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Compositional Studies of Rattle Box (*Crotalaria retusa* L.) Seeds Found in Nasarawa State, Nigeria

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Abstract: The rattle box (*Crotalaria retusa* L.) is a lesser known plant grown wild in Nigerian soil. Studies were conducted on its seed flour and oils to determine their suitability, using standard analytical techniques. The result of mineral composition revealed that magnesium was the most abundant mineral (50.82 mg/100 g) while the least was arsenic (0.03 mg/100 g). Lead, cadmium and chromium were not at detecting limit of AAS. The most concentrated fatty acids were stearic acid (11.02%) < oleic acid (15.50%) < linoleic acid (57.54%). Caprylic, margaric, capric, lauric, myristic, arachidonic, erucic and lignoceric acids were all determined but not detected. Unsaturated fatty acids predominated in all the samples with only linoleic (57.54%) available as essential fatty acid. The results of physicochemical parameters of the seed oils determined were: Colour (yellow), acid value (1.10 mg KOH/g), iodine value (46.34 g/100 g), saponification (161.28 mg KOH/g), peroxide value (5.26 meq/kg), specific gravity at 25°C (0.88), kinetic viscosity at 40°C (4.91), unsaponifiable matter (1.92%) and flash point (231.00). The results showed that *Crotalaria retusa* seed oils may not be used as edible oils due to their instability as frying oils but may be useful industrially for the manufacture of products such as paints and shampoos.

Key words: *Crotalaria retusa*, seed oils, fatty acids, physicochemical parameters

INTRODUCTION

The *Crotalaria retusa* L. is a genus of herbaceous plant and woody shrubs commonly known as rattle pods which attain a height about 1 m with ridged erect stem (Duffa, 1995). The leaves are simple, oblanceolate and have veins on each side of the midreins. The flowers have yellow petals with fine purple line near the base. The pods are dark brown to black in colour at maturity, 3-4 cm long, non-stipitate and glabrous and contain about 23 seeds per pod. The seeds are smooth and brown in colour and measure up to 4.5 mm in length (Evans, 2002). The common name rattle pod or rattle box is derived from the fact that the seeds become loose in the pod as they mature and rattle when the pod is shaken (Polhill, 1982).

Crotalaria species is the third largest genus of Papilionoidecae which distributed throughout the tropic and subtropics (Polhill, 1982). They are used as a source of fibres, silage and green manure which are its agronomic trails (Cook and White, 1996; Ramos *et al*., 2001; Sakale *et al*., 2000). *Crotalaria* species can be used as forage for horses and cattle owing to the large amounts of water soluble gums and proteins in their seeds (Purseglove, 1981; Pandey and Srinvastava, 1990). Their ability to fix nitrogen enables these plants to be used for enhancing soil fertility (Samba *et al*., 2002). Moreover, these leguminous plants develop a high resistance to main species of root knot nematodes which cause severe damage to crops (Kar, 2007). *Crotalaria retusa* L. supports nitrogen fixing bacteria and considered as a "soil builder", however it is poisonous to cattle due to the presence of the toxic alkaloid monocataline, a pyrrolizidine alkaloid (Kar, 2007).

There are limited information on the nutritional composition, utilization and physicochemical properties of the *Crotalaria retusa* L. seeds and seed oils. Therefore, the present study is aimed at investigating the mineral composition, physicochemical characteristics and fatty acid composition of *Crotalaria retusa* seed flour found in Keffi, Nasarawa State, Nigeria.

MATERIALS AND METHODS

Sample collection: The mature dried fruits were plucked from *Crotalaria retusa* plant growing wild at the premises of Nasarawa State University, Keffi, Nigeria during dry season (January, 2011). The seeds were removed from the pods by pounding them gradually and blowing by wind to separate the pods from the seeds. They were then dried in solar drier for 3-days and ground into fine powder using a manual grinding machine while powdered sample was kept in polythene bag and stored in the refrigerator at 4°C prior use.

Mineral analysis: The minerals were analyzed from solutions obtained by first dry-ashing the seed flour at 550°C. The ash obtained was boiled with 15 ml of 20%

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hydrochloric acid in a beaker, filtered into a 100 ml standard flask and made up to the mark with distilled water. Sodium and potassium were determined by using a flame photometer 4330 (Model 405, Corning UK). All other metals were determined by atomic absorption spectrophotometer (Perkin Elmer Model 403, Norwalk CT, USA). All determinations were done in triplicate while mineral values were reported in mg per 100 g sample.

Extraction of oils: Oils were extracted from the powdered sample according to the method described by Akintayo and Bayer (2002). Oven dried sample was extracted in Soxhlet apparatus with chloroform-methanol mixture (2:1) for 20 h under nitrogen atmosphere. Solvent was removed under reduced pressure in a rotary evaporator. Toluene was added to ensure removal of any water through azeotropic distillation with toluene.

