Original Research Article

ETHNOBOTANICAL SURVEY AND *IN-VITRO* ANTISICKLING EFFECT OF SOME SELECTED MEDICINAL PLANTS

Abstract:

Background: Sickle cell disease is a genetic disorder in which an individual inherits the sickle cell allele from both parents. The modern disease modifying therapies are quite expensive and often come with side effects, hence, there is need to search for natural alternatives from medicinal plants. This research was aimed at evaluating the antisickling effects of some selected medicinal plants.

Materials and Methods: Ethnobotanical survey was conducted on the medicinal plants used in Zuru Local Government Area of Kebbi State, Nigeria for the treatment/management of sickle cell di`sease. Five (5) most cited plants; *Carica papaya* leaf, *Prosopis africana* stem-bark, *Guiera senegalensis* leaf, *Syzygium aromaticum* seed and *Boswellia dalzielli* stem-bark were selected and their methanol extracts were subjected to *in vitro* antisickling activity using sodium metabisulphite. Phytochemical screening on the most active plants extracts was conducted using standard methods.

Results*:* The plant extracts and their combinations exhibited antisickling activities with varying degrees of efficacy. *C. papaya* leaf extract, *P. africana* stem-bark extract and *Guiera senegalensis* leaf extract were the most potent that caused reduction in the percentage sickling to 3.87± 2.73, 8.38±1.06 and 28.35±2.07% respectively. Phytochemical screening revealed the presence of Alkaliods and Tannins in all the three (3) plant extracts. Anthraquinones and glycosides were present only *C. papaya* and *G. senegalensis* leaves extracts, while Flavonoids and Saponins are only present in *G. senegalensis* leaf extract and *P. africana* stem-bark extracts. Phenols are present in *C. papaya* leaf extract and *P. africana* stem-bark extracts, while phlobatannins is only in *C. papaya* leaf extract.

Conclusion: The medicinal plant extracts were able to reduce the percentage of sickled cells. This may be due to the presence of some of the phytochemicals. Hence, these medicinal plants may be used as alternative to hydroxyurea in ameliorating the sickling in human HbS containing RBCs.

Keywords: Sickle cell disease; Antisickling; Red blood cells; Plant extracts; Hydroxyurea, Ethnobotanical survey.

I. Introduction

Sickle cell disease (SCD) also known as Drepanocytosis is a life-long blood disorder characterized by erythrocytes that assume an abnormal, rigid, sickle shape. It is a genetically inherited disease in which a single base substitution in the gene encoding the human β-globin subunit results in replacement of β6 glutamic acid by valine [1]. In SCD patients, sickle erythrocytes are rigid with decreased deformability and reduced life span resulting in hemolysis, vaso-occlusive disease, vasculopathy and subsequent inflammation and end organ damage.

Sickle cell disease affects millions of people worldwide. Today, with proper health care, many SCD patients have a good quality of life and are in fairly good health most of the time [2]. Medullar transplantation, repeated blood transfusion and the use of chemical agents that can interfere with the sickling process are the first-line clinical management of SCD. However, all proposed therapies are expensive for low income population [3]. Therefore, there is a need for more affordable and effective treatments for the disease.

Herbal extracts have been used in African traditional medicine for many years in the management of common ailments. Recent research has shown that various plants species traditionally used in managing SCD had in vitro antisickling activity which is mainly due to anthocyanins [3]. These secondary metabolites, as several other flavonoids, are natural products with a broad spectrum of pharmacological activities. The cost of managing SCD is

very high compared to the normal health care cost of non-sickle cell patient. The people living in the rural communities are mostly peasant farmers who may not afford the high cost of orthodox treatment for SCD. Due to the debilitating effects and the cost of managing SCD, researches are ongoing to determine the efficacy of the use of medicinal plants to tackle the multiple challenges of SCD. Very few ethnobotanical remedies for the treatment of Sickle Cell Anemia (SCA) have been reported due to the secrecy attached to the treatments of this disease [4].

Several therapies have been proposed and many chemical substances investigated for their possible role in the management of SCD. For instance, hydroxyurea has been shown to decrease the number and severity of sickle cell crises by increasing fetal hemoglobin production significantly in patients with sickle cell anemia [5]. In fact, there was no specific therapy available for sickle cell disease patients before the 1970s. However, subsequent studies have shown that patients with a higher concentration of fetal hemoglobin (HbF) in the red blood cell had less adverse clinical complications [6]. Hydroxyurea achieves this function by activating the production of fetal hemoglobin to replace the hemoglobin S that causes sickle cell anemia. One of the mechanisms for the action is based on its ability to inhibit the reaction that leads to the production of deoxyribonucleotides by acting on the enzyme of ribonucleotide reductase. The production of deoxyribonucleotides requires tyrosyl group (which is a free radical). So, hydroxyurea captures these tyrosyl free radicals thereby preventing the production of deoxyribonucleotides [7].

