

Evaluation of the Mineral Nutrients, Characterization and some Possible Uses of *Blighia unijugata* Bak. Seed and Oil

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ABSTRACT

The kernel (KL), aril (AL) and mixture of the kernel and aril (KAL, in a 1:1 ratio) of *Blighia unijugata* Bak. were analyzed for their proximate composition, physico-chemical characteristics and levels (ppm) of selected toxic trace metals (Fe, Mn, Cu, Pb, Cd and Zn) and macro nutrients (Na, K, Mg and Ca). The oil yield was 50.82% for AL, 14.15% for KL and 29.00% for KAL. The saponification and iodine value suggest their possible use in liquid soap, shampoo and oil-based ice cream production. The crude protein was 19.90% for AL, 14.00% for KL and 16.70% for KAL. The moisture content of AL (3.30%) was the lowest compared to those of KL (6.10%) and KAL (4.70%), suggesting that AL might exhibit the most stable shelf-life characteristics. Triacylglycerol was found to be the dominant lipid species in the oil with KL (90.80%) being higher than AL (88.30%). Sterol was also the major component of the unsaponifiable matter. The concentrations of the macronutrients were high with K (1559 ± 0.78 ppm) being the highest in KAL. The concentration of the trace metals also differs, with Zn (46.77 ± 0.01 ppm) being the highest in KL. The oils extracted from them were also found to be rich in macronutrients and had considerable amount of trace metals. Thus, the seeds could be considered as good source of minerals with the possibility of use as feed supplements and their oils for edible purpose and industrial applications.

Keywords: lipid, macronutrient, physico-chemical, proximate

INTRODUCTION

Seeds have high nutritive and caloric values which make them vital in human diets; they are also good sources of oils. *Blighia unijugata* Bak. is a tree that belongs to the *Sapindaceae* family, usually small but sometimes attaining 35 m in height. It is planted as a shade-tree and is attractive, especially when in fruit, which are red or pinkish yellow. The wood has use for buildings; the bark pulped in enema or macerated by draught is taken as febrifuge and purgative; the fruit has common use as a fish poison and the seed infusion is given in case of sickness and vomiting. It is also recognized for its sedative and analgesic properties in treatment of rheumatism (Burkill 2000).

Many species of trees serve as food for man and other lower animals in both humid and semi-arid tropics, but have received less attention from the scientific community (Cannel 1989). However, attention ought to shift in this direction, as there is need for investigation of the nutritive value of these plant species. *B. unijugata* falls into this group, as there is very little information available on it in the literature. Generally, there is no information on the physico-chemical properties and nutritional contents of the seed. It is underutilized but contains economically viable oil and meal from the fruit seed which could be utilized (Oderinde and Ajayi 2000). Edible oils are becoming scarce and there is a need to find seeds that are rich in oil as replacement, making the search for alternative oil sources, especially for developing countries of utmost importance.

A number of minerals such as Ca, K and Fe are required by humans in order to maintain good health (Oderinde and Ajayi 1998). Some of these essential minerals are accumulated in different parts of plants as these accumulate minerals essential for growth from the environment. Trace metals can be detected in plants and foodstuffs. Recently, plant species (*Moraceae* and *Euphorbiaceae*) have been identified that contain nutrients displaying new beneficial medic-

nal or therapeutic properties (Almoaruf *et al.* 2003). The purpose of this work was to characterize and determine the mineral nutrients and the possible uses of *B. unijugata* seed and seed oil.

MATERIALS AND METHODS

Sample preparation

Seeds of *B. unijugata* (Fig. 1) were collected from the University of Ibadan, Oyo State, Nigeria. They were identified the Herbarium Unit, Botany Department of the University. The seeds were manually separated from the aril (AL) and cracked in order to remove the kernel (KL). AL and KL were ground separately in a laboratory mill (Gallenkamph, 82942, Brit. Pat, England). KAL was prepared by mixing the Aril (AL) and Kernel (KL) in a 1:1 ratio.



Fig. 1 *Blighia unijugata* Bak. seeds

Physico-chemical analysis

Oil was extracted from KL, AL and KAL using a soxhlet extractor with petroleum ether (40-60°C) for 10 h (Esuoso and Odetokun 1995). The extracted oils were immediately analyzed for iodine value, peroxide value, saponification value, acid value and unsaponifiable matter by methods described by the Association of Official Analytical Chemists (AOAC 1984). Estimation of the percentage free fatty acids as oleic acid followed the method described by Cock and Rede (1986).

