

**PHARMACOGNOSTIC, NUTRITIONAL AND TOXICITY STUDIES ON THE
AERIAL PART OF *DAUCUS CAROTA* L. (APIACEAE)**

BY

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MAY, 2018

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B. TECH (HONS) BOTANY, (MAUTECH)2014
P15PHPD8008**

**A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE
STUDIES,
AHMADU BELLO UNIVERSITY ZARIA,**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD
OF MASTER DEGREE IN PHARMACOGNOSY**

**DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT,
FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA**

MAY, 2018

Declaration

I declare that the work in this dissertation entitled **Pharmacognostic, Nutritional and Toxicity Studies on the Aerial part of *Daucus carota* L. (Apiaceae)** has been performed by me in the **Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria**. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Emmanuel Ayodeji AYENI

Signature

Date

Certification

This dissertation entitled **Pharmacognostic, Nutritional and Toxicity Studies on the Aerial part of *Daucus carota* L. (Apiaceae)** by Emmanuel Ayodeji AYENI, meets the regulations governing the award of the degree of Masters of Science in Pharmacognosy and Drug Development of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

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Dedication

This dissertation is dedicated to my late mother Deaconess (Mrs.) Esther Moromoke Ayeni who brought me to the world with good intentions.

Acknowledgments

I am grateful to the Almighty God for the privilege, wisdom, strength and grace He has given me during the period of the research.

I am indebted to my supervisors Dr. A. Ahmed and Prof. G. Ibrahim for their enormous contributions, supervision, guidance and support during the research. I am grateful to the staff of the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria for their positive contributions during the period of the research.

I appreciate Professor D. J Adeyemo, Mr. Abdullahi Sirajudeen Babatunde and Mallam I. A Bappah for their guidance and assistance during the radioactivity analyses at the Centre for Energy Research Ahmadu Bello University Zaria, Nigeria. I am grateful to Mr. Ibrahim, Mal. Aliyu and Miss. Lucia Uhomoibhi of Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria during the toxicological studies for their technical assistance. I appreciate the positive contribution of Mr. Bamidele of the Department of Anatomy, Faculty of Medicine, Ahmadu Bello University Zaria during the histological studies, his suggestion and comments strengthened this work.

Thanks to my colleagues; Pharm. Atinga Vallada, Ekaette Ukut, Victoria Abioye, Zainab Muhammad, Mansur Lawal, Pharm. Abdulkarim Safiyanu, among others, I say thank you for the knowledge shared during course work. I am also grateful to Hajiya (Mrs.) H. Tanko, Pharm. (Mrs.) Nzekwe, Jide Famuwagun, Oginni Simon Oyewole (esq), Adejor John, Mal. Nura Umar, Isah Abraham, Lucia Uhomoibhi, Oche Dominic, Aliyu Nuhu and Ikechukwu Okoye for their good gesture and supports during the programme.

Abstract

Daucus carota (L.) commonly known as carrot belongs to the family of Apiaceae. The aerial part is used as component of livestock feed in some part of Northern Nigeria without any scientific evidence of safety. The present study was carried out to establish its pharmacognostic, nutritional potentials and safety to justify the use of *D. carota* aerial part. The pharmacognostic parameters, nutritional, acute and subchronic toxicity were determined by standard methods. The gross alpha, beta activities and the naturally occurring radioactive materials were carried out using spectrometry procedures. The results of the microscopy revealed wavy epidermal cells and several diacytic stomata on the lower and upper surfaces. The chemo-microscopy revealed presence of starch, tannins, lignins, cellulose, starch grains, aleurones grains, mucilage, suberin and cutins. The quantitative leaf microscopy of the lower epidermal layer showed stomatal number (14.80 ± 0.97), stomatal index ($31.22 \pm 3.09\%$) and palisade ratio (6.20 ± 0.37). The upper epidermal layer revealed stomatal number (10.60 ± 0.87), stomatal index ($32.55 \pm 1.25\%$), vein islet number (12.80 ± 1.98) and veinlet termination (31.80 ± 2.33). The physicochemical evaluation revealed moisture content (5.56%), alcohol extractive value (15.60%), water extractive value (21.80%), total ash (11.60%), acid insoluble ash (1.00%) and water soluble ash (5.67%). The proximate analysis revealed crude protein (14.59%), lipid (10.37%), crude fibre (9.07%) and carbohydrate (51.81%). The acute oral toxicity of the extracts on the wistar rats showed no sign of toxicity and mortality at 5000 mg/kg. The effect of n-hexane and methanol extracts on body weight of the wistar rats showed significant decreased ($p \leq 0.05$) at week 2. The ethylacetate extract showed no significance ($p \geq 0.05$) difference. The hematological effects of the extracts showed significant ($p \leq 0.05$) decreased in red blood cell in all the treated doses. The hexane and ethylacetate extract showed that MCV and MCH were also significant ($p \leq 0.05$). The *D. carota* aerial part extract showed significant decreased ($p \leq 0.05$) on ALP (IU/L) while the ALT (IU/L), AST (IU/L), TP (IU/L) and ALB (G/DL) had no significant differences ($p \geq 0.05$). The effects of oral administration of n-hexane extract on bicarbonate showed statistically significant at the treated groups and sodium was also significant at 1500 mg/kg ($p \leq 0.05$). The ethylacetate extract was not significant ($p \geq 0.05$) the kidney function parameters. The methanol extract showed that sodium and bicarbonate was significant ($p \leq 0.05$) at 500 mg/kg and 1500 mg/kg. The histopathological effects of the extracts showed moderate and slight tubular necrosis in the kidney at 500 mg/kg, while the liver had slight hepatic necrosis with moderate lymphocyte hyperplasia. The dose group at 1000 mg/kg and 1500 mg/kg showed moderate lymphocyte hyperplasia in the kidney and the liver revealed vascular congestion with kupfer cell hyperplasia. The effects of the extracts on the stomach revealed normal epithelium cells with slight necrosis when compared with the

control group. The gross alpha ($6.36 \pm 0.25 \text{ Bq/g}$) and beta activity ($2.37 \pm 0.32 \text{ Bq/g}$) concentration were above the acceptable limits. Thenaturally occurring radioactive materials level were ^{40}K ($389.52 \pm 134.52 \text{ Bq/kg}$), ^{226}Ra ($29.13 \pm 10.66 \text{ Bq/kg}$) and ^{232}Th ($84.85 \pm 64.85 \text{ Bq/kg}$). The findings from the study suggested that *D. carota* aerial part are not safe for man and livestock and that consumption of the aerial part is capable of causing liver, kidney tissue injury and other possible toxic related diseases.

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Abbreviations and Symbols

NORMS	Naturally Occurring Radioactive Materials
NaI (TI)	Sodium Iodide Detector crystal optically coupled to a photomultiplier tube
Bq/kg	Becquerel per kilogram
Bq/g	Becquerel per gram
OECD	Organization for Economic Cooperation Development
ALT	Alanine aminotransferase
ALP	Alkaline Phosphatase
AST	Aspartate aminotransferase
TP	Total protein
ALB	Albumin
^{40}K	Potassium-40
^{232}Th	Thorium
^{226}Ra	Radium
RBC	Red Blood Cells
WBC	White Blood Cells
HGB	Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MCH	Mean corpuscular haemoglobin
nGy/h	Nano Gray per hour
mSv/yr	milli Sieverts per year
Mg/kg	Milligram per kilogram
nGy/h	Nano Gray per hour
mSv/yr	milli Sieverts per year

CHAPTER 1

1.0 INTRODUCTION

1.1 Background of Study

Since ancient time, plants as a natural product have been the main sources of food, clothing, shelter, herbal beverages, supplements, insect repellent, natural cosmetics, livestock feed and drugs in managing health care in the developing countries including Nigeria (Etkins, 1993; Riewpaiboon, 2004; Elujoba *et al.*, 2005; Etkin., 2007). The World Health Organization defined medicinal plant as a plant, in which any of its part, in one or more of its organs contain substances that can be used for therapeutic purposes, or which are precursors for chemical and pharmaceutical synthesis (WHO, 2008).

It was established that about sixty-eighty percent of the world population solely depend on traditional medicines including medicinal plants to meet their health demands (WHO, 2008). This expresses the potential of medicinal plants or natural products in attaining and achieving the global statutory quotes of health for all towards total, quality and safe national health integration especially in Nigeria. Besides, many natural products are always and readily available, inexpensive, accessible, widely distributed, ecologically friendly in nature and their utilizations are relevant to peoples' cultures and origins, and also, many conventional drugs have their origin from plants (Sofowora, 1983; Gurnani *et al.*, 2014). Therefore, investigations and findings into medicinal and natural products research will continue towards providing great variety of lead compounds that could be used as models for many pharmaceutical industries (Borris, 1996) and also serve as templates in pharmaceutical research institutions.

Similarly, plants and other natural products have cultural relationship with many societies and origins in prevention of diseases (Thitiya, 2000) especially in African countries such as Nigeria, where the ratio of traditional practitioners outweighed trained medical doctors (Sofowora, 1983; Borokini *et al.*, 2013).

Medicinal plants constitute phytochemicals that are mainly primary and secondary metabolites which are essentially utilized as drug. In addition, they are pharmacologically active for their physiological actions. These chemical constituents are essential for the metabolic functions and they play vital roles in metabolism and life processes (Novak and Haslberger, 2000). They also satisfy the needs of human as food, drug and cosmetics (Etkins, 2007).

Toxicity can be seen as any substance that is capable to exert harmful effect on living organisms including humans and animals (Obidike and Salawu, 2013). Toxicity assessment guides utilization or condition of substances including natural products under short and long term effect and also provides adequate information about the product. Its assessment strengthens the knowledge about the natural product and proffer possible measures of utilization (Obidike and Salawu, 2013).

Toxicity can be measured by its effects on the host especially on organ, tissue or cells (Obidike and Salawu, 2013). The toxic effects of a substance on animal physiology can range from minor changes such as reduced weight gain, small physiological alteration or change in the levels of circulating hormones, to severe effects in organ functional loss leading to death. Intermediate levels of toxicity may cause pain and suffering (Pak *et al.*, 2004).

Radioactivity safety and other related radiation health hazards indices could guide toxicological evaluation of natural products for its effective utilization as herbal products, drugs, foods, pharmaceutical agents and other exploitations. The international food safety authorities network (INFOSAN, 2011) stated that plants are one of the primary linkages of radionuclides entering into the human body through the environmental and food chain. Some common radionuclides such as ^{40}K , ^{232}Th , ^{238}U and their progenies may be detected in medicinal plants or herbal drugs that may possibly cause damage to human body. Some of these natural radionuclides could come into the human body through the ecological cycles interaction (Elujoba *et al.*, 2005; Ali, 2008; Adewumi, 2011; Anekwe and Avwiri, 2016; Idoko *et al.*, 2016).

Naturally Occurring Radioactive Materials (NORMs) have been present in every part of the earth, tissue of animals, soil, public water, oils and mining areas and atmosphere (Ali, 2008). These NORMs can be primordial that is, existed from the creation of earth and through human activities such as environmental pollution, use of fertilizer, pollutions industry and factories and it can occur naturally through climatic changes or global warming (Njinga *et al.*, 2015; Idoko *et al.*, 2016).

The role of NORMs in animal and plant metabolism have been established, but their effects and influences on administration of medicinal plants had received relatively little attention without due regard to its possible side effects. Meanwhile, mankind has continually used traditional herbal medicine for the treatment of various diseases and ailments (Okoli *et al.*, 2007; Odugbemi and Akinsulire, 2008; Oladipo *et al.*, 2012; Njinga *et al.*, 2015; Idoko *et al.*, 2016).

However, during the process of photosynthesis both the stable elements and the unstable ones (radioactive element) find their way into plants through natural processes and between the various environmental compartment into the ecosystem and food chain either through direct or indirect contamination (Elujoba *et al.*, 2005; Adewumi, 2011). This makes it possible for plants to absorb radionuclides during nutrient absorption from roots and transport along other nutrients via their phloem to their active site. The rate of natural radionuclides uptake is highly dependent on its activity concentration in the soil. The root uptake depends on soil properties for instance the pH, mineralogical composition, organic matter content and nutrient status as well as metabolic and physiological characteristics of the plant species (IAEA, 2006).

1.2 Statement of the Research Problem

There are safety concerns over the use of botanical and botanical derived products use as food and medicines (WHO, 2011). *Daucus carota* aerial part is used as components of livestock feed in some part of Northern Nigeria and there is little or no scientific evidence on the safety or otherwise of the aerial part as a component of livestock feed for animals or as vegetables for human consumption.

1.3 Justification of the Study

Carrots are good source of antioxidant that are known to inhibit or reduced cardiovascular diseases, xerophthalmia and other inflammatory diseases (Bao and Chang, 1994; Doymaz, 2004; Arscott and Tanumihardjo, 2010; Sri-sudewi *et al.*, 2014).

Toxicological and safe exploitation of botanical derived products with scientific evaluation is significant to determine the safety and efficacy of medicinal plants (Salawu *et al.*, 2009; Sridhar and Hammed 2014). In addition, toxicity in herbs and other indiscriminate activities of feeding livestock with all substances may have been responsible for many diseases and illness especially with morbidity and prevalent increase in cancers cases in developing nations (Boyle and Levin 2008; Ferlay *et al.*, 2010).

Studies have shown that utilization of wastes peels from vegetables, fruits, citrus and their substrates are potential source of antioxidant and antimicrobial agent (Torrado *et al.* (2011); Ana-Maria *et al.* (2011); Sonia, (2014); Nabila *et al.* (2015); Yousefbek *et al.* (2014); Feumba *et al.* (2016); Alsnafi, 2017). This waste plant material could be converted to wealth and other values added products as nutraceuticals and food additives (Hanem, 2012 and Michalina *et al.*, 2014). The study was carried out to establish the pharmacognostic, nutritional potentials and toxicity of *Daucus carota* aerial part towards its further utilization.

1.4 Aim and Objectives of the Research

1.4.1 Aim of the Research

The aim of this study is to standardize the aerial part of *Daucus carota* (L.) and to provide scientific basis for its utilization as livestock feed and as vegetable for human consumption.

1.4.2 Specific Objectives of the Research

The specific objectives are:

- 1) to establish pharmacognostic standards of the aerial part of *D. carota*
- 2) to establish the nutritional potentials or otherwise of *D. carota* aerial part; and
- 3) to determine the safety profile of the aerial part *D. carota*.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Geographical and Botanical Descriptions of *Daucus carota*

Daucus carota is a native of Europe and Asia continents. This plant is cultivated in all parts of the world in Africa, Australia, North and South America and also in Mediterranean region (Heywood *et al.*, 2008). It belongs to the family Apiaceae which is synonymous to Umbelliferae family and comprises of about 455 genera with over 3,500 species. It is one of the largest taxon among higher plants (Pimenov and Leonov, 2004).

Daucus genus comprises about 20 species including wild and cultivated species (Ahmed *et al.*, 2005; Amirhossein and Mehrdad 2010). *Daucus carota* is a biennial plant mainly herbs, it has alternate leaves with sheathing bases and usually hollow internodes. Some of the species may possess antioxidant, antimicrobial activity and could also be used as flavorings for alcoholic beverages (Heywood *et al.*, 2008; Iorizzo *et al.*, 2013). The common names of *Daucus carota* include Wild carrot, Bird's nest, Bishop's lace and Queen Anne's lace (Heywood *et al.*, 2008). In Nigeria, carrot is called *Karas* in Hausa; *Karotior Atokain* Yoruba and *Karotuin* Igbo among other names in Nigeria languages.

2.1.1 Taxonomical Classification of *Daucus carota*

Kingdom: Plantae

Division: Angiosperm

Order: Apiales

Families: Apiaceae

Genus: *Daucus*

Specie: *Daucus carota* L.

Common name: Carrot (Heywood *et al.*, 2008).



Plate I: *Daucus carota* aerial part in its natural habitat(FADAMA farmland)of ABU-Zaria, Kongo campus Kaduna State

2.2 Ethnomedicinal Uses of *Daucus carota*

Ethnomedicinal uses of *D. carota* vary from culture to culture around the world. All the part of the plants has been used traditionally in many countries. The roots and seeds have documented historical uses, typically to promote menstruation or as a diuretic (Moerman, 1998). A medicinal infusion of the dried flowers is used in the treatment of various ailments including digestive disorders, kidney and bladder diseases and also in the treatment of dropsy. It supports the liver, stimulates the flow of urine and the removal of waste by the kidneys. The infusions of the leaves have been used to counter cystitis and kidney stone formation and to diminish stones that have already formed. The leaves contain furocoumarins that may cause allergic contact dermatitis from the leaves, especially when wet and later exposure to the sun may cause mild photodermatitis (Barnes, 1998).

In India, decoction of the fresh root is taken orally for jaundice and inflammation, as an anthelmintic, and externally for leprosy. The hot water extract of the dried root is taken orally as a tonic, expectorant, diuretic, stomachic and liver cleanser. Hot water extract of the leaf is taken orally as a uterine stimulant during parturition, abortifacient, emmenagogue and aphrodisiac and the dried seeds are used as a powerful abortifacient (Barnes, 1998).

In Iran, Canary Island, Pakistan, Peru and Philippines, hot water extracts of the leaf and seed are taken orally as stimulants of the uterus during parturition and dried aerial part are taken orally as a carminative, emmenagogue and cystitis (Barnes, 1998; Ross, 2005). In Rodrigues Islands decoction of the entire plant is taken orally for gout, jaundice, and mouth ulcers (Ross, 2005). In Lebanon and China, the plant is used for the treatment of cancer, uterus, gastric ulcer, diabetes, muscle and back pain, and for enhancing liver function and immune system. In European folk medicine, the volatile oil from wild carrot is used as an antiseptic and anti-inflammatory remedy for cystitis and prostatitis. The dried leaf is taken orally for *Diabetes mellitus* and hot water extract of the root is taken orally as an emmenagogue and anthelmintic. Thw wild carrot was also

reported to possess diuretic, carminative properties, and has been used traditionally to treat urinary infection, cystitis, gout, and lithuria (Barnes, 1998; Rossi *et al.*, 2007). Carrot seed oil is used as cosmetics to fight wrinkles and to rejuvenate the skin. It is also used in perfumes and aromatherapy industries due to essential oil. However, the leaves of the wild carrot can cause phyto-photodermatitis and folk-medicine beliefs that an infusion of the seeds will inhibit pregnancy (Sivanantham and Thangaraj 2015).

2.3 Pharmacological and Nutritional Activities of Carrot

Carrot is a rich source of carotene like vitamin A, vitamin B₁, vitamin B₂, vitamin B₃, vitamin B₆, vitamin C, vitamin E, vitamin K and contains other minerals elements such as potassium, cobalt, iron, magnesium, copper, iodine and phosphorus. Among 39 fruits and vegetables, carrot is ranked 10th in nutritional value (Ensminger *et al.*, 1995; Acharya, 2008). The aerial part (leaf and stem) could be investigated towards considering the nutritional values and substitutes for the roots.

Carrot is a good source of dietary fiber and also a good source of magnesium, manganese which are needed for bone, protein, making new cells, activating vitamins B, relaxing nerves and muscles, clotting blood, and energy production as reported by Guerrero, (2009). It also help in insulin secretion and its function also require magnesium (Bartlett and Eperjesi, 2008; Kim *et al.*, 2010). Manganese is helpful in carbohydrate metabolism, in coordination with enzymes in the body (Dias, 2012 and Dias, 2012). It is used by the body as a co-factor for the antioxidant enzyme and superoxide dismutase. Potassium and magnesium in carrots help in the functioning of muscles. The therapeutic effect of these medicinal plants for the treatment of various illnesses are based on the chemical constituent such as essential oil, vitamins and glycosides present in them (Desideri *et al.*, 2009; Lordford *et al.*, 2013) which could be evaluated in the aerial part of carrot to suggest its therapeutic effect.

Although, mineral elements such as calcium, selenium, sodium, copper, iron and magnesium have been considered as essential as the active constituent which are responsible for the curative properties of the medicinal plants including antioxidant activities (Serfor-Armah *et al.*, 2003; Zhang and Hamauzu, 2004; Dias, 2012 and Dias, 2012), they show anti-carcinogenic activities, reduce inflammatory, play roles in metabolism and physiological functions and control immune response.

Zaini *et al* (2011) studied the anti-carcinogenic effect of carrot juice extracts on myeloid and lymphoid leukemia cell lines and it was observed that carrot juice extract possessed the ability to induce apoptosis and cause cell cycle arrest in leukemia cell lines, It was reported that β -carotene and falcarinol present in the carrot juice extract may be responsible for this beneficial effect of inhibiting the leukemia cells and prevent their progression. Purup *et al*(2009) also reported that extracts of carrot contain different amounts of falcarinol, falcarindiol, and falcarindiol 3-acetate had significant inhibit normal and cancer cell proliferation which aliphatic C-17-polyacetylenes was suggested as the potential anti-cancer principles in the carrots. Other studies have also reported that falcarinol exerts cytotoxic activity against several human tumor cell lines in many *in vitro* studies. They were able to destroy pre-cancerous cells in the tumors (Matsunaga *et al.*,1990; Saita *et al.*,1993). It was also reported that carrot intake lowers the risk of lung cancer among smokers or other susceptible persons. The curative effect of carotenoids, antioxidant properties and dietary fibers against bladder cancer and other carcinomas have also been reported by Hung *et al* (2006).

Ekam *et al*(2006) assessed the immuno-modulatory effect of carrot-extracted carotenoid and the percentage variation showed a significant increase in lymphocytes, eosinophils, monocytes and platelet which may be due to α - and β -carotenoids. Dias (2012) studied carrots in protecting the eye especially night vision and provides protection against macular degeneration and development of senile cataract, the leading cause of blindness among aged people was reported

to be β -carotene. Consumption of carrots rich in β -carotene may restore good eye sight and prevent blindness even among children and young ones.

Carrots also contain other vitamins such as vitamin C and K, thiamin (B_1), riboflavin (B_2), pyridoxine (B_6) and folates (B_9) necessary for metabolism of carbohydrates, proteins and healthy growth (Dias (2012); Dias (2012) and USDHHS, (2010) that increases red blood cells and fight against infection.

Vitamin C promotes the absorption of non-heme iron and is required for fighting infections and vitamin K helps to prevent bleeding. Thiamin (B_1) has a high beneficial effect on nervous system and mental attitude; riboflavin is also necessary for cell respiration, and red blood cell formation; pyridoxine inhibits the formation of homocysteine and reduces the risk of heart disease; and folates may reduce the risk of heart attack by lowering homocysteine levels. Vitamin C also protects against birth defects in babies (Dias, 2012).

Coyne *et al* (2005) reported a significant association between vitamin A-rich carotenoids and diabetes status and the findings suggested that carrot and vitamin A-rich carotenoids might help diabetic patients to manage their condition. Chau *et al* (2005) concluded that the dietary fiber of carrot might help control post-prandial serum glucose level and dietary fiber intake which can lower risk of type II diabetes (Dias, (2012) and Dias (2012).

Griep *et al* (2011) examined the associations between fruit and vegetables of different colours and their subgroups and 10-year coronary heart disease incidence. It was concluded that eating more deep-orange-coloured fruits and vegetables is associated with a lower risk of coronary heart diseases and that a higher intake of deep orange fruit and vegetables especially carrots, may protect the heart.

Gilani *et al* (2000) also studied the anti-hypertensive effect of two coumarin glycosides (DC-2 and DC-3) from carrots. There was significant decrease in blood pressure which may be due to

the calcium channel blocking action of coumarin glycosides (DC-2 and DC-3) from carrots as reported by Silva-Dias (2014).

Mital *et al*(2011) studied the reno-protective activity of carrot root extract on renal ischemia reperfusion acute injury in rats and a significant increase in malondialdehyde level. The study revealed that carrot extract exerts reno-protective activity against ischemia reperfusion- induced kidney acute injury, by reducing free radical scavenging activity, one of the mechanisms behind ischemia reperfusion damage of kidneys. Muralidharan *et al*(2008) observed that carrot seed extract offers cardio protection and muscle contraction regulation in isoproterenol-induced myocardial infarction in rats by maintaining membrane bound enzymes. Carrot seed extracts have anti-inflammatory and analgesic effects and it was observed that the diseases condition and pain decreased in rats (Vasudevan *et al.*, 2006). Moreover, in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, luteolin showed greater radical scavenging activity (Silva- Dias, 2014) and Sri-sudewi (2014) also reported greater scavenging activity in the hexane extracts of *D. carota* aerial part qualitatively using DPPH assay.

Patil *et al*(2012) reported that animals treated with topical cream of ethanolic extract of carrot root, formulated at different concentrations, showed significant decreases in wound area, epithelization period and scar width when compared to control group animals in an excision wound model. Carrot seed extracts and its essential oil have been reported in many experimental studies to have cardio- and hepatoprotective, cognitive dysfunction, cholesterol lowering, anti-bacterial, anti-fungal, anti-inflammatory, analgesic, and wound healing benefits. Its wound healing activity and other pharmacological activity can also be carried out on the aerial part to assess its full utilization and substitute as bioprospecting.