Despite its wide acceptance, hydroxyurea is moderately toxic especially when administered long term [8]. In search of inexpensive but effective and readily available drugs, several investigations have been conducted on indigenous plant materials. Among the commonly used plants in Nigeria and other African nations for the management of many ailments including sickle cell disease is *Carica papaya*, *Piper guinesis*, Pterocarpa osun, Eugenia caryophlala and Sorghum bicolour. Potentially, medicinal plants could be used alongside pharmaceutical drugs for management of sickle cell disease. Because of the high number of sickle cell patients worldwide, especially in Nigeria, the high cost of pharmaceutical products, and the limited efficacy of the available drugs, there is a pressing need for the development of new drugs that are inexpensive but effective and readily available in rural communities as well as the world at large, for the management of sickle cell disease.

II. Material And Methods

Blood samples used in the evaluation of the antisickling activity of the plant extracts in this study were collected from known sickle cell disease patients attending Federal Medical Center, Birnin Kebbi, Kebbi State, Nigeria. None of the patients was transfused recently with Hb AA blood before taking the samples. All antisickling experiments were carried out with freshly collected blood. The blood was collected in EDTA bottles and stored in a refrigerator at 4^{o}C before use.

Study Location: The ethnobotanical survey was conducted in Zuru Local Government Area, Kebbi State, Nigeria from December, 2019 to March, 2020. While, the experimental work was performed in Biochemistry Research Laboratory, Department of Biochemistry, Faculty of Life Sciences, Kebbi State University of Science and Technology, Aliero, Nigeria.

Ethnobotanical Survey: Ethnobotanical information about the plant species selected for this study was obtained by interviewing traditional healers in Zuru Local Government Area of Kebbi State, Nigeria. A total of 25 traditional healers were interviewed. Informants were selected based on their authentic knowledge on the utilization of medicinal plants. Traditional healers were interviewed on a voluntary basis. They were asked on the local name of the plants, parts used, mode of preparation and administration, dosage and duration of administration. Number of citations was recorded and five (5) most cited plants were used to carry out the present study.

Collection and Identification of Plant Samples: All the plant samples; *Carica papaya* leaf, *Guiera senegalensis* leaf, *Prosopis africana* stem bark, *Boswellia dalzielli* stem bark and *Syzygium aromaticum* seeds were collected in the month of July 2020 from Zuru Local Government Area of Kebbi State. They were authenticated by a Taxonomist from the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero, Nigeria.

Plant Preparation and Extraction: The plant samples were washed with clean water and allowed to dry at room temperature for two (2) weeks. They were then pulverized into fine powder using mechanical blender. They were then extracted using methanol at room temperature with shaking at regular intervals for 72 hours after which each

one was filtered. Each methanol extract was completely dried. The extracts were stored in an air tight container and placed in a refrigerator at 4 ^{o}C and protected from light until further use.

***In vitro* Induction of Sickling**

The principle of this test is based on the morphological change of the HbSS blood cells when subjected to reduced oxygen environment by a reducing agent such as sodium metabisulphite solution during which the Red Blood Cells (RBCs) assumed the characteristic sickle shape.

Five milliliter (5ml) blood sample was added in a tube containing saline solution and centrifuged at 5000 rpm for 10 min for at least thrice, in order to obtain the RBCs which were resuspended in normal saline for further analysis. SS blood cell suspension was mixed with 0.2ml 2% sodium metabisulphite ($Na_2S_2O_5$) solution and incubated at 37 ^{o}C. RBCs were analyzed microscopically for sickling at a time span. In every single hour the number of cells was counted and percentage of sickling was calculated using the formula;

Percentage (%) of sickling = No. of sickling cells × 100/Total cells [9].

***In vitro* Antisickling Activity of the Extracts:** A 0.5millilitre of SS-RBCs preincubated with 2% $Na_2S_2O_5$ was added to 0.5millilitre of the extract. The mixture was incubated at 37 ^{o}C for 2 h which is necessary for obtaining maximum sickling. Sickle cells and normal cells were counted microscopically. The percentage (%) of sickling was calculated.

