SPECTROSCOPIC AND PHYSICOCHEMICAL CHARACTERIZATION OF *Plukenetia conophora* OIL

BY

OLOWOLAGBA, MARY ADENIKE

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BEING A DISSERTATION SUBMITTED TO THE DEPARTMENT OF INDUSTRIAL CHEMISTRY, FACULTY OF SCIENCE, FEDERAL UNIVERSITY OYE-EKITI, EKITI STATE, NIGERIA.

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CERTIFICATION

I hereby certify that this project work was carried out by **OLOWOLAGBA MARY ADENIKE** and submitted to the department of Industrial Chemistry having met the standard as required by the institution and approved by:

Supervisor

Dr. (Mrs) C.O AKINTAYO

Date

Head of Department

Dr. (Mrs) C.O AKINTAYO

3/11/2015

Date

DECLARATION

I, **OLOWOLAGBA MARY ADENIKE** hereby declare that this thesis was written by me and it is a record of all activities carried out during the research. All sources of information are clearly acknowledged by means of references.

Signature of student: _____

Date: 31/10/15

DEDICATION

This report is dedicated to the most awesome, wonderful, most faithful, everlasting, glorious, the most merciful, the Lion of the tribe of Judah, the most gracious and mighty God, the one who knows the end of a thing right from the beginning.

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ABSTRACT

The spectroscopic studies revealed that ***Plukenetia conophora*** oil is an unsaturated oil having linolenic acid as the predominant fatty acid, the elemental analysis (C = 77.75% carbon, 10.95% hydrogen and 11.18% oxygen) corresponds with the calculated (78.39% carbon, 10.62% hydrogen, and 10.99% oxygen). The proton NMR revealed characteristic peaks at δ = 2.7-2.8ppm for $=CCH_2C=$, 3.9-4.3ppm for $-OCH_2CHOCH_2O$ (glycemic group), 5.1-5.5ppm for -CH=C and so on. Physicochemical analyses that were carried out on the oil were organoleptic analysis, specific gravity, moisture content, smoke point, acid value, saponification value, ester value, peroxide value, iodine value, and free fatty acid value. The physicochemical report revealed that the oil is light- yellow in colour with a nutty smell, has smoke point of 240°C, specific gravity of 0.917g/cm^3 which makes it less dense than water, refractive index (at 29°C) of 1.42 and moisture content (%) of 0.49 ± 0.028 which shows that it can be preserved for a long time. The iodine value characterization of the oil (5.63g/100g) suggests that it is non-drying oil suitable for paint making and that it is less susceptible to oxidation. The saponification value (162.02 ± 0.042 mgKOH/g) obtained suggests that it can be used in saponification industries such as soap making industry, the free fatty acid value (3.12 ± 0.014) gotten as oleic acid suggests that it is good for consumption, and the acid value obtained for the oil was 28.08 ± 0.042mgKOH/g. The peroxide value (1.04 Meq/kg) suggests less susceptibility of the oil to oxidation and its stability and ester value(101.36 ± 0.283mgKOHg^{-1}) gotten for the oil falls between the range of oils good for consumption.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Agriculture which was used to be the pivot of Nigeria's economy showed greater decline immediately after independence due to the discovery of petroleum and its utility to the whole world. The revenue generated from petroleum appeared to have more effective impact on the development of the economy of Nigeria than agriculture which led to sudden neglect of agriculture. Petroleum, a non-renewable material and its products have constituted one of the most prevalent sources of environmental degradation in the industrialized world. In large concentrations, the hydrocarbon molecules that make up crude oil and petroleum products are highly toxic to living organisms including man. This has led to different environmental concerns, health effects from exposure to petroleum products vary depending on the concentration of the length of time that one is exposed. Breathing petroleum vapors can cause nervous system effects (such as headache, nausea, and dizziness) and respiratory irritation. Very high exposure can cause coma and death. Liquid petroleum products which come in contact with the skin can cause irritation and some can be absorbed through the skin. Hence there is need to move from non renewable materials to renewable materials which are environmental friendly. Agriculture has provided us varieties of plant from which edible oils can be extracted from. Oil extracted from different sources has been found out to have high nutritive value, medicinal uses and industrial applications.

According to Ilija Gawrilow 2004, vegetable oils were found to be an environmentally friendly lubricants that can be used as gear oil lubricants, metalworking fluids, hydraulic fluids,

textile lubricants, grease base fluids, food machinery lubricants and chain bar lubricants, instead of mineral oil. Mineral oil which is a liquid by- product of the distillation of petroleum to produce gasoline and other petroleum-based products from crude oil and of course derived from a non- vegetable (mineral) source can be replaced by vegetable oils in many areas. Despite the apparent popularity of petroleum products as raw materials in different areas of application, fats and oils are being greatly favoured for use in surface coatings, soaps, cosmetics, pharmaceuticals, lubricants, surfactants, and polymer processing (Asiagwu *et al.,* 2008). Their wide acceptance in these field of application is attributable to their being renewable resources and biodegradable, hence environmentally friendly.

Plukenetia conophora oil can be used as an essential ingredient in medicinal products, skin ointments, wood finishing products and as paint thinner instead of petroleum products.

1.2 Statement of problem

Due to environmental hazard caused by petroleum products, their non- renewability, scarcity, and fluctuation in price. The need to replace these petroleum products partially or totally by a non-toxic, biodegradable and renewable material arises. Petroleum is a useful chemical substance for many important purposes but a non- renewable resource with a highly toxic composition poses significant health problems. Petroleum products which are used by people at homes, manufacturing and petrochemical industries contain aliphatic, aromatic and a variety of saturated and unsaturated hydrocarbons (Henderson *et al,* 1993; Kato *et al,* 1993; Anderson *et al,* 1995) which have toxic effects on various organs and systems which include the respiratory, immune and nervous systems. The heart, lungs, skin, and kidneys are affected by these toxic compounds resulting in various diseases and different forms of genatoxic, mutagenic,

immunotoxic, carcinogenic and neurotoxic manifestations (Becker, 1985; Klassen 1990; d'Azevedo *et al,* 1996; Smith *et al,* 1996; Rabble and Wong, 1996; Ross 1996; Rothman *et al,* 1996).

There are many compelling reasons to decrease society's dependence on petroleum products because of their non-renewability, non-biodegradability, and their non eco-friendly characters. Thus there is need to produce alternative fuels or raw materials with little or no exhaust pollutants or green house gases that can be derived from plentiful renewable sources to serve the purpose of these petroleum products in some areas at homes and industries. Vegetable oils serve as ideal alternatives in this regard because of their biodegradability, non toxicity, and renewability.

1.3 Aim and Objectives of the study

This project work is aimed at elucidating the structure of walnut oil and studying its physical and chemical properties with the aim of totally or partially replacing petroleum and petroleum products in synthetic and manufacturing companies. Through the elucidation of the structure of walnut oil the reactive sites on the walnut oil will be revealed and can now be modified by synthetic chemists for several uses.

This project work will enable us to know the hidden properties and usefulness of ***Plukenetia conophora*** oil. To replace petroleum products by an eco- friendly and a renewable material, in order to avoid the hazards created by petroleum products on consumers. Spectroscopic and physicochemical characterization of the oil will enable us to establish fully the industrial use of this oil.

CHAPTER TWO

LITERATURE REVIEW

2.1.0 *Plukenetia conophora*

Plukenetia conophora was formerly known as *Tetracarpidium conophorum*. It belongs to Euphorbiacae family and commonly called the African walnut (Grin, 2010). It is a climbing shrub 10-20feet long known in the southern Nigeria as ukpa (Igbo), Western Nigeria as awusa or asala (Yoruba) (Dalzier, 2000). It is found in the wet parts of Eastern, Western Nigeria and Western Africa (Ekwe *et al.,* 2013). It is found in Uyo, Akamkpa, Akpabuyo, Lagos, Kogi, Ajaawa- Ogbomoso and Ibadan.

Plukenetia conophora plants are cultivated principally for the nuts which are usually cooked and consumed as snacks (Enujiugha and Ayodele 2003; Oke, 1995). A bitter taste is usually observed upon drinking water immediately after eating the nuts. The sensation of this bitter taste could be attributed to the presence of chemical substances such as alkaloid (Ayodele 2003).

Ayodele, (2003) reported the presence of oxalate, phylates and tannin in the raw *Tetracarpidium conophorum* nuts. Edem *et al*., 2009 reported the proximate composition, heavy metal contents and ascorbic acid of the nut. The work done by Oyenuga 1997, revealed the presence of amino acid and fatty acid components of the African walnut.

Onawumi *et al*., (2013) reported that when sample of ***Plukenetia conophora*** leaf (African walnut) was analysed for proximate composition, secondary metabolites, vitamins and mineral constituents. The result of proximate analysis shows that the leaf contained 29±0.71% moisture, 5.63±0.08% fat, 14.92±0.04% fibre, 16.62±0.30% protein, 12.89±0.02% Ash and 20.94±0.01%

carbohydrate. Also, the secondary metabolites screening and subsequent quantification revealed the presence of bioactive compounds such as tannin which is 0.560±0.01mg/kg, alkaloids, 2.670±0.02mg/kg, saponin, 1.080±0.01mg/kg and anthraquinones, 0.130±mg/kg. The mineral analysis revealed the presence of K which is 15937±0.02mg/kg, Na, 7980±0.01mg/kg, Ca, 18700±0.02mg/kg, Mg, 1766.25±0.1mg/kg, Fe, 4610±0.10mg/kg, Zn, 61.15±0.08mg/kg, Mn, 79.50±0.03mg/kg and Cu, 8.60±0.10mg/kg. Vitamin composition results showed that the leaf contained Thiamine (B1) 0.29±0.01µg/100g, Ascorbic acid (C) 16.28mg/100g, Riboflavin (B2) 0.34±0.01µg/g, Niacin, 0.12±0.3µg/1 00g and Cyanocobalamin (B12), 0.23±0.03µg/100g. The results obtained by Onawumi *et al.*, 2013 proved that ***Plukenetia conophora*** leaf is a food and could be a potential source of useful drug formulation.

2.2.0 Cultivation

The plant of ***Plukenetia conophora*** is planted under an indigenous tree that can provide strong support for the heavy weight of the climber when fully established on the crown of the tree (Ekwe and Ihemeje 2013). The climber of ***Plukenetia conophora*** takes over the crown of the tree which is used as support when fully established and thereby competes for sunlight and also affects fruiting of the host tree. Thus, trees that do not produce high economic fruits are mostly used as support for the climber. In cases where they cannot be harvested manually, they are left for full maturation so that the pod can fall off by itself and are picked , gathered, and allowed to rot after which the seeds are removed , washed and taken to market for sale. Knife and cutlass can be used to open the fruits in order to remove the nuts if the farmer is in need of money and cannot wait for the fruit to rot.

