Evaluation of Anti-inflammatory and Antisickling Potentials of Archidium ohioense (Schimp. ex Mull) Extracts

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Abstract: *The study investigated the possible anti-inflammatory and antisickling potential of a moss plant A. ohioense.The phytoconstituents of acetone, chloroform and ethylacetate extracts of the plant were analysed using standard methods. Membrane stabilizing, antisickling, xanthine oxidase and lipooxygenase inhibitory activities of the extracts of the plants on sickle and normal erythrocytes were conducted. Phytochemically, the ethylacetate, acetone and chloroform extracts of A. ohioense showed the presence of cardiac glycoside, flavonoids, saponin, steroid, alkaloids and triterpenes. The acetone and ethyl acetate extracts of the plant stabilized red blood cell membrane of normal and sickle erythrocytes at various concentrations except at 2.0 mg/ml while the chloroform extract exhibits profound protective effect on both normal and sickle erythrocytes at highest concentration used (2.0 mg/ml).All the A. ohioense extracts showed mild anti-lipoxygenase and xanthine oxidase inhibitory activities. As the concentrations of the A. ohioense chloroform and acetone extracts increased, the percentage inhibition of sickling significantly increased and compared favorably with Para-hydroxybenzoic acid. These two extracts also demonstrated significant (p ≤ 0.0001) dose dependent increase in antisickling reversal activity.*
This study indicates that A. ohioense could be valuable source of anti-inflammatory and antisickling agents.
Keywords: *Archidium ohioense, antiinflammatory, antisickling, erythrocytes.*

I. Introduction

Sickle cell disease (SCD) or drepanocytosisis a genetic disorder, characterized by the presence of the he- moglobin S (HbS).SCD is as a consequence of a single point mutation at the sixth codon of the normal haemoglobin genewhere valine replaces glutamic acid at the beta globin chain [1]. HbS molecules polymerize under low oxygen tension, causing the formation of rigid and sickle erythrocytes. The deformity of the sickled red blood cells results in their shortened survival since they become vulnerable to lysis as penetrate the interstices of the splenic sinusoids and hence severe hemolytic anemia ensues with hemoglobin values ranging from 6 to 10 g/L[2, 3].

SCD has been associated with acute and chronic clinical manifestations such as vaso-occlusive episodes (VOE), painful crisis, hemolysis, impaired blood flow as a result of intravascular sickling in capillary and vessels, inflammation processes and high susceptibility to infection, acute chest syndrome etc [4]

Plants are often employed as medicines for the treatment of diseases because they are known to synthesize bioactive compounds. The management or treatment goals for sickle cell disease aim to relieve pain, prevent infections and manage complications [5]. Some medicinal plant extracts have been reported to inhibits /decreased polymerization of HbS molecules [6, 7] and have been established to serve as potential antisickling chemotherapeutic preparations [8, 9].

A. ohioense is a short and tiny perennialplant that growabout2-20 mm high in clusters to form denseand short turfs. The colour ranges from green toyellow-green. *A. ohioense* stems are simple variable or often short and multi-branched by numerous innovations from axils of stem leaves or exterior perichaetial leaves. As the plant aged it become prostrate, fragile and often detached. [10]. This study is part of ongoing attempts to exploit the therapeutic potentials of a lower plant*(A. ohioense)* in treatment and management of inflammation and sickle cell disorder.

II. Materials and Methods

2.1 Collection of Plant
Fresh plants of*A. ohioense* was collected on a rock surface at road 8, Obafemi Awolowo University Senior Staff Quarters, Ile-Ife, Nigeria. The plant was identified and authenticated by Dr. A.M. Makinde of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.2Preparation of Plant Extracts
The fresh plant was harvested, rinsed in water and air dried at room temperature. The plant was milled using manual grinding machine and soaked separately with ethyl acetate, acetone and chloroform. The solution was separately decanted and filtered after 72 h. The residues were re-soaked in each of the solvents for another 72 h. The filtrate of each solvent was pooled and evaporated to dryness under reduced pressure at 35°C on rotatory evaporator.

2.3 Phytochemical Screening
The ethanolic extract was phytochemically screened for the presence of secondary metabolitesas described by Evans (2002) [12] and Sofowora (2006)[13]

2.4 *In vitro* Anti-inflammatory Activities
2.4.1Membrane Stabilization
2.4.1.1Preparation of reference drugs
Ibuprofen (a non-steroidal anti-inflammatory drug) tablet (500mg), a reference drug for membrane stabilizing activity, was purchased from Campus Pharmacy of Obafemi Awolowo University, Ile-Ife, Nigeria. The tablets were ground to powder and dissolved separately in normal saline to obtain concentrations of 1.0mg/ml and 2.0mg/ml respectively.

