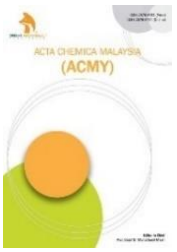
ZIBELINE INTERNATIONAL™ PUBLISHING

ISSN: 2576-6724 (Print)
ISSN: 2576-6732 (Online)
CODEN: ACMCCG

Acta Chemica Malaysia (ACMY)

DOI: http://doi.org/10.26480/acmy.01.2025.63.69



RESEARCH ARTICLE

CHEMICAL CHARACTERIZATION, *IN-VITRO* ANTISICKLING ACTIVITY IN HAEMOGLOBIN-SS AND HAEMATOLOGICAL INDICES IN WISTAR RAT FED WITH *Hibiscus sabdariffa* CALYCES EXTRACT

Iyekowa Osaro[a]*, Ogbeide Osahon Kennedy[a], Igbinumwen Osamwonyi[b], Innih Silvanus Olu[c], Oyelakin Oladele[d], Deborah Oluwatoyin Asiriuwa[e], Gilbert Izuagbe Osigbemhe[a] Adegbenga David Idowu[f] and Clifford Oyakhilomen Ehisuoria[e]

[a] *Department of Chemistry, Faculty of Physical Sciences University of Benin, Benin City, Nigeria*
[b] *Department of Haematology, Medical Laboratory Services, University of Benin Teaching Hospital, Benin City, Nigeria*
[c] *Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria*
[d] *Chemistry Unit, Division of Physical and Natural Sciences, School of Arts and Sciences, University of The Gambia.*
[e] *Department of Industrial Chemistry, Faculty of Science, Edo University, Iyamho, Edo State, Nigeria*
[f] *Department of Chemical Sciences, University of Johannesburg, Johannesburg, South*
Corresponding Author Email: osaro.iyekowa@uniben.edu

ARTICLE DETAILS

Article History:

Received 7 March 2025
Revised 5 March 2025
Accepted 10 April 2025
Available online 29 May 2025

ABSTRACT

A prominent medicinal plant used as condiment and in the traditional treatment and management of diseases like high blood pressure, high blood sugar level and anaemia is the *Hibiscus sabdariffa*. In this study, experimental research was conducted for the chemical constituents, *in* vitro antisickling activity in haemoglobin-SS and haematological indices in wistar rat fed with *hibiscus sabdariffa* calyces extract. Saponins, glycosides, steroids, among others were indicated with the exception of eugenols and tannins. There were no toxic signs/symptoms and mortality even after 72 hours (3 days) of treatment. The *in-vitro* antisickling studies of the extract given by photomicrographs indicated a decrease in the number of sickle cells from 38.24%, 22.58% down to 9.10% respectively with 100 mg/mL crude extract of *H. Sabdariffa* at time zero, 45 and 90 minutes. Hibiscetine (Rt. 11.050; 48.29 %), a flavonoid compound was detected as the major component among myrtillin (Rt. 12.166; 13.89 %), an anthocyanin flavonoid. These compounds have indicated physiological properties including antioxidation of cells and anti-inflammation of cells. There was an observed macrocytic morphology of the haemoglobin cells in the entire field examined but no sign of sickling was indicated. This suggest that the extract have antisickling activity.

KEYWORDS

Hibiscus sabdariffa, antisickling, phytochemicals

1. INTRODUCTION

Hibiscus sabdariffa belongs to the family:*Malvaceae* and is commonly called "Akenyen" (Edo), "Ile ago" (Igbo) and "Isapa" (Yoruba) in Nigeria. The plant calyxes have been applied as an infusion (herbal tea) (Aigbokhan, 2014). The plant is up to 2 m to 2.5 m (7 to 8 feet) in height and the leaves of about 35 lobed and 8-15 cm long with alternate arrangement on either side of the stem (Mathivann and Edwin, 2012). The plant leaves, seeds and calyxes are widely used as curries, salads and concoctions (Galib and Noor, 2010). The have also reported the presence of essential vitamins, minerals among other phytochemicals y (Ilondu and Iloh, 2007). The flower petals are usually prepared by decoction into "Zobo' in Nigeria (Chau al., 2000). They also reported the antisceptic, sedative and astringent potentials of the plant (Odigie et al., 2003). The leaves and calyces of Hibiscus sabdaiffa have been used as vegetables in many tropical countries especially in Africa (Adegunloyeet et al., 1996). In The Gambia and many other African countries, the calyces extract is prepared as a decoction and then fortified with ginger (which has anti-inflammatory activity), sugar to taste, and other spices. In Côte d'Ivoire, it is a vegetable food (Lépengué et al., 2009). Decoction of the calyxes is used for the traditional treatment of hypertension. However, in some parts of west Africa, the infusion of the mature red petals of the plant is used as a refrigerant drink in fevers and many authors have also reported the anti-inflammatory, cardioprotective and hepatoprotective activities (Obouayebaet al., 2014). Flavonoids, saponins, tannins, alkaloids and phenolics have also been reported by (Ugwu et al., 2020).

Sickle cell anemia (SCA) is an hereditary disease occuring as a result of the presence of mutant hemoglobin. It is a life threatening disease causing excruciating pain and other tissue challenges (Donald and Judith, 2004). Iron deficiency has been found to increase the effect of anemia and lack of Fe leads to low haemoglobin concentration which in turn causes anemia (Bertram et al., 2012). Individuals with SCA suffer from severe hemolytic anemia due to their erythrocytes life span which is less or halve of the normal 120-day life span of a normal cells (Carson-DeWitt, 2013).

