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Original Article

Evaluation of Phytochemical, Antioxidant, Anti-sickling, and Membrane Stabilization Properties of *Justicia carnea* Leaves and Stems Extracts

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ABSTRACT

Introduction: Sickle cell disease (SCD) is an autosomal recessive inherited genetic blood disorder resulting from the aberrant structure of hemoglobin (Hb) that results in the production of hemoglobin S (Hb S). **Objective:** This study aimed to investigate the phytochemical profiles, antioxidant, anti-sickling, and membrane stabilizing activities of crude extracts of *Justicia carnea* leaves and stems that used to treat SCD in Nigeria. **Methods:** The 70 % ethanol crude extracts of the dried, powdered leaves and stems of *J. carnea* were prepared by maceration. Reversal and inhibition activity of the plant materials were evaluated by using sodium-metabisulphite-sickled HB SS blood cells from two patients not in crisis. The membrane stabilization assays on rats' red blood cells (RBCs) and human sickled RBCs and antioxidant assays were determined following standard methods. Results were presented as Mean ± SEM. Data analysis and graph plotting were carried out using GraphPad Prism® version 8.01. **Results:** The antioxidant assays indicated that *J. carnea* leaves (JCL) had stronger DPPH activity (IC_{50} = 245.031 ± 0.228 µg/mL). While, the stems (JCS) exhibited higher NO scavenging (84.123 ± 0.497 µg/mL). The highest reversal and inhibition percentages observed at 60, 90, and 120-minute time points. At 60th minutes reading, JCL 20 mg/mL (78.9±0.13%) demonstrated the highest reversal activity, while JCL 10 mg/mL (66.9±0.82 %) revealed the strongest inhibition effect. The extracts showed dose-dependent membrane stabilization activity. **Conclusions:** The extracts demonstrated remarkable antioxidant, anti-sickling, and membrane stabilizing activities. The use of this plant could be a promising natural therapy for SCD.
Keywords: Erythrocytes, Hemoglobin, Medicinal Plants, Sickle Cell Disease

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INTRODUCTION

Sickle cell disease (SCD) is an autosomal recessive inherited genetic blood disorder resulting from the aberrant structure of hemoglobin (Hb) that results in the production of hemoglobin S (Hb S)[1,2]. Hb is the key constituent of red blood cells (RBCs) with the fundamental role of oxygen transport. Although there are several other forms of normal Hb that are known, the most prevalent form in adults is hemoglobin A (HbA)[3].

The mutation in chromosome 11 causes the abnormal gene that changed the amino acid. A single base-pair point mutation in the beta-globin chain at position six replaces the amino acid valine (GAG to GTG) for glutamic acid[4,5]. Children who receive the same mutated gene from both of their parents at birth are affected by the illness. If both parents are carriers the odds of a child getting both distinctive genes and developing the condition are 25%, and the likelihood of being a carrier is 50%[6].

The life span of normal RBCs is 120 days, and their biconcave disk shape with flexibility makes them free to move through the microvasculature. But, in hypoxia, there is an abrupt fall in the deformability of RBCs because of Hb polymerization and precipitation inside the cells[7]. Therefore, RBCs take the sickle shape with a 10–20 day lifespan that contributes to reduced microcirculation and hemolysis, leading to vaso-occlusion and disease complications, including, high susceptibility to infections, haemolytic anemia, splenomegaly, and elevated production of endogenous free radicals, primarily the OH radical[8,9].

SCD is still one of the most common inherited hemoglobinopathies among Africans, with Sub-Saharan Africa being the most affected[10]. To aid in the management of SCD several treatment interventions have been suggested. These include hydroxyurea, frequent blood transfusions, and hematopoietic stem cell transplantation[11,12,13]. However, these methods are either costly to the African community or ineffective, or they might introduce infectious diseases[14].

