ANTISICKLING PROPERTIES OF SOME PLANTS USED FOR THE MANAGEMENT OF ANAEMIA IN KATSINA STATE, NIGERIA

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Abstract: Medicinal plants have been a panacea for the management of various ailments including anaemia. In this study, phytochemical screening, X-RF analysis and *in vitro* antisickling properties of four ethnobotanicals; *Amaranthus hybridus, Manihot esculenta, Moringa oleifera* and *Jatropha tanjorensis* mostly used for the management of Anaemia in Katsina State were studied. The *in vitro* antisickling properties was studied by inducing sickling using 2% $Na_2S_2O_5$ followed by treatment with plants' extracts at 250 µg/mL, 500 µg/mL and 1000 µg/mL concentrations, while P-hydroxybenzoic acid and normal saline served as positive and negative controls respectively. The four investigated plants species showed the presence of Zinc and magnesium in various quantities. Aqueous extracts of *Manihot esculenta* and *Amaranthus hybridus* have highest percentage of Zinc and Magnesium (0.0095% and 1.23%, respectively). Statistically significant difference ($p < 0.05$) following treatment of the sickling induced erythrocytes with studied plants' extracts at different concentrations. Highest antisickling (95%) was observed in aqueous extract of *M. esculenta* at 1000 µg/mL while least antisickling (85.67%) was observed in ethanolic extract of *M. esculenta* at 250 µg/mL. This study provides baseline data for further researches on combating sickle cell anaemia and also revealed that *M. esculenta* exhibits antisickling properties more than the popular *M. oleifera.* Further studies aimed at evaluating the ethnobotanicals *in vivo* and formulating nutraceuticals from *M. esculenta* leaves is strongly recommended.

Keywords: Anaemia, antisickling, plants, Katsina State, X-Ray fluorescence

Introduction

Sickle cell disease is a hereditary genetic disorder with recessive autosomal transmission, characterized by alteration of normal haemoglobin, a protein that transports oxygen throughout the body tissue. It is as a result of a single mutation of the gene beta-globin located in chromosome 11 leading to the substitution in position 6 of the beta-globin of the glutamic acid, hydrophilic present in haemoglobin A (Normal) by hydrophobic valine resulting in haemoglobin with sickle shape (Yenon *et al.*, 2016). The rod like HbS polymers which distorts the red cell shape into the characteristic sickled appearance quensequently impeding flow through the microvasculature, leading to ischemia, pain and death, a feature of sickle cell crisis (Puppalwar *et al.*, 2015).

The mutation reduces the tendency of haemoglobin to transport oxygen to body tissues as well as its solubility in it deoxygenated form. Thus, when the partial pressure in oxygen decreases, haemoglobin S becomes less soluble. It polymerizes with other haemoglobin S molecules and crystallizes in the red blood cell and form a sickle shape (Mpiana *et al.*, 2008).

It is characterized by red blood cells that assume an abnormal, rigid, sickle shape and known to be of the diseases afflicting the population living in Africa, South America and Asia. It also occurs in other ethnic groups, including people who are Mediterranean and Middle Eastern descent (Nanfack *et al.*, 2013). As a genetic disease, no specific drugs are yet available however, several treatments have been investigated; Medullar transplantation, is not only expensive, but also faces incompatibility problems (Nanfack *et al.*, 2013).

Various proposed conventional drugs proposed drugs for its management (Hydroxyurea, Piracetam, Calcium antagonists) tested against this disease for inhibition of haemoglobin polymerization in order to increase the fetal haemoglobin rate (HbF) or reduce the sickling are toxic especially for long term usage (Mpiana *et al.*, 2008; Ibrahim *et al.*, 2010).

World health organization (WHO) estimated that more than 80% of the populace rely more often on traditional medicines, mainly plants serving as the main source of health care (Jamsid-ia *et al.*,2018). Medicinal plants have undoubtably been considered by human beings since time immemorial. It can be said that therefore, before the history and since the early humans recognized and exploited plants for fuel, clothing, shelter and food, they became aware of their properties more or less (Jamsid-ia *et al.*, 2018).

It is generally agreed that medicinal plants their products are relatively safer than their synthetic equivalent drugs medicinal plants have an intrinsic constituent which mimic closely the natural constituents of human somatic system (Igbinadwa *et al.*, 2011). In any case, the strong belief that medicinal plants are safety ought not to be utilized blindly. Furthermore, toxicological and pharmacological evaluation of these plants is very crucial on the ground that apparently harmless plants may end up being harmful (Gamaniel, 2000).

