BIOCHEMICAL ANALYSIS OF *Justicia carnea* leaves USED AS A HEMATINIC

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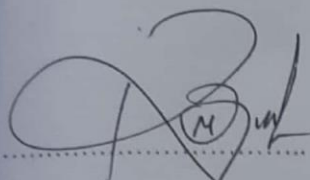
MAY, 2024.

CERTIFICATION

This is to certify that the thesis titled "**Some selected biochemical analysis of *justicia carnea* leaves used as a hematinic**", was carried out by **Andrew, Andrew Chimezie (20194198638)** in partial fulfilment for the award of Master of Science (M.Sc.) degree in Biochemistry.



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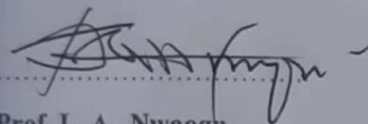

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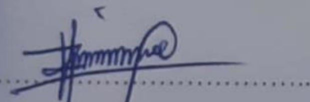

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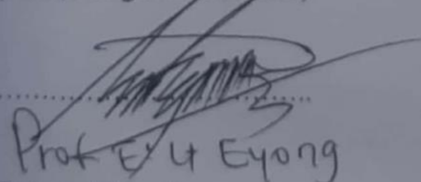

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DEDICATION

This work is dedicated to God Almighty and to my beloved parents' Chief and Lolo Ambassy Emezie.

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ABSTRACT

Justicia carnea is the largest genus of *Acanthaceae* and is a medicinal plant used widely in Nigeria which is reported to have diverse functions, including blood-boosting potential. The phytochemical, vitamin, mineral, amino acid and proximate compositions, and antioxidant effects of the leaf extract of Justicia *carnea* were determined using standard methods was the goal of this study. Results of the quantitative phytochemical analyses carried out on the leaves of *Justicia carnea* recorded appreciable presence of flavonoids, alkaloids, saponins, tannins, cyanogenic glycosides, oxalate, and phytate using GC-FID. Ribalinidine with 42.08±0.03 mg/kg was the highest alkaloid, Flavan -3-ol was 21.18±0.02 mg/kg, presented the highest amount of flavonoids, and phytate with 25.69±0.07 mg/kg as the highest antinutrient. Analysis of *Justicia carnea* leaves recorded iron (8.61 mg/kg) as the major element followed by potassium (5.29±0.11 mg/kg). The concentration of vitamins in *Justicia carnea* leaves shows vitamin C with the highest value of 232.32±12.26 mg/100g followed by Vitamin A (22.16±2.12 mg/kg. The results of proximate composition on *Justicia carnea* leaves showed a high concentration of carbohydrate (60.35±3.05 %) and appreciable amounts of ash (15.02±1.01 %), fibre (9.29±0.93 %), protein (8.40±0.41), and low amount of lipid (1.50±0.09 %). The amino acid results showed the presence of both essential and non-essential amino acids with their concentrations in increasing and decreasing order. The results of antioxidant activities assay of the *Justicia* leaf extract showed that the leaves had better ability to scavenge free radicals at a concentration of 5mg/ml and 10mg/ml but had a drop at 50mg/ml; the standard antioxidant was slightly higher than all the activities of the different concentrations of the extracts. The results of inhibition of lipid peroxidation of the leaf of *J. carnea* showed that the leaf extracts had a better ability to inhibit lipid peroxidation at a concentration of 5mg/ml and 10mg/ml but had drop at concentrations of 50mg/ml and 100mg/ml. The results of hydroxyl radical scavenging activity of the leaf of *J. carnea* Showed that the leaf extracts promoted inhibition of hydroxyl radical scavenging activity with increasing concentrations. These results obtained showed that leaves of *Justicia carnea* may serve as rich sources of natural antioxidants, free radical scavengers and should be recommended as a potential source of useful bioactive constituents as vegetable supplement and has no toxic effect and serve as an effective hematinic.

Keywords: Antioxidant activity, anemia, biochemical, blood tonic, blood disorders, herbal medicine, medicinal plants, *Justicia carnea* leaf.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Plants and herbs have shown excellent medicinal potentials and this have been discovered for decades and used in ethno medical practices ever since prehistoric times. Plants have shown capacity to synthesize hundreds of chemical and biochemical compounds for functions such as defense against insects, herbivorous mammals, fungi, and diseases. Great numbers of plant macronutrients, micronutrients and phytochemicals have established excellent potential biological activity and this have been identified in plants through various research endeavours (Ahn, 2017). Drug related research makes use of the ethnobotany to search for the pharmacologically active natural substances, and has in this manner discovered hundreds of beneficial compounds.

The place and roles of plants in medicine were radically altered in the nineteenth century by the application and use of chemical analyses. Alkaloids were extracted and isolated from succession of medicinal plants, beginning with morphine from the poppy, and soon followed by strychnos and ipecacuanha, quinine from the cinchona tree, and several others. As chemistry advanced, more classes of pharmacologically active compounds and substances were discovered in the medicinal plants (Atanasov *et al.,* 2015).

When plants are utilized with the intention of maintaining health, they are termed, medicinal plants. At such cases, the plants are administered for some specific health conditions, whether in traditional medicine or in modern medicine (Smith-Hall *et al.,* 2012; Ahn, 2017). The Food and Agriculture Organization of the UN estimated that more than 50,000 medicinal plants are in use worldwide (Schippmann *et al.,* 2002). In 2016, The Royal Botanic Gardens, Kew conservatively estimated that 17,810 plant species have medicinal use, out of about 30,000 plants for which a use of whichever kind is documented (Royal Botanic Gardens, 2016).

Medicinal plants have been identified to have bioactive molecules termed phytochemicals (Fasuyi,2006) and secondary metabolites that can protect humans against diseases, they have been

recognized as having beneficial characteristics utilized for the management of many ailments (Kumar *et al*., 2009).

Justicia carnea is a medicinal plant used widely in Nigeria which is reported to have diverse functions including blood-boosting potential. *Justicia carnea* has been used in traditional medicine in Nigeria in the treatment and management of various diseases which includes: inflammation, gastrointestinal disorders, anemia, respiratory tract infection, cancer, malaria, sickle cell disease, diabetes, diarrhea, typhoid, hepatitis, liver diseases, etc. (Badami *et al*.,2003; Correa and Alcantara, 2012). The leaves of plant *Justicia carnea* when soaked in boiled water in a closed container for about 15minutes, despite its green leaves, the boiled water turns into a purplish-red juice.

1.2 Statement of the problem

Medicinal plants are important potential sources of therapeutic compounds for minor and major disease conditions and potential material for maintaining good health and conditions. Studies have shown that two-third of the world's population depends on medicinal plants and herbs for primary health care. The reasons for wide and varied use of medicinal plants is due to their affordability, better cultural acceptability, better compatibility and adaptability with the human body and pose lesser side effects. However, the challenge has always been in the discovery of the medicinal value and bioactive components of plants.

1.3 Aim and objectives of the study

The aim of the study was to determine the biochemical composition of *Justicia carnea* leaf.

The objectives of the study include;

i. To carry out phytochemical analysis of the leaves using GC-FID.
ii. To elucidate the structure of some compounds present using GC-MS.
iii. To determine the amino acid compositions of the leaf using TSM amino acid analyser.
iv. To determine the vitamin compositions such as: Vitamin A (β-carotene), vitamin C Vitamin B_1, B_2, B_3, B_6, and B_9.

v. To determine the proximate composition.

vi. To determine the elemental (Mineral) composition such as Fe, Cu, Na, Mg, and Mn.

vii. To determine the antioxidants and free radical scavenging potential of the plants.

1.4 Significance of the study

The study will enrich research and provide data on the chemical composition of the leaves of *Justicia carnea* plant. This study will critically evaluate the phytochemical, amino acid, vitamins and minerals as well as the proximate content of the leaves. This will bring to the fore the potential medical and nutritional values of *Justicia carnea* leaf and the uses as a medicinal plant. It will also provide insight on the pharmacological and biochemical applications of the components of the leaves of *Justicia carnea*.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Justicia* plant

Justicia is a genus of flowering plants in the family *Acanthaceae*. *Justicia* is the largest genus in the family, which have estimated 700 species with enormous number yet unresolved (Daniel, 2011). They are native to tropical and to warm temperate regions of the Americas, India, and Africa. Common names include water-willow and shrimp plant, the latter from the inflorescences, which resemble a shrimp in some species. The generic name honours Scottish horticulturist James Justice (1698–1763) (Austin, 2004)

The plant genus *Justicia* has more than 600 species spread over the hemispheres; in the tropics and temperate regions, and it has found use in the treatment of numerous pathologies (Carneiro *et al*., 2023).

The genus Justicia (Figure 1) comprises herbaceous plants or erects ascending shrubs with opposite leaves of crenate or entire maple. Terminal or axillary inflorescences with sessile or pedunculated flowers, solitary or cymosal in the axils of the bracts, are arranged in spikes or panicles. The 4-5-parted calyx and corolla are of varying colors (purple, red, lilac, white, yellow, or orange), lipped limbus, rear inner lip in pre-flowering, usually narrow, erect or curved. Sometimes concave, with entire apex, bifid or frontal lip slightly more bilobed, wider, more or less patent or curved, trilobed (Carneiro *et al*., 2023).

Justicia species are used traditional medicine for the management and treatment of numerous diseases conditions, which include depression, anemia, epilepsy, kidney infection, respiratory problems, gastrointestinal diseases, arthritis and fever (Corrêa and Acântara, 2012). Plant extracts of *Justicia* have shown enormous biochemical activities such as antioxidant, antimutagenic, anticancer, anti-HIV, antimicrobial, antidiabetic, and others (Saran *et al*., 2019; Ameer et al., 2021; Naik et al., 2022). It is worthy to note that several and verities of special metabolites have been

identified in *Justicia*, which include alkaloids, steroids, tannins, terpenoids, lignans and flavonoids (Bafor *et al*., 2019; Yamoah *et al*., 2020; Naik *et al*., 2022)

2.1.1 Justicia carnea

Scientific classification are as follows: Kingdom: Plantae; Order: Lamiales; Family: Acanthaceae; Genus: *Justicia*; Species: *J. carnea*. *Justicia carnea*, is a Brazilian plume flower, Brazilian-plume, flamingo flower, or jacobinia, (USDA, NRCS, 2015). The perennial plant (Plate 2.1) is native to the Atlantic Forest ecoregions of eastern Brazil. It is cultivated and sold as a decorative potted plant and is planted in landscaping as a feature plant in warm temperate and subtropical climates.

Currently, a lot of attention has shifted to the use of plants with medicinal properties in the management and treatment of ailments such as anemia, diabetes and malaria. This is due mostly because of the local availability, ease of access and relatively affordable. Plants with medicinal properties are gaining attentions in health care programmes. Based on estimation by World Health Organization (WHO), about 80 to 90% of the world's population especially in developing countries depend on traditional system of medicine (Kone *et al*., 2012; Van Andel and Carvalheiro, 2013; Akintimehin *et al*., 2021).

Plate 2.1: ***Justicia carnea*** **(Asogwa *et al*., 2020).**

2.1.2 Antioxidant and anti-anaemic capacity of leaf extract of *J. carnea*

The *J. carnea* as a perennial plant propagated by stem cutting has an evergreen nature making it excellent for mass planting. The bright-coloured flower of Acanthaceae family is used as a source of therapeutic drugs (Correa *et al.,* 2011). The rural dwellers in Nigeria's eastern region boil *J. carnea* leaf in water and consume the fluid extract orally to boost blood levels, in a bid to treat disease including anaemia. *J. carnea* leaves and stalk extracts are boiled for 10 to 15 minutes to extract the bioactive compounds which present an appearance of a blood-like colour solution (Moswa *et al.,* 2015). The leaf of *J. carnea* is a rich source of micronutrient and natural antioxidants and reported to have the capacity to be deployed in the management of micronutrient deficiency diseases and oxidative stress (Asogwa *et al.,* 2020).

The plant, *J. carnea* is used as a treatment of anaemia in Congo by A report shows members of Jehovah's Witnesses in Congo Republic use the J carnea plant extract to treat anaemia because their belief is against blood transfusions (Pius *et al.,* 2010). Also, it has been reported by Ani *et al*. (2020) to be potent against diabetes.