Fatty acid analysis: The oil extracted was converted to the methyl ester. The fatty acid methyl esters were analyzed using a HP 6890 gas chromatograph powered with HP Chemstation Rev. A09.01 [1206] software fitted with a flame ionization detector and a computing integrator. Nitrogen was used as the carrier gas. The column initial temperature was 250°C rising at 5°C/min to a final temperature of 310°C while the injection port and the detector were maintained at 310°C and 350°C, respectively. A polar (HP INNO Wax) capillary column (30 m x 0.5 mm x 0.5 mm) was used to separate the esters. The peaks were identified by comparison with standard fatty acid methyl esters obtained from Signa Chemical Co. (St. Louis, MO, USA).

Physicochemical analyses: The physicochemical analyses of the seed oils for acid value, iodine value, saponification value, peroxide value, specific gravity, kinetic viscosity, unsaponifiable matter and flash point were carried out according to the methods of AOAC (1990) and standard procedures described by Plummer (1987). All the chemicals used were of Analar grade (British Drug Houses, London).

Statistical analysis: Sodium/potassium, calcium/ phosphorus and potassium/calcium and magnesium ratios were calculated for the samples (Nieman *et al*., 1992). Ester value was obtained by subtracting the acid value from the saponification value. Free fatty acid was calculated by multiplying acid value of each sample with a factor of 0.50 while heat of combustion was obtained by subtracting the values of iodine and saponification from 11380 (Norris, 1995).

RESULTS AND DISCUSSION

The mineral content (mg/100 g) of *Crotalaria retusa* seed flour is shown in Table 1. The least abundant mineral in the sample flour was arsenic (0.03 mg/100 g) while magnesium was found to be the most abundant mineral (50.82 mg/100 g). It has been reported that magnesium is an activator of many enzyme systems and maintains the electrical potential in nerves (Shills, 1973). Calcium was found to be the next highest mineral component with value of 41.74 g/100 g. Calcium in conjunction with magnesium, manganese, vitamin A, C and D, chlorine and protein are all involved in bone formation (Fleck, 1976). Calcium is also important in blood clotting, muscle contraction and in certain enzymes in metabolic processes. Harmful metals such as lead, cadmium and chromium were not at detection limit of AAS while nickel, copper, zinc, selenium and manganese were all having concentrated values less than 1.0 mg/100 g. Phosphorus had concentrated value (28.52 mg/100 g) next to calcium. Phosphorus assists calcium in many body reactions although it also has independent functions. Modern diets which are rich in animal proteins and phosphorus may promote the loss of calcium in the urine (Shills and Young, 1992). This has lead to the concept of the calcium to phosphorus ratio. If the Ca/P ratio is low (low calcium, high phosphorus intake) more than the normal amount of calcium may be lost in the urine. Food is considered "good" if Ca/P ratio is above one and "poor" if the ratio is less than 0.5 while Ca/P ratio above two helps to increase the absorption of calcium in the small intestine. The result of Ca/P ratio in the present study was 1.46. This value is comparable with the values obtained for processed cranberry bean (*Phaseolus coccineus* L.) (1.03-1.37) (Aremu *et al*., 2010). Sodium and potassium are required to maintain osmotic balance of the body fluids and pH of the body, to regulate muscle and nerve irritability and to control glucose absorption. The ratio of sodium to potassium in the body is of great concern for prevention of high blood pressure. Na/K ratio less than

Table 1: Mineral composition (mg/100 g) of *Crotalaria retusa* seed flour

Mineral	Concentration (mg/100 g)
Sodium	11.47
Potassium	8.20
Calcium	41.74
Magnesium	50.82
Iron	1.94
Nickel	0.93
Copper	0.75
Zinc	0.54
Lead	N.D
Cadmium	N.D
Arsenic	0.03
Selenium	0.62
Chromium	N.D
Manganese	0.96
Phosphorus	28.52
Sodium/Potassium ratio	1.40
Calcium/Phosphorus ratio	1.46
[K/(Ca + Mg)] ratio	0.04 meq*

ND = Not Detected; *meq = milliequivalent

one is recommended. The Na/K ratio in *Crotalaria retusa* seed flour was 1.40 comparable with that of processed kersting's groundnut (*Kerstingiella geocarpa*) seed flour (1.37-1.96) (Aremu *et al*., 2011a,b). This suggests that the sample considered in this study may promote high blood pressure if consumed. The observed value for [K/(Ca + Mg)] was 0.09 milliequivalent to prevent hypomagnesaemia. Marten and Andersen (1975) reported that the milliequivalent of [K/(Ca + Mg)] must be less than 2.2 hence *Crotalaria retusa* may have capacity not to lead to hypomagnesaemia.