Various studies have shown the antisickling activity of different plant-based compounds[15,16,17]. These can be a potential and safe alternative for the effective management of SCD in the quest for effective chemotherapy agents with minimal side effects[18]. *Justicia carnea* is a renowned medicinal plants of Acanthaceae Family[19]. This plant has been in high demand because of its anti-sickling, hematinic, antibacterial, and antihypertensive activity[20,21]. When soaked in boiled water in a closed container for about 15 minutes, despite its green leaves, the boiled water turns into a purplish-red juice[22]. In this study, the anti-sickling activity of 70% ethanol stem and leaf crude extracts of *J. carnea* were evaluated. This study aimed to investigate the phytochemical profiles, antioxidant, anti-sickling, and membrane stabilizing activities of crude extracts of *J. carnea* leaves and stems that used to treat SCD in Nigeria.

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MATERIALS AND METHODS

Plant Collection and preparation

Leaves and stems of *Justicia carnea* were collected from Ipara-Remo, Ogun State, Ibadan, Nigeria (Latitude: 7° 0'0" N. Longitude: 3°40' 0" E) on May 16, 2024. The plant was authenticated at the Forest Herbarium Ibadan (FHI), and the specimen voucher was deposited in the herbarium repository in the Department of Pharmacognosy, University of Ibadan, Ibadan, Nigeria. The plant materials were shade-dried for 3 weeks, grounded into powder, and 20 g was macerated in 500 mL of freshly distilled 70% ethanol for 72 hours with periodic stirring. The filtered extracts were concentrated at 40 °C using a rotary evaporator. The gel-like extracts weighed and stored in an air-tight glass container.

Chemicals and reagents

Sodium citrate, D-glucose, Sodium chloride (NaCl), citric acid, sodium phosphate buffer (pH 7.4) hyposaline, isosaline, indomethacin, DPPH (1, 1-diphenyl-2-picryl-hydrazyl), sodium hydroxide (NaOH), Sodium nitrate ($NaNO_2$), Aluminum chloride ($AlCl_3$), TPTZ (2,4,6-tripyridyl-s-triazine), Sodium acetate buffer, FeCl3, Dimethyl sulfoxide (DMSO), Liquid paraffin, SMP, PABA, 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid (AA), Tris-KCl buffer, Folin–Ciocalteu phenol reagent, trichloroacetic acid, thiobarbituric acid, 5,5'-dithiobis (2-nitrobenzoic acid) (DNTB)

Phytochemical screening

Determination of total phenolic content

The total phenolics of the crude extracts were quantified using the Folin–Ciocalteu method as described by Saeed et al. (2012) with slight modification[23]. A 1 mg/mL extract was incubated at room temperature with 200 µL Folin–Ciocalteu reagent and 800 µL of 7% Na_2CO_3. Absorbance measured at 765 nm after 2 hours. Results were expressed as a mg of GAE/mg sample from a gallic acid standard curve (duplicate analyses).

Determination of total flavonoid content

Total flavonoid content was determined by the aluminum chloride ($AlCl_3$) method in a 96-well plate. Each well contained 20 µL extract, 20 µL of 3% sodium nitrate, and 20 µL of 1% $AlCl_3$, incubated for 10 min, followed by 0.5 M NaOH addition. Absorbance was measured at 450 nm, and results expressed as a gram of Rutin Equivalent (RE)/g of extract using a rutin standard curve (triplicate assays).

Antioxidant activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging assay

A stock solution (5 mg/5 mL) of the extracts and ascorbic acid was prepared in 50% methanol. In a 96-well plate, 100 µL of each sample was serially diluted (1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL). Then, 100 µL of 0.002 M DPPH in methanol was added, and the plate was incubated in the dark for 30 minutes at room temperature. Absorbance was read at 540 nm. Assays were done in triplicate, with DH_2O as the blank.

Nitric oxide scavenging assay

Nitric oxide scavenging activity was assessed using sodium nitroprusside (30 mg/20 mL PBS) mixed with 50 µL of each extract in a 96-well plate. After 120 minutes of dark incubation, 100 µL of freshly prepared Griess reagent was added and incubated for another 15 minutes. Absorbance was measured at 540 nm. Extracts and ascorbic acid (reference) were tested in serial dilutions (1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL), with DH_2O as blank. All tests were done in duplicate.