In different parts of Africa, various species of *Justicia* have been adopted in traditional medicine for the treatment of anemia, inflammation, fever, diarrhea, liver diseases and arthritis respiratory and gastrointestinal disorder and J. carnea is one of them (Badami *et al*., 2003; Onyeabo *et al*., 2017). Recent reports indicate that *J. carnea* specie have cardioprotective properties, antioxidant and are excellently rich in vitamins and minerals (Medapa *et al*., 2011; Faiza *et al*., 2013; Radhika et al., 2013.)

The hematopoietic system presents sensitive target of toxic substances and a veritable index of physiological and pathological status in animals (Mukinda and Syce, 2007). Ethanol extract of *J. carnea* leaf have shown capacity to increase red blood cell, hemoglobin, packed cell volume and platelet count with significant increase at higher doses of extracts, indicating strength in to reverse anaemia – induced rat (Onyeabo et al., 2017; Anthonia *et al*., 2019). The anti-anaemic capacity property of *J. carnea* leaf is linked to the improved hemoglobin, PCV and RBC. Several medicinal

plants including *Xylopia aethiopica* (Oso *et al.,* 2019)*, Tectona grandis* (Diallo *et al*., 2008) and extracts of *M. indica*, *A. hybridus* and *T. occidentalis* (Ogbe *et al*., 2010) have as well shown the potentials to elevate RBC, hemoglobin and packed cell volume. The ability of leaf extracts of *J. carnea* to stimulate blood formation is attributable to the presence of bioactive constituents that stimulate activities of haematopoietic cells and stabilization of blood in circulation (Anthonia *et al*., 2019). Administration of leaf extracts of *J. carnea* have also lead to significant increase in platelet counts and this has been attributed to the capacity of the leaf extracts of *J. carnea* in blocking excessive blood loss (through blood clotting) and resistance of capillary membranes to leakage of red cells when blood vessels are damaged. Macrocytic and hypochromic anemia usually results due to increased mean corpuscular volume (MCV) and decrease in mean corpuscular hemoglobin concentration (Chanda *et al*., 2015).

2.2 Medicinal plants in disease conditions

Medicinal plants are considered essential sources of compounds, important in drug development pharmacopoeial, non-pharmacopoeial or synthetic drugs. Furthermore, medicinal plants are critical in the development of human cultures and tradition and play a key role in world health (Sandberg and Corrigan, 2001). Medicinal herbs or plants are important potential sources of therapeutics or curative compounds for minor and major disease conditions and potential material for maintaining good health and conditions. Studies have shown that two-third of the world's population depends on medicinal plants and herbs for primary health care. The reasons for wide and varied use of medicinal plants is due to their better cultural acceptability, better compatibility and adaptability with the human body and pose lesser side effects. Examples of plant derived drugs are; aspirin, atropine, artimesinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine etc. (Kaushik *et al*., 2021).

Medicinal plants possess significant antibacterial, antifungal, anticancer, antidiuretic, anti-inflammatory and anti-diabetic properties (Sule *et. al*., 2010; Timothy *et al.,* 2012; Oladeji, 2016). Medicinal plant derived drugs are applied in the management of mental illness, skin diseases,

tuberculosis, diabetes, jaundice, hypertension and cancer. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. Also, a lot of individuals die on daily basis from preventable or curable diseases due to the lack of basic health care solutions (Sofowora *et al*., 2013)

The importance of herbs and plants in medical care and as raw materials in pharmaceutical industries runs into millions of dollars worldwide and therefore cannot be overemphasized. The treatment of diseases with herbs and medicinal plants is of universal occurrence in non-industrialized societies. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitals and quinine (Mordeniz, 2019). The use of medicinal plants is increasing worldwide, in view of the tremendous expansion of traditional medicine and a growing interest in herbal treatments. Generally, medicinal whole plants and plant products are applied in medicine to maintain and augment health-physically, mentally and spiritually as well as to treat specific conditions and ailments.

Chemically or synthetically prepared drugs have shown their capacity to act quickly, however they present a lot of side effects which may adversely impair physiological and biochemical functions of the human body in the long run. However, plants with medicinal potentials work in an integrated or pro-biotic with little or no adverse effects on the body (Idu, 2009). The development of human culture, the use of medicinal plants has had magical-religious significance and different points of view regarding the concepts of health and disease which existed within each culture (Idu, 2009; Joshi *et al.,* 2009).

2.3 Plant phytochemicals

Phytochemicals are chemicals produced by plants through the plants' primary or secondary metabolism. They express biological activity in the plant host and play a significant role in plant growth and/or defense against competitors, pathogens, or predators (Molyneux *et al*., 2007; Breslin, 2017). Phytochemistry is the science responsible for the study of the compounds contained in plants. In this field, various techniques have been developed, ranging from the preparation of the plant

tissue sample to sophisticated techniques for the elucidation of organic structures. The search for new products for the application in the field of pharmaceuticals and agriculture is an important area of research (Dreyfuss *et al*., 1994).

Metabolism is a set of chemical reaction that occurs in the cells of living organisms to synthesize complex substances from simple substances, or the degradation of complexes substances to produce simple ones (Ávalos and Elena, 2009). Plants, and autotrophic organisms, exhibit two metabolisms, called primary and secondary metabolism. Primary metabolism involves and is present in all living organisms and secondary metabolism allows organisms to produce and accumulate compounds of diverse chemical structure and nature (Figure 2.1).

Biosynthesis of secondary metabolites is often restricted to specific stages of plant growth/development and periods of stress (Ávalos and Elena, 2009). Studies have shown that some plant cells express important secondary metabolites resulting from the interactions of the plant with the environment (protection against predators, pathogens or environmental stress) or some related to the reproductive mechanism of the plant (attraction of insects for the promotion of pollination) (Figure 2.2).

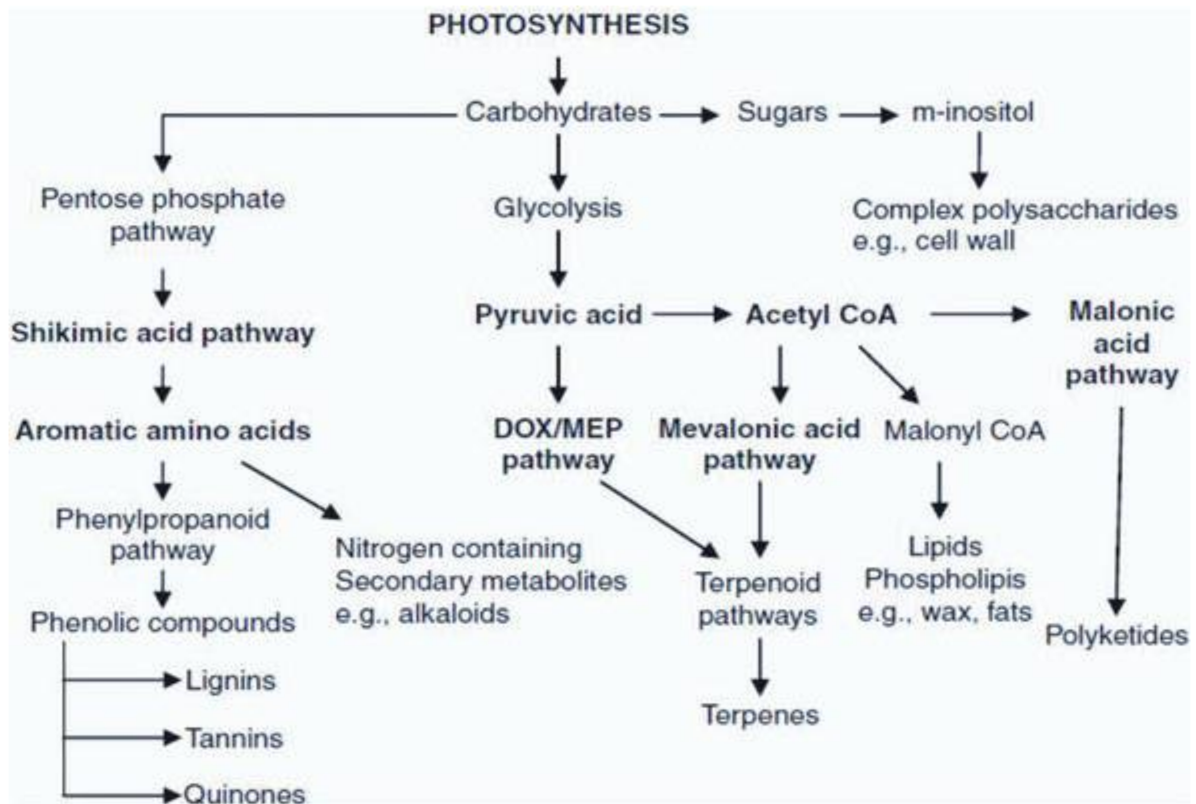

Figure 2.2. The metabolism of plants (Mendoza *et al*., 2018).

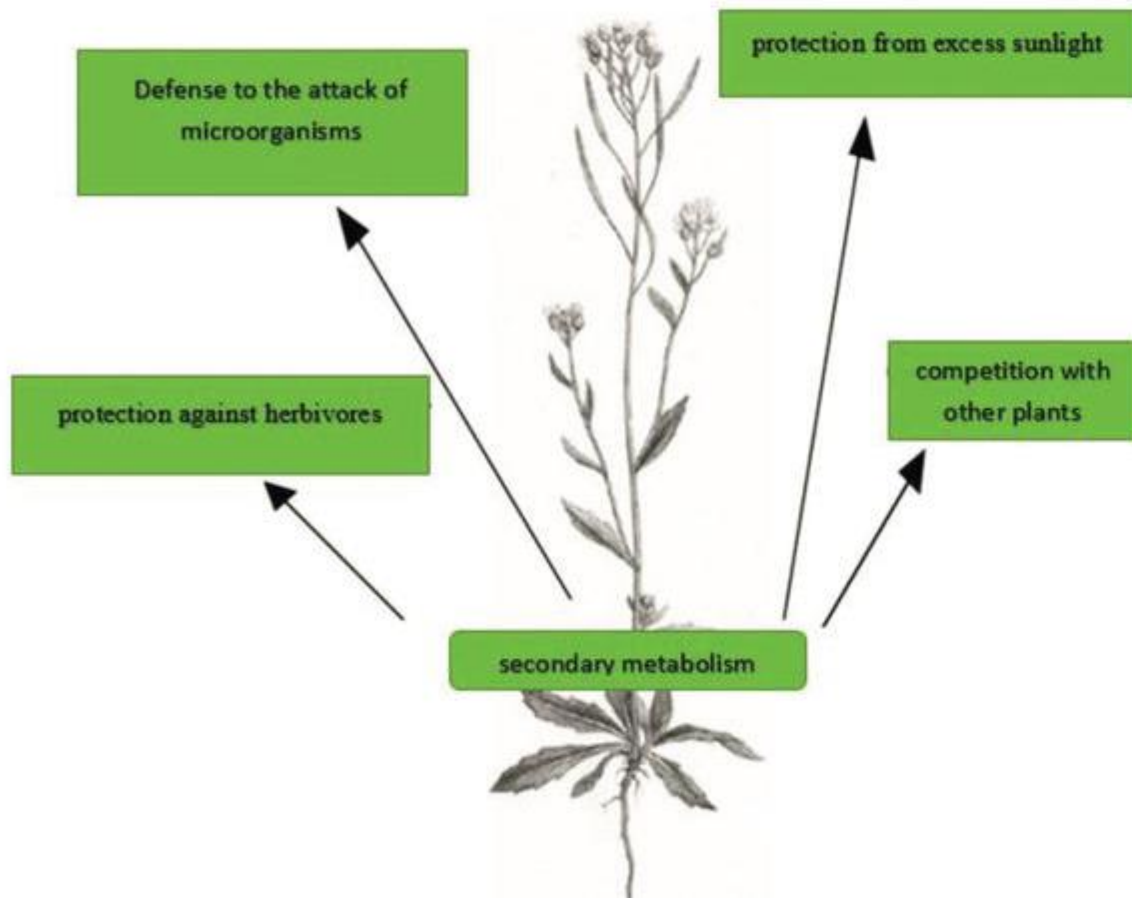

Figure 2.3: Production of plant secondary metabolites. (Mendoza *et al.,* 2018).

