UNIVERSITY OF NAIROBI

CHARACTERIZATION OF *TELFAIRIA PEDATA* (SMITH EX SIMS) HOOK., SEED KERNEL OIL FOR NUTRITIONAL VALUE AND ANTIOXIDANT ACTIVITY

BY

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2020

DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of a degree, or publication. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

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DEDICATION

This work is dedicated to my parents and all those who directly or indirectly contributed to its success.

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Lastly, Ms. Josephine Oluoch from the Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology (JKUAT) is acknowledged for guiding me on how to use GC-MS instrument in the institution to generate important data for my research work.

ABSTRACT

Some plant sources such as the seed kernel of *Telfairia pedata* contain edible vegetable oil but due to their limited prevalence around the world, little research has been done about them *Telfairia pedata* (Sm. Ex Sims) Hook seed kernel oil was characterized for its nutritional value as edible vegetable oil. Due to the limited research and availability of this species around the world, it is faced with the danger of extinction. As such, this research not only provides the nutritional information of *T.pedata* seed kernel oil but also has the potential of contributing to its preservation. Standard laboratory procedures were used for analyzing the oil's physicochemical parameters while its fatty acid and vitamin E composition were analyzed using gas chromatography mass spectrometer (GC-MS). The oil's antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The physico-chemical properties of the *T. pedata* seed kernel oil were determined and the results obtained were as follows: calorific value ($MJ.Kg^{-1}$) (44.66±0.32; 44.51±0.13; P = 0.5009), density (g/cm^3) (0.8439±0.0002; 0.8440±0.0002; P = 0.2524), moisture content (%) (0.0592±0.0140; 0.0517±0.0174; P = 0.5922), refractive index (1.455±0.001; 1.454±0.001; P = 0.2879), specific gravity (0.8752±0.0003; 0.8755±0.0002; P = 0.3039), viscosity (mPa.s) (55.6616±0.0465; 55.6675±0.1586; P = 0.9563), acid value (mg KOH/g) (0.6352±0.0330; 0.6235±0.0326; P = 0.6847), iodine value (g I_2/100g) (23.0058±2.2473; 24.2081±0.9563; P = 0.4565), peroxide value (meq O_2/Kg) (0.9641±0.2021; 1.2173±0.3124; P = 0.3235) and saponification value (mg KOH/g) (157.3007±2.4640; 157.2805±5.5738; P = 0.9958) for the *n*-hexane and mechanical pressing extracts, respectively. Monounsaturated, polyunsaturated and saturated fatty acids composition of the oil was found to be 0.13 %, 48.54 % and 51.33 %, respectively in the *n*-hexane and 0.13 %, 45.9 % and 53.97 %, respectively in the mechanical pressing extracts. The individual fatty acids found in *T. pedata* seed kernel oil included; myristic acid (14:0), palmitoleic acid (16:1), palmitic acid (16:0), margaric acid (17:0), linoleic acid (18:2), stearic acid (18:0), 10,13-octadecadienoic acid (18:2), 18-methylnonadecanoic acid (20:0) and behenic acid (22:0). Micronutrients such as vitamin E and squalene were found to be present in *T. pedata* seed kernel oil. The *n*-hexane and the mechanical pressing extracts of the oil were found to have half maximal inhibitory concentration (IC_{50}) values of 18.05 mg/mL and 17.36 mg/mL, respectively, which were significantly lower compared to that of ascorbic acid (IC_{50} 2.406 μg/mL), used as the standard. In conclusion, this study found out that *T. pedata* seed kernel oil has good nutritional value and recommends the oil to be commercialized as an edible vegetable oil.

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LIST OF ABBREVIATIONS AND ACRONYMS

ALA	Alpha-linolenic acid
BPX5	5 % Phenyl polysilphenylene-siloxane
CHD	Coronary heart disease
CVD	Cardiovascular disease
DPPH	2,2-diphenyl-1-picrylhydrazyl
EFAs	Essential fatty acids
FAO	Food and agriculture organization
FDA	U.S. food and drug administration
FT-IR	Fourier transform infrared
GC-MS	Gas chromatography–mass spectrometry
HDL	High-density lipoprotein
HPLC	High-performance liquid chromatography
LDL	Low-density lipoprotein
MUFAs	Monounsaturated fatty acids
PUFAs	Polyunsaturated fatty acids
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RSA	Radical scavenging activity
SFAs	Saturated fatty acids
T2D	Type 2 diabetes
UFAs	Unsaturated fatty acids

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CHAPTER ONE

INTRODUCTION

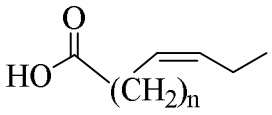
1.1 Background of the Study

Vegetable oils form an important part of our daily diet (Viola and Viola, 2009). Sunflower oil and coconut oil are some of the major vegetable oils in the commercial place today, among others (Gunstone, 2011). Focus has only been directed to certain vegetable oils depending on the prevalence of their plant sources around the world. Some plant sources such as the seed kernel of *Telfairia pedata* (Smith ex Sims) Hook. contain edible vegetable oil but due to their limited prevalence around the world, little research has been done about them.

Telfairia pedata (Smith ex Sims) Hook. is a vine plant and it belongs to the Cucurbitaceous family with 98 genera and 975 species. *T. pedata* species is indigenous to East Africa and is locally referred to as "Mukwini" by the Meru community in Kenya while the Wachaga people of Tanzania refer to the plant as "Mkwema" (Vossen and Mkamilo, 2007). Although the *T. pedata* seeds are not true nuts, they are called 'Oyster nuts' due to their relatively big size and the rough texture of their shells. The Oyster nuts are found within the *T. pedata* gourd-like fruits. In their characterization, these fruits weigh approximately 30 pounds and can grow to a length of between 12 to 20 inches, with a diameter ranging from 8 to 12 inches. The seed kernels from the Oyster nuts are edible while raw, cooked, or roasted. A single fruit by the *T. pedata* plant can have between 80 to 170 seeds (Vossen and Mkamilo, 2007).

T. pedata is not widely distributed around the world thus the plant has not been exhaustively exploited in terms of research. The present study determined the properties and composition of

the *T. pedata* seed kernel oil to establish its nutritional value as edible vegetable oil. Vegetable oils can contain both healthy and unhealthy fatty acids. Omega-3 **(1)** and omega-6 **(2)** fatty acids are considered healthy while the saturated fatty acids are considered unhealthy especially when consumed in higher amounts. Establishing the composition of *T. pedata* seed kernel oil in terms of fatty acids will provide crucial nutritional information regarding the oil. Vegetable oils are also known to contain minute components such as vitamin E **(3)** which is known to be a natural antioxidant. The present study also determined the antioxidant potential of the oil for its use as an alternative to synthetic antioxidants which have been associated with health concerns such as cancer, mutagenicity and toxicity effects (Shahidi, 2000).



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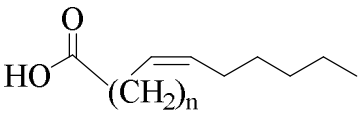

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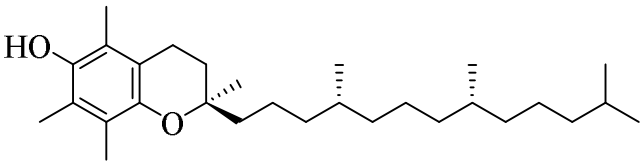
Where n = number of CH_2 bonds



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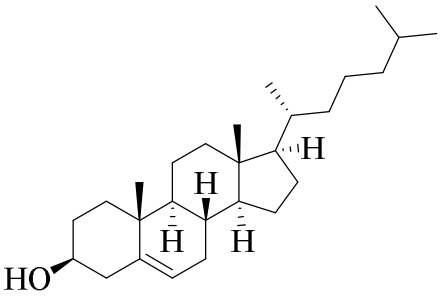
1.2 Statement of the Problem

There is lack of nutritional information about the *T. pedata* seed kernel oil. This information includes the physicochemical parameters, fatty acid composition, micronutrient, and antioxidant potential of the oil. The physicochemical parameters of vegetable oils determine whether the oil is compliant with set out standards for it to be considered edible. The fatty composition provides information about the essential fatty acids (EFAs) present in the oil, as well as the saturated fatty

acids (SFAs) present in the oil. EFAs have to be obtained from diet since humans do not synthesize them while SFAs are known to raise the cholesterol **(4)** levels in human beings, especially when consumed in large amounts. Micronutrients in vegetable oils serve as major nutritional components to human diet due to their associated health benefits. Antioxidants in food substances help in fighting oxidative stress in humans. Lack of this nutritional information about *T. pedata* seed kernel oil means that locals have been consuming the oil without making an informed decision.

T. pedata species is only found in East Africa. This limited distribution of the species around the world, the limited available research about it, and the lack of any commercial use or application implies that it if faced with possible extinction. For instance, one species of the *Telfairia* genus (*Telfairia batesii*) is known to be extinct (Ajayi *et al*., 2007). As such, a study like the current one has the potential of highlighting the usefulness of the species hence encouraging its preservation.



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1.3 Objectives

1.3.1 General Objective

To characterize *Telfairia pedata* (Smith ex Sims) Hook. seed kernel oil for nutritional value and antioxidant potential.

1.3.2 Specific Objectives

1. To determine the physico-chemical properties of *T. pedata* seed kernel oil.
2. To determine the fatty acid composition of the oil using Gas chromatography-Mass Spectrometer (GC-MS).
3. To determine the vitamin E **(3)** composition of the oil using GC-MS/HPLC.
4. To determine the antioxidant potential of the oil.

1.4 Research Hypothesis

This research hypothesizes that *T. pedata* seed kernel oil conforms to set out quality standards for edible vegetable oils and that it is a rich source of nutrition.

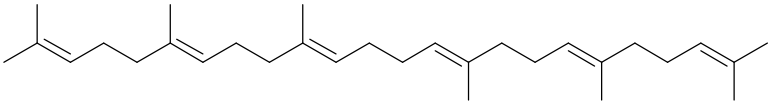
1.5 Justification

This study is important because it provides the nutritional information of the *T. pedata* seed kernel oil. By doing so, consumers of this oil will be able to make informed decision about the choice of their diet. For instance, the physicochemical parameters of the oil determine whether it conforms to the set out standards for edible vegetable oils. The fatty acid composition of the oil enables one to know the levels of both the EFAs and SFAs present. EFAs are required for good health while SFAs are harmful when consumed in high amounts. The micronutrient composition of the oil (vitamin E **(3)** and squalene **(5)**) is a measure of its nutritional importance. The information about the antioxidant potential of the *T. pedata* seed kernel oil establishes whether

the oil can be used as a natural antioxidant hence substituting the synthetic antioxidants such as Butylated hydroxytoluene **(6)** and butylated hydroxyanisole **(7)** which are associated with various health concerns on human beings (Pokorný, 2007).

Due to the lack of research data on the nutritional information and antioxidant potential of the *T. pedata* seed kernel oil, this work is important as it fills this gap in knowledge.

The species *T. pedata* is faced with possible extinction in the coming decades. This fact is occasioned by the limited distribution of the species around the world as it is only found in East Africa. Another contributor to this threat is the fact that the species has not been exploited commercially. To highlight the danger of extinction facing *T. pedata*, the genus *Telfairia* is only left with two species after the extinction of *Telfairia batesii* (Ajayi *et al*., 2007). As such, this research is important as it highlights the important of *T. pedata*, an aspect which has the potential of bolstering the preservation efforts of the species.



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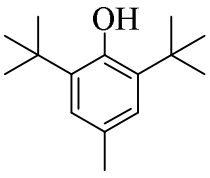

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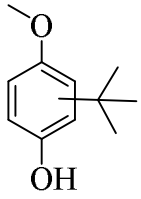

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CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical Information

2.1.1 The Family Cucurbitaceae

Telfairia pedata (Smith ex Sims) Hook. belongs to the family Cucurbitaceae, which is a gourd family that consists of flowering plants (Vossen and Mkamilo, 2007). The family Cucurbitaceae consists of 98 genera and more than 970 species of ornamental, as well as food plants. The common members of the family include pumpkins, melons, cucumbers, squashes and gourds, which are annual or perennial herbs mostly occurring in temperate and tropical areas (Schaefer and Renner, 2011).

2.1.2 The Genus *Telfairia*

The genus *Telfairia* is named after Charles Telfair, an Irish botanist (Ajayi *et al*., 2007). The genus *Telfairia* is little known scientifically and it belongs to the family of Cucurbitaceae. Nonetheless, the genus is known to be nutritionally important (Ajayi *et al*., 2007). The genus *Telfairia* comprises of two main species namely, *Telfairia occidentalis* Hook. F. and *Telfairia pedata* (Smith ex Sims) Hook. The species *Telfairia pedata* is also referred to as *Fevillea pedata* Sims. The common names assigned to both *T. pedata* and *T. occidentalis* species are oyster nut and fluted pumpkin, respectively (Ajayi *et al*., 2007). Another extinct species, *Telfairia batesii* Keraudren, which occurred in both Cameroon and Equatorial Guinea as a wild plant, is known to be the third member of the genus *Telfairia* (Ajayi *et al*., 2007). Traditionally, both *Telfairia*

occidentalis and *Telfairia pedata* have been used for their nutritious seeds as a source of food and vegetable oil for cooking (Kayode and Kayode, 2011).

2.1.2.1 Ethnomedicinal uses of the Genus *Telfairia*

Apart from being used as a source of food, the two species of the *Telfairia* genus have been used as part of folk medicine in the traditions of different communities. Nonetheless, the genus *Telfairia* is primarily used as a food genus thus it has limited accounts of ethnomedicinal uses. A notable example is the use of the seed kernel oil of *T. pedata* for the treatment of both rheumatism and stomach troubles in East Africa as reported by Vossen and Mkamilo (2007). The leaves of *T. occidentalis* have been used traditionally in Nigeria to treat anaemia. The leaf juice of *T. occidentalis* has been used traditionally by both pregnant women and patients suffering from anaemia, who drink it as a tonic to strengthen the blood (Eseyin *et al*., 2014).

2.1.2.2 Phytochemistry of the Genus *Telfairia*

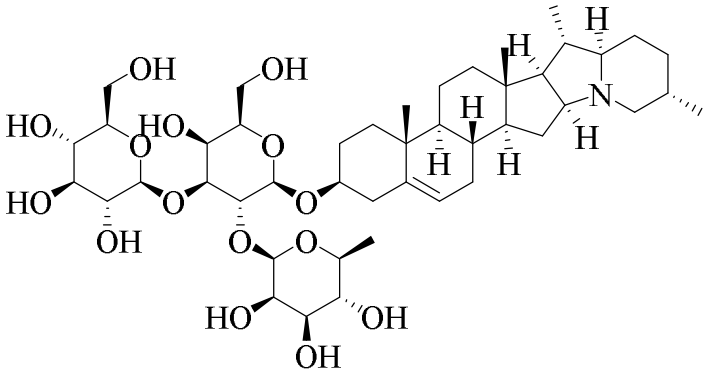
The genus *Telfairia* has a considerably diverse phytochemistry which is comprised of several classes of natural products. These natural products fall under the category of saponins, triterpenes, flavonoids and alkaloids (Eseyin *et al*., 2014). The phytochemistry of the *Telfairia* genus is attributed to the bioactivity of its species. *T. occidentalis* has been widely investigated for its bioactivity. Table 1 shows the bioactivity of the *T. occidentalis* species as reported by Imosemi (2018) and Eseyin *et al*. (2014).

Table 1: Bioactivity of *Telfairia occidentalis* species

Species	Part of plant investigated	Bioactivity	References
T. occidentalis	Seed	Antioxidant	Imosemi, 2018
T. occidentalis	Leaf	Antidiabetic	Imosemi, 2018
T. occidentalis	Seed	Anticancer	Eseyin *et al*., 2014
T. occidentalis	Root	Antimalarial	Eseyin *et al*., 2014
T. occidentalis	Leaf	Antimicrobial	Eseyin *et al*., 2014

2.1.2.2.1 Saponins from *Telfairia* genus

Saponins are a group of phytochemicals which naturally occur in most herbs and vegetables. The saponin solanine **(8)** has been isolated from the *Telfairia* genus (Eseyin *et al*., 2014).



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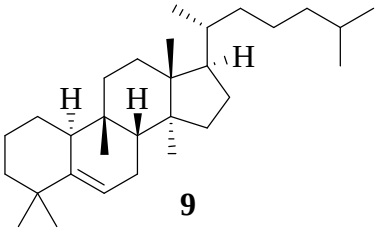
2.1.2.2.2 Triterpenes from *Telfairia* genus

Triterpenes are a group of natural compounds which primarily occur in animals, plants and fungi. Common triterpenes isolated from the *Telfairia* genus include squalene **(5)**, cucurbitacin **(9)**, and hopane **(10)** (Eseyin *et al*., 2014).



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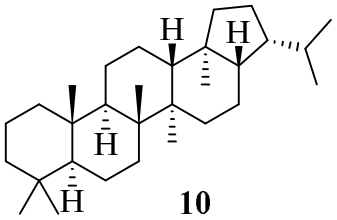

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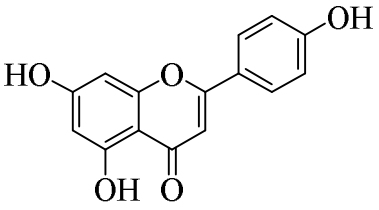
2.1.2.2.3 Flavonoids from *Telfairia* genus

Phytonutrients such as flavonoids occur in almost all fruits and vegetables (Panche *et al*., 2016). Flavones such as apigenin **(11)** and luteolin **(12)** and a flavonol such as quercetin **(13)** have been isolated from the *Telfairia* genus (Eseyin *et al*., 2014).



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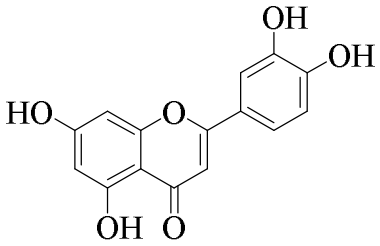

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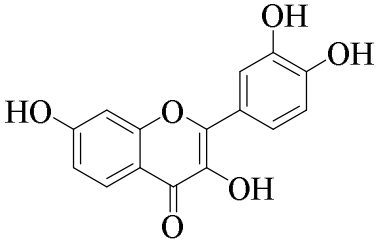

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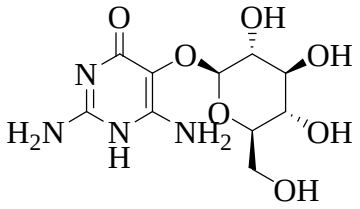
2.1.2.2.4 Alkaloids from *Telfairia* genus

Alkaloids are a group of compounds with an origin from plants animals, fungi and bacteria and are known to have pronounced physiological actions on humans. The alkaloid vicine **(14)** has been isolated from the *Telfairia* genus (Eseyin *et al*., 2014).



