COMPARATIVE IN-VITRO ANTISICKLING ACTIVITIES OF UNRIPE FRUIT AND LEAVES OF CARICA PAPAYA LINN

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ABSTRACT

Sickle cell sickness (SCD), otherwise called drepanocytosis is a genetic blood issue, described by a variation from the norm in the oxygen conveying hemoglobin molecule in red blood cells. Extracts of *Carica papaya* (Caricaceae) leaves and unripe fruits have been validated to have antisickling activities. This study analyzed the comparative *in vitro* antisickling properties of the extract and fractions of C. papaya leaves and unripe fruits. *In-vitro* antisickling studies were carried utilizing blood samples from sickle cell disease patients with normal saline and parahydroxybenzoic acid as negative and positive controls respectfully. Results confirmed the antisickling potency of both plant material used in this investigation. The extract and fractions reversed sickling in a dose-dependent manner. The unripe fruit was observed to be more potent having 2% (methanol), 3% (ethyl acetate), 4% (aqueous) percentage sickling in comparison with the leaf extract and fractions which has 3% (Methanol), 6% (Ethylacetate), 9% (aqueous) percentage sickling. Summarily, the unripe fruit of C. papaya has more antisickling activities when contrasted with the leaves. These outcomes demonstrate the possibility of C. papaya leaves and unripe fruits as an appealing potential possibility for the management of Sickle cell disease.

KEYWORDS: Antisickling, *Carica papaya*, Drepanocytosis, Haemoglobin, Parahydroxybenzoic acid.

1.0 INTRODUCTION

Sickle cell anaemia is an acquired ceaseless blood malady emerging from a point alteration in the β-globin gene that prompts the substitution of glutamic acid by valine at the 6th position of the β-chain of hemoglobin. At low oxygen pressure, the mutant haemoglobin, assumes a sickle shape, polymerizes inside the red blood cell into a gel or further into strands prompting an extreme increment in the red blood cell impairment. Accordingly, smaller scale vascular impediment emerges which may prompt genuine, some of the time deadly crisis (Mehanna, 2001)

A medication that extend the delay time before polymerization may be of helpful incentive in SCD because a more extended time diminishes the likelihood of sickling of HbS haemoglobin. Detailed antisickling compounds in this group incorporate Niprisan, MX-1520 and 5HMF (Iyamu *et al*., 2003; Chaojie Zhang *et al*., 2004; Abdulmalik *et al*., 2005), which change intracellular sickle hemoglobin and repress sickling of haemoglobin. Endeavours to discover elective, less expensive and less harmful regimen for SCD treatment, prompted the disclosure of sickle cell reversal activities of *Cajanus cajan* seeds (Ekeke and Shode, 1985) and root of Fagara (*Zanthoxylum macrophylla*) utilized locally by conventional healers in Nigeria (Sofowora *et al*., 1975; Elekwa *et al*., 2005).

Carica papaya Linn. (Caricaceae) is a lasting, herbaceous plant, with bountiful smooth latex coming to 6-10 meters in stature, the stem up to 30 cm thick, basic or stretched over the center and roughened with leaf scars. The unripe fruit is utilized generally among the Yoruba clan of Nigeria for treating jaundice and for the administration of sickle cell iron deficiency (Elujoba, 2001). Scientists have revealed the antisickling capability of *C. papaya* leaf and fruit. In one of the analyses, it was expressed that after fermenting the dried unripe fruit for a few days, the day 5 concentrate was found to have the most astounding sickle cell reversal activities with 93% inhibitory and 84% antisickling activities (Oduola *et al*., 2006). Oduola *et al*. (2012) have announced "Caricapinoside" as the powerful antisickling compound present in the *C. papaya* unripe fruit. This antisickling compound was observed to be in the ethyl acetate fraction of the extract (Oduola *et al*, 2012). The crude methanol extract and ethyl acetate fraction of *C. papaya* dried leaves have been found to have astounding antisickling properties (Imaga *et al*., 2009; Imaga and Adepoju, 2010; Imaga *et al*., 2011). There is no report on the comparative antisickling activities of the leaves and the unripe fruit. The point of the exploration work is to compare the antisickling impacts of *C. papaya* unripe fruit and leaves.

2.0 MATERIALS AND METHODS

2.1 Plant material

The leaves and unripe fruit of *C. papaya* samples were collected from the premises of the Lagos University Teaching hospital, Lagos, Nigeria and validated by Mr. O.O Oyegoke of the Herbarium unit, Department of Botany, University of Lagos. Voucher specimens (No.T6789/2011) was prepared and stored in the herbarium.