Though sickle cell anemia is a life-threatening and painful disease, medical intervention from the scientific community and traditional herbal medicines have played significant roles in the treatment and management of the disease to prolong the lives of sufferers. Among the herbal products developed in Nigeria is NIPRISAN™ (Ameh et al., 2012). The prophylactic intervention for the treatment of SCA is very expensive for many sufferers of Africa while new scientific approaches like gene editing by Casgevy, gene therapy and bone marrow transplantation are promising approaches but not with low cost to sufferers who are majorly from Africa and India.

Quick Response Code	Access this article online	
	Website: www.actachemicamalaysia.com	DOI: 10.26480/acmy.01.2025.63.69

Cite the Article: Iyekowa Osaro, Ogbeide Osahon Kennedy, Igbinumwen Osamwonyi, Innih Silvanus Olu, Oyelakin Oladele, Deborah Oluwatoyin Asiriuwa, Gilbert Izuagbe Osigbemhe Adegbenga David Idowu and Clifford Oyakhilomen Ehisuoria (2025). Chemical Characterization, *In-Vitro* Antisickling Activity in Haemoglobin-Ss And Haematological Indices in Wistar Rat Fed with *Hibiscus Sabdariffa* Calyces Extract. *Acta Chemica Malaysia, 9(1): 63-69.*

Drug intervention will remain the main and cost effective treatment option for sickle cell disease (Isoa, 2009).There is urgent need to investigate medicinal plants used by herbal practitioners to validate their traditional claims of improving the well being of sickle cell patients with anaemia. Thus, this study is aimed at chemical characterization, antisickling activity in haemoglobin-SS and haematological indices in Wistar rat fed with *Hibiscus sabdariffa* calyces extract.

2. Materials and Methods

2.1 Reagents and chemicals

Chemicals used in this research were of analytical grade and they included sodium metabisulphite, methanol, acetic anhydride, $FeCl_3$, saponin rein weiss (Merck), NaOH, HCl, ethanol, KOH, H_2SO_4, chloroform, picric acid, anthocyanins standards (cyanidin, delphinidin and cyanidin 3-O-glucoside) phenolic acids standards (gallic, cafeic, chlorogenic, ellagic, ferulic and p-coumaric), flavonoids standards (catechin, epicatechin, genistein, gossypin quercetin, isoquercetin, quercitrin, rutin and vanillin).

2.2 Plant Collection

The dried calyces of *H. sabdariffa* used were purchased from a rural market in Ovia North East local government area of Edo state, Nigeria. The plant sample was sorted to remove sand and debris. The plant samples were identified in the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria with herbarium voucher number: UBHH235 assigned. The plant samples were dried in the laboratory for 28 days and later pulverized to powdery form with electric blender and the mass of the powdered sample noted.

2.3 Extraction

Maceration method was adopted for the extraction of the calyces. Four hundred and ten grammes (410 g) of the plant sample were macerated in a beaker with 1L of methanol for 72 hours. The filtrate was recovered via separation by Whatman's number 4 filter paper and concentrated with a rotary evaporator at 50°C to afford a reddish dark crude extract. The resulting crude was then stored in the refrigerator below 4°C until further treatment.

2.4 Phyto constituents screening of *Hibiscus sabdariffa* calyces extract

A portion of the crude calyces extract was qualitatively screened using standard methods and procedures described by (Sofowora, 199; Trease and Evans, 1987). The chemical constituents analysed were terpenoids, tannins, saponins, glycosides among others.

3. Collection of Blood Samples

One confirmed sickle cell patient (HbSS) was chosen with consent from University of Benin Teaching Hospital (UBTH), Ugbowo, Benin City, Nigeria and five (5) mL of blood sample was obtained by vein puncture from the subject. The erythrocytes were isolated from the collected blood with centrifuge at 1500 rpm for 15 minutes while the plasma was separated from the sickle cell sediment with Pasteur pipette. Precaution was observed to note that the patient had not been transfused for twelve months with Hb AA blood before the antisickling analysis with the blood samples. Confirmation and characterization of the HbSS blood samples was done by hemoglobin electrophoresis on cellulose acetate gel (Mpiana et al.,2010). The sickle cell blood sample was then stored at ± 4 °C in a refrigerator with those presenting good sickling rate (≥ 90%) selected for antisickling study.

3.1 Antisickling Activity Evaluation:(Sodium metabisulphite(SMBS) Test)

A modified procedure was adopted for this analysis. Blood samples collected in EDTA bottles was centrifuged for serum removal (Sofowora and Isaac-Sodeye, 1979). The erythrocytes were washed thrice with 1mL sterile normal saline per mL blood. The samples were then centrifuged for 5 min at a speed of 2000 rpm to remove the supernatant, and 0.5mL of the washed erythrocytes were mixed each with, 0.5mL of the different extracts doses in uncovered test tubes. Sample were taken from the different mixtures and incubated at 37°C for three hours with intermittent agitation. A known volume of 0.2 mL of 2% sodium metabisulphite was added to deoxygenate the system, mixed thoroughly and sealed with liquid paraffin samples before incubating at 37°C and sample taken at 45 min interval until 3 readings were obtained.

The plant extract (100 mg/mL) sample was smeared on a microscope slide, fixed with 95% methanol, dried and stained with Leischman's stain. Immersion light microscope was used to count the red blood cells in each sample from five different fields of view across the slide. The counts of the numbers of sickle and unsickle red blood cells were noted. Negative control was obtained by sodium metabisulphite (5mg/mL) as a reducing agent (Sofowara and Isaac-Sodeye, 1979).