In view of all the genetic disorders associated with sickle cell disease and it enervating and the toxic effects associated with the orthodox drugs, threat of extinction faced by medicinal plants, prohibitively high cost of managing the disease, and larger quantities of O^{2-}, H_2O_2 and OH^- produced from sickled red blood cells than do normal red blood cells (Aslan *et al.*, 2000), hence the need for evaluation of some medicinal plants which have been suspected to possess antisickling properties in the study area. This work aimed at evaluating the antisickling potential of some plant species including, *Amaranthus hybridus, Moringa oleifera, Jatropha tanjorensis and Manihot esculenta* which have been cited to used for the management of anaemia in Katsina State.

Katsina, one of the poorest states in north western Nigeria, is among the most populous states in the country with the population of 5,801,584 (NPC, 2006). The existing number of hospitals in Katsina is not sufficient to cater for the secondary health requirements of the populace. Lack of accessibility of good health care by the rural majority, centralization of medical programs in urban centers, and the neglegence of rural areas in the allocation of socio-economic infrastructure have continued to underscore the magnitude of health-related problems facing the people of the state (State News Letter, 2005).

Few medicinal plants used for the management of sickle cell disease have been reported in literature due to the confidentiality associated with treatment of the disease (Egunyomi *et al.*, 2009). The leaves of *Telfairia occidentalis* have been claimed to be helpful to pregnant women (due to its high iron content) and sickle cell patients. Aqueous leaves extract of *Taminalia catapa* was shown to exhibit antisickling activity when tested against sodium metabisulphite induced sickling (Moody *et al.*, 2013). Adesanya *et al.* (1998), demonstrated the antisickling properties of *Adansonia digitata*. The plant's bark had been shown to reversed sickling though with little activity. In south western Nigeria, unripe fruit of *Carica papaya* is traditionally used for the treatment of sickle cell anaemia (Thomas and Ajani, 1987). The 48h evaluation of aqueous extracts of *Carica papaya* showed inhibition and reversing of HbSS erythrocytes. However, the 24h aqueous extract was not active. The antisickling effect observed was attributed to the action of organic acid produced by esters during fermentation of the fruit for 48h (Thomas and Ajani, 1987). The antisickling property of Lowsonia inermis was also reported. *Lowsonia inermis* which is used ethnomedicinally for the treatment of yellow fever, pains, jaundice, skin diseases and conditioning of hair was found to inhibit sickling of red blood cells and increase the oxygen affinity of HbSS blood (Chan and Suzuka, 1982).

Materials and method

This study was conducted in Katsina state, northern Nigeria. Katsina state, which covers an area of 23,938 sq. km, is located between latitudes 11 08' N and longitudes 6 52' E and 9 20' E. The state is bonded by Niger Republic to the north, Jigawa and Kano states to the east, Kaduna state to the south and Zamfara state to the west. The state has 34 Local Government Areas.

Ethical Clearance

Ethical clearance with reference number MOH/ADM/SUB/1152/1/332 was obtained from Katsina State Health Research Ethical Committee prior to the commencement of this study.

Informed consent form was issued to every respondent and was duly singed.

The aim of the study was revealed to the respondents.

Volunteers were told the participating in the study is voluntary and one could decline once the desire to do so arises.

Collection of Plant Material

Fresh samples of *Amaranthus hybridus* were obtained from Katsina Central market, *Manihot esculenta* and *Moringa oleifera* were obtained from Dabaibayawa of Bataagarawa local government, and *Jatropha tanjorensis* from Suleja of Niger state. The plants names were authenticated in the herbarium, department of Biology, Umaru Musa Yar' adua University Katsina and voucher numbers were given as UMYU222, UMYU240, UMYU253 and UMYU239 respectively.

Preparation of Plant Materials for Extraction

The leaves of the four plants species selected for the study were processed for extraction as described by Yenon *et al.* (2016). The leaves were plucked, washed with clean water thrice and dried under shade at room temperature for two weeks. The leaves were the pulverized using wooden mortar and pestle in order to obtain fine particles for easy extraction. The various samples were stored in air-tied container for later use.

Extraction

Each of the dried powdered leaves (100g) was macerated with 70 % 1 L of ethanol for 72 hrs. The same procedure above was repeated using 1 L of distilled water as a solvent. The mixture was filtered and concentrated to dryness. The extracts were stored at 4^0 C in freezer for later use (Nanafack *et al.*, 2013).

X-Ray Fluorescence

The resulting dried extracts of the four plant species investigated were sampled (2g each) after been pulverized using mortar and pestle. The samples were analyzed using X-Ray Fluorescence in accordance with the manufacturer' s instructions

Phytochemical investigation of the investigated plants species

Tests for alkaloids, phenolic compounds and saponin were conducted using standard procedures as described by (Sandeep *et al.*, 2014; Mohammed *et al.*, 2014).