2.2 Preparation of plant extracts

Dried leaves of *C. papaya* L. were ground in a cross beater mill equipped with a 1 mm sieve. The *C. papaya* leaves were extracted and partitioned into fractions as follows: 500g of the powdered sample was extracted with 1 litres of petroleum ether using a Soxhlet extractor for six hours. The marc was further extracted with 3 litres of aqueous methanol using the soxhlet extractor for another six hours. The obtained methanol extract was then evaporated to dryness using vacuum rotary evaporator.

Matured fresh unripe *C. papaya* fruit was peeled and the cream coloured seeds inside discarded, the unripe flesh of the fruit was chopped into little bits and rinsed with distilled water. The chopped unripe fruit was grinded using a blender, to create a better surface area for extraction. 1kg of the grinded unripe fruit was extracted by maceration with 2 litres of methanol at room temperature for 6days. On the sixth day the marc was separated and the resulting mixture was concentrated using the rotary evaporator.

2.3 Partitioning of the extracts

Extracts were partitioned with ethylacetate using a separating funnel. Separating funnel was left to stand for 15 minutes. A delineation was noticed between the two immiscible liquids. The upper layer (organic layer) was transferred into another clean beaker and continuous extraction was carried out on the lower layer (aqueous layer) by continuous addition of 25ml of ethyl acetate. This method was carried out on each 12g of crude extract measured.
All extracts and fractions were encoded as MEF- methanol fraction; EF-ethyl acetate fraction; AF- aqueous fraction remaining after the partitioning. All extract and fractions were concentrated using a rotary vacuum evaporator and stored at 4°C.

2.4 Antisickling activity

The HbSS blood acquired from patients were washed thrice in phosphate buffered saline to get the RBC which were then resuspended in ordinary saline and utilized for the examination as previously outlined by Acquaye *et al*. (1982), Ekeke *et al*. (1990) and Ogoda *et al*. (2002). The aqueous, methanol and ethyl acetate extracts of C. papaya leaves were utilized in this investigation, with para-hydroxybenzoic acid as the standard. 1 ml SS cell suspensions were pre-brooded with 5mg and10 mg/ml concentrations of the extracts after the induction of sickling using 2% sodium metabisulphite. Microscopic investigation of the time course of the impact of varying concentrations of the extract and fractions on the sickling of SS erythrocytes was carried out. A plot of percentage sickling inhibition against time at 5mg/ml and 10mg/ml concentrations of the extract and fractions was analysed for a possible explanation of the observed antisickling effect.

3.0 RESULTS

Results acquired from in vitro investigations on the antisickling action of the Leaf and unripe fruit extracts carried out on SS blood samples, uncover that treatment of SS cell suspensions with C. papaya leaf and unripe fruit extract and fractions repressed sickle cells formation under serious hypoxia at various rates and at 5mg/ml and 10mg/ml extract concentrations.

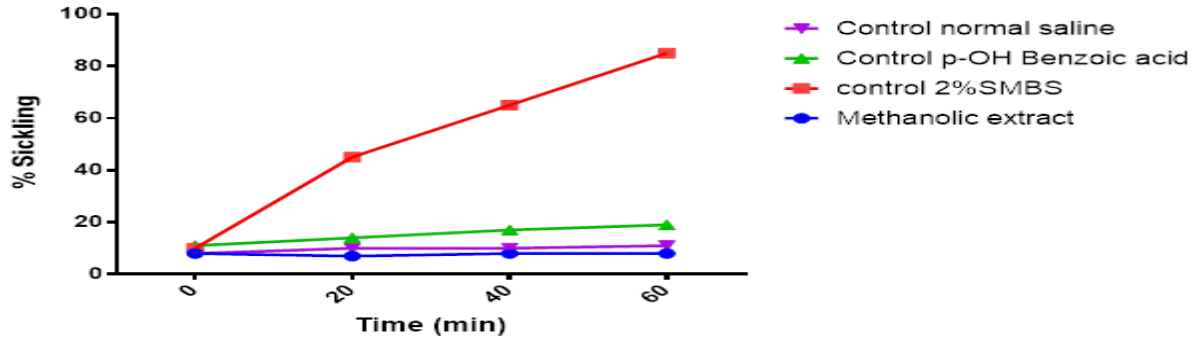

Fig.1: *In vitro* antisickling activity: % sickling of 5 mg/ml concentrations of *C. papaya*Methanol leaf extract.