Phytochemical Screening on the Most Active Plant: The Phytochemical screening was carried out according to the methods described by Harborne and Jeffrey [10], El-olemy *et al.,* [11], Trease and Evans [12], Kumar *et al.,* [13], Onwukaeme *et al.,* [14] and Mbatchou and Kossono [15]

Statistical analysis

The data generated from the study were presented as mean ± standard error of the mean and were subjected to one way Analysis of Variance (ANOVA) and statistical difference between the means was separated using new Duncan's multiple Range test at $P<0.05$ with the aid of a statistical package (IBM SPSS statistics 20).

III. Results

Ethnobotanical survey

A total of twenty five (25) herbal medicine practitioners were interviewed.The local name, common name and botanical name were sorted from online and hard copy literature. The most frequently cited plants are *Carica papaya* leaf, *Prosopis africana* stem bark, *Guiera senegalensis* leaf, *Boswellia dalzielii* stem bark and *Syzygium aromaticum* seed (Table 1).

Table 1: Medicinal Plants with Antisickling Activity Used in Zuru, Kebbi State, Nigeria.

S/N	Plant Botanical Name	Local Name (Hausa)	Part of the Plant Used	Number of Citation	Mode of Preparation	Mode of Administration
1	*Syzygium aromaticum*	Kanunfari/hancin kade	Seeds	7	Immersing of the seeds in a liquid in an air tight container	Drink twice daily for 5-6 days
2	*Sorghum bicolor*	Dawa	Seeds or leaves	5	Mixed with cloves and made into pap	One cupfull in the morning
3	*Psidium guajava*	Goobaa/Gwaiba	Leaves	4	Mix with custard apple and boil	Drink twice daily and inhale the vapour
4	*Annona senegalensis*	Gwandan daji	Leaves	3	Cut into small pieces and soak with small quantity of pottash	Take a shot three times a day for seven days
5	*Carica papaya*	Gwanda	Leaf	10	The leaves is squeezed and made into juice or boiled	Drink the juice and inhale the vapour every till relief
6	*Prosopis Africana*	Kirya	Stem Bark	5	The dried stem bark is powderd or boiled	Take two spoonful immediately and subsequently one spoonful daily.
7	*Boswellia dalzielii*	Hano	Stem Bark	6	The dried stem bark is powderd or boiled	Vapour inhalation and drink the juice untill relief
8	*Guiera senegalensis*	Sabara	Leaf	5	Dried leaf is boiled with pawpaw leaves and cloves	Drink twice daily and inhale the vapour till relief
9	*Nigella sativa*	Habbatus sauda	Seeds	4	Crush the seeds	Chew the seeds and swallowed within three (3) days
10	*Piper guinense*	Masoro	Seeds	1	Seeds are mixed with paw paw leaves ground into powder and mixed with cloves	Drink twice a day until relief

Effect of the Plants Extracts on the Morphology of Sickled Red Blood Cells

The antisickling activity of the test plants extracts and their combinations on the morphology of red blood cells are presented in plates 1-18. Plates 1 and 2 exhibited a high percentage of sickling indicating the blood samples were collected from sickle cell patients while Plate 3 shows the effect of hydroxyurea on the sickle cells indicating reversal of sickling to normal shape.

Plates 4-8 shows how individual plant extracts were able to inhibit sickling of the red blood cells. However, blood samples treated with *C. papaya* leaf possesses higher percentage of antisickiling effect.

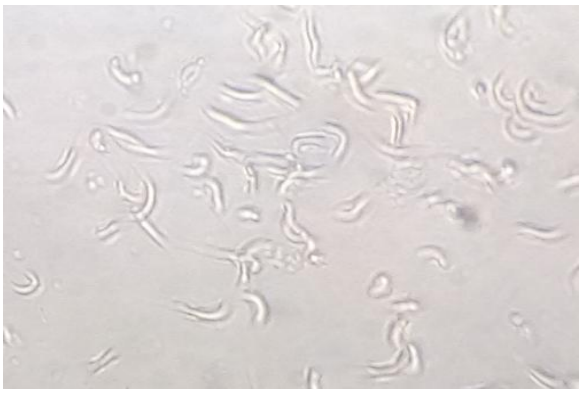

Plate 1: Photomicrograph of sickle cells alone (X40), [NaCl, 0.9%]