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2.1.3 *Telfairia pedata* (Smith ex Sims) Hook.

T. pedata is a fast-growing vine plant that occurs in the family of Cucurbitaceae and in the order of Cucurbitales. Oysternut is the common name given to the plant. Other common names for this plant include 'Zanzibar olivine' and 'Queen's nut' (Duke, 2000). Table 2 shows the scientific classification of *T. pedata*.

Table 2: The scientific classification of *Telfairia pedata* (Smith ex Sims) Hook. (Duke, 2000)

Scientific Classification	
Kingdom	Plantae
Class	Magnoliopsida
Order	Cucurbitales
Family	Cucurbitaceae
Genus	*Telfairia*
Species	*T. pedata*

The plant is dioecious, meaning that it does not experience self-fertilization since it has distinct male and female individual organisms. The male and the female reproductive structures of *T. pedata* are found on separate plants rather than on a single plant. This aspect of dioecy is described by Vossen and Mkamilo (2007), who argues that about 6 percent of angiosperm species are entirely dioecious. Figure 1 shows the *T. pedata* (Smith ex Sims) Hook. plant.

Figure 1: Pictorial representation of *Telfairia pedata* plant (Duke, 2000).

2.1.3.1 Geographic Distribution

T. pedata is natively found in Tanzania, northern Mozambique, as well as in the islands of Zanzibar and Pemba. In Kenya, the plant is exotic (Duke, 2000). The geographical distribution of *T. pedata* in Africa is as shown in Figure 2.

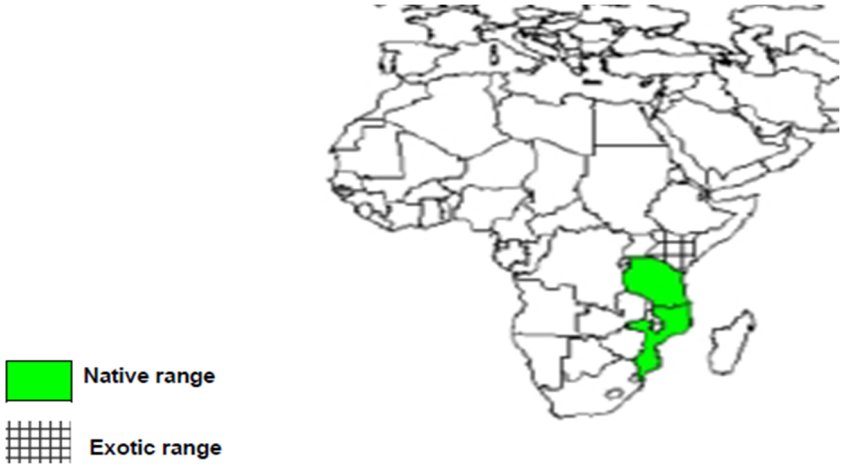


Figure 2: Geographic distribution of *Telfairia pedata* around the world (Duke, 2000).

2.1.3.2 Description

T. pedata can grow up to 30 m long. The plant has an initial herbaceous stem, which becomes woody with age and can reach a diameter of up to 10 cm. The leaves for this plant are arranged in a spiral manner. The plant bears fruits that can weigh up to 15 kg. The fruits contain numerous seeds that are enclosed in a fibrous, reticulate sheath (Duke, 2000). Figure 3 shows both the fruit and the seeds of *T. pedata* plant.

(a) (b)

Figure 3: *Telfairia pedata* fruit and seeds (Duke, 2000). Fig 3a shows the fruit of *Telfairia pedata* while Fig 3b shows the seeds of *Telfairia pedata*.

2.1.3.3 Ethnobotanical Uses

Telfairia pedata seed kernels are edible and can be eaten raw, cooked, or roasted. The kernels have similar tastes to almonds or Brazil nuts (Vossen and Mkamilo, 2007). In East Africa, where *T. pedata* is native, the seed kernels are used to facilitate lactation in nursing mothers. The East African people also use the oil from *T. pedata* seeds as medicine for both rheumatism and stomach troubles (Vossen and Mkamilo, 2007). In Tanzania, *T. pedata* seeds are used as a tonic after childbirth by the Wachagga people. The press-cake remaining after oil extraction is known to be rich in protein and therefore it makes valuable feed for livestock (Duke, 2000). A study conducted by Agoi (2010) established that the unmodified lignocellulosic biomass of *T. pedata* is a cost effective adsorbent material for the removal of dye from dye wastewaters.

2.1.3.4 Extinction Threat to the Species

The *Telfairia pedata* species is faced with possible extinction due to a number of aspects. The fact that the plant is dioecious means that it does not experience self-fertilization hence it requires both the male and female plants for production (Vossen and Mkamilo, 2007). This aspect is a discouragement to many farmers who are opting for other self-fertilizing crops. Another factor contributing to the possible extinction of *Telfairia pedata* is its limited geographical distribution around the world as the species is only found in East Africa (Duke, 2000). The threat of extinction to *Telfairia pedata* is amplified by the fact that one species of the *Telfairia* genus (*Telfairia batesii*) is known to be extinct (Ajayi *et al*., 2007).

2.2 Fatty Acids (FAs) in Vegetable Oils

According to Orsavova *et al*. (2015), fatty acids form the primary component of vegetable oils. Different fatty acids impact human health differently. Essential fatty acids (EFAs), for instance,

are considered vital for good health, hence have to be provided in the diet since the human body is unable to produce them (Orsavova *et al*., 2015). Saturated fatty acids (SFAs) have the potential to cause heart diseases. Table 3 shows the percent composition of polyunsaturated fatty acid (PUFA), monounsaturated fatty acid (MUFA) and SFA in selected vegetable oils.

Table 3: Summary composition of fatty acids in selected vegetable oils (Frančáková *et al*., 2015)

Summary composition of FA [%]	**Sesame oil**	**Flaxseed oil**	**Olive oil**	**Rapeseed oil**	**Pumpkin seed oil**
PUFA	41.24	71.13	7.69	29.23	46.57
MUFA	41.05	17.69	75.72	60.16	32.87
SFA	16.24	9.92	14.22	7.33	19.07

2.2.1 Saturated Fatty Acids (SFAs)

In terms of structure, saturated fatty acids contain no double bonds. According to Kabara (2008), palmitic acid (16:0) **(15)** and stearic acid (18:0) **(16)** are some of the saturated fatty acids commonly found in human diet. Most vegetable oils have low proportions of saturated fatty acids relative to other fatty acids. This aspect is exemplified in Table 3, as outlined by Frančáková *et al*. (2015). Also, a research conducted by Orsavova *et al*. (2015) revealed that the composition of SFA, MUFA and PUFA in several vegetable oils and represented by palmitic acid (16:0) **(15)**, oleic acid (18:1) **(17)** and linoleic acid (18:2) **(18)** ranged from (4.6 % - 20.0 %), (6.2 % – 71.1 %) and (1.6 % - 79.0 %), respectively.

Certain vegetable oils, however, contain higher levels of SFAs. According to Elson and Alfin (1992), these vegetable oils are collectively referred to as ‘tropical oils’. Table 4 shows a summary of the tropical oils’ fatty acid composition as outlined by Elson and Alfin (1992).

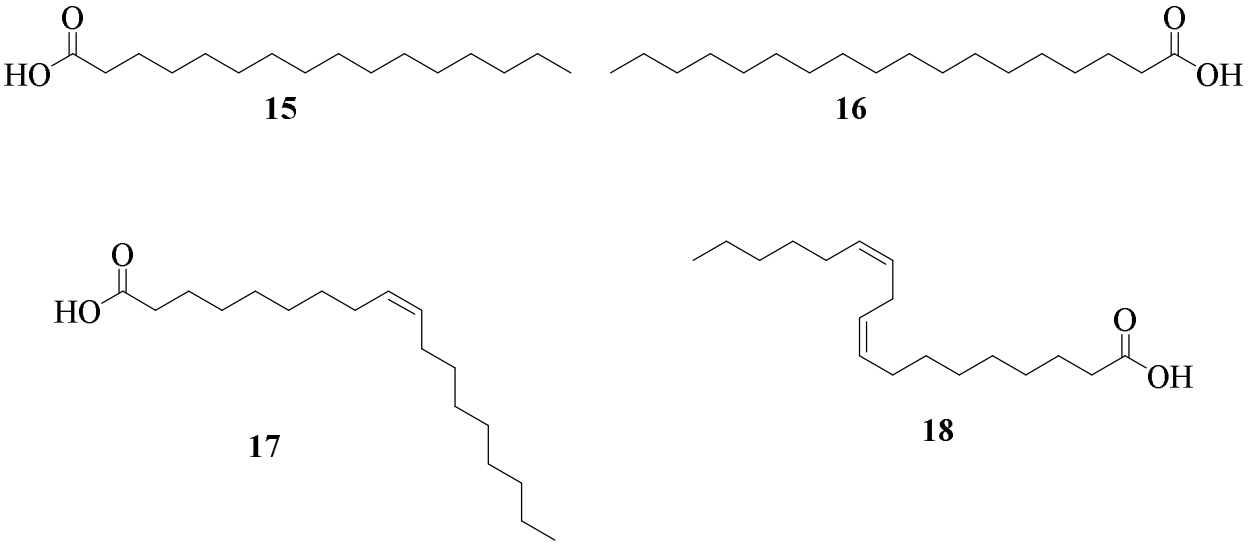


Table 4: Summary composition of fatty acids in tropical oils (Elson and Alfin, 1992)

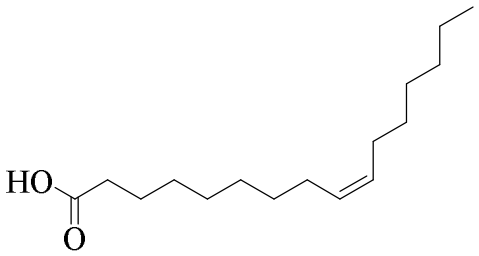
Summary composition of FA [%]	**Palm oil**	**Palm kernel oil**	**Coconut oil**
PUFA	10.6	2.5	2.0
MUFA	40.0	13.4	6.0
SFA	49.5	82.2	92.0

2.2.2 Monounsaturated Fatty Acids (MUFAs)

MUFAs contain a hydrocarbon chain with a single double bond (Gillingham *et al*., 2011). Most vegetable oils contain MUFAs in greater proportions than the SFAs, except for tropical oils (Orsavova *et al*., 2015). Table 4 shows the percent composition of MUFAs of some of the common vegetable oils as outlined by (Frančáková *et al*., 2015). Olive oil, peanut oil and canola oil are examples of edible vegetable oils notably high in MUFAs concentration in the proportion of 78.4 %, 58.5 % and 59.5 %, respectively (Kostik *et al*., 2013).

According to Schwingshackl and Hoffmann (2014), palmitoleic acid (16:1) **(19)** is one of the most common monounsaturated fatty acids in daily nutrition, along with oleic acid (18:1) **(17)**

and vaccenic acid (18:1) **(20)**. Dietary monounsaturated fatty acids such as palmitoleic acid (16:1) **(19)**, when used as a replacement for dietary saturated fatty acids, have been associated with improved blood lipids related to type 2 diabetes (T2D) and cardiovascular disease (CVD) (Hayes and Benson, 2016). The risk of both CVD and T2D gets greatly reduced by the replacement of dietary SFA with MUFA in healthy adults which in turn improves insulin responsiveness in insulin-resistant and T2D subjects (Hayes and Benson, 2016). Mediterranean diet is known to be rich in MUFAs. According to Hayes and Benson (2016), studies show that the Mediterranean eating patterns among people with diabetes improve CVD risk factors such as blood pressure and lipids, thus reducing CVD events and stroke.



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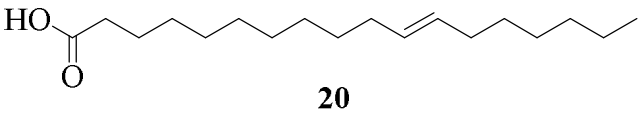

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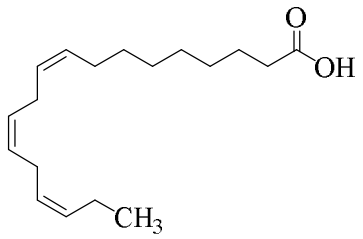
2.2.3 Polyunsaturated Fatty Acids (PUFAs)

PUFAs possess a hydrocarbon backbone that contains more than one double bond (Benatti *et al*., 2004). Some of the most essential classes of PUFAs to human health include the n-3 **(1)** and n-6 **(2)** fatty acids (FAs). Different vegetable oils have different PUFAs concentrations. Sunflower oil and soybean oil are some of the vegetable oils rich in PUFAs at the proportions of 59.5 % and 57.5 %, respectively (Kostik *et al.*, 2013). Other vegetable oils, however, contain notably low amounts of PUFAs. Examples include coconut oil and olive oil with PUFAs proportions of 0.5

% and 7 %, respectively (Kostik *et al*., 2013). Common examples of PUFAs include α - linolenic acid (ALA) (18:3) **(21)** and linoleic acid (18:2) **(18)** (Orsavova *et al*., 2015).

According to Ander *et al* (2003), PUFAs induce significant beneficial cardiovascular effects to humans when consumed as part of the diet. Such beneficial effects by PUFAs in humans are achieved via different mechanisms of action such as different effects on the heart, vasculature and blood. Due to this aspect, PUFAs can function as a therapeutic modality (Ander *et al*, 2003). According to Bentsen (2017), PUFAs also act as signal molecules, either directly or via metabolites.



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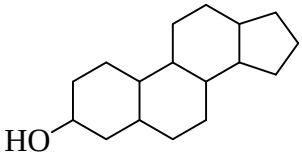
2.2.4 Health Aspects of Dietary Fatty Acids

EFAs are known to be beneficial to human health (Benatti *et al*., 2004). The EFAs have to be provided in the diet since humans are unable to synthesize them (Benatti *et al*., 2004). EFAs are associated with several health benefits such as good cardiovascular health (by decreasing cardiovascular disease factors), reduced inflammation, stress management and weight management (Di Pasquale, 2009). The main EFAs that occur in vegetable oils include the omega-3 **(1)** and omega-6 **(2)** fatty acids.

Unlike the EFAs, SFAs are known to be unhealthy when consumed in high amounts. According to Williams (2000), the SFAs have been proven to raise the levels of blood cholesterol **(4)** in human beings. Cholesterol **(4)** is a sterol **(22)** molecule that occurs in animal cell membranes as part of the structural components. Cholesterol **(4)** can be classified as either Low-density lipoprotein (LDL) cholesterol **(4)** or high-density lipoprotein (HDL) cholesterol **(4)**, depending on different carrier molecules in the body (Mensink *et al*., 2003). One of the important functions of cholesterol **(4)** is in the biosynthesis of vitamin D **(23)** and steroid **(24)** hormones where it serves as a precursor molecule. Nonetheless, not all SFAs are responsible for increasing low density lipoprotein (LDL) cholesterol **(4)** concentrations in the human body. For instance, Williams (2000) reports that the SFAs shown to raise the LDL cholesterol **(4)** concentrations in the human body mainly include lauric acid (12:0) **(25)**, myristic acid (14:0) **(26)** and palmitic acid (16:0) **(15)**. However, stearic acid (18:0) **(16)** has been reported to be a non-contributor to increasing the LDL cholesterol **(4)** concentrations in the human body (Williams, 2000).

According to Di Pasquale (2009), dietary fats are useful in ensuring good health and normal metabolism of the body. These dietary fats are necessary for the proper absorption of fat-soluble vitamins such as vitamins A, D, E and K, as well as their transportation and function. Other important uses of dietary fats are in the production of cellular components, hormones, as well as other essential components necessary for body functioning (Di Pasquale, 2009). In particular, linoleic acid (18:2) **(18)** undergoes a biological conversion when consumed to produce eicosanoids (hormone-like biochemical compounds) (Di Pasquale, 2009). These compounds act as signaling molecules and have various biological functions. Eicosanoids aid in vital organ

functioning and intracellular processes such as regulating blood pressure and inflammation. They also help in the regulation of gastrointestinal and kidney functions (Di Pasquale, 2009).



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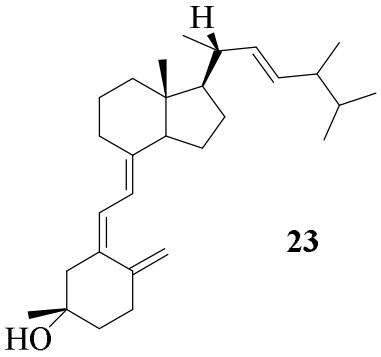

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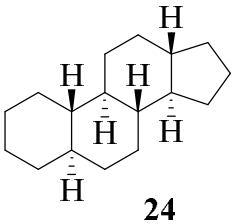

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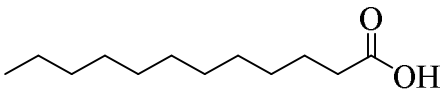

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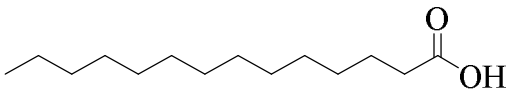

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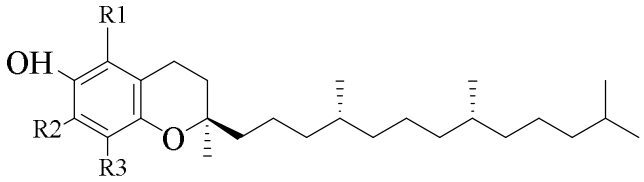
2.3 Micronutrients in Vegetable Oils

According to Fine *et al*. (2016) and Popa *et al*. (2014), vegetable oils can have different micronutrients such as vitamin E **(3)** and squalene **(5).** These micronutrients are vital to human health.

Vitamin E **(3)** occurs naturally in many vegetable oils (Gimeno *et al*., 2000). Tocopherols **(27)**, as well as tocotrienols **(28)**, are the main components that constitute vitamin E **(3)** and are known to be natural antioxidants (Grilo *et al*., 2018). Table 5, as reported by Kamal and Andersson (1997) shows the tocopherol **(28)** composition of various vegetable oils.



