FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 7 No. 6, December (Special Issue), 2023, pp 104 -109
DOI: https://doi.org/10.33003/fjs-2023-0706-2175

INVITRO MEMBRANE PROTECTION EFFECT OF *AGERATUM CONYZOIDES* METHANOLIC EXTRACT ON HUMAN SICKLED CELLS

***Ibrahim, M. D., Atawodi, S. E. and Sallau, A. B.**

Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria

*Corresponding authors' email: murjadanja@gmail.com

ABSTRACT

Hemolytic anemia caused by membrane damage is the major objective of treating sickle cell anemia in both mainstream and alternative medicine, which is a characteristic hallmark of the disease.One plant that is reportedly utilized in traditional medicine to treat sickle cell anemia is ageratum conyzoides. Prelimnary phytochemical investigation were carried out according to the standard protocols. In vitro spectrophotometric analysis was used to assess the methanolic extract of A. conyzoides' capacity to shield sickle red blood cells from lysis. Preliminary phytochemical screening of crude extracts of leaf, stem and root of *Ageratum conyzoides* tested positive for the presence of phytochemicals such as alkaloids, flavonoids, tannins, cardiac glycosides, saponins, steroids and triterpenes. The leaf extract caused significant ($P<0.05$) membrane protection with the value of 24.13±0.47% at 2.5*mg/ml* concentration, while the stem extract produced a significant ($P<0.05$) protective effect with the value of 29.13±0.47% at 2.5*mg/ml*.The root also gave a significant ($P<0.05$) membrane protective effect of 40.73±1.04% at 2.5*mg/ml*. These finding clearly support the traditional usage of A. conyzoides in the management of sickle cell anemia by demonstrating the functions that the plant's leaves, stem, and roots play in membrane protection.

Keywords: *Ageratum conyzoides*, *In vitro*, Red Blood Cells, Membrane protection, Sickle Cell Anemia

INTRODUCTION

Plants have played an important role in the fight for survival from man's evolution. Plants aren't just the principal food source for all animals and even humans, but there are a lot of plant species that have been shown to be very valuable medicinal products over time (Lam *et al.*, 2016). Medicinal plants are used to treat and diagnose diseases and infections. From ancient times, plants have been rich sources of effective and safe medicines (Misganaw 2022)

The use of medicinal plants in therapeutics throughout the world is currently experiencing an interest among individuals despite the advancement of modern medicine (Akakpo-Akue et.,al 2021). The active principle is what's known as the ingredients found in medicinal plants that contain plant healing properties. It differs from one plant to another and examples of active ingredients include: anthraquinones, flavonoids, glycosides, saponins, tannins etc. Other substances found in plants include morphine, atropine, codeine, steroids, lactones, and volatile oils, all of which have therapeutic applications in the treatment of various illnesses. (Ajlan, 2016). For low-income populations, the cost of patient care is often out of reach. Therefore, many people use medicinal plants to treat a range of illnesses, even chronic diseases such as sickle cell anemia (Akakpo-Akue *et al*.,2021). One primary benefits of medicinal plants are their accessibility. Many plants have therapeutic qualities that can be grown in gardens or bought from the local market, making them practical and affordable substitutes for medications (Rutherford 2023).

Sickle cell disease is a hereditary condition caused by the inheritance of two mutant allelomorphic genes that regulate the formation of hemoglobin (Hb) β chains (Louise-Oluwasanmi - 2020). This deficiency results in the production of hemoglobin S (HbS), a hemoglobin tetramer ($\alpha 2/\beta 2$) that is poorly soluble and polymerizes when deoxygenated (Alomari 2020). The sickle hemoglobin (HbS) disorder is caused by a point mutation affecting the coding sequence of the β-globin gene, causing a substitution of glutamic acid by valine. The solubility (gelation) of the deoxy-HbS molecule is drastically reduced as a result of this amino acid swap (Ferreira 2022).

Sickling of hemoglobin is the cause of precocious hemolysis of erythrocytes and various complications of HbSS subjects (Man *et.,a*l 2021). The disease's chronic discomfort and complications can affect a patient's quality of life, which includes their ability to learn, work, and develop psychologically (Alabdallat and Adam, 2016). SCA is linked to high rates of morbidity and death in developing nations (Bhattacharya *et.,a*l 2021).