Singh *et al*(2010) reported the hypolipidemic activity of carrot seeds in rats. It was observed that rats fed with carrot seeds showed a reduction in the total cholesterol and triglyceride HDL and VLDL as compared with the control group of rats.

Interestingly, Silva-Dias (2014) reported that carrots when consumed could improve cognitive skills which include all aspects of learning, intelligence and aid memory retention. Since, cognitive impairment is the leading cause of neurodegenerative diseases such as Alzheimer's disease, mental confusion and disturbance of thought and language among elderly individuals. It is usually accompanied with personality defects, brain disorder, hearing and speech impairments (Vasudevan and Parle, 2006) which might be helpful in the management of forgetfulness. Vasudevan *et al* (2006) showed that carrot seed extract reversed the memory deficits in scopolamine (or diazepam)-induced amnesia in young mice and ethanolic extract of carrot seeds improved the retention capacity of aged mice, when administered orally for 7 days.

Rossi *et al* (2007) reported that the essential oil obtained from aerial part of the wild carrot showed inhibitory action against entero-pathogen bacterial such as *Campylobacter jejuni*. Also, phenyl-propanoids, such as Methylisoeugenol and Elemicin, from essential oil also inhibit microbial effect on *Campylobacter coli* and *C. lari* strains and it was concluded that an aromatic ring with a double bond on the side chain of both methyl-Isoeugenol and elemicin might be responsible for the anti-microbial effects. Misiaka *et al* (2004) reported that carrot seed oil extracts exhibited moderate inhibitory effects on fungi growth, the action of carotol, β -caryophyllene, and daucol could be suspected as the active constituents responsible for the anti-fungal activity of carrot seed oil extracts. Kumarasamy *et al* (2005) carried out anti-bacterial assay using the compounds obtained from methanolic extract of wild carrot seeds. Luteolin, luteolin 3'-O- β -D-glucopyranoside, and luteolin 4'-O- β -glucopyranoside are the major flavones isolated from methanolic extract.

2.4 Economic Importance of *Daucus carota*

Carrot is a very important vegetable crop for its contribution to human health (Ensminger *et al.*, 1995). In KwaZulu-Natal, South Africa, because of the seed and root utilizations is probably one of the five most important vegetable crops grown (Sun *et al.*, 2009; Shelly, 2012). *Daucus carota*

is one of the most important vegetables grown in the world and the production has increased to over 33.7 million tons from the area of 1.16 million ha in 2010 (FAO, 2010). In global market, China is the largest carrot producing countries and currently producing about one-third of all carrots bought and sold worldwide while Russia is the second largest carrot producing and U.S. following a close third carrot producing nation.

Many European countries produce substantial amounts of carrots of over 400,000 metric tons. In addition, nations such as Mexico, India, Indonesia, Australia, Canada South Africa and Nigeria are also important countries in the worldwide production of carrots (US-FDA, 2000; FAO, 2005). Due to its abundance and diversities in many countries, its demand and utilization is universal. However, most of the carrots roots produced are eaten within the countries of production and almost ninety percent of the aerial part is underutilized thereby resulting to waste (FAO, 2010).

2.5 Some Compounds Isolated from *D. carota* Root and Leaves

Daucus carota was reported to contain carotenoid (α -carotene, β - carotene, γ - carotene, lycopene, lutein, kriptoxantin) and phenolic compounds (phenylpropanoid, eugenol, miricetin) (Ross, 2005), vitamin C (Kumar *et al.*, 2004), polyacetylenes (Baranska and Schulz, 2005; Purup *et al.*, 2009); flavonoid (quercetin, rutin, luteolin) (Muralidharan *et al.*, 2008). These flavonoids, phenolic compounds and vitamin C are reported to have antioxidant because they can donate hydrogen/electron to stabilize radical species (Lopez-lazaro, 2009) which could bind singlet oxygen or deactivate oxygen radical.

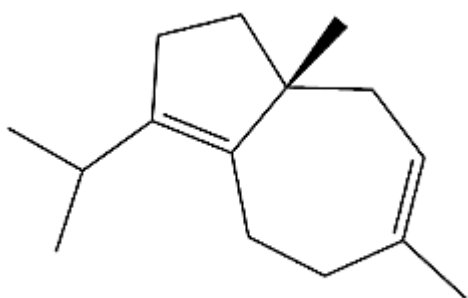
However, Hichem *et al* (2015); Dalvir *et al* (2016) reported potential sources of high value compound including essential oils in different part of *D. carota*. Carotol was reported in the seeds, leaves, stem, flowering umbels and the root (Ozcan and Chalchat 2007; Ram *et al.*, 2013; Smigielski *et al.*, 2014). Daucene (a) , myrcene and limonene (b)were reported in the leaves,

seeds, fruits, flowers, roots and stem (Ozcan and Chalchat 2007; Sokovic *et al.*, 2009; Ram *et al.*, 2013; Meliani *et al.*, 2013 and HaFlamini *et al.*, 2014) which could be explored as antimicrobial agents. Sabinene and α -pinene have also been reported in the ripe and unripe fruits, leaves, seeds, blooming umbels and stem (Marzouki *et al.*, 2010; Ram *et al.*, 2013; Meliani *et al.*, 2013; Smigielski *et al.*, 2014).

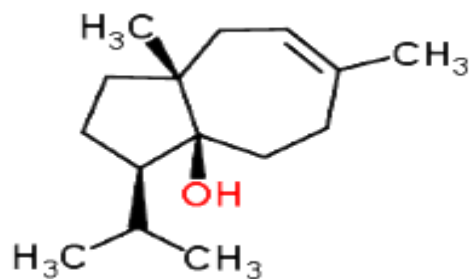
Sri-sudewiet *al*(2014) also reported antioxidant activities from the leaves of *D. carota* and lutein (c) was isolated as the compound responsible for the antioxidant activities. Carrots have also been reported to have unique combination of three flavonoids including kaempferol, quercetin (d) and luteolin (e) (Ching and Mohamed, 2001; Lila, 2004). Carrots are also rich in other phenolic compounds including chlorogenic, caffeic and p-hydroxybenzoic acids along with numerous cinnamic acid derivatives. Other hydroxycinnamic acid and its derivatives contain chlorogenic acid were presented in total phenolic compounds detected in different carrot tissues (Zhang and Hamauzu 2004). Daucoside and Daucosol which are mainly sesquiterpenoids have been isolated from the carrot seeds and reported to have cytotoxic effect against human gastric cell lines (Ahmed *et al.*, 2005; Fu, 2010).

Falcarinol and falcarindiol were also found in carrots which are bioactive polyacetylenes. This compound may stimulate cancer-fighting mechanisms in the human body (Lund and White, 1990) and might stop proliferation of the cancer cells. The presence of coumarins such as bitter coumarin compound has also been demonstrated in various biochemical analyses in carrots (Czepa, and Hofmann, 2003). Other compounds such as sesquiterpene, pyrrolidine (present in the leaves), 6-hydroxymellein, 6-methoxymellein, eugenin, 2, 4, 5-trimethoxybenzaldehyde (gazarin) or (Z)-3-acetoxy-heptadeca-1,9-diene-4,6-diin-8-ol (falcarindiol 3-acetate) can also be found in carrot and responsible for various biochemical pathways (Lund and White, 1990).

The structures of some compounds isolated in the *D. carota* are shown in plate 2.1

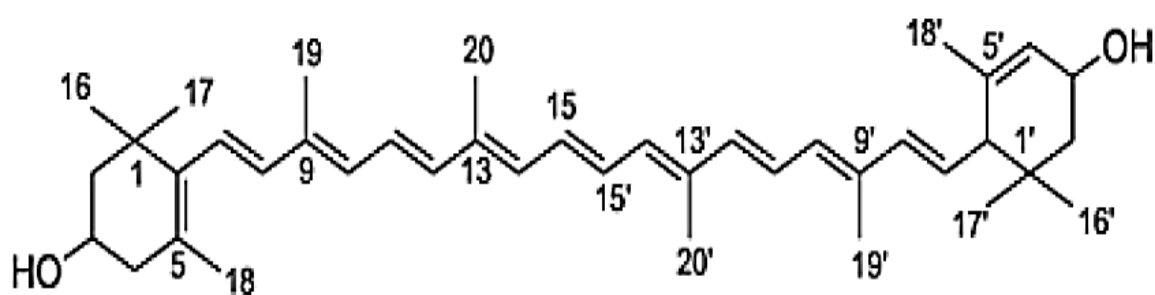


(a) **Daucene**

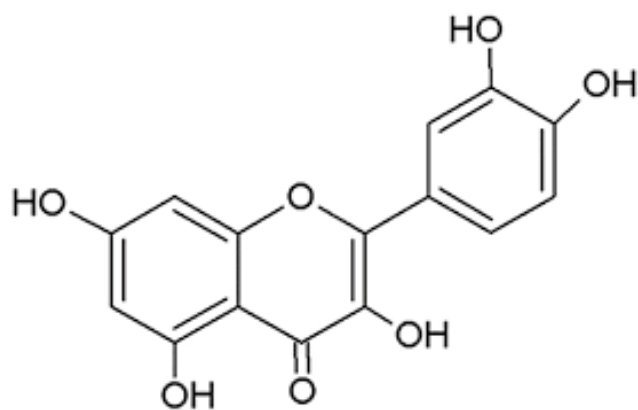


(b) **Limonene**

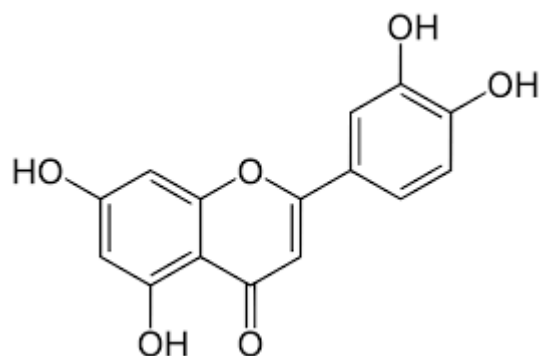
(Ram *et al.*, 2013; Meliani *et al.*, 2013; HaFlamini *et al.*, 2014)



(c) **Lutein**(Sudewi, 2014)



(d) **Quercetin**(Lila, 2004)



(e) **Luteolin** (Ching and Mohamed, 2001)

Plate 2.1: Structures of some compounds isolated from *Daucus carota*

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and Solvents

Chemicals and reagents used included n-hexane, ethylacetate, methanol, glacial acetic acid, diethyl ether, distilled water, de-ionized water, perchloric acid (HClO_4), magnesium chloride (MgCl_2), sodium hydroxide (NaOH), ferrous sulphate (FeSO_4), nitric acid (HNO_3), hydrochloric acid (HCl), acetic acid, phloroglucinol, zinc chloride, iodine solution, sudan red, potassium hydroxide, sulphuric acid (H_2SO_4), benzene, chloroform, ferric chloride, formalin, chloroform, glycerol, Mayer's reagent, Wagner's reagent, Dragendorff's reagent, acetic anhydride, Molisch reagent, Fehling solutions A and B, ammonia, bleaching agents (Sodium hypochlorite) and barium sulphate, all were of high standard analytical grade. The spraying reagents were prepared according to the standard operating practices (SOP) and it include, *p-anisaldehyde* general spray, Liberman- buchard reagent, Dragendorff's spray, Ferric chloride spray; Aluminum chloride spray and Bontrager's spray.

3.1.2 List of Equipment and Apparatus

Light Microscope (Rating: 85,65 V), glass slides, cover slips, camera lucida, Oven (Lightwater, Surrey, GU185TA, UK), analytical weighing balanced (Santorius ED-2245), TLC (pre-coated silica on aluminum plates), Silica gel for column (60-200 mesh) from Merck (Germany), maceration flask, Erlenmeyer's flask, fume cupboard, glass rod, separating funnel, rotary evaporator (Rota-vapor R110, BUTCH MKF6540/2), clamp and retort stand, crucibles, metal scrappers, water bath (HH-S: Digital Thermostatic water bath), desiccators, syringes and needles, razor blades, ruler, measuring cylinder, non-heparinized hematocrit capillary tubes, TLC tank, photographic camera, funnels, beakers, aluminum foils, conical flask, UV-visible (CAMAG; Type: 29000; Ger No.: 850459, UV spectrophotometer (Jen-way 6405), test tubes, cotton wool,

vaseline, candlewax, masking tape, binder, masking tapes, sample container, hydraulic machine, stage micrometer and ocular lens (Graticules LTD, Ton Bridge, Kent, England), flask shakers (Gallenkamp, App No: 6B8872E, made in England), WhatMan No 1 filter paper, metallic cages and feeding bottles for laboratory animals, disposable syringes (1mL, 5mL and 10 mL respectively), Atomic Emission Spectrophotometer (MP-AES) from Agilent Technologies Atomic Absorption Spectro-photometer (AAnalyst700, Perkin Elmer), HACH Spectrophotometry (DR/4200), Gamma Ray Spectrophotometer with NaI radioactive detector (Canberra, ModelNo:727-ORTEC MAESTRO-32), Proportional counter (Protean Instrument Corporation, Model No: MPC2000DP). Some of the listed equipment sourced were from Multiuser Laboratory, Department of Chemistry and Centre for Energy Research, Ahmadu Bello University Zaria, Nigeria.

3.2 Methods

3.2.1 Plant Collection, Preparation and Identification

The aerial part of *Daucus carota* was sourced from cultivated farmland at the outskirts of Samaru with coordinates 11° 10' 0" N and 7° 37' 60" E, Sabon Gari Local Government Area of Kaduna State in November to December 2016. The whole plant of *D. carota* (root inclusive) was identified and authenticated by Mallam Namadi Sanusi, a Taxonomist in the Herbarium Unit of the Department of Botany, Ahmadu Bello University Zaria, Nigeria and voucher specimen with number (12034) was deposited there for future references. The aerial part of *D. carota* was collected in large quantities, air dried under shade at room temperature, pulverized and stored in an air tight container for further use. The fresh leaves of the plant were collected and used for the microscopic studies.

3.2.2 Microscopic Studies of *Daucus carota* Leaf

The fresh leaves of *D. carota* plant was cut into section and mounted in dilute glycerol and observed under the light microscope using x40 objective lens as described by Evans (2009).

3.2.3 Quantitative Leaf Microscopy of *Daucus carota* Leaf

This involves counting the specific histological features of the *D. carota* leaf namely the stomata number, stomata index, palisade ratio, veinlet termination and vein-islet number.

3.2.3.1 Stomatal Number

This is the average number of stomatal per square millimetre of the upper and lower epidermis of the leaves of the plant. Section from the upper and lower epidermis of the plant was cleared by boiling in 70 % sodium hypochlorite solution and mounted on a clean microscope slide with dilute glycerol. The stomatal was photomicrographed and counted in the fields on a single section of the leaf of the plant and the average number of stomata per mm² of epidermis was recorded (Evans, 2009).

3.2.3.2 Stomatal Index

This is the percentage proportion of the number of stomatal form to the total number of epidermal cells of the leaf. Section of the epidermal portion of the leaf was mounted and examined base on the stomatal number counted, except that both stomatal and epidermal cells was counted. The stomatal index was calculated in accordance with Evans (2009).

$$\text{Stomatal Index} = \frac{\text{stomata number}}{\text{number of Epidermal cells} + \text{No. of stomata}} \times 100$$

3.2.3.3 Vein-islet Number

Vein-islet number is the number of vein-islet per unit square millimeter calculated from four contiguous square millimetres in the central part of the lamina midway between the midrib and the margin. It was determined by boiling pieces of leaf of the plants in a test-tube containing 70% sodium hypochlorite solution. It was then treated with 10% hydrochloric acid to remove calcium

oxalate crystals to enhance visibility. A camera lucida was used by means of a stage micrometer; the paper was divided into squares of 1 mm² using x10 objective lens. The veins were traced in four contiguous squares that is a rectangle (1mm x 4mm) and photomicrograph Evans (2009).

3.2.3.4 Vein-let Termination Number

It is the vein termination present in one square millimeter of leaf surface midway between midrib and margin. It was determined for *D. carota* leaves using camera Lucida set up as in vein-islet number but here the termination number in each square was counted (Evans, 2009).

3.2.3.5 Palisade Ratio

It is the average number of palisade cells beneath each epidermal cell of leaf. A section from the upper epidermis of the leaf was cleared with boiling in 70% chloral hydrate solution and mounted on a clean microscope slide with dilute glycerol and examined with x40 objective. A camera lucida was set up and the palisade ratio determined in groups of five and the average was recorded (Evans, 2009).

3.2.4 Chemomicroscopic Examination of *Daucus carota* Aerial Part

The histo-chemical evaluations of *Daucus carota* aerial part were carried out to observe the presence/ absence of cell walls materials and cell inclusions. The methods as described in WHO (2011) guidelines on quality control methods for medicinal plant materials were used.

3.2.4.1 Cell wall Materials

a) Cellulose cell walls: Two drops of iodinated zinc chloride was added to the powdered leaf sample and allowed to stand for a few minutes. It was then observed under the microscope. The presence of blue stain indicates cellulose cell wall.

b) Lignified cell walls: The powdered leaf material was moistened on a glass slide with two drops of phloroglucinol and allowed to stand for about two minutes or until almost dry. A drop of

concentrated hydrochloric acid was added and view under the microscope. The presence of pink or cherry red colour indicates the presence of lignin.

c) Suberin or cuticular cell walls: Two drops of sudan red was added to the powdered leaf sample and allowed to stand for a few minutes and observed under the microscope. The presence of orange red colour indicates the presence of suberins and cutins.

3.2.4.2 Cell Contents/Cell inclusions

a) Calcium carbonate deposit: The cleared sample was placed on a slide, covered with a cover slip was and observed under the microscope. The appearance of effervescences on addition of concentrated hydrochloric acid to the powdered leaf on the glass slide indicates the presence of calcium carbonate.

b) Calcium *oxalate crystals*: Two drops of hydrochloric acid was added to the powdered leaf sample on a glass slide and viewed under the microscope. There was no dissolution of crystals on the anatomical sections of the leaves indicates its absence.

c) Gums and Mucilage: A drop of ruthenium red was added to the dry powdered leaf on glass slide and viewed under the microscope. Mucilage showed up as transparent, spherically dilated fragments on a black background.

d) Starch: Two drops of iodine was added to the powdered leaf sample and viewed under the microscope. A blue-black colouration indicates the presence of starch.

e) Tannins: A drop of conc. ferric chloride was added to the powdered leaf sample and viewed under the microscope. The formation of greenish black colouration indicates the presence of tannins.

3.2.5 Fluorescence Analyses of *Daucus carota* Aerial Part

The *D. carota* aerial part powdered were observed for fluorescence characteristic using chemical and non-chemical treatment under UV-light. The colours were observed in day light and under ultra-violet light between 254 nm and 365 nm (Shivani *et al.*,2013).

3.2.6 Determination of Physico-chemical Constants of *Daucus carota* Aerial Part

Moisture content (loss on drying), total ash, water soluble ash and acid insoluble ash values, extractive values (water and alcohol) of the plant sample was determined as described in World Health Organization guidelines on methods of evaluation of crude drugs(WHO, 2011).

3.2.6.1 Determination of Moisture Content

The moisture content was determined by “Loss on drying” method (gravimetric determination). Three grammes of the air-dried leaves was weighed using KERN EW Electronic Balanced and in a dried and weighed crucible. The crucible was transferred into a hot air sterilizing oven at 105⁰C. After an hour, the crucible was removed and placed in a desiccator over phosphorous penta-oxide and under atmospheric pressure and at room temperature. After 30 minutes in the desiccator, the weight of the powder and crucible were determined and the crucible returned to oven. The heating and weighing was repeated until a constant weight was obtained and recorded (WHO, 2011).Five determinations were carried out and the average taken as the moisture content of the drugs and the standard deviation was calculated. The moisture content (loss of weight) was calculated using the following formula:

$$\% \text{ Moisture content} = \frac{\text{Initial Weight of Powder} - \text{Final Weight of Powder}}{\text{Initial Weight of Powder}} \times 100$$

3.2.6.2 Determination Total Ash Value

A platinum crucible was heated red hot, cooled in a desiccator and immediately weighed to know the initial weight of the crucible. The air-dried leaf powder (2g) was weighed into the previously

heated crucible. It was ignited gradually and the heat increased, until it turned white. The residue was cooled in a desiccator and weighed (WHO, 2011). The procedure was repeated in five determinations to obtain average value and the standard deviation was calculated. The total ash content of the air-dried powder was calculated in percentage, using the following formula:

$$\% \text{ Ash value} = \frac{\text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \times 100$$

3.2.6.3 Determination of Acid-insoluble Ash

To the crucible containing the total ash, 25mL of dilute hydrochloric acid was added and covered with a watch glass and then boiled gently for 5 minutes. 5mL of hot water was used to rinse the cover glass into the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. This was transferred into the crucible and dried on a hot plate and ignited to a constant weight and then ignited. The residue was allowed to cool in a desiccator for 30 minutes and immediately weighed (WHO, 2011). The procedure was repeated in five determinations to obtain average value and the standard deviation was calculated. The acid insoluble ash was calculated as follows:

$$\% \text{ Acid insoluble Ash} = \frac{\text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \times 100$$

3.2.6.4 Determination of Water Soluble Ash

To the crucible containing the total ash, 25mL of water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered glass crucible. It was then washed with hot water and ignited in a crucible for 15 minutes at 105°C. The weight of the residue was subtracted from the weight of the total ash (WHO, 2011). The procedure was repeated in five determinations to obtain average value and the standard deviation was calculated. The water soluble ash of air dried powder was calculated using the following formula:

$$\% \text{ Water Soluble Ash} = \frac{\text{Weight of Total Ash} - \text{Weight of Residual Ash}}{\text{Original Weight of Powder}} \times 100$$

3.3 Extractive Values of *Daucus carota* Aerial Part

3.3.1 Water Extractives Value of *Daucus carota* Aerial Part

The air-dried leaf powder (4g) was weighed into a 250 mL glass stoppered conical flask and 100 mL of chloroform water was added to macerate the powder for 6 hours with frequent shaking using flask shaker and allowed to stand for 18 hours. It was then filtered rapidly and 25 mL of filtrate was measured and transferred into a previously dried and weighed evaporating dish. It was evaporated to dryness on a hot water bath. This was further dried in the oven at 105°C for 6 hours, cooled in a desiccator for 30 minutes and was weighed immediately (WHO, 2011). The procedure was repeated in five determinations to obtain average value and the standard deviation was calculated. The percentage water extractive value was calculated using the following formula:

$$\% \text{ Water Extractive Value} = \frac{\text{Weight of Extract in 25ml}}{\text{original Weight of Powder}} \times 100$$

3.3.2 Alcohol Extractives Value of *Daucus carota* Aerial Part

The air-dried leaf powder (4g) was weighed into a 250 mL glass stoppered conical flask and 100 mL of ethanol was added to macerate the powder for 6 hours with frequent shaking by using flask shaker and was allowed to stand for 18 hours. It was then filtered rapidly and 25 mL of filtrate was measured and transferred into a previously dried and weighed evaporating dish and evaporated to dryness on a hot water bath. This was further dried in the oven at 105°C for 6 hours, cooled in a desiccator for 30 minutes and was immediately weighed (WHO, 2011). The procedure was repeated in five determinations to obtain average value and the standard deviation was calculated. The percentage water extractive value was calculated using the following formula:

$$\% \text{ Alcohol Extractive Value} = \frac{\text{Weight of Extract in } 25\text{ml}}{\text{original Weight of Powder}} \times 100$$

3.4 Extraction Procedure of *Daucus carota* Aerial Part

The powdered air-dried aerial part (1.2 kg) was extracted successively using n-hexane, ethylacetate and methanol with cold solvent maceration technique. It was macerated for 72 hours with intermittent shaking using flask shaker (Evans, 2009). The extracts obtained were evaporated to dryness and stored in a desiccator for further use. The successive extraction flow procedure is in Fig. 3.1

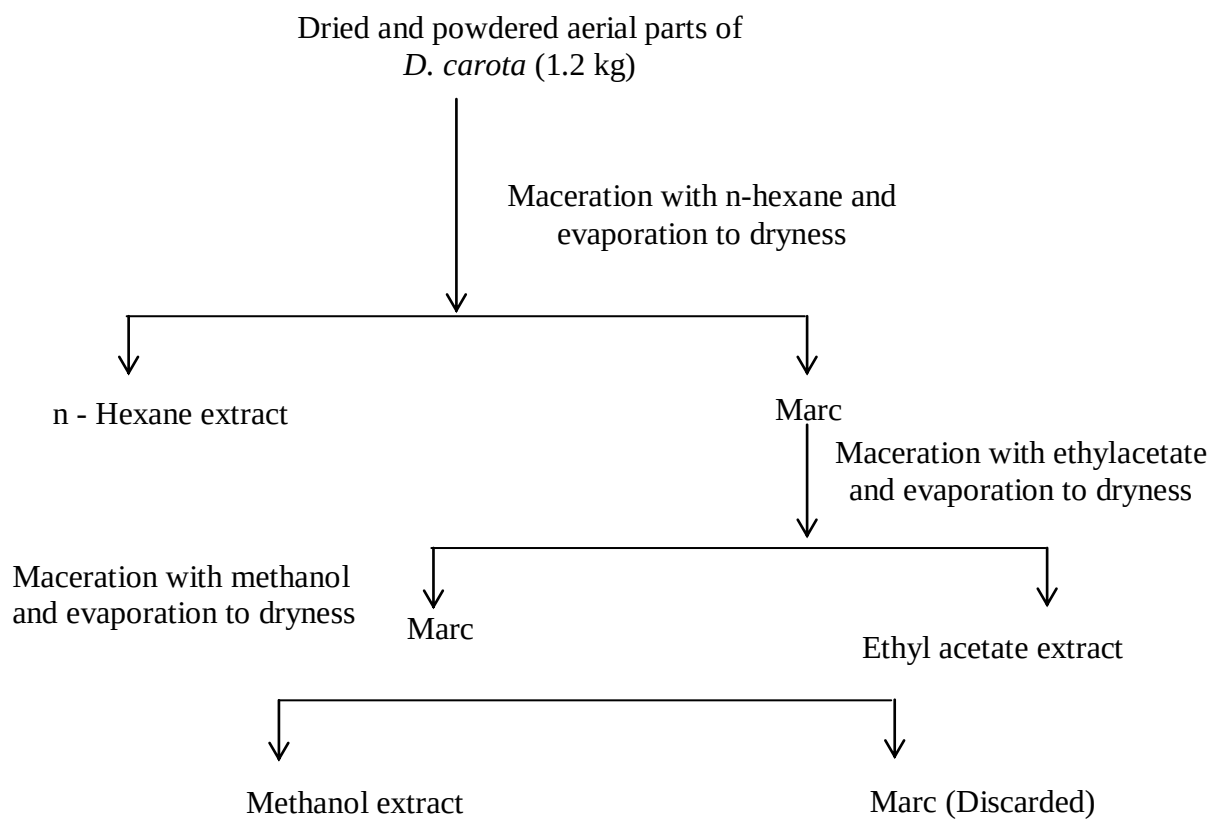


Fig. 3.1: Successive Extraction of *Daucus carota* Aerial Part adopted from Evans (2009)

3.5 Preliminary Phytochemical Screening of *Daucus carota* Aerial Part

The n-hexane, ethyl acetate and methanol extracts of *Daucus carota* aerial part were subjected to preliminary phytochemical screening using standard methods, as described by Sofowora (1993); Ashutosh (2007); Harborne (2009) and Evans (2009).

