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Proximate Characterisation and Physicochemical Properties of Raw and Boiled Milk Bush (*Thevetia Peruviana*) Seed

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Abstract: This study examined the powdered sample of the raw and boiled seeds of *thevetia Peruviana*, the seeds were analysed for proximate composition, mineral content, while the oil extracted from the seeds were evaluated for physicochemical properties using standard methods. The results of the study showed that the proximate composition (%) of the raw and boiled samples respectively are moisture (2.00 and 2.89), ash (3.33 and 2.96), crude protein (30.10 and 29.6), crude fibre (4.79 and 5.21), crude fat (58.3 and 59.20), carbohydrate (1.80 and 0.30), and energy (2524.5 and 2521.1) KJ/g. While the physiochemical properties of the extracted oil are acid value (1.71 and 1.41) mg/g, peroxide value (3.85 and 11.51) mg/g, iodine value (83.89 and 76.20) mgKOH/g, saponification value (224.26 and 193.78)mg/g, unsaponification value (2.04 and 1.59)mg/g, density (0.89 and 0.88) g/dm^3, specific gravity (0.91 and 0.93), refractive index at 32^{0}C (1.46 and 1.47) and viscosity (28.21 and 30.59) pal/sec. The mineral contents of both raw and boiled samples are in the order of P > K > Na > mg > Fe > Ca > Zn > Mn. Cd, Pb, Ca and Cr were detected in raw and boiled samples except Ca that was detected in raw sample. Generally, the high protein and fat contents show that the samples can be used in food and feed formulation while the saponification values, iodine values and the presence of some valuable minerals indicate that they can be used for other industrial purpose.

Keywords: Thevetia Peruviana, Phytochemical, Proximate, Seed Oil

Introduction

Seeds have nutritive and caloric values which make them necessary in diet. (Odeomelam, 2005). Africa, indeed the most tropical and sub-tropical, countries are blessed with numerous seeds and nuts, many of which are yet to be fully used due to insufficient information on their chemical, biological and industrial importance. One of such seed-bearing plants is *thevetia peruviana* commonly known as lucky-nut or milk-bush. *Thevetia peruviana* belongs to *Apocynaceae* family; Thevetia Genus; Yoruba name is called Olomi-Ojo. The plant is commonly known as yellow oleander, lucky-nut, be- still tree and milk bush. (Ibiyemi *et al*, 2002).

Thevetia peruviana is used medicinally throughout the tropics in spite of its toxicity. In Côte d'Ivoire and Benin the leaf sap is used as eye drops and nose drops to cure violent headaches; the seeds may be used as a purgative. The seed oil is applied externally in India to treat skin infections. In southern Africa and Cameroon the seeds are used as an arrow or ordeal poison. Other reports state the use of the seeds as an abortifacient. The seeds act as a contact poison; mashed with a soap solution they are used as an insecticide. In Ghana and Uganda the wood is used to make tool handles and building poles (Vandana D. and Varsha Z. 2014). It is also used as fuel. The fruit pulp is sometimes eaten. *Thevetia peruviana* is widely planted as an ornamental in gardens, and also as a hedge. In cooler climates it can be grown in tubs in the glasshouse and outdoors in summer.

It is a plant toxin insecticide for termites. *Thevetia peruviana* inhibited spermatogenesis in rats, indicating the possibility of developing a herbal male contraceptive. (Gupta R. *et al*, 2011).

The aim of this work is to evaluate the effect of boiling on the chemical constituents and physicochemical properties of the extracted oil. The research is expected to provide information on the usefulness of chemical constituents of the seed and serve as a resource in advancement of medicine and industrial applications.

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Materials and Methods

Preparation

The milk bush seed (Thevetia peruviana) also called olomiojo in Yoruba used in this study was obtained from Lafe Area in Akure Ondo State Nigeria. The fruits were air dried, cracked to remove the seed; some of the seeds were boiled while the remaining were left unboiled (raw). Both the raw and boiled dried seeds were ground to powder, packaged in airtight sample plastics and stored in the refrigerator at 4 ^{o}C prior to laboratory analysis.