Reagents

All the reagents used are of analytical grades including, ethanol, 2 % sodium metabisulphite ($Na_2O_5S_2$) para-hydroxybenzoic acid, dimethylsulfuoxide (DMSO), chloroform, distilled water and normal saline.

Bioassay

Washing of Red blood Cells:

Half millilitres EDTA blood samples obtained from volunteers were centrifuged at 3000 rpm for 10 minutes ssto remove the plasma. The resulting packed erythrocytes were washed three times with 1 mL sterile normal saline per 0.5 mL of blood.

The samples were then centrifuged each time to remove the supernatant. Washed red blood cells were the resuspended in the remaining suspension and used for the analysis (Nessrin and Ibrahim, 2016).

Test for antisickling activity

A method described by Oduola *et al.*, (2006) was adopted. A stock extract solution (10mg/ml) was prepared by dissolving 0.1 g of dry extracts each for aqueous and ethanolic extract of *A. hybridus, J. tanjorensis* and *M. esculenta, M. oleifera* in 1 mL of 100% dimethylsulfuoxide (DMSO) that was prior diluted with 10 mL of normal saline. Three concentrations were prepared as follows: 250 µg/ml, 500 µg/mL and 1000 µg/mL. 0.5 mL of washed red blood cells was mixed with an equivalent volume of 2 % sodium metabisulfite ($Na_2O_5S_2$). 10 µL from the above mixture was spotted on a grease free microscopic slide, and then 10 µL from the plant extract was added and mixed with the blood mixture. 10 µL normal saline was added to one of the slides instead of the plant extract which served as a negative control. A positive control was prepared by dissolving 0.5 g in 1 mL. All slides were covered with a cover slip gently. For each slide, a clean piece of cotton wool was placed at the edge of the slide after tilting it for the

excess mixture to be absorbed. The excess mixture was removed using cotton wool and the edges of the cover slip sealed with Vaseline to prevent air from coming in. The Slides were then incubated at 37°C for about two periods (30 and 60 minutes) (Oduola *et al.*, 2006). Percentage of sickling was calculated using the formula: Percentage sickling = No of sickling cells × 100/total cells (Nanfanck *et al.*, 2013).

Data analysis

The data obtained from the bioassay for the antisickling properties and the percentages of Zinc and Magnesium of the four investigated plants species were subjected to analysis of variance (ANOVA) using SPSS version 20.

Results

Table 1 shows the phytochemical screening of aqueous and ethanolic leaves extracts of the four investigated plant species revealed various bioactive principles. Aqueous and ethanolic extracts of *A. hybridus* showed the presence of alkaloid, saponin, tannin, phenolic compounds. Similarly, aqueous and ethanolic extracts of *M. oleifera* revealed the presence of alkaloid, saponin, phytosterol, tannin, phenolic compounds, flavonoid, terpenoid, and anthocyanin, except ethanolic extract of the *M. oleifera* which didn' t show the presence of saponin. Furthermore, alkaloid, saponin, phytosterol, tannin, phenolic compounds, flavonoid, terpenoid, and anthocyanin were also revealed in aqueous and ethanolic extracts of *J. tanjorensis*. However, saponin, phytosterols and terpenoids were absent in aqueous and ethanolic extracts of *M esculenta*. Moreover, cardiac glycoside was absent in all the four plant species investigated in the present study.

Table 1: Phytochemicals of the investigated plant species

Plants extracts	Alkaloids	Saponin	Phytosterols	Tannin	Phenolic compounds	Flavonoids	Terpenoid	Cardiac glycosides	Anthocyanin
Aq. Amar	+	+	+	+	+	+	+	-	-
Et. Amar	+	+	+	+	+	+	+	-	-
Aq. Mor	+	+	+	+	+	+	+	-	+
Et. Mor	+	-	+	+	+	+	+	-	+
Aq. Jat	+	+	+	+	+	+	+	-	+

Et. Jat	+	+	+	+	+	+	+	+	+
Aq. Man	+	-	-	+	+	+	-	+	+
Et. Man	+	-	-	+	+	+	-	-	+

Aq. Amar = Aqueous Amaranthus hybridus, Et. Amar = Ethanol Amaranthus hybridus, Aq. Mor = Aqueous Moringa oleifera, Aq. Jat = Aqueous Jatropha tanjorensis, Et. Jat = Ethanol Jatropha tanjorensis, Aq. Man = Aqueous Manihot esculenta, Et. Man = Ethanol Manihot esculenta
"+"=Present,"-"=Absent.

