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Determination of the Mineral Nutrients, Characterization and Analysis of the Fat-Soluble Vitamins of *Caesalpinia pulcherrima* and *Albizia lebbeck* Seeds and Seed Oils

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ABSTRACT

The seeds and seed oils of *Caesalpinia pulcherrima* (CP) and *Albizia lebbeck* (AL) 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 fatsoluble vitamins were also investigated using HPLC. These seeds are good sources of protein, carbohydrate, fat-soluble vitamins and minerals. Triglyceride was the dominant lipid species in the oils while sterol was the major component of the unsaponifiable matter. Potassium was the most abundant metal in the seeds and oil. The physico-chemical characterization of the oils suggests a good industrial application of these oils.

Keywords: extraction, glyceride, lipid, spectrophotometry, vitamin

INTRODUCTION

Seed oils represent one of the largest key materials that can be obtained from biomass and cheaply processed. They are of nutritional, industrial and pharmaceutical importance. The characteristics of oils from different sources depend mainly on their composition and no oil from a single source can be suitable for all purposes (Schneider 2001). There are many seeds, which are underutilized due to lack of information on their composition and utilization. Some of these underutilized seeds include *Caesalpinia pulcherrima* (commonly known as pride of Barbados; **Fig. 1**) and *Albizia lebbeck* (commonly known as 'woman's tongue' acacia; **Fig. 2**).

C. pulcherrima is a shrub, about 2-3 m tall, with prickles on the young twig and flowers. It is cultivated for its showy flowers; it is hard and will survive under rigorous conditions. *A. lebbeck* is a robust tree able to grow under a variety of climatic conditions. It is a tree of pleasing appearance and is commonly planted in gardens as an ornamental and as an avenue and a shade tree (Burkill 1995).

A number of minerals are required by the human body in order to maintain good health. Some of these essential minerals are accumulated in different parts of plants as it accumulates minerals essential for growth from the environment. It has also been reported that trace metals can be detected in plants and food stuffs (Liu e*t al*. 2005).

MATERIALS AND METHODS

Sample preparation

The seed samples were obtained from the surroundings of the Uni-

Fig. 1 Seed of *Caesalpinia pulcherrima*.

Fig. 2 Seed of *Albizia lebbeck*.

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versity of Ibadan, Oyo State, Nigeria. They were identified at the herbarium unit, Botany Department University of Ibadan, air dried at room temperature and subsequently ground in a laboratory mill (Gallenkamph, 82942, Brit. Pat, England) and stored in a cellophane bag at 4°C prior to analysis.

Physico-chemical analysis

Oil was extracted from *C. pulcherrima* and *Albizia lebbeck* using a Soxhlet extractor with petroleum ether (40-60°C) for 1 h (Ajayi *et al*. 2004). The extracted oils were immediately analyzed for their iodine, peroxide value, saponification and acid values and unsaponifiable matter by the methods described by the Association of Official Analytical Chemist (AOAC 1984). Estimation of the percentage of free fatty acids as oleic acid was done following the method described by Oderinde *et al*. (1990). The refractive indices of the oils (at 25°C) were determined with an Abbe refractometer (Oderinde and Ajayi 2000) and the specific gravity measurements were also carried out at 25°C using 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) × 1000 (Akintayo and Bayer 2002) where SV is the saponification value.

Proximate analysis

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

Mineral determination

Metals determined were lead, cadmium, copper, zinc, iron, magnesium, calcium, sodium, potassium and manganese. 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 (Oderinde and Ajayi 1998). These metals were analyzed by atomic absorption spectrophotometry (Perkin-Elmer, GMBH, Ueberlingen, Germany).

Lipid classes

Lipid classes were separated on 0.75 mm plates (20 × 20) 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%). They were developed according to the method described by Oboh and Oderinde (1988).

Isolation of unsaponifiables

Ten g of the oil was dissolved in 200 ml of 2 M ethanolic 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 unsaponifiable fraction was then extracted three times with 100 ml diethylether. The ether extract was first washed with 100 ml aqueous solution of 0.5 M KOH in order to remove any residual fatty acids. This was further washed and cleaned with 5 × 100 ml distilled water and dried over anhydrous sodium sulphate. The solution was filtered and dried.

Separation of unsaponifiables

A chloroform solution (50%) of the unsaponifiable matter (30 mg/plate) was then applied uniformly along the line from the edge of the 20 × 20 cm plate coated with 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 scraped and extracted with petroleum ether (Kayode *et al*. 2001).