Phytochemicals or chemicals from plants may affect health of consumers but are not essential nutrients. There is enormous evidence to support the health benefits of diets rich in plant and plant products such as fruits, vegetables, legumes, whole grains, and nuts. Since plant-based foods are complex mixtures of bioactive compounds, information on the potential health effects of individual phytochemicals is linked to information on the health effects of foods that contain those phytochemicals. (Achibat et al., 2015) Some classes of pharmacologically active phytochemicals include flavonoids, saponins, alkaloids tannins etc.

Primary metabolites which make up part of the proximate composition are necessary for plant life and include carbohydrates, amino acids, proteins, lipids, purines, and pyrimidines of nucleic acids. However, secondary metabolites are the remaining plant chemicals produced by the cells through metabolic pathways derived from the primary metabolic pathways (Hussein and El-Anssary, 2019). Plants secondary metabolites or chemical components are described as an antiviral, antifungal, and antibiotic, which has shown potency for protecting plants from pathogens. These chemicals also are critical UV absorbing chemical factors, preventing severe damage to plant leaf from sun light. Due to their enormous biological activities, plant secondary metabolites have found use for centuries in traditional medicine and the medicinal effects of plants have been implicated in these molecules (Jamwal *et al*., 2018). Moreover, various tissues and organs of medicinal plants could have peculiar medicinal properties at specific developmental phases (Rabizadeh, *et al*., 2022). Some classes of pharmacologically active phytochemicals include the secondary constituents such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumins, saponins, phenolics, flavonoids and glucosides (Saxena *et al*., 2013; Thakur *et al*., 2018). The main classes of the phytochemicals are presented in Figure 2.3. Furthermore, based on chemical structure, phytochemicals are classified into the above categories but most basically subdivided into three main categories such as phenolic acids, flavonoids and stilbenes or ligans. The flavonoids can be further subdivided into anthocyanins, flavones, flavanones, isoflavones as well as flavonols and flavanols (Wen and Walle, 2006).

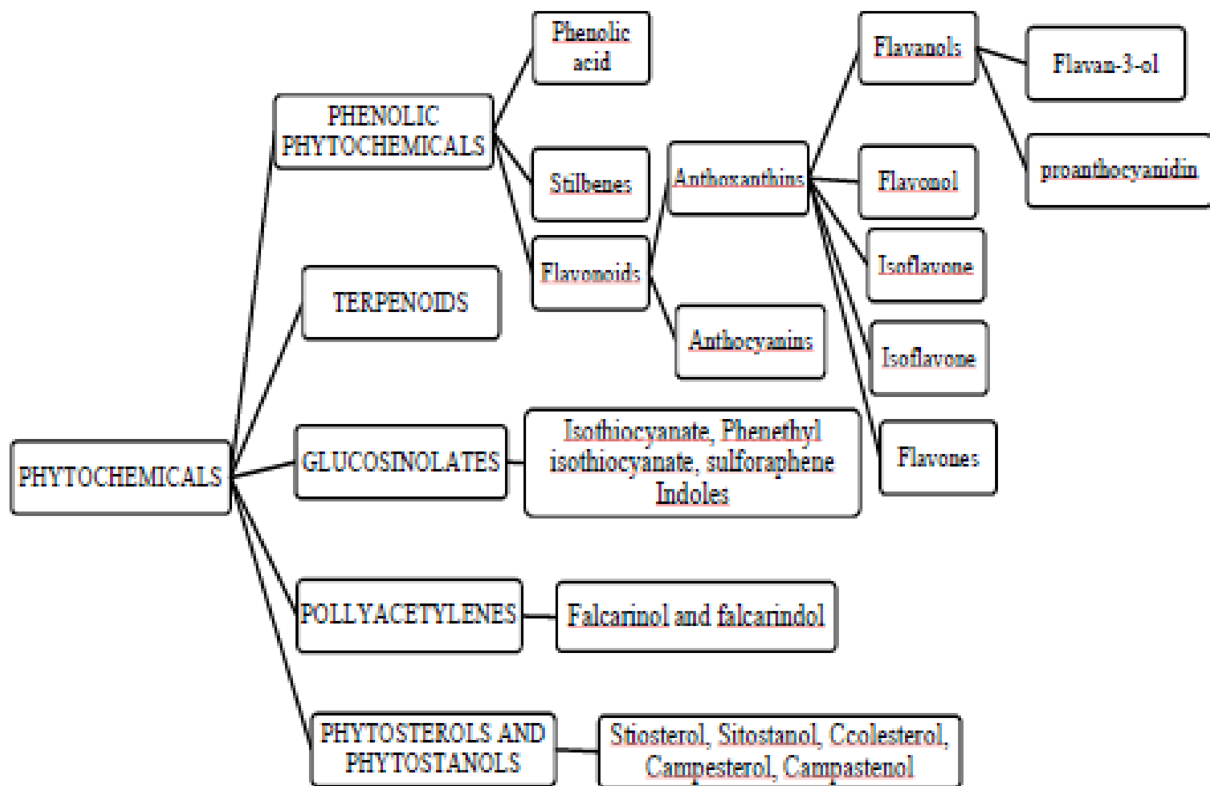

Figure 2.3 Classification of phytochemicals (Marta and Casado 2011)

2.4 Plants as source of amino Acids

Among the greater than three hundred amino acids (AAs) occurring in nature, 20 serve as building block of proteins (Wu, 2009). The essential AAs (EAAs), or indispensable AAs [(WHO, 2007), are those whose carbon skeletons cannot be synthesized or are inadequately synthesized de novo by the body based on their needs and must be obtained from the diet to satisfy optimal requirements, while the non-essential AAs (NEAAs) are those that can be synthesized de novo by the body in required sufficient amounts (Wu, 2009). Functional AAs, which include arginine, cysteine, glutamine, leucine, proline and tryptophan, help in the regulation of important metabolic pathways needed for maintenance, growth, reproduction and immunity.

The branched-chain AAs (BCAAs) comprising isoleucine (Ile), leucine (Leu) and valine (Val) are a sub-group of EAAs in humans (Yang *et al.,* 2007). Similar to other EAAs, they are mainly obtained through dietary protein intake (Ferguson and Wang, 2016) with rich amount present in meat, fish, dairy products and eggs (White and Newgard, 2019). However, studies have reported the possibility of the consumption of animal protein contributing to some human health-related problems such as heart disease, diabetes and obesity, among others (McDougall, 2002). Studies have also related a high regular intake of animal protein with increased adiposity; whereas the prevention of CVDs and some of their risk factors have been linked to plant-based diets (Olsen *et al*., 2018)

2.5 Proximate composition of plants

Proximate analysis is used for estimation of the quantitative content of food and food substance including moisture, crude protein, total fat, total carbohydrate, and dietary fiber (Thangaraj and Rainsford, 2016). Proximate composition ensures that consumers are well aware of the nutritional composition of foods so as to enable them to make informed and knowledgeable decisions about their diet.

Moisture is sometime used for estimation of the qualitative content of food. However, the amount of moisture content is one of the main factors in storage, due to the proliferation of microorganisms,

such as fungi and mold. Crude protein is the amount of total nitrogen multiplied by protein factors. Total nitrogen consists of protein nitrogen and a few nonprotein nitrogens. Different types of plants present different protein factors (Thangaraj and Rainsford, 2016). The protein factor of 6.25 is often used to convert nitrogen to protein. Total fat, ether extract, is the amount of fat including fatty acid, oil-soluble dyes, fat-soluble vitamins, and steroids (Thangaraj and Rainsford, 2016). Total carbohydrate is the amount of carbohydrate, which is one of the main components of structural materials of plants (Puwastien *et al*., 2011). This is often calculated based on difference method, and dietary fiber is the amount of total dietary fiber (Puwastien *et al*., 2011; Ganogpichayagrai and Suksaard, 2020). Ash content is the amount of total mineral residue left after incinerating the plant samples until constant weight. Protein, lipid, and carbohydrate each contribute to the total energy composition.

2.6 Plants as source of vitamins

Plants contain a complete spectrum of vitamins, minerals, aromatic oils, and phytonutrients that the human body can use. The vitamins and minerals extracted from plants come with other natural compounds, called co-nutrients, which work together with the vitamins and minerals to create beneficial processes in the body (Tardy *et al*., 2020).

Plant based vitamins are derived from natural sources as opposed to chemical sources. Natural sources for plant-based vitamins include herbs, flowers, leaves, stems, bark, seeds, nuts, spices, vegetables, fruits, and other naturally occurring botanical ingredients. vitamins are micronutrients, which are important as they help with growth and development by encouraging cellular functions and activity such as metabolism, digestion and also immunity. Vitamins also allow growth and development of the body (Tardy *et al*., 2020).

Vitamins are organic molecules (or a set of closely related molecules called vitamers) that are essential to an organism in small quantities for proper metabolic function. As essential nutrients vitamins are not synthesized in the organism in sufficient quantities for survival, and therefore must be obtained through the diet. For instance, vitamin C can be synthesized by some species but not

by others; it is not considered a vitamin in the first instance but is in the second. Most vitamins are not single molecules, but groups of related molecules called vitamers. There are a list of thirteen vitamins; vitamin A (all-trans-retinols, all-trans-retinyl-esters, as well as all-trans-beta-carotene and other provitamin A carotenoids); vitamin B_1 (thiamine); vitamin B_2 (riboflavin); vitamin B_3 (niacin); vitamin B_5 (pantothenic acid); vitamin B_6 (pyridoxine); vitamin B_7 (biotin); vitamin B_9 (folic acid and folates); vitamin B_{12} (cobalamins); vitamin C (ascorbic acid and ascorbates); vitamin D (calciferols); vitamin E (tocopherols and tocotrienols); vitamin K (phylloquinones, menaquinones, and menadiones) (WHO/FAO, 2004).

Vitamins have diverse biochemical functions. Vitamin A acts as a regulator of cell and tissue growth and differentiation. Vitamin D provides a hormone-like function, regulating mineral metabolism for bones and other organs. The B complex vitamins function as enzyme cofactors (coenzymes) or the precursors for them. Vitamins C and E function as antioxidants Both deficient and excess intake of a vitamin can potentially cause clinically significant illness, although excess intake of water-soluble vitamins is less likely to do so. (Bender, 2003).

For example, 60 mg of vitamin C is the recommended daily dose to help maintain tissue metabolism. Vitamin C can be found in many citrus fruits and in raw green vegetables. Deficiencies in vitamin C can lead to a disease known as scurvy. Another example of an essential vitamin would be vitamin B_2 also known as riboflavin. One point seven milligrammes of riboflavin is required daily and can be obtained from milk, eggs and leafy vegetable. Riboflavin is essential in the electron transport chain and deficiencies can lead to visual problems and skin fissures. Most vitamins are obtained from the diet, but some are acquired by other means: for example, microorganisms in the gut flora produce vitamin K and biotin; and one form of vitamin D is synthesized in skin cells when they are exposed to a certain wavelength of ultraviolet light present in sunlight. Humans can produce some vitamins from precursors they consume for example, vitamin A is synthesized from beta carotene; and niacin is synthesized from the amino acid tryptophan Vitamin B12 is the only vitamin or nutrient not available from plant sources. (Institute of Medicine (1998).

2.7 Plants as source of macro and micro minerals

Minerals are micronutrients, which are important for three main purposes these include building strong bones and teeth, for converting food into energy and for controlling body fluids (in and out of cells). For example, the mineral calcium, which is found in milk, is essential for strong bones. The mineral iron, also found in green leafy vegetables is essential for oxygen transportation in the body**.** minerals elements are very important for good health as different elements provide various functions and benefits to the body. There are two types of minerals needed by the body microminerals (trace minerals), which are needed in trace amounts, and macrominerals, which are needed in larger amounts. The absence of minerals in the diet can lead to many problems such as improper fluid balance, unhealthy teeth and bones and stunted growth. minerals elements are required by humans for important functions throughout life, with approximately 20 mineral elements discovered to be important for electrolyte balance, structural and functional roles (Zoroddu *et al*., 2019).