Ferric-reducing antioxidant power (FRAP)

Stock solutions (5 mg in 5% DMSO) of extracts and ascorbic acid were serially diluted (1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL) in 96-well plates. Fresh FRAP reagent (sodium acetate buffer (0.3 M, pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ in 10:1:1 ratio) was added (25 µL/well), and the plates incubated in the dark for 20 min at room temperature. Absorbance was measured at 620 nm, with distilled water as blank.

Phosphomolybdate assay (total antioxidant capacity)

Stock solutions (5 mg in 5% DMSO) of each extract and ascorbic acid were prepared and serially diluted (1000, 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL) in 96-well plates (50 µL per well). To each well, 50 µL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) was added. The plates were foil-wrapped and incubated at 95 °C for 15 minutes. After cooling, absorbance was read at 695 nm.

Anti-sickling assays

Blood sample collection and preparation

Four milliliters (4 mL) of blood were drawn from two male patients with HbSS (off transfusion, non-crisis, and hydroxyurea for over 3 months) in the Hematology Department, University College Hospital (UCH), Ibadan. The samples were collected in EDTA bottles, gently mixed, and were washed by centrifugation at 4000 rpm for 5 minutes. The experiments were conducted within 12 hours of blood sample collection. Ethical approval was granted by the UI/UCH Ethics Committee (UI/EC/24/0741), and questionnaire administered with informed consent prior to blood collection.

Reversal antisickling assay

The procedure employed by Atabo et al. (2016) was used with a little modification[24]. The extracts (10 and 20 mg/mL) and the standard PABA (5 mg/mL) were prepared as stock solutions. 50 µL of washed erythrocytes were mixed with 50 µL of 2% sodium metabisulphite and incubated at 37 °C for 30 minutes. Then, equal volumes of saline, extract, or PABA were added and incubated for another 30 minutes. Smears were prepared at 30-minute intervals for 2 hours, fixed in methanol, stained in 10% Giemsa. The RBCs were examined microscopically (×40). Sickled and non-sickled cells were counted in six fields for the percentage of sickling. RBCs that closely resembled the typical biconcave disk shape and exhibited central pallor are classified as normal. In contrast, abnormal cells, referred to as drepanocytes, displayed shapes such as elongated, crescent, star-like, rod-like, wrinkled, or bolt-like. All the assays were carried out in triplicate.

Inhibition antisickling assay

The sickling inhibition assay was performed using a modified method from Adejumo et al. (2012)[25]. Plant extracts (10 and 20 mg/mL) were prepared in normal saline. Blood samples (150 µL) were mixed with an equal volume of extract and incubated for 3 hours. Then, 60 µL of sodium metabisulphite (SMB) and a drop of liquid paraffin were added. Thin blood smears were prepared in triplicate at 0, 30, 60, 90, and 120 minutes for microscopic analysis. The Percentage of sickling calculated as described by Oyedapo et al. (2017)[26].

$$\% \text{ Sickling} = \frac{Mean\ Sickeled\ RBCs}{Mean\ total\ RBCs} \times 100$$

The percentage reversal/inhibition of sickling was determined using the formula:

$$\% \text{ reversal/inhibition} = \frac{T\infty - TS}{T\infty} \times 100$$

Where T∞ = Percentage sickle cells at time T on incubation with normal saline; Ts = Percentage sickle cells at time T on incubation with extract

Membrane stabilizing assay

Experimental animal

An Albino Wistar rat (180 g) was purchased from the central animal house, University of Ibadan. The experiment complied with the "Guide of the Care and Use of Laboratory Animals". The Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan approved the experimental protocol with approval number UI-ACUREC/140-0924/20.

Preparation of erythrocyte suspension

Hypotonic and heat-induced membrane stabilizing effect of two extracts was evaluated on rat and human sickled erythrocytes by using the method of Sadique et al. (1989) with slight modification by Ajayi et al. (2014)[27,28]. Alsever solution, prepared by dissolving D-glucose 2.05 g, Sodium citrate 0.8 g, citric acid 0.055 g, and sodium chloride 0.42 g in distilled water, was mixed in a plain tube with an equal volume of fresh whole blood. The blood was centrifuged twice at 4000 rpm for 10 mins, followed by the removal of the supernatant. The packed cells were washed with isosaline (0.85%, pH 7.2), and a suspension (10% V/V) was prepared.