2.4.1.2 Collection of Blood Samples
Fresh normal and sickle blood samples (5ml) each were collected by venipuncture into heparinized bottles at Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife.The sickle blood samples were collected from confirmed sickle cell anaemia patients who were in a steady state and attending the routine clinic. Ethical clearance was obtained from the Ethical Committee of the Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria.

2.4.1.3 Preparation of red blood cells
Red blood cells were prepared as described by Oyedapo *et al*., (2010) [14]. Freshly collected human normal and sickle blood samples were poured separately into clean dried centrifuge tubes and centrifuged at 3000 rpm for 15 minutes. The supernatant was carefully removed with the aid of dropping pipettes and residuewas re-suspended in fresh isosaline, mixed carefully and gently to prevent lyses of red blood cells. The process of centrifugation was repeated until a clear supernatant was obtained. To 2 ml of the packed cell was added 98 ml of isosaline to make a 2% (v/v) red blood cells.

2.4.1.4 Membrane stabilizing assay
The membrane stabilizing activity assay method was based on the procedure described by Oyedapo *et al*. (2004)[15]. The assay mixture was made up of hyposaline (2 ml), 0.1 M phosphate buffer, pH 7.4 (1.0 ml), varying volumes of extract (0.0 - 1.0 ml) at a concentration of 2.5 mg/ml and 2% (v/v, 0.5 ml) erythrocyte suspension to a total volume of 4.5 ml. The control was prepared as above without the drug while the drug control (4.5 ml) lacked fixed red blood cell.The reaction mixtures were incubated at 56°C for 30 min. The supernatants were collected into test tubes and the absorbance of the released haemoglobin was read at 560 nm against reagent blank.The percentage membrane stability was estimated using the expression:

Percentage Membrane Stability =100 - {Abs test drug - Abs drug control} x 100
     {Abs of blood control}

Where the blood control represented 100% lyses. The values represent the average of triplicates ± SEM

2.4.2 Assay of Anti-Lipoxygenase Activity
Anti-lipoxygenase activity of the extracts with linoloeic acid as substrate was measured as described by Malterud and Rydland (2000)[16]. The assay mixture consisted of 150 µl phosphate borate (1/15 M, pH 7.5), extract solution (50 µl), 50 µl enzyme solution (0.28 U/ml in phosphate borate). The reaction was initiated by the addition of 250 µl of the substrate solution (0.15 mM in distilled H_2O). Enzymatic kinetic was recorded at

234 nm for 2 min at 30s intervals. All the assay was performed in triplicate and lipoxygenase activity was calculated using the expression shown below. One Unit (U)will convert 1.0 μmole of xanthine to uric acid per seconds at pH 7.5 at 25ºC.

$$\text{Volume activity} = \frac{\Delta OD/\sec \times tv \times df}{12.5 \times l \times sv}$$

tv = Total volume (0.5 ml)   sv= Sample volume
12.5= Millimolar extinction coefficient of uric acid under the assay condition (n^{-1} cm^{-1})
 l = Light path length   df = Dilution factor
ΔOD/sec = Change in absorbance per seconds

2.4.3 Xanthine Oxidase Inhibitory Assay

Xanthine oxidase inhibitory activity of the extracts with xanthine as substrate was as described by Ferraz Feliah *et al*. (2006) [17].Theextracts were separately dissolved in phosphate buffer-MeOH (1%) and assayed for xanthine oxidase inhibitory activity at final concentration of 50μg/ml. The assay mixture consisted of 150μl phosphate buffer (1/15 M, pH 7.5), extract solution (50 μl) and 50μl enzyme solution (0.28U/ml in phosphate buffer.) The reaction was initiated by the addition of 250μl of the substrate solution. Enzymatic kinetic was recorded at 295 nm for 2 min at 30s intervals. All the assay was performed in triplicate. Xanthine oxidase inhibitory activity was calculated as volume activity using the equation below. One Unit U will convert 1.0 μmole of xanthine to uric acid per second at pH 7.5 at 25ºC.

$$\text{Volume activity} = \frac{\Delta OD/\sec \times tv \times df}{12.5 \times l \times sv}$$

tv = Total volume (0.5 ml)   sv= Sample volume
12.5= Millimolar extinction coefficient of uric acid under the assay condition (n^{-1} cm^{-1})
l = Light path length   df = Dilution factor
ΔOD/sec = Change in absorbance per seconds

