EVALUATION OF FATTY ACID, AMINO ACID AND PHYTOCHEMICAL COMPOSITES OF RAW AND BOILED MILK BUSH SEED (THEVETIA PERUVIANA)

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ABSTRACT

This study assessed the raw and boiled seeds of *Thevetia peruviana* for amino acid contents, fatty acids composition and phytochemical properties. The parameters of interest were determined using standard methods. The study revealed the main saturated and unsaturated fatty acids in raw and boiled *Thevetia peruviana* seed. The unsaturated fatty acids are palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3) and erucic acid (C22:1). Oleic acid is the most abundant unsaturated fatty acid followed by linoleic acid with values as follows, (54.75 % and 21.48 %) while for boiled (52.45 % and 18.95 %) respectively in each case. Myristic acid, Palmitic acid, Stearic acid and Behenic acid are the saturated fatty acid. Also, the value of oleic acid (C18:1) reduced (52.45 %) due to the effect of boiling, while the saturated fatty acid of palmitic acid (C16:0) increased (19.06 %) due to the effect of boiling. The study further revealed that all the essential amino acids required in human and animal diet were present in both raw and boiled seed of *Thevetia peruviana.* Glutamate acid has the most abundant in both raw (18.45 %) and boiled (19.99 %) sample respectively while the lowest of all in raw is Cysteine (1.18 %) and in the boiled Histidine (1.25 %) which is as a result of boiling. The Phytochemical screening on the oil revealed the presence of flavonoids, saponins, steroids, cardiac glycosides, terpenoids, and alkaloids. However, boiling has little reduction on the characteristics of boiled *Thevetia peruviana* seed.

Keywords: Fatty acid composition, phytochemical properties, milk bush seed and amino acids

INTRODUCTION

During the twentieth century when natural environment was uncovered, man made great discoveries that prompted the use of considerable number of natural resources (Zibbu and Bata, 2011). *Thevetia peruviana* was one of the earliest plant that was discovered and contained a milky sap with a compound called thevetin that is used as a heart stimulant but its natural form is extremely poisonous as are all parts of plants, especially the seeds (Gupta et al., 2011; Bandara et al., 2010; Kareru et al., 2010 and Eddleston et al., 2000). The plants toxins have been tested in experiments for uses in biological pest control. *Thevetia peruviana* seed oil was used to make paint with antifungal, antibacterial and anti-termite properties (Kareru et al., 2010).

This plant has been known for its diverse range of therapeutic importance in ethno medicine, Ayurvedic, Unani and in the recent system of medicine i.e. allopathy. These all remedial values to cure massive diseases are due to the presence of a diverse range of chemical constituents. Amino acids are biologically important organic compounds composed of amine ($-NH_2$) and carboxylic acid (-COOH) functional groups. Fatty acid is a carboxylic acid with a long aliphatic chain (tail), which is either saturated or unsaturated. Most naturally occurring

fatty acids have a chain of an even number of carbon atoms, from 4 to 28 and are usually derived from triglycerides or phospholipids.
A lot of studies have been carried out on *Thevetia peruviana* plant. Despite extensive literature research which revealed that in its natural form all parts of the plants is extremely poisonous, especially the seed (Ibiyemi et al., 2002; Hammed et al., 2011).

Akintelu and Amoo, (2016) presented the physicochemical properties of the oil extracted in their work and found that the sample contained high content of oil that can be used in industrial application. Ibiyemi et al, (2002) also affirmed that the high saponification value of the sample make the oil useful in the production of liquid soaps and shampoos.

However, no intensive information was found on Fatty acid composition, amino acid composition and on phytochemical properties of boiled thevetia peruviana seed which need to be investigated to provide more information about the usefulness and the effect of boiling on the amino acid contents, fatty acid profiles and phytochemical properties of milk bush seed.

MATERIALS AND METHODS

Chemical Reagents

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

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 (covered plate) and stored in the refrigerator at 4 ^{o}C prior to laboratory analysis.

Phytochemical Screening Analysis (Qualitative)

(a) **Alkaloid Determination:** 0.5 g of the sample was taken and 5 ml of 1 % aqueous HCl was added and placed on a steam water bath; 1ml of the filtrate was treated with a few drops of Dragendorf reagent, blue black turbidity was taken as preliminary evidence for the presence of alkaloid (Trease and Evans 1989; A.O.A.C, 2005).

