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Physicochemical Properties, Fatty Acids Composition and Antioxidant Potential of the Seed Kernel Oil of Oysternut *(Telfairia pedata)* Found in Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Author BMI conducted the experiments and wrote the first draft of the manuscript. Authors JMO and LKO conceived the research idea, designed the experiments and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Certain edible plant sources contain vegetable oils that have been under-exploited both commercially and in research. This study aimed to determine the physicochemical properties, fatty acids composition, and antioxidant potential of the oil from the seed kernels of *Telfairiapedata*, which are used as food by the local population of Tharaka-Nithi County in Kenya.

Materials and Methods: *Telfairia pedata* seeds were collected from farmers in the county of Tharaka-Nithi, Kenya. *n*-Hexane was used to extract the oil via soxhlet extraction. Standard laboratory protocols were used to characterize the oil's physicochemical properties, while fatty acids composition and antioxidant potential were characterized using gas chromatography mass spectrometry and 2, 2-diphenyl-1-picrylhydrazyl assay, respectively.

Results: The seed kernels of *Telfairia pedata* yielded more than 66% of oil. The oil's physicochemical properties were found to be within the Food and Agriculture Organization set limits

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and were as follows; moisture content (0.0592±0.0140%), peroxide value (0.9641±0.2021 meq O_2/Kg), iodine value (23.0058±2.2473 gI_2/100g) and acid value (0.6352±0.0330 mg KOH/g). Fatty acids such as myristic acid (14:0; 0.11%), palmitoleic acid (16:1n7; 0.13%), palmitic acid (16:0; 34.97%), margaric acid (17:0; 0.10%), linoleic acid (18:2n6; 48.46%), stearic acid (18:0; 15.33%), 10,13-octadecadienoic acid (18:2n5; 0.09%), 18-methylnonadecanoic acid (20:0; 0.68%), and behenic acid (22:0; 0.14%) were found in the oil. The antioxidant potential of the oil expressed in IC_{50} was found to be 18.05 mg/mL, in relation to that of ascorbic acid 2.406 μg/mL.

Conclusions: *Telfairia pedata* seed kernel oil can be economical to exploit commercially due to its relatively high yield. The determined properties of *Telfairiapedata* seed kernel oil present high nutritive value making the oil fit for edible applications.

Keywords: Telfairia pedata; physicochemical properties; essential fatty acid; antioxidant potential; Kenya.

1. INTRODUCTION

Human beings obtain vegetable oil from different plant sources. *Telfairiapedata*is a plant species in the family of Cucurbitaceae that is commonly found in some parts of East Africa. The species is considered endangered since one of the three species of the genus *Telfairia*(*T. batesii*) is already extinct [1]. The seed kernel of *T. pedata*is edible and can also be exploited for its oil content. *T. pedata*seed kernels can be eaten while cooked, roasted, or even raw [2]. Profiling of edible vegetable oil based on its physicochemical properties, fatty acids composition, and antioxidant potential provides important information about its nutritional quality, as well as its performance as a raw material in industrial applications [3]. Consuming vegetable oil from edible oil seeds has beneficial health effects due to the inherent nutritional aspects such as essential fatty acids (EFAs), vitamins, and antioxidants.

Vegetable oils are composed of different compounds such as triglycerides, phytochemicals such as tocopherols, phenolic compounds and carotenoids [4]. These components determine the value and properties of any vegetable oil. When consumed, different vegetable oils impact human health differently by exhibiting such qualities as anti-mutagenic and anti-inflammatory potentials, linked to their phytochemistry [5]. Research on different edible vegetable oils enables the determination of their inherent properties hence providing crucial nutritional information about them.

Other than edible applications, vegetable oils can be used for other industrial applications. Such industrial applications can include the manufacturing of biodiesel, lubricants, paints, and soaps. It is, therefore, paramount to determine the suitable industrial application of vegetable oils by determining their quality properties [6]. Oil production and processing for edible purposes is also guided by the quality properties of each individual vegetable oil [7]. Research on vegetable oils regarding their quality properties, therefore, help in deciding the best industrial application of any given oil.