The most abundant elements are carbon, hydrogen, oxygen and nitrogen, accounting for about 96% body weight of the humans. However, these elements are not listed as nutrient minerals, whereas macro minerals such as magnesium, sodium, calcium, sulfur, chlorine, phosphorus and potassium and micro minerals such as manganese, zinc, cobalt, copper, molybdenum, fluoride, iron, iodine and selenium make up the remaining percentage (Berdanier *et al*., 2016).

Several health benefits have been associated with micronutrients' (minerals and vitamins) intake attributed to their functions in the enzyme system as cofactors and coenzymes, healthy bones and teeth formation, maintenance of body tissues as well as other physiological and biochemical functions (Awuchi *et al*., 2020).

These elements are derived from different dietary sources (with plants making significant amount) required by humans at different levels (generally less than 100 milligrams per day) to prevent against deficiencies (Blancquaert *et al*., 2017; Awuchi *et al*., 2019) . Deficiency of trace element nutrients can only be met by eating a diet of plant sources. Adequate daily intake of trace elements

is crucial for maintaining healthy life. For example, copper is essential for maintaining cognitive function in elderly people and Selenium plays an important role in boosting the immune system (Pfrimer *et al*., 2018; Mravunac *et al*., 2019). Furthermore, microminerals can prevent deterioration of age-related diseases (Ponikowski *et al*., 2015).

Iron is an essential micromineral involved in many crucial body functions. It is found in diets mainly in meat, legumes and leafy vegetables. The iron mineral is primarily present in the body in the form of hemoglobin in red blood corpuscles, in oxygen transfer processes (Dev and Babitt, 2017). Overall, adult females typically require more iron than men; this is attributed to the fact that adult females loose blood during menstrual cycle (Nutrient Reference Values, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Collection and authentication of plant sample

Justicia carnea leaves used for the study were obtained from Obinze in Owerri West LGA, Imo State Nigeria. They were identified by Mr. Francis Iwueze, a Plant Taxonomist in the Department of Wildlife and Forestry, Federal University of Technology Owerri (FUTO), Nigeria. The leaves of *justicia carnea* were prepared and kept at the herbarium with voucher number **FUTO/FWT/HERB/2019/057**

3.1.2 Chemicals and Reagents

Methanol, ethanol, N-butanol, tap water, chloroform, and n-hexane, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene, alkaline DPPH in methanol, L-Ascorbic acid, Methylated spirit, Phenylalanine, catalyst mixture (Potassium sulphate, Copper sulphate and Selenium sulphate), Sodium hydroxide solution, Boric acid, Oxygen, Nitrogen. 1% Dilute hydrochloric acid, Mayer's reagent, Wagner's reagent, distilled water, Ethylacetate, Ammonium chloride, Aqueous Ammonia, concentrated Sulphuric acid, acetic anhydride, Fehling's solution A and B, ethylacetate, ethanol, chromogen solution, Hydrochloric acid, Potassium ferricyanide, aqueous Phosphomolybic solution, alkaline Copper reagent, Phospmolybdic acid, alkaline picrate solution, Helium, acid anhydride, TCA in chloroform,

3.1.3 Equipment

Gas Chromatography column (Agilent 6890N) and Mass spectrophotometer (5975B MSD) Spectrophotometer, water bathe, dessicator, Amino acid analyzer, micro Kjedahl distillation apparatus, Unicam Spectronic, 20-DR spectrophotometer, curvette Grinder, UV/VIS Spectrophotometer, HPLC.

3.2 Methods

3.2.1 Plant sample preparation

Freshly collected leaves were washed with tap water and then spread to air dry at room temperature for two weeks. The dried samples were ground into fine powder using industrial grinder and stored in an airtight container. Decoction of the dried powdered plant leaves were prepared in aqueous solution, filtered, and used for further analyses.

3.2.2 Determination of phytochemical content.

Phytochemical analysis was carried out using the method of Gas Chromatography-Mass Spectrometer (GC-MS) as described by Kelly and Nelson (2014).

3.2.2.1 Preparation of sample

The leaves of the plant were air dried at room temperature for 2 weeks. The dried leaves were ground, and the powdered sample was subjected to Soxhlet extraction method. Five hundred milliliters ml clean boiling flasks was dried in an oven at 105 - 110^{0}C for 30 min. It was transferred into a desiccator and allowed to cool. Weighed sample 100g was poured into the Soxhlet thimble plugged lightly with cotton wool to aid filter the extract. Then the boiling flask was filled with 300 ml of ethanol and Soxhlet set up was allowed to reflux for about 4 hr at 60^{0}C. Afterwards the thimble was removed carefully, and the extract poured into a volumetric flask and allowed to cool. Finally, the content of the volumetric flask was transferred into a rotatory evaporator to separate the solvent (n-hexane) from the oil at temperature until a semi-solid extract was obtained.

3.2.2.2 Extraction of phytochemicals

A weighed 1g of the extract from 3.2.2.1 was transferred in a test tube and 25 ml of ethanol was added. The contents of test tube were allowed to react in a hotplate at 60 ^{0}C for 90 min. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water and 3 ml of hexane, and all were transferred to the funnel. The extracts were combined and washed three times with 10 ml of 10% v/v ethanol aqueous solution. The solution was dried with anhydrous

sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000 µl of pyridine of which 200 µl was transferred to a vial for analysis.

3.2.2.3 Quantification by GC-MS

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with HP-5MS column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 mL/min. The initial temperature was set at 50 °C with increasing rate of 3 °C/min and holding time of about 10 min. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% of the extracts diluted with acetonitrile was injected in ansplitless mode. Relative quantity of the chemical compounds present in each of the extracts was expressed as percentage based on peak area produced in the chromatogram.

3.2.2.4 Identification of chemical constituents

Bioactive compounds of the plant extracts were identified based on GC retention time on HP-5MS column and matching of the spectra with computer software data of standards (Replib and Main lab data of GC–MS systems).

3.2.3 Determination of amino acid profile of the extracts

Amino acid profile of the extracts was determined using applied bio-systems PTH amino acid analyzer as described by AOAC (2005).

3.2.3.1 Hydrolysis of the sample

One gramme of the defatted sample was weighed into glass ampoule. Then, 10 ml of 4.2M NaOH was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $105^0C \pm 5^0C$ for 4 h. The ampoule was allowed to cool before breaking open at the tip and the content was filtered to remove the humins. The filtrate was neutralized to pH 7.00 and evaporated to dryness at 40 ^0C under vacuum

in a rotary evaporator. The residue was dissolved with 5 ml of borate buffer (pH 9.0) and stored in a plastic specimen bottle, and stored in the freezer.

Afterwards, 60 µl of the hydrolysate was loaded into TSM amino acid analyzer. This was dispended into the cartridge of the analyzer for a period of 45 min. An integrator attached to the analyzer calculated the peak area proportional to the concentration of each of the amino acids.

3.2.3.2 Determination of tryptophan

Introduction: Tryptophan is a difficult amino acid to determine in proteins and peptides because it chemically decomposes during acid hydrolysis. Tryptophan is destroyed by 6N HCl during hydrolysis.

Antioxidants such as thioglycolic acid or dodecanethiol have been used instead of 6 N HCl to preserve tryptophan. Alkaline hydrolysis has also been studied and was shown to produce higher tryptophan recovery than acid hydrolysis. The addition of phenol has also been reported. Alkaline hydrolysis was improved by using sodium hydroxide (NaOH) instead of barium hydroxide to prevent problems with both precipitation and adsorption of tryptophan.

Determination**:** The tryptophan in the sample was hydrolyzed with 4.2 M Sodium hydroxide (Maria *et al*, 2004). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

Defatting sample**:** A weighed 2 g of the dried sample was added into extraction thimble and the fat was extracted with chloroform/methanol (2:1 mixture) using Soxhlet extraction apparatus as described by AOAC (2005), The extraction lasted for 15h.

Nitrogen determination**:** Nitrogen was determined by micro Kjeldahl method according to AOAC (2005). The nitrogen of protein and other compounds are converted to ammonium sulphate by acid digestion with boiling Sulphuric acid.

Procedure: A weighed 250 mg of sample was placed in Kjeldahl flask and 200 milligram of catalyst mixture (potassium sulphate, copper sulphate and selenium powder) was added.

Ten milliliters of concentrated sulphuric acid was added to the content of the flask. Heat was applied gently for few minutes until frothing cease. Afterwards the heat was increased to digest for 1 hour. This was allowed to cool and 100 ml distilled water added. Distilled 10 ml of the aliquot of the dilute solution of the digest was obtained by pipetting the volume into distillation chamber of micro Kjeldhal distillation apparatus. Then 10 ml of 40% sodium hydroxide solution was added and steam distilled into 10 ml of 4% boric acid containing mixed indicator (note colour from red green) and titrated with standard 0.01N hydrochloric acid to grey end point.

$$\% N = \frac{(a-b) \times 0.01 \times 14.0057 \times c \times 100}{dxe}$$

a = Titre value for the sample

b = titre value for the blank

c = Volume to which digest is made up with distilled water

d = Aliquot taken for distillation

e = Weight of dried sample (mg)

3.2.4 Determination of mineral elements

Metal analysis was conducted using Agilent FS240AA Atomic Absorption Spectrophometer according to the method of APHA (1995) (American Public Health Association)

Working principle: atomic absorption spectrometer's working principle is based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelength, a source lamp composed of that element is used, making the method relatively free from spectral or radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

3.2.4.1 Sample digestion for elemental analysis (Adrian, 1973)

The sample digestion for elemental analysis was carried out by the method described by Adrian (1973). A weighed 2g of the dried sample was delivered into a digestion flask with addition of 20

ml of the acid mixture (650 ml conc. HNO_3; 80 ml perchloric acid; 20ml conc. H_2SO_4). Heat was applied to the flask until a clear digest was obtained. The digest was diluted with distilled water to the 100 ml mark.

3.2.4.2 Preparation of reference solutions

A series of standard metal solutions in the optimum concentration range were prepared, the reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5 ml concentrated nitric acid/litre. A calibration blank was prepared using all the reagents except for the metal stock solutions. Calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations.

3.2.4 Determination of proximate composition

This was carried out using the methods of Association of Official Analytical Chemist (AOAC, 1990).

3.2.5.1 Determination of moisture content

A petri-dish was washed and dried in the oven and 1 g of the sample was weighed into a petri dish. The weight of the petri dish and sample was noted before drying. The Petri dish and sample were put in the oven and heated at 105 ^{0}C for 2h and the result noted and heated another 1h until a steady result constant weight was obtained and the weight was noted. The drying procedure was continued until a constant weight was obtained.

$$\% \text{ Moisture content} = \frac{W1\text{-}W2 \text{ x}100}{\text{Weight of sample}}$$

Where W1 = weight of Petri dish and sample before drying

W2 = weight of Petri dish and sample after drying.

3.2.5.2 Determination of ash content

Principle: The ash of foodstuff is the inorganic residue remaining after the organic matter has been burnt away. However, the ash obtained is not necessarily of the composition as there may be some from volatilization.

Procedures

An empty platinum crucible was washed, dried and the weight was noted. A weighed 1 g of sample was transferred into the platinum crucible and placed in a muffle furnace at 550 ^{0}C for 3 h. The sample was cooled in a dessicator after burning and weighed.

Calculations

$$\% \text{ Ash content} = \frac{W3 - W1 \times 100}{W2 - W1 \times 1}$$

Where

W1 = weight of empty platinum crucible

W2 = weight of platinum crucible and sample before burning

W3 = weight of platinum and ash.

3.2.5.3 Determination of crude fibre

Procedure: A weighed 2g of material was defatted with petroleum ether (if the fat content if more than 10%). The sample was boiled under reflux for 30 min with 200 ml of a solution containing 1.25 g of H_2SO_4 per 100 ml of solution and the solution was filtered through linen. The extract was washed with boiling water until the washings are no longer acidic. The residue was transferred into a beaker and boiled for 30 min with 200 ml of a solution containing 1.25g of carbonate free NaOH per 100ml. The final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible. This was dried in an electric oven and weighed and then incinerated, cooled and weighed

The loss in weight after incineration x 100 is the percentage of crude fibre.

$$\% \text{ Crude fibre} = \frac{\text{weight of fibre} \times 100}{\text{Weight of sample}}$$

3.2.5.4 Determination of crude fat

Principles: Soxhlet fat extraction method

This method is carried out by continuously extracting a food with non- polar organic solvent such as petroleum ether for about 1 hour or more.