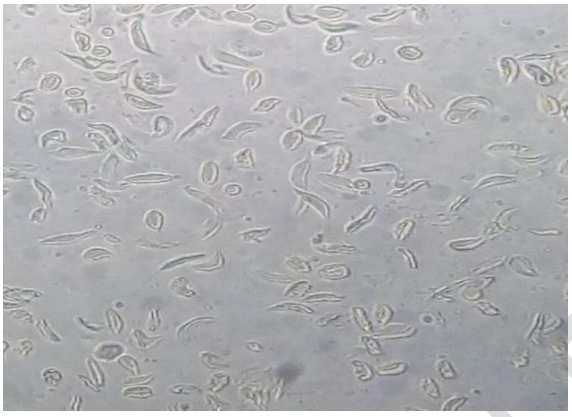

Plate 2: Photomicrograph of sickle cells (control) (x40) [NaCl, 0.9%; $Na_2S_2O_5$, 2%]

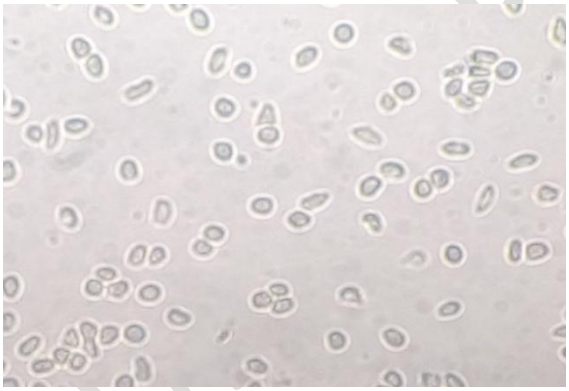

Plate 3: Photomicrograph of sickle cells treated with 1% hydroxyurea (X40), [NaCl, 0.9%; $Na_2S_2O_5$, 2%]

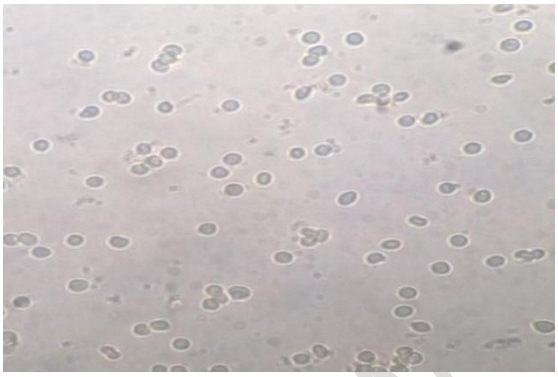

Plate 4: Photomicrograph of sickle cells treated with 1% *Carica papaya* leaf extract (X40), [NaCl, 0.9%; $Na_2S_2O_5$, 2%]

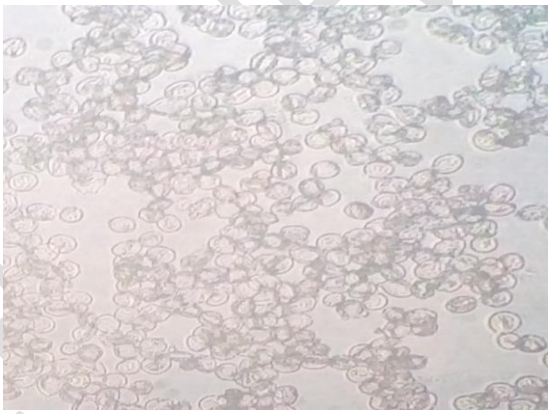

Plate 5: Photomicrograph of sickle cells treated with *G. senegalensis* leaf extract (X40), [NaCl, 0.9%; $Na_2S_2O_5$, 2%]

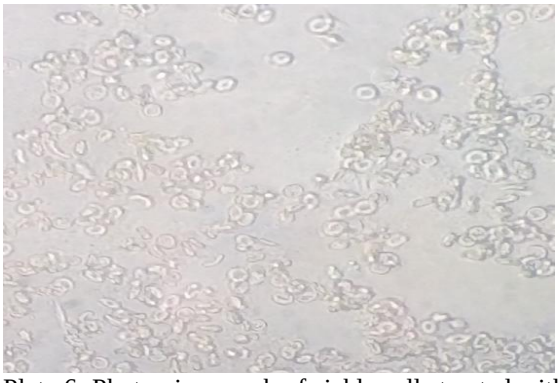

Plate 6: Photomicrograph of sickle cells treated with *B. dalzielii* stem-bark extract (X40), [NaCl, 0.9%; $Na_2S_2O_5$, 2%]

