CHARACTERISATION AND EVALUATION OF FLOURS AND OILS FROM SELECTED UNDERUTILISED PLANT SEEDS FOR FEEDS AND OLEOCHEMICALS PRODUCTION

By

Emmanuel Nnamdi **IFEDI**
B. Sc. (FUNAAB), M. Sc. (Ibadan)
Matric No.: 154049

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CERTIFICATION

I certify that this work was carried out by Emmanuel Nnamdi IFEDI in the Department of Chemistry, University of Ibadan, Ibadan, Nigeria.

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Supervisor
Ibironke A. Ajayi,
B. Sc. Hons, (Benin), M. Sc., Ph. D (Ibadan),
Senior Lecturer,
Department of Chemistry,
University of Ibadan,
Ibadan, Nigeria.

DEDICATION

This research is dedicated to the glory of God the Father for giving me the grace and opportunities to pursue this degree. It is also dedicated to my parents Chief Jerome Ifedi (late) and Mrs. Angelina Ifedi for their supports.

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ABSTRACT

Plant seeds are sources of readily available nutrients and raw materials for man and industries, respectively. Although commonly regarded as wastes, flours, oils and spent cakes from these seeds can be used in animal nutrition. While the chemical constituents of *Areca catechu*-(AC), *Enterolobium cyclocarpum*-(EC), *Polyalthia longifolia*-(PL), *Balanites aegyptiaca*-(BA) and *Neocarya macrophylla*-(NM) seeds are known, there is limited information on the use of their flours and cakes in dietary feed and their oils as biofuels. The aim of this study was to screen these seeds for the production of cakes and oils from seeds flours, characterise and evaluate their applications as feed and oleochemicals.

The AC-(FHI-113371), EC-(FHI-113391) and PL-(FHI-113392) seeds collected from Oyo; BA-(FHI-113393) (Kaduna) and NM-(FHI-113395) (Niger) were authenticated at FRIN (Ibadan) and separately pulverised into flours. Oils were extracted using n-hexane and cakes were obtained after extraction. Proximate compositions of all flours and cakes from only BA and NM were determined using standard methods. The mineral and amino acids contents were determined using ICP-OES and HPLC. Fatty-acid profiles of oils were evaluated by flame ionisation gas chromatography. Wistar rats were fed at 0-30% substitution for 56 days with AC, EC and PL flour formulations as well as BA and NM oils and cakes formulations, respectively. The control Wistar rats were maintained on conventional rations. Rat weights were assessed weekly. *In vivo* toxicity was determined using Red Blood Cell count (RBC) and tissue histopathology using standard procedures. The BA and NM oils were converted into biodiesel, biolubricant and biosurfactant following established procedures. Their chemical conversions were monitored with FTIR and ^{1}HNMR. Their physicochemical properties were evaluated and compared with standard specifications for automotive fuels-(EN-14214), lubricant-(ISO-VG-32) and surfactant-(SLS). Data were analysed using ANOVA at $\alpha_{0.05}$.

The protein contents of the flours were 4.20±0.08, 25.43±0.22, 12.40±0.25, 30.54±0.15 and 30.69±0.05% for AC, EC, PL, BA and NM, respectively. Their respective carbohydrate contents (%) were 52.58±0.64, 54.66±0.23, 59.65±0.19, 5.08±0.08 and 8.15±0.25. Predominant minerals (ppm) were K(2116.98±0.01-5767.62±0.11), Ca(503.63±0.03-1225.12±0.02) and Mg(674.33±0.57-3560.56±0.09). Essential amino acid ranged from 17.93-36.36 g/100g. The oils were rich in oleic and linoleic acids with cholesterol-lowering activity. A significant increase in weight (72.86±0.01-96.44±1.01 g) was recorded in Wistar rats maintained on 10% AC-modified feed compared to other treatments. The RBC ranging from 7.09±0.49 to 7.51±0.58×10^6 cells/μL compared favorably with the control (6.66±1.59 to 7.73±0.53×10^6 cells/μL). The BA intake caused a mild diffuse hydropic degeneration of rats' liver cells. The viscosity at 40°C (3.52-4.42; 19.21-26.64 mm^2/s), density at 15°C (0.88-0.89; 0.88-0.92g/cm^3) and flash point (139.30-146.51; 220-240.35^{o}C) of synthesised biodiesel, biolubricant and surface tension (27.45±0.02-27.90±0.03mN/m) of biosurfactant from BA and NM oils, respectively, compared favourably with standards. Peaks at 1617cm^{-1}($C{=}O_{amide}$) and 1060cm^{-1} ($C\text{-}N_{amide}$) indicated biosurfactant formation, while those at 3366-3442cm^{-1} ($O\text{-}H_{stretching}$) suggested biolubricant formation. A singlet at 3.70ppm (methoxy-protons) indicated biodiesel formation. These peaks were absent in the spectra of the oils.

Balanites aegyptiaca and *Neocarya macrophylla* seeds had good nutritional constituents and could serve as alternative sources of nutrients in feed formulations if purified. Their oils were potential raw materials for oleochemicals.

Keywords: Essential amino acids, Dietary feed, Biolubricant, Biodiesel

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LIST OF ABBREVIATIONS

AC: *Areca catechu*

ALB: Albumin

AST: Aspartate amino transferase

ASTM: American Society for Testing and Material

ATP: Alkaline amino transferase

AV: Acid value

BA: *Balanites aegyptiaca*

BASR: *Balanites aegyptiaca* seed residue

BASODEA: *B. aegyptiaca* seed oil diethanolamide

BASOEPOX: *Balanites aegyptiaca* epoxidised seed oil

BASOLUB: *Balanites aegyptiaca* seed oil biolubricant

CA: *Chrysophyllum albidum*

^{13}CMR: Carbon-13 nuclear magnetic resonance

DSC: Differential scanning calorimeter

EC: *Enterolobium cyclocarpum*

ECSR: *Enterolobium cyclocarpum* seed residue

EPOBASODEA: *B. aegyptiaca* epoxidised oil epoxidised diethanolamide

EPONMSODEA: *N. macrophylla* epoxidised oil epoxidised diethanolamide

ESI-MS: Electro spray ionisation mass spectroscopy

FT-IR: Fourier transform infrared spectroscopy

GC-FID: Gas chromatography coupled with flame ionisation detector

GC-MS: Gas chromatography coupled with mass spectrometry

Hb: Haemoglobin

HBBABS: Haematological and blood biochemistry analyses of blood samples

HST: Histopathological study of the tissues

HCl: Hydrochloric acid

HNMR: Proton nuclear magnetic resonance

HNO_3: Nitric acid

HPLC: High performance liquid chromatograph

ICP-OES:	Inductive couple plasma optical emission spectrometer
IV:	Iodine value
MWG:	Mean weight gain
NMSODEA:	*N. macrophylla* seed oil diethanolamide
NMSOEPOX:	*Neocarya macrophylla* epoxidised seed oil
NMSOLUB:	*Neocarya macrophylla* seed oil biolubricant
NM:	*Neocarya macrophylla*
NMSR:	*Neocarya macrophylla* seed residue
PL:	*Polyalthia longifolia*
PCV:	Packed cell volume
RBC:	Red blood cell
RGR:	Relative growth rate
SEM:	Scanning electron microscopy
SLS:	Sodium laurel surfactant
SV:	Saponification value
TLC:	Tin layer chromatograph
UV:	Ultra violet region
WHO:	Word Health Organisation

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CHAPTER ONE

INTRODUCTION

1.1 Background of study

Good and healthy nutrition are basic human right in our world today. The correlation among nutrition, food, as well as health in the growing population should be strengthened in other to have healthy populations that encourage development. An important way of actualising this in developing countries is by the application of local resources and plant materials that are available (Mohammed *et al.,* 2014; Frona *et al*., 2019). Nature preserves a number of vegetative growths which include plants of all kind, microscopic plants, fungi, grasses, herb shrubs and trees that can be of important uses. Tropical plant seeds are necessary components in the everyday meal in many communities, and these seeds are pulled together from desert trees and those cultivated locally. A countless number of these seeds are useful for different purposes and their plant products also meet a wide range of local non food purposes (Verma *et al*., 2017). Numerous tropical fruits remain relatively obscure and have not received sufficient attention. These seeds from tropical plants, despite their lack of familiarity, could serve as valuable dietary supplements. Furthermore, their utilisation has the potential to contribute to increased income generation and subsequently alleviate poverty (Asgar *et al*., 2010; Verma *et al*., 2017).

Plant-based proteins have a crucial role to fulfill in our dietary choices, particularly in developing countries where there is an insufficient intake of protein to meet the necessary requirements. Products obtained from plant rich protein are becoming more interesting and more useful as one of the qualities necessary in food production all over the Nations. Due to their significant chemical components such as protein, minerals, and carbohydrates, plant seeds have garnered increased attention and interest among a considerable portion of the population in developing nations (Singh and Singh, 1991; Santiya *et al.,* 2020).

Animal protein is a valuable reservoir of essential amino acids necessary for proper growth, tissue repair, and maintenance in the human body. It also provides vital nutrients such as vitamins, minerals, and healthy fats. However, too much of animal protein has been related with health problem for example an elevated likelihood of heart disease, certain cancers, and concerns regarding the environmental sustainability of intensive animal farming practices. Consequently, there has been a continuous pursuit for alternative protein sources that can be integrated into dietary foods as additives and essential components. This endeavor aims to ensure optimal nutrition while minimizing the potential negative consequences of excessive animal protein intake. Recently, there has been a rise in the direct utilisation and acceptance of diverse proteins derived from plant seeds in the formulation of diets and the creation of products due to their affordability (Asgar *et al.,* 2010; Verma *et al*., 2017; Sa *et al*., 2020).

The food scientists and nutritionists are putting more attention in developing beneficially stable proteineous nutriments for individuals in developing countries. In those developing countries, many agencies (both international and governmental) are faced with various task of lessening the protein calories malnutrition. To subdue this problem, important raw materials can be sourced from local plant seeds, oil seeds and grains to produce highly proteineous foods at less cost. The gradual increase in the application and usefulness of protein from various oil seeds and legume in the manufacture of several nutritional diets and food products might be because of the large amount of protein in them (Asgar *et al*., 2010; Verma *et al*., 2017).

With the recent industrialisation in developing countries, there is inadequate supply of petroleum base raw materials. World-wide reduction in crude oil reserves arising from the continual usage of non-renewable fossil materials (oil, gas, and coal) as well as the rate at which they are consumed, the high prices and unavailability of petroleum, energy insecurity and anxiety over environmental degradation, has increased global search for other sources of energy and interest in bio-based materials (Aldhaidhawi *et al.,* 2017). Vegetable oils from biomass are found as another source of materials for the manufacturing of renewable energy. More importantly, the production of industrial fluids (diesel, lubricants and other fuels) that are biodegradable, renewable and environmentally

friendly has raised the necessity and interest to search for other sources of renewable fuels (Yang *et al.,* 2012; Anjaneyulu, *et al*., 2016; Basumatary *et al*., 2018; Molino *et al*., 2018).

In relation to protein malnutrition, a lot of attempts have been made to provide solutions to nutritional problems in Africa. The need to research on newer and alternative sources of protein is on a high side due to insufficient protein in developing countries, especially Nigeria where several underutilised seeds such as *Afselia africana* (Ajiboye *et al.,* 2018), *Lupinus termis* (Khalid *et al*., 2016), *Sterculia urens* (Galla *et al.,* 2012) and *Dalium guineense* (Ogungbenle and Ebadan, 2014) have been assessed for their chemical and nutritional properties. In Nigeria, unusual proteins containing foodstuffs that are locally available have been evaluated for their nutritional potentials in other to reduce the dependence and competition between livestock and mankind. Seeds from these fruits could be of important uses to mankind to bridge the gap of oil deficiency, feed replacement due to their nutritional values, in the treatment of some ailment as in herbal medicine, symptoms and other infections. Oil from these seed could also be used in production of lubricants, biodiesels and biosurfactant, coating and pharmaceutical industries among other industrial applications. However, due to the fear of toxicity and lack of adequate information on their properties and applications, they are lying fallow in some location and area of human habitation.

Toxicity is the level at which a compound causes harm to animal or human beings. The three stages of toxicity are acute, sub-chronic and chronic. So, determining the toxicological profile of a substance, plants or seeds is a basic requirement and regulations for the use, marketing of product and also essential condition for guaranteeing public health (Vishnu *et al*., 2010). In order to effectively utilise plant seeds and their oils to their fullest potential, it is crucial to gather comprehensive information regarding their compositions, properties, and potential toxicity. Addressing these requirements, this study was conducted to explore the application of six underutilized seeds as novel sources of oils. The results were used to find out some suitable industrial applications of these seeds and their oils.

1.2 Statement of problem

Previous research conducted by various scholars has documented the application of plant seeds and their oils in the creation of feeds and oleochemicals, specifically biofuels. Majority of these oils possess both edible and versatile properties. Examples of such oils include soybean oil, coconut oil, sesame oil, and numerous others. The production of these biofuels holds great importance due to the fact that conventional fuels derived from petroleum are finite fossil fuels. Moreover, they are expensive, not environmentally friendly and have been discovered to pose environmental risks by releasing harmful gases like H_2S and other sulphur compounds into the atmosphere (Adewuyi *et al*., 2012a; Nath *et al*., 2019).

In many developing nations, a significant portion of the population with low income relies on plant seed flours as sources of dietary proteins due to limited access to animal proteins, which are often costly and can cause health problem if consume in excess. The global rise in population and the demand for affordable, economical dietary proteins within these low-income communities that will ensure optimal nutrition and minimizing the potential negative consequences of excessive animal protein intake have led to the exploration and usage of alternative vegetable protein sources.

1.3 Justification for the study

Biofuels (including biodiesels, biolubricants, and biosurfactants) from plant base are projected to serve as viable substitutes for petroleum-based chemicals, particularly in industries where their usage is extensive and for the manufacturing of renewable energy. The anticipated qualities of these biofuels are biodegradability, non-toxicity, and ecological friendliness. Tropical plant seeds are necessary components in the everyday meal in many communities, and could serve as valuable dietary source of protein that will ensure optimal nutrition without health problem. Despite the existing utilisation of certain plant seeds, there remain a considerable number of seeds that have not been fully exploited. Therefore, this research aims to examine the viability of selected under-utilised plant seeds and oils in the production of feeds and biofuels, with the goal of establishing them as green and cost-effective energy sources that are renewable, sustainable, non-toxic,

highly degradable, and environmentally friendly. Additionally, the aim of the study was to propose new feedstock options to ensure the sustainability of the food and oleochemical industries.

1.4 Aim of research

The study was designed to screen, characterise and evaluate underutilised seeds of *Areca catechu*, *Balanites aegyptiaca*, *Chrysophyllum albidum*, *Enterolobium cyclocarpum*, *Neocarya macrophylla* and *P. longifolia* for their applications as feed and oleochemicals.

1.5 Objectives of the research

In view of the above, the following specific objectives were to

i. evaluate the chemical properties (mineral composition, proximate analysis, amino acid composition and classification) of the various flours, residues and oils.
ii. characterise the extracted oils (physicochemical properties, fatty acid composition and distribution, lipid classes, unsaponifiable matter, molecular speciation, mineral composition).
iii. carry out the phytochemical screening of the seed flours.
iv. assess the toxicological status of the seed flours, residues and oils using rats and fishes.
v. evaluate the anti-microbial and wound healing activities of the seed oils.
vi. utilise the seed residues in dietary cake production;
vii. synthesis and characterise the fatty acid methyl esters, polyols and diethanolamide from the seed oils for production of biodiesel, biolubricant and biosurfactant.
viii. compare the biodiesel, biolubricant and biosurfactant properties with the standards E. N. (14214) and ASTM (D 6751) specifications

CHAPTER TWO

LITERATURE REVIEW

2.1 The various plants seeds

2.1.1 Areca nut (*Areca catechu*)

Areca catechu is a masticatories plant that is popularly known as betel nut and from *Arecaceae* family (Trease and Evan, 2009; Homey *et al.,* 2014). The *A. catechu* is found commonly in Asia and some part of Africa, including Nigeria. It is an average seed tree that grows 30 m tall and its leaves' length ranges from 1.5 cm to 2 cm. They were closely arranged on each side of a common stem. The fruits were strong and oblong, the riped ones were red-orange in colour while the unriped ones were green. The middle layer of the seed is fibrous which were coverred by a woody pericar layer (William *et al*., 2002; Manish, 2021). The *A. catechu* known as areca nut is a drupe which is always chewed, wrapped in betel leaves. They are sold in both dried and fresh forms. The nuts in the husk are easily sliced with knife when fresh (green), become strong, wood like and hard when dried (yellow or orange). In Nigeria, *A. catechu* is planted as a decorative plant along the street or in a compound and the seeds are not eaten.

The phytochemicals in *Areca catechu* were mainly found to be polyphenols which include tannins, flavonoids and alkaloids related compounds. The polyphenols which are mostly flavonols, include catechin, epicatechin, leucocyanidin, and portion of complex flavonoids in varying degrees of polymerisation. Arecaidine, guvacine, guvacoline and arecoline were the most important four alkaloids extracted from *Areca catechu* nut. Other nutritional components found also comprise of amino acids, minerals and carbohydrates (George and Robert, 2006; Srimany *et al.,* 2016).

It has been reported that tropical application of *A. catechu* extract in inhibiting the activity

of hyaluronidase enzyme on a retarded hypersensitiveness and croton oil induced ear edema on mice suggested that *A. catechu* extract is efficient in reducing inflammation. The *A. catechu* ethanol extract has been also regarded as potent and promising anti-inflammatory as well as anti-melanogenesis agents that might be useful in the cosmetics production (Kook *et al.,* 1999; D' Souza *et al*., 2021).

Oral administration (100 to 200 mg/kg) of aqueous extract of *A. catechu* nut has been reported to result in a significant reduction in hypertension (in rats). Tannins present in the aqueous extracts possessed the ability to control blood pressure (Khan *et al.,* 2008). Aqueous extracts of Areca nuts have also been found to be effective against tapeworms (causing paralysis). Additionally, a 1% decoction of *Areca* nut has been shown to eliminate blood flukes by affecting their nervous system (Peng *et al.,* 2015). Ethanolic extracts (40 - 80 mg/kg) of *A. catechu* nut have also been found to possess antidepressant activity – while the extract caused a reduction in the immobility time interval, it did not affect motor activity. Similarly, ethanol extracts of *A. catechu* nut have been found to possess anti-inflammatory (D' Souza *et al*., 2021); anti-oxidative (Dsouza *et al*., 2019), anithelmintic and anti-bacterial (Hai *et al*., 2019) free radical scavenging, and anti-hyaluronidase activities in rats (Bhandare *et al.,* 2010). The anti-oxidative effect of *A. catechu* extract is comparable with that of tocopherol but greater than that of ascorbic acid. Areca catechu is a medicinally significant plant used in the indigenous medicinal system to treat various diseases and disorders. It has been equally reported to have carcinogenic properties, leading to oral cancer when consumed for the long term (Manish, 2021).

2.1.2 *Balanites aegyptiaca*

The *Balanites aegyptiaca* is a spherical tree with multiple branches from *Balanitecea* family that grows approximately 10 m tall. Its trunk is short and branched at the base. The leaves of the tree consist of two separate leaflets that are asymmetric and measures between 2.5 to 6 cm in length. When young, the leaves are bright green, leathery and covered in fine hairs. The *B. aegyptiaca* fruits are long (ranging from 2.5 to 7 cm), with diameter of 1.5 to 4 cm. They are green when still young and become yellow upon maturity. The seed is 1.5 to 3 cm long; hard and makes up the larger part (60%) of the

fruit. The *B. aegyptiaca* tree is commonly found in some part of Africa including Sahel-Savannah region, Senegal and Mauritania (Daya *et al.,* 2011). It is a tree that can grow in numerous types of habitat with different type of soil that ranged from sandy soil to heavy clay. It can relatively tolerate flooding, livestock activity, and wildfire (Ndoye *et al.,* 2004; Idrissa *et al*., 2018). In Nigeria, *B. aegyptiaca* is called desert date (English), Adowa (Yoruba) and Aduwa (Hausa).

Through the spectrophotometric analysis of the stem-bark of the *B. aegyptiaca*, it was found to contain a range of mineral constitutents especially Ca and K. *Balanites aegyptiaca* stem-bark ethanolic extracts were found to possess good antimicrobial properties using disc-diffusion method. The bioactive compounds such as tannins, carbohydrates and glycosides and other metabolites found in the extracts could be useful in the synthesis and preparation of medicinal drugs for infections (Khan *et al.,* 2008). *Balanites aegyptiaca* seed ethanolic extracts was equally investigated for their anti-inflammatory activity in rat. Its oral administration (200-2000 mg/kg) was reported to show positive effect within 3 h without any case of death. This extract would be perfectly utilised in the treatment of inflammatory disorders (Mayba *et al*., 2010).

The antimicrobial properties of flavonoids extracted from the callus tissue of *B. aegyptiaca* investigated against *Escherichia coli, Proteus vulgaris, Pseudomonas aureginosa, Staphylococcus aureus, Micrococcus lylae, Bacillus subtilis and Sporolactobacillus* by disc diffusion method were reported (Shashi *et al*., 2011). The free flavonoids fraction of callus tissue of *B. aegyptiaca* possessed more anti-microbial activity against gram +ve bacteria while the ethyl ether and ethyl acetate bounded flavonoids extracts showed moderate activity against both the gram +ve bacteria as well as gram –ve bacteria (Shashi *et al*., 2011).

The *B. aegyptiaca* plants contain flavonoids and phenolic compounds, which have been observed to possess both antioxidants and hepatoprotective properties. The ethanolic extract of the plant contains saponins that act as antioxidants, protecting against oxidative damage to the liver. When combined, flavonoids and saponins work together to stabilise reactive oxygen species by reacting with them, ultimately, resulting in the formation of stable and less reactive radicals (Mayba *et al*., 2011).

2.1.3 *Chrysophyllum albidum*

Chrysophyllum albidum tree is a big tree of 8-30 m tall, with a short trunk of 1 m thickness, brown-hairy branchlets and white, gummy latex. *C. albidum* tree is from *Supotaceae* family, a forest plant, big tree that is mostly planted as a compound tree within the village. *C. albidum* tree produced fruits that are edible and the consumption of these fruits are common within Africa countries and mostly Nigeria. The leaves, nearly evergreen are elliptic or oblong-elliptic, while the fruits are round, oblate, ellipsoid or even pear-shaped with 5 cm to 10 cm in diameter. The skin or the peel of the fruit is from orange to gold yellow when it is ripe. The pulp within the peel is orange and there may be 6 to 10 flattened nearly oval seeds, pointed, hard which are not eaten. *C. albidum* (African star apple) is respectively identified as Agbalumo (Yoruba), ehya (Igala), Agwaluma (Hausa) and Udara (Igbo) in Nigeria. It is originally cultivated for its delicious juicy fruits, which were good source of vitamin C, flavour and K for diets. The ripe fruits also serve as a valuable raw material to various manufacturing industries, including resin production (Adisa *et al*., 2000). In Nigeria, the fruits are sold in local market between December and April and the fleshy part of the pulp is naturally consumed.

The *C. albidum* was reported to contain some antioxidants needed by the whole body to fight some free radicals activities (Adepoju and Adeniji, 2012). The proximate analysis of *C. albidum* fruit as well as its ascorbic acid and anti-nutrient contents has been carried out (Edem *et al.,* 2011). The results of proximate analysis of *C. albidum* flour documented compared favourably to those in wheat flour (Akubor *et al.,* 2013). Tannins, flavonoids, terpenoids, protein, carbohydrates and resins were reported as the the major secondary metabolites found in *C. albidum* seed flours (Akaneme, 2008).

2.1.4 *Enterolobium cyclocarpum*

Enterolobium cyclocarpum is well known in Western Mexico as parota. It is a long-lived tree from *Leguminosae* family and considered as one of the important type of legumes originated from America (Avoseh *et al*., 2021). The *E. cyclocarpum* was recently disseminated and spread all over the tropical areas and are now grown in a very large surface area (29 million hectares) world-wide (FAO, 2010). The *E. cyclocarpum* has a

reserve and potential source of carbohydrates, minerals, proteins and amino acids (Gamal El-Din *et al*., 2017; Avoseh *et al*., 2021). The *E. cyclocarpum* seeds are contained in ear shaped pads with nearly 86 kg of seeds per tree as yield (Andrade *et al.,* 2008). The *E. cyclocarpum* seeds are locally consumed while the leaves and pods are good sources of forage for cattle (Castro *et al.,* 2006). The *E. cyclocarpum* seeds are potential sources of protein. *E. cyclocarpum* leaves, seeds and back have been reported to exhibit a remarkably wide range of chemical diversity and a multiplicity of biological properties that ranged from anti-inflammatory to anti-tumor that make them to be useful raw material in pharmaceutics and medicine (Martinez *et al.,* 2021; Avoseh *et al*., 2021).

The *E. cyclocarpum* seeds were found to contain crude protein (26.3%), ether extracts (2.8%) and nitrogen free extractives (63.1%). They were equally reported to have good nutritional potentials for ruminants (Serratos *et al.,* 2008; Ojo *et al*., 2018). Ekanem *et al.,* (2020) assessed the effect of preservation methods on the chemical composition of *Enterolobium cyclocarpum* leaves and anti-nutritional factors such as tannins, saponins, oxalate, phytate and hydro cyanic acids were found in fresh and preserved leaves in moderayte concentrations. There were more saponins revealed in the leaves than in the seeds, roots, stem and bark. The high saponin content provoked its low *in vitro* gas production parameters which made it not to be suitably and perfectly accepted by livestock (Ekanem *et al.,* 2022). The *E. cyclocarpum* seeds and fruits might be useful as feed additives because of its high nutrients contents (Babayemi, 2006; Ojo *et al*., 2018; Ekanem *et al.,* 2022).

2.1.5 *Neocarya macrophylla*

Neocarya macrophylla formerly called *Parinari macrophylla* Sabine is a plant seed that grows in different regions (arid and semi-arid) and mostly in West Africa. *Neocarya macrophylla*, from *Chrysobalanaceae* family is a medium sized tree that is between 6 to10 m tall with brown stems. *N. macrophylla*, a semi-cultivated plant in Northern part of Nigeria is regarded and accepted as one of the indigenous plant seeds from Western Africa and Central America. The plant trees produce fruits which have an egg-shape, elongated, ellipsoidal in shape, smooth and light yellow brown with greyish lumps on the surface. The fruits are harvested from the ground and can be useful in many areas. They can be

eaten with cereals when still fresh or boiled. They are used in preparing fragrant syrups which are shown to be better than some fruit juice. The kernel is rich in protein, not well known, contains high oil and under-utilised (Amza *et al*., 2010). The innermost layer of the pericarp surrounding the seed in the fruits (endocarp) is 85% while the kernel, the edible part is 15% of the fruit. The kernel has been reported to contain up to 60% of oil, and 21-25% of protein (Amza *et al.,* 2010). The *N. macrophylla* tree known as Gingerbread plum, produces seeds that might be a potential source of energy, protein, minerals, amino acid, dietary fibre, fatty acids and other essential nutrients that might be required for human nutrition, growth and good health (Mohamed *et al.,* 2016).

Neocarya macrophylla seeds are often roasted and eaten like cashew nuts. Many are taken as light snacks, blended and added into prepared food while some others were hard-pressed to get oil. The leaves have ethno-medicinal properties as they are useful in the treatment of tooth pain and mouth odour (National Research Council, 2008; Yusuf *et al.,* 2019). The defatted seed meal contains up to 61% protein and some quality amino acids like lysine, valine among other necessary amino acids which are useful to compensate for the insufficiency and inadequacy of essential amino acids in foods or diets that are mostly cereal-based (Amza *et al.,* 2010). The chemical evaluation of oil from *N. macrophylla* showed the presence of some important phytochemicals which include saponins, steroids, tannins and flavonoils. *N. macrophylla* seed oil has been reported to contain 153.30 mg KOH/g for saponification value, 32.07 I_2/100 g for iodine value and 45.48 meq H_2O_2 for peroxide value (Warra *et al.,* 2013). The *N. macrophylla* seed methanolic extract has been reported to contain some secondary metabolites or phytonutrients (Yusuf *et al.,* 2015). *Neocarya macrophylla* leave, stem bark have been reported to possess various medicinal properties such as antimicrobial activitie (Yusuf *et al*., 2018 and Olowo-Okere *et al*., 2018), antibacterial and anti-biofilm activities (Yusu *et al.,* 2022), analgesic and antivenin activities (Yusuf *et al*., 2022).

2.1.6 *Polyalthia longifolia*

Polyalthia longifolia, from *Annonaceae* family and have almost 120 species that were mainly found within Africa, Asia, and some countries like Nigeria, India, Australia and New Zealand. It is a tall tree that has a straight trunk with branches that are horizontally

long. The leaves are dark green, glossy and narrow oblanceolate. *P. longifolia* is an evergreen tree that is planted because of its usefulness as ornamental street tree due to its effectiveness in combating noise pollution. *P. longifolia* fruits are green in colour when unripe, black when ripe, ovoid in shape and arranged in clusters of 10-20 or more (Chandaka *et al.,* 2018). Most of the studies on *P. longifolia* were carried out on the stem and bark while few works were already reported on their leaves and berries. Most samples studied were from India, China and some African countries including Nigeria. The phytochemicals such as steroids, terpenoids, alkaloids, flavonoids and phenolics were majorly seen and found in their leaves (Malairajan *et al.,* 2008; Chandaka *et al*., 2018).

The *P. longifolia* leaves' methanolic extracts (300, 600, 900 mg/kg) were reported to possess significant anti-inflammatory properties even similar to the standard used (Tanna *et al*., 2009). Sharma *et al*. (2011) also evaluated the anti-inflammatory activities of ethanol and aqueous extracts of *P. longifolia* leaves in Wistar rats. The aqueous extracts studied at 200 mg/kg of the animal body showed better anti-inflammatory properties than ethanol extracts of similar dose.

The *P. longifolia* seeds extracts were found to possess excellent antibacterial and antifungal activities (Marthanda *et al.,* 2006; Babatunde *et al*., 2023). Flavonoids fractions isolated from the bark of *P. longifolia* tree showed potent and positive antibacterial activity (Bose *et al*., 2010, Babatunde *et al*., 2023). Similarily, they were equally found to exhibit a concentration-dependant free radical scavenging ability which also confirmed *P. longifolia* bark with positive antioxidant properties (Bose *et al.,* 2010). Some other isolated compounds demonstrated promising antimicrobial activity against micro-organisms. Their effects were comparable to Gentamicin, Penicillin and Ketoconasol used as standard drugs. *P. longifolia* plant was suggested to be a versatile plant that could be useful and considered in treating several ailments like rheumatism, scorpions tung pain, diabetes, hypertension and skin disease among others. The assessment of several solvent extracts from *P. longifolia* plant illustrated and confirmed that the plant possessed antibacterial, anticancer, antifungal, antioxidant and anti-diabetic activities and can be medicinally useful (Atolani *et al.,* 2016).

2.2 Toxicity study

Aqueous extract of *Areca catechu* administered orally revealed no toxic effect in rats. The body weight, death rate and other properties were evaluated to establish a lethal dose (LD50) of higher than 15,000 mg/kg. The weight increased significantly and no mortality was recorded. Aqueous extract of *A. catechu* seed was not toxic and could be effectively utilised in pharmaceutical formulations (Sari *et al.*, 2014).

Balanites aegyptiaca seed oil has been reported to be successfully substituted for groundnut oil that was used in the preparation of rats feed at 5% level (Wilson *et al.*, 2009). The four weeks experiment for the study of dose toxicity was carried out with male Wistar rats with the aim to assess the harmful effect of crude *B. aegyptiaca* seed oil. No significant alteration was observed in the toxicological and biochemical parameters of the rat. It was equally found that the seed oil was not toxic at 5% level of incorporation as no negative effect, injury or damage was observed on liver and kidney of rats (Wilson *et al.*, 2009).

The effect of *C. albidum* leaves ethanol extracts on haematological and blood chemistry parameters on Wistar rats has been investigated (Adebayo *et al.,* 2010). The extract was found not to show any adverse effect on haematological and blood biochemistry indices and therefore not toxic. The ethanolic extracts obtained from *C. albidum* leaves were discovered to possess properties that inhibited platelet aggregation and lower blood sugar levels. These extracts were recommended for the potential use in treating myocardial infections and diabetes mellitus.

The lethal dose (LD50) of 283 mg/kg established through toxicological sutudy of crude methanol extract of *N. macrophylla* stem bark on mice emploring Lorke's method give suggested *N. macrophylla* not to be toxic (Yusuf *et al.*, 2015). The crude extracts from *N. macrophylla* stem bark was reported to contain some bioactive constituents with significant anti-venom activity which perfectly supports the medicinal usefulness of *N. macrophylla* seed in snake bite treatment (Yusuf *et al.*, 2015).

Oral toxicological study of *P. longifolia* leaves methanol extract was carried out with Wistar rats at dose range of 540 - 3240 mg/kg body weight. Parameters such as death rate,

feed intake, signs of toxicity and body weight gain were all evaluated daily after administering the extract orally for 14 days (Chanda *et al*., 2012). On the 15th day, the effect of various doses on organ weight and other blood parameters were determined. *P. longifolia* leaves methanol extract was found not to produce any toxic effect or death at 3240 mg/kg body weight. The rats weight, and other blood parameters evaluated displayed no significant change. *P. longifolia* leaves methanol extract seemed to be harmless at 3240 mg/kg body weight.

2.3 Hepatoprotective activity

The plant species *B. aegyptiaca* has been utilised in the management of jaundice, liver disorders, syphilis and yellow fever. Ethanol extracts from the plants' aerial parts was found to exhibit potent hepatoprotective properties, as evidenced by the alterations observed and recorded in blood biochemical parameters analyses when administered orally on Wistar rat at 100 and 200 mg/kg body weight (Mayba *et al*., 2011)

Polyalthia longifolia leaves methanolic extracts administered orally to rat at 300, 600 and 900 mg/kg per body weight demonstrated significant hepatoprotective activity. The findings suggested that the methanolic extract of *P. longifolia* leaves had a confirmed liver-protective effect, as evidenced by both biochemical and histopathological changes. Histological studies revealed a marked improvement in the liver's tissue structure, which supported the earlier biochemical observations. This research indicated that *P. longifolia* leaf extract could enhance the liver's antioxidant defense mechanisms, potentially safeguarding it against oxidative damage induced by paracetamol (Tanna *et al.,* 2009).

Adebayo *et al*. (2011) documented the hepatoprotective effects of *C. albidum* leaf extract on Wistar rats with liver damage induced by carbon tetrachloride (CCl_4). A Sgnificant difference ($p < 0.05$) in the hematological parameters of the rats following treatment with C. albidum leaf extract was observed. Rats treated without *C. albidum* leaf extract exhibited notable centrilobular fatty degeneration and necrosis. In contrast, the groups treated with *C. albidum* leaf extracts displayed no signs of necrosis. The *C. albidum* leaf extracts demonstrated positive potential hepatoprotective activity towards liver damage induced by CCl_4 in rats.

Pithayanukul *et al.* (2009) conducted a research on the hepatoprotective potentials of aqueous extracts obtained from *A. catechu*. Hepatoprotective activity towards the liver damage in stimulated by CCl4 in rats was equally studied. It was recorded that aqueous extracts from *A. catechu* showed positive potential hepatoprotective activity towards the liver damage in rats studied. Procyanidins, the most condensed form of tannins in *Areca catechu* extracts were then referred to as the most important metabolite taking part in the hindrance of the free radical mediated disorder. This information supported the use of *A. catechu* extracts in traditional medicines because of their anti-inflammatory and hepatoprotective properties.

2.4 Phytochemical

Phytochemicals are chemical compounds which are biologically active and are always found naturally not only in plants but also in fruits, vegetable whole grains and seeds in a substantial amount (Fasuyi, 2007). They have been reported to provide health benefits for humans as curative constituents and nutrients as well as protect human healthiness from hazards. They shield plants from different forms of infections and damages, improve plant physical properties (colour, aroma, flavour) and generally protect plants from ecological danger like pollution, stress and pathogenic attack among others. Phytochemicals possess valuable properties that help in fighting diseases (Sexenal *et al.,* 2013).

On the basis of their functions and relevance in plant metabolism phytochemicals were grouped into primary and secondary metabolites. The primary metabolite include common sugar, carbohydrate, protein, lipid, vitamins, amino acide while some of the secondary metabolite are steroid, essential oils, alkaloids, phenolics, toxins and saponins (Muthukrisnan and Manogaran, 2018). Other classes of plants' phytochemicals, their percentage of occurrence as well as their roles in human health care are presented on Table 2.1 and Figure 2.1.

2.4.1 Phenolics

Phenolics, the highest class of phytochemicals is found in different parts of plants. They are the most widely distributed within the plant kingdom. Phenolic have OH group unswervingly attached to an aromatic hydrocarbon. The simplest among this class of

Table: 2.1. Major classes of phytochemicals found in plant seeds and their functions

Cass of phytochemicals	Percentage occurrence as natural product in plant	Usefulness of various phytochemicals in health care
Phenolics	45	Antioxidant, anticancerous, cytotoxicants, antimicrobial and Vasodilaling
Terpenoids and steroids	27	Antimicrobial, detoxifying agents, Strengtheners, anti-rheumatics, anti-malarial and hepaticidal
Alkaloids	18	Neuropharmaceutical, anti-cancerous, sedatives, antimicrobial, insecticidal
Other chemicals	10	Anti-inflammatory, immune-stimulating

Source: Koche *et al.* (2016)

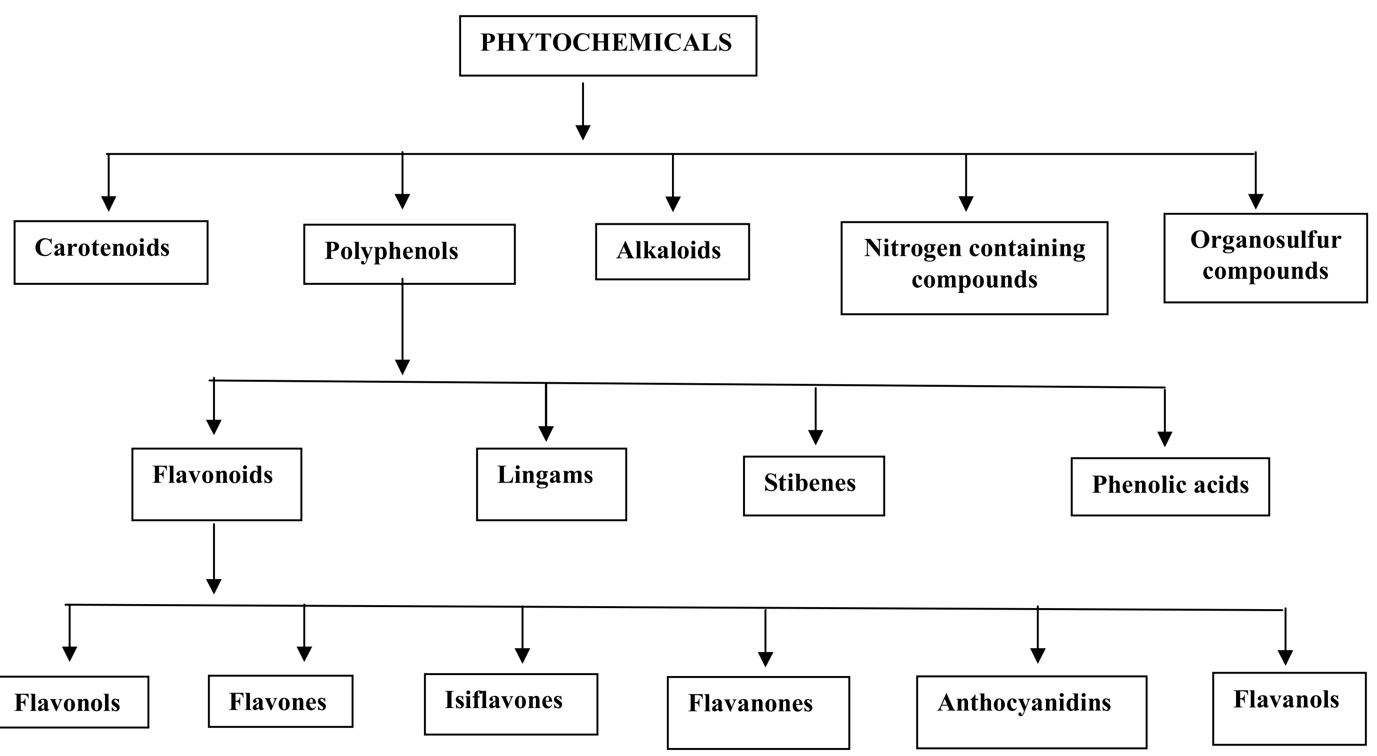


Figure2.1: Classification of phytochemsicals found in plants
Source: Ramos *et al*. (2011)

natural products is C_6H_5OH. The phenolics are secondary metabolites with several properties beneficiary to humans. They served as protective compounds with good antioxidant properties, protecting agents, antimicrobial and other major biological properties. Polyphenols, phenolic acids and flavonoids are the most important groups of dietary phenolics (Koche *et al.,* 2016).

2.4.1.1 Flavonoids

One of the extensively studied components of plant phenol is flavonoids, which encompass polyphenolic compounds occurring as aglycone, glycosides, and methylated derivatives. These compounds are commonly found conjugated with sugar and are characterised as monoglycosidic and diglycosidic forms. Recent research has demonstrated their diverse biological properties, including antimicrobial, anti-tumor, anti-inflammatory and anti-allergic activities, leading to increased interest in their study (Sexenal *et al.,* 2013). Figure 2.2 illustrates the fundamental structures of several pharmacologically significant flavonoids derived from plants (Sexenal *et al.,* 2013).

2.4.1.2 Phenolic acids

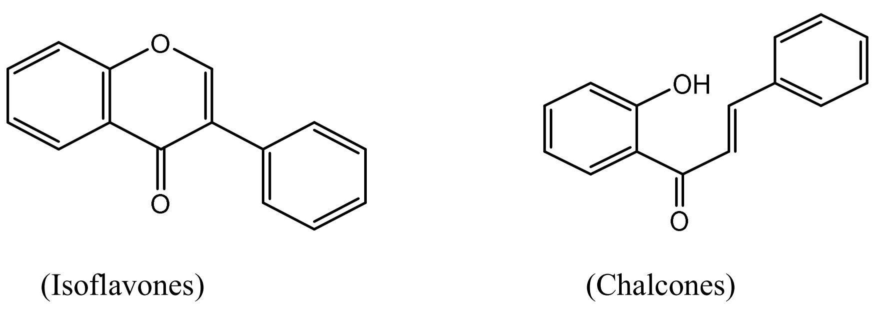
Phenolic acids belong to the category of phenols that contain a single COOH functional group. They exhibit two primary distinct carbon frameworks known as hydroxycinnamic and hydroxybensoic structures. These compounds are found in plant polyphenols as they serve as both bioprecursors and metabolites of polyphenols. Extensive research has been conducted on phenolic acids due to their diverse range of biological properties, including antimicrobial, anti-tumor, anti-inflammatory, and anti-allergic effects, as well as their ability to lower cholesterol and lipid levels in the bloodstream, increase bile secretion, and act as antidepressants, among others. Numerous reports have documented their roles in various fields such as agriculture, biology, chemistry, and medicine (Ghasemsadeh *et al.,* 2010).

2.4.2 Tannin

Tannins are a type of polyphenolic compounds that have a high molecular weight and are classified as phenolic polymers (Sexenal *et al.,* 2013). They have the potential to form

(Flavones) (Flavanonols)

(Flavanones) (Flavonols)



(Isoflavones) (Chalcones)

Figure 2.2: Basic structures of some pharmacological important flavonoids derived from plant

Source: Sexenal *et al*. (2013)

complexes with various substances such as proteins, polysaccharides, gelatin, alkaloids, nucleic acids, and minerals. Based on their structural characteristics, tannins can be categorised into condensed tannins, ellagitannins, complex tannins, and gallotannins (Koche *et al.,* 2016). Ellagitannins and gallotannins are known as hydrolysable tannins, which generate pyrogallic acid when heated and produce gallic acids and ellagic acid upon hydrolysis. Tannins are predominantly found in fruits and legume trees. They are utilised for their astringent properties, as well as for treating diarrhea, stomach and duodenal tumors. They also possess anti-inflammatory, antiseptic, antioxidant, and hemostatic properties, making them valuable in pharmaceutical applications. Furthermore, tannins are employed in the dye industry as caustic cationic dyes, for ink production, and for clarifying wine, beer, and fruit juices in the food industry. They are also used as textile dyes and coagulants in rubber manufacturing (Koche *et al*., 2016).

2.4.3 Alkaloids

Alkaloids are natural products containing heterocyclic nitrogen atoms. They are basic in nature and are naturally synthesised by a large number of organisms including animal, plants, bacteria and fungi. They are so much involved in a variety of molecular structure such that their classification seems to be difficult. They are grouped in different families based on the kind of heterocyclic ring system found in their molecule (Figure 2.3). These groups include pyrrolidine, pyridine, quinoline, isoquinoline, indole, quinasoline, steroids, diterpenoids and other alkaloids (Noureddine, 2018). Plants containing alkaloids are used as dyes, spices, drugs or poisons. Alkaloids possess several pharmacological properties that include anti-hypertensive effects, anti-arrhythmic, anti-malaria effect and anti-cancer actions among others. Some have stimulant property (caffeine and nicotine) and analgesic property (morphine). Quinines are mostly used as malarial drugs (Sexenal *et al.,* 2013).

2.5 Wound healing activity of seed extracts

Wound is referred as disruption of structural and physiological continuity of living tissue. It is an aperture in the epithelial of the skin tissue that might be caused by physical, chemical, thermal or mechanical injury (Ajayi *et al*., 2015). Wounds now constitute a major health burden and drain on healthcare resources globally. Majority of Africans

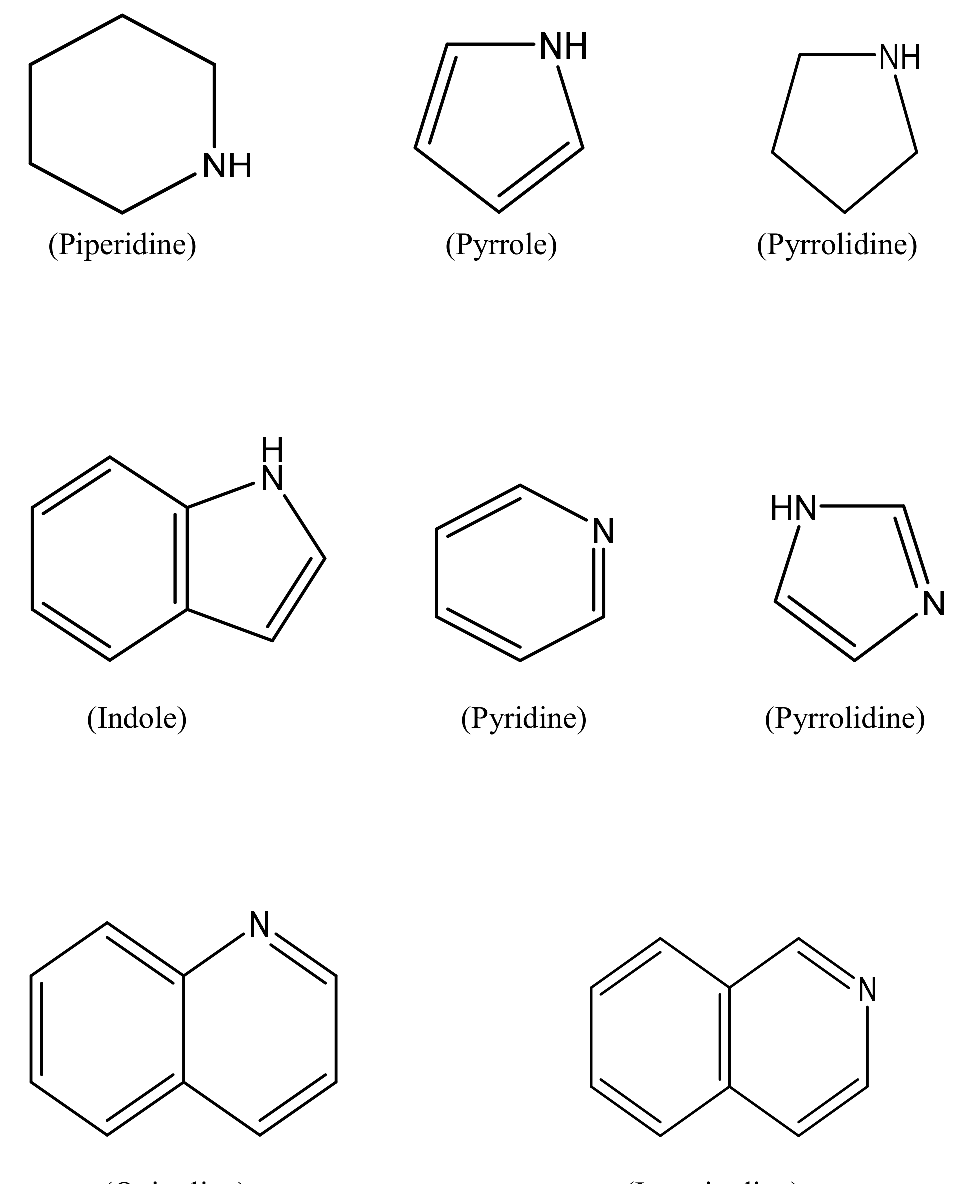


Figure 2.3: Basic heterocyclic structures of some skeleton constituting the group of important alkaloids derived from plant

Source: Noureddine, (2018)

employ traditional medicine for their health care needs including the treatment of different forms of wounds. The World Health Organisation has been promoting the use of traditional medicine as one of the sources of cheaper and comprehensive medical care in the developing countries. Approximately one-third of all traditional medicine in use are for the treatment of wounds and skin disorders as compared to only 1 - 3% from modern drugs (Pandey *et al*., 2014). Wound repair or healing is a normal process of epidermal tissue restoration. It is a complex and dynamic process of restoring tissue structure in damaged tissue as fast as possible to its normal form. It depends majorly on the ability of the tissue to be repaired, the category and the extent of damage and the general form of the hosts' health.

The healing process is charaterised by hemostasis, re-epithelialisation, granulation, remodeling of the structure matrix and formation of scar (Mary *et al*., 2002; Ajayi and Omole, 2020). Studies and application of herbal and traditional medicine in wound care management is increasing globally and the wound healing herbal extracted from plants are known to fight infections, promote blood clothing and accelerate the healing process of wounds (Kumarasamyraja *et al*., 2012). Many researchers have effectively reported the wound healing activity of several indigenous medicinal plants. These plants have been reported to contain necessary phytochemicals such as tannins, polyphenols, alkaloids, glycosides, flavonoids and saponins all of which play different roles either collectively or individually in wound healing (Sapna *et al.,* 2016). Quite a lot of plants employed as traditional healing remedies have been fully reported to treat skin disorder, including wounds (Nayak *et al*., 2009; Ghosh *et al*., 2012; Kuntal, 2013; Ajayi *et al*., 2015; Francis *et al*., 2018; Wubante *et al*., 2018; Ajayi and Omole, 2020 and Ekaye *et al*., 2022).

Kuntal Das (2013) researched on the wound healing activity of aqueous extract of the leaves of *Stevia rebaudiana* in mice. The leave extracts were found effective in the functional recovery of the wound healing by dose dependent manner. The efficacy of the extracts might due to the presence of phytoconstituents in them which may be either due to their individual or cumulative effect that enhanced wound healing. This result provides scientific evidence to ethno-medicinal properties of *Stevia rebaudiana.*

Francis *et al*. (2018) reported on wound healing activity of water leaf extract of *Sida rhombifolia.* He concluded that 80 % ethanolic extract and water preparation of *S. rhombifolia* leaves have potential benefit in enhancing wound healing process. This efficacy might be attributed to the presence of different compounds in the extracts which are known to contribute immensely in the wound healing properties of the plant.

Ajayi *et al.* (2015) showcased in their study, the wound healing benefits of *M. myristica* and *M. tenuifolia* seed extracts when applied topically on rats. The extracts proved effective in promoting wound healing due to their capacity to eliminate free radicals, inhibit inflammatory pathway mediators and target bacteria that could potentially infect the wound. Consequently, their research findings supported the potential use of *M. myristica* and *M. tenuifolia* seeds in pharmacology for wound management.

Ajayi and Omolere (2020) investigated also the wound healing potential of hexane and methanol extracts of *Azadirachta indica* on rats. The seed extracts accelerated the wound healing process better than the control with a short epithelialisation time. It was suggested that *Azadirachta indica* have the potential benefit in enhancing wound healing process.

Kupeli-Akkol *et al*. (2012) evaluated the wound healing and anti-inflammatory activities of *Ranunculus aonstanitinapolitanus* and *Ranunculus pedatus* by using in vivo experimental models. Methanol extracts of the plants containing major phytochemicals were found to possess anti-inflammatory activity and be active in wound healing activities evaluation essays. The study provided useful information and evidence for the ethnomedicinal features of *R. aonstanitinapolitanus* and *Ranunculus pedatus* in Turkey.

Wubante *et al*. (2018) carried out research on the wound healing activity of *Acanthus polystachyus* leaves methanolic extract on rats. The findings demonstrated that 80% methanolic extract of *Acanthus polystachyus* leaves possessed wound healing activity and justified their application in the treatment of wounds as claimed in the folklore literature. Many other plants have been reported by scientists for wound healing activities. Their applications are majorly due to the presence of active phytochemicals such as tannins, alkaloids, glycosides, flavonoids and saponins. *B. aegyptiaca* and *N. macrophylla* seeds hexane extracts have not been in anyway reported for wound healing activity.

2.6 Proximate composition of seed flours

The evaluation of food composition is necessary to both the theoretical and applied research within food science and technology. It is the basis for establishing the nutritional value and overall acceptance of food from consumers' end point (Wilson, 1979). The proximate composition parameters of the food items include protein, carbohydrate, ash content, fat content, crude fibre and moisture content.

2.7 Minerals

Mineral elements are required for body building, growth, control of body processes e.g. transmission of nerve impulses. They are extensively divided into major minerals (macro-minerals) and trace minerals (micro-minerals). Major minerals include calcium, Mg, K, Na and P while trace minerals also include Zn, Fe, Mn and Cu. For a good and balanced nutrition, different plant and animal sources can be consumed to receive a number of important minerals (Gharibsahedi and Jafari, 2017). Minerals act as co-factors in many biological reactions within the body including muscle contraction, neuro-transmission and production of hormones, digestion and utilisation of nutrients. They are involved in building strong bone, transmitting nerve impulses, healthy and lengthy life.

2.7.1 Calcium and phosphorus

Ca is needed to build strong bones, to guarantee proper muscle action, for a normal heart beat and for making Fe concentration both in and outsideof the body cells steady. It takes part in making the nerve and muscle to function normal and also in enzyme activation to maintain the components of teeth and bones. Ca and P combine to form $Ca_3(PO_4)_2$, which strengthening the bones and help in maintaining their rigid structure. P contributes essentially in the producing energy in the body and is frequently utilised as preservative in manufactured foods. Ca and P are absorbed better by the body if consumed together. A deficiency of calcium, phosphorus and other micronutrient in children leads to rickets and causes softening of the bones in adults. This affects more than 2 billion people globally leading to underweight and malnourished children with reduced productivity and intellectual capacity (Soetan *et al.,* 2010; Chongtham *et al*., 2020).

2.7.2 Magnesium

Magnesium is involved in protein production and in releasing energy. It is known to activate many enzymes responsible for Ca metabolism in bones and in the maintenance of electrical potential in nerves (Ishida *et al.,* 2000). Magnesium is very important mineral in the body, it helps in stabbilsing the function of the nerve and muscle, supports the body immune system, assists in keeping the heart beat steady, helps bones to be strong and regulates the blood glucose level. Magnesium helps hold Ca in the enamel of the teeth while deficiency may result in excess vomiting and diarrhea (Soetan *et al.,* 2010).

2.7.3 Iron

Iron is an essential micronutrient for plants, animals, and humans. In the human body, iron plays a vital role in numerous biological processes. Its primary function is to facilitate the transport of oxygen throughout the body as part of the hemoglobin molecule found in red blood cells (Mehas and Rodgers, 1997). Hemoglobin binds to oxygen in the lungs and carries it to tissues and organs, ensuring proper oxygenation and energy production. Iron is also a critical component of enzymes involved in various metabolic reactions. It is necessary for the production of new cells, including red blood cells, and supports the immune system, brain function, and energy metabolism. Insufficient iron levels can result in health problems such as anemia, fatigue, impaired cognitive function, and weakened immune response. Conversely, excessive iron levels can be detrimental, especially for individuals with certain genetic disorders, potentially causing organ damage (Tull, 1996).

2.8 Energy

The three main nutrients, protein, fat and carbohydrate all supply energy. Of the three, fat provide the most concentrated source of energy, weight for weight (Tull, 1996). Some food components, water, vitamins and minerals, do not provide any kilocalories or energy to the body (Mehas and Rodgers, 1997). Energy is measured in kilocalories (KCal) or kilojoules (kJ). A kilocalorie is the amount of heat energy that is required to raise the temperature of 1 kilogram of pure water by 1 ˚C. One gram of pure protein has an energy value of 4 KCal or 17 kJ. One gram of pure fat has an energy value of 9 KCal or 38 kJ.

One gram of pure carbohydrate has an energy value of 3.75 KCal (usually rounded up to 4 KCal or 17 kJ (Tull, 1996). The energy requirement per day for an adult was reported to be between 10,500 - 12,600 kJ depending on his physiological state while that of infants is 3094.68 kJ (FAO/WHO/UNU, 1985).

2.9 Fatty acid

Fatty acids are crucial components of dietary fats and oils. They consist of long carbon chains with a carboxyl group at one end. There are three main types: saturated, monounsaturated, and polyunsaturated fatty acids. Saturated fatty acids have no double bonds and are solid at room temperature. Examples include lauric acid, stearic acid, and palmitic acid. Monounsaturated fatty acids have one double bond and are typically liquid, like oleic acid found in olive oil. The two next carbon atoms in the chain that are bonded to either side of the double bond can occur in either *cis* or *trans* configuration. Polyunsaturated fatty acids (PUFAs) have multiple double bonds and include omega-3 and omega-6 fatty acids. The double bonds in PUFAs are separated from each other by a methylene group. These acids are essential and must be obtained from the diet. Fatty acids play roles in energy production, cell membranes and hormone production. Different types of fatty acids having varying effects on health, with excessive intake of some have been reported to link to cardiovascular disease (Rustan and Drevon, 2005). A balanced diet includes moderate amounts of unsaturated fats from sources like fish, nuts, and oils, while minimizing saturated and trans fats found in processed and fried foods (Rustan and Drevon, 2005). Saturated and unsaturated fatty acids found in plants are illustrated in Table 2.2.

2.10 Amino acid

Amino acids are known as organic compounds that have amino and carboxyl functional groups with a specific side chain to each one of them. They are building block or monomers that are involved in making or building proteins. Proteins are specifically formed from one or more linear chains of amino acids which are joined together by peptide bonds to form a long chain. Every amino acid possesses a fundamental structure characterised by a central carbon atom, commonly known as the alpha carbon atom.

Table 2.2: Saturated and unsaturated fatty acids fatty acids commonly found naturally in plant seeds

	Common name	IUPAC name	Structure		Possible source
			Saturated fatty acids		
1	Butyric acid	Butanoic acid	$CH_3(CH_2)_2COOH$	C4:0	Butter
2	Caproic acid	Hexanoic acidn	$CH_3(CH_2)_4COOH$	C6:0	Butter, palm oil, coconut oil
3	Caprylic acid	Octanoic acid	$CH_3(CH_2)_6COOH$	C8:0	Palm oil, coconut oil
4	Capric acid	Decanoic acid	$CH_3(CH_2)_8COOH$	C10:0	Palm oil, coconut oil
5	Lauric acid	Dodecanoic acid	$CH_3(CH_2)_{10}COOH$	C12:0	Palm of Lauraceae, palm oil
6	Myristic acid	Tetradecanoic acid	$CH_3(CH_2)_{12}COOH$	C14:0	Butter, coconut oil
7	Palmitic acid	Hexadecanoic acid	$CH_3(CH_2)_{14}COOH$	C16:0	Plant fat, palm oil, peanut
8	Stearic acid	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$	C18:0	Plant and animal fats
9	Arachidic acid	Eicosanoic acid	$CH_3(CH_2)_{18}COOH$	C20:0	Peanut oil
10	Behenic acid	Docosanoic acid	$CH_3(CH_2)_{20}COOH$	C22:0	Plant lipids
11	Lignoceric acid	Tetracosanoic acid	$CH_3(CH_2)_{22}COOH$	C24:0	Plant lipids
12	Cerotic acid	Hexacosanoic acid	$CH_3(CH_2)_{24}COOH$	C26:0	Bee wax, wool
			Unsaturated fatty acids		
1	Palmitoleic acid	9-hexadecenoic acid	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	C16:1	Sardine oil
2	Oleic acid	9-Octadecenoic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	C18:1	Olive, palm, linseed oil
3	Linoleic acid	9,12-Octadecadienoic acid	$CH_3(CH_2)_4(CH{=}CH\text{-}CH_2)_2(CH_2)_6COOH$	C18:2	Olive, peanut, linseed, soybean oil
4	α-linolenic acid	9,12,15-Octadecatrienoic acid	$CH_3CH_2(CH{=}CH\text{-}CH_2)_3(CH_2)_6COOH$	C18:3	Linseed oil
5	Ɣ-linolenic acid	6,9,12-Octadecatrienoic acid	$CH_3(CH_2)_4(CH{=}CH\text{-}CH_2)_3(CH_2)_3COOH$	C18:3	Plant lipid, Linseed oil
6	Parinaric acid	9,11,13,15-Octade-catetraenoic acid	$CH_3CH_2(CH{=}CH)_3(CH_2)_7COOH$	C16:3	Plant lipid
7	Erucic acid	13-docosenoic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$	C22:1	Rape seed oil
8	Arachidonic acid	5,8, 11,14-Eicosa-tetraenoic acid	$CH_3(CH_2)_4(CH{=}CH\text{-}CH_2)_4(CH_2)_2COOH$	C20:4	Peanut oil
9	Eicosenoic acid	11-Icosenoic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_9COOH$	C20:1	Plant, jojoba oils, nuts
10	Myristoleic acid	9-tetradecenoic acid	$CH_3(CH_2)_3CH{=}CH(CH_2)_7COOH$	C14:1	Plant seed oils

Source: Gopinath *et al*. (2010) with modification

This alpha carbon atom forms bonds with a carboxyl group, an amino group, a hydrogen atom, and a distinctive alkyl group that varies and distinguishes each amino acid. Amino acids differ from one another based on the chemical properties of the R group and they take part in several processes like the building of proteins, synthesis of hormones, transmittance of neutrons, food digestion, formation and functions of enzymes, neurotransmitters, biosynthesis among others (Sabina, 2018).

They are twenty types of amino acids that are commonly found in proteins among all the existing amino acids (Figure 2.4). They are classified with reference to their polarity as hydrophobic (non polar) and hydrophilic (polar) amino acids. Some of these amino acids could be naturally synthesised by the body system (non essential amino acids) and there is no need to take them from diets. They include alanine, arginine, aspargine, aspartic acid, cysteine, glutamine, glycine, glutamic acid, proline, serine and tyrosine. Others that cannot be synthesised from the body (essential amino acids) are indispensable and must be necessarily taken from diets. They include histidinne, isoleucine, leucine, lycine. methionine, phenyl alanine, threonine, tryptophan and valine (Akram *et al.,* 2011). Essential amino acids should be supplied through food rich proteins which are also known to contain amino acid and plant based product like beans, cabbage, nuts, fruits, chia seeds, carrots, cucumber, soybean, vegetable, whole grains and some dairy products such as egg, seafood, chicken, meat and many others. It is then important to take proper balance diet that containes the essential amino acids to maintain a healthy and proper function of the body system.

Insufficient or deficiency of proper amino acids in the body system might lead to a variety of pathological disorder such as edema, anemia, insomnia, diarrhea, depression, hypoglycemia, deposit of fat in the liver, hair related problems among others. Both essentials and non essential amino acids play important role. They help in maintaining healthy nervous system, promote muscle growth and proper functions of immune systems. They have been also reported to be involved in the production of vitamin B3 and stimulate the pancreas to synthesise insuline. They are also involved in the treatment of kidney stones, maintaining healthy skin, formation of antibodies and synthesis of nucleic acids DNA and RNA (Sabina, 2018). Some are involved in the development of human brain,

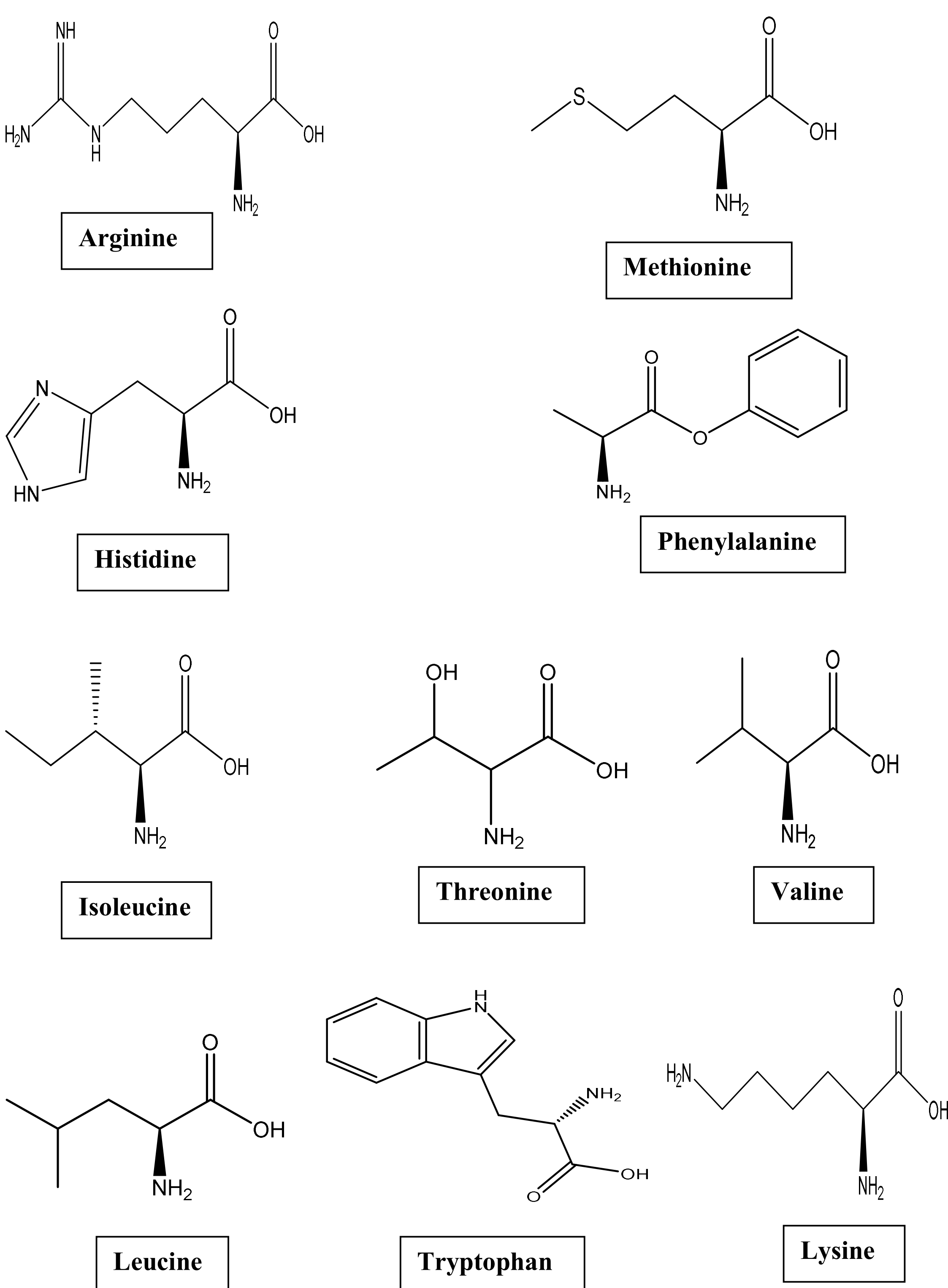


Figure 2.4: Structures of essential amino acids commonly found in protein
Source: Sabina, (2018)

healing of wounds, promoting the production of melani (natural pigment found in the eyes, hair and skin), transportation of nitrogen into our body cells, several enzymatic processes and synthesis of both red and white blood cells (Akram *et al.,* 2011).

2.11 Toxicological effect of seed meal

Toxicity refers to the harmful effects caused by a compound or material on animals or humans. It represents a state of being poisonous and indicates adverse reactions resulting from the interaction between toxic substances and cells (Sujatha *et al.,* 2003). This interaction can vary depending on the chemical properties of the toxic substances, whether on the cell surface, inside the cell, or in the surrounding tissues. These effects can occur even before the toxic substances bind to vital organs like the liver and kidneys. Evaluating the toxic properties of substances is crucial for public safety, as chemical exposure can be hazardous and lead to harmful effects on humans.

Typically, this evaluation involves three types or stages: acute, sub-chronic, and chronic. Acute toxicity refers to harmful effects caused by short-term exposure, while sub-chronic toxicity lasts for more than a year but less than the lifespan of the exposed organism. Chronic toxicity, on the other hand, refers to harmful effects caused by repeated or continuous exposure over an extended period.

Ajayi *et al.* (2013a) studied the amino acid composition and toxicological study of *G. mangostana* residues on rats. The study showed that *G. mangostana* seed cake had high chemical values, mineral content and no major alterations were detected in the blood haematological indices and organs histological analysis in the experimental rats. *Garcinia mangostana* seed cake was therefore suggested to be a good additive in feed supplement and free from toxicity at the level of incorporation.

Datta *et al.* (2011) carried out the safety evaluation of Garden cress (*Lepidium sativum L.*) seeds in Wistar rats. The research revealed that Garden cress seeds showed no sign of toxicity on tested rats. Garden cress seeds were well accepted and did not produce any poisonous effect even at 10% dietary inclusion level. Non existence of toxic effects and differences on the growth, body weight gain, organ weight, histology and blood haematology in Wistar rats is a clear evidence that Garden cress was not harmful at 10%.

Ewuola *et al.* (2012) evaluated the haematological parameters, serum biochemistry and the histopathological evaluation of the organs of growing rabbits that were fed with different concentrations of whole kenaf (*Hibiscus canabinus*) seed flour (0, 20, 40, 80, 100%) as a substitute of soy beans flour for feeding trial that lasted 12 weeks. The study demonstrated and showed that kenaf seed flour contains quality dietary protein for optimal growth. It was also found that feeding rabbit with feed prepared with concentration above 20% significantly altered the blood parameters; provoke anaemic conditions, destroy and dysfunction of instinctive organs which might be the cause of the low performance of the animal.

In a study by Ajayi *et al.* (2013b), a chemical analysis and toxicological evaluation were carried out on the residue of *G. suaveolens* seeds when used as a complete replacement for wheat in rat diets. No significant differences were observed in the growth and blood parameters of the animals. Thus, the study suggested that defatted *G. suaveolens* seeds could potentially be included in rat feed formulations due to their apparent lack of toxicity.

Ajanaku *et al.* (2010) investigated the histopathological effect of incorporating brewery spent grains (BSG) into the human food chain. The results indicated that BSG blends ranging from 1 - 3% could potentially serve as a beneficial protein supplement in human food products, such as confectioneries. Furthermore, the study revealed that a 3% inclusion of spent grains was identified as the threshold limit due to observed histological effects on the liver of rats. Consequently, it was recommended that blends within the range of 1 - 3% could be safely utilised in human food production without adverse effects on the liver, whereas blends exceeding 3% may not be advisable.

Determination of toxicological profile of a substance, plants or seed flours is a very important regulation for the marketing of product and also essential prerequisite for guaranteeing public health (Vishnu *et al*., 2010). The presence of toxic substances such as antinutrients or toxin contaminants in diets invariably leads to histological damage in critical organs, particularly the liver, spleen, and kidneys (Ewuola, 2009). Toxicological data that might be obtained in such study would be of significance importance in promoting the consumption of the plant seed flours for health benefits and remedial purposes worldwide (Tawatana *et al.,* 2010).

2.12 Vegetable oils

Vegetable oils are triglycerides extracted from plants, which fall into the category of fats and oils (Luo *et al.,* 2013). Lipids, including fats and oils, consist of various components such as sphingolipids, phospholipids, sulpholipids, cyanolipids, sterols, and complex glycolipids. Oils are obtained from plants, while fats are derived from animals, although some plants can also produce fats. The distinction between fats and oils lies in their physical state, with oils being in a liquid form at room temperature and fats being solid or semisolid. Vegetable oils can further be classified as essential oils and fixed oils. Essential oils are mixtures of low molecular weight volatile organic compounds, including alcohols, aldehydes, and ketones. On the other hand, fixed oils and fats are glyceryl esters of long-chain fatty acids. In these oils and fats, the three hydroxyl groups of glycerol are esterified with fatty acids, making them triglycerides. A glyceride molecule can contain either the same three fatty acids (simple triglyceride) or different fatty acids (mixed triglyceride) (Li *et al.,* 2014). Oils and fats are chemically composed of mixed triglycerides. Triglycerides can be hydrolyzed to produce monoglycerides and diglycerides. Controlled hydrolysis of triglycerides lead to the formation of fatty acids, which are high molecular weight compounds containing a -COOH functional group. Triglycerides have specific functional groups that can be chemically modified to yield useful products. Additionally, vegetable oil is sometimes used in non-food applications, such as cosmetics and biodiesel production.

2.13 Industrial uses of seed oils

Seed oils possess diverse industrial applications, which could be categorized as either edible or non-edible based on their nutritional and chemical properties. Edible seed oils are those that are safe for consumption and offer nutritional benefits. They are often used in cooking, food preparation, and as ingredients in various food products. These oils contribute essential fatty acids, vitamins, and other beneficial nutrients to the diet, promoting overall health and well-being. On the other hand, non-edible seed oils are not suitable for consumption due to their chemical properties or potential toxicity. These oils have diverse applications across various industries, including cosmetics, pharmaceuticals, lubricants, biofuels, other industrial products and they may possess specific properties,

such as high viscosity, stability at extreme temperatures, or resistance to oxidation, making them valuable for various industrial purposes. It is very necessary to be informed on the exact nutritional and chemical properties of seed oils to determine their appropriate application in different industries. Some of the industrial applications include emulsifiers, surfactant, bio-fuel, resin, plasticisers, lubricant, biopolymer, coating materials, paints, application in pharmaceutical and food industries (Meshrama *et al.,* 2013).

2.13.1 Biodiesel

Biodiesel refers to long-chain alkyl esters of fatty acids that are produced through the transesterification reactions of vegetable oil or animal fats with a monohydric alcohol inthe presence of a catalyst. Biodiesel derived from vegetable oils or animal fats has been reported to possess favourable fuel properties, including high flash point, good lubricity, biodegradability, renewability, low toxicity, and low emissions of gaseous and particulate pollutants (Adewuyi *et al*., 2012a). It also exhibits a higher cetane number compared to conventional fossil fuels. These properties make biodiesel a suitable alternative to conventional diesel fuel, as it can be used directly or blended with diesel in compression ignition engines. Biodiesel is considered a renewable and environmentally friendly energy option (Kouzu and Hidaka, 2012; Ahmed *et al.,* 2014) and free from sulfur and aromatic compounds. Due to its comparable properties, it is regarded as an excellent substitute for conventional diesel (Monteiro *et al.,* 2008).

2.13.2 Biolubricant

Lubricants are liquids used to reduce friction between moving surfaces, improving efficiency and minimizing wear (Jumat *et al.,* 2010). They also have additional functions such as heat transfer, contaminant suspension, liquid sealing, and corrosion protection. There are two main types of lubricants based on their source. Mineral oil lubricants, derived from crude oil, are considered harmful to the environment and human life. In contrast, bio-lubricants synthesised from plant oils and other environmentally friendly sources are biodegradable and safe (Ghazali *et al.,* 2006). Plant seed oils, renewable resources, can be used as a base stock for producing eco-friendly and rapidly biodegradable lubricants. These oils exhibit superior lubricant properties, viscosity index,

and anti-corrosion characteristics due to their affinity for metal surfaces. However, their application as lubricants is limited by low oxidation stability and high melting points caused by beta-carbons in the glycerol molecule and unsaturation (double bonds) in the fatty acid acyls (Kaya *et al.,* 2009). Epoxidising the double bonds in the unsaturated fatty acid acyls can enhance stability. Opening the epoxy ring through base or acid-catalyzed reactions produces poly-functional substances with multiple hydroxyl groups in the presence of suitable reagents (Narine *et al.,* 2007). Polyols derived from vegetable oils offer favourable properties such as low-temperature behavior, stability, evaporation ability, biodegradability, and compatibility with various materials, making them desirable as lubricants.

2.13.3 Biosurfactant

Seed oils serve as renewable and sustainable sources of essential fatty acids, making them viable raw materials to replace petrochemicals in industrial synthesis. These oils have significant industrial applications, particularly in the production of surfactants used in both edible (such as emulsifiers and thickening agents) and non-edible (like soap and detergent production) products. Surfactants play a vital role in various industries, including cosmetics, corrosion inhibitors, lubricants, oil drilling fluids, agrochemicals, and polymers. They can be categorised into different groups, including anionic, cationic, amphoteric, and non-ionic, based on their ionisation behavior in aqueous solutions. Typically, conventional surfactants consist of a hydrophilic group and a hydrophobic group in their molecular structure.

In contrast, biosurfactants are surfactants derived from plant materials like seed oil. They offer several advantages, such as high surface activity, cost-effectiveness, excellent performance in extreme temperature conditions, low toxicity, and ease of production (Saharan *et al.,* 2011). Seed oils provide fatty acids that can be easily converted into various derivative compounds with a wide range of applications. Through processes like amidation, esterification, and epoxidation, these fatty acids can be modified to yield specific surfactant structures with desired properties.

2.13.4 Other industrial uses of seed oils

Due to increasing concerns regarding environmental protection and energy conservation, coating technologists have been exploring renewable resources as alternatives to conventional petroleum-based polymeric binders. Vegetable oils extracted from plant seeds have emerged as active ingredients in the production of various polymeric binders, including alkyds, polyurethanes, epoxies, polyester amides, and polyether amides. The utilization of polymeric resins derived from seed oils offers a valuable opportunity to utilize eco-friendly and sustainable resources in the manufacturing of polymeric binders. These synthesized resins find application in diverse fields such as coatings, adhesives, binders, composites, and more (Meshrama *et al.,* 2013).