2.5 Antisickling Assay
2.5.1 Inhibitory assay

The evaluation of extracts*A. ohioense*for antisickling activities was carried out using procedures that was based on the methods of Egunyomi *et al.*(2009)and Sofowora *et al.* (1979) [18, 19].The washed erythrocytes (0.2 ml), phosphate buffered saline (0.2 ml) and the test extract (0.2 ml) were mixed together in a test tube. The mixture was overlaid with 1 ml of liquid paraffin. The mixture was then incubated in a thermostated water bath at 37°C for 4 hr. Freshly prepared 2% (w/v) sodium metabisulphite solution (0.6 ml) was carefully added to the mixture under the paraffin, mixed gently by rolling the test tube between the two palms and incubated for additional 11/2 h at 37°C in a water bath. The liquid paraffin was carefully removed using a Pasteur pipette and the cell was fixed with 3 ml of 5% w/v buffered formalin solution. The positive control was treated described above except parahydroxybenzoic acid (PHBA) was added instead of extract while the negative control lacked extract but normal saline. Each test was performed in triplicate. A drop of each reaction mixture was smeared on a microscope slide and viewed under high powered magnification (x 100) under the oil immersion. Cells were counted on five fields on each slide the numbers of sickled and unsickled cells were counted to determine the total number of cells. The percentage mean sickling as well as the percentage inhibition activity for each extract were estimated using the expression below:

% Mean Sickling = $\underline{\text{Mean sickled cells}}$ x 100
                                        Mean total cells

% Inhibition activity = $\underline{\text{Control - % Mean sickled}}$ x 100
                                        Control

2.5.2 Reversal Antisickling Assay

The antisickling activity of chloroform and acetone of *A. ohioense* extracts was carried out using modified methods of Egunyomi *et al.* (2009) and Sofowora *et al.* (1979) [18, 19]. Mixture of the washed erythrocytes (0.2 ml) and 0.2 ml phosphate buffered saline in a test tube was carefully overlaid with 1ml liquid paraffin and 0.6 ml of 2% (w/v) sodium metabisulphite was carefully introduced under the liquid paraffin. The mixture was then incubated at 37°C for ninety minutes. At the end of the incubation period, 0.2 ml of the extract was carefully added under the liquid paraffin and was further incubated at 37°C for additional 6 hr. The liquid paraffin layer was removed with a Pasteur pipette and the cells were fixed with 3 ml of 5% (w/v) buffered formalin solution, which was carefully mixed by rolling the test tube between the two palms. The positive

control also involved all the procedure described bove except that PHBA was added instead of extract while the negative control was normal saline. A drop of each reaction mixture was smeared on a microscope slide and viewed under high powered magnification (x 100) under the oil immersion. Cells were counted on five fields on each slide, the number of sickled and unsickled cell were counted to determine the total number of cells. The percentage mean sickling as well as the percentage reversal activity for each extract was estimated using the expression below:

% Reversal activity = $\underline{\text{Control- \%Mean sickled}}$ x 100
                                          Control

Total number of cells counted= No of sickled + No of Unsickled cells
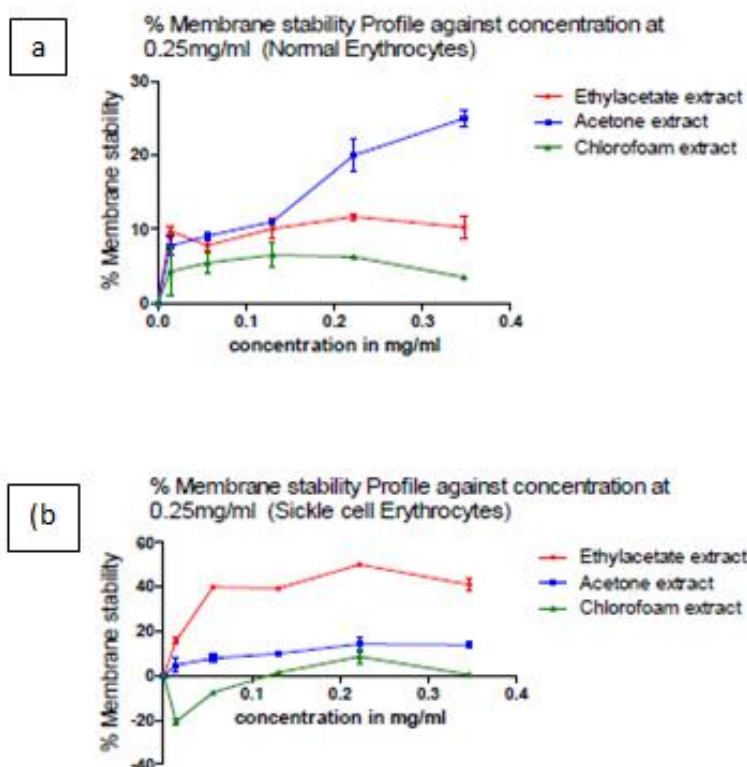
2.6 Statistical Analysis
Values are expressed as mean ± SEM of 3 consistent readings. The statistical significance differences were analyzed using Student-- Newman- Keuls Multiple Comparison test and analysis of variance. Values of $p < 0.0001$ were considered to be extremely significant.
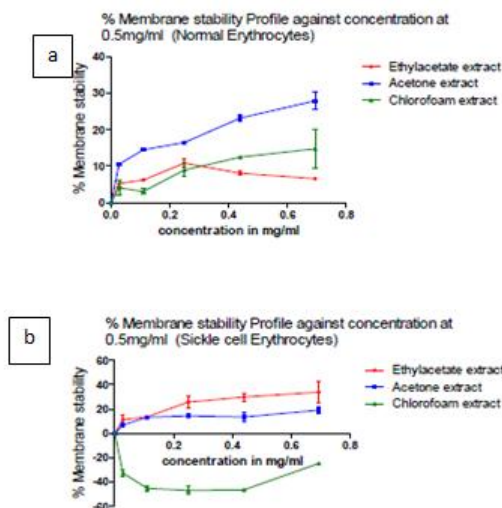
III. Results