3.2 Calculation of percentage of sickled red blood cell

$$\% \text{ sickled red blood cell} = \frac{\text{Sickle cells}}{\text{RBC count}} \times \frac{100}{1} \quad (1)$$

$5mg.mL^{-1} = 500mg.L^{-1}$

RBC = Red blood cell

4. Acute Toxicity Test

Fifteen mice weighing (27-36 g) were purchased for acute toxicity experiment. The mice were kept in cages and left to acclimatize in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin for 14 days. The animal house was well ventilated while pellet feed (Premier Feed Mills Co. Limited) was made available to the mice. Water was made accessible ad libitum. The research was carried out in the University of Benin in accordance with the laws governing the use of laboratory animals as accepted internationally.

At the end of the 14days acclimatization period, the mice were randomly shared into groups and acute toxicity test was evaluated using method with slight modification. Here, five groups were assigned three mice per group (Uwumarongie and Oyiana, 2017).

Group A received 5 mL/kg of water which is the diluent for the extract while group B,C,D and E were administered 1000, 1600, 2900 and 5000 mg/kg of the methanol extract of *H. sabdariffa* respectively. Observation were made after administration of the extract for the first 4 hours (for immediate effect) and then 24hrs (for delayed effect), for acute effects such as weakness, drowsiness, aggressiveness, food refusal, weight loss, diarrhoea lacrimation, noisy breathing, tremors and mortality.

The mice were furthered monitored for 2 weeks to observed if any delayed effects will emerged. The acute toxicity test was studied by modified approach to Acute Toxicity Testing according to Igbe *et al.* (2010). The median lethal dose (LD_{50}) value was estimated by the application of the equation below:

$$LD_{50} = \sqrt{\{LD_0 \times LD_{100}\}} \quad (2)$$

Where: LD_0 = Maximum dose without death;

LD_{100} = minimum dose with death

Those that survived were used for the sub acute toxicity test before haematological analysis.

5. Haematological Studies

5.1 Experimental grouping and dosage of methanol extract of *H. sabdariffa*

Group A: Control received 1 mL of distilled water

Group B: 1 mg/kg of methanol extract

Group C: 2 mg/kg of methanol extract

Group D: 3 mg/kg of methanol extract

Duration of studies: 14 days

Wistar rats were used for the sub-acute toxicity test and were fed with the respective doses of the calyces extract for 14 day period to evaluate the cumulative effects of repeated oral administration of the extract and this was done to detect early signs of organ toxicity. At the start and end of the 14- day period, the rats were weighed to the nearest grams using the Metler PE 6000(R) Digital weighing balance. Wistar rats that survived the acute toxicity test were regrouped into four: Group A: Control (received 1ml distilled water), Group B (received 1 mg/kg of *H. sabdariffa*extract), Group C (received 2 mg/kg of *H. sabdariffa* extract) and Group D (received 3 mg/kg of *H. sabdariffa*extract), At the end of the 14-day period of extracts administration, blood samples were collected for haematological assessment

5.2 Red Blood Cell (Rbc) Count

This was done using the automated haematology analyzer method by Kaznowska-Bystryk (2011). The tail vein of the rat was identified for blood collection. The blood sample was stored in EDTA bottles and analysed by automated hematology analyzer. The RBC Count was expressed as cells/µL or cells x 10^6/ µL

5.3 Haemoglobin (Hb)

Blood samples collected from the tail vein in EDTA bottles was analysed by the automated hematology analyzer and expressed in g/dL

5.4 Hematocrit (Hct)/ Packed Cell Volume (Pcv)

The PCV was determined by centrifuging blood sample of the rat in a capillary tube (known as micro haematocrit tube) at 10,000 revolutions per minute (rpm) for 5 minutes. After centrifugation, the blood sample was separated into three layers: bottom layer (packed red blood cells); middle layer (white blood cells and platelets) and top layer (plasma).

The volume of the packed red blood cells divided by the total volume of the blood sample gives the packed cell volume (PCV). This calculation was done automatically by the hematology analyzer.

5.5 White Blood Cell (Wbc)

Blood samples from Wistar rats were collected from the tail vein in EDTA bottles. Then automated hematology analyzer was used to count the WBC directly from the blood sample. Normal WBC count range for Wistar rat is expected to be 6,000 to 12,000 cells/µL of blood.

5.6 Platelet

This test is a critical part of haematological analysis conducted to evaluate blood clotting capacity and thrombocytopenia. Blood samples from Wistar rats were collected from the tail vein in EDTA bottles. Then automated hematology analyzer was then used to measure the platelet count, mean platelet volume (MPV) and platelets distribution width (PDW)). Normal range of platelet count for Wistar rat expected is given as 600,000 to 1,200,000/ µL

6. Ethical Consideration

Approval for the animal study was obtained from the Faculty of Life Science, Animal Use and Ethics Committee of the University of Benin with a permit reference number EC/FP/022/24.

7. Isolation of Plant Extract

7.1 Vacuum Liquid Chromatographic (VLC) Analysis

The crude methanol extract was uniformly packed with silica gel (1:1) and subjected to chromatographic separation using silica gel (60-120 mesh) as the stationary phase. The sample was eluted with the assistance of a vacuum pump using combined solvents consisting of 100% hexane, hexane : ethylacetate(1:1) and ethylacetate (100%) solvents as mobile phase. The three fractions eluted were concentrated respectively and monitored with thin layer chromatography (TLC). Fractions with similar Rf values were combined, dried and coded. Isolated brown oil coded sample D (R_f 0.79, solvent system: 100% ethyl acetate) was recovered from 100% ethyl acetate crude fraction. Other sample coded A, B and C which did not have R_f above 0.5 were not characterized.

7.2 Hplc Analysis

7.2.1 Preparation of sample

Ten grammes (10g) of solid sample D from ethyl acetate crude fraction were extracted with acetonitrile and the extract stabilized with ethyl acetate in 25 mL standard flask and made up to the mark.