Magnesium content of the four investigated plants species

Table 2 shows the result obtained from X-Ray fluorescence of the plants investigated. The plant extracts showed the presence of Magnesium in various quantities. Aqueous extract of *A. hybridus* had the highest magnesium content (1.215%), followed by aqueous extract of *J. tanjorensis* (1.023%). However, least magnesium content was observed in ethanolic extract of *M. esculenta* (0.536%).

Table 2: Magnesium content of the investigated plants species

Plant species	Aqueous extracts (%)	Ethanolic extracts (%)
Moringa oleifera	0.740 ± 0.000576^{a}	0.387 ± 0.000576^{b}
Amaranthus hybridus	1.215 ± 0.000576^{c}	0.453 ± 0.000576^{d}
Jatropha tanjorensis	1.023 ± 0.00576^{e}	0.384 ± 0.0010^{f}
Manihot esculenta	1.177 ± 0.000576^{g}	0.536 ± 0.4000^{h}

Values with different superscripts are statistically different ($p\geq0.05$), (n=3)

Zinc content of the four investigated plants species

The zinc content of the plants investigated was determined using X-Ray fluorescence and the various quantities are tabulated below (Table 3). Highest zinc content was observed in aqueous extract of *M. esculenta* (0.0095%) followed by aqueous extract of *M. oleifera* (0.0043%). The least zinc content was however observed in ethanolic extract of *A. hybridus* (0.0023%).

Table 3: Zinc content of the investigated plant species

Plant species	Aqueous extract (%)	Ethanolic extract (%)
Moringa oleifera	0.0043 ± 0.000577^{a}	0.0043 ± 0.000577^{a}
Amaranthus hybridus	0.0027 ± 0.000577^{b}	0.0023 ± 0.000577^{b}
Jatropha tanjorensis	00.027 ± 0.000577^{b}	0.00403 ± 0.000577^{a}

Manihot esculenta	0.0095±0.00867^{c}	0.0064±0.00567^{c}

Values with different superscripts are statistically different (p≥0.05), (n=3)

Antisickling property of the four investigated plant species

The antisickling property the aqueous and ethanolic extracts four investigated plant species, *M. oleifera, A. hybridus, J. tanjorensis, and M. esculenta* are presented in table 4 bellow. The results of the antickiling activity of the test extracts and the positive control (Para-hydroxybenzoic acid) at different concentrations (250 µg/mL, 500 µg/mL, and 1000 µg/mL) are expressed as mean percentage (%) of unsickle cell ± standard deviation. P values ≤ 0.05 were considered to be significant. Analysis of variance (ANOVA) revealed statistically significant different in antisickling property of aqueous and ethanolic extract of the four investigated plants species and the positive control (Para-hydroxybenzoic acid) at various concentrations, 250 µg/mL, 500 µg/mL, and 1000 µg/mL (P<0.05). There was statistically significant different between the samples treated with aqueous extract of *M. oleifera*, ethanolic extracts of *M. oleifera, A. hybridus, M. esculenta* and the positive control (P<0.05). However, there was no statistically significant different between the samples treated with aqueous extracts of *A. hybridus, J. tanjorensis, M. esculenta*, ethanolic extracts of *Jatropha tanjorensis* and the positive control at 250 µg/mL concentration (P>0.05).

At the concentration of 500 µg/mL, there was no statistically significant different between the samples treated with aqueous extract *A. hybridus, J. tanjorensis, M. esculenta,* ethanolic extracts of *M. esculenta* and the positive control (P>0.05). However, there was statistically significant different between the samples treated with aqueous extract of *M. oleifera*, ethanolic extracts of *Moringa oleifera, Amaranthus hybridus, Manihot esculenta* and the positive control at 500 µg/ mL concentration (P<0.05).

At the concentration of 1000 µg/mL, there was no statistically significant different between the samples treated with aqueous extract *Amaranthus hybridus, Manihot esculenta* and the control (P>0.05). However, there was statistically significant different between the samples treated with aqueous extract of *M. oleifera, J. tanjorensis*, ethanolic extracts of *J. tanjorensis, M. esculenta* and the control (P<0.05).