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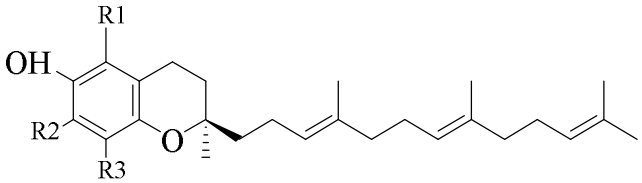

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Table 5: Various vegetable oils and their tocopherol composition (Kamal and Andersson, 1997)

Oil type	**Tocopherols (ppm)**			
	α – T	**β – T**	**γ – T**	**δ – T**
Sunflower	671	23	4	-
Groundnut	141	4	131	9
Soybean	116	17	578	263
Cottonseed	403	2	383	4
Olive	96	6	12	-
Palm	377	1	4	-
Rapeseed	180	-	340	-
Linseed	-	-	588	6

(-) means not detected

Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) **(5)** is an organic compound which is commonly found in shark liver oil but also occurs in vegetable oils (Popa *et al*., 2014). The compound is known to have health and pharmacological benefits when consumed as part of the human diet. It also has cosmetic applications due to its role in skin hydration (Popa *et al*., 2014). Several vegetable oils have been reported to have a considerable amount of squalene **(5)** in their composition. According to Popa *et al*. (2014), olive oil was the first plant oil to be discovered as having squalene **(5)** as part of its composition. Some of the edible vegetable

oils such as corn oil and soybean oil have been determined to have squalene **(5)** concentrations of 27.4 mg/100 g and 9.9 mg/100 g, respectively (Popa *et al*., 2014).

The presence of unsaturated fatty acids in the structure of many vegetable oils makes them susceptible to oxidative degeneration. Vegetable oils thus have natural mechanisms for countering this oxidative degeneration. These natural mechanisms involve the use of micronutrients known to be antioxidants. According to Fine *et al*. (2016), some of the micronutrients with antioxidant activity that can be found in oils include vitamin E **(3)** and squalene **(5)**.

Apart from the primary role of preventing the vegetable oils from autoxidation, these micronutrients are very useful to the human body for helping in the inhibition of the lipid peroxidation process which is associated with various health problems. Lipid peroxidation is the oxidative degeneration of lipids in the human body caused by oxidative stress (Peña *et al*., 2019). According to Peña *et al*. (2019), oxidative stress is the imbalance between free radicals and antioxidants in the human body. Reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are the common forms of free radicals and they are predominantly produced in the mitochondria from molecular oxygen and nitrogen (Peña *et al*., 2019). Oxidative stress can result in the oxidation of biomolecules such as protein, DNA, as well as lipids. The mechanism of lipid peroxidation is presented in Figure 4.

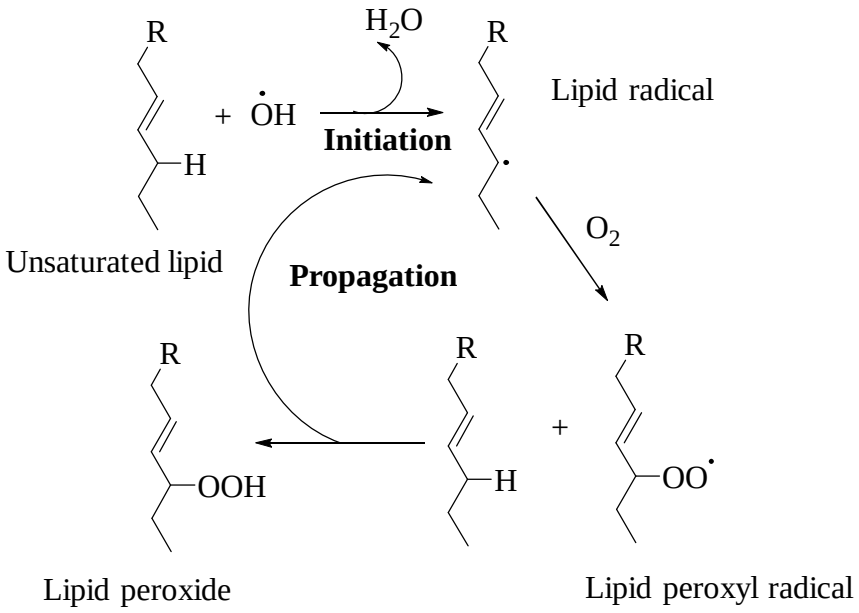


Figure 4: Mechanism of lipid peroxidation (Gaschler and Stockwell, 2017)

Lipid peroxides are known to mediate pathological states such as cancer, inflammation and neurodegenerative disease (Gaschler and Stockwell, 2017). In the human body, lipids are tasked with the main function of protecting the integrity of cellular membranes. The assembly, composition, structure and dynamics of lipid membranes can be altered by lipid peroxidation (Gaschler and Stockwell, 2017). Lipid peroxides are highly reactive compounds hence capable of propagating further ROS generation. The lipid peroxides can also degrade into reactive compounds capable of crosslinking DNA and proteins, therefore, inflicting health problems (Gaschler and Stockwell, 2017).

2.4 Vegetable Oils as Natural Antioxidants

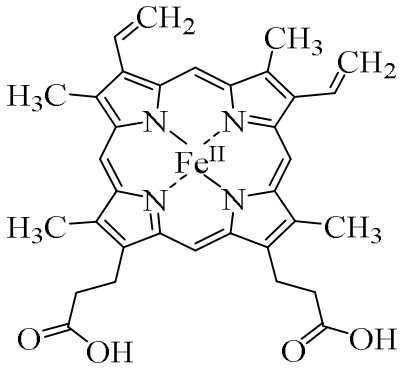
Vegetable oils are known to possess some antioxidant potential attributed to some of their micronutrient components (Marina *et al.*, 2009). Nonetheless, different vegetable oils have different antioxidant activities. For instance, the antioxidant potential of sunflower oil (34 μg of

Trolox equivalent/mg of oil) significantly differs from that of sesame oil (122 μg of Trolox equivalent/mg of oil), as reported by Dhavamani *et al*. (2014). Consumption of food substances with antioxidant activity helps humans in coping with oxidative stress.

Oxidative stress in human beings is caused by the presence of free radicals. These free radicals contain unpaired electrons which make them highly reactive. During a chemical reaction, these radicals can either donate or accept electrons thus behaving as oxidants or reductants, respectively. Nitric oxide radical, hydroxyl radical, as well as hydrogen peroxide, constitute some of the most common free radicals in many disease states, which contain oxygen atoms (Valko *et al*., 2007). In biological systems, these free radicals have the potential of damaging several molecules including proteins, carbohydrates, deoxyribonucleic acid (DNA) and lipids. According to Dröge (2002), homeostatic disruption can result from the damage caused to certain macromolecules by the free radicals. While free radicals can attack all kinds of molecules in the human body, DNA, lipids and proteins are the major targets (Halliwell, 1994).

Free radicals in our bodies can be generated via normal metabolic processes (Pham *et al*., 2008). They can also be derived from other external sources which include but not limited to air pollutants, smoking of cigarettes, as well as exposure to chemicals. Free radical formation in the human body takes place continuously, owing to the cells' enzymatic and non-enzymatic reactions (Halliwell, 2009). According to Halliwell (2009), some of the enzymatic reactions which involve free radical formation include; the ones involved in the synthesis of prostaglandins, such as in the cytochrome P-450 **(29)** system and phagocytosis. The free radical formation can also result from non-enzymatic reactions involving oxygen and other organic compounds (Pham *et al*., 2008).

For the proper physiological function in human beings, free radicals should be in balance with antioxidants (Lobo *et al.*, 2010). Oxidative stress can result in human beings given that the inherent ability of the body in regulating free radicals become overwhelmed by their presence (Lobo *et al.*, 2010). Oxidative stress is known to cause many diseases due to its adverse alteration of DNA, proteins, or lipids, which are biologically important molecules. The human body can be assisted in coping with oxidative stress through the intake of external sources of antioxidants. These external sources can be of natural or synthetic origin. Nonetheless, various synthetic antioxidants have been suspected to have some chronic health effects on human beings (Poljsak *et al.*, 2013). Butylated hydroxytoluene **(6)** together with butylated hydroxyanisole **(7)** constitutes the main synthetic antioxidants. Butylated hydroxyanisole **(7)** is composed of both 2-*tert*-butyl-4-hydroxyanisole **(30)** and 3-*tert*-butyl-4-hydroxyanisole **(31)**, which are isomeric compounds. Due to the health concerns associated with the synthetic antioxidants natural antioxidants are preferred.



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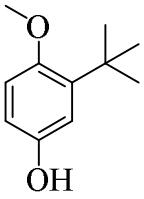

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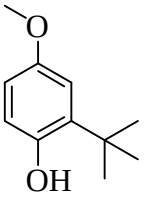

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2.5 Gap in Knowledge

Extensive research has been done on various commercial edible vegetable oils present in the market today. Such common vegetable oils include sunflower oil and coconut oil, among others. Literature about their fatty acid composition, micronutrient composition, as well as antioxidant activity, is widely available through many publications. Edible vegetable oil plants, such as *T. pedata* seed kernel oil have received little attention due to their limited geographical distribution and availability throughout the world. There is limited literature concerning the fatty acid composition, micronutrients content and antioxidant potential of *T. pedata* seed kernel oil, thus, the current research addresses this gap in knowledge.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample Collection and Identification

In Kenya, *Telfairia pedata* plant is commonly found in the Mt. Kenya region. *Telfairia pedata* seeds as well as sample leaves (for helping in species identification) were collected from Mugirirwa Sub-Loacation in Tharaka-Nithi County, Kenya. The map of the sampling site is shown in Figure 5. Figure 6 shows the photograph taken of *Telfairia pedata* during sample collection. After collection, the sample was taken to the University of Nairobi Herbarium in the School of Biological Sciences (SBS) for identity verification. The identities of the seeds together with sample leaves were verified to be those of *Telfairia pedata* plant by a botanist, Mr. Patrick Mutiso from the School of Biological Sciences (SBS), University of Nairobi. The identity verification of the sample was allocated a voucher number BMI/2019/001 and deposited at the University of Nairobi, School of Biological Sciences Herbarium.

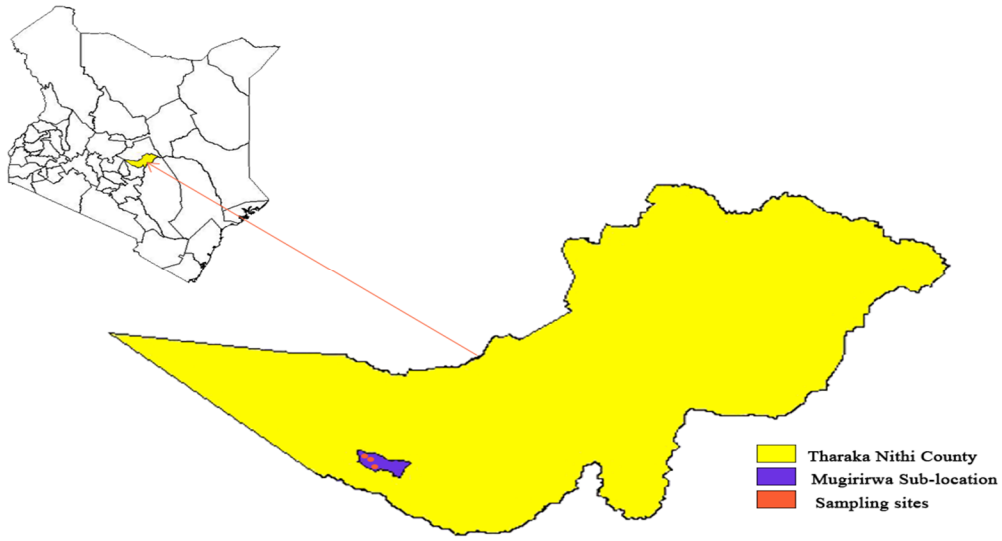


Figure 5: Map showing the sampling site for *Telfairia pedata*.

Figure 6: Photo of *Telfairia pedata* during sample collection.

3.2 Sample Preparation

T. pedata seed kernels are contained in enclosed shells which are usually very rigid. To obtain the seed kernels, the shells were cracked using a mechanical press machine in the Science Workshop, College of Biological and Physical Sciences (Figure 7). After the decortication process, the seed kernels were pounded into smaller pieces by the use of a mortar and pestle. This process was necessary to increase the kernels' surface area for optimum Soxhlet extraction of the oil. The oil obtained was directly subjected to physical analysis. For chemical analysis, standard laboratory procedures were used to prepare the sample before analysis.

Figure 7: Sample decortication by way of mechanical cracking.

3.3 Oil Extraction and Percent Yield Determination

Both the Soxhlet extraction and mechanical pressing were used as methods of extraction. Extraction of the oil using the Soxhlet method was done in triplicate using a modified protocol as outlined by Manirakiza *et al*. (2001). For every 50 g of the sample, 500 mL of *n*-hexane was used. The extraction temperature was set at 80 ℃ and the process took about 4 hours to complete. After extracting the oil using the soxhlet method, the solvent was recovered by use of rotary evaporation, after which the oil's weight was determined. The percent yield of the oil was determined using Equation (1).

$$\%\, oil\ yield = \frac{weight\ of\ extracted\ oil}{weight\ of\ seed\ kernel\ used} \times 100 \qquad (1)$$

For the mechanical pressing method of oil extraction, the seeds were pressed using a hydraulic pressing machine designed for that purpose. The oil was collected in a clean glass container as it oozed from the extracting machine (Figure 8).

The extracted oil was kept in an amber glass container with an airtight lid to prevent both light and oxidative degradation of the oil as it awaited analysis.

Figure 8: Oil extraction using mechanical pressing method.

3.4 Sample Analysis

After the extraction process of the oil, further sample preparation was conducted to enable the determination of the oil's physico-chemical properties, fatty acid composition, vitamin E **(3)** and antioxidant activity.

3.4.1 Physico-chemical Properties

Analysis of the oil's physico-chemical properties was done using standard laboratory procedures.

3.4.1.1 Calorific Value

A modified method outlined by Gravalos *et al*. (2008) was used in this research.

Before being placed in a bomb calorimeter, the oil sample was weighed to determine its weight. It was then enclosed in a capsule of known calorific value and the ignition of the sample was done electrically after the bomb was sealed with oxygen. The bomb was surrounded by a water bath whose temperature change was monitored as the complete oxidation of the sample took place. The change in temperature was recorded using a thermometer. Equation (2) was used for the determination of the oil's calorific value.

$$CV_s = \frac{C \times \Delta T - (E_1 + E_2 + E_3)}{M} \quad \ldots\ldots (2)$$

CVs = Sample's calorific value

C = Calorimeter's heat capacity

ΔT = Final rise in temperature (°C)

E_1 = Thread's calorific value

E_2 = Ignition wire's calorific value

E_3 = Capsule's calorific value

M = Sample's mass (grams)

3.4.1.2 Viscosity

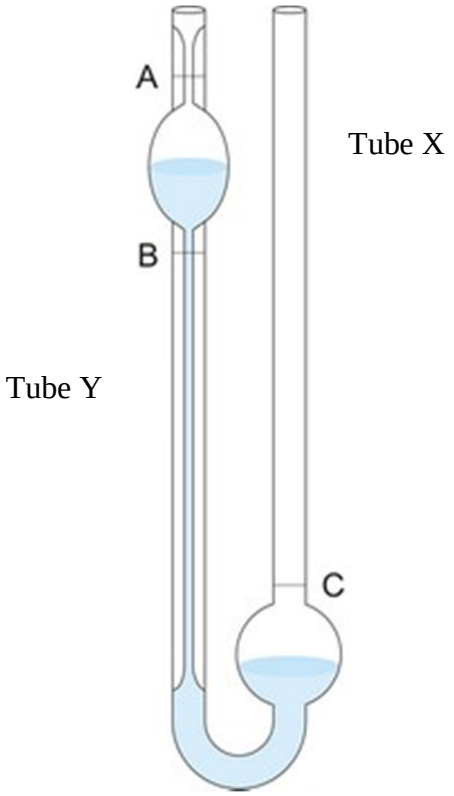


Figure 9: Ostwald's viscometer (glossary.periodni.com).

The method outlined by Ghani *et al*. (2018) was used in this research with modification as described below:

A previously washed and completely dried Ostwald's viscometer, as shown in Figure 9, was filled with the oil sample through tube X to slightly above the mark C. This filling was achieved using a long pipette to minimize the wetting of the tube above the mark. Suction pressure was applied at tube Y until the oil reached slightly above point A. The oil was allowed to flow down by gravity by releasing the suction pressure. The oil took a specific amount of time to flow from point A to point B and that time was recorded using a stop-watch.

Deionized water, a material of known viscosity, was used in calibrating the Ostwald's viscometer. Since the viscosity of water is known, the viscosity of the oil was calculated using Equation (3) as shown below.

$$\eta_1 = \eta_2 . \frac{\rho_1 t_1}{\rho_2 t_2} \quad \text{..... (3)}$$

η_1 = viscosity coefficient of sample

η_2 = viscosity coefficient of water

ρ_1 = density of sample

ρ_2 = density of water

t_1 and t_2 is the time in seconds taken by the oil and water, respectively, to flow from point A to point B.

3.4.1.3 Density

The method outlined by Bamgboye and Adejumo (2010) was used in this research with slight modification.

A clean and dry pycnometer was first weighed by use of an analytical balance to determine its precise weight. The oil sample was then added to the pycnometer to fill the stopper's capillary hole. Any oil overflow on the pycnometer was wiped dry before taking its new weight. The new weight was then obtained to allow the determination of the oil's weight as follows:

Weight of oil = (weight of pycnometer + oil) – weight of pycnometer

Equation (4) was used in the determination of the oil's density value.

$$density\ of\ oil = \frac{Weight\ of\ oil\ used}{volume\ of\ oil\ used} \quad \text{..... (4)}$$

The procedure was done in triplicate to obtain an average value of the oil's density.

3.4.1.4 Specific Gravity

The method outlined by Bamgboye and Adejumo (2010) was used in this research with slight modification.

The weight of an empty pycnometer was first taken and recorded using an analytical balance. The pycnometer was then filled with distilled water to the stopper's capillary hole. Any water overflow on the pycnometer was wiped dry before taking its new weight. The new weight was then obtained to allow the determination of the weight of water as follows:

Weight of water = (weight of pycnometer + water) – weight of pycnometer

The oil's weight was also determined using the same pycnometer procedure.

Weight of oil = (weight of pycnometer + oil) – weight of pycnometer

The specific gravity of the oil sample was calculated using Equation (5).

$$Specific\ gravity\ of\ oil = \frac{Weight\ of\ oil}{Weight\ of\ equal\ volume\ of\ water} \quad \text{..... (5)}$$

The procedure was done in triplicate to obtain an average specific gravity value of the oil.

3.4.1.5 Refractive Index

The method described by Kumar and PadmaJeeth (2005), was used in this research.