7.2.2 HPLC Procedure

The constituents of the solid sample D was characterized by HPLC analysis using the method described by (Drust and Wrolstad, 2001). The solid sample and the three standards (Anthocyanins phenolics and flavonoid and) were subjected to High Performance Liquid Chromatography using 600 series HPLC pump and 2487 dual wavelength UV detector-254 and 360 nm of bioazyemes, Bangalore having Reprobond C18 column-4.6x250mm and 7725 Rheodyne injectors. The instrument was operated at room temperature (23 ± 2°C). Ten grammes each (10 g) of the solid sample D was extracted with acetonitrile, and the extracts stabilized with ethyl acetate in 25 mL standard flask. Five micro liters (5 µL) of extracts was respectively injected in to the column at a flow rate of 2.0 mL/min and the peak area were reported and used for quantification. The compounds eluted with two solvents such as acetonitrile and 0.1% phosphoric acid in water were used for the detection of the external standards while the total run time of the program was 20 minutes.

8. Results and Discussion

8.1 Phyto constituents

The phytochemicals in the methanol extract of *H. sabdariffa* is given in Table 1.

Table 1: Phyto constituents in methanol extract of *H. sabdariffa*

S/N	Phytochemical constituents	methanol extract of *H. sabdariffa*
1	Alkaloids	+
2	Tannin	-
3	Flavonoid	+
4	Phenolics	+
5	Saponin	+
6	Eugenol	-
7	Steroid	+
8	Terpenoids	+
9	Glycosides	+

+ = present - = absent

In Table 1, tannins and eugenols were absent while terpenoids, saponins, glycosides, flavonoids, and alkaloids. These constituents are responsible for most physiological and pharmacological activities of medicinal plants (Sofowora, 1993). They reviewed the role of phytochemicals and opined that claims of wellness, antimicrobial potency, antioxidant activities and anti-inflammatory properties are attributed to their presence as metabolites in medicinal plants (Doughari, 2012). Meanwhile, according to the study, the leaves extract of *H. sabdariffa* contained glycosides, alkaloids, flavonoids and phenolics but in this study, the methanol extract of the calyces contained more components including glycosides, saponins, flavonoids among others (Adegunloye et al., 1996). The absence of tannins in this work was also reflected in the research who also adopted the same methanol extract of *H. sabdariffa* calyces (Okereke et al., 2015). The presence of phenolics, flavonoids and anthocyannins in the calyces of Zobo plant has been identified as strong antioxidants with potential of protecting the heart for optimal function (Guyton and Kensler 1993). The report was slightly different with the presence of tannins in hot aqueous extract of Zobo plant when compared to our findings with the absence of tannins in the cold methanol extract (Ugwu et al., 2020). This result could be attributed to temperature and polarity of extracting solvents with water being more polar than methanol.

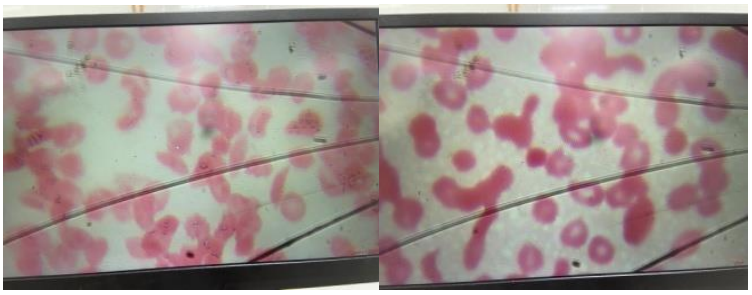
8.2 Antisickling Activity Result

Table 5: Antisickling result of methanol of *H. sabdariffa* extract on Hb-SS blood

Durationof exposure of extract (minutes)	Number of sickle cell (%)	Number of non sickle cell (%)	Morphology of cell
0	13 (38.24)	21 (61.76)	Hypochromic and macrocytic cell.
45	07 (22.58)	24 (77.42)	Mmacrocytic red cell (Abnormally large RBC)

Table 5 (cont): Antisickling result of methanol of *H. sabdariffa* extract on Hb-SS blood			
90	04 (9.10)	40 (90.90)	Normal RBC
135	03 (11.11)	24 (88.89)	Fragmented RBC.
Sodium metabisulphite (Placebo) (time, zero)	71		All sickle

RBC = Red blood cells



I II

Plate I: Optical micrographs of untreated HbSS blood erythrocytes (control), NaCl 0.9%, 2% $Na_2S_2O_5$, (Magnification x 100) .Plate II: Optical Micrograph of 100mg/mL of methanol extract of *H. sabdariffa* on sickle cell blood at 0 minutes (Magnification x 100).

III IV V

Plate III: Optical micrograph of 100mg/mL of methanol extract of *H. sabdariffa* on sickle cell blood at 45 minutes (Magnification x 100). Plate IV: Optical Micrograph of 100mg/mL of methanol extract of *H. sabdariffa* on sickle cell blood at 90 minutes (Magnification x 100). Plate V: Optical Micrograph of 100mg/mL of methanol extract of *H. sabdariffa* on sickle cell blood at 135 minutes (Magnification x 100)

The antisickling studies of *H. sabdariffa* (Zobo plant) (Plate I) shows an optical morphology of the sickle cell blood alone (control group) while plates II,III, IV and V are the morphology of sickle cell blood in the presence of 100mg/mL methanol extracts of *H. sabdariffa* at different duration of exposures in minutes. Plate II showed hypochromic (reduced amount of haemoglobin) and macrocytic red blood cells at the initial start of the experiment (zero minutes). From zero minutes (Plate II), 45 minutes (Plate III) to 90 minutes (Plate IV) there were significant reduction of the number of sickle cells with a percentage decrease from 38.24%, 22.58% down to 9.10% (Table 5) respectively. Macrocytic red blood cells were prominent in the field examined but with no sign of sickling and this suggest that the plant extract have antisickling activity.