The fruits are four winged ridged between wings and up to 3 inches in diameter with four round seeds (usually brown) in each fruit (Nuhu *et al.*, 2000).The seed is about 2.5cm long and has wooly materials that attach the nut to the shell when cracked open. Though the weights of walnut vary depending on the cultivar, location grown and irrigation rate (Caglarirmak, 2003; Ozkan and Koyuncu, 2005).

2.3.0 Uses

Apart from consuming African walnut as snacks, some studies on the plant have revealed that African walnuts have nutritive value (Oke and Fafunso 1995; Adebona *et al.*, 1988) medicinal uses and industrial applications. ***Plukenetia conophora*** plant is known in Africa for its antibacterial efficacy (Okerulu and Ani 2001).

2.3.1 Nutritional Uses of *Plukenetia conophora*

Walnut is one of the several highly nutritive seed that contains oxalates, phylates, tannins, as well as fibers, protein, carbohydrates, and vitamins (Savage *et al.*, 2001). The vitamin content is useful in the treatment of common cold and other diseases like prostate cancer and other minerals present in small amount are essential for body metabolism (Okwu and Okele 2003).

Walnut serves as a rich source of mineral elements such as calcium, magnesium, sodium, phosphorus and potassium (James 2009). Walnuts are edible even when raw and give a better taste and a stimulating effect like Kola. They can be cooked, sun dried, roasted and the roasted seeds could be grounded like melon seeds and used as thickner in soup preparation.

Dried walnuts can be ground and turned into flour which can be used as composite flour during baking or in-place of milk in tea preparation (Ekwe and Ihemeje 2013). The cake left after the extraction of the oil contains 45% protein which has other local uses for food and can be fed

to livestock which serves as a good source of vitamins (Aiyeoba and Fadare 2006). The consumption of 68g of walnuts per day reduced the total and low density lipoprotein cholesterol by five percent and nine percent respectively. These reductions would have some positive effect in reducing the risk of coronary heart disease (Abbey *et al.*, 1994).

2.3.2 Medicinal Uses of *Plukenetia conophora*

A diet supplemented with walnut or its oil has a beneficial effect on blood lipids and also helps to lower blood cholesterol present in the body (Savage, 2001). ***Plukenetia conophora*** seed have been shown to cure male infertility problems, fibroid and the leaves are used for the treatment of dysentery (Ajaiyeoba and Fadare, 2006). Alkaloids contained in walnut seeds are the most efficient plant substances used therapeutically. Pure isolated alkaloids and the synthetic derivatives are used as the basic medicinal agent because of their analgesic, antispasmodic and bacterial properties. The presence of tannins in both seed and leaf support their strong use for healing of hemorrhoids, frost bite and varicose ulcers in herbal medicine (Igboko, 1983).

Decoction of leaves and seeds serve as beverage which relives abdominal pains and fever (Malu *et al.,* 2009). An African walnut, *Tetracarpidium conophorum* is an example of such plants found to be rich in antioxidants and contains essential nutrients (Ayoola *et al.*, 2011). Stanner *et al.*, 2004 reported that plants rich in antioxidant are a group of compounds with quite different chemical structure which are able to neutralize free radicals generated in heavy metal toxicity during the aging process and have a potential role in preventing the onset of some chronic diseases such as cardiovascular disease, some neurological disorders or certain inflammatory processes. Several works have found that different parts of this plant have antioxidant properties (Amaeze *et al.*, 2011). *Tetracarpidium conophorum* belongs to the class of

plant foods, particularly nuts, that have a high fat content and at the same time exhibit considerable antioxidant capacity.

Some recent research determined through various methodologies by Torabians *et al.,* (2009); Sabate, (2010) revealed the antioxidant capacity of different nuts, such as almonds, brazil nuts, hazelnuts, macadamias, peanuts, pecans, pine nuts, pistachios, and walnuts. These studies confirmed that walnuts possess the greatest antioxidant capacity than other nuts and that this antioxidant property is presumably a product of phenolic compounds especially hydrolysable tannins, tocopherol, and melatonin.

The experiment carried out by Esther *et al.*, (2013) suggested that walnut oil could be effective as an antioxidant in ameliorating the toxic effects of cadmium.

2.3.3 Industrial Uses of *Plukenetia conophora*

The oil seeds containing uncommon fatty acids are industrially useful in the sense that they are used in dispersants, protective coating, pharmaceuticals, cosmetics and as stabilizers in plastic formulations (Hosamani and Sattigeri, 2003; Eganathan *et al.*, 2006).

Plukenetia conophora oil has been used in the formulation of wood varnish, stand oil, vulcanized oil for rubber and leather substitutes. Recent study carried out by Asiagwu *et al.*, (2008) confirmed that ***Plukenetia conophora*** oil can be used to synthesize an alkyd resin which was utilized in the production of white gloss paints and varnishes. The white gloss paint and varnishes produced had the right physical qualities such as brushability and good drying time. Alkyd resins are one of such products from oils used extensively in industries and they can be defined as the reaction product of an oil or fatty acids, polyol and polyacid (Asiagwu *et al.*, 2008).

2.4.0 Spectroscopy

The history of spectroscopy began with Isaac Newton's optics experiments (1666-1672) when Newton described 'spectrum' as rainbow of colours that combine to form white light and that are revealed when the white light is passed through a prism. Joseph Von Fraunhofer made experimental advances during the early 1800s, with dispersive spectrometers which enabled spectroscopy to become a more precise and quantitative scientific technique (Brand, 1995).

Spectroscopy is the analysis of the interaction between matter and any portion of the electromagnetic spectrum. Spectroscopy can be defined as the study of the absorption and emission of light and other radiation by matter, as related to the dependence of these processes on the wavelength of the radiation. Recently, the definition has been expanded to include the study of the interactions between particles such as electrons, protons, and ions, together with their interaction with other particle as a function of their collision energy. It is a term used to refer to the measurement of radiation intensity as a function of wavelength and often used to describe experimental spectroscopic methods. Spectral measurement devices are spectrometers, spectrographs, spectrophotometers or spectral analyzers.

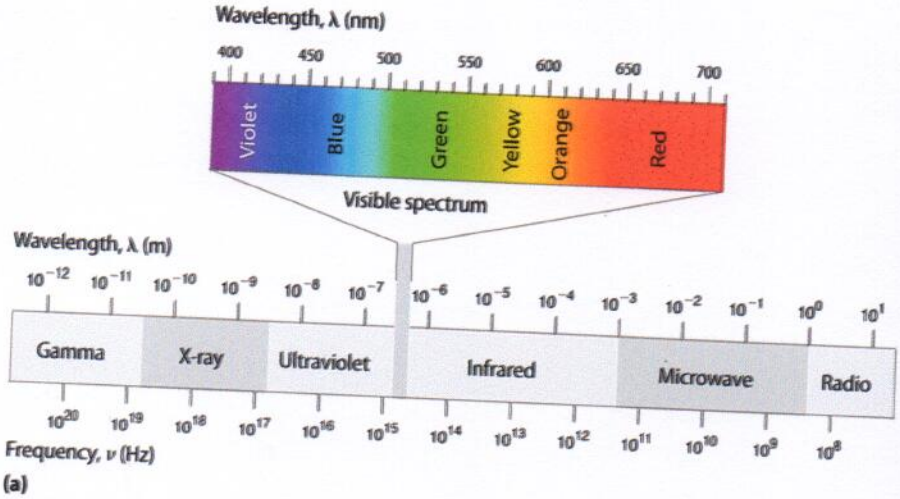
2.4.1 Electromagnetic radiations

Electromagnetic radiation is a form of radiant energy released by certain electromagnetic processes. It is composed of visible light and invisible electromagnetic radiations such as x- rays and radio waves. It is a radiant energy that exhibits wavelength- like behavior and travels through space at the speed of light in a vacuum (Atkins and Julio, 2006). Electromagnetic radiation (EMR) consists of electromagnetic waves, which are synchronized oscillations of electric and magnetic fields that propagate at the velocity of light. The oscillations of the two

fields are perpendicular to each other and perpendicular to the direction of energy as well as wave propagation, forming a transverse wave (Chang 2005). Electromagnetic waves can be characterized by either the frequency or wavelength of their oscillations to form the electromagnetic spectrum, which includes order of increasing frequency and decreasing wavelength of radio waves, microwaves, infrared radiation, visible light, ultraviolet radiation, X-rays and gamma rays.

Electromagnetic waves are produced whenever charged particles are accelerated, and these waves can subsequently interact with any charged particles. Electromagnetic waves carry energy, momentum and angular momentum away from their source particle and impart those quantities to matter with which they interact (Atkins and Julio 2006). Electromagnetic energy (such as visible light) has no detectable mass component. In other words, it can be referred to as "pure energy." Other types of radiation such as alpha rays, which consist of helium nuclei, have a detectable mass component and therefore cannot be categorized as electromagnetic energy (McQuarrie and Simon, 1997). The important parameters associated with electromagnetic radiation are energy (which is directly proportional to frequency and inversely proportional to wavelength), frequency, and wavelength.

$E = hc/\lambda$



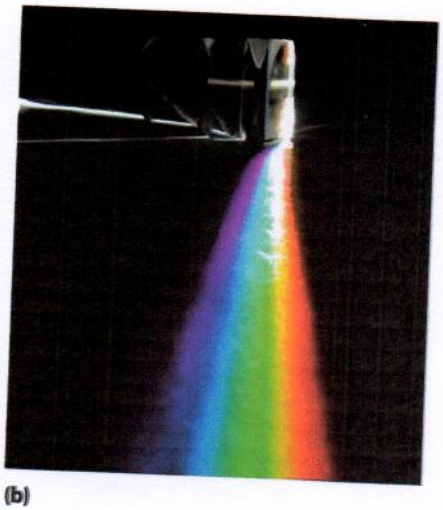


Figure 1: Electromagnetic Spectrum

Source: Wikipedia

The above diagram (a) shows the wavelength and frequency ranges of electromagnetic radiation. The visible portion of the electromagnetic spectrum is the narrow region with wavelengths between about 400 and 700 nm. Diagram (b) shows that when white light is passed through a prism, it is split into light of different wavelengths, whose colours correspond to the visible spectrum.

2.4.2 Spectroscopic Techniques

The four major spectroscopic techniques or methods in structural elucidation of organic molecules are mass spectroscopy, infrared spectroscopy, ultraviolet–visible spectroscopy, and nuclear magnetic resonance spectroscopy. These methods yield information about the characteristics of the molecule but does not provide the three-dimensional picture of the molecule.

