Physicochemical and Genetic Diversity Studies of *Vitellaria paradoxa* in Northern Nigeria

H. M. Ibrahim[1*], Z. Mohammed[2], A. A. Warra[3], Y. S. Abdulrahman[4], I. Sabo[1], U.M Jajere, G. Ibrahim[2], A. Victor[3], R. A. Oyi[1]

[1]Department of Pharmacognosy and Drug Development, Gombe State University-Nigeria
[1]Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria-Nigeria
[1]Centre for Entrepreneurial, Federal University Gusau-Nigeria[3]
[4]Nigerian Institute for Palm Oil Research, Date Palm Research Station, Dutse-Nigeria
[5]Department of Virology, University of Ibadan Teaching Hospital, Ibadan-Nigeria
[6]Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria-Nigeria

Submitted 15/01/2022; accepted 14/02/2022,; Published online 28/02/2022
https://doi.org/10.54117/jcbr.v2i1.4
Corresponding address: hadeezai161@gsu.edu.ng

Abstract

Shea tree is an economic plant with factors such as genetic variations and environmental conditions responsible for its variations. The present study was aimed at establishing the physicochemical properties and genetic variation of shea tree from northern Nigeria. The plant materials were harvested from Bosso (A), Ngaski (B) and Yamaltu-Deba (C). The sheanut B had the highest potassium content while sodium and magnesium were higher in C, with no significant difference between the calcium and copper contents of A and C. The percentage yield of sheabutter B (37.20%) was higher than A and C. Iodine values ranged from 22.32 ± 0.12^{a} mg/g to 58.67 ± 0.20^{c} mg/g. Sheabutter B have the highest saponification value at 198.51 ± 0.21^{c} mgKOH/g, with no significant difference in the peroxide values of B (0.72 ± 0.03^{a} meqO_2/g) and C (0.74 ± 0.03^{a} meqO_2/g). Stearic and oleic acid are the major fatty acids identified in the GC-MS profile of the sheabutters. The microsatellite markers used produced 71 % polymorphic and 19 % monomorphic bands with allele numbers ranging from two to four, with polymorphic information content range of 0.3092 to 0.5658 and genetic diversity ranging from 0.3378 to 0.6222. Fifteen shea tree varieties were divided into two clusters with cluster A made up of four subclusters and cluster B consisting of two subclusters. The Nei's genetic distance within the population was between 0.00% and 0.982 %, an indication of diversity within the individual trees and not between the populations across different geographical distance.

Keywords: *Vitellaria paradoxa*, physicochemical, microsatellite markers, Nigeria

Introduction

Molecular markers are presently utilized in the study of medicinal plants for the purpose of biodiversity protection, plant breeding, identification of desired traits in plants, validation of gene diversity and variations as well as authentication of medicinal plants. The employment of these molecular markers in medicinal

plant research is very important in the study of plant genetic variation (Swatantra *et al*., 2010; Gwali *et al*., 2015), as geographical conditions have been reported to affect the physical and chemical properties of plants.

Simple Sequence Repeat (SSR) also known as microsatellites refers to sets of repeated sequences found in eukaryotic genomes, consisting of sequences of repetitions including basic short motifs that are generally between 1 and 6 base-pairs long appearing in non-coding and coding regions (Sorkheh *et al*., 2016; He *et al*., 2020). Microsatellites are advantageous of being highly polymorphic, retaining co-dominant inheritance and existing in high quantity within the genome, with a vast extent of allelic diversity, which eases the evaluation of SSR size variation using polymerase chain reaction (PCR) with pairs of flanking primers and high reproducibility. These markers measures genetic diversity based on estimated gene flow, genetic distance and infer intraspecific genetic relationship. However, the major disadvantage of the marker is the development of microsatellites which involves having extensive knowledge on the DNA to be sequenced, and sometimes can underestimate genetic structure measurements (Vieira *et al*., 2016).

The genus *Vitellaria* is a monotypic genus which prevails naturally in the wild of the dry savannah belt of West African countries including Senegal, Burkina Faso, Cote d'Ivoire, Mali, Ghana, Togo, Benin, Nigeria, Cameroon, and Niger republic (El-Mahmood *et al*., 2008; Israel, 2015). In Nigeria, shea tree thrives in the wild mainly between the guinea and sudan sahel savanna zones of the country including states like Niger, Nasarawa, Kwara, Benue, Plateau, Kebbi, Sokoto, Zamfara, Katsina, Kogi, Taraba, Borno, Adamawa, Edo and Oyo states (Ololade and Ibrahim, 2014).

The geographical source and distribution range, age, climatic conditions, genetic variations and soil structures are some of the factors reported to have influence on the variation of shea tree and shea butter (Abdulai *et al*., 2015; Honfo *et al*., 2014). Intra-specific variations are reported to be high in populations of shea tree growing in the West African countries (Djekota *et al*., 2014; Kelly and Senou, 2017).

The molecular studies of shea tree using random amplified polymorphic DNA (RAPD) markers produced high genetic variation of 82% amongst individual within a population which indicates a correlation to the geographical distance covered by the trees within the study areas. The genetic data on shea trees population in Ghana exhibited high diversity producing a mean expected heterozygosity (He) of 0.667 with the species reported to be spatially structured across the ecozones following climatic gradient (Bouvet *et al*., 2004; Sanou *et al*. 2005; Abdulai *et al*., 2017). Fontaine *et al*., (2004) reported that the longitude or latitude of the shea tree populations across Senegal to Uganda does not have effect on its genetic diversity. The microsatellite markers used in the study of genetic diversity in the Ugandan ethno-varieties of shea tree populations produced microsatellite locus between 6 and 13, with high variation of 86.28 % occurring within the individual trees at 86.28% and a low variation of 11.25% among the individual trees. Also, based on the cluster analysis, fixation and

in-breeding index as well as the gene flow, the shea trees from the population studied represents a single out crossing population with very low genetic differentiation and high gene flow (Gwali *et al*., 2014).

In Nigeria, morphological markers have been employed to study the variations in shea fruits and nuts, nut length, leaf length and width as well as the leaf petiole which varied across agroecological zones, with significant variations in reported between the tree populations from the Northern Guinea Savanna and Southern Guinea Savanna (Ugese *et al*., 2010; Enabenue *et al*., 2014). The present study aims to evaluate the genetic diversity of shea trees growing in Nigeria at molecular level and identify whether or not it has effects on the physicochemical properties of shea butter.