In 1910 sickle cell disease was first reported by Dr. James Herrick and subsequent characterization by Linus Pauling. In 1952, he postulated the characteristics of sickle cell hemoglobin (HbS) and how it contributes to sickle cell anemia. Ever since, finding a cure and effective treatment for the illness have remained a challenge for all of humanity (Kunle and Egharevba, 2013).

The Plant *Ageratum conyzoides*

Ageratum conyzoides is a common tropical annual herbaceous weed (Motmainna et *a*l., 2021). It belongs to the family Asteraceae (Rolnik and Olas 2021). It is a branching herb that reaches a height of about one meter. The leaves are up to 7.5 cm long and ovate, with fine white hairs covering the stems and leaves. The flowers are purple to white, less than 6mm across and arranged in close terminal inflorescences (Chauhan and Rijhwani, 2015). The plant is commonly known as "billy goat weed." while its hausa name is "*Iccen ciniki*".

Traditional uses of *Ageratum conyzoides*

The leaves are applied to cuts, burns and sores and externally for body rash. Additionally, they are used to treat leprosy and other skin conditions, stomach problems, spasms, diarrhea, epilepsy, and sore throats (Shukla, Bhat & Chakravarty 2022). This research aims to investigate the role of *Ageratum conyzoides* in HbSS erythrocyte membrane protection

MATERIALS AND METHODS

Extraction of Plant material

Fresh plant samples were collected from Dumbi, Zaria Local Government Area of Kaduna state. The samples were authenticated at Herbarium Unit of Biological Sciences Department, Ahmadu Bello University Zaria, with Voucher Numbers V3611. The samples were shade dried, pounded and extracted using soxhlet extractor. The extracts were dried and reconstituted

Ethical clearance

Ethical clearance was obtained from Health Research Ethics Committee Ahmadu Bello University Teaching Hospital Shika with no ABUTH/HREC/GO7/2013. Informed consent form was issued to patients to obtain their permission for blood collection.

Preliminary Phytochemical Screening

Phytochemical screening was carried out on all the extracts according to the method of sofowara, (1993).

Test for Carbohydrates (Molisch's test)

Few drops of molisch's reagent was added to 2ml of the extract and then concentrated sulphuric acid was added down the side of the test tube to form a lower layer with reddish colored ring at the interphase which indicates presence of carbohydrates (Evans, 1989).

Test for free Anthracene Derivatives (Bontrager's Test)

To a portion of the extract in a dry test tube, 5ml of chloroform was added and shaken for at least 5 minutes. This was filtered and the filtrate shaken with equal volume of 10% ammonia solution, bright pink color in the aqueous (upper) layer indicates the presence of free anthraquinones (Evans, 1989).

Test for Steroids and Triterpenes (Lieberman-Burchard's Test)

To a portion of the extract, equal volume of acetic anhydride was added and mixed gently. One (1ml) of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Color changes were observed immediately and over a period of one hour. Blue to blue-green color in the upper layer and reddish, pink or purple color indicates the presence of triterpenes (Evans, 1989).

Test for Cardiac glycosides (Keller-Killani Test)

A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. It was observed carefully at the interface for purple-brown ring. This indicates the presence of deoxy sugars and a pale green color in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 1989).

Test for Saponin Glycoside (Frothing Test)

About 10ml of distilled water was added to a portion of the extract and was shaken vigourosly for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 minutes. A honeycomb froth that persists for 10-15 minutes indicates presence of saponins (Evans, 1989).

Test for Tannins (Ferric chloride Test)To a portion of the extract, 3-5 drops of ferric chloride solution was added. A greenish-black precipitate indicates presence of condensed tannins while hydrolysable tannins give to a blue or brownish blue precipitate (Evans, 1989).

Test for Flavonoids (Sodium hyroxide test)

Few drops of 10% sodium hydroxide were added to the extract. Yellow coloration indicates presence of flavonoids (Evans, 1989).

Test for Alkaloids (Dragendroff's Test)

To a portion of the extract, few drops of Dragendroff's reagent were added. A reddish brown precipitate indicates the presence of alkaloids (Evans, 1989).