Nuclear magnetic resonance spectroscopy: The use of NMR spectrophotometer in analysis of oil is a great success. NMR spectrometers are tuned to certain nuclei (e.g. ^{1}H, ^{13}C, ^{19}F, & ^{31}P) and for a given type of nucleus. This spectroscopy distinguishes and counts atoms in different locations in the molecule. Nuclear magnetic resonance can be in form of proton NMR, 13-carbon NMR or two dimensional (HETCOR, COSYetc)

^{1}H NMR: Information in proton NMR is limited in comparison with that of carbon -13 spectra. However detection of functional groups like alkene, double bond conjugation, conjugated double – triple bond, terminal vinyl groups is possible by their characteristic proton NMR signal.

^{13}Carbon NMR signals provide structural information on the individual fatty acids and lipid classes and even elucidation of the distribution of the different acyl groups on the glycerol backbone and the position of the unsaturated centres and functional group

Infrared spectroscopy: it is used in determining the functional groups in a molecule, by taking advantage of the vibrational and rotational energy changes that occur in molecules when they are exposed to infrared radiation ($10^{14} - 10^{13}$ Hz).

Most seed oils show similar IR spectra between 4000 and 660cm^{-1} hence, presence of additional IR absorption can be attributed to less common functional groups. For good spectra using a small quantity of oil in a short time Fourier transform IR spectrometer is recommended. Infra red is useful in getting stereo chemical information. This is based on the fact that the presence of trans double bonds can easily be proven by prominent absorption band(s) between 940 and 999cm^{-1}. The preliminary analysis for the presence of conjugated olefin (Akintayo *et al.*, 2013) and olefin acetylenic system in new seed oils can be proven using IR. Infra red can also be used to verify

lack of trans double bond because absence of trans double bond peaks shows that only cis double bonds are present (Grivello and Narayan,1972).

Elemental analysis is a process where a sample of some material such as bodily fluids, minerals, chemical compounds is analyzed for its elemental and sometimes isotopic composition. Elemental analysis is also known as elemental spectroscopy or atomic emission spectroscopy (AES). It can be qualitative (determining what elements are present) or/and quantitative (determining how much of each are present). For organic chemists, elemental analysis or "EA" almost always refers to CHNX analysis—the determination of the mass fractions of carbon, hydrogen, nitrogen, and hetero atoms (X) (halogens, sulfur) of a sample (Sowers 2001).

The method is used for analysis of pure organic compounds as well as for industrial and agricultural raw materials and intermediate and final products such as oils, pharmaceuticals. The high temperature created by the combustion of the tin capsules ensures a complete decomposition of metal salts and other refractory materials. This information is important to help determine the structure of an unknown compound, as well as to help ascertain the structure and purity of a synthesized compound.

Table 1: Spectroscopic techniques and obtained information for the analysis.

Radiation absorbed	Effect on the molecule / deduced information
Ultraviolet- visible λ, 190- 400nm 400-800nm	Changes in electronic energy levels within the molecules (extent of π-electron system, presence of conjugated unsaturation and conjugated with non- bonding electrons).
Infrared λ, 2.5- 25μm ῡ, 400- 4000cm^{-1}	Changes in the vibration and rotational movement of the molecule (detection of functional groups, which have specific vibrational frequencies e.g C=O, NH_2,OH)
Microwaves V, 9.5× 10^9Hz	Electro spin resonance or electron paramagnetic resonance: induced changes in the magnetic properties of unpaired electrons (detection of free radicals and the interaction of the electron with neighboring protons).
Radiofrequency V, 60- 600MHz	NMR; induced changes in the magnetic properties of certain atomic nuclei, notable that of hydrogen and ^{13}C isotopes (hydrogen and carbon atoms in different environment can be detected and counted)
Electron beam impact 70eV, 6000KJ/mol	Ionization and fragmentation of the molecule into a spectrum of fragment ions (determination of molecular weight and deduction of molecular structure from fragments produced)

2.5.0 Physicochemical Characterization

Physicochemical characterization of oil has to do with the physical and chemical characteristics of oil. Consideration of the physical and chemical characteristics of oil must be put in place as the characteristics of oil differ from one seed oil to another due to the composition.

For instance, when two-conventional seeds *Plukenetia conophora* (PKCP) and *Adenopus breviflorus* (ADB) were analysed for their proximate, sterols composition , fatty acids and physicochemical characteristics, it was found that the crude protein for PKCP is 25.65% and that of ADB is 28.25%(Akintayo and Bayer 2002).

The moisture content of PKCP and ADB was 8.0% and 4.5% respectively indicating that ADB has better shelf life than PKCP. The oil yield for PKCP was 49.58% and that of ADB was 56.22%. The major sterol in PKCP was found to be stigmasterol and the major sterol in ADB was beta-sitosterol (Akintayo and Bayer, 2002)

Akintayo and Bayer (2002) found out that PKCP oil had 98.8% unsaturated fatty acids with linoleic acid predominating (70.1%)while ADB had 85.1% unsaturated fatty acids with linoleic acid (65.3%). It was also found out that PKCP oil has a very high saponification and iodine value of (179±0.3mg KOH g^{-1}) and (7.31± 0.02gI $100g^{-1}$) respectively which suggest its usefulness in shoe polish, alkyd resin, liquid soap, and shampoo production (Isong *et al.*,2013; Akintayo and Bayer, 2002).

CHAPTER THREE

EXPERIMENTAL (MATERIALS AND METHODS)

3.1.0 Sample Collection

Matured walnuts were purchased from Sabo Irojo market in Ilesa East Local Government area in Osun State. The walnuts were dried with the shell for a month for easy removal of the seed from the shell. The walnut seeds were sun-dried for another two months, and after this the seeds were grinded into a jelly-like form with a grinding machine.

Figure 2: ***Plukenetia conophora*** seed in the pod.

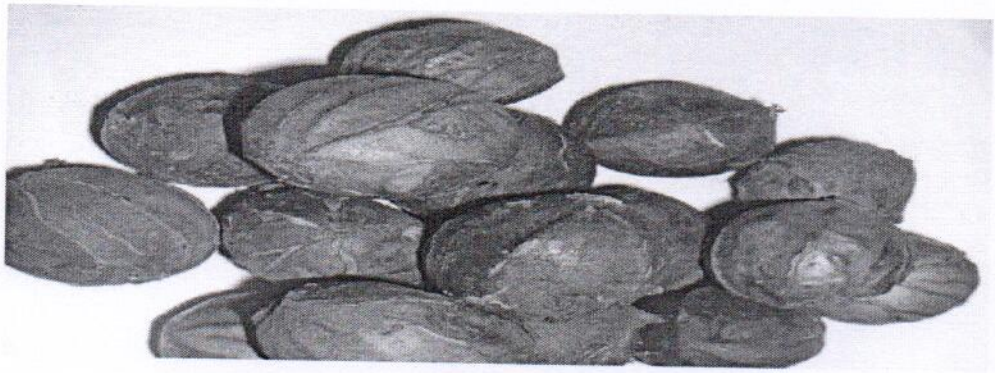


Figure 3: ***Plukenetia conophora*** released from the pod.

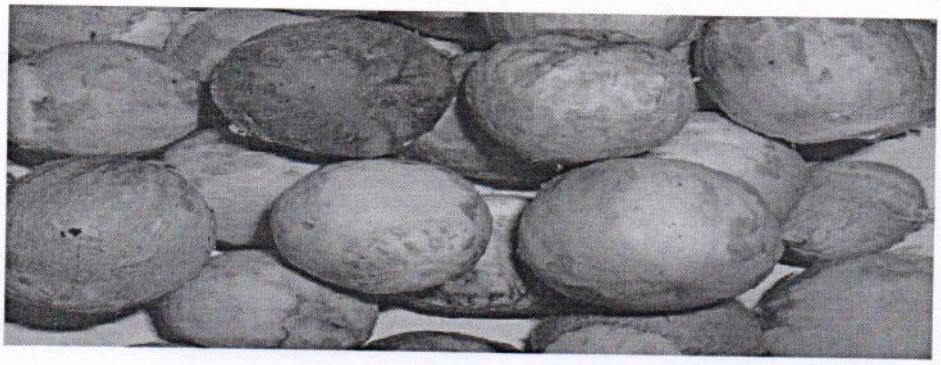


Figure 4: ***Plukenetia conophora*** seed released from the shell.

3.2.0 Preparation of Raw Material

For thorough and efficient extraction, each and every oil- bearing cell of the material is brought in contact with the solvent. Proper preparation of materials prior to extraction is very important to ensure this contact. The smaller the material size, the better is the penetration of the solvent into the oil-bearing cells.

3.3.0 Extraction

Solvent extraction is a process which involves extracting oil from oil- bearing materials by treating it with a low boiler solvent. Solvent extraction is basically a process of diffusion of a solvent into oil- bearing cells of the raw material resulting in a solution of the oil in solvent. The extraction process consists of treating the raw materials with n- hexane and recovering the oil by distillation of the resulting solution of oil in hexane. Hexane has boiling point of 67^0C/152^0F and the high solubility of oils and fats in it are the properties exploited in the solvent extraction process.

The grinded seed was placed in a semi permeable membrane before placing it in the solvent extractor. In soxhlet extraction, volatile hexane is vaporize into the top of the chamber containing materials to be extracted, where it is condensed and falls down on the bag containing the material, in the process dissolving and carrying the oil. The mixture is carried into the evaporation container when the level of the mixture of hexane and oil in the chamber increases to the arm connected to the evaporation container. A new cycle of evaporation of hexane begins leaving the oil in the container.

Figure 5: Oil extracted from *Plukenetia conophora* seed.

3.4.0 Purification of *Plukenetia conophora* oil

The crude oil was refined by agitating with 18M NaOH (1:30g/g of alkali: powder) for 15min. The resultant mixture was heated to 75- 80^0C to break the soap stock and the neutral oil separated by centrifugation. Perchloric acid (ACS reagent, 90% w/w), methylene chloride, sodium carbonate anhydrous magnesium sulphate, glacial acetic acid (100%), AR grade hydrogen peroxide (30wt%), Amberlite IR-I20H ion exchange resin, 62 % HBr in acetic acid (Merck, Darmstadt, Germany) and 1-butanethiol, 1-decanethiol, 1-octadecanethiol and deuteriated chloroform(99.8%), Sigma-Aldrich Chemie GmbH (Taufikirchen, Germany) were used as received.

3.5.0 Spectroscopic characterization of oil.

The spectroscopic characteristics that were determined in this research work were the Fourier Transform Infrared (FTIR), Proton Nuclear Magnetic Resonance (^{1}H NMR), and Carbon- 13 Nuclear Magnetic Resonance (^{13}C NMR) spectroscopy. Elemental analysis of ***Plukenetia conophora*** oil was also determined.