Table 4: Antisickling activity (%) of aqueous and ethanolic extracts of M. oleifera, A. hybridus, J. tanjorensis and M. esculenta on 2% sodium metabisulphite induced sickled red blood cells

	Concentration		
Treatments	**250 µg/mL**	**500 µg/mL**	**1000 µg/mL**
Aq. Mor + ISRBCs	88.33 ± 1.53^{bc}	87.67 ± 0.29^{c}	90.33 ± 1.26^{c}
Et. Mor + ISRBCs	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}	0.00 ± 0.00^{d}
Aq. Ama + ISRBCs	91.67 ± 2.08^{ab}	91.03 ± 0.06^{abc}	93.33 ± 1.53^{ab}
Et. Ama + ISRBCs	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}
Aq. Jat + ISRBCs	91.33 ± 1.53^{ab}	91.87 ± 1.25^{ab}	92.33 ± 1.53^{bc}
Et Jat + ISRBCs	90.00 ± 2.00^{ab}	92.02 ± 1.29^{ab}	91.67 ± 0.58^{bc}
Aq. Man + ISRBCs	90.00 ± 2.00^{ab}	91.00 ± 4.58^{abc}	**93.67 ± 2.31^{ab}
Et Man + ISRBCs	*85.67 ± 1.53^{c}	90.00 ± 2.00^{bc}	89.83 ± 0.76^{c}
C^{+} + ISRBCs	93.33 ± 1.15^{a}	94.67 ± 0.56^{a}	95.67 ± 0.56^{a}
C^{-} + ISRBCs	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}	0.00 ± 0.00 ^{d}

Values with the same superscripts (a-c) in the same column are significantly the same at $P < 0.05$ (n=3)
Note: " – " represent complete lysis of red blood cells.
ISRBCs = Induced sickled Red Blood Cells.
Aq. Mor = Aqueous *Moringa oleifera*
Aq. Ama = Aqueous *Amaranthus hybridus*
Aq. Jat = Aqueous *Jatropha tanjorensis*
Et. Jat = Ethanol *Jatropha tanjorensis*

Aq. Man = Aqueous *Manihot esculenta*
Et. Man = Ethanol *Manihot esculenta,* C^+ = Positive control, C^- = Negative control * = lowest activity, **= highest activity.

