas *L. alata* and *O. subscorpioidea* almost tied in their zinc content with values 57.25±0.46 and 57.44±1.49 mg/kg, respectively. Manganese was found to be 67.71±4.19 mg/kg in *L. alata*, 50.71±1.58 mg/kg in *A. djalensis*, and 30.94±2.13 mg/kg in *O. subscorpioidea*. The amount of copper present in *A. djalensis* was 22.67±2.08 mg/kg, 8.66±0.59 in *O. subscorpioidea* and 8.38±0.58 in *L. alata*. Lead tested negative in all the three samples screened. Mineral elements have been considered to be of great importance in the prevention of disease and in the general well-being of individuals (Nielsen, 2000) as they fulfil a critical function in physiological and biochemical processes. Moreover, these elements are our natural resources and are sourced from both plants and animals. The major elements are necessary for life processes; calcium and magnesium, for example, are essential for

teeth and bone formation, for healthy blood vessels, energy formation, and in the transmission of nerve impulses (Griffith, 1988; Soetan et al., 2010). Potassium and sodium are needed in the maintenance of body fluid, blood pressure, muscle contraction, nerve transmission, and metabolism (Anon., 2014). Phosphorus plays a key role in the absorption of carbohydrates, proteins, and fat and in the biochemical reactions in the body. Iron is important in haemoglobin formation and in the production of energy. In the regulation of immune system, iron and zinc are essential. Copper is involved in respiratory and red blood cell function, whereas zinc helps to maintain taste and sensitivity (Mayer and Goldberg, 1990; Saraf and Samant, 2013). Manganese features in enzyme activities, glucose, protein and fat metabolism and in the maintenance of healthy immune system (Anon., 2014).

Conclusion

This study has provided an assessment of the chemical composition of three antimalarial plants. *A. djalonensis*, *L. alata* and *O. subscorpioidea* contained major mineral elements and nutritive compounds. These substances will serve well as food supplements. They may help to prevent opportunistic infections associated with malaria, as well as help to manage metabolic diseases. Anti-nutritive compounds and heavy metal composition are negligible and as such make the plants safe for consumption.

Conflict of interest

The authors declare no conflict of interest.

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