3.5.1 Fourier Transform Infrared (FTIR) Analysis: FTIR of the samples were done on Nicolet 380 FTIR (Thermo Electron Corporation, Madison, USA) using KBr cell (Akintayo et al., 2013).

3.5.2 Proton Nuclear Magnetic Resonance(^{1}H NMR) Analysis: It was done on a Bruker Avance 400 (Bruker Instruments, Inc Karlsruhe, Germany) Fourier transform spectrometer operating at 400.6MHz. The gated decoupling pulse sequence was used with the following parameters; number of scans 256; acquisition time 1.366s; pulse width 10.3Ks. Free induction decay FID was transformed and zero filled to 300k to give digital resolution of 2Hz/point (Akintayo *et al.*, 2013)

3.5.3 Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR) Analysis: It was done in $CDCl_3$ on a Bruker AVANCE DPX 300 Fourier transform spectrometer operating at 300 MHz. The gated decoupling pulse sequence was used with the following parameters; number of scans, 256; acquisition time 1.366s; pulse width 10.3μs. Free induction decay FID was transformed and zero filled to 300K to give digital resolution of 2Hz/point (Akintayo *et al.*, 2013)

3.5.4. Elemental analysis: The technique used for the determination of CHN was based on the quantitative "dynamic flash combustion" method. The samples were held in a tin container, placed inside the autosampler drum where they were purged with a continuous flow of helium and then dropped at present intervals into a vertical quartz tube maintained at 1030^0C (combustion reactor). When the samples were dropped inside the furnace, the helium stream was temporarily enriched with pure oxygen and the sample and its container melt. The tin promotes a violent reaction (flash combustion) in a temporary enriched atmosphere of oxygen. Under these favourable conditions even thermally resistant substances were completely oxidized. Quantitative combustion was then achieved by passing the mixture of gases over a catalyst layer. The mixture plug of combustion gases is then passed over copper to remove the excess of oxygen and to reduce the nitrogen oxides to elemental nitrogen (UCL School of Pharmacy, 2015). The resulting mixture was directed to the chromatographic column (porapak PQS) where the individual components were separated and eluted as Nitrogen (N_2), Carbon dioxide (CO_2), Water (H_2O) with the help of a Thermal Conductivity Detector whose signal feeds the automatic workstation known as Eager 200. The instrument was calibrated with the analysis of standard compounds. All results for elemental analyses were calculated based on a known value of a standard by using the K value factors calculation. This K value is determined by analyzing an organic standard of a known elemental composition (UCL School of Pharmacy, 2015).

The standards used were all traceable back to NIST primary standards and the instrument was checked with NIST primary standards on a regular basis to assure day-to-day accuracy of results. The instrument was standardized with acetanilide.

3.6.0 Physicochemical characterization of oil

The physicochemical properties were divided into two parts:

Physical properties which include the colour, refractive index, specific gravity including the moisture content of the oil.

Chemical properties include the saponification, peroxide, ester, free fatty acid, and acid values using standard methods.

3.6.1 Physical properties

Colour: The colour of the oil was gotten by visual observation

Refractive index: The refractive index of the walnut oil was measured at room temperature using the Abbey refractometer (Prince Optical Works, Malka Ganj Delhi). (Bello *et al.*, 2011)

Specific gravity: Pycnometer was used in measuring the density/specific gravity. Cleaned, dried pycnometer was weighed. It was filled with water maintained at 20^0C and weighed again. The bottle was emptied, dried and filled with oil and then weighed. The value was calculated using the formula:

$$\text{Specific gravity} = \frac{\text{Weight of oil}}{\text{Weight of water at } 20^0\text{C}}$$

(Musa *et al.*, 2012)

Moisture content

Three dried crucibles were weighed and 10g of the oil was weighed into each crucible. The samples were dried to constant weights in an oven at 105^0C cooled in dessicator and weighed. The procedure was repeated thrice for each sample and the average value was determined. (Edcey, 1954)

Smoke point

Smoke point was determined by placing 20 ml of oil sample into a stainless steel crucible. A thermometer was inserted in the oil sample and the crucible with the oil was heated under strict temperature regulation using a thermostat-equipped hot plate (Bello *et al.,* 2011).

3.6.2 Chemical Properties

Acid value

Acid value is the number of mg of potassium hydroxide required to neutralize the free acid in 1 g of the substance. 25ml of ethanol was added to 1g of each oil extract in a conical flask. This was titrated against 0.1MKOH solution using phenolphthalein as indicator.

$$\text{Acid value} = \frac{\text{titre value} \times 0.00561 \times 1000}{\text{Weight (in g) of substance}}$$ (Martin and Onigbinde, 2013)

Iodine value

10 ml of 15%KOH (w/v) was added to a 250 ml flask containing 2grams of oil and 30ml of Hanus reagent mixture that was stationed initially for 30 min in the cupboard. The resulting solution was titrated with 0.12M sodium thiosulphate. The titration was carried out for the blank using similar parameters. The titre values obtained were employed to compute the iodine value.

Iodine value of oil = $\frac{(B-R) \times \text{molarity of } Na_2S_2O_3 (0.3 \times 12.69)}{W}$

B and R stand for blank and oil samples in term of titre values, respectively. (Garba *et al.*, 2014)

Peroxide value

1.0g of the sample was transferred into $250cm^3$ flask and 1g of powdered potassium iodide (KI) and a solvent mixture (2:1 of glacial acetic and trichloromethane) were then added. The solution was placed on a water bath for a few minutes for complete dissolution. $20cm^3$ of 50% potassium iodide were introduced and the sample titrated with 0.0020N $Na_2S_2O_3$. A regular starch solution was used as the indicator. Blank experiment was similarly performed.

Peroxide value = $\frac{(R \times B) \times \text{molarity of } Na_2S_2O_3}{W}$

Where, R and B take their usual meanings and notations as in the case above. (Garba *et al.*, 2014)

Saponification value

Saponification value can be defined as the amount of mg KOH required to completely saponify 1g of oil. 0.5g of oil was weighed in a quick- fit-reflux flask and 25ml alcoholic KOH was added. It was refluxed for 30minutes in order to get simmer. The flask was cooled and 1ml of phenolphthalein indicator was added and titrated against 0.5N HCl. The saponification value was calculated using:

$$\text{Saponification value} = \frac{56.1 \times (b-a) \times N}{W}$$

W= weight of sample= 0.5g

B= blank titre value

A= sample titre value

N= Normality of HCl (Sulaimon *et al.*, 2012)

Free Fatty Acid (%FFA)

10g of oil was boiled with 50ml ethanol, allowed to cool and 2 drops of phenolphthalein indicator was added. It was titrated against 0.1N NaOH until pink colour was obtained

The free fatty acid was calculated by:

$$\text{Free fatty acid} = \frac{\text{titre value} \times 2.82}{\text{Weight of sample}}$$

(Sulaimon *et al.*, 2012)

Ester value

5ml of the walnut oil was weighed in a quick-fit refluxing flask. 25ml of alcoholic KOH with 10 drops of phenolphthalein indicator were added. It was then cooled and titrated against 0.5N HCl. Blank titration was also carried out in the same manner without the oil

The value was calculated using equation

$$\text{Ester value} = \frac{(\text{Blank sample titre value}) \times 0.5 \times 56.1}{\text{Weight of oil}}$$

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Spectroscopic Characterization

4.1.1 Fourier Transform-Infrared Spectrum

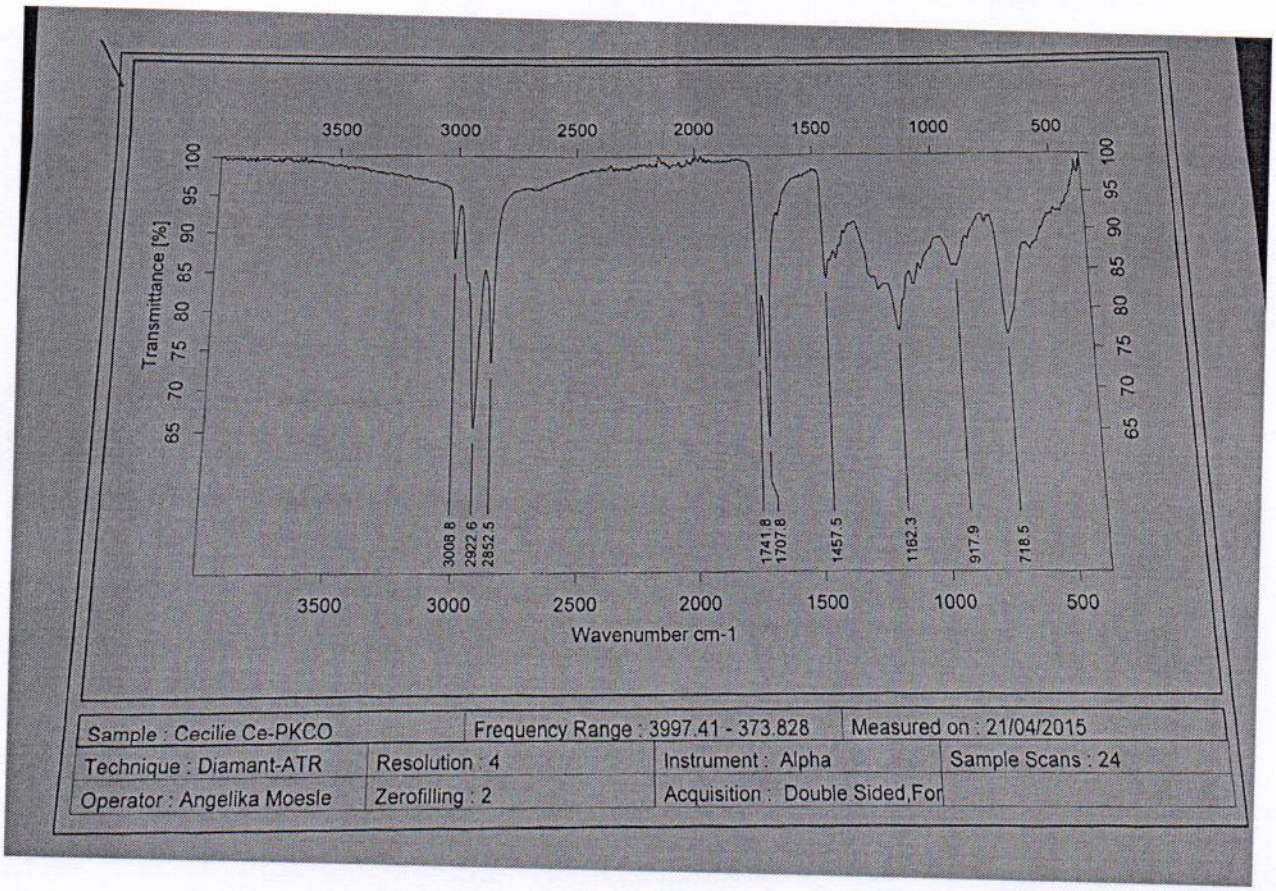


Figure 6: Fourier Transform Infrared Spectrum for ***Plukenetia conophora*** oil

Table 2: Description of the wave bands (cm^{-1}) obtained for ***Plukenetia conophora*** oil FTIR spectrum.