8.3 Acute Toxicity Result

The result of the acute toxicity of extract of *H. sabdariffa* at graded doses of 1000, 1600, 2900 and 5000 mg/kg body weight isshowed in Table 6 below:

The oral administration of the crude extracts at graded doses of 1000, 1600, 2900 and 5000 mg/kg body weight showed no indication of acute toxicity except the highest dose of *H. sabdariffa* extract at 5000 mg/kg after 24 hours. There were no effect, toxic signs/symptoms and mortality even after 72 hours (3 days) of treatment and cautious observation of theWistar rat groups. More so, there were no variations in shallow breathing, raised tails, salivation, paw licking and restlessness during the 24 hour period.

Table 6: Acute toxicity results of methanol extract of *H. sabdariffa*			
Test Group	**Doses (mg/kg)**	**Number of lethality**	**Percentage mortality**
Control	DW (5mL/kg)	0/3	0
Methanol	1000	0/3	0
Methanol	1600	0/3	0
Methanol	2900	0/3	0
Methanol	5000	1/3	33

DW = Distilled water

LD_{50} ≤ 1 mg/kg (Extremely toxic); 1 mg/kg ≤ LD_{50} ≤ 50 mg/kg (Highly toxic);

50 mg/kg ≤ LD_{50} ≤ 500 mg/kg (Moderately toxic);

500 mg/kg ≤ LD_{50} ≤ 5000 mg/kg (Slightly toxic)

5000 mg/kg≤LD_{50} ≤ 15000 mg/kg (Non-toxic or harmless).

Hodge and Sterner scale in Unuigbe *et al.* (2021).

From the Hodge and Sterner scale, only the highest dose of *H. sabdariffa* extract used in this study can be considered as relatively slightly toxic which gave 33% mortality after the 24 hours for the acute toxicity test.

9. Results of Haematological Studies

9.1 Red Blood Cells (Rbc)

The RBC Count at the end of the 14 day sub acute toxicity test expressed as cells/µL is given in Figure 1 below

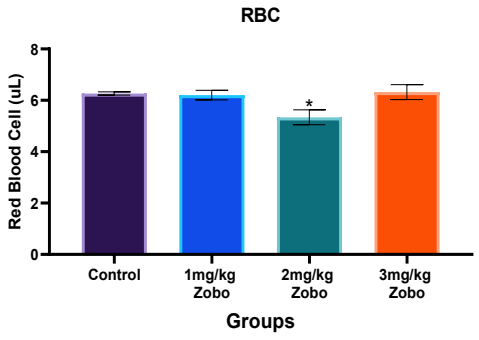


Figure 1: Red Blood Cell after administration. Values are given as mean ± SEM. *p< 0.05 compared with the control group.

According to the study, the reference value of RBC for Wistar rat is 6 to 9 x 10^6 cells/µL (Kaznowska-Bystryk, 2011). In this study, the control group which received 5 mL distilled water had lower RBC when compared to the

reference. The test group which received methanol extracts of *H. sabdariffa*root had higher RBC which fell within the normal range. This gave an indication that the extract raised the RBC of the rats. This increase suggestsome physiological and nutritional effects. The increase may be due to some bioactive compounds present in the calyces of the plant which are important for RBC synthesis. Although increase in RBC could cause increased blood viscosity which mayin turn cause circulatory problems

9.2 Haemoglobin

The haemogloblin expressed in g/dL is shown below in Figure 2

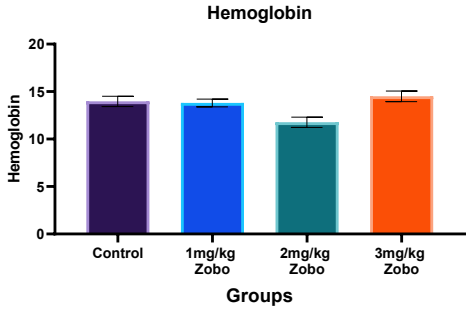


Figure 2: Haemoglobin level after administration. Values are given as mean ± SEM.

In Figure 2, the haemoglobin count increased as the group received the extracts at the assigned doses, however the count rate was not dose dependent as the moderate dose of 2mg/kg had a slightreduction of the haemoglobin. The rise of haemoglobin helps to correct anaemia.

9.3 Packed Cell Volume (Pcv) Or Hematocrit

The packed cell volume or hematocrit of the Wistar rat after the 14-day treatment period is shown in Figure 3 below

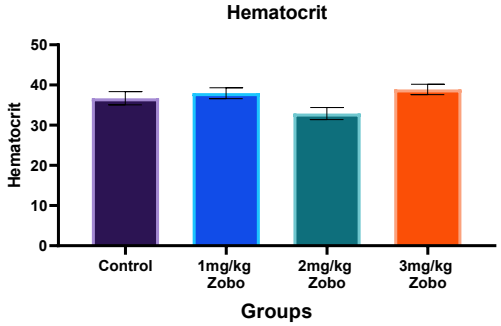


Figure 3: Hematocrit level after administration. Values are given as mean ± SEM.

The result of the PCV levels did not indicate a dose dependent activity. The highest dose group hadHigh PCV which suggest that the animals were not malnourished. The reference for PCV in Wistar rats fall within 35 to 50% and the last dose group had 47% (Figure 3)

9.4 White Blood Cells (Wbc)

The white blood cells determined from the blood samples of the Wistar rat is shown in Figure 4.