Materials and Methods

Collection, Identification and Preservation of Plant Materials

The leaves and fruits of *Vitellaria paradoxa* were harvested randomly from twenty (20) trees within the tree populations found in Ngaski (Latitude 10º 24' 26" North; Longitude 4º 43' 4" East), Bosso (Latitude 9º 36' 53" North; Longitude 6º 21' 57" East) and Yamaltu-Deba (Latitude 10º 14' 18" North; Longitude 11º 26' 30" East) local government areas of Kebbi, Niger and Gombe states of Nigeria respectively between the months of August-September, 2018. The plant materials were identified in the field using taxonomical characters and authenticated by a Taxonomist at the Herbarium unit of the Department of Botany, Ahmadu Bello University, Zaria-Nigeria. The young leaves collected were rapidly dried in silica gel contained in a sealable polythene bags before transporting to the Multi-User Laboratory of the University of Ibadan, Ibadan-Nigeria for further analysis. The shea fruits were depulped, nuts were air dried at room temperature and further dried in oven before pulverization for further use.

Elemental analysis

The elemental analysis of the shea nuts were carried at the Department of Biochemistry, Gombe State University, Nigeria. The nuts were washed with water, air dried at room temperature and placed in an oven for 6 hours 80ºC. The dried nuts were pulverized and 2 g of the samples were weighed in beakers. Digestion was carried out on the samples by the addition of 40 ml of Hydrochloric acid and Nitric acid in ratio the 30:10, before heating in fume cupboard for 150 minutes at 70ºC. The contents were kept to cool, and the filtrate was collected by filtering the samples into a volumetric flask with the aid of Whatman N0. 42 filter paper. Deionsied water was added into the volumetric flask to make up to 100 cm^3 of the samples. A blank was also prepared as standard and all the samples including the blank were analysed for calcium (Ca), potassium (K), sodium (Na), Iron (Fe), Manganese (Mn), copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb) and nickel (Ni) using Buck scientific model 210VGP Atomic Absorption Spectrophotometer (Helal Uddin *et al*., 2016).

Extraction of shea butter

The weight of 1.6 kg of shea nuts samples from the study locations were extracted in using hexane (2.5 litres) in a soxhlet apparatus (Konte USA) at 60ºC for 6 hours. The oil obtained were transferred into evaporating dishes and placed over water bath at 70ºC to remove excess

solvent. They were further dried in an oven, cooled in desiccator and transferred to airtight containers for further use (Warra *et al*., 2013).

Percentage yield, sensory characteristics, moisture content, specific gravity, peroxide, iodine and saponification values of Shea Butter

The percentage yield, colours and odours, moisture content, specific gravity, saponification, iodine and peroxide values were determined using standard procedures (AOAC, 2000; Akpan *et al*., 2006; Warra *et al*., 2013; Julius *et al*., 2013),

Fatty acid composition

The Gas-Chromatography/Mass Spectroscopy (GC-MS) analysis for sheabutter samples were carried out at the Multi-User laboratory, Department of Chemistry, Ahmadu Bello University, Zaria using the Shimadzu QP2010 plus series gas chromatography coupled with Shimadzu QP2010 plus mass spectroscopy detector (GC-MS) system. The spectra machine temperature was set between 70°C to 280°C and the carrier gas used was helium. The volume of 2 µL of the sheabutter samples were injected at 250°C with 1.80 mL/min as the column flow. The scan range between 30 to 700 amu at a speed 1478 was used in the ACQ mode scanner spectroscopy (Warra, 2015). The mass spectra were compared with the NIST05 mass spectral library (NIST, 2012).

Molecular Studies

DNA extraction and quantification

The dried leaves (100 mg) were grinded and 1000 µl of Dellaporta buffer was added to each samples. The mixtures were transferred into sterile eppendorf tubes and 40 µl of 20% SDS was added and the mixtures were briefly vortexed before incubation at 65^{o}C for 10 minutes and allowed to cool at room temperature. The mixtures were vortexed and centrifuged at 10,000 g for 10 minutes after addition of 160 µl of 5 M potassium acetate. The supernatant transferred into sterilized eppendorf tubes and kept for 60 minutes at -20^{o}C after the addition of 400 µl of cold iso-propanol. The samples were further centrifuged at 13,000 g for 10 minutes to precipitate the DNA and the supernatant were gently decanted. The DNA isolated was washed with 500 µl of 70 % ethanol by centrifuging at 10,000 g for 10 minutes. The solvent was decanted and the DNA air-dried at room temperature. Pellets were re-suspended in 50 µl of Tris EDTA buffer to preserve and suspend the DNA (Weigel and Glazebrook, 2009).

The evaluation of the quality of extracted DNA was carried out by running the isolated DNA on 0.8 % agarose gel stained with ethidium bromide. The purity and concentration of genomic DNA was also estimated by calculating the ratio of optical densities measured at 260-280 nm with a spectrophotometer (Thermo Scientific Type UV1, England). The DNA was diluted for amplification and polymerase chain reaction (PCR) analysis.

SSR-Primers

Polymorphic six (6) SSR markers were used for genotyping the entire genome of the 15 shea tree genotypes. The

primers were selected based on previously works of Allal *et al.,* (2008) and Gwali *et al.,* (2014) for shea tree as listed in Table 1.

Table 1: Forward and Reverse SSR Primers used for the Molecular Studies

Primer name	Forward primer sequence	Reverse Primer sequence
mCIRVp44	TCTCTTTCATCGTCCTTTG	AACACTTGGTCATCATCTTG
mCIRVp190	TTTACAAGTGACTCCTACAAC	TTCATTTCTTCTTCTCTCTG
mCIRVp134	CTCTTCTCCTCCCCTTCAAC	ACCATAATCCCTCAGCAATC
mCIRVp14	TCTCTTTCATCGTCCTTTG	AACACTTGGTCATCATCTTG
mCIRVp8	AATTCATTGGAGGACAGCA	ACACCAATCGCAACACAG
mCIRVp28	ATTGTTAGTTATGGTTTTGG	TGATTTGCTATTTTGCTTAC

Polymerase Chain Reaction (PCR) Amplification

The optimization of the PCR reaction was at 15 µl including 2 µl of 100 µg DNA template, 7.0 µl Dream Taq PCR master mix (Thermos Scientific Incorporation), 2 µl of each SSR primer, and 4.0 µl nuclease free water. The touch-down PCR protocol was employed consisting of an initial denaturation for 5 minutes 94˚C, followed by 9 cycles of denaturation of for 15 seconds at 94˚C with annealing for 20 seconds at 55˚C and extension for 30 seconds at 72˚C. Further denaturation for 15 seconds at 94˚C, annealing afor 20 seconds at 45˚C and extension for 30 seconds at and then a final extension for 7 minutes at 72˚C was carried out.

Agarose Gel Electrophoresis of the Amplified DNA

The agarose gel at 1.5 % was used to achieve separation of bands produced by each primer. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled for 5 minutes in a microwave and the molten agarose was allowed to cool to 60°C before staining with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify to form wells for 20 minutes. The 1XTAE buffer was poured into the gel tank to barely submerge the gel before addition of 7 µL of each PCR product were loaded into the wells after the 100 bp DNA ladder. The gel was electrophoresed at 120V for 45 minutes with visualization using ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100 bp molecular weight ladder that was ran alongside experimental samples in the gel.

