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Biochemical analysis of *Justicia carnea* leaves used as a hematinic

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Abstract

Justicia carnea is the largest genus of *Acanthaceae* and is a medicinal plant used widely in Nigeria, reported to have diverse functions, including blood-boosting potential. The phytochemicals, vitamins, minerals, amino acid and proximate compositions of the leaf extract of *Justicia carnea* were determined using standard methods. Results of the quantitative phytochemical analyses 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 (8.61mg/kg) as the major elemental content followed by potassium (5.29±0.11mg/kg). The concentrations of vitamins in *Justicia carnea* leaves showed 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 orders. These results obtained showed that leaves of *Justicia carnea* may serve as rich source of natural antioxidants, this may be attributed to its rich phytochemicals, nutrients and vitamin compositions and could be recommended as a potential source of useful bioactive constituents as vegetable supplement and has no toxic effect and as an effective hematinic supplement when prepared adequately.

Keywords: Antioxidant activity; Anemia; Biochemicals; Blood tonic; Blood disorders; Herbal medicine; Medicinal plants; *Justicia carnea* leaf

1. Introduction

Plants and herbs have shown excellent medicinal potentials and this has 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, and herbivorous mammals, fungi, and diseases. Great numbers of plant macronutrients, micronutrients and phytochemicals have shown excellent potential in biological activity and these has been identified in plants through various research endeavors [1]. 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 functions 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 later followed by strychnos and ipecacuanha, quinine from the cinchona tree, and several

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others. As the story of organic chemistry advanced, more classes of pharmacologically active compounds and substances were discovered in medicinal plants [2].

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 [3,1]. The Food and Agriculture Organization of the UN estimated that more than 50,000 medicinal plants are in use worldwide [4]. 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 [5].

Medicinal plants have been identified to have bioactive molecules termed phytochemicals/pharmaceutical [6] and secondary metabolites that can protect humans against diseases, they have been recognized as having beneficial characteristics utilized for the management of many ailments [7].

Justicia carnea is a medicinal plant used widely in Nigeria 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. [8, 9]. The leaves of the 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.

Figure 1 Leaves of *Justicea carnea*

2. Material and methods

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 Forestry and wildlife, 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. The leaves were removed from the stems, sorted, washed and pulverized to powder using an electric blender after air drying. The powdered leaves were stored in airtight containers until use. Extraction was performed by dissolving 1 kg of a powdered sample of its leaves in 6 L of ethanol and allowing them to stand for 48 hours with constant stirring. At the end of the extraction, the solution was filtered using Whatman No. 1 filter paper, and the extract was concentrated to a semi-solid residue in a water bath at 60°C for 48hurs. A total amount of 26.3 g of the extract was obtained.

2.1. Proximate analysis

The parameters determined include ash, moisture, crude protein, fat, fiber and carbohydrate. All of these were carried out using the method of analysis described by [10]. The carbohydrate content was estimated as the difference of all other nutrients. as follows:

$$\% \text{ carbohydrate} = 100 - (\%\text{crude protein} + \%\text{crude fiber} + \% \text{ ash} + \% \text{ lipid} + \% \text{ moisture})$$

2.2. Mineral elements analysis

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

2.3. 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).

2.4. Vitamins analysis

Vitamin A were determined by the method of Bayfield and Cole (1980). Vitamin B_1, B_2, B_3 , B_6 B_9, and vitamin C was determined using the Barakat titrimetric method described by Okwu and Ndu (2006)[11].

2.5. Phytochemical analysis

Some of the phytochemicals investigated in the leaves include phenols, saponins, steroid, flavonoids, alkaloids and tannins using the method of Gas Chromatography-Mass Spectrometer (GC-MS) as described by Kelly and Nelson(2014) [12].

2.6. Statistical analysis

The results were expressed as mean ±SD. Data were analyzed using one-way analysis of variance (ANOVA) using SPSS version 20.0. Differences between means were considered to be significant at ($p < 0.05$) using the post hoc test (Least Square Difference).

3. Results

3.1. Proximate composition of *Justicia carnea* leaves

The result (Figure 2) 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%).

3.2. Mineral composition of *Justicia carnea* leaves

The values obtained in the study of mineral composition of *Justicia carnea* leaves (Figure 3) shows that iron (8.61±0.24 mg/kg) has the highest concentration. Others are potassium (5.29±0.11mg/kg) > magnesium (4.72±0.16mg/kg) > sodium (3.89±0.15mg/kg) > copper (0.48±0.04mg/kg) > manganese (0.34±0.04mg/kg) in reducing order of presentation.