CHAPTER THREE

MATERIALS AND METHOD

3.1 Experimental design of the work

The experimental design of the research work is shown on Figure 3.1.

3.2 Sample collection and characterisation

3.2.1 Sample collection

The plant seeds employed in this experiment are *Areca catechu*, *Balanites aegyptiaca, Chrysophyllum albidum, Enterolobium cyclocarpum, Neocarya macrophylla* and *Polyalthia longifolia* (Table 3.1). *Neocarya macrophylla* seeds were purchased from Junju urban area in Niger State. *Balanites aegyptiaca* seeds were procured from Tundun wada, a small market in Zaria, Kaduna State of Nigeria. *Enterolobium cyclocarpum, Areca* catechu and *Polyalthia longifolia* seeds were collected within the University of Ibadan, Nigeria. *Chrysophyllum albidum* seeds were procured from Bode (a small market in the city of Ibadan, Oyo State of Nigeria). The fruits and seeds were first identified in the Herbarium Unit of the Botany Department, University of Ibadan and later authenticated by Mr. A. J. Egunjobi at the Forest Research Instutute of Nigeria (Ibadan) where voucher specimen were deposited in the Herbarium.

3.2.2 Chemicals and equipment

The reagents and chemicals used for this research are yellow paraffin, perchloric acid (70%), ethanolamine (99%), diethanolamine (99%), formaldehyde, KOH (99%), NaOH (80%), H_2O_2 (85%), $NaOCH_3$(99%), CH_3OH (99.80%), ethyl acetate (99%), n-hexane (99%), HCOOH (85%), HBF_4 (50%), Na_2SO_4 (98%), H_2SO_4 (98%), HNO_3 (65%), HCl (35%), N_2 (nitrogen gas), 2-ethylhexanol (99%), MgO (84%), NaCl (80%), ammonium molybdate (99%), ammonium metavanadate (99%), diethyl ether (99%), chloroform

EXPERIMENTAL DESIGN OF THE WORK

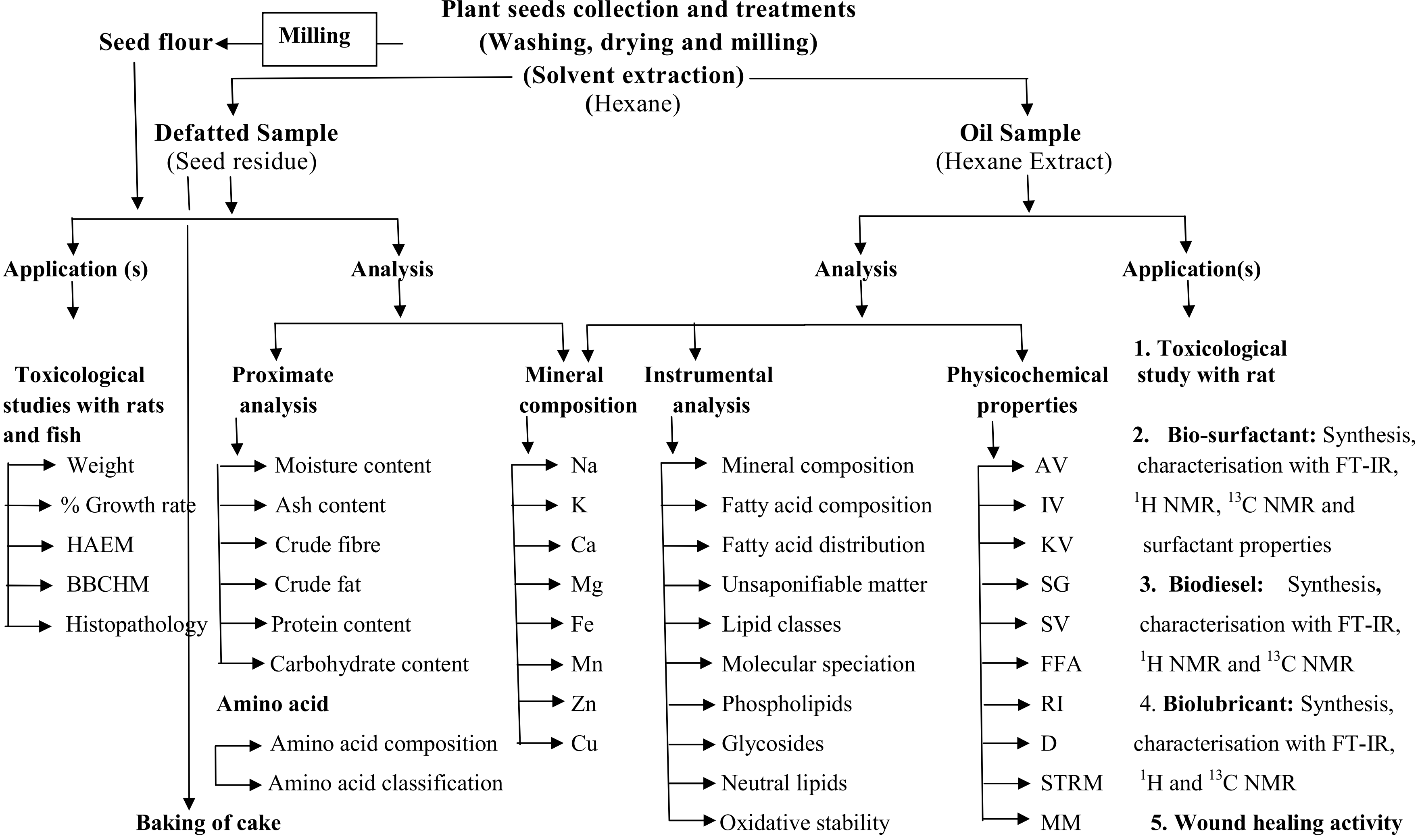


Figure 3.1: Experimental design of the research work

HAEM: Haematology BBCHM: Blood biochemistry

Table 3.1: Seed samples and their botanical names

	Seeds	Botanical name	Authority	Common name	Family name	Sample code	Vouher Specimen
1		*Areca catechu*	(Linn.)	Betel nut, Betel palm	*Arecaceae*	ACS	FHI-113371
2		*Balanites aegyptiaca*	(Linn.) Delile	Desert date	*Balanitaceae (Zygophyllaceae)*	BAS	FHI-113393
3		*Chrysophyllum albidum*	(G. Don)	Star apple	Sapotaceae	CAS	FHI-113374
4		*Enterolobium cyclocarpum*	(Jacq.) Griseb	Ear pod tree Devil's ear tree	*Fabaceae*	ENS	FHI-113391
5		*Neocarya macrophylla*	(Sab.) Prance	Gingerbread-plum	*Chrysobalanaceae*	NMS	FHI-113395
6		*Polyalthia longifolia*	(Sonn.) Thwaites	Ashoka, Indian mast tree	*Annonaceae*	PLS	FHI-113392

(99%), acetone (99%), ethanol (95%) among others. These reagents and chemicals were of analytical grade.

The major equipment employed during this research work for characterisation include rotary evaporator, FT-IR (Perking Elmer FtIR system spectrum BXLR64912C, Germany); rapid visco analyser 3C (RVA, model 3 C, Newport Scientific PTY Ltd, Australia); GCMS (Agilent 5977B with ionisation beam range 50-700 am); GC-FID (Agilent Technology 6890N with split mode 50:1); HPLC (Agilent Technology 1260 infinity); ICP-OES (Optima 4200DV equipped with ultrasonic nnebulizer, Cetal Technology, Inc, Omaha, NE, USA); NMR (400 MZ Brucker, Campbell, US) and ESI/MS (Waters e2695 Milford, MA, USA).

3.2.3 Physical characteristics

Physical characterisations of these various seeds were evaluated by employing a modified method of Ajayi *et al*. (2006). Twenty seeds of each sample were weighed and the result documented. The length and the width of each seed were also noted. The proportion of the kernel in the various seed samples and other discoveries were recorded in triplicate.

3.2.4 Sample preparation

The different seeds were collected, hand screened to remove the defective ones, cleaned, washed, air dried at room temperature and later dehulled. The kernels obtained were then broken with hammer and later milled to coarse flour with a domestic blender before the extraction. The flours obtained were later pulverised and further reduced to fine powder by passing through a 200 µm mesh sieve to increase the extent of extraction and later kept for the experiment. Oils were extracted from pulverised seed flour using n-hexane (67 ^{o}C - 68 °C as boiling point) continuously for 8 h. The solvent was distilled off completely from the oil after extraction and concentrated with rotary evaporator. The oils obtained were stored in a properly labeled container for further study. The seed residues, (also known as cakes) obtained after the extraction were air dried to allow the solvent evaporate from them, grinded once again before passing through a 200 µm mesh size and kept in a Ziploc brand nylon bags for further analyses.

3.3 Proximate composition of the seed flours and residues

The crude fat, crude fiber, moisture, crude protein and ash contents were assessed using the standard method of AOAC (2010) as the immediate parameters. The nitrogen content was determined using the Micro-kjeldahl method described in AOAC (2010). A multiplication factor of 6.25 was applied to the nitrogen content to calculate the crude protein. Carbohydrates, also referred to as nitrogen-free extract, were estimated by subtracting the sum of all proximate parameters from 100% (AOAC, 2010). The metabolic energy was determined following the approach outlined by Olaofe *et al.* (2009). The values for carbohydrate, protein, and crude fat were multiplied by Atwater factors of 17, 17, and 37, respectively to obtain the final results.

$$\text{CHB}\ (\%) = \left[100 - (\text{fat}\ \% + \text{protein}\ \% + \text{fibre}\ \% + \text{moisture}\ \% + \text{ash}\ \%)\right] \quad \text{Equ (3.1)}$$

Where CHB stands for Carbohydrate

3.4 Pasting properties

The viscosity properties of the seed residues of *B. aegyptiaca, E. cyclocarpum*, and *N. mycrophylla* were determined using a Rapid Visco Analyser 3C (RVA, model 3 C, Newport Scientific PTY Ltd, Australia) according to the method previously described by Kaur and Singh (2005). Viscosity profiles of the flours were recorded by preparing flour suspensions with a concentration of 10% (w/w; total weight of 28 g). Each sample, weighing 3 g, was placed in a weighing vessel, and a new test canister was filled with 25 ml of distilled water. The mixtures were allowed to equilibrate for 1 minute at a temperature of 50 °C. The temperature-time conditions involved heating the mixtures from 50 °C to 95 °C at a rate of 6 °C/min, with a holding time of 2 min, followed by cooling to 50 °C for an additional 2 min of holding time. The heating and cooling rates were constant at 10 °C/min. Peak viscosity, peak time; trough, breakdown, final viscosity, setback, and pasting temperature were obtained from the pasting profile using the method described by Ojo *et al.* (2017).

3.5 Analysis of mineral elements

The mineral analysis of the various seed flours, residues and oils was determined for Cu, Zn, Fe, Mg, Ca, Na, K and Mn. For the mineral analysis, 0.5 g of each sample was digested with 20 ml mixture of concentrated HNO_3 and $HClO_4$ (2:1 v/v) to decompose the organic matter completely until the solution became clear. Thereafter, it was filtered and transferred into a 100 mL standard flask, made up to the mark with deionised water and stored in a clean polyethylene bottle. Minerals elements contents were determined using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometer) for trace elemental analysis (Parvin *et al.,* 2015).

Phosphorus content in the various oils was determined by the method described by Mohan *et al.* (2016). Magnesium oxide (0.1 g) and oil sample (5 g) were perfectly weighed in a 50-100 mL silica crucible and ignited on a heater in a fume hood to burn the organic material. The crucible was transferred into muffle furnace and kept for 1 h and ignited to a white ash at 800 ^{o}C. The white ash was allowed to cool and dissolved in exactly 5 mL of HNO_3 solution via a 5 mL pipette. Then 20 mL of 1:1 aqueous ammonium molybdate and ammonium metavanadate solution was added to the sample. Blank solution was prepared following the same process without the oil sample. The absorbance of both the sample and the blank were recorded using a UV-visible spectrophotometer (Perkin Elmer, Lambda 35) at ƛmax of 460 nm and the corresponding concentrations were taken. Phosphorous content was determined using the formula:

$$\text{Phosphorus content (ppm)} = \frac{2.5 \text{ x C x } 10000}{\text{Mass of the sample (g)}} \qquad \text{Equ (3.2)}$$

Where C is the concentration of P in ppm

3.6 Phytochemical screening

The various seed flours and oils were screened for different metabolites including tannins, saponins, terpenoids, flavonoids, cardiac glycosides, anthraquinones and alkaloids. Chemical test were carried out on the extract of the seed flours using standard procedure as described by Ajayi *et al*. (2011) and Trease and Evans, (2009).

3.7 Amino acid profile and classification

The amino acid composition of each sample was determined using ion-exchange chromatography with automatic amino acid analyser, (Hitachi L8500, Tokyo, Japan) as described and outlined by Adeyeye and Afolabi, (2004) and Galla *et al.* (2012). Two grammes of milled sample flours were defatted using soxhlet extraction method. The residues were re-dried and milled into fine powder. Thirty milligrammes of each milled sample was weighed into glass ampoules; 5 mL of 6 M HCl and 5 µmL norleucine were added. The ampoules were evacuated with liquid nitrogen and sealed with burner flame and hydrolysed in an oven at 110 °C for 24 h. The ampoules were cooled, broken at the tip and the contents passed through a filter. The filtrates were dried in rotary evaporator at 40 ^{o}C under vacuum. The residues were dissolved to 5 µL (for acid and neutral amino acids) or 10 µL (for basic amino acids) with acetate buffer, pH 2.2. The solutions were dispensed into the cartridge of amino acid analyser. The peak area of each amino acid in the sample to the area of the corresponding standard amino acid of the protein hydrolysate was compared. The predicted protein efficiency ratio (PPER) was determined using the equation 3.3 (Ogungbenle *et al.,* 2014). The total acidic amino acids (TAAA), total essential amino acids (TEAA), total aromatic amino acids (TArAA), total sulfur amino acids (TSAA), total basic amino acids (TBAA) and total non essential amino acids (TNAA) were all deteramined on the basis of amino acids classification following equation 3.4 – 3.10.

$$\text{PPER} = -0.468 + 0.454\,(\text{Leu}) - 0.105\,(\text{Tyr}) \qquad \text{Equ (3.3)}$$

$$\text{TArAA} = (\text{Phenylalanine} + \text{Tyrosine}) \qquad \text{Equ (3.4)}$$

$$\text{TSAA} = (\text{Methionine} + \text{Cystine}) \qquad \text{Equ (3.5)}$$

$$\text{TAAA} = (\text{Glutamic acid} + \text{Aspatic acid}) \qquad \text{Equ (3.6)}$$

$$\text{TBAA} = (\text{Arginine} + \text{Lysine} + \text{Histidine}) \qquad \text{Equ (3.7)}$$

$$\text{TEAA} = (\text{Histidine} + \text{Isoleucine} + \text{Leucine} + \text{Lysine} + \text{Methionine} + \text{Phenylalanine} + \text{Threonine} + \text{Tryptophan} + \text{Valine}) \qquad \text{Equ (3.8)}$$

$$TNEAA = (Arginine + Alanine + Asparagine + Aspatic\ acid + Cysteine + Glutamine + Glycine + Glutamic\ acid + Proline + Serine + Tyrosine) \quad Equ\ (3.9)$$

$$TNAA = (Serine + Isoleucine + Leucine + Cystine + Methionine + Proline + Phenylalanine + Threonine + Tryptophan + Valine + Glycine + Alanine + Tyrosine) \quad Equ\ (3.10)$$

3.8 Chemical properties of the seed oils

3.8.1 Physicochemical analysis of the oils

The various oils extracted from the seed samples were analysed for iodine value, saponification value, acid value, specific gravity, mineral composition, unsaponifiable matter (AOAC, 2010).

a. ***Acid value*****:** The acid value of each oil sample was determined by dissolving one gramme (1 g) of oil in 25 mL of methanol in a 100 mL conical flask. The resulting mixture was warmed in a water bath for 5 min, cooled and was titrated against 0.1 M KOH using phenolphthalein as indicator. The titration was terminated at the appearance of the pink colour (Atolani *et al.,* 2016). The acid value was determined using the formula below:

$$\text{Acid value} = \frac{\text{Title value (ml) x M x 56.1}}{\text{Weight of sample (g)\}}} \quad \text{Equ (3.11)}$$

Where M is the molarity of KOH solution used.

b. ***Iodine value*****:** One gramme (1 g) of the seed oil was weighed into 25 mL CCl_4 in a conical flask. A 25 mL Wiji's solution was added from a dry pipette. The flask was shaken, closed with a stopper. The resulting mixture was allowed to stand in the dark for 1 h and the liberated iodine was titrated against 0.1 M $Na_2S_2O_3$ using starch indicator. A solution containing no sample was also titrated (blank titration) appropriately. Iodine value was determined using the expression in equation 3.12.

$$IV = \frac{W1 - W2}{Z} \quad \text{Equ (3.12)}$$

W1 is the volume of $Na_2S_2O_3$ (aq) solution needed for the blank, W2 is the volume of sodium thiosulphate used for the titration in the sample, Z is the weight of oil (g).

c. ***Saponification value*:** Saponification value is expressed as the number of milligrams of potassium hydroxide required to saponify one gramme (1 g) of the oil sample. One gramme of the seed oil was weighed into a 150 mL round bottom flask and 25 mL of ethanolic KOH was added. The mixture was refluxed for 1 h and titrated against 0.5 M of HCl using freshly prepared phenolphthalein as indicator until the pink colour disappeared. The saponification value was calculated as:

$$SV = \frac{[56.1 \text{ x M x } (E - F)]}{W \text{ (g)}} \qquad \text{Equ (3.13)}$$

Where

M is the molar concentration of KOH used.

E is the volume (ml) of 0.5 M of HCl used with the oil

F is the volume (ml) of 0.5 M of HCl used with the blank

W is the weight of oil (g) and SV is saponification value (mg/KOH/g)

d. ***Free fatty acids (FFA):*** One gramme (1 g) of each oil sample was boiled with 25 mL of ethanol for 5 min and allowed to cool. About 2 drops of phenolphthalein indicator was added to the mixture obtained and titrated immediately against 0.1 M of KOH with contant stearing until the appearance of a pink colour (Atiku *et al.,* 2014). The FFA was thereafter determined using the equation:

$$\text{\% free fatty acid} = \frac{(\text{V ml of KOH x M x 56.1 g/mol})}{W \text{ (g)}} \qquad \text{Equ (3.14)}$$

Where: 56.1 g/mol is the molecular mass of KOH;

V is the average titre value while M is the molarity of KOH

e. ***Refractive index at 40 ^oC*:** Refractive Index of the oil was determined using a refractometer (Anton Parr, RFM 870). The refractometer was calibrated at the same temperature with distilled water as a reference before use. Two to three drop of oil was

placed on the lower prism of the refractometer and closed firmly with the screw head. The oil was allowed to stand for 1 to 2 min until the oil attained the fixed temperature and the refractive index was measured in 5 digit of constant reading (Adewuyi *et al*., 2010).

f. ***Density and Specific gravity at 30 ^oC and 40 ^oC:*** The density of the oil samples were determined by an Automatic Density Meter (Anton Parr, DMA 4500) according to ASTM method (Adewuyi *et al*., 2010). The instrument with the sample tube was thoroughly cleaned and washed with hexane, air dried and set at 30 ^oC or 40 ^oC. A 5 mL of the oil was injected without any air gap and the values of the density as well as specific gravity were recorded automatically (ASTM 4052).

g. ***Kinematic viscosity at 40 ^oC*****:** The kinematic viscosity was obtained following D 445 (ASTM, 2010) outline method. The various viscosities of oils and biodiesel were measured by a falling ball viscometer. Thar falling-ball viscometer was used to measure the viscosity of liquid by measuring the time required for a ball to fall under gravity through a sample filled tube that is inclined at an angle. The average time of ten testes were taken in the experiment. The viscosity and kinematic viscosity can be determined by the two equations 3.15 and 3.16 (Aworanti *et al*., 2019).

$$n = \mathrm{C}\ [\mathrm{dball} - \mathrm{dmedium}) \qquad \mathrm{Equ.}\ (3.15)$$

$$\mathrm{Kv} = \frac{\mathrm{n}}{\mathrm{d}} \qquad \mathrm{Equ.}\ (3.16)$$

Where, n is the dynamic viscosity (g/cm.s); Kv is the Kinematic viscosity (g/cm.s)
C is the geometrical constant of the ball (m^2); t is the fall time through the tube (s)
dball is the density of the ball (g/cm^3)
dmedium is the density of the medium (g/cm^3)
d is the density of the sample (g/cm^3)

h. ***Oxidative stability*****:** The oxidative stability index was determined using a Rancimat with EN 14112 (2011) method. Oxidative stability of oils by DSC method was determined by a Setaram LABSYS evo DSC (France) under isothermal conditions where the accelerated oxidation of oil samples in an air flow of 60 ml/min in DSC cell set at isothermal temperatures (100-300 °C). Oil samples of 20 mg mg were weighed into open

aluminium pans without lid (open crucibles) to allow the samples to be in direct contact with the air stream and placed in the equipment's sample chamber. The aluminium reference pan as identical as possible to the oil sample pan was left empty. The DSC oxidative induction time (T0) of the oxidative reaction corresponded closely to the intersection of the extrapolated baseline and the tangent line (leading edge) of the exothermic curve and measured in minutes (Almoselhy, 2021).

3.8.2 Fatty Acid Composition by Gas Chromatography

Fatty acid profile of the seed oils was determined after converting these oils into their fatty acid methyl esters (FAME) by refluxing the oils at 70 ^{o} C for 3 h with 2% H_2SO_4 in CH_3OH (2% v/v). The esters were extracted using ethyl acetate as solvent, washed free from acid with distilled water and dried with anhydrous Na_2SO_4. The ethyl acetate extract obtained were further concentrated using a rotary evaporator to remove the solvent. The fatty acid methyl esters composition was evaluated using an Agilent 6890 N series gas chromatograph coupled to a flame ionisation detector (FID) which was equipped with a DB 225 capillary column (30 mm x 0.25 mm x 0.25 µm, (J and W Scientific, USA). The column temperature programme was 2 min at 160 °C, 5 °C/min to 230 °C and 20 min at 230 °C. The injector temperature was 230 °C with a split ratio of 10:1. The carrier gas was nitrogen at a flow rate of 1 mL/min. The detector temperature was 270 °C with air and hydrogen flow rates of 300 mL/min and 30 mL/min, respectively. The fatty acids were identified by comparing the retention times with mixture of standard FAMEs, C_4 - C_{24} (Supelco, USA). The area percentage was recorded with a standard Chemistation Data System (Anjaneyulu *et al*., 2016; Adewuyi *et al*., 2010).

3.8.3 Lipid classes and Fatty acid distribution in the oil samples

The extracted oils were separated into neutral lipids, glycolipids and phospholipids on 1 g scale by silica gel column chromatography using a glass column 20 cm x 2 cm OD packed with 30 g activated silica gel (60-120 mesh). Neutral lipids, glycolipids and phospholipids were eluted using chloroform, acetone and methanol, respectively. These fractions were separately screened by TLC and the various components identified with a mixture of hexane-ethyl acetate (90:10, v/v) as developing solvent for neutral lipids;

chloroform/methanol/water (65:25:4, v/v/v) for glycolipids and phospholipids. Eluted glycolipids and phospholipids spots were identified using different spray reagents (α-naphthol, ammonium molybdate and iodine vapour, respectively for glycolipids, phospholipids and neutral lipids (Ravinder *et al*., 2016). Fractions obtained were refluxed with 2 % solution of H_2SO_4 in CH_3OH for 3 h for fatty acid methyl esters. The esters obtained were extracted into ethyl acetate, washed with distilled water and dried over anhydrous sodium. The fatty acid profile was analysed using Gas chromatography (Adewuyi *et al.,* 2010).

3.8.4 Isolation and identification of unsaponifiable matter

The unsaponifiable matter in the oil samples were identified using GC-FID, an Agilent 6850 gas chromatograph coupled to a flame ionisation detector FID and equipped with a HP-1 capillary column (30 mm x 0.25 mm x 0.25 µm, 100% dimethyl polysiloxane stationary phase material; company, J and W Scientific, USA). The column temperature was programmed to 2 min at 150 °C, 10 °C/min to 300 °C and 20 min at 300 °C. The temperature of the injector was 280 °C with a split ratio of 50:1. The carrier gas was nitrogen at a flow rate of 1 mL/min. The temperature of the detector was 300 °C with air and hydrogen flow rates of 300 mL/min and 30 mL/min, respectively.

Two to five grammes of the oil were dissolved in 25 mL of methanolic KOH and refluxed for 1 h. The reaction mixture was later diluted to 150 mL with distilled water and transferred into a separating funnel. The unsaponifiable matter was extracted with 50 mL diethyl ether three times. The ether extracts, pulled together, were washed with 100 mL aqueous solution of 0.5 M KOH to remove any leftover fatty acids. The mixture was further washed with distilled water until free of KOH, dried over anhydrous Na_2SO_4 and concentrated through a rotary evaporator. The unsaponifiable matters were identified using a GC-MS. Structural assignments were made based on interpretation of mass spectrometric fragmentation and confirmation by comparison of retention time as well as fragmentation pattern of authentic compounds and the spectral data from the Wiley and NIST libraries (Anjaneyulu *et al*., 2016).

3.8.5 Triacylglycerol molecular species of the extracted oils

A reversed phased HPLC that is equipped with an Evaporative Light Scattering Detector (ELSD) with a quaternary pump was used to determine the molecular species of the oils. A 1 mg/mL of triacylglycerols were injected into the SGERP column (250 SS 4.6-W5C18-RS, Darmstadt, Germany). The molecular species of the triacylglycerols were eluted within 10 min using an isocratic mobile phase of 95:5 (v/v) of acetone/isopropanol at a flow rate of 1 mL/min. ELSD operating conditions include: drift tube temperature 50 º C and flow of nitrogen 50 psi with gain 100. The molecular species of oils were identified by their Equivalent Carbon Numbers (ECN) by injecting reference triacylglycerols mixture and comparing with literature data while the elution order was predicted according to Anjaneyulu *et al*. (2016) and Adewuyi *et al.* (2010).

3.9 Toxicological studies of seed flours, residues and oils

3.9.1 Utilisation of seed flours, residues and oils as additive in feed formulation and feeding of Wistar rats

3.9.1.1 Feed formulation with the seed flours and residues

Feeds employed in the research work were prepared to meet young rats' nutrient specifications using *A. catechu*, *C. albidum*, *E. cyclocarpum* and *P. longifolia* seed flours as well as *B. aegyptiaca* and *N. macrophylla* residues. The diets were formulated based on modified Souza *et al.* (2007) procedure. The major ingredients employed in the formulation were: soybeans, maize, groundnut cake, corn bran, wheat, palm kernel cake, bone, oyster shell and salt. This formulation was adopted in the control group, while in the experimental groups; 10, 20 and 30% of ingredients in the control groups were respectively replaced with each of the seed flours and residues. Table 3.2 showed more detailed composition of the diet. The feeds were made in pellet form, sun dried within 48 h and stored in different containers for the experiment.

3.9.1.2 Feed formulation with *B. aegyptiaca* and *N. macrophylla* seed oils

The feeds were prepared following the methods described by Toyomizu *et al.* (2003) and Ajayi *et al.* (2013a) with little modification. These feed ingredients were measured at

different percentages (Table 3.2), homogeneously and blended together to obtain diet A (100%) which served as control while 10% of groundnut oil (w/v), 10% and 20% of *B. aegyptiaca* and *N. macrophylla* seed oils (w/v) were incorporated into the diet A to obtain three other diets B, C and D which served as the experimental diets. The control diet (A), diet containing groundnut oil (B), diet made with *B. aegyptiaca* oil (C) and diet prepared with *N. macrophylla* oils (D) (w/v) were separately pelletised with Hobart A-200T pelleting machine, sun dried and stored.

3.9.1.3 Experimental animals, diets and feeding

Thirty two mature Wistar rats (eight weeks old) weighing between 70 and 100 g were selected for each of the experiment. The animals were aranged in 4 groups (A, B, C and D) containing 8 rats each according to their weight. The rats were procured at the Department of Anatomy within the University of Ibadan. They were acclimatised for one week, maintained on standard diet (Ladokun Feeds Limited, Ibadan, Nigeria) with water *ad-libitum* in the animal house under normal room temperature before starting the experiment. The method employed for the research was in conformity with current laboratory animal care and ethical guidelines, UI/EC/15/0208. They were kept at normal room conditions and fed with four different formulated meals for 56 days before being sacrificed. The weight of rats in A (control), B (fed with 10 % sample four), C (20 % seed sample) and D (30 % seed sample) were documentated at the beginnig of the research as day zero and then on 7 daays interval thereafter. The rats were fed with various diets prepared from seed flours, residues, BASO and NMSO compounded feeds.

The rats were physically examined daily during the feeding period for clinical symptoms and mortality before feeding, after and up to 24 h later. The feed intakes were also recorded daily while the body weight was documented weekly throughout the experimental period. Rats with odd behaviour or physical changes in the eyes, skin, posture or responsiveness to handling were noted and excluded from the groups. Animals were sacrificed on the last day of the experiment and blood samples collected for blood biochemistry and haematological studies. The liver, kidney and heart harvested from each sacrificed rats in each group were weighed and conserved in 10% formalin solution for histopathological study.

3.9.1.4 Effect of nutrition on growth performance, feed utilisation and survival rate

The evaluation of the growth parameters was based on the techniques described by Ajayi *et al.* (2013a). The parameters which include specific growth rate (SGR), feed conversion ratio (FCR), weight gain (WG), feed intake (FI), average daily growth (ADG), feed efficiency ratio (FER) and relative weight of the organs (RWO) were all determined. The equations were given as:

$$\text{Relative weight of the organs (RWO)} = \frac{100 \text{ x Organ weight (g)}}{\text{Body weight of the rat (g)}} \qquad \text{Equ (3.17)}$$

$$\text{Average daily growth (ADG)} = \frac{(\text{Initial weight} - \text{final weight})}{\text{Experimental period in days}} \qquad \text{Equ (3.18)}$$

$$\text{specific growth rate (SGR)} = \frac{(\ln W_2 - \ln W_1) \text{ x } 100)}{\text{Experimental period in days}} \qquad \text{Equ (3.19)}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{total weight of feed given (g)}}{\text{total weight gained by the rat (g)}} \qquad \text{Equ (3.20)}$$

$$\text{Feed efficiency ratio (FER)} = \frac{\text{Weight gain (g)}}{\text{total feed intake (g)}} \qquad \text{Equ (3.21)}$$

Where: W_1= mean weight at the beginning; W_2 = mean weight at the end

3.9.2 Utilisation of seed flours (ACSF, PLSF) and seed residues (BASR, NMSR) as additive in feed formulation and feeding of catfish

3.9.2.1 Experimental fish

Both fingerlings and post juvenile of *C. gariepinus* (catfish) were used in this experimental work. A known quantity (180) of fingerlings with average weight of 4.55 - 5.53 g and post juvenile with average weight ranging from 27.76 g to 31.90 g were bought from the Department of Aquaculture and Fisheries Management Research Farm,

University of Ibadan. The fish were housed in 12 round shape 40-litres plastic containers with 15 fish each and allowed to acclimatise for 7 days. They were given feed two times in a day at 5% body weight with an imported commercial feed brand during the acclimatisation period and divided after into four groups of 3 replicates. About 35 liters of de-chlorinated water (tap water exposed to air) was poured in each round shape plastic container. Synthetic nets were applied to wrap the plastic container to avoid fish escaping from it and equally to protect the fish from strange objects and invasion of insects. Water in each basin was changed at 3 days interval. All the fish were examined daily during the feeding period for clinical symptoms and mortality before feeding, after and up to 24 h later throughout the experimental period.

3.9.2.2 Feed formulation and feeding of the fish

The feed components used in this research work comprised of fish meal, soybean meal, maize, wheat offal, vitamin/mineral premix, millet, starch, calcium phosphate, salt, vegetable oil and groundnut cake (Table 3.2). These food items were homogeneously blended together. ACSF, PLSF, BASR and NMSR were separately added to the formulated feed at 0% (control diet), 10%, 20% and 30% (test diets). Each formulated feed was pelletised with Hobart A-200T pelleting machine and packaged separately. The pelletised diets were dried under the sun, broken mechanically into appropriate magnitude, repackaged in transparent container according to each group and kept for further usage. The fish were fed with experimental feeds every morning and evening at 5 percent of their body weight. Weights of fish were noted on weekly basis with a digital scale (model EHA 251) and feeding was always adjusted to the current body weights. Length of fish was equally recorded weekly with a 30 cm ruler for each test group.

3.9.2.3 Growth performance and feed utilisation efficiency

The evaluation of the growth parameters were done based on the techniques described by Ajayi *et al.* (2013c). The parameters which included specific growth rate (SGR), feed conversion ratio (FCR), weight gain (WG), feed intake (FI), average daily growth (ADG), feed efficiency ratio (FER) and relative weight of the organs (RWO) were all determined following the method descried in section 3.8.4.

Table 3.2: **Recipe used in the formulated diets for Wistar rats, catfish and baked cakes**

Ingredients (g)	Control (0%)	10%	20%	30%
	Wistar rats[a]			
Maize	1,320.00	1,188.00	1,056.00	924.00
Soya beans	600.60	540.54	480.48	420.42
Oyster Shell	72.60	65.34	58.08	50.82
Corn bran	234.30	210.87	187.44	164.91
Wheat	234.30	210.87	187.44	164.91
Palm kernel cake	234.30	210.87	187.44	164.91
Groundnut cake	468.60	421.74	374.88	329.82
Salt	26.40	23.76	21.12	18.48
Bone	108.90	98.01	87.12	76.23
Total **(g)**	3,300.00	3,300.00	3,300.00	3,300.00
Seed flour/residue	0.00	330.00	660.00	990.00
	***C. gariepinus* fish[b]**			
Fish meal	242.00	242.00	242.00	242.00
Soya bean	245.00	245.00	245.00	245.00
Groundnut cake	243.00	243.00	243.00	243.00
Corn meal	190.00	190.00	190.00	190.00
DCP	10.00	10.00	10.00	10.00
Vitamin premix	20.00	20.00	20.00	20.00
Chromium oxide	5.00	5.00	5.00	5.00
Starch	30.00	30.00	30.00	30.00
Fish oil	15.00	15.00	15.00	15.00
Total	1000.00	1000.00 g	1000.00 g	1000.00 g
Seed flours/residue	-----	100.00 g	200.00 g	300.00 g
	Baked cake[c]			
Wheat/seed cake ratio	100:00	90:10	80:20	70:30
Wheat flour	100	90	80	70
Seed residues	0.00	10	20	30
Fat (margarine)	50	50	50	50
Sugar	40	40	40	40
Baking powder	2	2	2	2
Salt	0.5	0.5	0.5	0.5
Eggs (whole)	2	2	2	2

The recipe formulation were according to the procedure described by [a] Souza *et al*. (2007), [b] Ajayi *et al*. (2013c) and [c] Onuegbu *et al*. (2013) with modification. DCP: di-calcium phosphate

3.9.3 Haematological, blood biochemical and histopathological analysis of rats blood and organs

3.9.3.1 Blood sample collection from rats

After feeding and monitoring of the weekly average weight for 56 days (8 weeks), the rats were made to fast overnight and blood was collected by heart puncture under diethyl ether anesthesia into two heparinized sample bottles containing EDTA to prevent blood coagulation. One sample bottle content was used for haematological study and the second for serum biochemistry analysis (Mbaka and Adeyemi, 2010).

3.9.3.2 Haematological analysis of rats' blood

For each of the blood sample collected, the underlisted parameters, the red blood cells (RBCs) count, total and differential white blood cells (WBCs) count, packed cell volume (PCV), erythrocyte sedimentation rate and haemoglobine (Hb) content were evaluated. The quantities of RBCs and WBCs were found with the improved Neubauer Haemocytometre. The packed cell volume (PCV) was analysed spectrophotometrically. The erythrocyte sedimentation rate was estimated by the descried method of Westergren (Mbaka and Adeyemi, 2010).

3.9.3.3 Packed cell volume (PCV) of rats' blood

The PCV was estimated following the standard method (Jain, 1986). The capillary tube containing blood sample was spinned at 25,000 rpm for 5 mn and the value was read on the microhaematocrit graphic. Measuring the height of this erythrocytes column and expressing this as a fraction of the height of the total blood column was subsequently used to determine the PCV of each blood sample (Ajayi *et al*., 2013a).

3.9.3.4 Haemoglobin

To perform a hematological analysis, a 5 mL volume of Drackins solution was added to a tube. The tube was then placed in a spectrophotometer cuvette. This step aimed to calibrate the spectrophotometer by using the Drackins solution after allowing it to stabilize for approximately 30 min. Subsequently, the absorbance of the solution was measured

using a colorimeter at a specific wavelength of 540 nm. For each sample, 20 mL was taken and combined with 5 mL of Drackins hemoglobin solution. The mixture was thoroughly mixed and left undisturbed for 15 min after which it was transferred to the spectrophotometer cuvette. The concentration of hemoglobin was determined by comparing the absorbance of the sample with a standard chart that had been prepared in advance, i.e standard calibration curve (Mbaka and Adeyemi, 2010).

3.9.3.5 White Blood Cell count (WBC)

The WBC fluid (0.5 mL) was taken through a graduated pipette into 5 mL bijour bottle, blood sample (25 mL) was also added using 25 uL automatic micropipette and was thoroughly mixed together. The cover slip was properly fixed on the counting chamber. A little amount of the diluted blood was dispensed under the cover slip and left for 3 mn after which it was mounted on the stage. Counting was done at x 40 objectives (Raphael *et al*., 2014).

3.9.3.6 Red Blood Cell count (RBC)

To facilitate the visual red blood count, a solution commonly utilised as diluents and known as formal citrate solution was prepared by mixing 10 mL of formalin (40% of formaldehyde) with one liter of 31.3 g/L solution of sodium citrate. The resulting solution known as formal citrate solution was filtered and stored in a clean glass container. A know quantity of 2 microliters of each blood sample was mixed with 40 mL of diluents, resulting in a final dilution of 1 in 20 mL. The diluted sample was then mixed and loaded onto the haemocytometer. After the cells had settled out of the suspension, the number of cells in 0.04 mm^2 area was counted and noted (Raphael *et al*., 2014).

3.9.3.7 Blood biochemistry analysis

The blood biochemistry parameters, namely aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), were examined (Chawla, 1999). Commercial kits from Randox Laboratories Co Atrium, UK, were utilised for this analysis. Additionally, other parameters such as serum creatinine, albumin, globulin, urea, total protein, and the albumin/globulin ratio (A/G) were also determined (Ajayi and

total protein, and the albumin/globulin ratio (A/G) were also determined (Ajayi and Aghanu, 2012).

3.9.3.8 Tissue collection for histopathology

After the removal of blood, individual rats were dissected consecutively to obtain several organs and tissues, including the heart, spleen, lungs, brain and liver. The weight of each organ harvested was measured immediately after collection. These organs and tissues were subsequently preserved for histopathological examination in 10% formalin solution (Ajayi and Aghanu, 2012).

3.9.3.9 Histopathology analysis

The internal organs, liver, heart and kidney harvested were observed for gross lesions. Small portions of each of these organs already stored in formalin were fixed and put through timed series of dehydration in graded concentrations of xylene (Ajayi and Aghanu, 2012). They were equally embedded in wax, sectioned at 5 uL and later transferred into a clean slide. The thin sections were stained with haematoxylene and eosin (H and E) dye for examination under light microscope for histological changes following the method outlined by Jain (1986).

3.9.4 Production and quality evaluation of cake from whole wheat flour, BASR, ECSR and NMSR blends

3.9.4.1 Preparation of BASR, ENSR and NMSR

Oil was extracted from BASF, ENSF and NMSF using soxhlet extractor with n-hexane (67 - 68 ^{o}C). After 6 h of extraction, the residues (BASR, ENSR and NMSR) obtained were air dried, pulverised, passed through a 200 µm mesh sieve to get fine powder of uniform particle size and stored at room temperature for analysis.

3.9.4.2 Preparation of composite flour blends for cake production

Wheat flour was separately mixed with 0%, 10%, 20% and 30% of BASR, ECSR and NMSR to make 5 different composite flour blends (100:0, 90:10, 80:20, 70:30 and 0:100)

with 100:0 (0% residue) wheat flour serving as control. A digital weighing balance and blender were used for weighing and mixing the flours respectively.

3.9.4.3 Production of dietary cake from composite flour blends

The composite flours were blended with other baking ingredients in a mixer as listed on Table 3.2. The cake samples were in ratios of wheat flour with either BASR or ECSR or NMSR at 100:0, 90:10, 80:20, 70:30 and 0:100. Other ingredients used along with the flour blends were fat (margarine), sugar, baking powder, salt and eggs. The creaming method of cake preparation was in accordance with that of Kiin-Kabari and Banigo (2015) was adopted with slight modification. Mixing was carried out for 7 mins using a wooden hand mixer in a clockwise direction. The resulting dough was cut into uniform sizes and passed through a series of molding, shaping and stamping. The mixed flour pastes were baked in a pre-heated oven at 240 ^{o}C for 40 min. The dietary cakes produced were allowed to cool, packaged with a cellophane wrapper and stored at room temperature for analytical investigation (Kiin-Kabari and Banigo, 2015).

3.9.4.4 Physical properties of the flour blends

The physical characteristics of the cakes produced (weight, height, volume and specific volume) were measured after baking (Gimai and Barker 2004). The cake volume was calculated with the equations 3.22 and 3.23.

$$\text{Volume of cake (cm}^3) = \pi h\,(d^2 + db + b^2) \qquad \text{Equ (3.22)}$$

Where d and b are upper and lower diameters of cake

$$\text{The specific volume} = \frac{\text{cake volume}}{\text{cake weight}} \qquad \text{Equ (3.23)}$$

The physical properties determined include:

a. ***Bulk Density*****:** The bulk density (loose and packed) of the flour samples was evaluated according to Chinma *et al.* (2008) procedure. A known weight of the flour was taken into a previously weighed (W_1) measuring cylinder and the weight of the cylinder (W_2) as well as the volume of the flour (V_1) was noted. Loose bulk density (LBD) was

then calculated as g/mL. The cylinder was tapped gently to eliminate air spaces between the particles of the flour. The new volume (V_2) of the sample and mass of the cylinder (W_3) was noted and packed bulk density (PBD) was calculated as g/mL.

$$\text{LBD} = \frac{(\text{W2} - \text{W1})}{\text{V1 g/mL}} \qquad \text{Equ (3.24)}$$

$$\text{PBD} = \frac{(\text{W3} - \text{W1}))}{\text{V2. g/mL}} \qquad \text{Equ (3.25)}$$

Where LBD and are loose and packed bulk densities

b. Swelling Index: The swelling index of each flour sample was determined as the ratio of swollen volume of a unit weight of each sample to its initial volume in a graduated measuring cylinder (Chinma *et al.,* 2008). One gram of each flour sample was weighed out with a weighing balance and dispensed into a 20 mL measuring cylinder in each case. Then, this was followed by the addition of 10 mL of distilled water in each measuring cylinder. The volume of each sample was noted. They were then allowed to stand undisturbed for 1 h before their volumes were taken again and recorded accordingly. The swelling index (SI) of each flour sample was calculated as:

$$\text{Swelling index (SI)} = \frac{\text{V1}}{\text{V2}} \qquad \text{Equ (3.26)}$$

Where V_1 = Initial volume occupied by sample and V_2 = Final volume occupied by the sample after swelling.

3.9.4.5 Sensory Evaluation of the cakes

The sensory evaluation of the dietary cakes was carried out after baking (Gimai and Barker (2004). Fifteen members, experienced panelists and regular producers of cakes were selected to evaluate the samples. The properties such as appearance, taste, aroma, crunchiness and overall acceptability were evaluated using 9-point hedonic scale with 1 representing the least score (extremely dislike) and 9 the highest score (extremely like). The panelists were selected based on their interest and ability to make a distinction in food

sensory properties. Panelists rinsed their mouth with table water after each evaluation as necessary precautions to prevent carryover of sensory attributes during profiling.

3.9.4.6 Effect of the cakes produced on blood haematology and weight of Wistar rat

A quantity of 25 matured eight weeks old Wistar rats weighing between 80 and 100 g used in this study were procured from Anatomy Department, University of Ibadan. The animals were classified into four different groups of five animals each, selected according to their weights. The animals which familiarised themselves with the environment for one week were maintained on standard normal diet (Ladokun Feeds Limited, Ibadan, Nigeria) with water *ad-libitum* in the animal house under normal room condition before the commencement of the experiment. After the acclimatisation period, they were nourished for three weeks with the dietary cakes produced. The rats in their different experimental groups were respectively fed with 100:0, 90:10, 80:20 and 70:30 prepared dietary cakes with unrestricted access to water. The physical appearance, survival rate and growth rate of each rat were monitored. The rats' body weights were documented weekly throughout the experimental period. Blood samples were collected for haematology at the end of the three weeks as described in Section 3.11.

3.10. Wound healing activities of *Balanites aegyptiaca* And *Neocarya macrophylla* seed oils (BASO and NMSO)

3.10.1 Antimicrobial activity

The antimicrobial activity of BASO and NMSO oils were carried out as follows:

i. Test of microorganisms

Aspergillus niger and *Candida albicans* clinical isolates with multiple drug resistance were used as experimental organisms while *Bacillus subtilis, Escherichia coli*, *Pseudomonas aerugonisa* and *Staphylococcus aureus* were employed as experimental bacteria organisms. The pure bacterial and fungal strains were supplied by the Department of Pharmaceutical Microbiology, University of Ibadan where all the cultures were performed.

ii. Antimicrobial activity assay of seed oils

The *B. aegyptiaca* and *N. macrophylla* seed oils antimicrobial activity were assessed against few pathogenic bacteria (*Bacillus subtilis, Escherichia coli*, *Pseudomonas aerugonisa* and *Staphylococcus aureus*) and fungi (*Aspergillus niger* and *Candida albicans*). The pure bacterial strains were cultured for 12 hrs at 37 ^{o}C in a nutrient agar while the fungal were prepared for 12 hrs at 28 ^{o}C using potato dextrose agar. By dilution procedure, the 12 hrs culture was evaluated for noticeable growth on agar plates or the wells of microplate with various oil concentrations (0-200 mg/ml) and standard antibiotics (Ajayi *et al.,* 2015). Sterile filter paper disks (6 mm width having 6.25-200 mg/disk of the extracts) were incubated for 12 hrs at 28 ^{o}C on the surface of the agar to evaluate the growth inhibition in the disk diffusion test.

iii. Minimum inhibitory concentrations (MIC) of seed oils

The MIC is known as the smallest extract concentration that can hinder the observable growth of bacteria or fungi on the surface of the agar. The MIC for the bacteria and fungi strains was quantified (Ajayi *et al.*, 2015).

3.10.2 Experimental animals

A known quantity of 28 Wistar female rats with mean weight that ranged from 125 to 245 g were seperately used for each seed extracts. These animals were categorised into 4 groups of seven (7) rats each, kept in transparent plastic cages and acclamaised for one week. The rats were fed with standard feeds throughout the eight weeks experimental periods. The protocols used were in agreement with the Ethical approval (15/0208/UI/ECRA) obtained from Institute of Advance Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan.

3.10.3 Evaluation of wound healing activity of BASO and NMSO oils

The wound healing activities were studied as follows:

a. Preparation of formulations

The ointments were formulated from each of the seed oils and yellow paraffin to evaluate

their efficacy. A 10% (w/w) ointment based extracts were prepared by mixing 5 g of each oil sample in 50 g of yellow paraffin (Ajayi *et al.,* 2015). The ointment used for positive control group was prepared from 5 g of povidone-iodine (standard ointment bought from Abimbola pharmacy shop, Agbowo, Ibadan) in 50 g of yellow paraffin. The ointments formulated were homogenously mixed together at ratio of 1:10 (oil to yellow paraffin).

b. Acute skin irritation test of the prepared ointments

The acute skin irritation test was carried out following Ghosh *et al*. (2012). About 200 mm^2 area was shaved on the dorsal fur of each animal (Figure 3.2). The shaved area was cleaned and the formulated ointments were applied to different groups of animals. The skin of each animal was observed for any sign of inflammation and infection after 4 h from when the ointments were applied.

c. Excision wound model

Wound excision model was used to investigate wound healing potential of BASO and NMSO. The formulated ointments were applied topically once a day. The animals in Group1 received ointment base (control), Group 2 were untreated while those in Group 3 were treated with 10% (w/w) of each formulated ointments. Wounds were created on rats according to the procedure of Ajayi *et al.* (2015). The dorsal fur of the rat was shaved with electrical clipper; the skin from the shaved area was cleaned with 70% alcohol, excised to its full thickness to about 200 mm^2 area with blade and scissors used for surgical operation (Figure 3.2). The wounds were excised on the dorsal thoracic region at 1.50 cm to the vertebral column on each side. Cotton swab previously soaked in normal saline was used to blot the wound to achieve complete haemostasis. The rats were anaesthetised using ketamine hydrochloride (100 mg/kg, i.p.) before and during the wound creation (Nayak *et al.,* 2009). The wound was left opened throughout the experimental period and all the wounded rats were treated with ointments formulated from the seed oils. The healing of wound was evaluated by tracing the wound closure on the first, fourth, eightieth, 12th, 16th and 20th post wounding days with transparent paper and marker. Percentage wound closure and epithelialisation time was evaluated (Ghosh *et al.,* 2012).

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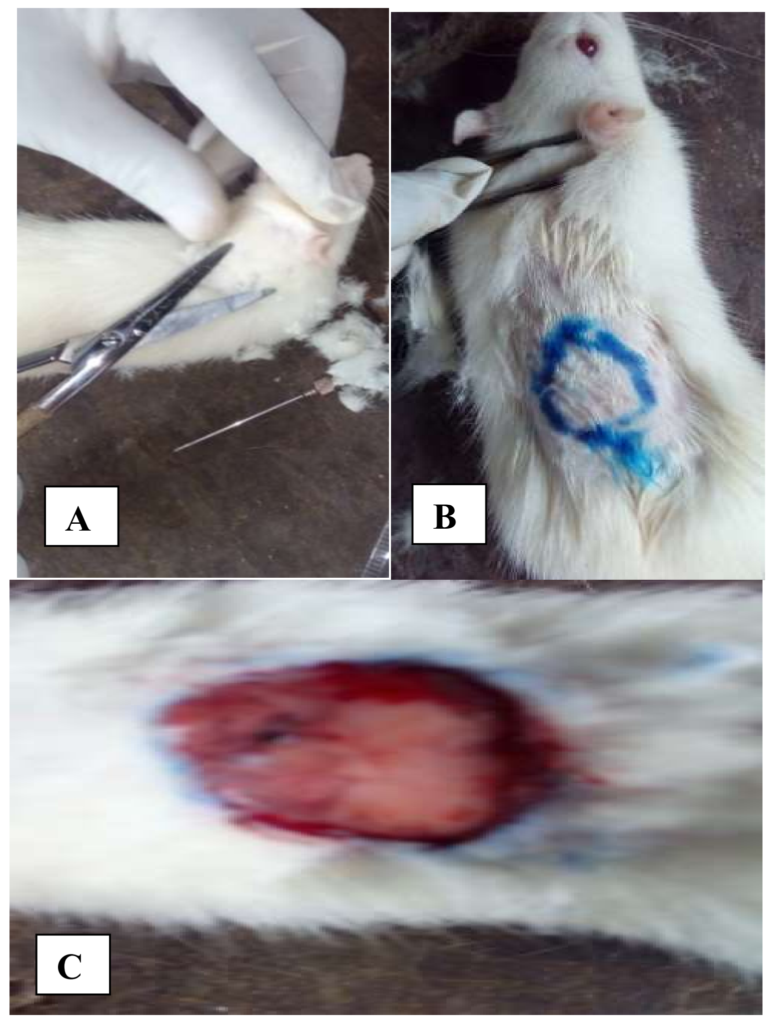


A. SHAVING OF HAIR

B. WOUND DIAMETER

C. WOUND INCISION ON RAT

Fig 3.2: Excision wound model

d. Wound healing activity

The wound healing capability of the extracts base ointments was assessed by checking the reduction in the wound area, rate of contraction of the wound and epithelialisation time as well as the histological study of the healed tissues (Ghosh *et al.,* 2012).

e. Rate of wound contraction and Epithelialisation time

The rate of wound contraction was determined as percentage reduction of size on a four-day basis (Ghosh *et al*., 2012). The number of days that the scar took to fall without any residual of the raw wound area was recorded as complete wound healing and was noted as period of epithelialisation (Nayak *et al.,* 2009).

$$(\%)\mathrm{WC} = \frac{\text{wound area on day (0)} - \text{wound area on day (n)}}{\text{wound area on day (0)}} \text{ x } 100 \qquad \text{Equ (3.27)}$$

Where WC stamds for wound contraction

f. Blood haematological and histological studies

Blood samples were collected for haematological analysis at the end of the experimental period as described in Section 3.11 while sections of the skin and some organs (liver, kidney and heart) were harvested for histological analysis (Ajayi *et al*., 2015).

3.11 Synthesis of oleochemicals (Biodiel, Biolubricant and Biosurfactant)

3.11.1 Synthesis and characterisation of biodiesel from BASO and NMSO

Biodiesel was prepared from seed oils following a two major step reaction mechanism. The first step involves using 2% H_2SO_4 in CH_3OH while the second step is a transesterification reaction using 1% KOH in methanol.

3.11.1.1 Transesterification

A round bottom flask with sampling outlet, well equipped with reflux condenser, magnetic stirrer and thermometer was used in the transesterification reaction. The various seed oils were tranesterified with CH_3OH in the presence of KOH as catalyst. The reaction temperature was kept at 60 - 65 ^{o}C and the contents were stirred constantly at 600 rpm for

90 min. The resultant mixture was cooled to 30 ^{o}C for the separation of two phases. The upper lighter phase of biodiesel and lower denser phase of glycerin (by-product) were separated by simple decantation. Crude biodiesel contains the excess CH_3OH, unused catalyst, soap formed, some entrained methyl esters and partially reacted glycerides. The excess catalyst was removed by successive rinsing with distilled water. The residual water content was removed by treatment with anhydrous Na_2SO_4 and filtered. Transparent yellow liquid obtained as the final product was kept for other characterisation and the yield of biodiesel formed was calculated by the expression described by Naureen *et al.* (2015).

$$\% \text{ Yield} = \frac{\text{Weight (g)of the methyl ester produced x 100}}{\text{weight of oil used (g)}} \qquad \text{Equ (3.28)}$$

3.11.1.2 Physicochemical characterisation and fuel properties of BASO and NMSO biodiesel

The following fuel properties (Flash point, pour point, cloud point, ester content, microcarbon residue, phosphorous content, trace metal in biodiesel, copper corrosion test, oxidative stability index, methanol ester content), were determined using standard official methods as described by the American Society for Testing and Material (ASTM) and the American Oil Chemists' Society (AOCS) as elaborated by Adewuyi *et al*. (2012a). The synthesised biodiesel was separated into triglycerides, diglycerides, monoglycerides, and free fatty acids on a 1 g scale by silica gel column chromatography with a glass column 20 × 2 cm OD. The column was perfectly packed with 30 g activated silica gel (60 - 120 mesh) as described by AOCS (2003). The kinematic viscosity was obtained following ASTM (D 445, 2010). The density of the biodiesel was also recorded at 40 °C using a densitometer. Free glycerol was determined by AOCS (1997). Phosphorus level was estimated using a colourimetric method, (AOCS, 2002). Pour point was established with the ASTM D97 and ASTM D 5949 (ASTM, 2010). Copper corrosion test was done according to ASTM D396 (ASTM, 2004). EN 14103 (2011) method was used to evaluate the methyl ester content in the various biodiesel. Oxidative stability index (OSI) was

measured in accordance with EN 14112 (2011) employing a Rancimat. Sulphur content was evaluated following (ASTM-1998).

The physico-chemical characterisation and fuel properties of BASO and NMSO biodiesel were determined as follows:

i. Kinematic viscosity

The kinematic viscosity was obtained following D 445 (ASTM, 2010) outline method. The various viscosities of oils and biodiesel were measured by a falling ball viscometer (Aworanti *et al*., 2019) as described in Section 3.8.

ii. Flash point: The temperature at which a fuel is been heated in such a way that the vapour and air mixture above the fuel can be ignited is referred as flash point. The flash point was evaluated according to ASTM D93 (2010) method. The biodiesel sample was put up to the prescribed mark in the interior of the test cup. The cup was mounted onto its position on the tester. Heat was supplied through a bunsen burner to the apparatus at the rate of 1 °C per minute with constant stirring. A small test flame was periodically projected into the cup. The flash point was recorded as the temperature when the flame caused the vapour above.

iii. Pour point: The lowest temperature at which a liquid stop flowing is referred to as pour point. The pour point of the synthesised biodiesel was investigated following ASTM D97 (2010) method. The sample was poured up to the mark of a test jar. A thermometer was then immersed at 3 mm below the sample surface and the jar was placed on the disk inside the bath jacket maintained at 6 °C. The jar was removed from the jacket and tilted at each decrease of 3 °C in temperature to confirm the flow of the sample. The process was carried out again until the sample stop to flow. The temperature at this point which the sample stopped flowing again was noted. The pour point was obtained by adding 3 °C in temperature already noted.

3.11.2 Synthesis and characterisation of polyols from *B. aegyptiaca* and *N. macrophylla* seed oils for biolubricant properties

The BASO and NMSO were used to synthesise polyols via epoxidation reaction of the oils.

3.11.2.1 Epoxidation of BASO and NMSO

Epoxidation was carried out in a 250 mL round-bottom flask coupled with a mechanical stirrer and a thermometer as described by Adewuyi *et al.* (2012b). The complete set up was immersed in an oil bath. A 43.25 g of methyl esters from the seed oils and 4.90 g of concentrated HCOOOH were mixed to the flask. The temperature of the medium was reduced to 15 ^{o} C with continuous stirring while 46.10 g of H_2O_2 was added slowly with constant stirring for about half an hour. The reaction temperature was later increased to 80 ^{o}C and remained constant for 3 h. Samples of the mixture were collected at each 30 min for FTIR analysis to confirm for the formation of the epoxide ring. The temperature of the mixture was reduced to room temperature after confirmation of the epoxidation of the oil samples. The epoxidised oil was then extracted with ethyl acetate, washed severally with water to remove the acid and decanted with a separating funnel. The product was finally run through Na_2SO_4 and concentrated in a rotary evaporator. The equation of reaction is shown in Figures 3.3.

3.11.2.2 Synthesis of polyol from the epoxidised BASO and NMSO

Epoxidised BASO and NMSO were employed in synthesising polyol through oxirane ring opening. The ring was opened with $CH_3CH(C_2H_5)(CH_2)_3CH_2OH$ using HBF_4 as catalyst at a ratio of 1 % by weight of $C_8H_{17}OH$ and epoxidised oil considered. The molar ratio of the epoxy group to the OH group was 1: 10. The catalyst and the alcohol were poured into a 500 mL round bottom flask with three-neck, equipped with a stirrer (mechanical), refluxing column and heat control meter. The flask was heated while in an oil bath. The epoxidised oil was added to the mixture while stirring. The reaction mixture was kept at 80 ^{o}C for 4 h and later cooled to normal 30 ^{o}C. NH_3 (30 % in water) was added to neutralise the effect of the catalyst. The reaction mixture finally obtained was washed several times with water, decanted with separating funnel and passed through sodium

(a)

$$R\text{-}COOH + MeOH \xrightarrow{H^+} R\text{-}COOMe + H_2O$$

Free fatty acid Methanol Methyl ester Water

(b)

$$\begin{array}{l} CH_2\text{-}O\text{-}CO\text{-}R_1 \\ CH\text{-}O\text{-}CO\text{-}R_2 \\ CH_2\text{-}O\text{-}CO\text{-}R_3 \end{array} + 3\ CH_3OH \underset{H_2SO_4}{\overset{KOH}{\rightleftharpoons}} \begin{array}{l} R_1COOCH_3 \\ R_2COOCH_3 \\ R_3COOCH_3 \end{array} + \begin{array}{l} CH_2\text{-}OH \\ CH\text{-}OH \\ CH_2\text{-}OH \end{array}$$

Triglycerides Methanol Methyl esters Glycerol

linoleic acid + methanol $\underset{H_2SO_4}{\overset{NaOH}{\rightleftharpoons}}$ methyl ester

Chemical Formula: $C_{19}H_{34}O_2$
Exact Mass: 294.26
Molecular Weight: 294.48
m/z: 294.26 (100.0%), 295.26 (20.5%), 296.26 (2.0%)
Elemental Analysis: C, 77.50; H, 11.64; O, 10.87

Figure 3.3: Reaction mechanism involved in the preparation of Biodiesel from seeds oils

(a) Esterificatsion of free fatty acid using 2% H_2SO_4 in methanol

(b) Transesterification of triglyceride using 1% KOH in methanol

sulphate. The product obtained was concentrated using a rotary evaporator and its reaction mechanism is presented in Figure 3.4 (C).

3.11.2.3 Lubricant properties of polyols from BASO and NMSO

The viscosity, density, copper corrosion test, flash point, oxidative stability, hydroxyl value, and pour point of the BASO and NMSO synthesised biolubricant were investigated using ASTM methods. The density measurement was conducted using a density meter (DMA 450M model) following ASTM 4052 (2009) guidelines. The determination of copper corrosion test followed ASTM D 4048 (2010). The flash point was evaluated using the AOCS modified method for closed-cup flash point determination (2010). The oxidative stability was determined according to ASTM D 2272 (2011), which is the standard test method for oxidation stability of steam turbine oils. The hydroxyl value (AOCS, 2003), four-ball test (ASTM D 2596, 2010), and Emulsion stability (Ware *et al.,* 2007), pour point (ASTM D 2596, 2010) were all determined accurately. Refractive index, specific gravity, and iodine value were determined following AOAC guidelines (2010). The colour was assessed through visual inspection. Analyses were recorded in triplicate.

3.11.3 Synthesis and surface active property of diethanolamide and epoxidised diethanolamide surfactant from BASO and NMSO

The surfactants were synthesised from *B. aegyptiaca* and *N. mycrophylla* oils via epoxidation of the oil which followed by amidation reaction.

3.11.3.1 Seed oils epoxidation reaction

The epoxidation was carried out in a necked round 250 mL round-bottom flask coupled with a mechanical stirrer and a thermometer as described by Adewuyi *et al.* (2012b). The technique and procedure described in section 3.11.2.1 was equally considered under this subsection. The reaction mechanism is presented in Figure 3.5.

A- Formation of peroxyacid

$$H_2O_2 + HCOOH \longrightarrow HCOOOH + H_2O$$

B- Epoxidation

$$\text{-CH=CH-} + HCOOOH \longrightarrow \text{-HC(O)CH-} + HCOOH$$

C- Epoxy ring opening with ethyl hexanol

$$\text{-HC(O)CH-} \xrightarrow[\text{Tetrafluoroboric acid (80 °C and 4h) Reflux}]{HX} \text{-HC(X)-CH(OH)-}$$

$$HX = HO\text{-}CH_2\text{-}(CH_2)_3\text{-}CH(CH_2CH_3)\text{-}CH_3$$

$$\text{-HC(O)CH-} \xrightarrow[\text{Tetrafluoroboric acid (80 °C and 4hrs)}]{HO\text{-}CH_2\text{-}(CH_2)_3\text{-}CH(CH_2CH_3)\text{-}CH_3} \text{-HC(O-CH}_2\text{-(CH}_2)_3\text{-CH(CH}_2\text{CH}_3\text{)-CH}_3\text{)-CH(OH)-}$$

Polyol

Figure 3.4: Reaction mechanism for the production of polyol from seed oils

Where A is formation of peroxyacid, B is epoxidation process, C is the opening of epoxiring with ethyl hexanol and D is the synthesis of polyols.

3.11.3.2 Diethanolamide production from BASO and NMSO

The reaction was carried out in round bottom flask that was equipped with a stirrer, condenser and thermometer. The flask itself was suspended in an oil bath. The reaction of diethanolamine with the oil was evaluated at a molar ratio of 6:1 (diethanolamine: oil) with sodium methoxide (2% by weight of diethanolamine and oil) as catalyst and temperature of 115 ^{o}C. The reaction mixture was monitored with TLC, allowed to cool at the end of the reaction and dissolved in diethyl ether in a separating funnel. The ether phase was washed with 5% aqueous hydrochloric acid. The resultant ether layer was separated, washed with water, passed through Na_2SO_4 and concentrated in a rotary evaporator (Adewuyi *et al*., 2011). The mechanism of the reaction is presented in Figure 3.5.

3.11.3.3 Production of epoxidised diethanolamide from epoxidised BASO and NMSO

The round bottom flask placed in an oil bath and well equipped with a mechanical stirrer, condenser and thermometer was used. Diethanolamine was reacted with the epoxidised oil at a molar ratio of 6:1 (diethanolamine:oil) using sodium methoxide at 2% by weight of diethanolamine and oil as the catalyst. The reaction was carried out as described above for the unepoxidised oil as shown in Figure 3.4

3.11.3.4 Assessment of surface-active properties of diethanolamide and deoxidised diethanolamide

The surface active properties include:

i. ***Emulsion stability***: The emulsifying power of the aqueous solutions of the surfactants was carried out with water/liquid paraffin system. Aqueous solutions (0.5%) of epoxidised diethanolamide, diethanolamide and sodium lauryl sulphate (SLS) used as reference surfactant were prepared. A 20 mL of each prepared aqueous solution was poured separately into a 100 mL graduated measuring cylinder. A 20 mL of paraffin was later added to each measuring cylinder containing the aqueous solution by the side of its wall.

A- Formation of peroxyacid

$$H_2O_2 + HCOOH \longrightarrow HCOOOH + H_2O$$

B- Epoxidation

-CH=CH- + HCOOOH ⟶ -HC(O)CH- + HCOOH

$$\text{Triglycerides} + 3\ \text{Diethanolamine} \xrightarrow[110\text{-}120^{o}C]{NaOMe} \text{Diethanolamide} + \text{Glycerol}$$

Triglycerides: $CH_2\text{-O-CO-}R_1$ / $CH\text{-O-CO-}R_2$ / $CH_2\text{-O-CO-}R_3$

Diethanolamine: $HN(CH_2CH_2OH)_2$

Diethanolamide: $R_1\text{-CO-}N(CH_2CH_2OH)_2$, $R_2\text{-CO-}N(CH_2CH_2OH)_2$, $R_3\text{-CO-}N(CH_2CH_2OH)_2$

Glycerol: $CH_2\text{-OH}$ / $CH\text{-OH}$ / $CH_2\text{-OH}$

Figure 3.5: Reaction mechanism for the synthesis of diethanolamide

R_1, R_2 and R_3 are alkyl chains with or without epoxide functional groups

The cylinder containing the mixture of the sample solutions and the paraffin was inverted for 30 times at a rate of 1 turn per 2 s and allowed to separate back. The time taken for 15 ml of the aqueous phase to separate from the mixture was noted (Ware *et al.*, 2007).

ii. ***Foaming power*****:** The foaming power of the surfactants prepared was determined with the method described by Adewuyi *et al.* (2012c). Aqueous solutions (0.5%) of epoxidised diethanolamide, diethanolamide and sodium lauryl sulphate were prepared. SLS was used as reference surfactant. A 20 mL of each prepared aqueous solution was poured separately in a 100 mL graduated measuring cylinder. The cylinder containing the solution was turned upside down (inversed) for 30 times at a rate of 1 turn per 2 s. The height of the foam produced in mm was measured immediately and after 5 min interval. Data recorded were used to estimate the foam ability and foam stability of the prepared surfactants.

iii. ***Surface tension*****:** The surface tension of 0.5% aqueous solution of the prepared and reference surfactants were carried out at 293 K with Kruss Processor tensiometer (K100) under atmospheric pressure by the ring method (ASTM D 1331-2020). The platinum ring was thoroughly cleaned, and the flame dried before each measurement. The measurements were done in such a way that the vertically hung ring was dipped into the liquid to measure its surface tension. It was then subsequently pulled out. The maximum force needed to pull the ring through the interface was then expressed as the surface tension, γ (mN/m). Measurements of the surface tension of pure water at 293 K were performed to calibrate the tensiometer and to check the cleanliness of the glassware. In all cases more than 10 successive measurements were carried out, the average value was noted (Janczuk *et al.*, 2021).

3.11.4 Instrumental and spectroscopic characterisation of synthesised products

The instrumental and spectroscopic characterisation of the each synthesised products was carried out by FT-IR and NMR.

a. ***FTIR analysis*****:** The BASO and NMSO samples, epoxidised oil, their corresponding biodiesels, biolubricants and biosurfactant were analysed and various functional groups documented with a Perkin Elmer FTIR system spectrum BX

LR64912C. The spectra of samples spread over NaCl discs were recorded between 4000 - 400 cm^{-1} (Anjaneyulu *et al*., 2016)

b. ***NMR analysis*****:** The BASO and NMSO samples, their corresponding biodiesels, biolubricants and biosurfactant were qualitatively examined via ^{1}HNMR and carbon ^{13}C NMR methods. This was done by comparing some important peak integration in the starting material and the final products. The spectra were recorded using a 400 MHs Bruker NMR spectrophotometer in $CDCl_3$ (deuterated chloroform) containing little quantity of TMS (tetramethylsilane) as internal standard (Tariq *et al*., 2011).

3.12 Statistical analysis

All results were analysed and reported as means and standard deviation. All other data from the rats study were analysed using one ways ANOVA. The least significant different level at $\alpha_{0.05}$ between the means of various treatments and analyses were determined by Duncan's multiple range test (JBM STSS Statistics 20). Results were expressed as means±SD.

3.13 Quality control protocol

In order to ascertain the consistency of the result obtained, the analyses were done in triplicate. All the glass wears and instruments employed were cleaned properly to avoid contamination. All chemicals utilised were of analytical grade.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Physical properties of the seed samples

The physical properties of *A. catechu* (ACS), *B. aegyptiaca* (BAS), *C. albidum* (CAS), *E. cyclocarpum* (ECS), *N. macrophylla* (NMS), and *P. longifolia* (PLS), seeds with regard to their length, width, weight and kernel percentage were presented on Table 4.1. The weight (g) of 20 pieces of each plant seed was from 18.66 g (*C. albidum*) to 75.60 g (*A. catechu*). *A. catechu* seed had the highest average weight (3.78 g) while *C. albidum* seed had the lowest average weight (0.93 g). The average values obtained for the seeds lengths and widths were 2.31 cm and 1.46 cm (CAS); 2.25 cm and 1.88 cm (PLS); 3.19 cm and 2.38 cm (BAS); 3.58 cm and 2.91 cm (ACS), 1.60 cm and 1.25 cm (ECS), 1.73 cm and 1.13 cm (NMS).

4.2 Proximate analysis of seed flours, seed residues and energy contribution

The proximate analysis of various seed flours is given on Table 4.2. The analysis showed that protein content (%) of the seed flours ranged from 4.20% (ACSF) to 30.69% (ECSF). These protein values obtained are lower than 39.40 % obtained for *Cucumeropsis mannii* seed flour (Eunice *et al.,* 2012) and 39.40% reported for *Albisia saman* (Adewuyi *et al.,* 2011). The protein content of BASF (30.54%), ECSF (30.69%) and NMSF (25.43%) are higher than that of ACSF (4.20%), CASF (8.14%) and PLSF (12.20%). The high protein contents of these seeds suggested them as potential protein sources that could be practically used as protein supplement in area where there are deficiencies of protein. The values of protein contents in BASF (30.54%), ECSF (30.69%) and NMSF (25.43%) were higher than wheat (14.51%) which served as control.

The carbohydrate content of seed flours were in the order of 63.94% (CASF) > 59.65% (PLSF) > 54.66% (ECSF) > 51.58% (ACSF) > 8.15% (NMSF) > 5.08% (BASF).

Table 4.1: Physical properties of ACS, BAS, CAS, ECS, NMS and PLS

Properties	ACS	BAS	CAS	ECS	NMS	PLS
Weight of 20 seeds (g)	75.60±1.60	56.90±1.05	18.66±1.69	19.10±1.52	8.86±1.05	21.80±0.03
Weight of a seed (g)	3.78±0.32	2.84±0.24	0.93±0.07	0.95±0.17	0.94±0.12	1.09±0.01
Seed length (cm)	3.58±0.28	3.19±0.86	2.31±0.37	1.73±0.25	1.60±0.15	2.25±0.18
Seed width (cm)	2.91±0.18	2.38±0.90	1.46±0.16	1.13±0.02	1.24±0.10	1.88±0.10
Kernel percentage (%)	69.30	38.81	50.00	64.20	98.24	89.90

Values are means±SD for triplicate analysis

Means with different letters on the same row are significantly different at ($P \leq 0.05$)

ACS: *Areca catechu* seed,

BAS: *Balanite aegyptiaca* seed

CAS: *Chrysophyllum albidum* seed

ECS: *Enterolobium cyclocarpum* seed

NMS: *Neocarya macrophylla* seed

PLS: *Polyathia longifolia* seed

Table 4.2: Proximate composition of ACSF, BASF, CASF, ECSF, NMSF, PLST, BASR, ECSR and NMSR

Parameters	ACSF	BASF	CASF	ECSF	NMSF	PLSF	Wheat flour
				Flours			
Moisture content	6.79±0.02^{c}	7.78±0.04^{c}	9.93±0.04^{a}	6.12±0.15^{c}	8.83±0.04^{b}	9.20±0.03^{b}	10.92±0.04^{a}
Protein content	4.20±0.08^{e}	30.54±0.15^{a}	8.14±0.13^{d}	30.69±0.05^{a}	25.43±0.22^{b}	12.40±0.25^{c}	14.51±0.11^{c}
Crude fat	1.40±0.08^{d}	46.95±0.64^{a}	12.82±0.04^{b}	2.64±0.03^{d}	45.96±0.10^{a}	4.26±0.01^{c}	0.19±0.01^{e}
Ash content	1.76±0.03^{c}	2.95±0.05^{a}	2.32±0.03^{b}	3.70±0.15^{a}	3.81±0.03^{a}	2.58±0.02^{b}	1.59±0.02^{c}
Crude fibre	34.25±0.01^{a}	6.70±0.03^{c}	2.84±0.23^{d}	2.20±0.01^{d}	7.82±0.04^{c}	11.90±0.04^{b}	0.01±0.01^{e}
Carbohydrate	51.58±0.64^{a}	5.08±0.14	63.94±0.12^{a}	54.66±0.23^{a}	8.15±0.25^{b}	59.65±0.19^{a}	72.75±0.11^{a}
Energy (Kj/100g)	1000.06	2342.69	1699.70	1547.64	2277.33	1382.47	1490.92
Dry mater (%)	93.21	92.22	90.07	93.87	91.17	90.80	89.07
				Seed residue			
Parameters	BASR	NMSR	ECSR				
Moisture content	8.45±0.03^{b}	12.32±0.53^{a}	8.56±0.03^{b}				
Protein content	51.31±0.13^{a}	56.04±0.01^{a}	37.18±0.05^{b}				
Crude fat	6.90±0.04^{a}	4.51±0.01^{b}	0.39±0.11^{c}				
Ash content	4.40±0.02^{b}	6.53±0.06^{a}	4.30±0.15^{a}				
Crude fibre	12.90±0.02^{a}	7.41±0.01^{b}	1.19±0.01^{c}				
Carbohydrate	16.03±0.18^{b}	13.19±0.49^{c}	48.21±0.27^{a}				
Energy (Kj/100g)	1400.08	1343.78	1470.30				
Dry mater (%)	91.55	87.68	91.42				

Values are mean±standard deviation of triplicate determinations
Means with different letters on the same row are significantly different at ($P \leq 0.05$)

ASF: *Areca catechu* seed flour,
BASF: *Balanite aegyptiaca* seed flour
BASR: *Balanite aegyptiaca* seed residue
CASF: *Chrysophyllum albidum* seed flour
ECSF: *Enterolobium cyclocarpum* seed flour
NMSF: *Neocarya macrophylla* seed flour
NMSR: *Neocarya macrophylla* seed residue
PLSF: *Polyathia longifolia* seed flour

The NMSF had the lowest value while CASF had the highest value. The values of carbohydrate obtained in CASF, PLSF, ECSF and ACSF were higher than 46.02% and 41.32% reported for *Tamarindus indica* and *Albisia saman* (Adewuyi *et al.,* 2011).

The hydrolysis and fermentation of this high carbohydrate containing seeds might make them also potential feeds stocks in the production of bio-ethanol fuel. The crude fibre contents obtained varied from 2.84% (CASF) to 34.25% (ACSF). The high carbohydrate and high crude fibre contents of some of the seed flours indicate that they could be regarded as source of roughage in preparing animal feeds. These flours could probably replace carbohydrate rich foods like wheat ovals and serve as alternative sources of energy if consumed by human and livestock.

Ash content is an estimation of inorganic materials remaining in the various seed flours when the organic materials and water which helped in determining the nutritional quality of the seeds have been removed. The ash content (%) found in the seed samples were from 1.76 (ACSF) to 3.81 (NMSF). These values obtained compared favourably with 4.00% (*Tamarindus indica*), and 2.10% (*Albisia saman*) but lower than 5.10% (*Millettia griffonianus*) (Adewuyi *et al.,* 2011).

Microbial growth is influenced by the moisture content of seeds, which also plays a critical role in determining their shelf life. When the moisture content is high, the shelf life decreases, leading to a greater proliferation of microorganisms. The moisture content of the seed samples were 6.12% (ECSF), 6.79% (ACSF), 7.78% (BASF), 8.83% (NMSF), 9.20% (PLSF) and 9.93% (CASF). They compared favourably with 10.92% obtained for wheat flour. However, these values were higher than 4.01% and 2.90% reported for *Trilepisium madagascariense* and *Antiaris Africana* (Adewuyi *et al.,* 2010). *B. aegyptiaca* and *N. macrophylla* with high protein content and low carbohydrate content could be regarded as protein rich seeds while *A. catechu*, *P. longifolia* and *C. albidum* were carbohydrate rich seeds.

The oil yield obtained in ACSF, BASF, CASF, ECSF, NMSF and PLSF samples varied significantly among each other from the lowest value of 1.40% (ACSF) to the highest value of 46.95% (BASF). Others include 45.96% (NMSF), 12.82% (CASF), 4.26%

(PLSF) and 2.64% (ECSF). BASF and NMSF with very high yield (46.95% and 45.96%) could be classified as oil rich seeds with oil yield higher than 34.71% and 39.10% reported for *A. breviflorus* and *C. colocynthis* (Akinnifesi, 2017) which was slightly lower than 54.05% reported for *Sesamum indicum* (Nzikou *et al.,* 2010) and compared favourably with 47.02% of *M. charantia* (Akinnifesi, 2017).