Chemical Reagents

All reagents/chemical were of analytical grades and they were obtained at the Department of Chemistry, Federal University of Technology Akure (FUTA).

Proximate Analysis: The proximate analysis was carried out using standard methods as indicated below

(a) Moisture content: A known weight of dried sample was oven-dried at 105^{0}C to a constant weight. The loss in weight during drying in percentage was taken to be the percentage moisture content (A.O.A.C, 1990).

$$\%\ \text{moisture content} = \frac{\text{Loss in weight}}{\text{Weight of sample}} X100$$

(b) Ash content: Clean crucibles were placed in a muffle furnace for about 15 minutes at 350 ^{0}C. The dishes were removed, allowed to cool in a desiccator, well labelled and each was weighed (W_1). 1g of the sample was placed in the dishes, weighed (W_2), pre-ashed to burn off the carbon present and transferred into the furnace for ashing at 550 ^{0}C for 4hrs. After complete ashing, the dishes were allowed to cool in a desiccator and reweighed (W_3) (A.O.A.C, 1990).

$$\%\text{Ash content} = \frac{\text{W3} - \text{W1}}{\text{W2} - \text{W1}} X100$$

where W_1 =empty crucible, W_2 = sample +crucible and W_3 =sample+ crucible after ashing

(c) Fat Determination: 3 g of the sample was added in a weighed filter paper, tightened very well with white tread and transferred into a thimble inside a soxhlet apparatus. A weighed (W_1) 500 ml round bottom flask was filled with n- hexane up to about 2/3 of the flask. The soxhlet extractor was then fitted up with a reflux condenser and the heat source of the extractor was adjusted so that the solvent boils gently and left to siphon for 8 hrs. The filter paper and defatted sample was removed and dried in an oven at 50 ^{0}C for about 30 minutes. The round bottom flask with the extract was removed, placed on the laboratory table to allow the solvent evaporate completely and weighed (W_2). (A.O.A.C, 1990).

$$\%\text{Fat content} = \frac{\text{W2} - \text{W1}}{\text{Weight of sample}} X100$$

(d) Crude Protein Determination: Crude protein determination was carried out, using the micro kjeldahl method. About 1.0 g of the sample was weighed and transferred into the micro-kjeldahl flask. One tablet of catalyst and 10ml of concentrated H_2SO_4 were added to the sample inside the flask. The flask with its content was heated on a heating mantle inside a fume cupboard for 3 hrs until the black solution turned colourless. The clear solution was diluted with distilled water and made up to 100 ml. This was followed by distillation. 10ml of resulting solution from the digest was measured and transferred into a distillation apparatus. Then, 25 ml of 40% NaOH was added to the digested sample solution in order to make it alkaline. The cloudy nature of the sample solution after the addition of the 40%NaOH was in excess. 25 ml of 2 % boric acid was pipetted into a receiving conical flask; to which 2 drops of mixed indicator was added to produce a pink colour solution. The distillation was carried out with all the joints tightened, making sure the end of delivery tube dipping below the boric acid solution. As the distillation proceeds, the pink colour solution of the receiver turned light green, indicating the presence of NH_3. Distillation was continued until the distillate was about 50 ml after which the delivery end of the condenser was rinsed with distilled water into the receiving flask. The third step was the titration stage where the received ammonia in the boric acid was titrated against standard 0.01HCl. A colour change of this solution from light green back to pink due to the presence of the mixed indicator that was earlier introduced in the distillation stage indicated the end point. The titre value was noted and recorded. The final step was to estimate the % Nitrogen in the sample and hence the crude protein by multiplying that value by general factor; 6.25

$$\%\ \text{Nitrogen} = X\frac{\text{M x T x 0.014}}{\text{W}} X \frac{\text{V1}}{\text{V2}}\ X\ 100$$

Where M = Molarity of the acid (HCl), T = Titre value, W = Weight of the sample

V_1 = Volume of the digest, V_2 = Volume of the digest used.