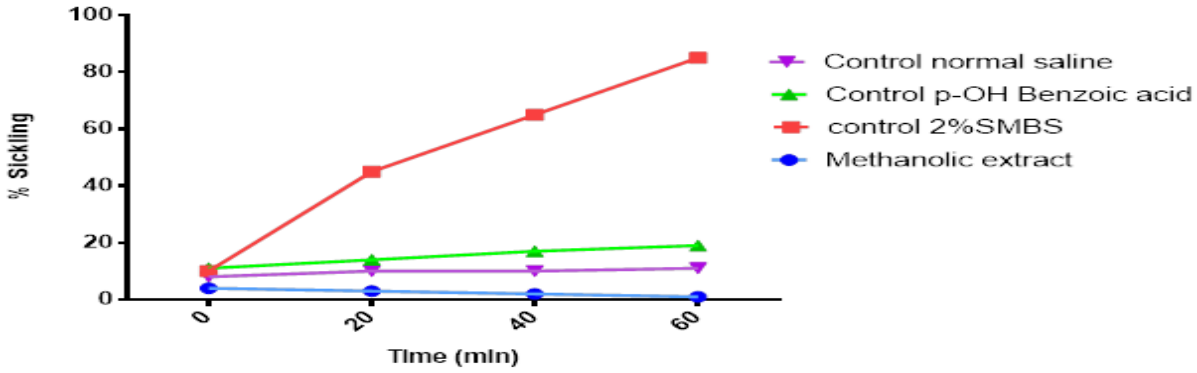

Fig. 2:*In vitro* antisickling activity: % sickling of 10 mg/ml concentrations of *C. papaya*Methanol leaf extract.

At 5mg/ml concentration of methanolic extract (fig 1), result showed 8% sickling at 60mins compared with 10mg/ml (fig. 2) with 3% sickling at 60mins. The negative control, normal saline had 11% sickling, the positive control, parahyrodroxybenzoic acid with 19% sickling and sodium metabisulphite, which induces sickling, had 85% sickling. The 10mg/ml leaf methanolic extract in comparison with the 5mg/ml shows more potent antisickling activities.

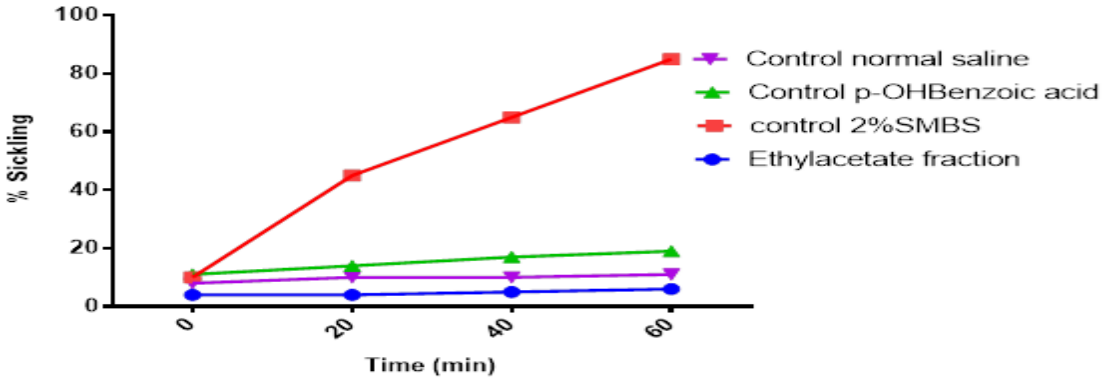

Fig.3: *In vitro* antisickling activity: % sickling of 5 mg/ml concentrations of *C. papaya leaf ethylacetate fraction*.