Procedure

Various concentrations of extracts (1000,500, 250, 125, and 62.5 µg/mL) were tested in a 5 mL assay mixture consisting of 2 mL hypotonic solution, 1 mL sodium phosphate buffer (pH 7.4), 0.3 mL of 10% suspension RBC, and 1 mL extract, covered with isotonic solution. Samples were incubated at 56 °C for 30 min, stored refrigerated, temporarily frozen, and spun at 4000 rpm for 10 min. Supernatants were pipetted into 96-well plates, and 540 nm absorbance was read. Indomethacin (0.1 mg/mL) served as standard. Duplicate testing was done within 12 h of blood collection.

Data analysis

Results were expressed as Mean ± SEM. Data analysis and graph plotting were carried out using GraphPad Prism® version 8.01 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Results of total phenolic and flavonoid content

Table 1 illustrates the 70% ethanol crude extract yields and phytochemical contents. *Justicia carnea* leaf (JCL) gave a higher yield (5.03 g, 25.15%) and also higher total phenolics (3.750 ± 0.024 mg GAE/mg) and flavonoids (0.226 ± 0.007 mg RE/mg) content.

Results of DPPH, nitric oxide scavenging, ferric-reducing antioxidant power and phosphomolybdate assay

Table 2 details IC_{50} values for DPPH, NO scavenging, FRAP, and TAC of the 70% ethanol crude extracts. JCL had stronger DPPH activity (IC_{50} = 245.031 ± 0.228 µg/mL) than JCS (332.662 ± 0.286 µg/mL). While, JCS exhibited higher NO scavenging (84.123 ± 0.497 µg/mL) and greater FRAP (160.097 ± 0.773 µg/mL) and TAC (321.890 ± 0.730 µg/mL) activities than JCL.

Result of reversal anti-sickling assay

Table 3 shows the effects of crude extracts on the reversal of sickled erythrocytes to the normal RBCs at different time intervals. The highest reversal percentages observed at 60, 90, and 120-minute time points. At 60 minutes reading, JCL 20 mg/mL (78.9±0.13%) demonstrated the

Table 1. Percentage yield, total phenolic and flavonoid content of 70% ethanol crude extracts of *Justicia carnea* leaves and stems.

Extracts	Yield (g)	Percentage yield (%)	Total phenolic content (mgGAE/mg sample)	Total flavonoid content (mgRE/mg sample)
JCL	5.03	25.15	3.750±0.024	0.226±0.007
JCS	3.04	15.20	1.191± 0.069	0.060±0.003

Values presented as: Mean ± SEM
Abbreviations: JCL: *Justicia carnea* leaf, JCS: *Justicia carnea* stem.

Table 2. IC_{50} values of DPPH, nitric oxide scavenging, ferric-reducing antioxidant power and phosphomolybdate assays of the 70% ethanol crude extracts of *Justicia carnea* leaves and stems.

IC_{50} (µg/mL)				
Extract	DPPH	NO	FRAP	TAC
JCL	245.031±0.228	605.383±23.258	576.956±1.139	438.472±0.33
JCS	332.662±0.286	84.123±0.497	160.097±0.773	321.89±0.73
AA	62.039±25.982	224.445±0.339	214.759±0.273	

Values presented as: Mean ± SEM
Abbreviations: DPPH: 2, 2-diphenyl-1-picrylhydrazyl radicals, NO: nitric oxide, FRAP: ferric-reducing antioxidant power, TAC: total antioxidant capacity, JCL: *Justicia carnea* leaf, JCS: *Justicia carnea* stem, AA: ascorbic acid.

Table 3. Percentage reversal of RBCs sickling by 70% ethanol crude extracts of *Justicia carnea* leaves and stems.

