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Evaluating the antimicrobial and hematological effect of *Justicia carnea* leaf extract on selected bacterial species and albino rats

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ABSTRACT
Antibiotic-resistance is becoming a worldwide public health concern especially in terms of food-borne illness and hospital-acquired infections, which has led to intensify research into medicinal plants with antimicrobial and therapeutic potentials. This study investigated the effects of Justicia carnea (locally known as "Ogwu Obara" in Igbo and "Edit Idung" or "Edit Udia" in Ibibio) ethanolic leaf extract on the antimicrobial and some haematological parameters of albino rats. Fresh leaves of the plant were collected, washed, shade-dried at room temperature for 10 days, pulverized into a fine powder using an electric blender and extracted using ethanol. Antimicrobial sensitivity testing was conducted using the agar well diffusion method against selected bacterial species including; Escherichia coli, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia, and Pseudomonas aeruginosa. Thirty healthy albino rats (15 males and 15 females) were divided into control and experimental groups, and administered varying doses (100 mg/kg, 250 mg/kg, and 500 mg/kg body weight) of Justicia carnea extract for 14 days. Haematological parameters including: packed cell volume (PCV), haemoglobin concentration (HGB), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and total white blood cell count (TWBC), as well as differential WBC counts were determined using standard manual techniques. Data were analyzed using SPSS version 25, with results expressed as mean + standard deviation. Statistical comparisons between groups were done using Student's t-test and ANOVA, with a significance level set at P<0.05. Results showed a dose-dependent increase in antimicrobial activity against

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test organisms, moreover, the phytochemical screening revealed the presence of flavonoids, tannins, phenols, alkaloids, glycosides, terpenoids and saponins. Furthermore, the results of antibacterial activity showed that the Justicia carnea extract exhibited significantly higher antimicrobial activity as shown by larger zones of inhibition and lower MIC values against the test organisms where Escherichia coli was the most susceptible organisms with a minimal inhibitory concentration at 50mg/ml zone of inhibition of 16.0 mm respectively. PCV, HGB, and RBC in the treated groups which showed statistical significance compared to controls ($P<0.05$), among treated groups, indicating improved erythropoiesis. Conversely, total WBC and lymphocyte counts decreased, while monocyte and basophil percentages increased in treated groups compared to controls. These findings suggest that Justicia carnea possesses haematinic properties that could be beneficial in improving blood health.

KEYWORDS: hemoglobin, Justicia carnea, hematology, albino rats, anemia, clinical isolates, antimicrobial sensitivity

INTRODUCTION

Medicinal plants have been defined as those plants which contain one or more substances that can be used for the synthesis of useful drugs (Anionye *et al.,* 2017). The 80% of African population use some form of traditional herbal medicine and herbal medicine is still the most abundant, affordable, and reliable, trusted and well understood form of health care in virtually all African villages (Abulaka *et al.,* 2009). Polyherbal medicines are administered in most disease conditions over a long period of time without proper monitoring and consideration of toxic effects that might results from such prolonged usage (Tedong *et al.,* 2007).

The antimicrobial compounds found in plants are of interest because antibiotic resistance is becoming a worldwide public health concern especially in terms of food-borne illness and nosocomial infections (Lee, 2025; WHO, 2025), where pathogenic bacteria like Escherichia coli, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia, and Pseudomonas aeruginosa have developed resistance to many conventional antibiotics, thereby increasing the need for alternative antimicrobial agent from plant origin. Naturally occurring antimicrobials are being sought as replacements for synthetic preservatives such as parabens (ethyl, methyl, butyl and propyl parabens), butylated hydro-xytoluene (BHT) and butylated hydroxanisole (BHA) that are under scrutiny because it's been suspected as cancer causing agents (Bergfeldet al., 2005; Byford et al., 2002; Sun et al., 2003 Wangensteen et al., 2004). The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals (Latif et al., 2025).

The increase demand for herbal products coupled with the erroneous impression by the people that herbal products are natural and thus less harmful to the body has brought concern and fear over the quality, efficiency and safety of some of the available natural herbs. Current trends showed that the inability to afford modern medical healthcare in developing countries has forced patients to seek traditional medical attentions (Watcho *et al.,* 2007).

Moreover, medicinal plants have long been used in traditional medicine for managing various health conditions, including anaemia. *Justicia carne* is a medicinal plant that is widely used in sub-Saharan Africa as natural remedy for improving blood health. This plant is often consumed as soups, herbal infusions, or decoctions by individuals suffering from anaemia or those seeking to enhance their haemoglobin levels. Despite their widespread usage, limited scientific studies have been conducted to validate their haematinic effects (Olaniyan *et al.*, 2020). *Justicia carnea*, also known as the "blood leaf" or "hospital leaf," is a member of the Acanthaceae family and is used in folklore medicine to treat anaemia and improve general health. The bioactive compounds in these plants, such as flavonoids, alkaloids, and saponins, are believed to contribute to their medicinal properties (Ujowundu *et al.*, 2017).

The genus *Justicia*, named after the 18th-century Scottish botanist James Justice, belongs to the

large family of Acanthaceae consisting of about 600 species of herbs and shrubs native to the tropics and subtropics (Onyeabo *et al* 2017). Justicia *carnea (Justicia carnea*) is a lowering plant, widely distributed in various parts of Africa. In Nigeria, the shrubs of Justicia *carnea* are grown around homesteads and act as fences, which are easy to grow and propagate from stem cuttings by pushing the stems 1 to 2 inches into the soil (Mabberley, 1997). A survey that was conducted among the Igbo local populace in Nigeria revealed that the plant under study is locally called "ogwu - obara" meaning blood - tonic. The deep red colored juice from the leaves of this plant is extracted either by soaking or boiling in water, which can be drunk as tea. In other localities in Nigeria, the raw leaves are chewed and used together with "nchu anwu" as culinary vegetables to garnish yam porridge. Traditionally, several species of *Justicia* are used in the management of inflammation, gastrointestinal disorders, respiratory tract infection, fever, pain, diabetes, diarrhea, liver diseases, rheumatism and arthritis (Corrêa and Alcantara, 2012).