3.5.1 Test for Saponins

- i. Frothing Test: About 0.5 g of the extracts was shaken with water in a test tube followed by warming on a water bath. Frothing which persists on warming after 5 minutes was taken as an evidence for the presence of saponins (Sofowora, 1993).
- ii. Haemolysis Test: 2 mL of sodium chloride (1.8% solution in distilled water) was added to two test tubes A and B. Then 2 mL of distilled water was added to test tube A, 2 mL of the extract was added to test tube B. 5 drops of blood was added to each tube and the tubes were inverted gently to mix the contents. Haemolysis in tube B containing the extract but not in tube A (i.e. control), indicated the presence of saponins in the extract (Brain and Turner, 1975).

3.5.2 Test for Steroids/Triterpenes

Lieberman-Buchard's Test: 2 mL of acetic anhydride was added to 0.5 g of the hexane extract dissolved in 2 mL of chloroform. 1 mL of sulphuric acid was then added gently by the side of the test tube to form lower layer and at the junction of the two liquids formation of reddish brown or violet brown ring, the upper layer bluish green or violet indicated the presence of sterols and/or triterpenes. The procedure above was repeated for ethyl acetate and methanol extracts (Evans, 2009).

Salkowski's Test: 2 mL of chloroform was added to 0.5 g of the hexane extract and 1 mL of sulphuric acid was added carefully from the side of the test tube to form a lower layer. The

formation of reddish brown coloration at the interface indicates the presence of steroidal ring. The procedure above was repeated for ethyl acetate and methanol extracts (Sofowora, 1993).

3.5.3 Test for Flavonoids

- i. Shinoda's Test: 0.5 g of the hexane extract was dissolved in 5 mL of 50% methanol. 2 mg of magnesium filings and 3 drops of hydrochloric acid were added and a pink coloration indicated the presence of flavonoids. The procedure above was repeated for ethyl acetate and methanol extracts (Evans, 2009).
- ii. Sodium hydroxide Test: 0.5 g of the hexane extract was dissolved in water and filtered; 2mL of 10% aqueous sodium hydroxide solution was then added. The presence of yellow colour, a change in colour from yellow to colourless on addition of dilute hydrochloric acid was used as an indication for the presence of flavonoids. The procedure above was repeated for ethyl acetate and methanol extracts (Evans, 2009).

3.5.3 Test for Tannins

- i. Ferric chloride Test: The extracts (0.5g) was stirred with 10 mL distilled water and filtered. Two drops of 1% ferric chloride solution was added to 2 mL of the filtrate. Formation of a blue-black colouration indicated the presence of tannins (Evans, 2009). The procedure above was repeated for ethyl acetate and methanol extracts.
- ii. Lead sub-acetate Test: To a small quantity of the extracts, three drops of lead sub-acetate solution was added. The solution observed the presence of creamy precipitate which confirmed the presence of tannins (Evans, 2009). The procedure above was repeated for ethyl acetate and methanol extracts.

3.5.5 Test for Alkaloids

The extract (0.5g) was stirred in 5 mL of 1% aqueous hydrochloric acid (v/v) on a water bath and filtered. Ammonia solution was added to the filtrate until it became basic, then chloroform was added gently shaken to allow separation. The chloroform layer was collected and dilute HCL was added and gently shaken to allow separation and the aqueous layer was divided into three portions. To the first portion, few drops of freshly prepared Dragendorff reagent was added and observed for formation of orange to brownish precipitates. To the second, one drop of Mayer's reagent was added and observed for formation of white to yellowish or cream color precipitates. To the third portion, 1mL of Wagner's reagent was added. The formation of reddish brown precipitates indicates the presence of alkaloids (Evans, 2009). The procedure above was repeated for ethyl acetate and methanol extracts.

3.5.6 Test for Anthraquinone

Borntrager's Test: The extracts (0.5g) with 10 mL of chloroform were shaken, the content was filtered and 5 mL of 10% ammonia solution was added to the filtrate, the mixture was shaken. Pink or slight red colour in the upper part of the aqueous layer indicated the presence of free anthraquinone (Evans, 2009). The procedure above was repeated for ethyl acetate and methanol extracts.

Modified Borntrager's Test: The extracts were boiled with 10 mL of dilute sulphuric acid and filtered hot. The filtrate after cooling to room temperature was shaken with 5 mL chloroform. The chloroform layer was separated and to half of its volume, 10% ammonium hydroxide was added. The formation of a pink coloration in the ammonia at the lower phase indicates the presence of combined anthraquinone or anthraquinone

derivatives (Evans, 2009). The procedure above was repeated for ethyl acetate and methanol extracts.

3.5.7 Test for Cardiac Glycosides

- i. Keller-Kiliani's Test: The extract (0.5g) was dissolved in 2 mL of glacial acetic acid containing one drop of ferric chloride solution. It was under layered with 1 mL of concentrated sulphuric acid. Formulation of a brown ring colouration at the interphase indicated the presence of deoxy sugar which is characteristic of cardenolides (Evans, 2009). The procedure above was repeated for ethyl acetate and methanol extracts.
- ii. Kedde's Test: Few drops of 2% solution of 3, 5- dinitrobenzoic acid in 95% alcohol was added to small portion of filtrate. Formation of a solution of 5% sodium hydroxide also added to make the solution alkaline appearance. The presence of purple-blue colour indicated the presence of cardenolides and the absence confirmed absence of cardenolides (Evans 2009). The procedure above was repeated for ethyl acetate and methanol extracts

3.6 Thin Layer Chromatography profiles of the *Daucus carota* aerial part extracts

The pre-coated TLC plate (Silica Gel 60 F₂₅₄ Merck, Germany) of 0.25mm thickness was used. The spotting of the extracts was done manually using non-heparinized micro-hematocrit capillary tubes. The thin layer chromatographic aluminum plates were developed at room temperature using ascending technique order inside a saturated chromatographic tank and the best solvent system was determined after several trial and errors. The chromatograms were allowed dried and then observed in iodine tank before spraying with *P- anisaldehyde* as general spraying agent and placed in the oven at 120°C for 2 minutes (Stahl, 2005). The best solvent systems were developed after several trials and errors by combination of different solvents with different polarities. The

solvent systems that give reasonable components of the extracts were documented. The n-hexane extract, was resolved using solvent mixture of hexane: ethyl acetate (9:1); the ethyl acetate extract using hexane: ethyl acetate (5:5) and the methanol extract using Butanol: Acetic acid: Water (8:1:1). The R_f value of each spot was calculated.

$$R_f = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

The chromatograms were photomicrograph and reported as plates.

3.7 Nutraceutical Potentialsof the *Daucus carota* Aerial part

Acid Digestion of the samples: *Daucus carota* aerial part(0.5g) was weighed into 10 different beakers each of 50 mL capacity. 6mL of a mixture of Perchloric Acid (HClO_4) and Nitric Acid (HNO_3) in 1:5 ratio was added to each beaker. The beakers were placed in an open space for 2 hours and a mixture of hydrochloric acid (HCL) and nitric acid (HNO_3) in the 1:1 ratio was added to each beaker. These were kept on a hot plate at 300°C for 4 hours. After the contents in beakers were dried, 5mL of Hydrochloric acid (HCL) was added to each beaker and was kept on the hot plate until the entire liquid content in the beakers were evaporated. De-ionized water (5 mL) was added to each beaker and the solutions were transferred into sterile bottles and used for the quantification of the metals.

The concentration of Fe, Mg, Zn, Cu, K and Na was read using the flame atomic absorption spectrophotometer (FAAS), AA 500 model. The HACH Spectrophotometry (DR/4200) was used for determination of phosphorus and Atomic Emission Spectrophotometer (MP-AES) was used for determination of other elements.

The elemental analyses of the aerial part were carried out in Multiuser Research Laboratory, Department of Chemistry, Ahmadu Bello University Zaria, Nigeria. The mineral elements estimations indicated the amount of macro, trace elements and heavy metals present in the *D.*

carota aerial part. The mineral elements analyzed include; Zinc (Zn), Magnesium (Mg), Lead (Pb), Manganese (Mn), Selenium (Se), Copper (Cu), Potassium (K), Sodium (Na), Iron (Fe), Cadmium (Cd), Phosphorus (P), Calcium (Ca), Arsenic and Nickel. Before determining the concentration of any element in the sample, calibration curve of the element in the sample was prepared using prepared standard stock solutions for the elements as reported by AOAC, (2000; 2005); Akpabio, and Ikpe (2013).

3.7.1 Quantitative Proximate Analysis of *Daucus carota* Aerial Part

The proximate analysis of the aerial part was carried out to know the proportion of carbohydrates, fats (lipid), crude proteins, moisture and ash content using the method of AOAC (2005). Percentage was used for results presentation and all samples were replicated three times for statistical analysis.

3.7.1.1 Determination of Moisture content

Aluminum dishes were washed and dried to a constant weight in an oven at 100 °C. They were later removed and cooled in a desiccator and weighed (W_1). *D. carota* aerial sample of (2 g) was placed in the weighed moisture dish (W_2). The dish containing the sample was kept in an oven for about 3 hours. The samples were removed and cooled in the desiccator and weighed (W_3) (AOAC, 2005). The percentage of moisture was calculated and three determinations were recorded using this formula:

$$\text{Percentage of moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

3.7.1.2 Determination of Ash content

Crucibles were cleaned and dried in the oven; they were cooled in the desiccator and weighed (W_1). The ground *D. carota* samples (2g) were placed in the crucibles and weighed (W_2). They were transferred into the Muffle Furnace for about 550 °C, then removed and cooled in the desiccator and weighed (W_3) (AOAC, 2005). The percentage ash was calculated and three determinations were recorded (using the formula:

The percentage of ash was calculated as: $\frac{W_2 - W_3}{W_2 - W_1} \times 100$

3.7.1.3 Determination of Crude Fibre

The sample (2g) was placed in a beaker containing 1.2 mL of H₂SO₄ per 100 mL of solution and boiled for about 30 minutes. The residue was filtered then washed with hot water and was transferred to a beaker containing 1.2 gram of NaOH per 100 mL of solution. This was boiled for about 30 minutes. The residue was washed with hot water and dried in an oven and weighed (C₂). The sample was incinerated in a Furnace at about 550 °C. It was then removed and allowed to cool, and weighed (C₃)(AOAC, 2005). The percentage ash was calculated and three determinations were recorded

The % of Fibre was calculated as: $\frac{C_2 - C_3}{W} \times 100$

3.7.1.4 Determination of Lipids (Fats)

Boiling flask (250 mL) was cleaned and oven dried. It was transferred into a desiccator and allowed to cool. A filter paper was weighed using analytical weighing balance and labelled (W₁). The ground *D. carota* sample (2g) was weighed and labelled in filter paper (W₂). It was transferred into a boiling flask and filled with petroleum ether (boiling range 40 – 60 °C) where it was refluxed for 8 hours in a Soxhlet apparatus. It was removed, dried in an oven and allowed to cool in a desiccator and then weighed (W₃)(AOAC, 2005). The percentage of fat was calculated and three determinations were recorded

The % of Fat was calculated as: $\frac{W_2 - W_3}{W_2 - W_1} \times 100$

3.7.1.5 Determination of Proteins

Digestion: The powdered *D. carota* (1g) was weighed into kjeldah flask and copper was added as catalyst with 15 mL of concentrated sulphuric acid (H₂SO₄). It was heated in the fume cupboard till the heat solution changes to green colour. It was allowed to cool and the residues were

washed down from the mouth and neck of the flask with distilled water. After cooling, the digested sample was washed down with 100 mL of distilled water.

Distillation: The digested *D. carota* sample was steamed through the Markham distillation apparatus for about 15 minutes and the condenser was placed. Boric acid (10 mL) was used as indicator. The digested sample (10 mL) was pipette into the apparatus via the small funnel aperture and wash down with distilled water followed by addition of 10 mL of 40% NaOH solution. This was steamed for about 5-7 minutes in order to collect the ammonium sulphate. The receiving flask of about 30-40 mL was removed and the tip of the condenser into the flask was washed down

Titration: The solution was titrated in the receiving flask using N/100 (0.01N) hydrochloric acid and the nitrogen content was calculated as the protein content of the sample (AOAC, 2005). However, blank solution was prepared along with the *D. carota* sample. The percentage of protein was calculated and three determinations were recorded

$$\% \text{ protein} = \frac{\text{Final reading} - \text{Initial reading blank (0.2)}}{\text{Initial weight (0.5)} \times \text{standard number of protein (6.25)}} \times \text{Standard No. of Nitrogen (1.4)}$$

3.7.1.6 Determination of Carbohydrate

The differential method was used to quantify the carbohydrate content in *D. carota* powdered sample (Okwu and Morah, 2004; AOAC, 2005). It was obtained by estimating all other fractions by proximate analysis.

$$\% \text{ Carbohydrate} = 100 - (\% \text{ of moisture} + \% \text{ of ash} + \% \text{ of protein} + \% \text{ of fat})$$

3.8 Toxicity Studies on *Daucus carota* Aerial Part

Safety margins of the n-hexane, ethyl acetate and methanol extracts of the *D. carota* aerial part were determined on experimental animals using oral route of administration for the acute and sub chronic toxicity studies. The acute toxicity study assessed the median lethal dose (LD₅₀) of the extracts that killed fifty percent of the experimental animals within 24 hours and were

observed for 14 days period of time for any toxic reactions according to Organization for Economic Cooperation Development (OECD, FDM 420). The sub chronic toxicity was designed for period of 28 days as recommended by OECD guidelines in order to assess longer term effects or potential toxicity (OECD, 2011). The body weight, haematological, biochemical parameters (liver and kidney function test) and histopathological effects on liver, kidney and stomach of the three extracts were investigated and reported.

3.8.1 Animal Ethical Consideration

Ethical approval on the use and care of laboratory animals was obtained from Animal Rights Ethical Committee, Ahmadu Bello University Zaria, Nigeria with the approval number ABUCAUC/2017/005 and the protocols were followed in accordance with the standard laboratory conditions and practices.

3.8.2 Experimental Design

Seventy-five healthy Wistar Rats (*Rattus albus*) of weight 90-100g were sourced from the Animal House Unit, Department of Pharmacology and Therapeutics of Ahmadu Bello University Zaria, Nigeria. The Wistar Rats were housed in well ventilated cages, fed and allowed free access to water *ad libitum*. The animals were fed with vital feed and allowed free access to standard commercial pelleted feed purchased from Grand Cereals and Oils Mills Ltd, Jos, Plateau State, Nigeria. The rats were maintained under standard laboratory conditions in accordance with the protocols approved by the ABU, Zaria Ethical Committee on use and care of experimental animals.

3.8.2.1 Route of administration

Oral route of administration was used throughout the study for the control and the treated groups during the acute and sub-chronic toxicity studies of *Daucus carota* aerial part extracts.

3.8.3 Acute Toxicity

The fixed dose method (FDM) was used for the acute oral toxicity testing (new OECD FDM test guideline 420). Fifteen healthy Wistar Rats were used for the oral acute toxicity testing of the extracts. Five laboratory Rats of 90-100 g body weight were administered orally doses 5mg/kg, 50mg/kg, 500mg/kg, 2000 mg/kg and 5000mg/kg body weight of the n-hexane, ethylacetate and methanol extracts. The Wistar Rats were observed for toxicity signs for the first 30 minutes, 1 hour and at interval of 24 hours for 14 days for mortality and toxic signs such as convulsion, hyperactive, dullness, diarrhoea, tiredness, weakness and excessive urination. The LD₅₀ was determined and reported (OECD 2011; Parasuraman, 2011; Parasuraman *et al.*, 2014).

3.8.4 Sub chronic Toxicity Studies

Sub-chronic toxicity study was carried out on the remaining sixty apparently healthy Wistar Rats for the period of twenty eight days. The study was carried out in accordance with OECD (2011); Olorunisola *et al*(2012) and Ugbogu *et al*(2016). The n-hexane, ethylacetate and methanol of *D. carota* aerial part extracts were tested at the selected oral doses of 500mg/kg, 1000mg/kg and 1500 mg/kg body weight. The selections of doses were based on 30 % of LD₅₀ of the oral acute toxicity study. The Wistar Rats strains were divided into four groups of five animals each. The experimental animals were housed in a cage, made of aluminum with the top covered iron mesh. The bedding was made of saw dust, maintained at room temperature and natural daylight/night conditions.

Group 0: Received 1 ml/kg of distilled water and served as control.

Group I: Received 500mg/kg of n- hexane extract.

Group II: Received 1000 mg/kg of n- hexane extract.

Group III: Received 1500 mg/kg of n- hexane extract

The, same grouping was used for the ethylacetate and methanol extracts respectively.

The extracts and distilled water were administered orally, once daily every morning for the period of twenty eight days during the study. The Wistar Rats were observed twice daily for toxicity signs and mortality, cannibalism, behavioural abnormalities and other toxicity signs as prescribed by OECD method (OECD, 2011). The body weight differences of all the experimental animals were monitored at regular intervals and recorded weekly. During the study, regular water intake and feed were ensured. On the 28th day of the sub chronic toxicity study, all the animals were euthanized chemically using chloroform and cotton wool in a euthanized container. The organs harvested (liver, kidney and stomach) for the gross pathology was observed in the experimental animals.

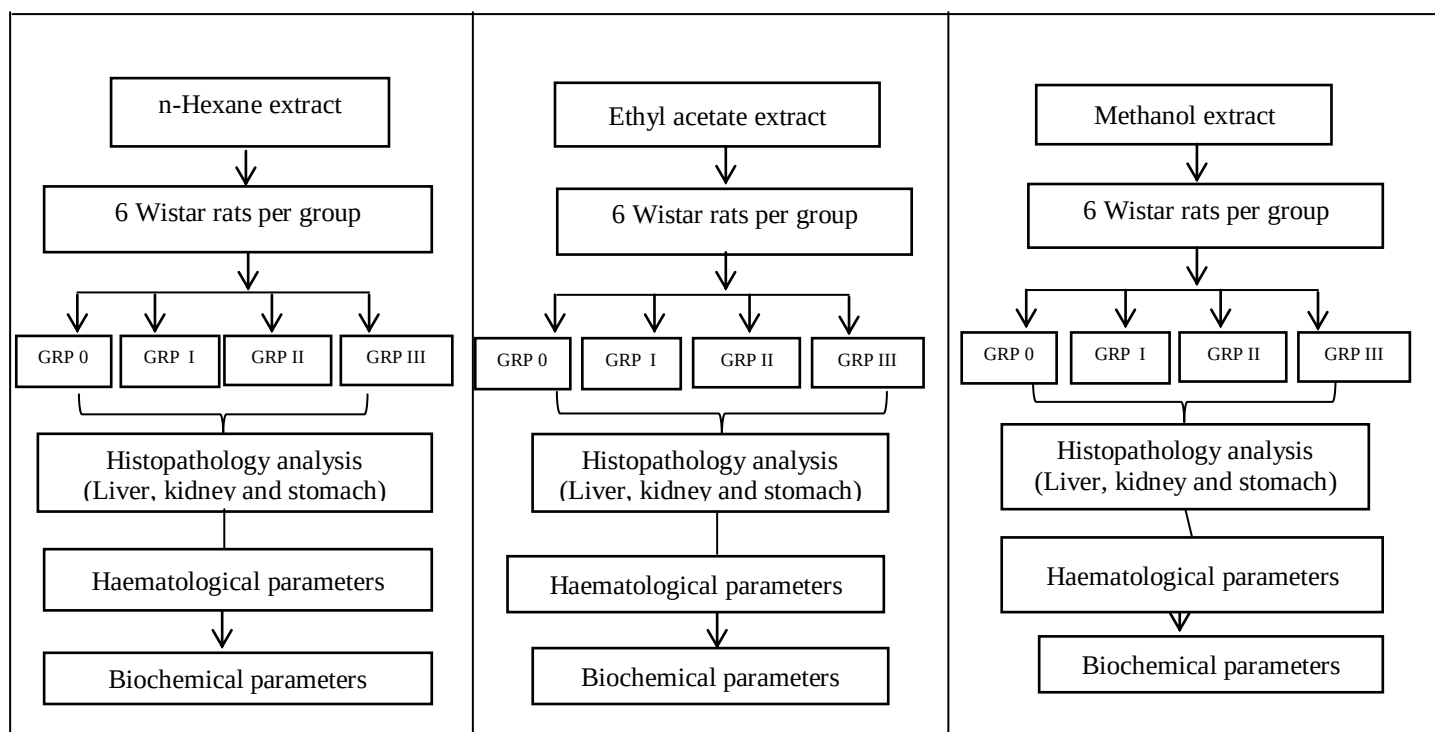


Fig. 3.2: Experimental animal Design of the *Daucus carota* aerial part extracts

3.8.4.1 Weekly Body Weight Differences

The body weight of each rat was measured once before commencement of dosing (Day 0) and once every week (week 1, week 2, week 3 and week 4) during the oral dose administration.

3.8.4.2 Collection of Blood Samples

The blood samples (5 mL) of the euthanized animals were collected by puncturing the prominent jugular vein with syringe needles into labelled heparinized (EDTA bottles) and non-heparinized bottles (without EDTA). The heparinized blood samples were used for haematological evaluation while the non-heparinized blood was allowed to coagulate centrifuged and the sera separated were analyzed for biochemical parameters. The remaining (1 mL) blood was allowed to clot at room temperature and the serum collected was centrifuge at 1000 revolution per minutes for 10 minutes. The aliquots of serum, plasma and erythrocyte samples were stored in a freezer below 4°C until required for the kidney and liver function tests. The kidney, liver and stomach of the euthanized animals were preserved in 10% formalin for histopathological analysis (Park *et al.*, 2011; Liu *et al.*, 2011 and Jaijoy *et al.*, 2011).

3.8.4.3 Haematological Parameters

The blood (5 mL) was collected after euthanasia, from the jugular vein of each rat. The blood samples were collected into vacuum tubes containing EDTA as anticoagulant and taken to the laboratory (Jaijoy *et al.*, 2011). The blood indices including the white blood cell (WBC), red blood cell (RBC), haemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), mean platelet volume (MPV) were assayed using automated Hematology Analyzer (Mind-ray-BC-3600).