% Crude protein = 6.25 x % N (6.25 is the factor). (A.O.A.C, 1990).

(e) Crude fibre determination: 200 ml of freshly prepared 1.25% H_2SO_4 was added to 5 g of

sample, which had been defatted by extraction with n-hexane, and brought to boiling quickly. Boiling was continued for 30 mins after which the mixture was filtered. The residue was washed free of acid with plenty of warm water. The residue was then transferred quantitatively into a digestion flask; 200 ml of 1.25 % NaOH was added and boiled for 30 mins. The mixture was then filtered and the residue washed free of alkali with warm water. The residue was then washed thrice with methylated spirit, thrice with petroleum ether and was allowed to properly drain. The residue was then transferred to a dried, weighed silica dish and dried to a constant weight at 105 ºC. The organic matter of the residue was burnt off by igniting for 30 mins in a muffle furnace at 600 ºC. The loss in weight on ignition was reported as crude fibre.

(f) Determination of carbohydrate

The total carbohydrate present in a sample is determined by difference i.e. % carbohydrate= 100% - (% moisture + % ash + % crude fibre + % crude protein + % crude fat). (A.O.A.C, 1990).

Physico-Chemical Analysis of Extracted Oil from *Thevetia Peruviana* Seed

(a) Determination of saponification value: 2 g of oil sample extracted from the seed was weighed into a conical flask and to this was added 25 ml of alcoholic potassium hydroxide solution. The flask was heated on a boiling water bath for 30 minutes with occasional shaking. 1ml of phenolphthalein indicator was added to the solution and titrated while hot with 0.5 M hydrochloric acid (aml). A blank titration was carried out (bml).

$$\text{Saponification value} = \frac{(a-b)\text{ ml x M x 56 V}}{\text{Weight of sample}}$$

Where: a = titre value, b= blank titre value, M = Molarity of acid used (A.O.A.C 1990).

(b) Determination of peroxide value: 1 g of the oil sample was weighed into a dry boiling tube, 1 g of powdered KI, 20 ml of mixed glacial acetic acid and 30 ml of chloroform were added into the tube and boiled for 60 seconds. The content was poured into the titration flask containing 20 ml of 5 % KI solution and 25 ml of distilled water. The contents were therefore titrated with 0.002 M sodium thiosulphate using starch indicator. (A.O.A.C,1990).

$$\text{Peroxide value} = \frac{(V2-V1)\text{ ml x M x 1000}}{\text{Weight of sample}}$$

Where: V_1 = Volume of the blank,V_2 = Titre value, M = Molarity of sodium thiosulphate

(c) Determination of acid value: 5 g of oil sample was weighed into conical flask. 25 ml of 95 % (v/v) alcohol and 1ml of phenolphthalein indicator were added. The solution was titrated with 0.1M potassium hydroxide (A.0.A.C, 1990).

$$\text{Acid value} = \frac{\text{V x M x 56}}{\text{Weight of sample}}$$

Where:V= Titre value, 56 = Molar mass of KOH, M = Molarity of potassium hydroxide used.

(d) Determination of Free Fatty Acid (FFA).

The acid value determined was used for the determination of free fatty acid (A.O.A.C, 1990).

Free fatty acid (FFA) x 2 = Acid value.

(e) Determination of Iodine value: About 0.2 g of oil sample was weighed on small squared of aluminium foil dropped into a dry 250 ml conical flask, 10ml of carbon tetrachloride (CCl_4) was added with measuring cylinder and the flask was shaken to dissolve the oil. About 20 ml of Wijs solution was pipetted into the flask in a fume cupboard and stoppered with moistened cotton wool dipped in potassium iodide (KI) solution. The flask was allowed to stand for 30 minutes in the dark. A blank was prepared with 10 ml of 5 % (v/v) potassium iodide and 100 ml distilled water were added.

