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Physicochemical and Molecular Characterization of *Telfairia occidentalis* Seed Oil

Ibitade Jawonisi* [1], Carrol Luka [2], Rainat Olayemi[1], Martha Akobe[1]

[1]*Department of Applied Chemistry, College of Science & Technology, Kaduna Polytechnic, P.M.B 2021, Kaduna, Kaduna State, Nigeria.*
[2]*Department of Biochemistry, Faculty of Medical Sciences, University of Jos, P.M.B 2084, Jos, Plateau State, Nigeria*

(*) Corresponding author: dribitadeolorun@kadunapolytechnic.edu.ng; 08035968663

Abstract

Lipids are of different qualities; some good for consumption while others are for pharmaceutical and other industrial purposes. Seeds are rich sources of oil. This study assesses the quatity and quanlity of oils extractable from *Telfairia occidentalis* seed and characterises the seed molecularly. Extraction of seed oil was done using petroleum ether (60-80^{0}C) via soxhlet extractor. Organoleptic properties were determined using sensory organs. Physicochemical parameters were analysed using standard laboratory procedures of AOCS. The lipid profile was determined by methylation of the oil subjected to gas chromatograph linked with mass spectrometer. Infrared spectrum was obtained using FTIR-8400S spectrophotometer. Deoxyribonucleic acid was extracted using genomic kits and characterization done via conventional methods. Percentage yield was 35.89%. Organoleptic properties revealed colour; brown, taste; tasteless, smell; pleasant, texture; non-viscous. The physicochemical parameters of *Telfairia occidentalis* obtained are specific gravity; 1.04, refractive index, 1.5267 at 20 ^{0}C, acid value; 8.42, iodine value; 3.53gI_2/100g, peroxide value; 41.4 meq/kg, saponification value; 53.30 mgKOH/g, unsaponifiable matter; 1.53g. Infrared spectrum revealed functional groups and molecular bond vibrations typical of vegetable oils. Fatty acid composition of oil from *T. occidentalis* seed revealed presence of linolenic acid; 7.92%, oleic acid;18.02%, palmitic acid; 7.05% and stearic acid; 14.79%. Amplified genomic DNA of the seed had 600 base pair. A significant alignment of queried sequence with those in the genbank gave 99.07% similarity.The results from this study shows that *Telfaria occidentalis* seed contains oil of appreciable quantity that can be explored as source of health-promoting supplements in pharmaceutical and cosmetic industries.

Keywords: fatty acids profile, infrared spectrum, molecular data, physicochemical parameters, seed oil.

Introduction

Telfairia occidentalis is cultivated in West Africa for diverse purposes such as economic, nutritional and medicinal benefits. It is commonly found in southern-eastern part of Nigeria, Imo state suggested as the center of it´s origin [1]. It is known as fluted pumpkin often cultivated in home gardens via seed. Ugwu (Igbo-Nigeria), apiroko (Yoruba-Nigeria), ubong (Eik-Nigeria), umeke (Edo-Nigeria) are common names of the

leaf. *Telfairia ocidentalis* is a perennial climbing plant having herbaceous stems, it supports itself via tendrils. It is a valuable crop for its edible leaf used for making vegetable soup found to be rich in nutrients. It has been described in traditional medicine for treatment of anaemia, diabetes, convulsion, fatigue, protein energy malnutrition, among others. The seed has also been documented to be of good nutritional value; high in protein and fat [2],[3].

Recent studies have shown that defatted seeds of *T. occidentalis* flour can be used as supplementation for nutritional improvement of composite flour of other cereals just as wheat flour for production of confectionaries [4], [5]. Species of the seed from Ogun state Nigeria and some other parts of Nigeria have been documented as rich source of edible oil and phytochemicals with evidence of antioxidant and antimicrobial activities among others [6], [7], [8]. Despite the recognised potential of *T. occidentalis* seed, the economic potential is yet to be fully explored for industrial purposes such as pharmaceutical and cosmeticeutical production. This study evaluated the oil yield, physicochemical parameters, fatty acid profile and molecular characterisation of *T. Occidentalis* seed with the aim of highlighting its economic potential and providing specie genomic identity for it.

Materials and Methods

Telfairia occidentalis seed used in this study were sourced from Ogoja Local Government Area of Cross River state of Nigeria. Extraction of the seed oil was done using petroleum ether (60-80^{0}C) via soxhlet extractor. Organoleptic properties were determined using sensory organs of small sample size of human subjects selected by random sampling who gave their consent to participate. Physicochemical parameters were analyzed using standard laboratory procedures of AOCS [9].