Wave band (cm^{-1})	Description
3008.6	=C-H stretch for sp^2 occurs at values greater than $3000cm^{-1}$
2922.6	-C-H stretch for sp^3
2852.5	-C-H stretch of an aldehyde
1741.8	C=O of an ester
1457.5	C=C stretch of an alkene
1162.3	C-O of an ether
718.5	four or more CH_2 groups in an open chain

The Fourier Transformed Infrared (FT-IR) spectrum of ***Plukenetia conophora*** oil shows the characteristic absorption band for =C-H stretching for alkene at $3008.6cm^{-1}$, -C-H stretching for alkane at $2922.6cm^{-1}$, wave band of $2852.5cm^{-1}$ shows –C-H stretching of an aldehyde, $1741.8cm^{-1}$ and $1457.5cm^{-1}$ shows C=O of an ester and C=C stretching of an alkene respectively. Absorption band of $1162.3cm^{-1}$ shows C-O-C of an ether and $718.5cm^{-1}$ shows four or more CH_2 groups in an open chain present in the oil (Akintayo et al., 2012).

4.1.2. Proton Nuclear Magnetic Resonance (^{1}HNMR) Spectrum

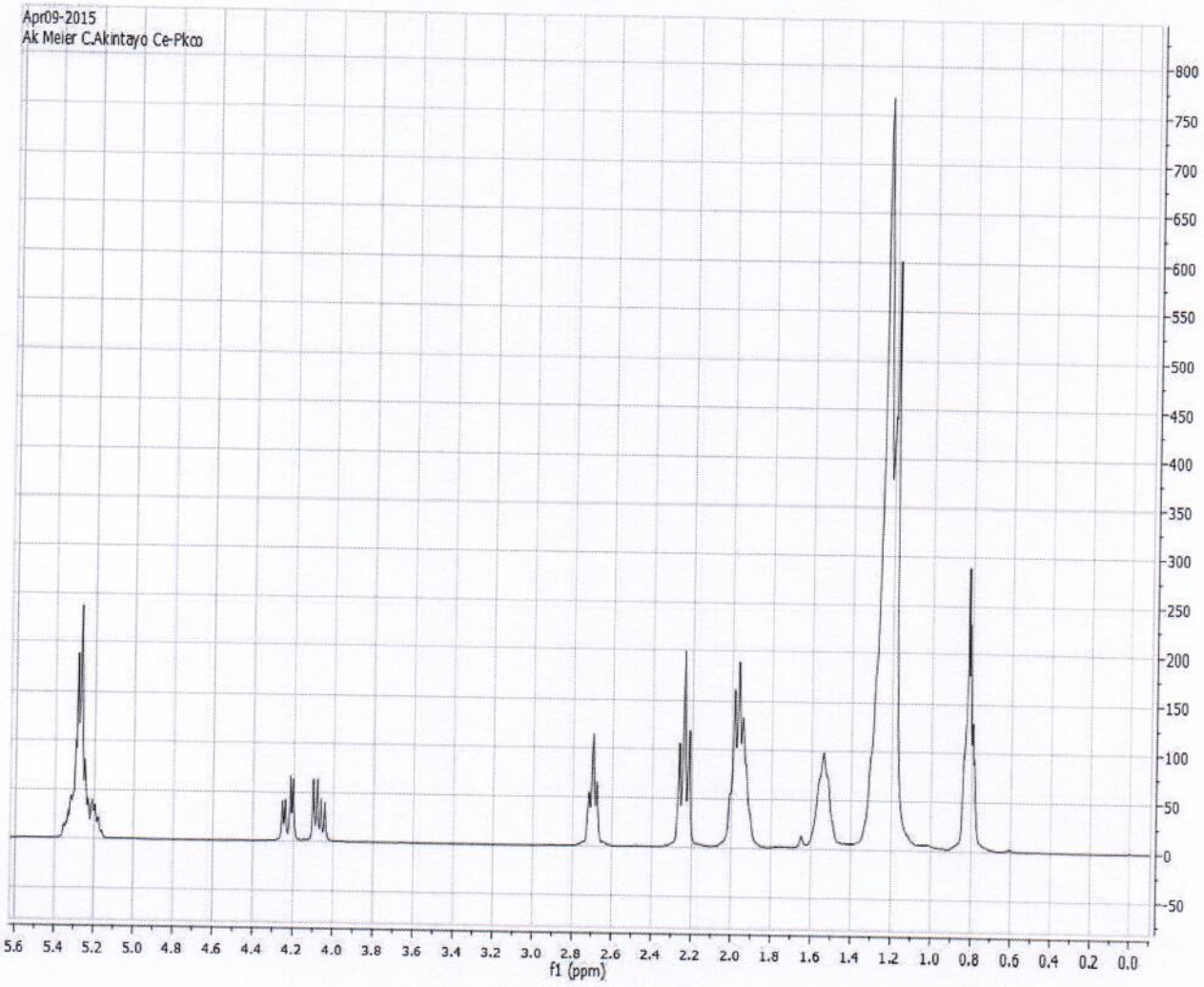


Figure 7: Proton Nuclear Magnetic Resonance (NMR) spectrum for ***Plukenetia conophora*** oil

Table 3: Description of the chemical shifts obtained for ***Plukenetia conophora*** oil ^{1}HNMR spectrum.

Chemical shifts (δ) in ppm	Description
0.8-0.9	-C<u>H</u>$_3$ terminal
1.2-1.4	-C<u>H</u>$_2$ chain stretching vibration
1.5-1.6	-C<u>H</u>$_2$CH$_3$
1.64	-CH$_2$C<u>H</u>$_2$CH$_2$COO
1.9-2.1	-C<u>H</u>$_2$C=C
2.2-2.3	-C<u>H</u>$_2$COO
2.7-2.8	=CC<u>H</u>$_2$C=
3.9-4.3	-OC<u>H</u>$_2$CHOC<u>H</u>$_2$O
5.1- 5.5	-C<u>H</u>=C

The chemical shifts (δ) given by ^{1}HNMR spectrum in parts per million (ppm) for ***plukenetia conophora*** oil are: 0.8-0.9, 1.2-1.4, 1.5-1.6, 1.64, 1.9-2.1, 2.2-2.3, 2.7-2.8, 3.9-4.1, 4.2-4.3, and 5.1- 5.5.

Singlet peak for terminal -C<u>H</u>$_3$ may be allocated to δ between 0.8-0.9ppm, peaks between 1.2-1.4ppm may be assigned to -C<u>H</u>$_2$- chain stretching vibration, the chemical shifts between 1.5-1.6ppm reveal that there is -C<u>H</u>$_2$ group is attached to -CH$_3$ terminal group and –CH$_2$C<u>H</u>$_2$CH$_2$COO peak is obtained at δ=1.64ppm. Peak at δ= 1.9-2.1ppm indicates that -C<u>H</u>$_2$ group is next to C=C, chemical shift at δ= 2.2-2.3 shows -C<u>H</u>$_2$ group attached to C=O of an ester, CH$_2$ sandwiched between two C=C appears at δ = 2.7-2.8ppm, OC<u>H</u>$_2$CHOC<u>H</u>$_2$O (glycemic) peak is gotten at δ= 3.9-4.3, and δ= 5.1- 5.5 indicates the –C<u>H</u> of a C=C (Akintayo *et al.*, 2013).

4.1.3. Carbon- 13 Nuclear Magnetic Resonance (^{13}CNMR) Spectrum

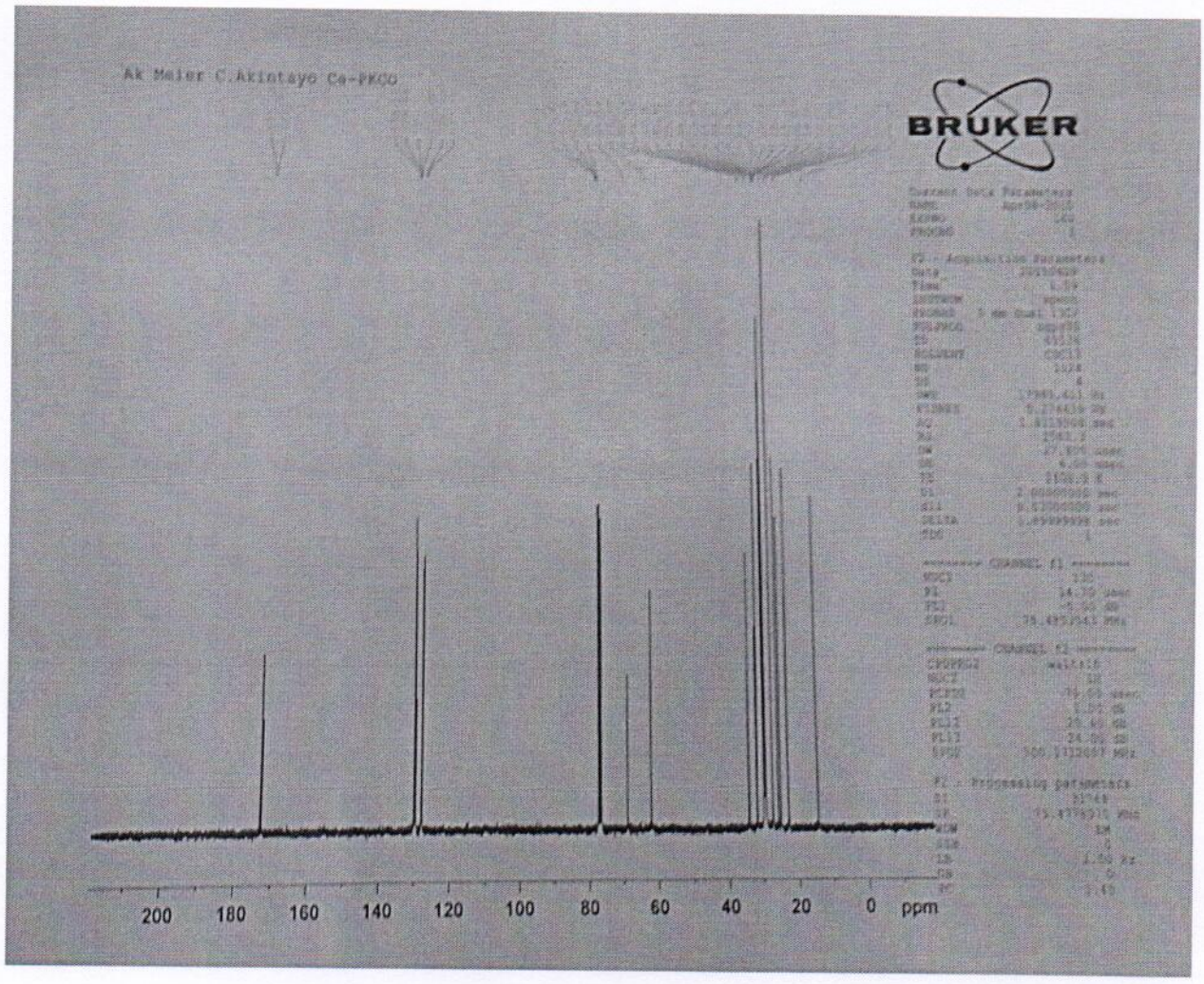


Figure 8: Carbon-13 Nuclear Magnetic Resonance (^{13}CNMR) spectrum for ***Plukenetia conophora*** oil