Statistical analysis

Analysis of variance (One-Way ANOVA) was used to compare the variation between the shea butter by generating the data using SPSS software version 17. Means and standard deviation were computed. Duncan multiple range test was used to compare the mean variance with significance level at $p< 0.05$.

Binary data was generated for each primer sets using 1 (presence of positive amplification at a particular band size) and 0 (absence of positive amplification at a

particular band size) the generated binary data was the used to create a data matrix which was analyzed using the Powermarker V2.35 software. Genetic diversity parameters such as major allele frequency, gene diversity and polymorphic information content where then generated using the power marker software. The genetic relationship among treated samples were also estimated by constructing a dendrogram through unweighed pair group method with arithmetic means (UPGMA) using the mega6 software and genetic distance where computed also using the mega6 software. The evolutionary history was inferred by using the Maximum Likelihood method (Tamura and Nei, 1993).

Results

Elemental analysis

The results from the elemental analysis revealed that shea nuts from Bosso (B) had the highest potassium (K) content at value of 1.84 ± 0.02 mg/L with no significant difference between the calcium (Ca) and copper (Cu) contents of shea nuts from Ngaski (A) and Yamaltu-Deba (C) (Table 2). shea nuts C had the highest concentrations of sodium (Na), magnesium (Mg), chromium (Cr) and Iron at 0.86±0.01 mg/L, 5.20 ± 0.05 mg/L, 0.09 ± 0.01 and 1.29 ± 0.06 respectively. There was no significant difference between the zinc (Zn) contents of all the shea nuts with lead (Pb), cadmium (Cd) and cobalt (Co) absent (Table 2).

Percentage yield, sensory characteristics, moisture content, specific gravity, peroxide, iodine and saponification values of Shea Butter

Sheanuts from Bosso (B) produced the highest percentage yield of butter (37.20%), while sheanuts from Yamaltu-Deba (C) produced the least yield of sheabutter (30.70 %) with shea nuts from Ngaski (A) producing 36.00% of the sheabutter (Table 3). The colours of the shea butter were creamy white, with smooth textures and characteristic smell. There was no significant difference in the moisture contents of shea butter A (8.23 ± 0.05) and C (8.41 ± 0.05) which were significantly higher than B at 4.98 ± 0.04. The iodine values for the shea butters was between the ranges of 22.32 ± 0.12 mg/g for sheabutter C to 58.67 ± 0.20 mg/g for shea butter A with significant difference between all the three samples. The saponification values of the shea butter varied significantly across the study areas with shea butter B having the highest value at 198.51 ± 0.21 mgKOH/g, while sheabutter A had saponification value of 161.29 ± 0.69 mgKOH/g and the lowest value was recorded for sheabutter C at 129.03 ± 0.24 mgKOH/g. There was no significant difference between the peroxide values of shea butter from B and C at 0.72 ± 0.03 meqO_2/g and 0.74 ± 0.03 meqO_2/g respectively, but they were significantly lower than the peroxide values of the shea butter from A at 0.93 ± 0.12 meqO_2/g (Table 3).

GC-MS spectra of all the shea butter samples revealed oleic and stearic acid as the major fatty acids with varying abundance. The abundance of oleic acid in shea butter A was 58.33 %, while shea butter B was 26.29 % and C 47.86 %. The abundance of stearic acid in shea butter A was 35.01 %, while B was 34.02 % and C 28.21 % (Tables 4, 5, 6).

Table 2: Elemental Analysis of the Shea Nuts from the Three Study Areas

S/No	Elements (mg/ L)	NA	NB	NC
1	Potassium (K)	1.41 ± 0.02^{b}	1.84 ± 0.02^{c}	1.35 ± 0.01^{a}
2	Calcium (Ca)	0.19 ± 0.01^{a}	0.18 ± 0.01^{a}	0.24 ± 0.01^{b}
3	Sodium (Na)	0.72 ± 0.02^{b}	0.32 ± 0.02^{a}	0.86 ± 0.01^{c}
4	Iron (Fe)	1.09 ± 0.02^{b}	0.68 ± 0.01^{a}	1.29 ± 0.06^{c}
5	Magnesium (Mg)	2.54 ± 0.19^{a}	3.43 ± 0.21^{b}	5.20 ± 0.05^{c}
6	Copper (Cu)	0.002 ± 0.01^{a}	0.002 ± 0.01^{a}	0.009 ± 0.01^{b}
7	Zinc (Zn)	0.05 ± 0.01^{a}	0.03 ± 0.01^{a}	0.04 ± 0.01^{a}
8	Lead (Pb)	0	0	0
9	Chromium (Cr)	0.01 ± 0.01^{a}	0.03 ± 0.01^{b}	0.09 ± 0.01^{c}
10	Cadmium (Cd)	0	0	0
11	Cobalt (Co)	0	0	0
12	Arsenic (As)	0.002 ± 0.003^{a}	0.004 ± 0.02^{b}	0.001 ± 0.003^{a}

Key: NA-Sheanuts from Ngaski, NB-Sheanuts from Bosso, NC-Sheanuts from Yamaltu-Deba

***SEM with same superscript across rows is not significantly different**

Table 3: Physical and Chemical Properties of Sheabutter from the Study Areas

S/no.	Physicochemical parameters	A	B	C
1	Colour	Cream white	Cream white	Cream white
2	Texture	Smooth	Smooth	Smooth
3	Yield (%)	37.20	36.00	30.70
4	Moisture content	8.23 ± 0.05^{b}	84.98 ± 0.04^{a}	8.41 ± 0.05^{b}
5	Specific gravity	0.92	0.90	0.91
6	Saponification values (mgKOH/g)	161.29 ± 0.69^{b}	198.51 ± 0.21^{c}	129.03 ± 0.05^{a}
7	Peroxide values (meqO_2/g)	0.93 ± 0.12^{b}	0.72 ± 0.03^{a}	0.74 ± 0.03^{a}
8	Iodine values (mg/g)	58.67 ± 0.20^{c}	31.29 ± 0.41^{b}	22.32 ± 0.01^{a}