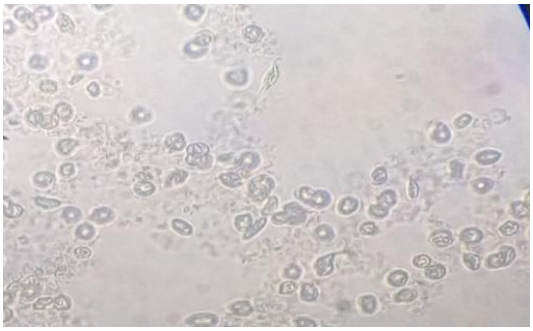

Plate 7: Photomicrograph of sickle cells treated with 1% *P. africana* stem-bark extract (X40), [NaCl, 0.9%; $Na_2S_2O_5$, 2%]

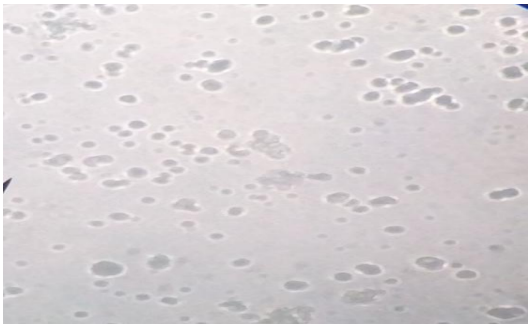

Plate 8: Photomicrograph of sickle cells treated with 1% *S. aromaticum* seed extract (X40)[NaCl,0.9%;$Na_2S_2O_5$,2%]

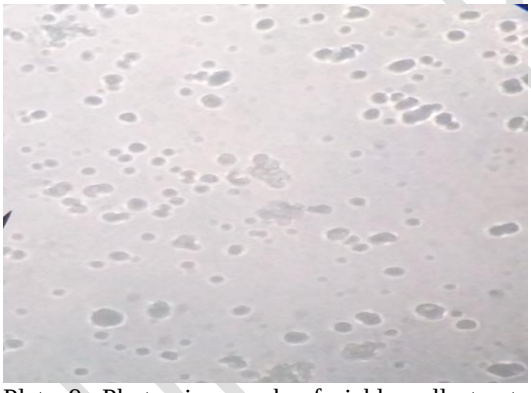

Plate 9: Photomicrograph of sickle cells treated with 1% *C. papaya* leaf extract and *G. senegalensis* leaf extract (X40), [NaCl, 0.9%; $Na_2S_2O_5$, 2%]

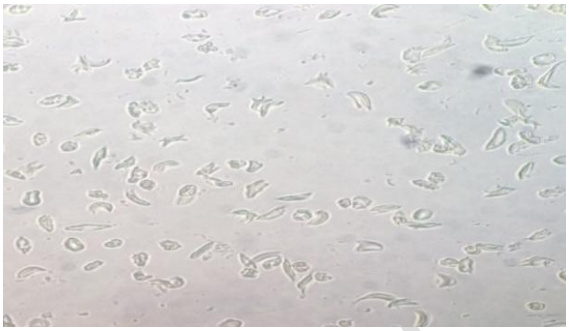

Plate 10: Photomicrograph of sickle cells treated with 1%C. papaya leaf extract and B. dalzielii stem-bark extract (X40), [NaCl, 0.9%; Na2S2O5, 2%]

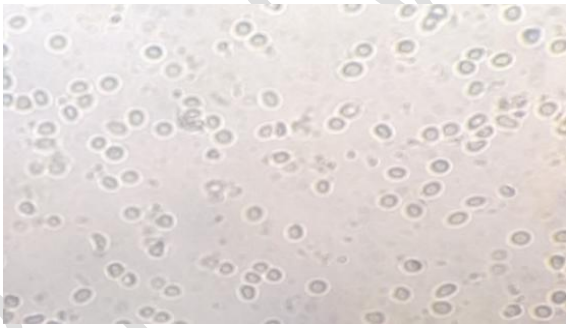

Plate 11: Photomicrograph of sickle cells treated with 1% *C.papaya* leaf extract and *P. africana* stem-bark extract (X40), [NaCl, 0.9%; $Na_2S_2O_5$, 2%]

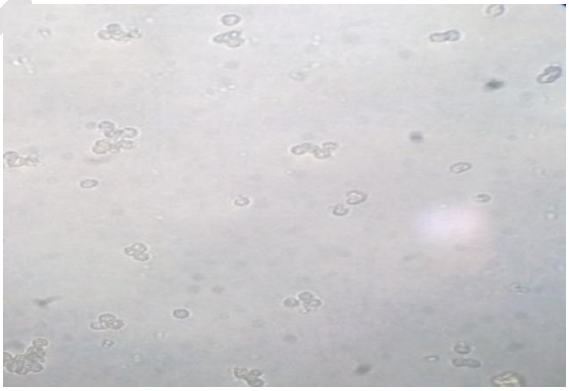

Plate 12: Photomicrograph of sickle cells treated with 1% *C. papaya* leaf extract and *S. aromaticum* seed extract (X40), [NaCl, 0.9%; $Na_2S_2O_5$, 2%]