This high oil yield might possibly suggest *B. aegyptiaca* and *N. macrophylla* as important seed oils that could be used in the synthesis of biodiesel, biolubricant, biosurfactant, biopolymer and other useful products in the industries. They are equally comparable to the vegetable oils that are presently employed for oleochemicals in the market such as soy bean (55 %) and ground nut (49-50 %) and could therefore serve as source of vegetable oils for industrial processes.

The proximate analysis of *B. aegyptiaca*, *E. cyclocarpum* and *N. macrophylla* seed residues (BASR, ECSR, and NMSR) was also presented on Table 4.2. The protein contents obtained in these seed residues were 37.17% (ECSR), 51.31% (BASR) and 56.04% (NMSR). The protein contents recorded in various seed residue were higher than those obtained in the seed flours. They compared favourably with 50.80±% obtained for moringa seed residue as revealed by Abiodun *et al.* (2012). Carbohydrate contents were very low in NMSR (13.19%) and BASR (16.03%). The ash content (%) found in the seed residues were from 4.30% (NMSF) to 6.53 (ECSR). These values obtained were than found in their respective seed flours. The energy values obtained were found higher in the flours than in the residues. This is possibly because of high crude fat content in the flours as compared to the lower values obtained for the seed residues.

The energy value obtained was 1470.30 kJ/100g, 1400.08 kJ /100g and 1343.78 kJ/100 g for ECSR, BASR and NMSR respectively. It ranged from 1000.06 kJ/100 g (ACSF) to 2277.33 kJ/100 g (NMSF) and 2342.69 kJ/100 g (BASF). The energy values of 2309 kJ/100 g, 1792.8 kJ/100 g and 2157 kJ/100 g were respectively reported for *Cucumeropsi mannii* (Eunice *et al*., 2012); *Propolis africana* (Aremu *et al.,* 2007) and *Lagenaria siceraria* (Olaofe *et al.,* 2009). These also compare favourably with 2277.33 kJ/100 g and 2342.69 kJ/100 g obtained in *N. macrophylla* and *B. aegyptiaca* seed flours respectively.

The various values of the total energy contributed by protein, fat and carbohydrate respectively were given on Table 4.3. It revealed that the total energy contributed by protein was higher (62.30 % and 70.89 %) in BASR and NMSR. This was majorly due to the high protein content in the seed residues after extraction of the oils. The total energy contributed by fat was higher in the seed flour that had high oil yield (BASF and NMSF) while that of carbohydrate was higher in the seeds with low oil yields and low protein contents (ACSF, CASF, ECSF). Most of the seed samples showed a high value of energy contributed by carbohydrate except BASF (3.69%) and NMSF (7.08%) which were lower than 17.5% observed in *L. siceraria* seed flour (Olaofe *et al.,* 2009). The seeds of ACS, BAS, CAS, ECS, NMS and PLS could definitely be good source of energy to human or livestock if found to be toxic free.

4.3 Mineral composition of various seed flours, oils and residues

The results of the mineral composition of the seed flours, oils and their residues were listed on Table 4.4. A total number of eight metals (Ca, Mg, Zn, Mn, Cu, Fe, Na and K) were determined using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The results obtained revealed that the various metals determined varied considerably in their concentrations in the seed flours, oils and their residues.

Potassium (K) was the highest in each group, followed by Mg and Ca while other elements were present in small amount. The same trend of results was observed in the seed flours, residues and oils. K was known as essential element in human nutrition and regarded as major cation in the animal cell. It remained therefore important in keeping the fluid and electrolyte balance in the body system as well as necessary in permitting the contraction of muscles (Adewuyi *et al.,* 2010). Calcium ion was reported as essential element in the organism, mostly in cell physiology where it's movement inside and outside the cytoplasm is used as signal for a lot of cellular processes (Remington, 2005). It was also involved in building and maintaining the bones healthy and stronger, in the maintenance of normal functions of the heart, muscles and nerves. Magnesium was considered as an important and useful mineral because of its healing action on a large number of diseases. It also reported to take part in maintaining the bones healthy. Calcium is equally involved in the hindrance of cardiovascular ailments; in the normalisatisation of

Table 4.3: The proportion of energy contributed by Carbohydrate, Fat and Protein in seed flours and residues

Proportion (%)	ASF	BASF	BASR	CASF	ECSF	NMSF	NMSR	PLSF
Proportion of total energy due to Protein	6.26	22.16	62.30	6.86	28.81	18.98	70.89	15.25
Proportion of total energy due to Fat	27.26	74.15	18.23	5.18	13.18	74.68	12.41	11.40
Proportion of total energy due to Carbohydrate	66.47	3.69	19.46	87.68	56.63	6.08	16.68	73.35

ASF: *Areca catechu* seed flour,
BASF: *Balanite aegyptiaca* seed flour
BASR: *Balanite aegyptiaca* seed residue
CASF: *Chrysophyllum albidum* seed flour,
ECSF: *Enterolobium cyclocarpum* seed flour
NMSF: *Neocarya macrophylla* seed flour
NMSR: *Neocarya macrophylla* seed residue
PLSF: *Polyathia longifolia* seed flour

Table 4.4: Mineral composition (ppm) of seed flours, oils and residues

Seed flour	ACSF	BASF	CASF	ECSF	NMSF	PLSF
Ca	723.88±0.06^{d}	1225.12±0.02^{a}	653.32±0.02^{e}	503.63±0.03^{f}	973.36±0.03^{b}	750.18±0.04^{c}
Cu	9.91±0.03^{c}	4.73±0.00^{f}	12.93±0.03^{b}	5.30±0.00^{e}	7.43±0.02^{d}	92.06±0.02^{a}
Fe	74.23±0.05^{c}	33.36±0.11^{f}	42.81±0.03^{e}	60.83±0.01^{d}	102.38±0.01^{a}	100.46±0.02^{b}
K	2116.98±0.05^{b}	5767.62±0.11^{a}	5100.06±0.05^{a}	5487.80±2.07^{a}	5179.90±0.02^{a}	5304.64±0.03^{a}
Mg	674.33±0.57^{f}	1845.83±0.20^{b}	1050.00±0.01^{d}	3560.56±0.09^{a}	1028.56±0.11^{e}	1071.75±0.25^{c}
Mn	18.03±0.05^{a}	12.69±0.01 ^{d}	17.20±0.01^{b}	13.28±0.02^{c}	9.36±0.03^{f}	10.03±0.05^{e}
Na	220.05±0.04^{a}	98.19±0.05^{d}	210.08±0.02^{b}	124.38±0.02^{c}	12.70±0.00^{f}	62.03±0.00^{e}
Zn	5.92±0.02^{f}	34.83±0.05^{b}	19.54±0.03^{e}	36.84±0.04^{a}	25.42±0.02^{d}	29.64±0.00^{c}
Seed oil	ACSO	BASO	CASO	ECSO	NMSO	PLSO
Ca	152.79±0.29^{d}	311.62±0.49^{c}	455.50±5.00^{b}	454.97±2.11^{b}	327.50±2.30^{c}	692.86±2.06^{a}
Cu	0.19±0.01^{c}	0.47±0.00^{a}	0.48±0.02^{a}	0.09±0.00^{d}	0.28±0.02^{b}	0.10±0.00^{d}
Fe	411.75±5.72^{b}	6.71±0.03^{c}	11.13±0.10^{c}	9.89±0.20^{c}	9.47±0.30^{a}	17.69±0.31^{c}
K	13.29±0.19^{f}	120.21±0.18^{c}	20.97±0.14^{d}	554.11±0.01^{b}	109.88±0.20^{e}	756.04±0.03^{a}
Mg	50.43±0.10^{f}	150.15±0.13^{c}	122.09±0.90^{d}	354.61±0.10^{a}	80.95±0.05^{e}	160.03±0.99^{b}
Mn	0.47±0.05^{f}	1.32±0.03^{e}	37.04±0.03^{a}	2.20±0.02^{c}	1.59±0.02^{d}	5.42±0.02^{b}
Na	45.89±0.09^{c}	65.51±0.07^{b}	24.53±0.03^{d}	124.63±0.45^{a}	0.65±0.05^{f}	16.28±0.07^{e}
Zn	2.37±0.65^{f}	3.69±0.09^{e}	4.23±0.03^{d}	9.55±0.05^{b}	8.64±0.05^{c}	11.03±0.03^{a}
Seed residue	NMSR	BASR	ECSR			
Ca	647.15±0.05^{b}	913.51±0.01^{a}	51.29±0.01^{c}			
Cu	7.17±0.00^{a}	4.25±0.05^{c}	5.20±0.05^{b}			
Fe	92.90±0.01^{a}	26.58±0.10^{c}	50.91±0.02^{b}			
K	5160.09±0.05^{a}	5647.32±0.02^{a}	5230.88±0.26^{a}			
Mg	947.49±0.05^{c}	1695.61±0.01^{b}	3205.90±0.05^{a}			
Mn	7.75±0.00^{c}	11.35±0.01^{a}	11.10±0.05^{b}			
Na	12.04±0.05^{b}	32.62±0.02^{a}	10.06±0.05^{c}			
Zn	25.40±0.05 ^{c}	31.11±0.01^{a}	27.12±0.02^{b}			

Values are mean±SD of three determinations. Means with different letters on the same row are significantly different at ($P \leq 0.05$).

high blood pressure and migraines among others (Soetan *et al.,* 2010).

Potassium concentration in the seed flours were 2116.98 ppm (ACSF), 5100.06 ppm (CASF)*,* 5179.90 ppm (NMSF), 5304.64 ppm (PLSF), 5487.80 ppm (ECSF) and 5767.62 ppm (BASF). In the seed oils, it ranged from 13.29 ppm (ACSF) to 756.04 ppm (PLSO). Potassium concentration of 120.21 ppm (BASO) and 109.88 ppm (NMSO) were equally noted. Potassium is discovered to be more concentrated in the seed flours than in the residues and the oils.

Magnesium concentration varied from 674.33 ppm (ACSF) to 3560.56 ppm (ECSF). Others include 1845.83 ppm (BASF), 1071.75 ppm (PLSF) 1050.00 ppm (CASF) and 1028.56 ppm (NMSF). Within the oil samples, ACSO had the lowest (50.43 ppm) Mg concentration while ECSO had the highest value (354.61 ppm). Mg concentration of 947.49 ppm, 1695.61 ppm and 3205.90 ppm were respectively obtained for NMSR*,* BASR and ECSR.

Calcium concentration obtained were 503.63 ppm (ECSF), 653.32 ppm (CASF), 723.88 ppm (ACSF), 750.18 ppm (ACSF), 973.36 ppm (NMSF), and 1225.12 ppm (BASF). The concentration of Ca in the oils varied from 152.79 ppm (ACSO) to 692.86 ppm (PLSO). Calcium concentration of 913.51 ppm, 1695.61 ppm and 3205.9 ppm were respectively recorded for NMSR, BASR and ECSR. This result showed that the seed flours, oils and residues were very rich in Ca, Mg and K. They also contained other important mineral elements such as Zn, Na, Mn, Cu and Fe in moderate concentration. The above identified minerals were necessary in breaking down foods and their transformation into energy, the carriage of nerve impulse, and making bones strong and healthy among other effects (Egwin *et al*., 2010).

The results of this study indicated that the various seed samples possessed low levels of trace metals (Cu, Zn, Mn, and Fe) while containing high levels of important macronutrients including Na, K, Mg, and Ca in their seed flours, oils, and residues. This implied that these seed varieties, namely ACS, BAS, CAS, ECS, NMS, and PLS, could be valuable sources of essential macronutrients such as Na, K, Mg, and Ca that could be utilised for nutritional purposes. In other words, these seeds have a favourable

composition of trace metals and macronutrients that could make them potentially beneficial for meeting dietary needs and promoting good health.

4.4 Phytochemical screening of sample flours

The results of the phytochemical screening of ACSF, BASF, CASF, ECSF, NMSF and PLSF were displayed on Table 4.5. It showed that the seeds contained majorly saponins, steroids, alkaloids, carbohydrate among others. These metabolites showed numerous pharmacological and biochemical activities when consumed by animals. They were biologically active compounds found in little amount in different plants. They also contributed majorly in the protection of degenerative diseases (Omale and Okafor, 2008). Alkaloids were secondary metabolites known for their medicinal and physiological activities. The absence of tannins, phenols and phlobatannins was observed in almost all the flours while saponins, carbohydrate and alkaloid were present in most of the sample. The presence of these phytochemicals in the flours suggested that these seed plants were pharmacologically active.

Cardiac glycosides were found to be very useful in the treatment of congestive heart failure (Schneider *et al.,* 2004), and flavonoids prevent allergies and ulcers (Okwu and Omodamiro, 2005). The results obtained were similar to the reported phytochemicals components which indicated the presence of alkaloids, flavonoids, saponins, cardiac glycosides in *Luffa cylindrical* seed flours (Oyetayo *et al.,* 2012), *G. mangostana* seed cake (Ajayi *et al.,* 2013b) and *P. thonningii* seed flour (Jimoh *et al.,* 2005). Flavonoids were known to possess antioxidant activity. The flavonoids, due to their pharmacological activities may be one of the bases for the medicinal properties shown by various plants. Saponins were also found to have both antimicrobial and antioxidant activities (Soetan *et al.,* 2006). They also possessed anti-inflammatory activities (Hassan *et al.,* 2012). They have been shown to possess beneficial effects on the level of the blood cholesterol as well as in the stimulation of the immune system (Cheeke, 2000). These various chemical components found in various seeds may be responsible for their therapeutic abilities.

Table 4.5: Phytochemical screening of the seed flours

PARAMETER	ACSF	BASF	CASF	ECSF	NMSF	PLSF
Saponin	Pr	Pr	Pr	Pr	Pr	Pr
Tannins	Pr	Ab	Pr	Ab	Ab	Pr
Akaloids	Ab	Pr	Pr	Pr	Pr	Pr
Reducing Sugar	Ab	Ab	Ab	Ab	Ab	Ab
Phenol	Ab	Ab	Ab	Ab	Ab	Ab
Anthraquinone	Ab	Ab	Ab	Ab	Ab	Ab
Glycoside	Ab	Ab	Ab	Pr	Ab	Ab
Flavonoids	Ab	Ab	Ab	Ab	Ab	Pr
Steroids	Pr	Ab	Ab	Ab	Ab	Pr
Cardiac Gycoside	Pr	Ab	Ab	Ab	Ab	Pr
Resin	Pr	Ab	Pr	Ab	Ab	Ab
Phlobatannins	Ab	Ab	Ab	Ab	Ab	Ab
Carbohydrate	Pr	Pr	Pr	Pr	Pr	Pr

P= Present
Ab= Absent

ASF: *Areca catechu* seed flour,
BASF: *Balanite aegyptiaca* seed flour
CASF: *Chrysophyllum albidum* seed flour,
ECSF: *Enterolobium cyclocarpum* seed flour
NMSF: *Neocarya macrophylla* seed flour
PLSF: *Polyathia longifolia* seed flour

4.5 Amino acids profile and classification

4.5.1 Amino acid composition

The estimation of protein requirement does not only consider the quantity of amino acids present but also their quality. It was majorly obtained by the composition of an amino acid and mostly essential amino acids. The amino acid composition of ACS, BAS, CAS, ECS, NMS and PLS were recorded as g/100 g of protein (Table 4.6). The total amino acid composition of the seeds ranged from 42.64 g/100 g of protein (ACSF) to 77.02 g/100 g (BASF). Others included 75.76 g/100 g of protein (ECSF), 71.81 g/100 g of protein (NMSF), 57.68 g/100 g of protein (CASF) and 57.40 g/100 g of protein (PLSF). The rest of the nitrogen could be considered as non protein nitrogen which included alkaloids, purines, ammonia, pyrimidines, amino sugars and vitamins (Galla *et al.,* 2012).

The high protein content seen in NMSF, BASF and ECSF were responsible for their high amino acids contents. Among all the amino acids analysed, glutamic acid was the most abundant with 11.36 g/100 g of protein (NMSF), 12.42 g/100 g of protein, 12.57 g/100 g of protein, 7.57 g/100 g of protein, 8.25 g/100 g of protein and 5.22 g/100 g of protein in NMSF, BASF, ECSF, CASF, PLSF and ACSF respectively. Other amio acids with high concentration included aspartic acid, leucine, lysine arginine and valine. This result showed good comparison with other reports stating that glutamic and aspartic acids were the most abundant amino acid (Ogungbenle *et al.,* 2014 and Olaofe *et al.*, 2013). The glutamic acid contents found in NMSF, BASF, ECSF, CASF, PLSF and ACSF was slightly lower than 14.8 mg/100 g in *Dalium guineense* pulp (Ogungbenle *et al*., 2014); 14.20 mg/100 g in *Sterculia urens* (Galla *et al.,* 2012); 13.8 g/100 g in *Moringa oleifera* (Olaofe *et al*., 2013); 19.00 mg/100 g in casein and 21.29 mg/100 g in soy protein isolate (Tang *et al.*, 2006).

The second most abundant amino acid in this study was aspartic acid with 7.63 g/100 g (NMSF), 8.21 g/100 g (BASF), 8.84 g/100 g (ECSF), 6.64 g/100 g (CASF), 6.20 g/100 g (PLSF) and 4.31 g/100 g (ACSF). Aspartic acid value of 1.73 to 2.1 g/100 g, 7.56 g/100 g, 9.86 g/100 g and 9.9 g/100 g were reported respectively by Mansouri *et al.* (2018), Ogungbenle *et al.* (2014), Galla *et al.* (2012) and Khalid *et al.* (2016). Leucine content

Table 4.6: Amino acid composition (g/100g of protein) of the various seed flours

Amino acid (g/100g of protein)	ACSF	BASF	CASF	ECSF	NMSF	PLSF	Khalid *et al.,* 2016	Galla *et al.,* 2012	Spi, Tang, 2006	Casein, Tang 2006	FAO/ WHO 1973	FAO/WHO,1985 (Requirements (g per day for 70 kg adult)
Leucine	3.33	7.62	4.90	8.32	7.21	5.17	6.90	4.72	7.00	8.40	7.00	2.73
Lysine	2.84	5.25	3.02	5.60	4.96	4.03	4.50	4.39	5.39	7.10	5.50	2.10
Isoleucine	1.93	4.32	3.11	3.96	4.03	2.62	4.00	2.93	4.48	4.90	4.00	1.40
Phenylalanine	1.60	3.99	2.84	4.08	3.73	3.02	3.60	3.25	5.30	4.50	----	1.75
Tryptophan	0.37	1.02	0.47	0.95	0.87	0.50	0.99	----	----	----	-----	
Valine	3.22	3.83	3.22	4.01	3.74	2.60	4.10	3.28	4.41	6.00	5.00	1.82
Methionine	0.80	1.26	1.02	1.55	1.12	1.02	0.34	1.44	0.93	2.60	----	1.05
Histidine	1.09	2.17	1.53	2.17	1.82	1.44	3.93	1.74	2.90	2.70		
Cystine	1.15	0.91	1.27	1.21	0.73	1.03	1.00	1.17	0.06	0.04		
Alanine	3.03	3.83	4.02	3.49	3.60	2.91	4.00	3.73	3.83	2.73		
Proline	3.05	3.86	2.94	3.35	3.66	2.34	4.80	4.11	5.29	NA		
Arginine	3.10	5.25	4.04	5.51	4.82	3.61	8.50	8.07	7.57	3.30		
Tyrosine	0.86	3.10	3.27	3.27	2.75	3.10	4.40	1.91	3.71	5.50		
Glutamic acid	5.22	12.42	7.57	12.57	11.36	8.25	24.60	14.20	21.29	19.90		
Glycine	2.26	3.09	2.52	3.47	3.35	4.23	4.20	3.27	3.86	1.60		
Threonine	1.83	3.22	2.30	3.41	3.00	2.30	4.00	2.54	4.41	6.60	4.00	1.05
Serine	2.65	3.67	3.00	3.75	3.43	3.03	4.33	3.87	5.48	4.60		
Aspartic acid	4.31	8.21	6.64	8.84	7.63	6.20	9.90	7.58	11.81	6.30		
Total amino acids	42.64	77.02	57.68	75.76	71.81	57.40	98.09	72.20	97.72	86.77		

content varied from 3.33 g/100 g (ACSF) to 8.32 g/100 g (ECSF). The values of leucine recorded in NMSF (7.21 g/100 g), BASF (7.62 g/100 g) and ECSF (8.32 g/100 g) were higher than 4.72 g/100 g in Galla *et al.* (2012) and compared favourably with both reported data from Ogungbnele *et al.* (2014) for *Dalium guineense* pulp and Khalid *et al.* (2016) for *Lupinus termis.* They were equally compared favourably with 7.00 g/100 g of protein reported by FAO/WHO, (1985). Significant quantities of lysine, isoleucine and valine were found in NMSF, BASF and ECSF and were comparable to soy protein isolate (Tang *et al.,* 2006). Methionine was considered as an indispensable amino acid that was found helpful in producing choline that leads to the formation of lecithin and other phospholipids in the body. Fat can be accumulated in the liver by formation of an insufficient choline which is due to the low content of protein in a diet. The methionine content that varied from 0.88 g/100 g (ACSF) to 1.55 g/100 g (ECSF) was in agreement with 0.93 g/100 g and 1.44 g/100 g revealed on soy protein isolate (Tang *et al.,* 2006) and *Sterculia urens* (Galla *et al.,* 2012).

The amount of the essential amino acids observed in NMSF, BASF and ECSF seeds protein were in agreement with those required for amino acids published by FAO/WHO, (1985).

4.5.2 Amino acid classification in the various seed flour proteins

Table 4.7 revealed the amino acids classification in ACS, BAS, CAS, ECS, NMS and PLS seed proteins. The amino acids, on the bases of classification, were grouped into total essential amino acids (TEAA), total non-essential amino acids (TNEAA), total acidic amino acids (TAAA), total basic amino acids (TBAA), total neutral amino acids (TNAA), total sulphur amino acids (TSAA) and total aromatic amino acids (TArAA). Their percentages were also calculated and recorded. The TAA (total amino acids) in the seed samples were 42.64 g/100 g (ACSF), 77.02 g/100 g (BASF), 57.68 g/100 g (CASF), 75.76 g/100 g (ECSF), 71.81 g/100 g (NMSF) and 57.40 g/100 g (PLSF) of protein. This TAA value obtained in NMSF, BASF and ECSF were higher than CASF, PLSF and ACSF. The TAA value of 77.22 g/100 g (Galla *et al.,* 2012), 98 g/100 g (Khalid *et al.,* 2016), 97.38 g/100 g (Tang *et al.,* 2006) and 102 g/100 g (Ogungbenle *et al.,* 2014) were already reported. The TNEAA values were 24.71 g/100 g of protein (ACSF) to 43.15 g/100g of

Table 4.7: Classification of amino acid found in ACSF, BASF, CASF, ECSF, NMSF and PLSF protein (g/100g of protein)

Amino acid classification	ACSF	BASF	CASF	ECSF	NMSF	PLSF	Khalid *et al.,* 2016	Galla *et al.,* 2012	Spi, Tang, 2006	Casein, Tang 2006
TEAA	17.93	34.52	25.42	36.36	32.24	25.39	33.83	25.63	35.69	45.64
(TEAA/TAA) %	42.05	44.82	44.00	47.99	44.83	44.23	34.48	35.50	36.52	52.59
TEAA with histidine	19.02	36.69	26.95	38.53	34.06	26.83	37.72	27.37	38.59	48.34
TNEAA	24.71	42.50	32.26	43.15	39.67	32.01	64.20	46.57	62.03	41.13
(TNEAA/TAA) %	57.95	55.18	55.92	56.95	55.16	55.76	65.45	64.50	63.47	47.40
TSAA	1.95	2.17	2.29	2.76	1.85	2.05	1.34	2.61	0.99	2.64
TArAA (phe + tyr)	2.46	7.09	6.11	7.35	6.48	6.12	8.00	5.16	9.01	10.00
TAAA % Glu + Asp	9.53	20.63	14.21	21.41	18.99	14.45	34.50	21.78	33.10	26.20
TBAA % Lys + Arg+ His	7.03	12.67	8.59	13.28	11.60	9.08	16.93	14.20	15.86	13.10
TNAA	26.08	43.72	34.88	41.07	41.32	33.87	46.66	36.22	48.76	47.47
PPER	0.95	2.59	1.41	2.96	2.42	1.55	2.20	1.47	2.32	2.76

TEAA: Total essential amino acids
TNEAA Total non essential amino acids
TArAA: Total aromatic amino acids
TSAA: Total sulphur containing amino acids
PPER: Predicted protein efficiency ratio
TNAA: Total neutral amino acids
TBAA: Total basic amino acids
TAAA: Total acidic amino acids
Khalid *et al*., 2016 (Bitter lupin seed flour)
Galla *et al*., 2011 (Stercalia urens seed)

protein (ECSF). The TEAA values ranged from 17.93 g/100 g of protein (ACSF) to 36.36 g/100 g of protein (ECSF). Percentage ratio of essential to non-essential amino acids in the seed samples ranged from 42.05 % (ACSF) to 47.99 % (ECSF). These values were very comparable with 45 % and 46.52 % as reported for *Sterculia urens* (Galla *et al.,* 2012) and *Lupinus termis* (Khalid *et al.,* 2016). The TAAA (%) obtained were greater than the TBAA (%) and this suggested that the plant seed proteins might be acidic in nature. Similar statements were made by Olaofe *et al.* (2013) and Ogungbenle *et al.* (2014).

The overall quality of the seed proteins might be evaluated by the high value of the essential amino acids content in the total amino acid present in the seeds. This study revealed that 100 g of the seed proteins provide good quantities of leucine, isoleucine, threonine, and lysine. Following FAO/WHO regulations, the quantity of essential amino acids in mg/kg body weight that is suggested for full-grown persons per day include isoleucine (20), leucine (39), lysine (30), methionine and cysteine (15), phenylalanine and tyrosine (25), threonine (15), tryptophan (4) and valine (26) (FAO/WHO/UNU, 2007). The predicted protein efficiency ratio (P-PER) is the weight gain per gramme of digested protein (Ogungbenle *et al.,* 2014). The P-PER is one of the parameters that is also considered in the protein evaluation. The values of P-PER recorded in the seed samples varied from 0.95 to 2.96 as shown on Table 4.6 while 1.49, 2.20 and 2.32 were earlier reported respectively for *Dalium guineense* (Olaofe *et al.,* 2009), *Lupinus termis* (Khalid *et al.,* 2016) and *Cannabis sativa* (Tang *et al.,* 2006).

This investigation revealed that BAS, ECS and NMS had better amino acid properties than CAS, PLS and ACS. It provided valuable information on their amino acids composition and classification. The BASF, ECSF, and NMSF have been identified as potentially excellent sources of amino acids for producing protein isolates, concentrates, and hydrolysates. These materials hold promise as raw ingredients in various industrial applications. Additionally, they could be utilised in the production of high-quality food products intended for human consumption. By incorporating these amino acid-rich sources into manufacturing processes, it might be possible to develop protein-based products with enhanced nutritional value and desirable properties. This highlights their

potential importance in both industrial and dietary contexts, as they offer opportunities to create protein-rich foods and ingredients

4.6: Pasting properties of BASR, ECSR and NMSR

Pasting properties describe the starch and starch-based products behaviour during heat processing when water is introduced. The pasting properties of BASR, ECSR and NMSR documented were significantly different from one another (Table 4.8). They are compared with those of wheat flour. The trough viscosity ranged from 780.60 RVU (ECSR) to 1366.00 RVU (NMSR). Trough viscosity was the lowest viscosity value which measures the ability of paste to withstand breakdown during cooling (Ribotta *et al.,* 2007). The BASR and ECSR would withstand breakdown better than others during cooling because of their lower values of trough viscosity. The breakdown viscosity refered to the ability of starch to break down when subjected to heat and shear force. It was reported to provide insights into the starch's tendency to become brittle under these conditions (Ribotta *et al.,* 2007). Additionally, breakdown viscosity was known to serve as an indicator of cooked starch sample's ability to undergo decomposition (Adebowale *et al.,* 2005). The breakdown viscosity in the various seed residues were 272.33 (BASR), 327.33 (ECSR) and 387.00 (NMSR). A low breakdown value was found to suggest the stability of starches under hot conditions. It equally indicates that the starch in the cake does not break down significantly when exposed to heat and shear force. As a result, the cake retained its structural integrity and does not become excessively crumbly or fragile (Ribotta *et al.,* 2007).

Peak viscosity value of 909.66 (ECSR), 1007.66 (BASR) and 1753.00 (NMSR) were recorded. Peak viscosity was the highest viscosity reached by starch sample or gelatinised one during heating in water. It was also found to be in connection with the degree of starch damage. The higher the peak viscosity, the higher the starch damage and starch binding capacity of the granules (Ribotta *et al.,* 2007). Final viscosity was used to determine the special starch based sample quality and the capability of a starch based material to form gel after cooking. It was high in NMSR (1883.33). Peak time is the required time (min) for any starch granules to reach the greatest paste viscosity during heating (Adebowale *et al.,* 2005). The peak time varied from 4.60 min (ECSR) to 6.30

Table 4.8: Pasting properties of BASR, ECSR and NMSR

Parameter	BASR	ECSR	NMSR	Wheat flour
Trough viscosity	802.33±1.15^{d}	780.60±1.73^{e}	1366.00±15.5^{b}	1290.00±0.57^{c}
Peak viscosity	1007.66±1.14^{d}	909.66±2.30^{e}	1753.00±25.98^{b}	1511.33±1.15^{c}
Break down viscosity	272.33±6.57^{d}	327.33±4.04^{c}	387.00±10.39^{b}	220.66±0.57^{e}
Final viscosity	956.00±0.00^{d}	879.33±4.61^{e}	1883.33±9.81^{b}	1811.33±1.15^{c}
Set back viscosity	154.33±1.15^{c}	98.33±2.88^{d}	520.66±5.77^{b}	520.66±0.57^{b}
Peak time (min)	6.30±0.38^{a}	4.60±0.00^{d}	5.44±0.03^{c}	5.88±0.10^{b}
Pasting temperature (^{o}c)	88.00±0.00^{a}	84.00±0.00^{b}	79.66±0.49^{c}	79.28±0.44^{c}

Values stand for the mean±SD of three determinations

Means followed by different letters on the same row are significantly different at ($P \leq 0.05$).

min (BASR). The pasting temperature was the lowest temperature needed to cook a given food sample. The pasting properties as obtained in Table 4.8 were significantly different from each other within the groups.

The pasting temperatures of 88.0 ^{o}C, 84.00 ^{o}C and 88.00 ^{o}C documented for BASR, ECSR and NMSR respectively, compared favourably with 79.28 ^{o}C found in wheat flour. The BASR, ECSR and NMSR, with their high protein content and good pasting properties might be used as supplement with a view to improve the nutritional quality of bakery products.

4.7 Chemical analysis of the oils

4.7.1 Physicochemical properties and phosphorous content of the seed oils

The physicochemical properties and the phosphorus (P) content of the various seed oils as presented on differ from one another significantly (Table 4.9). The oils were liquid at 30 ^{o}C. Their colour varied from light yellow (NMSO and ECSO), golden yellow (BASO) to deep brown (PLSO and CASO). The oxidative stability of the oils varied from 174.17 ^{o}C (PLSO) to 273.70 ^{o}C (NMSO). High oxidative stability is desirable as it indicates that the oil has a longer shelf life and can withstand higher cooking temperatures without deteriorating rapidly. Oils with good oxidative stability tend to have a milder flavor and aroma, as they are less prone to rancidity.

Acid value in the oil could be an index to measure the acidity of the lipid that involved from the fatty acids constituting the glyceride molecules (Adewuyi *et al.,* 2010). It is a way of estimating the amount of carboxylic acid that is present in the group. The acid values in mg KOH/g obtained for the oils samples were 5.37 (NMSO), 13.46 (ECSO), 6.27 (BASO), 8.76 (CASO) and 8.79 (PLSO). The acid values determined in the various seed oils compared favourably with 9.76 and 7.86 mg/KOH/g reported respectively for Moringa and melon oils (Afolayan *et al.,* 2014). Free fatty acid content is one of the specifications explored in evaluating the steadiness, freshness, quality and performance of oils. It also triggers the deterioration of oil in the presence of oxygen by chemical and enzymatic oxidation to produce off-flavour components (Kowalki, 1995). Both the acid value and free fatty acid contents obtained suggested that the various oils samples were of

Table 4.9: Physicochemical properties of the seed oils

Parameter	BASO	CASO	ECSO	NMSO	PLSO
AV (mg/KOH/g)	6.27 ± 0.29^{c}	8.76 ± 0.19^{cd}	13.46 ± 0.66^{a}	5.37 ± 0.45^{d}	8.79 ± 0.14^{b}
FFA (%)	3.12 ± 0.02^{c}	2.84 ± 0.40^{cb}	6.75 ± 0.31^{a}	2.68 ± 0.22^{d}	4.39 ± 0.70^{b}
SV (mg/KOH/g)	198.31 ± 2.71^{b}	209.15 ± 8.03^{b}	192.36 ± 1.65^{b}	191.82 ± 1.14^{b}	197.25 ± 0.55^{b}
IV (mg I_2/g)	102.71 ± 2.03^{b}	86.21 ± 0.50^{c}	107.97 ± 0.02^{a}	89.05 ± 1.15^{c}	64.04 ± 2.70^{d}
MMW (g/mol)	869.01 ± 0.67^{b}	869.67 ± 0.42^{b}	876.90 ± 0.05^{a}	974.59 ± 0.04^{a}	854.64 ± 0.05^{c}
RI @ 30 ^{o}C	1.45 ± 0.00^{b}	1.44 ± 0.00^{b}	1.43 ± 0.00^{b}	1.46 ± 0.00^{a}	1.48 ± 0.00^{a}
SG @ 30 ^{o}C	0.89 ± 0.00^{a}	0.89 ± 0.00^{a}	0.83 ± 0.00^{b}	0.89 ± 0.00^{a}	0.89 ± 0.00^{a}
SG@ 40 ^{o}C	0.88 ± 0.00^{a}	0.88 ± 0.00^{a}	0.82 ± 0.00^{b}	0.88 ± 0.00^{a}	0.88 ± 0.00^{a}
KV @40 ^{o}C	18.93 ± 0.01^{b}	15.95 ± 0.02^{c}	14.22 ± 0.02^{d}	34.79 ± 0.01^{a}	34.02 ± 1.19^{a}
UNSAP (%)	0.58 ± 0.00^{d}	17.57 ± 0.04^{b}	2.88 ± 0.01^{d}	0.56 ± 0.00^{d}	23.02 ± 1.17^{a}
Phosphorus	221.41 ± 0.69^{b}	118.51 ± 0.01^{d}	1267.83 ± 0.10^{a}	86.85 ± 0.71^{e}	155.45 ± 0.01^{c}
D @ 30 ^{o}C	0.88 ± 0.00^{a}	0.89 ± 0.00^{a}	0.82 ± 0.00^{b}	0.88 ± 0.00^{a}	0.88 ± 0.00^{a}
D@ 40 ^{o}C	0.88 ± 0.00^{a}	0.88 ± 0.00^{a}	0.81 ± 0.00^{b}	0.87 ± 0.00^{a}	0.89 ± 0.00^{a}
% Yield	46.95 ± 0.64^{a}	12.82 ± 0.04^{b}	2.64 ± 0.03^{d}	45.96 ± 0.10^{a}	4.26 ± 0.01^{c}
Oxidative stability ^{o}C	187.07	186.38	208.86	273.79	174.17
Colour	Golden yellow	Deep brown	Light yellow	Light yellow	Deep brown

Values are mean ± standard deviation of triplicate determination. Data in the same row with different letters are statistically different at ($P\leq 0.05$).

AV: Acid value (mgKOH/g)
MMW: Mean molecular weight
SV: Saponification value (mgKOH/g)
UNSAP: Unsaponifiable matter
IV: Iodine value (mg/I_2/g)
KV: Kinematic viscosity
D: Density
RI: Refractive Index
OS: Oxidative stability

good quality, stable to oxidation and as well confirmed the presence of unsaturation in the triglyceride of the various oils.

Iodine value measure the degree of unsaturation of the various fatty acids contained in the oil. It also quantifies the number of double bonds that are available in the oil that reflect its sensibility to oxidation (Afolayan *et al.,* 2014). Iodine value can be used to classify the oils as non-drying (iodine value below 100 mg I_2/g), semi drying oils (iodine values between 100 and 130 mg I_2/g) and drying oils (iodine values above 130 mg I_2/g), (Adewuyi *et al*., 2010). The iodine values of the oil samples varied from 64.04 mg I_2/g in PLSO to 107.97 mg I_2/g in ECSO. Iodine values of 89.05 mg I_2/g, 86.21 mg I_2/g, and 102.71 mg I_2/g were respectively obtained for NMSO, CASO and BASO. These values are significantly different from each other ($p \leq 0.05$). The ECSO and BASO with high level of unsaturation are classified as semi-drying oil while NMSO, CAS and PLS are non drying oil. Semi-drying and non-drying oils are equally liquid at 30 ^oC and useful in the manufacturing of soaps, lubricants and food (Adelaja *et al.,* 2018).

The saponification value is used to check for the adulteration of oils. When the saponification value of specific oil is high, it suggests that the fatty acids within the oil contain a greater number of carbon atoms. Consequently, the saponification value provides valuable insight into the average molecular weight and chain length of the fatty acids that are present in the oil (Ardabili *et al*., 2011). The saponification values of each of the oil sample in mg KOH/g are 191.82, 192.36, 197.25, 198.31 and 209.15 for NMSO, ECSO, PLSO, BASO and CASO respectively. These values agreed with those found in the literature (Ardabili *et al*., 2011). The saponification value for most of the edible oil falls within the range of 180 - 200 mg KOH/g. The values obtained in this study were in agreement with the value 194.67 mg KOH/g reported on *Passiflora* seed oil, (193.90 mg KOH/g) and olive oils (189.30 mg KOH/g), (Ramaiya, *et al.,* 2019). The high saponification value of the oil samples showed that they possessed the potential to be employed in industrial processes. This also suggested that these oils might be good material that could undergo modification to desirable products.

The refractive index denotes the relationship between the speed of light in a given substance and its speed in a vacuum. This index in oils is used for identification purposes

and for establishing purity or adulteration which can also be used to monitor the progress of fat and oil reaction, such as catalytic hydrogenation and isomerisation. No significant difference was observed at $P \leq 0.05$ in the refractive index of the oil samples. They varied from 1.43 (ECSO) to 1.48 (PLSO). The specific gravity of the oil samples at both 30 ^{o}C and 40 ^{o}C were givem on Table 4.9. The values obtained at 30 ^{o} C were 0.89 (BASO), 0.89 (CASO), 0.83 (ECSO), 0.89 (NMSO) and 0.89 (PLSO). The same trend of results was obtained at 40 ^{o} C for all the oil samples. There were no significant differences ($p \leq 0.05$) among the values. *Neocarya macrophyla* and *Balanite aegyptiaca* seeds with high oil yield might be good sources of vegetable oil for industrial applications.

4.7.2 Fatty acid composition (wt %) of the seed oils

Seed oils are one of the major and important materials obtained from biomass and are nutritionally, industrially and pharmaceutically useful. The properties of oil acquired from different sources depend on their compositions. Their usefulness depends also on their chemical and physicochemical properties which can be evaluated by the composition of the available fatty acid (Gouveia *et al.,* 2004). The composition of the fatty acid in various oil samples was presented on Table 4.10. Oleic acid, linoleic acid, stearic acid and palmitic acid denoted C18:1, C18:2, C18:0 and C16:0 in the same order were the prominent fatty acid present in the oils but at difference percentages. The C18:1 was the most abundant fatty acid in NMSO (57.363 g/100 g), PLSO (44.202 g/100 g) and CASO (40.957 g/100 g). C18:2 was the most abundant fatty acid present in BASO (42.998 g/100 g) and ECSO (47.076 g/100 g). The values of C18:1 was 29.101 g/100 g (BASO) and 21.758 g/100 g (ECSO) while that of C18:2 were 11.527 g/100 g fatty acids (PLSO) and 26.948 g/100 g CASO). Palmitic acid was relatively high in some of the seed oils. The value of palmitic acid obtained were 12.060 g/100 g fatty acids (NMSO), 12.873 g/100 g (ECSO), 16.102 g/100 g (BASO), 18.796 g/100 g (CASO) and 36.621 g/100 g (PLSO). The total unsaturation of the fatty acid was very high in the various seed oils. It ranged from 57.78 (PLSO) to 79.89 g/100 g (NMSO). Others include 69.43 g/100 g (CASO), 72.67 g/100 g fatty acids (ECSO) and 72.88 g/100 g (BASO).

Table 4.10: Fatty acid composition (wt %) of the oils

Fatty acid	Formula	Name	BASO	CASO	ECSO	NMSO	PLSO
C14:0	$C_{14}H_{28}O_2$	Myristic aci	0.08	0.18	0.09	0.05	0.90
C16:0	$C_{16}H_{32}O_2$	Palmitic acid	16.10	18.79	12.87	12.06	36.62
C16:1	$C_{16}H_{30}O_2$	Palmitoleic acid	0.19	0.08	1.27	0.19	0.27
C18:0	$C_{18}H_{36}O_2$	Stearic acid	10.50	7.68	9.72	7.43	4.13
C18:1	$C_{18}H_{34}O_2$	Oleic acid	29.10	40.96	21.76	57.36	44.20
C18:2	$C_{18}H_{32}O_2$	Linoleic acid	42.99	26.95	47.07	21.18	11.52
C18:3	$C_{18}H_{30}O_2$	Linolenic acid	0.52	0.80	2.57	0.61	0.38
C20:0	$C_{20}H_{40}O_2$	Arachidic acid	0.30	1.40	2.39	0.38	0.37
C20:1	$C_{20}H_{38}O_2$	Eicosenoic acid	0.08	1.43	ND	0.53	1.40
C22:0	$C_{22}H_{42}O_2$	Behinic acid	0.04	0.88	1.50	0.08	0.08
C24:0	$C_{24}H_{48}O_2$	Lignoceric acid	0.06	0.81	0.72	0.07	0.09
TUFA			72.88	69,43	72.67	79.87	57.77
TSFA			27.10	29.77	27.32	20.11	42.22
MUFA			29.37	42.48	23.03	58.05	45.87
PUFA			43.51	26.95	49.64	21.82	11.90

TSFA: Total saturated fatty acid
TUFA: Total unsaturated fatty acid
MUFA: Monounsaturated fatty acid
PUFA: Polyunsaturated fatty acid

This high level of unsaturation with the presence of C18:2 suggested that the oil samples and mostly NMSO and BASO (with high yield) would have good and wonderful application in oleochemicals industries. The unsaturated points, area with the double bonds were the areas where other functional groups could be introduced into oils so as to modify and fortify them in order to improve their properties for better performance. The C18:2 was also reported to be the most abundant fatty acid with high level of unsaturation in *B. nitida* and *G. sepium* (Adewuyi and Oderinde, 2013). *Neocarya macrophyla* and *Balanite aegyptiaca* seed oils with high yield (45% and above) could be very good sources of C18:1, C18:2, C18:0 and C16:0 which suggested them as very important oils with good nutritional quality and industrial application (oleochemistry) such as the production of polyurethane for surface coating, polyamide for corrosion studies and even in biofuels production.

4.7.3 Fatty acid distribution in the lipid classes of BASO, CASO, ECSO, NMSO and PLSO

The oil samples were separated into various lipid classes which were neutral lipids, glycolipids and phospholipids. The percentage composition of the lipid classes as well as the percentage distribution of each fatty acid in the lipid classes were lised on Table 4.11. The neutral lipids were found to be the dominating lipid classes in all the oil samples. They were 94.40 g/100 g oil (NMSO), 92.67 g/100 g oil (BASO), 95.40 g/100 g oil (ECSO), 93.52 g/100 g oil (PLSO) and 92.28 g/100 g oil (CASO). Phospholipids were the lowest lipid class, even lower than glycolipids. They were between 0.70 g/100 g (ECSO) to 1.34 g/100 g (BASO). Others include 1.05 g/100 g (CASO), 1.07 g/100 g (NMSO) and 1.08 g/100 g (PLSO). The same trend of results was reported for *B. nitida* and *G. sepium* (Adewuyi and Oderinde, 2013). The fatty acids in the lipid classes, as shown on Table 4.11, were distributed in different proportions. The C18:2, C18:1, C18:0 and C16:0 were the prominent fatty acid present in the lipid classes. It was generally observed that the level of unsaturation was higher in neutral lipid than in glycolipids and phospholipids. The same trend of results was also reported for *B. nitida* and *G. sepium* (Adewuyi and Oderinde, 2013).

Table 4.11: **Lipid classes of BASO, CASO, ECSO, NMSO, PLSO and their corresponding fatty acid composition (wt %)**

Sample	Lipid Classes	Fatty acids C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C24:0	TUFA	TSFA
BASO		0.079	16.102	0.198	10.505	29.105	42.998	0.529	0.303	0.078	0.041	0.056	79.90	20.10
NL	92.67±0.02[d]	0.028	16.506	0.297	11.230	28.670	34.697	4.735	0.349	0.273	0.185	0.161	68.67	31.33
GL	5.97±0.11[b]	ND	22.284	ND	11.743	32.069	34.173	ND	ND	ND	ND	ND	66.24	33.78
PL	1.34±0.08[a]	1.708	24.751	ND	38.707	20.507	12.245	ND	ND	ND	ND	ND	32.75	67.25
CASO		0.186	18.796	0.087	7.687	40.957	26.948	0.803	1.402	1.432	0.885	0.814	70.23	29.77
NL	92.28±0.37[e]	0.236	17.938	0.096	7.448	41.265	26.864	0.673	1.318	1.312	0.749	0.683	70.21	29.79
GL	6.65±0.12[a]	ND	12.819	ND	5.889	38.304	39.039	1.166	0.568	0.529	ND	ND	79.30	20.69
PL	1.05±0.24[b]	1.902	25.682	ND	37.265	20.702	20.258	ND	ND	ND	ND	ND	40.96	59.04
ECSO		0.090	12.873	1.271	9.722	21.758	47.076	2.575	2.396	ND	1.506	0.927	72.68	27.32
NL	95.40±0.26[a]	0.111	14.886	ND	6.521	6.516	62.389	ND	0.596	8.661	0.110	0.205	77.56	22.43
GL	3.90±0.36[e]	0.101	15.060	2.040	10.602	30.636	30.936	ND	5.171	5.117	0.369	ND	68.73	31.27
PL	0.70±0.10[c]	0.153	17.065	ND	11.555	29.464	36.040	5.103	0.359	0.257	ND	ND	70.86	29.13
NMSO		0.054	12.076	0.199	7.434	57.363	21.183	0.610	0.385	0.532	0.081	0.077	79.89	20.11
NL	94.40±0.02[b]	ND	17.776	ND	11.066	56.882	11.695	ND	0.635	0.567	ND	ND	69.14	30.85
GL	4.52±0.02[d]	ND	14.877	ND	8.151	56.772	16.969	ND	0.345	2.083	0.376	ND	75.82	24.18
PL	1.07±0.02[b]	0.058	21.502	1.444	20.502	34.360	20.101	0.890	0.760	ND	ND	0.345	56.79	43.20
PLSO		0.904	36.621	0.272	4.134	44.202	11.537	0.378	0.369	1.400	0.081	0.096	57.78	42.22
NL	93.52±0.11[c]	ND	11.644	ND	3.872	33.846	20.928	5.472	0.229	0.314	ND	0.583	60.56	39.44
GL	5.39±0.00[c]	ND	7.828	ND	3.155	34.806	18.468	3.972	0.303	0.314	ND	ND	57.56	42.44
PL	1.08±0.11[b]	0.997	21.150	ND	5.702	41.295	23.849	0.635	0.446	ND	ND	ND	65.78	34.22

NL: Neutral lipids, GL:
Glycolipids,
PL: Phospholipids, ND: Not determined,

4.7.4 Triacylglycerol molecular species of the extracted oils

Triglycerides are esters obtained from the combination of three fatty acids and glycerol. They are the major component of vegetable oils. The major molecular species in the oil samples were significantly different from one oil to another as shown on Table 4.12. The molecular species with equivalent carbon chain number (ECN) C_{42}, C_{44}, C_{46}, C_{48} and C_{50} were significantly present in different quantity in the oils. The most abundant molecular species in NMSO were $C_{44,}$ LOL/LPL (35.78%), $C_{48,}$ OOO/POO/POP/PPP/SLO/ (35.61%) and $C_{42,}$ LLL (23.21%). $C_{42,}$ LLL (52.49) and $C_{44,}$ LOL/LPL (40.23%) were predominant in BASO. The most abundant molecular species found in ECSO and CASO are C_{44} (LOL/LPL), C_{46} (LOO/LOP/LPP/LLS), C_{48} (OOO/POO/POP/PPP/SLO/SLP) while $C_{48,}$ OOO/POO/POP/PPP/SLO/SLP (77.26%) was found higher in PLSO. The molecular species C48, SLnS/OOO/POP (39.6%) and C52, BOL/BPL/SLB (39.00%) were reported as the most abundant molecular species in *G. nitida* while C48, SLnS/OOO/POP (33.00%) and C46, POL/OOL/PLP (33.40%) in *G. sepium* seed oils (Adewuyi and Oderinde, 2013).

4.7.5 Tocopherols and Tocotrienols

The tocopherols and tocotrienols composition in the seed oils were shown on Table 4.12. Alpha tocopherols were majorly seen in all the seed oils at different concentrations. The values were 42.2 ppm (NMSO), 146.2 ppm (PLSO), 317.0 ppm (BASO), 727.50 ppm (CASO) and 742.50 ppm (ECSO). Gamma tocopherols were observed only in ECSO (1100.0 ppm) and BASO (218.0 ppm). Alpha tocotrienols were only found in CASO (17.5 ppm). The total tocols in the oils were 1842 ppm (ECSO), 745.0 ppm (CASO), 535.0 ppm (BASO), 146.2 ppm (PLSO) and 42.2 ppm (NMSO).

4.7.6 Unsaponifiable composition of the oils

The composition (%) of the unsaponifiable matter in the oils was respectively 0.56, 0.58, 2.88, 23.02 and 17.57 in NMSO, BASO, ECSO, PLSO and CASO. The GC-MS results of the oils revealed mostly hydrocarbons and some other compounds that were listed on Table 4.13 below as the major constituents of the unsaponifiable matter in the oils. The unsaponifiable matter was very high in PLSO and CASO than others. The unsaponifiable matter obtained in NMSO, BASO were lower than those in ECSO, PLSO which were

Table 4.12: Triglyceride molecular composition of the oils

ECN	EMS	BASO	CASO	ECSO	NMSO	PLSO
C42	LLL	52.49	5.26	6.87	23.21	9.93
C44	LOL/LPL	40.23	19.73	26.59	35.78	6.16
C46	LOO/LOP/LPP/LLS	3.59	29.06	34.07	4.13	3.66
C48	OOO/POO/POP/PPP/SLO/SLP	2.14	40.48	26.42	35.61	77.26
C50	SOO/SOP/SLS/SPP	1.21	5.44	6.04	1.25	2.96
Tocopherols (ppm)						
α-T		146.2	742.5	317.0	727.5	42.20
γ-T		-------	1100.0	218.0	-------	-------
δ-T		-------	--------	--------	-------	-------
Tocotrienols (ppm)						
α-T_{ri}		-------	17.5	-------	-------	-------
γ-T_{ri}		-------	-------	-------	-------	--------
δ-T_{ri}		-------	-------	-------	-------	--------
Total Tocols (T+T_{ri}) (µg/mL)		146.2	1842.0	535.0	745.0	42.20

ECN: effective carbon number
EMS: expected molecular species
L: linoleic acid, O: Oleic acid, P: Palmitic acid, S: Stearic acid
T: Tocopherols
T_{ri}: Tocotrienols

Table 4.13: Unsaponifiable composition of ACSO, BASO. CASO, ENSO, NMSO and PLSO

Unsaponifiable matter	ACSO	BASO	CASO	ENSO	NMSO	PLSO
Aalpha cubebene	+	-	-	-	+	+
Cyclohexane	+	-	-	-	-	+
Naphthalene	+	-	-	+	+	-
Tricosane	+	+	-	-	-	+
Hexadecane	+	-	-	-	+	+
Octocosane	+	+	-	-	-	+
Pentacosane	+	-	-	-	-	+
Hexacosane	+	+	-	-	+	+
Tetracosane	+	+	+	-	+	+
Heptacosane	+	+	+	-	-	+
Eicosane	-	+	-	-	-	+
Docosane	-	+	-	-	+	+
Hexadecane	+	-	-	-	+	+
Octadecene	-	-	-	+	+	+
Pentadecene	+	+	+	+	+	-
Alpha Tocopherol	-	-	-	+	+	-
Beat tocopherol	-	-	-	+	+	+
Gamma Tocopherol	-	-	-	+	+	+
Campesterol	+	-	-	+	+	+
Stigmasterol	+	+	+	+	+	+
Stigmasta-7-en-3-ol	-	-	-	-	-	+
Stigmasta-7,16-dien-3-ol	+	+	-	-	-	+
Stigmasta-7,25-dien-3-ol	+	+	+	-	-	+
Phenol	+	+	-	-	-	+
Silane	+	+	+	-	-	-
Squalene	-	+	-	-	-	-
Alpha Sitosterol	-	-	-	+	+	-
Beta Sitosterol	+	-	+	+	+	-
Gamma Sitosterol	+	-	+	+	+	-
Ergosterol	-	-	-	-	+	-
Alpha ergostenol	-	+	+	-	-	+
Ergost-5-en-3-ol	+	-	+	+	+	-
Vitamin E	+	-	+	+	+	-
Cholesterol	+	+	+	+	+	+
Cholest-7-en-3-oen	-	+	+	-	-	+
Cholest-5-en-3-oen	-	-	+	+	-	-
Cholest-8-en-3-oen	-	-	-	+	-	-
Phytol	-	-	+	-	-	+
Ethyl oleate	-	+	+	+	+	-
Lanosterol	-	+	+	-	-	-
Lanost-7-en-3-one	-	+	-	-	-	-
Lanost-8-en-3-one	-	-	+	-	-	-

+ Identified
- Unidentifie

equally found to compare favourably with 2.14% and 2.13% respectively reported for *Trilepisium madagascariense* and *Antiaris africana* seed oils (Adewuyi *et al*., 2010).

4.9 Toxicological studies of seed flours, residues and oils

4.9.1 Application of seed flours and residues as food supplement in Livestock using Wistar rats as case study

4.9.1.1 Proximate composition and energy contribution of ACSF, BASR, CASF, ECSF, NMSR and PLSF formulated diet on rats

The proximate composition and the energy contribution of ACSF, BASR and CASF diets for rats were shown on Table 4.14 while those of ECSF, NMSR and PLSF were presented on Table 4.15. The proximate composition and the energy contribution of ACSF diets for rats were shown on Table 4.14. There was a gradual reduction in the protein content, ash and moisture contents of the prepared diets as compared to the control feed. There was also a decrease in the energy value and this might be related to the low protein content in ACSF. The proportion of energy contributed by protein decreased slightly with an increase in that contributed by carbohydrate. As shown on Table 4.14, the crude protein (%) of the BASR diets was 3.58 (0 %) 18.15 (10%), 22.78 (20%) and 29.30 (30%). The crude fat (%) also increased gradually from 5.82 (0%) to 6.76 (30%). The crude fibre value was 6.42%, 7.55, 7.73 and 8.45 in 0%, 10%, 20% and 30% respectively. The ash content reduced from 10.97 (0%) to 6.15 (10%). The carbohydrate value reduced as the level of inclusion of BASR increased. The energy values obtained were lower in 0% diet (1346.91 kJ/100 g) when compared with 10% (1423.65 kJ/100 g), 20% (1425.25 kJ/100 g and 30% (1418.04 kJ/100 g). The proportion of energy value contributed by protein increased gradually from 17.14 % (0 %) to 35.12 % (30 %). There was at the same time, a gradual decrease in the proportion of energy value contributed by carbohydrate from 66.87% (0%) to 47.23% (30%). The gradual increase in the proportion of energy value contributed by protein is majorly due to the high content of protein in BASR.

The crude protein value (%) of 22.84 (0%), 24.00 (10%), 21.52 (20%) and 19.25 (30% were recorded. There was a decrease in the crude fibre content, dry matter, and ash content while a slight increase was observed in the carbohydrate value.

Table 4.14: Proximate analysis of rat diets from ACSF, BASR and CASF

Parameters	0%	10%	20%	30%
		ACSF		
Moisture	10.14±0.01^{a}	09.12±0.02^{b}	8.80±0.06^{b}	8.39±0.05^{b}
Crude protein	19.23±0.05^{a}	20.06±0.13^{a}	16.93±0.50^{b}	14.39±0.13^{b}
Crude fat	15.46±0.60^{a}	13.60±0.03^{b}	13.68±0.01^{b}	13.64±0.04^{b}
Crude fibre	7.58±0.14^{d}	11.01±0.03^{c}	15.90±0.04^{b}	15.77±0.03^{b}
Ash	14.10±0.02^{a}	15.61±0.02^{a}	10.89±010^{b}	12.18±0.03^{b}
Carbohydrate	33.47±0.06^{b}	31.03±0.14^{b}	33.76±0.09^{b}	35.61±0.14^{b}
Dry matter	89.85±0.02^{a}	90.88±0.05^{a}	91.19±0.04^{a}	91.67±0.10^{a}
Energy (kJ/100g)	1467.92±0.15^{a}	1371.73±0.03^{b}	1367.89±0.5^{b}	1354.68±0.06^{b}
		BASR		
Moisture	10.22±0.05^{a}	9.48±0.02^{b}	9.23±0.05^{a}	9.77±0.02^{a}
Crude protein	13.58±0.07^{c}	18.15±0.05^{c}	22.78±0.02^{b}	29.30±0.10^{a}
Crude fat	5.82±0.51^{b}	6.15±0.05^{a}	6.45±0.05^{a}	6.76±0.05^{a}
Crude fibre	6.42±0.17^{b}	7.55±0.05^{a}	7.73±0.05^{a}	8.45±0.05^{a}
Ash	10.97±0.05^{a}	6.15±0.05^{b}	6.78±0.02^{b}	6.30±0.10^{b}
Carbohydrate	52.98±0.37^{a}	52.21±0.05^{a}	47.01±0.05^{a}	39.40±0.35^{b}
Dry matter	89.78±0.05^{a}	90.51±0.28^{a}	90.76±0.05^{a}	90.82±0.25^{a}
Energy (kJ/100g)	1346.91±13.90^{b}	1423.65±0.51^{a}	1425.25±0.93^{a}	1418.04±0.47^{a}
		CASF		
Moisture	10.83±0.02^{a}	10.94±0.4^{a}	10.15±0.01^{b}	9.12±0.02^{c}
Crude protein	22.84±0.12^{a}	24.00±0.13^{a}	21.52±0.12^{b}	19.25±0.05^{c}
Crude fat	4.80±0.01^{b}	4.56±0.02^{b}	4.80±0.02^{b}	4.60±0.02^{a}
Crude fibre	8.39±0.01^{b}	9.60±0.03^{a}	6.60±0.03^{c}	5.77±0.03^{c}
Ash	8.30±0.02^{a}	8.55±0.04^{a}	8.11±0.01^{a}	8.39±0.05^{a}
Carbohydrate	44. 82±0.14^{c}	42.35±0.06^{d}	48.80±0.12^{b}	52.86±0.15^{a}
Dry matter	89.17±0.02^{b}	89.06±0.02^{b}	89.84±0.01^{a}	90.87±0.03^{a}
Energy (kJ/100g)	1328.11±0.69^{b}	1296.67 ± 0.04^{b}	1373.04±0.39^{a}	1396.07±0.39^{a}

Values are mean±standard deviation of triplicate determination. Data in the same row with different letters are statistically different at ($P \leq 0.05$).

The metabolic energy ranged from 1328.11 kJ/100 g (0% CASF) to 1396.07 kJ/100 g (30% CASF).

The proximate composition as well as the energy contribution of ECSF, NMSR and PLSF formulated diets for rats were shown on Table 4.15. Crude protein was observed to be 22.67% in the control feed (0 %). A slight increase was noted in the protein content as well as in ash content, carbohydrate content and energy as the concentration of inclusion of ECSF in the prepared diets increased. The proportion of energy contributed by protein is slightly lower in 0% diet and remained constant between 20% to 30% inclusion of ECSF. The contribution of energy value based on carbohydrate was respectively 56.63%, 56.40%, 56.29%, and 56.00% in 0%, 10%, 20% and 30% level of inclusion of ECSF. The addition of NMSR in the diets increased the protein content from 16.23% (0 %) to 22.65 (30%). The metabolic energy value (kJ/100 g) of each of the groups was 1352.18 (0 %), 1367.87 (10%), 1331.78 (20%) and 1331.91 (30%) as shown on Table 4.15. The proportion of energy value contributed by protein increased gradually from 20.40% (0%) to 28.90% (30%). There was at the same time, a gradual decrease in the proportion of energy value contributed by carbohydrate within the groups (Table 16). The gradual increase in the proportion of energy value contributed by protein is majorly because of the high protein content in NMSR. In PLSF diets, the protein content (%) increased from 16.23±0.06 (0%) to 20.08 (10%) and later decreased to 14.76 (30%). This is majorly due to the lower protein value in PLSF. There was slight increase in carbohydrate value (%) from 55.61 (0%) to 58.29 (30%) level of inclusion of PLSF.

The ACSF, BASR, CASF, ECSF, NMSR and PLSF showed positive effect in the proximate compositions of the diets formulated from them. The high content of protein seen in BASR and NMSR as well as the high carbohydrate content in ACSF, CASF, ECSF and PLSF confirm them as sources of energy and nutrient in feed.

4.9.1.2 Effect of seed flours ACSF, CASF, ECSF, PLSF) and residues (BASR, NMSR) on the mean body weight and survival rate of rats

The effect of the seed flour (ACSF, CASF, ESCF and PLSF) and residues (BASR, NMSR) diets on the weight and weight gain percentages was commendable and

Table 4.15: Proximate analysis of rat diets from ECSF, NMSR and PLSF

Parameters	0%	10%	20%	30%
		ECSF		
Moisture	10.83±0.02^{c}	9.37±0.03^{b}	9.05±0.07^{a}	8.39±0.01^{a}
Crude protein	22.67±0.36^{a}	24.57±0.45^{b}	25.56±0.01^{c}	25.76±0.58^{c}
Fat	4.80±0.01^{a}	4.89±0.01^{b}	4.72±0.07^{d}	4.66±0.05^{c}
Crude fibre	8.40±0.03^{b}	6.40±0.01^{a}	5.33±0.04^{a}	5.86±0.03^{a}
Ash	8.30±0.02^{b}	9.13±0.00^{a}	9.17±0.02^{a}	9.15±0.04^{a}
Carbohydrate	44.82±0.14^{d}	45.62±0.45^{c}	46.15±0.15^{b}	45.82±0.31^{a}
Dry matter	89.17±0.02^{a}	90.62±0.03^{b}	90.95±0.17^{c}	91.60±0.15^{d}
Energy (kJ/100g)	1328.11±0.69^{c}	1374.40±0.72^{b}	1393.88±0.68^{a}	1390.98±6.44^{a}
		NMSR		
Moisture	10.52±0.23^{a}	10.09±0.14^{a}	10.46±0.10^{a}	10.23±0.21^{a}
Crude protein	16.23±0.06^{b}	18.84±0.01^{b}	20.58±0.01^{a}	22.65±0.36^{a}
Crude fat	3.40±0.00^{a}	3.19±0.01^{a}	3.23±0.06^{a}	3.33±0.04^{a}
Crude fibre	8.03±0.06^{a}	9.00±0.00^{a}	9.00±0.01^{a}	9.03±0.01^{a}
Ash	6.00±0.01^{a}	4.20±0.00^{b}	6.07±0.06^{a}	6.30±0.01^{a}
Carbohydrate	55.91±0.72^{a}	54.68±0.14^{a}	50.73±0.16^{a}	48.45±0.15^{b}
Dry matter	89.28±0.14^{a}	89.91±0.14^{a}	89.61±0.07^{a}	89.77±0.03^{a}
Energy(kJ/100g)	1352.18±0.24^{b}	1367.87±0.44^{a}	1331.78±0.10^{a}	1331.91±0.10^{a}
		PLSF		
Moisture	10.72±0.14^{a}	10.45±0.16^{a}	09.98±0.19^{b}	9.94±0.46^{b}
Crude protein	16.23±0.06^{b}	20.08±0.00^{a}	17.94±0.04^{b}	14.76±0.00^{b}
Crude fiber	8.03±0.06^{a}	8.41±0.01^{a}	9.07±0.06^{a}	9.65±0.00^{a}
Ash	6.00±0.01^{a}	4.17±0.15^{b}	3.61±0.01^{b}	3.76±0.10^{b}
Crude fat	3.40±0.00^{a}	3.11±0.01^{a}	3.21±0.01^{a}	3.59±0.01^{a}
Carbohydrate	55.61±0.21^{a}	53.79±0.16^{a}	56.20±0.12^{a}	58.29±1.66^{a}
Dry matter	89.28±0.01^{a}	89.55±0.04^{a}	90.02±0.01^{a}	90.06±0.03^{a}
Energy (kJ/100g)	1347.04±0.22^{a}	1370.84±0.12^{a}	1379.15±0.14^{a}	1373.67±0.15^{b}

Values are mean±standard deviation of triplicate determination. Data in the same row with different letters are statistically different at ($P \leq 0.05$).

Table 4.16: Percentage energy contribution of the experimental seed flours and residues formulated diets based on fat, protein and carbohydrate contents

Parameters	0%	10%	20%	30%
		ACSF		
Protein	22.27±0.05[a]	24.86±0.02 [a]	21.04±0.02[b]	17.56±0.03[C]
Fat	38.96±0.02[a]	36.68±0.01[b]	37.00±0.05[b]	37.25±0.12[a]
Carbohydrate	38.76±0.01[b]	38.45±0.02[b]	41.96±0.05[a]	44.68±0.02[a]
		BASR		
Protein	17.14±0.08[d]	21.66±0.06[c]	27.16±0.13[b]	35.12±0.06[a]
Fat	15.97±1.16[c]	15.97±0.13[c]	16.74±0.11[a]	17.64±0.21[a]
Carbohydrate	66.87±1.15[a]	62.33±0.42[a]	56.02±0.02[b]	47.23±0.39[c]
		CASF		
Protein	29.24±0.15[b]	31.46±0.05[a]	26.74±0.17[c]	23.44±0.03[d]
Fat	13.38±0.40[a]	13.01±0.03[ab]	12.99±0.07[ab]	12.19±0.03[b]
Carbohydrate	57.37±0.19[c]	55.52±0.10[d]	60.65±0.48[b]	64.36±0.15[a]
		ECSF		
Protein.	28.81±0.57[b]	30.40±0.54[a]	31.17±0.08[a]	31.49±0.84[a]
Fat.	13.18±0.30[a]	13.18±0.03[a]	12.53±0.20[b]	12.39±0.17[b]
Carbohydrate.	56.63±1.47[a]	56.40±0.16[a]	56.29±0.16[a]	56.00±0.32[a]
		NMSR		
Protein	20.40±0.00[c]	23.41±0.30[b]	26.27±0.00[a]	28.90±0.04[a]
Fat	9.30±0.01[a]	8.63±0.06[b]	8.97±0.04[b]	9.25±0.00[a]
Carbohydrate	70.29±0.10[a]	67.95±0.04[b]	64.75±0.14[b]	61.83±0.16[d]
		PLSF		
Protein	20.48±0.02[c]	24.90±0.01[a]	22.11±0.10[b]	18.26±0.03[d]
Fat	9.34±0.11[a]	8.39±0.01[b]	8.61±0.01[b]	9.66±0.05[a]
Carbohydrate	70.18±0.02[a]	66.70±0.01[c]	69.27±0.01[b]	72.13±0.05[a]

Values are mean±standard deviation of triplicate determination. Data in the same row with different letters are statistically different at ($P \leq 0.05$).

represented on Table 4.17 and Figures 4.1-2. The average initial weight of rats (g) fed with ACSF was 55.00 (0%), 46.42 (10%), 55.00 (20%) and 60.71 (30%), while the average final weight recorded in gramme was 139.28, 142.86, 135.00 and 133.57 in 0%, 10%, 20% and 30% inclusion of ACSF respectively, (Figure 4.1a). The rat weight gain was higher in 10% than 0% and decreased gradually within 20% and 30%. The survival rate was 100% in all the groups. The mean weight gain percentage was 153.23% (0%), 207.67% (10%), 145.45% (20%) and 120.10 (30%). The high mean weight gain of 207.76% in 10% inclusion proposed that ACSF may be good supplement in livestock feed at that level.

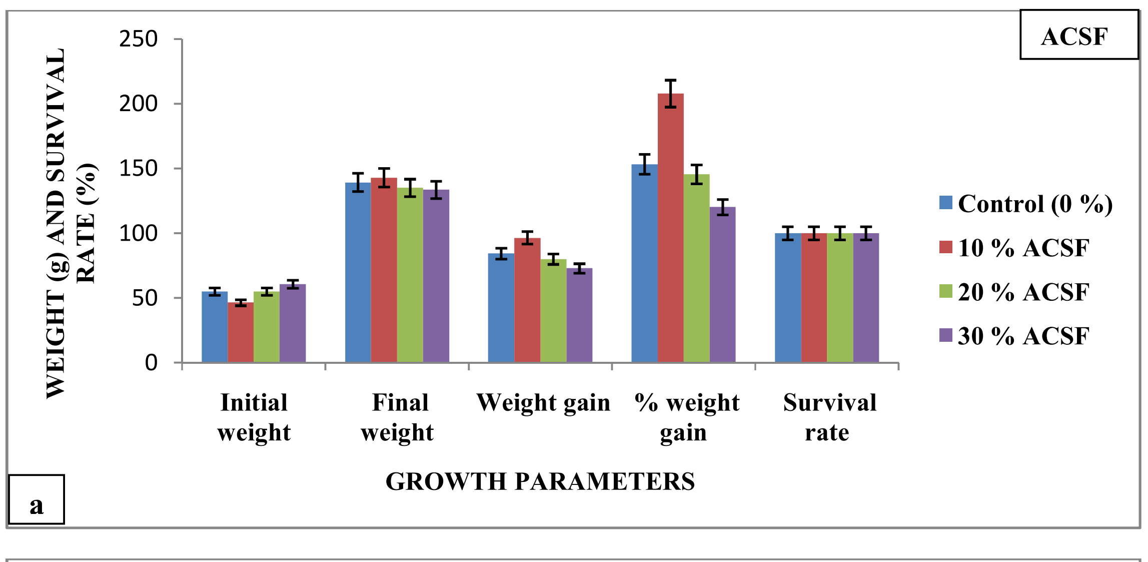
Rats fed with BASR had an average initial weight that ranged from 81.13 g (0%) to 84.26 g (10%). Others include 91.10 g (20%) and 100.16 g (30%) diet compounded with BASR (Figure 4.1b). A steady increase of 130.66 g, 136.40 g, 149.66 g and 174.26 g was respectively observed in the average final weight of 0%, 10%, 20% and 30% group of rats. The survival rate was 100% in the entire groups which signified that the seed residue might not be toxic to rats under this study. The percentage mean weight gain was observed to gradually increase within the groups. The mean weight percentages (%) of 49.53 in 0%, 51.96 in 10% and 58.56 in 20% with the highest value of 74.26 in 30% were recorded. This study revealed that BASR was a good supplement even at 30% with a high mean weight gain of 74.26 g against 49.53 g in the 0% and with no mortality (Figure 4.1b).

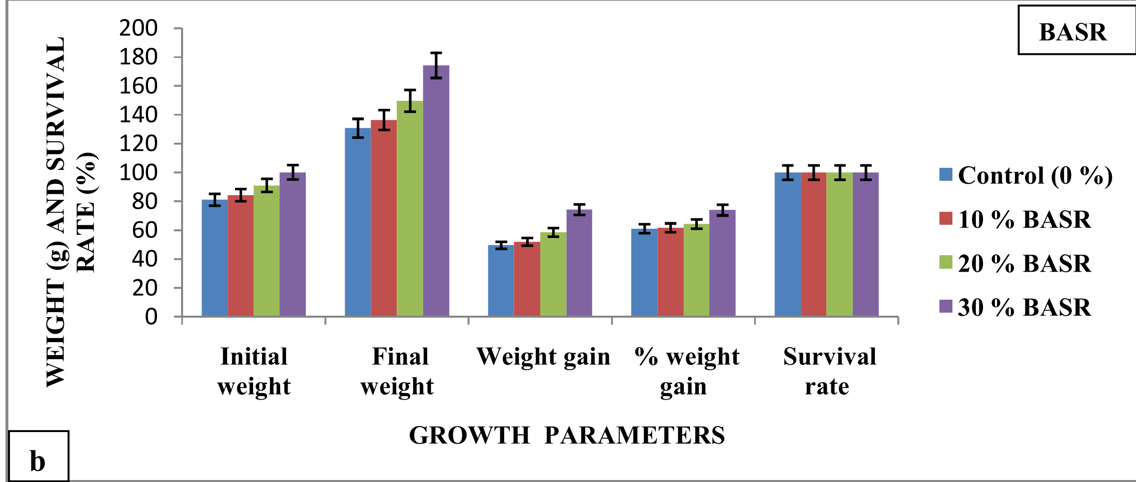
The average final weight of the rats fed with CASF obtained after the study period was 120.39 g, 102.85 g, 62.75 g and 55.40 g against 82.17 g, 73.57 g, 76.57 g and 73.57 g initially recorded weight for rat in the 0%, 10%, 20% and 30% groups respectively. There was a dramatic decrease in the weight gain across the experimental animal. The mean weight gain (%) observed was 38.21 (0%), 29.28 (10%), -13.82% (20%), and -28.09% (30%), inclusion of CASF (Figure 4.1c). The survival rate was also noticed to be very low with higher inclusion of CASF. Survival rate percentage was 100 (0%), 100 (10%), 50 (20%) and 30 (30%) inclusion levels of CASF. The results depict that CASF was not acceptable to the body system at above 10% inclusion level. The drastically reduction in weight of the rat as the concentration of CASF increased in the feed compounded was an indication that this seed flour might be toxic to human being when consumed in excess.

4.17: Mean body weight gain (g) and survival rate (%) of rats fed with diets formulated from ACSF, BASR, CASF, ECSF, NMSR and PLSF

Parameter	0%	10%	20%	30%
		ACSF		
Average initial weight (g)	55.00±1.19^{a}	46.42±5.06^{b}	55.00±9.12^{a}	60.71±1.17^{a}
Final average weight (g)	139.28±16.18^{a}	142.86±23.60^{a}	135.00±9.52^{b}	133.57±9.45^{b}
Weight gain/rat (g)	84.28±0.06^{b}	96.44±1.01^{a}	80.00±0.03^{b}	72.86±0.01^{c}
Weight gain (%)	153.23	207.76	145.45	120.09
Survival rate (%)	100	100	100	100
		BASR		
Average initial weight (g)	81.13±1.36^{c}	84.26±1.33^{c}	91.10±1.22^{b}	100.16±0.35^{a}
Final average weight (g)	130.66±1.01^{c}	136.40±3.73^{c}	149.66±0.66^{b}	174.26±2.67^{a}
Weight gain/rat (g)	49.53±1.06^{c}	51.96±2.34^{c}	58.56±1.42^{b}	74.26±2.67^{a}
Weight gain (%)	61.05	61.65	64.30	73.97
Survival rate (%)	100	100	100	100
		CASF		
Average initial weight (g)	82.17±6.84^{a}	73.57±2.46^{c}	76.57±5.75^{b}	73.57±2.40^{c}
Final average weight (g)	120.39±1.70^{a}	102.85±4.67	62.75±4.75^{a}	55.40±2.10^{c}
Weight gain/rat (g)	38.21±10.67^{a}	29.28±17.0^{a}	-13.82±1.41^{c}	-18.10±1.10^{c}
Weight gain (%)	46.01	39.79	-24.46	-28.09
Survival rate (%)	100	100	50.00	25.00
		ECSF		
Average initial weight (g)	78.63± 6.81^{a}	72.48±1.88^{b}	76.07±3.50^{ab}	72.76±1.66^{b}
Final average weight (g)	128.26±17.08^{a}	114.93±12.24^{ab}	118.36±5.04^{ab}	109.95±6.00^{b}
Weight gain/rat (g)	49.63±11.73^{a}	42.50±11.79^{a}	42.45±7.89^{a}	37.26±5.33^{b}
Weight gain (%)	62.78	58.53	56.35	51.11
Survival rate (%)	100	100	100	100
		NMSR		
Average initial weight (g)	82.61±10.21^{a}	82.68±10.67^{a}	82.48±6.78^{a}	82.06±12.86^{a}
Final average weight (g)	128.63±26.92^{b}	129.24±22.00^{b}	135.20±21.63^{a}	144.31±20.50^{a}
Weight gain/rat (g)	46.03±1.06^{c}	46.56±2.34^{c}	52.72±1.42^{b}	62.25±2.67^{a}
Weight gain (%)	55.72	56.31	63.92	75.86
Survival rate (%)	100	100	100	100
		PLSF		
Average initial weight (g)	82.61±10.21	119.76±3.59	112.78±10.21	110.50±5.02
Final average weight (g)	128.63±2.84	128.88±2.84	126.26±26.92	132.44±6.06
Weight gain/rat (g)	46.02±1.52^{a}	9.12±2.80^{c}	13.84±1.16^{c}	21.94±5.06^{b}
Weight gain (%)	55.71	7.62	12.27	19.85
Survival rate (%)	80.00	80.00	100	90.00

Values stand for the mean±SD of three determination (n=3) Means followed by different letters as superscripts on the same row are significantly different at ($P \leq 0.05$).