They also possess anti-inflammatory, anti-allergic, anti-tumoral, anti-viral and analgesic activities (Olaniyan *et al*., 2020). Hematological parameters, such as red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin concentration, and packed cell volume, are critical indicators of an organism's physiological and pathological state (Manna *et al.,* 2019). In biomedical research, the assessment of haematological parameters plays a pivotal role in understanding the physiological status and health conditions of organisms (Malomo *et al.,* 2017). Alterations in these parameters can signify underlying health issues, such as anaemia, infection, inflammation, or haematological disorders. Therefore, the evaluation of haematological parameters is crucial in both clinical practice and experimental research settings (Ojo *et al.,* 2019). Therefore, the aim of this study is to investigate the effects of *Justicia carnea* on haematological parameters of albino rats.,

MATERIALS AND METHODS

Study Area

The study was carried out in the Animal House, Medical Laboratory Science and Microbiology Laboratory Departments of Madonna University, Elele, Rivers State, Nigeria. The university has a well-equipped biomedical laboratory suitable for biochemical, microbiology and Hematological research. The area is located in the tropical rainforest belt, with environmental conditions conducive for laboratory animal care and research work.

Collection and Identification of the Plant

Fresh leaves of *Justicia carnea* was collected from a local farm in Elele, Rivers State, Nigeria, and authenticated by a plant taxonomist in the Faculty of Pharmacy, Madonna University.

Preparation of Plant Extract

The leaves were washed thoroughly with clean water to remove dirt and debris, then shade-dried at room temperature for 10 days. Once fully dried, the leaves were pulverized into a fine powder using an electric blender.

Approximately 5.5 g of the powdered leaves were macerated in 1000 ml of 95% ethanol for 48 hours with intermittent shaking. After extraction, the mixture was filtered first through muslin cloth and then through Whatman No. 1 filter paper. The resulting filtrate was concentrated using a rotary evaporator and then a water bath at 40–50°C to obtain a dark green ethanolic extract. The semi-solid extract was transferred to an airtight container and stored at 4°C in a refrigerator until further use.

Phytochemical Analysis

Qualitative phytochemical evaluation of extracts was conducted on the extracts to determine the presence of the bioactive phytochemical composition of the extract using standard procedures described by Trease et al., (2002); Sofowora (1993). The presence of alkaloid, flavonoids, phenols, saponins and

tannin and terpenoids was evaluated using different qualitative test, including Meyer's, Dragendorff's, ferric chloride, fronthing, keller-killiani and Salkowski tests, respectively.

Research Design

This study adopted an experimental design involving the administration of aqueous extract of *Justicia carnea* leaves to albino Wistar rats and clinical isolates, Test microorganisms mainly, which include: Salmonella typhi, Escherichia coli' Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae. These isolates were obtained from the microbiology laboratory, Madonna University Teaching Hospital, Rivers State. The isolates were identified using standard biochemical tests such as catalase test, coagulase test, citrate test, indole test, oxidase test, methyl-red, sugar fermentation test and urease test. The confirmed isolates were maintained on nutrient agar slants at 4^0C for further usage (CLSI,2021). The animals were randomly assigned into groups, with a control group receiving no treatment only dhistilled water and feed while the treatment groups received varying doses of the extract. The primary aim was to observe changes in haematological parameters specifically: packed cell volume (PCV), haemoglobin concentration (HGB), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and total white blood cell count (TWBC), as well as differential WBC counts were determined following extract administration over a defined period. This design allowed for the evaluation of cause-effect relationships between the extract and hemostatic status in the animal model.

Antimicrobial Susceptibility Screening

The Antibacterial activity of the extract was determined using the agar well diffusion method as described by Bauer et al., (1966), with little adjustments. Sterile Mueller-Hinton Agar (MHA) plates were prepared and allowed to solidify, the plates were inoculated with standard bacterial suspensions using a sterile swab, wells (6mm diameter) were bored aseptically into the agar using a sterile cork borer. An aliquot of the extract of ethanolic or ethyl acetate at different concentrations (i.e.,50mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, and 250 mg/ml) were introduced into the wells. DMSO (Dimethyl sulfoxide) was used as a control to validate the assay and compare effectiveness. The plates were allow6ed to pre-diffuse for an hour at room temperature before incubated at 37°C for 24 hours to allow bacterial growth and potential. Zones of clearance were measured in millimeters using a calibrated ruler. The larger the zone of clearance, the more effective the extract is at inhibiting the growth of the test bacteria.

Minimum Bactericidal Concentration (MBC) of *Justicia carnea*

The MBC of the seed of *Justicia carnea* extracts was determined using the standard broth dilution method. This procedure was conducted with the Minimum Inhibitory Concentration (MIC) determination to establish the lowest concentration of extracts that resulted in the death and inhibition of bacteria. After determining the MIC, organisms from the cleared zones were grown by streaking onto freshly prepared nutrient agar plates using a sterile wire loop. The plates were incubated at 37°Cfor 24 hours. The MBC was recorded as the lowest concentration of extract that produced no visible growth on the agar surface, indicating complete bactericidal activity. (CLSI, 2021).