Preparation of blood sample

About 5ml of blood was collected into anticoagulated tubes (Tripotassium EDTA bottles) under aseptic conditions. The blood sample was then collected and washed with normal saline

Osmotic fragility test

The stock erythrocyte (RBC) suspension (0.50 ml) was mixed with various concentrations (0.1, 0.2, 0.3, 0.5 0.6 and 0.9%) of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the extract (0.5-2.5 *mg/ml*). The control samples consisted of 0.5 ml SS washed erythrocytes mixed with hypotonic-buffered saline solution alone and 0.5ml AA erythrocytes mixed with only saline solution. The mixtures were incubated for 30 min at room temperature and centrifuged for 5 min at 3000 g and the absorbance of the supernatant was measured at 540 nm. The percentage lysis was calculated using the fomula below

$$\%\ lysis = \frac{Absorbance\ of\ Test}{Absorbance\ of\ 100\%\ lysis} \times 100$$

RESULTS AND DISCUSSION

Table 1 :Phytochemical Composition of the Different Parts of *A. conyzoides*.

Phytochemical	leaves	Stem bark	Root
Carbohydrates Molisch Test	+	+	+
Anthraquinone Bontrager's Test	-	-	-
Steroid and triterpens Liberman Bucchard Test	+	+	+
Cardiac Glycosides Keller Killiani Test	+	+	+
Saponin glycoside Froth Test	+	+	-
Tannins Ferric chloride Test	-	-	-

Flavonoids	+	+	+
NaOH Test			
Alkaloids	+	+	-
Dragendroff's test			

Key:
- = Absent
+ = Present

Table 2: Effect of methanolic extract of *A. conyzoides* stem bark on red blood cell membrane fragility

	Mean % RBC lysis					
NaCl %	**AA Blood**	**SS Blood**	**SS blood+0.5*mg/ml***	**SS blood+1.0*mg/ml***	**SS blood+2.0*mg/ml***	**SS blood +2.5*mg/ml***
0.1	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
0.2	76.20±0.92^{b}	98.63±0.39^{b}	91.13±0.24^{b}	77.60±0.42^{b}	76.30±0.76^{b}	73.80±0.49^{b}
0.3	69.17±1.54^{c}	95.90±0.20^{c}	61.00±1.11^{c}	61.70±2.01^{c}	60.13±2.49^{c}	53.37±1.17^{c}
0.5	30.80±0.40^{d}	81.30±0.36^{d}	36.27±1.51^{d}	30.93±1.26^{d}	30.93±1.26^{d}	29.13±0.47^{d}
0.6	4.87±0.57^{e}	40.57±0.28^{e}	16.20±1.01^{e}	12.23±0.84^{e}	12.23±0.84^{e}	12.60±0.35^{e}
0.9	0.40±0.12^{f}	12.97±0.34^{f}	5.00±0.31^{f}	3.27±0.64^{f}	3.27±1.11^{f}	2.93±0.03^{f}

Values are mean ± SD of the mean of triplicate experiments.

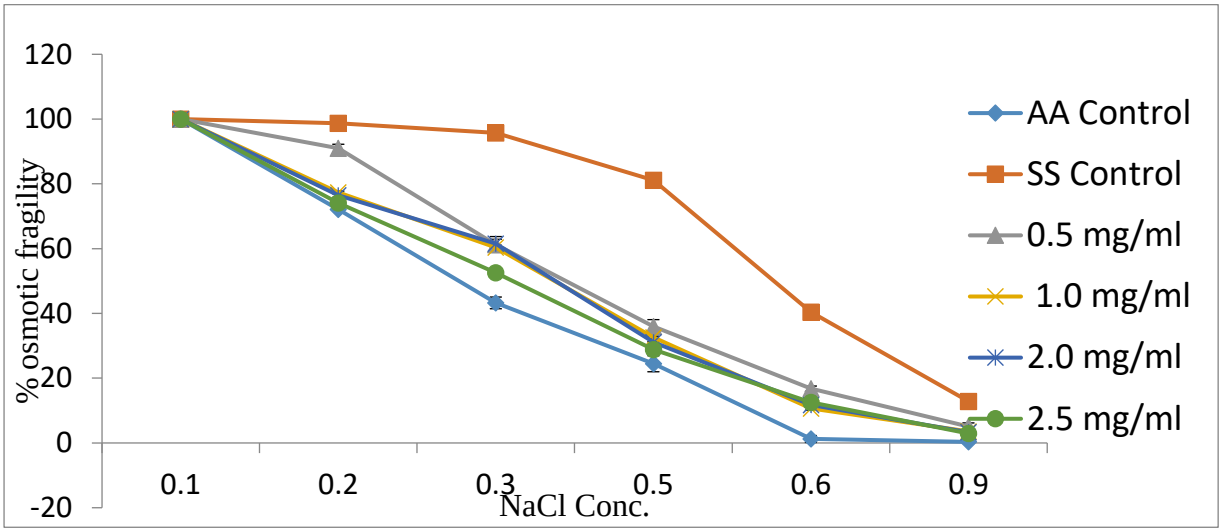

Figure 1: Effect of methanolic extract of *A. conyzoides* stem on human sickle cell *in vitro* at various NaCl concentrations (This is in line with the work of Oyenike *et al*., 2019)