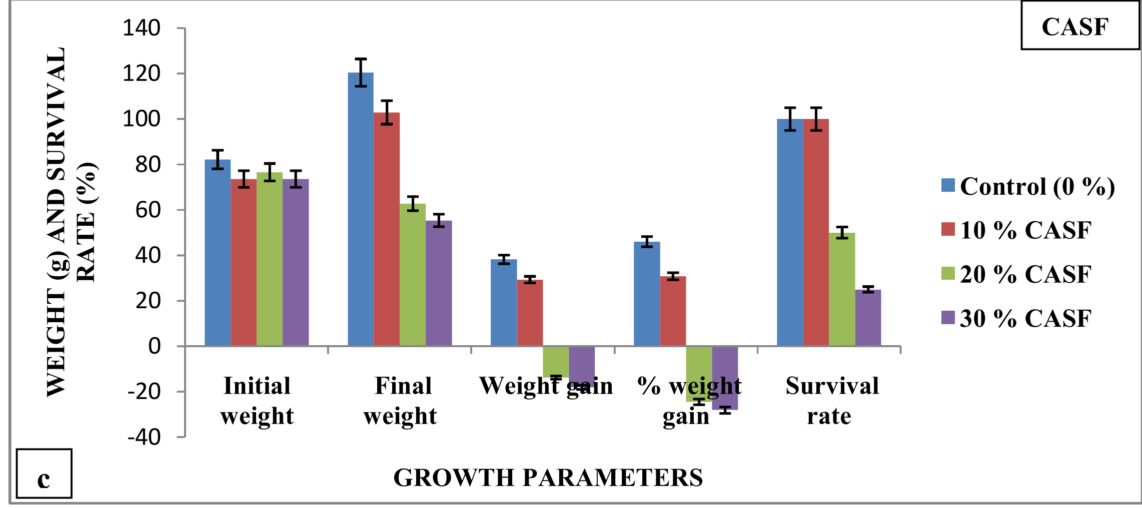


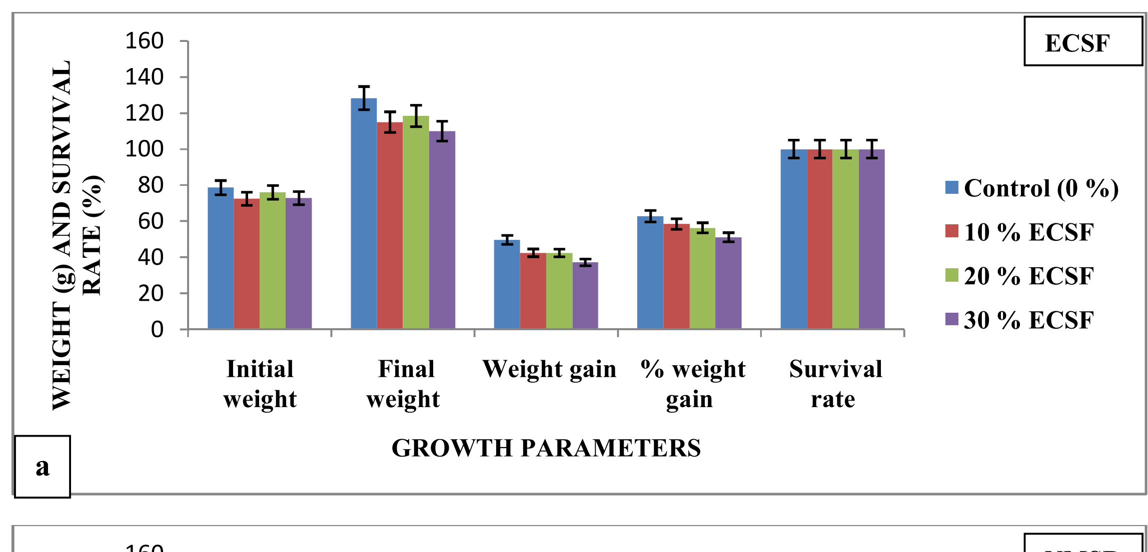
Figure 4.1: Graphical representations of the initial weight (g), weight gain (g), % weight gain and survival rate (%) of rat fed with ACSF (a), BASR (b) and CASF (c)

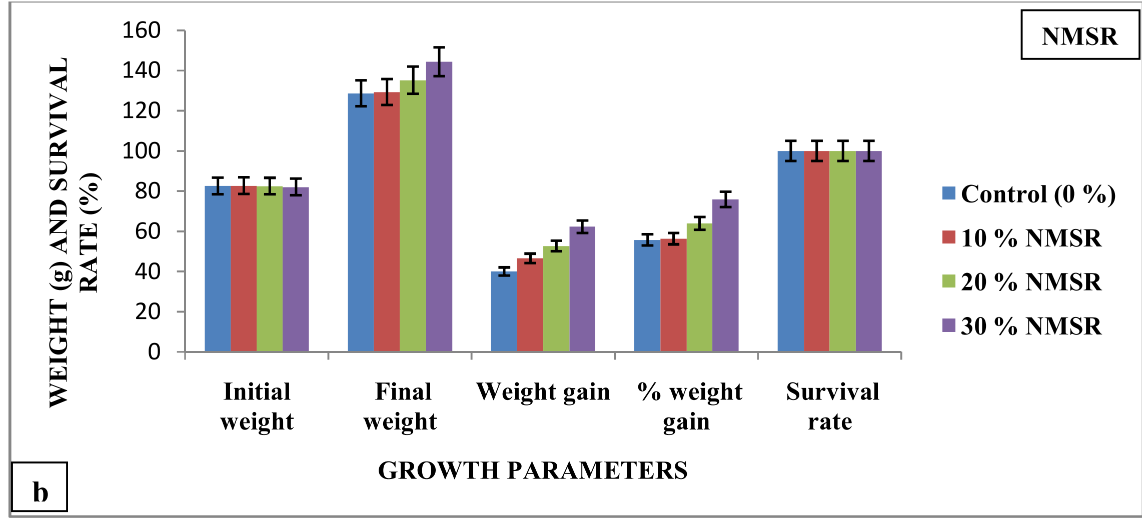
This might be due to the presence of some anti-nutrients that have traces of toxicity in the constituents of the seed flour. CASF might not be toxic at 10%.

The average initial weight of rat fed with ECSF was 78.63 g (0%) 72.48 g (10%), 76.07 (20%) and 72.76 g (30%); (Figure 4.2a). The average final weight of rat obtained was 128.26 g, 114.93 g, 118.36 g and 109.95 g respectively in 0%, 10%, 20% and 30%. The survival rate was 100% throughout the experimental period. This means that there was no loss of animal within the groups. A gradual reduction in the mean weight gain with a constant value within 10% and 20% inclusion levels was equally observed. The mean weight gain was 49.63 g (0%), 42.50 g (10%), 42.45 g (20%) and the lowerst value of 37.26 g (30%). This result reveals that ECSF incorporated at 30% will lead to a reduction in the weight of rat but will be better utilised as supplement at 20% with a mean weight gain of 42.45 g which is comparable to the one 0% (control).

Rats fed with NMSR had an average initial weight that was between 82.06 g (0%) and 82.68 g (30%). The average final weight recorded increased from 128.63 g (0%) to 144.31 (30%); (Figure 4.2b). A gradual increase was observed in the final weight across the experimental groups with 100% survival rate. The percentage weight gain obtained was 55.72 (0%), 56.31 (10%), 63.92 (20%) and 75.86 (30%). 30% inclusion of NMSR had a higher weight gain percentage of 75.86%. The high survival rate (100%) recorded within all the groups depict that there was no loss of rats in the entire groups. NMSR might not be toxic to the rats.

The average initial weight of the rats fed with PLSF ranged from 82.61 g (0%) to 110.50 g (30%). Others include 112.78 g (20%) and 119.76 g (30%), Figure 4.2c. The average final weight obtained was 128. 63 g (0%), 128.88 g (10%), 126.26 g (20%) and 132.44 g (30%). The survival rate was observed to be 80%, 80%, 100%, and 90% respectively across the groups. The loss and death of some rats might not have anything to do with the seed flour itself since 20% of the rats in the control (0%) died also. The values of 46.02%





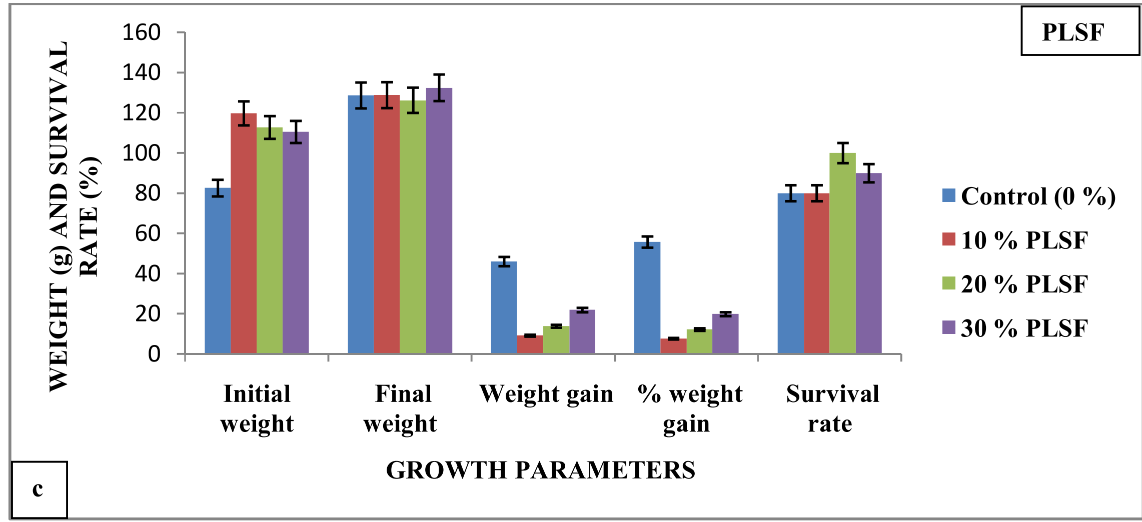


Figure 4.2: Graphical representations of the initial weight (g), weight gain (g), % weight gain and survival rate (%) of rat fed with ECSF (a), NMSR (b) and PLSF (c)

was recorded in 0% against 7.62% (10%), 12.27% (20%) and 21.94% (30%). The PLSF seems not to be toxic but might be a very good supplement when used at a concentration above 30%.

Alterations in the mean body weight have been employed as an indicator of adverse effects of drugs and chemicals on organs and tissues (Raphael *et al*., 2014). The determination of the body weight is a major factor in assessing the toxicity of a substance or chemical. The body weight reduction in the animals might be considered as sensitive index of toxicity after been exposed to a toxic substance. The considerable increase observed in the weight gain of rats fed with BASR and NMSR among the test groups suggested that they could be considered as suitable feed supplement with good properties up to 30% inclusion level, with 100% survival rate and showed no deteriorative effect or damage on the growth of the animals. The ACSF and PLSF showed a mean weight gain at 10% which compared favourable with 0% and a slight decrease in the 20% and 30%. This depicts that ACSF and PLSF with 100 % survival rate could be good supplement in livestock feed formulation at 10% inclusion level. The CASF had a pronounced effect on the rats' mean weight gain. The mean weight gain obtained in 10% compared favourably with 0% group with 100% survival rate. The final weight was lower than the initial weight in both 20% and 30%. This effect was more pronounced in 30%. The survival rate decreased drastically as the level of inclusion increases. This result suggested that CASF had deterious effect on the growth of the experimental animals. The CASF was found to contain tannins, flavonoids, terpenoids and other anti-nutritional factors that might be responsible for the reduction observed on Table 4.17.

4.9.1.3 Effect of seed flours and residues compounded diets on rats' organs

Relative weight of an organ is a necessary parameter to be considered in predicting the physiological and pathological reputation in animals. It is essential and considered in ascertaining if an organ was subjected to damage or not. Organs such as liver, kidney, spleen and heart studied in this research were the basic ones afflicted by metabolic reaction that are probably caused by toxic substance. The liver, highly accepted as major organ involved in the conversion of food intake to energy and in the purification of xenobiotics, is exposed to injury caused by toxic substances (Jothy *et al.,* 2001).

The effect of the seed flours and residues compounded diets on rats' organs was shown on Table 4.18 and Figures 4.3-4.4. There were no significant differences observed in the organs weight of rats fed with ACSF. Their weights appeared to be effectively similar to each other both in the tests and control groups. Only the weight of kidney in 20% was slightly lower than those in other groups. The ACSF might not be toxic to rats at 30% inclusion level.

The liver, spleen, kidney and heart weights recorded are comparable to each other within the groups but better in 20% and 30% inclusion level of BASR than in 10 and 0%. The kidney weight of 0.60 g, 0.66 g, 0.66 and 0.70 g were obtained respectively in 0%, 10%, 20% and 30% level of BASR. This similarity showed that BASR might not be toxic for body system. A similar result was reported by Vishnu *et al.* (2010) on pericarp extract of *Garcinia mangostana.*

The effect of CASF on rats' organ showed a gradual decrease in the weights of liver, kidney, spleen and heart recorded across the experimental groups when compared to 0% and to each other. The weights of liver (3.80 g), kidney (0.65 g) and spleen (0.50 g) in 10% inclusion of CASF were higher than those in 20% and 30% but remained closer to those in 0%. The observed reduction in the weights of liver and kidney of 20% and 30% inclusion depicts that CASF might not be safe to the animals at the levels of this study. They might be affected by some toxicants present in the seed flour (Edem *et al*., 2011).

The impact of ECSF on the organs of rats was illustrated on Table 4.18 and Figure 4.4d. The recorded kidney and heart weights were lower in the tests groups than in 0%. The weight (g) of 4.73, 0.90 and 0.43 obtained respectively for liver, kidney and heart of 10% inclusion of ECSF compared favourably with 4.26, 1.00 and 0.43 obtained in 0%. A slight decrease was observed in the spleen weight of 10% test group as compared to that of 0%. This similarity among 0% group and 10% shows that ECSF might not be toxic to the animal organs at 10% inclusion level.

Table 4.18: Mean internal organ weight (g) of the rat fed with diets formulated from ACSF, BASR, CASF, ECSF, NMSR and PLSF

Tissues	0%	10%	20%	30%
		ACSF		
Liver	4.41±1.08[a]	4.61±1.08[a]	4.35±0.81[a]	4.35±0.41[a]
Kidney	0.99±0. 19[a]	0.84±0.5[a]	0.68±0.5[b]	0.82±0.09[a]
Heart	0.60±0.15[a]	0.62±0.27[a]	0.67±0.27[a]	0.64±0.07[a]
Spleen	0.46±1.06[a]	0.53±0.15[a]	0.53±0.15[a]	0.53±01.5[a]
		BASR		
Liver	3.83±0.15[a]	3.86±0.11[a]	4.20±0.87[a]	4.63±0.15[a]
Kidney	0.60±0.60[c]	0.66±0.05[a]	0.66±0.05[a]	0.70±0.00[a]
Heart	0.36±0.05[a]	0.36±0.05[a]	0.40±0.05[a]	0.40±0.00[a]
Spleen	0.43 ± 0.05[b]	0.50±0.10[ab]	0.63±0.05[a]	0.66±0.05[a]
		CASF		
Liver	4.20±1.01[a]	3.80±0.32[b]	2.40±0.00[ab]	2.32±0.83[ab]
Kidney	0.67±0.09[a]	0.65±0.07[b]	0.40±0.00[b]	0.35±0.19[a]
Heart	0.60±0.05[a]	0.6±0.07[a]	0.58±0.07[a]	0.60±0.03[a]
Spleen	0.65±0.12[a]	0.50±0.14[b]	0.30±0.00[b]	0.30±0.09[a]
		ECSF		
Liver	4.26±0.05[b]	4.73±0.11[a]	4.76±0.11[a]	4.66±0.11[a]
Kidney	1.00±0.00[a]	0.90±0.00[b]	0.73±0.05[c]	0.70±0.00[c]
Heart	0.43±0.05[a]	0.43±0.05[a]	0.40±0.00[a]	0.40±0.00[a]
Spleen	0.83±0.11[a]	0.60±0.00[b]	0.50±0.00[b]	0.60±0.00[b]
		NMSR		
Liver	4.20±0.27[a]	4.43±0.06[a]	4.03±0.35[a]	4.20±0.04[a]
Kidney	0.63±0.06 [a]	0.67±0.12[a]	0.67±0.10[a]	0.65±0.01[a]
Heart	0.40±0.00[a]	0.43±0.06[a]	0.33±0.06[b]	0.40±0.02[a]
Spleen	0.50±0.35[a]	0.50±0.17[c]	0.80±0.36[b]	1.00±0.25[a]
		PLSF		
Liver	3.73±1.87[b]	4.57±0.06[a]	4.13±0.40[a]	4.35±0.41[a]
Kidney	0.63±0.32[b]	0.80±0.05[a]	0.77±0.06[a]	0. 72±0.09[a]
Heart	0.40±0.15[b]	0.50±0.01[a]	0.47±0.27[b]	0.60±0.07[a]
Spleen	0.50±0.38[b]	0.53±0.15[b]	0.70±0.10[a]	0.73±0.06[a]

Values stand for the mean±SD of three determination (n=3) Means followed by different letters as superscripts on the same row are significantly different at ($P \leq 0.05$).

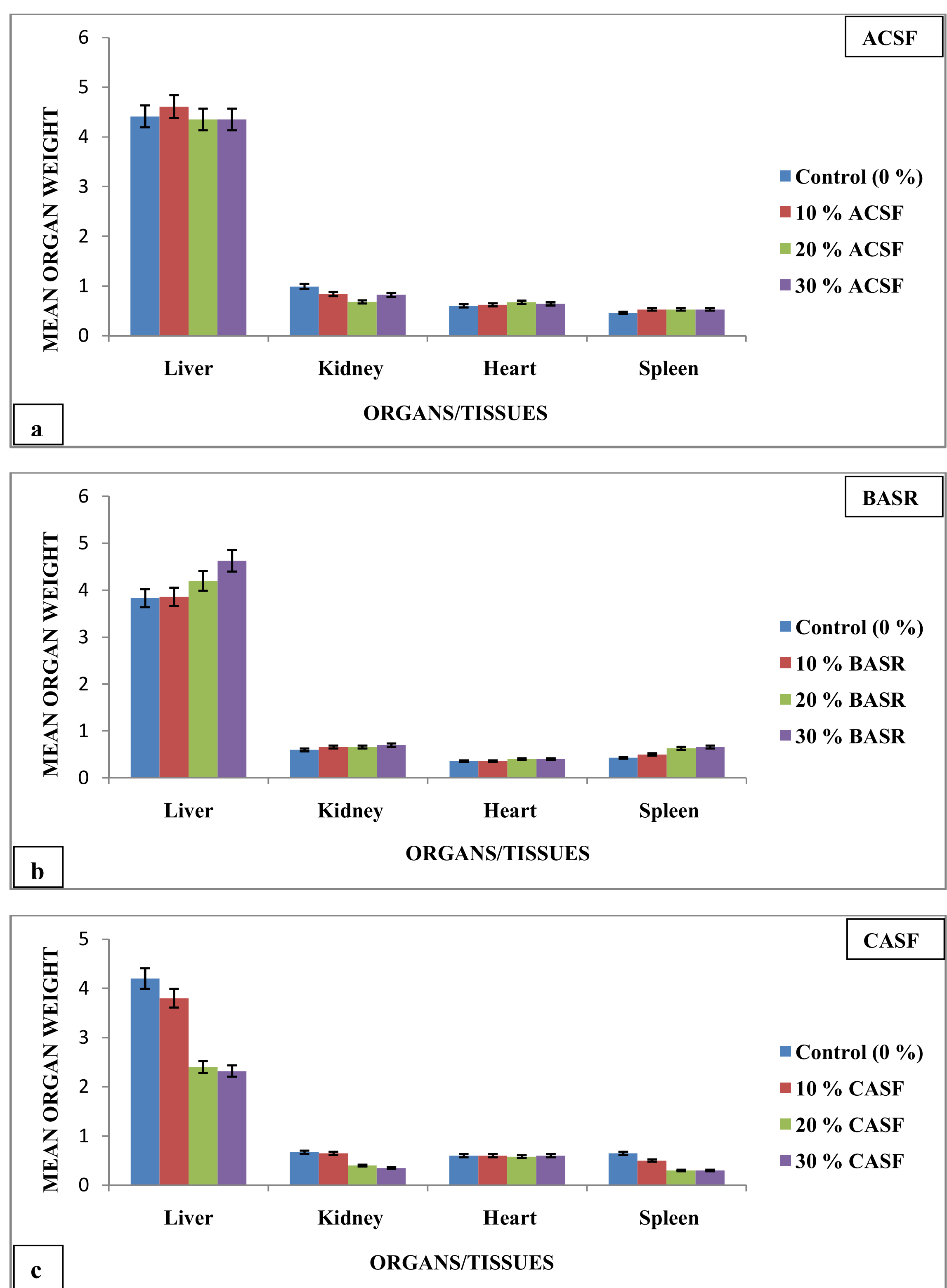


Figure 4.3: Graphical representation of mean weight (g) of rats' organs fed with ACSF (a), BASR (b) and CASF (c)

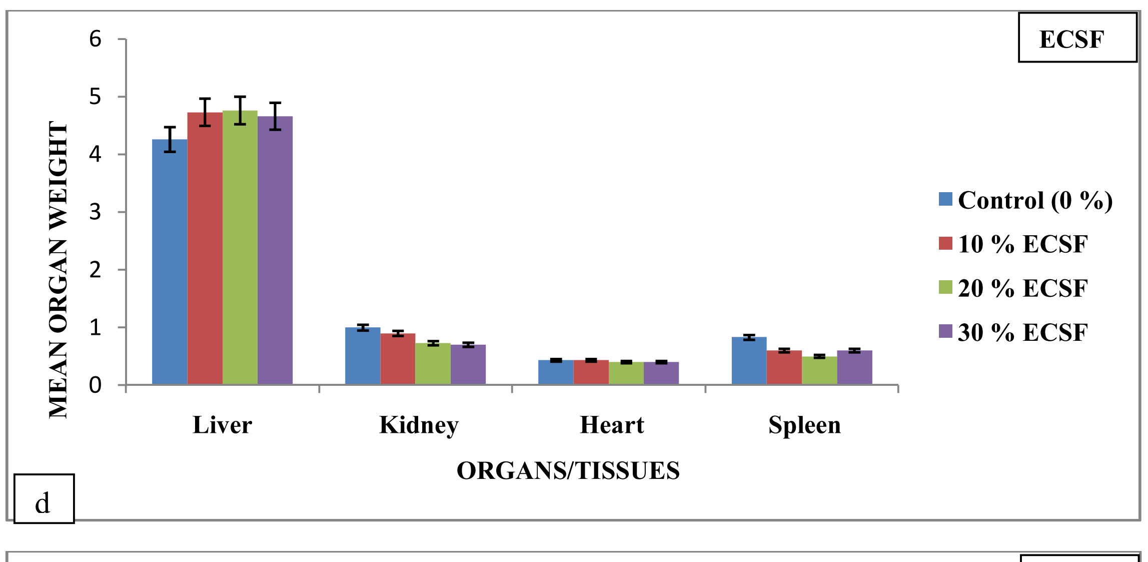
No significant differences were observed in weights of the liver, kidney and heart of the rats fed with NMSR. The organs weights obtained compared favourably with each other. The weight of spleen was higher in 20% and 30% inclusion level than those in 10% and 0% as shown on Table 4.18 and Figure 4.4e. The organs did not show any toxicological effect. They are therefore not affected by NMSR (Vishnu *et al*., 2010).

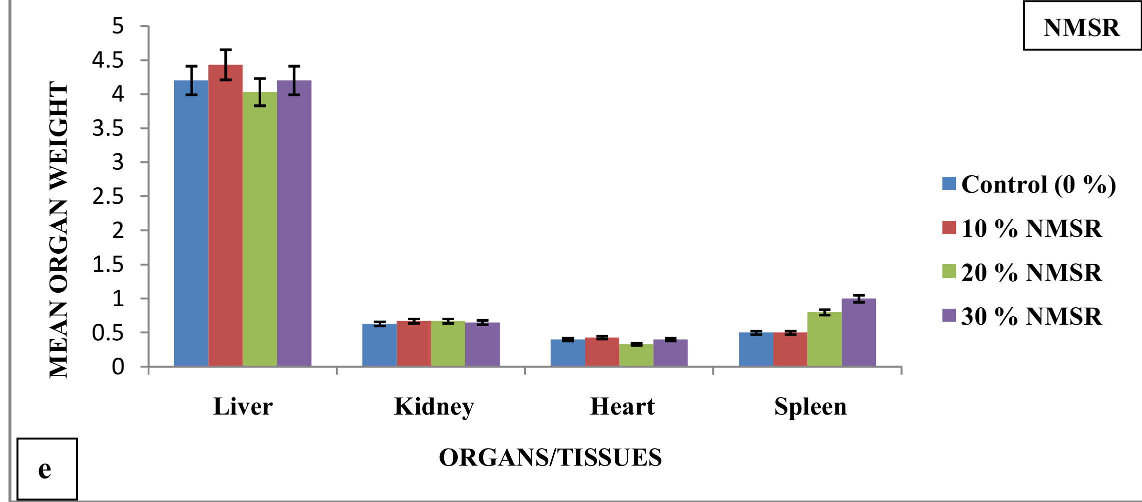
Represented on Table 4.18 and Figure 4.4f is also the effect of PLSF on rats' organ. The liver, spleen, kidney and heart weights recorded in the experimental groups (10%, 20%, and 30%) are slightly higher than those obtained in 0%. The weight of liver (4.35 g), kidney (0.72 g), heart (0.60 g) and spleen (0.73 g) obtained in 30% compared perfectly with those in 10% and 20%. The weight of 3.73 g (liver), 0.63 g (kidney), 0.40 g (heart) and 0.50 g (spleen) was found in 0%. The PLSF might not show any sign of toxicity on the organs even at 30%.

Changes in the various internal organ weights might be a sensitive index of toxicity in animal exposed to toxic chemicals. They have been confirmed and adopted as indicators or test that induced changes usually connected with treatment related to toxicological effects (Sellers *et al*., 2007). In this study, ACSF, BASR, ESCF, NMSR and PLSF showed no significant changes in the rat relative organs (liver, kidney, heart and spleen) weights. They might not affect the normal growth of the animal. The significant reduction observed with CASF depicts that the normal growth of the rat would be greatly affected when fed from 20% inclusion level and above.

4.9.1.4 Haematological and blood biochemistry analyses of blood samples from rats fed with ACSF, BASR, CASF, ECSF, NMSR and PLSF diets

Blood parameters analyses were relevant in evaluating the risk and the changes in haematological system. They also possessd high predictive value towards human toxicity especially when the data obtained were converted from animal studies (Ibrahim *et al.,* 2010). Evaluation of haematological parameter might be applicable in revealing the destructive effect of strange compounds which includes extracts from plants on the animals' blood constituents. They were also considered in the determination of possible





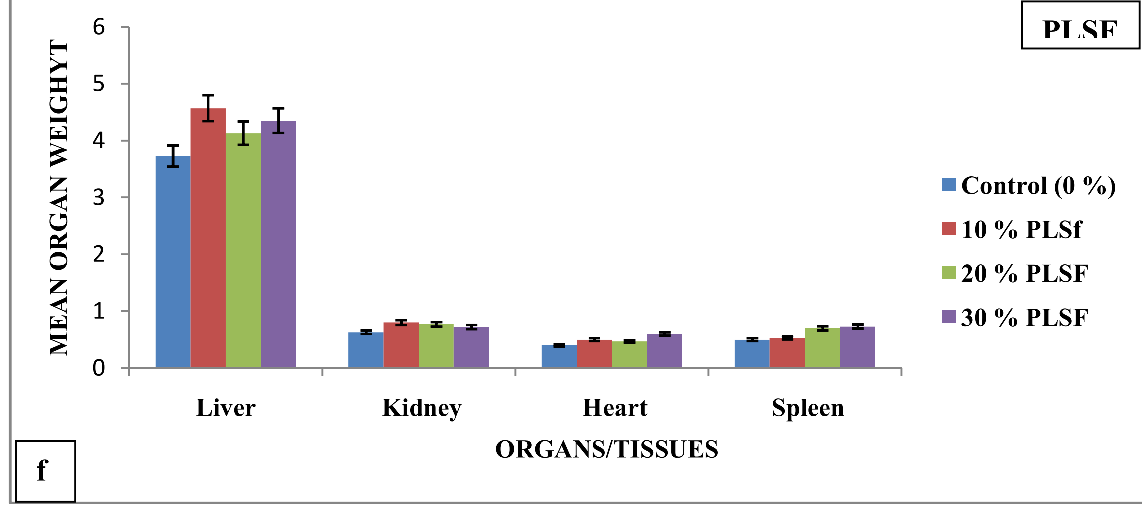


Figure 4.4: Graphical representation of mean weight (g) of rats' organs fed with ECSF (d), NMSR (e) and PLSF (f)

changes in the bimolecular level, metabolic products, abnormal performance and histomorphology of the organs (Jothy *et al.,* 2001). The results of the haematological analysis which included PCV, Hb, WBC, RBC, MCV, MCH, MCHC and blood biochemistry analysis (ALT, AST, ALP) of rats fed on graded levels of ACSF, BASR, CASF, ECSF, NMSR and PLSF were shown on Tables (4.19 - 4.21).

Table 4.19 showed the haematological and blood biochemistry analyses results of blood obtained from rats fed on various substitution level of ACSF and CASF. The PCV (%) values obtained increased from 35.67 (0%) to 40.33 (30%) (ACSF) while it reduced from 48.25 (0%) to 33.34 (30%) (CASF). The Hb (mg/dl) ranged from 12.17 (0%) to 13.60 (30%) in ACSF but still reduced from 15.80 (0%) to 12.40 (30%) in CASF. The RBC value range was comparable within the experimental groups in ACSF. The values were slightly lower in the experimental group than in the control with CASF. There were no significant differences observed among the groups in terms of other haematological parameters such as WBC, platelets and monocytes levels. Blood biochemistry parameters such as AST, ALT and ALP, urea and creatinine greatly compared with each other as no major significant difference was seen among them.

The haematological and blood biochemistry analyses results obtained from the rats fed with diets containing different concentrations (0-30%) of ECSF and PLSF were displayed on Table 4.20. The PCV (%) decreased from 45.00 (0%) to 41.66 (30%) with ECSF while it increased slightly from 36.67 (0%) to 38.67 (30%) in PLSR groups. There were no significant differences in Hb, RBC, platelets and other haematological parameters. Blood biochemistry parameters such as AST, ALT and ALP, urea and creatinine greatly compared with each other as no major significant difference was seen among them.

Table 4.21 showed the haematological and blood biochemistry analyses results obtained from the rats fed with graded level of BASR and NMSR (0-30%). The PCV (%) rose from 40.33 (0%) to 43.66 (30%) in BASR as well as from 41.33 to 42.00 (30%) in NMSR groups. The Hb (mg/dl) values obtained were in good agreement with each other in all the groups for both BASR and NMSR groups. Other haematological parameters such as MCV, MCH, MCHC, RBC, WBC and platelets compared favourably with each other within the test and control groups. The AST concentrations equally increased from 38.00

Table 4.19: Haematological and blood biochemistry analyses of blood samples of rats fed with ACSF and PLSF diets

Parameter	0%	10%	20%	30%
		ACSF		
PCV (%)	36.67±6.35[a]	40.33±5.51[a]	37.00±3.61[a]	40.33±3.51[a]
Hb (mg/dl)	12.37±1.80[a]	13.70±1.73[a]	12.57±0.90[a]	13.60±1.05[a]
RBC (10^6/μl)	3.92±0.38[a]	4.19±0.60[a]	3.97±0.13[a]	4.08±0.58[a]
WBC (10^3/μl)	12.90±4.17[b]	18.45±1.95[a]	14.32±1.57[ab]	16.56±1.39[a]
Platelet(cell)x10^5	1.37±0.38[a]	1.50±0.38[a]	1.36±0.48[a]	1.32±0.30[a]
Lymphocytes (%)	56.00±1.00[a]	52.67±3.79[a]	60.33±12.86[a]	61.33±2.08[a]
Heterophiles (%)	35.67±1.16[a]	40.00±2.65[a]	34.00±12.29[a]	31.67±3.79[a]
Monocytes (%)	4.00±1.00[a]	3.67±0.58[a]	3.33±1.16[a]	3.33±2.08[a]
Eosinophil (%)	4.00±1.73[a]	3.67±1.53[a]	3.00±1.00[a]	3.33±3.21[a]
Basilophils	0.33±0.58[a]	0.30±0.00[b]	0.37±0.58[a]	0.33±0.58[a]
Total protein	8.47±0.15[a]	8.17±0.29[ab]	7.63±0.12[c]	8.23±0.06[a]
Albumin	3.23±0.49[a]	2.93±0.51[a]	2.60±0.26[a]	2.53±0.06[a]
Globulin	5.57±0.15[a]	5.67±0.06[a]	5.10±0.30[a]	5.74±0.06[a]
A/G ratio	0.56±0.03[a]	0.53±0.11[a]	0.51±0.08[a]	0.44±0.01[b]
ALP (U/L)	124.7±5.57[ab]	134.00±3.46[a]	139.67±0.53[a]	136.67±0.58[a]
Creatinine	0.63±0.06[ab]	0.67±0.06[a]	0.63±0.12[ab]	0.67±0.06[a]
ALT (U/L)	25.33±2.08[a]	27.33±1.53[a]	27.67±2.52[a]	24.33±0.58[a]
AST (U/L)	217.3±31.94[a]	194.00±2.65[a]	209.3±15.50[a]	174.33±0.58[a]
BUN (mg/dl)	16.60±0.53[a]	15.83±0.57[a]	16.07±0.67[a]	18.07±0.12[a]
		PLSF		
PCV (%)	36.67±6.35[b]	41.33±3.51[a]	42.00±1.73[a]	38.67±0.58[a]
Hb (mg/dl)	12.37±1.80[a]	13.60±1.05[a]	13.93±0.67[a]	12.86±0.29[a]
RBC (10^6/μl)	3.91±0.38[ab]	4.08±0.58[ab]	4.42±0.05[a]	4.61±0.14[a]
Platelet(cell)x10^5	1.37±0. 38[a]	1.32±0.30[a]	1.58±0.28[a]	1.27±0.42[a]
Lymphocytes (%)	56.00±1.00[a]	61.33±2.08[a]	55.00±5.29[a]	55.33±3.51[a]
Heterophiles (%)	35.67±1.15[a]	31.67±3.79[a]	37.00±5.57[a]	38.00±3.00[a]
Monocytes (%)	4.00±1.00[a]	3.33±2.08[a]	4.00±1.73[a]	3.66±1.55[a]
Eosinophil (%)	4.00±1.73 [a]	3.33±3.21[ab]	3.67±1.53[a]	2.67±1.53[b]
Basilophils	0.33±0.59[a]	0.33±0.58[a]	0.33±0.58[a]	0.33±0.59[a]
Total protein/g/dL	8.47±0.15[a]	8.23±0.06[a]	7.67±0.12[a]	7.93±0.06[a]
Albumin (g/dL)	3.23±0.49[a]	2.53±0.06[b]	2.47±0.06[b]	2.47±0.12[b]
Globulin (g/dL)	5.57±0.15[a]	5.74±0.06[a]	5.20±0.20[a]	5.27±0.12[a]
A/G ratio	0.58±0.07[a]	0.44±0.01[a]	0.47±0.01[a]	0.46±0.10[a]
AST (U/L)	217.33±1.94[a]	174.33±0.58[b]	190.67±0.58[a]	196.01±1.80[a]
BUN (mg/dl)	16.60±0.53[a]	16.07±0.12[a]	15.07±0.05[a]	15.00±0.50[a]
ALT (U/L)	25.33±2.08[a]	24.33±0.58[a]	27.33±1.15[a]	27.30±1.45[a]
ALP (U/L)	124.67±5.37[b]	156.67±0.58[a]	162.67±0.58[a]	164.00±3.46[a]

Values = means±SD for triplicate determination. There are no significant differences at $P \leq 0.05$ in the values with similar superscripts in the same row.

Table 4.20: Haematological and blood biochemistry analyses of blood samples of rats fed with CASF and ECSF diets

Parameters	0%	10%	20%	30%
		CASF		
PCV (%)	48.25±3.40^{a}	35.50±6.35^{b}	34.00±0.00^{b}	33.34±1.10^{ab}
Hb (mg/dl)	15.80±1.03^{a}	11.40±2.01^{c}	11.40±0.00^{c}	12.40±0.20^{b}
RBC (10^{6}/µl)	7.73±0.53^{a}	5.72±1.26^{b}	5.27±0.00^{b}	7.69±0.11^{a}
WBC (10^{3}/µl)	5.31±0.70^{a}	4.91±1.1^{b}	5.40±0.00^{a}	5.22±0.4^{a}
Platelet(cell)x10^{5}	1.46±0.54^{a}	1.02±0.12^{c}	1.13±0.00^{ab}	1.22±0.23^{b}
Lymphocyte (%)	73.00±2.45^{a}	67.00±3.55^{b}	73.00±0.00^{a}	62.00±1.73^{c}
Heterophyl (%)	23.50±3.69^{b}	29.00±2.82^{a}	23.00±0.00^{b}	24.32±1.10^{b}
Monocyte (%)	1.75±0.95^{a}	1.75±0.50^{a}	1.00±0.00^{b}	1.70±0.10^{a}
MCH (pg)^{a}	20.46±0.94^{b}	20.07±0.93^{b}	21.63±0.00^{a}	18.78±1.20^{c}
MCV (fL)	62.36±2.43^{a}	62.50±3.86^{a}	64.52±0.00^{a}	56.34±0.21^{b}
MCHC (%)	32.75±0.44^{a}	32.36±1.47^{a}	33.53±0.00^{a}	33.21±0.33^{a}
Total protein (g/dL)	7.23±0.20^{ab}	6.63±0.11^{ab}	7.40±0.00^{a}	6.50±0.10^{b}
Albumin (g/dL)	3.20±0.10^{a}	2.40±0.26^{b}	3.00±0.00^{a}	2.56±0.15^{b}
Globulin (g/dL)	4.03±0.10^{ab}	3.83±0.40^{ab}	4.40±0.00^{a}	3.93±0.25^{a}
Albumin/Globulin	0.73±0.06^{a}	0.6±0.10^{a}	0.60±0.00^{a}	0.65±0.08
AST (U/L)	41.33±1.52^{a}	40.66±4.10^{a}	40.00±0.00^{a}	42.60±1.50^{a}
ALT (U/L)	30.00±0.10^{a}	29.33±3.78^{a}	28.00±0.00^{a}	30.33±1.15^{a}
ALP (U/L)	110.33±8.30^{a}	107.67±8.14^{a}	105.00±0.0^{a}	104.86±5.50^{a}
BUN (mg/dl)	16.87±0.28^{a}	16.20±0.26^{b}	15.20±0.00^{c}	16.00±0.40^{a}
		ECSF		
WBC (10^{3}/µl)	6.43±0.63^{a}	5.22±0.74^{b}	6.02±0.10^{ab}	5.40±0.46^{ab}
Hb (mg/dl)	14.90±0.43^{a}	14.00±0.50^{a}	14.46±0.28^{a}	15.53±1.81^{a}
Platelet(cell)x10^{5}	1.87±0.38^{b}	1.55±0.71^{b}	1.28±0.93^{a}	1.44±0.36^{b}
PCV (%)	45.00±1.00^{a}	43.00±2.00^{ab}	43.66±1.15^{ab}	41.66±1.52^{b}
RBC (10^{6}/µl)	7.33±0.11^{a}	7.09±0.49^{a}	7.39±0.12^{a}	7.51±0.58^{a}
MCV (fL).	61.37±1.53^{a}	60.75±2.19^{a}	59.08±1.49^{a}	55.74±5.97^{a}
MCHC (%).	33.10±0.41^{b}	32.73±0.47^{ab}	33.13±0.21^{ab}	37.29±0.26^{a}
Monocyte (%)	1.66±0.51^{a}	1.66±0.50^{a}	2.00±0.17^{a}	1.33±0.49^{a}
Lymphocyte (%)	70.66±2.51^{a}	66.33±4.16^{a}	61.00±1.73^{b}	67.66±2.08^{a}
Heterophyl (%)	23.00±3.60	30.66±2.51	34.33±1.15	28.00±2.64
MCH (pg)	20.33±0.49^{a}	19.85±0.48^{a}	19.61±0.33^{a}	20.71±0.60^{a}
Albumin.(g/dL)	3.13±0.57^{a}	3.36±0.11^{a}	2.56±0.15^{b}	2.33±0.20^{b}
Globulin (g/dL)	3.96±0.11^{a}	3.86±0.05^{a}	3.93±0.25^{a}	3.53±0.11^{b}
AST (U/L)	43.00±2.00^{a}	40.00±2.00^{a}	42.60±1.50^{a}	41.33±0.59^{a}
Total protein.(g/dL)	7.10±0.17^{a}	7.26±0.19^{a}	6.50±0.10^{b}	6.13±0.30^{c}
ALT (U/L)	31.33±1.52^{a}	27.66±2.51^{b}	31.33±1.15^{a}	29.66±1.52^{ab}
BUN (mg/dl)	17.00±0.60^{a}	15.86±1.15^{a}	16.23±0.40^{a}	16.30±0.52^{a}
Albumin/Globulin	0.78±0.00^{a}	0.87±0.04^{a}	0.65±0.08	0.65±0.03^{b}
ALP (U/L)	111.00±7.93^{a}	104.33±2.08^{a}	106.06±5.50^{a}	108.00±7.81^{a}

Values=means±SD for triplicate determination. There are no significant differences at $P \leq 0.05$ in the values with similar superscripts in the same row

Table 4.21: Haematological and blood biochemistry analyses of blood samples of rats fed with BASR and NMSR diets

Parameters	0%	10%	20%	30%
		BASR		
PCV (%)	40.33±0.51^{c}	41.33±0.51^{ab}	41.66±0.51^{b}	43.66±1.03^{a}
Hb (mg/dl)	13.70±0.15^{b}	14.06±0.41^{ab}	13.86±0.72^{b}	14.50±0.17^{a}
RBC ($10^{6}/\mu l$)	7.08±0.52^{a}	7.13±0.63^{a}	7.06±0.48^{a}	7.32±0.12^{a}
WBC ($10^{3}/\mu l$)	8.82±1.8^{a}	7.90±1.40^{ab}	6.93±0.34^{b}	8.40±0.18^{a}
Platelet(cell)x10^{5}	1.60±0.37^{a}	1.38±0.12^{ab}	1.37±0.23^{ab}	1.38±0.76^{ab}
Lymphocyte %	80.33±4.50^{a}	75.00±5.36^{a}	76.00±0.89^{a}	60.33±9.81^{b}
Heterophle (%)	16.00±3.57^{a}	23.33±6.28^{b}	20.66±2.73^{bc}	30.00±3.09^{a}
Monocyte (%)	2.10±0.89^{a}	0.33±0.51^{c}	1.33±1.36^{ab}	2.33±0.59^{a}
MCH	19.54±0.81^{a}	19.59±2.36^{a}	19.64±0.62^{a}	19.79±0.21^{a}
MCHC	33.72±0.46^{a}	34.02±0.58^{a}	33.26±1.36^{a}	33.21±0.87^{a}
Albumin (g/dl)	3.46±0.11^{b}	3.90±0.10 ^{b}	3.96±0.37^{b}	4.80±0.26^{a}
Total protein(g/dl)	6.40±0.10^{b}	6.76±0.50^{b}	6.76±0.15^{b}	7.46±0.41^{a}
Globulin (g/l)	2.93±0.05^{a}	2.86±0.50^{a}	2.86±0.03^{ab}	2.73±0.20^{ab}
A/G ratio	1.19±0.05^{b}	1.33±0.05^{ab}	1.36±0.20^{b}	1.76±0.05 ^{a}
ALP (U/L)	81.33±3.21^{a}	77.33±8.73^{a}	87.66±838^{a}	83.00±0.57^{ab}
AST (U/L)	38.00±2.66^{a}	41.33±0.57^{b}	41.66±3.21^{a}	41.66±2.08^{a}
ALT (U/L)	27.66±0.57^{a}	29.66±0.57^{a}	29.66±2.51^{a}	29.33±0.57^{a}
Creatinine	0.66±0.57^{ab}	0.56±0.57^{b}	0.73±0.11^{ab}	0.73±0.11^{ab}
		NMSR		
PCV (%)	41.33±0.58 ^{ab}	40.33±0.50^{ab}	40.33±1.15^{ab}	42.00±0.00^{a}
Hb (mg/dl)	13.53±0.41^{a}	13.53±0.11^{a}	13.03±0.45^{a}	13.96±0.25 ^{a}
RBC ($10^{6}/\mu l$)	7.10±0.33 ^{a}	6.60±0.60^{b}	6.59±0.37^{b}	6.96±0.36^{a}
WBC ($10^{3}/\mu l$)	7.38±2.29^{ab}	6.13±0.57^{b}	7.03±1.60^{ab}	7.33±0.99^{ab}
Platelet(cell)x10^{5}	1.26±0.32^{a}	1.19±0.30^{a}	1.23±0.20^{a}	1.18±0.13^{a}
Lymphocyte (%)	70.33±10.40^{a}	68.67±7.57^{a}	70.00±6.55^{a}	68.00±3.60^{a}
Heterophyl (%)	24.67±7.02^{a}	28.00±7.01^{a}	25.33±6.55^{a}	28.33±3.05^{a}
Monocyte (%)	2.66±1.53^{a}	2.33±1.50^{a}	2.00±0.01^{a}	2.40±1.05^{a}
MCH (pg)^{a}	19.07±1.15^{a}	20.50±0.19^{a}	19.76±0.58^{a}	19.58±0.74 ^{a}
MCV (fL)	60.91±3.70^{a}	61.11±1.22^{a}	61.16±0.49^{a}	58.28±1.09^{a}
MCHC (%)	32.73±0.60^{a}	33.56±0.76^{a}	32.23±0.49^{a}	33.25±0.59^{a}
Total protein(g/dL)	7.86±0.3 ^{a}	6.80±0.79^{b}	6.60±0.20^{b}	6.70±0.10^{b}
Albumin (g/dL)	5.07±0.22 ^{a}	4.03±0.97^{b}	3.73±0.15^{c}	3.78±0.05^{c}
Globulin (g/dL)	2.33±0.60^{b}	2.80±0.2 ^{a}	2.30±0.56^{b}	2.90±0.00 ^{a}
A/G ratio	2.17±0.57 ^{a}	1.44±0.46^{b}	1.67± 0.35^{b}	1.30±0.00^{b}
AST (U/L)	40.00±1.00^{b}	39.33±1.36^{b}	43.00±1.22 ^{a}	44.33±0.58 ^{a}
ALT (U/L)	28.33±0.94^{c}	28.67±0.58^{c}	30.30±0.58^{b}	32.00±1.00 ^{a}
ALP (U/L)	80.33±6.67^{a}	79.34±3.80^{a}	85.00±16.52^{a}	91.33±9.02^{a}
BUN (mg/dl)	14.67±0.71^{a}	14.33±0.58^{a}	14.33±0.58^{a}	14.33±0.57^{a}

Values=means±SD for triplicate determination. Means with different letters as superscripts on the same row are significantly different at ($P \leq 0.05$).

(0%) to 41.66 (30%) in BASR and from 40.00 (0%) to 44.33 (30%) in NMSR. Other haematological indices such as MCH, MCHC, platelets and WBC as well as biochemical parameter like ATP, ALT, creatinine and total protein were comparable with each other within the test and control groups in both BASR and NMSR as no major significant difference was seen among them.

Haematological parameters could be a useful tool to explain the blood relating function of a chemical compound, plant extracts and seed oils in an organism (Yakubu *et al.,* 2007). The assessment of these indices can reveal the deleterious effect of strange compound such as plant extract and blood constituents in animal. There was no significant difference in the RBC indices of ACSF, BASR, ECSF, NMSR and PLSF. This suggested that these seed flours and residues might have no effect on the morphology and osmotic fragility of the red blood cells as well as the Hb indices. The absence of significant differences in the WBC indices signified that there was no major tissue injury or any inflammation. The comparison of the lymphocyte, heterophils and monocytes among the groups of rats fed with the seed flours and residues indicated that these flours and residues might have not caused any damage on the animals' immune system (Mayur *et al*., 2017). The activities of liver enzymes (ALT, AST and ALP) helped to provide necessary notification on the liver functions. The AST was regarded as liver function indicator but not as effective as ALT. ALP was a membrane bound enzyme which affects the membrane permeability and produce disorder in the movement of metabolites when altered. Increase in the concentration of ALP was always seen as a characteristic effect showing traces of liver infection, hepatitis and bone illness (Aliyu *et al.,* 2006). Both creatinine and urea were accepted as considerable pointer in the kidney malfunction when they were in contact with dangerous substance or chemicals (Gnanami *et al*., 2008). The nonexistence of considerable difference in ALT, AST and ALP as well as in other biochemical parameter which are considered as good indicators of liver and kidney functions proposed that ACSF, BASR, ECSF, NMSR and PLSF did not affect the normal metabolism of the animals. Therefore, they might not be toxic to rat organs at the level of incorporation. PCV, Hb, RBC and platelet values of the control rats were significantly different from those in the experimental groups where rats were fed with various level of incorporation of CASF. Some biochemical indices such as ALP, urea and creatinine as well as

albumin/globulin ratio were found to be slightly lowered in 20% and 30% test groups when compared to the control group. The CASF might therefore not be safe at 10% level of incorporation.

4.9.1.5 Histopathological study of organs of rats fed with seed flours and residues

The results of the histopathological studies of selected organs (heart, kidney and liver) of rats fed with ACSF, BASR and CASF were shown on Table 4.22, while those of the rats fed with ECSF, NMSR and PLSF were given on Table 4.23. Alterations in the mean body weight were employed as parameter indicating negative effects of drugs, substances, as well as chemicals in organs and tissues (Raphael *et al*., 2014). The relative organs weights in toxicity studies are majorly based on their sensitivity to predict toxicity caused by a substance in the organs and it correlates well with the changes observed in the histopathological results.

Table 4.22 **s**ummarised the histopathological results of organs (liver, kidney and heart) of all experimental groups of rats that were fed with ACSF, BASR and CASF. No visible lesions were observed in the studied organs of both the control and 10% group of rats fed with ACSF. No visible lesion was also seen in the heart of all the groups fed with ACSF and the control. Moderate portal congestion was noted in kidney of rats fed with 20% while reasonable periportal and diffuse cellular infiltration by macrophages was witnessed in the liver of rats fed with 30%, but nothing else was noted in the rest of the organs in the control and other test groups.

The liver, heart and kidney sections of rats that were fed with BASR subjected to histopathological examination showed no visible lesions in the control group as well as in all the test groups. The lack of significant changes in the organs weights of these rats proposed that ACSF and BASR showed no abnormal effect or did not cause any damage on the organs. The CASF did not show any visible lesions in the histopathology of the liver, heart and kidney of 0% and 10% experimental groups. However, there is a focus of myofibre necrosis with cellular infiltration around the endocardium in the heart of 20% group, a severe interstitial congestion and hemorrhage in the kidney of 20% group of rats fed with CASF. In the kidney sections of rats that were fed with 30% CASF, there were

Table 4.22: Histopathological study of the tissues of rats fed with ACSF, BASR and CASF diets

Organ	Heart	Kidney	Liver
		ACSF	
0%	No visible lesions seen	No visible lesions seen	No visible lesions seen
10%	No visible lesions seen	No visible lesions seen	No visible lesions seen
20%	No visible lesions seen	Moderate portal and central venous congestion	No visible lesions seen
30%	No visible lesions seen	No visible lesions seen	A moderate and mild periportal as well as diffuse cellular infiltration by microphages.
		BASR	
0%	No abnormalities detected	No abnormalities detected	No abnormalities detected
10%	No abnormalities detected	No abnormalities detected	No abnormalities detected
20%	No abnormalities detected	No abnormalities detected	No abnormalities detected
30%	No abnormalities detected	No abnormalities detected	No abnormalities detected
		CASF	
0%	No abnormalities detected	No abnormalities detected	No abnormalities detected
10%	No abnormalities detected	No abnormalities detected	No abnormalities detected
20%	A focus of myofibre necrosis, with cellular infiltration was seen around the endocardium	A severe interstitial congestion and hemorrhage was observed	No visible lesions seen
30%	No abnormalities detected	A severe interstitial congestion and hemorrhage was observed	Severe portal congestion, moderate periportal cellular infiltration was seen

ACSF: *Areca catecu* seed flour

BASR: *Balanites aegyptiaca* seed residue

CASF: *Chrysophyllum albidum* seed flour

severe intestinal congestion and hemorrhage while severe portal congestion and moderate periportal infiltration were observed in the liver sections. These various lesions were also confirmed by the significant changes shown in the rats' organs and body weights recorded at 20% and 30%. This information depicts that *C. albidum* seed flour (CASF) might have caused damages to organs at 20% and 30% inclusion level.

Table 4.23 provided a summary of the histopathological outcomes of the organs of 0% and experimental groups that were given diets containing ECSF, NMSR and PLSF. No apparent abnormalities were detected in the heart, liver and kidney of the control group, as well as the 10% and 20% experimental groups that were fed with these seed flours. However, observations in the kidney sections of rats that were fed with 30% ECSF indicated the presence of a mild congestion and pink staining material in the collecting duck and renal tubules. On the other hand, the liver sections exhibited severe portal congestion and moderate periportal cellular infiltration. The kidney weight in the control group compared favourable better with those in 10% test group than those in 20% and 30% of ECSF. The mean weight gain obtained in 20 and 30% of ECSF test groups were lower than those in 10% which in turn, is greater than 0%. ECSF might not be toxic to the animals up to 30% but could be better at 10% inclusion level. The histopathological study of the sections of the kidney and heart of rat fed with NMSR showed no visible lesions in the control group as well as in 10% and 20% test groups. A reasonable periportal cellular permeation was seen on the liver sections of the control and all the test groups. The liver enzymes (AST, ALT, ALP and others) which were good indicators of liver functions showed no significant difference within the control and test groups (Table 4.22). The lack of significant changes in the organs weights of the rats was an indication that NMSR did not cause any damage on the organs. NMSR seemed safe, non toxic and could be good supplement at 30% inclusion levels. There was no visible lesions seen in the heart, liver and kidney of the rats fed with *P. longifolia* seed flour (PLSF) in all the groups as well as in the control group. No significant difference was observed in the organs. The PLSF seed flour was probably not toxic to the animal at 30%.

Table 4.23: Histopathological study of the tissues of rats fed with ECSF, NMSR and PLSF diets

Organ	Heart	Kidney	Liver
		ECSF	
0%	There was no observable lesion	There was no observable lesion	There was no observable lesion
10%	There was no observable lesion	There was no observable lesion	A mild to moderate diffuse vacuolar degeneration was observed in the hepatocytes
20%	No observable lesion was seen	There was no observable lesion	Moderate portal congestion, moderate periportal cellular infiltration
30%	There was no observable lesion	Pink staining material observed in the collecting ducts and renal tubules	Severe portal congestion, moderate periportal cellular infiltration.
		NMSR	
0%	No visible lesions seen	No visible lesions seen	The hepatocytes exhibited mild to moderate diffuse hydropic degeneration, accompanied by a moderate periportal cellular infiltration
10%	There was no abnormalities detected	No visible lesions seen	A severe diffuse vacuolar degeneration was observed in the hepatocytes
20%	There was no abnormalities detected	No visible lesions seen	There is no observable lesion
30%	There was no abnormalities detected	No visible lesions seen	A mild diffuse hydropic degeneration was observed in the hepatocytes
		PLSF	
0%	There was no observable lesion	There was no observable lesion	There was no observable lesion
10%	There was no observablelesion	There was no observable lesion	There was no observable lesion
20%	There was no observable lesion	There was no observable lesion	There was no observable lesion
30%	There was no observable lesion	There was no observable lesion	There was no observable lesion

ECSF: *Enterolobium cyclocarpum* seed flour
NMSR: *Neocarya macrophylla* seed residue
PLSF: *Polyalthia longifolia* seed flour

4.9.2 Application of BASO and NMSO as nutritional supplement

4.9.2.1: Effect of BASO and NMSO on rats' body weight, feed-intake, growth performance and survival rate

The effect of BASO on rats' body weight, feed-intake, growth performance and survival rate was carried out by incorporating the seed oils in diets prepared for rats. Four groups of rats were considered (rats in group one were fed with died prepared without BASO or NMSO and denoted 0% group, rats in group two were fed with diets compounded with 10% groundnut seed oil (GNSO) while rats in the remaining two groups were fed with diets prepared with 10% and 20% of each oil sample) and the results were displayed on Table 4.24 and Figure 4.5a.

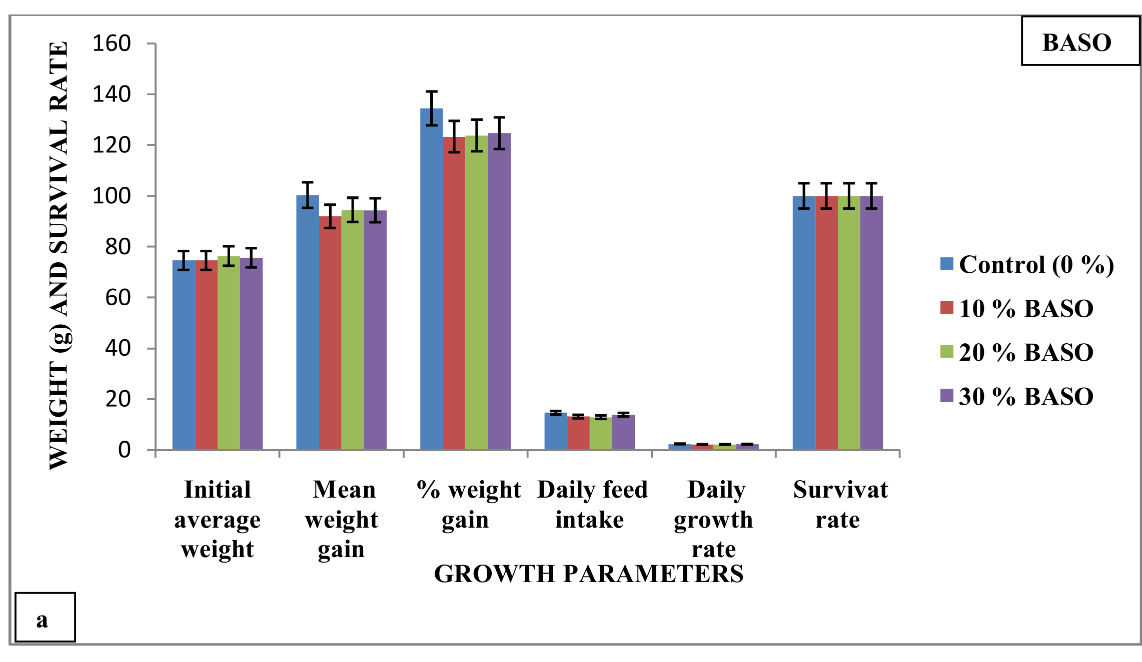
The average initial rats' weight recorded at the commencement of the experiment was respectively noted as 74.60 g (0%), 74.60 g (10% GNSO), 76.36 g (10% BASO) and 75.65 g (20% BASO). The weight gain (g) was 100.29 (0%), 91.96 (10% GNSO), 94.77 (10% BASO) and 94.33 (20% BASO) respectively. The highest percentage weight gain of 134.41, 123.29, 123.77 and 124.66 respectively obtained for 0%, 10% GNSO, 10% BASO and 20% BASO were comparable to each other. No significant different at $P \leq 0.05$ was observed in the daily feed intake, daily growth rate and survival rate as shown on Table 4.24 and Figure 4.5a. The survival rate was 100% within all the groups showing no loss of animal. This result with good comparison showed that *B. aegyptiaca* seed oil has a good and positive effect on rats' body weight, feed-intake, growth performance and survival rate with no mortality. This seed oil, if properly refined could be good nutritional supplement and toxic free to human.

The effect of NMSO on rats' body weight, feed-intake, growth performance and survival rate was noted respectively on Table 4.24 and Figure 4.5b. The initial average rats' body weights were 68.86 g (0%) to 76.57 g (10% GNSO), 77.71 g (10% NMSO) and 78.29 g (20% NMSO). The mean weight gain (g) recorded after the experimental period was 105.45, 102.03, 121.30 and 105.85 respectively in 0%, 10% GNSO, 10% NMSO and 20% NMSO. The highest percentage weight gain of 156.08 (10% NMSO) compared favourably with 153.12 (0%). The lowest percentage weight gain of 133.20 was recorded in 10% GNSO group which has good comparison with 135.16 (20% NMSO). There was

Table 4.24: Effect of BASO and NMSO on rats' body weight, feed-intake, growth performance and survival rate

Parameter	0%	10% GNSO	10%	20%
		BASO		
Initial weight	74.60±1.58^{a}	74.60±0.39^{a}	76.36±0.35^{a}	75.65±0.73^{a}
Feed intake/week	102.62±10.10^{a}	92.39±7.84^{b}	90.47±6.30^{b}	97.40±6.75^{a}
Final weight	174.89±4.43^{a}	166.57±4.81^{a}	170.88±3.77^{a}	169.98±4.94^{a}
Weight gain	100.29±2.86^{a}	91.96±5.19^{a}	94.51±3.88^{a}	94.33±4.94^{a}
SGR	2.02±0.07^{a}	1.88±0.08^{a}	1.92±0.04^{a}	1.93±0.08^{a}
Mean weight gain	100.29±2.86^{a}	91.96±5.19^{a}	94.51±3.88^{a}	94.33±4.94^{a}
% Weight gain	134.41±1.12^{a}	123.29±7.59^{b}	123.77±5.30^{ab}	124.66±5.54^{ab}
Feed intake/day/g	14.65±1.44^{a}	13.19±1.11^{a}	12.92±1.55^{a}	13.87±1.66^{a}
FCR	6.03±0.41^{a}	5.90±0.34^{a}	5.55±0.17^{a}	5.00±0.35^{a}
Daily growth rate	2.38±0.05^{a}	2.19±0.05^{a}	2.15±0.01^{a}	2.32±0.07^{a}
Survival rate	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
		NMSO		
Initial weight	68.86±17.12^{b}	76.57±3.18^{a}	77.71±7.63^{a}	78.29±9.16^{a}
Feed intake/week	113.03±0.02^{c}	126.86±0.01^{a}	119.61±0.01^{a}	123.11±0.01^{a}
Final weight	174.29±34.68^{b}	178.57±22.63^{b}	199.00±17.75^{a}	184.14±12.48^{a}
Weight gain	105.45±0.02^{b}	102.03±0.05^{d}	121.30±0.12^{a}	105.85±0.02^{c}
SGR	2.20±0.00^{b}	2.02±0.01^{d}	2.24±0.00^{a}	2.04±0.00^{c}
Mean weight gain	105.45±0.02^{b}	102.03±0.05^{d}	121.30±0.12^{a}	105.85±0.02^{c}
% Weight gain	153.12±0.02^{a}	133.20±0.00^{c}	156.08±0.01^{a}	135.16±0.05^{b}
Feed intake/day/g	16.13±0.02^{d}	18.11±0.01^{a}	17.09±0.00^{c}	17.59±0.00^{b}
FCR	6.43±0.01^{c}	7.46±0.03^{a}	5.91±0.01^{d}	6.98±0.01^{b}
Daily growth rate	2.50±0.00^{bc}	2.42±0.02^{c}	2.88±0.01^{a}	2.51±0.01^{bc}
Survival rate	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

Values are mean±SD of three determinations. Means with and having the same letter as superscripts on the same row are not significantly different ($P \leq 0.05$)
SGR: Specific growth rate



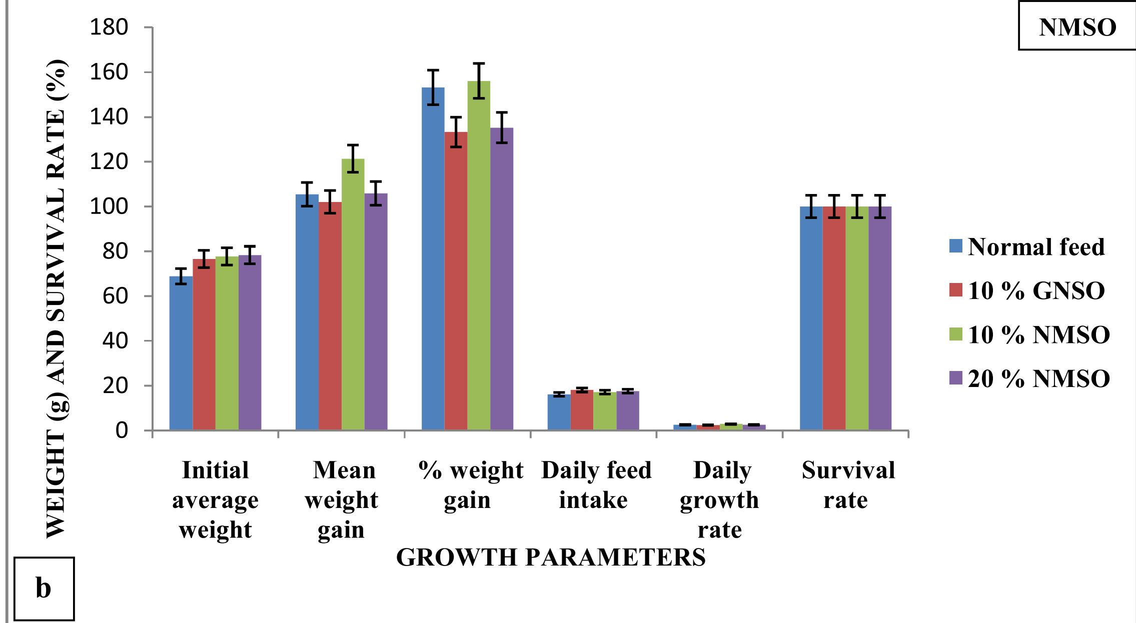


Figure 4.5: Graphical representations of the initial weight (g), mean weight gain (g), percentage weight (%), daily feed intake (%), daily growth rate (%) and survival rate (%) of rat fed with BASO (a) and NMSO (b)

no significant difference at $P \leq 0.05$ in the daily growth rate and feed intake per day across the groups (Table 4.23 and Figure 4.5b). The survival rate of 100% was found in all the experimental groups and the control. This result depicts that NMSO might have a very good effect on rats' body weight, feed-intake, growth and survival rate when used as supplement.

4.9.2.2: Effect of BASO and NMSO on internal organs weights of rats

The effect of BASO and NMSO on the organs mean weights of the rats was shown on Table 4.25 and Figure 4.6 a and b. The kidney weights of 1.04, 0.97, 1.11 and 1.17 g were obtained respectively for 0%, 10% GNSO, 10% BASO and 20% BASO. Similar results were recorded for the liver and brain. Generally, with this result, the organs weights recorded appeared to compare favourably with both 0% and (10% GNSO). This depicts that there was no significant difference in the organ weights in all the groups.

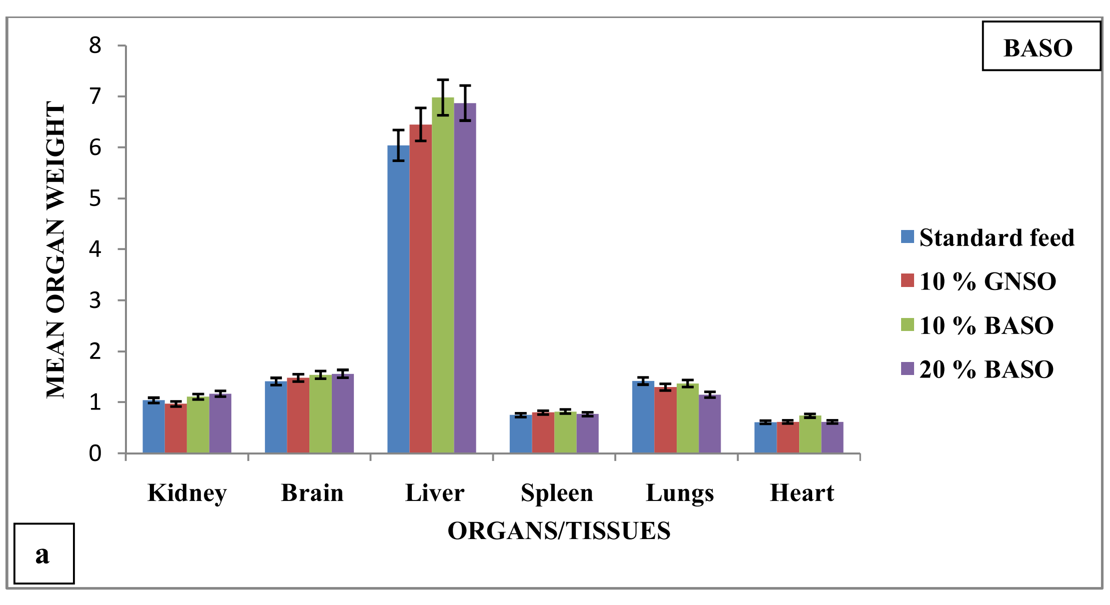
As shown in 4.25 and Figure 4.6b, the kidney weights were 1.21, 1.28 and 1.29 g respectively for 10% GNSO, 10% NMSO and 20% NMSO. These values obtained were equally comparable with 1.11 g (0%). The liver mean weight of 7.73 g obtained was higher in 10% NMSO than 6.81 g, (0%), 6.79 g (10% GNSO) and 6.87 g (20% NMSO. The value recorded in 20% NMSO compared perfectly with that of 10% GNSO. The weight of spleen obtained in 10% GNSO and 10% NMSO were comparable to each other but higher than that of 20% NMSO which was in turn higher than the value obtained in 0% (Figure 4.6b).

The absence of major significant difference at $P \leq 0.05$ in the weights of kidney, liver, spleen of rats fed with BASO and NMSO suggested through this study that BASO and NMSO had no major deleterious effect on the rats' organs weights. These seed oils could be good and useful nutritionally if properly refined or purified to remove some unwanted particles that might be present in them.

Table 4.25: Mean internal organs weight of rat fed with BASO and NMSO

Tissue	0%	10% GNSO	10%	20%
		BASO		
Kidney	1.04 ± 0.78^{ab}	0.97 ± 0.13^{b}	1.11 ± 0.06^{ab}	1.17 ± 0.48^{a}
Brain	1.41 ± 0.08^{b}	1.48 ± 0.69^{ab}	1.54 ± 0.53^{a}	1.56 ± 0.07^{a}
Liver	6.04 ± 0.51^{c}	6.45 ± 0.20^{b}	6.98 ± 0.15^{a}	6.87 ± 0.95^{a}
Spleen	0.75 ± 0.09^{a}	0.80 ± 0.08^{a}	0.82 ± 0.01^{a}	0.77 ± 0.04^{a}
Lungs	1.42 ± 0.07^{a}	1.30 ± 0.81^{a}	1.37 ± 0.04^{a}	1.15 ± 0.53^{a}
Heart	0.61 ± 0.03^{b}	0.62 ± 0.09^{b}	0.74 ± 0.05^{a}	0.62 ± 0.04^{b}
		NMSO		
Kidney	1.11 ± 0.17^{b}	1.21 ± 0.07^{ab}	1.28 ± 0.10^{a}	1.29 ± 0.04^{a}
Brain	1.56 ± 0.10^{a}	1.50 ± 0.16^{a}	1.60 ± 0.11^{a}	1.54 ± 0.17^{a}
Liver	6.81 ± 0.94^{a}	6.79 ± 0.81^{a}	7.73 ± 0.69^{a}	6.87 ± 0.77^{a}
Spleen	0.74 ± 0.17^{a}	0.91 ± 0.23^{a}	0.90 ± 0.30^{a}	0.80 ± 0.11^{a}
Lungs	1.50 ± 0.51^{a}	1.36 ± 0.21^{a}	1.30 ± 0.17^{a}	1.19 ± 0.18^{a}
Heart	0.60 ± 0.16^{ab}	0.57 ± 0.05^{ab}	0.65 ± 0.05^{a}	0.50 ± 0.08^{b}

Values are mean±SD of three determinations. Means having similar letters as superscripts on the same row are not significantly different ($P \leq 0.05$)



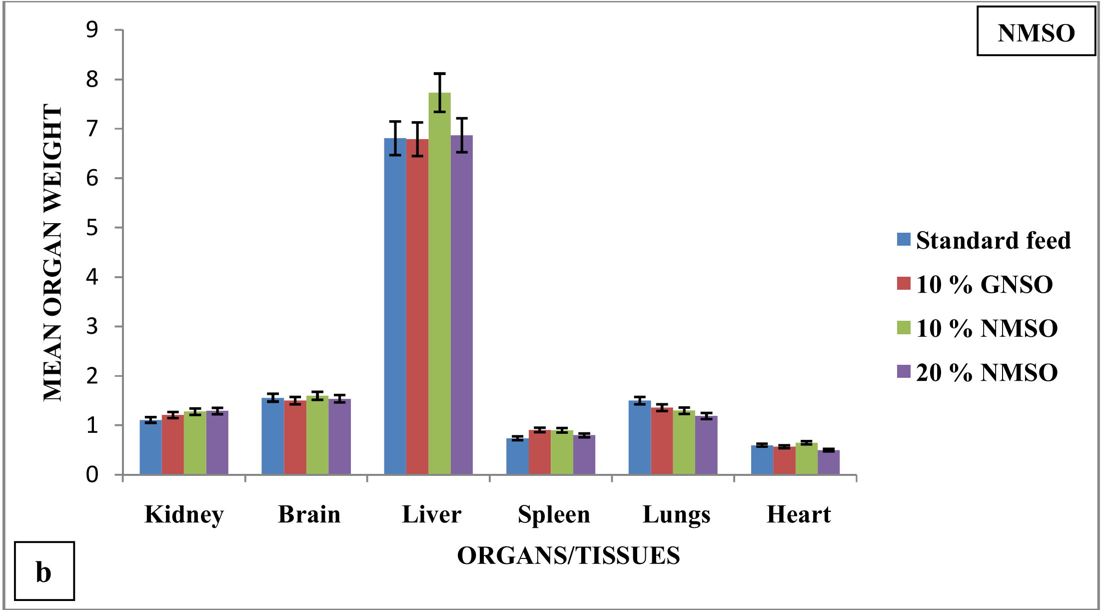


Figure 4.6: Graphical representation of mean weight (g) of rats' organs fed with BASO (a) and NMSO (b)

4.9.2.3: Blood haematological analysis of the rats' fed with BASO and NMSO

The effect of BASO and NMSO on haematological indices of the rats' blood is shown on Table 4.26. The Hb (haemoglobine concentration), PVC (packed cell volume) and platelet are comparable to each other in the group fed with groundnut oil (10% GNSO), 10% BASO and 20% BASO but these values obtained are lower than those in 0%. WBC value obtained is slightly lower within 10% BASO and 20% BASO when compared with those in the group fed with 0% and groundnut oil (10% GNSO). The MCH (mean corpuscular haemoglobine), MCV (mean corpuscular volume) and MCHC (mean corpuscular haemoglobine concentration) values recorded in the experimental and control group were also comparable to each other. The comparativeness and absence of any significant difference at $P \leq 0.05$ in this study gave an opinion that *B. aegyptiaca* seed oil might not have any negative effect on the blood haematological indices of rats. The effect of NMSO on blood haematological indices of rats was also shown on Table 4.26. PCV, Hb, RBC, MCV, MCH, and MCHC values were found to be comparable among the experimental group, control group, and the group fed with normal feed. However, there was a significant difference in the values of WBC and platelets observed across the groups. These results showed that NMSO did not have any adverse effects on the blood of the rats studied. The haematological outcomes of this study were consistent with those reported in a toxicity study of defatted *Garcinia mangostana* seeds on rats (Ajayi and Aghanu, 2012).

The NMSO and BASO showed no major adverse effect on the haematological parameter of the blood of rat at both 10% and 20% inclusion levels. They might be considered as good nutritional supplement in human food.

4.9.2.4: Effect of BASO and NMSO on biochemical parameters and lipid profile of rats' blood

The results of the effect BASO and NMSO on biochemical indices such as AST, ALP, ALT, albumin, globulin, total protein, creatinine and lipid profile of rats' blood were illustrted on Table 4.27 and Figures 4.7a-b. The evaluation of ALT, AST and ALP activities supplies important facts on liver functions. An increase of ALT in the liver leads to hepatocellular or liver cells damage. ATP is a membrane bound and changes in its

concentration would likely affect the membrane permeability and cause disorder in the metabolites movements. An increase in the level of ALP was always a parameter in the determination of liver infection, hepatitis and bone sickness (Aliyu *et al.,* 2006).

No significant differences were observed at $P \leq 0.05$ in the albumin, total protein and globulin. The value of AST enzyme obtained was 44.67, 42.10, 42.33 and 40.67 μL in (0%), 10% GNSO, 10% BASO and 20% BASO. The enzyme ALT value recorded ranged from 28.33 (0%) to 29.33 (10% GNSO), 29.33 (10% BASO) and 31.33 μL (20% BASO). The values of 83.33, 84.00, 84.33 and 89.33 μL were obtained correspondingly for ALP in 0%, 10% GNSO, 10% BASO and 20% BASO. A slight increase was observed in ALP values going from 83.33 μL in 0% to 89.33 μL in the group fed with 20% BASO. There were no significant differences at $P \leq 0.05$ in ALT as the values recorded compared favourably with each other within the various groups. A slightly decrease was observed in AST from 44.67 μL in the normal feed to 40.67 in 20% BASO. These slight increase and decrease respectively observed in ALP and AST are not too significant to effect any change on the animal as the values are still close to each other in the various groups. There were also no significant differences in the urea and creatinine obtained.

The results of blood biochemistry analyses and lipid profile of rats fed with NMSO were also shown on Table 4.27. The value of AST enzyme (μL) obtained was 40.67 (0%), 40.33 (10% GNSO), 40.00 (10% NMSO) and 41.67 (20% NMSO). The enzyme ALT value (μL) recorded were 28.00 (0%), 29.00 (10% GNSO), 28.67 (10% NMSO) and 30.00 μL (20% NMSO). The values of 76.00, 92.00, 103.00 and 89.00 μL were obtained respectively for ALP in 0%, 10% GNSO, 10% NMSO and 20% NMSO. A slight increase was observed in ALP values going from 76.00 μL (0%) to 103.00 μL (10% NMSO) and later reduced to 89.00 μL in the group fed with 20% NMSO. No significant differences at ($P \leq 0.05$) were obtained in both AST and ALT as the values obtained compared favourably with each other within the various groups under study.