Study Population

A total of 30 albino rat which were divided into 15 males and 15 females weighing 130 to 176 were used for the study. The animals were procured from a reputable animal breeder and housed in well- ventilated standard plastic cages. The rats were allowed to acclimatize for two weeks under controlled temperature and humidity, with a 12- hour light/dark cycle. They were fed a standard pellet diet and provided water ad libitum. Animal handling and experimentation were carried out in accordance with institutional ethical guidelines for animal research.

Experimental design / Experimental Grouping and Administration

Thirty (30) albino rats which were divided into 15 males and 15 females employed in this study were further divided into eight (8) 'groups of four (4) animals except the control which were three (3) rats each:

- Group A (control for male): Receiveddistilled water
- Group B (low Dose for male): Received 100 mg/kg (0.3ml) of extract
- Group C (medium Dose for male): Received 250 mg/kg (1.75 mls) of extract
- Group D (High Dose for male): Received 500 mg/kg (3.5 mls) of extract
- Group E (control for female): Received distilled water
- Group F (low Dose for female): Received 100 mg/kg (0.3ml) of extract
- Group G (medium Dose for female): Received 250 mg/kg (1.75 mls) of extract
- Group H (High Dose for female): Received 500 mg/kg (3.5mls) of extract.

The extract was administered orally using an orogastric tube once daily for 14 consecutive days. On the 15th day, the animals were sacrificed under light chloroform anesthesia, and blood was collected via cardiac puncture into tri-sodium citrate anticoagulated tubes for coagulation analysis.

Collection of Blood Samples

At the end of the experiment, five milliliters (5 mL) of blood were collected from each rat through venipuncture into EDTA containers and labeled. The samples were taken to the laboratory for hematological analysis.

Length of Rd Cell Count (mm)

Length of Total (mm)

Hematological Analysis

The following hematological parameters were evaluated using the standard method as describe by the recent laboratory animal studies adopted by Saleem et al., (2025):

- Haemoglobin levels (Hb)
- Red blood cell count (RBC)
- Packed cell volume (PCV).
- White blood cell count (WBC)
- Mean corpuscular volume (MCV).
- Mean corpuscular haemoglobin (MCH).

Total White Blood Cell Count

The total white blood cell count determined of according to method described by McPherson & Pincus (2021). **Procedure:** The counting chamber and cover slip were cleaned with water, dried and mounted on a mechanical stage of the microscope. The blood sample was pipetted from the EDTA bottle up to 0.5 mark, followed by drawing of diluted fluid up to 11 marked from the watch glass by keeping the pipette in a vertical position. The blood and diluting fluid were allowed to mix well by rolling the pipette horizontally in between the palms. The cells were allowed to settle for 3 minutes, and then counted with a microscope, using x10 objective. The numbers of white blood cells per liter of blood was calculated as:

$$WBC = \frac{\text{Total Number of Cell counted}}{20}$$

The number obtained was multiplied by a factor of 10^9, giving the white blood cell count.

Estimation of Packed Cell Volume (PCV): Packed cell volume (PCV) was determined by the method of McPherson & Pincus (2021). A plain capillary tube was filled with un-clotted blood from EDTA tube up to three quarters of the capillary tube. The end of the tube was sealed with a plasticine sealant. The capillary tubes were arranged according to their number in the micro-hematocrit, followed by centrifuging for 5 minutes at RCF 3000 rpm. Immediately after centrifugation, the PCV was read using micro – hematocrit reader by aligning the base of the red cell column above the sealant on the 0 line and the top of the plasma column on the 100 lined.

Determination of Haemoglobin (Hb) Concentration

Hemoglobin (Hb) concentration was determined using hemoglobin-cyanide (HICN) technique as described by McPherson & Pincus (2021). Twenty micoliter (20 µl) of capillary blood was dispensed into a tube containing 4 ml of Drabkin's neutral diluting fluid. The tube was stopped, mixed well and then left at room temperature for minutes. The colorimeter was adjusted to 540 nm, followed by zeroing it with Drabkin's fluid. The absorbance of the solution was read. With the aid of a table prepared from the calibration graph, the hemoglobin values were read.

Determination of Red blood cell

The method described by Chesbrou'gh (2006), was used, the blood sample was mixed gently to ensure even distribution of cells. Using a pipette, 20 microlitres (20 µL) was added to a test tube containing 4ml of red blood cell (RBC) diluting fluid, resulting in a 1:200 dilution. A small amount of the diluted blood was taken into a clean pipette and the hemocytometer was filled by allowing the diluted blood to flow into the chamber by capillary action, ensuring no air bubbles were present. Placing the hemocytometer on the microscope stage, the grid area was focused on at low magnification (10x objective) and then switched to higher magnification (40x objective) to count the RBCs in five small squares of the large central square of the grid, only counting cells touching the top and left borders. Finally, the RBC count was calculated using the formula: RBC count=$\frac{\text{Number of cells counted} \times \text{dilution factor,}}{}$ where the volume of the counted area was 0.02 µL (since each small square represents 0.004 µL).

Statistical Analysis

The data was analyzed using Statistical Package for Social Science (SPSS) version 25. The results were presented as mean ± standard deviation. Analysis of variance was used to compare means. The value of $p \leq 0.05$ was considered significant.

RESULTS

Table 1 shows the result of the phytochemical analysis of *Justicia carnea* leaf extract and it revealed the presence various important bioactive compounds which include: saponins, tannins, flavonoids, alkaloids, phenols, glycosides, terpenoids, steroids, saponins. These phytochemicals are known to possess antimicrobial, antioxidant and hematopoietic properties.