3.8.4.4 Biochemical Parameters

The blood (3 mL) was further collected from the euthanized rats without an anticoagulant. The blood samples for biochemical analyses were collected into plain universal bottles and allowed to clot and centrifuged at 3500 rpm for 10 minutes.

The sera was separated and stored at -4 °C. The biochemical parameters evaluated include; alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, Sodium, Potassium, Creatinine, Chloride, Urea and Bicarbonate as liver and kidney function tests by colorimetric method using Randox assay kits and automated Biochemistry analyzer (Jaijoy *et al.*, 2011).

3.8.5 Histopathological Effects of the *Daucus carota* Aerial Part Extracts

The tissue of the Wistar Rats were sliced in 3 to 4 cm thick for the kidney, liver and stomach of the euthanized Rats and fixed in labelled containers containing 10% formalin. They were dehydrated for 2 hours in each of the following ascending grades of alcohol: 85%, 90% and 100% v/v.

The dehydrated tissues were then cleared in toluene for 2 hours, after which the tissue slices were embedded in paraffin wax and left to cool. The blocks were trimmed on the microtome at 5 microns. The ribbon of sections was dehydrated in xylene and rehydrated in the following grades of alcohol: 100%, 90% and 70% v/v and subsequently infiltrated with molten paraffin wax. They were then stained in Haematoxylin for about 5 minutes, differentiated in 1% acid alcohol, blued in Scotch's tap water and stained with Eosin for 3 minutes. They were later rinsed, dehydrated in ascending grades of alcohol: 70%, 90% and 100% v/v and finally cleared. The slides were then examined microscopically for pathological lesions (Parasuraman, 2011; Liu *et al.*, 2011; Jaijoy *et al.*, 2011).

Photomicrographs of the lesions were taken at $\times 40$ objective lens. Three Wistar Rats were randomly selected in each group and observed for similar histopathological effects. The tissues necrosis was photomicrograph respectively for the n-hexane, ethyl acetate and methanol extracts.

3.8.6 Gross Alpha, Beta and Natural Occurring Radioactive Materials (NORMs) of *Daucus carota* Aerial part

3.8.6.1 Determination of Alpha and Beta Activity in the aerial part

Alpha-beta spectrometry (Protean Instrument Corporation Lenoir City, Tennessee; Model: MPC-2000-DP (ASTM 1995; IAEA, 2007) was used in counting the gross alpha and beta activity concentrations of *D. carota* aerial part. This instrument was sourced and carried out in Centre for Energy Research, Ahmadu Bello University Zaria, Nigeria.

The *Daucus carota* aerial part were air dried to obtain a constant dry weight and it was pulverized, sieved to grain size of less than 0.63mm and sealed in a plastic container. Analytical weighing balance was used to measure 0.5 grammes portions, each being held by a binder (PolyVinyl Chloride) and fused into pellets with the help of a die and hydraulic press and finally dropped into planchet for proportional counting.

For gross alpha counting the high voltage was set at 1600 volts and samples were counted for 3 cycles of 3600 sec per cycle. The results were displayed as raw counts, count rate (count/min) activity and the standard deviation were also recorded. The data were acquired from alpha mode only and the alpha activity concentration was calculated as reported by ASTM (1995).

Also, gross beta counting was set at high voltage for 1700 volts and samples were counted for 3 cycles of 3600 sec per cycle in beta mode. The count rate and the activity concentrations were calculated according to ASTM (1995). The international standard recommended permissible limits were obtained for proper recommendation on its alpha and beta activity (UNSCEAR, 2000; IAEA, 2007).

3.8.6.2 Determination of the naturally occurring radioactive materials (NORMs) in *D. carota* aerial part

This analysis was carried out using a gamma spectrophotometer (76×76mm sodium iodide detector) crystal optically coupled with photomultiplier tube (PMT- Canberra, Model: 727). The instrument was sourced from the Centre for Energy Research, Ahmadu Bello University Zaria, Nigeria. The samples of *D. carota* aerial part were air dried under room temperature $\pm 25^{\circ}\text{C}$ and pulverized into fine powder under aseptic conditions. The samples were packed into radon-impermeable cylindrical plastic containers which were selected based on the space allocation of the detector vessel which measures (7.6 by 7.6 cm) in geometry dimension. The sealed packed containers prevented radon-222 from escaping in order to target the progenies of the unstable radio isotopes of ^{40}K , ^{226}Ra and ^{232}Th . The sealing process included smearing of the inner rim of each container lid with vaseline jelly, filling the lid assembly gap with candle wax to block the gaps between lid and container, and tight-sealing lid-container with masking adhesive tape. Radon and its short-lived progenies were allowed to reach secular radioactive equilibrium by storing the samples for 30 days prior to gamma spectroscopy measurements. The packaging in each case was triple sealed for replicates and statistical analysis (IAEA, 2006; Tettey-Larbi *et al.*, 2013; Njinga *et al.*, 2015; Idoko *et al.*, 2016). The assembly has a pre-amplifier incorporated into it and a 1 kilovolt external source. The detector is enclosed in a 6cm lead shield with cadmium and copper sheets. This arrangement aimed at minimizing the effects of background and scattered radiation. The data acquisition software used was ORTEC Maestro-32 by Canberra Nuclear Products. The samples were measured for a period of 29,000 seconds (8 hours). The peak area of energy in the spectrum was used to compute the activity concentrations in each sample (IAEA, 2006). Three replicates of *Daucus carota* aerial part were carried out for average determination of naturally occurring radioactivity concentration.

3.9 Statistical Analysis

The data obtained were analyzed using Statistical Package for Social Sciences (SPSS), IBM version 20 and Microsoft Excel 2010. The results were expressed as mean $\pm$ standard deviation (SD) and percentages except where otherwise stated. The sub-chronic toxicity study data were analyzed using one way analysis of variance followed by *Bonferroni posthoc* test for mean separation at ($p \leq 0.05$) as significant (Duncan, 1955).

CHAPTER 4

4.0 RESULTS

4.1 Some Pharmacognostic Standards of *Daucus carota* Aerial Part

Organoleptic and other morphological features showed that the *Daucus carota* aerial part had pleasant odour, characteristic taste, and smooth hairy surface along the stem and also may be yellowish during spoilage. The average leaf size was $2.52 \pm 0.20 \times 0.44 \pm 0.04$ cm), height (39.00 ± 1.34 cm). The *Daucus carota* leaf has serrated leaf blade, acute shape, parallel venation and tri-pinnate leaf arrangement (Table 4.1).

4.1.1 Microscopic Features of *Daucus carota* Fresh Leaf

The microscopy of the lower and upper epidermis surface of *Daucus carota* leaf revealed diacytic stomata, osteole, wavy epidermal shape, subsidiary cell, guard cell and sheath covering layer (Plate III and IV).

4.1.2 Quantitative Microscopy of *Daucus carota* Leaf

The quantitative microscopy revealed that the lower epidermis (31.40 ± 1.86), stomata number (14.80 ± 0.97), stomata index (31.22 ± 3.09) and palisade ratio (6.20 ± 0.37) while in the upper epidermis (21.80 ± 0.97), stomata number (10.60 ± 0.87) and stomata index (32.55 ± 1.25). The average vein islet number (12.80 ± 1.98) and veinlet termination (31.80 ± 2.33) were also quantified (Table 4.2).

4.1.3 Chemo-microscopical of the Powdered *Daucus carota* Aerial Part

The chemo-microscopy of the powdered *Daucus carota* aerial part revealed the presence of starch, tannins, lignin, cellulose, aleurone grains, mucilages, suberin and cutins and calcium oxalate crystal was absent (Table 4.3).

4.1.4 Physicochemical Constants of the Powdered *Daucus carota* Aerial Part

The physicochemical parameters of the powdered *Daucus carota* aerial part showed percentage moisture content (5.57%), total ash (11.60%), acid insoluble ash (1.00%), water soluble ash (5.67%), ethanol extractive value (15.60%) and water extractive value (21.80%) (Table 4.4).

4.1.5 Fluorescence Analyses *Daucus carota* Aerial Part

The fluorescence analyses of the *Daucus carota* aerial part revealed fluorescence characteristics under daylight and under UV light at 254 and 365 nm (Table 4.5).

4.2 Preliminary Phytochemical Screening of *Daucus carota* Aerial Part Extracts

The preliminary phytochemical screening of the n-hexane extracts revealed the presence of steroids/triterpenes and other phytochemical were found to be absent. The ethyl acetate and methanol extracts showed the presence of saponins, flavonoids, tannins and steroid/triterpenes. Secondary metabolites such as alkaloid, cardiac glycosides and anthraquinones were absent in the methanol extract (Table 4.6).

4.3 Chromatogram of *Daucus carota* Aerial Part Extracts

The thin layer chromatographic analysis of n-hexane, ethyl acetate and methanol extracts of *Daucus carota* aerial part with the R_f values. The separations were carried out using suitable solvent systems and sprayed with *p*-anisaldehyde (Plate V-VII).

4.4 Nutritional Potentials of *Daucus carota* Aerial Part

The proximate composition revealed that the aerial part contain carbohydrate (51.81%), crude protein (14.59%), lipid (10.37%), fibre (9.07%), moisture content (5.67%) and ash content (11.60%) (Fig. 4.4)

4.5 Elemental Composition of the Aerial Part of *Daucus carota*

The aerial part of *D. carota* was found to contain elements such as Zn (0.26 ± 0.02), Fe (3.19 ± 0.02), Cu (0.13 ± 0.02), Mn (1.15 ± 0.06), Se (0.40 ± 0.04), Mg (1.02 ± 0.02), Na (5.38 ± 0.02), P (11.00 ± 0.05), K (2.25 ± 0.01), Ca (2.02 ± 0.02), As (0.83 ± 0.02), Pb (0.17 ± 0.00), Cr (0.02 ± 0.00), Ni (0.04 ± 0.01) and Cd (-0.00 ± 0.00) was absent at various concentration as presented in Fig. 4.5.

Table 4.1: Organoleptic and Morphological Features of *Daucus carota* Aerial Part

Organoleptic/Macroscopic features	Physical characteristics
Colour	Green leafy
Odour	Pleasant
Taste	Characteristic
Appearance/texture	• Smooth hairy surface along the stem • Yellowish during storage
Dimension of leaf size	$2.52 \pm 0.20 \times 0.44 \pm 0.04$ cm
Aerial part height	39.00 ± 1.34 cm
Leaf shape and blade	Acute/serrated leaf blade
Venation	Parallel
Leaf arrangement	Tri-pinnate



Plate III: Air-dried powdered aerial part of *Daucus carota*

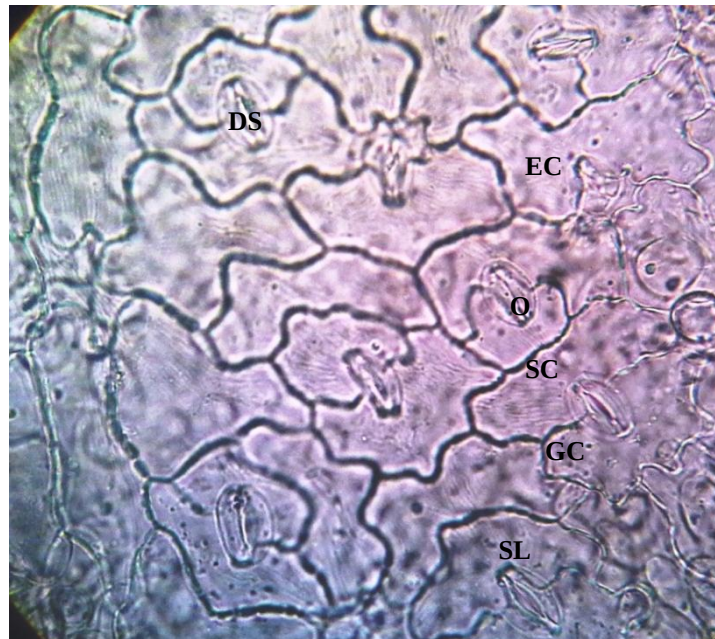
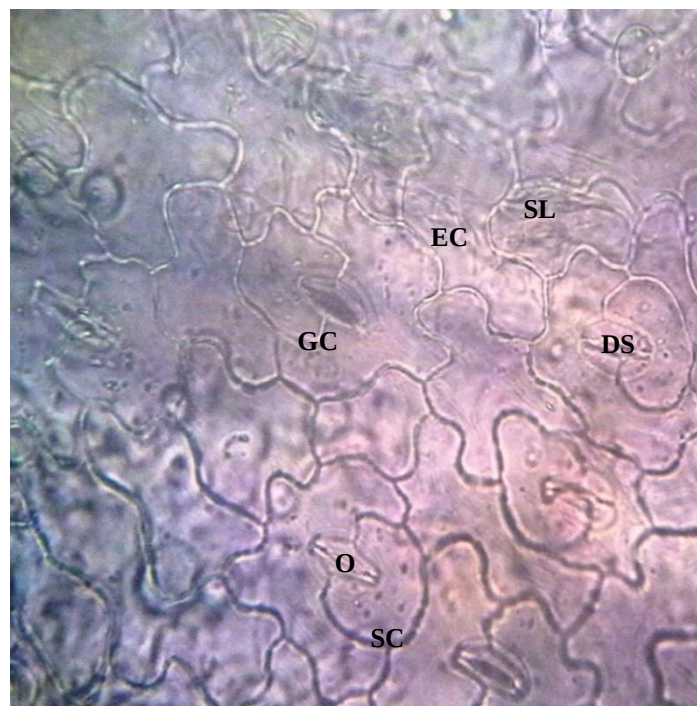


Plate III: Lower Epidermal Layer of *Daucus carota* Leaf (x40)



Key:
 DS =Diacytic stoma;
 O = Osteole;
 EC = Epidermal cell;
 GC = Guard cell;
 SC = Subsidiary cell;
 SL = Sheath layer

Plate IV: Upper Epidermal layer of *Daucus carota* Leaf (x40)

Table 4.2: Quantitative Leaf Microscopic Features of *Daucus carota*

Standards	Lower Epidermal Mean±SEM	Upper Epidermal Mean±SEM
Epidermal cells	31.40±1.86	21.80±0.97
Stomata number	14.80±0.97	10.60±0.87
Stomata index (%)	31.22±3.09	32.55±1.25
Palisade ratio	6.20±0.37	4.00±0.05
Vein islet number	12.80±1.98	12.80±1.98
Vein termination number	31.80±2.33	31.80±2.33
Average values of five determinations		

Table 4.3: Chemo-microscopical Features of Powdered *Daucus carota* Aerial Part

Constituents	Detecting reagents	Observation	Inference
Starch	N/50 iodine	Blue-black colour on grains within the cell.	Starch present
Lignin	Phloroglucinol	Red-pink colour on the walls of lignified cell.	Lignin present
Tannins	5% FeCl ₃	Greenish-black colour in some parenchyma cells.	Tannin present
Mucilage	Ruthemium red	Dilated fragments	Mucilage present
Calcium oxalate	HCl	Dissolution of shining crystals on the anatomical sections of the Leaf.	Calcium oxalate absent
Calcium carbonate	HCl	Effervescence in the cell.	Calcium carbonate present
Cellulose	Chlor-Zinc- Iodine	Blue coloration of the cell wall	Cellulose present
Suberin	Sudan red	Orange red colour on cell wall.	Suberins present
Aleurone grains	Iodine in ethanol	Yellowish brown	Aleurones grains present

Table 4.4: Some Physicochemical Parameters of Powdered *Daucus carota* Aerial Part

Physico-chemical Parameters	Values (% w/w) $\pm$ SD
Moisture content	5.57 $\pm$ 0.51
Total ash value	11.60 $\pm$ 1.02
Acid Insoluble ash	1.00 $\pm$ 0.90
Water Soluble ash	5.67 $\pm$ 0.76
Ethanol Extractive	15.60 $\pm$ 0.55
Water Extractive	21.80 $\pm$ 1.10
Meanvalues of 5 counts	

Table 4.5: Fluorescence Analysis of *Daucus carota* Aerial Part

Reagents/solvent Used	Daylight		Short Wavelength (254 nm)	Long Wavelength (365 nm)
	Immediately	After one hour		
Conc. HCL	Green	Green	Deep green	Dark green
Dilute HCL	Brown	Brown	Brown	Black
Dilute NaOH	Light green	Greenish yellow	Deep green	Dark green
H ₂ SO ₄	Dark green	Greenish black	No fluorescence	Green
n-Hexane	Yellowish green	Yellowish Green	Light green	Green
NH ₄ OH	Yellow	Greenish yellow	Deep green	Dark green
Water	Green	Green	No fluorescence	No fluorescence
Ethyl acetate	Green	Green	Light green	Brown
Nitric acid	Deep brown	Deep brown	Brown	Deep brown
Methanol	Blue green	Greenish black	Light green	Green
Picric acid	Light green	Light green	Light green	Dark green
Acetic acid	Green	Green	Dark green	Dark green
Iodine	Green	Green	Green	Green
Magnesium chloride	Green	Green	Green	Dark green
Ferric chloride	Blue-black	Blue-black	Dark green	Black
Ferrous sulphate	Blue-black	Black	Black	Black
Benzene	Green	Green	Green	Dark green

Table 4.6: Preliminary Phytochemical Screenings of Extracts of *Daucus carota* Aerial Part

Phytochemical constituents	N-hexane extracts	Ethyl-acetate extracts	Methanol extracts
Alkaloids			
<i>Dragendorff test</i>	Absent	Absent	Absent
<i>Wagner's test</i>	Absent	Absent	Absent
<i>Mayers test</i>	Absent	Absent	Absent
Cardiac glycosides			
<i>Keller-killani</i>	Absent	Absent	Absent
<i>Keddes test</i>	Absent	Absent	Absent
Saponins			
<i>Frothing test</i>	Absent	Present	Present
<i>Haemolysis test</i>	Absent	Present	Present
Phenolic compounds			
Flavonoids	Absent	Present	Present
<i>Shinodas test</i>	Absent	Present	present
<i>Lead acetate test</i>			
Anthraquinones			
<i>Bontragers test</i>	Absent	Absent	Absent
<i>Modified bontragers test</i>	Absent	Absent	Absent
Tannins			
FeCl_3	Absent	Present	Present
Lead acetate test	Absent	Present	Present
Steroid/triterpenes			
Lieberman buchard	Present	Present	Present
Salkwoski test	Present	Present	Present

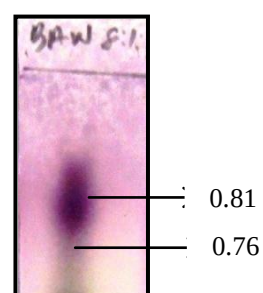
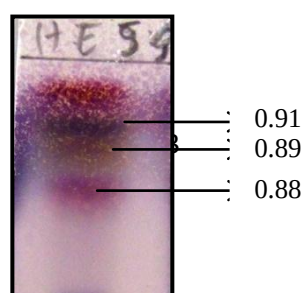
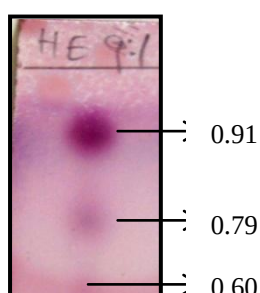


**Plate V: Hexane
Extract**

**Plate VI: Ethyl
acetate Extract**

**Plate VII: Methanol
Extracts**

Plate V: TLC Profile of *D. carotaaerial* part n-hexane extract using solvent system Hexane: Ethyl acetate (9:1)

Plate VI: TLC Profile of *D. carotaaerial* part ethyl acetate extract using solvent system Hexane: Ethyl acetate (5:5)

Plate VII: TLC Profile of *D. carotaaerial* part methanol extract using solvent system Butanol: Acetic Acid and Water (8:1:1)

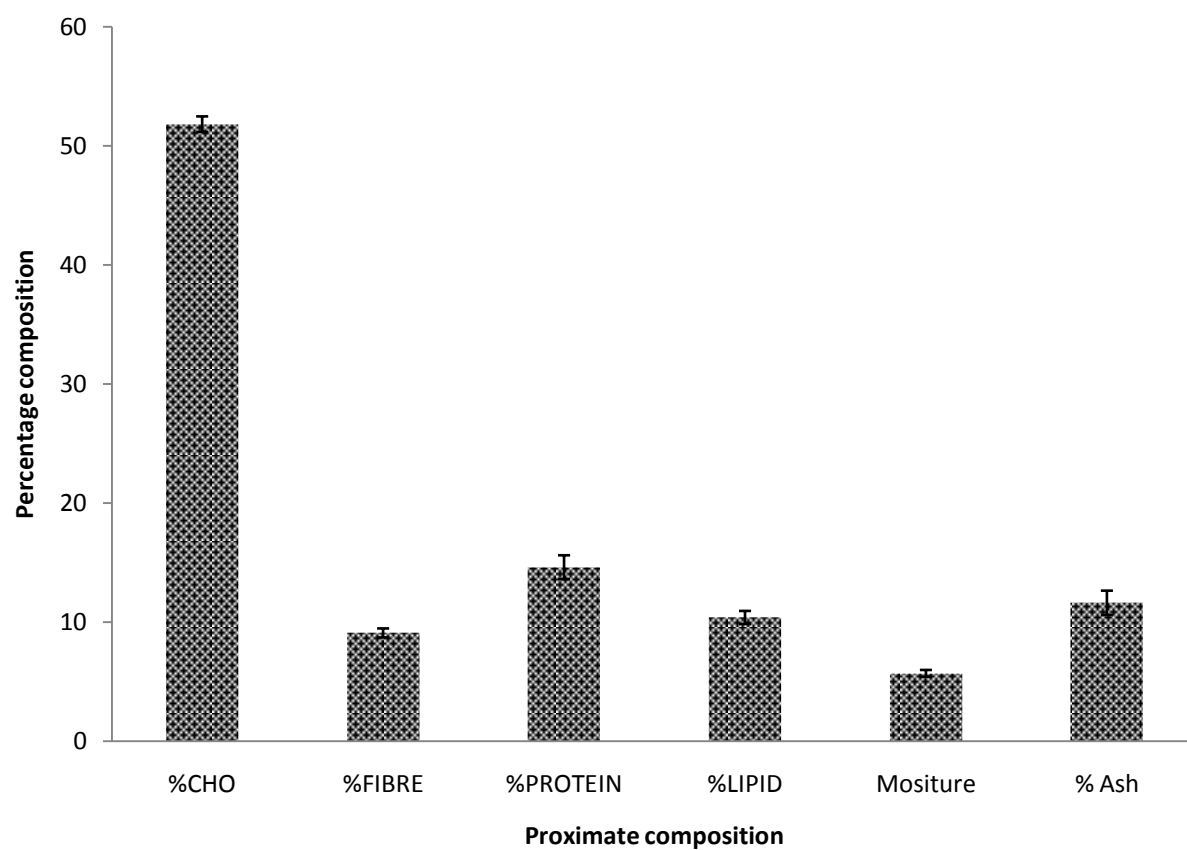


Fig. 4.4: Proximate Composition of *Daucus carota* Aerial Part

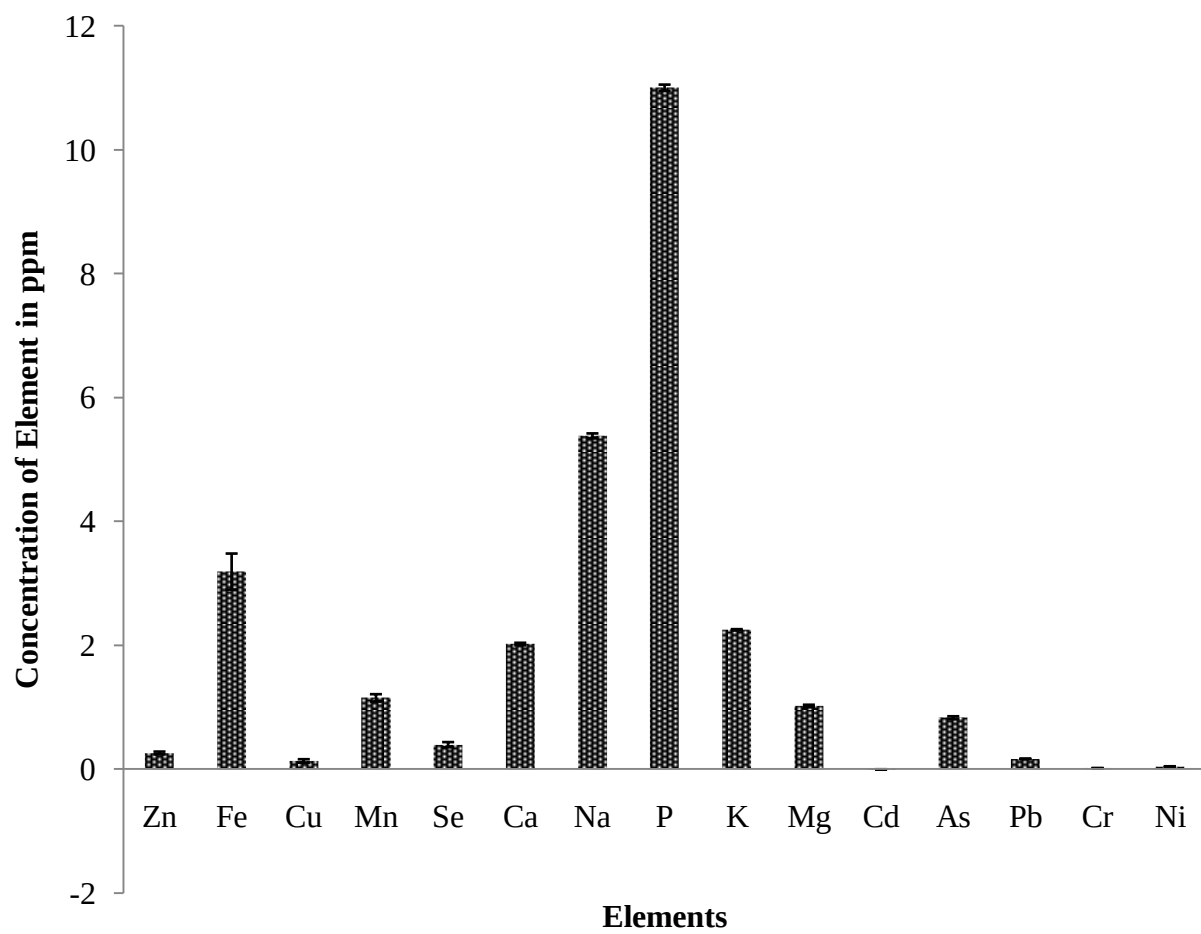


Fig. 4.5: Mineral Elements Compositions of *Daucus carota* Aerial Part

4.6 Safety Margins Evaluation of *Daucus carota* Aerial Part Extract on Wistar Rats

4.6.1 Acute toxicity of *Daucus carota* aerial part extracts

The acute fixed oral dose toxicity of the n-hexane, ethyl acetate and methanol extracts was carried out on healthy Wistar Rats observed for 14 days showed no mortality or any toxicity signs such as tiredness, weakness, convulsion, hyper activeness, dullness, diarrhea and diuresis were noticed at the high dose of 5000 mg/kg.