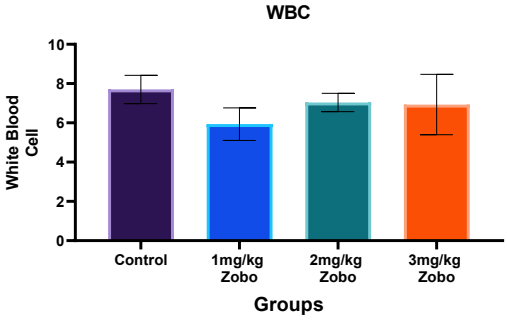


Figure 4: Level of White Blood Cell (x 10^3 cells/µL) after administration. Values are given as mean ± SEM. (Normal WBC count for Wistar rat: 6 x 10^3 to 12 x 10^3 cells/µL of blood)

The white blood cells count in Figure 4, indicated a slightly dose dependent result. Low dose of extract (1mg/kg) had a slightly normal WBC range of 5.9 x 10^3 cells/µL, while the intermediate and high dose gave 6.5 x 10^3 cells/µL respectively. This stable level of WBC as the administration of extract progresses suggests no immune suppressive issues that may arise from the plant's toxicity.

9.5 Platellet

Blood clotting capacity determined by the platelet count is shown in Figure 5

below:

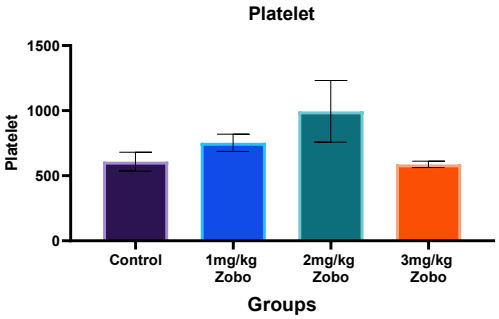


Figure 5: Platelet Level after administration. Values are given as mean ± SEM. $^*p< 0.05$ compared with the control group.

The normal range of platelet count is given within 600 to 1200 x 10^3 cells/µL while the control group had low platelet count; the test groups had varying count with the intermediate dose having the highest count of 1000 x 10^3 cells/µL.The test groups which received the various doses of the extract had platelet counts within the normal range but the upsurge observed in the intermediate dose group might be as a result of inflammation, infection or stress. More so, lower platelet counts which normally impair the clotting of blood was not observed in this study.

9.6 Quantification of chemical constituents by HPLC

The characterised constituents by HPLC chromatogram of the solid sample coded sample D from methanol extract of *H. sabdariffa* is indicated in Figure 6 while the chemical constituents are givenin Table 7.

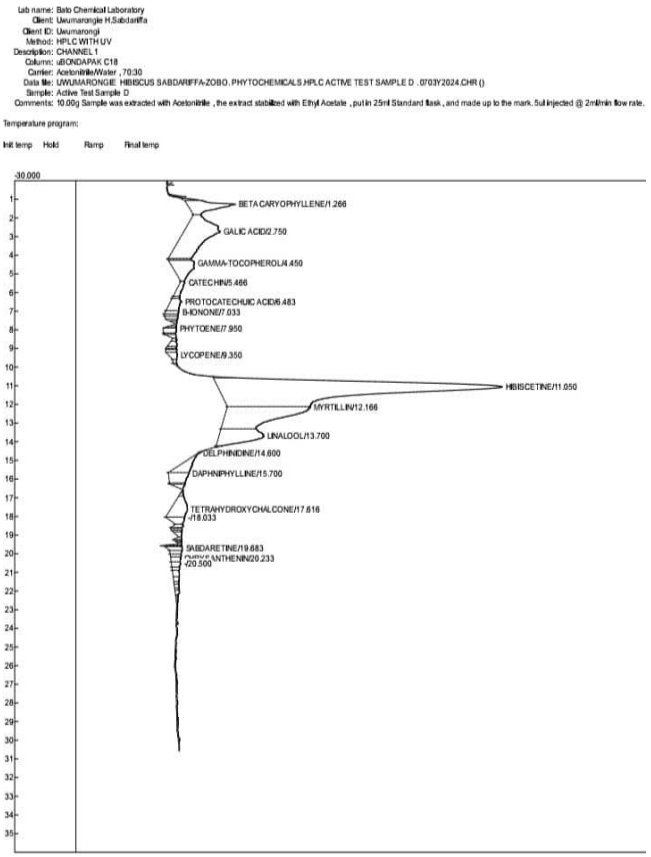
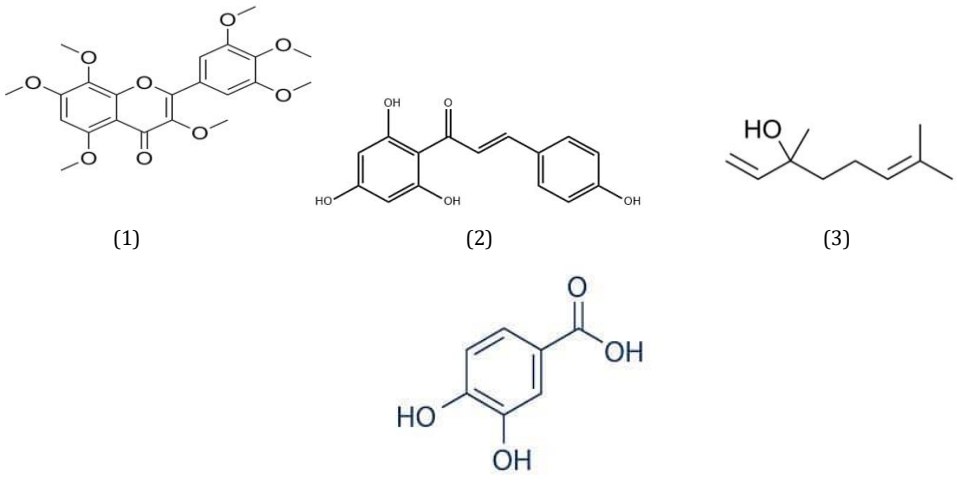