The value of creatinine recorded ranged from 0.73 (0%) to 0.77 (10% GNSO), 0.77 (10% NMSO) and 0.77 mg/dL (20% NMSO). The values of 15.53, 16.67, 15.13 and 16.93 mg/dL were obtained correspondingly for urea in 0%, 10% GNSO, 10% NMSO and 20% NMSO. No significant difference was observed in both the values of urea and creatinine

Table 4.26: Result of haematological and blood biochemical analyses of rats fed with BASO diet

Parameters	0%	10% GNSO	10 %BASO	20% BASO
		Haematological analysis and lipid profile		
PCV (%)	42.67±1.53[a]	35.67±3.05[b]	35.00±1.00[b]	35.00±2.00[b]
Hb (g/dL)	14.30±0.70[a]	11.83±0.65[b]	11.93±0.49[a]	11.93±0.40[b]
RBC (10^6/µL)	4.21±0.55[a]	3.46±0.03[c]	3.40±0.07[c]	5.76±0.42[a]
WBC (10^6/µL)	1.73±0.21[a]	1.79±0.10[a]	1.49±0.24[a]	1.56±0.22[a]
Platelet (x 10^4)	12.30±0.96[a]	11.50±0.80[a]	11.33±0.94[a]	8.13±1.59[a]
Lymphocyte (%)	57.33±11.84[a]	59.66±3.05[a]	57.66±8.32[a]	63.33±5.68[a]
Neutrophyl (%)	39.33±3.78[a]	31.00±5.57[b]	36.00±8.88[a]	29.00±5.29[b]
Monocyte (%)	3.33±0.57[a]	3.66±0.57[a]	3.33±1.57[a]	3.33±0.58[a]
Eosinophyl (%)	4.00±1.00[a]	3.00±1.00[a]	2.33±1.52[a]	3.67±1.57[a]
MCV (fL)	102.20±10.58[a]	103.12±9.42[a]	102.82±0.68[a]	92.80±2.33[a]
MCHC (%)	33.51±0.52[a]	33.23±1.03[a]	35.05±0.90[a]	30.74±0.88[a]
MCH (pg)	34.20±3.05[a]	34.21±2.12[a]	34.09±0.18[a]	19.75±0.13[a]
		Biochemistry analysis and lipid profile		
Total protein (g/dL)	7.73±0.11[a]	7.73±0.11[a]	7.80±0.20[a]	8.00±0.20[a]
Albumin (g/dL)	3.26±0.76[a]	3.06±0.14[a]	3.30±0.26[a]	3.00±0.20[a]
Globulin (g/dL)	2.69±0.20[a]	2.60±0.20[a]	2.73±0.11[a]	2.53±0.23[a]
Albumin/Globulin	1.22±0.05[a]	1.12±0.09[a]	1.20±0.12[a]	1.18±0.13[a]
AST (µL)	44.67±0.57[a]	42.10±1.00[ab]	42.33±2.51[ab]	40.67±0.57[b]
ALT (µL)	28.33±0.57[b]	29.33±0.57[ab]	29.33±3.79[ab]	31.33±1.15[a]
ALP (µL)	83.33±2.30[b]	84.00±2.00[b]	84.33±1.52[b]	89.33±1.15[a]
Urea (mg/dL)	15.00±1.00[a]	15.67±0.57[a]	15.00±1.00a	15.67±0.57[a]
Creatinine (mg/dL)	0.69±0.05[a]	0.73±0.05[a]	0.73±0.57[a]	0.77±0.05[a]
Total Cholesterol	55.33±3.05[a]	59.33±1.15[a]	56.66±2.30[a]	58.66±1.15[a]
Triglyceride	54.02±2.00[b]	64.33±2.00[a]	48.67±1.15 [c]	52.62±1.15[bc]
HDL	31.33±1.15[b]	30.67±1.15[b]	33.33±1.15[a]	34.00±0.00[a]

Values are mean±SD of three determinations. Means having similar letters as superscripts on the same row are not significantly different ($P \leq 0.05$)

Table 4.27: Result of haematological and blood biochemical analyses of rats fed with NMSO diet

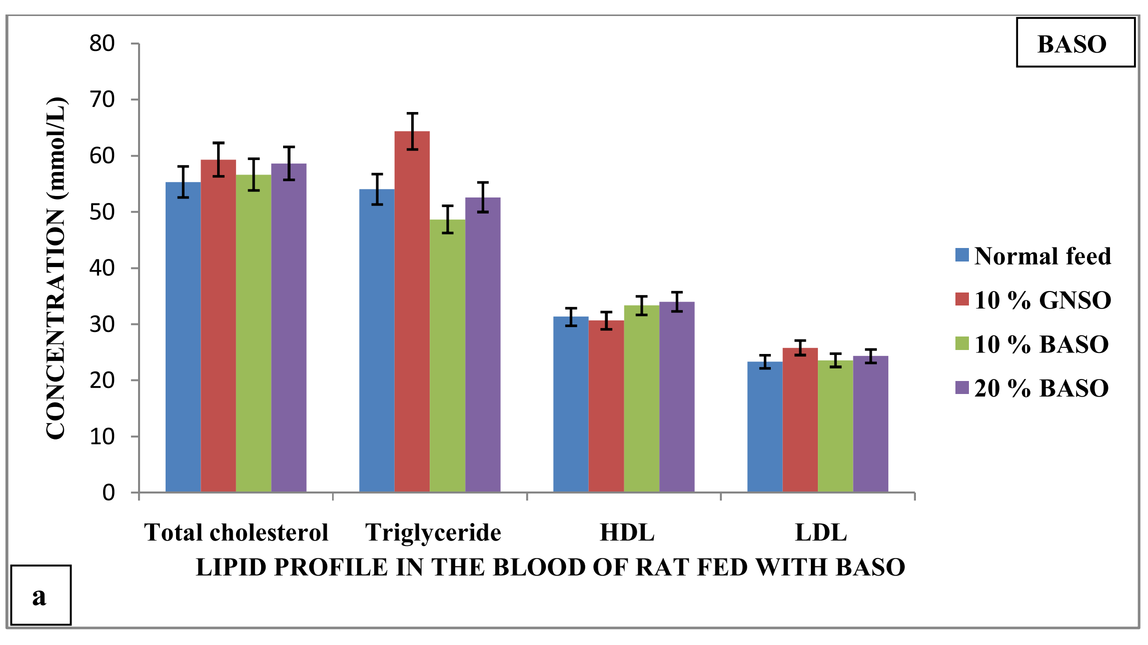
Parameters	0%	10% GNSO	10% NMSO	20% NMSO
Haematological analysis and lipid profile				
PCV (%)	40.25±3.59^{a}	39.75±6.55^{a}	45.25±5.25^{a}	42.25±3.59^{a}
Hb (g/dL)	13.35±0.97^{a}	13.08±2.40^{a}	14.85±1.75^{a}	13.83±0.89^{a}
RBC (10^6/μL)	6.66±0.41^{a}	6.55±1.43^{a}	7.58±0.94^{a}	7.00±0.46^{a}
WBC (10^6/μL)	4.64±0.53^{a}	5.51±0.76^{a}	4.63±0.99^{a}	5.38±0.10^{a}
Platelet (x 10^4)	28.35±2.98^{a}	22.10±6.0^{ab}	16.40±9.98^{b}	17.05±4.14^{b}
Lymphocyte (%)	65.50±5.32^{a}	68.00±3.74^{a}	70.25±2.99^{a}	65.75±4.35^{a}
Neutrophyl (%)	31.00±6.27^{a}	28.00±3.16^{a}	26.25±3.10^{a}	30.25±4.65^{a}
Monocyte (%)	1.75±0.50^{a}	2.00±0.82^{a}	1.75±0.50^{a}	2.50±1.00^{a}
Eosinophyl (%)	1.75±0.96^{a}	2.00±0.82^{a}	1.75±0.50^{a}	3.00±0.00^{a}
MCV (fL)	60.37±1.79^{a}	61.40±4.34^{a}	59.78±0.98^{a}	60.30±1.28^{a}
MCHC (%)	33.21±0.78^{a}	32.82±0.78^{a}	32.81±0.25^{a}	32.77±0.80^{a}
MCH (pg)	20.03±0.25^{a}	20.13±1.09^{a}	19.60±0.18^{a}	19.75±0.13^{a}
Biochemistry analysis and lipid profile				
Total protein (g/dL)	7.90±0.66^{a}	7.97±0.38^{a}	7.97±0.29^{a}	8.07±0.32^{a}
Albumin (g/dL)	3.10±0.72^{a}	2.93±0.76^{a}	2.90±0.82^{a}	2.63±0.85^{a}
Globulin (g/dL)	4.80±0.10^{a}	5.03±0.38^{a}	5.07±0.55^{a}	5.10±0.89^{a}
Albumin/Globulin	0.60±0.17^{a}	0.57±0.21^{a}	0.53±0.25^{a}	0.50±0.26^{a}
AST (μL)	40.67±1.52^{a}	40.33±2.52^{a}	40.00±1.73^{a}	41.67±3.22^{a}
ALT (μL)	28.00±2.65^{a}	29.00±3.46^{a}	28.67±3.79^{a}	30.00±3.00^{a}
ALP (μL)	76.00±7.21^{b}	92.00±19.47^{ab}	103.00±3.00^{a}	89.00±6.25^{ab}
Urea (mg/dL)	15.53±1.17^{ab}	16.67±0.57^{a}	15.13±0.38^{b}	16.93±0.64^{a}
Creatinine (mg/dL)	0.73±0.23^{a}	0.77±0.06^{a}	0.77±0.06^{a}	0.77±0.21^{a}
Total Cholesterol	50.66±1.15^{c}	69.33±1.15^{a}	58.67±1.15^{b}	68.67±1.15^{a}
Triglyceride	37.33±1.15^{c}	57.31±1.15^{a}	47.66±2.51^{b}	49.33±3.00^{b}
HDL	29.67±1.52^{c}	33.00±2.00^{b}	29.33±1.15^{c}	38.67±1.15^{a}
LDL	26.33±1.15^{ab}	29.00±1.73^{a}	24.47±0.50^{b}	25.10±0.52^{ab}

Values are mean±SD of three determinations. Means having similar letters as superscripts on the same row are not significantly different ($P \leq 0.05$).

as these values were compared to each other within the groups. The absence of any major change in the blood biochemistry indices was perfect evidence that there was no hepatocellular damage in the liver, the membrane permeability was not affected and no derangement was observed in the transport of metabolites in ALP. The NMSO might not be toxic on the kidney, liver and other blood parameters.

Figure 4.7a revealed the graphical representation of the lipid profile in the blood of rats fed with BASO. The total cholesterol value recorded was from the lowest in the control group to the highest in 10% GNSO. The total cholesterol values obtained in 10% and 20% BASO were lower than those of 0% group. There was a slight decrease in the triglyceride values in the test groups as compared with 10% GNSO. HDL (High-Density Lipoprotein) wss found higher in 10% and 20% BASO (experimental groups) and lower in 0% and 10% GNSO. The low-Density-Lipoprotein (LDL) found in experimental groups (10% and 20% BASO) was between the LDL value observed in the group fed with 0% and 10% GNSO.

Figure 4.7b presented the graphical representation of the lipid profile in the blood of rats fed with NMSO. The total cholesterol obtained was comparable within the 10% GNSO, 10% NMSO and 20% NMSO but higher than that in the rat fed with 0%. The triglyceride obtained is higher in 10% GNSO; it compared with those between 10% and 20% NMSO while lower in 0%. HDL was found highest in 20% NMSO group when compared with other groups. LDL was lowest in the 10% NMSO group than in others. Total cholesterol, Low LDL, Triacylglycerol and low HDL were useful and important indices in the cardiovascular disorders such as hypertension, arteriosclerosis, stroke and heart failure (Ghasi and Ofili, 2000). The increase in concentrations of cholesterol may be due to the slow uptake of chylomicron remnants originating from the saturated fatty acids in the oils (Ghasi and Ofili, 2000). In summary, the parameters obtained compared very well with each other as they were no major significant difference between them at $P \leq 0.05$. These seed oils seem to be very good nutritionally if properly refined to remove unwanted materials and could be useful raw material in several chemical industries.



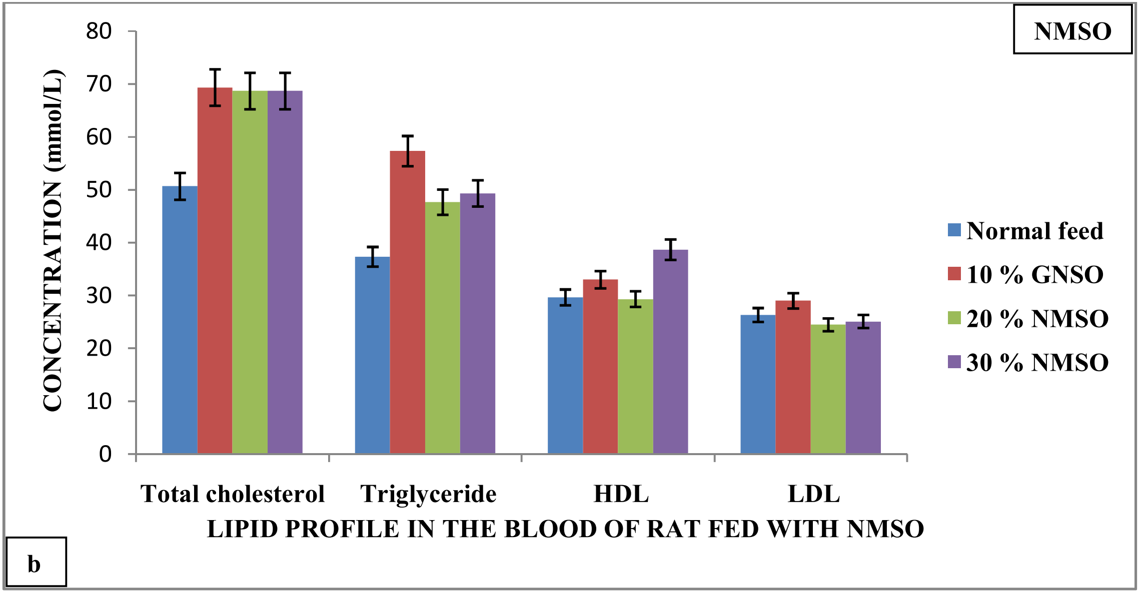


Figure 4.7: Graphical representation of the lipid profile in the blood of rats fed with BASO (a) and NMSO (b)

4.9.2.5: Histopathological studies of organs of the rats fed with BASO and NMSO

Table 4.28 showed the results of the histopathological examination of the kidney, heart, and liver of rats fed with BASO and NMSO. No abnormalities were detected in the heart and kidney sections of 0% group, as well as 10% and 20% BASO groups. In the liver section, the hepatic plates were closely packed with moderate thinning and no visible lesion in 0% and 10% GNSO groups. However, random foci of single-cell hepatocellular necrosis were observed in the experimental groups (10% and 20% BASO).

No abnormalities were detected in the heart section of the rat fed on NMSO. The glomeruli, tubules and renal interstitial appear normal in the kidney sections of the rats in 0% and experimental groups (10% and 20% NMSO) while locally extensive foci of mild sloughing off and flattening of epithelium of tubules in the renal medulla cortico-medullary junction was observed in the section of the kidney of the rats fed with 10% GNSO. Within the sections of the liver samples, 0% and 10% GNSO group displayed several areas where hepatocytes exhibited mild vacuolar changes, as well as widespread marked vacuolar changes. On the other hand, in the experimental groups (10% and 20% NMSO), multiple areas showed moderate thinning of hepatic plates, resulting in the dilation of hepatic sinusoids.

The study of the histological examination of the tissues was useful information that identifies the kind of lesion was caused by xenobiotics and it was acknowledged as the important end point for detecting organ toxicity (Sellers *et al.,* 2007). Kidney and liver were major organs that were mostly targeted by toxic chemicals. Because of their essential roles in the process of removing injurious substances from the body and excretion process, susceptibility to harmful compound and their ability in predicting toxicity, they were majorly useful in toxicity studies (Sellers *et al.,* 2007).

The histological examination result given on Table 4.28 revealed no major changes in the architecture of the internal organs of both the controls and the test rats. The minor differences observed might not be attributed to BASO and NMSO since they were also seen in the control groups. The observation could be from the commercial feed employed

Table 4.28: Histopathology analysis of tissues from rats fed with BASO and NMSO

Organ	Heart	Kidney	Liver
		BASO	
Normal	The observed congestion of coronary blood vessels is moderate	The glomeruli, tubules and renal interstitium appear normal. Visible lesion not seen	There was a dense arrangement of hepatic plates, and no visible lesions are apparent
Control (10% GNSO)	The observed congestion of coronary blood vessels was moderate	There was no observable lesion	Hepatic plates are moderately thinning.
10% BASO	There was no observable lesion. Cardiomyocytes appear normal.	There was no observable lesion	Single-cell recrosis of the hepatocytes were observed in random foci, but no visible lesions are apparent
20% BASO	There was no observable lesion	There was no observable lesion	Single-cell recrosis of the hepatocytes were observed in random foci. No visible lesion
		NMSO	
Normal	The observed congestion of coronary blood vessels is moderate	The glomeruli, tubules and renal interstitium appeared normal	There was a dense arrangement of hepatic plates. There are multiple foci of mild vacuolar change of hepatocytes
Control (10% GNSO)	The observed congestion of coronary blood vessels is moderate	Locally extensive foci of flattening epithelium of tubules was seen in the renal medulla cortico-medullary junction	Hepatic plates were moderately thinning. There was widespread marked vacuolar change of hepatocytes
10% NEMSO	Visible lesion not seen as the cardiomyocytes appeared normal	No visible lesion was seen in the glomeruli, tubules and renal interstitium as they appeared normal	There were multiple foci of moderate thinning of hepatic plates with consequent dilation of hepatic sinusoids
20% NEMSO	Visible lesion not seen as the cardiomyocytes appeared normal	There were few foci of flattening epithelium of tubules. The glomeruli, tubules and renal interstitium appeared normal	There were multiple foci of moderate thinning of hepatic plates with consequent dilation of hepatic sinusoids

BASO: *Balanites aegyptiaca* seed oil
NMSR: *Neocarya macrophylla* seed oil

in feeding the rats during the acclimatisation period. The absence of major alterations or changes in the levels of ALT, AST, ALP, creatinine and urea which are good indicators of liver and kidney functions (Jothy *et al*., 2001) suggested that BASO and NMSO did not alter the normal metabolism in the animals and supported the detected histopathological examinations.

The relative organ weight as reported by Mayur *et al*. (2017) is necessary and important in establishing whether an organ is exposed to damage or not. This always correlates well with histopathological changes that might be observed. The resemblance observation in the relative organ weight of rats in control and experimental groups showed that none of the organs were adversely affected and had no signs of toxicity as revealed in the histopathological examination. The BASO and NMSO showed no adverse effect of chemicals on the organs (liver and kidney) of the experimental rats. Therefore, they might not be toxic or harmful when used by human nutritionally.

4.9.3 Application of seed flours (ACSF, PLSF) and residues (BASR, NMSR) as additive in catfish feed formulation and feeding (Nutritional application)

4.9.3.1 Proximate composition of fish diets containing seed flours (ACSF and PLSF) and seed residues (BASR and NMSR)

The proximate composition of diets formulated with seed flours (ACSF, PLSF) and seed residues (BASR, NMSR) were shown on Table 4.29. The diets were comparable in moisture, dry matter, crude fibre and ash contents within the control and experimental groups. There was a gradual increase in the carbohydrate contents with increase in inclusion level of ACSF and PLSF across the groups because of the high carbohydrate values of ACSF and PLSF. Table 4.29 equally revealed a steady increase in the values obtained for protein across the group with 30% of BASR and NMSR having the highest concentration. This increase was as result of high protein content in BASR and NMSR. The energy values in cal/100g were similar to each other within the control and experimental groups. Proteins were essential component of the diet that is needed for survival of animals, humans and of which basic function in nutrition was to supply adequate amounts of required amino acids (Ajayi *et al.,* 2013c). It was observed from Table 4.29 that there were significant differences ($p \leq 0.05$) among the various feed

Table 4.29: Proximate composition of diets formulated from ACSF, PLSF, BASR and NMSR for catfish (*C. gariepinus*)

Parameters	Seed sample	0%	10%	20%	30%
			ACSF		
Carbohydrate	57.55 ± 0.01^{a}	35.67 ± 0.12^{b}	36.42 ± 0.06^{b}	43.26 ± 0.12^{c}	45.08 ± 0.24^{c}
Crude protein	6.42 ± 0.13^{d}	37.94 ± 0.13^{a}	36.48 ± 0.127^{a}	32.03 ± 0.16^{b}	30.28 ± 0.13^{b}
Moisture	14.54 ± 0.04^{a}	10.07 ± 0.01^{c}	10.57 ± 0.035^{b}	10.50 ± 0.02^{b}	10.86 ± 0.05^{b}
Crude fibre	18.28 ± 0.02^{a}	4.53 ± 0.03^{e}	7.28 ± 0.03^{d}	9.10 ± 0.02^{c}	10.47 ± 0.01^{b}
Ash	3.22 ± 0.00^{d}	10.63 ± 0.06^{a}	10.93 ± 0.16^{a}	9.28 ± 0.03^{b}	9.07 ± 0.07^{c}
Crude fat	0.27 ± 0.01^{d}	5.68 ± 0.03^{a}	5.59 ± 0.03^{a}	4.92 ± 0.03^{b}	4.71 ± 0.03^{c}
Dry matter	85.46	89.93	89.43	89.50	89.14
Energy(Cal/100g)	1097.48	1461.53	1446.13	1461.97	1455.39
			BASR		
Moisture	8.45 ± 0.03^{a}	7.90 ± 0.05^{a}	7.95 ± 0.04^{a}	8.17 ± 0.02^{a}	8.21 ± 0.03^{a}
Protein	51.31 ± 0.13^{a}	40.71 ± 0.28^{b}	41.40 ± 0.12^{b}	42.12 ± 0.10^{b}	44.40 ± 0.60^{a}
Fat content	6.90 ± 0.04^{d}	8.36 ± 0.04^{bc}	8.40 ± 0.00^{bc}	8.45 ± 0.10^{a}	8.64 ± 0.02^{a}
Ash content	4.40 ± 0.02^{b}	10.21 ± 0.07^{a}	10.93 ± 0.30^{a}	11.04 ± 0.25^{a}	10.98 ± 0.05^{a}
Fibre content	12.90 ± 0.02^{a}	5.70 ± 0.03^{c}	5.39 ± 0.0^{c}	7.40 ± 0.03^{b}	7.66 ± 0.02^{b}
Carbohydrate	16.03 ± 0.18^{a}	27.04 ± 0.30^{a}	27.08 ± 0.01^{a}	22.80 ± 0.01^{b}	20.11 ± 0.06^{c}
Dry matter	91.55	92.10	92.05	91.43	91.79
Energy(Cal/100g)	1400.08	1461.07	1474.96	1416.29	1416.35
			PLSF		
Protein	12.40 ± 0.25^{e}	41.74 ± 0.25^{a}	37.18 ± 0.05^{b}	36.05 ± 0.13^{c}	34.70 ± 0.57^{d}
Moisture	9.20 ± 0.03^{a}	7.90 ± 0.05^{c}	8.20 ± 0.21^{b}	8.21 ± 0.15^{b}	8.22 ± 0.06^{b}
Crude Fibre	11.90 ± 0.04^{a}	5.78 ± 0.30^{d}	5.68 ± 0.03^{e}	6.38 ± 0.03^{c}	7.48 ± 0.11^{b}
Crude Ash	4.26 ± 0.01^{a}	8.36 ± 0.04^{a}	7.86 ± 0.05^{b}	7.09 ± 0.02^{c}	6.56 ± 0.42^{d}
Fat	2.58 ± 0.02^{e}	10.31 ± 0.07^{a}	8.73 ± 0.10^{b}	8.37 ± 0.76^{c}	7.86 ± 0.40^{d}
Carbohydrate	59.66 ± 0.19^{a}	25.90 ± 0.29^{e}	32.28 ± 0.26^{d}	33.89 ± 0.23^{c}	35.24 ± 1.29^{b}
Dry matter	90.80	92.10	91.80	91.79	91.78
Energy(Cal/100g)	1320.48	1531.35	1503.83	1498.67	1479.80
			NMSR		
Moisture	12.32 ± 0.53^{a}	10.30 ± 0.26^{b}	10.15 ± 0.05^{a}	10.03 ± 0.15^{a}	10.08 ± 0.07^{a}
Protein	56.04 ± 0.01^{a}	34.91 ± 0.08^{d}	36.05 ± 0.08^{c}	37.51 ± 0.07^{c}	39.97 ± 0.20^{a}
Fat content	4.51 ± 0.01^{a}	6.35 ± 0.30^{a}	6.20 ± 0.10^{bc}	5.98 ± 0.10^{bc}	5.76 ± 0.06^{d}
Ash content	6.53 ± 0.01^{a}	10.76 ± 0.25^{a}	9.95 ± 0.29^{a}	8.93 ± 0.79^{b}	8.05 ± 0.13^{c}
Fibre content	7.41 ± 0.01^{a}	5.18 ± 0.07^{c}	5.85 ± 0.05^{b}	5.97 ± 0.06^{b}	6.16 ± 0.08^{a}
Carbohydrate	13.19 ± 0.42^{a}	32.16 ± 0.85^{a}	31.80 ± 0.31^{a}	31.58 ± 0.61^{a}	30.02 ± 0.43^{b}
Dry matter	87.68	89.70	89.85	89.97	92.98
Energy(Cal/100g)	1343.78	1375.14	1382.85	1395.79	1402.95

Values = means±SD for three samples (n=3). Means followed by different letters superscripts on the same row are significantly different at ($P\leq 0.05$).

ACSF: *Areca catechu* seed flour

BASR: *Balanites aegyptiaca* seed residue

NMSR: *Neocarya macrophylla* seed residue

PLSF: *Polyalthiaa longifolia* seed four

compounded for this experiment in their protein, fat, crude fibre, ash, moisture and carbohydrate contents.

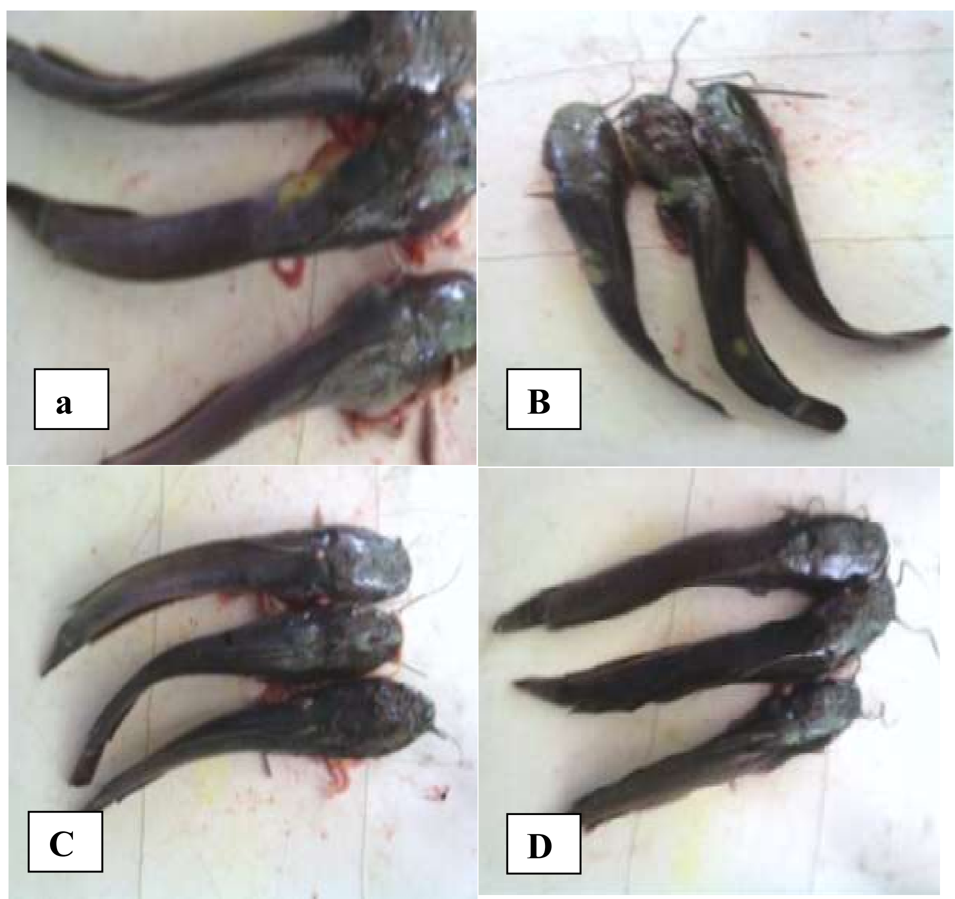
4.9.3.2 Proximate composition of the fish after experiment

The results of the proximate analysis of the fish after the experiment (plate 1) were given on Table 4.30. All the fish in the experimental diets had high protein content in their tissues. The protein and fat contents obtained in the fish fed with ACSF, BASR, NMSR ad PLSF were comparable with each other in all the groups. The higher values were obtained in the 30 % inclusion levels. Values (%) of 79.55 (ACSF), 73.96 (PLSF), 74.73 (BASR) and 78.80 (NMSR) were recorded.

Fat content was found highest in the 30% inclusion within all the samples while other parameters were comparable. These values compared favourably with the control and this suggested that the various seed flours and residues contributed perfectly and positively to the growth and nutrition of the fish. They could serve as sources of nutrients.

4.9.3.3 Effect of ACSF, BASR, NMSR and PLSF on growth performance and protein utilisation efficiency of fish

The result of growth performance and nutrients utilisation of ACSF and BASR were summarised on Table 4.31 and Figures 4.8 (a-b) while those of NMSR and PLSF were recapitulated on Table 4.32 and Figures 4.8 (c-d). As reported on Table 4.31 and Figure 4.8a, the catfish with initial weight that ranged from 4.55 g (0% ACSF) to 5.53 g (30% ACSF) were used. Fish fed with 0% diet had the best body weight gain of 8.14 g, followed by 7.88 g in 30% (ACSF) diet with the least value of 7.20 recorded in 10% (ACSF). The fish responses to the formulated diets indicated that there were significant differences in the growth and nutrient utilisation among the various treatments. The mean weight gain, nitrogen metabolism and the survival rate were better in 30% (ACSF) diets than in 10% (ACSF) and 20% (ACSF) diets. The condition factor increased from 0.80 (10%) to 0.95 (30%) with 0.82 at 0% (ACSF). Condition factor above 1 was reported to indicate better feed utilisation by fish for growth and development (Edward *et al.*, 2010). There was no significant difference in other studied parameters. These results confirmed that ACSF might be used as as additive in fish feed.



A. Fish fed with diet containing 0% of sample after experiment
B. Fish fed with diet containing 10% of sample after experiment
C. Fish fed with diet containing 20% of sample after experiment
D. Fish fed with diet containing 30% of sample after experiment

Plate 4.1: Sample of *C. gariepinus* after experiment

Table 4.30: Proximate analysis of catfish (*C. gariepinus*) fed on diets formulated with ACSF, BASR, PLSF and NMSR

Parameters	0%	10%	20%	30%
		ACSF		
Moisture	4.22±0.01^{b}	5.37±0.03^{a}	3.88±0.03^{d}	4.13±0.03^{c}
Crude Protein	80.43±0.08^{a}	75.67±0.13^{d}	78.22±0.13^{c}	78.55±0.05^{b}
Fat content	9.05±0.03^{a}	9.07±0.03^{c}	9.24±0.01^{b}	6.07±0.02^{d}
Ash content	6.20±0.03^{d}	9.70±0.02^{b}	7.92±0.03^{c}	10.24±0.02^{a}
Crude fibre	0.00	0.00	0.00	0.00
		BASR		
Moisture	10.49±0.10^{e}	11. 26±0.05^{c}	11.70±0.10^{b}	11.85±0.05^{b}
Protein	76.43±0.15^{a}	72.91±0.02^{d}	73.63±0.32^{c}	74.73±0.05^{a}
Fat content	8.80±0.10^{c}	10.10±0.10^{a}	9.40±0.15^{b}	8.70±0.32^{c}
Ash content	4.23±0.05^{e}	5.31±0.16^{b}	4.84±0.08^{c}	4.40±0.26^{d}
Crude fibre	0.23±0.05^{b}	0.42±0.10^{a}	0.34±0.2^{ab}	0.36±0.10^{ab}
		PLSF		
Moisture	11.71±0.05^{a}	11. 46±0.03^{b}	12.00±0.02^{a}	12.12±0.03^{a}
Crude Protein	71.88±0.07^{e}	72.26±0.05^{b}	73.03±0.03^{c}	73.96±0.01^{b}
Ash content	8.94±0.08^{a}	8.91±0.01^{a}	8.01±0.02^{b}	8.12±0.02^{b}
Crude fibre	0.28±0.03^{c}	0.33±0.02^{b}	0.36±0.01^{ab}	0.38±0.01^{a}
Crude fat	7.16±0.05^{a}	7.01±0.05^{b}	6.58±0.11^{c}	5.41±0.01^{d}
		NMSR		
Moisture	8.56±0.05^{a}	9. 63±0.11^{b}	9.68±0.02^{a}	9.13±0.05^{c}
Protein	80.13±0.05^{d}	75.72±0.16^{ab}	76.13±0.06^{ab}	78.80±0.20^{b}
Fat content	7.06±0.05^{d}	9.11±0.02^{a}	8.87±0.19^{ab}	7.32±0.76^{c}
Ash content	3.86±0.05^{e}	5.10±0.02^{b}	4.93±0.04^{c}	4.36±0.05^{d}
Crude fibre	0.33±0.02^{b}	0.38±0.10^{a}	0.3±0.02^{b}	0.30±0.00^{b}

Results are means±SD for three samples. Means followed by different letters as superscripts on the same row are significantly different at ($P \leq 0.05$).
ACSF: *Areca catechu* seed flour
BASR: *Balanites aegyptiaca* seed residue
NMSR: *Neocarya macrophylla* seed residue
PLSF: *Polyalthiaa longifolia* seed four

Table 4.31 and Figure 4.8b also showed the growth performance and protein utilisation efficiency of fish fed with BASR. Catfish with initial weigh that were from 27.06 g (0% BASR) to 31.90 g (30% BASR) were used. The body weight gain (g) recorded increased gradually from 19.50 (0%) to 32.00 (30%) diets. The specific growth rate, protein efficiency ratio, average growth rate, relative growth rate, condition factor and nitrogen metabolism increased gradually with the inclusion level of BASR. The lowest was obtained in 0% BASR diet while the highest in 30% BASR. Feed formulated with BASR at 30% showed better growth performance than the control and other experimental groups. This showed that BASR could be considered as good additive in formulating feed for fish.

The growth performance and protein utilisation efficiency of fish fed with NMSR was recapitulated on Table 4.32 and Figure 4.8c. The initial average body weight of the catfish used was between 27.83 g (0% NMSR) and 30.67 g (30% NMSR). The body weight gain (g) of 21.63; 22.59; 27.30 and 38.10 were respectively found for 0%, 10%, 20% and 30%. The experimental diets showed a gradual increase in parameters such as weight gain percentage, protein efficiency ratio, specific gravity, average and relative growth rate, survival rate and nitrogen metabolism. The highest values were observed at the 30% inclusion level while the lowest values were seen at the 0% inclusion level. The food conversion ratio (1.99) was found higher in 10% than in any other group. NMSR revealed better growth performance at 30%. This might be due to high palatability and attractive odour of the seed residue.

The growth performance and protein utilisation efficiency of fish fed with PLSF is also summarised on Table 4.32 and Figure 4.8d. Catfish with initial weigh between 27.76 g (0 % PLSF) and 28.98 g (30% PLSF) were used. The body weight gain (g) recorded increased gradually in the experimental groups with the highest in the control diets. 38.38 g (0% PLSF) obtained is followed by 32.29 g (30% PLSF) while 22.97 g and 20.11 g recorded in 10% PLSF and 20% PLSF are low. The specific growth rate, protein efficiency ratio, average growth rate, relative growth rate, condition factor and nitrogen metabolism increased gradually with the inclusion level and compared favourably at 30% with the control (0%) group. The PLSF might not have any deleterious effect on the growth of fish and the results obtained suggested that this seed flour might be employed

Table 4.31: Growth performance and nutrients utilisation of *C. gariepinus* fed with diets formulated from ACSF and BASR

Parameter	0%	10%	20%	30%
		ACSF		
Initial weight (g)	4.99±0.62[a]	4.66±0.43[a]	4.55±0.25[a]	5.53±1.00[a]
Final weight (g)	13.14±1.52[a]	11.87±0.83[a]	11.77±0.80[a]	13.45±1.46[a]
Weight gain (g)	8.14±1.79[a]	7.20±1.12[b]	7.39±0.72[b]	7.88±1.05[a]
% Weight gain	166.22±49.05[a]	156.66±38.74[a]	164.39±12.96[a]	145.32±29.45[b]
Relative growth rate	166.22±49.05[a]	156.66±38.74[a]	164.39±12.96[a]	145.32±29.45[b]
Daily growth rate (g/day)	0.15±0.03[a]	0.13±0.02[a]	0.13±0.01[a]	0.14±0.01[a]
Specific growth rate	0.75±0.14[a]	0.73±0.11[a]	0.74±0.05[a]	0.69±0.01[a]
Food conversion ratio	1.32±0.27[a]	1.38±0.12[a]	1.32±0.12[a]	1.41±0.01[a]
Protein efficiency ratio	0.27±0.06[a]	0.24±0.03[a]	0.25±0.02[a]	0.26±0.03[a]
Nitrogen metabolism	278.70±22.68[a]	254.20±9.92[a]	251.02±14.53[a]	307.23±53.84[a]
Condition factor (K)	0.82±0.05[a]	0.80±0.14[a]	0.92±0.20[a]	0.95±0.28[a]
Length gain (cm)	3.51±0.29[a]	3.37±0.43[a]	2.83±0.36[a]	3.57±1.28[a]
% Survival rate (SR)	93.33	91.43	88.02	92.50
		BASR		
Initial weight (g)	29.53±0.76[a]	27.06±0.25[c]	28.21±012[a]	28.06±0.35[b]
Final weight (g)	49.03±1.13[c]	48.83±1.20[c]	51.06±0.77[c]	60.06±0.23[b]
Weight gain (g)	19.50±1.90[c]	21.76±1.10[c]	22.85±0.73[c]	32.00±0.55[b]
% weight gain	66.16±8.21[d]	80.41±3.90[c]	80.92±2.57[c]	114.04±3.38[b]
Relative growth rate	66.16±8.21[d]	80.41±3.90[c]	80.92±2.57[c]	114.04±3.38[b]
Specific growth rate	0.90±0.08[d]	1.00±0.03[c]	1.05±0.02[c]	1.36±0.03[b]
Average daily growth	0.35±0.03[d]	0.39±0.20[c]	0.40±0.01[a]	0.57±0.01[b]
Food conversion ratio	1.82±0.08[a]	1.60±0.05[b]	1.58±0.03[b]	1.20±0.00[c]
Protein efficiency ratio	0.48±0.04[b]	0.50±0.02[b]	0.54±0.01[b]	0.72±0.01[a]
NM $x10^3$	1.20±0.00[cd]	1.20±0.02[d]	1.22±0.01[c]	1.35±0.00[b]
Condition factor (K)	0.50±0.01[b]	0.53±0.03[b]	0.56±0.04[a]	0.57±0.03[ab]
Length gain (cm)	7.93±0.58[a]	7.43±0.60[a]	7.06±0.46[a]	6.96±0.20[a]
% Survival rate (SR)	93.30±0.00[b]	93.33±0.00[b]	95.55±0.00[a]	95.95±0.00[a]

NM= Nitrogen metabolism,
Values= means±SD for three samples (n=3). Means followed by different letters as superscripts on the same row are significantly different at ($P \leq 0.05$).
ACSF: *Areca catechu* seed flour
BASR: *Balanites aegyptiaca* seed residue

Table 4.32: Growth performance and nutrients utilisation of *C. gariepinus* fed with diets formulated from NMSR and PLSF

Parameter	0%	10%	20%	30%
		NMSR		
Initial weight (g)	28.70±0.60^{b}	27.98±0.89^{b}	27.83±070^{b}	29.23±0.90^{b}
Final weight (g)	50.33±1.04^{b}	50.56±0.05^{c}	55.16±3.01^{c}	67.33±3.88^{b}
Weight gain (g)	21.63±1.62^{c}	22.59±0.91^{c}	27.30±2.61^{c}	38.10±3.81^{b}
% weight gain	75.47±7.24^{d}	80.91±5.92^{bc}	98.17±8.53^{b}	130.43±13.90^{a}
Initial standard length (cm)	12.43±0.40^{b}	14.36±0.30^{a}	14.03±0.46^{a}	14.26±0.87^{a}
Final standard length (cm)	21.33±0.50^{b}	19.76±1.56^{c}	20.43±0.40^{bc}	21.06±0.37^{bc}
Length gain (g)	8.90±0.55^{a}	5.40±1.27^{c}	6.13±0.11^{c}	6.13±0.15^{c}
Specific growth rate	1.00±0.07^{c}	1.05±0.05^{c}	1.21±0.07^{b}	1.48±0.10^{a}
Average daily growth	0.38±0.03^{c}	0.40±0.17^{c}	0.48±0.05^{c}	0.68±0.06^{b}
Relative growth rate	75.47±7.24^{d}	80.91±5.92^{bc}	98.17±8.53^{b}	130.43±13.90^{a}
Condition factor (k)	0.50±0.02^{c}	0.67±0.16^{a}	0.64±0.05^{bc}	0.72±0.07^{a}
Food conversion ratio	1.56±0.12^{c}	1.50±0.05^{a}	1.24±0.12^{b}	1.04±0.11^{d}
NM x10^{3}	2.21±0.01^{d}	2.20±0.02^{d}	2.32±0.10^{c}	2.70±0.11^{b}
Protein efficiency ratio	0.62±0.05^{a}	0.63±0.01^{a}	0.72±0.01^{b}	0.95±0.05^{a}
% Survival rate (SR)	93.30±0.00^{b}	93.33±0.00^{b}	95.55±0.00^{a}	95.95±0.00^{a}
		PLSF		
Initial weight (g)	27.76±0.64^{b}	28.98±1.30^{b}	28.18±0.48^{b}	28.35±0.87^{b}
Final weight (g)	66.15±5.61^{b}	48.95±5.13^{c}	51.16±6.73^{c}	60.69±5.02^{b}
Weight gain (g)	38.38±5.08^{ab}	20.11±4.72^{c}	22.97±4.72^{c}	32.29±5.76^{b}
% weight gain	138.02±5.08^{a}	69.72±15.80^{b}	81.52±24.18^{b}	113.89±23.43^{a}
Initial standard length (cm)	15.87±5.51^{a}	14.92±0.96^{a}	12.45±0.47^{a}	15.00±1.04^{a}
Final standard length (cm)	21.85±1.03^{a}	20.50±1.07^{b}	19.43±0.23^{b}	21.32±0.58^{a}
Length gain (g)	8.50±0.33^{a}	5.57±1.30^{c}	6.97±0.33^{ab}	6.32±1.32^{b}
Specific growth rate	1.54±011^{a}	0.93±0.16^{b}	1.03±0.22^{b}	1.35±0.19^{a}
Average daily growth	0.68±0.01^{b}	0.36±0.10^{c}	0.41±0.10^{c}	0.61±0.03^{b}
Relative growth rate	138.02±5.08^{a}	69.72±15.80^{b}	81.52±24.18^{b}	113.89±23.43^{a}
Condition factor (k)	0.64±0.15^{a}	0.57±0.07^{a}	0.70±0.09^{a}	0.67±0.05^{a}
Food conversion ratio	1.38±0.06^{b}	1.99±0.29^{a}	1.87±0.35^{a}	1.48±0.13^{b}
NM x10^{3}	1.37±0.18^{a}	1.18±0.10^{a}	1.29±0.06^{a}	1.36±0.06^{a}
Protein efficiency ratio	0.92±0.12^{a}	0.54±0.12^{b}	0.64±0.18^{b}	0.98±0.16^{a}
% Survival rate (SR)	95.55±3.14^{a}	93.30±0.00^{b}	91.10±3.14^{a}	91.10±3.14^{a}

NM= Nitrogen metabolism,
Values= means±SD for three samples (n=3). Means followed by different letters as superscripts on the same row are significantly different at ($P \leq 0.05$).
NMSR: *Neocarya macrophylla* seed residue
PLSF: *Polyalthiaa longifolia* seed four

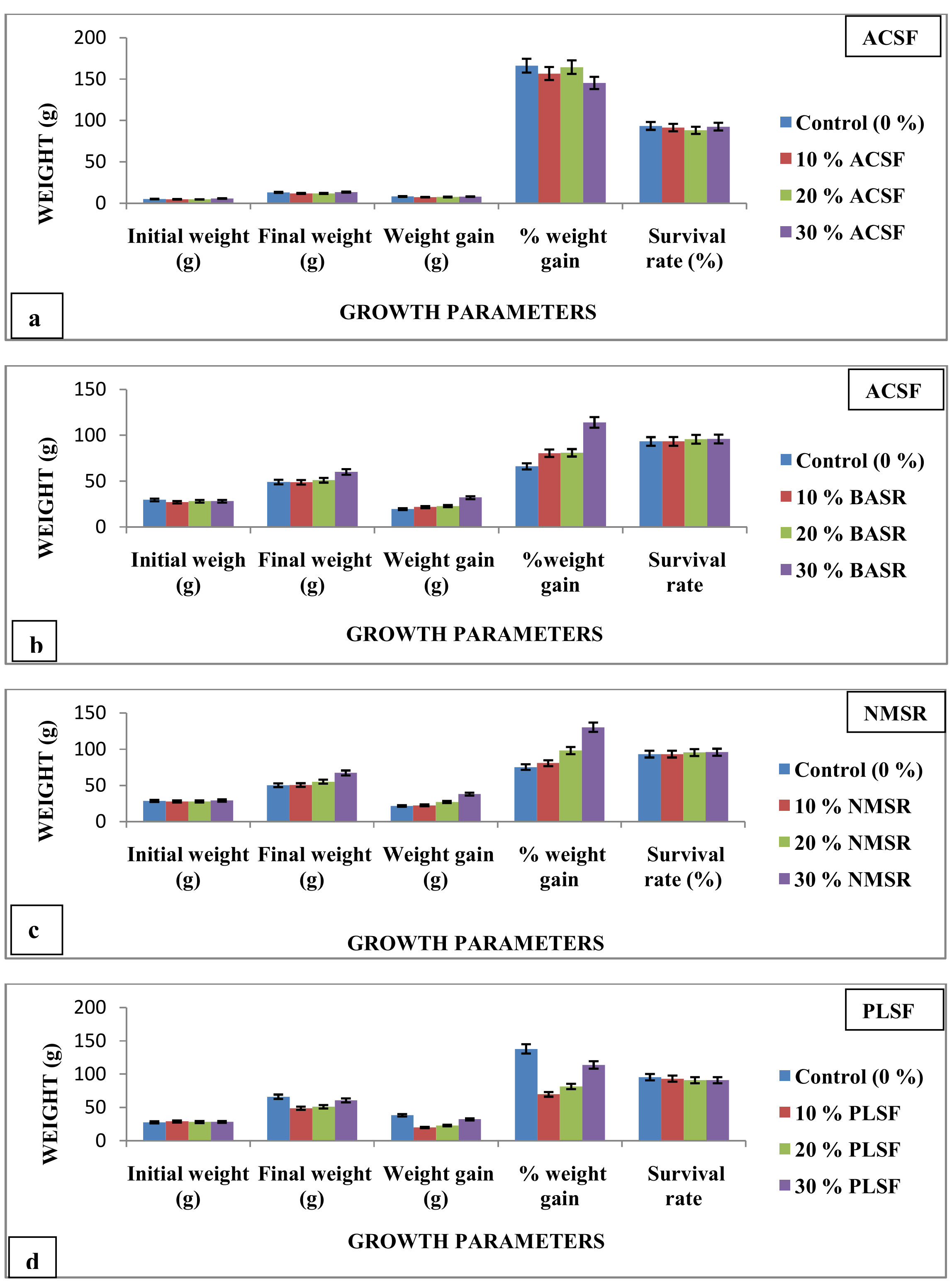


Figure 4.8: Graphical representations of the initial weight (g), final weight, weight gain (g), percentage weight (%) of fish fed with ACSF (a), BASR (b), NMSR (c) and PLSF (d)

as source of nutrient for Catfish (post juvenile) at 30 % and higher inclusion levels.

The common increase observed in the fish weight gain within all the treatment was an indication that they responded optimistically to all the prepared diets. The responses of fish to the different diets showed that growth and nutrient utilisation differed significantly ($p \leq 0.05$) among the treatments. The protein contents of the experimental diets adequately promoted the growth and dietary energy supply to the fish. The weight gain and the growth rate are usually considered as the most important measurement of productivity of diets (Ajayi *et al.,* 2013). ACSF, BASR, NMSR and PLSF could be used as additives in feed formulation.

4.9.3.4 Effect of ACSF, BASR, NMSR and PLSF on weight of organ and tissues of *C. gariepinus*

The weights of organs of fish fed with diets containing different concentrations of ACSF, BASR, NMSR and PLSF were prsented on Tables 4.33. The result revealed no significant differences at $P \leq 0.05$ in the weights of gills, liver, kidney and heart recorded. The values obtained in the experimental groups compared perfectly well with the control. The organ weight is an important index in animals' studies. It was therefore suggested that the organs studied might not be affected by the seed flours and residues used in this study.

4.9.3.5 Haematological and blood biochemistry analyses of blood samples of *Clarias gariepinus* fed with diet from ACSF, BASR, NMSR and PLSF

Table 4.34 showed the haematological parameters and blood biochemistry analyses of fish fed with ACSF and PLSF. The results revealed no significant differences at $p \leq 0.05$ in PVC, Hb, WBC, monocytes, heterophils, lymphocytes, MCHC, MCH and MCH values across all the groups. RBC values in the blood of fish fed on diet containing ACSF were found lower across the experimental groups than the control groups with the lowest in 30% group. The ALP varied significantly at $p \leq 0.05$ in the fish fed on PLSF with the highest values (325.33) recorded at 30% inclusion levels. The PCV, Hb and RBC were significantly different in all the experimental groups with the highest in 10%. PCV (%) of 25.67 (0%), 30.33 (10%), 24.66 (20%) and 26.00 (30%) were obtained in the rats fed with PLSF.

Table 4.33: Weight of organs of *Clarias gariepinus* fish fed with diet from ACSF, BASR, NMSR and PLSF

Organs	Control	10%	20%	30%
		ACSF		
Gill	3.10±0.81[a]	2.86±0.50[a]	3.16±0.64[a]	3.50±0.53[a]
Liver	1.70±0.70[ab]	1.96±0.56[ab]	1.93±0.16[ab]	1.60±0.50[ab]
Kidney	0.40±0.10[a]	0.53±0.10[a]	0.30±0.00[b]	0.36±0.05[ab]
Heart	0.10±0.00[a]	0.10±0.00[a]	0.10±0.00[a]	0.13±0.05[a]
		BASR		
Gill	3.13±0.32[a]	3.23±0.20[a]	3.36±0.50[a]	3.60±0.36[a]
Liver	1.43±0.11[bc]	1.60±0.58[a]	1.50±0.58[bc]	1.53±1.00[bc]
Kidney	0.40±0.00[ab]	0.46±0.05[a]	0.36±0.05[ab]	0.40±0.10[ab]
Heart	0.10±0.00[a]	0.10±0.00[a]	0.10±0.00[a]	0.10±0.00[a]
		NMSR		
Gill	3.10±0.82[ab]	2.90±0.62[b]	2.70±0.30[b]	3.40±0.30[a]
Liver	1.76±0.70[ab]	1.50±0.10[b]	1.53±0.21[b]	1.30±0.64[c]
Kidney	0.40±0.10[a]	0.36±0.15[a]	0.36±0.57[a]	0.43±0.15
Heart	0.10±0.00[a]	0.13±0.07[a]	0.10±0.00[a]	0.11±0.03[a]
		PLSF		
Gill	3.10±0.81[a]	2.86±0.51[a]	2.96±0.15[a]	3.50±0.36[a]
Liver	1.76±0.72[b]	1.96±0.50[a]	2.13±0.60[a]	2.13±0.40[a]
Kidney	0.40±0.10[ab]	0.50±0.17[a]	0.40±0.10[ab]	0.50±0.10[a]
Heart	0.10±0.00[a]	0.10±0.10[a]	0.10±0.00[a]	0.13±0.00[a]

The values are presented as mean±SD of three determinations. Means sharing the same letter as superscripts in the same row are not significantly different ($P \leq 0.05$).

ACSF: *Areca catechu* seed flour

BASR: *Balanites aegyptiaca* seed residue

NMSR: *Neocarya macrophylla* seed residue

PLSF: *Polyalthiaa longifolia* seed four

Table 4.34: Haematological and blood biochemistry analyses of blood samples of *Clarias gariepinus* fish fed with diet from ACSF and PLSF

Parameter	0%	10%	20%	30%
		ACSF		
PVC (%)	29.33± 0.58[a]	25.67±0.58[b]	25.33±0.58[b]	23.33±2.88[b]
HB (g/dl)	9.20±0.69[a]	8.60±0.10[ab]	8.50±0.00[ab]	7.93±0.98[b]
RBC ($x10^{12}$/L)	3.49±0.06[a]	2.55±0.13[b]	2.54±0.06[b]	2.26±0.37[b]
WBC ($x10^{12}$/L)	17.52±0.81[c]	18.12±0.39[bc]	17.48±0.20[c]	18.60±0.43[b]
PLAT (x 10^{12}/L)	172.67±56.58[b]	189.00±1.73[a]	173.50±1.17[ab]	221.33±28.87[a]
MCV (fl)	84.04±2.96[a]	101.28±2.90[a]	99.48±0.20[a]	103.62±4.48[b]
MCH	36.38±2.40[a]	33.55±1.38[a]	33.75±0.84[a]	35.23±1.76[b]
Monocyte (%)	3.68±0.58[ab]	2.33±1.15[b]	3.33±0.58[ab]	4.33±1.15[a]
Eosinophil (%)	1.67±1.15[a]	2.67±1.15[a]	3.00±0.00[a]	2.67±0.58[a]
MCHC	32.26±1.70 [a]	33.39±0.89 [a]	33.96±076 [a]	34.00±0.00 [a]
Lymphocyte (%)	66.68±6.35[a]	68.33±2.89[a]	75.33±2.89[a]	66.00±10.39[a]
Total Protein	4.23±0.32[a]	4.40±0.53[a]	4.10±0.20[a]	4.29±0.31[a]
Albumin	1.83±0.58[a]	1.90±0.43[a]	0.96±0.84[c]	1.46±1.52[ab]
Globulin	2.40±0.26[ab]	2.50±0.17[ab]	2.70±0.20[a]	2.63±0.29[ab]
A/G ration	0.76±0.06[a]	0.76±0.15[a]	0.54±0.60[c]	0.57±0.12[bc]
AST	167.67±51.28[ab]	208.00±47.28[a]	137.33±27.39[c]	199.00±7.21[ab]
ALT	34.33±1.5[a]	21.33±3.51[c]	23.33±4.16b[c]	19.67±0.57[c]
ALP	227.33±12.22[a]	242.67±38.7[a]	240.00±44.03[a]	206.80±65.25[a]
Creatinine	0.50±0.1[a]	0.60±0.1[a]	0.46±0.11[a]	0.50±0.10[a]
		PLSF		
PCV (%)	25.67±0.57[c]	30.33±5.03[a]	24.66±2.51[c]	26.00±0.00[ab]
Hb (mg/dl)	8.43±0.25[ab]	9.90±1.55[a]	8.00±1.00[a]	8.66±0.10[ab]
RBC (10^6/μl)	2.73±0.05[a]	3.35±0.02[a]	3.01±0.62[a]	2.49±0.05[a]
WBC (10^3/μl)	19.10±0.10[d]	22.71±2.07[ab]	21.33±0.70[c]	24.48±2.67[a]
Platelets (10^3/μl)	255.66±66.67[a]	286.66±79.93[a]	215.00±10.14[a]	259.00±33.00[a]
MCV (%)	93.67±0.75[ab]	91.04±8.55[ab]	91.85±8.85[ab]	104.45±3.20[a]
MCH (%)	30.77±0.35[a]	29.76±3.17[a]	29.86±4.10[a]	34.55±0.88[a]
MCHC (%)	32.85±0.58[a]	32.67±0.48[a]	32.94±7.53[a]	33.07±0.38[a]
Lymphocyte (%)	69.56±4.51[a]	63.01±9.84[a]	68.10±2.01[a]	65.66±3.54[a]
Monocytes (%)	5.00±0.00[a]	3.00±1.00[cd]	4.00±1.00[a]	2.00±0.00[a]
Eosinophils (%)	3.33±0.10[ab]	2.33±0.58[c]	2.67±0.05[d]	4.00±1.00[a]
Total protein	6.00±0.10[b]	5.30±1.17[c]	5.70±0.20[b]	6.80±0.20[a]
Albumin	3.06±0.05[b]	2.16±0.15[d]	2.90±0.10[b]	3.60±0.20[a]
Globulin	2.86±0.05[b]	2.13±0.11[c]	2.80±0.20[b]	3.86±0.15[a]
Alb/Glo ratio	0.97±0.16[a]	1.02±0.01[a]	1.04±0.11[a]	0.93±0.90[a]
AST	175.00±1.10[c]	180.00±3.96[b]	184.33±2.10[b]	180.20±0.00[b]
ALT	27.00±1.10[c]	25.00±1.00[c]	27.60±1.00[c]	32.66±2.51[a]
ALP	184.66±4.50[d]	237.00±36.38[b]	327.66±6.80[a]	325.33±5.10[a]
Creatinine	0.60±0.10[a]	0.50±0.00[a]	0.56±0.05[a]	0.53±0.72[a]

NM= Nitrogen metabolism, Values=means±SD for three samples (n=3). Means followed by different letters as superscripts on the same row are significantly different at at ($P \leq 0.05$). ALB: Albumin
AST: Aspartate aminotransferases, ALT: Alanine aminotransferases, ALP: Alkaline phosphatase

Most of the blood parameters (MCV, MCH, MCHC, PVC, Hb) recorded in the blood of fish fed on diet containing BASR and NMSR as presented on Table 4.35 was comparable across the experimental groups with the highest values in 30%. No significant difference was observed in the values of AST, ALP and ALT. The BASR and NMSR might not affect the fish negatively.

The haematological analysis together with blood chemistry will reinforce the fish culture by helping in early detection of infectious diseases and identification of sublethal condition that could disturb the production performance (Saleh *et al*., 2015). The results displayed on Table 4.35 revealed that the administration of BASR and NMSR induced a considerable increase in some measured blood parameters such as PVC, Hb, RBC, WBC and platelets which was found to be similar to the observation reported by Kalvankar *et al*. (2013). Increase in some of the blood parameters might be due to the presence of some constituents in the seed flours or residues that play important role in the stimulation of the immune system as well as in the performance of blood cell formation organs which might include spleen, thymus and bone marrow (Saleh *et al*., 2015). The reduction in blood parameters might also be connected to some antinutrients in the feeds (Ajayi *et al*., 2013c). The PVC, RBC and Hb decreased gradually from control diet to 30% diet with the highest in the control and 10% while the lowest in 30% diets prepared with ACSF and PLSF. The various blood parameters are important indexes in the assessment of the quality and suitability of feed ingredients for livestock. These ACSF and PLSF were therefore suggested to be good additives at 10% inclusion while BASR and NMSR seem to be good additives even abbove 30%.

4.9.3.6 Histological analysis of the fish organs

The histological analysis of the fish organs such as kidney, liver, gill and heart harvested in this study was given on Tables 4.36 and 4.37. No lesions were observed in the heart and gill tissues across all groups of fish after the experimental periods. The major observed changes were exhibited by the kidney and the liver. This might be because they were the major organs always affected by metabolic reactions that might be caused by toxicants.

Table 4.35: Haematological and blood biochemistry analyses of blood samples of *Clarias gariepinus* fish fed with diet from BASR and NMSR

Parameter	0%	10%	20%	30%
		BASR		
PCV (%)	23.60±1.52^{d}	27.67±0.57^{bc}	28.60±1.15^{b}	31.30±1.52^{a}
Hb (mg/dl)	7.76±0.77^{d}	8.50±0.20^{bc}	9.13±0.75^{ab}	10.27±0.64^{a}
RBC (10^6/µl)	2.33±1.05^{c}	3.22±0.37^{ab}	3.55±0.24^{a}	3.48±0.29^{a}
WBC (10^3/µl)	16.00±2.50^{d}	20.06±1.28^{ab}	21.30±3.16^{ab}	22.33±3.04^{a}
Platelets (10^3/µl)	233.30±25.18^{a}	207.33±41.47^{b}	204.00±40.59^{b}	210.67±30.02^{b}
MCV (%)	120.53±3.70^{a}	86.50±8.70^{b}	81.57±1.32^{c}	90.09±8.10^{b}
MCH (%)	40.43±1.50^{a}	26.57±2.76^{c}	27.74±3.92^{c}	32.07±1.48^{ab}
MCHC (%)	32.78±1.94^{a}	30.72±0.36^{a}	31.82±1.30^{a}	32.75±0.54^{a}
Lymphocyte (%)	60.67±9.29^{a}	65.00±2.60^{a}	65.00±3.01^{a}	57.33±6.11^{a}
Eosinophils (%)	2.00±1.10^{a}	3.00±0.00^{a}	2.66±0.05^{a}	2.67±1.52^{a}
Monocytes (%)	2.67±0.57^{a}	3.00±1.10^{a}	2.67±0.50^{a}	2.33±0.50^{a}
Total protein	5.76±0.25^{d}	6.26±0.46^{b}	6.93±0.30^{b}	7.56±0.11^{a}
Albumin	2.30±0.79^{a}	2.76±0.91^{a}	3.60±0.20^{a}	3.20±0.75^{a}
Globulin	3.50±0.51^{a}	3.63±0.60^{a}	3.90±0.05^{a}	4.23±0.78^{a}
Alb/Glo ratio	0.67±0.30^{a}	0.80±0.32^{a}	0.90±0.52^{a}	0.76±0.25^{a}
AST	246.66±67.10^{a}	209.00±48.50^{a}	198.66±8.08^{a}	193.60±4.00^{a}
ALT	32.00±3.00^{a}	35.00±4.58^{a}	36.33±3.50^{a}	35.60±7.37^{a}
ALP	242.66±77.35^{ab}	238.30±78.14^{a}	298.56±42.25^{a}	259.00±59.80^{a}
Creatinine	0.56±0.05^{a}	0.70±0.10^{a}	0.63±0.05^{a}	0.80±0.15^{a}
		NMSR		
PCV (%)	25.66±0.60^{d}	28.33±1.52^{ab}	27.00±2.51^{bc}	30.30±2.52^{a}
Hb (mg/dl)	8.50±0.26^{c}	9.26±0.87^{ab}	8.86±0.60^{ab}	9.90±1.15^{a}
RBC (10^6/µl)	2.53±0.27^{c}	3.19±0.55^{a}	2.95±0.15^{ab}	3.50±0.25^{a}
WBC (10^3/µl)	22.65±4.07^{a}	23.25±2.00^{a}	22.97±1.98^{a}	22.88±2.11^{a}
Platelets (10^3/µl)	207.67±17.15^{a}	230.00±24.57^{a}	201.33±67.95^{a}	231.00±24.57^{a}
MCV (%)	102.08±8.96^{a}	90.75±8.53^{ab}	91.73±6.85^{ab}	86.74±8.26^{ab}
MCH (%)	33.62±2.65^{a}	29.30±3.05^{ab}	30.07±1.94^{ab}	28.00±3.52^{ab}
MCHC (%)	33.11±0.33^{a}	32.32±1.61^{a}	32.86±1.82^{b}	32.22±1.13^{a}
Lymphocyte (%)	62.33±3.05^{a}	63.66±2.08^{a}	62.33±4.04^{a}	64.66±3.05^{a}
Moncytes (%)	3.67±1.57^{a}	2.66±1.50^{a}	3.30±0.57^{a}	2.67±0.57^{a}
Eosinophils (%)	3.30±0.57^{a}	3.00±0.05^{a}	3.00±0.00^{a}	3.30±0.58^{a}
Total protein	6.00±0.00^{b}	6.17±0.30^{b}	6.63±011^{a}	6.53±0.60^{a}
Albumin	2.87±0.06^{a}	3.47±0.11^{a}	2.95±0.81^{a}	3.43±0.06^{a}
Globulin	3.03±0.06^{a}	3.60±0.00^{a}	3.73±0.75^{a}	3.43±0.06^{a}
Alb/Glo ratio	0.96±0.05^{a}	0.92±0.02^{a}	0.85±0.36^{b}	0.96±0.12^{a}
AST	233.33±5.57^{a}	173.33±3.05^{b}	179.00±4.58^{b}	177.10±3.00^{b}
ALT	32.30±3.7^{c}	42.00±3.05^{b}	32.00±1.73^{c}	36.00±6.55^{b}
ALP	244.66±48.27^{ab}	237.66±18.50^{ab}	261.00±28.68^{a}	278.66±48.01^{a}
CREATINE	0.60±0.00^{a}	0.63±0.15^{a}	0.70±0.26^{a}	0.56±0.05^{a}

NM= Nitrogen metabolism, Values=means±SD for three samples (n=3). Means followed by different letters as superscripts on the same row are significantly different at ($P \leq 0.05$). ALB: Albumin, AST: Aspartate aminotransferases, ALT: Alanine aminotransferases, ALP: Alkaline phosphatase

In the kidney of fish that were fed ACSF (Table 4.36), no visible abnormalities were observed in the groups that received 0% and 10% inclusion levels. However, congestion of the blood vessels was observed in the groups that received 20% and 30% inclusion levels. In the liver section, a mild diffuse hepatic vacuolation was seen in the 10% group, while a more severe diffuse vacuolation and mild portal congestion were observed in the 20% and 30% groups.

When fish were fed PLSF, mild congestion of the blood vessels and mild diffuse vacuolar degeneration were observed in the 0% and 10% inclusion levels, respectively. No visible abnormalities were seen in the 20% and 30% inclusion levels. In the liver, 20% and 30% groups showed moderate portal congestion and diffuse vacuolar degeneration, while no lesions were observed in the 0% and 10% groups. At the 0% and 10% inclusion levels of ACSF, the fish were able to compensate for the presence of antinutrients in the seed flours through a physiological mechanism. Therefore, the negative effects of these antinutrients may not be evident. However, at higher inclusion levels (above 10%), when the limit may have been exceeded, the negative effects of these antinutrients become more noticeable. Based on these findings, it was recommended to administer ACSF to fish at a 10% inclusion level. The findings suggested that low inclusion levels of ACSF and PLSF did not have noticeable negative effects, but higher levels can lead to congestion and vacuolar degeneration in the organs. The recommendation was to use a 10% inclusion level of ACSF.

In the kidney of fish that were fed BASR according to Table 4.37, mild congestion of the blood vessels and mild diffuse vacuolar degeneration were observed at 0% and 10%, respectively. However, no visible lesions were seen at 20% and 30%. In the liver, moderate portal congestion and diffuse vacuolar degeneration were observed in the control and 20% groups, but no lesions were noticed at 0% and 30%. In the kidney of fish fed NMSR, mild congestion of the blood vessels was equally seen at 0% and 10%, while mild diffuse hepatic vacuolation was perceived in the liver section of both the control (0%) and 10% groups. No visible lesions were seen in either the kidney or the liver of the 20% and 30% groups.

Table 4.36: Histological study of fish fed on diets formulated with ACSF and PLSF

Organs	0%	10%	20%	30%
		ACSF		
Liver	No observable lesions	A mild diffuse diffuse vacuolar degeneration seen without the nuclei been displaced by the vacuoles	A severe diffuse vacuolar degeneration with a mild portal congestion	A severe diffuse vacuolar degeneration with a mild portal congestion
Kidney	There was a pink staining fluid in the interstitial space. No visible lesions seen	No visible lesions seen	Severe congestion of the blood vessels	There was severe congestion of the blood vessels
Gills	No observable lesions	No observable lesions	No observable lesions	No observable lesions
Heart	No observable lesions	No observable lesions	No observable lesions	No observable lesions
		PLSF		
Kidney	A mild congestion of the blood vessels was observed	A mild diffuse vacuolar degeneration seen	No visible lesions seen	No observable lesions
Liver	A moderate portal congestion and diffuse vacuolar degeneration seen	No observable lesions	A moderate portal congestion and diffuse vacuolar degeneration	No observable lesions
Gill	No observable lesions	No observable lesions	No observable lesions	No observable lesions
Heart	No observable lesions	No observable lesions	No observable lesions	No observable lesions

ACSF: Areca cathechu seed flour
PLSF: Polyalthia longifolis seed flour

Table 4.37: Histological study of fish fed on diets formulated with BASR and NMSR

Organs	0%	10%	20%	30%
		BASR		
Kidney	A mild congestion of the blood vessels was observed	A mild diffuse vacuolar degeneration seen	No observable lesions	No observable lesions
Liver	moderate portal congestion and diffuse vacuolar degeneration seen	No observable lesions	moderate portal congestion and diffuse vacuolar degeneration seen	No observable lesions
Gill	No observable lesions	No observable lesions	No observable lesions	No observable lesions
Heart	No observable lesions	No observable lesions	No observable lesions	No observable lesions
		NMSR		
Kidney	A mild congestion of the blood vessels was observed	A mild congestion of the blood vessels	No observable lesions	No observable lesions
Liver	A mild diffuse hepatic vacuolation was seen	A mild diffuse hepatic vacuolation was observed	No observable lesions	No observable lesions
Gill	No observable lesions	No observable lesions	No observable lesions	No observable lesions
Heart	No observable lesions	No observable lesions	No observable lesions	No observable lesions

BASR; *Balanites aegyptiaca* seed residue

NMSR: *Neocarya macrophylla* seed residue

The observed changes in the liver could be attributed to its role in processing various chemicals and eliminating toxins from the body (Pathan *et al.,* 2010). Additionally, Ajayi *et al.* (2013b) reported moderate sinusoidal congestion and moderately severe vacuolar degeneration of hepatocytes in the liver of fish fed diets containing 45% fluted pumpkin seed residue. It was important to note that vacuolation was a common response to the presence of chemicals in the fish's system (Shaw and Handy, 2006). The absence of major alterations and damage in both the kidney and liver of the fish fed with BASR and NMSR suggested that BASR and NMSR did not have any adverse effects on these organs. Therefore, they do not appear to be harmful at 30% levels.

4.9.4 Production of dietary cake from wheat flour, BASR, ECSR and NMSR blends (nutritional application)

4.9.4.1 Proximate composition of flour samples

The proximate composition of the flour samples prepared from wheat flour with BASR, ECSR and NMSR were given on Table 4.38. The results showed that parameters such as moisture, fat, carbohydrate decreased while ash, crude fibre and protein contents increased with the increasing concentration of ECSR substitution. Similarly, moisture and carbohydrate decreased while protein content, fat, fibre and ash contents increased with the increasing concentration of BASR and NMSR substitutions. This increase recorded in the protein content is probably due to the high protein content observed in BASR, ECSR and NMSR. The protein contents of 51.31%, 37.18% and 56.04% were recorded for BASR, ECSR and NMSR respectively. In all flour samples, carbohydrate decreased gradually from 72.33%, 72.75% and 72.05% in 100% wheat to 60.55%, 62.20% and 59.36% in 30% substitution while protein increased from 14.76%, 14.51% and 14.8% to 24.93%, 23.56% and 25.50% equally in 30% BASR, ECSR and NMSR blends. The inclusion of the various seed flours and residues caused the protein contents in the composites prepared to increase.

4.9.4.2 Functional properties of wheat flour with BASR, ECSR and NMSR blends.

The functional properties of the wheat flour with BASR, ECSR and NMSR blends were displayed on Table 4.39. The results showed significant differences among the values

Table 4.38: Proximate composition (%) of wheat flour with BASR, ECSR and NMSR blends

Parameters	Wheat flour	90:10	80:20	70:30	100% Sample
			BASR		
Dry 1matter	89.43±0.05^{d}	89.50±0.01^{d}	90.13±0.05^{c}	90.33±0.06^{b}	91.41±0.03^{a}
Moisture	10.56±0.05^{a}	10.50±0.01^{a}	9.86±0.05^{b}	9.67±0.05^{c}	8.45±0.03^{d}
Protein	14.76±0.06^{e}	18.03±0.20^{d}	20.40±0.10^{c}	24.93±0.15^{b}	51.31±0.13^{a}
Fat	0.28±0.30^{e}	1.43±0.05^{d}	1.56±0.05^{c}	1.85±0.08^{b}	6.90±0.04^{a}
Crude fibre	0.43±0.05^{e}	0.78±0.03^{d}	0.93±0.03^{c}	1.16±0.06^{b}	12.90±0.02^{a}
Ash	1.61±.030^{c}	1.63±0.02^{c}	1.66±0.02^{c}	1.83±0.05^{b}	4.40±0.02^{a}
Carbohydrate	72.30±0.15^{a}	67.63±0.15^{b}	65.53±0.11^{c}	60.55±0.20^{d}	16.03±0.18^{e}
Energy	1.49±0.00^{c}	1.50±0.00^{b}	1.51±0.01^{ab}	1.52±0.00^{a}	1.40±0.00^{d}
			ECSR		
Dry matter	89.07±0.41^{f}	89.05±0.47^{e}	89.29±0.05^{d}	89.73±0.03^{c}	91.42±0.03^{c}
Moisture	10.92±0.04^{a}	10.94±0.05^{a}	10.71±0.05^{b}	10.27±0.04^{c}	8.56±0.03^{d}
Protein	14.51±0.11^{f}	16.23±0.05^{e}	18.80±0.51^{d}	23.56±0.04^{c}	37.18±0.05^{a}
Fat	0.19±0.01^{e}	0.68±0.02^{b}	0.39±0.02^{c}	0.29±0.01^{d}	0.39±0.11^{c}
Crude fibre	0.01±0.01^{d}	0.19±0.00^{c}	0.18±0.01^{c}	1.19±0.01^{b}	1.19±0.01^{b}
Ash	1.59±0.02^{f}	1.70±0.15^{e}	2.11±0.51^{d}	2.39±0.17^{c}	4.30±0.15^{a}
Carbohydrate	72.75±0.11^{a}	70.24±0.10^{b}	67.80±0.02^{c}	62.20±0.04^{d}	48.21±0.27^{f}
Energy	1.49±0.42^{b}	1.49±6.95^{b}	1.48±6.14^{b}	1.47±2.37^{c}	1.47±3.91^{c}
			NMSR		
Dry matter	89.30±0.10^{c}	89.66±0.05^{c}	90.66±0.05^{b}	90.88±0.02^{a}	87.68±0.00^{d}
Moisture	10.70±0.0^{b}	10.33±0.05^{b}	9.33±0.05^{c}	9.11±0.02^{d}	12.32±0.53^{a}
Protein	14.85±0.05^{e}	17.22±0.02^{d}	20.53±0.05^{c}	25.50±0.43^{b}	56.04±0.01^{a}
Fat	0.31±0.03^{e}	1.78±0.02^{b}	1.97±0.05^{c}	2.15±0.05^{d}	4.51±0.01^{c}
Crude fibre	0.45±0.05^{d}	0.85±0.05^{c}	1.06±0.06^{c}	1.26±0.05^{b}	7.41±0.01^{b}
Ash	1.65±0.05^{f}	1.94±0.04^{e}	2.08±0.03^{d}	2.26±0.05^{c}	6.53±0.06^{a}
Carbohydrate	72.05±0.14^{a}	67.87±0.06^{b}	65.00±0.08^{c}	59.36±0.24^{d}	13.19±0.49^{f}
Energy (kcal)	1.49±1.85^{a}	1.51±1.00^{a}	1.53±1.45^{a}	1.52±9.71^{a}	1.34±0.00^{b}

Values=mean±SD of three determinations. Means with same letter as superscripts on the same row are not significantly different ($P \leq 0.05$).

BASR: *Balanites aegyptiaca* seed residue

NMSR: *Neocarya macrophylla* seed residue

ECSR: *Enterolobium cyclocarpum* seed residue

Table 4.39: Functional properties of wheat flour with BASR, ECSR and NMSR blends

Parameters	0%	10%	20%	30%
		BASR		
LBD (g/cm^3)	$0.41±0.05^a$	$0.41±0.03^a$	$0.40±0.00^a$	$0.42±0.01^a$
PBD(g/cm^3)	$0.63±0.01^a$	$0.62±0.01^a$	$0.63±0.01^a$	$0.64±0.01^a$
SI (g)	$1.10±0.01^c$	$1.16±0.04^c$	$1.26±0.05^b$	$1.32±0.02^{ab}$
WAC	$1.45±0.05^c$	$1.46±0.02^c$	$1.50±0.00^b$	$1.55±0.05^b$
OAC	$1.33±0.05^c$	$1.35±0.05^c$	$1.38±0.02^c$	$1.40±0.01^b$
		ECSR		
LBD (g/cm^3)	$0.39±0.00^c$	$0.47±0.01^a$	$0.39±0.01^c$	$0.43±0.03^b$
PBD (g/cm^3)	$0.60±0.03^b$	$0.64±0.04^a$	$0.55±0.01^c$	$0.59±0.03^b$
SI (g)	$1.05±0.00^d$	$1.43±0.57^a$	$1.33±0.00^b$	$1.18±0.07^c$
WAC	$1.38±0.04^b$	$1.40±0.05^b$	$1.43±0.02^b$	$1.45±0.04^b$
OAC	$1.23±0.04^a$	$1.24±0.05^a$	$1.23±0.03^a$	$1.22±0.01^a$
		NMSR		
LBD (g/cm^3)	$0.36±0.05^b$	$0.48±0.01^a$	$0.40±0.02^c$	$0.45±0.03^a$
PBD (g/cm^3)	$0.61±0.00^a$	$0.51±0.01^c$	$0.60±0.02^a$	$0.55±0.01^b$
SI (g)	$1.08±0.02^d$	$1.45±0.05^a$	$1.23±0.00^c$	$1.28±0.02^{bc}$
WAC	$1.56±0.02^d$	$1.53±0.02^d$	$1.62±0.01^c$	$1.68±0.02^b$
OAC	$1.37±0.03^b$	$1.37±0.02^b$	$1.39±0.02^b$	$1.40±0.03^b$

Values are mean±SD of triplicate determinations. Means with same letter as superscripts ion the same row are not significantly different ($P \leq 0.05$)
BASR: *Balanites aegyptiaca* seed residue
NMSR: *Neocarya macrophylla* seed residue
ECSR: *Enterolobium cyclocarpum* seed residue

recorded in the control and the experimental groups. In the wheat flour and ECSR blends, the loose bulk density was higher in 90:10 substitutions than other groups while the lowest was seen at the control and 80:20 groups. The packed bulk density compared favourably with each other within the groups. The water absorption capacity increased gradually from 1.38 g/g in the control (100:0) to 1.45 g/g in 70:30. Onuegbu *et al*. (2013) reported an increase in water absorption capacity that ranged from 2.05 g/g (100% wheat flour) to 2.52 g/g (80:20) in wheat/maize blend. The oil absorption capacity decreased gradually as the level of substitution increased. The water absorption capacity values of 1.23 g/g, 1.24 g/g, 1.23 g/g and 1.22 g/g were correspondingly found for 100% wheat flour, 90:10, 80:20 and 70:30 in wheat flour and ECSR blends.