The lipid composition of the oil was determined by sulphuric acid-methanol methylation of the fatty acids in the oil. The fatty acid methyl esters obtained were subjected to gas chromatography coupled with mass spectrometer using GCMS-QP2010 Plus. The quantitative data of the specific constituents of the oil were obtained by peak area normalization using GC-FID. Identification was done by comparison of both mass spectra and GC-retention data with authentic compounds analyzed and stored in the data system. Infrared spectrum of the oil was obtained using FTIR-8400S spectrophotometer The seed genomic deoxyribonucleic acid was extracted by using plant genomic DNA mini prep kit. Amplification of genomic DNA was by using real time polymerase chain reaction. The amplification was carried out in a DNA thermal cycler using both forward and reverse versions of primers. Strands of amplified genomic DNA was separated using agarose-gel electrophoresis, sanger

sequencer for sequencing. NCBI genbank was used for blasting of obtained nucleotide.

Results and Discussion

The percentage yield of *Telfairia occidentalis* seed oils was 35.89%. Observed Organoleptic properties of *T. occidentalis* extracted seed oil are as follows: colour; brown, smell; pleasant, taste; tasteless, texture; non-viscous. The reported values for physicochemical parameters of *Telfairia occidentalis* are mean of triplicate determinations, specific gravity; 1.04, refractive index, 1.5267 at 20 ^{0}C, acid value; 8.42, iodine value; 3.53gI_2/100g, peroxide value; 41.4 meq/kg, saponification value; 53.30 mgKOH/g, unsaponifiable matter; 1.53g (Table 1). As shown in Table 2, the fatty acid composition of oil from *T. occidentalis* seed revealed presence of linolenic acid; 7.92%, oleic acid;18.02%, palmitic acid; 7.05% and stearic acid; 14.79%.

Telfairia occidentalis (Pumpkin seed) analyzed contains an appreciable amount of oil. The acid value of an oil has effect on its use, various limits are set for these uses, either as edible, for cosmetic or pharmaceuticals by codex Alimentarius Commision[10]. The maximum acid value for edible oils is 4.0mg/KOH/g [10]. The acid value of the extracted oil is higher than edible value and seem to be in the range of biodiesel ASTM,this may limit its use to industrial purposes.

The peroxide value obtained is on the high side, this may reduce the shelf life ,oils with high peroxide values have been described as prone to oxidation which can affect it´s stability [11]. The amount of alkali required to saponify a given oil reflects the purposeful application of the oil and suggest the length of its fatty acid chain, saponification value of 53.30mg/KOH/g is a relatively low value compared to other oils. This oil will be suitable for industrial applications such as soap making or lubricant production [10].

The unsaponifiable matter content of the seed analysed indicates presence of sterols and other hydrocarbons, the GCMS results shows presence of a triterpene identified as squalene in little amount, a precursor in sterol biosynthesis, it has application in cosmetics and pharmaceutical industries [12],[13], [14]. The measure of degree of saturation;iodine value indicates a low value of unsaturation, the GCMS result complements this as over 60 % of saturated fatty acids were detected in this specie of seed used. Another studies identified presence of unsaturated fatty acids in a specie of the plant grown in Ogun state, Nigeria [6]. The optical property of the oil; refractive index seems to indicate it will be of industrial benefit.

Infrared spectrum showed evidences of functional groups and molecular bonds typical of vegetable oils such as strong absorption bands; 2850-3000cm^{-1} C-H stretch vibrations of fatty acids; 2500-3500 cm^{-1} O-H stretch of carboxylic acids;1740-1755 cm^{-1} C=O stretch of triglyceride esters and 1650-1680 cm^{-1} C=C stretching vibrations of unsaturated fatty acids and 1650-1680 cm^{-1} C=C stretching vibrations of unsaturated fatty acids were obtained, many of

Density	1.04g/cm^3
Specific gravity	1.04
Refractive index	1.5267
Acid value	8.415
Iodine value	3.53gI_2/100g
Peroxide value	41.4mlMPerkg
Saponification value	53.295
Unsaponifiable matter	1.525g

these bonds were also detected in oil of olive seed used as reference standard while running the IR of extracted oil(Figure 3; Table 3) [15].

Fatty acid composition revealed presence of monounsaturated fatty acid; 18.02% of oleic acid that has varied health benefits such as improving heart health and anti-inflammatory effects. Linoleic acid, an essential fatty acid was also found in the seed oil in little amount. These findings provide an insight to the potential of the oil for applications in food, cosmetics or pharmaceutical industry, which is in agreement with earlier findings of Gunstone, 2011[12],[13].