Table 3: Effect of methanolic extract of *A. conyzoides* leaf on red blood cell membrane fragility

	Mean % RBC lysis					
NaCl %	**AA Control**	**SS Control**	**SS blood+0.5*mg/ml***	**SS blood+1.0*mg/ml***	**SS blood+2.0*mg/ml***	**SS blood +2.5*mg/ml***
0.1	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
0.2	76.20±0.92^{b}	76.20±0.92^{b}	88.27±0.75^{b}	79.33±0.29^{b}	75.30±0.70^{b}	75.23±1.94^{b}
0.3	69.17±1.54^{c}	69.17±1.54^{c}	61.57±1.04^{c}	60.30±0.79^{c}	58.27±2.26^{c}	61.80±1.22^{c}
0.5	30.80±0.40^{d}	30.80±0.40^{d}	34.90±1.10^{d}	32.27±1.05^{d}	31.53±1.90^{d}	24.13±0.47^{d}
0.6	4.87±0.57^{e}	4.87±0.57^{e}	16.33±1.73^{e}	13.53±0.87^{e}	17.00±1.73^{e}	11.70±0.57^{e}
0.9	0.40±0.12^{f}	0.40±0.12^{f}	4.27±1.18^{f}	2.90±0.52^{f}	7.53±0.90^{f}	7.40±0.74^{f}

Values are mean ± SD of the mean of triplicate experiments

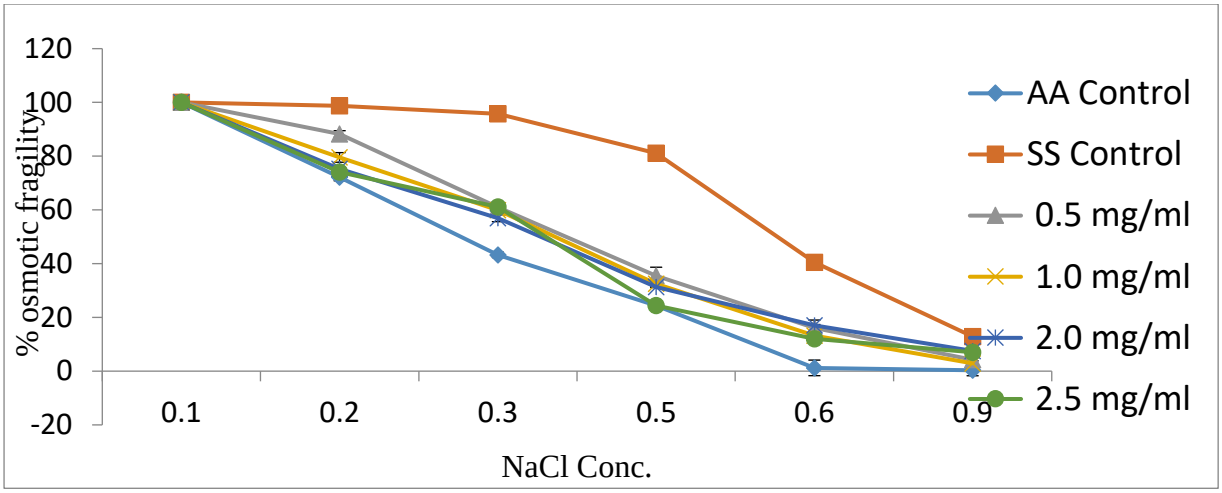

Figure 2: Effect of methanolic extract of *A. conyzoides* leaf on human sickle cell *in vitro* at various NaCl concentrations (This is in line with the work of Oyenike *et al*., 2019)

Table 4: Effect of methanolic extract of *A. conyzoides* root on red blood cell membrane fragility