Procedure: A clean boiling flask of 250ml volume was dried in an oven at 105 - 110^{0}C for 30 min and transferred into a dessicator and allowed to cool and the weight noted. The boiling flask was filled with 300 ml of petroleum ether (boiling point 40 – 60 C). An extraction thimble was lightly plugged with cotton wool. The Soxhlet apparatus was assembled and allowed to reflux for about 6 h. Furthermore, the thimble was carefully removed, and the petroleum ether top was collected and drained into a container for re – use. When flask is almost free of petroleum ether, it was removed and dried at 105 C – 110 C for 1h. Finally, this was transferred from the oven into a dessicator and allowed to cool and the weight noted.

$$\% \text{ Fat} = \frac{\text{Wt of flask} + \text{oil} - \text{Wt of flask} \times 100}{\text{Wt of sample}}$$

3.2.5.5 Determination of crude proteins

Principle: The method is the digestion of sample with hot concentrated sulphuric acid in the presence of a metallic catalyst. Organic nitrogen in the sample is reduced to ammonia. This is retained in the solution as ammonium sulphate. The solution is made alkaline, and then distilled to release the ammonia. The ammonia is trapped in dilute acid and then titrated.

Procedures: Exactly 0.5 g of sample was weighed into a 3 0ml Kjehdal flask (gently to prevent the sample from touching the walls of the side of each) and then the flasks were stoppered and shaken. Then 0.5 g of the Kjedahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appeared.

The clear solution was allowed to stand for 30 min and allowed to cool. After cooling it was made up to 100 ml with distilled water added to avoid caking and then 5 ml was transferred to the Kjedahl distillation apparatus, followed by 5 ml of 40% sodium hydroxide. A 100 ml receiver flask containing 5 ml of 2% boric acid and indicator mixture containing 5 drops of Bromocresol blue and 1 drop of methylene blue was added under a condenser of the distillation apparatus so that the tap was about 20 cm inside the solution and distillation commenced immediately until 50 drops got into the receiver flask, after which it was titrated to pink colour using 0.01N hydrochloric acid.

Calculations

% Nitrogen =Titre value x 0.01 x 14 x 4

% Protein = % Nitrogen x 6.25

3.2.5.6 Determination of available carbohydrate content

Available carbohydrate content was determined by the Differential method:

100% – (%Protein + %Moisture + %Ash + %Fat + %Fiber)

3.2.5 Determination of vitamins

3.2.5.1 Determination of Vitamin A

Vitamin A was determined by the method of Bayfield and Cole (1980).

***Principle*:** The assay is based on the spectrophotometric estimation of the color produced by vitamin A acetate or palmitate with TCA.

Reagents: Saponification mixture (2N KOH in 90% alcohol), Petroleum ether, anhydrous sodium sulphate, chloroform, Vitamin A palmitate, TCA reagent (60% TCA in chloroform) – prepared fresh.

Procedure: All procedures were carried out in the dark to avoid the interference of light. One gramme (1 g) of sample was mixed with 1.0ml of saponification mixture and refluxed for 20 min at 60^0C in the dark. The tubes were cooled, and 20 ml of water was added and mixed well. Vitamin A was extracted twice with 10 ml of (60 C) petroleum ether. The two samples were pooled and washed thoroughly with water. Anhydrous sodium sulphate was added to remove excess moisture. An aliquot of the sample (1.0 ml) was taken and evaporated to dryness at 60^0C. The residue was dissolved in 1.0 ml chloroform. Standards (vitamin A palmitate) of concentrations ranging from 0-7.5 µg were pipetted out into a series of test tubes.

The volume in all the tubes was made up to 1.0ml with chloroform. The TCA reagent (2.0 ml) was added rapidly, mixed and the absorbance was read immediately at 620 nm in a spectrophotometer (Genesys 10UV). The same procedure was repeated for other sample tubes also. Vitamin A content was expressed as mg/kg.

3.2.5.2 Determination of vitamin B_1 and B_2

This was determined using the Barakat titrimetric method described by Okwu and Ndu (2006). One gramme (1g) of sample was weighed into a conical flask. This was dissolved with 100ml of deionized water, shaken thoroughly and heated for 5 min and allowed to cool and filtered. The filtrate was poured into cuvette and their respective wavelength for the vitamins set to read at the appropriate absorbance using spectrophotometer. Vitamin B_1 = 261nm and Vitamin B_2 = 242 nm

Calculations:

$$\text{Concentration (mg\%)} = \frac{\text{A x D.F x volume of cuvette (5)}}{\text{E}}$$

Where A = absorbance

E = extinction coefficient = 25 for B_1 and B_2

DF = dilution factor

3.2.5.3 Determination of Niacin

This was determined using the Barakat titrimetric method described by Okwu and Ndu (2006). Five gramme (5g) of sample was dissolved in 20 ml of anhydrous glacial acetic acid and warmed slightly. Then, 5 ml of acetic anhydride was added and mixed. Afterwards 2 drops of crystal violet solution was added as indicator and this was titrated with 0.1M perchloric acid to a greenish blue colour.

Calculation:

$$\text{Niacin} = \frac{\text{titre value x 0.0122}}{0.1}$$

3.2.5.4 Determination of folic acid

This was determined using the Barakat titrimetric method described by Okwu and Ndu (2006). A weighed 5 g of sample was dissolved in a mixture of 5 ml of anhydrous glacial acetic acid and 6ml of 0.1M mercury II acetate solution. Two (2) drops of crystal violet was added as indicator and titrated with 0.1 m perchloric acid to a green color end point. Calculation: each meal of 0.1 M perchloric acid is equivalent to 0.02056 g of folic acid.

3.2.5.5 Determination of vitamin B_6

Vitamin B_6 was determined spectrophotometrically by coupling reactions with pyridine.

Sample preparation*:* A weighed equivalent 0.1 ml of sample was taken into a separator. In separator, 5 ml of water was added, mixed well and extract with 5 ml chloroform. Discard the water layer then taken chloroform in dry 50 ml volumetric flask by passed through anhydrous sodium sulphate and made up to 50 ml with chloroform.

Procedure*:* A weighed 2 ml sample and blank solution was taken into test tube. In each test tube, 2 ml of 0.2% solution of phenyl hydrazine (in hydrochloric acid and alcohol in ratio of 1:5 v/v) was added and mixed well. Afterwards the setup was placed in a water bath and heated until almost dryness, cooled at room temperature. Furthermore, 2 ml solution mixture (ammonia and alcohol in ratio of 1:1) was added in each test tube and 1 ml pyridine. Absorbance recorded at 635 nm against blank. Standard vitamin B_6 was also analyzed and treated same as sample. Calibration curve was plotted, and the concentration of sample extrapolated.

3.2.6. Antioxidant Activity

3.2.6.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity of the samples was assessed through their ability of scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radicals. The DPPH assay was performed as described by Mimica-Dukic *et al* (2003). The samples (from 5 to 500 µg mL^{-1}) were mixed with 1 mL of 90 µM DPPH solution and made up with 95% methanol, to a final volume of 4 mL. Synthetic antioxidant, BHT was used as control. After 1 h incubation period at room temperature, the absorbance was recorded at 515 nm. Percent radical scavenging concentration was calculated using the following formula:

$$\%\ DPPH\ Scavanging\ activity = 100 - \frac{A518(sample) - A518(blank) \times 100}{A518(blank\ positive\ control)}$$

3.2.6.2 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of extracts from *justicia carnea* was determined according to the method described by Kim *et al.* (2020). In order to generate hydroxyl radical 1.0 mL of 1,10-

phenanthroline was mixed with 2.0 mL of 0.2 M sodium phosphate buffer (pH 7.4) and 1.0 mL of 0.75 mM $FeSO_4$ and 1.0 mL of H_2O_2. The mixture was incubated with 1 ml of sample for 10 min at 37 °C. Gallic acid was used as standard. Absorbance was measured at 510 nm and the hydroxyl radical scavenging activity was calculated using the following formulae below:

% **Hydroxyl radical scavenging activity** $= (A_0 - A_1) \times 100$

where Ao = Absorbance of control

A_1 = Absorbance in the presence of sample

3.2.6.3 Lipid peroxidation inhibition

The lipid peroxidation inhibition of *Justicia carnea* extract was ascertained using a method previously described by Bajpai *et al*. (2015). In both the absence and addition of *Justicia carnea* extract (5-250 mg/mL) or a control substance, the reaction mixture of 1 mM $FeCl_3$, 50 µl of bovine brain phospholipids (5 mg/L), and 1 mM ascorbic acid in 20 mM phosphate buffer was incubated at 37 °C for 60 minutes. Malondialdehyde (MDA), which was measured by the 2-thiobarbituric acid (TBA) reaction, was created as a byproduct of the process as hydroxyl radicals, which led to lipid peroxidation and lipid peroxidation. The proportion of inhibitory activity was calculate using the following formula.

$$\text{MDA } (\mu\text{mol/ml}) = \frac{\text{Abs x total reaction volume}}{\text{Sample volume x extinction coefficient}}$$

Extinction Coefficient = 0.156 μmol^{-1} cm^{-3}

Sample volume = 0.5 ml

Total reaction volume = 2.51

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Phytochemical composition of *Justicia carnea* leaves using GC-FID

Table 4.1 reveals that the quantitative phytochemical analyses of the ethanol extract of *Justicia carnea* leaves recorded appreciable presence of flavonoids, alkaloids, saponins, tannins, cyanogenic glycosides, oxalate, and phytate using GC-FID. Ribalinidine with 42.08±0.03 mg/kg was the highest alkaloid, Flavan -3-ol was 21.18±0.02 mg/kg, presented the highest amount of flavonoids, and phytate with 25.69±0.07 mg/kg as the highest antinutrient.

4.1.2 GC-MS result of *Justicia carnea* leaves

The results of GC-MS study (Table 4.2abc) revealed the presence of cyclohexane, primary alcohols, esters, alkanoic acids as well as epoxide and haloalkanes.

4.1.3 Amino acid profile (essential) of *Justicia carnea* leaves

Figure 4.1 presents the essential amino acid content of *Justicia carnea* leaves. It shows an appreciable concentration of leucine (6.47± 0.04 g/100g) > Phenylalanine (5.14±0.07 g/100g) > valine (4.27±0.07 g/100g) > lysine (3.71±0.13 g/100g) > isoleucine (3.4±0.17 g/100g) > threonine (3.11±0.08 g/100g). The least essential amino acid was tryptophan (0.89±0.17 g/100g).

4.1.4 Amino acid profile (non-essential) of *Justicia carnea* leaves

Figure 4.2 presents the non-essential amino acid content of *Justicia carnea* leaves**.** Appreciable concentrations of non-essential amino acids were recorded in the leaves of *Justicia carnea*. Glutamic (11.35±0.12 g/100g) and aspartic acids (8.67±0.10 g/100g) were the more abundant non-essential amino acids in the plant extract. Others are arginine (5.33±0.07 g/100g) > proline (4.97±0.03 g/100g) > glycine (4.51±0.16 g/100g) > alanine (3.90±0.11 g/100g) > serine (3.40±0.11 g/100g). The least non-essential amino acids are tyrosine (2.41±0.15 g/100g)> cystine (0.91±0.08 g/100g).

4.1.5 Total amino acids and amino acid groups (g/100g protein) in of *Justicia carnea* leaves.

Table 4.3 presents total amino acids and amino acid groups of leaves of *J. carnea.* Total amino acid of 75.9 unit was recorded. It also reveals that *Justicia carnea* leaves contain more non-essential amino acids (45.45g/100g protein) than essential amino acids (30.45 g/100g protein). Also, *Justicia carnea* leaves contain more acidic amino groups (20.02 g/100g protein) than basic amino groups (11.27 g/100g protein). Furthermore, the leaves of *Justicia carnea* show that the ratio of leucine to isoleucine in *Justicia carnea* is greater than 1, indicating higher leucine content**.**

4.1.6 Percentages of amino acids and amino acid groups in *Justicia carnea* leaves

Table 4.4 presents percentage amino acids amino acids and amino acid groups of leaves of J. carnea. It reveals that *Justicia carnea* leaves also contain more percentage non-essential amino acids (59.9) than essential amino acids (40.12). Also, *Justicia carnea* leaves contain more percentage acidic amino groups (26.38) than basic amino groups (14.85). Furthermore, the leaves of *Justicia carnea* show that the percentage ratio of leucine to isoleucine in *Justicia carnea* is greater than 1, indicating higher leucine content.