Determination of vitamins

The procedures of Bogumila and Marek (2003) were adapted for separation and quantification of the fat soluble vitamins (A, D and E) in the oil using HPLC (1100 series, Agilent) equipped with a thermostated column compartment (G1316A, Germany), variable wavelength detector (G1314A), quaternary pump (G1311A, Germany) and a degasser (G1379A). The automated system (HPLC) is driven by a Chemstation software. The mobile phase was water and acetonitrile (5: 95, v/v). The flow rate was 1.5 ml/min. The vitamins were monitored using a UV detector at 210 nm and 35°C.

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

Physico-chemical properties

The physico-chemical characteristics of the oils are shown in **Table 1**. Both *C. pulcherrima* and *A. lebbeck* are light brown in colour. There was no significant difference in all the values obtained except for the acid, free fatty acid and saponification values. The acid values were high with that of *C. pulcherrima* being 38.00 ± 0.60 mg KOH/g and that of *A. lebbeck* being 2.24 ± 0.10 mg KOH/g. The free fatty acids which stimulates oxidative deterioration of oils by enzymatic and chemical oxidation to form off-flavour components is also high. The saponification value of these oils are fairly high with that of *A. lebbeck* (167.00 ± 0.50 mg KOH/g) being significantly higher than that of *C. pulcherrima* (83.00 ± 0.80 mg KOH/g). This high saponification value is an indication of high molecular weight which suggests the application of these oils in the cosmetic industries (Akintayo and Bayer 2002). The slightly high iodine values of 71.00 ± 3.50 mg iodine/g (*C. pulcherrima*) and 67.01 ± 1.00 mg iodine/g (*A. lebbeck*) indicates the preponderance of unsaturated fatty acids. This value could be used to quantify the amount of double bonds present in the oil which reflects the susceptibility of oil to oxidation. The unsaponifiable matters were found to be 4.16 ± 0.20% for *C. pulcherrima* and 2.11 ± 0.50% for *A. lebbeck*. The peroxide values were found to be 11.60 ± 0.80 mgO_2/g oil for *C. pulcherrima* and 12.00 ± 0.50 mgO_2/g oil for *A. lebbeck*. Although these values are high they are lower than values stipulated for rancid oil which ranges from 20.00 to 40.00 mg O_2/g oil (Pearson 1976).

Proximate composition

The summary of the proximate composition of *C. pulcherrima* and *A. lebbeck* is presented in **Table 2**. There is significant difference in the crude protein, ash and carbohydrate contents. The oil content of the seeds was quite low, 7.20 ± 0.40% for *C. pulcherrima* and 6.40 ± 0.80% for *A. lebbeck*. The moisture content of the seeds is low indicating a good shelf life characteristic. The moisture content of *C. pulcherrima* (3.10 ± 0.50%) is the same as that of *A. lebbeck* (3.10

Table 1 Physico-chemical characteristics of oils from *Caesalpinia pulcherrima* and *Albizia lebbeck*.

Parameter	*Caesalpinia pulcherrima*	*Albizia lebbeck*
Colour	Light brown	Light brown
Acid value (mg KOH/g)	38.00 ± 0.60 a	21.20 ± 0.10 b
Free fatty acid (%)	15.21 ± 0.30 a	10.31 ± 0.10 b
Saponification value (mg KOH/g)	83.00 ± 0.80 a	167.00 ± 0.50 b
Iodine value (mg iodine/g)	71.00 ± 3.50 a	67.01 ± 1.00 a
Unsaponifiable matter (%)	4.16 ± 0.20 a	2.11 ± 0.50 a
Peroxide value (mg O_2/g oil)	11.60 ± 0.80 a	12.00 ± 0.50 a
Mean molecular mass	674.69 a	335.33 b
Refractive index (25°C)	1.4100 a	1.3260 a
Specific gravity (25°C)	0.9580 ± 0.06 a	0.9100 ± 0.05 a
State at room temperature	Liquid	Liquid

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 2 Proximate composition (%) of *Caesalpinia pulcherrima* and *Albizia lebbeck*.

Assay	*Caesalpinia pulcherrima*	*Albizia lebbeck*
Crude fat	7.20 ± 0.40 a	6.40 ± 0.80 a
Crude protein	47.40 ± 0.80 a	38.60 ± 0.40 b
Crude fibre	4.03 ± 0.10 a	2.11 ± 0.20 a
Ash	3.60 ± 0.10 a	5.60 ± 0.20 b
Moisture	3.10 ± 0.50 a	3.10 ± 0.10 a
Carbohydrate	34.67 ± 0.60 a	43.19 ± 1.20 b

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

± 0.10%) showing the possibility of a long shelf life of *C. pulcherrima* and *A. lebbeck*. Generally, the moisture content of these seeds is lower than those of similar legumes such as *Cassia floribunda* (Vidivel and Janardhanen 2001) which might be advantageous in keeping the quality of the seeds.

