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PROXIMATE COMPOSITION, PHYTOCHEMICAL PROFILE, ANTIOXIDANT, ANTIDIABETIC AND ANTI-INFLAMMATORY PROPERTIES OF JUSTICIA CARNEA LEAF POWDER

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Abstract: Determining the proximate composition, phytochemical profile, antioxidant, anti-diabetic and anti-inflammatory effects of *Justicia carnea* leaf powder (JLP) is the goal of this study. The results revealed that the crude fat (8.19%) had the lowest content in JLP and the nitrogen-free extract (37.85%) had the greatest. JLP included 9.98 percent crude protein, 17.54 % crude fibre, and 18.18 % ash, respectively. Phenol had the highest concentration (383.15 mg/g), whereas flavonoids (1.84 mg/g) had the lowest concentration. Alkaloids, tannins, saponins, and steroids all had concentrations of 24.03 mg/g, 84.81 mg/g, 188.13 mg/g, and 231.3 mg/g, respectively. JLP had a 62.21 % inhibition of lipid peroxidation and a 28.49 % scavenging of ABTS radicals, respectively. The percentages for the DPPH radical scavenging, Fe chelation, and hydroxyl radical inhibition were 54.05 %, 42.81 %, and 54.35 %, respectively. JLP's alpha-glucosidase and alpha-amylase inhibition activities were 65.96 and 65.82 %, respectively. JLP had a 35.51 % albumin denaturation inhibition and a 61.58 % antiprotease activity. Thus, these results suggested that JLP possesses antioxidant, antidiabetic and anti-inflammatory activities.

Keywords: Anti-oxidant, Anti-diabetic, Anti-inflammatory, *Justicia carnea*, Phytogens, Dietary supplements

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1. Introduction

The discovery of botanicals as significant sources of biologically functioning medicine has piqued researchers' curiosity, leading them to investigate the chemical make-up of medicinal plants and determine if they might be used as nutraceuticals (Dillard and German, 2000). Medicinal plants have been widely used as a source for the discovery of novel drug compounds because they are a rich source of secondary metabolites with interesting bioactivity. All portions of the plant are potential sources of bioactive substances because secondary metabolites are produced in all sections of the plant body, including the stem, bark, leaves, flowers, roots, fruits, etc. (Madhayan et al., 2022).

Varieties of phytochemicals are used in veterinary, human, and other scientific research studies, and recently, plants such as *Litsea floribunda* Gamble (Madhayan et al., 2022), *Momordica charantia*, *Ocimum gratissimum* (Oloruntola et al., 2021), *Ficus carica, F. exasperata, F. thonningii* (Osowe et al., 2021), *Anacardium occidentale* (Oloruntola, 2021), wild sunflower, goat weed (Adeyeye et al., 2020), mucuna (Oloruntola et al., 2022), *Tithonia diversifolia* (Hemsl.) A. Gray (Dada and Oloruntola, 2016) among others were reported for their neutraceutical values such as antioxidant, anti-inflammatory, antimicrobial, anti-plasmodial, and anti-diabetic properties among others.

Acanthaceae family, which encompasses over 600 species of shrubs, herbs, and delicate perennials, includes *Justicia carnea*, which is frequently found in tropical and subtropical regions (Correa, 2012). According to Uroko et al. (2017), the bioactive profile of *Justicia carnea* was linked to its medicinal properties, which included antioxidant, anti-cancerous, antimicrobial, and hypocholesterolemic effects. The plant species is also used in folk medicine to treat respiratory, gastrointestinal, inflammatory, and anaemic conditions

(Correa, 2021, Anthonia et al., 2019).

The evidence supporting the use of Justicia leaf to treat anaemia is sparse, however, there may be further health benefits from using *Justicia carnea* leaf as nutraceuticals to treat physiological issues and prevent the development of chronic illnesses (Wood et al., 2020). There is also a need for ongoing and further investigation or characterization of the bioactive composition profile of *Justicea cornea* leaf, as dietary supplements are considered nutraceuticals when they are used for health-related purposes rather than nutrition. Nutraceutical supplements are defined as products that have at least a vitamin, an amino acid, a mineral, a medicinal herb, a concentrate metabolite, extracts or a blend of these aforementioned ingredients (Nasri et al., 2014). Therefore, the goal of this study is to assess the *Justicia carnea* leaf powder's proximate composition, phytochemical profile, antioxidant, antidiabetic, and anti-inflammatory properties.