	Mean % RBC lysis					
NaCl %	**AA Control**	**SS Control**	**SS blood+0.5*mg/ml***	**SS blood+1.0*mg/ml***	**SS blood+2.0*mg/ml***	**SS blood +2.5*mg/ml***
0.1	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
0.2	76.20±0.92^{b}	98.63±0.39^{b}	95.73±0.52^{b}	89.57±0.68^{b}	79.40±5.46^{b}	83.17±0.23^{b}
0.3	69.17±1.54^{c}	95.90±0.20^{c}	84.30±0.71^{c}	72.33±1.49^{c}	66.10±0.64c	54.03±5.30^{c}
0.5	30.80±0.40^{d}	81.30±0.36^{d}	50.80±1.27^{d}	45.23±1.91^{d}	44.13±2.62^{d}	40.73±1.04^{d}
0.6	4.87±0.57^{e}	40.57±0.28^{e}	9.27±1.36^{e}	8.27±1.77^{e}	10.83±1.30^{e}	8.60±1.78^{e}
0.9	0.40±0.12^{f}	12.97±0.34^{f}	5.43±1.45^{f}	4.13±1.33^{e}	5.67±1.98^{e}	4.30±1.36^{e}

Values are mean ± SD of the mean of triplicate experiments

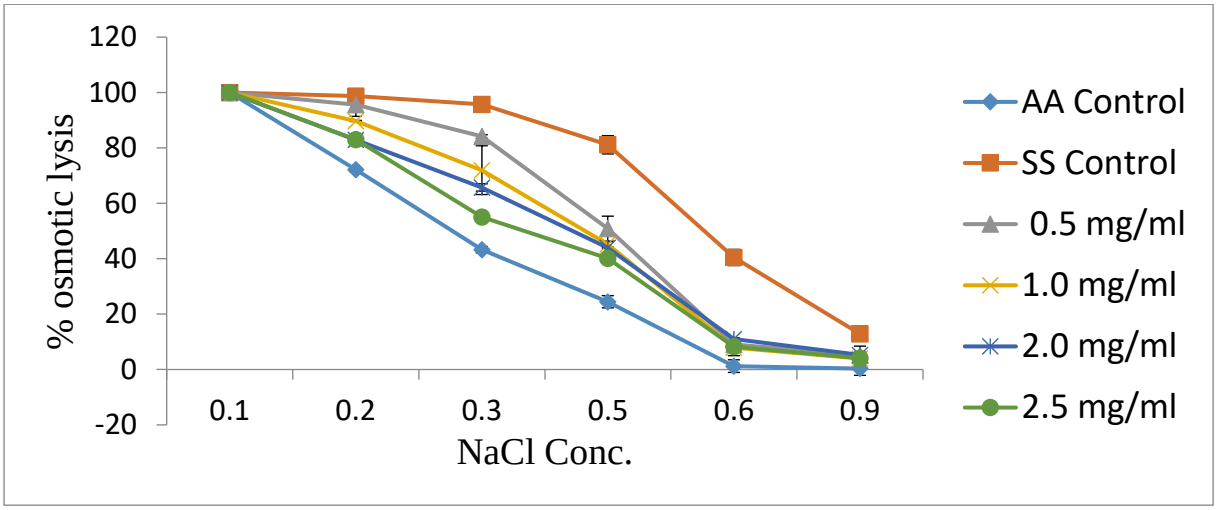

Figure 3: Effect of methanolic extract of *Ageratum conyzoides* root on human sickle cell *in vitro* at various NaCl concentrations (This is in line with the work of Oyenike *et al*., 2019)

Discussion

Research on phytotherapy is currently being done to manage diseases like typhoid fever, cancer, hypertension, and genetic disorders like SCA. The goal is to find more affordable, alternative medications that the majority of the population, who are from lower socioeconomic classes, can take immediately. (Imaga *et al.*, 2009). For the pharmacological discovery of novel drugs, the primary essential information regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts (Suntar, 2020).

Phytochemical qualitative screening of various plants in the current study revealed the presence of multiple groups of bioactive molecules, including anthraquinones, triterpenes, alkaloids, glycosides, saponins, flavonoids, and tannins. This result is similar to that reported by (Egunyomi *et al.*, 2009). The presence of alkaloids in the stem, leaf and root of both plants is an indication that they may be useful in alleviating pains associated with SCA (Ejele and Aneke, 2011).