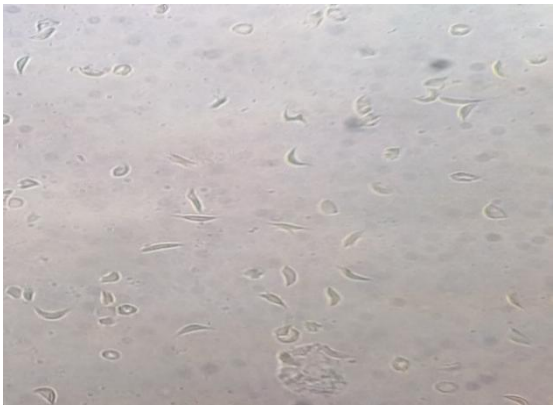

Plate 13: Photomicrograph e of sickle cells treated with 1% *G. senegalensis* leaf extract and *B. dalzielii* stem-bark extract (X40), [NaCl, 0.9%; $Na_2S_2O_5$, 2%]

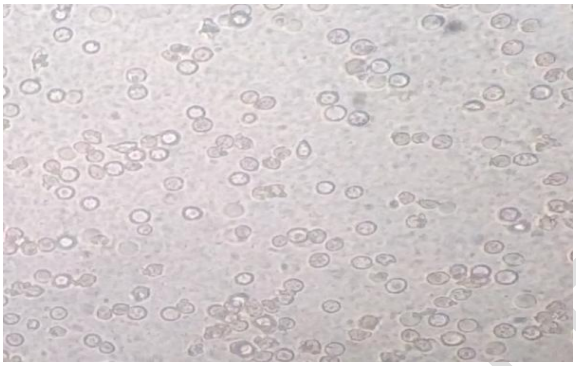

Plate 14: Photomicrograph of sickle cells treated with 1% *G. senegalensis* leaf extract and *P. africana* stem-bark extract (X40), [NaCl, 0.9%; $Na_2S_2O_5$, 2%]

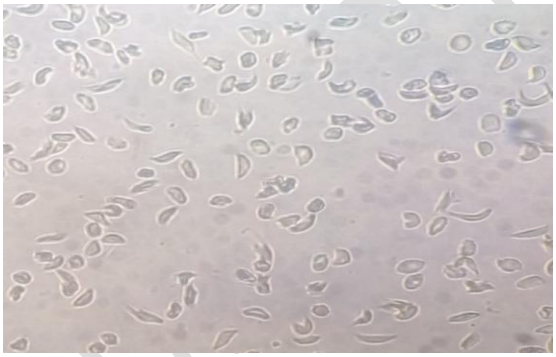

Plate 15: Photomicrograph of sickle cells treated with 1% *G. senegalensis* leaf extract and *S. aromaticum* seed extract (X40), [NaCl, 0.9%; $Na_2S_2O_5$, 2%]

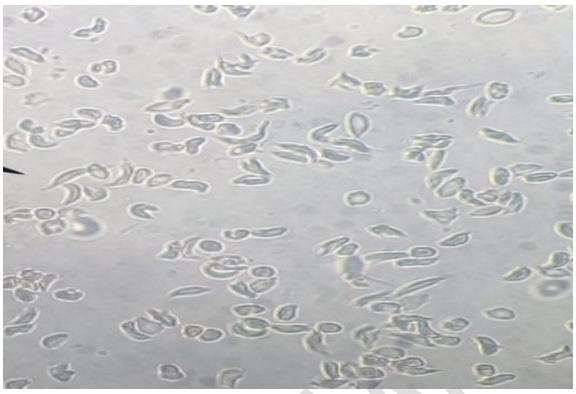

Plate 16: Photomicrograph of sickle cells treated with 1% *B. dalzielii* stem-bark extractand *P.africana* stem-bark extract (X40),[NaCl,0.9%;$Na_2S_2O_5$,2%]