2. Materials and Methods

2.1. *Justicia carnea* Leaf Powder and Reagents

The *Justicia carnea* fresh leaves were gathered from a private garden in Akure, Nigeria. The plant was verified by a plant scientist from the Adekunle Ajasin University in Akungba Akoko, Nigeria's Department of Plant and Biotechnology. The samples were carefully cleaned with fresh water, drained, and shade dried for 14 days. They were then milled into *Justicia carnea* leaf powder (JLP) and kept at 4°C until used for analysis. Three duplicates of the parameters were analysed. The JLP samples underwent three iterations of analyses for each parameter. The analytical reagent grade chemicals utilised for chemical analysis were all acquired from Sigma-Aldrich.

2.2. *Justicia carnea* Leaf Proximate Analysis

JLP was analyzed for moisture, crude fat, crude fibre, crude protein, ash, and nitrogen-free extract using the AOAC method (AOAC, 2010).

2.3. Quantitative Phytochemical Analysis of JLP

Oloruntola (2021) described and reported the methods for determining phenols, saponins, flavonoids, and tannins; while the procedures for determination of steroids were reported by Madhu et al. (2016).

2.3.1. Phenols

A total of 2000 ml of 70% ethanol was added to 400 g of JLP, which was shaken for six hours, and then allowed to stand motionless for a further 48 hours before filtering through Whatman No 1 filter paper. The JLP ethanolic extract was vacuum condensed at 35–40 °C using a rotary evaporator. Whatman No. 1 filter paper was used to filter 200 g of JLP after it had been submerged in 1000 cc of 70% ethanol and vibrated continuously for six hours.

Using the Folin-Ciocalteau method described by Otles and Yalcin (2012), the phenolic content of JLP was measured. 250 mL of Folin-Ciocalteau reactive was added to 50 µL of JLP extract or standard solution. This mixture was left at room temperature in a dim setting for five minutes. At the conclusion of this time, a 750 microlitre solution of 7 percent Na_2CO_3 was added. The mixture was diluted to 5 mL using distilled water. The combination was then allowed to react for 120 minutes at room temperature in a dark area. At 760 nm, the absorbance of the standards and samples was measured. An 80 percent methanol solution (50µ l) was added to the blank solution in place of the 50 µl of extract. Using standards that are comparable to gallic acid, a calibration curve was used to determine the total phenolic content.

2.3.2. Alkaloids

To ascertain the alkaloid content of the leaf sample, the gravimetric method was employed (Adeniyi et al., 2009). 50 ml of acetic acid solution in ethanol (10% w/v) were mixed with 5 g of the JLP. Before being sieved, the mixture was vibrated and left alone for around 240 minutes. On a heated plate, the filtrate was reduced to one-fourth of its original volume. Then, by adding droplets of highly concentrated ammonium hydroxide, the alkaloids were precipitated. The precipitate was filtered through the filter paper and then washed with a 1 percent solution of ammonium hydroxide. The precipitate was then moved to desiccators and reweighed until it attained a constant weight after being oven-dried for 30 minutes at 60°C. Alkaloids' weight as a proportion of the sample weight was calculated.

2.3.3. Saponins

Saponin was measured using the vanillin and concentrated sulfuric acid colourimetric method (He et al., 2012). The 0.1 ml of JLP extract was combined with 0.5 ml of 50% ethanol, 4.0 ml of 77 percent sulfuric acid, and 0.5 ml of freshly made vanillin solution. The mixture was heated in a water bath to 60 °C for 15 minutes after being allowed to cool to room temperature. The absorbance at 545 nm was measured using a UV/Vis spectrophotometer. Utilizing a tea saponin calibration curve, the total amount of saponin in each sample was calculated and expressed as mg tea saponin equivalent per g (TSE/g DW).

2.3.4. Steroids

According to Madhu et al., (2016) reports, steroids concentration in JLP was identified. Steroids were determined as reported by Madhu et al. (2016). 10 ml volumetric flasks were filled with 1 ml of JLP steroid extract. Following the addition of potassium hexacyanoferrate (III) solution (0.5 percent w/v, 0.5 ml), sulphuric acid (4N, 2 ml) and iron (III) chloride (0.5 percent w/v, 2 ml) were added. The mixture was heated for 30 minutes at 70–20 °C in a water bath with periodic shaking before being diluted with distilled water to the proper concentration. At 780 nm, the absorbance was measured in comparison to a reagent blank.