Concentration (mg/mL)	0 min	30 mins	30 mins (2)	60 mins	90 mins	120 mins
JCL1	68.6±0.57	49.5±1.34	79.5±0.99	77.7±0.98	72.0±0.78	70.4±1.04
JCL2	44.7±0.25	21.1±0.11	76.5±0.79	78.9±0.13	63.1±0.32	73.4±0.60
JCS1	70.7±0.82	76.5±0.50	67.2±0.10	69.7±0.31	63.6±0.71	69.5±0.31
JCS2	55.1±0.53	76.6±0.40	39.3±0.53	78.3±0.87	76.6±0.50	79.8±0.29
PABA	61.9±0.60	30.6±1.10	83.2±0.51	89.7±1.90	86.3±0.87	86.0±0.62
NS	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00

Values presented as: Mean ± SEM of percentage reversal
Abbreviations: JCL: *Justicia carnea* leaf, JCS: *Justicia carnea* stem, PABA: Para-hydroxybenzoic acid, NS: Normal Saline: 1 (10mg/mL), 2 (20mg/mL), 30mins (after 30 minutes of blood samples + SMB incubation), 30 mins (2) (after 30 minutes of blood samples + SMB + extracts incubation).

highest activity followed by JCS 20 mg/mL (78.3±0.87%) (Figure 1).

Result of inhibition antisickling assay

The 70% ethanol crude extracts of *J. carnea* leaf and stem demonstrated the highest inhibition percentages at the mid-time points (Table 4). JCL 10 mg/mL (66.9±0.82 %) demonstrated the highest activity, followed by JCS 10 mg/mL (67.2±0.40 %) at 60 minutes reading. PABA maintained the highest inhibition across all time points, reaching 95.7 ± 1.03% at 120 min (Figure 2).

Results membrane stabilizing assay

JCL and JCS extracts showed dose-dependent membrane stabilization in rat (RRBC) and human sickled (HRBC) RBCs. In RRBCs, JCL was more active, ranging from the lowest activity of 44.85±1.01% (0.156 mg/mL) to the highest activity of 63.04±0.05% (2.5 mg/mL). In HRBCs, JCS slightly outperformed JCL, ranging from the lowest activity of 27.34±1.41% (0.156 mg/mL) to the highest activity of 76.50±0.25% (2.5 mg/mL). The IC_{50} values detailed in Table 5 and Figure 3.

Table 4. Percentage inhibition of RBCs sickling by 70% ethanol crude extract of *Justicia carnea* leaves and stems.

Concentration (mg/mL)	0 min	30 mins	60 mins	90 mins	120 mins
JCL1	62.5±2.13	53.5±2.14	66.9±0.82	63.5±2.34	52.0±1.84
JCL2	66.1±1.03	71.5±0.23	66.3±1.61	80.5±2.64	66.7±1.39
JCS1	74.3±1.18	56.4±1.12	67.2±0.40	41.0±1.71	77.9±0.98
JCS2	80.3±0.47	79.1±0.87	57.6±1.96	78.2±1.07	67.2±0.23
PABA	82.5±0.80	86.1±3.00	90.1±3.48	93.8±2.08	95.7±1.03
NS	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00

Values presented as: Mean ± SEM of percentage inhibition
Abbreviations: JCL: *Justicia carnea* leaf, JCS: *Justicia carnea* stem, PABA: Para-hydroxybenzoic acid, NS: Normal Saline: 1 (10mg/mL), 2 (20mg/mL).

Table 5. IC_{50} values of 70% ethanol crude extract of Justicia carnea leaves and stems on rat and human erythrocyte membrane stabilization activity.

IC_{50} (µg/mL)		
Extract	RRBC	HRBC
JCL	0.533±0.127	0.841±0.321
JCS	0.940±0.417	0.389±0.439
Indo	0.532±0.619	0.597±1.888

Values presented as: Mean ± SEM
Abbreviations: RRBC: Rat red blood cells, HRBC: Sickled human red blood cells, JCL: *Justicia carnea* leaf, JCS: *Justicia carnea* stem, Indo: Indomethacin.