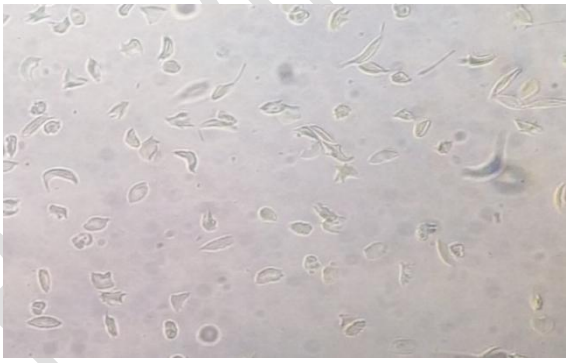

Plate 17: Photomicrograph of sickle cells treated with 1% *B. dalzielii* stem-bark extract and *S. aromaticum* seed extract (X40), [NaCl, 0.9%; $Na_2S_2O_5$, 2%]

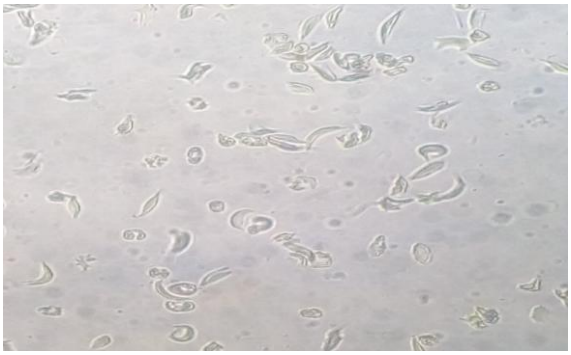

Plate 18: Photomicrograph of sickle cells treated with 1% *P. africana* stem-bark extract and *S. aromaticum* seed extract (X40), [NaCl,0.9%; $Na_2S_2O_5$, 2%]

Antisickling Activity of the Selected Plant Extracts

The antisickling activity of all the test plants and conventional drug (Hydroxyurea) against sickled red blood cells are presented in Table 2. Sickled cells treated with conventional drug (Hydroxyurea) revealed a high antisickling effect when compared to untreated sickled red blood cells ($P<0.05$). Also, sickled red blood cells treated with *Carica papaya* leaf extract, *Prosopis africana* leaf extract, *Syzygium aromaticum* seed extract, combination of *Carica papaya* leaf extract and *Guiera senegalensis* leaf extract revealed significant antisickling effect when compared to untreated sickled red blood cells ($P<0.05$) and are not significantly different ($P>0.05$) when compared with conventional drug (Hydroxyurea). Meanwhile, *Boswellia dalzielii* stem-bark extract, *G. senegalensis* leaf extract, combination of *C. papaya* leaf extract and *Prosopis africana* stem-bark extract and combination of *C. papaya* leaf extract and *S. aromaticum* seed extract showed a moderate antisickling activity when compared to untreated sickled red blood cells ($P<0.05$) but significantly different from conventional drug (Hydroxyurea) ($P<0.05$). However, combination of *C. papaya* leaf extract and *B. dalzielii* stem-bark extract, combination of *G. senegalensis* leaf extract and *B. dalzielii* stem-bark extract, combination of *G. senegalensis* leaf extract and *S. aromaticum* seed extract, combination of *B. dalzielii* stem-bark extract and *P. africana* stem-bark extract, combination of *B. dalzielii* stem-bark extract and *S. aromaticum* seed extract and combination of *P. africana* stem-bark extract and *Syzygium aromaticum* seed extract showed a very low antisickling effect when compared to untreated sickled red blood cells ($P<0.05$) and are significantly different when compared with conventional drug (Hydroxyurea).

Table 2: Antisickling Activity of the Selected Plants Extracts.

Treatment	% Sickling
Sickled cells	96.57 ± 1.23^{f}
Sickled cells + A	3.87 ± 2.74^{a}
Sickled cells + B	28.35 ± 2.07^{b}
Sickled cells + C	43.94 ± 10.84^{c}
Sickled cells + D	8.38 ± 1.06^{a}
Sickled cells + E	10.30 ± 1.40^{a}
Sickled cells + A+ B	6.60 ± 1.05^{a}
Sickled cells + A+ C	75.38 ± 9.47^{d}
Sickled cells + A+ D	39.70 ± 3.41^{c}
Sickled cells + A + E	34.34 ± 15.14^{bc}
Sickled cells + B+ C	71.60 ± 7.15^{d}
Sickled cells + B+ D	10.90 ± 2.13^{a}
Sickled cells + B + E	67.20 ± 3.31^{d}
Sickled cells + C + D	67.41 ± 8.70^{d}
Sickled cells + C + E	71.94 ± 4.02^{d}
Sickled cells + D + E	77.90 ± 5.14^{d}
Sickled cells + F	2.26 ± 0.33^{a}

Values are presented as mean ± SEM (n = 3) Values having similar superscript are not significantly different at (P>0.05) analysed using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0. A= *C. papaya* leaf extract, B= *G. senegalensis* leaf extract, C= *B. dalzielii* stem-bark extract, D= *P. africana* stem-bark extract, E= *S. aromaticum* seed extract, F = Hydroxyurea (Standard drug).