2.3.5. Flavonoids

The concentration of flavonoids in JLP was measured according to Surana et al (2016) method. 0.1 ml of aluminium chloride solution, 1.50 ml of methanol, 0.1 ml of potassium acetate solution, and 2.8 ml of distilled

water were added to a test tube containing 0.50 ml of JLP extract. The same procedure was used to create sample blanks for extract and rutin standard dilutions (10-100 g/ml), but distilled water was used in place of aluminium chloride solution. The solutions were then filtered using Whatman filter paper after that (No. 1). At 510 nm, absorbance ratios were recorded in comparison to blanks. Following that, it was found that the overall flavonoid concentration was equal to 1 mg of rutin per gramme of the ethanolic JLP extract.

2.3.6. Tannins

Total tannins concentration was measured using the Folin-Ciocalteau method (Biswas et al., 2020). 1 ml of the JLP ethanolic extract was diluted with 49 ml of distilled water, 1.7 ml of 75% ethanol, 0.1 ml of metaphosphoric acid, 10 ml of 1.0 mol/ml Na_2CO_3, and 2.5 ml Folin-Ciocalteu in a volumetric flask (100 ml). After completely blending, the mixture was allowed to sit at room temperature for 15 minutes. The absorbance of the standard solution and JLP extract was then measured at 680 nm in a spectrophotometer in comparison to a blank. Tannic acid (TA) mg TA/g dry weight was utilised to express the sample's total tannin content as a reference against the standard curve (R^2 = 0.9972).

2.4. Antioxidant Activity

2.4.1. Lipid peroxidation inhibition

The lipid peroxidation inhibition of JLP extract was ascertained using a method previously described by Bajpai et al. (2015). In both the absence and addition of JLP extract (50-250 g/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 calculated (Equation 1).

$$\% \text{ inhibition} = \frac{(\text{AC} - \text{AT})}{(\text{AC})} \text{x}100 \quad (1)$$

here, AC= absorbance of control, AT= absorbance of test.

2.4.2. 2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic acid (ABTS)

The modified ABTS assay was conducted using a method outlined by Turkoglu et al. (2010) and Ozgen et al. (2006). ABTS was produced with potassium persulfate, dissolved in 20 mM sodium acetate buffer (pH 4.5), and then diluted in an acidic solution to achieve an absorbance of 0.700. (0.01 at 734 nm). Then, 3 ml of JLP extract in ethanol were combined with 1 ml of ABTS + solution at 100 g/ml concentrations. Thirty minutes after mixing, the absorbance was measured, and for each concentration, the radical scavenging percentage was determined in relation to a blank that had no scavenger. The percentage reduction of absorbance is used to determine the degree of decolourization. Different ABTS + concentrations were employed to create a standard curve. Using the following equation, the test compounds' ability to scavenge was determined (Equation 2):

$$\% \text{ inhibition} = \frac{(\text{AC} - \text{APJLP})}{(\text{AC})} \text{x}100 \quad (2)$$

here, AC= absorbance of control, APJLP= Absorbance in presence of JLP extract.

2.4.3. Ferrous chelating activity

Ebrahimzadeh et al. (2008) reported the procedures used for estimating the ferrous chelating activity of JLP. In a nutshell, 1 ml of various doses of the JLP extract (0.2, 0.4, 0.8, 1.6, and 3.2 mg/ml) were combined with 50 µl of 2 mM FeCl2. Thereafter, 0.2 ml of a 5 mM ferrozine solution was added to start the reaction. After giving the mixture a good shake, the mixture was given 10 minutes to stand at room temperature. The solution's absorbance was subsequently determined at 562 nm. . Na_2EDTA served as the positive control (Equation 3).

$$\% \text{ inhibition} = \frac{(\text{AC} - \text{AJLP})}{(\text{AJLP})} \text{x}100 \quad (3)$$

here, AC= absorbance of control, AJLP= Absorbance of JLP extract.

2.4.4. Hydroxyl radical inhibition

Outlines of Tijani et al. (2012) were followed to assess the hydroxyl radical inhibition activity of JLP extract. The reaction mixture included different quantities of the extract concentrations (50-350 g/ml) and 1.0 ml of reagent (3.0 mM deoxyribose, 0.1 mM EDTA, 2 mM H2O2, 0.1 mM L-Ascorbic acid, and 0.1 mM $FeCl_3$.6H2O in 10 mM phosphate buffer, pH 7.4). After the reaction mixtures had been incubated at 37 °C for 1 hour, 1.0 ml of 1 percent (w/v) TBA (in 0.25 N HCl) and 1.0 ml of 10 percent (w/v) TCA were added. The reaction mixtures were heated in a bain-marie of boiling water for 20 minutes at 100 °C, and the pink chromogen (malondialdehyde-(TBA) adduct) was extracted into 1.0 ml of butan-1-ol before the absorbance was measured at 532 nm against reagent blank (Equation 4).