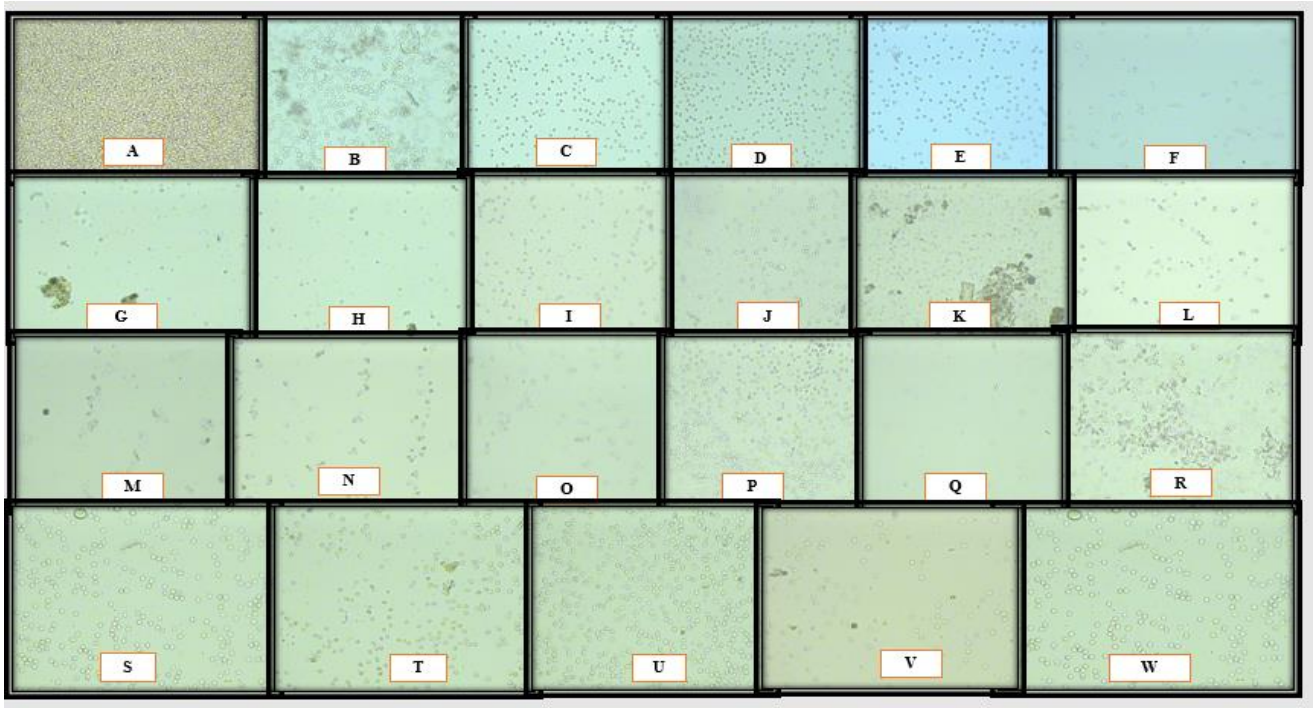

Figure 1: Photomicrographs showing the effects of the various plant extracts on sodium metabisulphite RBCs induced sickling: A-=Negative control, B=Positive control, C=aqueous A. hybridus at 250 µ g/mL, D= aqueous A. hybridus at 500 µ g/mL ,E= aqueous A. hybridus at 1000 µ g/mL, F= ethanol A. hybridus µ g/mL , G= ethanol A. hybridus at 500 µ g/mL , H= ethanol A. hybridus at 1000 µ g/mL , I= aqueous M. oleifera at 250 µ g/mL , J= aqueous M. oleifera at 500 µ g/mL , K= aqueous M. oleifera at 1000 µ g/mL , L= ethanol M. oleifera at 250 µ g/mL , M= ethanol M. oleifera at 500 µ g/mL , N= ethanol M. oleifera at 1000 µ g/mL , O= aqueous M. esculenta at 250 µ g/mL , P= aqueous M. esculenta at 500 µ g/mL , Q= aqueous M. esculenta at 1000 µ g/mL , R= ethanol M. esculenta at 250 µ g/mL , S= ethanol M. esculenta at 500 µ g/mL , T= ethanol M. esculenta at 1000 µ g/mL , U= aqueous Jatropha tanjorensis at 250 µ g/mL , U= aqueous J. tanjorensis at 500 µ g/mL , W= aqueous J. tanjorensis at 1000 µ g/m

Discussion

The phytochemical screening of aqueous and ethanolic extracts *Manihot esculenta* revealed various secondary metabolites including alkaloid, tannin, phenolic compounds, flavonoid, and anthocyanin .This result is partly in conformity with the findings of Kay and Phyu (2018), who demonstrated that the qualitative phytochemical analysis of ethanolic extract of *Manihot esculenta* revealed the presence of flavonoids and phenolic compounds and in addition, steroids, terpenoids, saponins and glycosides were also present which contradicts the result of the findings. Similarly, Mustarichie *et al.* (2019) reported the presence of flavonoids and polyphenols in ethanolic and aqueous extracts of *M. esculenta* which also concurs with our findings, however, saponin was also detected which contradicts the result of our findings. The presence of flavonoids and phenols was also reported by Quartey *et al.* (2016). This result also agrees with our findings. The various differences observed in the phytochemical contents might be due to differences in geographical regions, season of harvesting and soil nutrients.

The result of the phytochemical screening of the aqueous and ethanolic extract of *M. oleifera* revealed the presence of alkaloids, saponin, phytosterols, tannins, phenolic compounds, flavonoids terpenoids, and anthocyanin. The phytochemicals detected in this plant extracts have been reported to be the major bioactive principles of the plant and responsible for various physiological activities in human body (Abdulkadir *et al.*, 2015). The result obtained in this finding is in agreement with the work of Abdulkadir *et al.* (2015) who also found out that ethanolic extract of *M.*

oleifera contains alkaloids, flavonoids and saponins. Aliyu *et al.* (2008) and Dahiru *et al.* (2006) however, reported contrary result demonstrating lack of alkaloids and tannins in the ethanolic extract of *M. oleifera* leaves. This could be due to differences in the developmental stage of plant used for the studies. In another similar work, the phytochemical screening of aqueous *M. oleifera* leaves showed the presence of alkaloids, flavonoids, saponins, steroids, tannins and glycosides Amabye and Tadesse (2016) this agrees with our findings.

Preliminary phytochemical test on the powdered leaf of *J. tanjorensis* indicated the presence of alkaloids, saponin, phytosterols, phenolic compounds, flavonoids, terpenoids, and anthocyanin. This result agrees in part with the findings of Igbinaduwa *et al.* (2011) found out and reported the biogenic compounds contained in the leaves of *Jatropha tanjorensis* to be alkaloids, saponins tannins, Flavonoids. However Cardiac glycoside was also detected in the study does not correspond to our findings. Furthermore, Ebe *et al.* (2019) showed that *J. tanjorensis* possess the phytochemicals; Flavonoids, alkaloids, tannins, saponins and cardiac glycosides which wasn't detected in the present study.

The qualitative phytochemical screening of *A. hybridus* revealed important biogenic compounds including, alkaloids, saponin, Phytosterols, flavonoids, tannins, phenolic compounds. However, cardiac glycoside and athocyanin were not detected. Francis (1999) reported that anthocyanin is replaced by betalain in Amaranth. Betalains are a class of indole derived pigments found in plants of the order caryophyllales where they replace anthocyanin pigments. Strack *et al.* (2003).