Dietary importance of edible vegetable oils is of much concern to researchers. Many vegetable oils possess crucial nutritive components necessary for human health. Around the world, a considerable number of people, especially children, are reported to be deficient in one or more essential nutritional components that can be obtained via the consumption of seed oils [8]. Some of the nutritional components of edible vegetable oils associated with their dietary importance include the presence of EFAs and phenolic compounds exhibiting antioxidant properties [6].

The types of fatty acids present in vegetable oils constitute a crucial piece of dietary information to consumers. There are three major classifications of fatty acids that can be found in vegetable oils. That is, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). Higher consumption of SFAs increases the risk of cardiovascular disease while higher consumption of MUFAs and PUFAs reduces the risk of cardiovascular disease [9]. EFAs are required by the human body for good health except the body does not synthesize them. EFAs have to be obtained from dietary sources. Edible vegetable oils such as seed oils are good natural sources of the EFAs. Common EFAs found in vegetable oils include alpha-linolenic acid (ALA) and linoleic acid (LA) [10].

Research on edible oil seeds such as that of *Telfairiapedata*has the potential to uncover their potential in terms of nutrition and medicinal value. There is limited literature on the physicochemical properties, fatty acids composition, and antioxidant potential of *T. pedata*seed kernel oil. This research sought to fill this gap in knowledge. The research findings of this study can be helpful to the industrial applications of *T. pedata*seed kernel oil.

2. MATERIALS AND METHODS

2.1 Sample Collection, Sample Pre-paration and Oil Yield Determina-tion

*Telfairiapedata*seeds used in this research were collected in Kenya at a place known as Chuka within Tharaka-Nithi County. The University of Nairobi Herbarium was used for the identification of the species. Fig. 1 shows the photography of *T. pedata.* The seeds were decorticated mechanically to obtain the kernels. The seed kernels were pounded using a mortar and pestle to increase the surface area for maximum oil yield during soxhlet extraction. *n*-Hexane was used as a solvent and the extraction process took about four hours to complete. Solvent recovery was done using a rotary evaporator. The percent oil yield of the *T. pedata*seed kernels was determined using the equation below [11]:

$$\%\,oilyield = \frac{weightofextractedoil}{weightofseedkernelused} \times 100$$

2.2 Analysis of Physicochemical Properties

The physicochemical properties analyzed in this research include moisture content, peroxide value, iodine value, and acid value. Standard laboratory protocols for the determination of physicochemical properties in vegetable oils were used in this research [11].

2.3 Moisture Content

About five g of the oil sample was dried in an oven while placed in a previously weighed crucible for about two hours until a constant weight was achieved. The oven temperature was set at 105℃. After drying, the sample was allowed to cool in a desiccator before the weight difference was determined using the following equation [11].

$$\%Moisture = \frac{W1 \times 100}{W2}$$

Where, W1 = weight (g) of the oil sample after drying, W2 = weight (g) of the oil sample before drying.

2.4 Peroxide Value

Acetic acid/chloroform (3:2) solution was used to dissolve 10 mL of the oil sample before using 0.5 mL of 15% potassium iodide (KI) to further react with the solution. 0.1 N sodium thiosulphate solution was then used to titrate the liberated iodine, using a 0.5 mL starch solution as indicator. Blank titration was also performed. The following equation was used in the determination of the oil's peroxide value [11].

$$Peroxidevalue = (B - S) \times W \times N$$

Where, B = volume of sodium thiosulphate used for blank, S = volume of sodium thiosulphate consumed by the oil sample, W = weight of the oil sample, N = the normality of sodium thiosulphate.