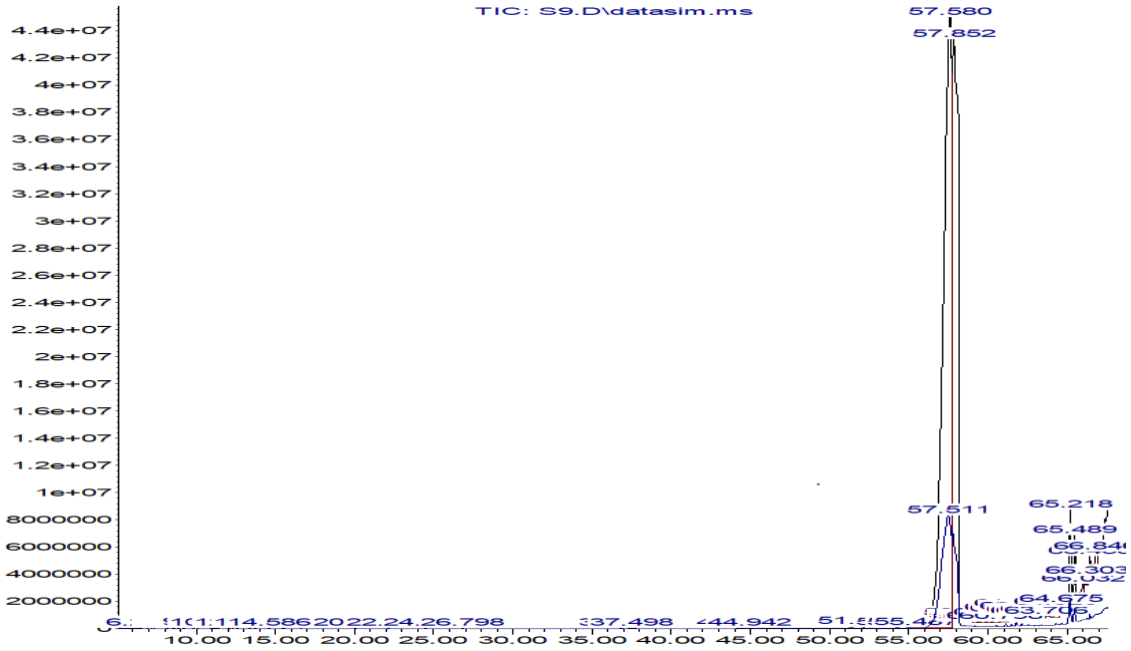


Figure I: Gas Chromatogram of Shea Butter from Ngaski (A)

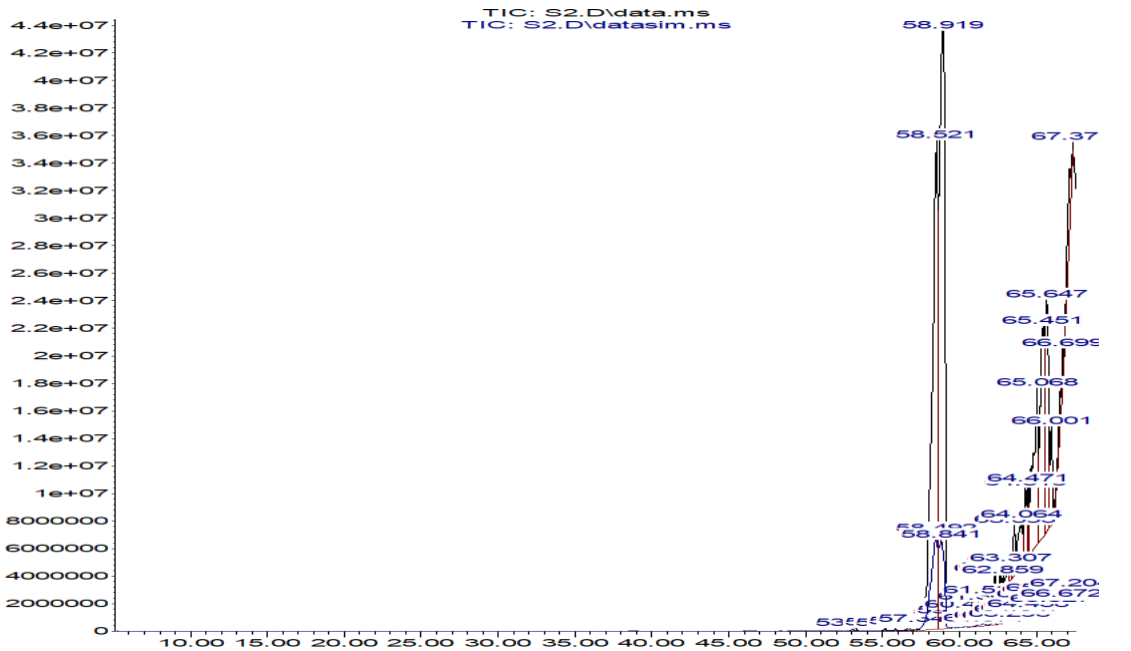


Figure II: Gas Chromatogram of Shea Butter from Bosso (B)

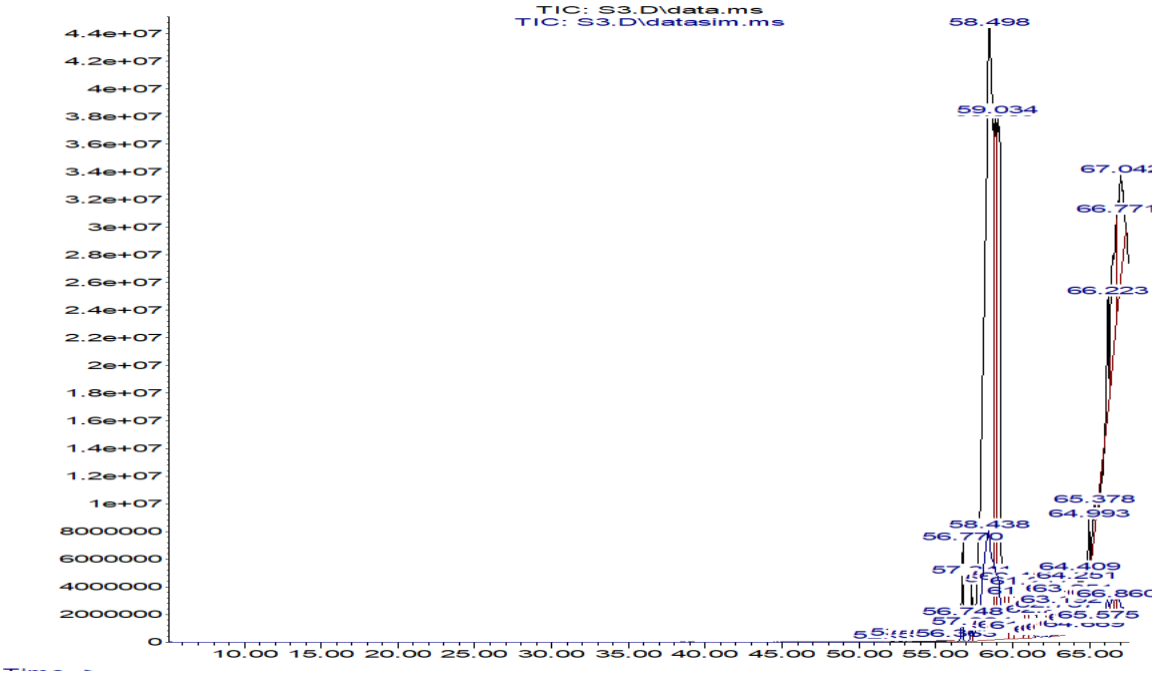


Figure III: Gas Chromatogram of Butter from Yamaltu-Deba (C)

Table 4: Major Compounds Identified in Shea Butter from Ngaski (A)