3.3. Amino acid profile (essential) of *Justicia carnea* leaves

Figure 4 presents the essential amino acid content of *Justicia carnea* leaves. It shows an appreciable concentration of leucine (6.47± 0.04g/100g) > Phenylalanine (5.14±0.07 g/100g) > valine (4.27±0.07g/100g) > lysine (3.71±0.13 g/100g) > isoleucine (3.4±0.17g/100g) > threonine (3.11±0.08g/100g). The least essential amino acid was tryptophan (0.89±0.17g/100g).

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

Figure 5 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±0.12g/100g) and aspartic acids (8.67±0.10g/100g) were the more abundant non-essential amino acids in the plant extract. Others are arginine (5.33±0.07g/100g) > proline (4.97±0.03g/100g) > glycine (4.51±0.16g/100g) > alanine (3.90±0.11g/100g) > serine (3.40±0.11g/100g). The least non-essential amino acids are tyrosine (2.41±0.15 g/100g)> cystine (0.91±0.08 g/100g).

3.5. Vitamin composition of *Justicia carnea* leaves.

Table 1.0 shows the concentration of vitamins in *Justicia carnea* leaves. Vitamin C shows the highest value of (232.32±12.26mg/100g) followed by Vitamin A (22.16±2.12mg/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.

3.6. Phytochemical composition of *Justicia carnea* leaves using GC-FID

Table 2 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±0.03mg/kg was the highest alkaloid, Flavan -3-ol was 21.18±0.02mg/kg, presented the highest amount of flavonoids, and phytate with 25.69±0.07mg/kg as the highest antinutrient.

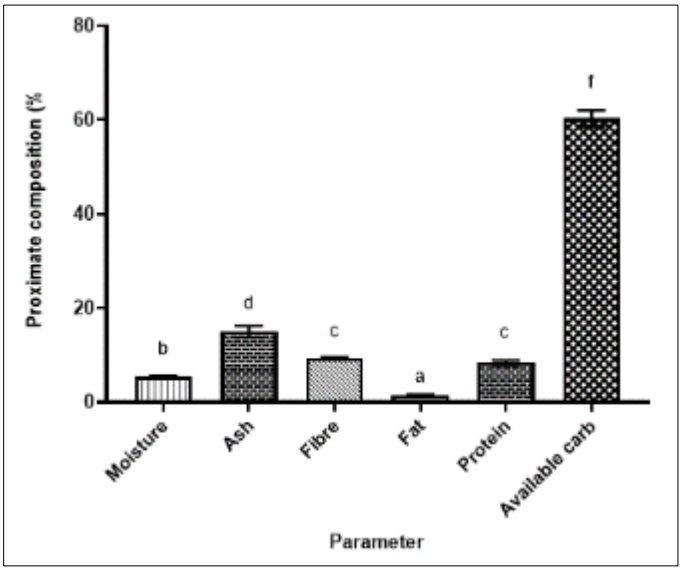

Figure 2 Proximate composition of *Justicia carnea leaves.* Values are means ± standard deviation of triplicate determinations. Bars with different alphabets are statistically significant at $P \leq 0.05$

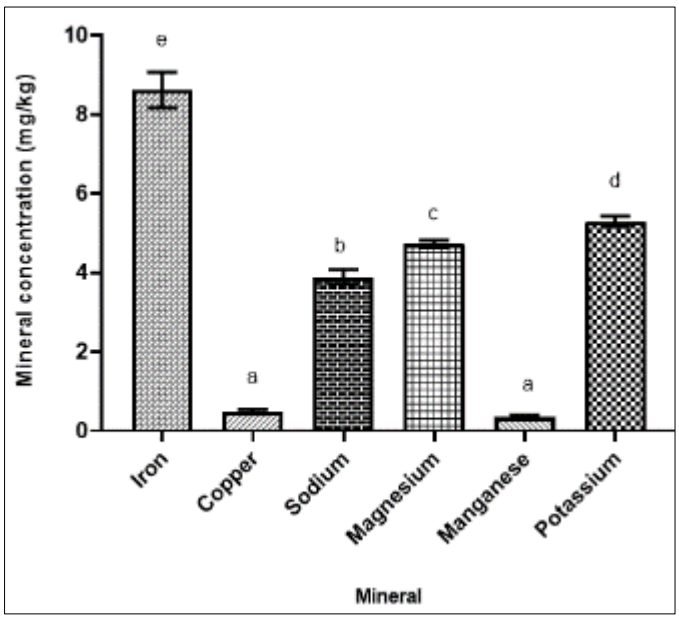

Figure 3 Mineral composition of *Justicia carnea* leaves. Values are mean± standard deviation of triplicate determinations. Bars with different alphabets are statistically significant at $P \leq 0.05$