$$\% \text{ inhibition} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \text{x}100 \quad (4)$$

2.4.5. 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical degradation activity method (Otles and Yalcin, 2012) was employed to assess the antioxidant activity of the leaf sample. Pure methanol was used to develop the DPPH radical, and 2 µl of a methanolic DPPH solution was added to 100 µl of sample extract or standard solution. For 20 minutes, this combination was left in the dark. After then, the sample absorbance was ascertained at 515 nm. It was done with a 100% methanol blank

solution. In the control solution, 100 microliters of clean water were used in place of the 100 microliters of extract. Using a calibration curve created with different gallic acid solution concentrations (10-100 ppm), the antioxidant properties of sample extracts were assessed.

2.5. Antidiabetic Properties

2.5.1. Alpha-amylase inhibitory activity

The α-amylase inhibition study was carried out using the 3,5-dinitrosalicylic acid (DNSA) method (Wickramaratne et al., 2016). To create concentrations ranging from 10 to 1000 g/mL, the JLP extract was treated with at least 10 percent dimethylsulfoxide and then diluted in buffer ((NaCl (0.006 M, Na_2HPO_4/NaH_2PO_4 (0.02 M, at pH 6.9). 200 µL of extract and 200 µL of -amylase solution were mixed and incubated at 30 °C for 10 minutes. After that, each tube received 200 µL of the starch solution (1 percent in water (w/v)) and was incubated for 3 minutes. The reaction was stopped by adding 200 µL DNSA reagent (12 g sodium potassium tartrate tetrahydrate in 8.0 mL 2 M NaOH and 20 mL 96 mM 3,5-dinitrosalicylic acid solution) to a water bath at 85–90 °C and boiling for 10 minutes. The mix was cooled to room temperature and diluted with 5 mL distilled water before being analysed with a UV-Visible spectrophotometer at 540 nm. By substituting 200 µL of buffer for the plant extract, a blank with 100% enzyme activity was created. In the absence of the enzyme solution, a blank reaction was generated using the plant extract at each concentration. As a positive control sample, acarbose (100–200 µg/mL) was employed, and the reaction was conducted in the same manner as the plant extract reaction. Using the equation below, the inhibitory activity of -amylase was calculated and reported as a percentage of inhibition. By plotting the percentage of α-amylase inhibition versus the extract concentration, the IC50 values were determined (Equation 5).

$$\%\ \alpha - \text{amylase inhibition } = 100\ \text{X}\frac{\text{A\%C} - \text{AS}}{\text{A\%C}} \quad (5)$$

Here, A%C= absorbance 100% control, AS= absorbance sample.

2.5.2. Alpha-glucosidase inhibitory activity

An assay for assessing the glucosidase inhibitory activity of JLP was described by Dej-adisai and Pitakbut (2015). The glucosidase enzyme converts the substrate, p-nitrophenol-D-glucopyranoside (pNPG), into the yellow product, p-nitrophenol (pNP), which is used to analyse the glucosidase reaction. 50µL of a 10 mM phosphate buffer solution (pH 7) containing 0.2 mg/mL sodium azide and 2 mg/mL bovine serum albumin were added to a well plate.

One unit/mL of Saccharomyces cerevisiae α-glucosidase and 50 L of an 8 mg/mL sample solution were added to the phosphate buffer solution (Type I, lyophilized powder, Sigma, EC 3.2.1.20). The solvent control was a 5 percent DMSO solution, and the positive control was 8 mg/mL of acarbose in each well. The mixes were incubated at 37o C for 2 minutes. 50 microlitres of 4 mM pNPG were then put into the well. The mixture has to incubate for a further five minutes in the same circumstances. For 5 minutes, the pNP was carried out and timed using a microplate reader at 405 nm every 30 seconds. The following linear relationship equation between absorbance and time was used to calculate the velocity (V) (Equation 6).

$$\text{Velocity} = \frac{\Delta Absorbance\ at\ 405\ nm}{\Delta\ Time} \quad (6)$$

Each sample's initial reaction's highest velocity was gathered, and the equation below was used to calculate the percentage of inhibition (Equation 7).

$$\%\ \text{Inhibition} = \frac{\text{V control} - \text{V sample}}{V\ control} X\ 100 \quad (7)$$

2.6. Anti-inflammatory Activities

2.6.1. Albumin denaturation inhibition

The assay was carried out as outlined by Osman et al. (2016). Ibuprofen and diclofenac, two positive standards, were produced at a concentration of 0.1 percent each (1.0 mg/ml), along with the JLP extracts. Each mixture's reaction vessel was made up of 1000 µl of the test extract, 1400 µl of phosphate-buffered saline, and 200 µl of egg albumin. As a negative control, distilled water was utilised in place of the extracts. The mixtures were then heated for 5 minutes at 70 °C after 15 minutes of incubation at 37 °C. Their absorbances at 660 nm were measured after cooling. This formula was used to determine the protein denaturation inhibition percentage (Equation 8):

$$\%\ \text{DI} = \left(1 - \frac{\text{ARTS}}{\text{ARTS}\ (-\text{ve control})}\right) * 100\% \quad (8)$$

DI= denaturation inhibition, ARTS= absorbance reading of the test sample.