The refractive indices of the oils (35°C) were determined with an Abbe refractometer (Pearson 1976) and the specific gravity measurements were also carried out at room temperature (35°C) using specific gravity bottles. Visual inspection was used to note the state and colour of the oils at room temperature. The mean molecular mass was estimated from the relation $(56/SV) \times 1000$ (Akintayo 2002).

Lipid classes

Lipid classes were separated on 0.75 mm plates (20 × 20 cm) coated on a silica gel (Merck). Plates were developed vertically in a 80: 20: 1 volume mixture of petroleum ether (95%): diethylether (92%): acetic acid (99%) obtained from Sigma-Aldrich Chemical Co., Steinheim, Germany. They were developed according to the method of Oboh and Oderinde (1988).

Isolation of unsaponifiables

Ten (10) grammes of the oil was dissolved in 200 ml of 2 M-ethanol potassium hydroxide and refluxed for 1 h. The reaction mixture was later diluted to 400 ml with distilled water and transferred into a 1 L-separating funnel. The unsaponifiables were then extracted three times with 100 ml diethylether. The ether extract was first washed with 100 ml aqueous solution of 0.5 M potassium hydroxide in order to remove any residual fatty acids. This was further washed and cleaned 5 times with 100 ml distilled water and dried over anhydrous sodium sulphate. The solution was filtered and dried (Adebowale and Adedire 2006).

Separation of unsaponifiables

Chloroform solution (50%) of the unsaponifiable material (30 mg/plate) was then applied uniformly along the line from the edge of the 20 × 20 cm plate coated with a 0.55 mm layer of silica gel and developed three times with hexane/ethylacetate (6:1, v/v) as mobile phase. The developed plates were dried and irradiated at 254 nm with ultraviolet radiation. Three zones corresponding to *n*-alkanes, triterpene alcohols and sterols were marked carefully

scrapped and extracted with petroleum ether (Kayode *et al.* 2001).

Proximate analysis

Proximate analysis was carried out as described by the Association of Official Analytical Chemists (AOAC 1990).

Mineral determination

Metals - lead, cadmium, copper, zinc, iron, magnesium, calcium, sodium, potassium and manganese were determined. This was achieved by digesting the samples using 5 ml (2:1) of 69.40% (w/w) nitric acid and 90.00% (w/w) perchloric acid (Almoaruf *et al.* 2003). These metals were analyzed by atomic absorption spectrophotometry (Perkin-Elmer, GMBH, Ueberlingen, Germany).

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA) and least significant differences between treatment means were determined by Duncan's multiple range test (Duncan 1955) with significant differences measured at $P < 0.05$.

RESULTS AND DISCUSSION

The proximate compositions of KL, AL and KAL are presented in **Table 1**. There is significant difference among the values obtained for KL, AL and KAL except for the Ash content where there is no significant difference among the values obtained. The oil content of AL was high, $50.82 \pm 1.21\%$, indicating that processing of the seed for industrial or edible purposes would be economical. The yield of KL and KAL are $14.15 \pm 0.91\%$ and $29.00 \pm 2.11\%$, respectively. The moisture content of KL, AL and KAL is low indicating a good shelf life characteristic. AL has the lowest moisture content ($3.30 \pm 0.21\%$) followed by KAL ($4.70 \pm 0.11\%$) and KL having the highest ($6.10 \pm 0.32\%$). The ash and crude fibre content were high, as indicated in **Table 1**. The carbohydrate content of KL, AL and KAL were found to be 54.02, 9.27 and 36.86%, respectively (**Table 1**). The high ash, crude fibre and carbohydrate content of KL could suggest its suitability in the compounding of animal feeds (Abighor *et al.* 1997).

The protein content of AL, KL and KAL were respectively 19.90 ± 0.71 , 14.00 ± 0.28 and $16.70 \pm 0.37\%$ (**Table 1**). These values are comparable with those reported for some conventional oil seeds (*Adenopus breviflorus* and *Cucumeropsis edulis*), which ranged from 15–22% (Esuoso and Bayer 1998). However, they are higher than the ranges

Table 1 Proximate composition (%) of *Blighia unijugata* Bak.