Table 2, shows the result of the biochemical test conducted on the selected test organisms to confirm the ideal clinical organism.

Antimicrobial Activity of *Citrillus lanatus* Extracts on Selected Test Organisms

Tables 3 shows the antibacterial activity of *Justicia carnea* extracts against five organisms: Salmonella, Escherichia coli, Pseudomonas, Staphylococcus aureus, Klebsiella pneumoniae. The inhibition zones, recorded in millimeters (mm) at various extracts concentration (250 mg/ml, 200 mg/ml, 150 mg/ml, 100 mg/ml, 50 mg/ml), demonstrate variable susceptibility. The ethanolic extracts were moderately effective against staphylococcus and while highly effective against Escherichia coli, suggesting the

presence of strong antibacterial gram-negative bacteria. For the Salmonella spp., it showed moderate antibacterial activity while observed to be ineffective against Klebsiella. It showed moderate sensitivity to Pseudomonas.

The outcome of the comparative hematological data between female rats administered 100 mg/kg of ethanolic extract of *Justicia carnea* and their non-treated counterparts is presented on (Table 4) A Student's T-test was used to assess the difference in mean values between the groups, to determine if statistical significance exists between groups. Significant improvements were observed in erythrocyte indices, with treated female rats exhibiting elevated packed cell volume (PCV) values (38.67 ± 1.16%) compared to controls (36.50 ± 0.58%; P = .021), hemoglobin concentration (HGB) increased from 11.93 ± 0.35 g/dL to 12.87 ± 0.46 g/dL (P = .027), and red blood cell (RBC) count rose from 5.95 ± 0.21 $\times 10^{12}$/L to 6.43 ± 0.23 $\times 10^{12}$/L (P = .033). However, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) did not show statistically significant alterations.

Regarding white blood cell parameters, no significant changes were observed in total white blood cell count (TWBC), lymphocyte (LYM), or monocyte (MONO) percentages. A near-significant reduction in neutrophil (NEUT) percentage was noted (P = .052). Notably, a statistically significant increase in basophil (BASO) count was detected in treated rats (1.27 ± 0.08%) compared to controls (1.25 ± 0.08%; P = .020), while eosinophil (EOS) values remained unaffected (P = .721).

In this research, the results of the comparative assessment of erythrocytic and leukocytic indices between non-treated male rats and those administered 100 mg/kg of ethanolic extract of *Justicia carnea is shown in* (Table 5). A Student's T-test was used to assess the difference in mean values, which indicated a significant increase observed in red blood cell parameters among treated males, including packed cell volume (PCV: 39.25 ± 0.96% vs. 37.00 ± 1.00%; P = .029), hemoglobin concentration (HGB: 13.19 ± 0.24 g/dL vs. 12.17 ± 0.15 g/dL; P = .002), and red blood cell count (RBC: 6.55 ± 0.13 $\times 10^{12}$/L vs. 6.07 ± 0.06 $\times 10^{12}$/L; P = .002). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) remained statistically unaltered ($P > .05$).

In the leukocyte parameters, a significant reduction in total white blood cell count (TWBC) was observed in treated rats (8.88 ± 0.21 $\times 10^{9}$/L) compared to controls (9.77 ± 0.35 $\times 10^{9}$/L; P = .008). Lymphocyte percentage decreased significantly (P = .000), and neutrophil levels also showed a modest but significant reduction (P = .045). Although monocyte and basophil counts were slightly elevated in the treated group, these changes did not reach statistical significance (P = .065 and P = .229, respectively). Eosinophil percentage remained unaffected (P = .677).

Table 6 summarizes the effects of ethanolic extract of *Justicia carnea* (250 mg/kg) on red and white blood cell parameters in female rats. A Student's T-test was used to assess the difference in mean values between the groups. Statistically, there is a significant rise in erythrocytic indices, including packed cell volume (PCV: 41.25 ± 1.71% vs. 36.50 ± 0.58%; P = .002), hemoglobin concentration (HGB: 13.73 ± 0.56 g/dL vs. 11.93 ± 0.35 g/dL; P = .002), and red blood cell count (RBC: 6.85 ± 0.26 $\times 10^{12}$/L vs. 5.95 ± 0.21 $\times 10^{12}$/L; P = .002). On the other hand, no significant difference was observed in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), or mean corpuscular hemoglobin concentration (MCHC), indicating that red cell morphology remained unaffected. White blood cell indices showed a significant reduction in total white blood cell count (TWBC: 8.40 ± 0.36 $\times 10^{9}$/L vs. 9.38 ± 0.17 $\times 10^{9}$/L; P = .003), lymphocyte percentage (P = .001), and neutrophil count (P = .000). Basophil and eosinophil counts were significantly elevated in treated rats (P = .007 and P = .004, respectively), while monocyte levels showed a modest, non-significant increase (P = .076).

Table 7 presents the hematological profile of male rats administered 250 mg/kg of ethanolic extract of *Justicia carnea*. A Student's T-test was used to assess the difference in mean values between the groups, from the results obtained, treated rats exhibited marked improvements in erythroid parameters, with significant increases in packed cell volume (PCV: 40.25 ± 1.71% vs. 37.00 ± 1.00%; P = .034),

hemoglobin concentration (HGB: 13.75 ± 0.31 g/dL vs. 12.17 ± 0.15 g/dL; *P* = .000), and red blood cell count (RBC: 6.95 ± 0.21 ×10¹²/L vs. 6.07 ± 0.06 ×10¹²/L; *P* = .001). No statistically significant changes were observed in red cell indices such as MCV, MCH, and MCHC. Leukocytic parameters showed significant reduction in total white blood cell count (TWBC: 8.43 ± 0.38 ×10⁹/L vs. 9.77 ± 0.35 ×10⁹/L; *P* = .005), lymphocytes (*P* = .001), and neutrophils (*P* = .000), similar to trends observed in female counterparts. Monocyte percentage was significantly increased (*P* = .019), while changes in basophils (*P* = .053) and eosinophils (*P* = .203) did not reach statistical significance.