2.5.2. Antiproteinase activity

The test was performed as outlined by Rajesh et al., (2019). 1 ml of 20 mM Tris-HCl buffer (pH 7.4), 0.06 mg of trypsin, and 1 ml of the test sample with varying concentrations (100–500 g/ml) were all included in the reaction mixture (2 ml). For five minutes, the mixture was kept heated at 37°C. 1 ml of 0.8 percent (w/v) casein was then added to the mixture. A further 20 minutes were spent keeping the mixture heated. To stop the process, 2 ml of 70% perchloric acid was added to the mixture. The murky suspension was then centrifuged after that. The supernatant's absorbance was then measured at 210 nm using a buffer as a blank. Three times the experiment was conducted. The following formula was used to calculate the % inhibition of proteinase inhibitory activity (Equation 9):

% inhibition = (Abs control-Abs sample)*100/Abs control (9)

3. Results

Figure 1 shows the proximate composition of JLP. The crude fat (8.19%) had the lowest content in JLP and the nitrogen-free extract (37.85%) had the greatest. JLP included 9.98 percent crude protein, 17.54 percent crude fibre, and 18.18 percent ash, respectively.

Phenol, one of the phytochemicals examined, had the highest concentration (383.15 mg/g), whereas flavonoids (1.84 mg/g) had the lowest concentration. Alkaloids, tannins, saponins, and steroids all had concentrations of 24.03 mg/g, 84.81 mg/g, 188.13 mg/g, and 231.3 mg/g, respectively (Figure 2).

The antioxidant activity of JLP is displayed in Figure 3. JLP had a 62.21 percent inhibition of lipid peroxidation and a 28.49 percent scavenging of ABTS radicals, respectively. The percentages for the DPPH radical scavenging, Fe chelation, and hydroxyl radical inhibition were 54.05 percent, 42.81 percent, and 54.35 percent, respectively.

JLP's alpha-glucosidase and alpha-amylase inhibition activities were 65.96 and 65.82 percent, respectively (Figure 4). JLP had a 35.51 percent albumin denaturation inhibition and a 61.58 percent antiprotease activity, respectively (Figure 5).

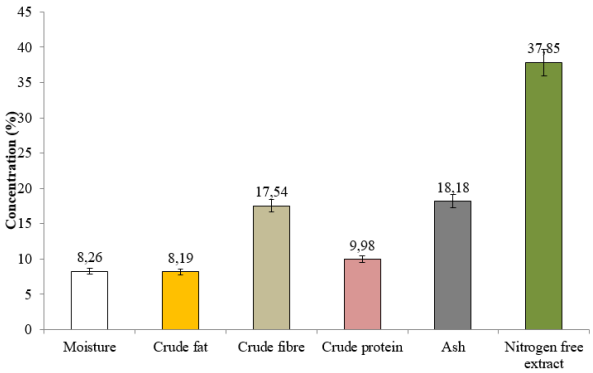

Figure 1. Proximate composition of *Justicia carnea* leaf powder.

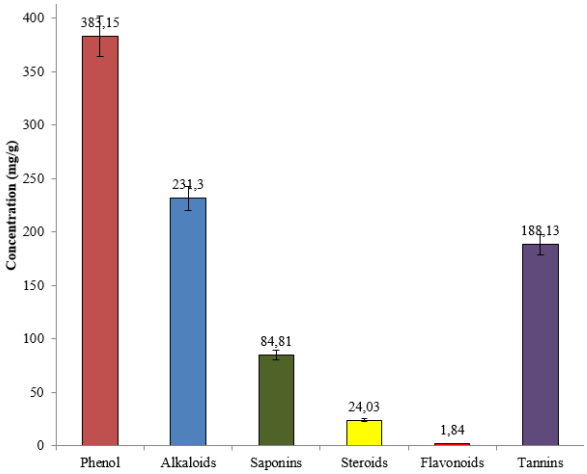

Figure 2. Phytochemical composition of *Justicia carnea* leaf powder.