Assay	AL	KL	KAL
Crude fat	50.82 ± 1.21 a	14.15 ± 0.91 b	29.00 ± 2.11 c
Crude protein	19.90 ± 0.71 a	14.00 ± 0.28 b	16.70 ± 0.37 c
Crude fibre	11.21 ± 0.60 a	6.33 ± 1.10 b	8.14 ± 0.71 c
Ash	5.50 ± 0.31 a	5.40 ± 0.16 a	5.60 ± 0.62 a
Moisture	3.30 ± 0.12 a	6.10 ± 0.11 b	4.70 ± 0.22 a
Carbohydrate by difference	9.27 a	54.02 b	36.86 c

Values are mean \pm standard deviation of triplicate determinations. Data in a row with different letters are statistically different according to DMRT ($P \leq 0.05$).

Table 2 Physico-chemical characteristics of oils from *Blighia unijugata* Bak.

Parameter	AL	KL	KAL
Colour	Light brown	Green	Light brown
Acid value (mg KOH/g)	15.00 ± 0.30 a	18.00 ± 0.10 a	15.10 ± 0.10 a
Free fatty acid (%)	7.00 ± 0.10 a	9.10 ± 0.30 b	7.20 ± 0.10 a
Saponification value (mg KOH/g)	300 ± 0.40 a	217 ± 0.10 b	234 ± 0.50 c
Iodine value (mg iodine/g)	8.70 ± 1.50 a	7.00 ± 1.00 a	9.00 ± 0.50 b
Means molecular mass	186.67 a	258.86 b	239.32 b
Unsaponifiable matter (%)	3.04 ± 0.03 a	0.73 ± 0.01 b	3.11 ± 0.07 a
Refractive index (25°C)	1.5580 a	1.5530 a	1.5570 a
Specific gravity (25°C)	0.8500 a	0.8480 a	0.8520 a
State at room temperature	Liquid	Liquid	Liquid
Peroxide value (mg/g oil)	10.50 ± 0.61 a	6.80 ± 0.20 b	8.00 ± 0.41 ab

Values are mean \pm standard deviation of triplicate determinations. Data in a row with different letters are statistically different according to DMRT ($P \leq 0.05$).

found for cereal seeds and wheat and animal protein (Heger and Eggum 2000). These values fall within the range for important legumes and high protein animals (Bhuiyan *et al.* 1986). They could be recommended as protein supplements, though, the suitability of any plant materials as food supplement depends on factors like the presence of anti-nutritional factors and the digestibility of its nutrients. These aspects still need to be investigated.

The physico-chemical characteristics of KL, AL and KAL oils are shown in **Table 2**. KAL and AL were light brownish in colour. The saponification values (300 ± 0.40 , 217 ± 0.10 and 234 ± 0.50 mg KOH/g for AL, KL and KAL, respectively) of the oils as shown in **Table 2** were high and this suggests their possible utilization in production of liquid soap, shampoos and latter shaving creams. The iodine values were low indicating the presence of saturated fatty acids and these places the oils in the non-drying group (Kayode *et al.* 2001). The oil could be utilized for cooking and may find application as a raw material in industries for the manufacture of vegetable oil-based ice-cream (Ibiyemi *et al.* 1992). The small amount of alkali required to saponify the oils confirms the mean molecular mass (Akintayo 2002). Acid value is the expression of the total acidity of the lipid involving contributions from all the constituent fatty acids that makes up the glyceride molecule (Ekpa and Ekpe 1995). The acid value of these oils was high, ranging from 14 to 18 mg KOH/g.

There is no significant difference in the acid value, specific gravity and iodine value of all the studied oils. Free fatty acids can stimulate oxidative deterioration of oils by enzymatic and/or chemical oxidation to form off-flavour components. It is desirable that the free fatty acid content of cooking oil lies within limits of 0-3.0% (Basisir 1971; Onyike and Acheru 2002). The high free fatty acid values of these oils implies that refining would be required to make them suitable for edible purposes and may be better utilized for industrial purposes. The peroxide values were found to be 10.50 ± 0.61 , 6.80 ± 0.20 and 8.00 ± 0.41 mg/g oil for AL, KL and KAL, respectively. The peroxide value of AL was significantly different from that of KL while that of KAL is not significantly different from those of AL and KL. These values are lower than values stipulated for rancid oils. Oils with peroxide value ranging from 20 to 40 mg/g oil are considered rancid (Cock and Rede 1986). The peroxide values obtained were below this range, the oils are therefore considered to be stable (Ojeh 1981).