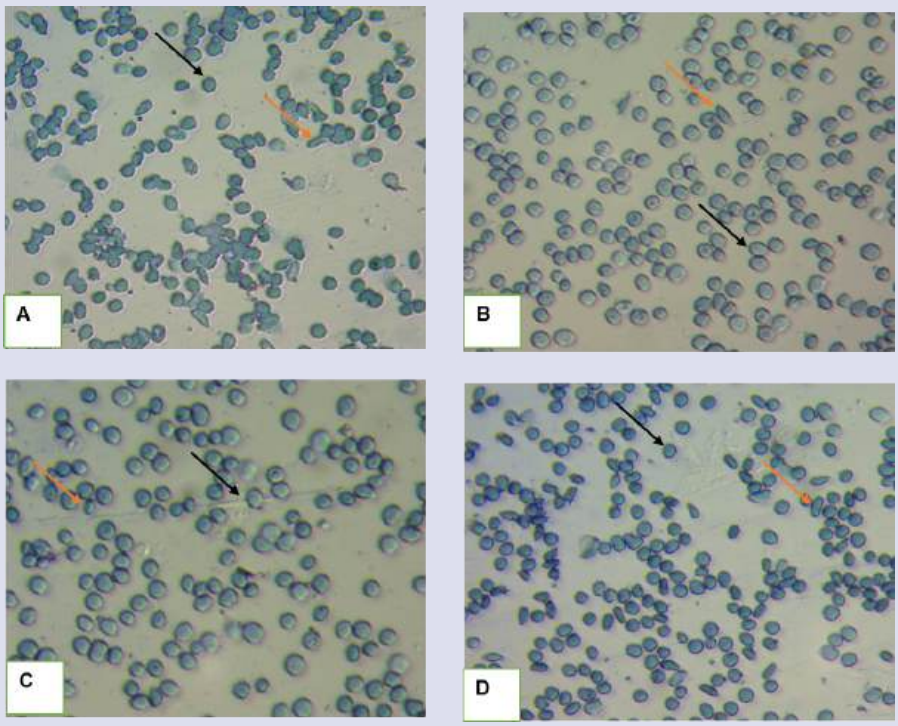

Figure 1. Photomicrographs of erythrocyte morphology under different treatments at 60th minute reading, Mag.x40. The sickled shape of red cells is illustrated by a yellow arrow, and normal RBCs by a black arrow. (A) - The reversal effect of NS on SS-RBC morphology. (B) - The reversal effect of PABA on SS-RBC morphology. (C) - The reversal effect of JCL 20 mg/mL on SS-RBC morphology. (D) - The reversal effect of JCS 20 mg/mL on SS-RBC morphology.

Abbreviations: SS-RBC: sickled red blood cells, JCL: *Justicia carnea* leaf, JCS: *Justicia carnea* stem, PABA: Para-hydroxybenzoic acid, NS: Normal Saline.

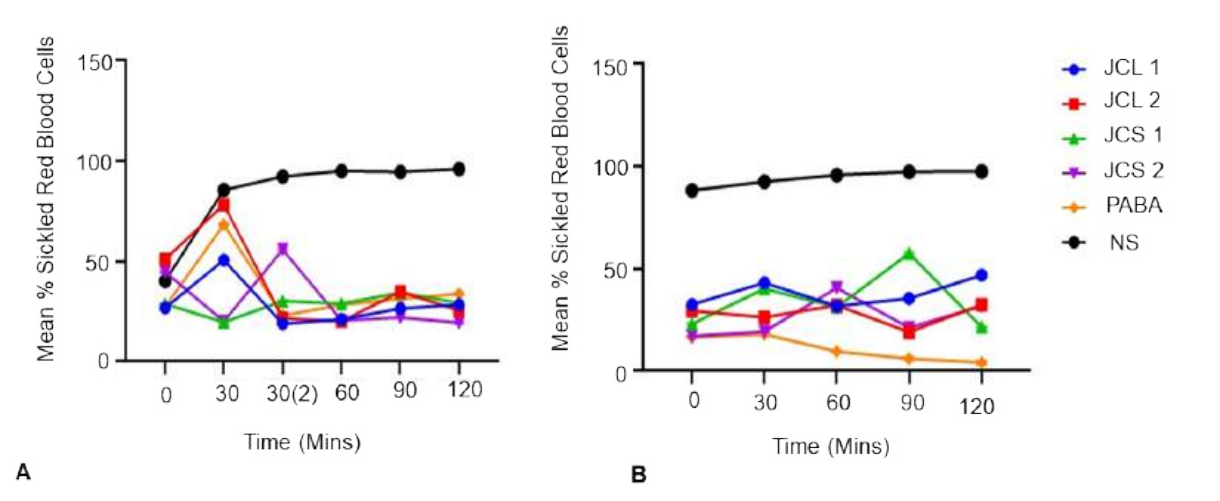

Figure 2. Mean of % Sickled RBCs of (A) reversal and (B) inhibition anti-sickling assay for 10 mg/mL and 20 mg/mL concentration of 70% ethanol crude extract of **Justicia carnea** leaves and stems.