The result of fatty acid composition of the oils from *Crotalaria* seed is presented in Table 2. The saturated fatty acids were palmitic (C16:0), stearic (C18:0), arachidic (C20:0) and behenic (C22:0). The monounsaturated acids were palmitoleic (C16:1 n-7) and oleic (C18:1 n-9) while the polyunsaturated component was linoleic (C18:2 n-6). The highest concentration was linoleic (57.54%). This value is favourably compared with pigeon pea (54.8%) (Oshodi *et al*., 1993), soyabean (52.0%) (Paul and Southgate, 1985), sponge luffa oils (54.32%) (Aremu and Amos, 2010) and pinto bean (58.7%) (Audu and Aremu, 2011). Linoleic and oleic acids are major fatty acids in peanut, soyabean, chide pea, garden pea, broad bean and lentil. Cowpea, black-eyed pea, kidney and California small white bean have linoleic and linolenic acids as the major fatty acids (Adeyeye *et al*., 1999; Aremu *et al*., 2007a). Many lipids from legume seeds contain substantial amounts of saturated fatty acids, especially palmitic acid (Lee and Mattick, 1961). A higher proportion of either linoleic or linolenic acid is associated with legumes containing insignificant amounts of lipids (Salunkhe *et al*., 1985). The behenic acid (C22:0) had the least value of 0.38%. This is contrary to the report of Audu and Aremu (2011) who found lauric acid (C12:0) as the least concentrated value in pinto bean seeds. The total lipid content of legume values varies with variety, origin, location and climate as well as seasonal and environmental conditions and the type of soil in which they are grown (Worthington *et al*., 1972; Adeyeye *et al*., 1999; Aremu *et al*., 2007a).

Table 2 also depicts fatty acids distribution into TSFA, MUFA, PUFA, TUFA and O/L ratio. The Total Unsaturated Fatty Acid (TUFA) value which makes up n-7, n-9 and n-6 fatty acids was 80.29%. The n-6 and n-3 fatty acids have critical roles in the membrane structure (Lynch and Thompson, 1984; Kinsella, 1990; Aremu *et al*., 2007a) and as precursors of eicosanoids, which are potent and highly reactive compounds. Since they compete for the same enzymes and have different biological roles, the balance between the n-6 and the n-3 fatty acids in the diet can be of considerable importance (WHO/FAO, 1994) but n-fatty acid was not present in the present study. However, the high content of TUFA in this study is of great concern because it has been reported that fats and oils which contain more unsaturated fatty acids are particularly susceptible to oxidation and intake of food containing oxidized lipid increased the concentration of secondary peroxidation products in the liver (Hegsted *et al*., 1993). Linoleic and α-linolenic acids are the most important essential fatty acids required for growth, physiological functions and body maintenance (Salunkhe *et al*., 1985). In the present study only linoleic constituted available essential fatty acid which was 57.54%, therefore *Crotalaria retusa* seed will participate well in this function. It has also been reported that polyunsaturated linoleic acid moderately reduced serum cholesterol and Low Density Lipoprotein (LDL) levels (WHO/FAO, 1994). The Oleic/Linoleic (O/L) acids ratio has been associated with high stability and potentiality of the oil for deep frying fat (Branch *et al*., 1990). The O/L level of the sample was 0.27 (Table 2) which is lower than peanut oil (1.48) (Branch *et al*., 1990) hence *Crotalaria retusa* seed oils may be less stable compared with peanut oil and may also not be useful as frying oil.

Table 2: Fatty acid composition (%) of *Crotalaria retusa* seed oils

Fatty acid	Concentration (%)
Palmitic acid (C16:0)	3.48
Stearic acid (C18:0)	11.02
Arachidic acid (C20:0)	4.83
Behenic acid (C22:0)	0.38
Palmitoleic acid (C16:1 n-7)	7.25
Oleic acid (C18:1 n-9)	15.50
Linoleic acid (C18:2 n-6)	57.54
Total Saturated Fatty Acid (TSFA)	19.71
Monounsaturated Fatty Acid (MUFA)	22.75
Polyunsaturated Fatty Acid (PUFA)	57.54
Total Unsaturated Fatty Acids (TUFA)	80.29
O/L level	0.27