The iodine liberated was back titrated with 0.1M sodium thiosulphate using starch solution as indicator. (A.O.A.C, 1990).

$$\text{Iodine value} = \frac{12.69\text{ x M (V2}-\text{V1)}}{\text{Weight of sample}}$$

Where: 12.69 = molecular weight of iodine, M = Molarity of sodium thiosulphate

V_1 = Blank titre value, V_2 = Titre value of the sample

(f) Determination of Unsaponifiable Matter: 1 ml of 3M KOH solution was added to the titrated (neutralized) liquid in the sample flask from the earlier saponification value analysis in order to make it alkaline again. This alkalised solution was then transferred to a separating funnel and washed once with distilled water. It was then extracted 3 times with 50 ml of petroleum ether for each extraction. The combined ether extracts were washed with 100 ml distilled water and filtered. The residue was then oven-dried to a constant weight at 80 ºC for about 3 hrs.

$$\%\text{ Unsaponifiable matter} = \frac{\text{Wf x 100}}{\text{Wi}}$$

Where: W_f = weight of washed, oven- dried extract,

W_i = weight of oil taken for the determination of saponification value

(g) Determination of Relative Density (specific Gravity): This is the ratio of the weight of the oil sample in grams to that of equal volume of water. A density bottle with its stopper was weighed (w_1), filled with the oil, covered with its stopper and re-weighed (w_2). The density bottle was washed, drained, filled with water and weighed (w_3).

$$\text{Relative density} = \frac{w2-w1}{W3-W1}$$

Where: w_1 = weight of density bottle with stopper , w_2 = weight of density bottle + oil,
w_3 = weight of density bottle + water

(h) Determination of Viscosity: The oil of the sample was filled into the upper glass bulb of a jackets viscometer, whose temperature was maintained at 40 ºC, to the upper calibration mark with the aid of syringe and needle. The device stopered with a rubber bung at the end near the lower bulb to prevent the flow until the upper bulb was filled to the mark. After noting the temperature, the rubber bung was removed and a stopwatch was started simultaneously. The time, in seconds, required for the volume of liquid between the upper and lower calibration marks to drain from the upper bulb into the lower bulb was measured. The time elapsed was used in conjunction with the formula (as shown below) supplied by the manufacturer of the viscometer bulb to determine the viscosity in centipoises.

$$\text{Viscosity} = \frac{\text{flow time (mins) x oil relative density x 1.002}}{17.186}$$

(i) Determination of refractive Index: The refractive index was determined with a refractometer. The prism of the refractometer was wiped clean with a tissue paper and moistened with acetone. A drop of the oil sample was placed on the prism surface and clamped. Viewing through the telescope, the control knob was adjusted so that the path of view coincided with the dark part as indicated in the cross wire. The refractive index was read directly from the calibrated scale as observed through the telescope and recorded.

Mineral content determination: The ashed content of the sample was dissolved in 20 ml 10%HCl solution, heated and filtered. The filtrate was then made up to 50 ml with distilled water. The solution obtained was used to determine the mineral content. Sodium and potassium contents were determined using JENWAY Flame Photometer, the vanadomolybdate method was used for phosphorus determination while the other minerals were determined using Atomic Absorption Spectrophotometer (AAS).

Results and Discussion

Table 1: Proximate composition for both raw and boiled thevetia peruviana seed.

Parameters	Raw (%)	Boiled (%)
Moisture	2.00± 0.00	2.89± 0.19
Ash	3.33± 0.00	2.96± 0.01
Crude Protein	30.10± 0.00	29.6± 0.00
Crude fibre	4.79± 0.01	5.21± 0.46
Crude Fat	58.30± 0.00	59.20± 0.00
Carbohydrate	1.80± 0.00	0.30±0.00
Energy KJ/g	2524.50	2521.10

Results are the means of triplicate determinations ± standard deviation.