Abbreviations: JCL: *Justicia carnea* leaf, JCS: *Justicia carnea* stem, PABA: Para-hydroxybenzoic acid, NS: Normal Saline: 1 (10mg/mL), 2 (20mg/mL); 30 mins (after 30 minutes of blood samples + SMB incubation), 30 (2) mins (after 30 minutes of blood samples + SMB + extracts incubation).

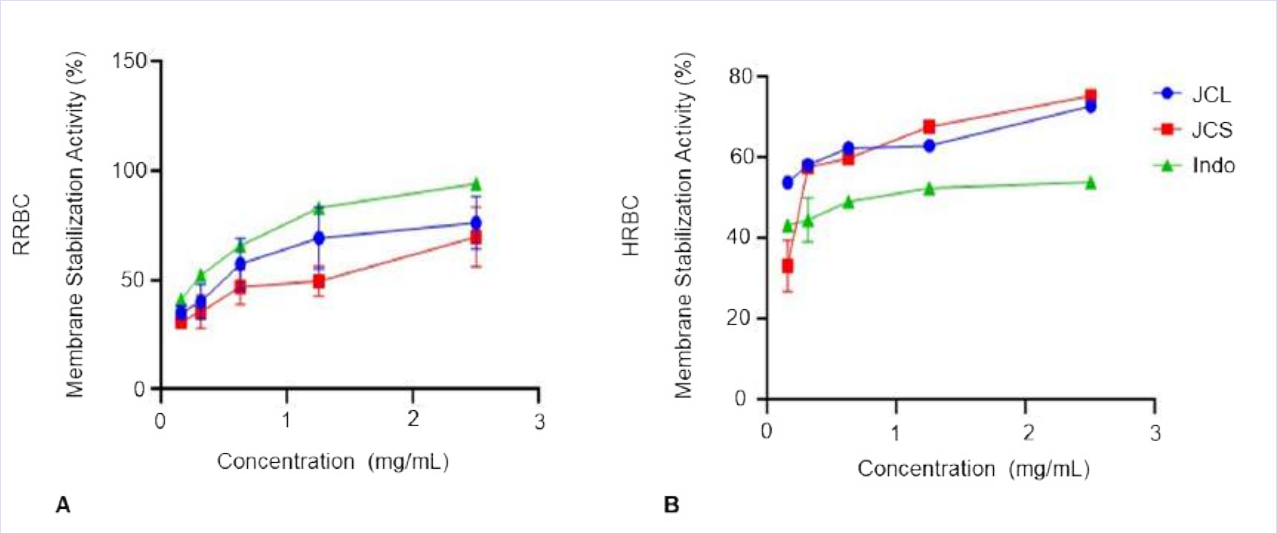

Figure 3. Concentration-dependent (A) RRBC and (B) HRBC membrane stabilizing activity of 70% ethanol crude extracts of **Justicia carnea** leaves and stems.

Abbreviations: RRBC – Rat red blood cells, HRBC – Sickled human red blood cells, JCL – *Justicia carnea* leaf, JCS – *Justicia carnea* stem, Indo– Indomethacin.

DISCUSSION

Sickle cell disease (SCD) is a major health problem worldwide because of its relatively high incidence and far-reaching social consequences. More than 200,000 children are born annually with SCA in Africa alone[29]. Being a genetic disorder, SCD has not been easy to treat; however, there is increasing evidence that the condition is treated both with conventional and traditional medicine[30,31,32]. Since few conventional therapies are found to be effective, it is essential to embark on a comprehensive study of alternative treatments. Phytomedicines are potential alternatives because of the ability of some plants to reverse sickling, stabilize membranes of erythrocytes, halt hemolysis, and reduce the occurrence of sickling crises[33].