Telfairia occidentalis contains decanoic acid (capric acid; 0.75%) which is used in the manufacture of esters for artificial fruit flavours and perfume, as food additives and active pharmaceutical ingredients. Another fatty acid present in T. *occidentalis* seed oil is eicosanoic acid (arachidic acid;1.97%). Arachidic acid is used for the production of detergents, photographic materials and lubricants[12].

Electrophoretic profile showed the amplified DNA has 600base pair. Blasting on NCBI tool gave an e-value of 0.0, percentage identification of 99.07 with *T. occidentalis* nucleotides deposited in the genbank database having accession numbers NC_067979.1 and DC535773.1 ,this implies significant alignment between the nucleotide of this study with the two referenced nucleotides in the genbank database of NCBI[16]. The alignment authenticates the seed of the plant used, e-values of close to zero are said to be accurate for phylogenetical analysis [17].

Table 1: Physicochemical Properties of *Telfairia occidentalis* Seed Oil

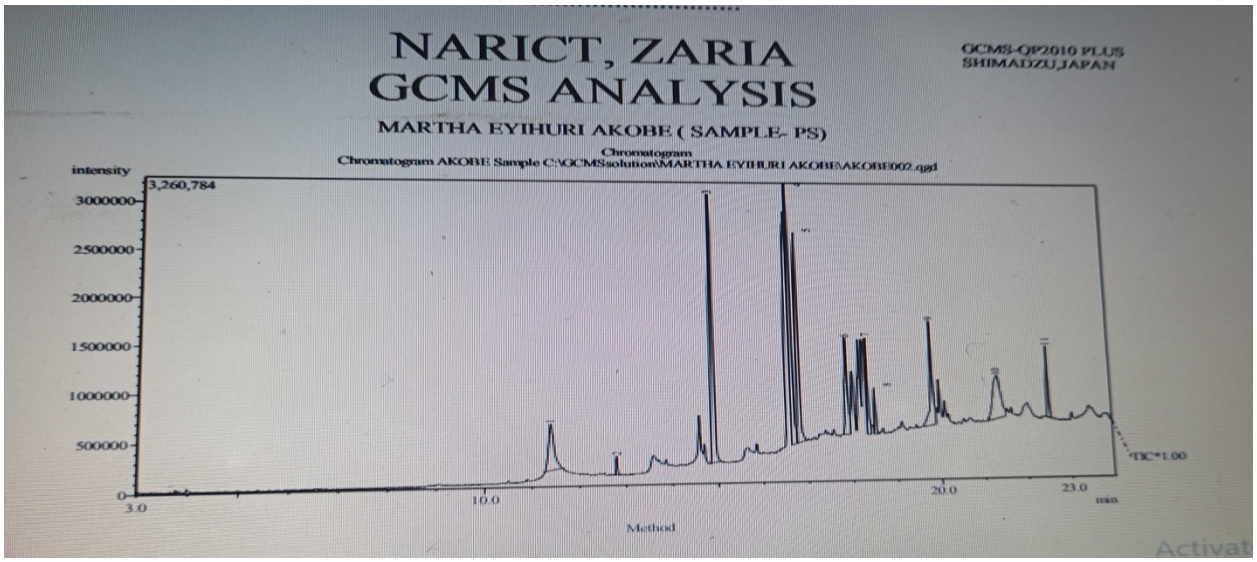

Figure 1: Chromatogram of *Telfairia occidentalis* Extracted Seed Oil

Table 2: Fatty Acid Composition of *Telfairia occidentalis* Seed Oil

Peak Number	Retention time (Minutes)	IUPAC name	Common name	Molecular formula	Area %
1	11.394	Hexadecanoic acid	Palmitic acid	C15H31COOH	7.05
2	12.794	Decanoic acid	Capric acid	C9H19COOH	0.75
3	14.919	Hexadecanoic acid	Palmitic acid	C15H31COOH	20.47
4	16.664	9- Octadecanoic acid	Oleic acid	C17H33COOH	18.02
5		Octadecanoic acid	Stearic acid	C17H35COOH	14.79
6	17.902	9,12,15-Octadecatrienoic acid	Linolenic acid		7.92
7	18.363	Hexadecanoic acid			5.19
8	18.526	Eicosanoic acid	Arachidic acid	$C_{19}H_{39}COOH$	1.97

Area % is proportional to concentration.