Table 1 shows the values of proximate composition (%) of the samples for raw and boiled seed of Thevetia peruviana. The moisture content for raw and boiled seeds are 2.00±0.00 and 2.89±0.19 respectively. The moisture content of boiled sample is significantly higher than that of the raw sample indicating that the seed can be stored without growing mould or any other organisms for many days. These results are lower than that of 4.66± 0.28 reported for *Hura crepitans* seed by Jokotagba and Amoo (2012). The ash content for raw and boiled seeds are 3.33±0.58 and 2.96±0.01 respectively. The ash content for boiled sample is lower than that of the raw, which may be due to leaching of nutrients in the boiling water or chemical interaction during boiling. This indicates that the inorganic residue of the seed is very low compared to the organic matter which had been burnt off before evaluating the ash content. These results are lower than 3.55± 0.10% reported for *Hura crepitans* seed by Muhammed *et al,* (2013). It was also discovered that the protein content (30.10±0.00) of the raw seed was significantly higher than that of the boiled seed (29.6±0.00), which could be as a result of denaturation of the protein by heat during boiling process. These values are close to the protein values reported for some other melons like three varieties of *Lageneria siceraria* with protein contents (27.71, 32.70 and 34.64) Ogundele and Oshodi (2010). The higher crude fibre in boiled sample may be due to the dissolution effect of boiling on the sample. The crude fibre is an index for digestivity of food sample. These results are higher than 1.14 % reported for *Trichosanthes cucumerinia* seed by Adeniyi and Amoo (2013). The fat content of the seeds showed that the oil seed contained high level of fat in boiled (59.20±0.00) than in raw (58.30±0.00). The results obtained are higher than 51.33 % reported for *Trichosanthes cucumerina*

seeds by Adeniyi and Amoo (2013); it is also quite higher than 43.5 % and 47.7 % reported by Amoo and Owoeye (2002) for snake gourd seeds. The carbohydrate by difference are given as raw (1.80±0.00) and boiled (0.30±0.00 %). These values are very low compared to the high values (3.60±0.09) for six varieties of dehulled African yam bean flour reported by Aletor *et al*, (2007).

Table 2: Physicochemical parameter of the extracted oil for both raw and boiled thevetia peruviana seed oil

Parameters	Raw (%)	Boiled (%)
Acid Value (mg/g)	1.71± 0.00	1.41± 0.00
Peroxide Value (mg/g)	3.85± 0.00	11.51± 0.00
Iodine Value mgKOH/g)	83.89± 1.22	76.20± 2.81
Saponification Value (mg/g)	224.26± 0.00	193.78± 0.00
Free fatty acid (mg/g)	0.86± 0.00	0.71± 0.00
Unsaponification Value (mg/g)	2.04± 0.00	1.59±0.00
Relative density	0.89±0.00	0.88±0.00
Specific gravity	0.91±0.00	0.93± 0.00
Refractive index (32ºC)	1.46± 0.00	1.47± 0.00
Viscosity (pal/sec)	28.21± 0.00	30.59± 0.00

Results are the means of triplicate determinations ± standard deviation.

Table 2 presents the physicochemical properties of the oil extracted from the sample. The refractive index values for raw (1.46± 0.00) and boiled (1.47± 0.00) are very close. These compare with 1.449 reported for groundnut oil by Atasie *et al*, (2009) which shows that the oil may contain double bonds in their fatty acid composition. Raw seed has low specific gravity (0.91) than the boiled seed (0.93). The results are very close to (0.929 to 0.932) as reported by Ibiyemi *et al*, (2002) for purple and yellow flowered varieties of the plant. The acid values for both boiled and raw samples respectively are (1.40) and (1.71). These values are in conformity with Esuoso and Odetokun (1995) who reported that the acid value of oil suitable for edible purposes should not exceed 4.0mgKOH/g. The iodine value unit (83.89±1.22) of raw sample is higher than the boiled (76.21±2.81). These may be due to loss of unsaturation in the fatty acid of the oils by heat of boiling. The results are higher than (12.34±1.02) value reported for *Trichosanthes cucumerina* seeds by Adeniyi and Amoo (2013). Peroxide value unit (3.85±0.00) for raw is lower than that of boiled (11.51±0.00). Oils with peroxide values ranging from 20.00 to 40.00 mg/g are considered rancid (Cock and Rede, 1986). The peroxide values obtained are below this range. The peroxide value is usually used as an indicator of deterioration of fats or oil (Asiedu, 1989).