The prisms of the Abbe refractometer used were thoroughly cleaned using a piece of cotton wool. A drop of the oil was then removed from the reservoir using a glass tube and put on the lower prism, where it was spread evenly using a fingertip. The prism was then closed and the prism holder rotated using the refractive index (RI)-adjustment knob, looking through the eyepiece. A half-circular dark spot appeared while the knob was being turned slowly. The boundary between the bright and dark spots was adjusted to coincide with the cross wires. The focusing knob was adjusted until the boundary became visibly clear. The oil's refractive index was then read from the refractometer scale using the eye-piece.

3.4.1.6 Moisture Content

The moisture content of *T. pedata* seed kernel oil was determined using a modified method (Odoom *et al*., 2014).

An empty crucible was washed, dried in the oven and then its weight recorded. 5 g of the oil sample was then put in the crucible. The oil sample in the crucible was then oven dried at 105 ℃ for about five hours. A desiccator was then used to allow both the sample and the crucible cool down at room temperature without reabsorbing moisture. After the sample and the crucible totally cooled down, their weight was measured and recorded. Equation (6) enabled the determination of the oil's moisture content.

$$Moisture\ Content\ (\%) = \frac{(WD_C + WW_S) - (WD_C + WD_S)}{WW_S} \times 100 \quad \ldots\ldots (6)$$

Where:

WD_C = dried crucible's weight

WW_S = wet sample's weight

WD_S = dried sample's weight

3.4.1.7 Iodine Value

The method outlined by Yildiz *et al*. (2019) was used in this research with slight modification.

A 250 mL conical flask was used to mix 0.25 g of the oil sample with 10 mL of chloroform. The formed solution was topped up with a 30 mL Hanus solution (Iodine Monobromide) before using a Parafilm to completely seal the flask. 30 minutes were allowed for complete reaction to occur. Preparation of the Hanus solution was done by mixing glacial acetic acid (1 L) with bromine water (3 mL) and then dissolving 18.2 g of iodine into the solution. After leaving the solution for about half an hour, a solution of distilled water (100 mL) with 10mL potassium iodide (15 %) was added and shaken to mix well. The contents of the flask were then titrated using 0.1 N sodium thiosulfate until a yellow color appeared. 2 mL starch solution (1 %) was added, turning the solution into a blue color. Further titration was then performed till the disappearance of the blue color. For the blank, this procedure was repeated. Equation (7) enabled the determination of the oil's iodine value.

$$Iodine\ value\ of\ sample = \frac{(B-S)\times N\times 0.1269\times 100}{W}\ \left(\frac{gI_2}{100g}\right) \quad \text{..... (7)}$$

Where:

B = $Na_2S_2O_3$ titre for the blank

N = $Na_2S_2O_3$ Normality

S = $Na_2S_2O_3$ titre for the sample

W = sample's weight in grams

0.1269 = equivalent weight of iodine

3.4.1.8 Saponification Value

T. pedata seed kernel oil's saponification value was determined following a method outlined by Ghani *et al*. (2018).

A clean and dry beaker was used to mix 2 g of the oil sample with 0.5 N alcoholic KOH (25 mL). A round-bottomed flask was used for the transferring of the mixture before being attached to a reflux condenser for refluxing. 30 minutes were allowed for the refluxing of the mixture, then allowed to cool down. Titration of the resulting solution once it cooled down was done using 0.5 N HCl. Phenolphthalein indicator was used to mark the titration endpoint. For the analysis of the blank, the same procedure was used. The saponification value of *T. pedata* seed kernel oil was then calculated using Equation (8).

$$Saponification\ Value\ = \frac{(B-S)\times 28.05}{Weight\ of\ Sapmle\ (g)} = mg\ KOH/g \quad \text{..... (8)}$$

B = Blank titre

S = Sample titre

3.4.1.9 Acid Value

The method outlined by Odoom *et al*. (2014) was used in this research with slight modification.

A neutral solvent was first prepared. The solvent was made of 1 % phenolphthalein solution (1 mL), 25 mL diethyl ether, as well as 95 % alcohol (25 mL). 0.1 N alkali (KOH) was then used to neutralize the solvent.

A clean and dry 250 mL conical flask was used to prepare a solution between 5 g of the oil sample and 50 mL of the neutral solvent. The formed solution was titrated using 0.1 N potassium hydroxide. A small amount of phenolphthalein (2-3 drops) was used to indicate the reaction endpoint. The contents of the flask were shaken constantly during titration. Equation (9) enabled the determination of the oil's acid value.

$$Acid\ value\ \left(\frac{mg\ KOH}{g}\right) = \frac{Titre\ value \times Normality\ of\ KOH \times 56.1}{Weight\ of\ sample\ (g)} \quad \text{..... (9)}$$

3.4.1.10 Peroxide Value

The method outlined by Ghani *et al*. (2018) was used in this research.

A 250 mL conical flask was used to prepare a mixture between 5 g of the oil sample and 25 mL of acetic acid/chloroform solution (3:2). Saturated potassium iodide solution (1 mL) was added to the prepared mixture and stirred until complete dissolution of the oil. The contents of the flask were then incubated at room temperature for 1 hour in the dark. 75 mL of distilled water was added into the flask after the incubation period to form the final solution. The final solution was titrated with 0.01 N sodium thiosulphate while using starch solution (1 %) as the indicator. Equation (10) enabled the determination of the oil's peroxide value.

$$PV = \frac{N \times V}{W} \quad \text{..... (10)}$$

Where:

PV = Peroxide value of the oil sample in milliequivalents (meq) of peroxide O_2 per kilogram of the oil sample

N = Normality of $Na_2S_2O_3$

V = Volume of $Na_2S_2O_3$ used

W = Weight of sample in Kg

3.4.1.11 FTIR Characterization

For the Fourier Transform Infrared **(**FTIR) characterization of *T. pedata* seed kernel oil, no further sample preparation was required. A Shimadzu IRAffinity-1S FTIR Spectrometer was used. After initializing and running a background spectrum for the FTIR instrument, only a small portion of the oil sample was required for analysis. A small drop of the oil sample was placed in the sample compartment of the FTIR instrument and analyzed for its different absorption bands. The analysis was done in the 500-4000 cm^{-1} range.

3.4.2 Fatty Acid Composition

The method used by Ichihara and Fukubayashi (2010) to prepare fatty acid methyl esters was used in this research.

HCL and methanol were mixed in a beaker to form a 1:3 ratio solution. For every 10 mL of HCL used, 30 mL of methanol was used. 5 mL of the oil sample was then introduced to the solution before being transferred into a round-bottomed flask which was then connected to a condenser for refluxing. Methanol was present in the solution in excess to favor the forward reaction hence achieving a high yield of fatty acid methyl esters (FAMEs) during the trans-methylation process. The solution was refluxed for about four hours at 100^oC after which the top lipid layer was separated from the bottom polar layer. Traces of HCL and methanol from the lipid layer were removed by use of distilled water (Bateni *et al*., 2017). The lipid layer and the aqueous layer were further separated to allow the drying of the lipid layer over anhydrous sodium carbonate.

The resulting supernatant formed the final sample which was analyzed by use of a gas chromatography – mass spectrometer (GC-MS).

990 µL of high performance liquid chromatography (HPLC) grade *n*-hexane was used to dilute 10 µL of the oil sample in an auto-sampler vial for analysis using a GC-MS. A Shimadzu QP 2010-SE instrument was used. The instrument had an auto-sampler coupled to it. The carrier gas (Ultrapure Helium) flow rate was set at 1mL/minute. The column used was non-polar (BPX5), 30 m long with 0.25 mm ID and 0.25 µm film thickness. 60 ℃ (1 minute); 10 ℃ /min to 250 ℃ (10 minutes) was the temperature programming applied to the GC. Other temperatures were set as following: 200 ℃ (injection temperature), 250 ℃ (interface temperature) and 200 ℃ (EI ion source). 50-500 *m/z* range was the scanning range for the analysis. Each analysis took a total of 45 minutes.

3.4.3 Vitamin E Composition

T. pedata seed kernel oil was analyzed for its vitamin E **(3)** composition using a modified method outlined by Matthäus and Musazcan (2015).

N-hexane (5 mL) was used to dissolve 5 mg of the oil sample. The obtained solution was injected into a GC-MS instrument to detect vitamin E **(3)** and any other micronutrient present in the oil such as squalene **(5)**. A Thermo Scientific™ ISQ™ 7000 instrument was used. The instrument utilized a capillary column which had the following dimensions: 100 m long, 0.25 mm ID and 0.2 µm film thickness. Hydrogen was used as the carrier gas and the flow rate was set at 36 cm/s. The temperature set up used for the analysis included 250 ℃ for both the injector and the detector. For the GC, the initial temperature was set at 155 ℃ which was then heated to 220 ℃ at a rate of 1.5 ℃/min.

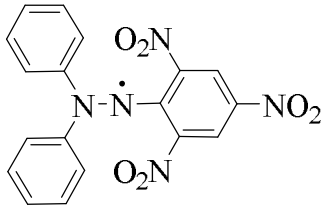
3.4.4 Antioxidant Activity

As outlined by Wang *et al*. (2012), a preliminary thin layer chromatography (TLC) test was carried out to establish whether the *Telfairia pedata* seed kernel oil extracts possessed any antioxidant potential. Both the *n*-hexane and the mechanical pressing extracts were first spotted on a TLC plate. A solvent system of *n*-hexane-chloroform (1:1) was then used to develop the spots. The TLC plate was then air-dried for 10 minutes and compounds detected under a UV lamp. The R_f values of the compounds were calculated by obtaining the ratio of the distance moved by the compounds and the distance moved by the solvent. The plate was then sprayed with 2,2-diphenyl-1-picrylhydrazyl (DPPH) **(32)** reagent (1 mM) to detect any color change from purple to yellow, an indication of antioxidant activity.

A modified method outlined by Prevc *et al*. (2013) which uses DPPH (2,2-diphenyl-1-picrylhydrazyl) **(32)**, a stable free radical, to measure the hydrogen donating ability of a substance was then used to quantify the antioxidant potential of the oil.

Ethyl acetate (10 mL) in a 25 mL conical flask was used to dissolve 12.5 g of the oil sample. The solvent was then used to top up the solution to the mark hence making a 500 mg/mL solution. Serial dilutions (dilution factor of 2) from this solution were made to produce concentrations of 250 mg/mL, 125 mg/mL, 62.5 mg/mL and 31.25 mg/mL. In 2 mL of each newly prepared solution, 0.1 mM solution of DPPH **(32)** in ethyl acetate (1 mL) was added. A blank which represented 0.0 mg/mL of the oil sample was prepared by mixing 2 mL of ethyl acetate with 1 mL 0.1 mM DPPH **(32)** solution. Solutions were incubated in darkness for 30 minutes for the reaction to occur. The resulting solutions were then analyzed using a UV-Vis spectrophotometer at 517 nm. Ascorbic acid was used as the standard (methanol was used as a solvent). Since

ascorbic acid has a higher antioxidant activity, the working concentrations were 100 μg/mL, 50 μg/mL, 25 μg/mL, 12.5 μg/mL and 6.25 μg/mL. Equation (11) was used to compute the percentage amount of DPPH **(32)** inhibited. The IC_{50} values for the sample extracts and ascorbic acid were determined by performing a nonlinear regression analysis using the GraphPad Prism 7.03 software.



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$$\%\ RSA = \frac{(A_{con} - A_{test})}{A_{con}} \times 100 \quad \text{..... (11)}$$

A_{con} = Absorbance of the control.

A_{test} = Absorbance of the sample/standard.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Percent Weight of *Telfairia pedata* Seed Kernels from Different Seeds with Different Weights

After sample collection, it was observed that the seeds of *Telfairia pedata* were not uniform in size and weight. The same observation was also true for the obtained seed kernels. This study was therefore conducted to determine the weight of seed kernels from different seeds of different weight so as to establish how they are related. The weight of the seed kernels was expressed as a percentage of the total weight of the seed. The weight of the investigated seeds ranged from 4.64 g to 13.22 g. Figure 10 shows the non-linear relationship observed between the weight of the seed kernel and the total weight of the seed.

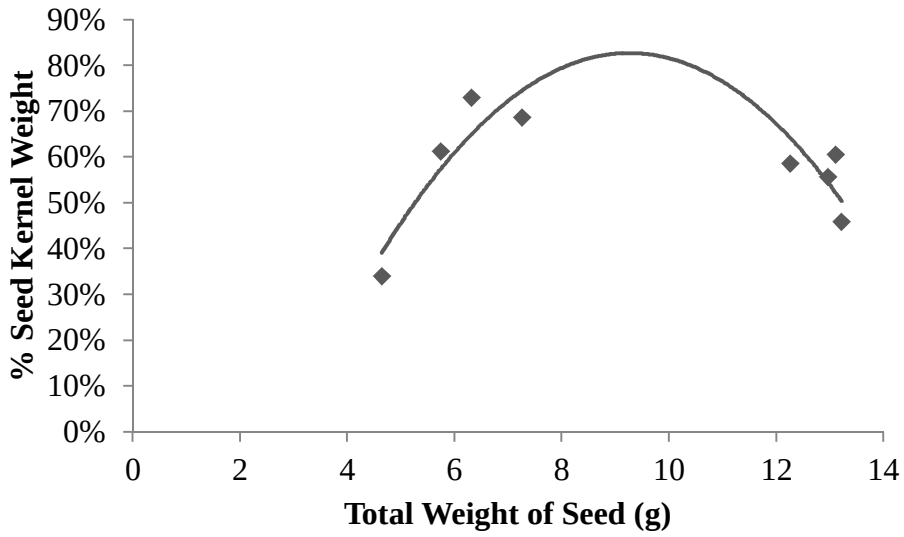


Figure 10: Plot of % seed kernel weight from *Telfairia pedata* seeds with different weights.

This observation can be attributed to the fact that *T. pedata* seeds with the lowest weight have smaller seed kernels while those with the highest weight have a high weight of the seed shell which reduces the percentage weight of the seed kernels produced.

4.2 Percent Oil Yield of *Telfairia pedata* Seed Kernel

4.2.1 Soxhlet Extraction Method

The average % oil yield of *T. pedata* seed kernel obtained via Soxhlet extraction is presented in Table 6.

Table 6: Average % oil yield of *Telfairia pedata* seed kernel obtained via Soxhlet extraction.

Weight of Sample Used (g)	Weight of Oil Obtained (g)	% Oil Yield	Average % Oil Yield ±SD
100.45	65.70	65.41	66.35±3.17
102.47	71.60	69.87	
50.78	32.39	63.78	

Each value is a mean ± standard deviation of three determinations. SD means standard deviation

As shown in Table 7, more than 50 % of the weight of *T. pedata* seed kernel is composed of oil. This finding implies that the seed kernel of *Telfairia pedata* plant is richly endowed with vegetable oil. In comparison to other locally available plant seed oils, the average % oil yield of *T. pedata* seed kernel closely relates to that of sesame seed oil (57 %) as reported by El-Hamidi and Zaher (2018). Nonetheless, the seed kernel of *T. pedata* contains more oil than those of *Lepidium sativum* L. seed oil (21.54 %) and *Telfairia occidentalis* Hook F. seed oil (13 %), as reported by Diwakar *et al*. (2010) and Agatemor (2006), respectively.

4.2.2 Mechanical Pressing Extraction Method

Table 7 presents the average % oil yield of *T. pedata* seed kernel extracted by mechanical pressing method.

Table 7: Average % oil yield of *Telfairia pedata* seed kernel obtained via mechanical pressing method.

Weight of Sample Used (g)	Weight of Oil Obtained (g)	% Oil Yield	Average % Oil Yield ±SD
513.27	161.97	31.56	32.74±4.23
410.56	120.01	29.23	
550.72	206.19	37.44	

Each value is a mean ± standard deviation of three determinations. SD means standard deviation

Unlike the case of Soxhlet extraction, this method of extraction recorded a significantly lower percentage yield of the oil. There was a significant difference between the values obtained at p = 0.05 ($p = 0.0004 < 0.05$) (Appendix 1)

4.2.3 Comparison Between the two Methods of Oil Extraction

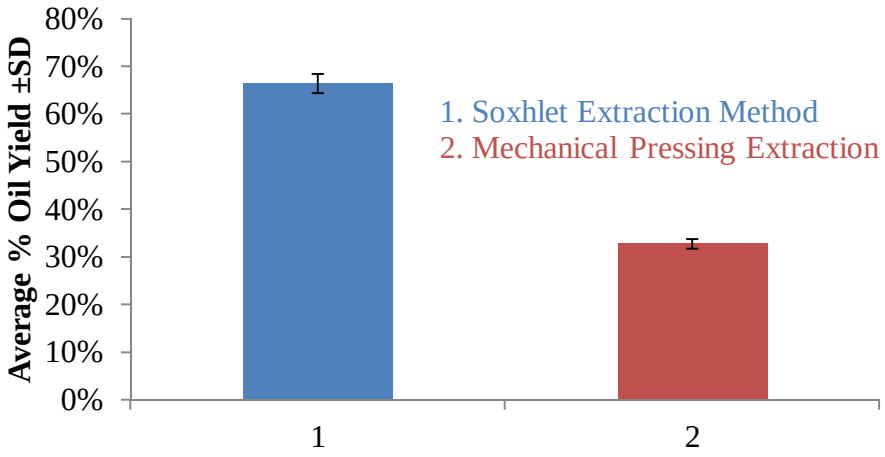
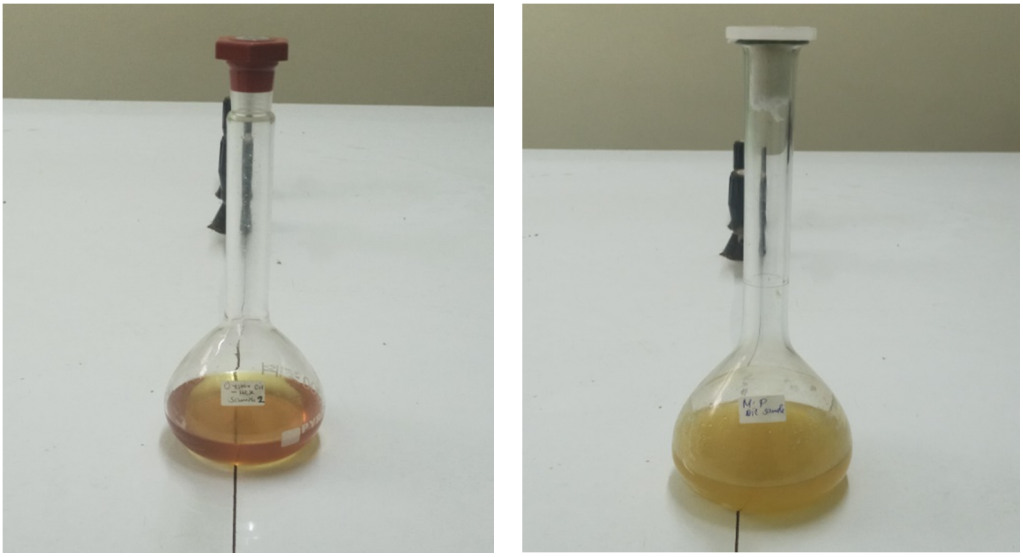


Figure 11: Comparison between Soxhlet extraction method and mechanical pressing method of oil extraction.