This study evaluated the pharmacological potential of the 70% ethanol crude extracts of *Justicia carnea* leaf (JCL) and stem (JCS) through a combination of quantitative phytochemical analysis, antioxidant, antisickling, and membrane-stabilizing assays. The findings highlight the therapeutic relevance of these extracts, particularly in the management of SCD, where oxidative stress, hemolysis, and sickling of erythrocytes play central roles.

The present study indicated that JCL extracts have the highest phenolic and flavonoid content, which is consistent with the previous study[34]. In the antioxidant assays, JCL showed stronger DPPH radical scavenging compared to JCS, in line with its higher phenolic and flavonoid content. JCS had larger NO scavenging, FRAP, and TAC than JCL. This

might mean that different classes of phytochemicals may dominate leaf and stem extracts and confer selective antioxidant activities. For instance, Phenolics likely drive the hydrogen donating activity of JCL, and phytochemicals in JCS may favor electron transfer and nitric oxide modulation. Although the two extracts were less active than the standard, ascorbic acid, their activities might be therapeutically meaningful given the involvement of oxidative stress in SCD[9,35]. DPPH scavenging potential of JCL has been evaluated in the previous study as well[36].

Oxidative stress has also been found to increase sickling as well as hemolysis in SCD patients[37]. It is reported that the higher antioxidant property of an antisickling agent better the likelihood of exerting an antisickling effect by inhibiting oxidative stress[38]. Therefore, the antioxidant potential of *J. carnea* extracts could provide an added protection advantage via inhibition of cellular damage and improvement in RBCs survival. The results support the hypothesis that antisickling activity of *J. carnea* is meditated to a certain degree by its antioxidant mechanism.

This study depicts the effect of 70 % ethanol crude extracts of JC leaves and stems on reversing or inhibiting sickled erythrocytes using different time intervals of incubation, showing activities that are comparably effective. A large number of studies have established that tropical plants provide an effective alternative therapy for SCD due to the presence of bioactive compounds responsible for reversing the sickle cell[39]. In line with this study, the extracts successfully restored sickled cells to their normal shape after induction with 2% sodium metabisulfite[24,40]. Which suggested that active compounds responsible for the antisickling activity are probably distributed in similar manners between the two plant parts.

Another major complication of SCD is inflammation[41]. Anti-inflammatory actions of extracts were investigated by membrane-stabilizing assay. Hypotonic solution leads to swelling and additional cell lysis by extreme osmotic trans-movement of water. In RBCs, this process is referred to as hemolysis[8]. JCL was more effective against rat RBCs, closely resembling indomethacin. Against human RBCs, JCS was superior, outperforming both JCL and indomethacin. This shows that *J. carnea* possesses bioactive compounds that are capable of protecting erythrocytes from lysis under stress conditions, which plays a role in reducing hemolysis in SCD. Because RBC membranes share structural similarities with lysosomal membranes, this assay provides an indirect measure of a drug's potential to prevent lysosomal enzyme release, further supporting its stabilizing effects[28].

CONCLUSION

This study evaluated the antioxidant, antisickling, and membrane stabilizing effects of leaves and stems of *J. carnea*, used in Nigerian folk medicine for SCD treatment. The quantitative phytochemical studies have shown that the JCL presents higher levels of phenolic compounds and flavonoids. These types of bioactive compounds are reported to possess an intense antioxidant activity playing a crucial role in their therapeutic use. The extracts also showed remarkable antioxidant activity (DPPH, NO, FRAP, TAC). Antisickling activity was evaluated by using two models. The extracts demonstrated notable potential in preventing and reversing sickling. The anti-inflammatory activity was also assessed based on the extracts' rat and human sickled erythrocyte membrane stabilizing potential, showing comparable effects with both extracts. Markedly, the current study represents therapeutic capability of *J. carnea* stem and leaf against SCD through their antisickling, anti-inflammatory, and antioxidant properties. The finding therefore suggested that such plant could be a promising natural therapy for SCD and warranted further investigations to isolate active constituents and establish efficacy and safety *in-vivo*.

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