Table 1. Phytochemical Constituents in the Ethylacetate, Acetone and Chloroform Extracts of *Archidium Ohioense*

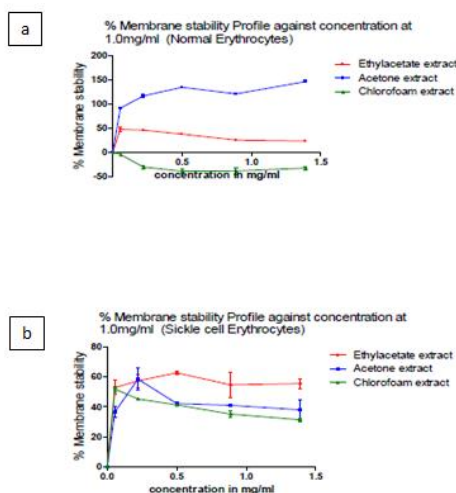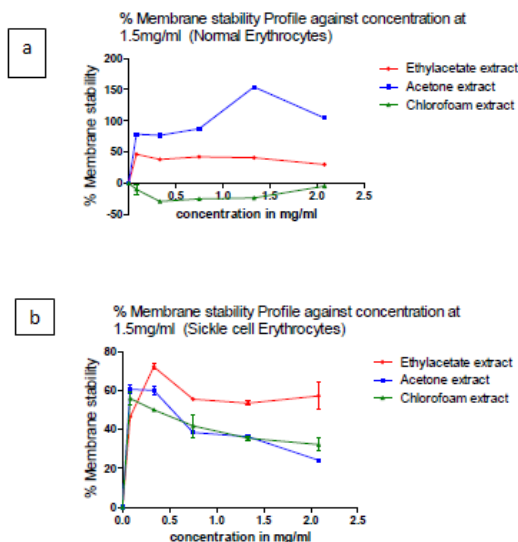|	Ethylacetate Extract	Acetone Extract	Chloroform Extract
Alkaloids	+	+	-
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	-	-
Anthaquinones	-	-	-
Cardiac glycosides	+	+	+
Phlobatanins	-	+	-
Steroids	+	+	+
Triterpenes	+	+	+
Xanthoproteins	-	-	-

+   Present
- Absent

Fig. 1(a, b): Membrane stabilizing profiles of ethylacetate, acetone and chloroform extracts of *A. ohioense* on normal (a) and sickle (b) erythrocytes at 0.25 mg/ml. Each value represented the mean ± SEM of readings

Fig. 2 (a, b): Membrane stabilizing profiles of ethylacetate, acetone and chloroform extracts of *A. ohioense* on normal (a) and sickle (b) erythrocytes at 0.5 mg/ml. Each value represented the mean ± SEM of readings

Fig. 3(a, b): Membrane stabilizing profiles of ethylacetate, acetone and chloroform extracts of *A. ohioense* on normal (a) and sickle(b) erythrocytes at 1.0 mg/ml. Each value represented the mean ± SEM of readings