Figure 11 shows that the Soxhlet method is able to extract oil from *T. pedata* seed kernel with a higher efficiency than the mechanical pressing method. In this case, the efficiency difference between the two methods is by a factor of two. This fundamental difference between the two methods of oil extraction is due to the fact that organic solvent is able to penetrate the sample matrix and dissolve nearly all the oil contained in it hence achieving an extraction efficiency of about 99 % (Onyeji and Momoh, 2010). In comparison, the mechanical pressing method is not able to drive out all the oil contained in the sample. A comparison between the two samples of *T. pedata* seed kernel oil extracted by the two extraction methods is shown in Figure 12.



(a) (b)

Figure 12: Comparison between *Telfairia pedata* seed kernel oil extracted using (a) Soxhlet method and (b) mechanical pressing method.

4.3 Physico-chemical Parameters

The quality of *Telfairia pedata* seed kernel oil was determined by analyzing its various physico-chemical parameters.

4.3.1 Physical Parameters

Both the *n*-hexane and mechanical pressing extracts were examined separately. Table 8 presents the values for the physical parameters obtained in this research for the two extracts.

Table 8: Physical parameters of *Telfairia pedata* seed kernel oil

Physical Parameter	**Value**			
	***n*-Hexane Extract (**±SD)	**Mechanical Pressing Extract (**±SD)	**P value**	**FAO Limit**
Density (g/cm^3)	0.8439±0.0002	0.8440±0.0002	0.2524	-
Moisture Content (%)	0.0592±0.0140	0.0517±0.0174	0.5922	< 0.2
Refractive Index	1.455±0.001	1.454±0.001	0.2879	-
Specific Gravity	0.8752±0.0003	0.8755±0.0002	0.3039	-
Viscosity (mPa.s)	55.6616±0.0465	55.6675±0.1586	0.9563	-

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"

The Food and Agriculture Organization (FAO), a body of the United Nations that issues international food standards, does not have general set limits for all crude vegetable oils regarding their physical parameters except for the moisture content. As a result, this research compared the obtained physical parameters values of *T. pedata* seed kernel oil with other known edible vegetable oils.

4.3.1.1 Density

The density of any given substance is presented as the ratio of the mass of that substance to its unit volume (Gooch, 2011). This research determined the density of *T. pedata* seed kernel oil *n*-hexane extract to be 0.8439±0.0002 g/cm^3 while that of the mechanical pressing extract as

0.8440±0.0002 g/cm^3. There was no significant difference between the values obtained at p = 0.05 (p = 0.2524 > 0.05) (Appendix 2). Relative to safflower oil, soybean oil and sesame oil with density values of 0.940, 0.916 and 0.912 (g/cm^3), respectively as reported by Katkade *et al*. (2018), *T. pedata* seed kernel oil is slightly less dense. This low density of the oil is attributed to its low moisture content as determined by this research, which implies that the oil can stay for long without microbial degradation.

4.3.1.2 Moisture Content

According to Gooch (2011), the moisture content of a material is measured as the content of water that the material contains. It is, therefore, presented as the percentage of weight loss when the material is dried at more than 100 °C until a constant weight is obtained. This research found out that the moisture content of *T. pedata* seed kernel oil *n*-hexane extract was 0.0592±0.0140 % while that of the mechanical pressing extract was 0.0517±0.0174 %. There was no significant difference between the values obtained at p = 0.05 (p = 0.5922 > 0.05) (Appendix 3). The determined moisture content value of *T. pedata* seed kernel oil closely relates to that of virgin coconut Oil (0.06±0.01 %), as reported by Mansor *et al*. (2012). The determined value is also within the acceptable limit set out by FAO (Codex Alimentarius, 2020), as shown in Table 9. The amount of moisture content present in any given vegetable oil determines how long the oil can stay on shelf while maintaining quality. Higher moisture content can accelerate the deterioration of the oil's quality due to microbial growth (Akinoso *et al*., 2010). The low moisture content value of *T. pedata* seed kernel oil as determined by this research implies that the oil has the potential for a longer shelf life while maintaining the integrity of its quality.

4.3.1.3 Refractive Index

According to Gooch (2011), a medium's refractive index is defined as a measure of light's velocity in a vacuum in relation to its velocity in that medium. It describes the speed with which light propagates through a certain medium (Gooch, 2011). This research found out that the refractive index of *T. pedata* seed kernel oil *n*-hexane extract was 1.455±0.001 while that of the mechanical pressing extract was 1.454±0.001. A refractive index of 1.455 implies that light travels through the oil 1.455 times slower than it does in a vacuum. There was no significant difference between the values obtained at $p = 0.05$ ($p = 0.2879 > 0.05$) (Appendix 4). Since the value of the refractive index of the oil indicates the speed of light through the medium, it, therefore, hints on the density of the medium. In this case, *T. pedata* seed kernel oil has a slightly lower refractive index value of around 1.455 in relation to those of safflower oil (1.469), soybean oil (1.476) and sesame oil (1.468) as reported by Katkade *et al*. (2018). This aspect implies that *T. pedata* seed kernel oil (with a density value of around 0.844 g/cm^3) is not as dense as the aforementioned vegetable oils which have density values of 0.940, 0.916 and 0.912 (g/cm^3), respectively (Katkade *et al*., 2018). This phenomenon is so because the refractive index and the density of any given medium are directly proportional (Gooch, 2011). Nonetheless, *T. pedata* seed kernel oil's refractive index was found to be within the range of other edible oils' refractive indices.

4.3.1.4 Specific Gravity

According to Gooch (2011), a material's specific gravity is the measure of the ratio between its density and that of a reference substance. This research found out that the specific gravity of *T. pedata* seed kernel oil *n*-hexane extract was 0.8752±0.0003 while that of the mechanical pressing

extract was 0.8755±0.0002. There was no significant difference between the values obtained at p = 0.05 (p = 0.3039 > 0.05) (Appendix 5). The oil's specific gravity was measured against deionized water as the reference material whose density value was 0.9641±0.0002 g/cm^3. A specific gravity value which is lower than the density value of the reference material indicates that *T. pedata* seed kernel oil is lighter than water. When compared to the specific gravity values of other edible oils, *T. pedata* seed kernel oil's specific gravity closely relates to those of safflower oil, soybean oil and sesame oil with specific gravity values of 0.917, 0.917 and 0.926 respectively as reported by Katkade *et al*. (2018).

4.3.1.5 Viscosity

A fluid's viscosity is measured by its thickness or stickiness resulting from the fluid's internal friction and is expressed in millipascal seconds (mPa.s) (Gooch, 2011). This research found out that the viscosity of *T. pedata* seed kernel oil *n*-hexane extract was 55.6616±0.0465 mPa.s while that of the mechanical pressing extract was 55.6675±0.1586 mPa.s at room temperature (25 ℃). There was no significant difference between the values obtained at p = 0.05 (p = 0.9563 > 0.05) (Appendix 6). In vegetable oils, a higher viscosity value indicates a high average molecular weight (Diamante and Lan, 2014). The determined viscosity value of *T. pedata* seed kernel oil closely relates to that of garden cress seed oil (55.5 ± 0.37) as determined by Diwakar *et al*. (2010).

4.3.2 Chemical Parameters

Both the *n*-hexane and mechanical pressing extracts were examined separately. Table 9 presents the values of the chemical parameters obtained for the oil. A Fourier transform infrared technique (FT-IR) was also used to characterize the oil's chemical composition.

Table 9: Average values for *Telfairia pedata* seed kernel oil's chemical parameters.

Chemical Parameter	Value			
	***n*-Hexane Extract (±SD)**	**Mechanical Pressing Extract (±SD)**	**P Value**	**FAO Limit**
Calorific Value ($MJ.Kg^{-1}$)	44.66±0.32	44.51±0.13	0.5009	-
Acid Value (mg KOH/g)	0.6352±0.0330	0.6235±0.0326	0.6847	< 4.0
Iodine Value (g I_2/100g)	23.0058±2.2473	24.2081±0.9563	0.4565	-
Peroxide Value (meq O_2/Kg)	0.9641±0.2021	1.2173±0.3124	0.3235	< 15
Saponification Value (mg KOH/g)	157.3007±2.4640	157.2805±5.5738	0.9958	-

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"

4.3.2.1 Calorific Value

Speight (2005) defines the calorific value of a material as the heat due to the material's unit quantity combustion when using a bomb calorimeter. The energy value of any given material such as vegetable oil is directly indicated by its calorific value, which can be expressed in mega joules per kilogram of the material ($MJ.Kg^{-1}$) (Speight, 2005). The calorific values of both the *n*-hexane and mechanical pressing extracts of *T. pedata* seed kernel oil were determined to be 44.66±0.32 $MJ.Kg^{-1}$ and 44.51±0.13 $MJ.Kg^{-1}$, respectively. There was no significant difference between the values obtained at $p = 0.05$ ($p = 0.5009 > 0.05$) (Appendix 7). In comparison with other vegetable oils, *T. pedata* seed kernel oil has a slightly higher calorific value. For instance, Antolin *et al*. (2002) reports the gross calorific value of sunflower to be 40.0 $MJ.Kg^{-1}$. Oliveira and Da Silva (2013) also report the calorific value of soybean oil to be 39.46 $MJ.Kg^{-1}$. The higher calorific value of *T. pedata* seed kernel oil implies that it is a high-calorie food and thus can be recommended for under-weight people.

4.3.2.2 Acid Value

According to Gooch (2011), oil's acid value is measured as the content of that oil existing in the free fatty acid form as opposed to the triglyceride form. The acid value of oils is expressed as the number of milligrams of potassium hydroxide (KOH) neutralized by the free fatty acid present in 1 g of the oil (Gooch, 2011). This research found out that the *T. pedata* seed kernel oil's acid value of the *n*-hexane extract was 0.6352±0.0330 mg KOH/g while that of the mechanical pressing extract was 0.6235±0.0326 mg KOH/g. There was no significant difference between the values obtained at p = 0.05 (p = 0.6847 > 0.05) (Appendix 8). In comparison to other vegetable oils, *T. pedata* seed kernel oil's acid value is comparable to that of Soya bean oil (0.561 mg KOH/g) as reported by Belsare and Badne (2017). Other vegetable oils can have an acid value as high as 1.12 mg KOH/g such as that of coconut oil as determined by Belsare and Badne (2017). According to Codex Alimentarius (2020), FAO recommends acid values below 4.0 mg KOH/g for all unrefined vegetable oils for them to be classified as edible oils (Table 10). The determined acid value of *T. pedata* seed kernel oil falls within this limit.

4.3.2.3 Iodine Value

A material's iodine value, also referred to as the iodine number, is defined by Gooch (2011) as the amount of iodine that can be absorbed by 100 g of that material under arbitrary conditions. A low iodine value of a vegetable oil implies a low degree of unsaturation while a higher one implies a higher degree of unsaturation (Gooch 2011). This research found out that the iodine value of *T. pedata* seed kernel oil *n*-hexane extract was 23.0058±2.2473 g I_2/100g while that of the mechanical pressing extract was 24.2081±0.9563 g I_2/100g. There was no significant difference between the values obtained at p = 0.05 (p = 0.4565 > 0.05) (Appendix 9). Certain

vegetable oils contain a relatively higher value of iodine number due to high unsaturation such as that of Roselle seed oil (111.2 g I_2/100g), as determined by Bamgboye and Adejumo (2010). This aspect implies that the *T. pedata* seed kernel oil's iodine value is considerably low, implying that the oil is not highly unsaturated. This relatively low iodine value of the oil also implies that it is relatively stable to oxidative rancidity due to a slow reaction with atmospheric oxygen. Nonetheless, some other vegetable oils contain even lower iodine values such as that of coconut oil (4.18±0.04 g I_2/100g) as determined by Mansor *et al*. (2012), implying that they are even more saturated than *T. pedata* seed kernel oil.

4.3.2.4 Peroxide Value

Warner and Eskin (1995) defines oil's peroxide value as the amount of oxygen consumed in the reaction that reduces all the unsaturated (C=C) bonds in that oil during autoxidation. This value is an important indication of rancidity that occurs in unsaturated fats and oils (Warner and Eskin, 1995). The peroxide value is, therefore, no doubt an important measure of how much unsaturated oils have sustained oxidative degradation. This research found out that the peroxide value of *T. pedata* seed kernel oil *n*-hexane extract was 0.9641±0.2021 meq O_2/Kg while that of the mechanical pressing extract was 1.2173±0.3124 meq O_2/Kg. There was no significant difference between the values obtained at $p = 0.05$ ($p = 0.3235 > 0.05$) (Appendix 10). The peroxide value of *T. pedata* seed kernel oil compares to those of palm oil (1.05 meq O_2/Kg) and soybean oil (1.17 meq O_2/Kg) as determined by Hasan *et al*. (2016). It is also comparable to that of sunflower oil (1.18 meq O_2/Kg) as determined by Popa *et al*. (2017). Rancidity of vegetable oils is not desirable as it results in the quality deterioration of the oils. The low peroxide value obtained for *T. pedata* seed kernel oil indicates that the oil is relatively stable to autoxidation

thus implying a longer shelf-life. According to Codex Alimentarius (2020), FAO recommends peroxide values below 15 meq O_2/Kg for all unprocessed edible vegetable oils (Table 10). *T. pedata* seed kernel oil's peroxide value is within this limit. Peroxide value is related with iodine value since a low iodine value results into a low peroxide value.

4.3.2.5 Saponification Value

Gooch (2011) defines vegetable oil's saponification value as the measure of certain groups in the oil that are capable of reacting with an alkali. This value is expressed as the amount of potassium hydroxide in terms of milligrams able to react with 1g of the oil. The saponification value is also referred to as the Koettstorfer Number (Gooch, 2011). This research found out that the saponification value of *T. pedata* seed kernel oil *n*-hexane extract was 157.3007±2.4640 mg KOH/g while that of the mechanical pressing extract was 157.2805±5.5738 mg KOH/g. There was no significant difference between the values obtained at $p = 0.05$ ($p = 0.9958 > 0.05$) (Appendix 11). When comparing to the saponification values of coconut oil (170.3 mg KOH/g) and ground nut oil (185.0 mg KOH/g) as reported by Belsare and Badne (2017), *T. pedata* seed kernel oil has a considerably lower value. This lower value implies a higher concentration of long-chain fatty acids in the oil.

4.3.2.6 FT-IR Characterization

Analysis of *T. pedata* seed kernel oil was also done by the use of FT-IR to determine its chemical nature in terms of the bonding present throughout the structure of its molecules. The *n*-hexane extract and mechanical pressing extract spectra resulting from the FT-IR analysis are presented in Figures 13 and 14, respectively.

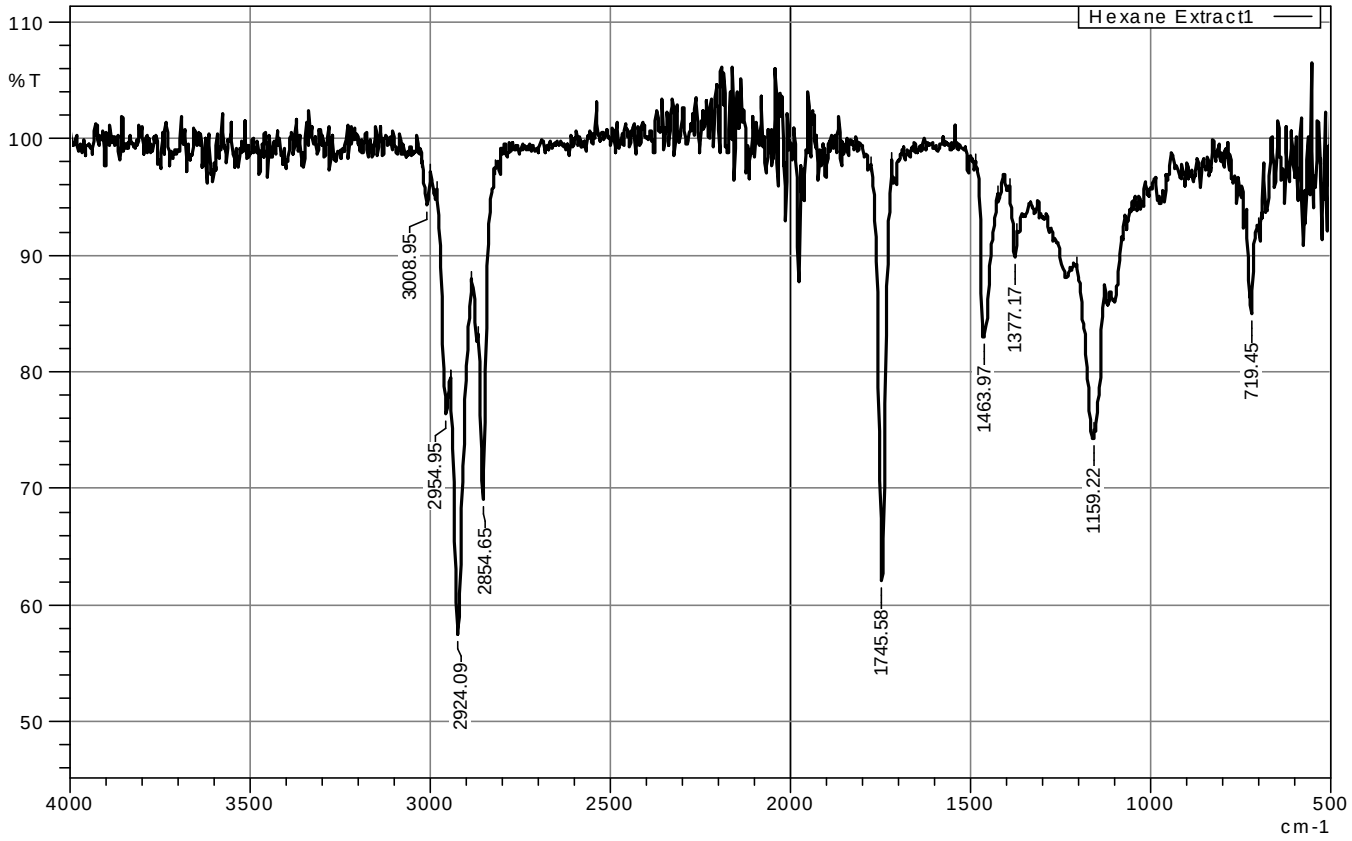


Figure 13: FT-IR spectrum for *T. pedata* seed kernel oil extracted by *n*-hexane