Triacylglycerol was the dominant lipid species in AL (88.30%) and KL (90.80%). Different concentrations of hydrocarbon, polar lipids, sterols, diacylglycerols, monoacylglycerols and free fatty acids were also detected in the oil (**Table 3**). The unsaponifiable matter consisted mainly of triterpene alcohols, sterols and *n*-alkanes with sterols having higher percentage composition in both cases, $40.10 \pm 0.60\%$ in AL and $38.50 \pm 0.20\%$ in KL (**Table 4**). There was no significant difference in the lipid classes of the studied oils except for the polar lipids and diacylglycerol content. However, work is already in progress to characterize the unidentified peaks obtained in this study as well as to determine the nature of the sterols and triterpenes.

A total of 10 elements (Na, Mg, K, Ca, Fe, Mn, Zn, Cu, Cd and Pb) were determined in AL, KL and KAL and also from their oils by Atomic Absorption Spectrophotometry (AAS). **Table 5** shows the mean concentration of various metals in the seed while **Table 6** shows the mean concentration of various metals in the oils extracted from AL, KL and KAL. This study shows that AL, KL, and KAL and their oils are rich in Ca, K, Zn, Na and Mg. The concentration of Cu ranges from 0.70 to 4.00 ppm. KL has the highest concentration being 4.00 ppm while AL oil has the least, 0.70 ppm. Cu and Zn are considered micronutrients.

Bowen and Allaway (1968) reported these elements range in agricultural products between 4 and 15 ppm for Cu and 15 to 200 ppm for Zn. However, the concentrations of Cu and Zn in all the samples in this study were found to be within this range for Zn and low in the case of Pb. The sta-

Table 3 Lipid classes (%) of the oil from *Blighia unijugata* Bak.

Class	AL	KL
Polar lipids	1.20 ± 0.90 a	0.60 ± 0.70 b
Sterols	3.60 ± 0.50 a	2.00 ± 0.30 a
Diacylglycerols	0.90 ± 1.20 a	1.80 ± 0.90 b
Monoacylglycerols	2.00 ± 0.40 a	0.80 ± 0.50 a
Triacylglycerols	88.30 ± 0.80 a	90.80 ± 0.20 a
Hydrocarbons	0.80 ± 0.60 a	1.90 ± 0.40 b
Free fatty acids	3.20 ± 0.20 a	2.10 ± 0.70 a

Values are mean ± standard deviation of triplicate determinations. Data in a row with different letters are statistically different according to DMRT ($P \leq 0.05$).

Table 4 Composition of the unsaponifiable matters of the oil of *Blighia unijugata* Bak.

Unsaponifiable	AL (%)	KL (%)
Triterpene alcohol	20.80 ± 0.60 a	18.10 ± 1.10 a
<i>n</i> -Alkanes	40.10 ± 1.50 a	38.50 ± 0.80 a
Unidentified	25.70 ± 0.40 a	30.80 ± 1.00 a

Values are mean ± standard deviation of triplicate determinations. Data in a row with different letters are statistically different according to DMRT ($P \leq 0.05$).

Table 5 Result of the metal contents from the analyzed seed samples.

Metal	AL	KL	KAL
Ca	990.87 ± 0.02 a	933.87 ± 0.14 a	1488.90 ± 0.65 b
K	874.73 ± 1.64 a	1216.66 ± 9.61 b	1559 ± 0.78 c
Na	473.20 ± 0.05 a	384.72 ± 0.77 b	331.56 ± 0.01 b
Mg	255.46 ± 0.11 a	239.13 ± 0.28 b	258.07 ± 0.03 a
Mn	168.23 ± 0.01 a	85.00 ± 0.11 b	100.33 ± 0.09 c
Pb	2.87 ± 0.05 a	4.57 ± 0.03 b	1.23 ± 0.01 c
Cd	6.40 ± 0.01 a	1.17 ± 0.01 b	4.37 ± 0.01 c
Zn	36.07 ± 0.01 a	46.77 ± 0.01 b	36.27 ± 0.01 a
Cu	4.67 ± 0.01 a	4.00 ± 0.01 a	4.60 ± 0.02 a
Fe	102.70 ± 1.56 a	31.40 ± 0.01 b	64.67 ± 0.01 c

Average concentration ± standard deviation of triplicate determinations (ppm) (mg/kg). Data in a row with different letters are statistically different according to DMRT ($P \leq 0.05$).

Table 6 Result of the metal contents from the analyzed samples of oils.