4.1.7 Mineral composition of *Justicia carnea* leaves

The values obtained in the study of mineral composition of *Justicia carnea* leaves (Figure 4.3) shows that iron (8.61±0.24 mg/kg) has the highest concentration. Others are potassium (5.29±0.11 mg/kg) > magnesium (4.72±0.16 mg/kg) > sodium (3.89±0.15 mg/kg) > copper (0.48±0.04 mg/kg) > manganese (0.34±0.04 mg/kg) in reducing order of presentation.

4.1.8 Proximate composition of *Justicia carnea* leaves

The result (Figure 4.4) of proximate composition studies on *Justicia carnea* leaves showed a high concentration of available carbohydrate (60.35±3.05 %) and appreciable amounts of ash (15.02±1.01 %), fibre (9.29±0.93 %), protein (8.40±0.41), and low amount of lipid (1.50±0.09 %).

4.1.9 Vitamin composition of *Justicia carnea* leaves.

Table 4.5 shows the concentration of vitamins in *Justicia carnea* leaves. Vitamin C shows the highest value of 232.32±12.26 mg/100g followed by Vitamin A (22.16±2.12 mg/kg). vitamin B_6 with a concentration of 3.47±0.16 mg/100g. and vitamin B_3 (0.61±0.06 mg/100g) recorded..

4.1.10 Free radical scavenging potential

The results of DPPH radical scavenging activity of the leaf of J. carnea and the reference standard antioxidants (BHT) is presented in table 4.6. The leaf extracts and the standard antioxidants promoted inhibition of DPPH radical scavenging activity at a concentration of 5mg/ml and 10mg/ml, but but had a drop at a concentration of 50 mg/ml and increased slightly at 100mg/ml. However, the percentage inhibition of the DPPH radical of the leaf extract was lower when compared with the standard antioxidant (BHT).

4.1.11 Hydroxyl radical scavenging activity

The results of hydroxyl radical scavenging activity of the leaf of *J. carnea* presented in table 4.7. Shows that the leaf extracts promoted inhibition of hydroxyl radical scavenging activity with increasing concentrations. However, the percentage inhibition of the hydroxyl radical scavenging activity of the leaf extracts was lower at concentration of 5mg/ml and higher at concentration of 100mg/ml.

4.1.12 Inhibition of lipid peroxidation

The percentage inhibition of lipid peroxidation of the leaf of *J. carnea* and standard shows that the leaf extracts have a better ability to inhibit lipid peroxidation at a concentration of 5mg/ml and 10mg/ml but had a drop at a concentration of 50mg/ml and 100mg/ml as shown in the table 4.8.

Table 4.1: GC-FID phytochemical constituents of *Justicia carnea* leaves

Phytochemicals	Concentration (mg/kg)	Type
Ribalinidine	42.08 ± 0.03^{r}	Alkaloid
Spartein	8.92 ± 0.03^{g}	Alkaloid
Epihedrine	18.09 ± 0.02^{n}	Alkaloid
Cardiac glycoside	6.09 ± 0.02^{c}	Antinutrient
Cyanogenic glycoside	22.88 ± 0.02^{p}	Antinutrient
Phytate	25.69 ± 0.07^{q}	Antinutrient
Oxalate	13.65 ± 0.07^{j}	Antinutrient
Rutin	14.09 ± 0.04^{k}	Flavonoid
Naringin	18.18 ± 0.05^{n}	Flavonoid
Flavan -3-ol	21.18 ± 0.02^{o}	Flavonoid
Anthocyanin	10.91 ± 0.01^{h}	Flavonoid
Naringenin	2.64 ± 0.07^{a}	Flavonoid
Flavonones	16.44 ± 0.08^{l}	Flavonoid
kaempferol	7.99 ± 0.02^{f}	Flavonoid
Epicatechin	7.05 ± 0.03^{d}	Flavonoid
Flavone	7.52 ± 0.07^{e}	Flavonoid
Resveratol	11.67 ± 0.08^{i}	Flavonoid
proanthocyanin	7.63 ± 0.07^{e}	Polyphenol
Sapogenin	5.77 ± 0.07^{b}	Saponin
Steroids	17.31 ± 0.08^{m}	Steroid

Values are means ± standard deviation of triplicate determinations. Column with different superscripts are statistically significant at $P \leq 0.05$

Table 4.2a: % composition of bioactive compounds identified from the GC-MS of *Justicia carnea* leaves

Class of Compound	RT	AREA (%)	BIOACTIVE COMPOUND
Alkane	9.059	1.19	Tetradecane
	8.876	3.38	Hexadecane
	10.268	4.51	Heptadecane
	11.71	0.07	Octadecane
	20.087	0.46	Pentadecane
	12.803	1.73	Dodecane
Alkene	23.743	0.31	Centene
	28.09	2.66	1 Octadecene
	28.553	0.15	Octadecene
	5.455	0.17	1 docosene
Aromatic hydrocarbon	18.963	0.33	Naphthalene
	29.799	0.47	Anthracene

Table 4.2b: Percentage (%) composition of bioactive compounds identified from the GC-MS of *Justicia carnea* leaves

Class of Compound	RT	AREA (%)	BIOACTIVE COMPOUND
Carboxyl group	9.938	4.71	Carbonic acid
Terpenoid	19.354	0.23	Citronellol
Fatty Acid	29.928	0.2	Oleic acid
	33.229	0.4	N-hexadecenoic acids
	32.929	0.31	Eicosanoic acids
	33.103	0.53	Cis- Vaccenic acid
	34.001	0.25	Tetra decanoic acids

Table 4.2c: Percentage % composition of bioactive compounds identified from the GC-MS of *Justicia carnea* leaves

Class of Compound	RT	AREA (%)	BIOACTIVE COMPOUND
Steroid	31.551	1.43	9,12 –octadecadienoic acids
Aromatic hydrocarbon	15.873	0.83	Naphthalene
Fatty aldehyde	6.885	1.05	9-octadecenal, (z)
	12.584	0.95	E -14-Hexadecenal
	17.662	0.19	13-octadecenal, (z)

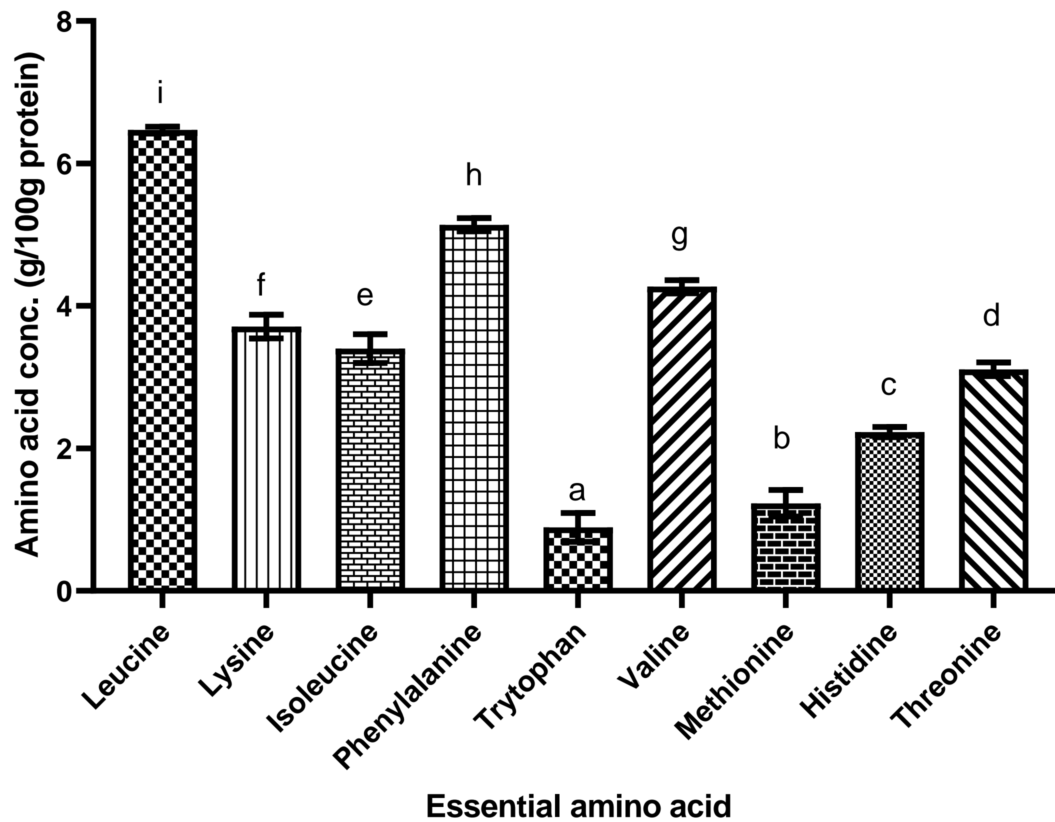

Figure 4.1: Essential amino acid profile of *Justicia carnea* leaves. Values are mean± standard deviation of triplicate determinations. Bars with different alphabets are statistically significant at $P \leq 0.05$

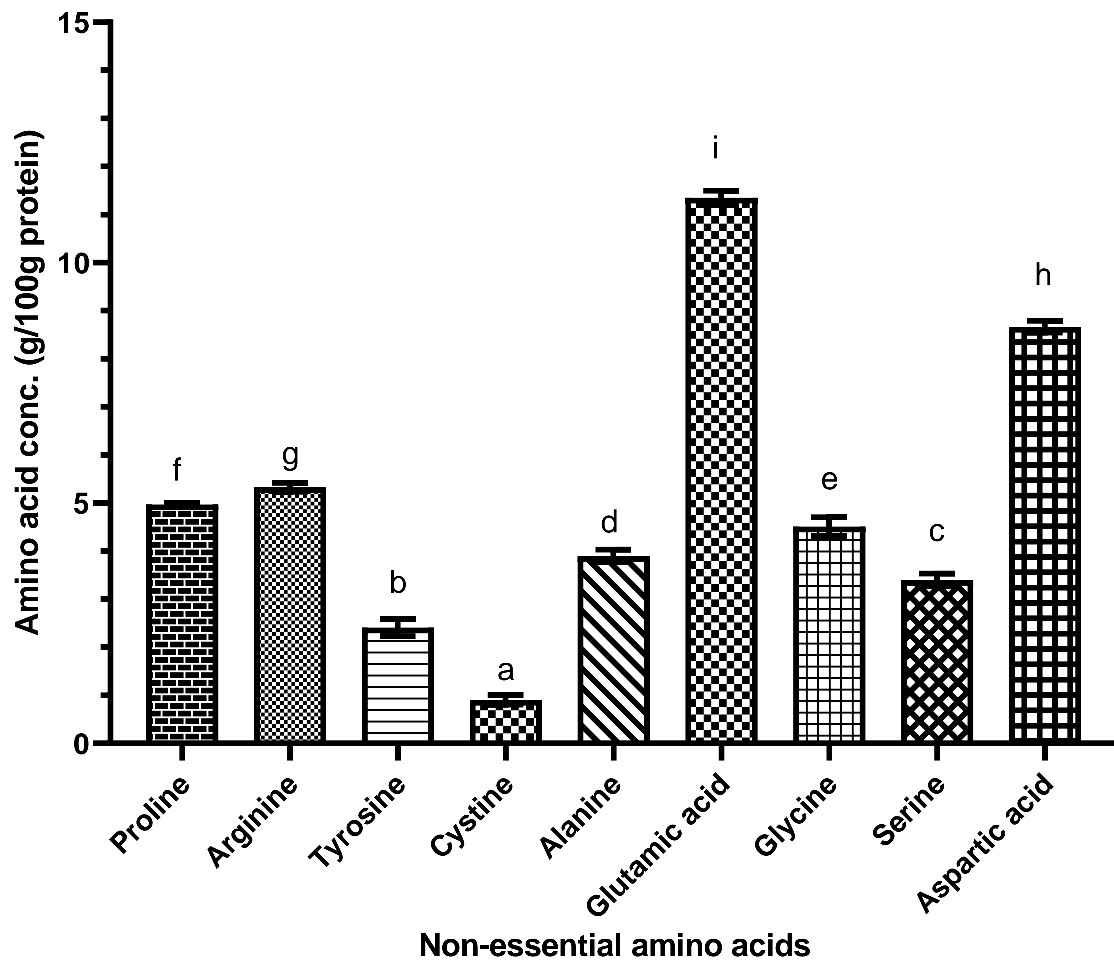

Figure 4.2: Non-essential amino acid profile of *Justicia carnea* leaves. Values are mean± standard deviation of triplicate determinations. Bars with different alphabets are statistically significant at *P*≤ 0.05