4.6.2 Sub-chronic Toxicity Evaluation of *Daucus carota* Aerial Extracts on Wistar Rats

4.6.2.1 Weekly body weight index of the extracts on wistar rats

The effects of n-hexane and methanol extracts on the body weight of Wistar Rat showed significant decrease ($p \leq 0.05$) at 500 mg/kg, 1000 mg/kg 1500 mg/kg after week 2 when compared with the control group (1 mg/kg distilled water). The ethyl acetate extract showed no significance difference ($p \geq 0.05$) on the body weight (Table 4.7).

4.6.2.2 Haematological parameters of the extracts on wistar rats

The effects of oral daily administration of the n-hexane and ethyl acetate extracts showed significant statistical difference at ($p \leq 0.05$) on red blood cell (RBC) at different doses. The n-hexane and ethyl acetate extract showed that MCV was significant ($p \leq 0.05$) at 1000 mg/kg and 1500 mg/kg in the n-hexane extract and at 500 mg/g in ethyl acetate. The MCH was only significant ($p \leq 0.05$) at 500 mg/kg in the ethyl acetate (Table 4.8).

4.6.2.3 Liver function parameters of the extracts on wistar rats

The effect of oral daily administration of the three extracts on the liver function parameters showed significant statistical difference at ($p \leq 0.05$) on ALP (IU/L) at 500 mg/kg, 1000 mg/kg and 1500 mg/kg when compared with the control group (1 mL/kg distilled water). The ALT (IU/L), AST (IU/L), TP (IU/L) and ALB (G/DL) showed no significance difference ($p \geq 0.05$) (Table 4.9).

4.6.2.4 Kidney function of the extracts on wistar rats

The effects of n-hexane extract on bicarbonate showed statistically significant difference ($p \leq 0.05$) on 500 mg/kg, 1000 mg/kg and 1500 mg/kg. The n-hexane extract showed significant difference ($p \leq 0.05$) on sodium at 1500 mg/kg. The ethyl acetate extract showed no significant difference in any of the kidney function parameters. The methanol extract showed that there was significant decreased on sodium at 500 mg/kg group and bicarbonate was significant at 1000 mg/kg and 1500 mg/kg ($p \leq 0.05$) when compared with the control group (Table 4.10).

Table 4.7: Effects of Oral Administration of n-Hexane, Ethyl acetate and Methanol extracts of *Daucus carota* aerial part on the Body weights of Wistar Rats after 28 days

Body weight (mg/kg)	Doses (Oral Administration)± SD			
	(1 mL/kg) Distilled water	500mg/kg	1000mg/kg	1500mg/kg
Hexane Extract				
Weight at day 0	92.00±8.37	96.00±5.48	94.00±5.48	94.00± 5.48
Weight at week 1	96.60±8.91	88.00±7.31	85.00±11.55	96.60± 24.88
Weight at week 2	98.00±15.38	97.40 ±9.48*	88.20±7.29*	80.80± 20.39*
Weight at week 3	102.60±20.43	132.00±10.79	110.40±26.41	100.40±19.75
Weight at week 4	115.00±26.56	138.00±12.83	118.80±23.32	118.00± 21.01
Ethylacetate extract				
Weight at day 0	92.00±8.37	92.00± 4.47	100.00±0.00	94.00±5.48
Weight at week 1	96.60±8.91	95.00±11.02	109.60±11.13	98.00±10.52
Weight at week 2	98.00±15.38	90.6±8.44	105.80±8.79	98.60±10.48
Weight at week 3	102.60±20.43	99.00±11.77	114.60±10.81	104.80±12.09
Weight at week 4	115.00±26.56	111.40±6.35	122.20±12.62	116.60±13.97
Methanol extract				
Weight at day 0	92.00±8.37	90.00±0.00	90.00±0.00	92.00±4.47
Weight at week 1	96.60±8.91	92.20±9.68	90.20±18.87	90.20±10.94
Weight at week 2	98.00±15.38	89.00±14.19*	85.00±13.98*	75.40±6.20*
Weight at week 3	102.60±20.43	86.40±7.89	95.60±11.93	97.60±10.26
Weight at week 4	115.00±26.56	101.40±13.52	102.00±12.96	104.20±12.52

The mean difference is statistically significant * ($p \leq 0.05$) compared to control. Mean ± SD (n = 5)

Data were analyzed using one way ANOVA followed by Bonferroni post hoc.

Table 4.8: Effects of Oral Administration of n-Hexane, Ethyl acetate and Methanol extracts of *Daucus carota* aerial part on the haematological parameters of Wistar Rats after 28 days

Haematological parameters		Doses (Oral Administration) $\pm$ SD			
Hexane extract	(1 mL/kg) Distilled water	500 mg/kg	1000 mg/kg	1500 mg/kg	
WBC ($\times 10^9/L$)	8.63 $\pm$ 2.65	7.87 $\pm$ 2.29	7.27 $\pm$ 3.41	5.93 $\pm$ 18.35	
RBC ($\times 10^9/L$)	7.62 $\pm$ 0.40	4.62 $\pm$ 0.28*	3.17 $\pm$ 1.05*	3.21 $\pm$ 1.35*	
HGB (g/dL)	14.82 $\pm$ 1.33	11.37 $\pm$ 2.48	8.20 $\pm$ 2.45	8.53 $\pm$ 3.12	
HCT (%)	45.07 $\pm$ 3.80	35.47 $\pm$ 6.43	25.17 $\pm$ 8.93	27.17 $\pm$ 10.08	
MCV(μm^3)	59.33 $\pm$ 6.86	76.80 $\pm$ 13.18	80.13 $\pm$ 5.03*	84.57 $\pm$ 2.26*	
MCH (pg)	19.50 $\pm$ 2.39	24.63 $\pm$ 5.15	26.27 $\pm$ 2.16	26.87 $\pm$ 1.39	
MCHC (g/dl)	32.87 $\pm$ 0.25	31.87 $\pm$ 1.36	32.70 $\pm$ 1.78	31.77 $\pm$ 0.85	
Ethyl acetate extract					
WBC ($\times 10^9/L$)	8.63 $\pm$ 2.65	5.53 $\pm$ 0.55	7.97 $\pm$ 3.60	7.77 $\pm$ 3.33	
RBC ($\times 10^9/L$)	7.62 $\pm$ 0.40	4.06 $\pm$ 0.29*	3.93 $\pm$ 1.35*	3.73 $\pm$ 1.14*	
HGB (g/dL)	14.82 $\pm$ 1.33	11.53 $\pm$ 0.95	10.87 $\pm$ 2.10	14.70 $\pm$ 2.09	
HCT (%)	45.07 $\pm$ 3.80	37.93 $\pm$ 0.40	33.83 $\pm$ 6.95	43.6 $\pm$ 7.02	
MCV(μm^3)	59.33 $\pm$ 6.86	84.37 $\pm$ 3.82*	54.60 $\pm$ 3.57*	56.60 $\pm$ 7.46*	
MCH (pg)	19.50 $\pm$ 2.39	28.53 $\pm$ 1.50*	23.63 $\pm$ 4.11	19.07 $\pm$ 1.77	
MCHC (g/dl)	32.87 $\pm$ 0.25	31.23 $\pm$ 1.27	32.17 $\pm$ 0.55	33.77 $\pm$ 1.27	
Methanol extract					
WBC ($\times 10^9/L$)	8.63 $\pm$ 2.65	8.80 $\pm$ 14.33	5.67 $\pm$ 0.93	5.37 $\pm$ 0.23	
RBC ($\times 10^9/L$)	7.62 $\pm$ 0.40	3.90 $\pm$ 1.63*	3.76 $\pm$ 0.69*	2.89 $\pm$ 2.15*	
HGB (g/dL)	14.82 $\pm$ 1.33	6.83 $\pm$ 2.47	9.83 $\pm$ 1.55	9.39 $\pm$ 4.79	
HCT (%)	45.07 $\pm$ 3.80	22.57 $\pm$ 9.16	30.77 $\pm$ 5.68	28.53 $\pm$ 15.03	
MCV(μm^3)	59.33 $\pm$ 6.86	74.23 $\pm$ 10.37	81.83 $\pm$ 2.70	69.53 $\pm$ 30.73	
MCH (pg)	19.50 $\pm$ 2.39	22.90 $\pm$ 4.41	26.20 $\pm$ 0.79	48.40 $\pm$ 34.56	
MCHC (g/dl)	32.87 $\pm$ 0.25	30.70 $\pm$ 1.67	32.03 $\pm$ 0.86	31.43 $\pm$ 2.21	

The mean difference is statistically significant * ($p \leq 0.05$) compared to control. Mean $\pm$ SD (n = 5)

Data were analyzed using one way ANOVA followed by Bonferroni post hoc

Table4.9: Effect of Oral daily administration of n-Hexane, Ethyl acetate and Methanolic extracts of *Daucus carota* Aerial part for 28 days on the liver function parameters of the control and treated rats in the sub-chronic toxicity study

Liver Injury Parameters	Doses (Oral Administration) $\pm$ SD			
	(1 mL/kg) Distilled water	500 mg/kg	1000 mg/kg	1500 mg/kg
Hexane extract				
ALT (IU/L)	18.67 $\pm$ 3.06	6.33 $\pm$ 4.16	12.33 $\pm$ 7.00	15 $\pm$ 4.51
AST (IU/L)	21.33 $\pm$ 1.89	28.33 $\pm$ 1.53	28.33 $\pm$ 5.86	27.33 $\pm$ 4.61
ALP (IU/L)	72.26 $\pm$ 14.20	17.14 $\pm$ 5.74*	16.49 $\pm$ 0.68*	11.29 $\pm$ 2.06*
TP (G/DL)	7.31 $\pm$ 0.76	182.76 $\pm$ 21.30	5.21 $\pm$ 0.78	6.42 $\pm$ 1.31
ALB (G/DL)	2.59 $\pm$ 0.13	2.59 $\pm$ 0.36	2.68 $\pm$ 0.54	2.43 $\pm$ 0.15
Ethyl acetate extract				
ALT (IU/L)	18.67 $\pm$ 3.06	18.67 $\pm$ 0.58	20.00 $\pm$ 5.19	17.33 $\pm$ 2.52
AST (IU/L)	21.33 $\pm$ 18.90	21.33 $\pm$ 4.04	26.33 $\pm$ 4.62	28.00 $\pm$ 3.61
ALP (IU/L)	72.26 $\pm$ 14.20	42.52 $\pm$ 2.22*	32.88 $\pm$ 4.61*	28.21 $\pm$ 12.93*
TP (G/DL)	7.31 $\pm$ 0.76	6.09 $\pm$ 0.26	6.07 $\pm$ 0.42	7.58 $\pm$ 1.67
ALB (G/DL)	2.59 $\pm$ 0.13	2.68 $\pm$ 0.22	2.75 $\pm$ 0.16	2.59 $\pm$ 0.40
Methanol extract				
ALT (IU/L)	18.67 $\pm$ 3.06	21.67 $\pm$ 5.03	20.67 $\pm$ 4.04	23.67 $\pm$ 2.51
AST (IU/L)	21.33 $\pm$ 18.90	31.00 $\pm$ 8.54	23.33 $\pm$ 2.08	27.00 $\pm$ 3.00
ALP (IU/L)	72.26 $\pm$ 14.20	12.15 $\pm$ 3.77*	15.18 $\pm$ 4.63*	11.28 $\pm$ 13.86*
TP (G/DL)	7.31 $\pm$ 0.76	7.24 $\pm$ 1.97	7.71 $\pm$ 2.89	6.58 $\pm$ 0.55
ALB (G/DL)	2.59 $\pm$ 0.13	2.31 $\pm$ 0.26	2.27 $\pm$ 0.15	2.53 $\pm$ 0.30

The mean difference is statistically significant * ($p \leq 0.05$) compared to control. Mean $\pm$ SD (n = 5)
Data were analyzed using one way ANOVA followed by Bonferroni post hoc

Table4.10: Effect of daily administration of n-Hexane, Ethyl acetate and Methanolic extracts of *Daucus carotaaerial* part for 28 days on Kidney function parameters of the control and treated rats in the sub-chronic toxicity study.

Kidney function Parameters	Doses Oral Administration) $\pm$ SD			
	1 mL/kg Distilled water	500 mg/kg	1000 mg/kg	1500 mg/kg
Hexane extract				
Urea (umol/I)	37.41 $\pm$ 10.68	32.48 $\pm$ 4.63	34.48 $\pm$ 8.22	33.71 $\pm$ 1.87
Sodium (mmol/I)	116.89 $\pm$ 14.81	110.04 $\pm$ 10.03	102.96 $\pm$ 11.61	38.69 $\pm$ 6.45*
Potassium (mmol/I)	8.08 $\pm$ 1.97	6.74 $\pm$ 0.76	6.92 $\pm$ 2.60	8.13 $\pm$ 4.74
Creatinine(umol/I)	1.3 $\pm$ 0.44	0.83 $\pm$ 0.25	1.00 $\pm$ 0.30	1.33 $\pm$ 0.25
Chloride(mmol/I)	83.33 $\pm$ 7.57	80.00 $\pm$ 1.00	72.33 $\pm$ 11.24	80.67 $\pm$ 2.08
Bicarbonate(mmol/I)	29.33 $\pm$ 2.52	20.00 $\pm$ 2.65*	20.67 $\pm$ 5.51*	21.33 $\pm$ 2.52*
Ethyl acetate extract				
Urea (umol/I)	37.41 $\pm$ 10.68	44.72 $\pm$ 6.30	30.92 $\pm$ 12.53	34.73 $\pm$ 5.97
Sodium (mmol/I)	116.89 $\pm$ 14.81	100.68 $\pm$ 4.53	115.33 $\pm$ 13.47	89.68 $\pm$ 60.9
Potassium (mmol/I)	8.08 $\pm$ 1.97	6.14 $\pm$ 0.70	9.39 $\pm$ 3.11	6.78 $\pm$ 1.69
Creatinine (umol/I)	1.3 $\pm$ 0.44	1.07 $\pm$ 0.15	1.13 $\pm$ 0.31	1.10 $\pm$ 0.20
Chloride (mmol/I)	83.33 $\pm$ 7.57	80.67 $\pm$ 1.52	84.33 $\pm$ 1.15	87.33 $\pm$ 2.52
Bicarbonate (mmol/I)	29.33 $\pm$ 2.52	24.00 $\pm$ 9.54	30.33 $\pm$ 1.15	21.00 $\pm$ 1.73
Methanol extract				
Urea (umol/I)	37.41 $\pm$ 10.68	39.18 $\pm$ 0.42	41.81 $\pm$ 6.37	44.28 $\pm$ 4.09
Sodium (mmol/I)	116.89 $\pm$ 14.81	82.90 $\pm$ 8.32*	104.67 $\pm$ 16.03	104.62 $\pm$ 6.89
Potassium (mmol/I)	8.08 $\pm$ 1.97	10.04 $\pm$ 1.72	7.58 $\pm$ 3.45	5.99 $\pm$ 2.15
Creatinine (umol/I)	1.3 $\pm$ 0.44	1.00 $\pm$ 0.35	1.10 $\pm$ 0.35	1.03 $\pm$ 0.06
Chloride (mmol/I)	83.33 $\pm$ 7.57	81.33 $\pm$ 3.70	80.67 $\pm$ 3.51	83.67 $\pm$ 7.23
Bicarbonate (mmol/I)	29.33 $\pm$ 2.52	29.00 $\pm$ 2.65	27.33 $\pm$ 2.08*	28.33 $\pm$ 2.52*

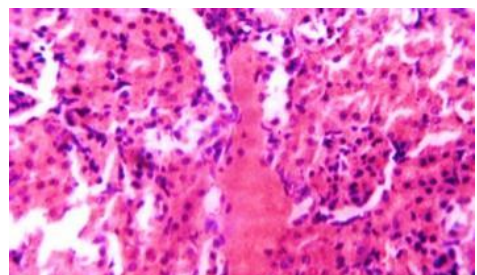
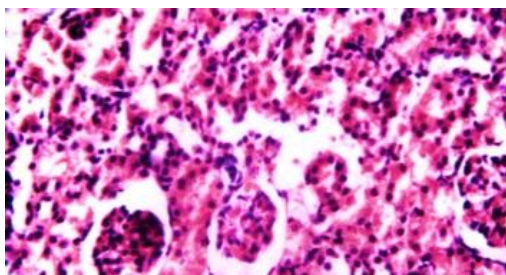
The mean difference is statistically significant * ($p \leq 0.05$) compared to control. Mean $\pm$ SD (n = 5)

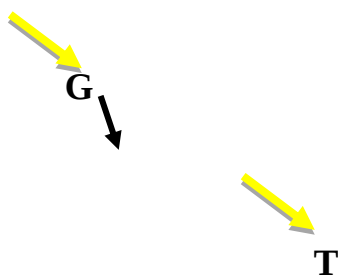
Data were analyzed using one way ANOVA followed by Bonferroni post hoc

4.6.3 Histopathological effects of the *Daucus carotaaerial* partn-hexane extracts

The histopathological effects of the control group with the n-hexane extract showed normal tubules (T) and glomerulus (G) in the kidney. Normal hepatocyte was observed in the liver and normal mucosa epithelium (E) in the stomach.

In the doses group, at 500 mg/kg there was moderate and slight tubular necrosis (TN) in the kidney, the liver had slight hepatic necrosis (N) with moderate lymphocyte hyperplasia (LH) and the stomach had normal features while at 1000 mg/kg there was moderate lymphocyte hyperplasia (LH) in the kidney, vascular congestion (VC) with kupfer cell hyperplasia (KH) in the liver and slight apical epithelium necrosis (N) in the stomach. Also at 1500 mg/kg, moderate tubular necrosis with slight lymphocyte hyperplasia (LH) occurred in the kidney. The liver showed slight hepatocellular necrosis with kupfer cell hyperplasia (KH) and the stomach had slight epithelium necrosis (N) (Plate VIII-XVI).

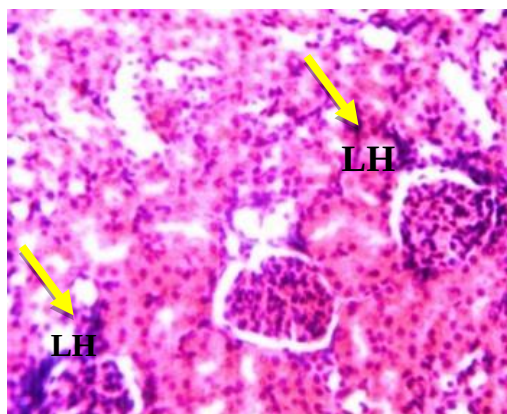




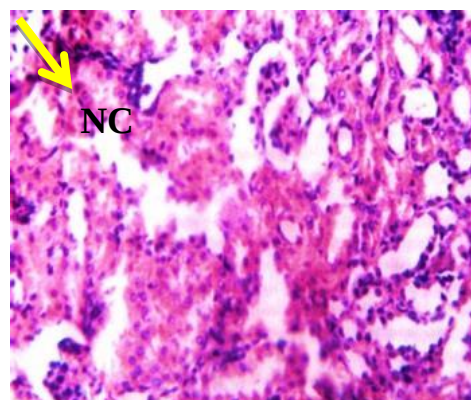
a). Section from the kidney of Wistar Rat from the control group (1 mL/kg distilled water) show the normal architecture tubules (T) and glomerulus



b). Section from the kidney of Wistar Rat treated with 500 mg/kg of n-hexane extract show moderate and slight tubular necrosis



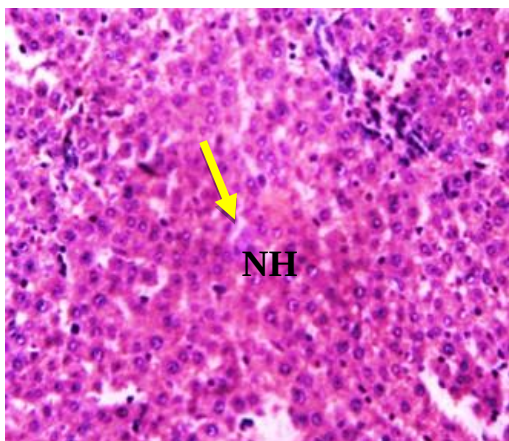
c). Section from the kidney of Wistar Rat treated with 1000 mg/kg of n-hexane extract show moderate lymphocyte hyperplasia



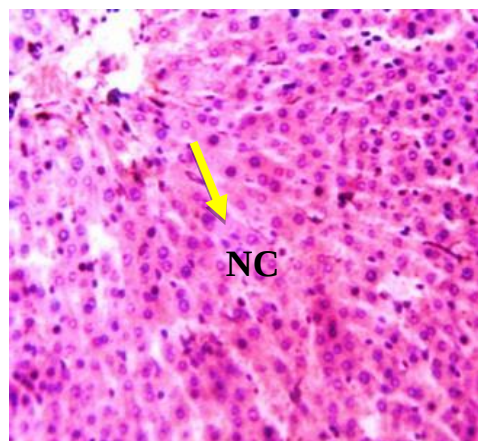
d). Section from the kidney of Wistar Rat treated with 1500mg/kg of n-hexane extract show tubular necrosis moderate and slight lymphocyte hyperplasia necrosis

KEY: **G** = Glomerular; **T** = Normal tubules; **TN**= Tubular necrosis; **LH** = Lymphocyte hyperplasia; **NC** = Slight hepatic necrosis

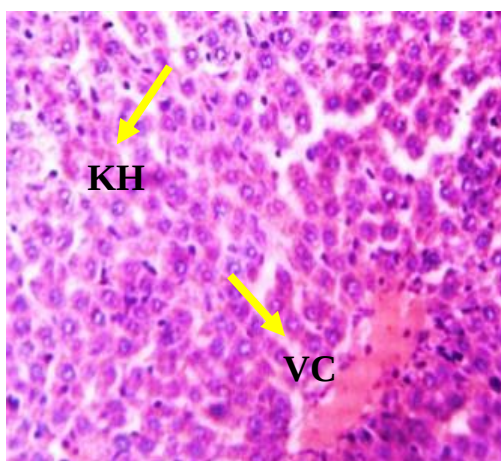
Plate VIII-X (a-d): Photomicrographs of the kidney section of Wistar Rats for the control and treated groups using the n-hexane extract of *Daucus carota* (x40)