S/no	Retention Time	Peak Area (%)	IUPAC Name	Molecular Weight (g/mol)	Molecular Formular
1	57.580	58.62	9-octadecenoic acid (Oleic acid)	282	$C_{18}H_{34}O_2$
2	57.852	35.01	Octadecanoic acid (Stearic acid)	284	$C_{18}H_{36}O_2$
3	59.991	0.35	Lauroyl peroxide	398	$C_{24}H_{46}O4$
4	64.326	0.28	Dodecanoic acid-1-methyl ester	214	$C_{13}H_{26}O_2$
5	65.218	2.13	Undecenal	168	$C_{11}H_{20}O$
6	65.489	0.86	Cis-vaccenic acid	282	$C_{18}H_{34}O_2$
7	66.132	0.48	Methoxyacetic acid, tetradecyl ester	286	$C_{17}H_{34}O_3$

Table 5: GC-MS Spectra of the Shea Butter from Bosso (B)

S/no	Retention Time	Peak Area (%)	IUPAC Name	Molecular Weight (g/mol)	Molecular Formular
1	58.511	26.29	9-octadecanoic acid (Oleic acid)	282	$C_{18}H_{34}O_2$
2	58.860	34.02	Octadecanoic acid (Stearic acid)	284	$C_{18}H_{36}O_2$
3	62.271	1.94	Docosanoic acid (Behenic acid)	340	$C_{22}H_{44}O_2$
4	63.229	7.79	2-Heptanol, 4-methyl	130	$C_8H_{18}O$
5	65.647	8.20	3-methyl-5-methoxymethyl-1,2-oxazole	127	$C_6H_9NO_2$
6	67.377	3.28	3-triisobutyloxyhex-4-yne	296	$C_{18}H_{36}OSi$

Table 6: GC-MS Spectra of the Shea Butter from Yamaltu-Deba (C)

S/no	Retention Time	Peak Area (%)	IUPAC Name	Molecular Weight (g/mol)	Molecular Formular
1	56.770	47.86	9-Octadecanoic acid (Oleic acid)	286	$C_{18}H_{34}O_2$
2	58.433	0.22	Cis-vaccenic acid	282	$C_{18}H_{34}O_2$
3	58.869	28.21	Octadecanoic acid (Stearic acid)	284	$C_{18}H_{36}O_2$
4	59.945	2.34	Lauroyl peroxide	398	$C_{24}H_{46}O_4$
5	66.771	10.97	Dodecanoic acid, 1,2,3-propanetriyl ester	639	$C_{39}H_{74}O_6$

Molecular analysis

The allele size ranges for all the six SSR primers used in the present study ranged between 150-350 bp with primer mCIRVp190 producing a least allele size of 150 bp while primers mCIRVp8 and mCIRVp28 produced the highest allele size range of 300-350 bp.

A total number of 19.00 % monomorphic bands and 71.00 % polymorphic bands were produced by the six SSR primers. The SSR primer mCIRVp28 produced the highest monomorphic bands (10) with the least polymorphic bands (5) and percentage polymorphism of 33.3%. Primers mCIRVp44 and mCIRVp8 produced monomorphic bands (1) with 14 polymorphic bands and 93.3 % polymorphism. The primer mCIRVp190 produced 15 polymorphic bands, thereby producing 100.0 % polymorphism. The primers mCIRVp14 and mCIRVp34 produced 3 and 4 monomorphic bands, thereby having 80.0 % and 73.0 % polymorphism. The six SSR primers produced a mean Polymorphic Information Content (PIC) of 0.4337 with mCIRVp8 producing the highest value of 0.5658. This was followed by the PIC for mCIRVp14 with value of 0.5011, while

mCIRVp44 produced the least PIC value of 0.3092. Primers mCIRVp190 and mCIRVp134 have PIC values of 0.4403 and 0.4122 respectively, while mCIRVp28 produced PIC value of 0.3737.

The allele number produced from the six primers ranged from 2 to 4 with an average of 3.1667. Primers mCIRVp44, mCIRVp190 and mCIRVp134 having the allele value of 3. The primers mCIRVp14 and mCIRVp8 produced the highest allele number of 4 while mCIRVp28 produced the least allele number of 2. The major allele frequency ranged from 0.800 to 0.5333 with an average of 0.6222. Primer mCIRVp44 have the highest major allele frequency of 0.8000, while primers mCIRVp8 and mCIRVp28 have the least major allele frequency values of 0.5333. Primers mCIRVp190 and mCIRVp14 have major allele values of 0.6000 and primer mCIRVp134 have a value of 0.6667. The gene diversity produced by the six SSR primers ranged from 0.6222 to 0.3378 with an average of 0.5037. The highest gene diversity value of 0.6222 was produced by mCIRVp8. This was followed by primer mCIRVp14 with value of 0.5600. Primers mCIRVp190 and mCIRVp28 have gene diversity values of 0.5244 and 0.4978 respectively, while mCIRVp44 produced the lowest gene diversity of 0.3378 (Table 7).

The genetic distance based on the Dendogram constructed classified the fifteen (15) shea tree genotypes into two (2) clusters A and B, with cluster A made up of four (4) subclusters and cluster B made up of two (2) subclusters. Subclusters AI and BII are made up of four (4) genotypes namely, A1, A3, C4, C5 and A4, B3, B5, C2 respectively (Figure V). Subclusters AII and BI are made up of one genotype each namely, B1 and A2 respectively. Genotypes A5 and C1 are clustered together in subcluster AIII while genotypes B2, B4 and C3 fall under the subcluster AIV.

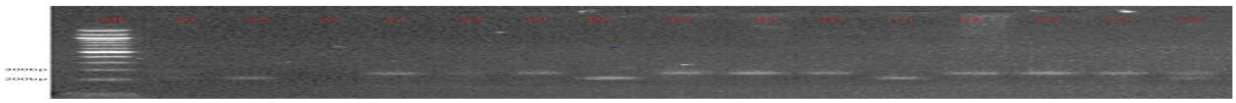
The values of genetic distance based on Nei's genetic distance ranged from 0.928 to 0.00. The highest genetic distance value of 0.928 is between genotypes B1 and C1. This is followed by the genetic distance of 0.854 between genotypes A4 and A2 and genotypes C1 and B4. The genetic distance value of 0.777 is observed between genotypes A2 and C2, B1 and C3 and C1 and C3. The lowest genetic distance value of 0.00 is observed between genotypes B3 and B5 (Table 8).

mCIRVp44

mCIRVp190

mCIRVp134

mCIRVp14



mCIRVp8

mCIRVp28

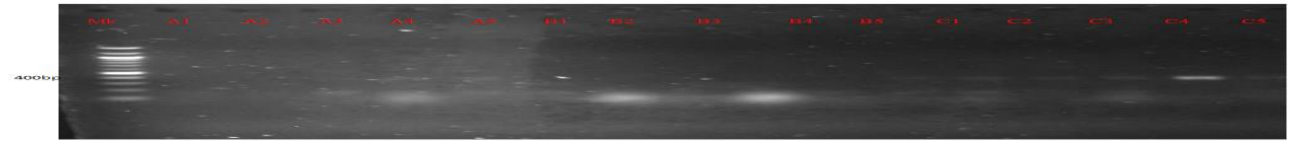


Figure IV: PCR amplification using six SSR markers in the fifteen (15) shea tree individuals