O/L = Oleic: Linoleic ratio

The results of some physicochemical parameters of *Crotalaria retusa* seed oils are shown in Table 3. The colour of the oil is yellow resembles that of cream coat bambara groundnut seed oils (Aremu *et al*., 2006). The acid value was 1.10 mg KOH/g. This value is much lower when compared with *Plukenetia conophora* oil (11.5 mg KOH/g) as reported by Akintayo and Bayer (2002). Acid value is used as an indicator for edibility of an oil and suitability for use in the paint and soap industries (Aremu *et al*., 2006). This acid value obtained in this report showed that the oils may not be suitable for use as edible oil and soap production, but may however, be useful for the production of paint and shampoos (Akintayo, 1997). Also appreciable amount of acid value of the seed oils was an indication that the seed might be poisonous for livestock and this explains the reason why cattle, sheep and goats do not browse the seeds. The saponification value of the oils was 161.28 mg KOH/g which is below the values obtained for some vegetable oils ranging from 188-196 mg KOH/g (Aremu *et al*., 2006). However, there are some vegetables with higher

Table 3: Physicochemical characteristics of *Crotalaria retusa* seed oils

Parameter	Oils value
Colour	Yellow
Acid value (mg KOH/g of oils)	1.10 (0.10)
Iodine value (g/100 g of oils)	46.34 (2.14)
Saponification (mg KOH/g of oils)	161.28 (2.50)
Peroxide value (meq/kg)	5.26 (3.40)
Specific gravity (at 25°C)	0.88 (0.05)
Kinetic viscosity (at 40°C)	4.91 (1.10)
Unsaponifiable matter (%)	1.92 (0.20)
Flash point (°C)	231.00 (4.25)
[w]Free fatty acid (as % of oleic acid)	0.55
[x]Ester value (mg KOH/g)	160.18
[y]Heat of combustion (g cal/g)	9757.95

Numbers in parentheses are standard deviation for triplicate determinations.
[w]Calculated free fatty acid = 0.50 x acid value.
[x]Ester = was obtained by subtracting the acid value from the saponification value.
[y]Heat of combustion = 11380 - Iodine value - 9.15 (saponification value) (Norris, 1995)

saponification values such as coconut oil (253 mg KOH/g), palm kernel oil (247 mg KOH/g) and butter fat 225 (mg KOH/g). It has been reported by Pearson (1976) that oils with higher saponification values contain high proportion of lower fatty acids. Therefore the value obtained for *Crotalaria retusa* seed oils indicated that the oils contained higher proportion of higher fatty acids. The iodine value (46.34 mg Iodine/g) of *Crotalaria retusa* seed oils is comparable with cashew nut oil (44.4 g Iodine/g) (Aremu *et al*., 2007a), *Citrullus vulgaris* oil (38.1 g Iodine/g) (Achinewhu, 1990) and Hausa melon oil (38.50 g Iodine/g) (Oladimeji *et al*., 2001). In view of the fact that drying oils have an iodine value above 100 (Duel, 1951), *Crotalaria retusa* seed oils could only be categorized as non-drying oils. The peroxide value (5.26 meq/kg) obtained for the seed oils is lower than the recommended value of 10 meq/kg (WHO/FAO, 1994). Peroxide value is an indicator for deterioration of oils (Akumbugwo *et al*., 2008). The peroxide value of the sample is far below the value reported for *Bilphia sapida* seed oil which is only recommended for industrial use (Akintayo and Bayer, 2002). The specific gravity at 25°C (0.88) indicating that the oil is less than water and compared favourably with oils of varieties of bambara groundnut (0.87-0.88) (Aremu *et al*., 2011b). The unsaponifiable matter (1.92) is low and lower than the values reported for varieties of bambara groundnut (2.39-2.47%) (Aremu *et al*., 2011b) and Malagasy legume seed oils (Gaydolel *et al*., 1983). High unsaponifiable matter content of fats and oils have been reported to be an indication of adulteration or contamination (Aremu *et al*., 2007b). The kinetic viscosity at 40°C (4.91) and flash point (231°C) are comparable with the values reported for varieties of bambara groundnut, 2.95 to 4.94 and 210 to 220°C, respectively. Free fatty acid (0.55%) is comparable with cream coat bambara groundnut (0.42%) as reported by Ferrao *et al*. (1987). Free fatty acids can stimulate oxidative determination of oils by enzymatic and/or chemical oxidation to form off-flavour components (Akintayo and Bayer, 2002). Calculated ester and combustion values were 160.18 mg KOH/g and 9757 g cal/g, respectively. The heat of combustion for *Crotalaria retusa* was found to be greater than the recommended value (9500 g cal/g) for edible oils (Norris, 1995).

Conclusion: The seeds of *Crotalaria retusa* L. have been shown to be high in magnesium content and contained harmful metals such as nickel, arsenic and selenium. Result of fatty acid composition on the seed oils revealed appreciable quantity of polyunsaturated fatty acids particularly linoleic acid. The acid and iodine values showed that the seed oils can be categorized as non-drying oils and that the oils may not be suitable as edible oil and soap production, but may however, be useful for the production of paint and shampoo.

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