Fig. 4(a, b): Membrane stabilizing profiles of ethylacetate, acetone and chloroform extracts of *A. ohioense* on normal (a) and sickle (b) erythrocytes at 1.5 mg/ml. Each value represented the mean ± SEM of readings
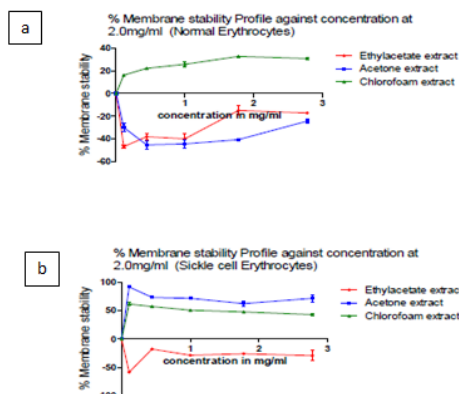
Fig. 5(a, b): Membrane stabilizing profiles of ethylacetate, acetone and chloroform extracts of *A. ohioense* on normal (a) and sickle(b) erythrocytes at 2.0 mg/ml. Each value represented the mean ± SEM of readings
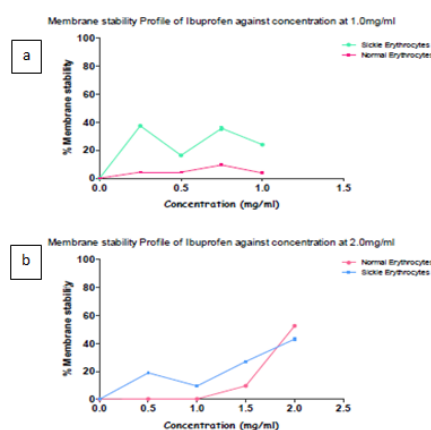

Fig. 6 (a, b): Membrane stabilizing Ibuprofenon normal and sickle erythrocytes at 1.0 mg/ml (a) and 2 mg/ml (b). Each valuerepresented the mean ± SEM of readings

Table 2: Lipooxygenase and Xanthine Oxidase Inhibitory Activities of Chloroform,Acetone and Ethylacetate of *A. ohioense*.

	Lipoxygenase Activity U/ml	Xanthine oxidase activity U/ml
Chloroform extract	$2.7 \times 10^{-3} \pm 0.005$	$1.08 \times 10^{-3} \pm 0.0004$
Acetone extract	$3.6 \times 10^{-3} \pm 0.001$	$3.6 \times 10^{-2} \pm 0.008$
Ethylacetate extract	$2.0 \times 10^{-3} \pm 0.002$	$2.4 \times 10^{-2} \pm 0.0000$
Quercetin	$7.2 \times 10^{-3} \pm 0.001$	ND

The values represent the means of triplicate absorbance + SEM
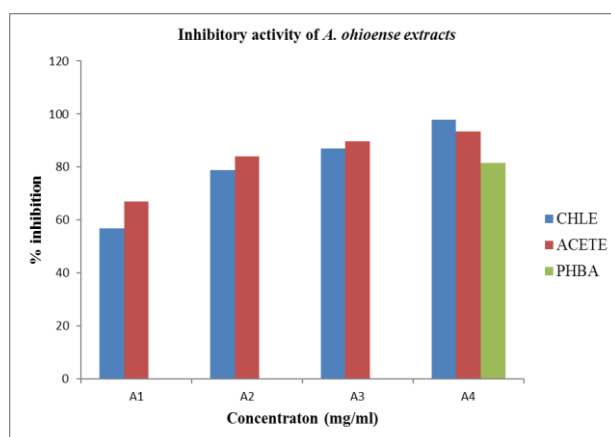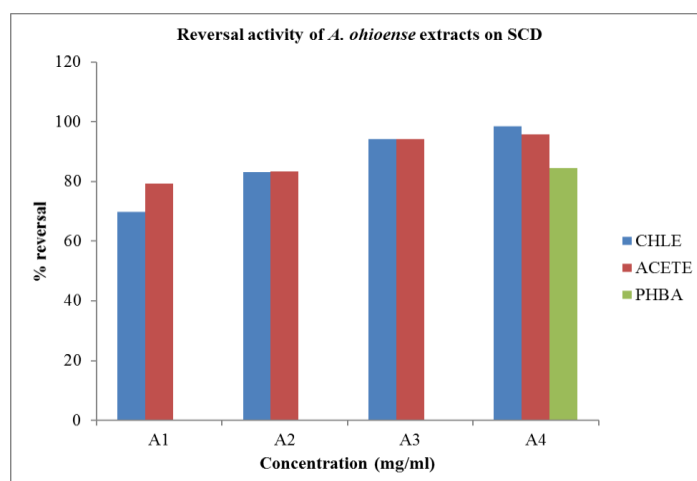ND – Not determined

Fig.7: The relative inhibitory activities of the chloroform and acetone leaf extracts of *A ohioense*

Keys: CHLE= Chloroform Extract, ACETE= Acetone Extract
A1=0.5mg/ml, A2=1.0 mg/ml, A3=2.0 mg/ml, A4= 4.0 mg/ml and PHBA (Positive control) = Para-hydroxybenzoic acid, Data are expressed as ±SEM (n=3) for each group. All of them are significant at p ≤ 0.0001 degree.