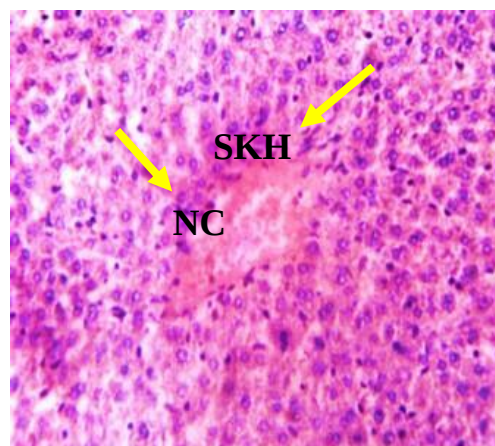
a). Section from the liver of Wistar Rat from the control group (1 mL/kg distilled water) show the



b). Section from the liver of Wistar Rat treated (500mg/kg) of n-hexane had slight hepatic necrosis with moderate lymphocyte hyperplasia



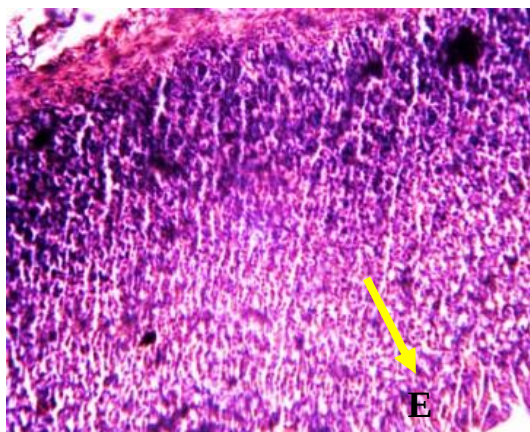
c). Section from the liver of Wistar Rat treated with 1000mg/kg of n-hexane extract had vascular congestion with kupfer cell hyperplasia



d). Section from the liver of Wistar Rat treated with 1500mg/kg of n-hexane extract show slight hepatocellular necrosis with kupfer cell hyperplasia

KEY: VC = Vascular congestion necrosis; NC = Slight hepatic necrosis; NH= Normal hepatocytes; SKH= Slight kupfer cell hyperplasia

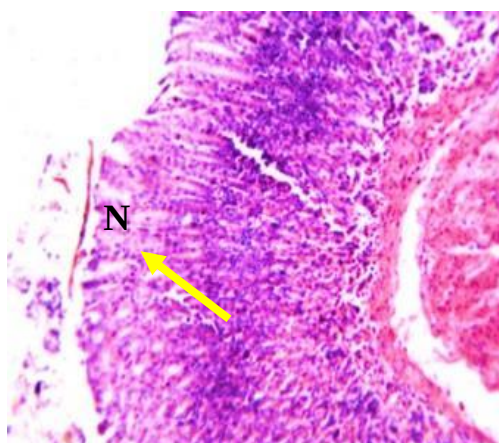
Plate XI-XIII (a-d): Photomicrographs of the liver section of Wistar Rats for the control and treated groups using the n-hexane extract of *Daucus carota* (x40)



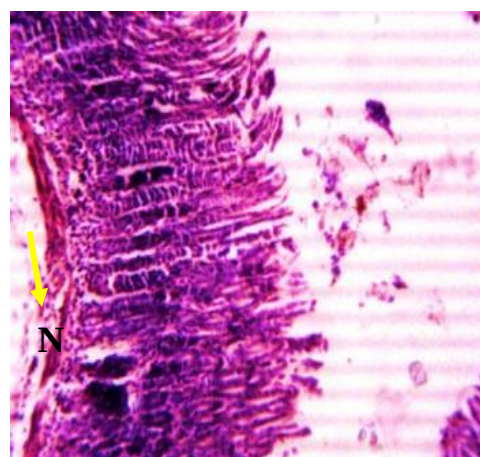
a). Section from the stomach of Wistar Rat from the control group (1 mL/kg distilled water) show the normal mucosa epithelium



b). Section from the stomach of Wistar Rat treated with (500mg/kg) of n-hexane had normal features.



c). Section from the stomach of Wistar Rat from the treated (1000mg/kg) of n-hexane had slight apical epithelium necrosis



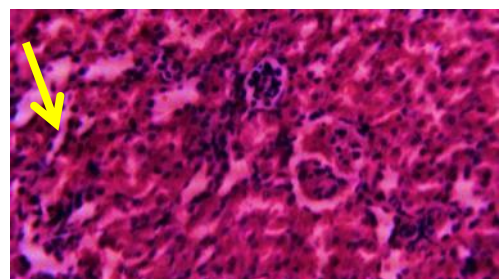
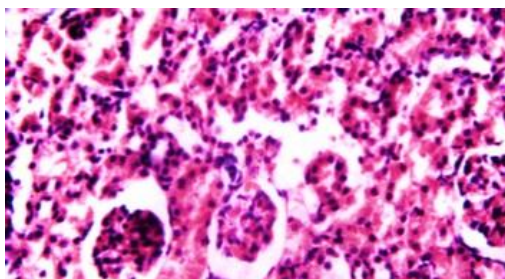
d). Section from the stomach of Wistar Rat treated with (500mg/kg) of n-hexane had slight epithelium necrosis

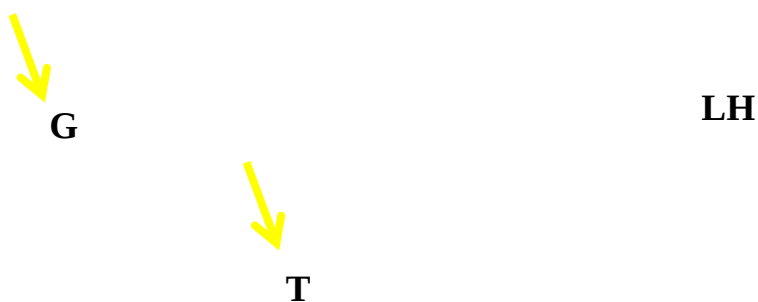
Key: E = Normal Mucosa Epithelium; N = Slight apical epithelium necrosis

Plate XIV-XVI (a-d): Photomicrographs of the stomach section of Wistar Rats for the control and treated groups using the n-hexane extract of *Daucus carota*(x40)

4.5.4 Histopathological effects of the *Daucus carota* aerial part ethylacetate extract

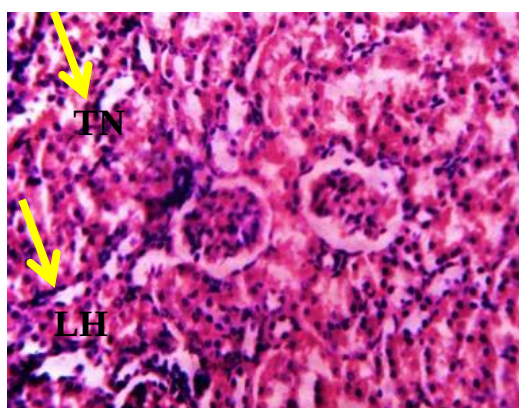
The histopathological effects of the control group of the ethyl acetate extract showed normal tubules (T) and glomerulus (G) in the kidney, normal hepatocytes in the liver and normal mucosa epithelium (E) in the stomach. In the dose group at 500 mg/kg there was moderate and slight lymphocyte hyperplasia (LH) in the kidney. The liver showed moderate pyknosis (PK) of hepatocytes nucleus and the stomach had normal features. While at 1000 mg/kg, it showed slight lymphocyte hyperplasia (LH) in the kidney. The liver showed vascular congestion (VC), sinusoidal congestion (SC) and slight vacuolation and necrosis (VCN). The stomach showed slight lymphocyte hyperplasia (LH). Also at 1500 mg/kg, the kidney showed slight tubular necrosis (TN), slight glomerular necrosis (GN) with lymphocyte hyperplasia (LH). The liver showed slight kupfer cell hyperplasia (SKH) and the stomach had moderate lymphocyte hyperplasia (LH) (Plate XVII-XXVI).



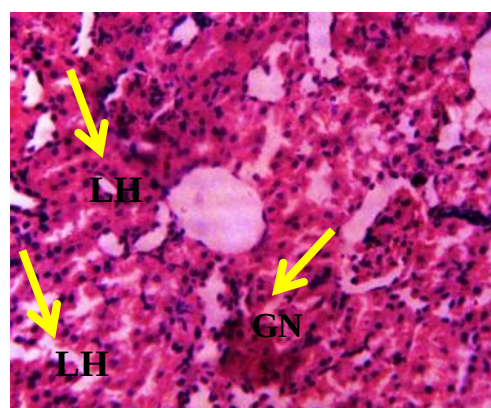


a). Section from the kidney of Wistar Rat from the control group (1 mL/kg distilled water) show

b). Section from the kidney of Wistar Rat treated with 500mg/kg of ethylacetate extract show moderate and slight lymphocyte hyperplasia



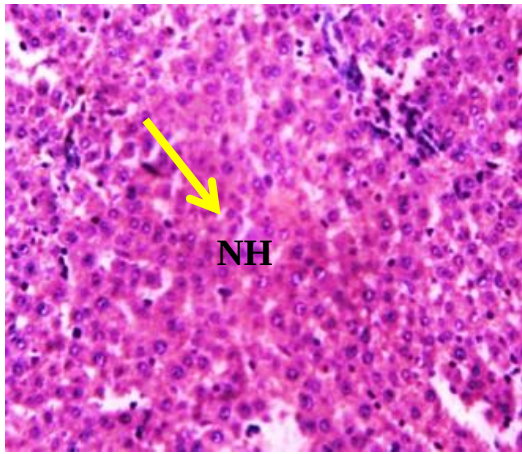
c). Section from the kidney of Wistar Rat treated with 1000mg/kg of ethylacetate extract show slight lymphocyte hyperplasia



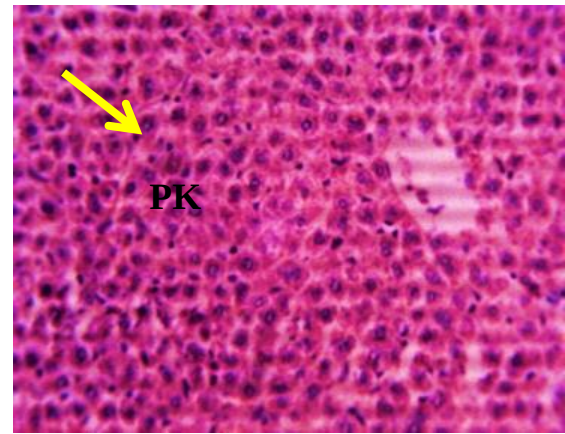
d). Section from the kidney of Wistar Rat treated with 1500mg/kg of ethylacetate extract show slight tubular necrosis, slight glomerular necrosis with lymphocyte hyperplasia

KEY: G = Glomerular; T = Normal tubules; LH = Lymphocyte hyperplasia; TN = Tubular necrosis; GN = Glomerulusnecrosis

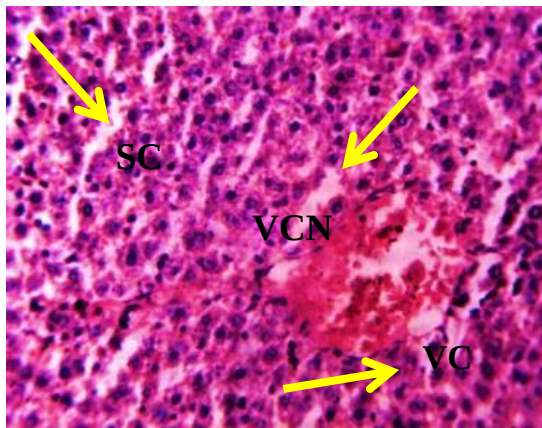
Plate XVII-XX (a-d): Photomicrographs of the kidney section of Wistar Rats for the control and treated groups using the ethylacetate extract of *Daucus carota* (x40)



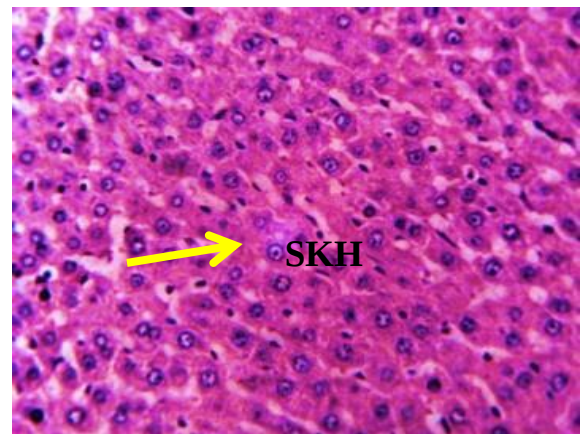
a). Section from the liver of Wistar Rat from the control group (1 mL/kg distilled water) show the normal hepatocytes



b). Section from the liver of Wistar Rat treated with (500mg/kg) show moderate pyknosis of hypertocytes nucleus



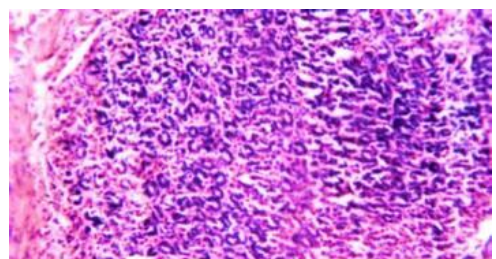
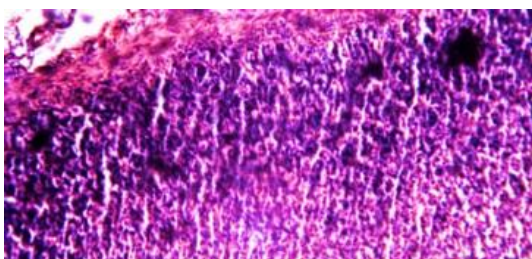
c). Section from the liver of Wistar Rat from the treated with 1000mg/kg show vascular congestion sinusoidal congestion and slight vacoulation necrosis

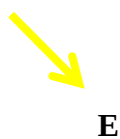


d). Section from the liver of Wistar Rat with treated (1500mg/kg) show slight kupfer cell hyperplasia

KEY: NH= Normal hepatocytes;VCN = Vascular congestion necrosis; SC = Sinusoidal congestion; PK = Pyknosis of hptocytes nucleus
SKH = Kupfer cell hyperplasia; VC = Slight vacoulation necrosis

Plate XXI-XXIII (a-d): Photomicrographs of the liver section of Wistar Rats for the control and treated groups using the ethylacetate extract of *Daucus carota*(x40)





E

a). Section from the stomach of Wistar Rat from the control group (1 mL/kg distilled water) show the normal mucosa epithelium



NC

b). Section from the stomach of Wistar Rat treated with 500mg/kg of ethylacetate extract show the normal stomach features



c). Section from the stomach of Wistar Rat treated with 1000mg/kg of ethylacetate extract show slight lymphocyte hyperplasia



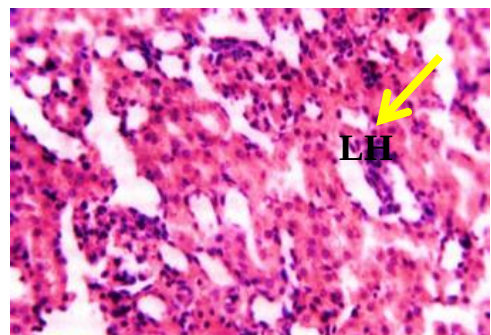
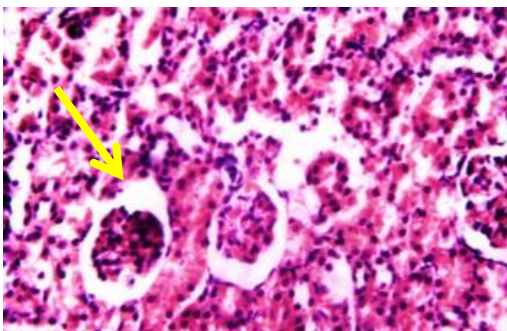
d). Section from the stomach of Wistar Rat treated with 1500mg/kg ethylacetate extract show moderate lymphocyte hyperplasia

Key: E = Normal Mucosa Epithelium; NC= Slight apical epithelium necrosis; LH = Lymphocyte hyperplasia

Plate XXIV-XXVI (a-d): Photomicrographs of the stomach section of Wistar Rats for the control and treated groups using the ethylacetate extract of *Daucus carota* (x40)

4.5.5 Histopathological effects of the *Daucus carota* aerial part methanol extract

The histopathological effects of the control group of the methanol extract showed normal tubules (T) and glomerulus (G) in the kidney, normal hepatocytes in the liver and normal mucosa epithelium (E) in the stomach. The 500 mg/kg of the kidney was observed to show slight tubular distortion (TD) and lymphocyte hyperplasia (LH). The liver showed slight hepatocellular necrosis (HN) with kupfer cell hyperplasia (KH). The stomach showed slight lymphocyte hyperplasia (LH). The kidney revealed slight glomerular necrosis (GN), vascular congestion (VC) with lymphocyte hyperplasia (LH) at 1000 mg/kg. The stomach showed moderate lymphocyte hyperplasia (LH). There was, slight tubular distortion (TD) at 1500 mg/kg was observed in the kidney. The liver showed vascular congestion (VC) and lymphocyte hyperplasia (LH) and slight hepatocellular necrosis (HN). The stomach showed normal feature (Plate XXVII-XXXII).



G



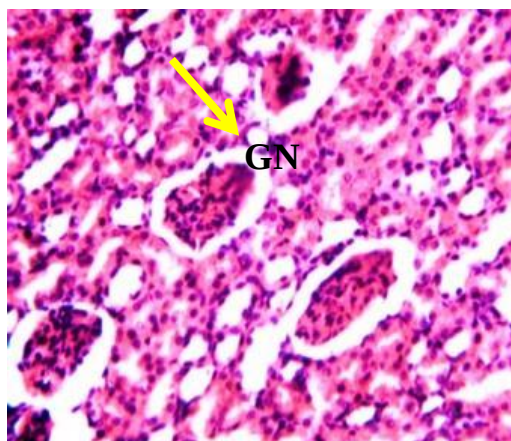
T

a). Section from the kidney of Wistar Rat from the control group (1 mL/kg distilled water) show the normal architecture tubules (T) and glomerulus

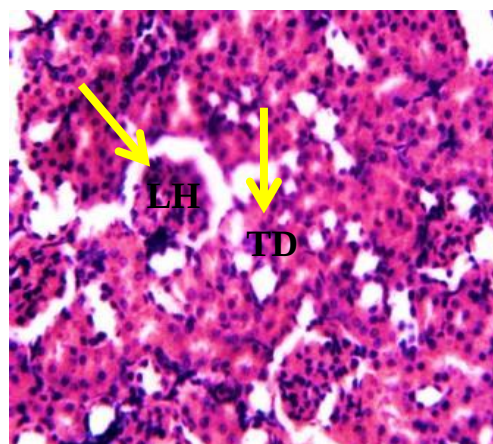


TD

b). Section from the kidney of Wistar Rat treated with 500mg/kg methanol extract slight tubular distortion and lymphocyte hyperplasia



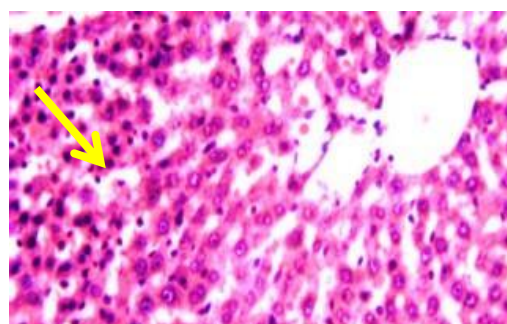
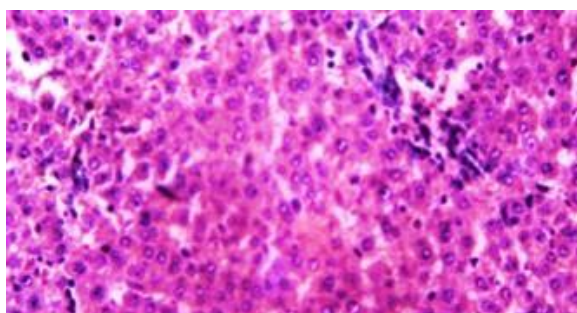
c). Section from the kidney of Wistar Rat treated with 1000 mg/kg methanol extract show slight glomerular necrosis



d). Section from the kidney of Wistar Rat with 1500mg/kg methanol extract show slight tubular distortion and lymphocyte hyperplasia

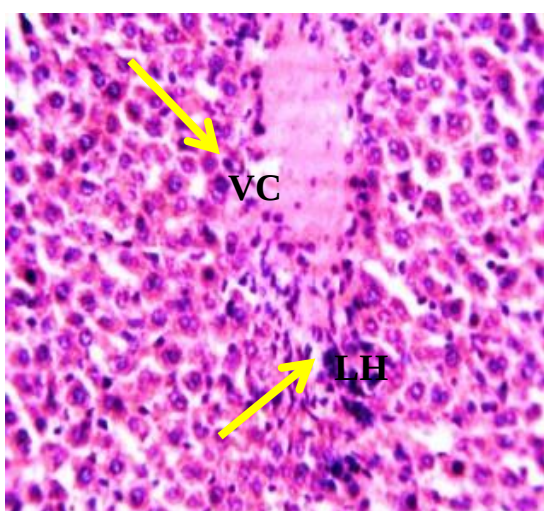
KEY: GN = Glomerular necrosis; T = Normal tubules; TD = Slight tubular distortion; NC = Slight hepatic necrosis; LH = Lymphocyte hyperplasia; HN = Slight hepatocellular necrosis; G = Glomerular;

Plate XXVII-XXVIV (a-d): Photomicrographs of the kidney section of Wistar Rats for the control and treated groups using the methanol extract of *Daucus carota*(x40)





a). Section from the liver of Wistar Rat from the control group (1 mL/kg distilled water) show the normal hepatocytes

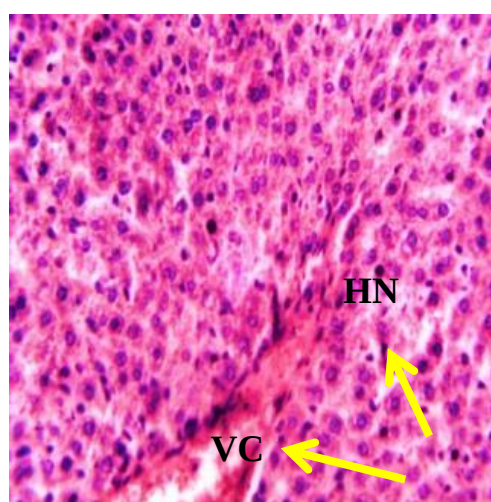


c). Section from the liver of Wistar Rat treated with 1000mg/kg methanol extract shows vascular congestion with lymphocyte hyperplasia.

KH



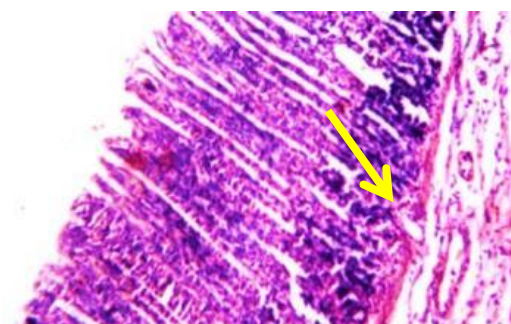
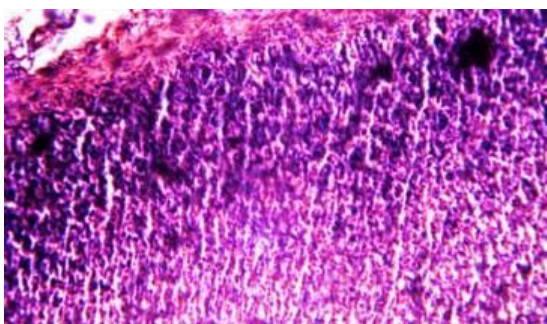
b). Section from the liver of Wistar Rat treated with 500mg/kg methanol extract shows slight hepatocellular hyperplasia



d). Section from the liver of Wistar Rat treated with 1500mg/kg methanol extract shows vascular congestion with lymphocyte hyperplasia and slight hepatocellular necrosis.