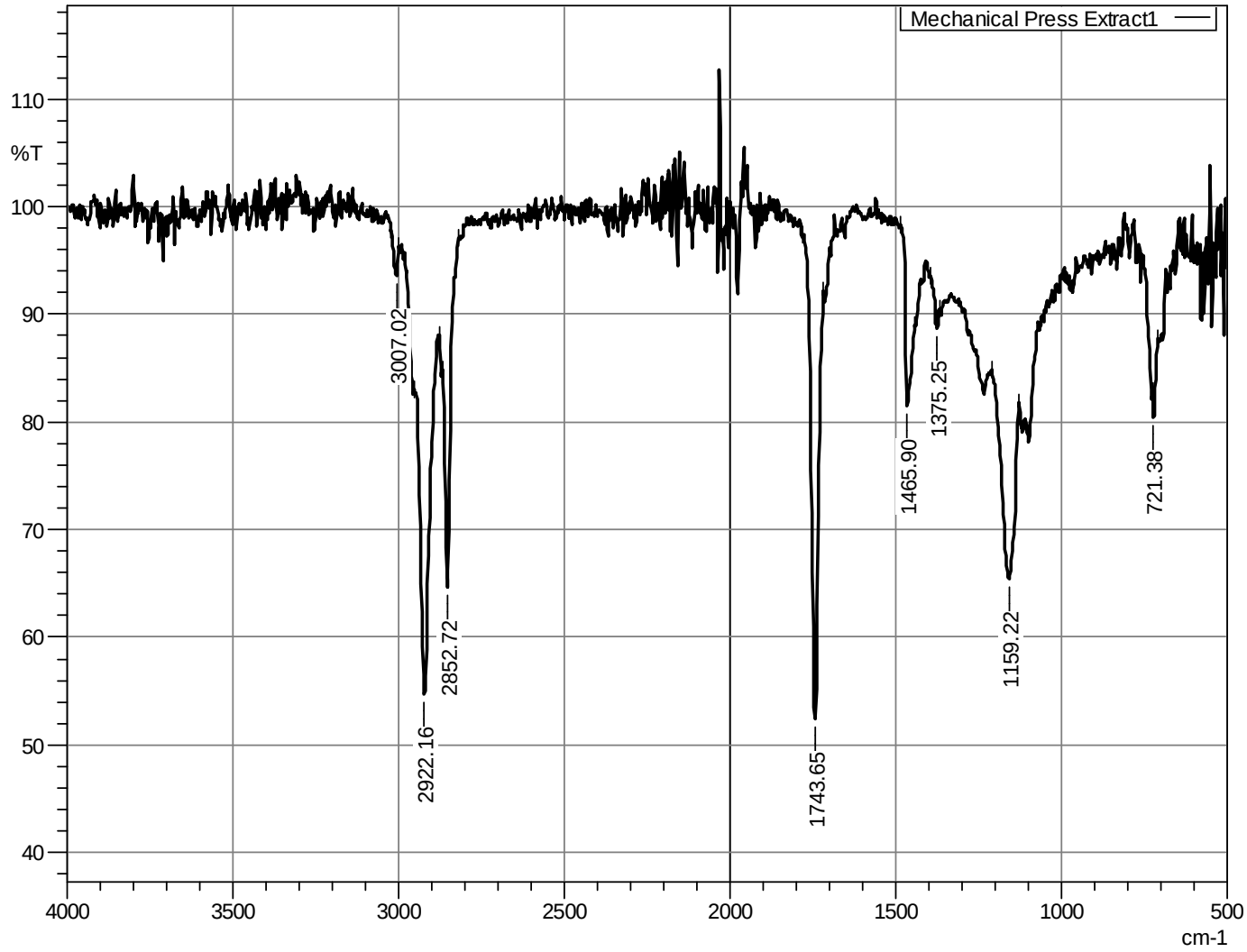


Figure 14: FT-IR spectrum for *T. pedata* seed kernel oil extracted by mechanical pressing.

Appendix 12 presents some of the common IR absorptions corresponding to various functional groups. The obtained spectra for the two extracts of *T. pedata* seed kernel oil contain similar absorption bands thus the *n*-hexane extract is used for illustration. The observed absorption bands are as follows: 3008.95 cm^{-1} assigned to sp^2 C-H stretch, 2924.09 cm^{-1} and 2854.65 cm^{-1} assigned to sp^3 C-H stretch, 1745.58 cm^{-1} assigned to C=O stretch, 1463.97 cm^{-1} and 1377.17 cm^{-1} assigned to sp^3 C-H bend, 1159.22 cm^{-1} assigned to C-O stretch and 719.45 cm^{-1} assigned to

sp^2 C-H bend (Silverstein *et al.*, 2005). The summary of the main FT-IR absorption bands for *T. pedata* seed kernel oil extracts is presented in Table 10.

The presence of sp^3 C-H stretch, sp^3 C-H bend, sp^2 C-H stretch, and sp^2 C-H bend absorption bands imply that *T. pedata* seed kernel oil is made of both saturated and unsaturated fatty acids. The C=O stretch absorption band observed is characteristic of the carbonyl carbon of the fatty acids found in *T. pedata* seed kernel oil. The observed C-O stretch absorption band is characteristic of ester bonds found in the oil's triglyceride molecules.

Table 10: Summary of the main FT-IR absorption bands for both *n*-hexane and mechanical pressing oil extracts of *Telfairia pedata* seed kernel.

Absorption Band		**Bond Vibration**
***n*-Hexane Extract (cm^{-1})**	**Mechanical Pressing Extract (cm^{-1})**	
3008.95	3007.02	sp^2 C-H stretch
2924.09	2922.16	sp^3 C-H stretch
2854.65	2852.72	sp^3 C-H stretch
1745.58	1743.65	C=O stretch
1463.97	1465.90	sp^3 C-H bend
1377.17	1375.25	sp^3 C-H bend
1159.22	1159.22	C-O stretch
719.45	721.38	sp^2 C-H bend

4.4 Fatty Acid Composition

In both the *n*-hexane and the mechanical pressing oil extracts of *T. pedata* seed kernel, the FA composition was analyzed using a GC-MS. Figures 15 and 16 presents the obtained spectra.

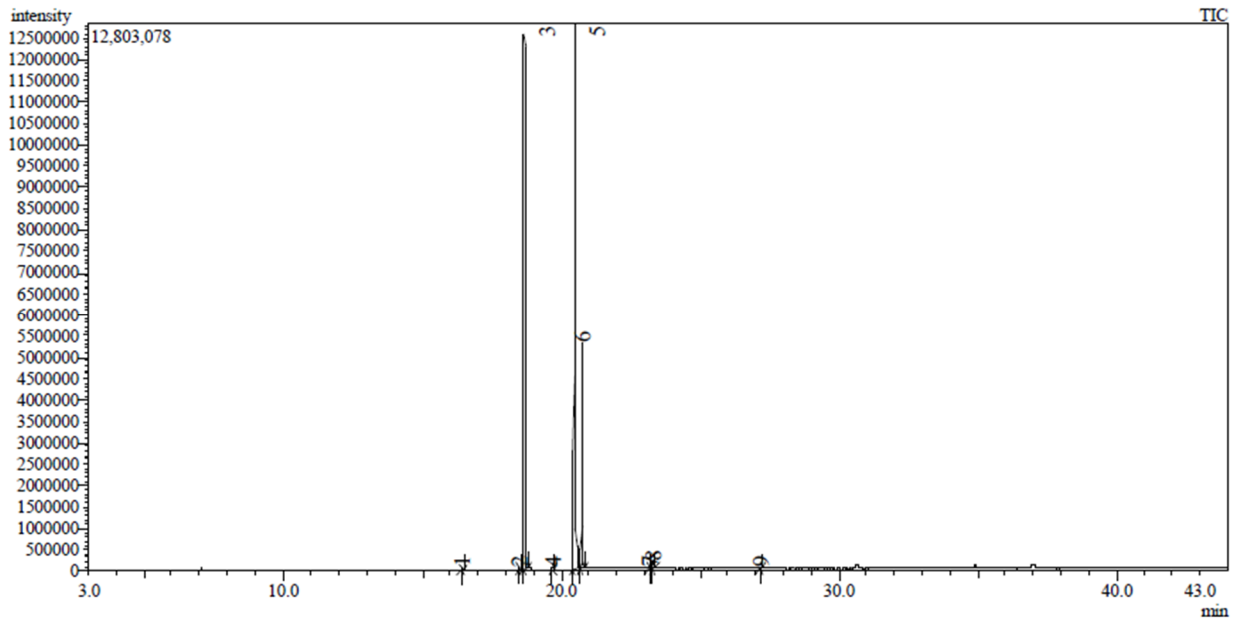


Figure 15: GC-MS spectrum of *T. pedata* seed kernel oil extracted by *n*-hexane

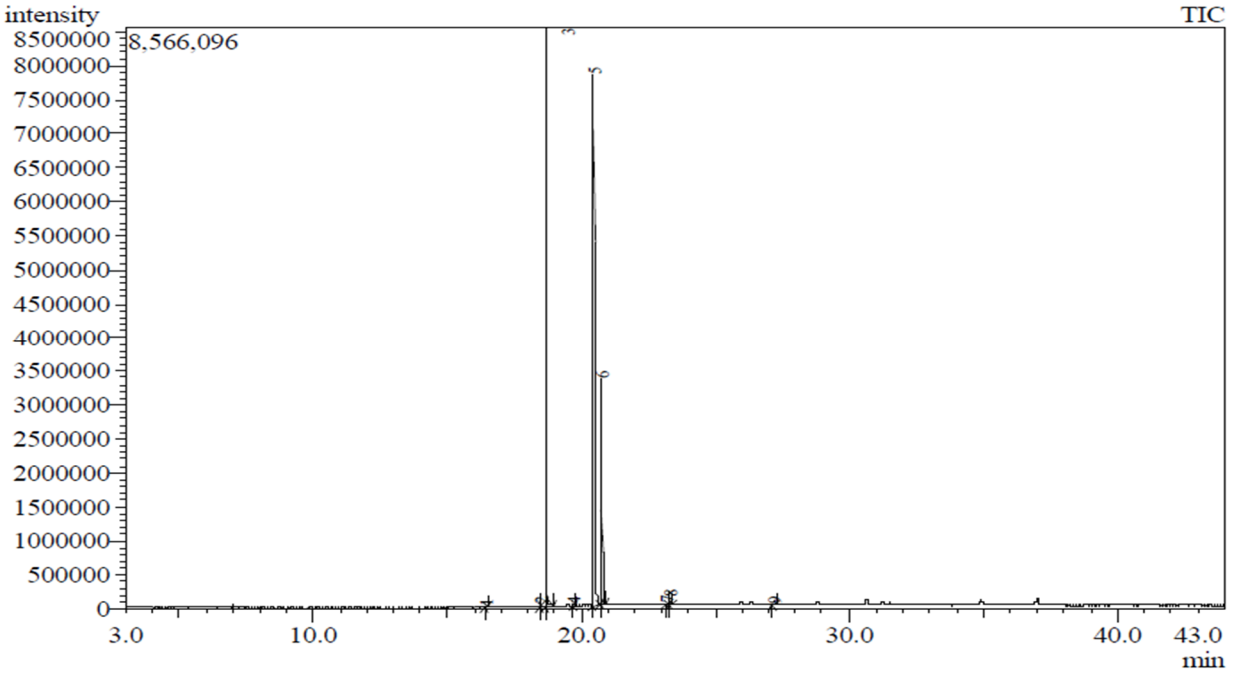


Figure 16: GC-MS spectrum of *T. pedata* seed kernel oil extracted by mechanical pressing

Tables 11 and 12 summarize the fatty acids identified from Figures 15 and 16, respectively.

Table 11: A summary of fatty acids identified from *T. pedata* (*n*-hexane extract).

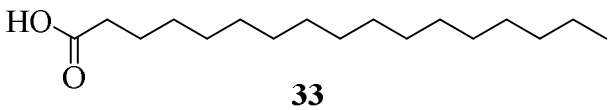
Peak Number	**Retention Time (min)**	**Area**	**%Area**	**FAME**	**Fatty Acid**	**ω-n**
1	16.435	83837	0.11	Tetradecanoic acid, methyl ester	Myristic acid (14:0) **(26)**	–
2	18.484	95816	0.13	Palmitoleic acid, methyl ester	Palmitoleic acid (16:1) **(19)**	ω–7
3	18.688	26138986	34.97	Hexadecanoic acid, methyl ester	Palmitic acid (16:0) **(15)**	–
4	19.716	73455	0.10	Heptadecanoic acid, methyl ester	Margaric acid (17:0) **(33)**	–
5	20.484	36218994	48.46	9,12-Octadecadienoic acid, methyl ester	Linoleic acid (18:2) **(18)**	ω–6
6	20.760	11454831	15.33	Octadecanoic acid, methyl ester	Stearic acid (18:0) **(16)**	–
7	23.173	63591	0.09	10,13-octadecadienoic acid, methyl ester	10,13-octadecadienoic acid (18:2) **(34)**	ω–5
8	23.319	507503	0.68	Nonadecanoic acid, 18-methyl, methyl ester	18-Methylnonadecanoic acid (20:0) **(35)**	–
9	27.184	101331	0.14	Docosanoic acid, methyl ester	Behenic acid (22:0) **(36)**	–
		74738344	100.00			

(–) implies a saturated fatty acid while ω stands for omega.

Table 12: A summary of fatty acids identified from *T. pedata* (mechanical pressing extract).

Peak Number	**Retention Time (min)**	**Area**	**%Area**	**FAME**	**Fatty Acid**	**ω-n**
1	16.439	37777	0.08	Tetradecanoic acid, methyl ester	Myristic acid (14:0) **(26)**	–
2	18.488	59408	0.13	Palmitoleic acid, methyl ester	Palmitoleic acid (16:1) **(19)**	ω–7
3	18.688	17308768	37.61	Hexadecanoic acid, methyl ester	Palmitic acid (16:0) **(15)**	–
4	19.721	46215	0.10	Heptadecanoic acid, methyl ester	Margaric acid (17:0) **(33)**	–
5	20.482	21079513	45.80	9,12-Octadecadienoic acid, methyl ester	Linoleic acid (18:2) **(18)**	ω–6
6	20.762	7078801	15.38	Octadecanoic acid, methyl ester	Stearic acid (18:0) **(16)**	–
7	23.186	46190	0.10	10,13-octadecadienoic acid, methyl ester	10,13-octadecadienoic acid (18:2) **(34)**	ω–5
8	23.325	308419	0.67	Nonadecanoic acid, 18-methyl, methyl ester	18-Methylnonadecanoic acid (20:0) **(35)**	–
9	27.203	56533	0.12	Docosanoic acid, methyl ester	Behenic acid (22:0) **(36)**	–
		46021624	100			

(–) implies a saturated fatty acid while ω stands for omega.



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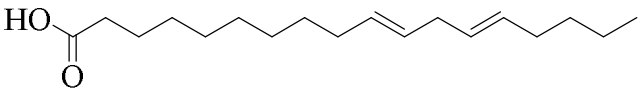

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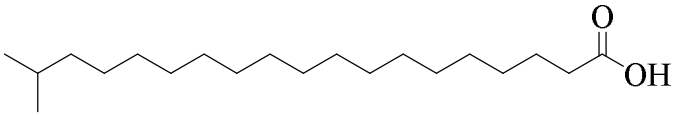

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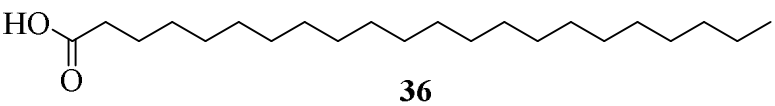

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Both extracts of *T. pedata* seed kernel oil were found to have the same fatty acid composition. Linoleic acid (18:2) **(18)**, an omega-6 fatty acid and an essential fatty acid is the most common in the two extracts of *T. pedata* seed kernel oil. The other essential fatty acid for humans that was not found in *T. pedata* seed kernel oil is alpha-linolenic acid (ALA) (18:3) **(21)**, which is an omega-3 **(1)** fatty acid (Blondeau *et al*., 2015).

In both extracts of *Telfairia pedata* seed kernel oil, monounsaturated fatty acid, polyunsaturated fatty acids, as well as saturated fatty acids have been found to be present. In the *n*-hexane extract, the total composition of monounsaturated fatty acid is 0.13 %, polyunsaturated fatty acids is 48.54 %, while that of saturated fatty acids is 51.33 %. In comparison, the mechanical pressing

extract of *T. pedata* seed kernel oil is composed of 0.13 % monounsaturated fatty acid, 45.9 % polyunsaturated fatty acid and 53.97 % saturated fatty acid.

Monounsaturated fatty acids (MUFAs) are known to have health benefits when consumed as part of diet (Schwingshackl and Hoffmann, 2014). Palmitoleic acid (16:1) **(19)**, which is an omega-7 fatty acid, is the only monounsaturated fatty acid that was detected in both extracts of *T. pedata* seed kernel oil. Nonetheless, the percent composition of palmitoleic acid (16:1) **(19)** in both extracts of *T. pedata* seed kernel oil was determined to be 0.13 %, implying that the oil is not richly endowed with monounsaturated fatty acids.

Polyunsaturated fatty acids (PUFAs) such as linoleic acid (18:2) **(18)** and 10,13-octadecadienoic acid (18:2) **(34)** were found to be present in both extracts of *T. pedata* seed kernel oil. The total composition of these fatty acids in the *n*-hexane extract of *T. pedata* seed kernel oil was found to be 48.54 % and that of the mechanical pressing extract as 45.9 %. This percent composition of the polyunsaturated fatty acids in the *T. pedata* seed kernel oil is considerably high thus making the oil a good natural source of polyunsaturated fatty acids. When consumed as part of the diet, polyunsaturated fatty acids have positive effects on cardiovascular disease (CVD), brain function and mental health (Ander *et al*, 2003 and Bentsen, 2017).