2.5 Iodine Value

The oil sample (0.5 mL) was mixed with 10 mL chloroform before being reacted with a 25 mL iodine solution. The reaction was allowed to last for 30 min to achieve a complete reaction between iodine and the unsaturated bonds of oil. Light exposure to the reactants was avoided by covering the flask with aluminium foil. The unreacted iodine was then converted to iodide by adding 20 mL of 15% aqueous KI and 100 mL of water to the solution. 0.1 N sodium thiosulphate ($Na_2S_2O_3$) solution was then used to titrate the final content in the flask while using starch as an indicator. The equation below was used to calculate the oil's iodine value [11].

$$Iodinevalue = \frac{(A - B) \times N \times 0.127 \times 100}{W}$$

Where, A = mL of 0.1 N sodium thiosulphate required by oil sample, B = mL of 0.1 N sodium thiosulphate required by the blank, N = normality of sodium thiosulphate, W = weight of oil in grams, 1 mL 1 N $Na_2S_2O_3$ = 0.127 g I_2.

Telfairia pedata

***Telfairia pedata* seeds**

Fig.1. Photography of *Telfairia pedata*

2.6 Acid Value

The oil sample (10 mL) was first mixed with 100 mL ethyl-alcohol. The mixture was heated in a hot water bath to its boiling point. The hot content was let to cool at room temperature before being titrated with a 15% KOH solution. Phenolphthalein was used as an endpoint indicator. The acid value of the oil was calculated using the following equation [11]:

$$Acidvalue = \frac{V \times N \times M.wt}{W}$$

Where, V = volume of standard KOH solution in mL, N = normality of standard KOH solution, W = weight of oil sample in grams, M.wt (molecular weight) of KOH = 56.1 g/mol.

2.7 Analysis of Fatty Acids Composition

The oil's fatty acids (FAs) composition was determined following the ISO 5509:2000 method [12]. About 50 mL of the oil sample was mixed with 5 mL methanolic sodium hydroxide and refluxed for 10 min while using a boiling aid. Methanolic boron triflouride (5 mL) was then added to the solution and refluxed for 10 more minutes. About 3 mL of isooctane was also added. After refluxing, a solution of 20 mL sodium chloride was immediately added to the contents of the flask and vigorously shaken for 15 s. More sodium chloride solution was added to top-up the contents of the flask to the mark before allowing the two phases to separate. The upper isooctane layer was dried over anhydrous sodium sulfate to remove any traces of water. The final supernatant was diluted with *n*-Hexane to form a 1 ppb solution for analysis using gas chromatography–mass spectrometry (GC-MS).

A Shimadzu QP 2010-SE instrument was used. The following specifications and conditions applied: 1 mL/minute carrier gas flow rate, 30 m long column with 0.25 mm internal diameter and 0.25 μm film thickness, 200℃ injection temperature, 250℃ interface temperature, 200℃ electron ionization, and 60℃ (1 minute); 10℃ /min to 250℃ (10 minutes) temperature programming.

Identification of the peaks was done by the use of mass spectrometry, where each fatty acid methyl ester (FAME) produced a unique fragmentation pattern which was compared against a library of predetermined compounds for true identity. Each compound was quantified statistically using the instrument's preinstalled integration software which expresses each peak area as a percentage relative to the other peaks.

2.8 Analysis of Antioxidant Potential

The antioxidant potential of the oil sample was determined using a modified DPPH (2,2-diphenyl-1-picrylhydrazyl) assay [13]. Ascorbic acid was used as standard. Concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL, and 31.25 mg/mL were made by dissolving the oil sample in ethyl acetate. 1 mL 0.1 mM DPPH was added to 2 mL of each concentration and incubated in darkness for 30 min to allow full reaction. For the standard, the same procedure was repeated with 100 μg/mL, 50 μg/mL, 25 μg/mL, 12.5 μg/mL, and 6.25 μg/mL concentrations of ascorbic acid dissolved in methanol. In both cases, a blank solution representing 0.0 mg/mL was also prepared. After the reaction with DPPH, solutions were analyzed using a UV-Vis spectrophotometer at 517 nm. The obtained absorbance was recorded and used to determine the percent radical scavenging activity (% RSA) as shown in the following equation:

$$\% RSA = \frac{(A_{blank} - A_{test})}{A_{blank}} \times 100$$

Where, A_{blank}= Absorbance of the blank sample, A_{test} = Absorbance of the test sample and the standard sample.