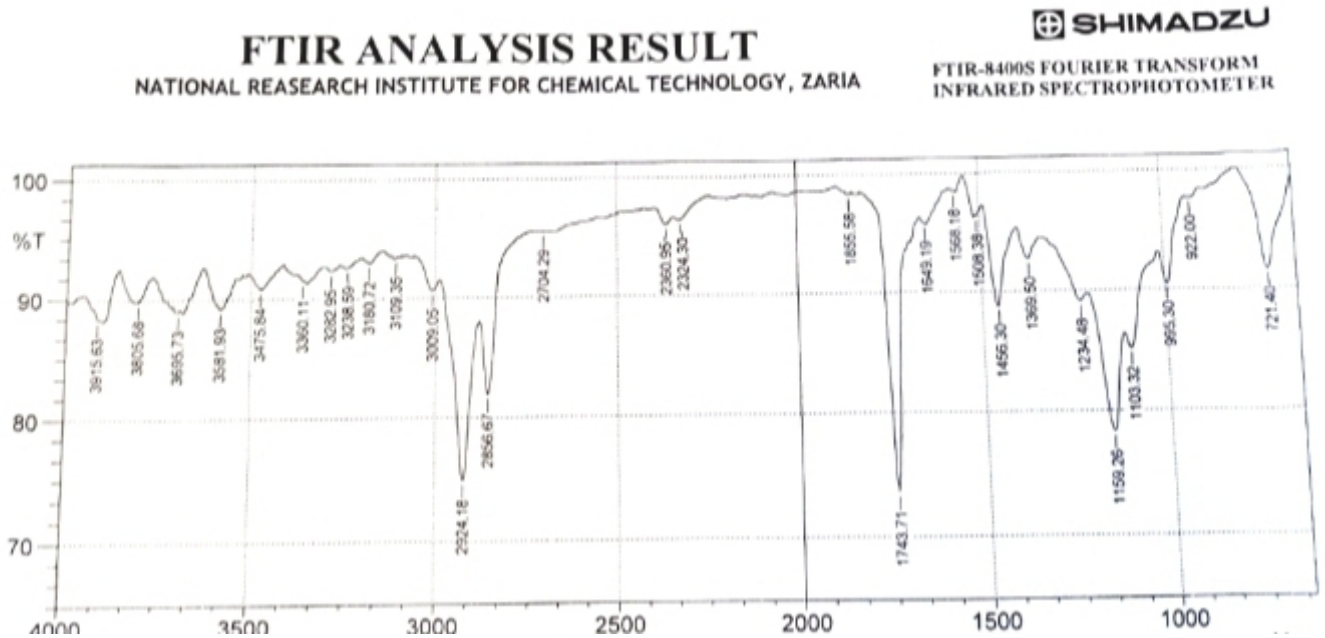

Figure 2: FTIR Spectrum of Extracted Seed Oil of *Telfairia occidentalis.*

Table 3: Functional Groups and Molecular Bond Vibrations present in Extracted Seed Oil of *Telfairia occidentalis*

Peak number	Wavenumber(cm^{-1})	Intensity	Class of Compounds/ Molecular bonds
17-19	2850 – 3000	s	Alkanes [C – H stretch] &
8	1450 – 1470	s	Alkyls[C – H bend]
22-26	3200 – 3600	s	Alcohols [O – H stretch]
5	1125 - 1205	m-s	C – O stretch
6	1180 – 1260	m-s	C – O stretch
16	2700 – 2725	s	Aldehydes [H – C = O stretch]
12	1740 – 1755	s	Ketones [C = O stretch]
26	2500 – 3500	s	Carboxylic Acids[O-H stretch]

Key: s denotes strong bonds indicating high concentration,m-s denotes medium –strong.

Conclusion

There is underutilization of the seed of *Telfaria occidentalis* for purposes such as health-promoting Supplements, cosmeticeuticals, and other industrial uses. This study highlights the potential of the seed as valuable source of oil for beneficial fatty acids such as oleic acid and linoleic acid. The seed oil could be a viable alternative source of oil for food, pharmaceticals and other industrial uses. The genomic data obtained serves as a crucial information providing insights into the evolutionary relationship and genetic diversity of the seed studied. Scarce information on this is available, this information can be used for improvement of agronomic traits of *Telfaria occidentalis* from cross river.

Further research can focus on optimizing extraction methods and evaluating the oil´s stability and shelf life in order to explore its highlighted potential for large-scale cultivation and commercialization.

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