The other important category of fatty acids found in *T. pedata* seed kernel oil is the saturated fatty acids (SFAs). According to the obtained results, the most common form of fatty acids found in *T. pedata* seed kernel oil are the SFAs. The SFAs found in both extracts of *T. pedata* seed kernel oil include myristic acid (14:0) **(26)**, palmitic acid (16:0) **(15)**, margaric acid (17:0) **(33)**, stearic acid (18:0) **(16)**, 18-methylnonadecanoic acid (20:0) **(35)** and behenic acid (22:0) **(36)**. The total concentration of SFAs in *T. pedata* seed kernel oil was determined to be 51.33 % for

the *n*-hexane extract and 53.97 % for the mechanical pressing extract. A study conducted by Hu *et al*. (1999) found out that a higher dietary intake of long-chain saturated fatty acids (12:0 and above) was associated with an increased risk of coronary heart disease (CHD), unlike the short- to medium-chain SFAs (10:0 and below). These saturated fats increase the LDL cholesterol **(4)** by inhibiting LDL receptor activity and enhancing apolipoprotein (apo) B-containing lipoprotein production (Siri-Tarino et al., 2010).The SFAs found in *T. pedata* seed kernel oil fall under the category of long-chain saturated fatty acids.

The information about the fatty acid profile obtained for *T. pedata* seed kernel oil show that the oil is composed of long-chain fatty acids since the shortest carbon chain fatty acid obtained is Myristic acid (14:0) **(26)** while the longest is Behenic acid (22:0) **(36)**.

4.5 Vitamin E Composition

Other than the fatty acids present in *T. pedata* seed kernel oil, the oil was also analyzed for its vitamin E **(3)** composition, as well as any other micronutrient present such as squalene **(5)**. The micronutrients present in the *T. pedata* seed kernel oil were analyzed using a GC-MS. The chromatograms resulting from this analysis are presented in Figures 17 and 18.

Library Search Report

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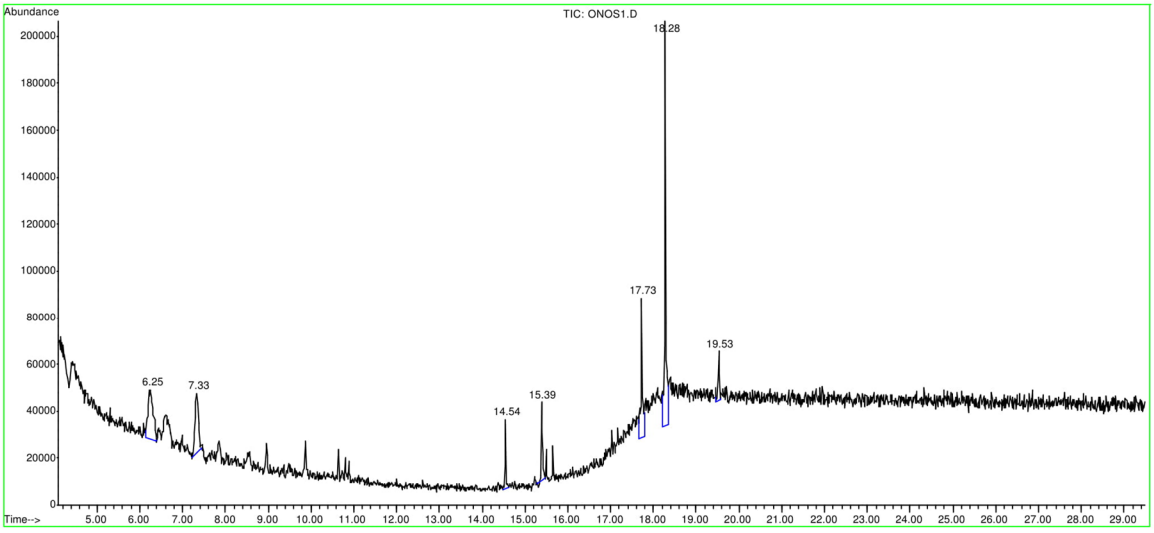

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Figure 17: GC-MS spectra showing micronutrients present in the *n*-hexane oil extract of *T. pedata* seed kernel.

Library Search Report

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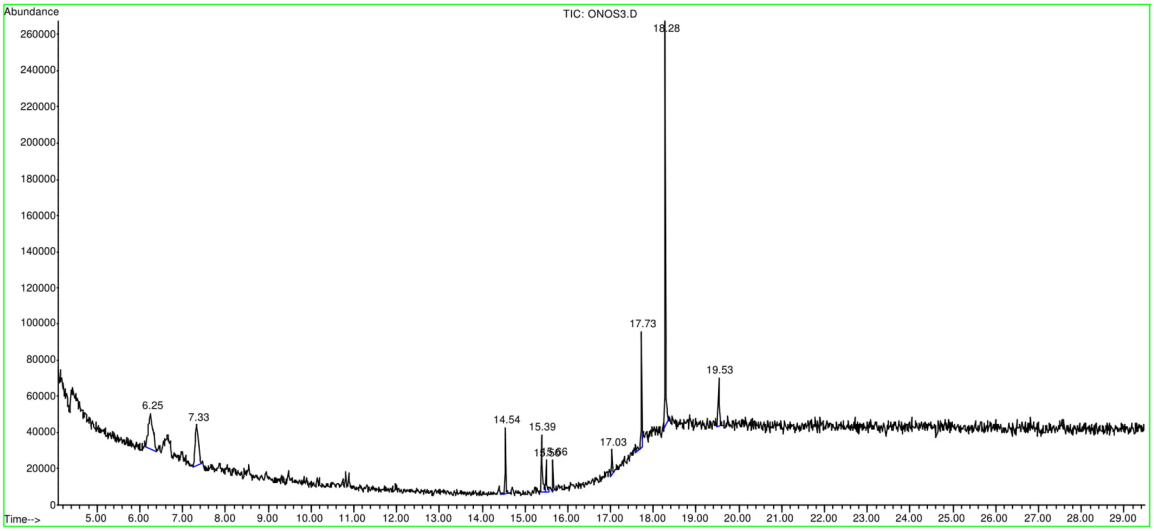

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Figure 18: GC-MS spectra showing micronutrients present in the mechanical pressing oil extract of *T. pedata* seed kernel.

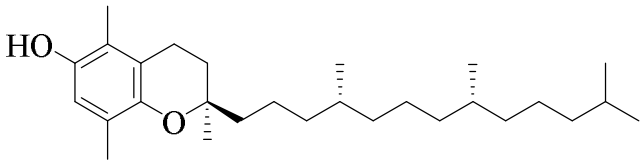
The peaks in Figures 17 and 18 representing micronutrients found in *T. pedata* seed kernel oil are summarized in Tables 13 and 14 respectively.

Table 13: Micronutrients composition in the *n*-hexane oil extract of *Telfairia pedata* seed kernel

Retention Time (min)	Area	%Area	Micronutrient
18.28	3316170	35.09	Squalene **(5)**
19.53	479534	5.07	Beta–tocopherol **(37)**

Table 14: Micronutrients composition in the mechanical pressing oil extract of *Telfairia pedata* seed kernel

Retention Time (min)	Area	%Area	Micronutrient
18.29	2918322	33.80	Squalene **(5)**
19.53	502178	5.82	Beta–Tocopherol **(37)**



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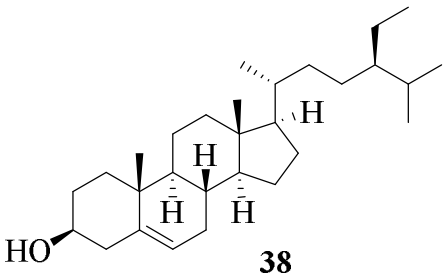

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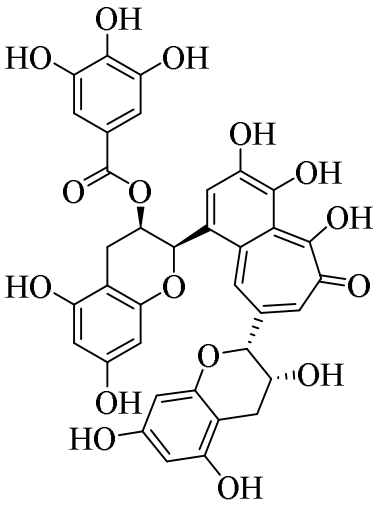

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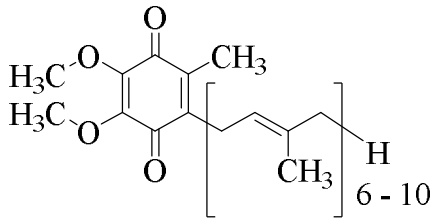

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According to Fine *et al*. (2016) and Popa *et al*. (2014), vegetable oils are composed of several minute components referred to as micronutrients, besides the main components which are the triglycerides. Such micronutrients can be tocopherols **(27)**, phytosterols **(38)**, polyphenols **(39)**, coenzymes Q **(40)**, as well as squalene **(5)**. Other than the nutritional value the micronutrients have on vegetable oils, they also determine their organoleptic properties (Fine *et al*., 2016). Tocopherols **(27)** belong to the vitamin E **(3)** family and occur in the alpha-, beta-, gamma- and delta- organic molecular forms. As evident in table 14 and table 15, beta-tocopherol **(37)** and squalene **(5)** are the only micronutrients that were detected in both extracts of *T. pedata* seed kernel oil.

One of the important micronutrient found in *T. pedata* seed kernel oil is beta-tocopherol **(37)** which belongs to the vitamin E **(3)** family. Vitamin E **(3)** as a micronutrient is known to possess several health benefits to humans. According to Miyazawa *et al*. (2011), all vitamin E **(3)** homologs have antioxidant properties thereby being able to protect biological membranes from lipid peroxidation by acting as protective agents. Other than being an antioxidant agent, vitamin

E **(3)** is also an anticancer agent, an antiaging agent and also protects the body from cardiovascular diseases (Mutalip, 2019). Vitamin E **(3)** has also been proven to improve male reproductive health (Mutalip, 2019).

Squalene (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) **(5)** is commonly found in shark liver oil but it also occurs in some vegetable oils (Popa *et al*., 2014). The peak for this compound appearing at retention time of 18.28 minutes on the *n*-hexane extract and 18.29 minutes on the mechanical pressing extract is the most intense peak in both extracts implying that squalene **(5)** is present in *T. pedata* seed kernel oil in a relatively higher amount. According to Popa *et al*. (2014), some of the vegetable oils which contain squalene **(5)** include olive oil, soybean oil, grape seed oil, corn oil, as well as hazelnuts oil. As determined by this research, *T. pedata* seed kernel oil is one of the vegetable oils that contain squalene **(5)**. Consequently, this finding has the potential to offset the burden posed on marine conservation efforts as a result of the overfishing of sharks for their liver oil which has been the main source of squalene **(5)** globally (Popa *et al.,* 2014).

Like other micronutrients, Squalene **(5)** is beneficial to human health. Fabrikov *et al*. (2019), reports that squalene **(5)** has the potential to reduce free radical oxidative damage in humans. Animal experimentation also suggests that squalene **(5)** is able to protect against cardiovascular/heart disease, a phenomenon attributed to the compound's ability to inhibit lipid peroxidation that is induced by isoprenaline (Fabrikov *et al*., 2019). Squalene **(5)** is also used in cancer therapy as a potentiating agent for anticancer drugs (Fabrikov *et al*., 2019).

4.6 Antioxidant Activity

A preliminary TLC test on the ability of *T. pedata* seed kernel oil to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) **(32)** radical confirmed that the oil has some antioxidant potential. After spotting the oil extracts on a TLC plate and developing the plate using a suitable solvent system, the R_f values obtained for both the hexane extract and mechanical pressing extract were R_f = 0.83 (hexane-chloroform mixture {1:1}) and R_f = 0.82 (hexane-chloroform mixture {1:1}), respectively, indicating that the sample was highly non-polar. The plate was then sprayed with a 1mM solution of DPPH **(32)** in ethyl acetate to observe whether a redox reaction between the radical and the oil samples took place. The change of the sprayed DPPH **(32)** solution's color from purple to yellow on the sample spots was an indication that the oil has some antioxidant potential. The outcome of the preliminary TLC test is shown in Figure 19.

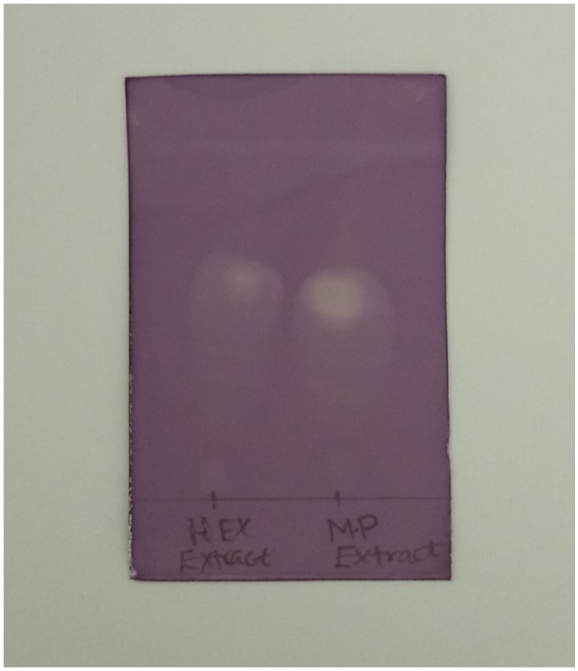

Figure 19: Preliminary TLC test on the ability of *Telfairia pedata* seed kernel oil to scavenge the DPPH radical.

After the preliminary TLC test, both the *n*-hexane and the mechanical pressing oil extracts of *T. pedata* seed kernel were tested for their *in vitro* percent radical scavenging activity (% RSA) using the DPPH **(32)** assay. The % RSA of a standard material (ascorbic acid) was also determined for comparison. Before the spectrophotometric analysis of the sample and the standard at 517 nm, an absorbance scan for DPPH **(32)** in methanol and ethyl acetate at the visible region was conducted to ascertain that the maximum absorbance was indeed at 517 nm as reported by other researchers (Appendix 13 and Appendix 14). Results on the % RSA of the *n*-hexane extract, mechanical pressing extract, as well as ascorbic acid (standard) are presented in Tables 15, 16 and 17, respectively. The determined IC_{50} values of the oil extracts and ascorbic acid are also presented on the respective Tables.

Table 15: *In vitro* DPPH scavenging activity of *Telfairia pedata* seed kernel oil extracted using *n*-hexane.

Conc.	**UV Absorbance at 517 nm and % RSA**						**Average**	**IC_{50}**
(mg/mL)	**1st Replicate**	**% RSA**	**2nd Replicate**	**% RSA**	**3rd Replicate**	**% RSA**	**% RSA**	**(mg/mL)**
500	0.116	78.0303	0.116	77.98861	0.117	77.79886	77.93926	18.05
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 16: *In vitro* DPPH scavenging activity of *Telfairia pedata* seed kernel oil extracted by mechanical pressing.

Conc. (mg/mL)	UV Absorbance at 517 nm and % RSA						Average % RSA	IC_{50} (mg/mL)
	1st Replicate	% RSA	2nd Replicate	% RSA	3rd Replicate	% RSA		
500	0.117	77.71429	0.118	77.48092	0.118	77.48092	77.55871	17.36
250	0.135	74.28571	0.136	74.0458	0.135	74.23664	74.18939	
125	0.165	68.57143	0.165	68.51145	0.166	68.32061	68.46783	
62.5	0.184	64.95238	0.185	64.69466	0.184	64.8855	64.84418	
31.25	0.211	59.80952	0.212	59.54198	0.212	59.54198	59.63116	
0	0.525	0	0.524	0	0.524	0	0	

Table 17: *In vitro* DPPH scavenging activity of ascorbic acid.

Conc. (μg/mL)	UV Absorbance at 517 nm and % RSA						Average % RSA	IC_{50} (μg/mL)
	1st Replicate	% RSA	2nd Replicate	% RSA	3rd Replicate	% RSA		
100	0.019	97.57033	0.018	97.53762	0.017	97.85624	97.65473	2.406
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	

After performing a nonlinear regression analysis for average % RSA against concentration using the GraphPad Prism 7.03 software (Appendices 15, 16 and 17), the half-maximal inhibitory concentration (IC_{50}) values for *T. pedata* seed kernel oil's *n*-hexane extract, mechanical pressing

extract and ascorbic acid were found to be 18.05 mg/mL, 17.36 mg/mL and 2.406 μg/mL, respectively. A low IC_{50} value of any given material implies a high antioxidant potential of that material and vice-versa (Espín *et al.*, 2000). The mechanical pressing extract of *T. pedata* seed kernel oil was found to be slightly more active than the *n*-hexane extract with no significant difference. In comparison to ascorbic acid's IC_{50} (standard), *T. pedata* seed kernel oil extracts were less active. This aspect can be attributed to the fact that the oil extracts used were crude while the ascorbic acid used was a pure compound.

With all the health concerns associated with oxidative stress, it is important for humans to regularly feed on a diet rich in antioxidants. This research has established that *T. pedata* seed kernel oil has some antioxidant potential, thus making it a good natural antioxidant.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

a) The physico-chemical properties of *Telfairia pedata* seed kernel oil obtained in this research were found to be closely related to those of many other edible vegetable oils. The determined properties also conform to the Food and Agriculture Organization's (FAO) "Standard for Edible Fats and Oils not Covered by Individual Standards." This study concludes that *T. pedata* seed kernel oil meets the quality requirements as edible vegetable oil and can be used as such.

b) The fatty acid composition analysis for *T. pedata* seed kernel oil revealed that it is composed of the monounsaturated fatty acids, polyunsaturated fatty acids, and saturated fatty acids. However, in terms of individual fatty acid composition, Linoleic acid (18:2) **(18)**, which is an essential fatty acid (EFA), was found to be the most abundant fatty acid occurring at 48% of the total fatty acid composition of the oil. Given that the human body is unable to synthesize EFAs, this study concludes that *T. pedata* seed kernel oil is a good nutritional source for the essential fatty acids.

c) The vitamin E **(3)** composition of *T. pedata* seed kernel oil was determined by use of a GC-MS, a technique which also led to the discovery of another important micronutrient in the oil known as squalene **(5)**. These micronutrients are paramount in human nutrition due to their health benefits. This research concludes that *T. pedata* seed kernel oil is a good source of nutrition micronutrients.

d) This research established that *T. pedata* seed kernel oil has some antioxidant potential. Due to the health issues associated with oxidative stress in humans, the search for antioxidant sources that augment the body mechanisms on neutralizing the oxidative stress has been ongoing. Synthetic antioxidants such as butylated hydroxytoluene **(6)** and butylated hydroxyanisole **(7)** can be used but are not widely encouraged due to their suspected health side effects. As a result, natural dietary substances with antioxidant potential are preferred over the synthetic antioxidants. This research, therefore, concludes that *T. pedata* seed kernel oil can be relied upon as a natural antioxidant.