4.1.4. Elemental analysis

Elemental analysis performed on ***Plukenetia conophora*** oil revealed that the percentage composition of carbon in the oil is 78.39%, that of hydrogen is 10.62% while the percentage composition of oxygen is 10.99%. These correspond to the calculated composition of 77.75% carbon, 10.95% hydrogen and 11.18% oxygen. Confirming the structure of ***Plukenetia conophora*** oil as

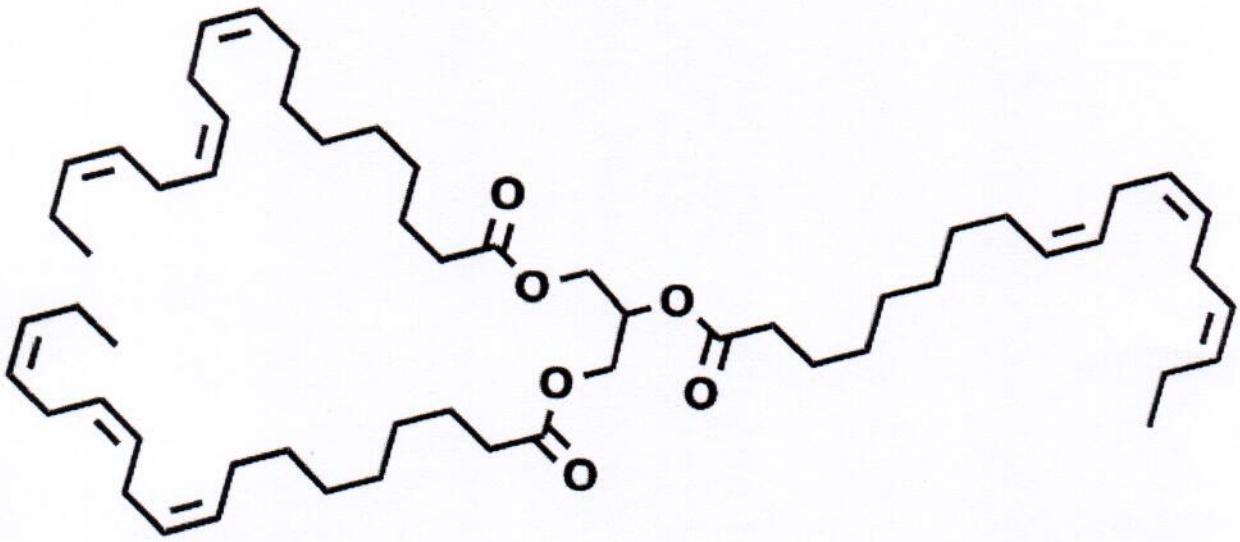


Figure 9: Schematic structure of the oil

4.2 Physicochemical Characterization

Table 4: Physical properties of ***Plukenetia conophora*** oil

Physical parameters	Values
Moisture content (%)	0.49± 0.028
Refractive index(at 29°C)	1.42
Specific gravity(at 20°C)	0.917 ± 0.001
Smoke point(°C)	204

Table 5: Chemical properties of ***Plukenetia conophora*** oil

Chemical parameters	Values
Acid value(mgKOH/g)	28.08 ± 0.042
Peroxide value(Meq/kg)	1.04
Free fatty acid(as oleic acid)	3.12 ± 0.014
Iodine value(g/100g)	5.63
Saponification value(mgKOH/g)	162.02 ± 0.042
Ester value(mgKOH/g)	101.36 ± 0.283

Values are means of triplicate determination ± standard deviation of mean.

4.2.1 Physical properties of *Plukenetia conophora* oil

The oil colour is light yellow which is in line with most oils. The colour is from the presence of chlorophylls and carotenoids (Fereidoon 2005) and consistently liquid at room temperature. It produces a nutty flavor or aroma.

Plukenetia conophora oil has a moisture content of (0.49 ± 0.028%) which shows that it can be preserved for a long time and does not create problem in trans esterification, increases shelf life and cannot cause corrosion in internal combustion engine (Ibeto *et al.*, 2012). The value obtained is lower than those reported for castor (8%), sheer butter (10%), and rubber seed (8.6%) (Asuquo, 2008)

Specific gravity is the ratio of the mass of a given volume of a substance to the mass of an equal volume of water. The specific gravity obtained for the oil was (0.917 ± 0.001g/cm^3) indicating that the oil is less dense than water. The specific gravity of ***Plukenetia conophora*** oil was in the same range of (0.86-0.98g/cm^3) reported for landophila seed oil by Karmakar *et al.*, 2010.

The refractive index of oil actually depends on their molecular weight, fatty acid chain length, degree of unsaturation, and degree of conjugation (Ibeto *et al.*, 2012). The refractive index obtained for ***Plukenetia conophora*** oil was 1.42 which is similar to that reported for soyabean oil (1.466-1.470) and palm kernel oil (1.449- 1.451). The refractive index of the oil analyzed falls within the range for many edible oils (Egbekun and Ehieze, 1997; Ilesanmi *et al.*, 1990). The high refractive index of this oil seems to conform to the high number of carbon atoms in their fatty acids (Falade *et al.*, 2008).

Smoke point obtained for ***Plukenetia conophora*** oil was 240°C which is the temperature at which it stops shimmering and starts sending out some serious smoke signals.

4.2.2 Chemical properties of *Plukenetia conophora* oil

Saponification value refers to the number of milligrams of potassium hydroxide required to saponify 1g of fat under the conditions specified. It is a measure of the average molecular weight (or chain length) of all the fatty acids present. The saponification value obtained for ***Plukenetia conophora*** oil in this study was ($162.02 \pm 0.042 mgKOHg^{-1}$) which is lower than $194 mgKOHg^{-1}$ reported for liquid red palm oil (Osita, 2007), $213 mgKOHg^{-1}$ in neem seed oil (Akpan, 2000), and $190.34 mgKOHg^{-1}$ for *Psophocarpus tetragonolobus* seed oil (Ibeto *et al.*, 2012), but higher than $159.33 mgKOHg^{-1}$ reported for *Demeltia tripatala* fruit oil (pepper fruit) (Nwinuka and Nwiloh 2009) and $143.76 mgKOHg^{-1}$ obtained for African pear oil which was reported to be good for soap making (Ikhuoria and Maliki, 2007). The saponification value for ***Plukenetia conophora*** oil in this study indicates the presence of high percentage of fatty acids in the oil and therefore implies its possible tendency and its suitability for industrial soap making.

The iodine value is a measure of the degree of unsaturation in oil and could be used to quantify the amount of double bonds present in the oil which reflects the susceptibility of oil to oxidation. Iodine value determines the degree of unsaponifiable matter of fats and oils. Nkafamiya et al., (2010) reported that lowering the iodine value improves the stability and good yield of oil. The iodine value of ***Plukenetia conophora*** oil was found to be 5.63gI/100g. Hildtch and seavell, (1980) reported that oils with iodine value less than 1.30 are non drying oil and are not suitable for paint making. Thus iodine value of 5.63gI/100g declares the potential of ***Plukenetia conophora*** oil being a suitable raw material for paint industries. The iodine value is also used as an index for evaluating the ability of oil to go rancid (Eka 1980; Amoo *et al.*, 2004). The iodine value of ***Plukenetia conophora*** oil which was found to be 5.63gI/100g indicates relative unsaturation in the oil and its less vulnerability to oxidation. The iodine value of oil does not

indicate the position of double bonds or amount of olefinic carbon but rather provides an overall status of unsaturation of the oil, so it is not possible to point out the position of double bond(s) which are more susceptible to oxidation (Knothe and Dunn, 2003). Thus iodine value of 5.63gI/100g declares the potential of ***Plukenetia conophora*** oil being a drying oil and a suitable raw material for paint industries.

Free fatty acid is the percentage by weight of a specified fatty acid (e.g. percent oleic acid) (Weiss, 1983; Derise *et al.*, 1974). It is an important parameter in determining the suitability of oil as edible oil because the lower the free fatty acid the better the quality of oil (Isong *et al.*, 2013). High levels of free fatty acids especially linoleic acids are not desirable in finished oils because they can cause off-flavour and reduce the shelf life of oils (sulaiman *et al.*, 2012). An excessive amount of free fatty acids lowers the smoke point of oil and will cause 'popping' of the oil during cooking. High quality oils are low in free fatty acids (AOCS 1973). The quantity of free fatty acid in oil is an indicator of its overall quality. The acceptable limit of free fatty acid value for edible oils is ≤ 10 (Balley, 1982). The free fatty acid of ***Plukenetia conophora*** oil obtained ($3.12 \pm 0.014\%$) in this study is lower than that of ginger bread plum (15.10%) (Ajayi, 2010), but higher than that reported for sesame oil (0.82%) (Elleuch *et al.*,2007) and 2.35% for camelina oil (Zubr, 1997) which were reported to be good edible oils. Thus, ***Plukenetia conophora*** oil can be considered to be edible oil has it contains a reasonable low percentage of free fatty acid which is necessary for oils intended for human dietary purpose.