Table 8 illustrates the hematological impact of 500 mg/kg ethanolic extract of *Justicia carnae* in female rats, revealing significant erythropoietic stimulation and leukocytic alterations. A Student's T-test was used to assess the difference in mean values between the groups to determine if statistical significance exists between groups. A significant elevation in erythroid parameters was observed in treated rats, including packed cell volume (PCV: 44.40 ± 1.67% vs. 36.50 ± 0.58%; *P* = .000), hemoglobin concentration (HGB: 14.86 ± 0.40 g/dL vs. 11.93 ± 0.35 g/dL; *P* = .000), and red blood cell count (RBC: 7.40 ± 0.19 ×10¹²/L vs. 5.95 ± 0.21 ×10¹²/L; *P* = .000).

However, red cell indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) showed no significant differences statistically (*P* > .05). Leukocyte analysis revealed a significant decrease in total white blood cell count (TWBC: 7.88 ± 0.16 ×10⁹/L vs. 9.38 ± 0.17 ×10⁹/L; *P* = .000), lymphocyte percentage (*P* = .001), and neutrophil percentage (*P* = .000) Conversely, significant increase was noticed in monocyte (*P* = .001), basophil (*P* = .000), and eosinophil (*P* = .033).

Table 9 presents the hematological effects of 500 mg/kg ethanolic extract of *Justicia carnae* in male rats, showing marked erythropoietic stimulation alongside significant immunological modulation. A Student's T-test was used to assess the difference in mean values between the groups to determine if statistical significance exists between groups. Administration of the extract resulted in a statistically significant increase in hemoglobin concentration (HGB: 14.80 ± 0.20 g/dL vs. 12.17 ± 0.15 g/dL; *P* = .000) and red blood cell count (RBC: 7.40 ± 0.10 ×10¹²/L vs. 6.07 ± 0.06 ×10¹²/L; *P* = .000), indicating enhanced erythropoiesis. Although packed cell volume (PCV) and red cell indices—mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH)—increased in the test group, these changes did not reach statistical significance (*P* > .05). Leukocyte analysis revealed a significant decline in total white blood cell count (TWBC: 7.93 ± 0.15 ×10⁹/L vs. 9.77 ± 0.35 ×10⁹/L; *P* = .001). Differential counts demonstrated a reduction in lymphocyte (*P* = .006) and neutrophil (*P* = .001) percentages, while there was a significant increase in monocyte (*P* = .001), basophil (*P* = .014), and eosinophil (*P* = .026) levels.

TABLE 1: Qualitative Screening of Phytochemical Analyses of Seeds of *Citrillus Lanatus* Extracts

Phytochemical Components	Results
Flavonoids	+
Alkaloids	+
Saponins	+
Tannins	+
Phenols	+
Glycosides	+
Terpenoids	+
Steroids	+

Key:
(+) = Present;
(-) = Absent

Table 2: Biochemical Tests Results of selected organisms

Organism	Catalase Test	Coagulase Test	Citrate Test	Indole Test
Eschericia coli	Positive	Negative	Negative	Positive
Klebsiella spp	Positive	Negative	Positive	Negative
Staphylococcus spp	Positive	Positive	Negative	Negative
Salmonella spp	Positive	Negative	Positive	Negative
Pseudomonas	Positive	Negative	Positive	Negative

Table 3: Antibacterial Activity of *Justicia Carnea* Ethanolic Extracts on Selected Test Organisms

S/N	Probable Isolates	Concentration(mg/ml)						MIC
		250	200	150	100	50	C	
1.	Staphylococcus aureus	20.0	16.0	12.0	10.0	9.0	20.0	100.0
2.	Escherichia coli	18.0	12.0	19.0	8.0	16.0	20.0	100.0
3.	Salmonella typhi	12.0	9.0	9.0	9.0		10.0	50.0
4	Klebsiella pneumonia	0.0	0.0	0.0	0.0	0.0	0.0	>250
5	Pseudomonas aeruginosa	14.0	11.0	11.0	7.0	10.0	0.012.0	50.0

Table 4. Comparison of Red Blood Cell and White Blood Cell parameters between female rats not treated and female rats treated with ethanolic extract of *Justicia carnea* at 100mg/kg

S/N	Parameters	Control	Test	T-Value	P-Value
1	PCV	36.50 ± 0.58	38.67 ± 1.16	1.896	.021
2	HGB (g/dl)	11.93 ± 0.35	12.87 ± 0.46	3.864	.027
3	RBC ($x10^{12}$/L)	5.95 ± 0.21	6.43 ± 0.23	1.509	.033
4	MCV (g/dl)	61.33 ± 1.45	60.10 ± 0.35	0.354	.220
5	MCHC (g/dl)	32.88 ± 0.78	33.23 ± 0.23	0.230	.478
6	MCH (pg)	20.00 ± 0.14	20.00 ± 0.00	0.002	.999
7	TWBC ($x10^{9}$)	9.38 ± 0.17	9.23 ± 0.67	0.553	.691
8	LYM (%)	69.25 ± 0.96	68.00 ± 1.00	0.712	.153
9	NEUT (%)	27.50 ± 0.58	26.00 ± 1.00	0.935	.052
10	MONO (%)	1.78 ± 0.10	1.93 ± 0.21	0.348	.228
11	BASO (%)	1.25 ± 0.08	1.27 ± 0.08	2.996	.020
12	EOS (%)	1.50 ± 0.58	1.67 ± 0.58	0.198	.721