Fig. 8: The relative reversal activities of the chloroform and acetone leaf extracts of *A ohioense*

Keys: CHLE= Chloroform Extract, ACETE= Acetone Extract
A1=0.5mg/ml, A2=1.0 mg/ml, A3=2.0 mg/ml, A4= 4.0 mg/ml and PHBA (Positive control) = Para-hydroxybenzoic acid. Data are expressed as ±SEM (n=3) for each group. All of them are significant at p ≤ 0.0001 degree

IV. Discussion

Phytochemical screening of the extracts (ethylacetate, acetone and chloroform) of *A. ohioense*gave a positive test for the presence of alkaloids, cardiac glycoside, flavonoids, phlobatanin, saponins, steroid, tannin and triterpene. These phytoconstituents have been demonstrated to possess a wide range of activities. Tannins are astringent polyphenols, though considered as anti-nutritional due to its decrease in the digestibility of proteins, it had been employed as antidiarrheal, hemostatic, and anti-hemorrhoidal compounds [20]. Saponins have various biological activities such as the expectorant, diuretic and adaptogenic activities associated with them. They are also responsible for the characteristic bitter taste of most plants [21, 22]. Flavonoids and phenolic compounds have been reported to exert multiple biological effects which include antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenicetc.[23]. The biological importance of cardiac glycosides primarily is in the treatment of cardiac failure. They result in an increase in cardiac output by increasing the force of contraction as a result of their ability to increase intracellular calcium concentrations [24]. Thus, the presence of these phytochemicals might be responsible for the reported anti-inflammatory and antisickling activities of the plant.

Plants derived drugs have been reported to promote stability of biological membranes when stressed [25]. Several investigations have backed up the possibilities that stability of various humanerythrocytes (HbAA,HbAS andHbSS) membranes varied [26 - 28].

Figs. 1-6 showed the membrane stabilizing profiles of human normal and sickle erythrocytes exhibited by *A. ohioense* extracts at different concentrations (0.25mg/ml to 2.0mg/ml) and Ibuprofen (1.0mg/ml and 2.0mg/ml) on red blood cells exposed to heat and hypotonic induced lyses.

At 0.25 mg/ml, it was observed that all the extracts protected normal erythrocytes (Fig.1a). Acetone extract showed monophasic mode of protection while the remaining two extracts protected better at lower concentrations. On sickle erythrocytes (Fig.1b), ethyl acetate and acetone extracts protected better while chloroform extract stabilizedthe red blood cells slightly. At 0.25 mg/ml acetone extract protected normal erythrocytes better whileethylacetate extract stabilizedthe sickled erythrocytes better.

On normal erythrocyte at 0.5 mg/ml (Fig. 2a), as the mode of protection increased as the acetone and chloroform extracts concentration increases extracts and ethyl acetate mode of protection decreases. In Fig. 2b, of the three extracts, only ethylacetate and acetone extracts protected sickle erythrocytes. At this concentration, just as in 0.25mg/ml ethylacetate extract protected sickle erythrocytes better than normal erythrocytes.

In Fig.3a, it was observed that ethylacetate and acetone extracts protected normal erythrocyte better at low concentration in biphasic manner at 1.0mg/ml. All the three extractswereobserved to protect sickle erythrocytes

effectively at lower concentrations (Fig.3b).The extracts protected both normal and sickled erythrocytes against heat and induced lyses and compared favourably with Ibuprofen at 1 mg/ml (Fig.6a).

The membrane stabilizing profiles of normal and sickled erythrocytes at 1.5mg/mlwere as shown in Fig. 4a and b. Acetone extractwas observed to exhibits highest mode of protection on normal erythrocytes (Fig.4a) and ethyl acetate extract on sickle cell erythrocytes (Fig.4b). Chloroform extractwas observed not to protect normal erythrocyte at 1.5 mg/ml (Fig.4a)

At 2.0 mg/ml, only chloroform extract stabilizes normal erythrocytes (Fig.5a) while acetone and chloroform extracts protected sickle erythrocytes (Fig.5b). The extracts that protected at 2 mg/ml compared favourable with a standard anti-inflammatory drug ibuprofen (Fig. 6). It could be surmised that ethyl acetate extract the was toxic to erythrocytes membrane at higher concentration.