Ash content is significant in food for various reasons. Among others, it is an index for the quality of feeding materials used for poultry and cattle feeding, already established by Pomeranz and Clifton (1981). It is also a measure of the inorganic components of the seeds; these inorganics are the majority of mineral elements which could be the macro- or microelements (Alabi and Alausa 2006). The ash content of these studied seeds was 3.60 ± 0.10% for *C. pulcherrima* and 5.60 ± 0.20% for *A. lebbeck*. The carbohydrate content of the seeds is fairly high. *C. pulcherrima* had a lower carbohydrate content (34.67 ± 0.60%) than *A. lebbeck* (43.19 ± 1.20%). The crude fibre content of *C. pulcherrima* is higher than that of *A. lebbeck* and this result compared favorably with those reported for commonly cultivated pulses, such as chick pea (*C. arietinum*) and horse gram (*Macrotyloma uniflorum*) (Premakumari *et al*. 1984). The presence of fairly high crude fibre in food material has been reported to decrease the dry matter digestibility in animals. These values suggest a good indication of nutritive value of feed material (Devendra 1995). The values of the ash, crude fibre and carbohydrate contents of these seeds indicate their suitability in the compounding of animal feeds (Abighor *et al*. 1997).

The crude protein is high (47.40 ± 0.80%) for *C. pulcherrima* and 38.60 ± 0.40% for *A. lebbeck*. This value corresponds with those obtained for some *Mucuna* (*M. monosperma*) seeds (Bressani 2002). Therefore, based on the recommended average human protein intake of 23-50 g by the National Research Council (1974), these seeds could contribute to alleviating the problem of protein malnutrition in the third world and developing countries. The values are higher than the range found for cereal seeds and protein animals (Heger and Eggum 1991). They could be recommended as protein supplements, though the suitability of any plant material as food supplement depends on factors like the presence of antinutritional factors and digestibility of its nutrients.

Table 3 Lipid classes of the oil from *Caesalpinia pulcherrima* and *Albizia lebbeck*.

Parameter	*Caesalpinia pulcherrima* (%)	*Albizia lebbeck* (%)
Polar lipids	5.60 ± 0.20 a	6.80 ± 0.60 a
Sterols	2.80 ± 0.40 a	0.90 ± 0.50 b
Diacylglycerols	1.70 ± 0.50 a	0.40 ± 0.80 b
Monoacylglycerols	0.60 ± 0.10 a	1.20 ± 0.10 b
Triacylglycerols	86.00 ± 1.00 a	86.90 ± 1.40 a
Hydrocarbons	1.80 ± 0.20 a	0.80 ± 0.10 b
Free fatty acids	1.50 ± 0.50 a	3.00 ± 0.10 b

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 unsaponifiables of the oil of *Caesalpinia pulcherrima* and *Albizia lebbeck*.

Composition	CP	AL
n-Alkanes	12.45 ± 1.30 a	12.50 ± 0.80 a
Triterpene alcohols	15.20 ± 0.50 a	11.30 ± 0.60 a
Sterols	40.30 ± 1.00 a	41.20 ± 0.70 a
Unidentified	32.00 ± 1.00 a	35.00 ± 0.80 a

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

Lipid classes and composition of the unsaponifiable matter of *C. pulcherrima* and *A. lebbeck*

Triglyceride was the dominant lipid species in the oils (*C. pulcherrima* has 86.00% and *A. lebbeck* has 86.90%) as shown in **Table 3**. The samples also contain varying concentration of hydrocarbons, free fatty acids, diacylglycerols, sterols, monoacylglycerols and polar lipids. Our study shows that the unsaponifiable matter consists mainly of *n*-alkane, triterpenoids and sterols. There is no significant difference in the polar lipids and triacylglycerols. The result of the unsaponifiable matter content is presented in **Table 4**. The fairly high unsaponifiable matter is an advantage for use as a natural insecticide. This is because unsaponifiable matter contains sterols and triterpene alcohols which are responsible for the insecticidal properties of fixed oils (Adebowale and Adedire 2006).