Table 4.3: Total amino acids and amino acid groups (g/100g protein) in leaves of *J. carnea*

Amino acids and groups	Concentration (g/100g protein)
Total amino acids (TAA)	75.9
Total non-essential amino acid (TNEAA)	45.45
Total essential amino acid (TEAA) with His	30.45
Total essential amino acid (TEAA) without His	28.22
Total neutral amino acids (TNAA)	44.61
Total acidic amino acids (TAAA)	20.02
Total basic amino acids (TBAA)	11.27
Total sulphur-containing amino acids (TSAA)	2.14
Total aromatic amino acids (TArAA)	8.44

Table 4.4 Percentages of amino acid groups and amino acids

Parameter	Value
% Total non-essential amino acids (%TNEAA)	59.9
% Total essential amino acid (%TEAA) with His	40.12
% Total essential amino acid (%TEAA) without His	37.18
% Total neutral amino acids (%TNAA)	58.77
% Total acidic amino acids (%TAAA)	26.38
% Total basic amino acids (%TBAA)	14.85
% Total sulphur-containing amino acids (%TSAA)	2.82
% cys in TSAA	42.52
% Total aromatic amino acids (%TArAA)	11.12
% tyr in TArAA	28.55

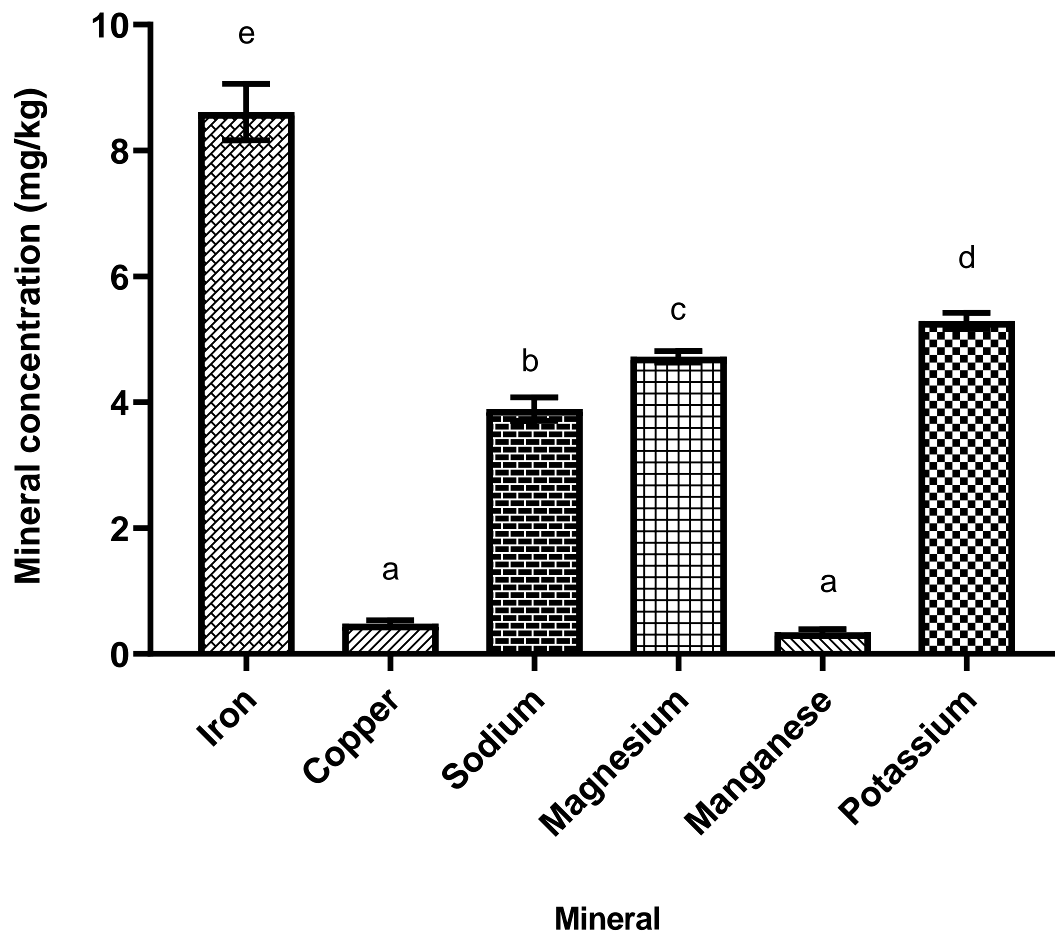

Figure 4.3: Mineral composition of *Justicia carnea* leaves. Values are mean± standard deviation of triplicate determinations. Bars with different alphabets are statistically significant at $P \leq 0.05$

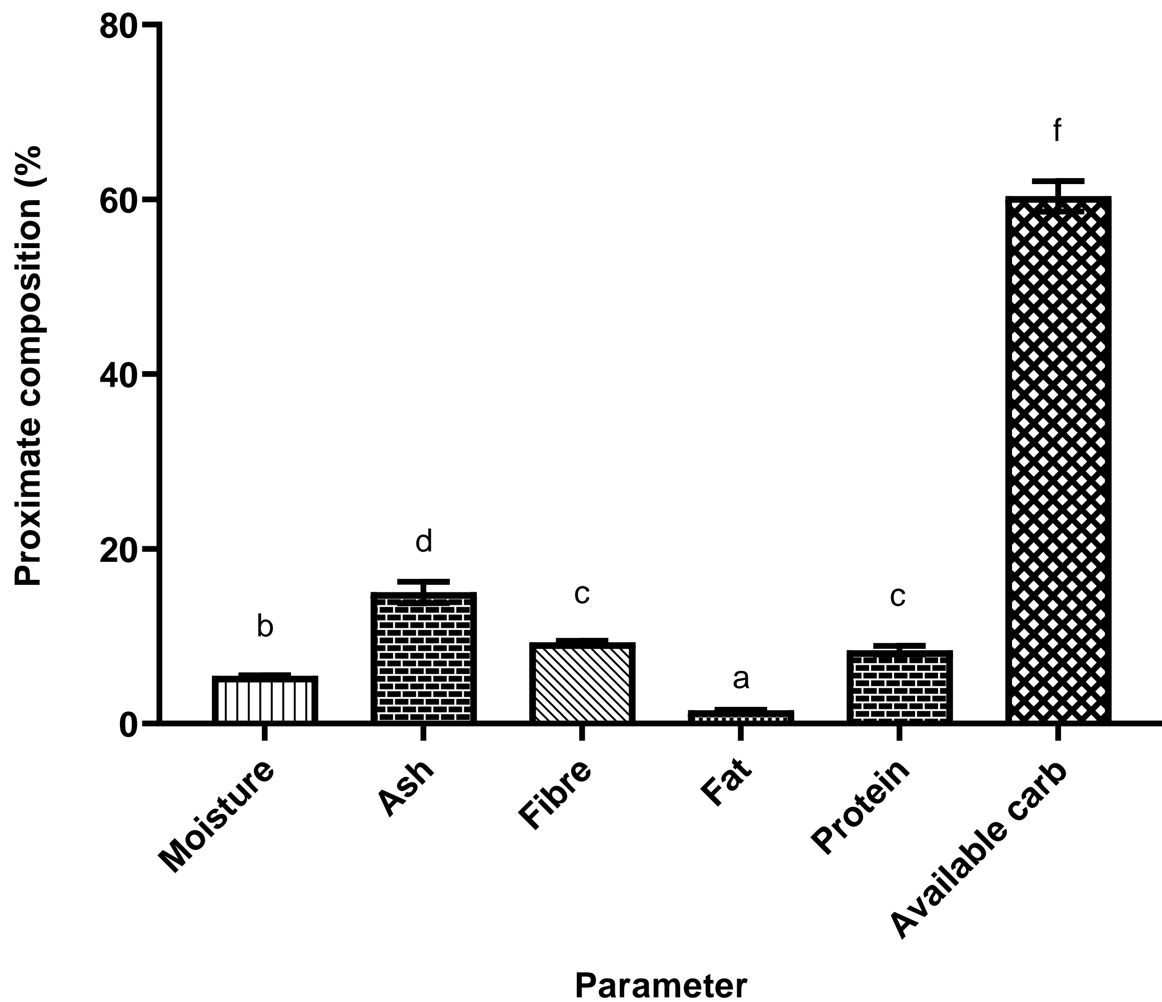

Figure 4.4: Proximate composition of *Justicia carnea leaves.* Values are means ± standard deviation of triplicate determinations. Bars with different alphabets are statistically significant at $P \leq 0.05$

Table 4.5: Vitamin compositions of *Justicia carnea* leaves

Vitamins	Concentrations
Vitamin A, mg/kg	22.16 ± 2.12^{c}
Vitamin B_1, mg/100g	0.02 ± 0.01^{a}
Vitamin B_2, mg/100g	0.02 ± 0.01^{a}
Vitamin B_3, mg/100g	0.61 ± 0.06^{a}
Vitamin B_6, mg/100g	3.47 ± 0.16^{b}
Vitamin B_9, mg/100g	0.25 ± 0.05^{a}
Vitamin C, mg/100g	232.32 ± 12.26^{d}

Values are means ± standard deviation of triplicate determinations. Column with different superscripts are statistically significant at $P \leq 0.05$

Table 4.6: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Concentration of extracts	% Scavenging activity
5mg/ml	85.87±0.14
10mg/ml	87.21±0.16
50mg/ml	80.90±0.05
100mg/ml	84.47±0.16

The mean value ±SD is the mean of the % DPPH scavenging activity from triplicate results.

Table 4.7: Hydroxyl radical scavenging activity

Concentration of extracts	% Scavenging
5mg/ml	19.10±0.00
10mg/ml	25.10±0.00
50mg/ml	28.00±0.00
100mg/ml	33.00±0.00

The mean value ±SD is the mean of % hydroxyl radical scavenging activity from triplicate results.

Table 4.8: Lipid peroxidation

Concentration of extracts	Concentration µg/ml
5mg/ml	6.1625±0.0485
10mg/ml	7.2245±0.1125
50mg/ml	4.6347±0.1290
100mg/ml	4.875±0.048

The mean value ±SD is the mean of the lipid peroxidation from triplicate results

4.2 DISCUSSION

Phytochemicals have been shown to have enormous impact on the health care system and can provide medical health benefits such as the prevention and treatment of diseases and physiological disorders. The result of the phytochemical studies of *Justicia carnea* leaves using GC-FID showed alkaloid, flavonoids, and other phytochemical such as sapogenin, proanthocyanin and steroids and antinutrient occurring at different levels. Saponins protect plants from other predator species (Lacaille – Dubois & Wagner, 2000). Phytochemicals have proven health benefits beyond those attributed to macronutrients and micronutrients (Hasler & Blumberg 1999). Saponins present anticholesterolemic and hypoglycaemic potentials through intra-lumenal physiochemical interaction (Price *et al.,* 1987, Ujowundu *et al.,* 2017). Saponins have been shown to have antifungal and antiviral properties (Lacaille Dubois & Wagner, 2000; Traore *et al.*, 2000; Ujowundu, 2017).