Table 7: Allele Size and Polymorphism obtained from the Six SSR Primers in the Fifteen Shea Tree Genotypes

S/No	Primer	Allele size range (Bp)	Monomorphic band	Polymorphic band	Polymorphism (%)
1	mCIRCp44	250	1.00	14.00	93.30
2	mCIRVp190	150	0.00	15.00	100.00
3	mCIRVp134	250	4.00	11.00	73.00
4	mCIRVp14	200-250	3.00	12.00	80.00
5	mCIRVp8	300-350	1.00	14.00	93.30
6	mCIRVp28	350	10.00	5.00	33.30
	Total		**19.00**	**71.00**	**532.90**
	Average		**3.17**	**11.83**	**88.82**

Table 8: Major Allele Frequency, Allele Number, Genetic Diversity and Polymorphic Information Contents values among the 15 Shea Tree Genotypes Based on Amplified DNA Fragments Data from Six (6) SSR Primer Pairs.

Markers	Major Allele Frequency	Allele Number	Gene Diversity	Polymorphic Information Content (PIC)
mCIRVp44	0.8000	3.0000	0.3378	0.3092
mCIRVp190	0.6000	3.0000	0.5244	0.4403
mCIRVp134	0.6667	3.0000	0.4800	0.4122
mCIRVp14	0.6000	4.0000	0.5600	0.5011
mCIRVp8	0.5333	4.0000	0.6222	0.5658
mCIRVp28	0.5333	2.0000	0.4978	0.3739
Mean	0.6222	3.1667	0.5037	0.4337

Table 5: Nei's Genetic Distance (GD) Between Fifteen (15) Genotypes of the Shea Tree

	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4
A1														
A2	0.512													
A3	0.065	0.512												
A4	0.065	0.854	0.065											
A5	0.512	0.512	0.065	0.065										
B1	0.065	0.694	0.512	0.065	0.065									
B2	0.122	0.04	0.04	0.606	0.606	0.777								
B3	0.065	0.694	0.023	0.023	0.065	0.065	0.122							
B4	0.04	0.606	0.122	0.04	0.04	0.01	0.694	0.04						
B5	0.065	0.694	0.023	0.023	0.065	0.065	0.122	0	0.04					
C1	0.606	0.04	0.122	0.606	0.606	0.928	0.023	0.606	0.854	0.606				
C2	0.122	0.777	0.04	0.01	0.04	0.122	0.512	0.01	0.065	0.01	0.512			
C3	0.065	0.694	0.512	0.023	0.023	0.023	0.777	0.065	0.01	0.065	0.777	0.04		
C4	0.606	0.606	0.04	0.04	0.04	0.606	0.065	0.04	0.512	0.04	0.065	0.023	0.122	
C5	0.122	0.606	0.122	0.04	0.122	0.606	0.065	0.04	0.512	0.04	0.065	0.023	0.122	0.023

A1-A5 (Genotypes from Ngaski), B1-B5 (Genotypes from Bosso), C1-C5 (Genotypes from Yamaltu-Deba)

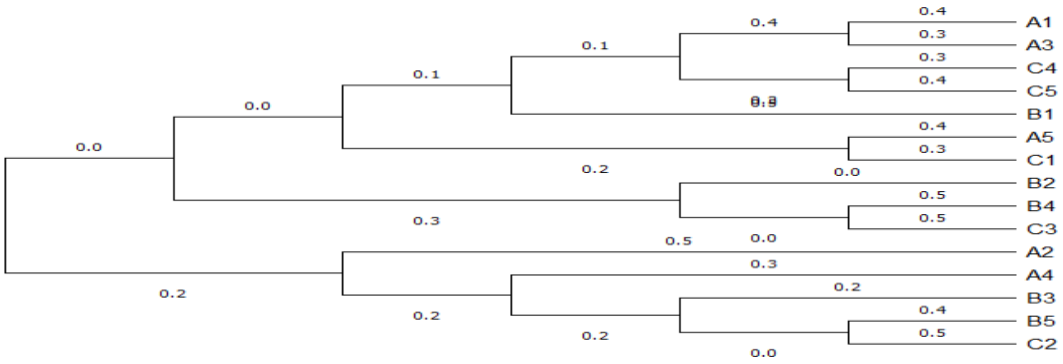


Figure V: Dendogram Constructed for 15 Shea Tree Genotypes Based on Genetic Distance using Six (6) SSR Primers.
A1-A5 (Genotypes from Ngaski), B1-B5 (Genotypes from Bosso), C1-C5 (Genotypes from Yamaltu-Deba)

Discussion

The elemental analysis of the shea nuts conforms with the report that climatic conditions as well as the level of absorption of mineral elements varies in shea tree (Aguzue *et al*., 2013; Quainoo and Augbatey, 2016) as it was observed that shea nuts from C had significantly higher concentrations for Ca, Na, Fe, Mg, Cr and Cu as compared to A and B. The handling techniques may also contribute to the accumulation of heavy metals such Pb, as shea nuts from the present study areas collected directly from the field had no Pb, Cd, and As. This is in contrast to the report of Ibrahim *et al*., (2019) who reported high amount of Pb in all the shea nuts sourced from the locals. All the nuts had values for K, and Ca within the range reported for the sheanuts across different shea tree distribution locations (Fasola and Ayoade, 2010; Abdul-Mumeen *et al*., 2013; Honfo *et al*., 2014) and the level of Na, Mn, Fe, Cu and Zn for all the nuts is lower than the levels reported in literatures (Abdul-Mumeen *et al*., 2013; Honfo *et al*., 2014; Manikuu and Peker, 2017).

The results of percentage yield of the shea butter extracted from all the shea nuts were lower than those reported in literatures (Julius *et al*., 2013; Warra *et al*., 2013; Honfo *et al*., 2014), although the percentage yield of the all the shea butters falls within the desirable range of 30% suitable for industrial application (Julius *et al*., 2013) with the butter C amongst the least yield reported for shea butter at 30.70 %. The colours, textures and odour of the shea butter extracted from the three study areas falls within the colour arrays described for shea butter (Honfo *et al*., 2014). The moisture contents of the shea butters from the present study are higher than the moisture contents for the shea butter reported in Nigeria (Julius *et al*., 2013; Audu and Awulu, 2016) but lower than the sample reported from Ghana at 12.04 ± 1.30 (Quainoo *et al*., 2012).