Nutritional and trace metal composition of the seed and seed oil of *C. pulcherrima* and *A. lebbeck*

The results of the nutritionally valuable minerals and trace metals are presented in **Table 5**. There is no significant difference between *C. pulcherrima* and *A. lebbeck* for the macro mineral nutrients (Ca, K, Mg and Na) studied in these seeds. The result of the mineral content of the oil from these seeds showed some levels of significant between *C. pulcherrima* and *A. lebbeck*, except for Ca and Cu. K was the most abundant mineral in the seeds and oils. *C. pulcherrima* has a higher value (765.30 ± 1.80 ppm) than *A. lebbeck* in the seed and in the oil. The concentration of Cu

Table 5 Nutritionally valuable and trace metals (ppm) of CP and AL.

Metal	Seed		Oil	
	Caesalpinia pulcherrima	*Albizia lebbeck*	*Caesalpinia pulcherrima*	*Albizia lebbeck*
Na	300.50 ± 1.00 a	370.50 ± 0.20 a	201.80 ± 0.30 a	290.40 ± 1.00 b
K	765.30 ± 1.80 a	620.60 ± 0.50 a	550.50 ± 0.05 a	420.30 ± 0.20 b
Ca	335.00 ± 0.70 a	380.10 ± 1.20 a	280.60 ± 1.00 a	295.40 ± 0.40 a
Mg	55.80 ± 0.05 a	88.00 ± 0.40 a	46.20 ± 0.70 a	65.90 ± 0.50 b
Fe	178.50 ± 1.50 a	98.30 ± 0.80 b	98.00 ± 0.80 a	57.00 ± 0.30 b
Cu	1.70 ± 0.05 a	0.80 ± 0.20 b	0.20 ± 0.20 a	0.20 ± 0.30 a
Zn	52.20 ± 0.05 a	21.78 ± 0.05 b	35.30 ± 0.50 a	16.90 ± 0.80 b
Mn	10.35 ± 0.08 a	22.30 ± 0.30 b	6.00 ± 0.01 a	12.80 ± 0.00 b
Pb	0.07 ± 0.10 a	0.20 ± 0.50 b	0.02 ± 0.10 a	ND
Cd	0.05 ± 0.50 a	0.02 ± 0.20 a	ND	ND

Average concentration ± standard deviation of triplicate determinations (ppm) (mg/kg)
ND; Not detected. Data in a row with different letters are statistically different according to DMRT ($P \leq 0.05$).

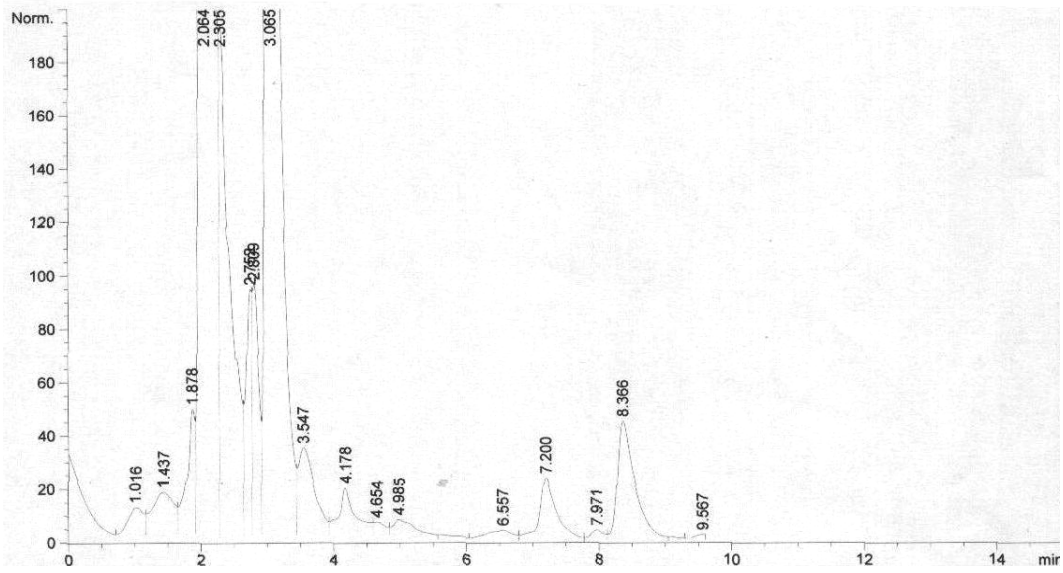

Fig. 3 HPLC of the fat soluble vitamins of *Caesalpinia pulcherrima*. Vitamin A (retention time) = 3.547 min; Vitamin E (retention time) = 8.366 min.