Osmotic fragility of erythrocytes demonstrates the integrity of red cell membranes in pathological and normal states (Salvagno, *et al.*, 2020). The results of osmotic fragility tests performed on sickle cell blood demonstrated that the plant

extracts had appreciable protective effect on membranes and inhibited hemolysis of red blood cells. The percentage of hemolysis of the control and test samples showed a sigmoidal relationship with increasing concentrations of saline solution. There was significant decrease ($p<0.005$) of percentage lysis from 95.70% of the negative control at 0.3% of NaCl solution to 52.60% at 2.5mg/ml *A.conyzoides* stem. This result is similar to that reported by (Imaga *et al.,* 2009 and Arokoyo *et al.,* 2015). Ohiri & Ohanador (2022) reported that a higher propensity for decreased mechanical and osmotic stability was linked to higher levels of free radicals in human sickle erythrocytes. As a result, the buildup of oxidants accelerates the deterioration of the sickle cell erythrocyte membrane and causes these cells to senescent (Esperti et.,al 2023). These extracts may have the ability to protect erythrocyte membranes because they contain flavonoids, which have been shown to possess antioxidant properties. (Ahur *et al.*,2014). The effect of varied concentrations of *A. conyzoides* plant extracts on the red blood cell membrane, analyzed using the Osmotic fragility test, revealed appreciable membrane protective effects of the extracts and their inhibitory action on hemolysis of red blood cells. The plant's stem, leaf, and root extracts were all found to exhibit significant antihemolitic activity for A. conyzoides. This suggests that the extract may be useful in delaying the in vivo polymerization of sickle hemoglobin and influencing the sickling time course.

CONCLUSION

From the study, it can be concluded that the methanolic extracts of *A. conyzoides* helps in maintaining erythrocyte membrane integrity, and hence justify its uses in traditional medicine for the management of sickle cell anemia. Further work on the screening of the secondary metabolites of these plants for biological and pharmacological studies will be necessary as well as the isolation of active compounds and their structural elucidation using spectroscopic techniques and subsequent *in vivo* trials for maximal exploitation of the medicinal properties of these medicinal plant.

REFERENCES

Ahur, V.M; Yahaya, A.A; Saganuwan, A.S. & Ikye-Tor, J.T. (2014): Ameliorative properties of aqueous extract of *Ficus thonningii* on erythrocyte osmotic fragility induced by acetaminophen in *Rattus norvegicus. Veterinary Research Forum*; **4** (4) 207 – 212

Ajlan A, (2016): Medicinal Plants: A Review.*Natural Products: An Indian Journal of Natural Products* **12**(3): 1-6

Akakpo-Akue Joël, Ahon Gnamien, Félix Houphouët-Boigny, Kplé Tatiana Kangah Mireille, Félix Houphouët-Boigny, Fofié Yvette, Ibrahime Sanogo, Yapo-Crezoit Antoinette Claire Chiyae & Kra Adou Koffi Mathieu (2021). Assessment of Radical Scavenging and Sickling Inhibitory Activities of the Bark of Disthemonanthus Benthamianus (Caesalpiniaceae)*EuropeanScientificJournal,ESJ*,17(10),121 . https://doi.org/10.19044/esj.2021.v17n10p121

Alabdallat Nessrin Ghazi & Adam Ibrahim A. (2016): *In vitro* Antisickling Activity of *Zingiber officinale Roscoe* (Ginger) Methanolic Extract on Sickle Cel l Disease. *British Journal of Medicine & Medical Research* **12**(12): 1-7.

Alomari, E. A. A. M. (2020). Protein therapeutics for clinical applications: Hemoglobin-based oxygen carriers and modulation of their oxygen-binding properties.

Arokoyo, D.S; Seriki, A.L; Babatunde, L.D; Ojo, A. & Bamidele, O. (2015): The In-Vitro Effect of Aqueous Leaf Extract of *Basella Alba* L. On Osmotic Fragility of Red Blood Cells in Hbss Subjects. *European Journal of Biotechnology and Bioscience*.**3** (10); 05-10.

Bhattacharya, P., Sarmah, D., Dave, K. R., Goswami, A., Watanabe, M., Wang, X., & Alvarez, O. (2021). Stroke and stroke prevention in sickle cell anemia in developed and selected developing countries. *Journal of the neurological sciences*, *427*, 117510.

Chauhan Anita & Rijhwani Shilpi (2015): A Comprehensive Review on Phytochemistry of Ageratum conyzoides linn. (Goat weed). *International Journal of Engineering Technology, Management and Applied Sciences*.**3,** 348-358.