The saponification value unit (244.26±0.00 mg/g) of the raw oil is higher than that of the boiled (193.76±0.00), High saponification value make the oil useful in the production of liquid soaps and shampoos (Ibiyemi *et al*, 2002) these implies that saponification of the oil seed of thevetia peruviana will be useful in industrial applications.

Table 3: Mineral composition of both raw and boiled thevetia peruviana seed.

Parameters	Raw (%)	Boiled (%)
Calcium (mg/l)	0.32	0.29
Iron (mg/l)	0.63	0.00
Zinc (mg/l)	0.26	0.26
Copper (mg/l)	0.06	0.00
Manganese (mg/l)	0.17	0.15
Magnesium (mg/l)	2.00	2.70
Sodium (mg/l)	3.50	3.10
Potassium (mg/l)	22.80	17.60
Phosphorus (mg/l)	11088.09	12657.06

Results are the means of triplicate determinations.

Table 3 shows the mineral content (mg/l) in the oil of *Thevetia peruviana* seed oil. The results showed that the seed contained both macro and micro minerals. Phosphorus is the most abundant with 11,088.09 in the raw sample. The implication of this is that Phosphorus in the form of adenosine (ADP) is important for production of energy in the cells (Sharma, 2006). Next in abundance to phosphorus is

potassium, with a raw value 22.80 and boiled value of 17.60. Sodium ranks next to potassium in the sample with a raw value of 3.50 and boiled value of 3.10. Evidence has showed that the boiled sample contain the lowest sodium concentration. The result also indicates that magnesium is the next to sodium in the sample with boiled sample of 2.70 which exceeded that of the raw sample value at 2.00. Magnesium is a mineral that is essential to a variety of cellular metabolic reactions and sometimes has the ability to replace calcium in the body. (Encyclopaedia Britannica, 2008). Processing of the sample by boiling causes an appreciable increase in magnesium content. The result further showed that the raw mineral composition value of Iron content is 0.63 against the boiled value which was 0.60. This implies that processing of the sample by boiling does not cause any appreciable increase in the content. The concentration of zinc for both samples is the same with (0.26). Sharma (2006) has reported that zinc is a crucial nutrient for immune function, healing nervous system, brain function, blood sugar, balanced reproduction and optimal health. Manganese content of the raw sample is 0.17 while that of the boiled sample is 0.15. The manganese in the raw is higher than the manganese in boiled sample. The raw sample only contains copper with 0.06 while the boiled sample did not detect the concentration of copper. Other minerals such as cadmium, lead and chromium were not detected while the rest minerals were present in trace amounts. The values for sodium, calcium and potassium are lower than those obtained for *Hura crepitans* seeds by Muhammed *et al*, (2013). The magnesium and iron are lower than those obtained for *Hura crepitans* seeds as reported by Muhammed *et al*, (2013). Thus, the seed is a good source of phosphorus and potassium.

Conclusion

This research work has given insight into proximate composition, mineral content and physicochemical parameters of the seed of *thevetia peruviana*. This shows that thevetia peruviana seed contained both macro and micro mineral elements which are essential for normal functioning of the body. The result had shown that boiled thevetia peruviana seed has little reduction in ash, protein, carbohydrate and increase in fat content. This was due to the effect of boiling on the seeds which is normal for industrial application and in curing of disesases. In conclusion, the high protein and fat contents show that the samples can be used in food and feed formulation while the saponification values, iodine values and the presence of some valuable minerals indicate that they can be used for other industrial purpose.

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