In the wheat flour and NMSR blends, the loose bulk density ranged from 0.48 g/cm^3 (90:10 substitutions) to 0.36 g/cm^3 (100% wheat flour). The packed bulk density was higher in 100% wheat flour with the lowest in 90:10 substitutions. The water and oil absorption capacities increased gradually with increase in level of NMSR in the sample. Comparable results were also observed with wheat and BASR blends. Similar observations were reported by Onuegbu *et al*. (2013) for wheat and maize flour blends with the highest value at 80:20. The water capacity is an important parameter in packaging and material handling. This is because materials with higher bulk density are able to occupy less volume, allowing for a larger quantity of material to be stored (Onuegbu *et al*., 2013).

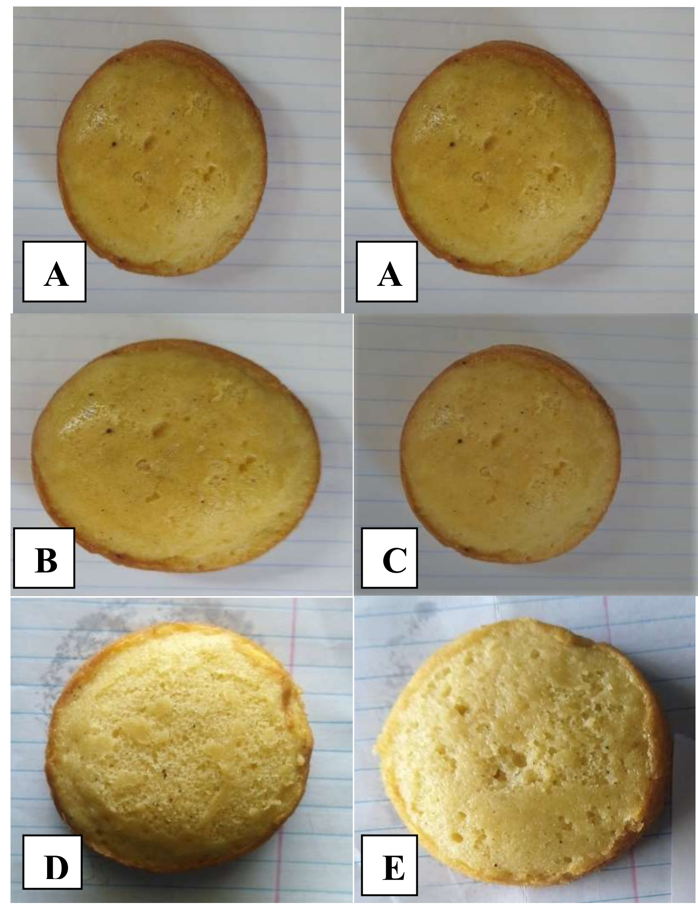
4.9.4.3 Physical properties of dietary cakes produced from wheat flour blended with BASR, ECSR and NMSR

The physical properties of dietary cakes produced from wheat flour blended with BASR, ECSR and NMSR were given on Table 4.40. The cake samples were presented in Plates 4.2, 4.3 and 4.4. The height of the cakes obtained from wheat flour and BASR blends ranged from 1.80 cm (30% BASR) to 2.04 cm (100% wheat). The weight of 26.00 g (30% BASR) was lower than 27.07 g (100% wheat). There was a gradually decrease in weights of the experimental cake with the increase in the substitution of BASR. With increase in the substitution level, there was also a decrease in the cake volume (cm^3) from 708.65 in (100%) wheat flour to 633.45 in 30% BASR substitutions. Similar results were recorded

Table 4.40: Physical properties of dietary cakes made from wheat flour blended with BASR, ECSR and NMSR

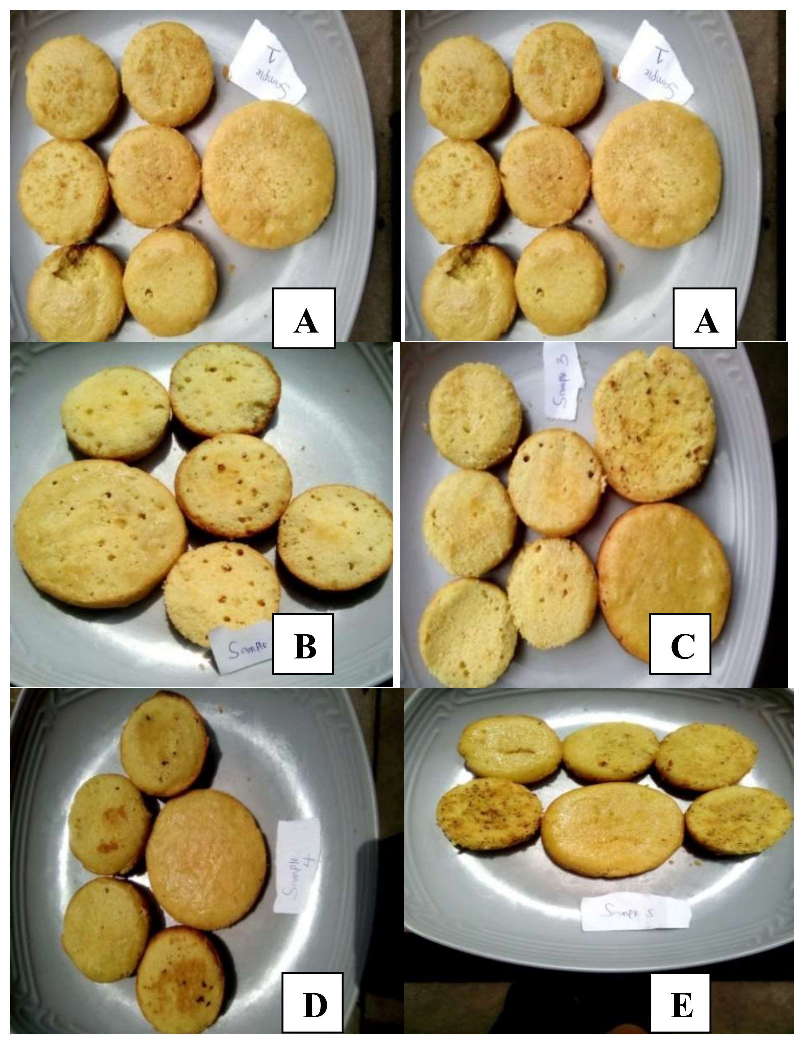
Parameters	0%	10%	20%	30%
		BASR		
Weight (g)	27.07±0.23^{a}	26.78±0.08^{a}	26.40±0.30^{b}	26.00±0.33^{c}
Height (cm)	2.04±0.08^{a}	1.96±0.08^{a}	1.82±0.11^{b}	1.80±0.07^{b}
Volume (cm^3)	710.96±28.60^{a}	699.31±36.45^{a}	616.72±38.86^{b}	613.85±25.96^{b}
Specific volume (cm^3/g)	26.44±1.06^{a}	24.98±135^{b}	23.51±1.30^{c}	23.56±0.95^{c}
		ECSR		
Weight (g)	25.02±0.48^{b}	24.83±0.63^{b}	25.12±0.74^{b}	25.31±0.40^{a}
Height (cm)	2.28±0.12^{a}	2.15±0.18^{a}	2.10±0.12^{a}	2.10±0.12^{b}
Volume (cm^3)	606.28±19.98^{a}	606.21±31.04^{a}	604.96±32.18^{a}	600.45±49.43^{b}
Specific volume (cm^3/g)	24.23±0.74^{b}	24.41±1.74^{a}	24.06±1.04^{bc}	23.71±1.39^{bc}
		NMSR		
Weight (g)	27.10±0.16^{a}	26.75±0.05^{b}	26.00±0.08^{c}	25.48±0.07^{d}
Height (cm)	2.06±0.16^{a}	1.90±0.12^{b}	1.88±0.11^{b}	1.98±0.05^{b}
Volume (cm^3)	708.65±53.61^{a}	694.39±35.97^{b}	671.50±38.38^{b}	633.45±18.06^{b}
Specific volume (cm^3/g)	26.15±1.96^{a}	25.94±1.34^{b}	25.82±1.41^{ab}	24.86±0.68^{b}

Values are mean±SD of three determinations. Means with same letter as superscripts on the same row are not significantly different ($P \leq 0.05$).
BASR: *Balanites aegyptiaca* seed residue
NMSR: *Neocarya macrophylla* seed residue
ECSR: *Enterolobium cyclocarpum* seed residue



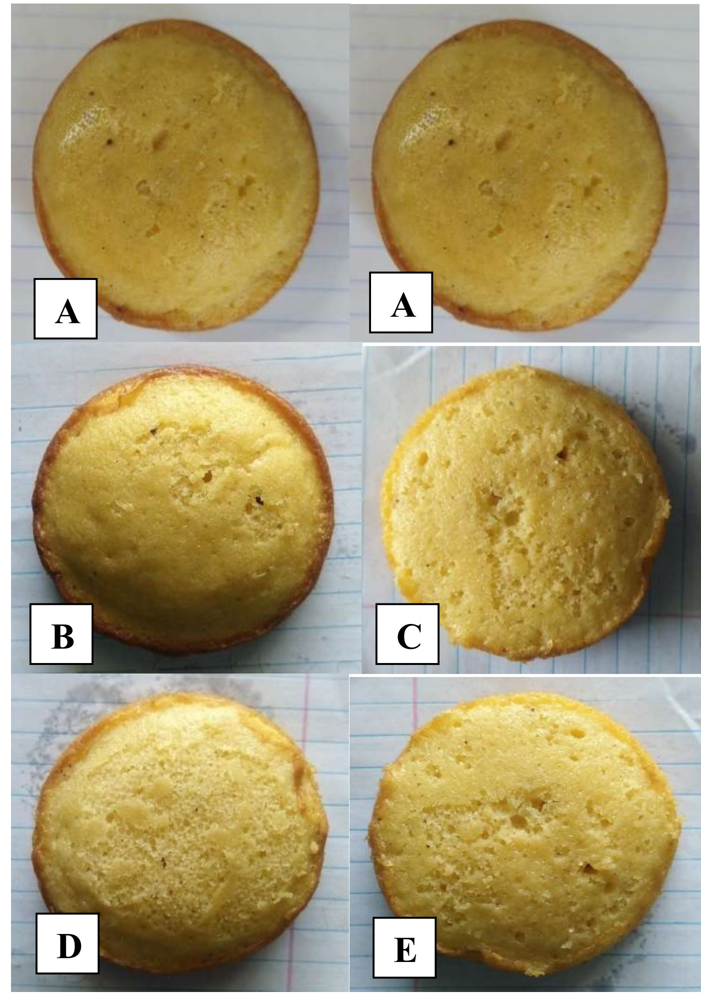
A. Cake produced from 100% Wheat flour
B. Cake produced from (90:10%) Wheat/BASR
C. Cake produced from (80:20%) Wheat/BASR
D. Cake produced from (70:30%) Wheat/BASR
E. Cake produced from (100%) BASR

Plate 4.2: Sample of dietary cakes produced from wheat flour and BASR blends



A. Cake produced from 100% wheat flour
B. Cake produced from (90:10%) Wheat/ECSR
C. Cake produced from (80:20%) Wheat/ECSR
D. Cake produced from (70:30%) Wheat/ECSR
E. Cake produced from 100% ECSR

Plate 4.3: Sample of dietary cakes produced from wheat flour and ECSR blends



A. Cake produced from 100% Wheat
B. Cake produced from (90:10%) Wheat/NMSR
C. Cake produced from (80:20%) Wheat/NMSR
D. Cake produced from (70:30%) Wheat/NMSR
E. Cake produced from 100% NMSC

Plate 4.4: Sample of dietary cakes produced from wheat flour and NMSR blends

for the specific volume of the cakes. The height of the cakes obtained from wheat flour and ECSR ranged from 1.80 cm (30% ECSR) to 2.28 cm (100% wheat flour). The weight of 27.07 g in 100% wheat is comparable with 26.78 g, 26.40 and 26.00 g recorded for 10%, 20% and 30% substitutions respectively. There was gradual decrease in the volume and the specific volume of the cake with the highest in 100% wheat and lowest in 30% ECSR. There were significant differences at $P \leq 0.05$ within the substituted cake. Cake with 10% ECSR substitution compared favourably with the control (100% wheat).

The height of wheat flour and NMSR cake ranged from 1.88 cm (20% NMSR) to 2.06 cm (100% wheat). Weight of 27.07 g in 100% wheat cake was comparable with 26.75 g (10%), but higher than 26.00 g (20% NMSR) and 25.48 g (30% NMSR). There was gradual decrease in the weight of the cake produced, the volume and the specific volume of the cake with the increasing concentration of NMSR. The highest values were obtained in 100% wheat while the lowest in 30% wheat/NMSR blend. The 10% wheat/NMSR blends compared favourably with the control (100% wheat). The differences observed in experimental produced cakes (cakes with different substitutions) might be due to the properties of BASR, ECSR and NMSR.

4.9.4.4 Sensory evaluation of the cakes prepared

The sensory evaluation and general acceptability of different cakes produced from wheat flour blended with BASR, ECSR and NMSR blends were shown on Table 4.41. The parameters studied such as taste, appearance, aroma and overall acceptability were significantly different at $p \leq 0.05$ in various cake samples in the sensory attributes observed. The cake with 30% ECSR substitution had high taste, appearance, and texture while the lowest was obtained in cake prepared with 100% wheat flour. The overall acceptability was 7.55 (30% ECSR); 7.20 (20% ECSR); 6.90 (100% wheat flour); and 6.70 (10% ECSR). The highest overall acceptability of 8.50 and 7.83 were recorded respectively in 30% substitutions of BASR and NMSR. The incorporation of ECSR resulted in better aroma, taste, appearance and overall acceptability of the cake at 30% inclusion levels. The same trend was obtained in the cake produced from wheat flour with BASR and NMSR. The parameters such as appearance, texture and aroma were high in

Table 4.41: Sensory evaluation of cakes produced from wheat flour with BASR, ECSR and NMSR blends

Parameters	0%	10%	20%	30%
		BASR		
Taste	7.67±0.49^{a}	6.75±0.45^{b}	6.91±0.50^{b}	7.66±0.70^{a}
Appearance	7.50±0.52^{b}	6.83±0.57^{c}	7.16±0.70^{bc}	8.33±0.65^{a}
Softness	7.58±0.51^{b}	6.88±0.66^{c}	7.41±0.51^{b}	8.25±0.45^{a}
Crumb colour	7.41±0.66^{ab}	6.67±0.49^{c}	7.25±0.70^{b}	8.08±0.50^{a}
Crush colour	7.58±0.51^{b}	6.66±0.50^{d}	7.38±0.65^{b}	8.00±0.42^{a}
Aroma	7.50±0.66^{ab}	6.80±0.50^{c}	7.16±0.57^{b}	7.75±0.45^{a}
Overall acceptability	7.83±0.38^{b}	7.08±0.9^{c}	7.25±0.62^{c}	8.50±0.52^{a}
		ECSR		
Taste	6.10±1.74^{ab}	6.90±2.07^{ab}	7.50±1.23^{a}	7.80±1.6^{a}
Appearance	7.00±1.65^{a}	7.15±1.35^{a}	7.15±1.09^{a}	7.60±1.18^{a}
Softness	5.70±1.68^{b}	6.15±1.81^{ab}	6.70±1.68^{ab}	7.25±1.37^{a}
Crumb colour	6.70±1.78^{b}	6.80 ±1.67^{b}	7.30±1.30^{a}	7.50±1.14^{a}
Crust colour	6.70±1.28^{c}	7.10±1.55^{ab}	8.00±1.02^{a}	7.90±1.16^{a}
Aroma	6.60±0.34^{a}	6.25±1.86^{a}	6.25±1.97^{a}	6.65±1.26^{a}
Overall acceptability	6.90±1.41^{ab}	6.70±1.59^{ab}	7.20±1.28^{ab}	7.55±1.14^{a}
		NMSR		
Taste	7.41±0.79^{a}	6.25±0.62^{b}	6.58±2.79^{b}	7.41±1.37^{a}
Appearance	7.33±0.77^{ab}	6.58±0.99^{b}	6.75±0.75^{b}	7.58±0.79^{a}
Softness	7.08±0.79^{ab}	6.08±0.80^{cd}	6.75±0.75^{bc}	7.58±0.51^{a}
Crumb colour	7.08±0.66^{ab}	6.08±0.51^{cd}	6.58±1.16^{bc}	7.25±0.75^{a}
Crust colour	7.25±0.96^{a}	6.41±0.99^{a}	6.83±0.80^{ab}	7.50±0.67^{a}
Aroma	7.33±0.65^{a}	6.08±0.79^{b}	6.25±1.13^{b}	7.33±0.88^{a}
Overall acceptability	7.50±0.52^{a}	6.16±0.72^{bc}	6.58±0.51^{b}	7.83±0.71^{a}

Values are mean±SD of three determinations. Means having the same letter as superscripts on the same row are not significantly different ($P \leq 0.05$).

BASR: *Balanites aegyptiaca* seed residue

NMSR: *Neocarya macrophylla* seed residue

ECSR: *Enterolobium cyclocarpum* seed residue

70:30 inclusion when compared to others groups.

The overall acceptability found with cake produced from wheat flour and NMSR were respectively 7.83 (30%); 7.50 (100% wheat); 6.58 (20%) and 6.15 (10%). Similar trend of results were obtained for BASR. The *N. macrophylla* seed had been reported to contain some essential amino acids which are important in diets (Amza *et al*., 2010). The fruits are consumed while fresh, boiled with cereal, roasted like cashew or almonds (Amza *et al*., 2010). The seed cakes from BASR, ECSR and NMSR blends have good nutritional elements and sensory properties that suggest them as potential sources of industrial raw material.

4.9.4.5 Effect of dietary cakes produced from wheat flour blended with BASR, ECSR and NMSR blends on the body weight and survival rate of rat.

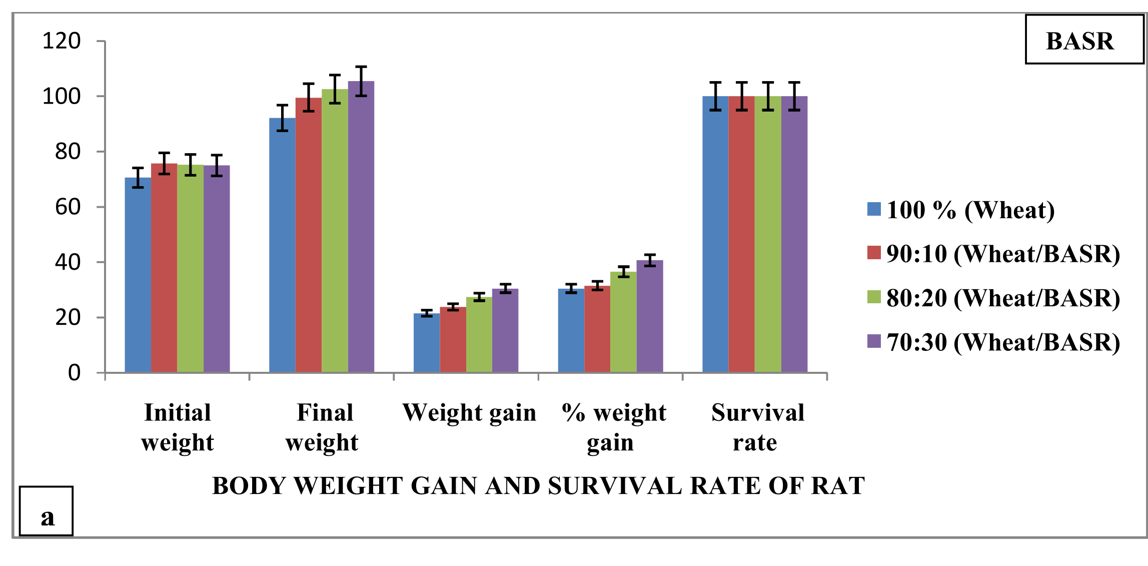
The study investigated the impact of cakes made from a blend of wheat flour with three different ingredients (BASR, ECSR, and NMSR) on the performance of rats. The findings were presented in Table 4.42 and Figures 4.9 (a, b, c). No significant differences were observed in the survival rate of the rats across all experimental groups, as none of them died. When comparing the weight gain of rats fed with dietary cakes made from wheat flour and ECSR, it was found that the control group had the highest weight gain, which gradually decreased in the other experimental groups. The group with 30% ECSR had the lowest weight gain, indicating that ECSR might contain some antinutrients that adversely affected the rats' weight when present in higher concentrations in the cake. Therefore, it is not recommended to use ECSR at 30% substitution level in the production of dietary cakes.

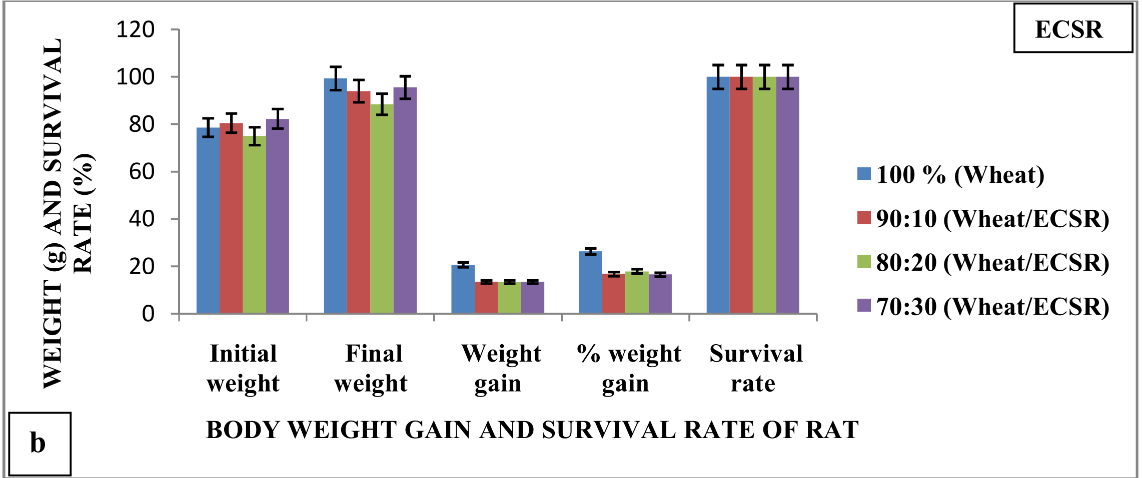
On the other hand, rats fed with dietary cakes made from wheat flour blended with BASR and NMSR showed a gradual increase in body weight gain. The control group (0% blend) had the lowest weight gain, while the groups with 30% BASR and NMSR had the highest weight gain. This suggested that higher concentrations of BASR and NMSR in the dietary cakes resulted in a greater percentage of weight gain in the rats. These findings revealed that BASR and NMSR may possess some beneficial dietary protein qualities that promote optimal rate growth. Based on these results, it was suggested that ECSR could be used at a

4.42: Mean body weight gain (g) and survival rate (%) of the rat fed with cakes produced from wheat with BASR, ECSR and NMSR blends

Parameter	0%	10%	20%	30%
		BASR		
Average initial weight (g)	70.60±0.17[c]	75.70±0.10[a]	75.17±0.10[a]	74.96±0.10[b]
Final average weight (g)	92.15±0.20[b]	99.53±0.77[a]	102.60±0.50[a]	105.46±0.20[a]
Weight gain/rat (g)	21.55±0.05[c]	23.85±0.70[c]	27.05±0.05[b]	30.50±0.10[a]
Weight gain (%)	30.50	31.50	36.53	40.40
Survival rate (%)	100	100	100	100
		ECSR		
Average initial weight (g)	78.63±1.06[c]	80.50±0.03[c]	75.00±0.02[b]	82.30±0.05 [a]
Final average weight (g)	99.33±0.01[c]	94.00±31.60[c]	88.45±0.06[b]	95.50±2.03[a]
Weight gain/rat (g)	20.70±1.00[c]	13.50±0.30[c]	13.45±1.02[b]	13.50
Weight gain (%)	26.33	16.77	17.93	16.4
Survival rate (%)	100	100	100	100
		NMSR		
Average initial weight (g)	71.00±2.23[b]	76.00±0.18[c]	74.10±2.8[b]	75.50±3.73[a]
Final average weight (g)	93.01±4.47[b]	98.05±5.70[b]	98.00±4.47[b]	105.00±1.00[a]
Weight gain/rat (g)	22.01±2.73[c]	22.00±2.47[c]	26.00±4.18[b]	29.80±2.93[a]
Weight gain (%)	30.98	28.95	35.13	39.73
Survival rate (%)	100	100	100	100

Values are mean±SD of three determinations. Means having the same letter as superscripts on the same row are not significantly different at ($P \leq 0.05$).





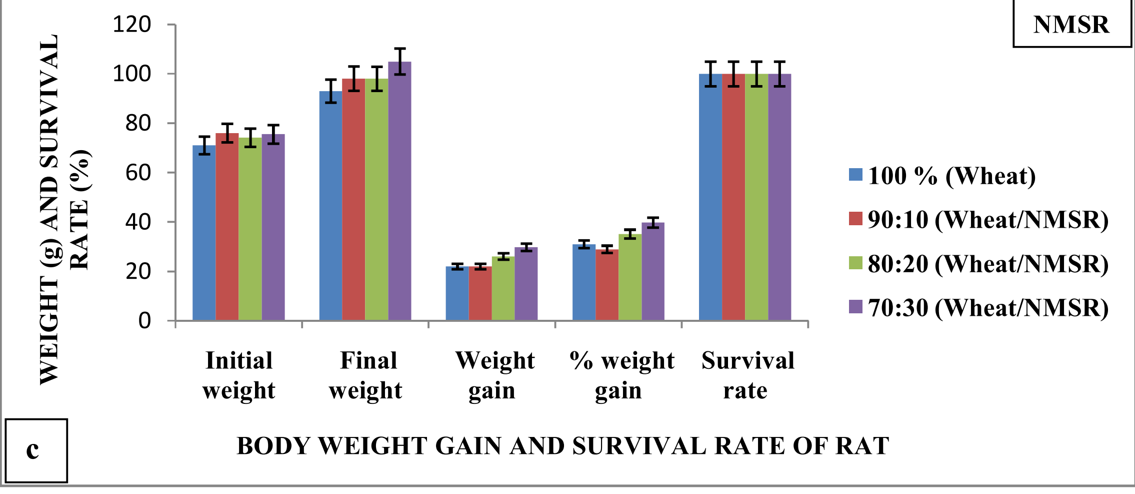


Figure 4.9: Effect of cake from wheat flour and BASR (a), ECSR (b) and NMSR (c) blends on rat body weight and survival rate

10% substitution level, while BASR and NMSR could be used successfully at substitution levels of up to 30% in the production of dietary cakes

4.9.4.6 Haematology analysis of the blood of rat fed on cakes produced from wheat flour with BASR, ECSR and NMSR blends

The cakes produced from wheat with BASR, ECSR and NMSR blends were used to feed rats and their effect on the blood haematology of the rats was recorded on Table 4.43. The parameter investigated include, PVC, Hb, RBC, WBC, Platelets, MCV, MCH and MCHC. The study revealed no significant discrepancies among the tested groups regarding the parameters mentioned above, as they had good comparison. In the dietary cakes from wheat flour with ECSR blends, PVC values of 41.33%, 41.33%, 42.33% and 43.33% were respectively recorded for 100% wheat, 10, 20 and 30% ECSR. There was a slight increase in PVC from 41.33 (10%) to 43.33 (30%). Similar results as obtained above were also recorded with cakes made from wheat flour with BASR and NMSR blends. The similarity observed from this haematology results revealed that the cakes produced from wheat flour with ECSR, BASR and NMSR blends had no abnormal effect on the blood as well as on the organs of the rats. The BASR, ECSR and NMSR might be therefore considered as a potential substitute for nutritionally enrich baked products.

4.9.5 Antimicrobial and wound healing activities of BASO and NMSO

4.9.5.1 Antimicrobial activity of BASO and NMSO

The results of antimicrobial activity of BASO and NMSO on the tested bacterial and fungi are shown on Table 4.44. These two oils (BASO and NMSO) were observed to perfectly inhibit organism growth at varied concentrations when put side by side with the controls (both negative and positive). The *P. aerugonisa* and *E. coli* showed higher susceptibility than other bacteria (*S. aureus*, *B. subtilis*) and fungi (*C. albicans* and *A. niger*) in BASO while *B. subtilis* and *P. aerugonisa* were recorded with higher susceptibility in NMSO. The growth of the tested organisms was inhibited by the oils at different concentrations and even at 12.5 mg/L and 6.25 mg/mL.

Table 4.43: Haematology of the blood samples of rat fed with cakes produced from wheat flour/BASR, ECSR and NMSR blends

Parameters	0%	10%	20%	30%
		BASR		
PCV (%)	42.66±0.57^{a}	41.66±0.57^{a}	42.33±0.60^{a}	41.33±0.57^{a}
Hb (mg/dl)	14.46±0.10^{a}	14.43±0.37^{a}	14.26±0.41^{a}	14.30±0.17^{a}
RBC (10^6/μl)	7.47±0.12^{a}	7.44±0.09^{a}	7.53±0.08^{a}	7.38±0.05^{a}
WBC (10^3/μl)	8.46±0.15^{a}	8.33±1.25^{a}	7.05±077^{a}	8.18±0.30^{a}
Platelets(10^3/μl)	121.33±12.05^{a}	122.67±21.38^{a}	135.33±25.06^{a}	126.00±31.42^{a}
MCV (%)	57.10±1.50^{a}	55.98±0.34^{a}	56.22±1.06^{a}	55.96±1.22^{a}
MCH (%)	19.45±0.45^{a}	19.39±0.29^{a}	18.96±0.75^{a}	19.34±0.22^{a}
MCHC (%)	34.06±0.65^{a}	34.63±0.44^{a}	33.69±0.97^{a}	34.59±0.78^{a}
		ECSR		
PCV (%)	41.33±0.57^{a}	41.33±1.52^{a}	42.33±0.57^{a}	43.33±3.70^{a}
Hb (mg/dl)	13.70±0.60^{a}	13.87±0.56^{a}	14.43±0.75^{a}	14.13±1.33^{a}
RBC (10^6/μl)	6.31±1.54^{a}	6.84±0.31^{a}	7.24±0.22^{a}	7.06±0.49^{a}
WBC (10^3/μl)	7.76±2.1^{a}	6.16±1.46^{a}	7.20±2.30^{a}	6.80±1.61^{a}
Platelets(10^3/μl)	141.00±36.59^{a}	125.67±5.50^{a}	159.67±32.13^{a}	134.00±7.21^{a}
MCV (%)	68.30±17.63^{a}	59.63±2.30^{a}	58.83±1.38^{a}	61.00±1.73^{a}
MCH (%)	22.53±5.31^{ab}	20.24±0.15^{a}	19.93±0.40^{a}	19.96±0.55^{a}
MCHC (%)	33.13±1.00^{a}	33.55±0.79^{a}	34.08±1.29^{a}	32.59±0.29^{a}
		NMSR		
PCV (%)	42.33±0.60^{a}	41.67±1.50^{a}	42.67±1.15^{a}	43.33±1.15^{a}
Hb (mg/dl)	14.43±0.20^{a}	14.26±0.50^{ab}	14.67±0.60^{a}	14.20±0.64^{ab}
RBC (10^6/μl)	7.40±0.17^{a}	6.30±1.05^{a}	7.03±0.65^{a}	7.13±0.46^{a}
WBC (10^3/μl)	6.35±0.3^{ab}	6.03±1.55^{ab}	7.92±0.50^{a}	6.43±1.06^{ab}
Platelets(10^3/μl)	113.66±10.50^{a}	132.00±28.83^{a}	121.53±18.92^{a}	124.33±6.80^{a}
MCV (%)	57.23±1.80^{a}	60.46±2.40^{a}	61.10±7.57^{a}	59.43±2.30^{a}
MCH (%)	19.50±0.45^{a}	22.76±4.36^{a}	20.92±4.36^{a}	20.70±2.03^{a}
MCHC (%)	34.10±0.94^{a}	33.76±0.37^{a}	34.39±0.85^{a}	33.60±0.03^{a}

Values are mean±SD of three determinations. Means having the same letter as superscripts on the same row are not significantly different at ($P \leq 0.05$)

BASR: *Balanites aegyptiaca* seed residue

NMSR: *Neocarya macrophylla* seed residue

ECSR: *Enterolobium cyclocarpum* seed residue

Table 4.44: Antimicrobial activity of BASO and NMSO

Conc.	Zone of inhibition diameter(mm) Test organisms						
	S. aureus	*E. coli*	*B. subtilis*	*P. aerugonisa*	*C. albicans*	*A. niger*	Groups
200%	26.00±0.02[a]	28.00±0.01[a]	26.00±0.01[a]	27.00±0.00[a]	24.00±0.02[a]	24.00±0.01[a]	
100%	24.00±0.00[a]	26.00±0.00[a]	24.00±0.01[a]	26.00±0.00[a]	20.00±0.03[a]	20.00±0.00[a]	
50%	20.00±0.00[a]	22.00±1.00[a]	20.00±0.04[a]	22.00±0.00[a]	18.00±0.00[a]	18.00±0.01[a]	BASO
25%	18.00±0.00[a]	18.00±0.00[a]	16.00±0.05[a]	18.00±0.00[a]	16.00±0.00[a]	14.00±0.01[a]	
12.5%	14.00±0.01[a]	14.00±0.00[a]	14.00±0.02[a]	14.00±0.00[a]	14.00±0.00[a]	12.00±0.00[a]	
6.25%	12.00±0.00[a]	12.00±0.04[a]	10.00±0.00[a]	12.00±0.00[a]	10.00±0.00[a]	10.00±0.01[a]	
200%	26.00±0.02[a]	26.00±0.01[a]	27.00±0.01[a]	26.00±0.02[a]	24.00±0.01[a]	24.00±0.00[a]	
100%	22.00±0.01[a]	24.00±0.01[a]	26.00±0.01[a]	24.00±0.01[a]	22.00±0.00[a]	22.00±0.01[a]	
50%	20.00±0.01[a]	24.00±0.01[a]	24.00±0.01[a]	22.00±0.01[a]	20.00±0.00[a]	20.00±0.00[a]	NMSO
25%	18.00±0.00[a]	20.00±0.01[a]	22.00±0.01[a]	20.00±0.00[a]	18.00±0.00[a]	16.00±0.00[a]	
12.5%	14.00±0.01[a]	14.00±0.02[a]	18.00±0.02[a]	16.00±0.00[a]	14.00±0.02[a]	14.00±0.01[a]	
6.25%	12.00±0.01[a]	10.00±0.01[a]	14.00±1.00[a]	12.00±0.00[a]	10.00±0.00[a]	12.00±0.02[a]	
Negative Control	-	-	-	-	-	-	
Positive Control	38.00±0.00[a]	38.00±0.00[a]	36.00±0.00[a]	38.00±0.00[a]	28.00±0.00[a]	28.00±0.00[a]	

Values are mean±SD of triplicate determinations. Means having similar letters as superscripts on the same row are not significantly different at ($P \leq 0.05$)

+ve control- Gentamicin (for bacteria), 70 % Triconazole (for fungi)

-ve control – hexane (Solvent of dilution)

Comparatively, the oils exhibited considerable antibacterial and antifungi activities in contrast to the standard antibiotics employed as control. The seed oils equally displayed minimum inhibition of concentration (MIC) of 6.25 mg/mL. *Balanites aegyptiaca* seed oil showed higher activity against *E. coli* with 28.00 mm as zone of inhibition followed by *E. coli* and *S. aureus* both with 26.00 mm and 26.00 mm as respective zone of inhibitions. *Neocarya macrophylla* seed oil showed higher activity against *B. subtilis* with 27.00 mm as zone of inhibition followed by *E. coli* and *S. aureus* both with 26.00 mm as zone of inhibition. With the fungi, the extracts showed higher activity against *P. aerugonisa* with 26.00 mm and 27.00 mm as zone of inhibition followed by *C. albicans* and *A. niger* with 24.00 mm as zone of inhibition respectively in BASO and NMSO. The results obtained here was comparable to those reported on wound healing potentials of *M. myristica, M. tenuifolia* and *Azadirachta indica* seed extracts (Ajayi *et al*., 2015 and 2020).

Table 4.45 revealed the minimum inhibition concentration (MIC) of BASO and NMSO against some bacteria and fungi. It was generally observed that BASO and NMSO possessed some antibacterial and antifungal activity. Further assays were prepared to test the sensitivity of the extract at differet concentration. *Balanites aegyptiaca* and *Neocarya macrophylla* seed oils fully inhibited growth of the organisms at a concentration of 12.5 %.

4.9.5.2 Acute skin irritation test of ointment for wound healing

After being applied to a pre-shaved and cleaned area of the dorsal fur of each rat, the ointments made from BASO and NMSO, along with yellow paraffin, did not cause any irritation or observable inflammation, swelling, rashes or other changes on the skin even after 4 h of application.

4.9.5.3 Effects of BASO and NMSO on weights of rats used for wound healing experiment

The effect of BASO and NMSO on the weight of rats used or wound healing experiment was highlighted on Table 4.46. The mean weight of rats as observed at the beginning of the experiment was 198.45 g (Group 1), 207.14 g (Group 2) and 244.00 g (Group 3) for BASO. The mean weight of rats used for NMSO was 198.45 g (Group 1), 228.00 g

Table 4.45: Minimum inhibition concentration of BASO and NMSO

Conc.	Test organisms					
	200 %	**100 %**	**50 %**	**25 %**	**12.5 %**	**6.25 %**
			BASO			
Staphylococcus aureus	-	-	-	-	+	+
Escherichia coli	-	-	-	-	+	+
Bacillus subtilis	-	-	-	-	+	+
Pseudomonas aerogonisa	-	-	-		+	+
Candida albicans	-	-	-	+	+	+
Aspergillus niger	-			+	+	+
			NMSO			
Staphylococcus aureus	-	-	-	-	+	+
Escherichia coli	-	-	-	-	+	+
Bacillus subtilis	-	-	-	-	+	+
Pseudomonas aerogonisa	-	-	-	-	+	+
Candida albicans	-	-	-	+	+	+
Aspergillus niger	-	-	-	+	+	+

Key: (+): signifies inhibition (no growth of organisms), (–): no inhibition (growth of organism)

Table 4.46: Mean body weight of rat treated with BASO and NMSO

Groups	Weight (g)					
	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
			BASO			
+ ve Control	198.45 ± 1.96^{cd}	191.11 ± 1.74^{e}	183.42 ± 2.37^{f}	199.58 ± 1.06^{cd}	210.06 ± 4.93^{b}	219.85 ± 0.75^{a}
- ve Control	207.14 ± 4.88^{c}	200.00 ± 4.08^{de}	191.71 ± 2.56^{f}	198.83 ± 2.14^{de}	205.12 ± 4.35^{be}	222.14 ± 7.56^{a}
10 % BASO	244.00 ± 3.46^{ab}	225.00 ± 4.08^{de}	214.28 ± 5.34^{f}	223.68 ± 6.23^{d}	232.56 ± 8.76^{c}	249.28 ± 3.45^{ab}
			NMSO			
+ ve Control	198.45 ± 1.96^{cd}	191.11 ± 1.74^{e}	183.42 ± 2.37^{f}	199.58 ± 1.06^{cd}	210.06 ± 4.93^{b}	219.85 ± 0.75^{a}
- ve Control	228.00 ± 18.35^{def}	239.33 ± 16.43^{def}	232.67 ± 22.53^{def}	254.75 ± 21.26^{c}	267.33 ± 7.71^{b}	283.50 ± 2.45^{a}
10 % NMSO	211.28 ± 28.87^{c}	212.25 ± 15.77^{b}	193.00 ± 6.97^{e}	199.30 ± 38.47^{d}	215.00 ± 29.63^{b}	233.76 ± 3.18^{a}

The values are presented as mean±SD, and those sharing the same letter as superscripts in the same row are not significantly different at ($P \leq 0.05$).

(Group 2) and 211.28 g (Group 3). A gradual decrease was observed in the body weight of the rats from the excision day of the wound (day 0) to day 8. This reduction in the body weight was most likely to be as a result of the animals adjusting to the new body morphology probably due to the wound created on them. The rats' weight later increased gradually from the 8th days to the end of the experiment (day 21). The increase in the weight after day 8 might be resulted from the reduction in pain due to the healing process of the wound and free movement of the rats. The final weights recorded in each group of treated animal were higher than the initial weights. This showed that BASO ad NMSO probably had no adverse or toxic effect on the aimals

Alterations in the mean body weight have been employed as an indicator of adverse effects of drugs and chemicals in organs and tissues (Raphael *et al*., 2014). The weight gain observed in this study, coupled with the 100% survival rate recorded in each group were indications that BASO and NMSO exerted no harmful effect on the universal well being of the animal and efficiently contributed in healing the wound created on them. Similar results were observed and reported by Ajayi *et al*. (2015) and Musila *et al*. (2017).

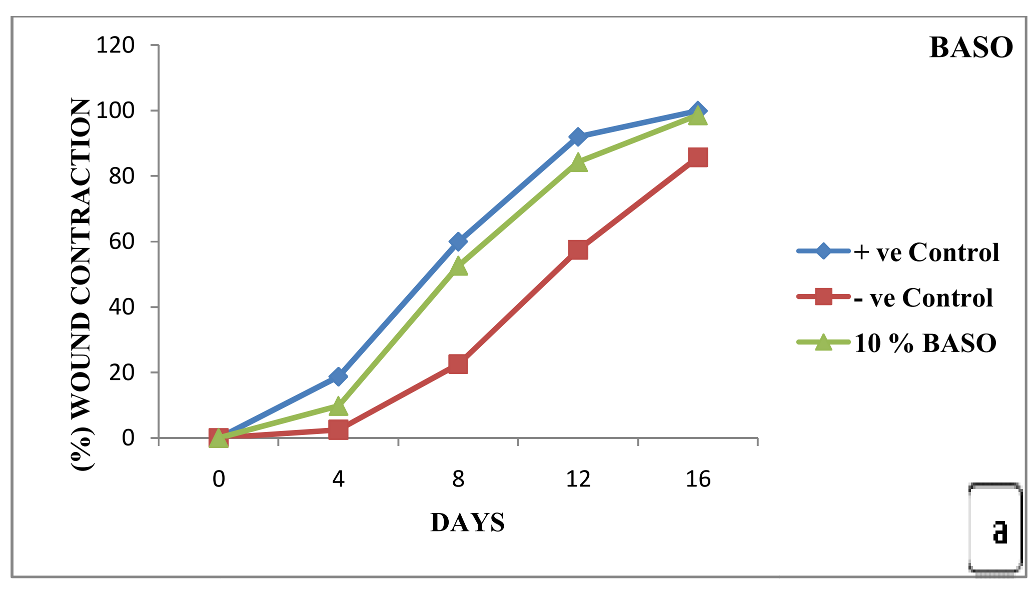
4.9.5.4 Wound contraction and epithelialisation time

The contraction of the wound area is an indication of the rate of reduction of uncured area of the wound during the healing process. This process depends majorly on the type of tissue, the extent of the damage and the state of the tissue health (Ghosh *et al*., 2012). The progressive contraction in wound area of various groups of rats for 21 days experimental period, by BASO and NMSO was given on Table 4.47. It was discovered that the animals in group 1 treated with ointment prepared from 10% povidone-iodine which served as positive control gave the fastest wound healing or wound contraction with 0.14% (as unhealed area at16th day) and better epithelialisation time of 16.28 days (Table 4.47 and Plates 4.5-4.7). Animal in group 3 treated with 10% of BASO showed wound contraction of 3.63% (as unhealed area) and epithelialisation time (day) of 17.14 while the rats in group 1 (untreated) had 28.71% (as unhealed area) on the 16th day with epithelialisation time (day) of 20.14 days. The same trend of result was obtained for both BASO and NMSO. Similar observation was reported by Ghosh *et al*. (2012) where 15.50 day was

Table 4.47: **Effect of topical application of BASO and NMSO on wound contraction and epithelialisation time**

Animal groups	Wound healing area (mm ^{2})						**Epitheliasation Time (day)**
	Day 0	**Day 4**	**Day 8**	**Day 12**	**Day 16**	**Day 20**	
				BASO			
+ ve Control	200.42±0.78^a	162.57±2.07^b	80.14±2.67^c	16.14±1.06^{de}	0.14±0.37^f	0.00±0.00^g	16.28±0.48^{de}
- ve Control	200.28±0.48^a	195.00±0.80^b	155.00±5.00^c	85.14±3.80^d	28.71±3.67^e	0.00±0.00^g	20.14±0.37^f
10% BASO	200.40±0.80^a	180.42±2.40^b	95.00±4.08^c	35.28±3.63^d	3.63±1.37^f	0.00±0.00^g	17.14±0.37^e
				NMSO			
+ ve Control	200.42±0.78^a	162.57±2.07^b	80.14±2.67^c	16.14±1.06^{de}	0.14±0.37^f	0.00±0.00^g	16.28±0.48^{de}
- ve Control	200.00±0.00^a	193.00±7.59^b	160.00±10.00^c	96.00±4.76^d	37.00±4.73^e	0.00±0.00^g	19.07±1.86^f
10% NMSO	200.00±0.00^a	193.33±7.59^b	130.00±10.00^c	86.67±4.76^d	7.00±4.73^e	0.00±0.00^g	17.83±2.34^f
			Percentage (%) wound contraction				
				BASO			
+ ve Control		18.71± 1.03^e	59.92±1.33^d	91.92±0.53^c	99.85±0.24^{ab}	100.00±0.00^{ab}	
- ve Control		2.50±0.40^e	22.50±2.50^d	57.43±1.90^c	85.67±1.85^b	100.00±0.00^a	
10 % BASO		9.78±1.20^e	52.64±1.84^d	84.28±1.88^c	98.57±0.34^b	100.00±0.00^a	
				NMSO			
+ ve Control		18.71±1.03^e	59.92±1.33^d	91.92±0.53^c	99.85±0.24^{ab}	100.00±0.00^{ab}	
- ve Control		3.50±4.26^e	20.00±5.00^d	52.00±2.38^c	81.50±2.36^b	100.00±0.00^a	
10 % NMSO		3.50±2.22^e	35.00±10.80^d	56.67±5.77^c	91.50±11.64^b	100.00±0.00^a	

The values are presented as mean±SD, and those sharing the same letter as superscripts in the same row are not significantly different at ($P \leq 0.05$).
+ ve Control: Povidone iodine
- ve Control: untreated wound



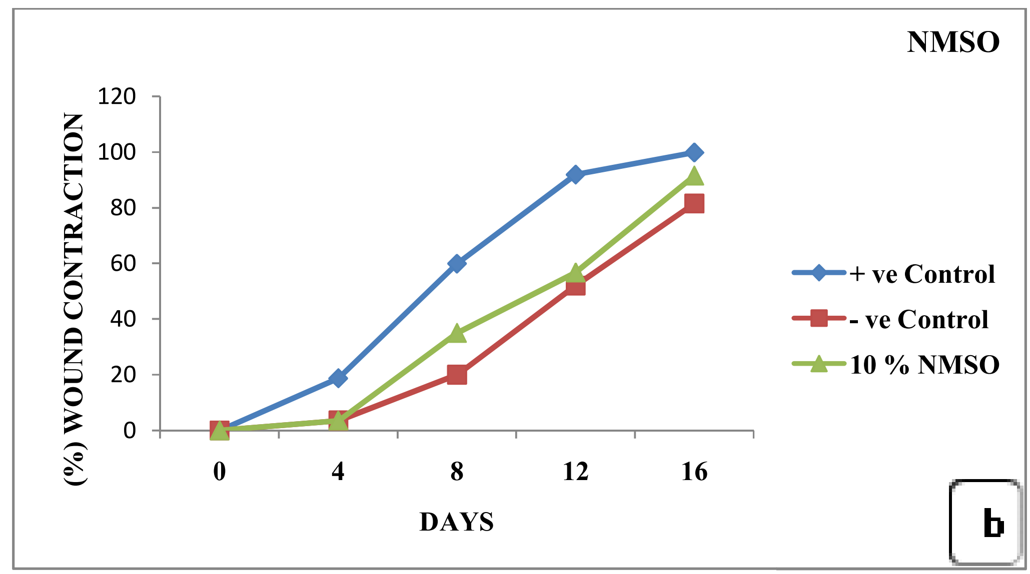


Figure 4. 10: The effect of BASO (a) and NMSO (b) on wound contraction

obtained as better epithelialisation time than 19.50 and 20.00 days obtained respectively for 5% and 2.5% of *Pedilanthus tithymaloides* extracts.

These wound healing studies carried out revealed the improved rate of wound contraction in the rats treated with ointment prepared from BASO and NMSO as compared with the control group. This was because the epithelialisation was reached in a shorter time in the terst groups. Ghosh *et al*. (2012) reported that for epithelialisation to be attained in a shorter time, the seed extracts might have promoted it either by facilitating the proliferation or by increasing the viability of epithelial cells. The above result was a perfect indication that BASO and NMSO contributed adequately in the healing of the wounds. They might possess some chemical constituents which quicken the healing time of the wounds. This equally supported their usefulness and applications in the production of some ointment for skin disorder, rashes and other possible medicinal purpose.

4.9.5.5 Effect of topical application of BASO and NMSO oils on percentage wound contraction

The effect of BASO and NMSO on percentage wound contraction as presented on Table 4.47 and Figures 4.10 (a and b) confirmed the adequate contribution of the oils in the healing of wounds on the 16th day as observed. The highest percentage wound contraction of 99.85 was recorded for the group of rat treated with 10% povidone-iodine ointment. It was followed by 98.57 for the group treated with 10% BASO while 85.67 was noted for the group with untreted animals. Similar results were obtained with NMSO (Table 4.47). This high and comparable percentage wound contraction between 10% povidone-iodine ointment which was a standard prescribed ointment for wound healing purpose and ointments formulated from 10% of BASO and NMSO studied might reveal that these oils participated fully in wound healing process.

4.9.5.6 Effect of topical application of BASO and NMSO on blood haematological analyses of rats

The haematological analysis result of the blood parameters of rats used in the wound healing study was shown on Table 4.48. The PVC (%) value in the group treated with 10% Povidone-iodine was higher than those in the other test groups. The PVC values of

45.33% (group treated with 10% Povidone-iodine), 41.66% (untreated group), 41.33% (group treated with 10% BASO) and 38.61 (group treated with 10% NMSO) were obtained. Other parameters such as WBC, Hb, Platelets, Lymphocytes, Heterophils, MCHC, MCH and MCV obtained in both oils were comparable to each other within the groups. Haematological parameters can be a useful tool to explain the blood relating function of chemical substances, plant extracts (oils) in an organism (Yakubu *et al.,* 2007). The assessment of these indices can reveal the deleterious effect of strange compound such as oil and blood constituents in animal (Jothy *et al*., 2001). The haematological analysis result of the blood parameters indicated that there was no appreciable difference between the test and control groups which suggested that topical application of the seed extract did not have a negative effect on the blood system of the rats as shown on Table 4.48.

The absence of significant differences within the blood parameters between the test and control groups was an indication that BASO and NMSO did not have negative effect on the blood system of the animals.

4.9.5.7 Histopathology analysis of the heart, kidney and liver of rats and healed skin

Histopathology analysis of the organs (heart, kidney and liver) was displayed on Table 4.49 while those of the healed skin area were recorded on Table 4.50 and Plate 4.7. As shown on Table 4.49, no lesions were observed in the heart, kidney and liver but the hepatic plates were closely packed together in the group treated with povidone-iodine. Moderate coronary blood vessels congestion, normal appearance of tubules and moderate thinning of hepatic plates were respectively seen in the heart, kidney and liver of the group with untreated animals. No major lesions were recorded in the group treated with 10% BASO and NMSO. Moderate congestion of coronary blood vessels and random foci of single-cell hepatocellular necrosis were seen in the heart and liver of the test group treated with 10% BASO based ointments. The absence of major injury in the studied organs and tissues was evidence that BASO and NMSO did not have any effect on rats' organs. Ajayi *et al*. (2015) recorded similar observations on organs of the rats treated with *M. monodora* and *M. tenuifolia* extract based ointments.

Table 4.48: Haematological analyses of blood samples of rats treated with BASO and NMSO

Parameter	+ ve Control	- ve Control	10% Sample
	BASO		
PVC (%)	45.33±0.57[a]	41.66±0.60[b]	41.33±1.50[b]
Hb (mg/dl)	14.76±0.05[a]	13.50±0.40[ab]	13.40±0.40[a]
RBC (W^6/ul)	7.32±0.17[a]	6.85±0.30[a]	6.85±0.45[a]
WBC (w^3/ul)x10^3	15.76±3.08[a]	13.91±1.04[a]	16.73±2.60[a]
Platelet(cell)x10^5/ml	1.46±2.40 [a]	1.37±2.22[a]	1.37±2.96[a]
Lymphocytes (%)	67.00±4.30[a]	67.33±3.51[a]	71.66±1.15[a]
Heterophiles (%)	26.30±4.04[a]	26.70±4.60[a]	25.00±1.00[a]
MCHC (%)	32.55±0.36[a]	32.21±0.94[a]	32.40±0.70[a]
MCH (%)	20.17±0.50[a]	19.70±0.50[a]	19.60±0.30[a]
MCV (%)	61.94±2.30[a]	60.82±2.08[a]	60.42±1.94[a]
	NMSO		
PVC (%)	45.33±0.57[a]	41.00±2.12[a]	38.61±0.99[b]
Hb (mg/dl)	14.76±0.05[a]	13.63±6.79[a]	12.70±0.57[b]
RBC (W^6/ul)	7.32±0.17[a]	6.79±0.42[a]	6.60±0.11[a]
WBC (w^3/ul)x10^3	15.76±3.08[a]	17.96±1.98[a]	17.62±1.18[a]
Platelet(cell)x10^5/ml	1.46±2.40[a]	1.98±1.24 [a]	2.02±0.67[a]
Lymphocytes (%)	67.00±4.30[a]	70.25±3.86[a]	65.67±4.51
Heterophiles (%)	26.30±4.04[a]	25.25±3.30[b]	30.67±3.06[a]
MCHC	32.55±0.36[a]	33.24±0.06[a]	32.84±0.05[a]
MCH	20.17±0.50[a]	20.70±0.04[a]	19.24±0.01[a]
MCV	61.94±2.30[a]	60.38±0.17[a]	58.59±0.04[ab]

The values are presented as mean±SD, and those sharing the same letter as superscripts in the same row are not significantly different at ($P \leq 0.05$)
+ ve Control: Povidone iodine
- ve Control: untreated wound

Histological examination of the skin reveals the healing process of the wounded tissue in rats treated with BASO and NMSO. The healing was better in the test groups, closer to the group treated with povidone iodine than in the group with untreated animals. This histopathology examination provided more proof for the wound healing activities of seed extracts based on the contraction of the wound area and the epitheliasation time (Kuntal Das, 2013). Healing of wound is complex in nature and dynamic process which involves tissue structure restoring back to its normal state (Ajayi *et al.,* 2015). In all, a scar composed of dense fibrous connective tissues, moderator thickened epidermis, complete epithelialisation and a dense irregular collagen arrangement in the dermis were observed in the groups treated with 10% BASO and NMSO based ointments. This observed result is comparable to those obtained with the rats treated with povidone-iodine ointment (positive control). *Balanites aegyptiaca* and *Neocarya macrophylla* seed oils might be recommended for the treatment of wounds and other related skin disorder.

Table 4.49: Summary of histopathology of organs from rat heated with BASO and NMSO

Sample	Heart	Kidney	Liver
-ve control	Visible lesion not seen	There was no observable lesion	There was a dense arrangement of hepatic plates, and no observable lesion
+ ve control	The coronary blood vessels are moderately congested.	There was no observable lesion	Hepatic plates atre moderately thinning
10% BASO	The coronary blood vessels are moderately congested.	There was no observable lesion	Single-cell recrosis of the hepatocytes were observed in random foci, but no observable lesion
10% NMSO	Visible lesion not seen	Single-cell recrosis of the hepatocytes were observed in random foci, but no visible lesion seen	Single-cell recrosis of the hepatocytes were observed in random foci, but no visible lesion seen

Table 4.50: A synopsis of histological examination conducted on the skin tissues of rats healed with BASO and NMSO

Sample	Observation on the skin
-ve control	Plate 4.7a: This plate shows a photomicrograph of a healed skin wound on rat (with wound left untreated) (H&E x100). The epidermis appears as stratified squalors keratinsed tissue and there are several sebaceous glands present with complete epithelialisation
+ ve control	Plate 4.7b: This is a photomicrograph (H&E x100) of a healed skin wound on rat treated with povidone- iodine ointment. There is a mild degeneration of the epidermal cells and keratinisation present. New immature collagen fibers are present with complete epithelialisation
10% w/w of BASO	Plate 4.7c: Displays a photomicrograph (H&E x 100) of a healed skin wound on a rat healed with 10% w/w BASObase ointment. The epidermi appear thickened with a few atreas of epidermal depressions and only a few visible sebaceous glands. Epithelialisation complete and immature and moderately collagen fibers present.
10% w/w of NMSO	Plate 4.7d: Exhibits a photomicrograph of a healed skin wound on a rat treated with 10% w/w NMSO base ointment (H&E x100). The photomicrograph shows numerous hair follicles and a dermis (indicated by a star) composed of dense fibrous connective tissue. The epidermis appears thickened with complete epithelialisation. Additionally, there is a dense irregular arrangement of collagen fibers in the dermis.

0 day

Control **10% NMSO**

4 day

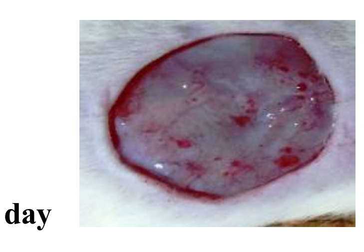
8 day

12day

16 day

20 day

Plate 4.5: Pictures of various steps of NMSO wound healing activity (Excision wound model)



0 day

Control **10% BASO**

4 day

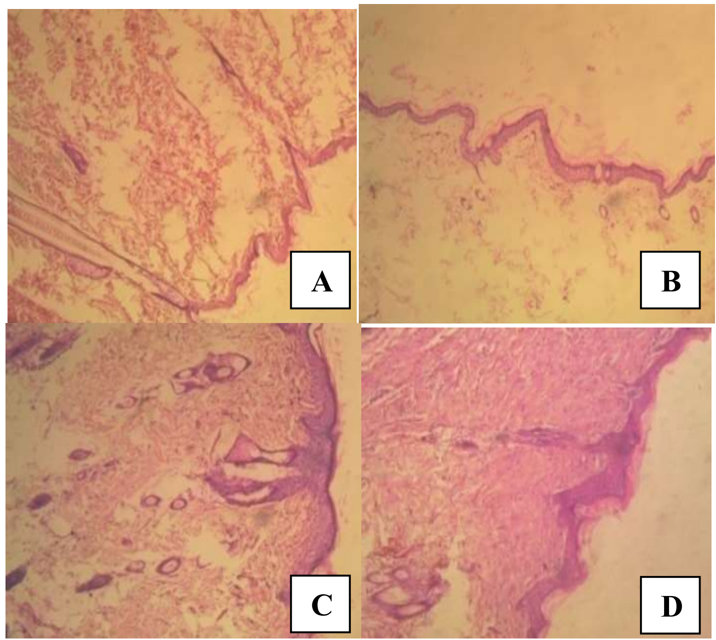
8 day

12day

16 day

20 day

Plate 4.6: Pictures of various steps of BASO wound healing activity (Excision wound model)



A. Skin area of untreated rat
B. Skin area of rat treated with povidone- iodine
C. Skin area of rat treated with 10 % w/w of BASO base ointment
D. Skin area of rat treated with 10 % w/w of NMSO base ointment

Plate 4.7: Photomicrograph of skin of the rats treated with control, BASO and NMSO (H&E x400)

4.10 Synthesis of oleochemicals (Biofuels) from baso and nmso

4.10.1 Application of BASO and NMSO in the synthesis and characterisation of (methyl ester) biodiesel

4.10.1.1 Synthesis of biodiesel from BASO and NMSO

Biodiesel fuel according to the American Society of Testing and materials (ASTM) was defined as mono-alkyl esters of long chain fatty acids that are derived from a renewable lipid feedstock such as vegetable oil or animal fat. Biodiesel is a diesel fuel, a renewable fuel obtained from biological source in contrast to the traditional diesel obtained from petroleum. The use and production of bio-based diesel is in increasing level recently in many countries in the world because of its advantages over the petroleum base diesel which are expensive, not environmental friendly, non renewable and non biodegradable. Some advantages of biodiesel over petro-based diesel are domestic origin, renewability, environmental friendly, biodegradable, inherent lubricity, higher cetane number and very safe in handling due to higher flash point (Adewuyi *et al.,* 2012a). The quest of readily available biodiesel led to its production from a variety of other possible sources which includes algae, non edible oils, soap stock, used oils which are discarded as waste oils, animal fats, cheap and underutilised seed oils among others. The use of the above sources as raw materials for the synthesis and preparation of biodiesel is an effective way towards the reduction of the high price of diesel fuel (Adewuyi *et al.,* 2012a).

The percentage yield was 90.85% and 84.86% respectively for *B. aegyptiaca* and *N. macrophylla* seed oils biodiesel (BASOBIO and NMSOBIO). These values are higher than 83.30% for rocket seed oil biodiesel as reported by (Tariq *et al.*, 2011). Naureen *et al.* (2015) reported 85.10% as observed percentage yield on sunflower biodiesel. Adewuyi *et al.* (2012b) reported a percentage yield of 98% for biodiesel produced from *Blighia unijugata* and *Luffa cylindrica* seed oils while Anjaneyulu *et al.* (2017) reported a percentage yield of 97.6% for biodiesel produced *Ailanthus excelsa* seed oil. This value is slightly higher than 90.85% and 84.86% recorded for biodiesel produced from *B. aegyptiaca* and *N. macrophylla* seed oils in this study.

4.10.1.2 Physical and fuel properties of biodiesel from BASO and NMSO

The results of the physical and fuel properties of biodiesel from BASO and NMSO were given on Table 4.51 together with the (EN-14214) recommendation and (ASTM-D6751) recommended values for biodiesel. There are various standards specified to check the biodiesel quality since it is important for engine functionality. The density values of 0.87 g/cm^3 determined for both BASOBIO and NMSOBIO at 40 ^oC are much comparable to EN-14214 and ASTM-D6751 recommendations. It is also in accordance with 0.89 g/cm^3 rocket seed oil (Tariq *et al.,* 2011). At 15 ^oC, the density values of 0.88 g/cm^3 and 0.89 g/cm^3 were recorded in the order mentioned for BASOBIO and NMSOBIO respectively. These values are also in agreement with 0.88 g/cm^3 and 0.89 g/cm^3 noted for *Blighia unijugata* and *Luffa cylindrica* seed oil biodiesels by (Adewuyi *et al.,* 2012b). The value of 0.89 g/cm^3 was obtained for *Momordica charantia* biodiesel (Rashid *et al.,* 2014). The specific gravity determined in BASO and NMSO biodiesel at 15 ^oC were 0.88 g/cm^3 and 0.89 g/cm^3 while 0.89 g/cm^3 and 0.86 g/cm^3 were obtained at 40 ^oC. These values were comparable to 0.88 which iwas the recommended value by ASTM-D6751 and 0.98 g/cm^3 for rocket seed oil biodiesel (Tariq *et al.,* 2011).

Viscosity is a property of biodiesel that affect the operation and the function of the fuel injection equipment. Increasing the viscosity at a lower temperature affects the quality of the diesel fuel and its ability to be able to flow freely as a fluid. The observed viscosities at 40 ^oC were respectively 4.42 mm^2 s^1 and 3.52 mm^2 s^1 BASOBIO and NMSOBIO respectively. High viscosity will lead to poorer atomisation of the fuel spray (Naureen *et al.,* 2015). The determined viscosity in BASOBIO and NMSOBIO were within the specified limit of EN-14214 recommendation (3.50-5.00 mm^2 s^1) while 4.48 mm^2 s^1 was for *Momordica charantia* biodiesel (Tariq *et al.,* 2011). Iodine value is a necessary index that promotes the evaluation of the fuels' degree of unsaturation. It influences greatly the oxidation of the fuel, the kind of aging products and the deposits that are accumulated in the diesel engines injectors (Adewuyi *et al.,* 2012a). The resulting iodine values obtained in BASOBIO and NMSOBIO (88.59 g iodine/100 g and 101.19 g iodine/100 g) were slightly lower than 120 (max) recorded in EN-14214 standard. This indicated that these biodiesel prepared will be less susceptible to degradation with a good shelf life.

Table 4.51: Physical and fuel properties of biodiesel from BASO and NMSO

Fuel properties	BASOBIO	NMSOBIO	EN-14214 recommendation	ASTM D6751
Specific gravity @ 15 °C	0.88±0.00	0.89±0.00		
Specific gravity @ 40 °C	0.88±0.00	0.86±0.00		0.88
Density @ 15 °C	0.88±0.00	0.89±0.00	--------	------
Density @ 40 °C	0.87±0.00	0.87±0.00	0.86 - 0.90	0.86 - 0.90
Micro carbon residue	0.01±0.00	0.01±0.00	0.20 (max)	0.05 (max)
Refractive index @ 25 °C	1.44±0.02	1.45±0.03	---------	--------
Ester content (%)	96.70±003.	97.29±0.02	96.50 (min)	--------
Monoglyceride	0.16±0.03	0.14±0.00	0.80 (max)	0.60 (max)
Triglyceride	0.06±0.03	0.01±0.00	0.20 (max)	0.20 (max)
Diglyceride	0.11±0.01	0.15±0.00	0.20 (max)	0.20 (max)
FFA (%)	0.53±0.02	0.62±0.02	0.25	0.25
Methanol content	0.10±0.00	0.09±0.00	0.20 (max)	-------
Viscosity @ 40 °C	3.52±0.01	4.42±0.02	3.50 - 5.00	1.9 – 6
Iodine value (mg I_2/g)	88.59±0.00	101.19±0.51	120 (max)	--------
Flash point (°C)	139.30±0.06	146.51±3.38	120 (min)	130 (min)
Cupper corrosion test	1A	1A	1A	1A
Sulphur (ppm)	< 0.001	< 0.001		
Oxidative stability (h)	9.8	10.2	6.00	3.00
Phosphorous content	< 1ppm	< 1ppm	10 (max)	10 (max)
Free glycerides	0.00134	0.00145	0.02 (max)	------
Cloud point (°C)	3	3		-3 to 12
Pour point (°C)	6	6	-------	-15 to 10

Values are expressed as mean±SD of three determinations
(EN-14214) recommendation and (ASTM-D6751) recommended values for biodiesel.
BASOBIO: *Balanites aegyptiaca* seed oil biodiesel
NMSOBIO: *Neocarya macrophylla* seed oil biodiesel

Similar results were obtained by Adewuyi *et al.,* (2012a). Triglyceride, diglyceride, free glyceride, free fatty acid content and Cu corrosion test were all within the limit values of EN-14214 and ASTM-D6751 recommendations.

The phosphorus content of the produced biodiesel was lesser than 1 ppm. This value was much lower than the recommended 10 ppm of EN-14214. The temperature at which the fuel can no longer flow due to the presence of enough wax to gel the fuel was known as the pour point of biodiesel (the minimum temperature below which the fuel loses its flow characteristics). Pour point of 6 ^{o}C was obtained for both BASOBIO and NMSOBIO. Sun flower oil biodiesel has a pour point of -5 ^{o}C (Naureen *et al.,* 2015). The recommended minimum value of the oxidative stability by EN-14214 specification is 6.00 h. BASOBIO and NMSOBIO oxidative stability found were 9.8 h and 10.2 h. These values were slightly higher than 6.00 h recommended as minimum value by EN-14214. This implies a better use of the prepared biodiesel in a little higher temperature environment. When dealing with flammable substances, such as fuels, the flash point is a critical factor to take into account in terms of storage, handling, and overall safety. The recommended limit of flash point by EN-14214 is minimum of 120 ^{o}C and between 100 and 170 ^{o}C for ASTM D 6751 specification. The flash point of 139.30 ^{o}C (BASOBIO) and 146.51 ^{o}C (NMSOBIO) were within the specified range. These values were higher than the lowest limit in the standard specifications and were comparable to the values reported by Adewuyi *et al.* (2012a and 2012b).

The BASO and NMSO obtained from Nigeria were with high oil yield. The fuel properties of biodiesels synthesised from these two oils were in good comparison with both EN-14214 and ASTM D6751 specifications. The above results revealed that BASO and NMSO have potential that suggest them to be properly employed in synthesising fatty acid methylesters that could be successfully used as biodiesel with good fuel properties as a replacement for the highly expensive petro-based diesel.

4.10.1.3 Chemical properties of *B. aegyptiaca* and *N. macrophylla* seed oils biodiesel (BASOBIO and NMSOBIO)

4.10.1.4 FT-IR spectrometry

Functional groups as well as bands corresponding to various stretching and bending vibrations observed in the oil and biodiesel samples were identified from FT-IR spectra in the mid-infrared. There were two major strong characteristics absorption bands detected in the esters. They arose from carbonyl (C=O) that are always around 1750-1730 cm^{-1} and C-O (anti-symmetric and asymmetric axial stretching) also seen within 1300-1000 cm^{-1}. The CH_3, CH_2, and CH stretching vibrations appeared generally at 2980-2950 cm^{-1}, 2950-2850 cm^{-1} and 3050-3000 cm^{-1}, while the bending vibrations appeared respectively at 1475-1350 cm^{-1}, 1350-1150 cm^{-1} and 722 cm^{-1} (Tariq *et al.,* 2011).

The results of FT-IR spectra of BASO and NMSO as well as their respective biodiesels are summarised on Table 4.52 and Appendices 17-20. The methoxy group in BASO and NMSO biodiesels was seen at 1741.90 cm^{-1} and 1741.53 cm^{-1} respectively while those in the oils were around 1744.05 cm^{-1} and 1742.90 cm^{-1} respectively. The small shift in the carbonyl frequency could potentially be attributed to electron-donating impact of the methyl group that exists alongside the C=O group in the biodiesels. The methyl group (-CH_3) stretching band are seen at 2923.31 cm^{-1} and 2923.47 cm^{-1} in the biodiesels while they appeared correspondingly at 2922.44 cm^{-1} and 2922.47 cm^{-1} in BASO and NMSO. The methylene group (-CH_2-) stretching bands appeared at 2856.71 cm^{-1} and 2855.80 cm^{-1} in BASO and NMSO biodiesels while they appeared at 2855.58 cm^{-1} and 2855.48 in BASO and NMSO. The CH_3 bending vibrations are seen around 1450.30 cm^{-1} and 1450.38 cm^{-1} in the biodiesels and around 1456.68 cm^{-1} and 1456.72 cm^{-1} in the oils. The methylene bending vibrations were displayed around 1233.51 cm^{-1}, 1233.26 cm^{-}1, 1243.60 cm^{-1} and 1246.99 cm^{-1} in BASO, NMSO and their respective biodiesels. CH groups bending vibrations were seen around 721.91 cm^{-1}, 725.26 cm^{-1}, 721.22 cm^{-1} and 720.62 in BASO, NMSO, BASOBIO and NMSOBIO. FT-IR spectra of oils and biodiesels were almost similar to each other but some various differences were observed for identification purposes. Some peaks disappeared in the FT-IR spectra of the oils (1744.05 cm^{-1}, 1456.31 cm^{-1} and 1158.93 cm^{-1}) and some others are formed in the FT-IR spectra of biodiesel (1741.90 cm^{-1}, 1449.59 cm^{-1} and 1174.07 cm^{-1}) indicated total conversion of oils

Table: 4.52: FT-IR analyses (cm^{-1}) of BASO, NMSO and their respective biodiesels

BASO	NMSO	BASOBIO	NMSOBIO	Functional group
3007.45	3007.55	3005.05	3005.47	C=C-H unsaturation
2922.44	2922.75	2923.31	2924.43	Stretching vibration of C-H (CH_3)
2855.58	2855.76	2855.71	2856.72	Stretching vibration of C-H (CH_2)
1744.05	1742.90	1741.90	1741.16	C=O stretching of ester
1456.68	1456.31	1450.30	1449.59	Bending vibration of C-H (CH_2)
1233.51	1233.96	1243.60	1246.99	Bending vibration of C-H (CH_3)
1157.92	1158.93	1171.52	1174.07	C-O stretching of ester
721.22	724.16	721.91	724.61	Bending vibration of C-H

BASOBIO: *Balanites aegyptiaca* seed oil biodiesel
NMSOBIO: *Neocarya macrophylla* seed oil biodiesel

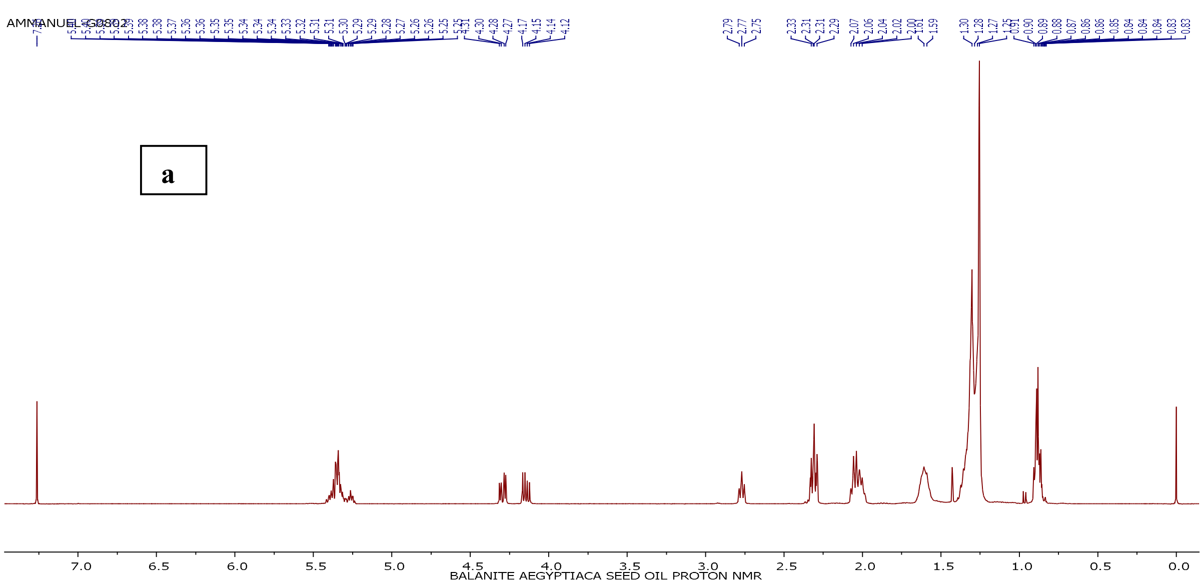
into biodiesels. This result is in agreement with the reported findings on rocket seed oil biodiesel (Tariq *et al.,* 2011).

4.10.1.5. ^{1}H NMR analysis

Figure 4.11a-b showed the ^{1}HNMR spectrum of BASO and its corresponding biodiesel (BASOBIO), while Figures 4.12a-b depicted the ^{1}HNMR spectrum of NMSO and NMSOBIO. The biodiesel samples exhibited specific peaks that were not in the oils, such as an intense singlet peak of methyl ester proton at δ = 3.67 ppm in both biodiesels, which was not present in their oils. Additionally, a triplet peak at 2.30 ppm, which corresponded to the alpha methylene protons (-CH_2-) adjacent to double bond carbon atoms (-CH_2-CH=CH-CH_2-) in the biodiesel spectra was observed. These two peaks were distinct feature that confirmed the presence of methyl esters in biodiesel. Other notable peaks include the terminal proton (-CH_3) of the fatty acid chains, which resonate at δ = 0.88 ppm, and the internal protons (-CH_2-) of the fatty acid chains, which were represented by signals at δ = 1.30 ppm. The beta carbonyl methylene proton of the carbon chain exhibit a signal at δ = 1.59 ppm, while the olefinic protons are observed at δ = 5.36 ppm in both BASOBIO and NMSOBIO. The peaks at δ = 5.24-5.42 ppm correspond to (-CH-) of glycerol backbone and =CH of unsaturated carbon in the fatty acid, while glycerol (-CH_2-) proton appears at δ = 4.14-4.30 ppm in both oils. The absence of glycerol (-CH_2-) proton at δ = 4.14-4.30 ppm in the methyl esters (biodiesel) indicated that the glycerol backbone in the triglyceride of the oil had been broken down to form different straight chain fatty acids in the methyl esters formed. Finally, the intense peak observed at δ = 3.67 ppm in both BASOBIO and NMSOBIO was attributed to the methyl ester protons, confirming the conversion of the various oils into corresponding methyl esters (biodiesel). This observation was in agreement and similar with the results accounted for biodiesels from *Treculia africana* seed oil (Adewuyi *et al.,* 2012a), rocket seed oil (Tariq *et al.,* 2011), sunflower oil (Naureen *et al*., 2015) and *Ailanthu excelsa* oil (Anjaneyulu *et al.,* 2017).