The obtained % RSA values were then used to determine the IC_{50} values for both the test sample and the standard sample by performing a nonlinear regression analysis using the GraphPad Prism 7.03 software.

3. RESULTS

The seed kernels of *Telfairiapedata*were found to have an average of 66.35±3.17% oil yield. The determined physicochemical properties of the oil are summarized in Table 1. These include moisture content (0.0592±0.0140%), peroxide value (0.9641±0.2021 meq O_2/Kg), iodine value (23.0058±2.2473 gI_2/100g), and acid value (0.6352±0.0330 mg KOH/g). The obtained values for the oil's physicochemical properties were found to be within the limits of the Food and Agriculture Organization's (FAO) "Standard for Edible Fats and Oils not Covered by Individual Standards."

Fig. 2 shows the chromatogram of the FAMEs obtained after analyzing the oil sample by the use of a GC-MS. A total of nine FAMEs were detected and identified. The fatty acids (FAs) composition of the oil is summarized in Table 2 with the structures presented in Fig. 3. *T. pedata*seed kernel oil constitutes of nine fatty acids namely myristic acid (14:0; 0.11%), palmitoleic acid (16:1n7; 0.13%), palmitic acid (16:0; 34.97%), margaric acid (17:0; 0.10%), linoleic acid (18:2n6; 48.46%), stearic acid (18:0; 15.33%), 10,13-octadecadienoic acid (18:2n5; 0.09%), 18-methylnonadecanoic acid (20:0; 0.68%), and behenic acid (22:0; 0.14%). Six FAs are saturated, accounting for 51.33% of the total FAs, two FAs are polyunsaturated (48.54%), and only one FA is monounsaturated (0.13%).

The % RSA results of both the oil sample and the standard material (ascorbic acid) are summarized in Table 3 and Table 4 with their IC_{50} values presented in Fig. 4 and Fig. 5, respectively. At the concentration of 500 mg/mL, *T. pedata*seed kernel oil recorded an average % RSA value of about 80 (Table 3), while ascorbic acid recorded an average % RSA value of about 98 at the concentration of 100 μg/mL (Table 4). Fig. 4 shows that the IC_{50} value of *T. pedata*seed kernel oil is 18.05 mg/mLwhile that of ascorbic acid is 2.406 μg/mL (Fig. 5).

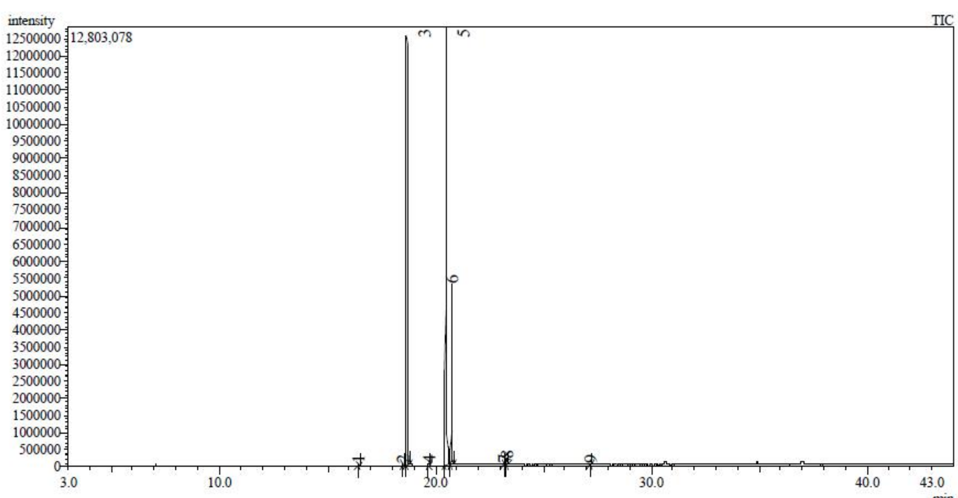

Fig.2. Chromatogram of fatty acid methyl esters from *Telfairiapedata*seed kernel crude oil analyzed by gas chromatography (GC)