The iodine values for shea butter A at 58.67 ± 0.20^{c} mg/g indicated it contains higher amount of unsaturated fatty acids when compared with that of B and C at 31.29 ± 0.41^{b} mg/g and 22.32 ± 0.01^{a} mg/g respectively. The saponification values for shea butter B at 198.51 ± 0.21^{c} mg KOH/g

is an indication of its suitability in production of soap when compared to B at 161.29 ± 0.69^{b} mg KOH/g and C at 129.03 ± 0.24^{a} mg KOH/g. The peroxide values for all the shea butter samples in the present study were lower than those reported in the shea butter from Benue, and Kwara of Nigeria (Shahidi, 2005; Julius *et al*., 2013; Animasaun *et al*., 2019).

The percentage of saturated to unsaturated fatty acids across the study areas varied with A and C containing higher ratio of unsaturated fatty acids comprising mainly of oleic acids at 58.62 % and 47.86 % respectively. The amount of saturated fatty acid in A is mainly stearic acid at 35.01% and palmitic acid. While C had the lowest concentration of saturated fatty acids with stearic acid at 28.21%. Another fatty acids found in A and C is Cis-Vaccenic acid at 0.86 % and 0.22 % respectively. The percentage of the oleic acid for A and C conforms to the range of oleic acid at 37.2 % to 60.7 % reported in literature while the percentage of stearic acid in C is lower. (Honfo *et al*., 2014; Victoria and Ajayi, 2015). The presence of stearic and oleic acid in the shea butter is of pharmaceutical importance as both have been reported suitable for use in oral formulations, as binders as well as vehicles for controlling the release of formulation drugs (Hernandez *et al*., 2009; El Kacimi *et al*., 2016).

The allele sizes for all the six SSR microsatellite primers are within the range reported by Allal *et al*., (2008). All the microsatellite loci exhibited high polymorphism of 33.3 % to 100 % with a total number of 71 % polymorphism, which falls within the range (37-85 %/) reported from previous studies, and it is an indication of wide and diverse genetic data within the population studied (Allal *et al*., 2008; Gwali *et al*., 2014).

The numbers of alleles observed within the microsatellite loci are between 2 to 4 with an average of 3.1667, which is lower than the range reported from previous study. All the microsatellite markers used in the present study are highly informative and can be used as a powerful genetic marker in genetic variation studies (Allal *et al*., 2008).

Molecular markers with high Polymorphic Information Content (PIC) values of 0.5 and above are said to be suitable in identification of genetic diversity among plant species. The PIC values from the present study ranges between 0.3092 and 0.5658 which is an indication that primer mCIRVp14 and mCIRVp8 are highly informative and are suitable for genetic variation study of shea tree.

The results from UPGMA dendogram indicates that shea trees from the study areas are classified into three (3) clusters regardless of the geographical locations. This result is an indication of little or no genetic differentiation within the shea tree populations. Also, the appearance of the individual trees from the same location in different cluster is an indication of large gene pool as it was observed that regardless of the phenotypic differences of the shea tree growing in Uganda, the trees are interspersed with each other. The present findings is in support of the report that genetic diversity of shea tree exist within the individual trees and not between the population across different geographical distance (Gwali *et al.*, 2014).

The high genetic diversity from the Nei's genetic distance with the highest value of 0.928 % is in accordance with the report from previous studies on shea tree population across different African countries (Lovett and Haq, 2000; Kelly *et al*., 2008; Gwali *et al*., 2014). The existence of high genetic diversity is commonly attributed to out-crossing species such as *Vitellaria paradoxa* and *Olea europeaea*. These out-crossing species exhibit low variability and high gene flow which is commonly attributed to the roles of animals such as insects, bats, monkeys and human that are mainly responsible for their pollination due to the edible fruits the plants produce (Hall *et al*., 1996; Kelly *et al*., 2008).

Conclusion

There is variation in the physicochemical and genetic makeup of the shea tree population with genetic differences in the individual species of the plant and not among the plant population based on genetic distances.

Acknowledgement

The authors will like to appreciate the efforts of the laboratory staff of the Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria-Nigeria.

Conflict of interest

The authors wish to declare that there is no conflict of interest.

Ethical approval

This article does not contain any studies on animal or human subject.

References

Abdulai A, Akwasi A, Iddrisu A (2015). Effect of Soil Variation on Quality of Shea Butter in Selected Areas of the Northern Region in Ghana. Journal of Agricultural Biotechnology and Sustainable Development 7(5):43-50.

Abdul-Mumeen I, Beauty A, Adam A (2013). Biochemical and Microbiological Analysis of Sheanut Cake: A Waste Product from Shea Butter Processing. Journal of Agricultural Biotechnology and Sustainable Development 5(4): 61-68.

Aguzue OC, Akanji FT, Tafida MA, Kamal MJ (2013). Nutritional and Some Elemental Composition of Shea (*Vitellaria Paradoxa*) Fruit Pulp. Archives of Applied Science Research 5 (3):63-65.

Akpan UG, Jimoh A, Mohammed AD (2006). Extraction, Characterisation andModification of Castor Seed oil. Leonardo Electron Journal of Practical Technology 8:43-52.

Allal F, Vaillant A, Sanou H, Allaye KB (2008). Isolation and Characterization of New Microsatellite Markers in Shea Tree (*Vitellaria paradoxa* C.F. Gaertn): PermanentGenetic Resources. Molecular Ecology Resources 8(4):822-4.

Animasaun D, Oyedeji S, Olorunmaiye KS, Azeez MA, Tijani IA, Morakinyo J (2019). Morpho-chemical Divergence and Fatty Acid Profile of Shea Tree Seeds (*Vitellaria paradoxa*) Collected from Different Locations in Kwara State, Nigeria. Acta Botanica Croatica.

Association of Official Analytical Chemists (AOAC, 2000). Official

methods of analysis, Washington, DC. 17th Edition.

Audu J, Awulu JO (2016). Effect of Extraction Methods on Food Biodiesel Properties of Sheanut oil (*Vitellaria paradoxa*). Journal of Postharvest Technology 5(1):17-26.

Bouvet JM, Fontaine C, Sanou H, Cardi C (2004). An Analysis of the Pattern of Genetic Variation in *Vitellaria paradoxa* using RAPD Markers. Journal of Agroforestry System 60(1):61-69.

Djekota C, Diouf D, Sane S, Mbaye MS, Noba K (2014). Morphological Characterization of Shea Tree (*Vitellaria paradoxa* subsp *paradoxa*) Populations in the Region of Mandoul in Chad. International Journal of Biodiversity Conservation 6(2): 184-193.

El-Kacimi R, Kamil N, Bennajah M, Kitane S (2016). Extraction of Oleic Acid from Moroccan Olive Mill Wastewater. Biomedical Research International 1397852: 1-9.

El-Mahmood AM, Doughari JH, Ladan N (2008). Antimicrobial Screening of Stem Bark Extracts of *Vitellaria paradoxa* against Some Enteric Pathogenic Microorganisms. African Journal of Pharmacy and Pharmacology 2(5): 89-94.