The membrane stabilizing profile (Figs. 1 - 6)revealed that acetone and ethyl acetate extracts of *A. ohioense* protected red blood cell membrane at various concentration except at 2.0mg/ml. However, the chloroform extract of the plant exhibits a profound protective effect on both normal and sickle l erythrocytes only at higher concentration.

Lipoxygenase and xanthine oxidase are key enzymes implicated in many mediated inflammation diseases such as cancer, arthrosclerosis, hypertension and diabetes [29]. Their inhibition could be a way for finding new anti-inflammatory agent/ compound.Table 2 shows the summary of lipoxygenase and xanthine oxidase inhibitory activities of chloroform, acetone and ethylacetate of *A. ohioense*. All the extracts showed low inhibitory activities compared to Quercetin.

Furthermore, *A ohioense* chloroform and acetone extracts exhibitedappreciable anti-sickling activities. There is linear increase in the inhibitory activity of the two extracts with higher inhibitory activity in chloroform extract having 97.75% while acetone extract has inhibitory potential of 93.51% (Fig.7). This compared favorably with positive control Para-hydroxybenzoic acidwith percentage inhibitory activity of 81.50. Also, as shown in Fig.8, *A. ohioense* chloroform and acetone extractsdemonstrated significant (P< 0.001) increased in antisickling reversal activity as the concentrations of the extracts increases.

Some medicinal plants such as *Piper guineensis*, *Pterocarpus osun*, *Eugenia caryophyllala* and *Sorghum bicolor* extracts had been reportedin the treatment of sickle cell disorder[30]. Also, the extracts of *Pterocarpus* and *Aloe vera* were also reported to increase the gelling time of sickle cell blood and inhibits sickling *in vitro*[31].This study indicates the potential of A. *ohioense* extract in the management of sickle cell disorder.

V. Conclusion

The result of this study suggested that A. *ohioense* possess anti-inflammatory and antisickling activities.Therefore*A. ohioense* could be regarded as a potential anti-inflammatory plant that can play essential role in the management and treatment of sickle cell disorder and offer stability to the membrane of already reversed Hbs molecules. However, the need to carefully investigate the plant toxicity, isolate compound(s) responsible for the observed anti-inflammatory effect and develop suitable formulation beneficial for anti-inflammatory related disorder is thereby suggested.

References

[1]. Steinberg, M.H. (2008) Sickle cell anemia, the first molecular disease: Overview of molecular etiology, patho- physiology, and therapeutic approaches. The Scientific World Journal, 8, 1295-1324.
[2]. Martins, D.W. (1981). Porphyrins and bile pigments. Harper's Review of Biochemistry, 212-214.
[3]. Karayakin, H.N. (1979). Cholelithiasis in children with sickle celldisease. American Journal Dis Child 133:306-307.
[4]. Steinberg, M.H. (2005) Predicting clinical severity in sickle cell anaemia. British Journal of Haematology129: 465-481.
[5]. Diabetes Research 2015. Complications and TreatmentsSickle cell disease. Http://www.cailsilorin.com.
[6]. Chikezie, P.C. (2006). Studies on the anti-sickling effects of *Azadirachta indica* and Vernonia *amygdalina* on HbSS erythrocytes. Inter. J. Nat. Appl. Sci.2:24–8.
[7]. Chikezie, P.C. (2007). Effect of ethanolic extracts of *Garcina kola, Cola nitida* and *Cola acuminata* on *in vitro* haemoglobin S polymerization.Inter. J. Trop. Agric. Food Sys.;1:220–5.
[8]. Ekeke, G.I. and Shode, F.O. (1985). The reversion of sickled cells by *Cajanus cajan*. Planta Medica 6:504–7.
[9]. Okpuzor, J., Adebesin, O., Ogbunugafor, H. and Amadi, I. (2008). The potential of medicinal plants in sickle cell disease control: A review. Inter. J. Biomed. Health Sci.4:47–55.
[10]. Flora of North America (2008). Missouri Botanical Garden 27:315-318
[11]. Akinpelu, B.A., Makinde, A.M., Isa, M.O., Taiwo, O.P., Ojelabi, O.M. and Oyedapo, O.O. (2015). *In vitro* Evaluation of Membrane Stabilizing Potential of Selected Bryophyte Species. European Journal of Medicinal Plants6 (3): 181-190.
[12]. Evans, W.C. (2002). Phenols and Phenolic glycosides. In Trease andEvans Pharmacognosy, Elsevier Science Limited, pp. 214-252.
[13]. Sofowora, A. (2006). Phytochemical screening: In "Medicinal plants and TraditionalMedicine in Africa", Second Edition, Spectrum BooksLimited Ibadan, Nig., pp. 150-153.
[14]. Oyedapo, O.O., Akinpelu, B.A., Akinwunmi, K.F., Adeyinka, M.O., Sipeolu, F.O. 2010Red blood cell membrane stabilizing potentials ofextract of *Lantana camara* and itsfractions. Int. J. Plant Phy. and Biochem.2(4);46-51.
[15]. Oyedapo, O.O., Akinpelu, B.A., Orefuwa, S.O. (2004). Anti-inflammatoryeffects of *Theobroma cacao*, L. root extract. J.Tropical med. Plants(Malaysia) 592): 161-166.
[16]. Malterud, K.E and Rydland, K.M. (2000). Inhibitors of 15 -lipoxygenase from orange peel. Journal of Agriculture and Food Chemistry 48, 5576 – 5580.