Table 1. Physicochemical properties of crude oil from the seed kernels of *Telfairiapedata*

Physicochemical Property	Value (±SD)	FAO Limit
Moisture Content (%)	0.0592±0.0140	< 0.2
Peroxide Value (meq O_2/Kg)	0.9641±0.2021	< 15
Iodine Value (gI_2/100g)	23.0058±2.2473	-
Acid Value (mg KOH/g)	0.6352±0.0330	< 4.0

Each value is a mean ± standard deviation of three determinations. SD means standard deviation. FAO stands for Food and Agriculture Organization. – means not specified. < stands for "less than"

Table 2. Fatty acids composition (%Area) of crude oil from the seed kernels of *Telfairiapedata*

Fatty Acid	Retention Time (min)	Peak area	% Peak area
Myristic acid (14:0)	16.435	83837	0.11
Palmitoleic acid (16:1n7)	18.484	95816	0.13
Palmitic acid (16:0)	18.688	26138986	34.97
Margaric acid (17:0)	19.716	73455	0.10
Linoleic acid (18:2n6)	20.484	36218994	48.46
Stearic acid (18:0)	20.760	11454831	15.33
10,13-Octadecadienoic acid (18:2n5)	23.173	63591	0.09
18-methylnonadecanoic acid (20:0)	23.319	507503	0.68
Behenic acid (22:0)	27.184	101331	0.14
		74738344	100.00