Figure 6: HPLC chromatogram of fraction D of methanol extract of *H. sabdariffa*

Table: 7: HPLC profile of ethyl acetate fraction of methanol extract of *H. sabdariffa*

Peak number	Retention time (Rt) (mins)	Name of compound	Peak area	Area percent (%)
1	1.266	Beta caryophyllen	562.3820	3.84
2	2.750	Galic	1706.1570	11.65
3	4.450	Gamma Tocopherol	527.0050	3.60
4	5.466	Catechin	124.5610	0.85
5	6.483	Protocatechuic acid	204.8600	1.40
6	7.033	Beta-ionone	71.9155	0.49
7	7.950	Phytoene	100.7250	0.69
8	9.350	Lycopene	87.7320	0.60
9	11.050	Hibiscetine	7069.9670	48.29
10	12.166	Myrtillin	2033.0015	13.89
11	13.700	Linalool	934.3970	6.38
12	14.600	Delphinidine	305.7540	2.09
13	15.700	Daphniphylline	294.0955	2.01
14	17.616	Tetrahydroxychalcone	454.0275	3.10
15	19.683	Sabdaretine	87.9300	0.60
16	20.233	Chrysanthenin	75.9640	0.52
Total			**14,640.4740**	**100**

Sixteen chemical constituents detected from the analysis includes sabdaretine (Rt 19.683; 0.60 %), Galic acid (Rt. 2.750; 11.65 %), and myrtillin (Rt. 12.166; 13.89 %) which occur in varying percents. However, hibiscetine (Rt. 11.050; 48.29 %), a flavonoid compound was detected as the highest peak from the chromatogram. Flavonoid and other organic compounds have been reported in H.sabdariffa (Muller et al., 1992). Among the isolated compounds from the calyces of the plant are hibiscetine, protocatechuic acid, sabdaretineand small amount of myrtillin which is a delphinidin 3- monoglucoside. These compoundshave indicated potential cardiovascular benefits, antiiflammatory and antioxidant properties in both in-vitro and in-vivo studies (Pegu et al.,2021). Anti-inflammatory properties of most medicinal plants have been reported to alleviate oxidative stress associated with sickle cell disease (Osamwonyi et al., 2024). More so, the presence of Ca, K, Mg, Fe, Na and Cu and lipid soluble antioxidant, tocopherol have been reported in recently (Iyekowa et al., 2024; Pegu et al., 2021).



(1) (2) (3)

(4)

Figure 7: Some chemical constituents detected in *Hibiscus sabdariffa*isolates: (1) Hibiscetin(2) Tetrahydro chalcone (3) Linalool (4) Protocatechuic acid.

10. Conclusion

This study investigated the phyto constituents characterization, *in-vitro* antisickling potential studies in Hb-SS and haematological parameters in Wistar rat fed with extracts of *Hibiscus sabdariffa*. The result indicated a significant reduction of sickle cells as the time of exposure of 100 mg/mL methanol extracts of *H. sabdariffa* increased from zero, 45 minutes to 90 minutes duration. The study further revealed *Hibiscus sabdariffa* have bioactive components like hibiscetine, sabdaretine, protocatechuic acid and myrtillin that possess antioxidant and antinflammatory properties which help to alleviate oxidative stress associated with sickle cell disease

Acknowledgement

The authors wish to appreciate the grant given for this research work from IBR, 2023 of the University of Benin, Benin City, Nigeria and funded by Tertiary Education Trust Fund (TETFUND), Nigeria.

Conflict of Interest

The authors declare no conflict of interest.

References

Adegunloye, B.J., Omoniyi, J.O and Ajabonna, O.P., 1996. Mechanism of blood pressure lowering effects of the calyx extract of Hibiscus sabdariffa in rats. Journal of science, Pp. 235- 238.

Aigbokhan EI., 2014. Anotated checklist of vascular plants of Southern Nigeria- a quickreference guide to the Vascular plants of southern Nigeria: a systematic approach. Uniben Press, Benin City. Pp. 63

Ameh, S.J., Tarfa, F.D andEbeshi, B.U., 2012. Traditional herbal management of Sickle Cell Anemia: Lessons from Nigeria. Anemia 9. DOI:10.1155/2012/607436.

Bertram, G.K, Susan B.M and Anthony, J.T., 2012. Basic and clinical pharmacology International edition. Asia Publishing, Asia. Pp. 581–582.

Carson-DeWitt, M.D., 2013. Sickle Cell Disease. EBSCO Publishing, New York. Pp. 228

Chau, J.W., Jin, M.W., Wea, L.L., Chia, Y.C., Fen, P.C. and Tsui, H.T., 2000. Protective effect of Hibiscus anthocyannins against tertbutylhydroperoxide-induced hepatic toxicity in rats. Food and Chemical Toxicology, 38 (15): Pp. 412-417.

Donald, V and Judith, GV., 2004. Biochemistry. (3rdedn) U.S.A publishing, U.S.A. Pp. 183

Doughari, J. H., 2012. Phytochemicals, extraction methods, basic structures andmodes of action as potential chemotherapeutic agents. Phytochemicals- a globalperspective of their roles in nutrition and health. Intech: Pp. 1-5.

Drust RW, Wrolstad RE., 2001. Separation and characterization of Anthocyanins by HPLC. Current Protocols in Foods Analytical Chemistry. Pp. 1-13. doi: 1001002/0471142913.FAF0103S00

Galib, A.M.A and Noor, M.W., 2010. Comparative study on diet supplementation with a mixture of herbal plants dendelion.As a source of prebiotic on the performance of broilers.Pakistan Journal of Nutrition. Pp. 91-71.