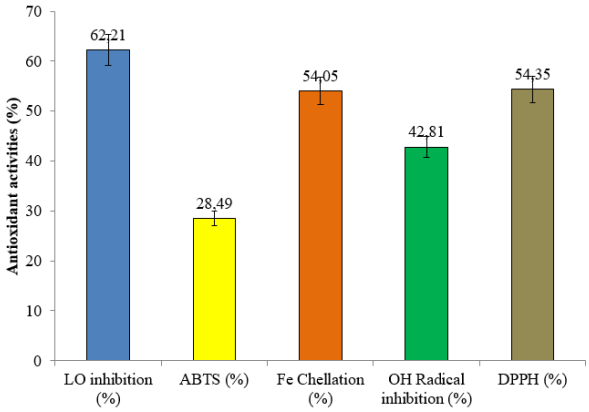

Figure 3. Antioxidant assays of *Justicia carnea* leaf powder (LO= Lipid peroxidation, ABTS= 2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid, OH= Hydroxyl, DPPH= 2,2-diphenyl-1-picrylhydrazyl).

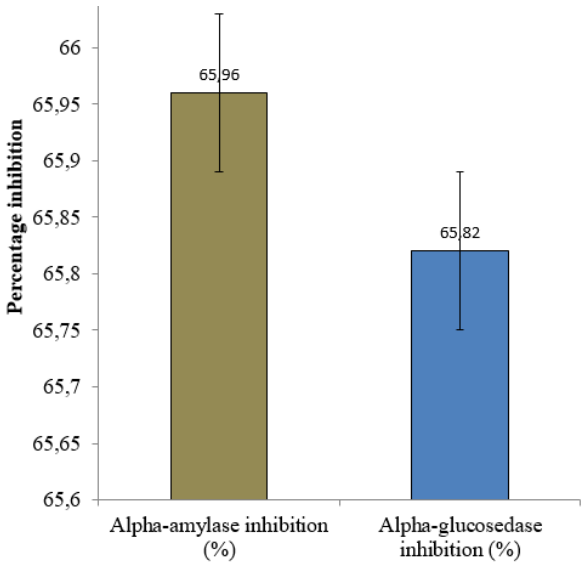

Figure 4. Antidiabetic properties of *Justicia carnea* leaf powder.

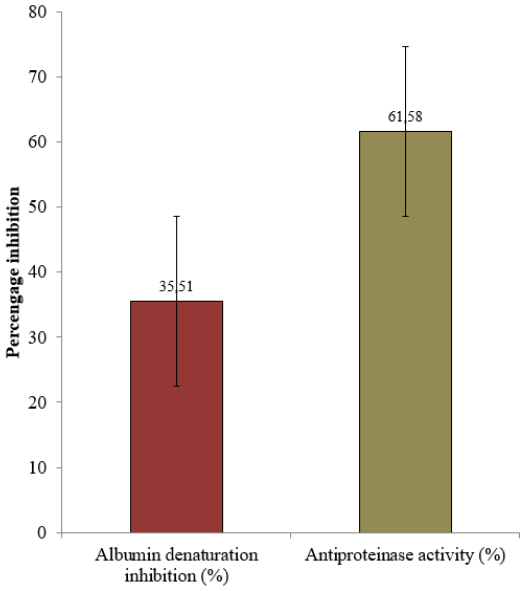

Figure 5. Anti-inflammatory properties of *Justicia carnea* leaf powder.

4. Discussion

Investigating the proximate composition can help determine whether certain foods, feeds, or supplements are appropriate to include in diets (Wu and Wu, 2017; Oloruntola, 2022). When taken as a dietary/food supplement, JLP's relatively high ash level suggests that it might augment the minerals present in foods or diets. Leaf meals were previously described by Somarsiri et al. (2010) as a dependable recipe for supplying dietary minerals. Additionally, the substantial amounts of crude fat and crude protein found in JLP reveal its nutritional value, indicating that when used as a feed ingredient or supplement, JLP, like several other phytogens or phytosupplements, may complement or contribute to the levels of fat and protein in diets (Sugiharto et al., 2019). When compared to other compositions, the nitrogen-free extract, which was noticeably more concentrated in JLP, also demonstrates its potential contribution to delivering dietary energy, especially when utilised at a macro level in feed or food formulation (Oloruntola, 2022). The 17.54 percent crude fibre content of JLP demonstrates that when added at a macro level in the diet, there may be a rise in feed bulkiness, which could affect protein intake, impair digestibility, and impair some monogastrics' performance (Buragohain 2016).