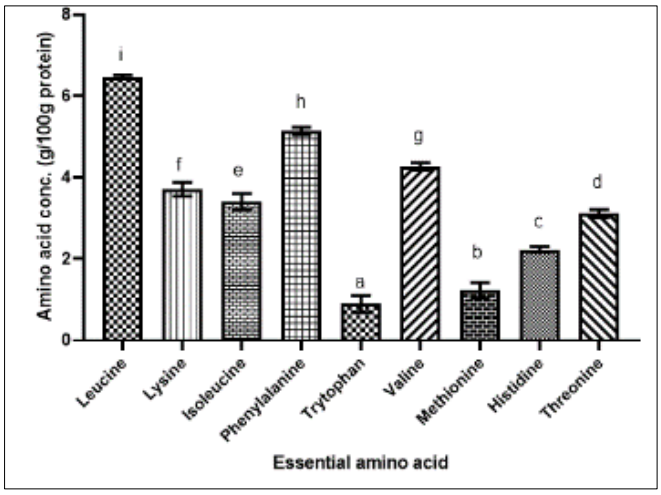

Figure 4 Essential amino acid profile of *Justicia carnea* leaves. Values are mean± standard deviation of triplicate determinations. Bars with different alphabets are statistically significant at *P*≤ 0.05

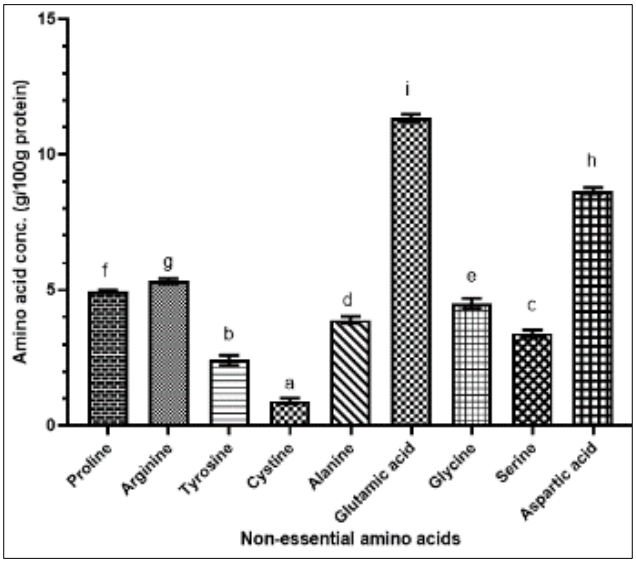

Figure 5 Non-essential amino acid profile of *Justicia carnea* leaves. Values are mean± standard deviation of triplicate determinations. Bars with different alphabets are statistically significant at *P*≤ 0.05

Table 1 Vitamin compositions of *Justicia carnea* leaves

Vitamins	Concentrations
Vitamin A, mg/kg	22.16±2.12c
Vitamin B1, mg/100g	0.02±0.01a
Vitamin B2, mg/100g	0.02±0.01a
Vitamin B3, mg/100g	0.61±0.06a
Vitamin B6, mg/100g	3.47±0.16b
Vitamin B9, mg/100g	0.25±0.05a
Vitamin C, mg/100g	232.32±12.26d

Values are means ± standard deviation of triplicate determinations. Column with different superscripts are statistically significant at *P*≤ 0.05

Table 2 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 $P \leq 0.05$

4. Discussion

The proximate composition of *Justicia carnea* leaves showed carbohydrates, protein, lipids, crude fibres, ash and moisture at appreciable amounts (Figure 2). 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) [13], as well as *Phyllanthus amarus* (45.52%, 6.10% and 6.03% , respectively) [14]. 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^{0}C for 2-4 hours. The high ash content *Justicia carnea* leaves reflected the quality of the mineral content of a plant.

Studies have shown the important metabolic roles of minerals as in the living organisms [15]. 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.

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 [16]. 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 [17]. 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 [17].

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 1). 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 [18]. 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 [19].

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 [20].

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 [21, 22].

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 [23].

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 [24]. Phytochemicals have proven health benefits beyond those attributed to macronutrients and micronutrients [25]. Saponins present anticholesterolemic and hypoglycaemic potentials through intra-lumenal physiochemical interaction [26, 27]. Saponins have been shown to have antifungal and antiviral properties [24, 28, 29].

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 2.0). Flavonoids are plant phenols bearing low molecular weight and possessing a wide spectrum of occurrence [30]. 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 [31, 32, 33, 34]. 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 [35, 36], due to its ability reduce the resistance of cancer cells to anti-cancer drugs such as vinblastine and paclitaxel [37]. 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 [38].

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 [39, 40]

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 [41]. 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 [42].

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. 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 [43]

5. Conclusion

This chemical evaluation of the leaves of *Justicia carnea* has shown its enormous composition of a variety of biologically active Phyto compounds and a good blend of nutrient. The leave extracts exhibited significant pharmacological activities 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.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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