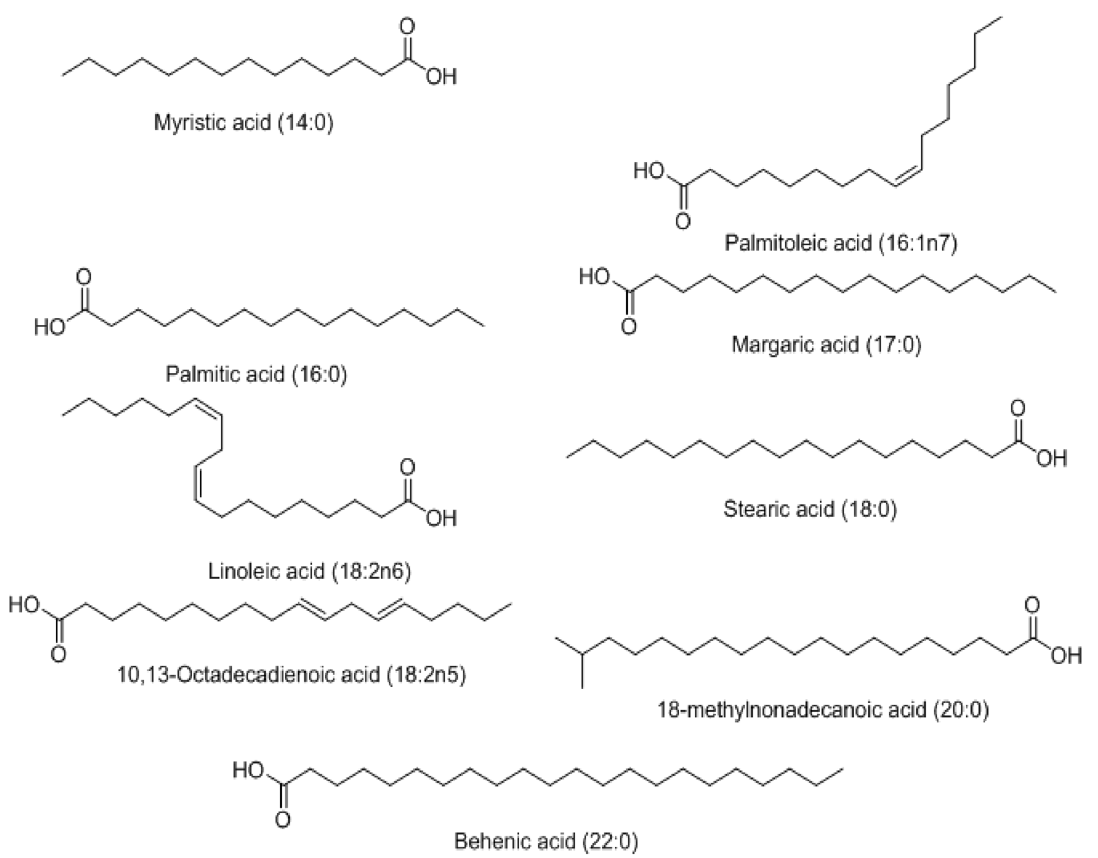

Fig. 3. Structures of fatty acids found in *Telfairia pedata* seed kernel oil

Table 3. *In vitro* DPPH scavenging activity of crude oil from the seed kernels of *Telfairiapedata*

Conc.(mg/mL)	**UV Absorbance at 517 nm and % RSA**						**Average % RSA**
	1st Replicate	% RSA	2nd Replicate	% RSA	3rd Replicate	% RSA	
500	0.116	78.0303	0.116	77.98861	0.117	77.79886	77.93926
250	0.131	75.18939	0.129	75.52182	0.132	74.95256	75.22126
125	0.169	67.99242	0.171	67.55218	0.171	67.55218	67.69893
62.5	0.188	64.39394	0.188	64.32638	0.189	64.13662	64.28565
31.25	0.219	58.52273	0.219	58.44402	0.217	58.82353	58.59676
0	0.528	0	0.527	0	0.527	0	0

Table 4. *In vitro* DPPH scavenging activity of ascorbic acid

Conc. (μg/mL)	**UV Absorbance at 517 nm and % RSA**						**Average % RSA**
	1st Replicate	% RSA	2nd Replicate	% RSA	3rd Replicate	% RSA	
100	0.019	97.57033	0.018	97.53762	0.017	97.85624	97.65473
50	0.018	97.69821	0.018	97.53762	0.019	97.60404	97.61329
25	0.023	97.05882	0.023	96.85363	0.026	96.72131	96.87792
12.5	0.060	92.32737	0.058	92.06566	0.057	92.81211	92.40171
6.25	0.226	71.09974	0.216	70.45144	0.287	63.80832	68.45317
0	0.782	0	0.731	0	0.793	0	0

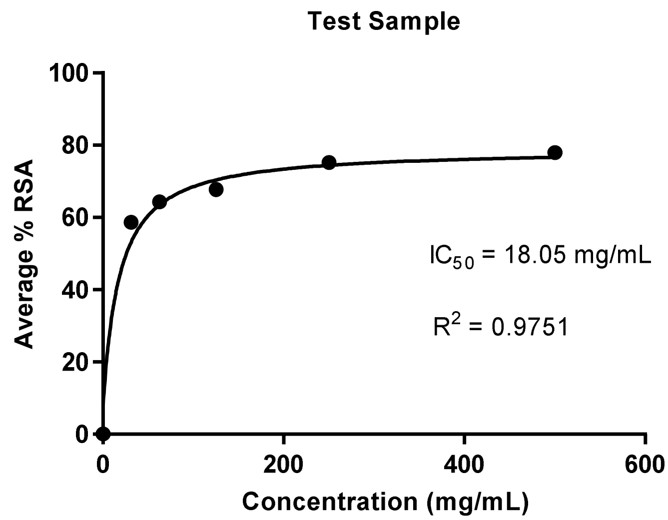

Fig.4. Nonlinear regression curve showing the IC_{50} value of crude oil from the seed kernels of *Telfairiapedata*