Ejele, A. E., & Aneke, N. J. (2011). Anti-Sickling Potential of Aloe Vea Extract Iii: Gas Chromatography/Mass Spectrometry Characterization of Neutral Metabolite. *International Journal of Tropical Agriculture and Food Systems*, *5*(1), 31-36.

Esperti, S., Nader, E., Stier, A., Boisson, C., Carin, R., Marano, M., ... & Connes, P. (2023). Increased retention of functional mitochondria in mature sickle red blood cells is associated with increased sickling tendency, hemolysis and oxidative stress. *Haematologica*, *108*(11), 3086.

Ferreira, J. A. G. (2022). *Genetic variability, disease severity and therapeutic response in a cohort of Angolan Sickle Cell Disease patients* (Doctoral dissertation).

Imaga, W.O; Gbenle, G.O; Okochi, V.I; Akanbi, S.O; Edeoghon S.O; Oigbochie, V; Kehinde, M.O. & S.B., Bamro, (2009): Antisikling property of *Carica papaya*leaf extract. *African Journal of Biochemistry*. **3**(4): 102-106.

Kunle O. F. & Egharevba H. O. (2013): Chemical constituents and biological activity of medicinal plants used for the management of sickle cell disease - A Review: *Journal of Medicinal plants Research***7**(48), 3452-3476.

Lam, K.Y; Ling, A.P; Ko, R.Y; Wong, Y.P. & Say Y.H. (2016): A Review on Medicinal Propertiesof Oretin. *Advances Pharmacological and Biological Sciences.* Vol 2016, Article ID 4104595, 9 pages.

Louise-Oluwasanmi, O. (2020). *Targeting of Bisphosphoglycerate Mutase in Sickle Cell Anemia* (Doctoral dissertation, Howard University).

Man, Y., Kucukal, E., An, R., Bode, A., Little, J. A., & Gurkan, U. A. (2021). Standardized microfluidic assessment of red blood cell–mediated microcapillary occlusion: Association with clinical phenotype and hydroxyurea responsiveness in sickle cell disease. *Microcirculation*, *28*(2), e12662.

Misganaw Gedlu Agidew 2022. Phytochemical analysis of some selected traditional medicinal plants in Ethiopia.Bulletin of the National Research Centre (2022) 46:87 https://doi.org/10.1186/s42269-022-00770-8.

Motmainna, M., Juraimi, A. S., Uddin, M. K., Asib, N. B., Islam, A. M., Ahmad-Hamdani, M. S., & Hasan, M. (2021). Physiological and biochemical responses of Ageratum

conyzoides, Oryza sativa f. spontanea (weedy rice) and Cyperus iria to Parthenium hysterophorus methanol extract. *Plants*, *10*(6), 1205.

OhirI, R. C., & Ohanador, R. A. (2022). Membrane Stabilizing Effect and Anti-sickling Potentials of Trametes hirsuta (Hairy bracket mushroom) Extract on Human HbS Erythrocytes. *Journal of Biochemistry International*, 10-17.

Oyenike, M. A., Akpan, H. B., Otulana, O. J., Adefule, A. K., Adedokun, K. A., Oluogun, W. A., ... & Ojokuku, H. O. (2019). In-vitro anti-sickling and membrane stability potentials of Mishenland polyherbal extract on sickle red blood cells. *The Egyptian Journal of Haematology*, *44*(1), 65-71.

Rolnik, A., & Olas, B. (2021). The plants of the Asteraceae family as agents in the protection of human health. *International journal of molecular sciences*, *22*(6), 3009.

Rutherford M. 2023, Healing Properties and Challenges Associated with the Use of Medicinal Plants. Journal of Medicinal & Aromatic Plants Volume 12 Issue 1

Salvagno, G. L., Demonte, D., Dima, F., Bovo, C., & Lippi, G. (2020). Stability of refrigerated whole blood samples for osmotic fragility test. *Hematology, transfusion and cell therapy*, *42*, 134-138.

Shukla, G., Bhat, J. A., & Chakravarty, S. (2022). Species richness and folk therapeutic uses of ethnomedicinal plants in West Bengal, India–A meta-analysis. *Phytomedicine Plus*, *2*(1), 100158.

Süntar, I. (2020). Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. *Phytochemistry Reviews*, *19*(5), 1199-1209.