[17]. Ferraz Filha, Z.S., Vitolo, I.F., Fietto, L.G.,Lombardi, J.A. and Saude-Guimares, D.A. (2006). Santhine oxidase Inhibitory activity ofLychnophora species from Brazil("Arnica). Journal of Ethnopharmacology 107, 79 – 82.

[18]. Egunyomi, A.J., Moody, O. and Eletu,O. (2009). Antisickling activities of two ethnomedicinal plant recipes used for the management of sickle cell anaemia Ibadan, Nigeria, African Journal of Biotechnology Vol. 8 (1), pp. 020-025.

[19]. Sofowora, E.A., Isaac-Sodeye, W.A., Ogunkoya, L.O. (1979). Isolation and Characterization of an antisickling agent from the root of *Fagaraxanthoxyloides*. African Medicinal Plants. Proceedings of a Conference Ed. Sofowora EA, pp. 89-97.

[20]. Lamy, E., Graça, G., da Costa, G., Franco, C.E., Silva, F.C., Baptista, E.S. and Coelho, A.V. (2010). Changes in mouse whole saliva soluble proteome induced by tannin-enriched diet. Proteome Science 8(65): 1-12.

[21]. Kensil, C.R. (1996). Saponins as vaccine adjuvants. Critical Reviews in Therapeutic DrugCarrier Systems 13: 1–55.

[22]. Barr, I.G., Sjolander, A. and Cox, J.C. (1998). ISCOMs and other saponin based adjuvants (Review). *Advanced Drug Delivery Reviews* 32: 247-271.

[23]. Lalitha, T. P. and Jayanthi, P. (2012). Asian journal of. Plant Science Res., 2(2), 115-122**.**

[24]. Xie, Z. and Askari, A. (2002). Na+/K+-ATPase as a signal tranducer. European Journal ofBiochemistry 269 (10): 2434-2439.

[25]. Sadique J., Al-Rqobahi W.A., Bughaith M. F. and El-Gindy A. R. (1989). The bio-activity of certain medicinal plants on the stabilization of RBC membrane system. Fitoterapia 60 (6), 525-532.

[26]. Onah J. O, Akubue P. I, and Okide J. F. (2002). The kinetics of reversal of pre-sickled erythrocytes by the aqueous extract of *Cajanus cajan*; Phytother. Res. 16(8): 748-750

[27]. Elekwa I, Monanu M. O and Anosike E. O (2003) Studies on the effect of aqueous extracts of *Garcinia kola* seed on the human erythrocytes adenosine triphosphatase of HbAA, HbAS, and HbSS genotypes. Global J. Med Sci. 2(2). 107-114.

[28]. Okpuzor J, Adebesin O. Membrane stabilizing effect and antisickling activity of S*enna podocarpa* and *Senna alata*. 31st congress for European biochemical societies, Instanbul, Turkey, July 25-29, 2006.

[29]. Osher, E., Weisinger, G., Limor, R., Tordjman,K. and Stern, N. (2006). The 5-lipoxygenase system in the vasculature: Emerging rolein health and disease. Molecular and Cellular endocrinology 252 :201-206. Molecular and Cellular Endocrinology ·

[30]. Wambebe C, Khamof,. H and Momoh J. A. (2001). Double-blind, placebo-controlled, randomised cross-over clinical trial of NIPRISAN in patients with sickle cell disorder Phytomedicine8(4):252-61.

[31]. Ugbor, C. (2001). The effect of vegetable extracts on the antisickling potential of *Aloe vera*. 2006 www.biochemistry.org/meetings/abstracts/BS2006/BS20060567.pdf.