(b) **Saponin Determination**: 0.5 g of sample was shaken with distilled water in a test tube frothing which persist on warming was taken as preliminary evidence for the presence of saponin (Trease and Evans 1989; A.O.A.C, 2005).

(c) **Tannin Determination**: 0.5 g of the sample was stirred with 100 ml of distilled water in a beaker, filtered and ferric chloride reagent was added to the filtrate. A blue black green or blue green precipitate was taken as evidence for presence of tannin (Trease and Evans 1989; A.O.A.C, 2005).

(d) **Phlobatannin Determination**: 0.5 g of an aqueous extract of the sample was boiled with 1 % aqueous HCl and deposition of red precipitate was taken as evidence for the presence of phlobatannin (Trease and Evans 1989; A.O.A.C, 2005).

(e) **Anthraquinone Determination**: Borntrager's test was used for the detection of anthraquinone. 0.5 g of the extract was shaken with 10 ml of benzene, filtered and 5ml of 10 % ammonia solution was added to the filtrate. The mixture was shaken and the presences of pink red or violet colour in the ammonia layer indicate the presence of free anthraquinone (Trease and Evans 1989; A.O.A.C, 2005).

(f) **Flavonoid Determination**: 0.5 g of the sample was stirred with 20 ml of dilute ammonia solution. The disappearance of the yellow colour after the addition of 1 ml Conc. H_2SO_4 indicates the presence of flavonoid (Trease and Evans 1989; A.O.A.C, 2005).

(g) **Steroid Determination**: 20 ml of acetic anhydride was added to 0.5 g of the sample and filtered and 2 ml of Conc. H_2SO_4 was added to the filtrate. There was a colour change from violet to blue or green which indicates the presence of steroid (Trease and Evans 1989; A.O.A.C, 2005).

(h) **Terpenoid Determination**: 0.5 g of the sample was mixed with 20 ml of chloroform and filtered and 3 ml of Conc. H_2SO_4 was added to the filtrate to form a layer. A reddish brown colour at the interface was observed which indicate the presence of terpenoid (Trease and Evans 1989; A.O.A.C, 2005).

(i) **Cardiac Glycosides**: Keller- killiani's test- 0.5 g of the extract was dissolve in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. 1 ml of Conc. H_2SO_4 was added and a brown colour was obtained at the interface indicating the presence of a deoxy sugar. Characteristic of Cardenolides: a violet ring may appear below the brown ring while in the acetic acid layer; a green ring may form just above the brown ring and gradually spread throughout this layer (Trease and Evans 1989; A.O.A.C, 2005).

Phytochemical Analysis (Quantitative)

(a) **Determination of saponin**: The spectrophotometric method of Brunner (1994) was used for saponin determination. 2.0 g of the finely grinded sample was weighed into a 250 ml beaker and 100 ml of Isobutyl alcohol was added. Shaker was used to shake the mixture for 5hours to ensure uniform mixing. The mixture was filtered into 100 ml beaker containing 20 ml of 40 % saturated solution of magnesium carbonate ($MgCO_3$). The mixture obtained was again filtered to obtain a clean colourless solution. 1ml of the colourless solution was taken into 50 ml volumetric flask using pipette, 2 ml of 5 % iron (III) chloride ($FeCl_3$) solution was added and made up to the mark with distill water. It was allowed to stand for 30 min for the colour to develop. The absorbance was read against the blank at 380 nm.

(b) **Determination of total flavonoid**: The total flavonoid content of the sample was determined using a colourimeter assay developed by (Bao et al., 2005). 0.2 ml of the sample was added to 0.3 ml of 5 % $NaNO_3$ at zero time. After 5min, 0.6ml of 10% $AlCl_3$ was added and after 6 min, 2 ml of 1M NaOH was added to the mixture followed by the addition of 2.1 ml of distilled water. Absorbance was read at 510 nm against the reagent blank.

(c) **Determination of Terpenoid**: Procedure described by Sofowora (2006) was used. 0.5 g of finely grounded sample was weighed into a 50 ml conical flask and 20 ml of chloroform: methanol (2:1) was added and the mixture was shaken thoroughly and allowed to stand for 15min at room temp. The suspension was centrifuge at 3000 rpm and the supernatant was discarded. The precipitate was re-washed with 20 ml chloroform: methanol 2:1, then re-centrifuged again and the precipitate was dissolved in 40 ml of 10 % SDS solution. 1ml of 0.01M ferric chloride was added and allowed to stand for 30min before taken the absorbance at 510nm.