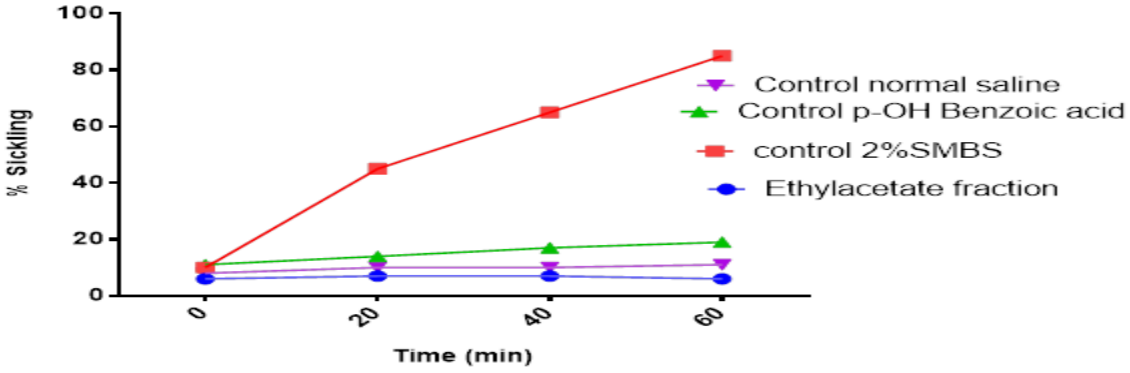

Fig.4: *In vitro* antisickling activity: % sickling of 10 mg/ml concentrations of *C. papaya*Leaf ethylacetate fraction

At the concentration of 5mg/ml concentration, the ethyl acetate fraction revealed a percentage sickling of 6% and the percentage sickling at 10mg/ml concentration is 5%. The ethyl acetate 10mg/ml is, therefore, more potent than the 5mg/ml leaf extract concentration.

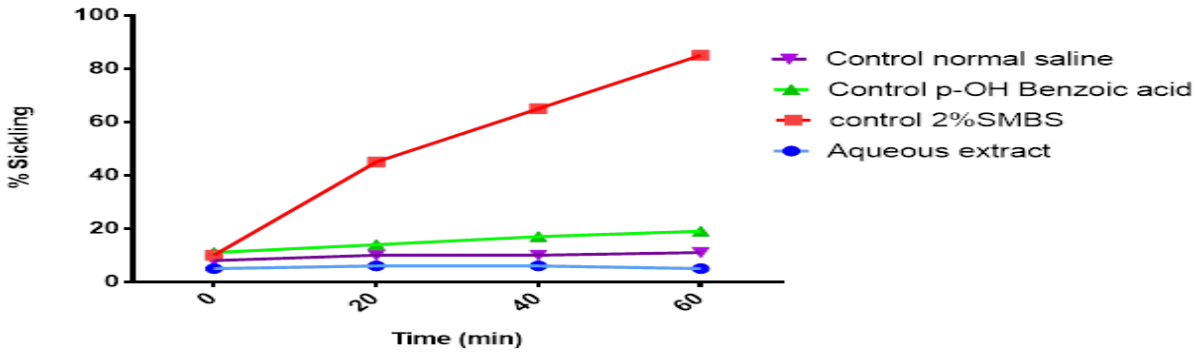

Fig. 5: *In vitro* antisickling activity: % sickling of 5 mg/ml concentrations of *C. papaya aqueous leaf extract*

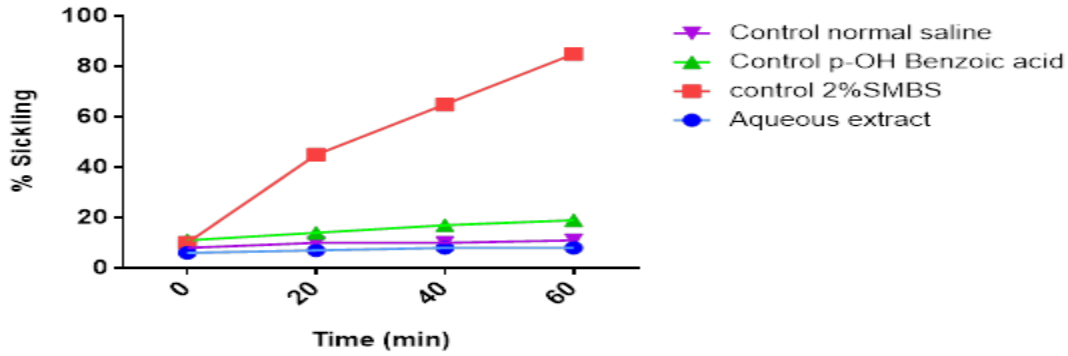

Fig.6: *In vitro* antisickling activity: % sickling of 10 mg/ml concentrations of *C. papaya*
Aqueous leaf extract.

According to the graph results, concentration of 5mg/ml of aqueous leaf extract is more potent with 5% sickling compared with that of 10mg/ml with 9% sickling.