The use of phytochemicals or dietary supplements with secondary metabolites as feed supplements is presently in vogue due to the present global legal prohibition of the use of antibiotics as a growth promoter in diets of meat animals (Valenzuela-Grijalva et al., 2017; Kurek, 2019). The phenol and alkaloids, which are substantially more abundant in JLP than the other compounds identified in this study, have health benefits because it has been linked to a decreased risk of degenerative diseases like diabetes, Alzheimer's disease, cancer, and cardiovascular disease (Gutierrez-Grijalva et al., 2016; Kurek, 2019). Phenols are thought to have greater antioxidant potential, even than the widely consumed dietary vitamin E (Hollman, 2001). The hydroxyl groups connected to the phenyl ring of phenols are thought to be responsible for their antioxidative properties (Hollman, 2001). The alkaloids demonstrate antioxidant activities by inhibiting NADPH-oxidase activity by activation of the nuclear factor Nrf2 pathway (Macakova et al., 2019). According to reports, phenols and alkaloids are the two most significant compounds in phytogens, with alkaloids having a stronger link with antioxidant activity than phenols (Gan et al., 2017). The presence of saponins at a worthwhile proportion inJLP is of health importance because, along with their use in treating hypercalciuria, as an antidote for acute lead poisoning, and as an inhibitor of dental cavities, saponins are another substance with a reputation for strengthening immune function and so lowering cancer risks, lowering blood sugar and blood lipids (Shi et al., 2004). Tannin, a phenolic molecule with a high molecular weight that is also present in JLP, is well-known for its capacity to form complexes with proteins, alkaloids, carbohydrates, and gelatin as well as its antibacterial and antioxidant properties (Widsten et al., 2014). In addition, tannins and related compounds were reported to exhibit moderate cytotoxicity against tumour cell lines (Li et al., 2013). Steroids change the fluidity of membranes and serve as signalling chemicals. Because of the immune-modulating and anti-inflammatory effects of steroids, the presence of steroids in JLP in this study adds to its nutritional and health benefits. (Ericson-Neilsen and Kaye, 2014). The prevention of disease may be aided by flavonoids, secondary metabolites with a benzopyrone ring carrying phenolic or polyphenolic groups at various locations (Cavalcante et al., 2018). For instance, the total flavonoids or subclasses were said to have qualities that protect the cardiovascular system, are anti-inflammatory and antioxidant, and fight cancer, obesity, and diabetes (Ballard and Maróstica, 2019). Flavonoids, albeit having the least concentration, compared to other chemicals evaluated in this study, its presence in JLP also contributed to the nutraceutical quality of the phytogens.

The loss of free radical electrons from the lipid cell membranes during the process of lipid peroxidation resulted in a decrease in physiological performance, an increase in the permeability of the cell membranes, and a loss of membrane fluidity. Lipid peroxidation generally has negative effects on the nutritional value, texture, flavour, and appearance of food and food products (Balu et al., 2005). Since superoxide and hydroxyl radicals, which produce peroxy radicals that enhance the lipids' chain reaction, start the process of lipid oxidation, antioxidants with the ability to scavenge peroxi radicals could inhibit the process (Bajpai et al., 2015). Medicinal plants have various phytochemical components (e.g. flavonoids, tannins and phenyl propanoids and phenolic acids) that have antioxidant and free radical scavenging efficacy (Bajpai et al., 2015). In this study, the JLP showing 62.21% inhibitory effects on lipid peroxides may be a result of its hydroxyl radical scavenging abilities. Some other botanicals were also reported for having protection against lipid oxidation (Geetha and Vasudevan, 2004; Bajpai et al., 2015).

Since the production of free radical species is inhibited by the addition of antioxidants, methods being used to measure the free radical scavenging capacity are typically based on the inhibition of the accumulation of oxidised products, which gives rise to a reduction of the endpoint by scavenging free radicals, regardless of the individual compounds which contribute towards the total capacity of a phytogens or botanicals in scavenging free radicals. The 2,2-azino-bis (3-ethylbenzenthiazoline-6-sulphonic) acid radical (ABTS) assay is a technique for assessing a plant's ability to scavenge free radicals (Turkoglu, 2009). According to this study, the JLP has the potential to be a phytogenic antioxidant due to its 28.49 percent ABTS radical scavenging activity.