The mean percentage of the unsickle red blood cells ranges from 88.33% ± 1.53 (aqueous extract of *Moringa oleifera*) to 90.00% ± 2.00 (Ethanol extract of *Jatropha tanjorensis* at 250 µg/mL respectively. At 500 µg/mL, the mean percentage of unsickle red blood cells ranges from 87.67% ± 0.29 aqueous extract of *M. oleifera* to 92.02% ± 1.29 (Ethanol extract of *J. tanjorensis*). At 1000 µg/mL, the mean values range from 89.83% ± 0.76 (aqueous extract of *M. oleifera*). The mean percentage of red blood cells treated with P-hydroxy benzoic acid (positive control) are 93.33% ± 1.15, 94.67% ± 0.56, 95.67% ± 0.56 at 250 µg/mL, 500 µg/mL, 1000 µg/mL respectively.

The contribution of secondary metabolite to the antisickling action of medicinal plants used for the management of sickle cell disease can't be overemphasize. Many studies showed that these plants have the tendency to prevent sickling due to the possession of various groups of secondary metabolites (Olufumilayo *et al.*, 2012). In this study, qualitative phytochemical screening of the four investigated plants species revealed various phytochemicals; flavonoids, including anthocyanin, alkaloids, saponin, phytosterols, phenolic compounds, flavonoids, terpenoids. The antisickling properties observed in the four investigated plant species could be due to presence of secondary metabolites such as flavonoids (anthocyanin), alkaloids, phenolic compounds, phytosterols and tannins. The result of this work concurs with the findings of Mpiana *et al.* (2009), who demonstrated that a class of flavonoid, anthocyanin reduces the gelation (Polymerization) of sickled haemoglobin (HbS) and stabilizes the membrane of erythrocytes. Similarly, Nanafack *et al.* (2013) also demonstrated the antisickling activity of aqueous *Zanthoxyllum heitzii* extract and attributed it to the ability of the various bioactive principles like phenol, flavonoid and saponin to inhibit polymerization of haemoglobin *in vitro*. Moreover, Zinc supplementation has been reported to drastically reduce verso-occlusions in patient with sickle cell anaemia (Ballas, 2007). Fung *et al.* (2002) also found out that there was an improvement in growth of children with sickle cell anaemia after being supplemented with Zinc. The plants species investigated showed antisickling activity in time and dose dependent manner. The first period of incubation showed no change in the morphology of the erythrocytes, but subsequent incubation for further 30 minutes showed morphological changes (figure 12 A) in both treatment and control tests. However, there was complete lysis of erythrocytes in the samples treated with ethanolic extracts of *M. oleifera* and *A. hybridus* at all the working concentrations (250 µg/mL, 500 µg/mL, and 1000 µg/mL) over 60 minutes period of incubation (figure 12 F, G, H, L, M, and N). This may be that the ethanolic extracts of *M. oleifera* and *A. hybridus* at 250 µg/mL, 500 µg/mL, and 1000 µg/mL had cytotoxic effects on the erythrocytes. This is similar to the finding of Samuel *et al.* (2016) who also reported similar findings where complete lysis of erythrocytes was observed when sickling induced red blood cells sample were treated with 10 mg/mL methanolic extract of *Telfairia occidentalis* seeds and incubated for the period of 120 minutes. In the sample treated with only 2% sodium metabisulphite (negative control), there was 100% change in the morphology of erythrocytes (disfigured) from normal biconcave to sickle or C-shape (figure 12A). The samples treated with aqueous and ethanolic extracts of the four investigated plants species at different concentrations showed antisickling activity of different percentages. At 250 µg/mL, highest antisickling activity was observed in sample treated with aqueous extract of *A. hybridus* (91.67% ± 2.08) followed by aqueous extracts of *Jatropha tanjorensis* (91.33% ± 1.53) and that of *M. esculenta* (90.0%0 ± 2.00).

At the concentration of 500 µg/mL, highest antisickling activity was observed in sample treated with ethanol extract of *J. tanjorensis* (92.02% ± 1.29), followed by aqueous extracts of *J. tanjorensis* (91.87% ±1.25) although, at 0.05 confidence interval, there is no significant difference between the percentage mean of unsickle erythrocytes treated with aqueous *A. hybridus* (91.03 ± 0.06), aqueous extracts of *J. tanjorensis* (91.87% ±1.25), and aqueous extracts of *M. esculenta* (91.00%± 4.58 which compared favorably with the positive control (94.67% ± 0.5).