Guyton K. Z and Kensler T. W, 1993. The effect of sour tea (Hibiscus sabdariffa) on essential hypertension. Ethnopharmacology. 65: Pp. 231-236.

Igbe I, hing F.P and Eromon A., 2010. Anti-inflammatory activity of aqueous fruit pulp extract of Hunteria umbellate K. Schum in acute and chronic inflammation. ActaPoloniae Pharm - Drug Res. 67(1): Pp. 81-85.

Ilondu, E.M. and Iloh, A.C., 2007. Inhibition of three fungal isolates from sorrel drink (Zobo) using hurdle technique. World Journal of Agricultural Sciences 3(3): Pp. 339-343.

Isoa, EM., 2009. Current trends in the management of sickle cell disease: an overview. Benin Journal of postgraduate medicine. 11: Pp. 50-64.

Iyekowa, O., Oviawe, A.P., Igbinumwen, O., Oluyide, J.A., Odigie, A.J., Ogbonwan, O.G., Asiriuwa, D.O and Anokhaoya, S.P., 2024. Mineral composition and chemical characterization of the oil constituents of Hibiscus sabdariffa calyxes (Zobo calyxes) by Gas chromatography mass spectrometry (GC-MS). Benin journal of physical sciences 1(2): Pp. 42-51

Kaznowska-Bystryk, I., 2011. The automated hematologyanalyzers. Annales Universitatis Mariae Curie-Sklodowska Lublin-Polonia. 25(3): Pp. 63-70

Lépengué NA, Mouaragadja I, M'batchi B, Aké S., 2009. Sciences et Nature; 6 (2): Pp. 95-105.

Mathivann, R and Edwin, SC., 2012. Hematological and Serum Biochemical Parameters of Broiler Fed with Andrographispaniculata as an alternative to antibiotic growth promoter. Journal of Medicinal Plant Research, 6(44): Pp. 5647 – 5650.

Mpiana PT, Ngbolua KN, Bokota MT, Kasonga TK, Atibu EK, Tshibangu DS, Mudogo V., 2010. In vitro effects of anthocyanin extracts from Justicia secunda Vahl on the solubility of haemoglobin S and membrane stability of sickle erythrocytes. Blood Transfusion. 8 (4): Pp. 248-254. doi: 10.2450/2009.0120-09

Muller BM, FranzG.Chemical structure and biological activity of polysaccharidesfrom Hibiscussabdariffa.PlantaMedica.1992; 58 (1): Pp. 60-67.

Obouayeba AB, Djyh NB, Diabate S, Djaman AJ, N'guessan JD, Kone M and Kouakou TH., 2014. Phytochemical and Antioxidant Activity of Roselle (Hibiscus Sabdariffa L.) Petal Extracts. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 5(2): Pp. 1453-1465

Odigie, I. P., Ettarh, R.R. and Adigun, S.A., 2003. Chronic administration of aqueous extract of Hibiscus sabdariffa attenuates hypertension and reverses cardiac hypertrophy in 2K-1 C hypertensive rats. Journal of Ethnopharmacology, 86: Pp. 181- 185

Okereke C.N, Iroka F.C and Chukwuma M.O., 2015. Phytochemical analysis and medicinal uses of Hibiscus sabdariffa.International Journal of Herbal Medicine. 2(6): Pp. 16-19

Okwuowu, I. E., Uchegbu, M. N and Obiorah, C. E., 2008. Chemical composition of Hibiscussabdariffa L. Journal of Sustainable Agriculture. 32(3), Pp. 147-157.

Osamwonyi, I., Iyekowa, O., Ebengho, M.I., Edema, M.O., Oviawe, A.P., Momoh, S.M., Eduwuirofo, L.O., Iyekekpolor, R., Michael, O.T., Oghomwen, R.O , and Umeodinka, A.A., 2024. Chemical characterizations and anti-sickling potential of methanol extract of Justicia carnea(flamingo plant). Mediterr J Pharm Pharm Sci. 4 (3): Pp. 65-75.

Pegu F, Paul A, Chetia A and Zaman K Md. An updated review on phytochemistry and therapeutic use of Hibiscus sabdariffa L., CurrTrends Pharm Res,2021; 8(1): Pp.41-72.

Sofowora A., 1993. Medicinal Plants and Traditonal Medivine in Africa. Spectrum Books limited Ibadan 289.

Sofowora, E.A and Issac, W.A., 1979. Reversal of sickling and crenation in erythrocytes by the root of Fagara zanthoxyloides. LIoydia. 34 (4): Pp. 383-385. PMID: 4950991

Trease G. E and Evans W.C., 1989. Pharmacognosy. 15th Edition published by Saudners 142: Pp. 333-337

Ugwu, DO, Jiwuba, PC, UboguVR andAkazue RC., 2020. Phytochemical properties of Hibiscus sabdariffa Calyx and the effects of its aqueous extract supplementation on haematological and serum biochemical indices of broiler birds. Nigerian J. Anim. Sci. 2020 Vol 22 (3): Pp. 165-172

Unuigbe C.A, Unula C.F, Aiwonegbe A, Uadia A.O, Akhigbe I, Asakitikpi E and Ogbeide O.K., 2021. Bioactive chemical constituents, acute toxicity and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of Polyalthialongifoliaroot.GSC Bio Pharm Sci. 14(01): Pp. 018–026

Uwumarongie, O.O. and Oyiana, L.A., 2017. Phytochemical screening and antioxidant activity of methanolic extract of Chromolaena odorata. Journal of Pharmacy and Pharmacology. 5(3): Pp. 231-238