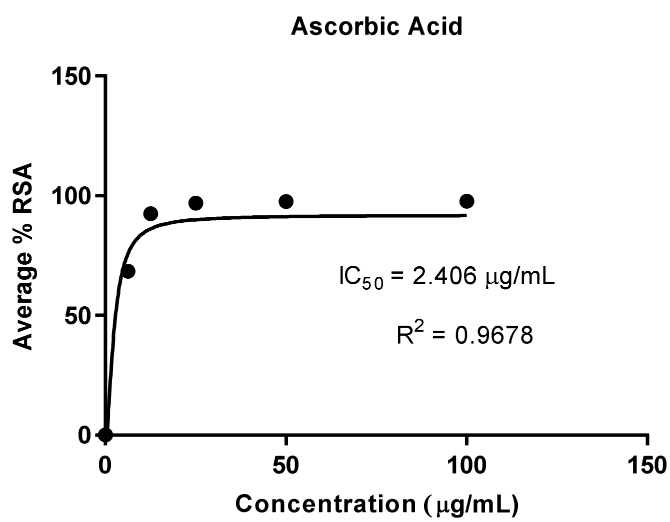

Fig.5. Nonlinear regression curve showing the IC_{50} value of ascorbic acid

4. DISCUSSION

The percent oil yield of *Telfairiapedata*seed kernel is 66.35±3.17%. In comparison to other seed oils, the percent oil yield of *T. pedata*seed kernel is higher than that of safflower seed (39.53%) and *Moringastenopetala* seed (44.30%) [14,15]. The higher percent oil yield of *T. pedata*seed kernel implies that the species is a reliable source of edible vegetable oil which would be economical to exploit commercially.

The determined physicochemical properties of *T. pedata*seed kernel oil play an important role in determining the value of the oil over an extended period while on the shelf. The moisture content of *T. pedata*seed kernel oil was determined to be 0.0592±0.0140%. This value is within the 0.2% limit set by FAO for edible crude vegetable oils not covered by individual standards [16]. The low moisture content of *T. pedata*seed kernel oil ensures its quality while on storage as it is not conducive for microbial growth [17]. Hydrolytic rancidity which is the production of unpleasant odor due to the hydrolysis of triglycerides is another quality aspect associated with the amount of moisture present in oils [18]. A higher moisture content of oil favors hydrolytic rancidity but the low moisture value observed in *T. pedata*seed kernel oil indicates that the oil is not susceptible to such quality degradation.

Lipid peroxidation in oils is measured by the determination of peroxides which form as the primary reaction products during oil oxidation. Peroxide value is, therefore, used as an indication of oxidative rancidity in unsaturated fats and oils [19]. As determined by this research, the peroxide value of *T. pedata*seed kernel oil is 0.9641±0.2021 meq O_2/Kg. This value is within the 15 meq O_2/Kg limit set by FAO for edible crude vegetable oils [16]. The low peroxide value observed in *T. pedata*seed kernel oil shows little evidence of oxidative rancidity in the oil. This observation can be attributed to either of the following facts regarding the oil; either the oil has antioxidant compounds such as vitamin E [20] which prevents the oxidation of unsaturated fatty acids or it is composed of a lower ratio of unsaturated fatty acids to that of saturated fatty acids. As determined by this research, *T. pedata*seed kernel oil exhibited some antioxidant activity and it has a considerably higher amount of saturated fatty acids.

The peroxide value of oil is closely related to its iodine value in the sense that iodine value is a measure used to indicate the extent of unsaturation in fatty acids [21]. The iodine value of *T. pedata*seed kernel oil was found to be 23.0058±2.2473 gI_2/100g. In comparison with other vegetable oils, the iodine value of *T. pedata*seed kernel oil is considerably lower than those of tropical almond oil (85.12 gI_2/100g), fluted pumpkin oil (101.73 gI_2/100g), and palm oil (56.10 gI_2/100g) [22]. This lower iodine value is an indication of lower levels of unsaturation in the oil's fatty acids. This observation is consistent with the higher levels of saturated fatty acids found in the oil. Due to higher levels of unsaturation, certain vegetable oils easily undergo oxidative rancidity when subjected to deep-frying [21]. *T. pedata*seed kernel oil would, however, be prime oil for deep-frying applications as implied by its low iodine value.