Phytochemical Composition of the Most Active Plant Extracts.

From the results of the antisickling activity, Carica papaya leaf extract, Guiera senegalensis leaf extract and *Prosopis africana* stem-bark extract were selected for both qualitative and quantitative phytochemical screening. The qualitative phytochemical compositions of the selected plants are presented in Table 3 while those of quantitative composition are presented in Table 4.

Table 3: Qualitative Phytochemical Composition of the Selected Plant Extracts

Phytochemicals	***C. papaya* leaf extract**	***G. senegalensis* leaf extract**	***P. africana* stem bark extract**
Flavonoids	–	+	+
Phenols	+	–	+
Tannins	+	+	+
Saponins	–	+	+
Alkaloids	+	+	+
Terpenoids	–	–	–
Steroids	–	–	–
Anthraquinones	+	+	–
Glycosides	+	+	–
Phlobatannins	+	–	–

+ = Present, - = Not detected

Table 4: Qualitative Phytochemical Composition of the Selected Plant Extracts

Phytochemicals	***C. papaya* leaf extract**	***G. senegalensis* leaf extract**	***P. africana* stem-bark extract**
Alkaloids (mg %)	3.48 ± 0.03	5.16 ± 0.01	7.49±0.01
Flavonoids (mg %)	_	1.52 ±0.00	11.05 ± 0.02
Tannins (mg %)	92.16 ± 0.08	91.98±0.04	94.37±0.01
Saponins (mg %)	_	6.50±0.01	14.79±0.01
Glycosides (mg %)	4.84 ± 0.01	2.49±0.00	_
Phenols (mg %)	3.47. ± 0.01	_	5.20±0.01
Phlobatannins (mg %)	93.14± 0.11	_	_

Values are expressed as Mean ± SEM., n = 3 (in triplicate).

IV. Discussion

Medicinal plants are widely used virtually in the treatment of all diseases in many parts of the world [16]; thus, various studies have documented many medicinal plants used to treat sickle cell conditions, although many rural dwellers assumed that sickle cell disease is spiritually dependent [17-20]. Indigenous people around the world have used oral tradition without detailed knowledge regarding the use of medicinal plants and this information has been passed from generations to generations [21]. In the present study, Ten (10) plants belonging to different families were documented from the Ethnobotanical survey conducted in Zuru Local Government Area of Kebbi State. It is well understood that Kebbi State is blessed with abundant indigenous traditional medicines of which many have not been scientifically documented.

Phytotherapy is the new and emerging area for providing the most effective, efficient, easily accessible and cheaper alternative to the chemically synthesized medicines for tropical disease and genetic disorders like sickle cell anaemia. Erythrocytic defects in sickle cell disease (SCD) resulted from the valine to glutamic acid substitution at position 6 on the Beta chain of hemoglobin forming sickled hemoglobin HbS [22].The deoxygenated state of abnormal sickle erythrocyte is more susceptible to oxidative damage of erythrocyte membrane components, erythrocyte deformation and membrane rigidity. Sodium metabisulfite act as a reductant, as HbS molecules undergo gelation when deprived of oxygen. The effect of inducing cells with 2% sodium metabisulphite *in vitro* is that it causes deoxygenation of red blood cells (RBCS) thereby causing aggregation and polymerization of individual Hb molecule [23]. In the present study, the medicinal plant extracts were capable for inhibiting the polymerization of Hb, where, *C. papaya* leaf extract was found effective, this agrees with the findings of Dash *et al.,* [24] and Mojisola *et al*.,[25].

Phytochemical constituents have been reported with potentials to act as a source of useful drugs or serve to improve the health status of biological system, secondary metabolites present in extracts such as phenols, tannins, flavonoids,

saponins and alkaloids have been reported to have strong anti-sickling effect [26]. In the current study, antisickling properties might be due to synergy of the secondary metabolites. This is inline with the findings of Singh *et a*l., [27] who also reported that phenols, tannins, flavonoids, saponins and alkaloids have strong antisickling potential.

V. Conclusion

Due to the presence of some of the tested phytochemicals in the three (3) plant extracts; C. papaya leaf extract, G. senegalensis leaf extract and P. africana stem-bark extract. The medicinal plant extracts were able to reduce the percentage of sickled cells. Therefore, they may be used as alternative to hydroxyurea in ameliorating the sickling in human HbS containing RBCs.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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