Acid value is the mass of KOH in mg needed to neutralize the organic acids present in 1g of fat and a measure of the free fatty acids (FFA) present in the fat or oil. That is, it measures the amount of carboxylic acid groups in a chemical compound such as fatty acid or in a mixture of compounds. Acid value is an important moiety of physicochemical property of oil that indicates

the quality, age, edibility and suitability of oil use in industries such as paint (Akubugwo *et al.*, 2008). Demain, (2008) reported that acid values are used to measure the extent to which glycerides in the oil has been decomposed by lipase and other physical factors such as light and heat. Thus, the high acid value of walnut oil suggests that the oil is more susceptible to lipase action. This acid value obtained for ***Plukenetia conophora*** (28.08 ± 0.042mg/g) in this study is much higher compare to 0.6mg/g proposed by (Usoro *et al.*, 1982) for edible vegetable oils.

Peroxide value is a measure of the extent to which rancidity reactions have occurred during storage. It measures the initial stages of oxygen absorption in oil and is considered satisfactory at values ≤10. The peroxide value is very useful criteria for indicating the deterioration of oils. Values of fresh oils are less than or equal to 10 Meq/g. Values between 20 and 40Meq/g result in rancid taste.The low peroxide value of ***Plukenetia conophora*** oil obtained in this study (1.04MeqO_2 /g) indicated less susceptibility of the oil to oxidation and its stability. Again, the value falls within the range of 1-10mEq/kg stipulated for freshly prepared oil (Cooks and Reds, 1966). High peroxide value of oil shows that oil can easily go rancid and therefore has short shelf life while low peroxide value shows that oil has a longer shelf life. Oils having high percentages of peroxide are unstable and go rancid easily (Nzikou *et al.*, 2007). The peroxide value of walnut oil which was obtained to be (1.04Meq/kg) suggests that walnut oil has a longer shelf life. The low moisture content gotten in this study corresponds to the peroxide value and therefore confirms that the oil has a long shelf life.

The ester value (101.36 ± 0.283mgKOHg^{-1}) obtained in this study is lower than that obtained from castor oil (174.09mgKOHg^{-1}) (Asuquo 2008). However it is higher than that reported for bay laurel oil (22.44mgKOHg^{-1}) (Sayyah *et al.*, 2003) indicating that the oil is suitable for consumption.

CHAPTER FIVE

CONCLUSION

Plukenetia conophora oils are simple triglycerides and they possess double bonds in their structure. The spectroscopic characterization of the ***Plukenetia conophora*** oil also revealed that the oil contains ester and glycemic functional groups. The availability of these functional groups (reactive sites) makes the oil a good substance for chemical modification and can therefore be exploit in many industries. ***Plukenetia conophora*** oil compares favourably with existing oils and is similar to other existing edible oils in physicochemical properties. The result of the physicochemical properties suggest the suitability of the oil for domestic use (cooking), varnishes, surface coating, soap making, paint making and useful for other industrial purposes.

REFERENCES

- Abbey, M., Noaks, M., Belling, G. B., and Nestel, P. J. (1994). "*Partial replacement of saturated fatty acids with almonds or walnut lowers total cholesterol and low–density lipoprotein cholesterol*". Am. J. Clin. Nutr., 59: 995 - 999.
- Adebona, M. B., Ogunsua A. O., and Ologunde M. O. (1988). "*Development of conophor nut-based cereal snack food I- Biscuits*". Journal of Food and Agric. 2: 123-136.
- Ajaiyeoba, E. O. and Fadare, D. A. (2006). "*Antimicrobial potential of extracts and fractions of the African walnut – Tetracarpidium conophorum*". African Journal of Biotechnology Vol. 5 (22), pp. 2322-2325.
- Ajayi, I. A. (2010). "*Physicochemical attributes of soils from seeds of different plants in Nigeria*". Bull. F. Chem. Soc. Ethiopia, 24: 145 –149.
- Akintayo, C. O., Akintayo, E. T., and Ziegler Thomas. (2013). "*Synthesis and characterization of renewable monomers - bromoacrylated and acrylated hydroxyl brominated oils from Jatropha curcas oil*". Natural Journal Vol 17, No. 6.
- Akintayo, C.O., Akintayo, E.T, and Azeez, M.A. (2012). "*Albizia Benth Oil Maleinised Polyesteramides: Synthesis, Structure, and Characteristics*". International Scholarly Research Network. doi:10.5402/2012/708520
- Akintayo, E.T. and Bayer, E. (2002). "*Characterization and some possible uses of plukenetia conophora and Adenopus breviflorus seeds and seed oils*". Biores. Technol., 85, 95-97.

- Akpan, U. G. (2000). ''*Extraction and characterization of neem seed oil*''. In: Eyo A. A., Aluko P. O., AOAC 2010. Official Methods of Analysis.19th edition. Association of Analytical Chemists, Washington DC.
- Akubugwo, I.E., Chinyere G.C., Ugbogu A.E. (2008). ''*Comparative studies on oils from some common plant seeds in Nigeria*''. Pak. J. Nut. 2008; 7:570-573.
- Amaeze, A.O., Ayoola, G.A., Sofidiya, M.O., Adepoju-Bello, A.D., Adegoke, A.O., Coker H.A.B. (2011). ''*Evaluation of antioxidant activity of Tetracarpidium conophorum (Mill-Arg HUIch &Dalziel leaves*''. Oxidative medicine and Cellular Longevity, 2011:1155-62.
- Amoo, I.A., Eleyinmi, A.F., Ilelaboye, N.A.O. and Akoja, S. S. (2004). ''*Characteristics of oil extract from gourd (Cucurbita maxima) seed*''. *Food, Agric. and environ.,* 2, 38-39 (2004)
- Amos-Tautua, Bamidele Martin W and Onigbinde, Adebayo O. (2013). ''*Physicochemical Properties and Fatty Acid Profiles of Crude Oil Extracts from Three Vegetable Seeds*''. Pakistan Journal of Nutrition Vol. 12 (7): 647-650.
- Anderson, D., Yu, T. W. and Schmeizer, P. (1995). ''*An investigation of the DNA-damaging ability of benzene and its metabolites in human lymphocytes using the comet Assay*''. *Environ. Mol. Mutat.* 26: 305-314.
- AOCS 1973. Official and Tentative methods of American Oil Chemists Society, 3rd ed.
- Asiagwu, A. K., Omuku, P. E., Okoye, P. A. C., Olisa, M. A. & Ajiwe, V. C. E. (2008). ''*Evaluation of the suitability of conophor oil for the production of alkyd resins and surface coatings*''. Oriental Journal of Chemistry Vol. 24(2), 405-408.

- Asuquo, J. E. (2008). ''*Studies on the absorption of some selected metallic soaps onto hematite*''. Portharcourt, Nigeria: University of Port Hacourt, Ph. D. Dissertation.
- Atkins Peter and Julio de paula. (2006). ''*Physical Chemistry for the Life Sciences*''. New York: Oxford University Press.
- Ayodele , O. B. 2003. Nutrition in Ibadan. Catoon Publishers, Nigeria.
- Ayoola, P.B., Adeyeye, A., Onawumi, O. O. E. and Faboya, O. O. P. (2011) ''*Phytochemical and Nutrient Evaluation of Tetracarpidium conophorum (Nigerian walnut) Root*''. International Journal of Research and Review in Applied Sciences. 7(2), pp : 198-203 .
- Balley A.E. (1982). ''*Industrial Oil and Fat Product*''. John Wiley-Inter science, New York, NY, USA, 3rd edition.
- Becker, C. E. (1985). ''*Principles of occupational Medicine*''. In; Cecil Textbook of Medicine, 17th ed. (J. B. Wyngaarden and L. H. Smith, Jr. eds.) pp. 2277-2279. W. B. Saunders Co, Philadalphia.
- Brand John C.D (1995). ''*Lines of light: The sources of Dispersive Spectroscopy*''. 1800-1930. Gordon and Breach Publishers.p.57.
- Caglarirmak N (2003). ''*Biochemical and physical properties of some walnut genotypes (Juglans regia L)*''. Nahrung Food 47:28–32.
- Chang, Raymond. (2005). ''*Physical Chemistry for the Biosciences*''. USA: University Science Books.
- Cooks, K.J, and Reds, B.C. (1966). ''*Laboratory Handout for Oils and Fats Analyst*''. (U.S. Edition). Academy Press Inc. London; 1966:419-421.

- d'Azevedo, P. A., Tannhauser, A. L., Tannhauser, S. L. (1996). ''*Haematological alternations in rats from xylene and benzene*''. *Vet. Human Toxicol* 38 (5): 340-344.
- Demain, M.J, (2008). ''*Principles of food chemistry*''. Van Nostrond Reinhold International Company Limited, London, England.2nd Ed; 2008; 37-38.
- Derise, N. L., Lau, H. A., Ritchey, S. J., and Murphy, E. W. (1974). ''*Yield, Proximate Composition and Mineral Elemental Content of three cultivars of raw and roasted peanuts*''. J. Food Sci., 39: 264 – 266.
- Dosumu M.I., and Ochu C. (1995). ''*Physicochemical properties and fatty acid composition of lipids extracted from some Nigerian fruits and seeds*''. *Global J. Pure Appl. Sci.,* 1, 45-47 (1995).
- Edcey, E.W. (1954). ''*Vegetable Fats and Oils*''. American Chemical Society Monograph Series, Reinhold Publication Company, New York pp 582-584.
- Edem, C. A., Dosunmu, M. I. & Bassey, F. I. (2009). ''*Determination of Proximate Composition, Ascorbic Acid and Heavy Metal Content of African Walnut (Tetracarpidium conophorum)*''. Pakistan Journal of Nutrition, 8(3), 225-226.
- Eganathan, P., Subramania, H. M., Latha, R. and Shrinivasa, R. C. (2006). ''*Oil analysis in seeds of Salicorniabrachiata*''. Industrial Crops and Products, 23: 177.
- Eka, O.U. (1980). ''*Proximate composition of bush mango tree and some properties of dika fat*''. Nig. J. Nutr. Sci., 1, 33-36 (1980).
- Ekwe, C.C., Ihemeje,A. (2013). ''*Evaluation of physiochemical properties and preservation of African walnut (Tetracarpidium conophorum)*''. Academic Research International, Vol. 4 No. 6 November 2013.