KEY: G = Glomerulus; T = Normal tubules; HN = Slight hepatocellular necrosis; VC = Vascular congestion; LH = Lymphocyte hyperplasia; NH= normal hepatocytes

Plate XXX-XXXII (a-d): Photomicrographs of the liver section of Wistar Rats for the control and treated groups using the methanol extract of *Daucus carota*(x40)

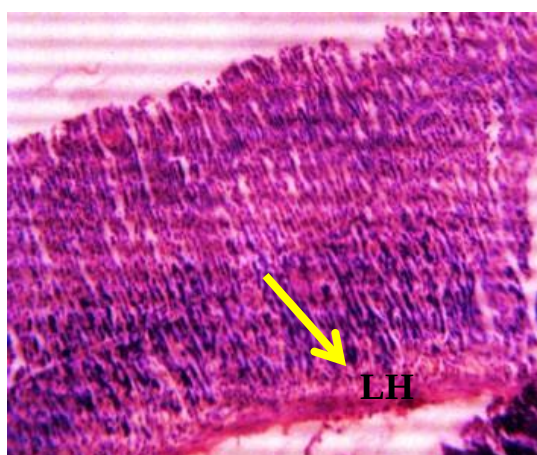


LH

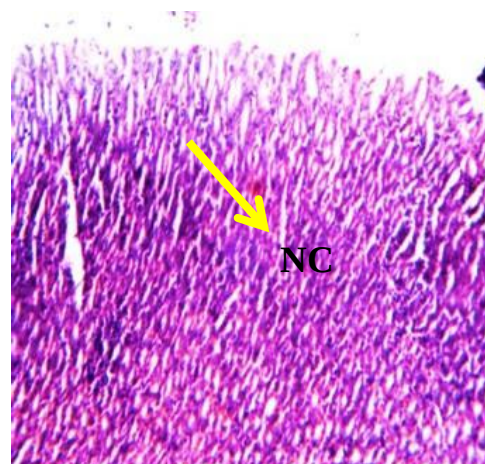


a). Section from the stomach of Wistar Rat from the control group (1 mL/kg distilled water) show the normal mucosa epithelium

b). Section from the stomach of Wistar Rat from the methanol treated 500mg/kg show slight lymphocyte hyperplasia



c). Section from the stomach of Wistar Rat from the methanol treated 1000mg/kg show moderatelymphocyte hyperplasia



d). Section from the stomach of Wistar Rat from the methanol treated 1000mg/kg show normal features

Key:LH = Lymphocyte hyperplasia; E = Normal Mucosa Epitheliu; NC= Slight apical epithelium necrosis; E= normal mucosa epithelium

Plate XXXIII-XXXV (a-d): Photomicrographs of the stomach section of Wistar Rats for the control and treated groups using the methanolextract of *Daucus carota*(x40)

4.6 Radioactivity Assessment of *Daucus carota* aerial part

The average gross alpha activity concentration of *Daucus carota* aerial part was $6.36 \pm 0.25 \text{ Bq/g}$ and the beta activity was $2.37 \pm 0.32 \text{ Bq/g}$ (Fig 4.6).

The average mean of the three naturally occurring radioactivity concentration detected in the *D. carota* aerial part showed the (Potassium-40) ^{40}K ($389.52 \pm 6.27 \text{ Bq/kg}$), (Thorium) ^{232}Th ($84.85 \pm 2.39 \text{ Bq/kg}$) and (Radium) ^{226}Ra ($29.13 \pm 2.66 \text{ Bq/kg}$) (Fig 4.7).

The radiation health hazard indices in the *D. carota* aerial part were calculated. The average results of three determinations showed D (82.39 nGy/h), AEDE (0.10 mSv/y), Ra_{eq} (180.46 Bq/Kg), H_{ex} (0.49 mSv/y), H_{in} (0.57 mSv/y), I_{γ} (0.52 Bq/Kg), I_{α} (0.15 Bq/Kg), AGED (573.66 mSv/yr) and ELCR (1.98 E-03) (Table 4.11).

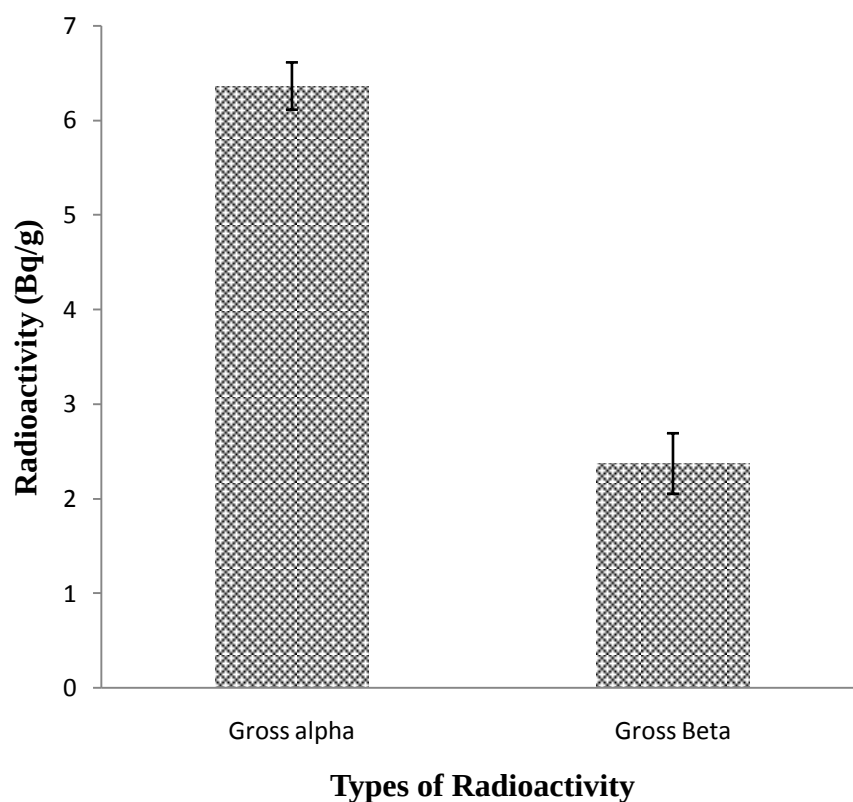


Fig. 4.6: Alpha and Beta Radioactivity Concentration of *Daucus carota* Aerial Part

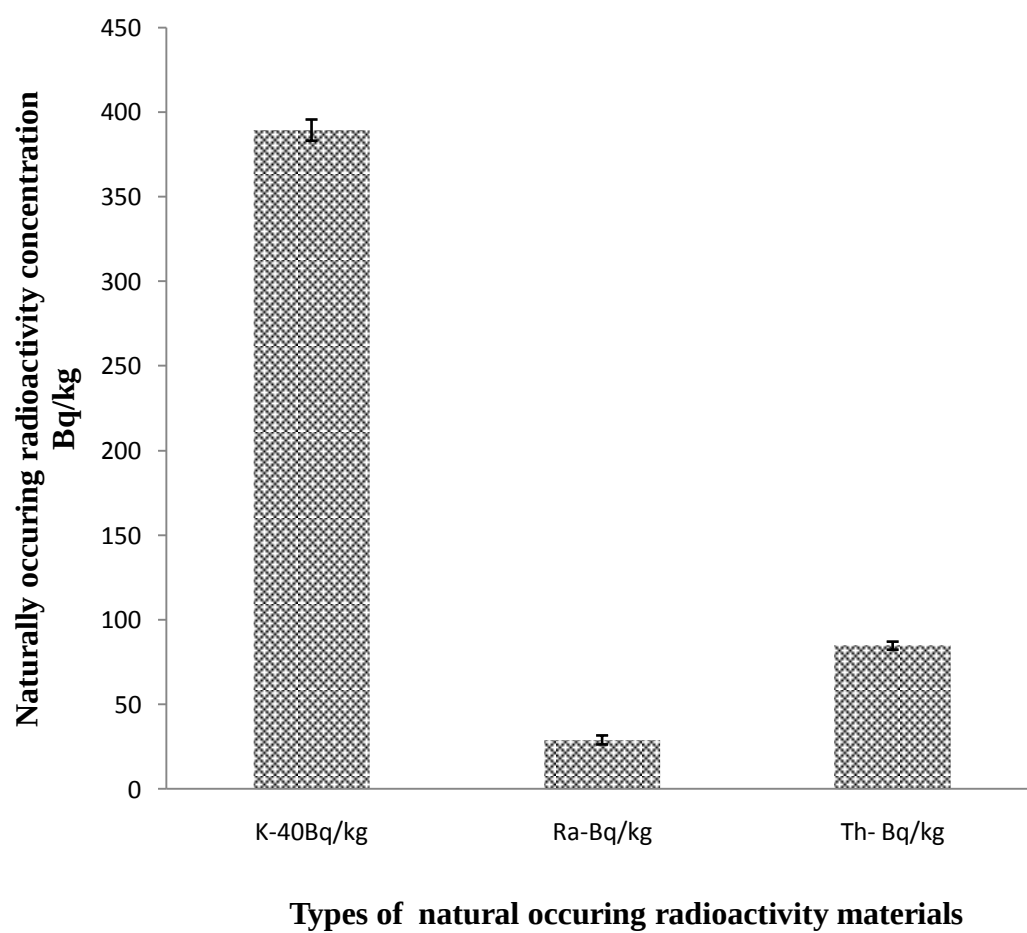


Fig. 4.7: Naturally Occurring Radioactive Material in *Daucus carota* Aerial Part

Table 4.11: Radiation Health Hazard indices of *Daucus carota* Aerial part

Health hazard indices	Mean Hazard
Absorbed dose rate D (nGy/h)	82.39
Annual effective dose rate AEDE (mSv/y)	0.10
Radium equivalent activity index Ra_{eq} (Bq/Kg)	180.46
External hazard index H_{ex} (mSv/y)	0.49
Internal hazard index H_{in} (mSv/y)	0.57
Representative level index I_{γ} (Bq/Kg)	0.52
Representative level index I_{α} (Bq/Kg)	0.15
Annual gonad equivalent dose AGED mSv/yr	573.66
Excess lifetime cancer risk (E-03)	1.98

Average of three determinations

CHAPTER 5

5.0 DISCUSSION

The morphological and organoleptic features showed some important features that are unique to the *Daucus carota* aerial part when compared with other member in the family of Apiaceae (Table 1). These morphological features would serve as a tool for its proper identification from similar plant species. These results are in line with Heywood *et al* (2008) and Alina *et al* (2009) that reported similar morphological features in some species in the Apiaceae family.

Daucus carota leaf has numerous diacytic type stomata on the lower epidermal surface and frequent on the upper epidermal surface. It has a wavy epidermal layer, lateral covering sheath, possession of osteole, subsidiary and guard cells which make it an organized crude drug and also revealed its potentials for transpiration and water propensity (Plate III-IV). Alina *et al* (2009) reported similar microscopic features on *Anethum graveolens* (Dill.) in the Apiaceae family and reported diacytic stoma as the stomata type present in the leaf. This could be the major stomata type in the Apiaceae family. But Santhan (2014) reported paracytic stomata type in *Centella asiatica* in the Apiaceae family, these differences may be attributed to different geographical distributions and wide evolutionary taxonomical relationship in the family between the two species. These results were in conformity with Ghani (1990); WHO (2011); Kunle *et al* (2012)

and Santhan (2014) that reported the need to establish some diagnostic features in some medicinal plants species in order to prevent adulterations.

The quantitative leaf microscopy of *Daucus carota* revealed different numerical standards on lower and upper surface. These standards could be used as baseline for its standardization and preparation of its monograph (Kunle *et al.*, 2012). However, there could be variation based on geographical distribution, time of harvesting, inter or intra species variation, postharvest factors and other environmental conditions (Table 4.2).

The powdered aerial part revealed some cell inclusion and cell contents materials (Table 4.3). These include cellulose, mucilage, starch, lignin, tannins, suberins, calcium carbonate and aleurone grains. Calcium oxalate crystal was found to be absent (Table 4.3).

The physicochemical parameters of the *Daucus carota* aerial part assessed the moisture content, total ash, acid insoluble ash, water soluble ash, ethanol extractive and water extractive values in order to serve as a reference standards in assessing the quality and purity of aerial part as a crude drug. The low moisture content would discourage bacteria, yeast, mould and fungi growth and probably could be stored for long period without losing its natural character since the British Herbal Pharmacopoeia (1990) and WHO (2011) reported that the percentage of moisture content in any crude drug should be within 12-14%. This moisture content percentage was similar to the work of Fazal and Singla, (2012) on *Apium graveolens* a member of *Daucus* genus. The total ash content was attributed to the presence of organic material like carbonate, silicate and other impurities in *D. carota* aerial part. The total ash content suggested a load of contaminations that could be directly or indirectly deposited on the aerial part. This could be used as criteria to judge the purity of drug (Mukherjee, 2008; Prasad *et al.*, 2012). The acid insoluble ash indicated some levels of contamination of *D. carota* aerial part with earthy material, sand and other contaminants in the crude drug. However, the result is within the permissible standard in evaluation of crude drug (WHO, 2011). The water soluble ash could be used to

estimate the amount of inorganic compound present in the crude drugs (Table 4.4). This results was in agreement with B. H. P (1990); WHO (2011); (Kanerla and Chanda, 2011) and (Sumitra, 2014) that have reported permissible limits of ash contents in crude drugs.

However, factors such as the use of fresh plants, age and part of plant collected, period, time and method of collection, temperature of processing, exposure to light, availability of water, nutrients, drying, packing, transportation of raw material and storage, can greatly affect the quality, purity and as well as the therapeutic value of herbal medicines (Kunle *et al.*, 2012).

Apart from these criteria above, factors such as the method of extraction, contamination with microorganisms, heavy metals contents and pesticides residues could also affect the quality, safety, and efficacy of herbal drugs (Roberts and Tyler, 1997; Blumenthal *et al.*, 1998; Barnes *et al.*, 2007 and Kunle *et al.*, 2012). The water extractive value ($21.80 \pm 1.10\%$) showed the highest extractive solvent value when compared with the alcohol extractive value ($15.60 \pm 0.55\%$). The high water extractive value indicated that water extract have the ability to extract more phyto constituents than alcohol extract base on their polarity. This is in agreement with the work of Ajazuddin and Shailendra (2010) that stated that, water extractive value is a better solvent of extraction than ethanol because water is a universal solvent and its use mostly as solvent by traditional healers and individual in crude drug preparation. Although, alcohol extraction is still choice of solvent when it comes to medicinal plant researches due to the fact it does not accommodate or proliferate microorganism growth and may easily be evaporated and handle. These results are similar to the findings of Pathak *et al.* (2011); Fazal and Singla (2012); Kunle *et al.* (2012). The solvent choice in a research involving plants depend on many factors among which include the diversity of different phytochemicals to be extracted and also what is intended to carried out with the extract as well as the cost and availability of the solvents (Tiwari and Mishra, 2010).

Furthermore, the fluorescence analyses revealed the nature of the *D. carota* aerial part under daylight, UV light at short (265nm) and under long wave length (365nm). The results showed different visible fluorescence region ranging from green to dark green (Table 4.5). Brown fluorescence was also observed when the aerial part was mixed with dilute HCL while there was no fluorescence when *D. carota* aerial part was mixed with water under short and long wavelength. Similar findings were reported by Kokashi *et al*(1958); Kunle *et al*(2012) and Shivani *et al* (2013). This result could also be used as guide in pharmacognostic standardization and its utilization as crude drug (African Pharmacopoeia, 1985; Sumitra, 2014).

The preliminary phytochemical screening revealed the absence of alkaloids, cardiac glycosides, saponins, flavonoid and anthraquinone in both the n-hexane and ethylacetate extracts (Table 4.6). This could be attributed with the evidence that secondary metabolites are based on solvent contact, polarity, procedures and duration (Kokate *et al.*, 2003; Evans, 2009; Ayinde and Agbakwuru, 2010). The n-hexane extract being non polar constituents extract extraneous material, fatty components in the plant material while the ethyl acetate and methanol extracts that is mainly moderate and polar phyto-constituents extract more phyto-constituents (Kokate *et al.*, 2003). This could be attributed to the non-polar nature of n-hexane (Harborne, 2009); however, the steroids/triterpenes in the n-hexane and ethylacetate may be investigated for possible pharmacological activities such as antioxidant, analgesic and anti-inflammatory (Danjuma *et al.*, 2010). The ethylacetate and methanol extracts showed the presence of saponins, flavonoids, tannins and steroid/triterpenes while anthraquinone and cardiac glycosides were absent the extracts. This result is similar to Vidita *et al* (2013) that reported similar phytochemical constituents in some members of the Apiaceae family. The absence of alkaloid, cardiac glycosides and anthraquinone in the three extracts suggested that the *D. carota* aerial part may have no cardiac glycosides or anthracene derivatives since they are metabolites that are found in few plant families (Farnsworth *et al.*, 1991; Cragg and Newman 2005; Harborne, 2009).

The presence of flavonoids and steroid/triterpenes in the methanol and ethylacetate extracts indicated an antioxidant activity, antimicrobial activity, hepatoprotective; anti-inflammatory activity and other pharmacological actions that could be evaluated and explored. This result is related with the work of Ray (2004); Soetan and Oyewole (2009); Danjuma *et al* (2010) and Dalvir *et al* (2016) that reported similar secondary metabolites and suggested possible pharmacological activities. The presence of tannins in the ethyl acetate and methanol extracts showed that the aerial part may possess anti-nutritional factors especially when the intake is high, though the tannins could be investigated for anti-diarrhoea, cardiovascular protection and anti-inflammatory properties. These findings were similar to the work of Sivanantham and Thangaraj (2015) that reported similar phyto constituents in *Daucus carota* root. Results from the present investigation shows that *D. carota* aerial part is rich in phytochemicals and specific biologically important compounds have been identified in extracts from other studies (Akinmoladun *et al.*, 2007; Danjuma *et al.*, 2010). Also, the phytochemical screening results could be a useful guide as chemotaxonomic markers and could also be used in the chemotaxonomical classification system of Apiaceae family (Jonathan and Tom, 2008). These results may guide further phylogenetic studies in Apiaceae family.

Similarly, the thin layer chromatographic analysis of the n-hexane, ethylacetate and methanol extracts were spotted on TLC plate precoated with analytical silica gel. The n-hexane extract showed eleven spots alongside with their R_f values of 0.09, 0.17, 0.23, 0.36, 0.40, 0.46, 0.50, 0.57, 0.60, 0.79 and 0.91 (Plate V), the ethyl acetate extract showed seven spots with R_f values of 0.16, 0.28, 0.65, 0.81, 0.88, 0.90 and 0.91 (Plate VI) and the methanol extract showed thirteen spots with R_f values of 0.16, 0.24, 0.29, 0.36, 0.42, 0.47, 0.51, 0.54, 0.57, 0.63, 0.66, 0.76 and 0.81. The methanol extract revealed highest number of spots that could be promising for new compounds towards drug development (Plate VII).

The nutritional potential of *D. carota* aerial part showed that the percentage proximate compositions of moisture content of the aerial part may be stored for longer period and may not be susceptible to microbial attack (Figure 4.4). The percentage composition of carbohydrate (51.81%) was moderate and similar to the (55.67%) in *T. terrestris* and in water spinach leaves (54.20%) as reported by Imran *et al.* (2007). This result is between the recommended dietary allowance of 130 g/d (FAO/WHO, 2004) which shows it could be good sources of food and contribute to daily carbohydrate requirement of a healthy individual. The crude protein (14.59%) was higher when compared to (2.11%) in *Amaranthus viridis*, (2.90%) in *C. murale* leaves, (2.76 %) in *N. officinale*, (6.30%) in water spinach and (6.40%) in *Mormordica foecida* leaves (Imran *et al.*, 2007) but lower than (24.80%) in sweet potatoes leaves as reported by Imran *et al.* (2007) and Asaolu *et al.* (2012). The crude fibre (9.07%) is similar to (7.20%) in sweet potato leaves and (13.00%) in *Tribulus terrestris* leaves but lower when compared to *A. viridis* (1.93%) (Akindahunsi and Salawu, 2005). This suggested lower deposit of lipid content in the aerial part. These results are comparable to similar studies carried out by Idoko *et al.* (2014); Mgbemena and Obodo (2016) and Feumba *et al.* (2016) on proximate and mineral compositions of some leafy vegetables consumed in Nigeria and other nutraceutical potential of some fruits peels as sources of vegetables.

The mineral elements detected at different concentrations (ppm) in the *D. carota* aerial part are rich sources of macro and trace elements that function in human during muscle contraction, bone formations, growth, metabolism, osmotic balance, regulatory processes activation and other organic bimolecular activities (Rabia *et al.*, 2012). This result was compared with FAO/WHO (2004); Asaolu *et al.* (2012) on required permissible limits of elements in edible plants and they were found within the permissible limits (Fig. 4.5). The high amount of phosphorus in the *D. carota* aerial part could be as a result of its relative abundance in nature and its deposit could be through atmosphere and other activities in the soil. Phosphorus is essential component of bone

mineral (Rabia *et al.*, 2012). The deficiencies of phosphorus and calcium imbalances could result in osteoporosis, arthritis, rickets and tooth decay (Asaolu *et al.*, 2012). Potassium is an essential element (Jwanbot *et al.*, 2012) that is controlled homeostatically in human cells (Shanthi *et al.*, 2012). Potassium and magnesium also helps in the functioning and contraction of muscles (Asaolu *et al.*, 2012). Since carrot root help in insulin secretion and its function also require in magnesium (Guerrera, 2009; Bartlett and Eperjesi, 2008; Kim *et al.*, 2010) Manganese also help in carbohydrate metabolism and coordination with enzymes in the body. It is used by the body as a co-factor for the antioxidant enzyme, superoxide dismutase (Dias, 2012 and Dias, 2012).

The presence of iron content in the *D. carota* aerial part could play crucial roles in haemopoietic and also control infection and cell-mediated immunity (Bhaskaran, 2001). The deficiency of iron has been described as the most prevalent nutritional and iron-related to anemia diseases and was estimated to affect more than one billion people worldwide (Trowbridge & Martorell, 2002).

Some certain mineral elements such as calcium, selenium, sodium, copper, iron, magnesium and sodium are also essential elements that are responsible for the curative properties in medicinal plants including antioxidant activity (Serfor-Armah *et al.*, 2003; Zhang and Hamauzu, 2004). They show anti-carcinogenic activities, reduce inflammation, play roles in metabolism and have physiological functions on their host and also control immune response.

The presence of heavy metals might be attributed to the toxic components that are present in the *D. carota* aerial part, although, they were in lower concentrations but may have effects on man when consumed or ingested as prescribed by WHO (2007). Its accumulation may have adverse effects over long period of time. But with good agricultural practices, lower use of chemicals, and suitable source of irrigation the toxic components could be prevented. However, there may be possible anti-nutritional potentials in the *D. carota* aerial part, this includes tannin, saponins and phytate which when ingested over a long period of time could cause stomach

cramps and intestinal disorders. The bioavailability of the nutrients demands more studies such as digestibility of the proteins and fats and existence of anti-nutrients, like metal chelators and interaction of these anti nutritional (phytate and oxalates) and protease inhibitors (Gholamreza *et al.*, 2015) that could reduce the risk that may be associated with it.

The fixed acute oral toxicity for *D. carota* aerial part extracts showed no mortality or any toxic signs such as tiredness, abdominal constrict convulsion, hyperactive, weakness, diarrhoea or increased diuresis even at a high dose of 5000 mg/kg. These extracts could be assumed practically safe and non-toxic. This result is in agreement with Olorunisola *et al.* (2012); Parasuraman *et al.* (2014); Ugbogu *et al.* (2016) and Adesegun *et al.* (2016) that reported acute toxicity of plants could be considered practically nontoxic and safe above oral administration of 5000 mg/kg. However, Osilon (2000); Pak *et al.* (2004); Maikai *et al.* (2008); Obidike and Salawu (2013) concluded that, further toxicity assay should be carried out in order to reveal possible long term toxicity effect on the physiology and organs for proper recommendation on its utilization.

The effects of oral administration of n-hexane and methanol extracts on the Wistar Rats showed statistically significant decrease ($p \leq 0.05$) on body weight at 500 mg/kg, 1000 mg/kg and 1500 mg/kg at week 2 when compared with the initial body weight of Wistar rats at week 0 and other weekly weights in the extracts (Table 4.6). This decrease may be attributed to presence of some phytoconstituents that are present in the n-hexane and the methanol extracts. Furthermore, the laboratory rats may have been able to metabolize the toxic component in the n-hexane and methanol extracts that could interfere with their appetite and food consumption (Kasimu, 2014). This result is comparable with similar studies carried out by Chokshi (2007); Rhiauani *et al.* (2008); Salawu *et al.* (2009); Nandy and Datta (2012).

The ethylacetate extract showed no statistically significant difference ($p \geq 0.05$) on the body weight of the Wistar Rats. This suggested the extract may have no chemical effect on the appetite

and food consumption of the Wistar Rats (Grance *et al*, 2008; Nandy and Datta 2012) since the ethyl acetate extract contain presence of moderate class of secondary metabolites (Table 4.7).