The acid value in oils measures the amount of free fatty acids (FFAs) present and can be used as an indication of the hydrolysis of triglycerides [23]. As determined by this study, the acid value of *T. pedata*seed kernel oil is 0.6352±0.0330 mg KOH/g. FAO recommends that for edible fats and oils, the acid value of virgin fats and oils/cold-pressed fats and oils should not exceed 4.0 mg KOH/g, while that of refined fats and oils should not exceed 0.6 mg KOH/g [16]. The low acid value obtained for *T. pedata*seed kernel oil shows that little effort would be required in its refinement to meet FAO guidelines for refined edible fats and oils. The obtained acid value of the oil is also consistent with its low moisture content which contributes to very little hydrolysis of triglycerides if any. High acid value in oils implies low quality since FFAs are prone to oxidation leading to breakdown products such as organic acids, ketones, aldehydes, and alcohols which have characteristic flavors and aromas [23].

Of the nine FAs found in *T. pedata*seed kernel oil, linoleic acid, which is an EFA [24], was found to be the most abundant FA. The human body is unable to make EFAs such as linoleic acid and thus has to be obtained from the diet [24]. EFAs play an important role in ensuring normal metabolism and good health in humans. When consumed, linoleic acid undergoes biological conversion to produce eicosanoids, necessary for vital organ functioning and intracellular processes such as regulating blood pressure and inflammation [24].

SFAs in *T. pedata*seed kernel oil were found to be more than 50%, implying that the oil is saturated. Like other saturated oils, the use of *T. pedata*seed kernel oil for edible purposes should be limited due to the health concerns associated with saturated fats and oils. A higher intake of SFAs in the diet increases the risk of cardiovascular disease (CVD) by raising plasma's low density-lipoprotein (LDL)-cholesterol [25]. The Dietary Guidelines for Americans recommend that people should observe a daily dietary intake of SFAs of not more than 10% of energy [26]. The total PUFAs and MUFAs in *T. pedata*seed kernel oil were found to be about 49%. These FAs are considered to be healthier in comparison to SFAs [27].

While ascorbic acid (standard material) was found to be more active than *T. pedata*seed kernel oil in terms of antioxidant activity as expected, the oil was found to have some antioxidant potential hence its possibility of being used as one of the natural food substances able to fight oxidative stress in humans. Oxidative stress is the imbalance between reactive oxygen species (ROS) in the body and the biological ability to detoxify them [28]. ROS in the body are produced via different mechanisms such as normal oxygen metabolism and environmental stressors and are responsible for attacking cellular structures, such as membranes, lipids, proteins, lipoproteins, and deoxyribonucleic acid (DNA) [28]. The antioxidant potential observed in *T. pedata*seed kernel oil can be attributed to the phenolic compounds commonly found in vegetable oils [29]. Other than the beneficial health effects of oil antioxidants on humans, they help in protecting the oils from autoxidation [29]. This aspect implies that *T. pedata*seed kernel oil can maintain the integrity of its quality by resisting oxidative degradation.

5. CONCLUSIONS

*Telfairiapedata*seed kernels have high oil yield which can be economical to exploit commercially. The physicochemical properties of *T. pedata*seed kernel oil determined by this research were found to be within the set limits by FAO for unrefined edible vegetable oils. The oil can, therefore, be used in various industrial applications for edible purposes. Determination of linoleic acid as the most abundant FA in *T. pedata*seed kernel oil leads to the conclusion by this research that the oil is a rich source of EFAs. Nonetheless, the consumption of the oil should be limited due to the health concerns associated with the relatively high amounts of saturated fatty acids found in the oil. These saturated fatty acids in the oil can, however, be reduced or minimized by means of refining the oil. The antioxidant activity observed in *T. pedata*seed kernel oil allows the oil to be used for the purpose of fighting oxidative stress in humans. The *in-vivo* antioxidant potential of the oil should be investigated.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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