(d) **Determination of Alkaloid**: 5.0 g of the sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added, allowed to stand for 4min and filtered. The filtrate was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The precipitate was collected, washed with dilute ammonium hydroxide and then filtered. The residue (alkaloid) was dried and weighed (Harbone, 1973).

$$\%\ Alkaloid = \frac{W3-W2}{W1} X100$$

Where:

w_1= weight of sample, w_2 = weight of filter paper, and w_3 = weight of filter paper after drying

(e) **Determination of Cardiac Glycosides**: The procedure described by Sofowora (2006) was used. 10 ml of the sample was pipetted into a 250 ml conical flask, 50 ml chloroform was added and shaken on vortex mixer for 1hour. The mixture was filtered into 100ml conical flask, 10 ml of pyridine and 2 ml of 29% of sodium nitroprusside were added then shaken thoroughly for 10 min. 3 ml of 20 % NaOH was added to develop a brownish yellow colour. A concentration of glycosides standard (digitoxin) which ranges from 0 – 50 mg/ml were prepared from stock solution and the absorbance was read at 510 nm.

Fatty Acid Composition

The fatty acid composition of raw and boiled *Thevetia peruviana* raw and boiled respectively was determined using their methyl ester on a gas chromatograph (Hewlett packard 6890 powered with HP chemstation Rev. A 09.01 [1206]) with 30m long HP- INNowax (cross- linked PEG) type column, while a flame ionization detector was used as GC detector and Nitrogen was used as the carrier gas. The injection and detection temperatures were 250ºC and 350ºC respectively. The inside diameter of the column was 0.2mm and film thickness was 0.5µm.

Amino Acid Composition

The amino acid composition of raw and boiled *Thevetia peruviana* raw and boiled respectively was determined using the (Hewlett packard 6890 powered with HP chemstation Rev. A 09.01 [1206]) with 30m long HP- INNowax (cross- linked PEG) type column, while a flame ionization detector was used as GC detector and Nitrogen was used as the carrier gas. The injection and detection temperatures were 250ºC and 350ºC respectively. The inside diameter of the column was 0.2mm and film thickness was 0.5µm.

RESULTS AND DISCUSSION

Table 1: Phytochemical screening analysis (Qualitatitive) for both raw and boiled Sample of *Thevetia peruviana.*

Parameter	Raw	Boiled
Alkaloid	+	+
Saponin	++	++
Tannin	-	-
Phlobatannin	-	-
Flavonoid	+	+
Steroid	+	+
Terpenoid	+	+
Cardiac glycoside	+	+
Anthraquinone	-	-

++: High level of presence; +: Moderate level presence; - : Absence

Table 1 showed the Phytochemical screening of *Thevetia peruviana* seeds. The Phytochemical analysis of the extract from the oil revealed the presence of Flavonoids, Cardiac glycosides, Steroids, Saponins, Terpenoids and Alkaloids in both raw and boiled while Tannins, Phlobatannins and Anthraquinones were absent. The results showed that the sample contains phytochemical properties as reported by Daniel *et al*, (2012) for *Azadirachta indica* seed.

Table 2: Phytochemical analysis % (Quantitative) for both raw and boiled *Thevetia peruviana* seed.

Sample	Flavonoid	Terpenoid	Alkaloid	Saponin	Cardiac Glycoside
Raw	0.39	1.34	12.08	39.80	0.00013
Boiled	0.26	5.01	8.46	46.33	0.00090

Results are the means of triplicate determinations.

Table 2 as shown above revealed the quantitative analysis for phytochemical present in raw and boiled. The flavonoid for raw (0.39 %) is higher than boiled (0.26 %), saponin for raw (39.8 %) is lower than boiled (46.33 %), terpenoid for raw (1.34 %) is lower than boiled (5.01 %), alkaloid for raw (12.08 %) is higher than boiled (8.64 %) and cardiac glycosides for raw (0.00013 %) is lower than boiled (0.00090 %). The results show that boiling increases the amount of saponin, terpenoid and cardiac glycoside compared with the raw sample. This substantiate the findings of Elegbede (1998) that processing may cause the loss of some nutrient and as a result, boiling significantly reduce the content of the antinutritional factors thereby improving the nutritional and functional properties of the *Thevetia peruvinana* seed.

Table 3: Fatty acid composition (%) for raw and boiled *Thevetia peruviana* seed.