At the concentration of 1000 µg/mL, highest antisickling activity was observed in sample treated with aqueous extract of *A. hybridus* (93.33% ± 1.53) followed by aqueous extract of *J. tanjorensis* (92.33% ± 1.5). Sample treated with aqueous *A. hybridus* (93.33% ± 1.53), aqueous *M. esculenta* (93.67% ± 2.31) and sample treated with control (95.67%± 0.56) are statistically the same at 0.05 confidence interval. The antisickling activities of the four plants extracts studied could be due the presence of flavonoids and magnesium. In a similar research, De Franceschi *et al.* (1997) reported that magnesium supplements when administered to ten patients with sickle cell disease, there was reduction in dense sickle erythrocytes, and K-Cl cotransporter. However, there was an increase in sickle erythrocyte magnesium and potassium content. Sickle cell dehydration is majorly determined by K^+-Cl^-cotransporter. Potassium-chloride cotransporter, a volume reduction mediator in normal reticulocytes is pathologically increased in HbS Red blood cells (Clinton and Franco, 2001). Potassium-chloride cotransporter, K^+-Cl^- is an electroneutral-coupled transport of K^+ and Cl^- across membranes and are believed to be crucial in cell volume regulation (Adragna *et al.*, 2004). In individual with HbAS and HbSS, which are considered normal, the K^+-Cl^- cotransporter activity is high in immature red blood cells (reticulocytes) and absent in mature red blood cells. The activity of K^+-Cl^- cotransporter however is increased in individual with HbSS disorder thereby resulting to red blood cells dehydration. The dehydration of red blood cells causes HbSS polymerization (Joiner *et al.*, 2004). Mineral analysis of the four investigated plants species also revealed the presence of Zinc in various percentages. Natta *et al.* (1992) reported that intestinal synthesis of metallothionein was stimulated when high Zinc intake was maintained over weeks. Metallothionein is a copper binding protein responsible for trapping copper within intestinal cells which consequently result to the blockage in its absorption. This is necessary because excess copper absorption may lead to free radical production and oxidative damage in sickle erythrocytes. Puppalwar *et al.* (2015) also reported that copper has been found at increased level in plasma of HbSS patients, which has inverse relation with plasma Zinc.

Olufumilayo *et al.* (2012) reported that the leaves, seeds, and flower, extracts demonstrated antisickling activity with the seeds and flower extracts having higher activity. However, in the present study, the antisickling activity of the aqueous and ethanolic extract of *M. oleifera* was compared with that of aqueous and ethanolic extracts of *J. tanjorensis, A. hybridus, and M. esculenta.* Interestingly, this study is the first to evaluate the in vitro antisickling activity *Jatropha tanjorensis, Amaranthus hybridus,* and *Manihot esculenta* which present a stance to further explore them for the management of sickle cell disease. On the whole, all the plants investigated showed antisickling property. The highest activity was however observed in the sample treated with 1000 µg/mL aqueous extract of *Manihot esculenta* (93.6%) while the least activity was observed in ethanolic extract of *Manihot esculenta* (85.67%). The highest activity observed in aqueous extract of *M. esculenta* could be due to the presence of vitamin B1 B12, magnesium zinc and carotenoids. This concurs with the findings of Wobeto *et al.* (2006) who reported that *M. esculenta* leaves is rich in iron, zinc, vitamin B1, B12, magnesium and carotenoid. Although, all the percentages of un-sickle red blood cells at corresponding concentrations of the plant extracts compared favorably with the positive control (p-hydroxybenzoic acid). Latif and Muller (2015) also reported that the levels potassium, manganese, magnesium, phosphorous and zinc decreases while calcium, sodium and iron and calcium increase with leaf maturity. Similarly, Kay and Phyu (2018) also reported that *Manihot esculenta* leaves are rich in flavonoids, anthocyanin, catechins, and hydrobenzoic and hydroxycinnamic acids. The high activity of this plant extract could also be due to the synergistic effect aforementioned minerals and bioactive principles including hydroxybenzoic acid, which similar to the compound used as the positive control in the present study (Para-hydroybenzoic acid).

Conclusion

Various phytochemicals have been detected in the plant extracts of the four species studied. However, the ones reported in literature associated antisickling potentials are; flavonoids (anthocyanin), alkaloids, phenolic compounds, phytosterols and tannins.

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X-Ray fluorescence analysis of the four investigated plants species showed that *A. hybridus* (1.2152%) and aqueous *M. esculenta* (0.0095%) had highest percentage of magnesium and zinc respectively.

In the *in vitro* antisickling assay of the plants investigated, aqueous extract of *M. esculenta* at 1000 µg/mL showed highest antisickling activity (93.33%). The antisickling property of the plants can be attributed to the synergistic effect of Zinc, Magnesium and anthocyanin. On the whole, the antisickling properties of the plants identified in this study would establish their candidature for any future *in vitro* research and possible formulation of nutraceuticals.

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