Metal	AL	KL	KAL
Ca	240.50 ± 0.05 a	858.73 ± 0.03 b	1241.43 ± 0.58 c
K	354.00 ± 0.21 a	866.66 ± 0.58 b	949.33 ± 0.44 c
Na	396.72 ± 0.02 a	348.40 ± 0.04 b	275.03 ± 0.82 c
Mg	192.57 ± 0.11 a	228.50 ± 0.08 b	241.51 ± 0.22 c
Mn	103.33 ± 0.52 a	55.00 ± 0.03 b	96.93 ± 0.61 c
Pb	1.57 ± 0.04 a	2.90 ± 0.05 b	1.07 ± 0.01 c
Cd	1.00 ± 0.01 a	0.73 ± 0.01 b	2.10 ± 0.01 c
Zn	31.17 ± 0.01 a	33.40 ± 0.01 b	34.37 ± 0.02 c
Cu	0.70 ± 0.01 a	1.00 ± 0.01 a	1.50 ± 0.01 a
Fe	99.07 ± 0.09 a	24.17 ± 0.01 b	58.67 ± 0.01 c

Average concentration ± standard deviation of triplicate determinations (ppm) (mg/l). Data in a row with different letters are statistically different according to DMRT ($P \leq 0.05$).

tistical evaluation reveals that the concentration of these metals differ significantly among the extracted oils except for Cu where there is no significant difference among the oils analyzed. The distribution of these minerals also varies significantly in the different parts of the seed. Of all the metals determined, only Cu was also found not to vary significantly in the seed just like in the case of the extracted oils. This also reflected in the transfer factor where the values obtained for Cu was also not significantly different among the analyzed samples.

The result obtained indicates that the samples contain a large amount of nutrients and are rich in Mg, Ca, Na and K. The abundance of K, Mg and Ca noted in this study is in agreement with previous findings that these three metals represent the most abundant metal constituents of many plants (Canel and Saura 1982). Pb concentration ranged from 1.23-4.57 ppm. The permissible limit for plants, based on ADI (Acceptable Daily Intake) for Pb is 10 ppm (Cui *et al.* 2004). KL, AL and KAL and their respective oil under investigation accumulated this metal at a level appreciably below the permissible level.

Table 7 Transfer factor (TF) of the movement of metals from seed to oil.

Metal	AL	KL	KAL
Ca	0.2427 a	0.9195 b	0.8334 c
K	0.4047 a	0.9908 b	0.6089 c
Na	0.8384 a	0.9056 b	0.8295 c
Mg	0.7536 a	0.9555 b	0.9358 b
Mn	0.6142 a	0.6471 a	0.9661 c
Pb	0.5470 a	0.6346 b	0.8699 c
Cd	0.1563 a	0.6239 b	0.4805 c
Zn	0.8642 a	0.7141 b	0.9476 c
Cu	0.1499 a	0.2500 a	0.3261 a
Fe	0.9647 a	0.7697 b	0.9072 c

Data in a row with different letters are statistically different according to DMRT ($P \leq 0.05$).

Transfer factor (TF) = $\frac{\text{Concentration of metal in oil}}{\text{Concentration of metal in seed}}$

Though much is known about the functional role of a number of elements, mineral nutrition lies in obtaining the correct amount of supplementation in the right form at the right time. Mg and Zn have an important role in the metabolism of cholesterol as well as heart diseases. The presence of Mn may be correlated with therapeutic properties against diabetic and cardiovascular diseases (Schwart 1975). Deficiency or excess Cu, Mn, Zn, Ca, Mg and K may cause a number of disorders; they also take part in neurochemical transmission and also as a cofactor for various enzymes and in variety of different metabolic processes (Oderinde and Ajayi 1998).

Transfer factor (FT) is an indicator of the leaching of metal from the seed to the oil (Cui *et al.* 2004). When the FT = 1, it shows that the leaching of the metal into the oil is very high which also indicates the affinity of the metals to the oil and also the probability of the metal to form a complex in the oil. From **Table 7**, the FT values of Cu (0.1499) and Cd (0.1563) are very low. In all the extracted oils Cu is the lowest (0.1499) in AL while K is the highest (0.9908) in KL. This is also expected since the macronutrients (Ca, K, Na and Mg) were expected to be high as a result of their nutritional role.

CONCLUSION

This present study has shown that the seed of *Blighia unijugata* Bak. is a good source of protein, carbohydrate, minerals and crude fibre. The oils extracted from them were also found to be rich in macronutrients and had considerable amount of trace metals. Thus, the seeds could be considered as a good source of minerals with the possibility of use as feed supplements and their oils for edible purpose and industrial applications.

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