4.10.1.6 ^{13}C NMR analysis

The ^{13}C NMR spectra of BASOBIO and NMSOBIO were presented on Figures 4.13a-b. The spectra showed the characteristics peaks of ester carbonyl (-COO-) and (C-O) which were observed at δ = 174.30 ppm, δ = 174.34 ppm and 51.42 ppm, δ=51.46 ppm



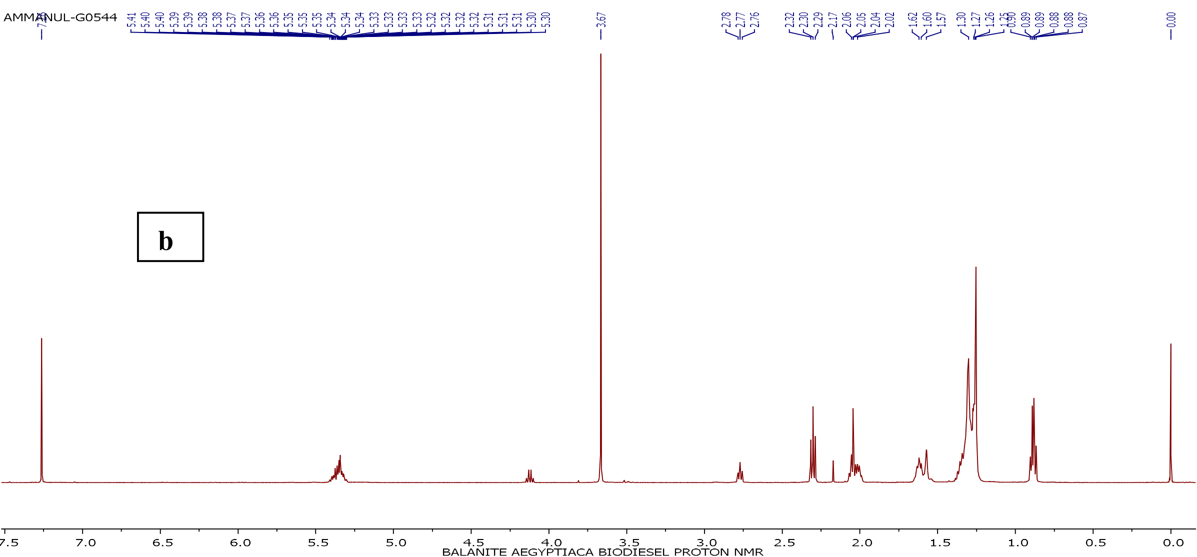
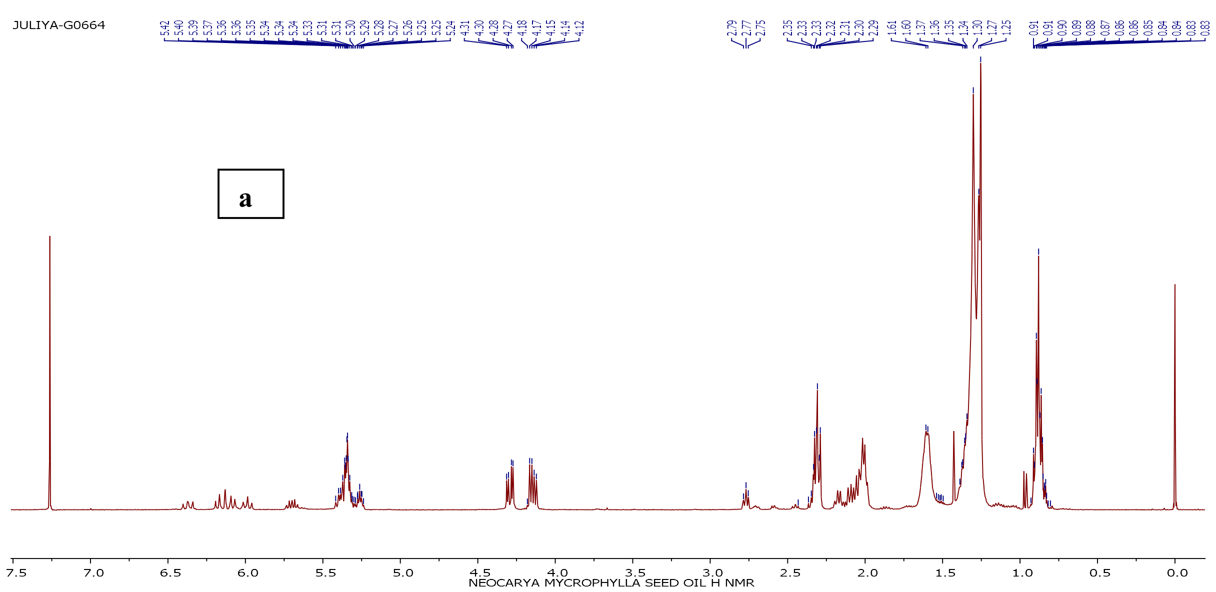


Figure 4.11: **^{1}H NMR spectra of triglycerides in (a) *B. aegyptiaca* seed oil and of (b) *B. aegyptiaca* biodiesel**



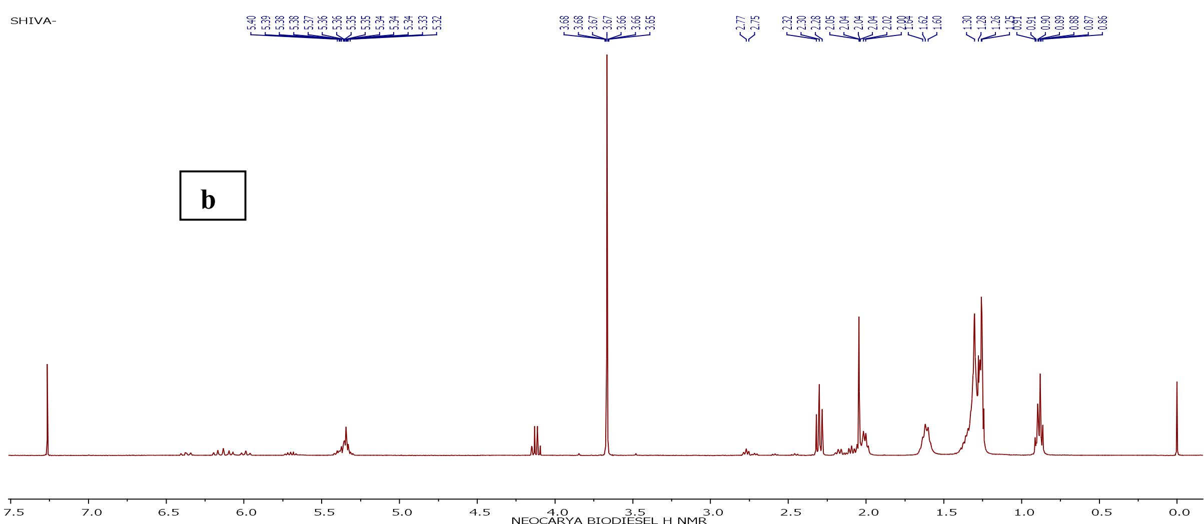


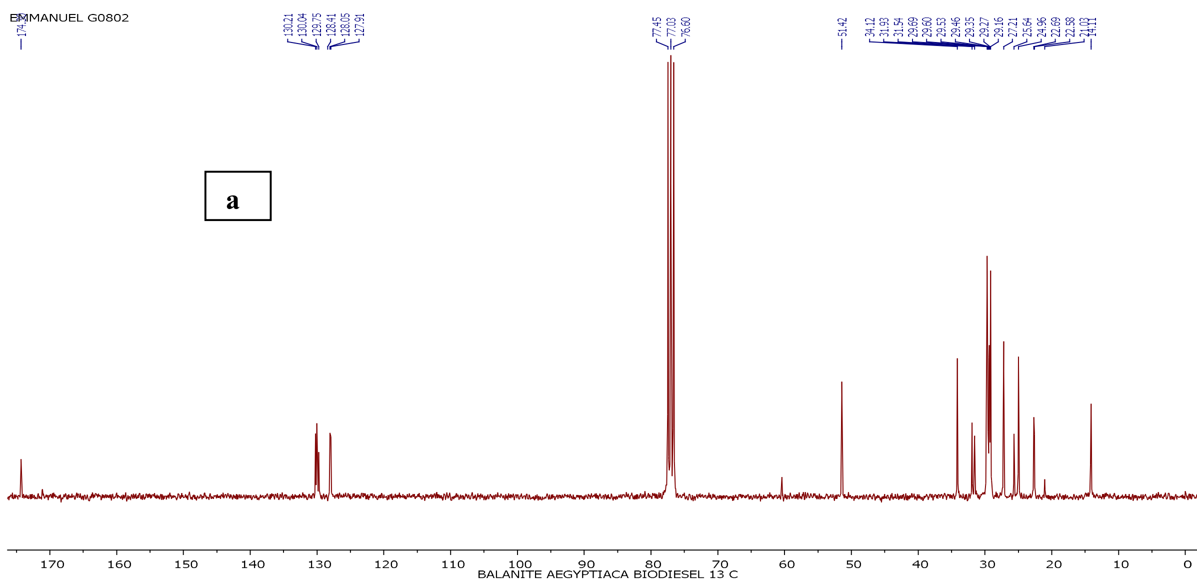
Figure 4.12: **^{1}H NMR spectra of triglycerides in (a) *N. macrophylla* seed oil and (b) *N. macrophylla* biodiesel**

respectively in BASOBIO and NMSOBIO. The peaks that fell within the range of 125.93 - 135.32 ppm and 127.91 - 130.21 ppm showed the unsaturation in the BASO and NMSO methyl esters (biodiesels) respectively. The peaks around 14.11 ppm are attributed to the terminal carbon of methyl groups while those between 22.73 and 34.13 ppm and from 21.03 to 34.42 ppm were also attributed to methylene carbons of long carbon chain in the methyl esters.

The methyl esters (biodiesels) from BASO and NMSO were also subjected to ESI-MS (Mass Spectrometry analysis by Electrospray Ionisation (Direct: Single Quad analyser) test using Liquid Chromatography/Mass Spectrometer (Single Quadrupole) for the determination of the mass of the methyl esters synthesised. The molecular formula of the methyl ester produced from NMSO is $C_{19}H_{36}O_2$ which is majorly oleic acid while the one from BASO is $C_{19}H_{34}O_2$ which is majorly linoleic acid. The exact mass of 296.27 g/mol was expected as calculated in *N. macrophylla* methyl ester while 294.26 g/mol was also expected from *B. aegyptiaca* methyl ester. ESI-MS analyses revealed the masses of 297.25 g/mol and 295.20 g/mol as the most abundant peaks respectively in the *B. aegyptiaca* and *N. macrophylla* methyl esters (biodiesels). Each of the spectra corresponds to [M+1] and this further confirms the structure of the synthesised methyl esters from the NMSO and BASO (Appendices 34-35).

4.10.2 Synthesis and characterisation of biosurfactant from BASO and NMSO

Fatty acids have been reported to be suitable for the production of surfactants and other similar products which are in turn discovered to possess numerous industrial applications such as detergents, dispersants, emulsifier, softeners and wetting agents (Heidrich, 1984). Surfactants are used in a variety of industries because of their physicochemical qualities, particularly their capability to create supramolecular structures such as liquid crystals, micelles, monolayers, bilayers, and multilayers via adsorption and aggregation (Zhao and Zhu, 2003). They can also be used to make both edible and non-edible end products. They may be useful as wetting agents, detergent, emulsifiers, foaming agents and dispersants. They are also regarded as active agents that bring down the surface tension of liquids (Adewuyi, 2019). Surfactants are regarded as essential components in the formulations of



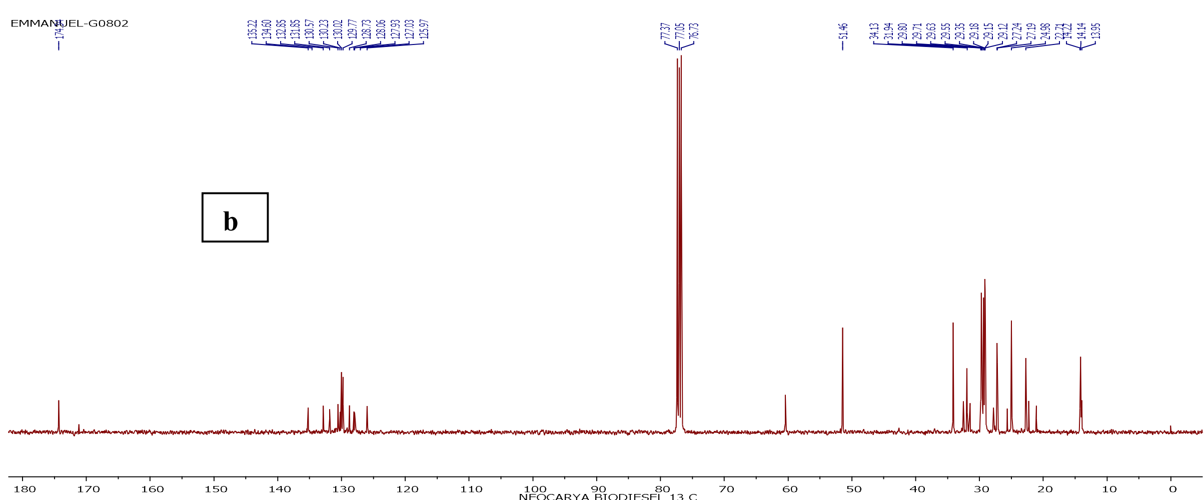


Figure 4.13: ^{13}**C NMR spectra of (a)** ***B. aegyptiaca*** **biodiesel and (b)** ***N. macrophylla*** **biodiesel**

agrochemicals, corrosion inhibitors, cosmetics, detergents, lubricants, metal working, drilling fluids, polymers and textile finishes. These alkanolamides find a variety of applications as foam boosters, viscisity builders, emulsifying agent, wetting and conditioning agent (Kamalakar *et al*., 2014). The BASO and NMSO were used to synthesise diethanolamide and epoxidised diethanolamide.

4.10.2.1: Assessment of the surface-active properties of diethanolamide and epoxidised diethanolamide (surfactants)

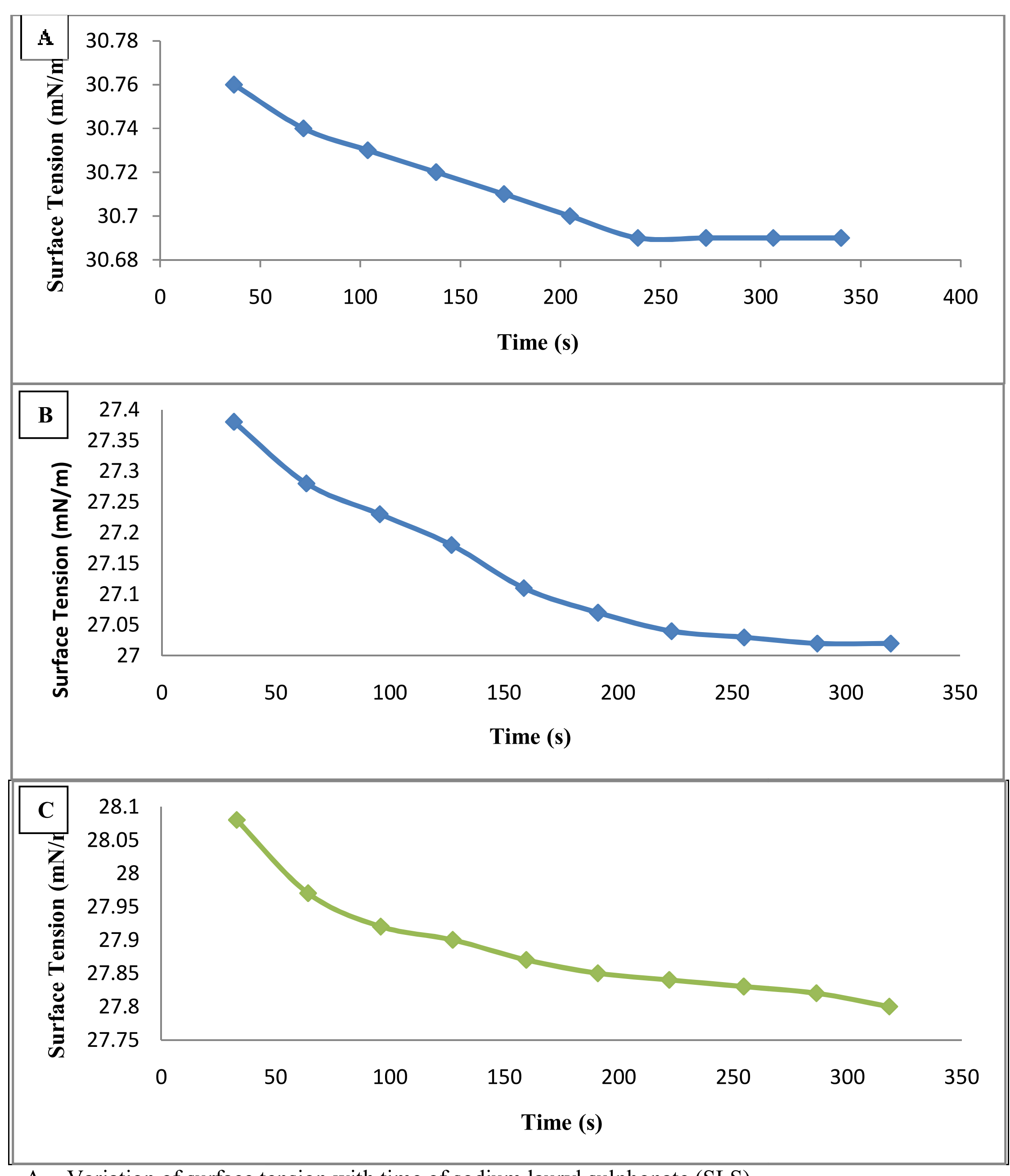
The various properties of the diethanolamide (BASODEA and NMSODEA) synthesised from BASO and NMSO and their cooresponding epoxidised oils (EPOBASODEA and EPONMSODEA) were shown on Table 4.53. These properties were compared with a reference standard popularly known as (SLS) Sodium Lauryl Surphate.

The surface tension of a liquid substance is an internal pressure that is due to the molecular attraction between the molecules at the bottom of the surface from those at the upper surface of the liquid at the interface. The attractions between these molecules give rise to an inward pull, inner force or internal pressure. The internal force (pressure) tends to limit the ability of the liquid substance to flow and form a wide interface with another substance. Figures 4.14 and 4.15 were representative graphs showing the variation of surface tension (mN/m) with time (sec). The surface tension values obtained as shown on Table 4.53 were comparable to each other among the diethanolamide and the epoxidised diethanolamide produced. The surface tension in diethanolamide prepared was 27.04 mN/m and 27.83 mN/m respectively from *B. aegyptiaca* and *N. macrophylla* seed oils. Those in the epoxidised diethanolamide are respectively 27.45 mN/m and 27.90 mN/m for BASODEA and NMSODEA respectively while surface tension value of 30.70 mN/m was obtained in sodium lauryl sulphate used as standard and control. Sodium lauryl sulphate was found to have higher surface tension than the epoxidised diethanolamide. Diethanolamide and the epoxidised diethanolamide from the seed oils were found to reduce the surface tension of water better than what was reported for the diethanolamide of rice bran, soybean and rapeseed protein (Toliwal and Patel, 2006). The surface tension of the synthesised surfactants was found lower than that of the standard (SLS) used to compare them. When surface tension is reduced at the air or water interface, the interfacial

Table 4.53: Assessment of surface-active properties of diethanolamide and epoxidised diethanolamide (surfactants) from BASO and NMSO

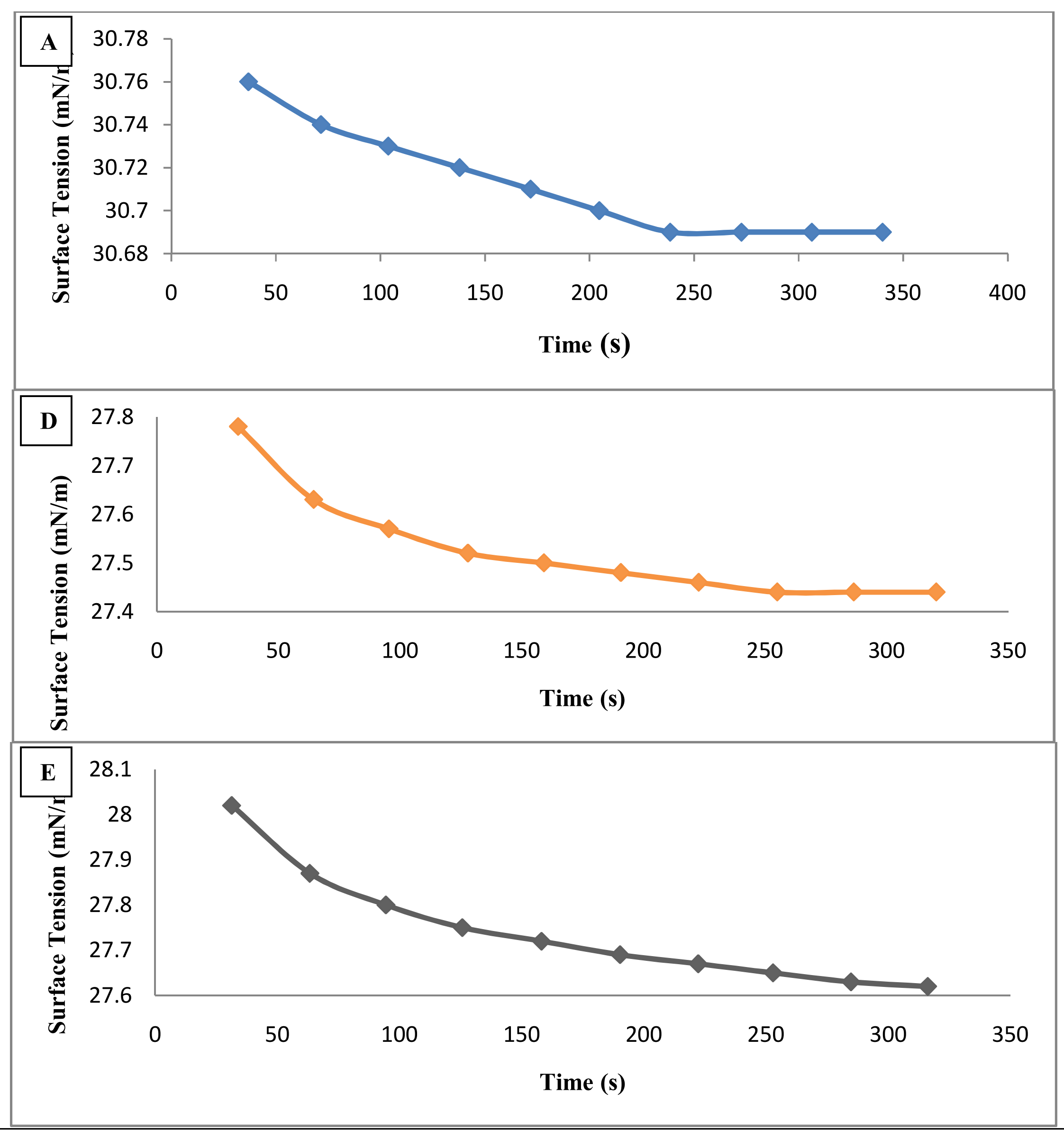
SAMPLE	Surface tension (mN/m)	Emulsion phase	Aqueous Separation	Foam	Power	
		10 ml	15 ml	Initial	After 5mn	Diff
BASODEA	27.04±0.02	72.43±0.75	197.60±1.24	8	6.5	1.5
EPOBASODEA	27.45±0.02	90.40±4.21	153.20±1.44	8	6	2
NMSODEA	27.83±0.02	73.06±0.30	216.46±1.81	8	7	1
EPONMSODEA	27.90±0.03	92.33±0.23	140.40±2.61	8	6	2
SLS	30.70±0.01	94.93±1.20	252.33±0.51	30	28	2

NMSODEA: *N. macrophylla* seed oil diethanolamide (surfactant)
EPONMDEA: Epoxidised *N. macrophylla* seed oil diethanolamide (surfactant)
BASODEA: *B. aegyptiaca* seed oil diethanolamide (surfactant)
EPOBASODEA: Epoxidised *B. aegyptiaca* seed oil diethanolamide (surfactant)
SLS: Sodium lauryl sulphate



A. Variation of surface tension with time of sodium lauryl sulphonate (SLS)
B. Variation of surface tension with time of the BASO diethanolamide
C. Variation of surface tension with time of the NMSO diethanolamide

Figure 4.14: Variation of surface tension with time of SLS (A), BASO diethanolamide (B) and NMSO diethanolamide (C) (surfactants)



A. Variation of surface tension with time of sodium lauryl sulphonate (SLS)
D. Variation of surface tension with time of epoxidised BASO diethanolamide
E. Variation of surface tension with time of epoxidised NMSO diethanolamide

Figure 4.15: Variation of surface tension with time of SLS (A), epoxidised BASO diethanolamide (D) and epoxidised NMSO diethanolamide (E) (surfactants)

tension will be depending directly on the displacement of liquid molecules on the interfacial surface by surfactant molecules (Ware *et al.,* 2007). The *B. aegyptiaca* and *N. macrophylla* seed oils surfactants tend to behave better as surface tension reducing agents.

Emulsions are dissipations that are made stable by the use of an emulsifier that engross up at liquid-liquid interface and thereby lead to the reduction of the interfacial tension. This reduction causes the decrease of the free energy due to the increase in the interfacial area within the two phases. The emulsifier reduces the rate at which the emulsified droplets amalgamate together by the formation of electrical barriers around them. The ability of the emulsifier was judged from the time that was necessary for the separation of 15 ml aqueous layer of an emulsion prepared under the same situations (Adewuyi *et al.,* 2014). The emulsion stability (the aqueous phase separation) carried out at 15 ml was higher than that of 10 ml. The emulsion stability of the surfactants from the oils was found higher than those from the epoxidised oils and even comparable with the standard (SLS) at 15 ml. It ranged from 197.60 sec to 216.46 sec in BASODEA and NMSODEA surfactants while those in the epoxidised oils (EPOBASODEA and EPONMSODEA) were from 140.40 sec to 153.20 sec respectively. The high emulsion stability tendency of the surfactant from the oils indicates their usefuness as good emulsifier and emulsion stabilizers.

The foam power of the diethanolamide and the epoxidised diethanolamide were crosschecked with the one obtained from she standard (odium lauryl sulphate). The foam power of both epoxidised and unepoxidised diethanolamide were reduced after 5 min, the reduction observed in them was found comparable to that of sodium lauryl surfactant. In this study, it was discovered that the epoxidised diethanolamide from BASO and NMSO had better foam properties than those from unepoxisidised BASO and NMSO.

In the epoxidised, unepoxidised diethanolamide and in the standard sodium lauryl sulphate, the graphs showing the variation of surface tension with time (Figures 4.14 and 4.15) were the same and similar to each other. The BASO and NMSO surfactant have been found to be exhibiting comparable surfactant properties with the standard, SLS.

4.10.2.2 FT-IR spectra of the oil, epoxidised oil and diethanolamide (surfactants)

The FT-IR spectra of BASO and NMSO, epoxidised seed oils and their various surfactants (diethanolamide) were displayed on Table 4.54 and Appendices 23 - 26. The spectra peaks results, shown in the Table 4.54 were different from one another. Peaks observed at 3007.45 cm^{-1} (BASO) and 3007.55 cm^{-1} (NMSO) were assigned to the C-H stretching vibration of H-C=C-H in the oils which reveal the existence of unsaturation in the oil. These peaks at 3007.45 cm^{-1} and 3007.55 cm^{-1} which were not present in epoxidised oil and ethanolamide prepared showed that epoxidation really took place in the various oils. It equally revealed that oxidation has taking place and -C=C- has taken part in the epoxidation reaction.

The bands at 2922.46 cm^{-1} and 2922.75 cm^{-1} found respectively in the FT-IR spectra of BASO and NMSO were common to all other (epoxidised oil, diethanolamide and epoxidised diethanolamide). These bands are due to C-H stretching vibrations of CH_3. The bands found at 2855.58 cm^{-1} (BASO) and 2855.76 cm^{-1} (NMSO) also common to epoxidised oil, diethanolamide and epoxidised diethanolamide were due to C-H stretching vibrations of CH_2. The bands seen around 1744.05 cm^{-1} and 1742.90 cm^{-1} in both oils are only seen at 1743.76 cm^{-1} and 1744.01 cm^{-1} in the epoxidised oils but were not seen in the diethanolamide and epoxidised diethanolamide. These bands seen between 1744.05 cm^{-1} and 1742.90 cm^{-1} were majorly due to C=O stretching of ester and were not found in the diethanolamide and epoxidised diethanolamide. This showed the total conversion of the ester to amide.

The bands at 1456.68 cm^{-1} and 1456.31 cm^{-1} were common to the oil, epoxidised oil, diethanolamide and epoxidised diethanolamide. These peaks were assigned to the C-H bending frequency of the saturated alkane. The bands at 1157.92 cm^{-1} and 1158.93 cm^{-1} respectively in BASO and NMSO, also at 1160.42 cm^{-1} and 1161.01 cm^{-1} in their epoxidised oils were not witnessed in the diethanolamide and epoxidised diethanolamide. These bands were due to the C-O stretching frequencies of the ester. These bands were not present in the diethanolamide and epoxidised diethanolamide which ascertain the conversion of the esters to amides.

Table 4.54: FT-IR analyses (cm^{-1}) of BASO, NMSO, epoxidised oils and their respective diethanolamide (biosurfactant)

BASO	NMSO	BAEPO	NMEPO	BASURF	NMSURF	BAEPOS	NMEPOS	Functional group
3007.45	3007.55	--------	-------	--------	-------	--------	-------	C=C-H unsaturation
-------	-------	--------	-------	3372.51	3371.64	3356.63	3386.06	OH Stretching vibration
2922.44	2922.75	2922.91	2922.78	2922.50	2924.21	2922.50	2923.64	Stretching vibration of C-H (CH_3)
2855.58	2855.76	2855.95	2855.42	2855.54	2856.87	2855.69	2856.40	Stretching vibration of C-H (CH_2)
1744.05	1742.90	1743.76	1744.01	--------	-------	--------	-------	C=O stretching of an ester
---------	---------	---------	---------	1617.58	1617.62	1617.65	1619.46	C=O stretching of a tertiary amide
1456.68	1456.31	1456.11	14.57.41	1462.31	1461.54	1462.29	1462.77	Bending vibration of C-H (CH_2)
1233.51	1233.96	1234.03	1235.23	1207.88	1265.73	1206.38	1205.37	Bending vibration of C-H (CH_3)
1157.92	1158.93	1160.42	1161.01	-------	--------	--------	--------	C-O stretching of an ester
-------	-------	--------	-------	1058.17	1055.54	1058.70	1062.18	C-N Stretching vibration
721.22	724.16	721.39	722.82	720.48	732.56	720.26	724.40	Bending vibration of C-H

NMEPO: *N. macrophylla* epoxidised oil
BAEPO: *B. aegyptiaca* epoxidised oil
NMSURF: *N. macrophylla* seed oil surfactant
BASURF: *B. aegyptiaca* seed oil surfactant
NMEPOS: *N. macrophylla* epoxidised oil surfactant
BAEPOS: *B. aegyptiaca* epoxidised oil surfactant

Characteristic bands found around 3372.51 cm^{-1} and 3371.64 cm^{-1} in diethanolamide are equally present at 3356.63 cm^{-1} and 3356.06 cm^{-1} in epoxidised diethanolamide but not in the oils and epoxidised oils. These bands can be assigned to the vibrational frequency of OH group. The peaks at 1617.58 cm^{-1} and 1617.62 cm^{-1} respectively for BASO and NMSO diethanolamide, also seen at 1617.65 cm^{-1} and 1619.46 cm^{-1} in epoxidised diethanolamide are not present in other spectra (oils and epoxidised oils). They are attributed to the C=O frequency of a tertiary amide. These peaks are obtained as the peaks around 1742.90-1744.05 cm^{-1} (C=O frequency) of esters in the oils disappeared. This also confirms the conversion of the oils to amides. The peaks at 1058.70 cm^{-1} - 1062.18 cm^{-1} found in both diethanolamide and epoxidised diethanolamide were not seen in other spectra (oils and epoxidised oils). They were attributed to C-N vibrational frequency of an amide.

The presence of the peaks around 3372.51-3386.06 cm^{-1}, 1617.58-1619.46 cm^{-1} and 1058.17-1062.18 cm^{-1} present in both diethanolamide and epoxidised ethanolamide (absent in oils and epoxidised oils) are assigned respectively to the frequency of vibration of OH group in the amide, the C=O frequency of a tertiary amide and to C-N vibrational frequency of an amide really substantiate the conversion of oils to amide. BASO and NMSO might be utilised in the synthesis of diethanolamide and epoxidised diethanolamide which can be applied as biosurfactant

4.10.2.3 ^{1}H NMR, ^{13}C NMR and ESI-MS spectra of the oil and diethanolamide

The spectra of BASO and NMSO and diethanolamide proton NMR are respectively presented in Figures 4.16a-b, 4.17a-b and 4.18a-b). The ethylene protons in the oils are seen between 4.14 ppm to 4.29 ppm (BASO) and 4.12 ppm to 4.31 ppm (NMSO). These signals were not seen in diethanolamides which indicate the presence of unsaturation in the oils. Bands seen at 7.27 ppm and 7.17 ppm in diethanolamide which were not present in the oils are attributed to the protons of the amide formed in the diethanolamide. The bands found around 3.48 to 3.57 ppm and 3.50 to 3.57 ppm in BASO and NMSO diethanolamide were absent in the spectra of the oils. These signals are attributed to the (-CH_2-) in (-N-$\underline{CH_2}$-) in diethanolamide.

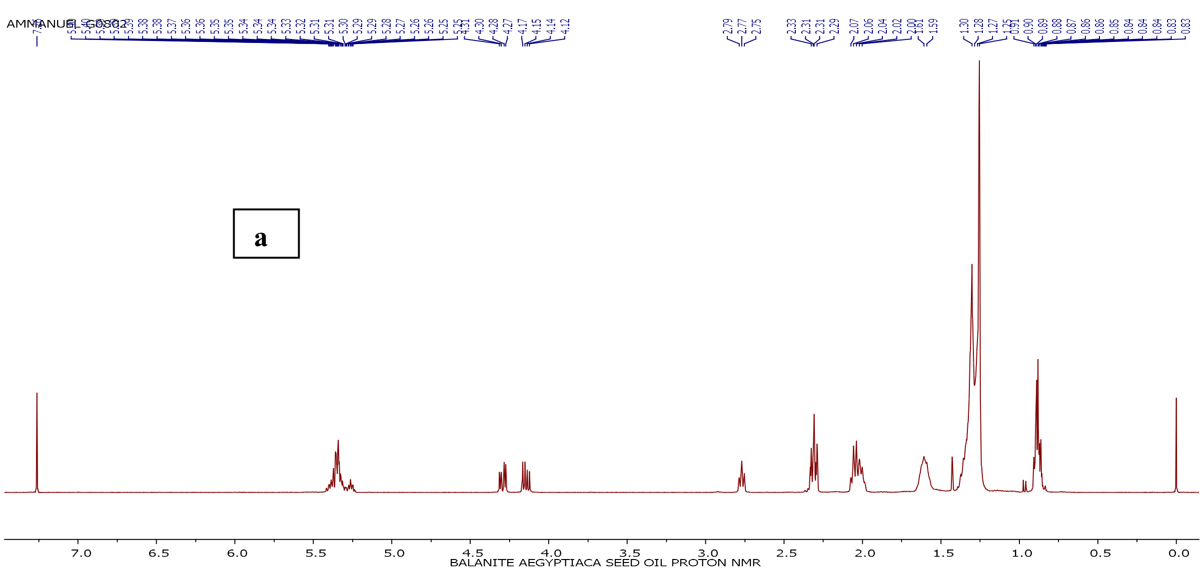
The bands found around 3.68-3.86 ppm and 3.77-3.87 ppm in diethanolamide are attributed to ($-CH_2-$) in ($-N-CH_2-\underline{CH_2}-OH$). The band found at 4.12 ppm in diethanolamide is assigned to the terminal proton in the OH group of the amide ($-N-CH_2-CH_2-O\underline{H}$). The terminal methyl group in both the oil and the diethanolamide is seen between 0.86 ppm and 0.90 ppm. The bands seen around 2.77 ppm, common to oil and the diethanolamide are due to $-CH_2-$ in ($-CH-\underline{CH_2}-CH=CH-$).

The carbon (^{13}C) NMR spectra of BASO and NMSO diethanolamide are shown respectively in Figures 4.18 (a and b). The signals observed at 175.85 ppm in both BASO and NMSO diethanolamide were assigned to C ($-\underline{C}O-N-$). The signals observed between 125.98 ppm to 128.71 ppm are attributed to the frequencies of carbon in ($HC=\underline{C}H-CH_2-$), 129.78 ppm to 130.4 ppm stand for ($-H\underline{C}=CH-CH_2-$), 52.27 ppm to 50.55 ppm represent ($-N-\underline{C}H_2-CH_2-OH$), 60.47 ppm to 61.74 ppm for ($-N-CH_2-\underline{C}H_2-OH$). The signal observed at 14.14 ppm is for terminal methyl group ($-\underline{C}H_3$) while those from 21.08 ppm to 33.64 ppm represent the carbon atoms in the $-CH_2-$ of the long chain. The diethanolamide synthesised were charaterised with ESI-MS and the calculated masses compared with the obtained masses. The expected masses of BASO and NMSO diethanolamide are respectively 369.32 g/mol and 367.31 g/mol with a molecular formula of $C_{22}H_{43}NO_3$ and $C_{22}H_{41}NO_3$ respectively.

4.10.3 Synthesis and characterisation of biolubricant from BASO and NMSO

4.10.3.1: Characterisation of biolubricant from BASO and NMSO

The FT-IR and ^1H NMR analyses of BASO and NMSO biolubricant are presented respectively on Table 4.55 and Appendices 21-22. Table 4.55 shows respectively the FT-IR analyses of BASO and NMSO, epoxidised oils and polyols or biolubricant produced. The spectra obtained at 3007.55 cm^{-1} and 3007.45 cm^{-1} are assigned to the C-H stretching vibration of (C=C-H). The band around 3007 cm^{-1} shows the existence of unsaturation in the oils. This band was not found in the epoxidised oils and in the polyol or biolubricant produced. The absence of this band in the FT-IR spectra of the produced biolubricant shows that oxidation has taken place in the epoxidised oils and that the C=C bond took part in the epoxidation reaction.



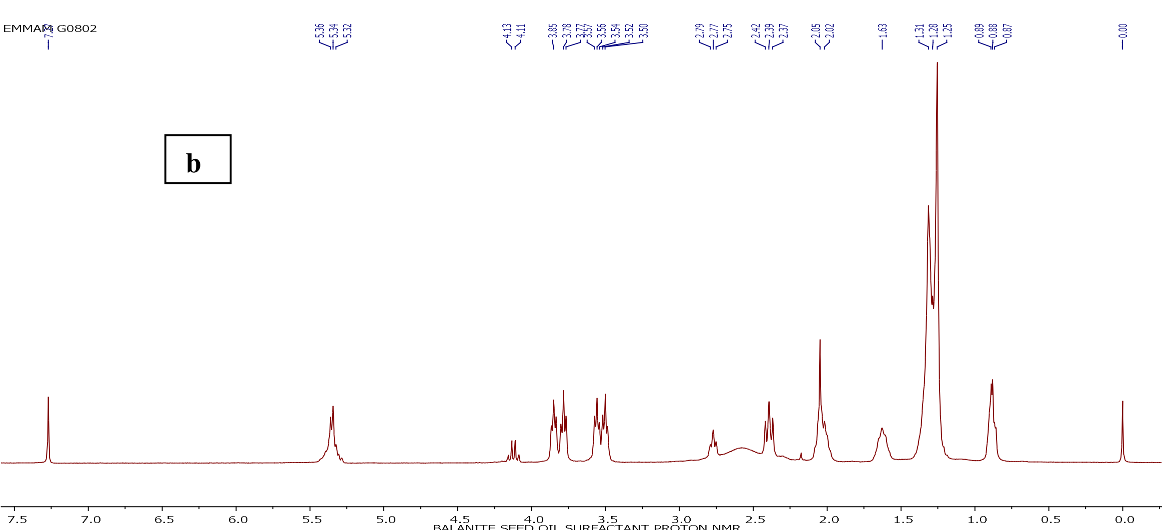
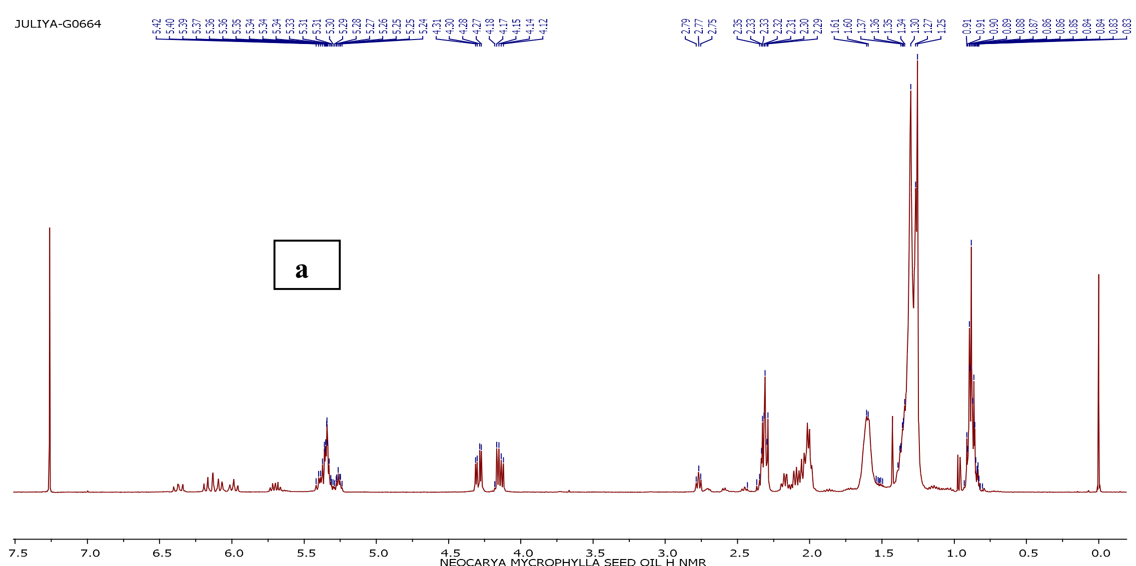


Figure 4.16: (a) ^{1}H NMR spectrum of BASO and (b) corresponding diethanolamide (surfactant)



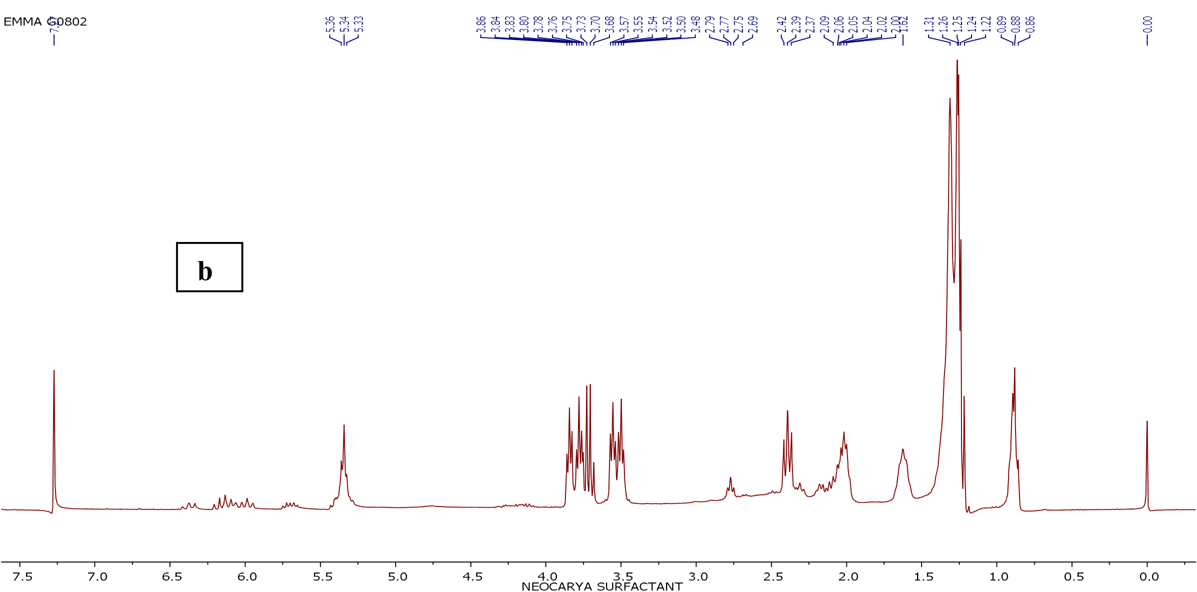
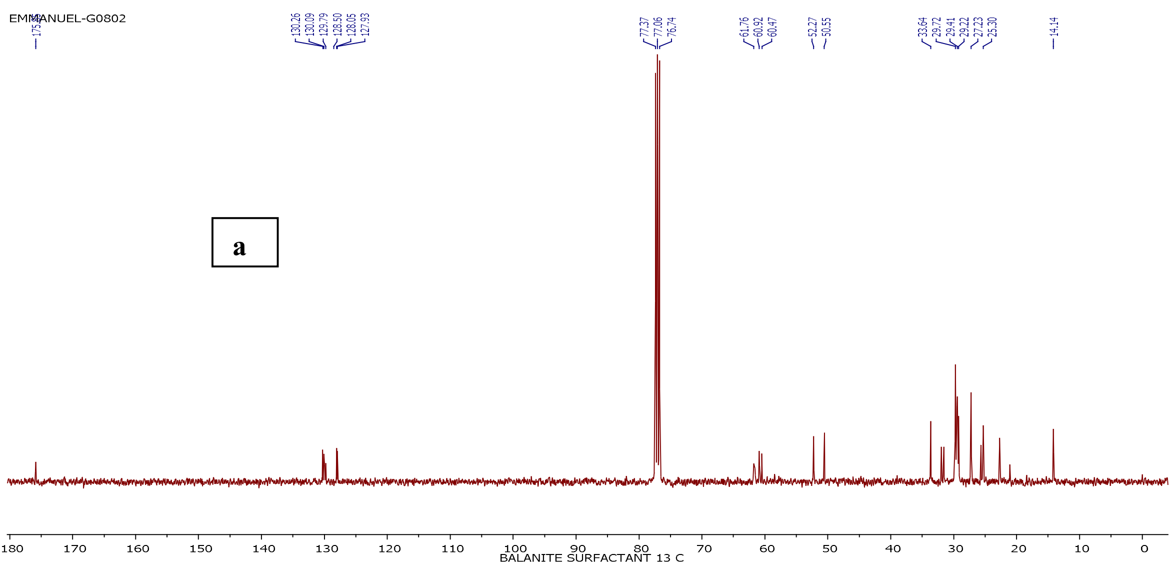


Figure 4.17: (a) ^{1}H NMR spectrum of NMSO and (b) corresponding diethanolamide (surfactant)



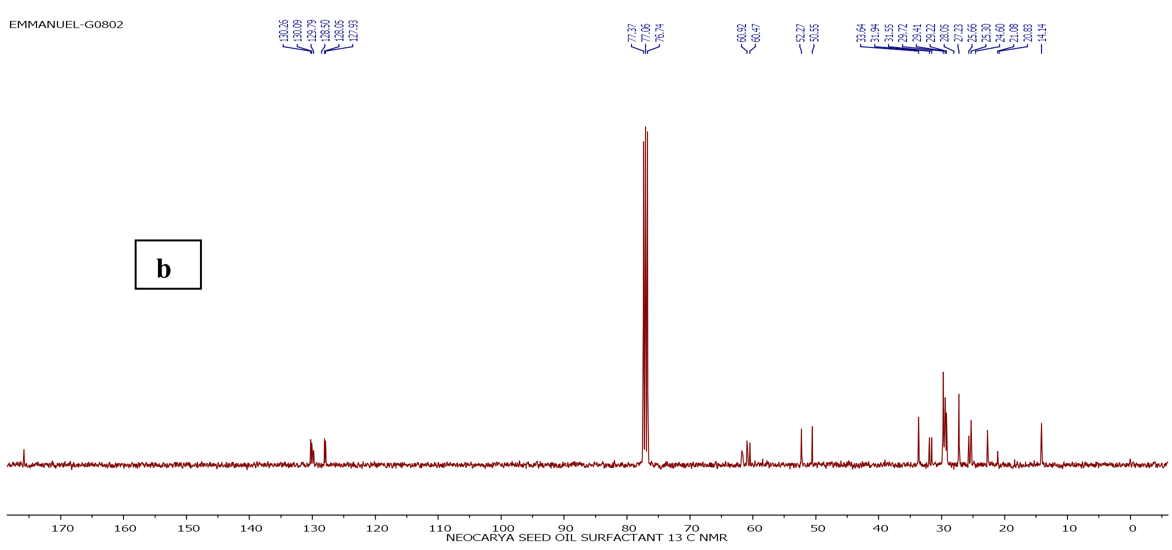


Figure 4.18: (a) ^{13}C NMR spectrum of BASO diethanolamide and (b) NMSO diethanolamide (surfactant)

The bands or peaks at 2922.44 cm^{-1}, 2855. 58 cm^{-1}, 1744.05 cm^{-1}, 1456.68 cm^{-1}, 1157 cm^{-1} and 721.92 cm^{-1} are common to the oils, the epoxidised oils and the biolubricant. The signal observed at 2922.58 cm^{-1} is assigned to C-H stretching vibration of $-CH_3$ and 2855.58 cm^{-1} is that of the methylene (CH_2) group stretching band. The band at 1744.05 cm^{-1} is assigned to the C=O stretching vibration of ester functional group. The band shown at 1456.68 cm^{-1} is the C-H bending frequency of saturated alkane while the one at 1157.92 cm^{-1} is the C-O stretching frequency of the ester. The characteristic peaks 3366.50 cm^{-1} and 3442.95 cm^{-1} are only shown in the spectra of the polyols or biolubricant but were not seen in the spectra of the oils and epoxidised oils. This peak is due to the formation of the polyols and are the results of the ring opening reaction of the epoxy group form in the oils. It stands for the broad O-H stretching vibration of the hydroxyl group present in the biolubricant produced (Adewuyi *et al.,* 2012c).

The ^{1}HNMR spectra of the synthesised biolubricant from BASO and NMSO are displayed on Figures 4.19a-b. The signal at 5.35 ppm is common to the oils but not seen in the biolubricant. It shows the confirmation of unsaturation in the oils which disappeared in the biolubricant. The peaks around 0.88 ppm and 0.90 ppm are the chemical shift of the terminal methyl group in both the oils and biolubricant. The peaks around 2.28 - 2.32 ppm, 1.58-1.62 ppm, 2.05 ppm and 1.26 - 1.40 ppm were all common to the oils and the biolubricant. The peaks seen around 2.28 - 2.32 cm^{-1} show the presence of CH_3 group of the esters while those within 1.26-1.40 ppm reflect the saturated methylene groups. The C-H back bone of the methane proton from b-glycerol carbon was seen in the oil at 4.10 ppm. The characteristics peaks at 3.55 ppm and 3.48 ppm observed respectively in BASO and NMSO biolubricant were assigned to the hydroxyl functional group.

4.10.3.2: Biolubricant properties of the synthesised BASO and NMSO polyols.

Table 4.56 presents the lubricant properties of the synthesised biolubricant from BASO and NMSO. The iodine values of 102.71 g iodine/100 g and 89.05 g iodine/100 g were respectively obtained in BASO and NMSO while 0.41 g iodine/100 g and 0.46 g iodine/100 g were respectively obtained in BASO and NMSO biolubricant. This reduction in the unsaturation serve as an evidence confirming that hydroxylation reaction had taken place.

Table 4.55: FT-IR analyses of BASO, NMSO and their respective biolubricants

BASO (cm^{-1})	NMSO (cm^{-1})	BAEPOS (cm^{-1})	NMEPOS (cm^{-1})	BASOLUB (cm^{-1})	NMSOLUB (cm^{-1})	Functional group
3007.45	3007.55	--------	-------	-------	-------	C=C-H unsaturation
--------	--------	--------	-------	3366.50	3442.95	OH Stretching vibration
2922.44	2922.75	2922.91	2922.78	2924.59	2926.80	Stretching vibration of C-H (CH_3)
2855.58	2855.76	2855.95	2855.42	2863.42	2862.58	Stretching vibration of C-H (CH_2)
1744.05	1742.90	1743.76	1744.01	1732.32	1734.53	C=O stretching of ester
1456.68	1456.31	1456.11	14.57.41	1459.70	1457.07	Bending vibration of C-H (CH_2)
1233.51	1233.96	1234.03	1235.23	-------	-------	Bending vibration of C-H (CH_3)
1157.92	1158.93	1160.42	1161.01	1179.49	1173.52	C-O stretching of ester
--------	----------	--------	--------	1040.18	1093.04	C-O-C Stretching vibration
721.22	724.16	721.39	722.82	726.48	727.85	Bending vibration of C-H

BAEPOS: *B. aegyptiaca* epoxidised seed oils
BASOLUB: *B. aegyptiaca* biolubricants
NMEPOS: *N. macrophylla* epoxidised seed oil
NMSOLUB: *N. macrophylla* biolubricants

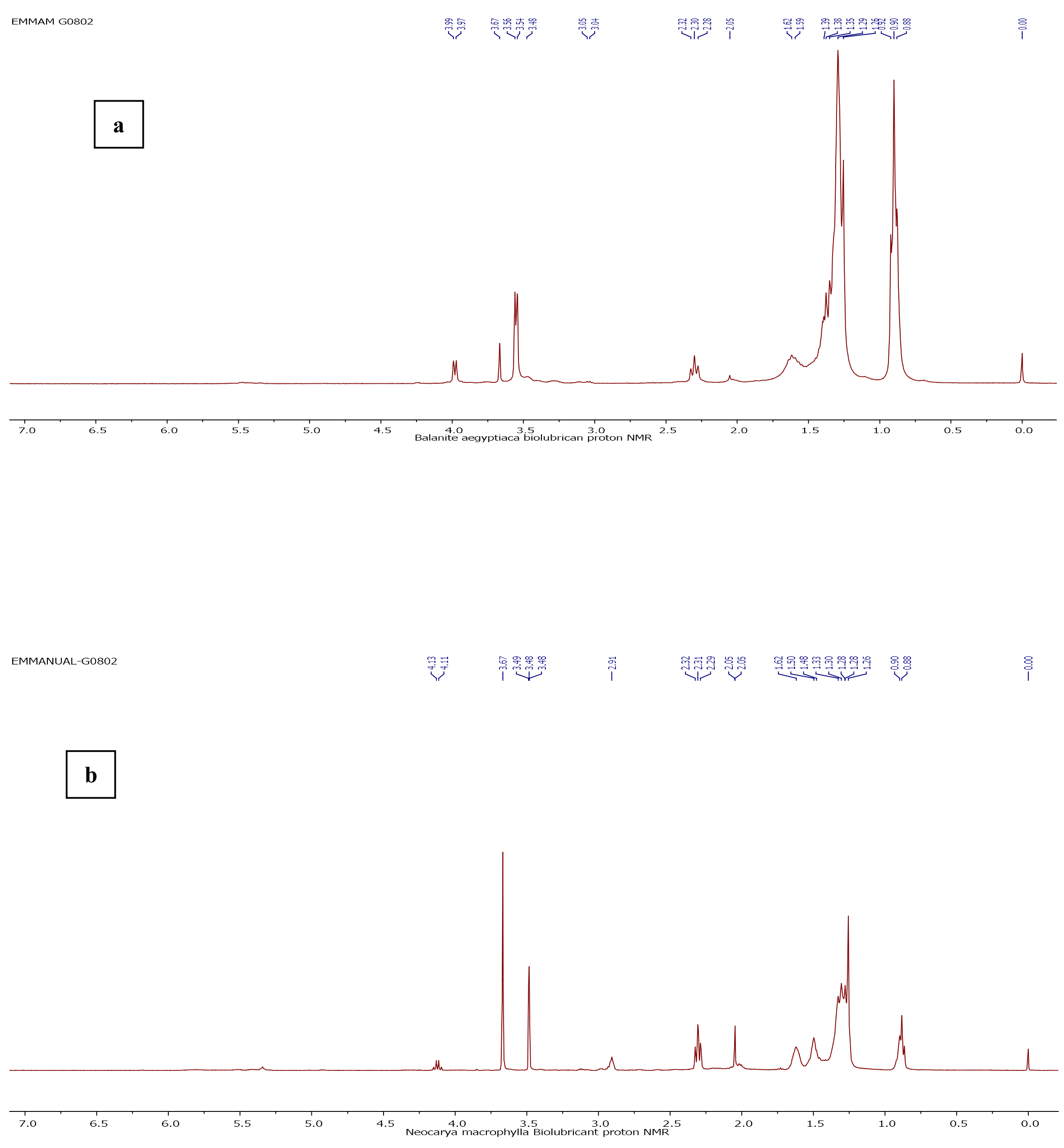


Figure 4.19: (a) ^{1}H NMR spectra of BASO biolubricant and (b) NMSO biolubricant

A 0.41 g iodine/100 g was reported for *L. cyanescens* seed oil biolubricant (Adewuyi *et al.,* 2012b). A result of (1 A) was recorded for the copper strip corrosion test in both BASO and NMSO biolubricant. This implies that the biolubricant produced will be stable towards corrosion.

The physical observation of the colours revealed that NMSO was light yellow while BASO was golden yellow. Golden and golden green colours were respectively found in BASO and NMSO biolubricant produced. This change in colour is also evidence that reaction took place within the oils. The viscosity of lubricant at 40 ^{o}C and 100 ^{o}C are very important lubricity properties employed in checking the ability of the lubricant to flow at low and high temperature as well as in showing the thermal stability of any lubricant (Bilal *et al.,* 2013). The viscosities of 19.21 and 26.64 obtained at 40 ^{o}C in *B. aegyptiaca* and *N. macrophylla* biolubricant are higher than 3.51 and 5.11 respectively obtained at 100 ^{o}C. This reduction discloses a better flow rate of the biolubricant as the temperature increases. The evaluation of emulsion stability is another test that provides a guide in knowing the level of interaction of the polyols, water molecules and turbulence. This emulsion stability was found to be 9.01 and 9.03 in BASO and NMSO biolubricant. The pour point was found to be - 9 ^{o}C and - 6 ^{o}C for BASO and NMSO biolubricant respectively. This value compared favourably with those observed in some other plant oil (Bilal *et al.,* 2013). The flash point determined was 220.20 ^{o}C for *B. aegyptiaca* biolubricant and 240.35 ^{o}C for *N. macrophylla* biolubricant. These values are in a very good agreement with 260.20 ^{o}C discovered for *L. cyanescens* biolubricant (Adewuyi *et al.,* 2012b). BASO and NMSO were found useful in the synthesis of polyols which are properly characterised as biolubricant with good lubricant properties. They are very promising seed oils in the production of biolubricant.

Table 4.56: Lubricant properties of the polyols from BASO and NMSO

Biolubricant Properties	BASO Biolubricant	NMSO Biolubricant	*L. cyanescens*	CPL ISO-VG-32
Specific gravity @ 15 °C	0.88±0.00	0.92±0.00		0.87
Specific gravity @ 30 °C	0.87±0.00	0.91±0.00	0.98±0.20	0.90
Specific gravity @ 90 °C	0.82±0.00	0.86±0.00	0.92±0.10	
Density @ 15° C	0.88±0.00	0.92±0.01		
Refractive index @ 25 °C	1.44±0.02	1.46±0.01		
Refractive index @ 40 °C	1.43±0.01	1.45±0.01		
Viscosity @ 40 °C (mm^2/s)	19.21±0.06	26.64±0.01	16.90±0.10	31.40
Viscosity @ 100 °C(mm^2/s)	3.51±0.01	5.11±0.58	3.00±0.10	5.40
Dynamic viscosity (Cst)	16.72±0.01	24.30±0.02		
Iodine value (mg I_2/g)	0.41±0.01	0.46±0.01	0.43±0.05	
Flash point (°C)	220.20±0.03	240.35±0.01	260.35±0.01	207
Cupper corrosion test	1A	1A	1A	1A
Oxidative stability (h)	3.03 h	3.02 h	1.50 h	
Cloud point (°C)	3	3		-5
Pour point (°C)	-9 °C	-6 °C		-30
Colour	Golden	Golden green		

L. cyanescens: Adewuyi *et al.* (2012)
Values are mean±SD of tree determinations

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

The various components of the plant seeds studied such as their flours, oils and residues were fully characterised and have shown different properties which suggested *A. catechu*, *Polyalthia longifolia*, *Balanites aegyptiaca*, *Neocarya macrophylla* and *Enterolobium cyclocarpum* seeds as useful raw materials in industries for numerous applications as feeds and oleochemicals.

5.2. Conclusion

The proximate analyses of the seeds showed that *B. aegyptiaca, N. macrophylla* and *E. cyclocarpum* seeds had high protein content while *A. catechu* and *P. longifolia* were high in carbohydrate. They might be used as energy food. The mineral composition revealed that these plant seeds contained some essential mineral like calcium, potassium, magnesium and sodium. They could be good sources of these mineral elements. They had low moisture content which could make them less prone to spoilage and their storage easy. They all contained eight out of the nine essential amino acids; this is of great nutritional importance. The *B. aegyptiaca, N. macrophylla* and *E. cyclocarpum* seeds were found to be rich in protein with high total essential amino acids. They could be good source of nutrients.

The seed oils have good physicochemical properties, high content of unsaturated fatty acid with linoleic and linolenic acids as major fatty acids. They could be useful in lowering cholesterol level in human and be subjected to a variety of chemical modification towards desired products. The *B. aegyptiaca* and *N. macrophylla* seeds oils had high oil yield (46.95 and 45.96%) among all the oil samples. They have the potential of serving as

replacement for other well known seed oils of similar composition.

The *A. catechu, B. aegyptiaca, N. macrophylla* and *P. longifolia* seed flours were effectively used to feed rat with good survival rate and weight gain. No sign of toxicity was seen in the rats when fed with 30% of the seed flours in their diets. The toxicological studies revealed high weight loss with *Chrysophyllum albidum* and *E. cyclocarpum* seed flours when consumed up to 30%. The flours could pass through other processes to remove other extraneous materials which they contained to make them suitable for consumption. *A. catechu* and *P. longifolia* seed flours were applied in fish with better results at 10% substitution while *B. aegyptiaca* and *N. macrophylla* seed residues were employed at 30% substitution with good survival rate, growth performance and weight gain in both rats and fish feeding.

The preliminary toxicological analysis carried out on *B. aegyptiaca* and *N. macrophylla* seed oils showed that they were toxic free and might be good for consumption after proper refining. They might not be toxic even if used in syntheses. The *B. aegyptiaca* and *N. macrophylla* seed oils showed good antimicrobial properties and were successful used as ointments to heal wound on rats. This will contribute positively to their pharmaceutical properties.

The *B. aegyptiaca* and *N. macrophylla* residues obtained after the extraction of oil with high protein contents (51.31 - 56.04%) were successfully blended with wheat flour up to 30% to produced dietary cakes with better sensory properties and higher overall acceptability than the control produced from wheat flour alone. These cakes have no abnormal effect on both the blood and the growth of rats fed on them. The *B. aegyptiaca* and *N. macrophylla* seeds might really be sources of nutrients and raw materials for man and industries. *E. cyclocarpum* seed residue was palatable at 10% substitution.

The *B. aegyptiaca* and *N. macrophylla* seed oils with good physicochemical properties were utilised to successfully synthesise methyl esters, polyols and fatty-amide which effectively displayed good biodiesel, biolubricant and biosurfactant properties that compared favourably with EN-14214, ASTM and ISO-VG-32 specifications. The oils from *B. aegyptiaca* and *N. macrophylla* seeds could be an alternative to crude oil. They

could be employed to develop an environmentally friendly, biodegradable and non toxic oleochemicals.

The successful application of these seed flours, residues and oils on livestock feeding and the positive contribution of *B. aegyptiaca* and *N. macrophylla* seed oils in the synthesis of biofuels revealed some of the possible usage of the seeds. Their potentials will definitely prevent them from being environmental pollutants and therefore contribute immensely to the economy. The *B. aegyptiaca* and *N. macrophylla* seed oils might also be suitable for oleochemicals.

5.3. Recommendations

The properties and composition of underutilised seed flours and their oils showed that they are really good sources of industrial raw materials. The *B. aegyptiaca* and *N. macrophylla* seeds have high oil yield, high protein content and peculiar properties that can placed them as one of the essential industrial raw materials. They are recommended by this research work to be included in the often planted seeds.

Complete study of the edibility of the seed flours, seed residues and oils employed in this research work, (most especially *B. aegyptiaca* and *N. macrophylla* seed oils) are well recommended. Establishing their edibility as animal or human feed will go a long way in creating more employment for many people, reducing the dependence on expensive protein sources and making the environment free from them as wastes.

The *B. aegyptiaca* and *N. macrophylla* seed oils were utilised to produce biodiesel and biolubricant with good properties. The properties of these biofuels are recommended to be improved upon for better performance either by addition of additives or antioxidants. The use of these oils in the synthesis of biodiesel, biolubricant and biosurfactant should be really encouraged. It might reduce the unemployment in the country at large and the products that will be obtained from them as industrial raw material will be biodegradable and will cost no harm to the environment. Further research can be carried out on some other less utilised seed oils to contribute to the sustenance of the oleochemical industries. This will largely contribute to the achievement of the various green chemistry policies.

The *B. aegyptiaca* and *N. macrophylla* seed oils because of their high oil yield and good physicochemical properties are recommended to be novel materials that are green, cheap, environmental friendly, biodegradable and can meet the unexpected demands for environmental friendly chemicals. They can possibly be used to replace petroleum based chemical in the synthesis of oleochemicals which might find usefulness as cosmetics, fuel, cleaners, bioplastics, encapsulating materials, adhesives, vanishes, surgical equipment and surface coating materials. The *B. aegyptiaca, N. macrophylla* and *E. cyclocarpum* seeds have high protein content. It was therefore recommended that their protein should be extracted, characterised and compared with other standard and well known protein for better applications.

5.4. Contributions to knowledge

Six under-utilised seeds have been characterised and applied industrially for nutritional, medicinal and biofuels purposes thereby bringing them to lime light, turning waste to wealth and giving room for job creation opportunity. This research work provides valuable information to the global community that *Neocarya maycrophyla* and *Balanites aegyptiaca* seed residues with amino acid content of 71.81% and 77.02% respectively contain high content of essential amino acids, high protein (56.04 g/100 g and 51.31 g/100 g), important and necessary nutritional elements and are not toxic. They could favourably be fully integrated as food supplement or as suitable raw material in various food industries. They were used as feed supplements between 10 to 30% with good weight gain. They might be considered as good sources of dietary proteins

This research also provides important and notable information to global community that *Neocarya maycrophylla* and *Balanites aegyptiaca* seeds from Nigeria have high oil yield (45.96% and 46.95%) and high unsaturated fatty acids (79.89% and 72.90%) with oleic acid (C18:1) and linoleic acid (C18:2) as major fatty acids. With good physicochemical properties and no sign of toxicity, they might replace other expensive oil for industrial applications. They could perfectly be considered as renewable resources for the synthesis of biofuels with good properties and plausible replacement of fossil fuel due to their overwhelming properties that are comparable to those of the fossil fuel in addition to their biodegradability, non-toxicity and eco-friendliness.

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APPENDICES

View of the seed samples and experimental house

Dried *Areca catechu* seed

Milled *Areca catechu* flour

1: *Areca catechu* dried and milled seeds

Balanites aegyptiaca dried seed

Balanites aegyptiaca seed cake

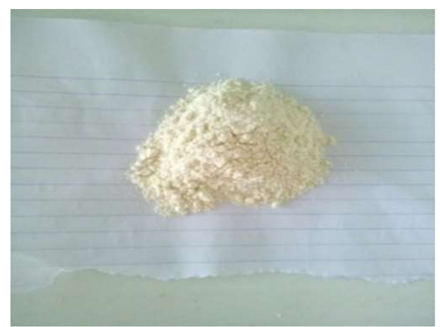
2: *Balanites aegyptiaca* dried and milled seeds

Dried *Chrysophyllum albidum* seed

Milled *Chrysophyllum albidum*

3: *Chrysophyllum albidum* dried and milled seeds

Enterolobium cyclocarpum seeds



Milled *Enterolobium cyclocarpum*

4: *Enterolobium cyclocarpum* dried and milled seeds

Neocarya macrophylla seeds

Neocarya macrophylla seed cake

5: *Neocarya macrophylla* dried and milled seed cake

Dried *Polyalthia longifolia* seed

Milled *Polyalthia longifolia*

6: *Polyalthia longifolia* dried and milled seeds

Balanites aegyptiaca seed oil

Neocarya macrophylla seed oil

7: Samples of *B. aegyptiaca* and *N. macrophylla* seed oils

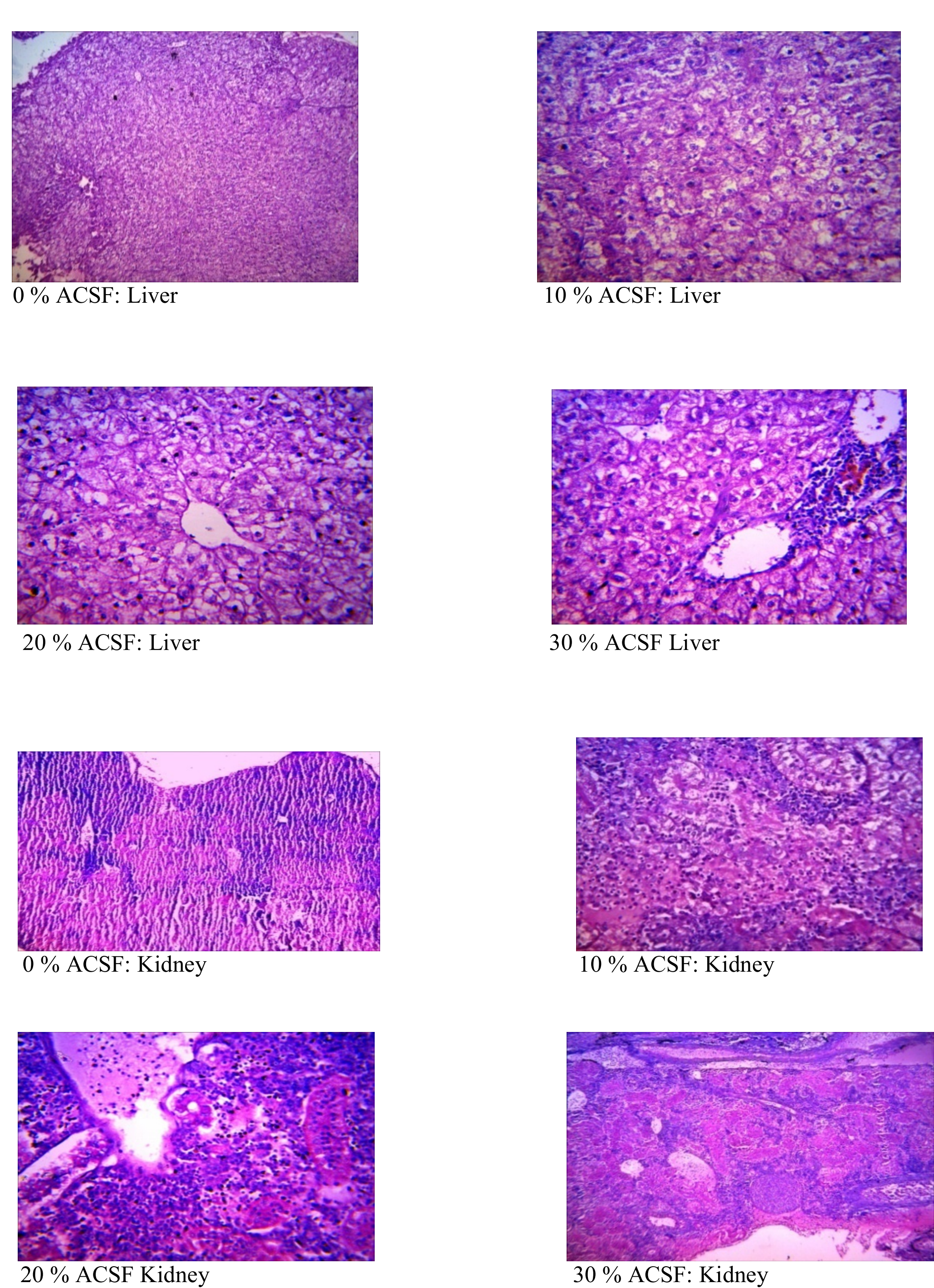
8**:** Front view of the Experimental animal house

9: View of the experimental animals in the cages well arranged

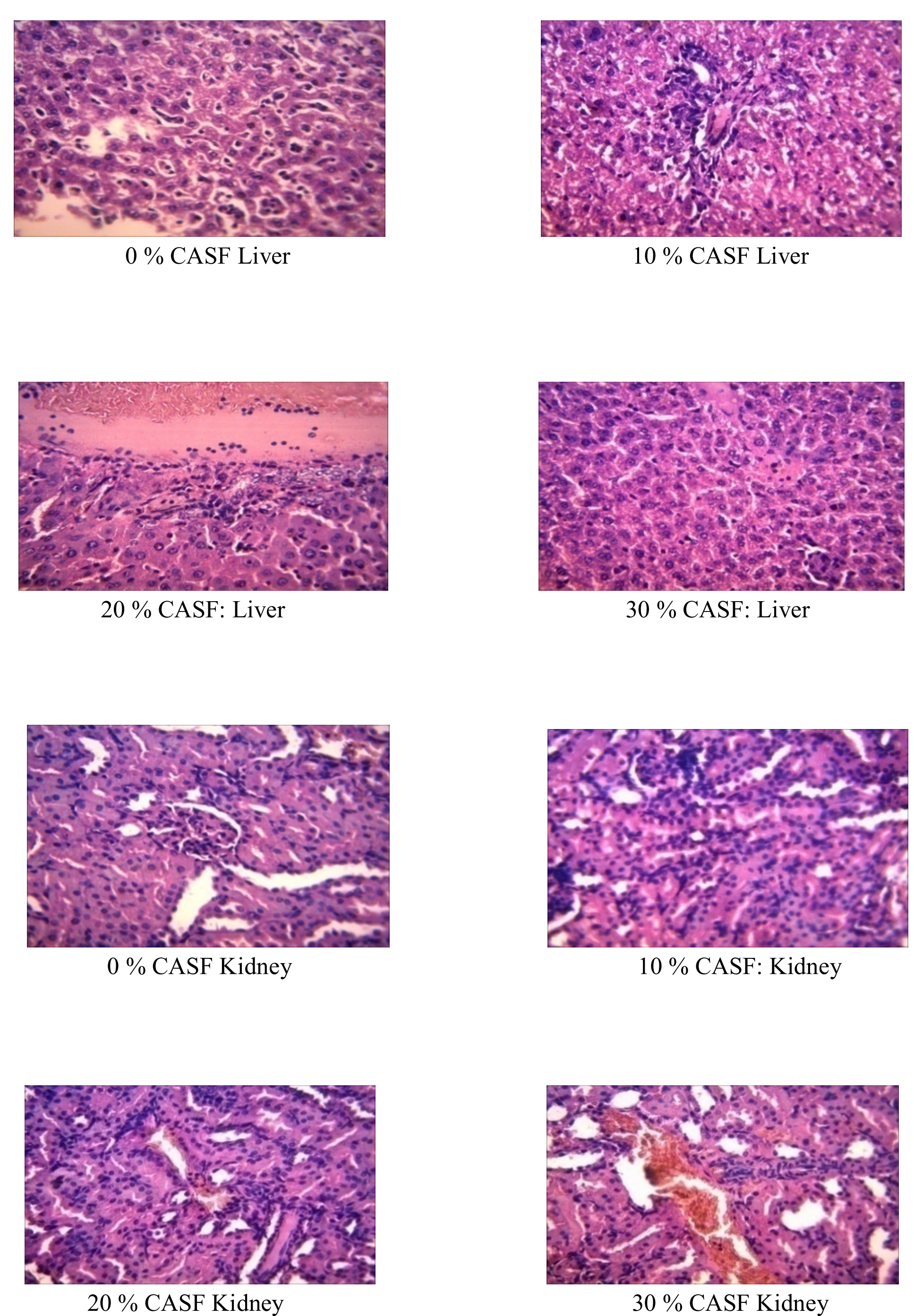
10: View of the experimental animals in their cages

Organs collected and stored in formali   Blood samples collected in EDTA bottles

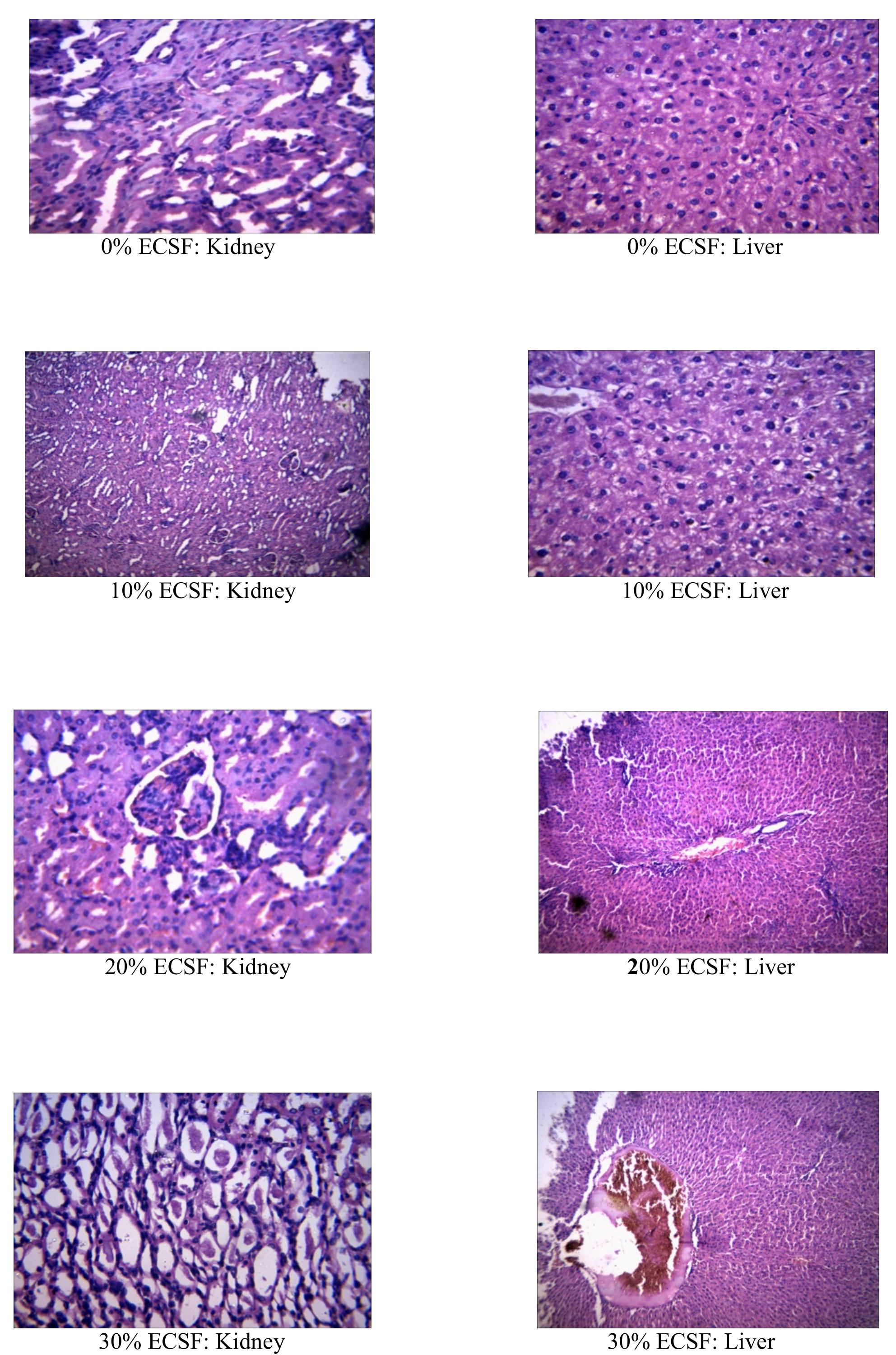
11: Samples of the organs and blood collected for analysis



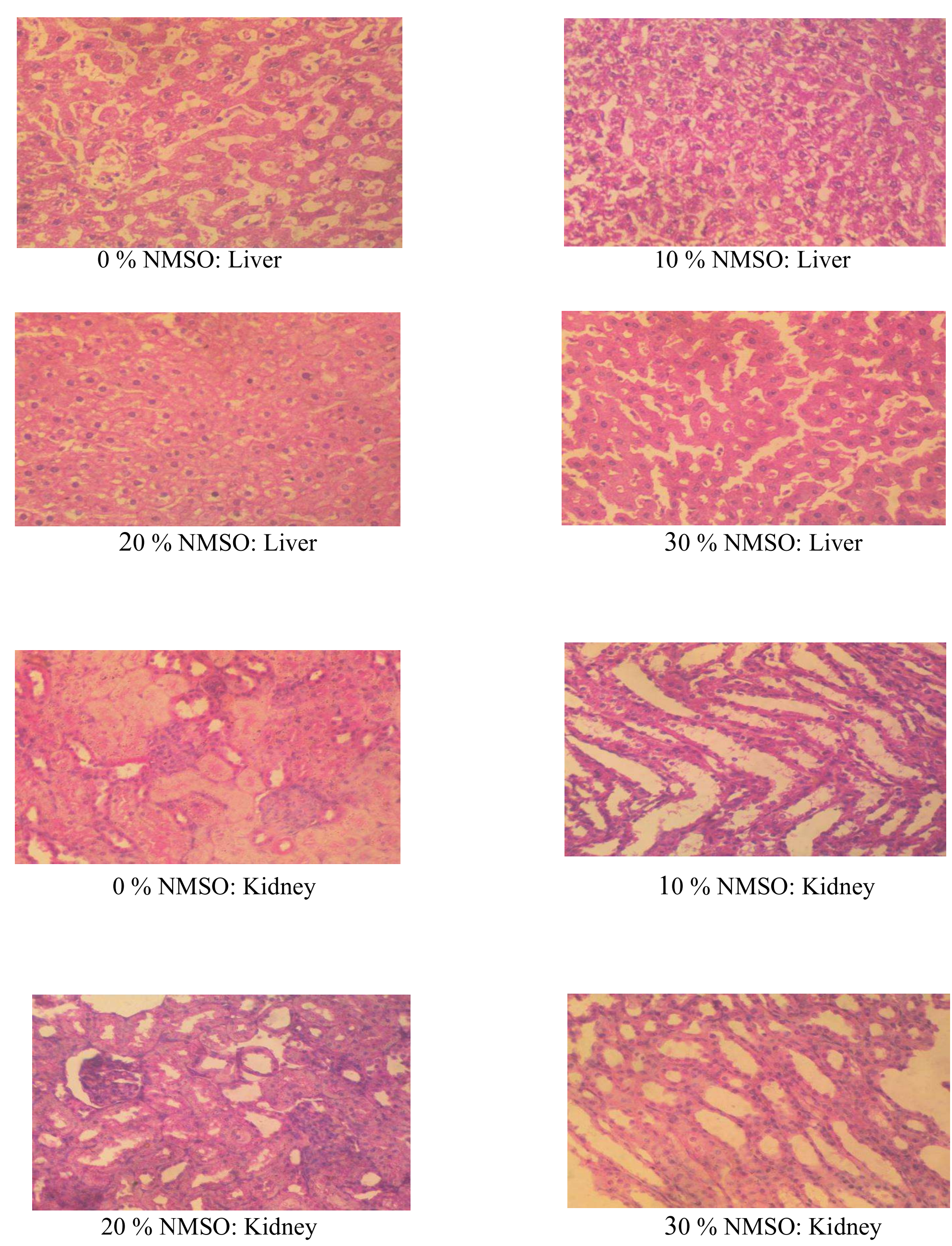
12: Histological examination of the Liver and kidney of fish fed with ACSF (x550).