5.2 Recommendations

a) *T. pedata* seed kernel oil has been found to be of good nutritional value. The seed kernels of *T. pedata* have also been found to have a higher percent oil yield. This high yield of the oil implies economic viability hence this research recommends the commercialization of the oil as edible vegetable oil. Commercialization of *T. pedata* seed kernel oil would also incentivize the cultivation of the species, an aspect which has the potential of saving the species from possible extinction.

b) Saturated fatty acids have been found to be more than 50 % of the total fatty acids found in *T. pedata* seed kernel oil. Due to the health concerns associated with high consumption of saturated fats, this study recommends that the consumption of the unprocessed *T. pedata* seed kernel oil should be limited. This drawback can, however, be remedied by processing the oil.

c) The antioxidant activity observed in the *T. pedata* seed kernel oil implies potential bioactivity by the species in general. This research, therefore, recommends that further research be done on bioactivity of extracts from different parts of the species.

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APPENDICES

Appendix 1: Data for average % oil yield significant difference test

Average % Oil Yield

Sample	Value	
	Hexane Extract	Mechanical Pressing Extract
1	65.41	31.56
2	69.87	29.23
3	63.78	37.44

t-Test: Two-Sample Assuming Unequal Variances

	Variable 1	*Variable 2*
Mean	66.35333333	32.74333333
Variance	9.939433333	17.90123333
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	11.03289092	
P(T<=t) one-tail	0.000191843	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.000383685	
t Critical two-tail	2.776445105	

Appendix 2: Data for density significant difference test

Density

Sample	Value	
	Hexane Extract	Mechanical Pressing Extract
1	0.8439	0.844
2	0.8437	0.8439
3	0.844	0.8442

t-Test: Two-Sample Assuming Unequal Variances

	Hexane Extract	*Mechanical Pressing Extract*
Mean	0.843866667	0.844033333
Variance	2.33333E-08	2.33333E-08
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-1.33630621	
P(T<=t) one-tail	0.126200274	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.252400549	
t Critical two-tail	2.776445105	

Appendix 3: Data for moisture content significant difference test

Moisture Content

Sample	Value	
	Hexane Extract	Mechanical Pressing Extract
1	0.0438	0.0596
2	0.0712	0.0317
3	0.0625	0.0637

t-Test: Two-Sample Assuming Unequal Variances

	Hexane Extract	*Mechanical Pressing Extract*
Mean	0.059166667	0.051666667
Variance	0.000196023	0.000303203
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	0.58139729	
P(T<=t) one-tail	0.296080359	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.592160718	
t Critical two-tail	2.776445105	

Appendix 4: Data for refractive index significant difference test

Refractive Index

Sample	Value	
	Hexane Extract	Mechanical Pressing Extract
1	1.454	1.455
2	1.456	1.453
3	1.455	1.454

t-Test: Two-Sample Assuming Unequal Variances

	Hexane Extract	*Mechanical Pressing Extract*
Mean	1.455	1.454
Variance	0.000001	0.000001
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	1.224744871	
P(T<=t) one-tail	0.143932067	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.287864135	
t Critical two-tail	2.776445105	

Appendix 5: Data for specific gravity significant difference test

Specific Gravity

Sample	Value	
	Hexane Extract	Mechanical Pressing Extract
1	0.8754	0.8753
2	0.8749	0.8755
3	0.8754	0.8756

t-Test: Two-Sample Assuming Unequal Variances

	Hexane Extract	*Mechanical Pressing Extract*
Mean	0.875233333	0.875466667
Variance	8.33333E-08	2.33333E-08
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-1.237436867	
P(T<=t) one-tail	0.151974956	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.303949913	
t Critical two-tail	3.182446305	

Appendix 6: Data for viscosity significant difference test

Viscosity

Sample	Value	
	Hexane Extract	Mechanical Pressing Extract
1	55.6211	55.7436
2	55.7124	55.7738
3	55.6514	55.4852

t-Test: Two-Sample Assuming Unequal Variances

	Hexane Extract	*Mechanical Pressing Extract*
Mean	55.66163333	55.66753333
Variance	0.002162463	0.025162093
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-0.061821007	
P(T<=t) one-tail	0.478163827	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.956327654	
t Critical two-tail	4.30265273	

Appendix 7: Data for calorific value significant difference test

Calorific Value

Sample	Value	
	Hexane Extract	Mechanical Pressing Extract
1	44.31	44.53
2	44.72	44.62
3	44.95	44.37

t-Test: Two-Sample Assuming Unequal Variances

	Hexane Extract	*Mechanical Pressing Extract*
Mean	44.66	44.50666667
Variance	0.1051	0.016033333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	0.76307174	
P(T<=t) one-tail	0.250468941	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.500937882	
t Critical two-tail	3.182446305	

Appendix 8: Data for acid value significant difference test

Acid Value

Sample	Value	
	Hexane Extract	Mechanical Pressing Extract
1	0.6595	0.6047
2	0.6485	0.6612
3	0.5977	0.6047

t-Test: Two-Sample Assuming Unequal Variances

	Hexane Extract	*Mechanical Pressing Extract*
Mean	0.635233333	0.623533333
Variance	0.001086813	0.001064083
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	0.436955003	
P(T<=t) one-tail	0.342348891	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.684697781	
t Critical two-tail	2.776445105	

Appendix 9: Data for iodine value significant difference test

Iodine Value

Sample	Value	
	Hexane Extract	Mechanical Pressing Extract
1	25.0791	25.0678
2	20.6177	23.1781
3	23.3207	24.3783

t-Test: Two-Sample Assuming Unequal Variances

	Hexane Extract	*Mechanical Pressing Extract*
Mean	23.00583333	24.20806667
Variance	5.050378253	0.914476063
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-0.852608135	
P(T<=t) one-tail	0.2282682	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.4565364	
t Critical two-tail	3.182446305	

Appendix 10: Data for peroxide value significant difference test

Peroxide Value

Sample	Value	
	Hexane Extract	Mechanical Pressing Extract
1	0.7751	0.9558
2	0.9401	1.5633
3	1.1772	1.1329

t-Test: Two-Sample Assuming Unequal Variances

	Hexane Extract	*Mechanical Pressing Extract*
Mean	0.964133333	1.217333333
Variance	0.040854303	0.097610803
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-1.178566674	
P(T<=t) one-tail	0.161769505	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.323539011	
t Critical two-tail	3.182446305	

Appendix 11: Data for saponification value significant difference test

Saponification Value

Sample	Value	
	Hexane Extract	Mechanical Pressing Extract
1	157.0989	162.7974
2	154.9438	157.3927
3	159.8594	151.6514

t-Test: Two-Sample Assuming Unequal Variances

	Hexane Extract	*Mechanical Pressing Extract*
Mean	157.3007	157.2805
Variance	6.07132327	31.06777063
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	0.005741116	
P(T<=t) one-tail	0.497889853	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.995779706	
t Critical two-tail	3.182446305	

Appendix 12: Characteristic IR absorption frequencies of organic functional groups

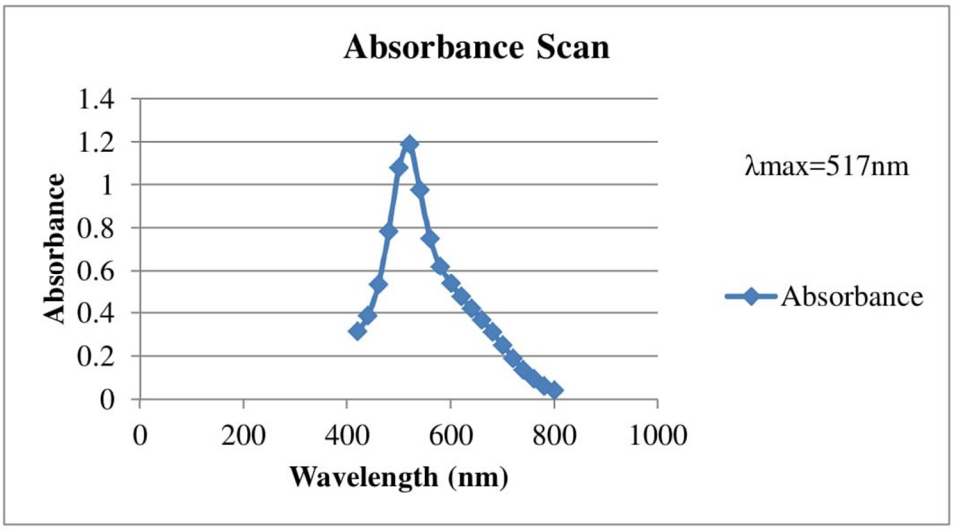
Characteristic IR Absorption Frequencies of Organic Functional Groups

Functional Group	Type of Vibration	Characteristic Absorptions (cm-1)	Intensity
Alcohol			
O-H	(stretch, H-bonded)	3200-3600	strong, broad
O-H	(stretch, free)	3500-3700	strong, sharp
C-O	(stretch)	1050-1150	strong
Alkane			
C-H	stretch	2850-3000	strong
-C-H	bending	1350-1480	variable
Alkene			
=C-H	stretch	3010-3100	medium
=C-H	bending	675-1000	strong
C=C	stretch	1620-1680	variable
Alkyl Halide			
C-F	stretch	1000-1400	strong
C-Cl	stretch	600-800	strong
C-Br	stretch	500-600	strong
C-I	stretch	500	strong
Alkyne			
C-H	stretch	3300	strong,sharp
–C≡C–	stretch	2100-2260	variable, not present in symmetrical alkynes
Amine			
N-H	stretch	3300-3500	medium (primary amines have two bands; secondary have one band, often very weak)
C-N	stretch	1080-1360	medium-weak
N-H	bending	1600	medium
Aromatic			
C-H	stretch	3000-3100	medium
C=C	stretch	1400-1600	medium-weak, multiple bands
Analysis of C-H out-of-plane bending can often distinguish substitution patterns			
Carbonyl	Detailed Information on Carbonyl IR		
C=O	stretch	1670-1820	strong
(conjugation moves absorptions to lower wave numbers)			
Ether			
C-O	stretch	1000-1300 (1070-1150)	strong
Nitrile			
CN	stretch	2210-2260	medium

Appendix 13: DPPH absorbance scan in methanol

DPPH Absorbance Scan in Methanol

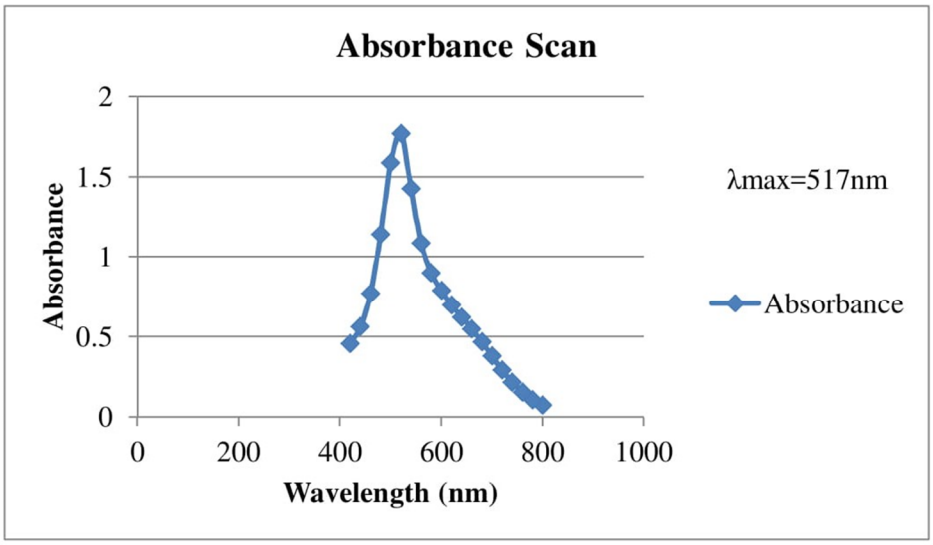
Wavelength	Absorbance
800	0.042
780	0.063
760	0.096
740	0.138
720	0.191
700	0.252
680	0.313
660	0.37
640	0.423
620	0.477
600	0.54
580	0.62
560	0.751
540	0.978
520	1.188
500	1.081
480	0.784
460	0.533
440	0.388
420	0.316



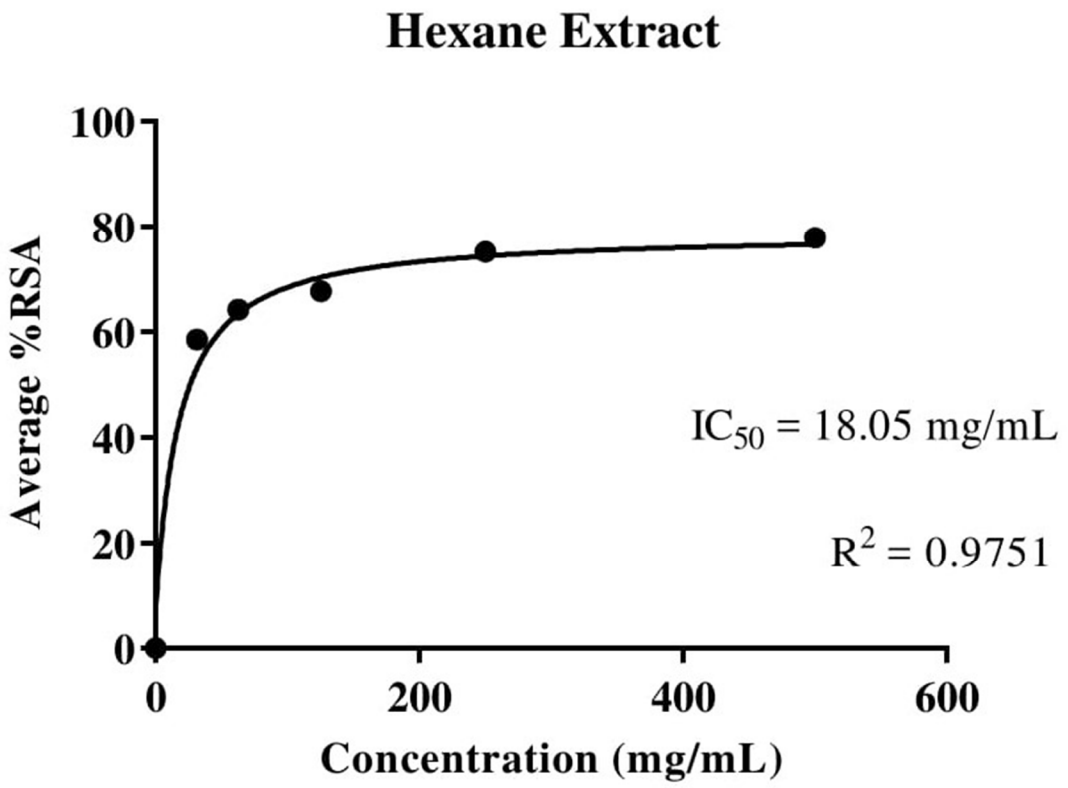
Appendix 14: DPPH absorbance scan in ethyl acetate

DPPH Absorbance Scan in Ethyl Acetate

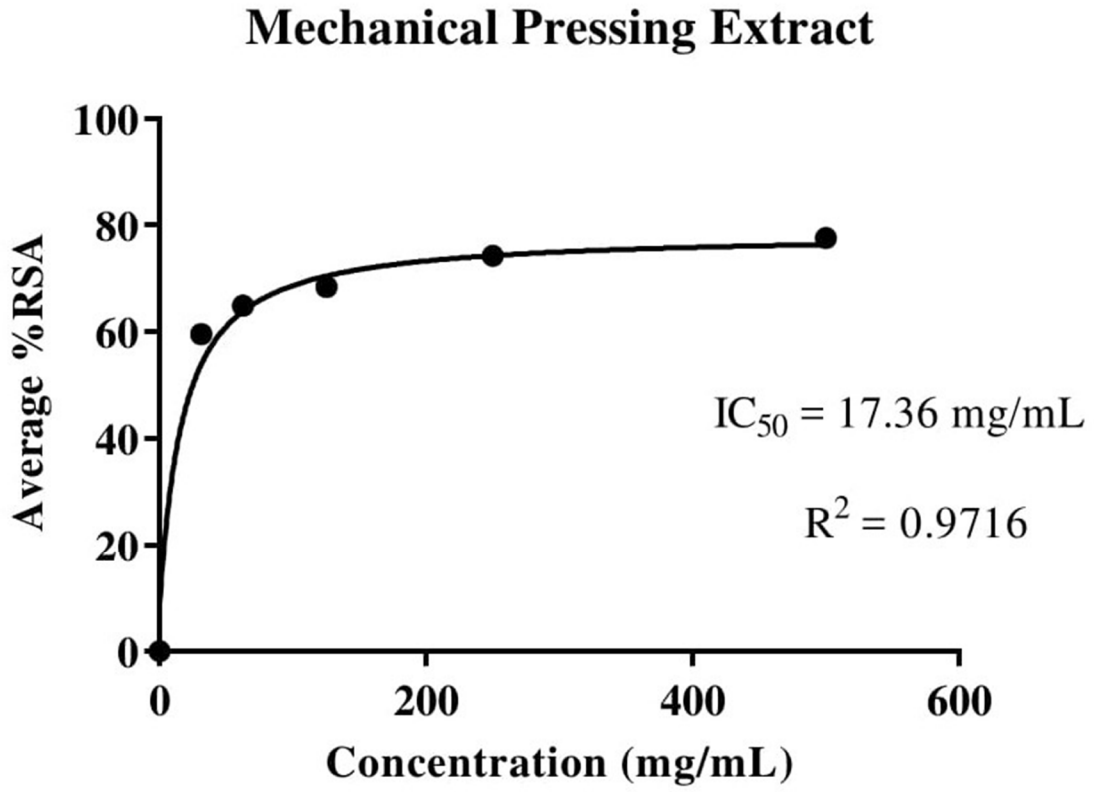
Wavelength	Absorbance
800	0.073
780	0.106
760	0.154
740	0.216
720	0.292
700	0.38
680	0.468
660	0.55
640	0.624
620	0.7
600	0.79
580	0.902
560	1.086
540	1.429
520	1.771
500	1.59
480	1.144
460	0.773
440	0.564
420	0.46



Appendix 15: Nonlinear regression analysis for *Telfairia pedata* seed kernel oil's *n*-hexane extract IC_{50} using GraphPad Prism software



Appendix 16: Nonlinear regression analysis for *Telfairia pedata* seed kernel oil's mechanical pressing extract IC_{50} using GraphPad Prism software



Appendix 17: Nonlinear regression analysis for ascorbic acid IC_{50} using GraphPad Prism software