Key: Results are expressed as Mean + Standard Deviation. PCV= Packed Cell Volume RBC = Red Blood Cell Count, HGB = Haemoglobin, MCHC = Mean Cell Haemoglobin Concentration, MCH = Mean Cell Haemoglobin, TWBC = Total White Blood Cell count, MCV = Mean Cell Volume, NEUT = Neutrophil, EOS = Eosinophil, BASO = Basophil, MONO = Monocytes, LYM = Lymphocytes, $P<0.05$ is statistically significant.

Table 5. Comparison of Red Blood Cell and White Blood Cell parameters between male rats not treated and male rats treated with ethanolic extract of *Justicia carnae* at 100mg/kg

S/N	Parameters	Control	Test	T-Value	P-Value
1	PCV	37.00 ± 1.00	39.25 ± 0.96	1.896	.029
2	HGB (g/dl)	12.17 ± 0.15	13.19 ± 0.24	4.553	.002

3	RBC (x10^{12}/L)	6.07 ± 0.06	6.55 ± 0.13	3.887	.002
4	MCV (g/dl)	60.60 ± 0.60	60.38 ± 0.52	0.222	.619
5	MCHC (g/dl)	32.87 ± 0.67	33.25 ± 0.25	0.456	.329
6	MCH (pg)	20.03 ± 0.06	20.00 ± 0.08	0.002	.576
7	TWBC (x10^{9})	9.77 ± 0.35	8.88 ± 0.21	5.437	.008
8	LYM (%)	70.67 ± 0.58	66.50 ± 0.58	4.776	.000
9	NEUT (%)	28.67 ± 0.58	26.50 ± 1.29	1.162	.045
10	MONO (%)	1.70 ± 0.10	1.88 ± 0.096	0.848	.065
11	BASO (%)	1.25 ± 0.08	1.27 ± 0.08	0.227	.229
12	EOS (%)	2.00 ± 0.00	2.25 ± 0.96	0.273	.677

Key: Results are expressed as Mean + Standard Deviation. PCV= Packed Cell Volume RBC = Red Blood Cell Count, HGB = Haemoglobin, MCHC = Mean Cell Haemoglobin Concentration, MCH = Mean Cell Haemoglobin, TWBC = Total White Blood Cell count, MCV = Mean Cell Volume, NEUT = Neutrophil, EOS = Eosinophil, BASO = Basophil, MONO = Monocytes, LYM = Lymphocytes, P<0.05 is statistically significant.

Table 6. Comparison of Red Blood Cell and White Blood Cell parameters between female rats not treated and female rats treated with ethanolic extract of *Justicia carnae* at 250mg/kg

S/N	Parameters	Control	Test	T-value	P-Value
1	PCV	36.50 ± 0.58	41.25 ± 1.71	2.896	.002
2	HGB (g/dl)	11.93 ± 0.35	13.73 ± 0.56	4.064	.002
3	RBC (x10^{12}/L)	5.95 ± 0.21	6.85 ± 0.26	3.370	.002
4	MCV (g/dl)	61.33 ± 1.45	60.68 ± 0.29	0.364	.246
5	MCHC (g/dl)	32.88 ± 0.78	33.23 ± 0.10	0.233	.400
6	MCH (pg)	20.00 ± 0.14	20.03 ± 0.05	0.102	.750
7	TWBC (x10^{9})	9.38 ± 0.17	8.40 ± 0.36	2.553	.003
8	LYM (%)	69.25 ± 0.96	65.00 ± 0.82	1.733	.001
9	NEUT (%)	27.50 ± 0.58	22.00 ± 0.82	3.305	.000
10	MONO (%)	1.78 ± 0.10	2.05 ± 0.24	0.218	.076
11	BASO (%)	1.25 ± 0.08	1.27 ± 0.08	2.300	.007
12	EOS (%)	1.50 ± 0.58	3.25 ± 0.50	3.656	.004

Key: Results are expressed as Mean + Standard Deviation. PCV= Packed Cell Volume RBC = Red Blood Cell Count, HGB = Haemoglobin, MCHC = Mean Cell Haemoglobin Concentration, MCH = Mean Cell Haemoglobin, TWBC = Total White Blood Cell count, MCV = Mean Cell Volume, NEUT = Neutrophil, EOS = Eosinophil, BASO = Basophil, MONO = Monocytes, LYM = Lymphocytes, P<0.05 is statistically significant.

Table 7. Comparison of Red Blood Cell and White Blood Cell parameters between male rats not treated and male rats treated with ethanolic extract of *Justicia carnae* at 250mg/kg

S/N	Parameters	Control	Test	T-Value	P-Value
1	PCV	37.00 ± 1.00	40.25 ± 1.71	2.334	.034
2	HGB (g/dl)	12.17 ± 0.15	13.75 ± 0.31	3.768	.000
3	RBC (x10^{12}/L)	6.07 ± 0.06	6.95 ± 0.21	1.889	.001
4	MCV (g/dl)	60.60 ± 0.60	58.00 ± 2.64	0.387	.162
5	MCHC (g/dl)	32.87 ± 0.67	34.18 ± 1.56	0.420	.237