Enabenue LO, Obisesan IO, Okolo EC, Akinwale RO, Aisueni NO, Ataga CD (2014). Genetic Diversity of Shea Butter Tree (*Vitellaria paradoxa* C.F Gaernt) in the Guinea Savannah of Nigeria Based on Morphological Markers. American Eurasian Journal of Agricultural and Environment Science, 14(7): 615-623.

Fasola TR, Ayoade O (2010). Physico-chemical Analysis and Odour Improvement of Traditional Shea Butter (*Vitellaria paradoxa* Geartn. F.). Zonas Aridas 14(1):192- 195.

Gwali S, Agaba H, Balitta P, Hafashimana D (2015). Tree Species Diversity and Abundance in Coffee Farms Adjacent to Areas of Different Disturbance Histories in Mabira Forest System, Central Uganda. International Journal of Biodiversity Science, Ecosystem Services and Management 9:1-10.

Gwali S, Vaillant A, Nakabonge G, Okullo JBL, Okullo L, Eilu G, Muchugi A, Bouvet J (2014). Genetic Diversity in Shea tree (*Vitellaria paradoxa* subspecies nilotica) Ethno-varieties in Uganda Assessed with Microsatellite Markers. Forest Trees and Livelihood 24 (3). Doi: 10.1080/14728028.2014.956808.

Hall JB, Aebischer HF, Tomlinson E, Hindle JR (1996). *Vitellaria paradoxa*: A Monograph. School of Agricultural and Forest Science Publication No. 8. University of Wales, Bangor, UK, 105.

He D, Zhang J, Zhang X, He S, Xie D, Liu Y, Li C, Wang Z, Liu Y (2020). Development of SSR Markers in *Peonia* Based on De Novo Transcriptomic Assemblies. PLoS ONE 15(1):e2=0227794.

Helal-Uddin ABM, Khalid RS, Alaama M, Abdualkader AM, Kasmuri A, Abbas SA (2016). Comparative Study of Three Digestion Methods for Elemental Analysis in Traditional Medicine Products using Atomic

Absorption Spectrometry. Journal of Analytical Science and Technology 7(6):10.1186.

Hernandez BL, Lean HA, Robles VL (2009). Effect of Stearic Acid and Properties of Metronidazole Method K4M Floatign Matrices. Brazilian Journal of Pharmaceutical Sciences 45(3): 55-61.

Honfo FG, Akissoe N, Linnemann AR, Soumanou M, Van Boekel MA (2014). Nutritional Composition of Shea Products and Chemical Properties of Shea Butter:A Review. Critical Revised Journal of Food Science and Nutrition 54: 673-680.

Ibrahim HM, Ibrahim G, Mohammed Z, Oyi, RA (2019). Studies on the Physicochemical Variations of Shea Nuts/Butter. Specialty Journal of Chemistry 4(4):1- 7.

Israel MO (2015). Effects of Topical and Dietary use of Shea Butter on Animals.American Journal of Life Sciences 2(5):303-307. Doi:10.11648/j.ajls.20140205.18

Julius KI, Umenger LN, Ayangealumun I (2013). Effect of Extraction Methods on the Yield and Quality Characteristics of Oils from Shea Nut Journal of Food Resource Science 2(1): 1-12 Doi:10.3923/jfrs.2013.1.12

Kelly BA, Senou O (2017). Variability of Leaf and Fruit Characteristics of *Vitellaria paradoxa* (Shea Tree) According to Agronomical Performance Along South-North Climatic Gradient in Mali. African Journal of Plant Science 1(5): 142-150.

Kelly BA, Bouvet JM, Picard N (2004). Size Class Distribution and Spatial Pattern of *Vitellaria paradoxa* in Relation to Farmers Practice in Mali. Agroforestry System 60: 3-11.

Lovett PN, Haq N (2000). Evidence for Anthropic Selection of the Shea nut Tree(*Vitellaria paradoxa*). Agro-forestry System Journal 48:73-88.

Maanikuu J, Peker A (2017). Medicinal and Nutritional Benefits from the Shea Tree (*Vitellaria paradoxa*). Journal of Biology, Agriculture and Healthcare 7(22):51-58.

NIST (2012). GC-MS NIST Library Information Manual.

Ololade LS, Ibrahim FA (2014). Assessment of the Contribution of Shea ButterProcessing to Poverty Reduction among Women in Kwara State, Nigeria. Journal of Sustainable Development in African 16(3): 1-12.

Quainoo AK, Bouvet J, Ja Y, Abubakari A (2012). Determination of Biochemical Composition of Shea (*Vitellaria paradoxa*) Nut using Near Infrared Spectrsocopy (NIRS) and Gas Chromatography. International Journal of Biology, Pharmacy and Allied Science 1(2):84-98.

Quainoo AK, Dugbatey JG (2016). Effect of Age of the Shea Tree (*Vitellaria paradoxa*) On the Flow of Shea Latex. UDS International Journal of Development 3(1): 33-40. D

Sanou H, Lovett PN, Bouvet JM **(**2005). Comparison of Quantitative and MolecularVariation in Agroforestry

Populations of the Shea Tree (*Vitellaria paradoxa* C. F. Gaertn) in Mali. Journal of Moecular Ecology, 14: 260-2610.

Shahidi F (2005). Quality Assurance of Fats and Oils. Edible Oil and Fats Product: Chemistry, Properties and Health Effects.

Sorkeh K, Kiani S, Sofo A (2016). Wild Almond (*Prunus scoparia* L.) as Potential Oilseed Resource for the Future: Studies on the Variability of its Oil Content and Composition. Food Chemistry 212 (5): 160-165.

Swatantra KSK, Neelottama K, Neeleshwar M, Rai AK (2010). Role of Markers in the Standardization of Herbal Drugs. A Review. Archives of Applied Sciences Research 2(1):225-229.

Ugese FD, Baiyeri P, Mbah BN (2010). Agroecological Variation in the Fruits and Nuts of Shea Butter Tree (*Vitellaria paradoxa* C.F.Gaertn) in Nigeria. Agroforestry System 79(2):201-211.

Victoria A, Ajayi OO (2015). Comparative Study of the Physicochemical Properties of Oils of Shea nut (*Vitellaria paradoxa*) and Cocoa (*Theobroma cocao*). Current Journal of Applied Science and Technology. 7(5): 496-501.

Vieira MLC, Santini L, Diniz AL, Munhoz CF (2016). Microsatellite Markers: What They Mean and Why They are Useful. Genetics and Molecular Biology.

Warra AA (2015). GC-MS Analysis of Various Extraxts of Sheanut Fats. American Journal of Biological Chemistry 3(6):67-73.

Warra AA, Jega S.A, Ahmad AJ, Abbas MY (2013). Comparative Physico-chemical Analysis of Traditional and Hexane Extracts of Shea nut Fat. Applied Science Report 3(1):100-102.

Wiegel D, Glazebrook J (2009). Quick Miniprep for Plant DNA Isolation. Cold Spring Harbor Protocols, 3:prot5179.