0 % CASF Liver
10 % CASF Liver
20 % CASF: Liver
30 % CASF: Liver
0 % CASF Kidney
10 % CASF: Kidney
20 % CASF Kidney
30 % CASF Kidney

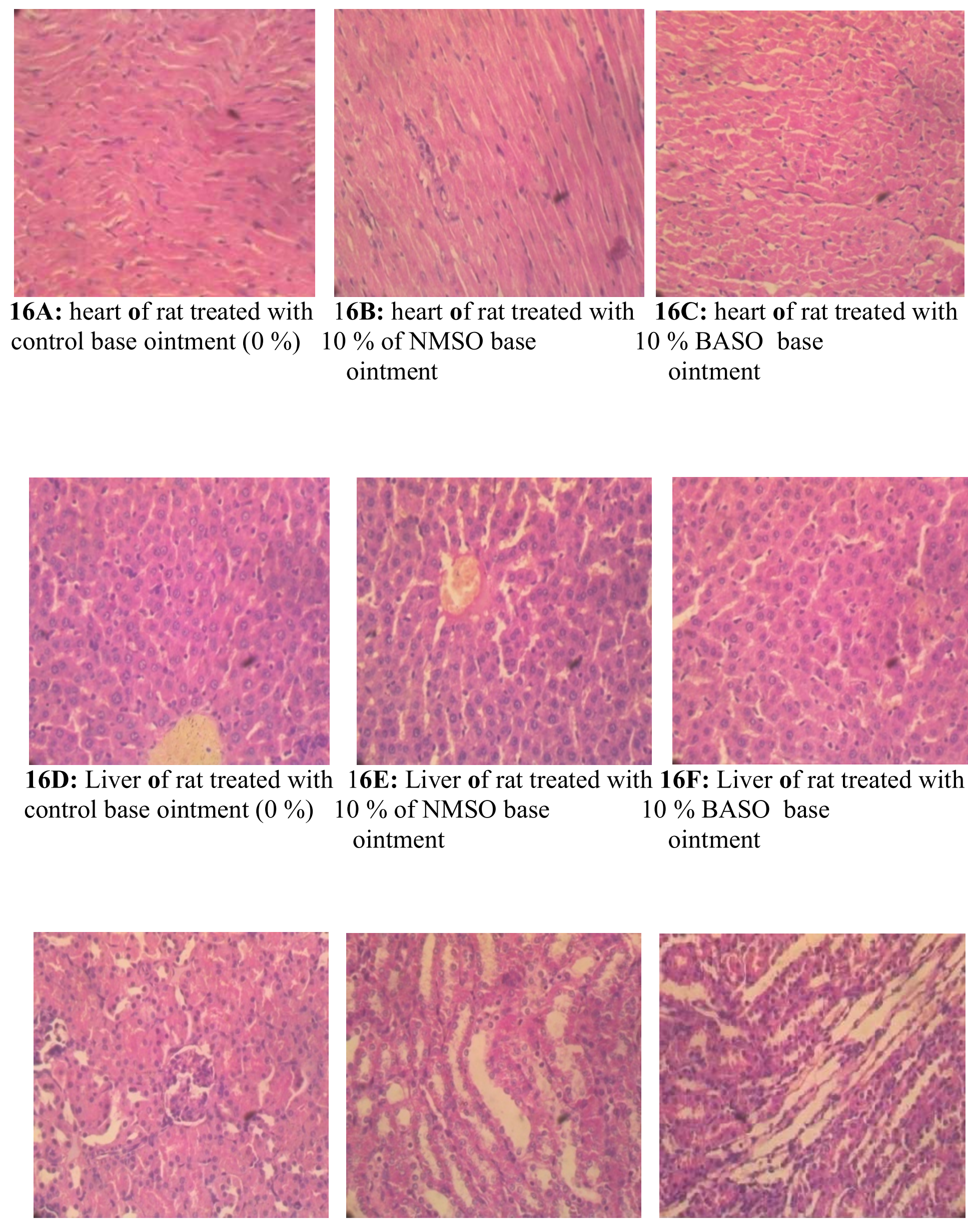
13: Photomicrographs of the histological examination of sections of the liver and kidney of rats fed with CASF (x550).



14: Photomicrographs of the histological examination of section of liver and kidney of rat fed with ECSFsupplemented diets (x550).



15: Photomicrographs of the histological examination of the liver and kidneys of the rat fed with NMSO (x550)



16A: heart **o**f rat treated with control base ointment (0 %)

16B: heart **o**f rat treated with 10 % of NMSO base ointment

16C: heart **o**f rat treated with 10 % BASO base ointment

16D: Liver **o**f rat treated with control base ointment (0 %)

16E: Liver **o**f rat treated with 10 % of NMSO base ointment

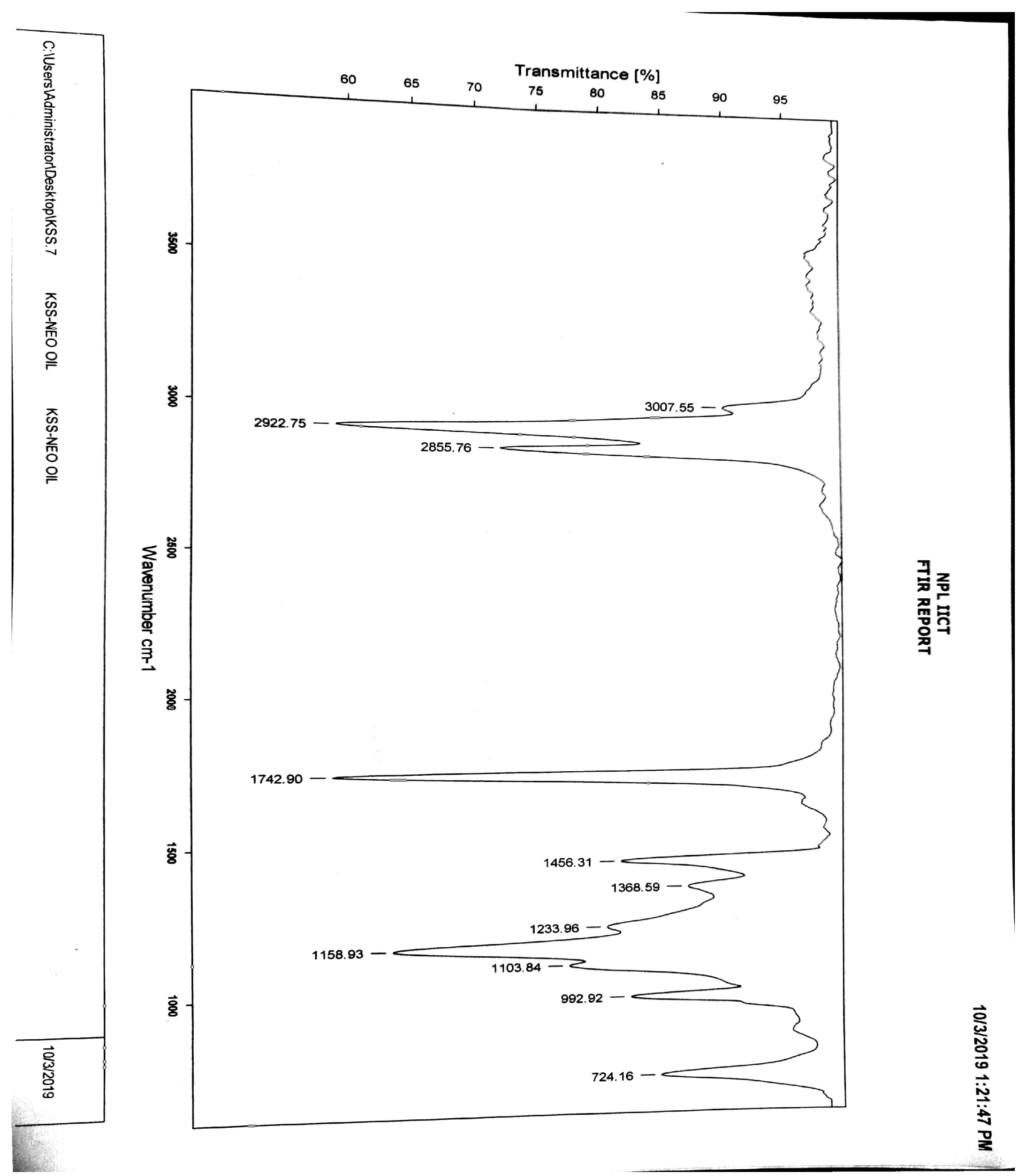
16F: Liver **o**f rat treated with 10 % BASO base ointment

16A: heart **o**f rat treated with control base ointment (0 %)

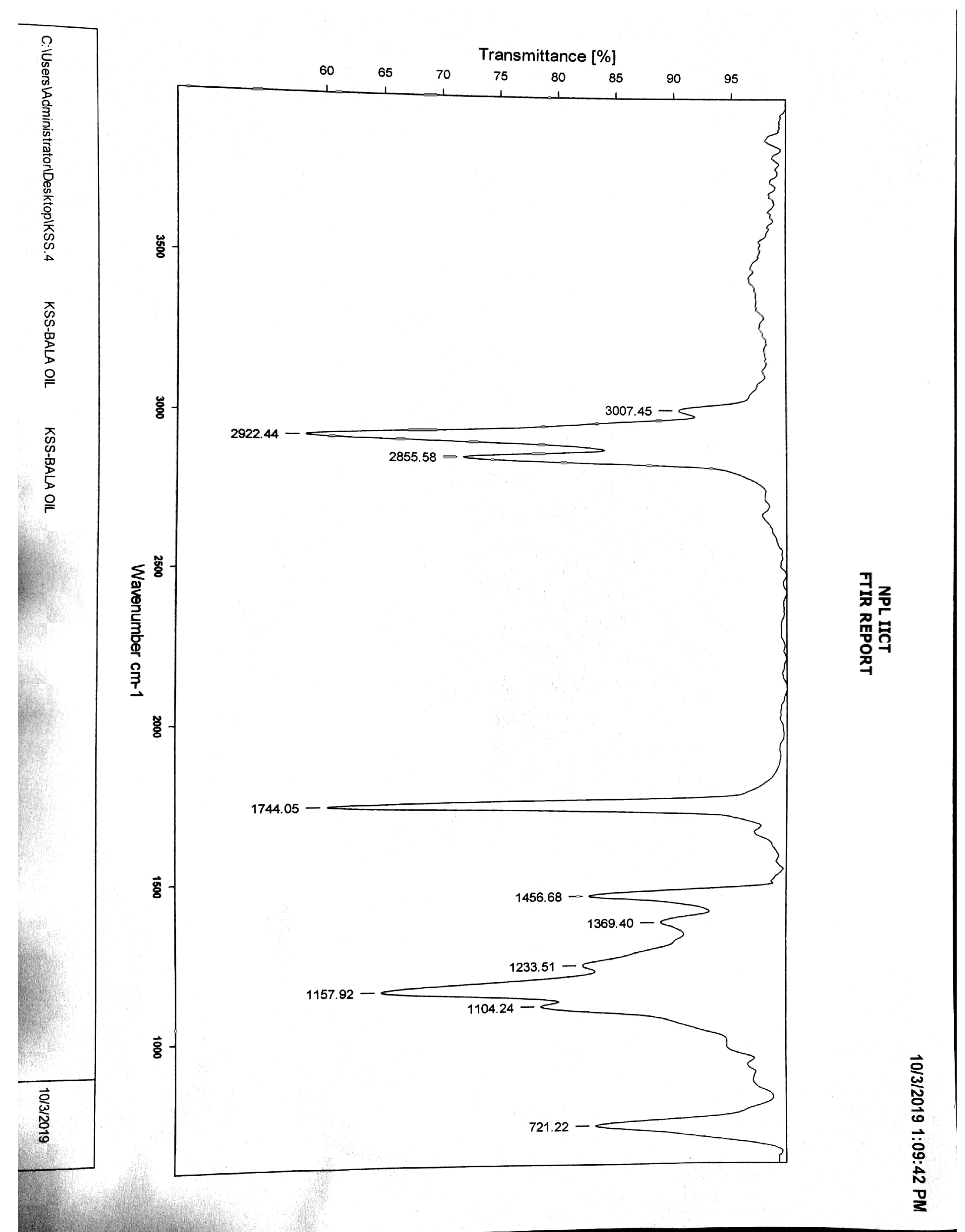
16B: heart **o**f rat treated with 10 % of NMSO base ointment

16C: heart **o**f rat treated with 10 % BASO base ointment

16: Photomicrographs of the histological examination of Heart, Liver and kidney of rat treated with BASO and NMSO (H&E x400).
Wound healing

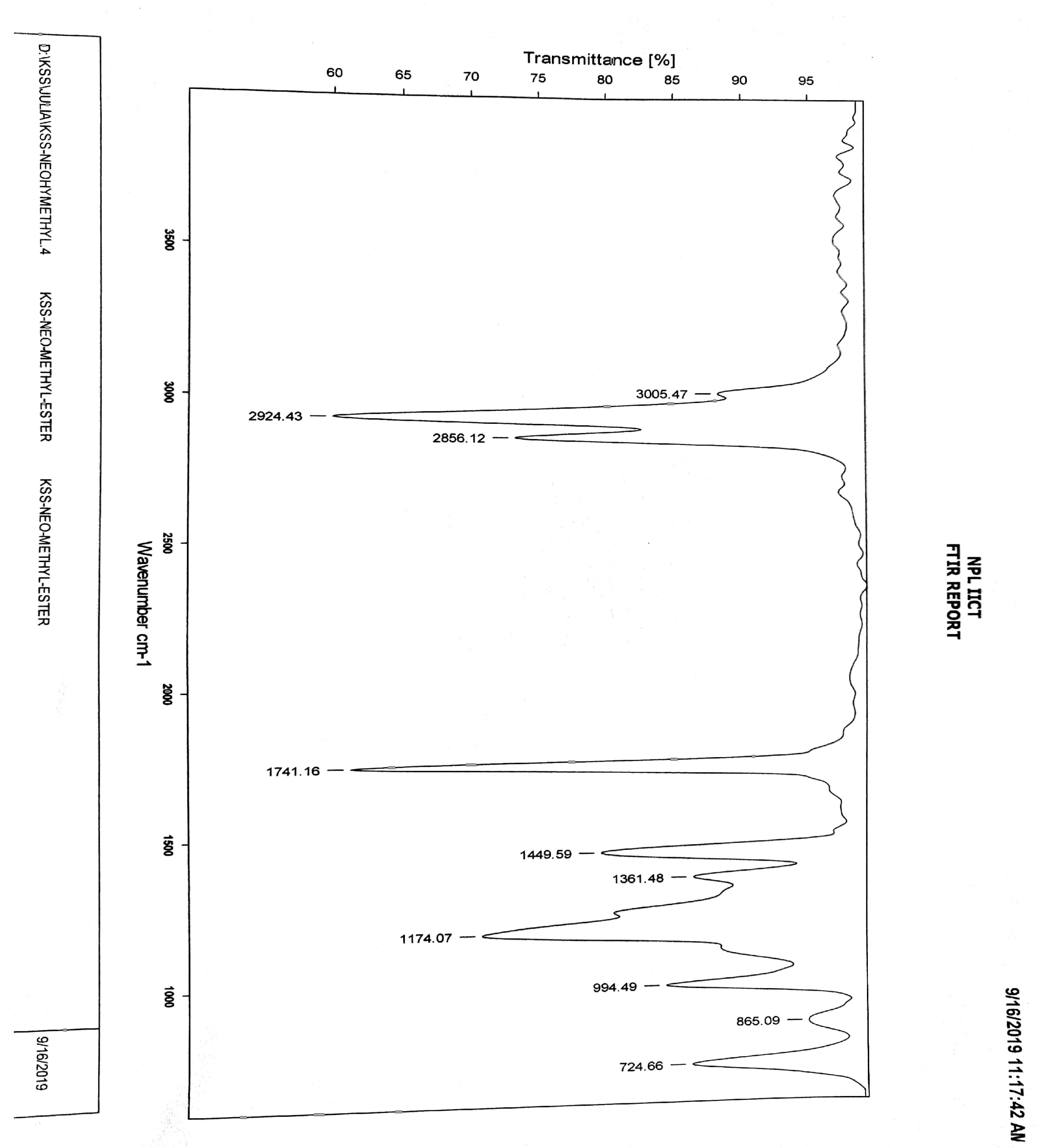


17. FT-IR spectrum of triglycerides in *Neocarya macrophylla* seed oil

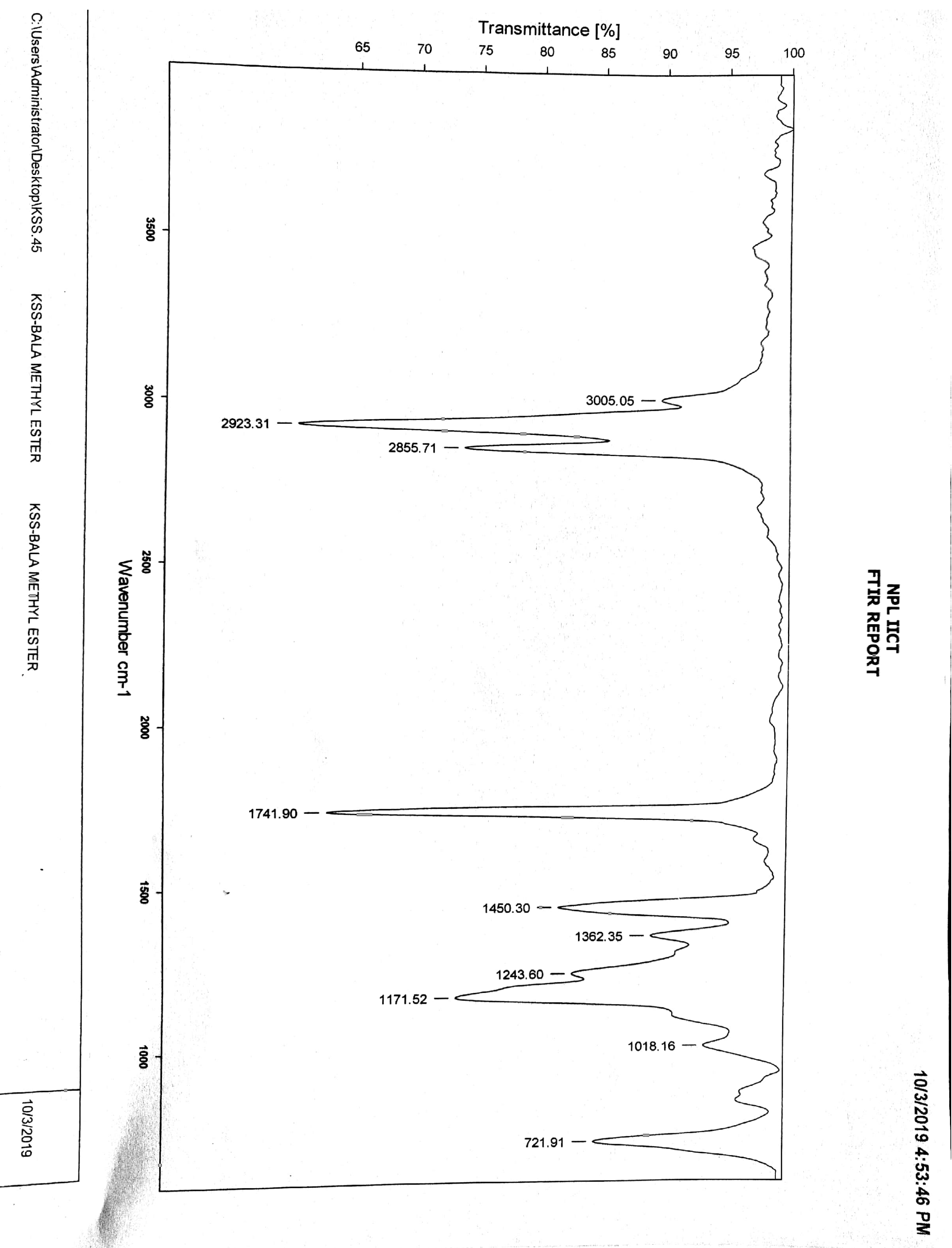


18. FT-IR spectrum of triglycerides in *Balanites aegyptiaca* seed oil

FT-IR spectrum of seed oils methyl ester (biodiesel)



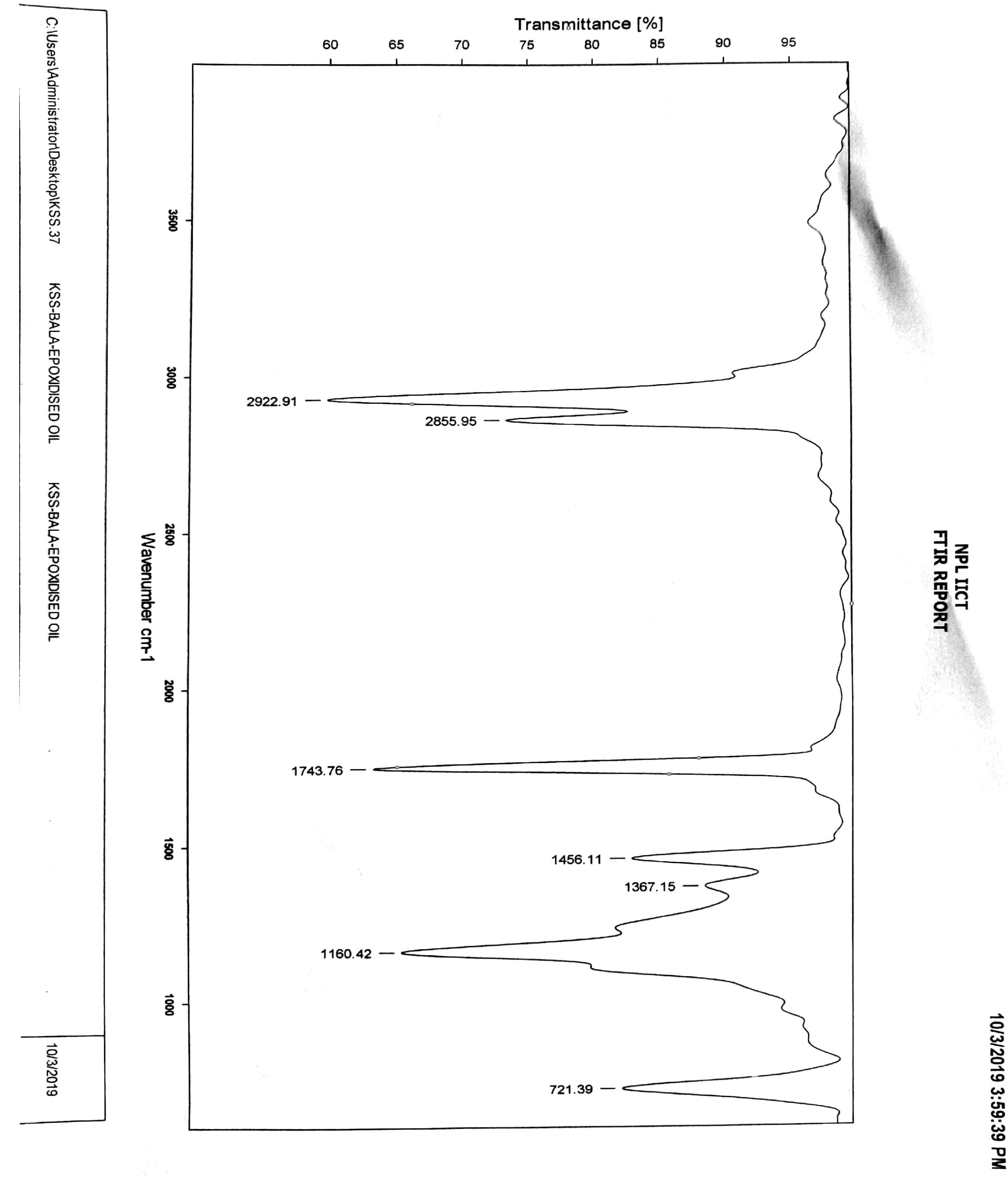
19. FT-IR spectrum of *Neocarya macrophylla* oil methyl ester (biodiesel)



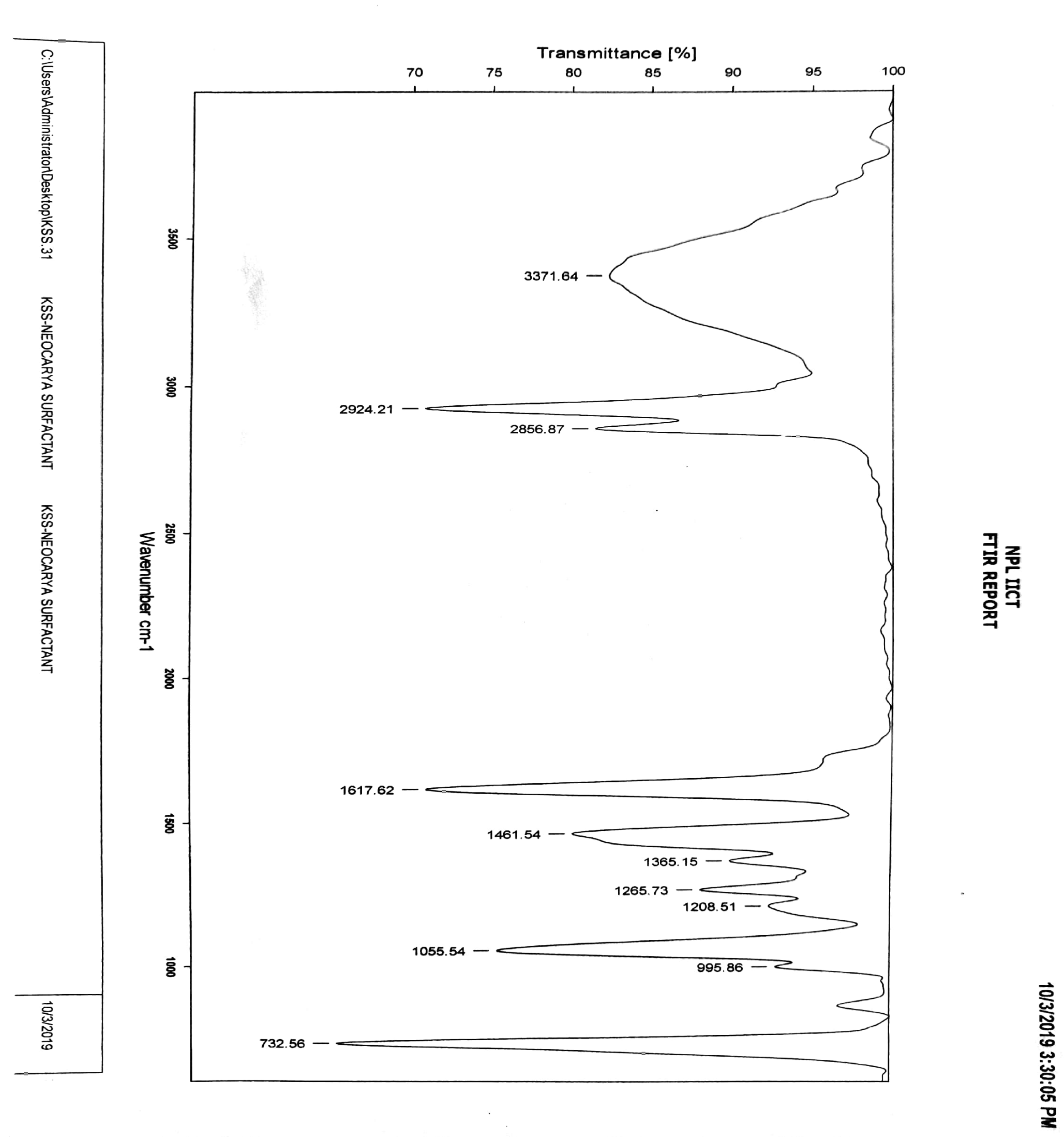
20. FT-IR spectrum of *Balanites aegyptiaca* seed oil methyl ester (biodiesel)

FT-IR spectrum of epoxidised seed oils

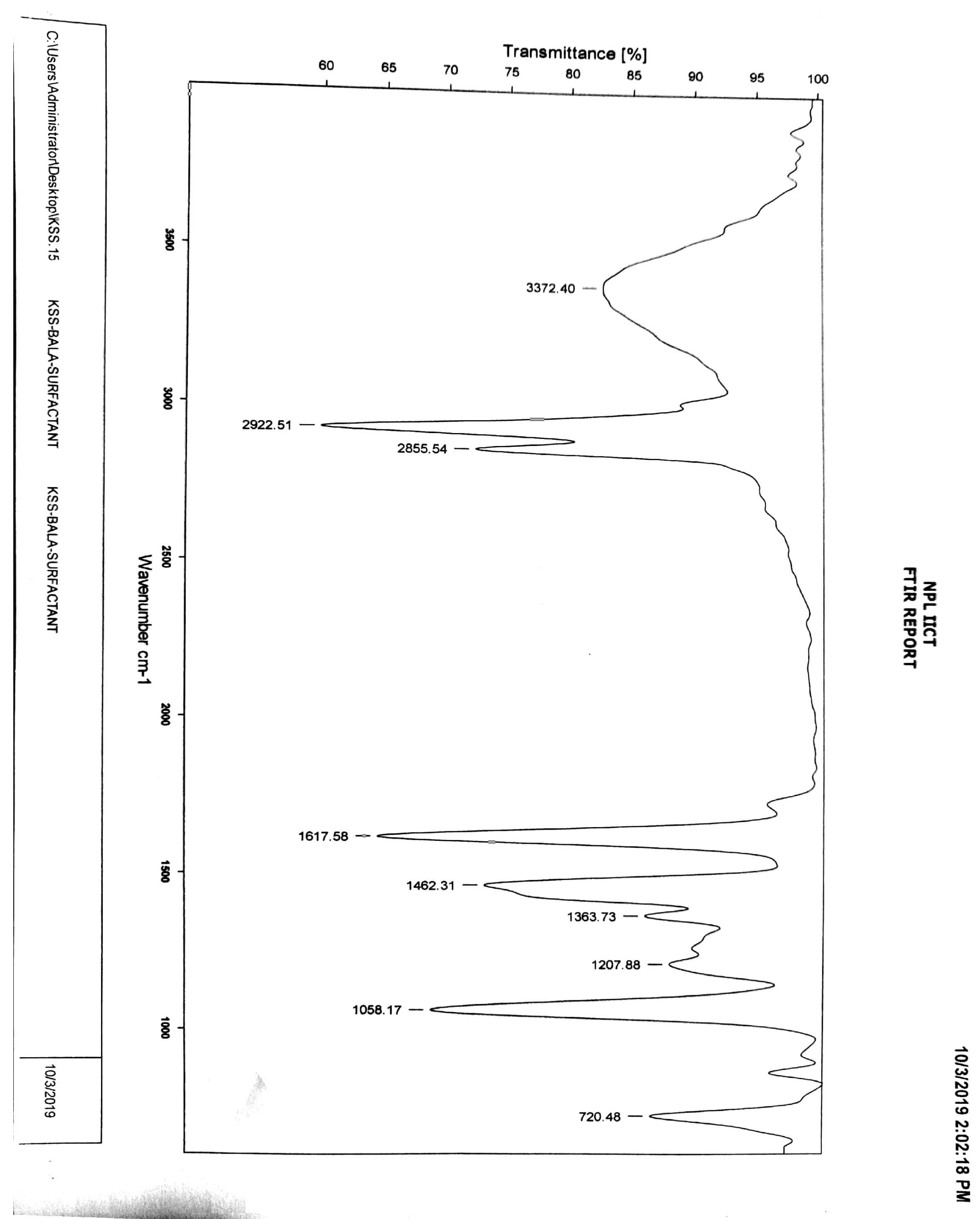
21. FT-IR spectrum of *N. macrophylla* epoxidised oil



22. FT-IR spectrum of *B. aegyptiaca* epoxidised oil



23. FT-IR spectrum of *N. mycrophylla* surfactant



24. FT-IR spectrum of *B. aegyptiaca* surfactant

FT-IR spectrum of epoxidised oils surfactants

Transmittance [%]

65 70 75 80 85 90 95 100

3386.06

2923.64

2856.40

1619.45

1462.77

1364.04

1205.37

1062.18

996.33

724.40

3500 3000 2500 2000 1500 1000

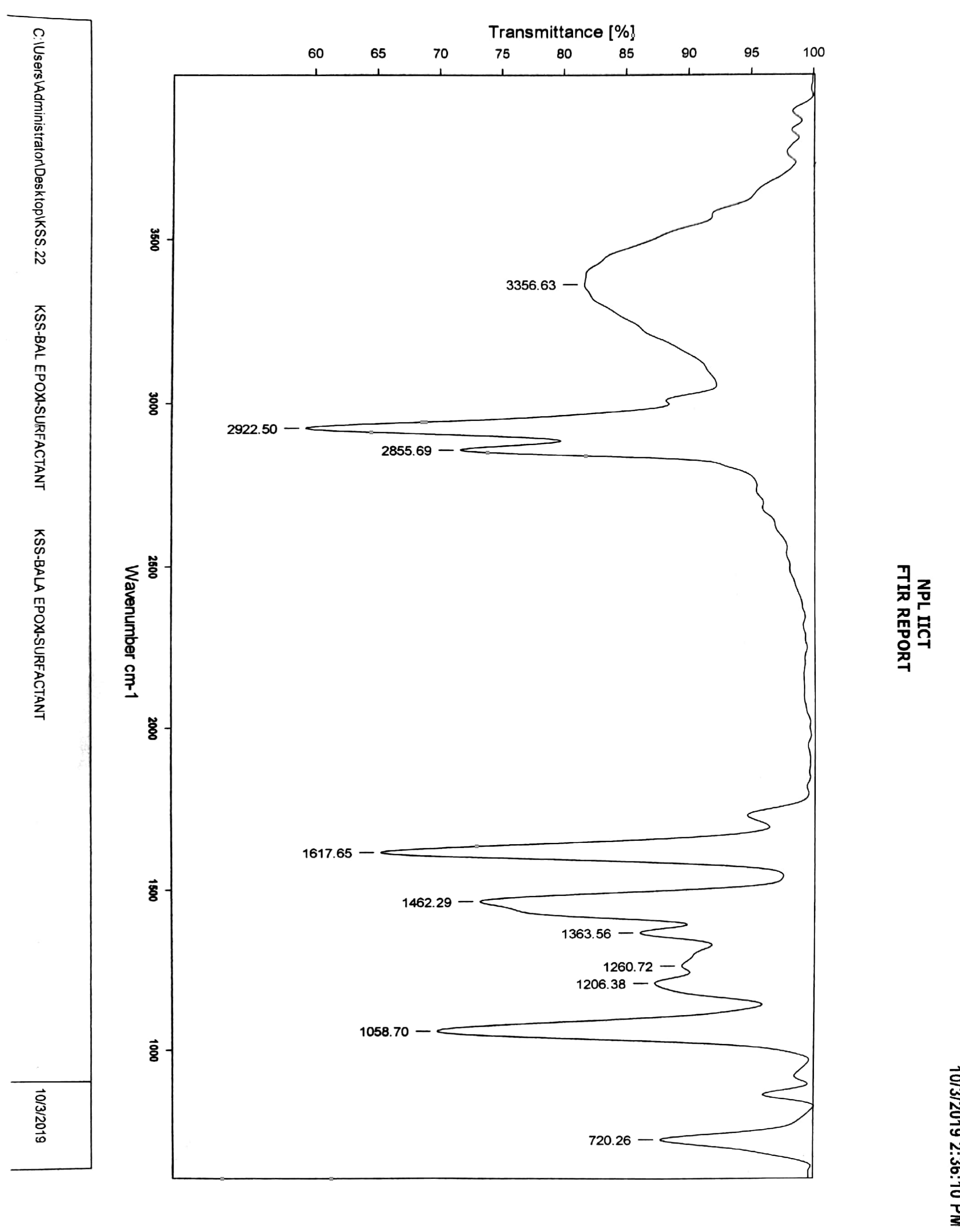
Wavenumber cm-1

C:\Users\Administrator\Desktop\KSS.19 KSS-NEO EPOXI-SURFACTANT KSS-NEO EPOXI-SURFACTANT 10/3/2019

NPL IICT
FTIR REPORT

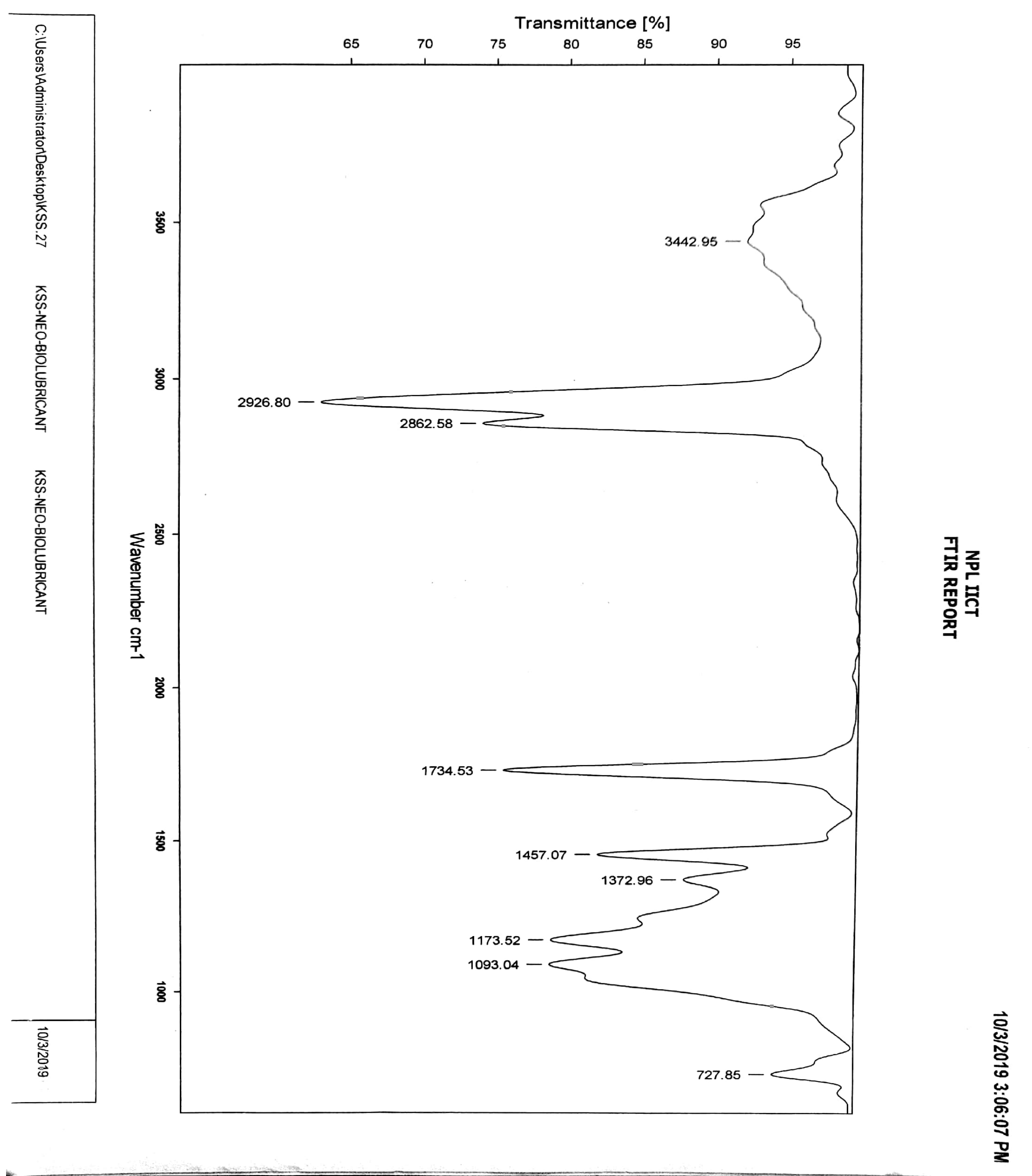
10/3/2019 2:22:39 PM

25. FT-IR spectrum of *N. mycrophylla* epoxidised oil surfactant

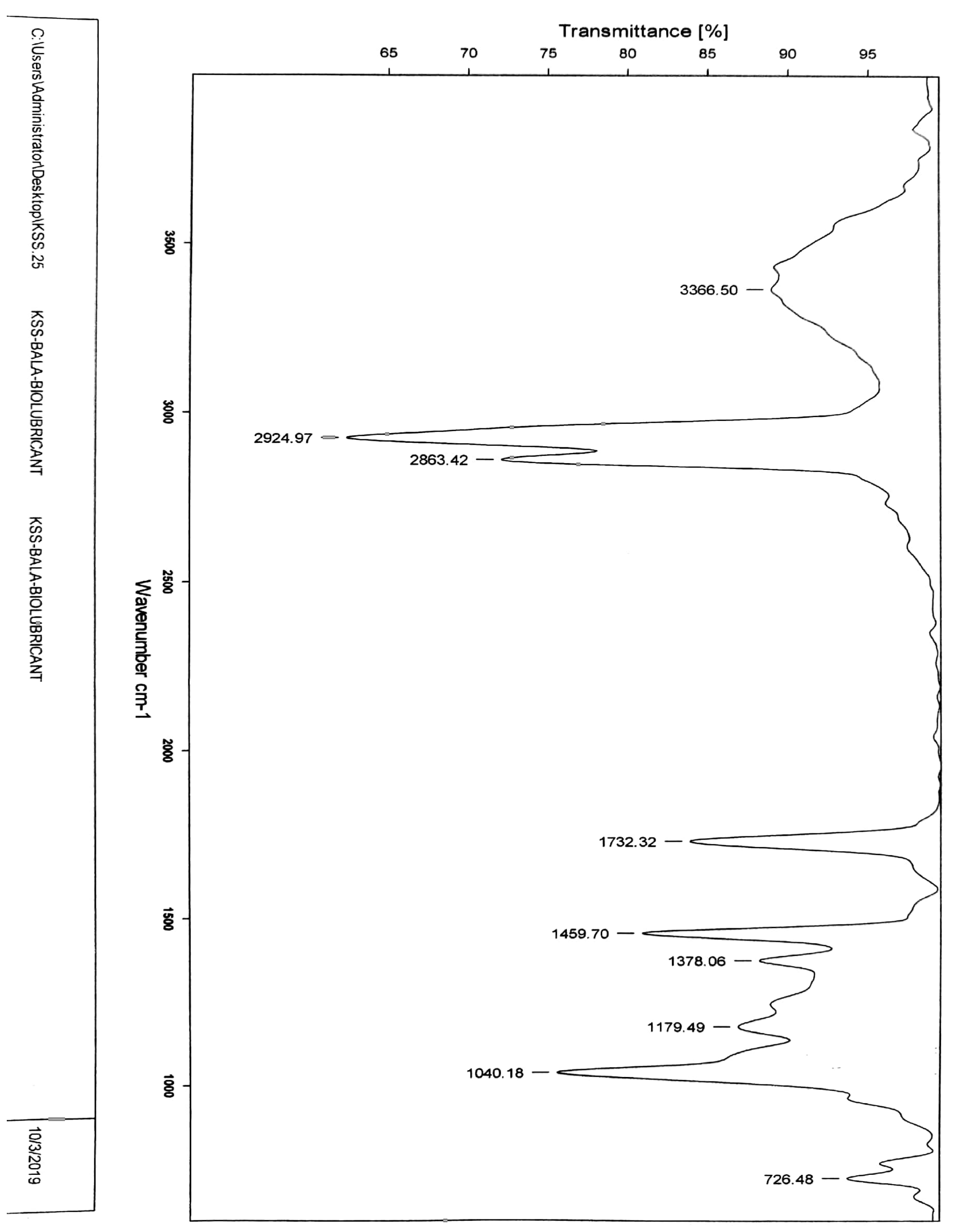


26. FT-IR spectrum of *B. aegyptiaca* epoxidised oil surfactant

FT-IR spectrum of seed oils biolubricants

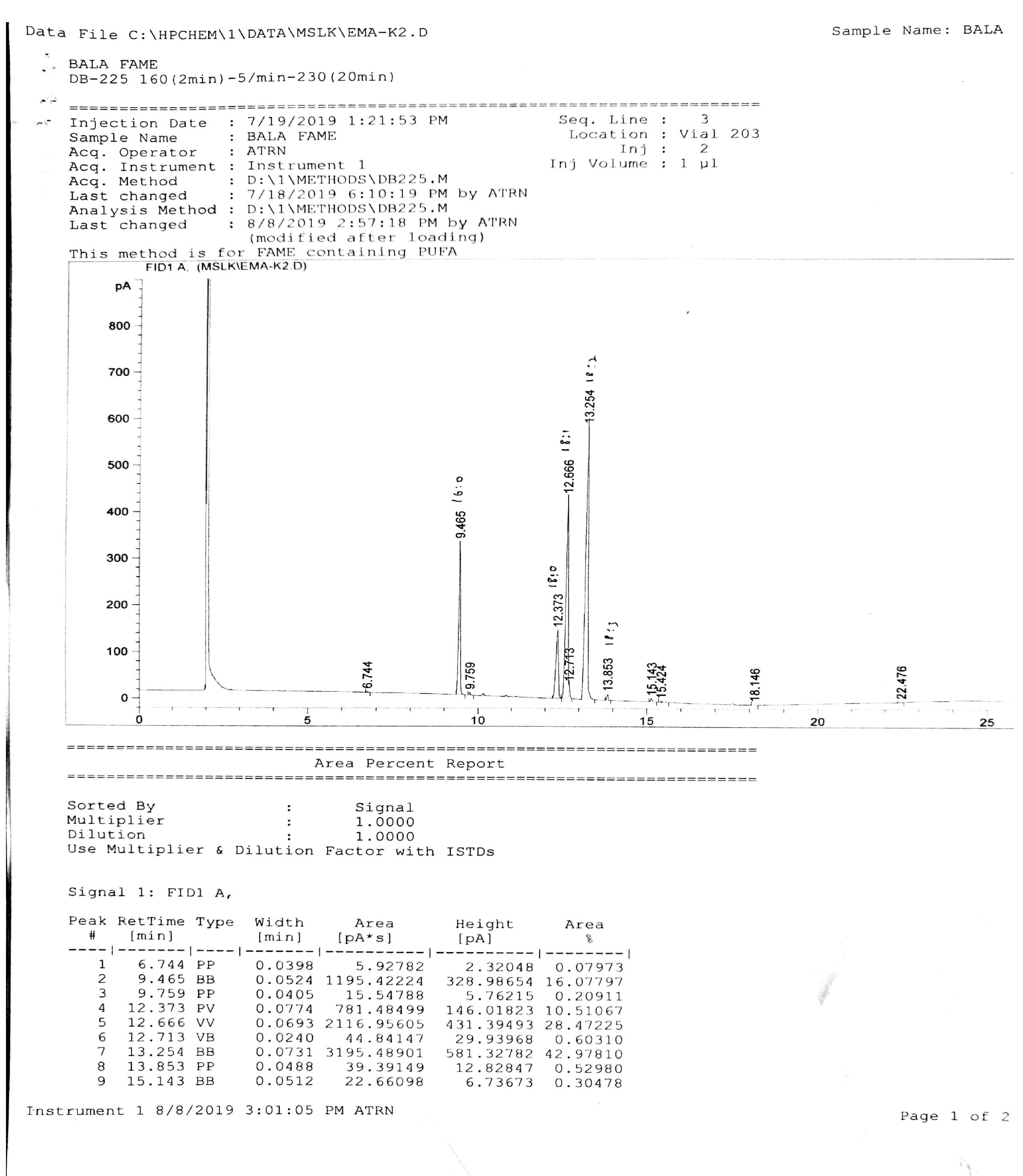


27. FT-IR spectrum of *N.macrophylla* seed oil biolubricant



28 FT-IR spectrum of *B. aegyptiaca* seed oil biolubricant

HPLC spectrum of the seed oils fatty acids



Data File C:\HPCHEM\1\DATA\MSLK\EMA-K2.D Sample Name: BALA

BALA FAME
DB-225 160(2min)-5/min-230(20min)

===

Injection Date : 7/19/2019 1:21:53 PM		Seq. Line :	3
Sample Name : BALA FAME		Location :	Vial 203
Acq. Operator : ATRN		Inj :	2
Acq. Instrument : Instrument 1		Inj Volume :	1 µl

Acq. Method : D:\1\METHODS\DB225.M
Last changed : 7/18/2019 6:10:19 PM by ATRN
Analysis Method : D:\1\METHODS\DB225.M
Last changed : 8/8/2019 2:57:18 PM by ATRN
(modified after loading)
This method is for FAME containing PUFA

===
Area Percent Report
===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	6.744	PP	0.0398	5.92782	2.32048	0.07973
2	9.465	BB	0.0524	1195.42224	328.98654	16.07797
3	9.759	PP	0.0405	15.54788	5.76215	0.20911
4	12.373	PV	0.0774	781.48499	146.01823	10.51067
5	12.666	VV	0.0693	2116.95605	431.39493	28.47225
6	12.713	VB	0.0240	44.84147	29.93968	0.60310
7	13.254	BB	0.0731	3195.48901	581.32782	42.97810
8	13.853	PP	0.0488	39.39149	12.82847	0.52980
9	15.143	BB	0.0512	22.66098	6.73673	0.30478

Instrument 1 8/8/2019 3:01:05 PM ATRN Page 1 of 2

29. HPLC spectrum of *B. aegyptiaca* seed oil fatty acids

OGUN FAME
DB-225 160(2min)-5/min-230(20min)

```
Injection Date      : 7/19/2019 10:48:53 AM          Seq. Line :   1
Sample Name         : OGUN FAME                       Location : Vial 201
Acq. Operator       : ATRN                                 Inj :   2
Acq. Instrument     : Instrument 1                  Inj Volume : 1 µl
Acq. Method         : D:\1\METHODS\DB225.M
Last changed        : 7/18/2019 6:10:19 PM by ATRN
Analysis Method     : D:\1\METHODS\DB225.M
Last changed        : 8/8/2019 2:57:18 PM by ATRN
                      (modified after loading)
This method is for FAME containing PUFA
```

FID1 A, (MSLK\EMA-I2.D)

Area Percent Report

```
Sorted By          :      Signal
Multiplier         :      1.0000
Dilution           :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	6.753	PB	0.0406	86.72504	33.01355	0.91170
2	9.530	BV	0.0761	3485.79883	634.04138	36.64449
3	9.778	VB	0.0591	26.00805	6.30638	0.27341
4	12.378	PV	0.0861	394.19025	65.58647	4.14393
5	12.705	VV	0.0971	4205.07764	618.40747	44.20592
6	13.200	VV	0.0620	1097.18066	278.30124	11.53412
7	13.854	VB	0.0563	37.13131	9.77855	0.39034
8	15.150	BP	0.0520	35.23684	10.81231	0.37043
9	15.427	PP	0.0575	15.53320	4.07383	0.16329

nstrument 1 8/8/2019 2:58:32 PM ATRN Page 1 of 2

30. HPLC spectrum of *P. longifolia* seed oil fatty acids

ata File C:\HPCHEM\1\DATA\MSLK\EMA-L2.D Sample Name: ENTERO FAME

ENTERO FAME
DB-225 160(2min)-5/min-230(20min)

===

Injection Date : 7/19/2019 2:38:26 PM Seq. Line : 4
Sample Name : ENTERO FAME Location : Vial 204
Acq. Operator : ATRN Inj : 2
Acq. Instrument : Instrument 1 Inj Volume : 1 µl
Acq. Method : D:\1\METHODS\DB225.M
Last changed : 7/18/2019 6:10:19 PM by ATRN
Analysis Method : D:\1\METHODS\DB225.M
Last changed : 8/8/2019 3:01:29 PM by ATRN
(modified after loading)
This method is for FAME containing PUFA

FID1 A, (MSLK\EMA-L2.D)

pA 500 400 300 200 100 0; 0 5 10 15 20 25 min; peaks: 6.745, 9.417, 9.753, 12.303, 12.584, 12.671, 13.168, 13.839, 15.144, 18.153, 22.465

===

Area Percent Report

===

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	6.745	MM R	0.0502	1.62411	5.39054e-1	0.09456
2	9.417	BB	0.0442	220.95291	75.36463	12.86393
3	9.753	PP	0.0423	21.62375	7.81412	1.25894
4	12.303	BB	0.0521	167.02306	49.86797	9.72412
5	12.584	BV	0.0507	322.01633	97.14460	18.74787
6	12.671	VB	0.0485	52.02884	17.11320	3.02913
7	13.168	BB	0.0505	808.29321	227.20177	47.05904
8	13.839	PB	0.0466	44.18700	14.46104	2.57258
9	15.144	BB	0.0527	40.97198	12.33907	2.38540

nstrument 1 8/8/2019 3:03:10 PM ATRN Page 1 of 2

31. HPLC spectrum of *E. cyclocarpum* seed oil fatty acids

Data File C:\HPCHEM\1\DATA\MSLK\EMA-M2.D Sample Name: NEO FAME

NEO FAME
DB-225 160(2min)-5/min-230(20min)

```
=====================================================================
Injection Date   : 7/19/2019 3:55:00 PM            Seq. Line :    5
Sample Name      : NEO FAME                         Location : Vial 205
Acq. Operator    : ATRN                                  Inj :    2
Acq. Instrument  : Instrument 1                   Inj Volume : 1 µl
Acq. Method      : D:\1\METHODS\DB225.M
Last changed     : 7/18/2019 6:10:19 PM by ATRN
Analysis Method  : D:\1\METHODS\DB225.M
Last changed     : 8/8/2019 3:01:29 PM by ATRN
                   (modified after loading)
This method is for FAME containing PUFA
```



```
=====================================================================
                     Area Percent Report
=====================================================================

Sorted By              :      Signal
Multiplier             :      1.0000
Dilution               :      1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A,
```

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	6.751	MM R	0.0425	1.42965	5.61019e-1	0.05383
2	9.427	BB	0.0453	320.44260	108.88573	12.06629
3	9.755	PB	0.0422	5.23672	1.95752	0.19719
4	12.321	BP	0.0623	197.39702	49.69886	7.43300
5	12.639	PV	0.0649	1489.42322	348.35468	56.08434
6	12.691	VB	0.0278	32.00462	17.91920	1.20514
7	13.163	BV	0.0552	563.19299	155.65631	21.20707
8	13.846	BP	0.0498	17.22208	5.31355	0.64850
9	15.145	BB	0.0516	10.40022	3.06352	0.39162

Instrument 1 8/8/2019 3:05:35 PM ATRN Page 1 of 2

32. HPLC spectrum of *N. macrophylla* seed oil fatty acids

Data File C:\HPCHEM\1\DATA\MSLK\EMA-H2.D Sample Name: EKITA FAME

EKITA FAME
DB-225 160(2min)-5/min-230(20min)

Injection Date	: 7/19/2019 3:46:26 AM	Seq. Line :	8
Sample Name	: EKITA FAME	Location :	Vial 208
Acq. Operator	: ATRN	Inj :	2
Acq. Instrument	: Instrument 1	Inj Volume :	1 µl
Acq. Method	: D:\1\METHODS\DB225.M		
Last changed	: 7/18/2019 6:10:19 PM by ATRN		
Analysis Method	: D:\1\METHODS\DB225.M		
Last changed	: 8/8/2019 2:56:05 PM by ATRN (modified after loading)		

This method is for FAME containing PUFA

FID1 A, (MSLK\EMA-H2.D)

Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

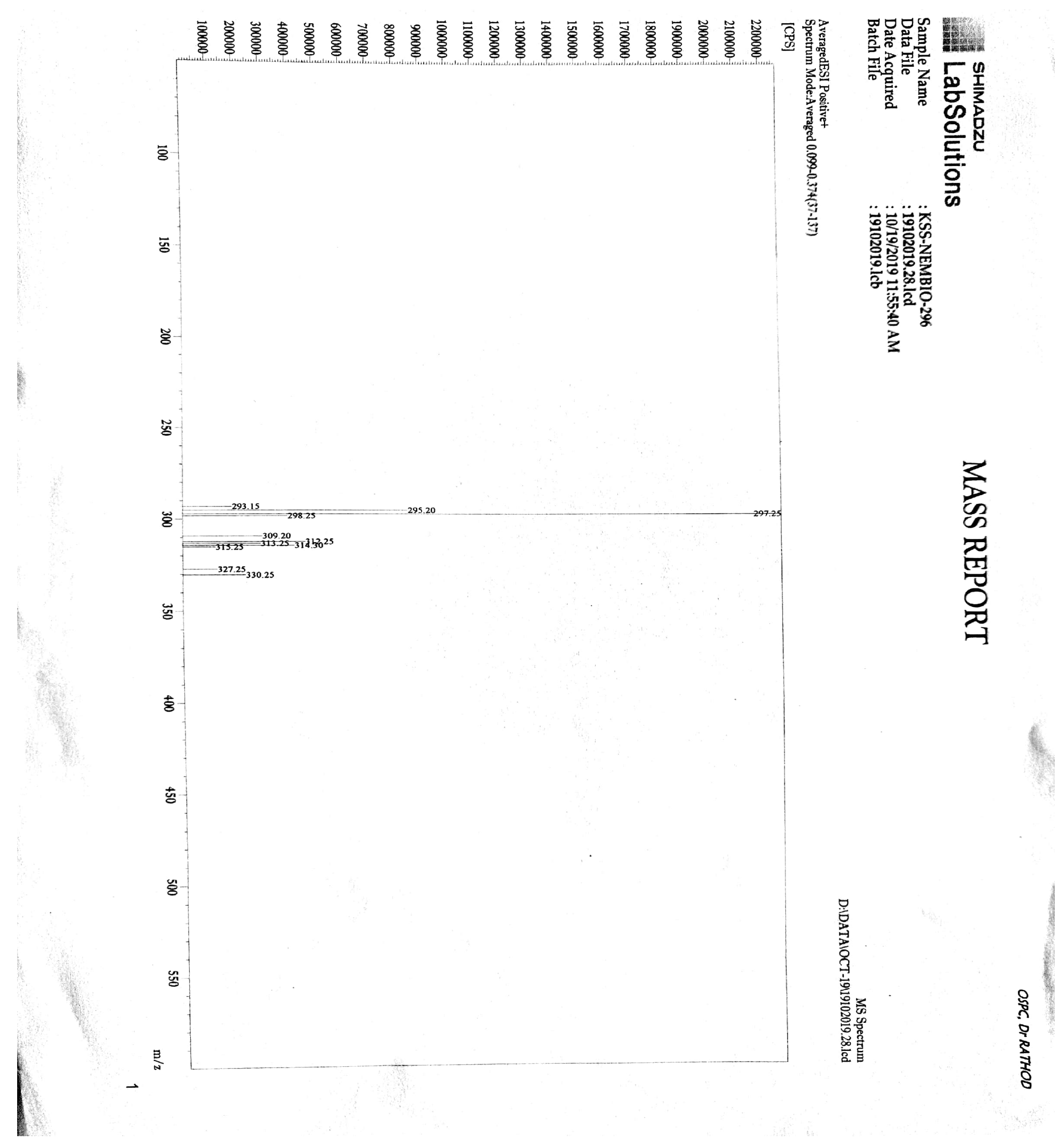
Signal 1: FID1 A,

Peak #	RetTime [min]	Type	Width [min]	Area [pA*s]	Height [pA]	Area %
1	6.747	PB	0.0379	3.66971	1.50999	0.18775
2	9.428	BB	0.0453	367.33905	121.41299	18.79391
3	9.754	MM R	0.0500	2.32706	7.75001e-1	0.11906
4	12.307	BV	0.0557	150.42404	42.05974	7.69604
5	12.608	VB	0.0568	801.95288	213.53444	41.02976
6	13.158	BB	0.0482	526.07025	156.61920	26.91497
7	13.838	PB	0.0470	15.75186	4.96912	0.80590
8	15.143	BP	0.0500	27.32426	8.38330	1.39797
9	15.426	PP	0.0506	27.90622	8.21567	1.42775

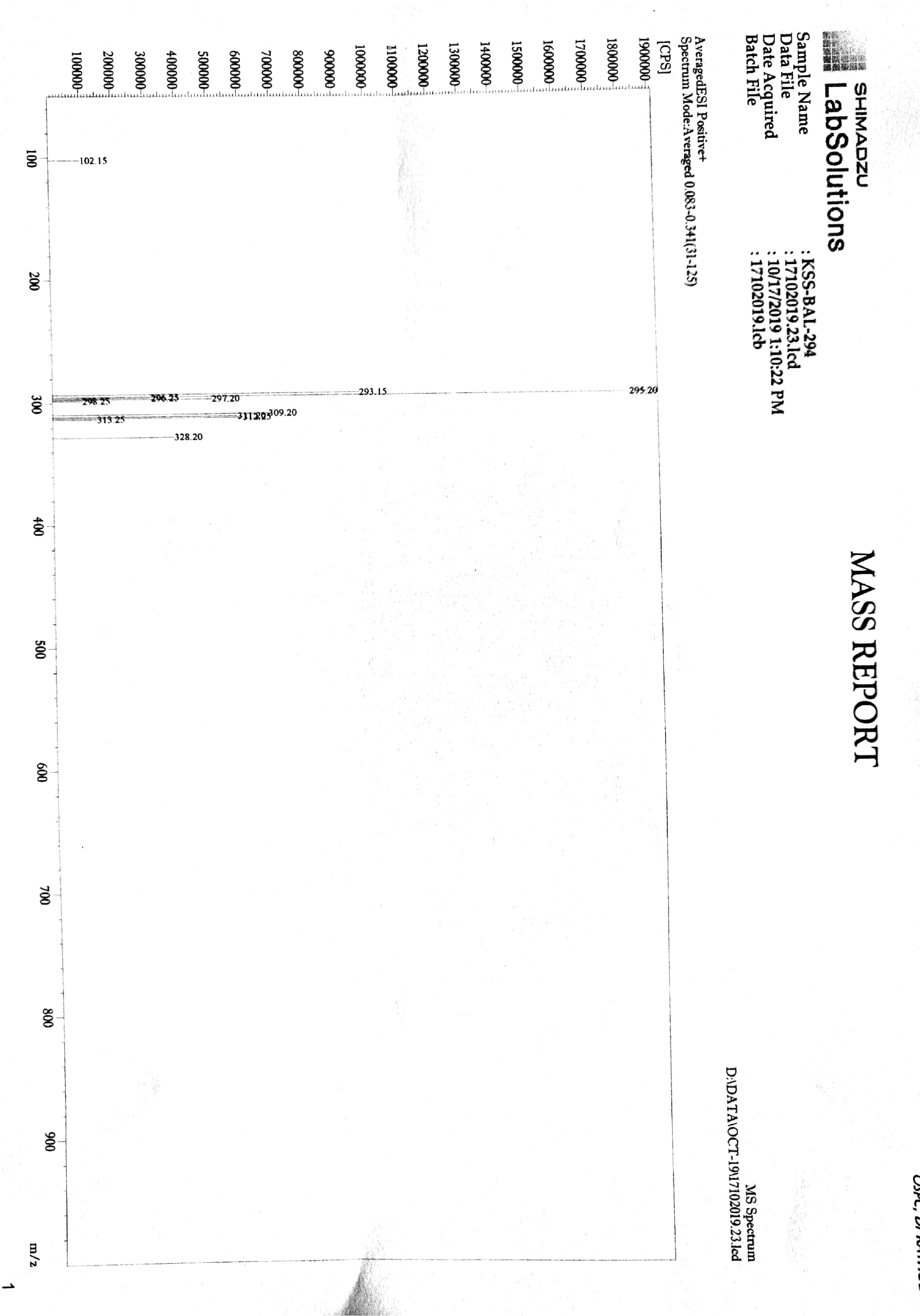
Instrument 1 8/8/2019 2:56:49 PM ATRN Page 1 of 2

33. HPLC spectrum of *C. albidum* seed oil fatty acid

The ESI/MS spectrum of seed oils methyl esters (biodiesel)

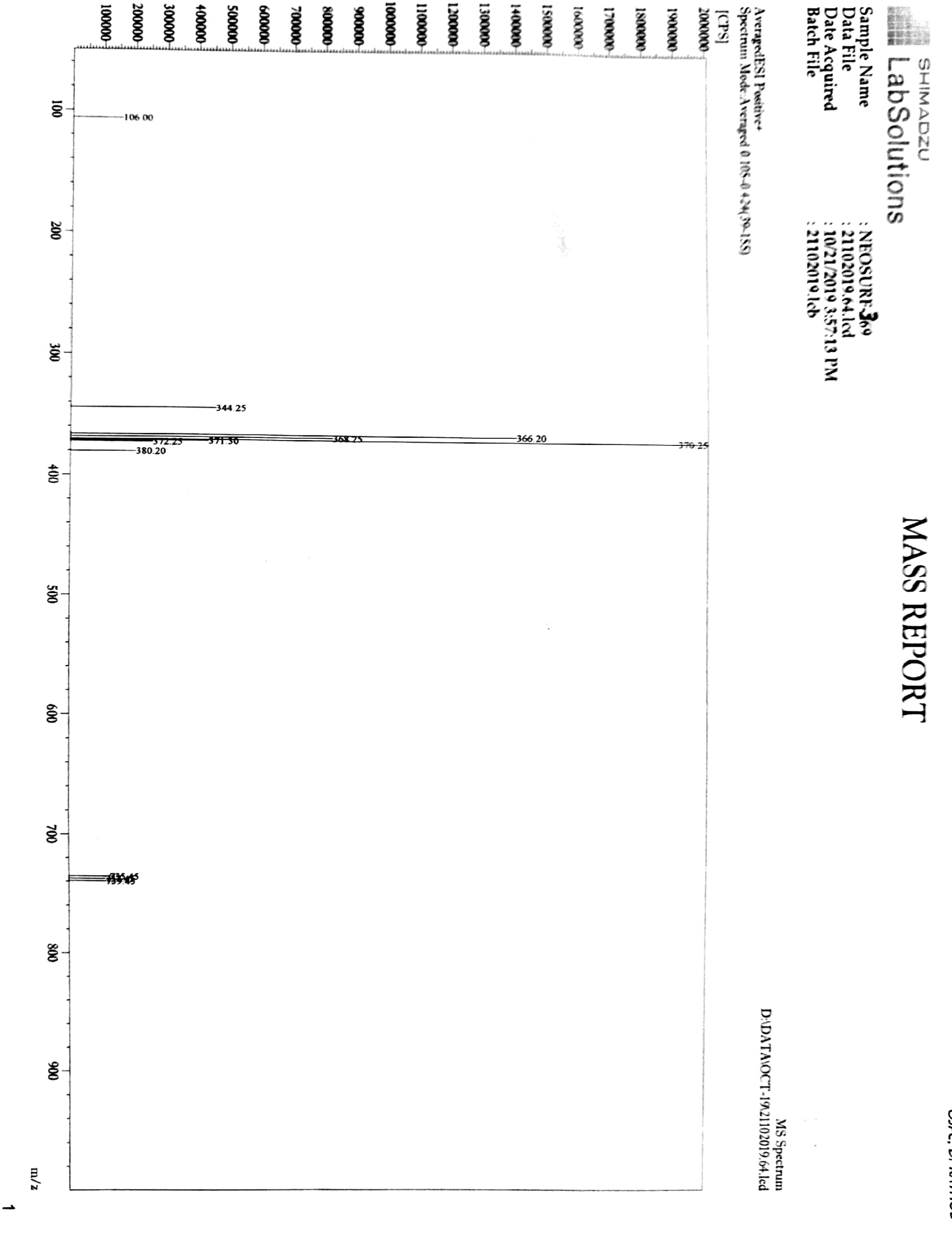


34. The ESI/MS spectrum of *Neocarya macrophylla* seed oil methyl esters (biodiesel)

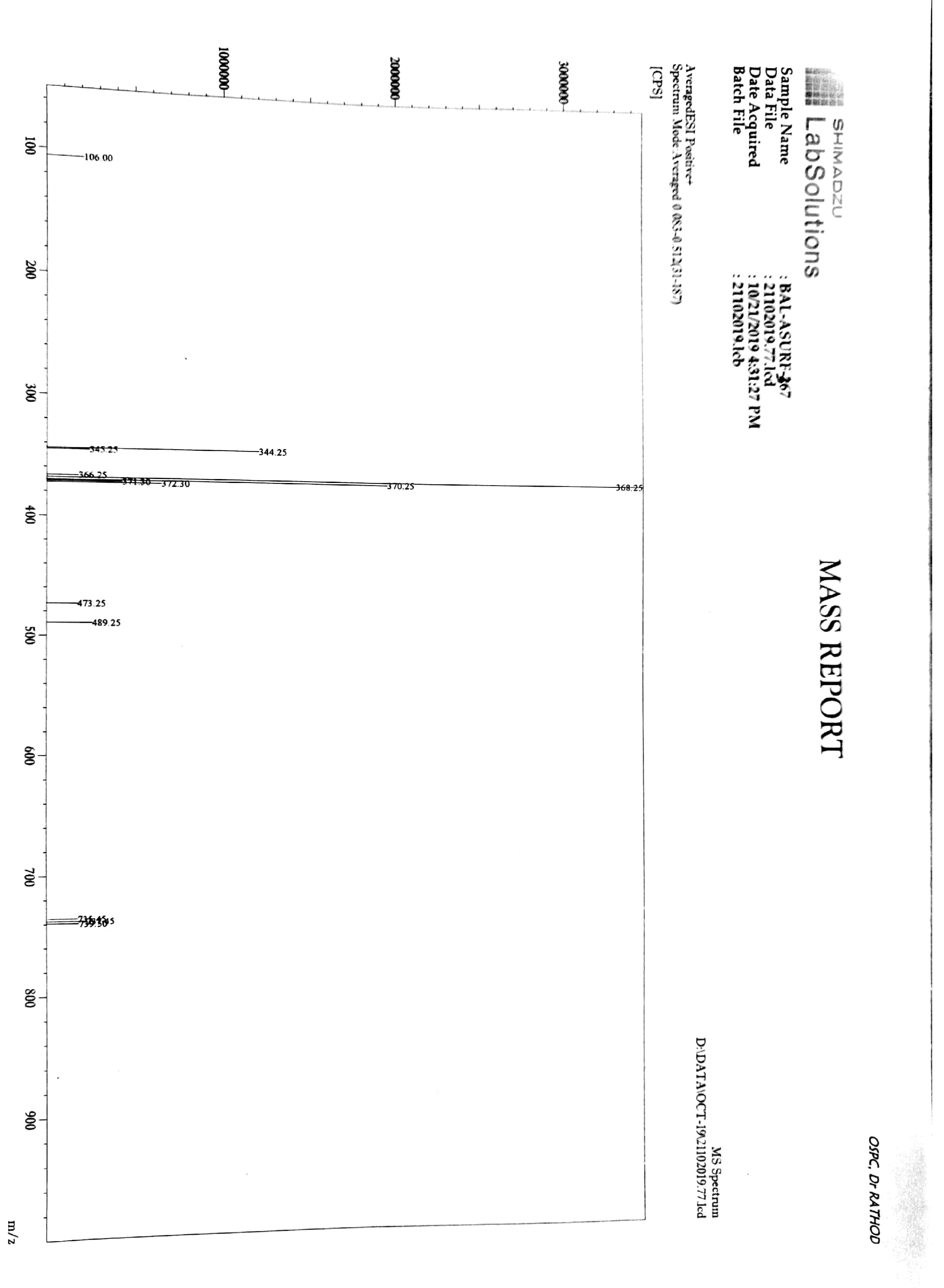


35. The ESI/MS spectrum of *Balanites aegyptiaca* seed oil methyl ester (biodiesel)

The ESI/MS spectrum of seed oils surfactants



36. The ESI/MS spectrum of *N. macrophylla* oil surfactant



37. The ESI/MS spectrum of *Balanites aegyptiaca* oil surfactant

INSTITUTE FOR ADVANCED MEDICAL RESEARCH AND TRAINING (IAMRAT)

College of Medicine, University of Ibadan, Ibadan, Nigeria.

Director: **Prof. Catherine O. Falade,** *MBBS (Ib), M.Sc, FMCP, FWACP*
Tel: 0803 326 4593, 0802 360 9151
e-mail: cfalade@comui.edu.ng lillyfunke@yahoo.com

UI/UCH EC Registration Number: **NHREC/05/01/2008a**

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Chemical and Preliminary Toxicological Evaluation of some selected Nigerian underutilized seed Flours and Cakes in Dietary Formulation of Albino Rats

UI/UCH Ethics Committee assigned number: UI/EC/15/0208

Name of Principal Investigator: **Emmanuel N. Ifedi**

Address of Principal Investigator: Department of Chemistry,
Faculty of Science,
University of Ibadan, Ibadan

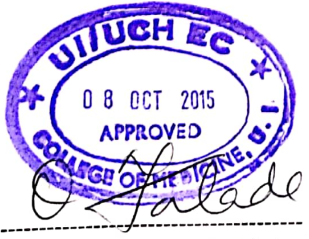
Date of receipt of valid application: 16/07/2015

Date of meeting when final determination on ethical approval was made: **08/10/2015**

This is to inform you that the research described in the submitted protocol and other information materials have been reviewed and *given full approval by the UI/UCH Ethics Committee.*

This approval dates from **08/10/2015 to 07/10/2016.** If there is delay in starting the research, please inform the UI/UCH Ethics Committee so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the* UI/UCH EC *assigned number and duration of* UI/UCH EC *approval of the study.* It is expected that you submit your annual report as well as an annual request for the project renewal to the UI/UCH EC early in order to obtain renewal of your approval to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the UI/UCH EC. *No changes are permitted in the research without prior approval by the* UI/UCH EC *except in circumstances outlined in the Code. The* UI/UCH EC *reserves the right to conduct compliance visit to your research site without previous notification.*



Professor Catherine O. Falade
Director, IAMRAT
Chairperson, UI/UCH Ethics Committee
E-mail: uiuchec@gmail.com

Research Units • Genetics & Bioethics • Malaria • Environmental Sciences • Epidemiology Research & Service • Behavioural & Social Sciences • Pharmaceutical Sciences • Cancer Research & Services • HIV/AIDS