Parameters	**Raw**	**Boiled**
Myristic Acid Methyl Ester (C14:0	0.144703	0.191223
Palmitic Acid Methyl Ester (C16:0)	15.223434	19.056761
Palmitoleic Acid Methyl Ester (C16:1)	0.504481	0.248288
Stearic Acid Methyl Ester (C18:0)	5.382776	7.080253
Oleic Acid Methyl Ester (C18:1)	54.750903	52.451223
Linoleic Acid Methyl Ester (C18:2)	21.481933	18.946108
Linolenic Acid Methyl Ester (C18:3)	0.608621	0.379214
Arachidic Acid Methyl Ester (C20:0)	0.265182	0.506958
Behenic Acid Methyl Ester (C22:0)	0.236087	0.316626
Erucic Acid Methyl Ester (C22:1)	0.657561	0.229352

Comparison of the essential amino-acids content of the samples.

Table 3 shows the fatty acid composition for both raw and boiled *Thevetia peruviana.* The main saturated and unsaturated fatty acids in raw were found to be 21.49 % and 78.00 % and boiled are 27.15 % and 72.85 % respectively. The unsaturated fatty acids are Palmitoleic acid (C16:1), Oleic acid (C18:1), Linoleic acid (C18:2), Linolenic acid (C18:3) and Erucic acid (C22:1).

Oleic acid was shown to produce the most abundant unsaturated fatty acid (54.75 % and 21.48 %) followed by Linoleic acid (52.45 % and 18.95 %) for both raw and raw seed respectively in each case. Myristic acid, Palmitic acid, Stearic acid and Behenic acid are the saturated fatty acid.

It was found that there was reduction in the value of Oleic acid (C18:1) (52.45 %) due to the effect of boiling while the Palmitic acid (C16:0) (19.06 %) which is a saturated fatty acid increased in boiled seed. This means that the degree of unsaturation was reduced as a result of boiling. This also support the findings of Dobargances *et al*, (1993) which affirmed that the milk bush seed oil is rich in Oleic acid (59 %) when compared with other popular frying oils. Such as Sunflower, Olive and Soya bean oils. The difference in the amounts of fatty acids may be due to the nature of oil and possibly of deterioration.

Table 4: Amino acid composition (%) for raw and boiled *Thevetia peruviana* seed.

Parameters	Raw	Boiled
1 Glycine	6.13333	6.39267
1 Alanine	5.85741	6.04225
1 Serine	5.45775	5.61280
1 Proline	4.04633	3.72525
1 Valine	4.56480	4.48310
1 Threonine	5.11364	4.60407
1 Isoleucine	4.42103	4.06809
1 Leucine	6.63838	7.39964
1 Aspartate	8.59335	8.42266
1 Lysine	3.18905	3.88686
1 Methionine	8.46445	5.27017
1 Glutamate	18.41502	19.99145
1 Phenylalanine	6.19045	6.05436
1 Histidine	2.63461	1.24722
1 Arginine	7.24462	6.38042
1 Tryosine	3.23684	3.00388
1 Cystine	1.18298	5.88751

Table 4 shows the amino acid composition on raw and boiled seed. It reveals that all the essential amino acids required in human and animal diet were present in both raw and boiled seed of *Thevetia peruviana*. Glutamate acid has the most abundance in both raw and boiled sample (18.45 %) (19.99 %) respectively while the lowest of all in raw and boiled are Cysteine (1.18 %) and Histidine (1.25 %) respectively.

These findings commensurate with WHO/FAO (1985) standard of daily requirements for essential amino acid contents which states that Isoleucine (4.2), Lysine (4.2), Phenylalanine (2.8) and Tyrosine (2.8) in the untreated and treated cake were found to be adequate. Thus the quantity of Cysteine (2.0), Threonine (2.8), and Valine (4.2) in all the samples corresponds favourably to the WHO/FAO standard. However, there were differences in the amount of some of the amino acid parameters which may be due to the effect of boiling.

CONCLUSION

This work has given insight into fatty acid and amino acid composition of the seed of *Thevetia peruviana*. It shows that these seeds contain high fat content and energy that serve as source of nutrients for the body. The high protein content and presence of essential amino acid can help to enhance proper growth in children and to meet daily amino acid requirements for adults at calculated quantities.

The predominant fatty acid in the oil is oleic acid, the high oil level ratio and high saponification values presents in the oil will result to a long shelf-life and will be suitable for industrial applications.

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