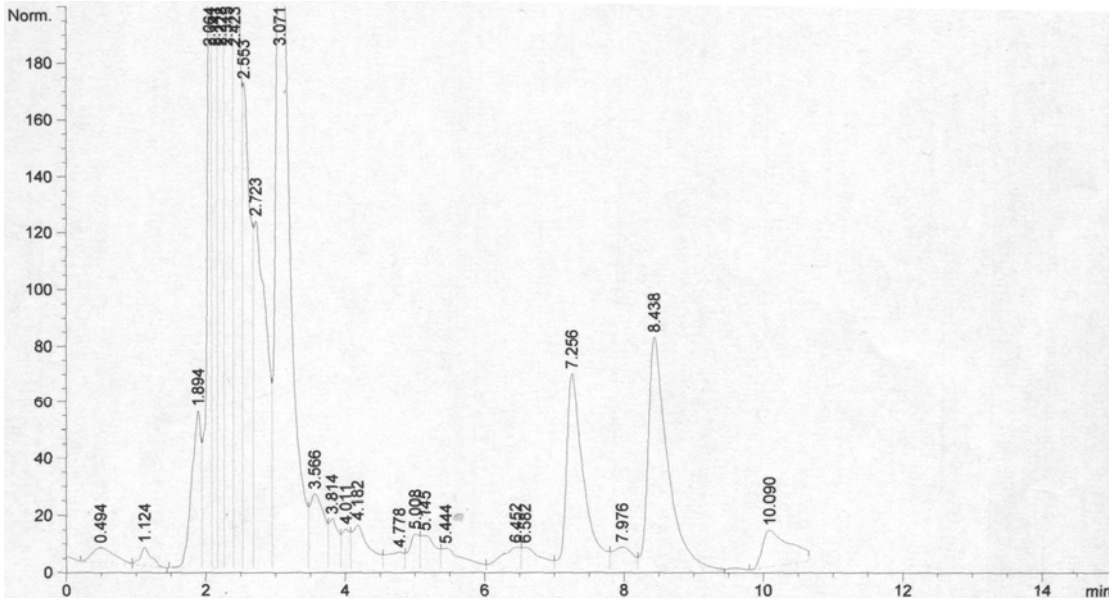

Fig. 4 HPLC of the fat soluble vitamins of *Albezia lebbeck*. Vitamin A (retention time) = 3.566 min; Vitamin E (retention time) = 8.438 min; Vitamin D (retention time) = 7.976 min.

and Zn has been reported to range from 4 to 15 ppm for Cu and 15 to 200 ppm for Zn (Allaway 1988). The values obtained from our studies falls within this range with *C. pulcherrima* having 1.70 ± 0.05 ppm and *A. lebbeck* 0.80 ± 0.20 ppm of Cu in the seed and 0.20 ± 0.20 ppm for *C. pulcherrima* and 0.20 ± 0.30 ppm for *A. lebbeck* in the oil. Pb was detected to be 0.07 ± 0.10 ppm in the seed of *C. pulcherrima* and 0.20 ± 0.50 ppm in *A. lebbeck*. Pb was not detected in the oils of *A. lebbeck* but was found at 0.02 ± 0.10 ppm in the oil of *C. pulcherrima*. Cd was not detected in any of the oils from these seeds. The abundance of K, Na and Ca in the result of this analysis is in agreement with previous findings that these three metals represent the most abundant metal constituents of many plants (Canellas and Saura-Calixto 1982).

Vitamin content of the oil of *C. pulcherrima* and *A. lebbeck*

The result of the fat soluble vitamins of *C. pulcherrima* and *A. lebbeck* are presented in **Figs. 3** and **4**. Vitamin E, which is the most important lipid-soluble antioxidant, protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain (Herrara and Barbas 2001) was found to be 14.61 mg/L in *C. pulcherrima* and 30.00 mg/L in *A. lebbeck*. These values are good enough for the Recommended Daily Amount (RDA) by The U.S Dietary Reference Intake (DRI) which was 15 mg/day. The value of vitamin A obtained was higher in *C. pulcherrima* (39.25 mg/l) than in *A. lebbeck* (25.51 mg/l). The values are in accordance with values obtained in some conventional seed oils (Tang *et al*. 2005). The value of Vitamin D was very low in *A. lebbeck* but was not detected in *C. pulcherrima*. The presence of vitamin E at these concentrations in these oils shows their possibility of being used as antioxidants.

CONCLUSION

The present study has shown that the seed of *C. pulcherrima* and *A. lebbeck* are good sources of protein, carbohydrate, fat-soluble vitamins (A and E) and minerals. In view of all the overall nutrient, physicochemical properties and proximate chemical composition, these seeds may be an economic and alternative protein, mineral and carbohydrate source that could alleviate malnutrition in developing countries and

improve overall nutritional status of functional food in the developed countries.

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