6	MCH (pg)	20.03 ± 0.06	19.80 ± 0.27	0.510	.211
7	TWBC (x10^9)	9.77 ± 0.35	8.43 ± 0.38	2.331	.005
8	LYM (%)	70.67 ± 0.58	64.50 ± 1.29	4.712	.001
9	NEUT (%)	28.67 ± 0.58	23.00 ± 0.82	2.359	.000
10	MONO (%)	1.70 ± 0.10	2.18 ± 0.22	2.212	.019
11	BASO (%)	1.25 ± 0.08	1.27 ± 0.08	0.987	.053
12	EOS (%)	2.00 ± 0.00	2.50 ± 0.58	0.176	.203

Key: Results are expressed as Mean + Standard Deviation. PCV= Packed Cell Volume RBC = Red Blood Cell Count, HGB = Haemoglobin, MCHC = Mean Cell Haemoglobin Concentration, MCH = Mean Cell Haemoglobin, TWBC = Total White Blood Cell count, MCV = Mean Cell Volume, NEUT = Neutrophil, EOS = Eosinophil, BASO = Basophil, MONO = Monocytes, LYM = Lymphocytes, P<0.05 is statistically significant.

Table 8. Comparison of Red Blood Cell and White Blood Cell parameters between female rats not treated and female rats treated with ethanolic extract of *Justicia carnae* at 500mg/kg

S/N	Parameters	Control	Test	T-Value	P-Value
1	PCV	36.50 ± 0.58	44.40 ± 1.67	2.242	.000
2	HGB (g/dl)	11.93 ± 0.35	14.86 ± 0.40	4.140	.000
3	RBC (x10^{12}/L)	5.95 ± 0.21	7.40 ± 0.19	2.365	.000
4	MCV (g/dl)	61.33 ± 1.45	59.94 ± 0.93	0.374	.124
5	MCHC (g/dl)	32.88 ± 0.78	33.42 ± 0.61	0.237	.271
6	MCH (pg)	20.00 ± 0.14	20.06 ± 0.05	0.111	.407
7	TWBC (x10^9)	9.38 ± 0.17	7.88 ± 0.16	3.514	.000
8	LYM (%)	69.25 ± 0.96	62.20 ± 2.39	2.213	.001
9	NEUT (%)	27.50 ± 0.58	18.60 ± 0.55	2.901	.000
10	MONO (%)	1.78 ± 0.10	2.30 ± 0.16	2.844	.001
11	BASO (%)	1.25 ± 0.08	1.27 ± 0.08	3.054	.000
12	EOS (%)	1.50 ± 0.58	3.00 ± 1.00	1.776	.033

Key: Results are expressed as Mean + Standard Deviation. PCV= Packed Cell Volume RBC = Red Blood Cell Count, HGB = Haemoglobin, MCHC = Mean Cell Haemoglobin Concentration, MCH = Mean Cell Haemoglobin, TWBC = Total White Blood Cell count, MCV = Mean Cell Volume, NEUT = Neutrophil, EOS = Eosinophil, BASO = Basophil, MONO = Monocytes, LYM = Lymphocytes, P<0.05 is statistically significant.

Table 9. Comparison of Red Blood Cell and White Blood Cell parameters between male rats not treated and male rats treated with ethanolic extract of *Justicia carnae* at 500mg/kg

S/N	Parameters	Control	Test	T-Value	P-Value
1	PCV	37.00 ± 1.00	41.33 ± 3.214	0.312	.090
2	HGB (g/dl)	12.17 ± 0.15	14.80 ± 0.20	2.864	.000
3	RBC (x10^{12}/L)	6.07 ± 0.06	7.40 ± 0.10	2.509	.000
4	MCV (g/dl)	60.60 ± 0.60	55.83 ± 3.63	0.354	.088
5	MCHC (g/dl)	32.87 ± 0.67	35.90 ± 2.26	0.230	.090
6	MCH (pg)	20.03 ± 0.06	20.00 ± 0.00	0.162	.373
7	TWBC (x10^9)	9.77 ± 0.35	7.93 ± 0.15	4.553	.001
8	LYM (%)	70.67 ± 0.58	62.67 ± 2.52	4.712	.006

9	NEUT (%)	28.67 ± 0.58	19.00 ± 1.73	3.935	.001
10	MONO (%)	1.70 ± 0.10	2.53 ± 0.12	4.348	.001
11	BASO (%)	1.25 ± 0.08	1.27 ± 0.08	1.996	.014
12	EOS (%)	2.00 ± 0.00	4.00 ± 1.00	0.470	.026

Key: Results are expressed as Mean + Standard Deviation. PCV= Packed Cell Volume RBC = Red Blood Cell Count, HGB = Haemoglobin, MCHC = Mean Cell Haemoglobin Concentration, MCH = Mean Cell Haemoglobin, TWBC = Total White Blood Cell count, MCV = Mean Cell Volume, NEUT = Neutrophil, EOS = Eosinophil, BASO = Basophil, MONO = Monocytes, LYM = Lymphocytes, $P<0.05$ is statistically significant.

DISCUSSION

The elementary phytochemical screening revealed the presence of several bioactive compounds contained in the *Justicia carnea* extracts, these include: tannins, saponins, flavonoids, phenols, glycosides, alkaloids, terpenoids and steroids. The discovery of the presences of these bioactive compounds in *Justicia carnea* extracts collaborated with the earlier reports of Onyekachi, (2021), who discovered similar phytochemicals components in *Justicia carnea* and linked them to plant derived secondary metabolites that are responsible for the antimicrobial, antioxidant, anti- inflammatory and haematinic properties of *Justicia carnea*.