The myocardium, spleen, endocrine glands, and liver accumulate ferritin and hemosiderin due to the body's inability to eliminate iron released from the breakdown

of transfused red blood cells, which causes tissue damage and complications like hypothyroidism, liver failure, diabetes, heart failure, and early death (Ebrahimzadeh et al., 2008). According to the results of this study, JLP had a significant amount of iron chelating activity (50%) which suggests the phytogens might be used as dietary iron chelators to mobilise tissue iron by creating soluble, stable complexes that are then eliminated in urine and/or faeces. Chelation therapy is said to lessen issues associated with iron, according to earlier reports (Ebrahimzadeh et al., 2008).

Being the most reactive free radical, the hydroxyl radical can be created by combining hydrogen peroxide and superoxide anion in the presence of Fe2+ and Cu2+. The hydroxyl radicals are recognised for generating DNA strand breaks, lipid oxidation stimulation, and mutagenicity, carcinogenicity, and cytotoxicity as a result of connecting nucleotides in DNA (Valko et al., 2007; Bajpai et al., 2015). In this study, JLP demonstrated substantive radical scavenging activity with a radical inhibition capacity of 42.81%. The hydroxyl radical scavenging activity of *Terminalia chebula*, *Terminalia belerica* and *Emblica officinalis* was reported (Hazra et al., 2010). The DPPH radical scavenging of JLP being 54.35% in this study shows that the phytogens have antioxidant and free radical scavenging properties. This agreed with the previous report on DPPH radical scavenging activity in some selected medicinal plants (Amari et al., 2014; Kaur and Mondal, 2014).

By selectively blocking pancreatic α-amylase and intestinal α-glucosidase, hyperglycemia in type 2 diabetes cases caused by hydrolysis of starch by uptake of glucose by intestinal α-glucosidases and pancreatic α-amylase can be effectively managed. The inhibition of these enzymes causes a delay in the breakdown of carbohydrates and lengthens the time it takes for carbohydrates to be completely digested. As a result, the rate of glucose absorption is slowed and the postprandial rise in blood sugar is also slowed (Kwon et al., 2007). JLP demonstrated a 65.96 percent and 65.82 percent inhibition of α-amylase and α-glucosidases in this investigation, demonstrating the phytogens' ability to block hydrolyzing enzymes. This was in line with earlier research showing that white and ginger suppressed *in vitro* α-amylase activity (Oboh et al., 2010).

Inflammatory factors, such as heat, toxic chemical irritants, microbial infections, and physical injuries by inflammation, elicit responses in living tissues. The inflammatory response of the cells results in a variety of pathological reactions such as swelling, redness, discomfort, and heat as well as some diseases like cancer, arthritis, and stroke (Osman et al., 2016). Because there is a correlation between tissue damage and the denaturation of protein cells or intercellular components, the ability of phytogens or metabolites to prevent protein denaturation indicates apparent potential for anti-inflammatory effect (Osman et al., 2016). JLP's capacity to prevent albumin protein denaturation (35.51 percent inhibition) in this investigation so supports the claim that it has anti-inflammatory characteristics. Furthermore, arthritic responses have been linked to proteinases and leukocyte proteinase, in particular, is crucial for the occurrence of tissue damage during inflammatory responses (Rajesh et al., 2019). This study's observation of JLP's antiproteinase activity (61.58 percent) further demonstrates or supports the anti-inflammatory properties of JLP.

5. Conclusion

Thus, these results suggested that JLP could be a source of protein, energy and vitamins. In addition, JLP possesses antioxidant, anti-diabetic and anti-inflammatory activities. The JLP could be suitable as a dietary phytogenic supplement and is recommended for use as a dietary supplement in feeding trials with an animal model.

Author Contributions

Concept: O.D.O (35%), S.O.A. (30%) and C.O.O. (30%), Design: O.D.O (35%), O.S.F. (30%) and T.O.G. (30%), Supervision: O.D.O (100%), Data collection and/or processing: O.D.O (20%), S.O.A. (20%), S.A.A. (20%), C.O.O. (20%) and T.O.G. (20%), Data analysis and/or interpretation: O.D.O (20%), S.O.A. (20%), S.A.A. (20%), C.O.O. (20%) and T.O.G. (20%), Literature search: O.D.O (20%), S.O.A. (20%), S.A.A. (20%), C.O.O. (20%) and T.O.G. (20%), Writing: O.D.O (15%), S.O.A. (15%), S.A.A. (15%), C.O.O. (15%), O.S.F. (20%) and T.O.G. (20%), Critical review: O.D.O (15%), S.O.A. (15%), S.A.A. (15%), C.O.O. (15%), O.S.F. (20%) and T.O.G. (20%). Submission and revision: O.D.O (15%), S.O.A. (15%), S.A.A. (15%), C.O.O. (15%), O.S.F. (20%) and T.O.G. (20%). All authors reviewed and approved final version of the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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