The haematological parameters of the extracts of *D. carota* aerial part showed statistically significant difference ($p \leq 0.05$) on the RBC at 500 mg/kg, 1000 mg/kg and 1500 mg/kg when compared with the control group (Table 4.8). However, there was a mean decreased of the RBC on n-hexane and ethyl acetate extracts. This reduction of RBC could be as a result of fatty acid or invasion of other foreign components present in the extracts, since red blood cells are involved in the transportation of oxygen and carbon dioxide into the body (Isaac *et al.*, 2013). Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Soetan *et al.*, 2013; Isaac *et al.*, 2013). The extracts probably contained some classes of secondary metabolites that could interfere with the hematopoietic system. This result is comparable with the work of Guyton and Hall (2000) that reported similar reduction in the red blood cells in some plant species. The hematological parameters of the methanol extract of *Daucus carota* aerial part showed no statistically significant difference ($p \geq 0.05$) when compared with the control group but may have some anaemic actions due to reduction in the red blood cell in hexane and ethyl acetate extracts. This probably showed that the extracts contained some hematopoietin-like principle that could reduce the red blood cell (Adeneye, 2008; Palani *et al.*, 2009; Wararutet *et al.*, (2012). The haematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in man and animals (Adeneye *et al.*, 2006).

However, there was no statistically significant difference ($p \geq 0.05$) in the WBC, HGB and HCT in the extracts; this probably did not affect the immune systems but might be susceptible to infection. However, the results of hematological parameter are within the internationally accepted reference range for each hematological parameter (Osilon *et al.*, 2000; Daramola *et al.*,

2005; Research Animal Resources, 2009; Uboh *et al.*, 2012; NseAbasi *et al.*, 2014; Kifayatullah *et al.*, 2015).

The liver function test showed alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP) and albumin (ALB) levels in the experimental animals respectively. They are commonly used indicators of liver injury and alanine aminotransferase (ALT) and aspartate aminotransferase (AST) often showed the assessment of liver damage by drugs or any hepato-toxin (Jaijoy *et al.*, (2011); Salihu (2011) and Kasimu (2014). The liver injury test revealed that the *Daucus carota* aerial part extracts showed statistically significant difference ($p \leq 0.05$) on the ALP of the n-hexane, ethyl acetate and methanol extracts. The decrease in the ALP may be as a result of hepatocellular injury on the animals or caused blood leakages in the hepatocytes (Aboudoulatif *et al.*, 2010). There was no statistically significant difference ($p \geq 0.05$) in the ALT, AST, TP and ALB. (Table 4.9).

The effect of oral administration of the n-hexane extract on the kidney function test showed that there was no statistically significant difference ($p \geq 0.05$) on the urea, potassium, creatinine and chloride. Potassium is the major intracellular monovalent cation in the body and is filtered freely at the glomerulus of the kidney, reabsorbed in the proximal tubule and secreted into the distal segment of the nephron (Schwartz and Garrison 2008). However, the n-hexane and methanol extracts showed statistically significant difference ($p \leq 0.05$) on bicarbonate (mmol/L) at 1500 mg/kg when compared with the control group. This could be attributed to dose dependent in the extracts and probably have influenced the regulation of other kidney parameters (Table 4.10).

There was no statistically significant difference ($p \geq 0.05$) on the urea, sodium, potassium, creatinine and chloride of the n-hexane and ethyl acetate extracts but sodium was statistically significant ($p \leq 0.05$) in the methanol extract at 500mg/kg. Since sodium is the principal cation of the extracellular fluid and plays a large part in the therapy of fluid and electrolyte disturbances (Wararut *et al.*, 2012). The result was different with the work of Salawu *et al.* (2008); Nandy and

Datta (2012) and Jaijoy *et al.* (2011). Wararut *et al.* (2012) reported increased doses increases the kidney function parameters when compared with control group in *Securinega virosa*(Salihu, 2011)and maycause renal failure and malfunctions of the kidney.

The histopathological effects of the n-hexane, ethyl acetate and methanol extracts at 500 mg/kg showed moderate and slight tubular necrosis in the kidney, while the liver had slight hepatic necrosis with moderate lymphocyte hyperplasia. At 1000 mg/kg and 1500 mg/kg moderate lymphocyte hyperplasia was observed in the kidney and vascular congestion with kupfer cell hyperplasia was observed in the liver. The 500 mg/kg dose of the extracts showed that the lowest dose produces slight detectable toxic reaction (Leclair and Willard, 1970). The stomach revealed normal epithelium cells when compared with control group (Gregory and Cheryl, 1998) with few apical necroses on epithelium cells (Plate VIII- XXXII). This probably explains the significant difference in the liver function test in spite ofthe hepatocellular degeneration and necrosis observed with the histopathological findings (Kirchain and Allen,2008; Salihu, 2011; Kasimu; 2014).

Generally, the n-hexane, ethylacetate and methanol extracts may be dose dependent and not be safe at high doses or at high consumption as vegetable. Since, the toxicity target organs (the liver and kidney)led to alteration or disorder in the histological function of animals and man (Tulsiani *et al.*, 1998; Wararut *et al.*, 2012)or could cause glomerula nephritis or other liver and kidney diseases when consumed even at high doses. The histopathological differences in the treated groups of the three extracts might have probablyinfluenced the low level of RBC, WBC and HGB that could cause haemolytic anaemia or weak defensive mechanisms (Soetan *et al.*, 2013) and that low adaptability to local environmental could cause disease prevalent conditions (Kabir *et al.*,2011; Okunlola *et al.*, 2012; Isaac *et al.*, 2013).This suggested the toxicities on the liver and kidney in the extracts are probably cytotoxic and not haematotoxic since there was no significant difference ($p \geq 0.05$) in the heamatological parameters.

The *Daucus carota* aerial part radioactivity concentration indicated that the gross alpha has high activity concentration ($6.36 \pm 0.25 \text{ Bq/g}$) and beta activity ($2.37 \pm 0.32 \text{ Bq/g}$). This high radiation could be as a result of direct and indirect contamination during agricultural activities. Other environmental components deposit from the river source, poor irrigation system, effluents or other contaminants discharged could trigger the radioactivity concentration. This result was compared with UNSCEAR, (2000) and Adewumi (2011) that report permissible limits of gross alpha activity to be 0.1 Bq/g and beta activity to be 1.0 Bq/g .

However, Adewumi (2011) established the needs to quantify gross alpha and beta particle contents of ingestible materials due to gross ionization energy that its deposits could damage body tissues and ingestion could lead to alteration of genetical material in human body. Avwiri *et al.* (2011); Adewumi (2011); Shanthi *et al.* (2012); Jwanbot *et al.* (2012); Njinga *et al.* (2016) and Idoko *et al.* (2016) reported that geographical locations have different radionuclides activities concentration.

The naturally occurring radioactive materials (NORMs) of *Daucus carota* aerial part showed high activity concentration of potassium-40 ($389.52 \pm 134.52 \text{ Bq/kg}$) when compared with 412.00 Bq/kg , while Thorium ($84.85 \pm 64.85 \text{ Bq/kg}$) was also high when compared with 45.00 Bq/kg and Radium ($29.13 \pm 10.66 \text{ Bq/kg}$) was low when compared 32.00 Bq/kg (Shittu *et al.*, 2015). The NORMs permissible limit was Potassium is an essential element that is controlled homeostatically in human cells (Shanthi *et al.*, 2012; Jwanbot *et al.*, 2012). However, these results were compared with worldwide average estimated specific activity of NORMs in the earth crust. This showed that ^{40}K and ^{226}Ra were within the permissible limits but and ^{232}Th was above the permissible limit. Also, (^{232}Th), an alpha particle emitter can settle in the lining of bones and caused bone cancer or bone marrow disorder (Cherry *et al.*, 2012). This result was similar to the work of Ortega *et al.* (1996); Jwanbot *et al.* (2012); Shanthi *et al.* (2012); Tchokosa *et al.* (2013); Jibiri and Fasae (2013) and Njinga *et al.* (2015) that reported that leafy vegetables seem to absorb

more radionuclide concentration in some food crops and medicinal plants. However, *Daucus carota* aerial part and even carrot may not be safe for human and livestock in highly deposited NORMs environment since there is cycle chain uptake from the soil to root and to aerial part. Jwanbotet *al.* (2012) also studied NORMs in some food crops in Jos Plateau state using gamma ray spectrometry and it was observed that ^{40}K , ^{226}Ra and ^{228}Th are relatively high which may be attributed to tin mining activities that have taken place in those areas in past decades.

Herbal preparations in relation to the level of NORMs in medicinal plants are associated with most forms of leukemia and with cancer of many organs such as the bone, in the tissues of the lung, breast and thyroid in the long term (UNSCEAR, 2000).

The radiation health hazard indices of the *Daucus carota* aerial part showed high average absorbed dose (82.39 nGy/h) when compared with the 60.00 nGy/h estimated in the world average. The annual effective dose rate (0.10 mSv/y) was low when compared with 1.00 mSv/y, radium equivalent activity (180.46 Bq/kg) when compared 370.00 Bq/kg while the external hazard index (0.49 mSv/y) and internal hazard index (0.57 mSv/y), representative level gamma index (0.52 Bq/kg) and representative level alpha index (0.15 Bq/kg) were within acceptable limits with 1.00 mSv/y and 1.00 Bq/kg in the world average estimate indices. But the annual gonad equivalent dose (573.66 mSv/yr) showed higher dose when compared with 300 mSv/yr which its accumulation could suggest threat to sensitive cells like the gonad, bone marrow or thyroid (Anekwe and Avwiri 2016). The excess lifetime cancer risk (1.98×10^{-3}) showed higher cancer risk when compared with 0.29×10^{-3} estimated in the world average permissible limits of 70 years life expectancy (Table 4.11).

The high absorbed average dose, high annual gonad value and high excess lifetime cancer risk values could be attributed to indiscriminate activities of human and other poor agricultural practices. Nevertheless, the radiocativity concentration may be locally dependent however but with good quality agricultural practices, lower use of fertilizer and other quality control measures

(Jibiri and Fasae 2013) its accumulation may produce minimal adverse effect but with carefulness if to be use.

CHAPTER 6

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Some pharmacognostic standards of the *D. carota* leaf and aerial part were established to guide towards its proper identification, quality and purity as a crude drug for further pharmaceutical utilizations. The result also shows distinct organoleptic and morphological features that could be used as diagnostic features from similar species in the Apiaceae family.

The microscopical features revealed wavy epidermal cells and several diacytic stomata on lower and upper surfaces that could enhance water transpiration. The chemo-microscopy revealed the presence of cell wall and cell inclusion materials. The quantitative leaf microscopy of the lower epidermal layer revealed that the stomata number (14.80 ± 0.97), stomata index ($31.22 \pm 3.09\%$) and palisade ratio (6.20 ± 0.37), while the upper epidermal layer revealed stomata number (10.60 ± 0.87), stomata index ($32.55 \pm 1.25\%$), vein islet number (12.80 ± 1.98) and veinlet termination (31.800 ± 2.33). The physico-chemical parameters showed that the moisture content (5.56%), alcohol extractive value (15.60%), water extractive value (21.80%), total ash (11.60%), acid insoluble ash (1.00%) and water soluble ash (5.67%).

The nutritional compositions revealed that the aerial part potential as sources of vegetables but some anti-nutritional factors present in the aerial part include saponins, tannins, alkaloids and hydrogen cyanide glycoside. The preliminary phytochemical screening revealed the presence of steroid/triterpenes, flavonoids, saponins and glycosides among others that could be explored for its pharmaceutical potentials. The thin layer chromatography profile also confirmed the different classes of secondary metabolites present in the crude extracts.

The fixed oral acute toxicity carried out on healthy Wistar Rats showed that the n-hexane, ethyl acetate and methanol extracts are practically safe and non toxic at 5000 mg/kg. The sub chronic toxicity study effect of oral administration of n-hexane and methanol extracts on the body weight of Wistar Rats were statistically significant ($p \leq 0.05$) at 500 mg/kg and 1000 mg/kg at week 2 but the ethyl acetate extract showed no significance difference ($p \geq 0.05$). The hematological parameters on the n-hexane and ethyl acetate extracts showed statistically significant difference ($p \leq 0.05$) and decreased the red blood cell (RBC) in all the treated groups. The methanol extract was not statistically significant ($p \geq 0.05$) and the n-hexane and ethylacetate extracts showed that MCV and MCH were significant ($p \leq 0.05$).

The effect of oral daily administration of the extracts on the liver function parameters showed statistically significant difference ($p \leq 0.05$) on ALP (IU/L) at 500 mg/kg and 1000 mg/kg. The ALT (IU/L), AST (IU/L), TP (IU/L) and ALB (G/DL) showed no significant difference ($p \geq 0.05$). The effects of oral administration of n-hexane extract on bicarbonate showed statistically significant difference ($p \leq 0.05$) at the treated groups and sodium was also significant ($p \leq 0.05$) at 1500 mg/kg. The ethylacetate extract was not significant in any of the kidney function parameters ($p \geq 0.05$). The methanol extract showed that sodium was significant ($p \leq 0.05$) at 500 mg/kg and bicarbonate at 1500 mg/kg. The histopathological effects of the n-hexane, ethyl acetate and methanol extracts at 500 mg/kg showed moderate and slight tubular necrosis in the kidney, while the liver had slight hepatic necrosis with moderate lymphocyte hyperplasia. At 1000 mg/kg and 1500 mg/kg moderate lymphocyte hyperplasia was observed in the kidney and vascular congestion with kupfer cell hyperplasia was also observed in the liver. The 500 mg/kg dose of the extracts showed the lowest dose that produces slight detectable toxic reaction. The stomach revealed normal epithelium cells when compared with the control group. The gross alpha activity concentration of *D.carota* aerial part was 6.36 ± 0.25 Bq/g and beta activity (2.37 ± 0.32 Bq/g). The three naturally occurring radioactive materials showed that ^{40}K

mean activity concentration ($389.52 \pm 134.52 \text{ Bq/kg}$), ^{232}Th ($84.85 \pm 64.85 \text{ Bq/kg}$) and ^{226}Ra ($29.13 \pm 10.66 \text{ Bq/kg}$), The radiation health hazard indices showed average absorbed dose (82.39 nGy/h), radium equivalent activity (180.46 Bq/kg), external hazard index (0.49 mSv/y), internal hazard index (0.57 mSv/yr), representative level gamma index (0.52 Bq/kg) and representative level alpha index (0.15 Bq/kg). The annual gonad equivalent dose (573.66 mSv/yr) showed higher dose and the excess lifetime cancer risk of 1.98×10^{-3} also indicated high cancer risk.

6.2 Conclusion

The studies had established:

- i. some pharmacognostic standards particularly the microscopic, chemomicroscopic, physicochemical, phytochemicals and thin layer chromatographic profiles of *Daucus carota* aerial part.
- ii. the proximate and the mineral element compositions showed that it contains macro and trace elements that support its utilization as livestock feed and vegetables for humans.
- iii. the acute toxicity profile of the extract and it was safe up to 5000 mg/kg . The sub-chronic toxicity study showed significant dose dependent toxicities, that high doses of the aerial part could cause significant liver and kidney injury in the tissues. Also, the alpha, beta and naturally occurring radioactivity materials activity concentrations as well as the radiation health hazard indices in the *D. carota* aerial part showed that the aerial part was not safe and may cause tissue damage and other toxicity related diseases to livestock and man due to its high ionizing radiation.

6.3 Recommendations

The following recommendations were suggested based on the findings of the present study that:

- i. the quantity of the aerial part given to livestock should be carefully measured and kept low to avoid induced hepatotoxicity or other adverse effects on man and livestock.
- ii. the carrot aerial part should not be a major livestock feed if it is to be used, in order to avoid its accumulation in the tissue of animal and potential transfer through food chain process to man.
- iii. carrot root and the aerial part should be produced with good agricultural and standard quality practices. This will prevent or reduced the toxic components in the soil, tissues of plants or through polluted irrigation system entering indirectly into human body.

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APPENDIX

Appendix I: Determination of moisture content of powdered

Description	1	2	3	4	5
Constant weight of crucible (g)	46.31	39.49	55.93	46.31	39.49
Initial weight with the powder (g)	49.31	42.49	58.93	49.31	42.49
Final weight of powder	49.14	42.32	58.75	49.14	42.32
Loss in weight (g)	0.17	0.17	0.18	0.17	0.17
Moisture content (%)	5.67	5	6	5.67	5
Average mean (%)			5.57		
SD			0.51		

$$\% \text{ Moisture content} = \frac{\text{InitialWeight of Powder} - \text{FinalWeight of Powder}}{\text{InitialWeight of Powder}} \times 100$$

$$\frac{49.31 - 49.14 \times 100}{3}$$

$$= 5.57 \%w/w$$

Appendix II: Determination of alcohol – soluble extractive value

Description	1	2	3	4	5
Constant weight of dish (g)	49.91	44.25	32.04	44.25	32.04
Weight of dish and content after heating (g)	50.07	44.4	32.2	44.4	32.2
Alcohol extractive content (g)	0.16	0.15	0.16	0.15	0.16
Alcohol extractive Value (%)	16	15	16	15	16
Average mean (%)	15.6				
SD	0.55				

Sample calculation

$$\text{Alcohol extractive value} = \frac{\text{Wt of dish \& content after heat (g)} - \text{Constant wt.of dish (g)} \times 4}{\text{Initial weight of drug}} \times 100$$

$$\text{Alcohol extractive value} = \frac{(50.07 - 49.91)}{4} \times 100$$

$$= 15.60 \%w/w$$

Appendix III: Determination of Water Extractive value

Description	1	2	3	4	5
Constant weight of dish (g)	30.87	30.85	39.79	30.87	30.85
Weight of crucible and content after heating (g)	31.10	31.06	40	31.1	31.06
Water extractive content (g)	0.23	0.21	0.21	0.23	0.21
water extractive Value (%)	23	21	21	23	21
Average mean (%)			21.8		
SEM			0.49		
SD			1.1		

Sample calculation

$$\text{Water extractive value} = \frac{\text{Wt of dish \& content after heat (g)} - \text{Constant wt.of dish (g)} \times 4}{\text{Initial weight of drug}} \times 100$$

$$\text{Water extractive value} = \frac{(31.10 - 30.87)}{4} \times 100$$

$$= 21.80 \%w/w$$

Appendix IV: Determination of Total Ash

Description	1	2	3	4	5
Constant weight of crucible (g)	31.85	29.83	23.51	31.85	29.83
Weight of crucible and powder (g)	33.85	31.83	25.51	33.85	31.83
Weight of crucible and Ash (g)	32.06	30.08	23.75	32.06	30.08
Weight of Ash (g)	0.21	0.25	0.24	0.21	0.25
Ash Value (%)	10.5	12.5	12	10.5	12.5
Average mean (%)			11.6		
SD	1.02				

Sample calculation= Weight of residue= final weight of crucible + residue – weight of crucible

Weight of Residue = 32.06 – 31.85 = 0.21

Ash value = $\frac{\text{weight of Ash}}{\text{Initial weight of drug}} \times 100$

Ash Value = $\frac{0.21}{2} \times 100$
= 10.50 % w/w

Appendix V: Determination of Acid Insoluble Ash

Description	1	2	3	4	5
Constant weight of crucible (g)	31.85	29.83	23.51	31.85	29.83
Weight of crucible and powder (g)	33.85	31.83	25.51	33.85	31.83
Weight of crucible and Ash (g)	32.06	30.08	23.75	32.06	30.08
Weight of Acid insoluble ash	31.86	29.85	23.54	31.86	29.85
Acid insoluble Ash Value	0.01	0.02	0.03	0.01	0.02
Acid insoluble Ash Value (%)	0.50	1.00	1.50	0.50	1.00
Acid Insoluble Average	1.00				
SD	0.90				

Sample calculation

$$\text{Acid Insoluble Ash value} = \frac{\text{weight of acid insoluble ash}}{\text{Initial weight of drug}} \times 100$$

$$\text{Acid Insoluble Ash value} = \frac{0.01}{2} \times 100$$

$$= 0.50 \%w/w$$

Description	1	2	3	4	5
Constant weight of crucible (g)	31.85	29.83	23.51	31.85	29.83
Weight of crucible and powder (g)	33.85	31.83	25.51	33.85	31.83
Weight of crucible and Ash (g)	32.06	30.08	23.75	32.06	30.08
Weight of Ash (g)	0.21	0.25	0.24	0.21	0.25
Water soluble ash	31.95	29.96	23.62	31.95	29.96
Water soluble Ash value	0.1	0.13	0.11	0.1	0.13
Water soluble ash %	5	6.5	5.5	5	6.5
Average Water soluble ash %		5.67			

Sample calculation

$$\text{Water soluble Ash value} = \frac{\text{Wt of total ash} - \text{Wt of Water Insoluble Ash}}{\text{Initial weight of drug}} \times 100$$

$$\text{Water soluble Ash value} = \frac{(0.21-0.10)}{2} \times 100$$

Sample ID	K-40Bq/kg	Error Bq/kg	Ra-Bq/kg	Error Bq/kg	Th- Bq/kg	Error Bq/kg
NOIDC001	533.4906	1.9842	22.0562	1.4784	159.5172	3.6567
NOIDC002	367.9949	10.3502	41.3953	3.8359	52.4122	2.2412
NOIDC003	267.0671	6.4354	23.9434	2.6771	42.6218	1.2582
Average NOIDC	389.5175	6.2566	29.1316	2.6638	84.8504	2.385367
World Average	412.0000		32.0000		45.0000	

Values are means $\pm$ Error (Bq/kg) of triplicate samples

CPS = Count per second

Bq/Kg = Becquerel per kilogram

Calibration factors: K-40 = 0.000643, Ra-226 = 0.000863, Th-232 = 0.000877

Conversion factor: $\times 10^{-4}$ cps/BqKg-1

Sample ID	Alpha Radioactivity concentration Bq/g	Error	Beta Radioactivity concentration Bq/g	Error
Sample A	1.243E-03	7.69E-03	2.598E-03	1.35E-03
Sample B	9.926E-03	6.34E-03	2.139E-03	9.83E-03
Sample C	7.896E-03	5.48E-03	BDL	BDL
Average	6.355E-03	6.503E-03	2.3685E-03	5.59E-03

Detector Efficiency: Alpha= 87.95%; Beta = 42.06%

Detector Background: Alpha = 0.50 cpm; Beta = 0.73cpm

Detection limit: Alpha = 0.21cpm; Beta = 0.22cpm

Calibration sourced used: Srontium (**Sr-90**) a beta source; Plutonium (**Pu- 239**) an alpha source

Appendix IX: Elemental Composition of *Daucus carota* Aerial Part

Element	Mean conc (ppm) $\pm$ SD	Spectroscopy methods
Zn	0.26167 $\pm$ 0.02	MP-AES
Fe	3.18967 $\pm$ 0.29	MP-AES
Cu	0.13267 $\pm$ 0.03	MP-AES
Mn	1.15133 $\pm$ 0.06	MP-AES
Se	0.396 $\pm$ 0.04	MP-AES
Ca	2.02 $\pm$ 0.02	Flame emission
Na	5.38 $\pm$ 0.04	Flame emission
P	11.00 $\pm$ 0.05	Flame emission
K	2.25 $\pm$ 0.01	Flame emission
Mg	1.02 $\pm$ 0.02	AAS
Cd	-0.0023 $\pm$ 0.00	MP-AES
As	0.83067 $\pm$ 0.02	MP-AES
Pb	0.16567 $\pm$ 0.00	MP-AES
Cr	0.016 $\pm$ 0.004	MP-AES
Ni	0.041 $\pm$ 0.01	MP-AES

Average of three count determinations

Appendix X: ABU, Animal ethical consideration Approval



Committee On Animal Use And Care

Directorate of Academic Planning & Monitoring
Ahmadu Bello University, Zaria

Appl No.: ABUCAUC/2017/Pharmacognosy & Drug Dev./005
Approval No: ABUCAUC/2017/005

24th July, 2017

Dr. A. Ahmed
Department of Pharmacognosy & Drug Development,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
Zaria.

Sir,

APPROVAL OF RESEARCH STUDY 'PHARMCOGNOSTIC, TOXICITY AND ANTIOXIDANT PROPERTIES OF THE AERIAL PART OF *DAUCUS CAROTA* L. (*APIACEAE*)

This is to convey the approval of the ABUCAUC to you for the aforesaid study domiciled in the Department of Pharmacognosy & Drug Development. The approval is predicated on the assumption that you shall maintain and care for the Experimental Animals as approved after the visitation of the Committee.

Monitoring of the Research by spot checks, invitations or any other means the Committee deems fit shall be undertaken at the convenience of the Committee.

This approval can and shall be revoked should a significant breach in the terms and condition of the approval occur. It is hence your responsibility to ensure that the agreed terms are maintained to the end of the Study.

The said approval shall be posted on the ABUCAUC Page on the University's website.
Note upon completion of the research, ethical clearance certificate will be issued.

U.D. Abdullahi

For: Chairman, ABUCAUC.

Cc. Director, DAPM
Director, IC & ICT
Dean, Faculty of Pharmaceutical Sciences,
HOD, Pharmacognosy & Drug Dev.,
Dr. Jamilu Ya'u

Director: Professor M. I. Sule B Pharm, MSc, PhD, (ABU), MPSN Tel: 08036184581

Chairman: Prof C. A. Kudi DVM, M.Sc, PhD (ABU), PhD (Lei.) PGDE (PLY) 08065978003 | Secretary: U.D. Abdullahi, 08034619772

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