- Elleuch, M., Besbes, S., Roiseux, O., Blecker, C. and Attia, H. (2007). "*Quality characteristics of sesame seed and by-products*". Food Chemistry, 103: 641 – 650.
- Enujiugha, V. N. & Ayodele, O. O. (2003). "*Evaluation of nutrients and anti nutrients in lesser known under-utilized oil seeds*". Int. J. Food Sci. and Technol., 38, 525-528.
- Falade, O.S, Adekunle, S.A, Aderogba, M.A, Atanda, O.S, Harwood, C, Adewusi, S.R.A. (2008). "*Physicochemical properties, total phenol and tocopherol of some Acacia seed oils*". J. Sci. Food Agric. 88, 2008, 263-268.
- Fereidoon, S. (2005). "*Bailey's Industrial Oil and Fat Products*". Sixth edition, six volume set. At John Wiley and Sons Inc; 2005:7-8.
- GRIN. (2010). "*Plukenetia conophora Müll. Arg. Germplasm Resources Information Network (GRIN) Taxonomy for Plants*". United States Department of Agriculture (USDA) and Agricultural Research Service (ARS).
- Grivello, J.V. and Narayan, R. (1972). "*Epoxidised triglycerides as renewable monomers in photoinitiated cationic polymerisation*". Chem. Mater., 4, pp. 692- 694
- Henderson, R. F., Sabourin, P. J., Bechntold, W. E., Steinberg, B. and Chang, I. Y. (1993). "*Isobutene (2-methylpropene)*". *Toxicol. Appl. Pharmacol, 123*: 50 - 61.
- Hilditch, J. P. and Seavell, R. (1980). "*The constitution of natural fats*". Champson and Hall, London. p571.
- Hosamani, K. M. and Sattigeri, R. M. (2003). "*Industrial utilization of Rivea Ornata seed oil: A moderate source of vernolic acid*". Ind.Crops Prod.,12: 93.
- Igboko, D.O. (1983). "*Phytochemical studies on Garcinia kola Heckel*". M.sc. Thesis. University of Nigeria Nsukka., pp: 202.

- Ikhuoria, E. U. and Maliki, M. (2007). ''*Characterization of avocado pear (Persea americana) and African pear (Dacryodesadulis) extracts*''. African J. Biotechnol., 6 (7): 950 - 952.
- Ilesanmi, J., Okusanya, B.A.O., and Tanko, S. (1990). ''*Characterization of oil extracts from Vegetable Seeds*''. National Conference on Application of Science for National Development. Mubi Press. Niger State, Nigeria, pp: 717-729.
- Ilija Gawrilow (2004). ''*Vegetable oil usage in lubricants*''. Oleochemicals: Volume 15 (11).
- Isong, N., Alozie, Y. E., and Ekwere, Y. (2013). ''*Physicochemical properties of African Walnut (tetracarpidium conophorum) oil and its suitability for domestic and industrial uses*''. Nigerian Journal of Agriculture, Food and Environment. 9(3):12-15
- James, N. R. (2009). ''*Volatile components of green walnut husks*''. J. Agric. Food Chem., 48 (7): 2858 - 2861.
- Karmarkar, A., Karmakar, B. and Mukherjee, S. (2010). Bioresource Technology, 101 :7201.
- Kato, M., Rocha, M. L., Carvallio, A. B., Chaves, M.. E., Rana, M. C., and Oliverra, F. C. (1993). ''*Occupational exposure to neutratoxicants- preliminary survey in five industries of camacari petrochemical complex*''. Brazil, *Environ.. Res.* 61: 133-139.
- Klassen, C. D. (1990). ''*Non metallic environmental toxicant: Air pollutants, solvents, vapour and particles*''. In: Goodman and Gillman's Textbook, The Pharmacological Basis of Therapeutics 8th ed,, A. G. Gilman, T. W. Rall, A. S Niuo and P. Taylor (eds.) NY, Pergamon Press, Pp 1596-1614.

- Knothe G, Dunn R. (2003). ''*Dependence of oil stability index of fatty compounds on their structure and concentration and presence of metals*''. J. Am. Oil Chem. Soc.; 2003(80): 1021-1026.
- Malu, S. P., Obochi, G. O., Edem, C. A. & Nyong, B. E. (2009). ''*Effects of methods of extraction on phytochemical constituents and antibacterial properties of Tetracarpidium conophorum seeds*''. Global journal of pure and applied sciences, 15(3), 373-376.
- McQuarrie Donald and Simon John. (1997). ''*Physical Chemistry: A Molecular Approach*''. Sausalito, C.A: University Science Books.
- Muibat Olabisi Bello, Temitope Lorine Akindele, Deborah Olubunmi Adeoye and AbdulKabir Oladele Oladimeji. (2011). ''*Physicochemical Properties and Fatty Acids Profile of Seed Oil of Telfairia occidentalis*'' Hook, F. International Journal of Basic & Applied Sciences IJBAS-IJENS Vol: 11 No: 06.
- Musa M. Sulaiman, Bello, A.U., Itumoh J. E., Bello K., Bello A. M., Arzika, A. T. (2012). ''*Physicochemical properties of some commercial groundnut oil products sold in sokoto metropolis, northwest Nigeria*''. Journal of Biological Science and Bioconservation. Volume 4.
- NkafamiyaII, M. H. M., Osemeahon, S. A., and Modibbo, U. U. (2010). ''*Percentage oil yield and physiochemical properties of different groundnut species (Arachishypogaea)*''. Afri. J. Food Sci., 4 (7): 418 - 421.
- Nuhu, A. M., Mshelia, M. S. & Yakubu, Y. (2000). ''*Antimicrobial screening of the bark extract of Pterocarpus erinaceous tree*''. Journal chemical society of Nigeria, 25, 85-92.

- Nwaoguikpe, R.N., Ujowundu, C.O and Wesley, B. (2012). ''*Phytochemical and Biochemical Compositions of African walnut (Tetracarpidium conophorum)*''. Journal of Pharmaceutical and Biomedical Sciences. 20(9):1-4.
- Nwinuka, N. M. and Nwiloh, B. I. (2009). ''*Physico-chemical Properties and Fatty Acid Composition of Dennettiatripetala Fruit Oil (Pepper Fruit)*''. Nig. J. Biochem Mol. Biol., 24 (1) 42 – 46
- Nzikou, J.M., Mvoula-Tsieri, M., Matos, L. (2007). "*Solanum nigrum L. seeds as an alternative source of edible lipids and nutriment in Congo Brazzaville*". Journal of Applied Sciences, vol. 7, no. 8, pp. 1107–1115, 2007.
- Oke O. L., and Fafunso M. A. (1995). ''*Lesser known oilseeds: the nutritive value of conophor seeds in vitro*''. Nutrition Report International. 12: pp 41-49.
- Oke, O.L., (1995). Leaf protein research in Nigeria Ibadan, University of Ibadan Press.
- Okerulu, I. O. & Ani, C. J. (2001). ''*The phytochemical analysis and antibacterial screening of extracts of Tetracarpidium Conophorum*''. Journal of Chemical Society of Nigeria, 26(1), 53-55.
- Okwu, D.E., and Okele, O. (2003). ''*Phytochemical Screening and Mineral Composition of Chewing Sticks in South Eastern Nigeria*''. *Global J. Pure Appl. Sci.,* 9: 235-238. 2003.
- Onawumi,O.O.E., Faboya, O.O.P., Ayoola, P.B. (2013). ''*Chemical evaluation and nutritive values of African walnut leaf (Plukenetia conophora Mull.arg.)*'' International Journal of Herbal Medicine, Volume 1, Issue 3
- Osita, B. U. (2007). ''*Physicohemical properties of "eketeke"(hard red palm oil)*''. Nigeria Journal of Nutritional Sciences, 1:157 - 162.

- Oyenuga, V.A. (1997). "*Nigeria Food and Feeding Stuffs Ibadan*". University Press, Ibadan.
- Ozkan, G. and Koyuncu, M. A. (2005). "*Physical and chemical composition of some walnut (Juglanregia L.) genotype grown in Turkey*". Grasas y Aceites, 56 (2): 142.
- Rabble, G. K. and Wong, O. (1996). "*Leukemia mortality by cell type in petroleum workers with potential exposure to benzene*". Environ. Health Perspect 104: 1381 – 1392.
- Ross, D. (1996). "*Metabolic basis of benzene toxicity (Review)*". *Euro. J. Haematol,* 60: 111 – 118.
- Rothman, N., Li, G. L., Dosemeci, M. Bechtold, W. E., Marti G. E, Wang, Y. Z. (1996). "*Haematotoxicity among Chinese Workers-heavily exposed to benzene*". Am, J. ind. Med. 29 (3): 236-246.
- Sabate J. Keiji O, Emilio R. (2010). Archives of Internal Med; 170 (9): 821-827.
- Savage, G. P. (2001). "*Chemical composition of walnuts (Juglan regia), grown in New Zealand*". Plant Foods Human Nutr., 56: 75 - 82.
- Savage, G. P., McNiel, D. L., and Dutta, P. C. (2001). "*Some nutritional advantages of walnuts*". Acta Hort., 544: 557-563.
- Sayyah, M., Saroukhani, G., Peirovi, A. and Kamalinejad, M. (2003). "*Analgesic and anti-inflammatory activity of the leaf essential oil of Laurusnobilis*". Phytother Res., 17 (7):733 – 736.
- Smith, J. H., Mallet, A. K., Brantom, P. G. (1996). "*Ninety days feeding study in Fischer – 344 rats of highly refined petroleum-derived food grade white oils and waves*". Toxicol Pathol 24: 214-230.

- Sowers, J. (2001). "Use Statistical Analysis to Create Wear Debris Alarm Limits." Practicing Oil Analysis. November-December, p. 38-41.
- Stanner, S.A., Hughes J., Kelly, C.N., Buttriss J. Public Health Nutr 2004; 7 (3): 407–22.
- Syeda Farina Asghar, Habib-ur-Rehman, M. I. Choudahry and Atta-ur-Rahman. (2011). ''*Gas chromatography-mass spectrometry (GC-MS) analysis of petroleum ether extract (oil) and bio-assays of crude extract of Iris germanica''*. International Journal of Genetics and Molecular Biology Vol. 3(7), pp. 95 -100.
- Torabian, S., Haddad, E., Rajaram, S., Banta, J., Sabate, J. J. (2009). ''*Human Nut and Diet*'' 22: 64-71.
- University College London School of Pharmacy- Gower Street - London - WC1E 6BT. 2015.
- Usoro, E.U., Suyamsothy, E., Sani, G.A. (1982). ''*Manual of chemical methods of food analysis*''. Bencox International Ltd. Lagos, Nigeria. Pp: 24-27.
- Weiss, T. J. (1983). ''*Physical and Chemical properties of fats and oils. Infood oils and their uses"*. AVI publ. Co. Inc. Westport.com. USA 2ndedition,Pp. 25 – 84.
- Zaharaddeen N. Garba, A. Galadima and Abdulfatai A. Siaka. (2014). '*Mineral Composition, Physicochemical Properties and Fatty Acids Profile of Citrullus Vulgaris Seed Oil*'. Research Journal of Chemical Sciences Vol. 4(6), 54-57.
- Zubr, J. (1997). ''*Oil Seed Crop: Camelina sativa''*. Ind. Crop Prod., 6:113 – 119.