The GC-FID analysis showed that the leaves of *Justicia carnea* contain flavonoids such as Naringin, Flavan -3-ol, Anthocyanin, Naringenin, Flavonones, kaempferol, Epicatechin, Flavone, and Resveratol in appreciable amounts (Tables 4.4). Flavonoids are plant phenols bearing low molecular weight and possessing a wide spectrum of occurrence (Dai and Mumper, 2010). Flavonoids express antioxidant properties and inhibitory role in various stages of tumour development in animal studies. Flavonoids exert these varieties of biological effects by specific interaction with molecular targets (Middleton *et al.,* 2000; Williams *et al.,* 2004; Calabro *et al.,* 2005; Kanakis *et al.,* 2005;). The molecular structure with varying position of OH groups which confers it with antioxidant capacities. These antioxidants and free radical scavenging capacities are seen in flavones and flavonols (kaempferol). Studies have shown kaempferol as a potential agent for cancer treatment (Calderon-Montario *et al*., 2011; Donnapea *et al*., 2014), due to its ability reduce the resistance of cancer cells to anti-cancer drugs such as vinblastine and paclitaxel (Batra & Sharma, 2013). The anthocyanins content of *Justicia carnea leaves* are responsible for the red colour of the leave extract and are reported to possess significant anti-inflammatory properties (Cliffrod, 2000).

The leaves of *Justicia carnea* presented the following alkaloids; ribalinidine, spartein and epihedrine. Alkaloids are used as pain killers (morphine), stimulants (caffeine), muscle relaxers (cocaine), tranquilizers (curare), anti-cancer (vincristine, Vinplastine), an aesthetics (cocaine), etc (Russo *et al*., 2013; Kittakoop *et al*., 2014)

Phytate, oxalate, cardiac glycoside and cyanogenic glycoside were recorded in the leaves of *Justicia carnea.* These compounds are antinutrient because of the possibility of their interference in the utilization of important nutrients by living organism. Phytate interferes with proteolytic digestion (Oboh *et al.,* 2005). Phytate is an antinutritional factor which complexes with essential minerals such as C a^{2+}, Fe^{2+}, Mg^{2+} and Zn^{2+}, rendering them unavailable from the diet. Oxalate works in similar way like phytate, it binds some divalent metal ions such as Ca^{2+} and Mg^{2+}. The binding of mineral renders them unavailable from the diet. Furthermore, excessive intake of oxalate could cause hypocalcaemia, muscular weakness or paralysis, development of urinary calculi, blockage of the renal tubules by calcium oxalate crystals and gastrointestinal irritation (O'Kell *et al*., 2017).

Knowledge of the chemical constituents of plants is desirable not only for the discovery of new therapeutic agents, but also disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and determining the actual significance of folkloric remedies (Milne *et al.,*1993). The GC-MS analysis was used to identify compounds with varying molecular weights, molecular formular and structures in *Justicia carnea* leaves. These compounds have numerous medicinal properties such as Alkane (Tetradecane, Hexadecane), Alkene (Centene, Octadecene), Aromatic hydrocarbon, Terpenoid (Citronellol) which have shown antimicrobial, antifungal, antioxidant and anti-inflammatory activity (Zeinab *et al*., 2022).

Amino acids are building blocks (or monomeric constituents) of proteins and peptides. Amino acids are involved in variety of biochemical and physiological functions in living organisms. A total amino acid presented by *Justicia carnea* leaves is more than 57.8 g/100g reported for *C. dolichopentalum* leaves (Ujowundu *et al*., 2015). Amino acids such as alanine, valine, leucine and isoleucine tend to cluster together within proteins; stabilizing protein structure by means of hydrophobic interactions. The hydroxyl group of tyrosine can form hydrogen bonds and tyrosine plays significant roles in the formation of thyroid hormones, epinephrine, norepinephrine and melanin. The disulphide bonds cysteine stabilizes protein structure by the formation of covalent links between parts of a protein molecule or between different polypeptide chains (Nelson & Cox, 2005). Glutamate, cysteine and glycine form the antioxidant- glutathione. Furthermore, glutamate and aspartate (acidic amino) are involved in the synthesis of pyrimidines and purines. Methionine in S – adenosylmethionine (active methionine) by transmethylation transfers methyl group to various substances. The amount of essential amino acid indicated that *Justicia carnea* leaves can be a good source of essential amino acids. Essential amino acids are not synthesized by the body and must be obtained from food. The most hydrophilic R groups are those that are either positively (basic) or negatively (acidic) charged. In many enzymes catalyzed reactions, histidine residues facilitate the reaction by serving as a proton donor/acceptor. Histidine has also shown its ability as a buffer in plasma (Nelson & Cox, 2005).

Studies have shown the important metabolic roles of minerals as in the living organisms (Enechi & Odonwodo, 2003). Analysis of *Justicia carnea* leaves recorded iron as the major element. This was followed by potassium, magnesium and sodium. Sodium and potassium affect muscular activity and also in acid-base balance, neuromuscular irritability and nerve conduction process. Potassium is a cofactor of pyruvate kinase and some other enzymes. Iron, zinc and manganese strengthen the immune system possibly as either antioxidants or apoenzymes of enzymatic antioxidants.

The proximate composition of *Justicia carnea* leaves showed carbohydrates, protein, lipids, crude fibres, ash and moisture at appreciable amounts (Figure 4.1). The leaves of *Justicia carnea* present high concentration of carbohydrate, ash and protein than C. *dolichopentalum* contains fairly good quantities of carbohydrates, proteins and lipids than *Boerhavia diffusa* (10.56 %, 2.26 % and 1.16 %, respectively) and *Commelina nudiflora* (5.67 %, 1.69 % and 1.44 %, respectively) (Ujowundu *et al.,* 2008), as well as *Phyllanthus amarus* (45.52 %, 6.10 % and 6.03 %, respectively) (Igwe *et al.,* 2007). The leaves of *Justicia carnea* presented higher ash content compared to *Spondias mombin* leaves (0.09±0.01 %), but less fibre and moisture content when compared to *S. mombin* leaves with 10.51±0.84 % and 15.13±0.57%, respectively. Ash content of a material is the residue remaining after ignition at 500 – 600^0C for 2-4 hours. The high ash content *Justicia carnea* leaves reflected the quality of the mineral content of a plant.

The leaves of *Justicia carnea* showed vitamins C, A, and the B-vitamins (B_1, B_2, B_3, B_6, and B_9) in appreciable quantities (Table 4.3). Vitamins are important coenzymes in numerous energy yielding biochemical reactions. The B-vitamins are water soluble which are generally lost from the body daily due to the limited capacity of systemic storage. The amount B-vitamins in the leaves of *Justicia carnea* may serve as replenishing source. Vitamin B1 (thiamine) is a coenzyme to these enzymes: pyruvate dehydrogenase, α–ketoglutarate dehydrogenase and transketolase in translocation reaction in hexose monophosphate pathway (HMP). Deficiency of vitamin B_1 results in beriberi (Wardlaw *et al.,* 2004). Vitamin B_2 (riboflavin) is coenzymes to succinate dehydrogenase and glutathione reductases with deficiency resulting in glossitis and inflammation of the throat, eye nervous system disorders (Berg *et al.,* 2002).

Vitamin B_3 (niacin) include two co-enzymes such as nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). These two coenzymes participate actively in the oxidation-reduction activity of ATP generation. Some examples of enzymes in which niacin participate include: alcohol dehydrogenase (ADH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), G-3-P dehydrogenase, and pyruvate dehydrogenase. The

deficiency of vitamin B_3 leads to pellagra, characterised by dermatitis, diarrhoea and dementia (FNB, 1998).

Furthermore, vitamin B_6 with pyridoxal phosphate (PLP) as the active coenzyme, participate in amino acid metabolism such as aminotransferase, decarboxylases. Deficiency of vitamin B_3 leads to microcytic hypochromic anaemia.

Similarly, vitamin B_7 (biotin) functions as the co-enzyme for carboxylases, involved carboxylation (CO_2 fixation) in biological systems such as acetyl CoA carboxylase, propionyl CoA carboxylase and pyruvate carboxylase. Vitamin B_7 deficiency is not common; However, it can result to hair loss, skin rash, convulsions, neurological disorders and impaired growth, vitamin B_9 (folate) exists in its active form called tetrahydrofolate which functions as a co-enzyme. It is concerned with one carbon metabolism, involving the transfer and utilization of one carbon moiety either as methyl ($-CH_3$), formyl (-CHO), formate (H-COOH), forminino group (-CH=NH) or hydroxylmethyl ($-CH_2OH$) group. Deficiency results in megaloblastic (or macrocytic) anaemia and, neural tube defects in fetuses (Giovannucci, 2002; Green, 2002).

The plant leaf had an ample content of vitamin A, whose biochemical actions are involved in vision, growth and development of many types of tissues (mucus forming cells) and immunity. Its deficiency leads to night blindness, diminished neutrophils and follicular hyperkeratosis (Byers *et al.,* 2002).

The results of DPPH radical scavenging activity of the leaf of *J. carnea* and the standard antioxidant (BHT) is shown in table 4.6. The leaf extracts and the standard antioxidants promoted inhibition of DPPH radical with increasing concentrations at 5mg/ml and 10mg/ml but had a drop at the concentration of 50mg/ml and slightly increased at 100mg/ml. However, the results suggested that the methanolic extract of the leaf extract showed strong antioxidant activity when compared with the standard antioxidant (BHT). The effect of antioxidants on DPPH is thought to be due to their hydrogen-donating ability (Unuofin *et al*., 2017). The results obtained in this study suggested that

the extracts showed free radical scavenging activity by their electron transfer or hydrogen-donating ability since DPPH mechanism is based on single-electron transfer (SET) or hydrogen atom transfer pathways. DPPH radical is known to be used as the model system to investigate the scavenging activities of most natural compounds (Baskar et al., 2007). DPPH is scavenged by antioxidants through the donation of proton forming the reduced DPPH which can be quantified by the decreased absorbance (Houcine *et al.,* 2017)

The results of hydroxyl radical scavenging activity of the leaf of *J. carnea* presented in table 4.8. Showed that the leaf extracts promoted inhibition of hydroxyl radical scavenging activity with increasing concentrations. However, the percentage inhibition of the hydroxyl radical scavenging activity of the leaf extracts was lower at concentration of 5mg/ml and higher at concentration of 100mg/ml. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells and is a potent initiator of lipid peroxidation process (Sharifi-Rad *et al.,* 2020) . These extracts showed appreciable hydroxyl radical scavenging activity and could serve as potential antioxidant agents by inhibiting the interaction of hydroxyl radicals with DNA. The ability of the extracts to quench hydroxyl radicals might directly relate to the prevention of lipid peroxidation.

The result of the inhibition of lipid peroxidation activities of *Justicia carnea* leaf is shown in table 4.7. The percentage inhibition of lipid peroxidation of the leaf of *J. carnea* and standards showed that the leaf extracts had a better ability to inhibit lipid peroxidation at a concentration of 5mg/ml and 10mg/ml but had a drop at a concentration of 50mg/ml and 100mg/ml as shown in the table. Peroxidation of lipid is a natural phenomenon and occurs on its exposure to oxygen. Recently, free radicals-induced lipid peroxidation has gained much importance because of its involvement in

several pathological conditions such as ageing, wound healing, oxygen toxicity, liver disorders, inflammations, etc (Elekofehinti *et al*., 2013)

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This chemical evaluation of the leaves of *Justicia carnea* has shown its enormous composition of a variety of biologically active phytocompounds and a good blend of nutrient, that serve as a link to its antioxidant capacity. The biochemical and physiological beneficial roles of these bioactive components can be harnessed and used in the pharmaceutical and food industries for the production of drugs and raw materials for industrial purposes.

5.2 Recommendation for further studies.

The leaves of *Justicia carnea* have been found to have diverse functions, including blood-boosting potential. However, further studies are recommended on the leaves to determine other bioactive components present in *Justicia carnea* leaves that could be responsible for anti-anemic effects and for drug formulation especially for sickle cell disease patients.

5.3 Contributions to knowledge.

1. The research work identified that *Justicia carnea* leaves are a rich in vitamin C and iron both of which support overall health.
2. Due to high concentration of iron (a major component of hemoglobin) and Vitamin C in the leaves, both works together to boost blood production. with all these findings from the research work, it can be proven that the leaves of *Justicia carnea* have blood boosting potentials.

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