Furthermore, the antimicrobial evaluation carried out indicated that the extract showed different levels of inhibition against the test bacteria isolates; Staphylococcus aureus, Escherichia coli, Salmonella typhi, Klebsiella pneumonia, and Pseudomonas aeruginosa which is in collaboration with earlier finding by Ijeoma et al., (2025), who reported that *Justicia carnea* extract shows a reasonable inhibitory effect against both gram negative and positive bacterial isolates. The highest susceptibility was seen in Staphylococcus aureus (20.0mm), confirming the extract contains potent antimicrobial constituents capable of inhibiting bacterial growth. The antimicrobial potency of the extract is attributed to the presence of phytochemicals such as tannins, saponins, flavonoids, phenols, glycosides, alkaloids, terpenoids and steroids, which have been reported widely to destroy the cell wall of bacterial isolates, interfere with enzyme systems and disrupt microbial biochemical processes microbial. The broad-spectrum activity of extract against the test isolates indicates that *Justicia carnea* could serve as a major source of natural antimicrobial agent for treating and managing bacterial infections. This research supports the traditional use of plant and shows its potential in relevance in pharmaceutical development.

The findings from this study demonstrates that administration of 100 mg/kg ethanolic extracts of *Justicia carnea* has a significant positive effect on certain hematological parameters in albino rats. Specifically, the treated groups showed significant increase in packed cell volume (PCV), hemoglobin concentration (HGB), and red blood cell (RBC) count compared to the control group. These results support traditional claims of the plant's blood-boosting potential and at the same time suggests that the extract demonstrated haemopoietic which has the capacity of stimulating erythropoiesis and bringing about the formation of blood, these effects may be associated with the presence of phytochemicals such as flavonoids, alkaloids, tannin and phenolic compounds which possess the antioxidant and blood boosting activities.

These findings corroborated with the results of Ebhohon et al., (2023) who reported significant increase in hemoglobin concentration, packed cell volume and red blood cell count in Wistar rat treated with Justicia carnea extract. The increase in erythrocytic indices indicates that the bioactive compounds in *Justicia carnea* — including iron, flavonoids, and other phytochemicals — may stimulate erythropoiesis or enhance iron availability and utilization in the body. The slight or non-significant changes in mean corpuscular indices (MCV, MCH, MCHC) suggest that the size and hemoglobin content of individual red blood cells remained stable despite the overall increase in RBC numbers.

However, the study also revealed a significant reduction in total white blood cell count (TWBC) and lymphocyte percentages in rats treated ethanolic extract of *Justicia* extract when compared with the control group. This could imply a mild suppressive effect on certain immune components or reflect a shift in leukocyte distribution. The increase in monocytes and basophils in treated rats may indicate compensatory immune modulation. These observations suggest that while *Justicia carnea* enhances erythropoiesis, its impact on the immune system needs to be carefully assessed to rule out any potential immunosuppressive effects during prolonged use. These results algins with the results of Yahaya *et al*., (2024), who observed a decrease in white blood cell parameters in albino rats exposed to bioactive compounds, pointing at the fact that some phytochemical compounds confer suppressive impact on the immune -related blood cell base on the dosage and exposure period.

The hematological profile of male rats administered 250 mg/kg ethanolic extract of *Justicia carnea* indicated improvement in erythroid attributes, majorly a significant rise in packed cell volume when compared with the control group, which suggest erythropoiesis and indicates that the extract exhibits hematinic properties which have the capability of stimulating the formation of red blood cells, thereby improving the overall blood quality. The improvement in erythroid parameters may be associated with the availability of certain phytochemical such as flavonoids, alkaloids, tannin and phenolic which can help in promoting the formation of blood and protecting erythrocytes from oxidative damage. These findings corroborated with the pervious study of Ebhohon et al., (2023) and Ojeaburu & Uanseoje (2025), who reported crucial rise in packed cell volume and other hematological indices following the administration of the extract on the rats. The results therefore encourage the traditional use of the plant as a blood tonic and suggest it efficacy in managing shortage of blood and related hematological disorders.

The administration of *Justicia carnea* at moderate doses will lead to erythropoiesis while higher doses like (500 mg/kg), will result in immune modulation or suppression of white blood cells. These results align with the work of Akintimehhin *et al*., (2021); Ebhohon *et al.,* (2023) and Ojeaburu & Uanseoje (2025). The rise in the red blood cell indicates erythropoiesis and improved oxygen-carrying capacity of the blood, showing that the extract exhibits hematinic ability. In contrast, the decline in the white blood cell count suggests a possible dose-dependent immunomodulatory or mild immunosuppressive effect of the extract at a higher concentration. This may show a low leukocyte activity or altered immune cell production, likely influenced by the concentration of the bioactive substance present in the extract. In summary, the administration of 500 mg/kg extract in both female and male rats enhance erythropoiesis and improves red blood cell attributes, it may at the same time modulate immune function by reducing the level of the white blood cells at higher dosages. These results highlight both the hematinic potential and dose-dependent immunological effect of the plant extract, which support the need for controlled dosage in therapeutic applications.

CONCLUSION

The results gotten from this study shows that Justicia carnea leaf extract possesses a meaningful antimicrobial and hematopoietic activity against selected bacterial species and favorable hematological effect in albino rats. The observed antibacterial activity can be associated to the presence of bioactive phytochemicals with the ability of inhibiting the growth of pathogenic microorganisms. Furthermore, the extract showed a positive influence on the hematological parameters by increasing the red cell count, hemoglobin concentration and packed cell volume, thereby reinforcing its traditional use as a blood enhancing plant. There were no toxic effects on the measured blood indices which suggest that the extract is some how safe at a tested dose.

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