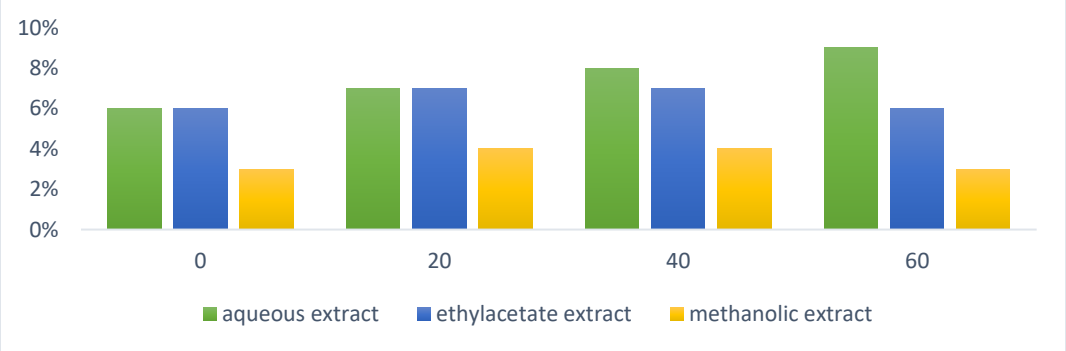

Fig. 7: In vitro antisickling of the 5mg/ml concentraton C. papaya extract and fractions.

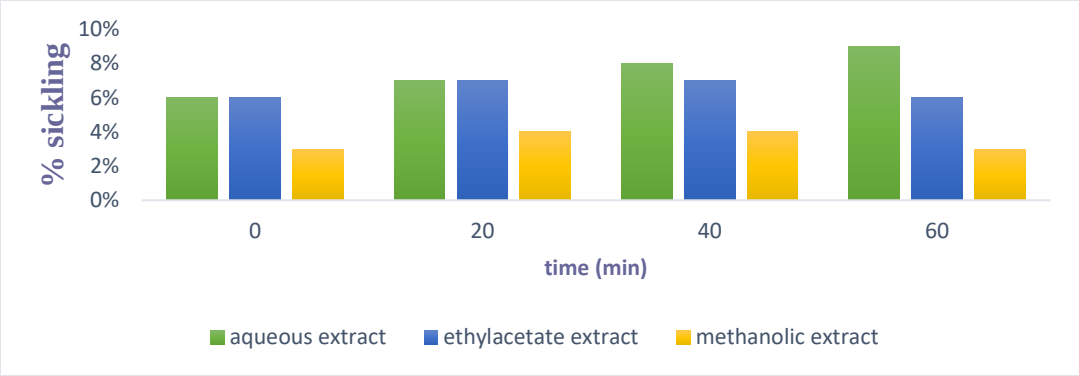

Fig.8: In vitro antisickling of the 10mg/ml concentraton C. papaya extract and fractions.

The 10 mg/ml concentration of *C. papaya* leaf fractions were more effective in inhibiting sickling than the 5 mg/ml concentrations at longer incubation periods of 60 min as illustrated apart from the aqueous fraction which has the 5mg/mg fraction to be more potent. The methanolic (10mg/ml) fraction showed the highest antisickling activity followed by the ethyl acetate (5mg/ml) fraction followed by the ethyl acetate (10mg/ml) fraction then methanolic 5mg/ml) fraction then the aqueous (5mg/ml) fraction and the aqueous (10mg/ml) fraction.

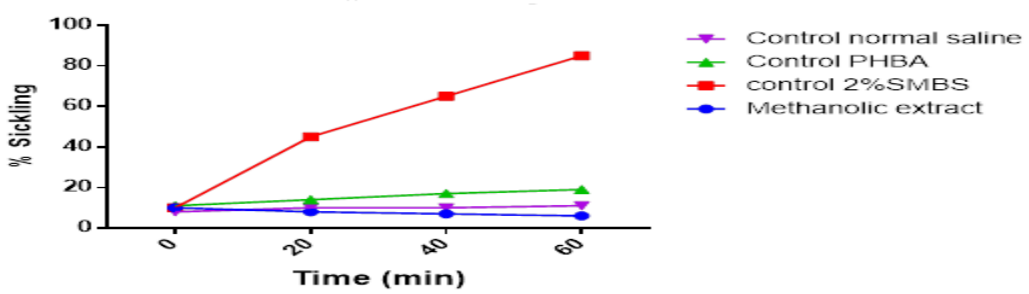

Fig.9: *In vitro* antisickling activity: % sickling of 5 mg/ml concentrations of *unripe papaya fruit* Methanol extract.

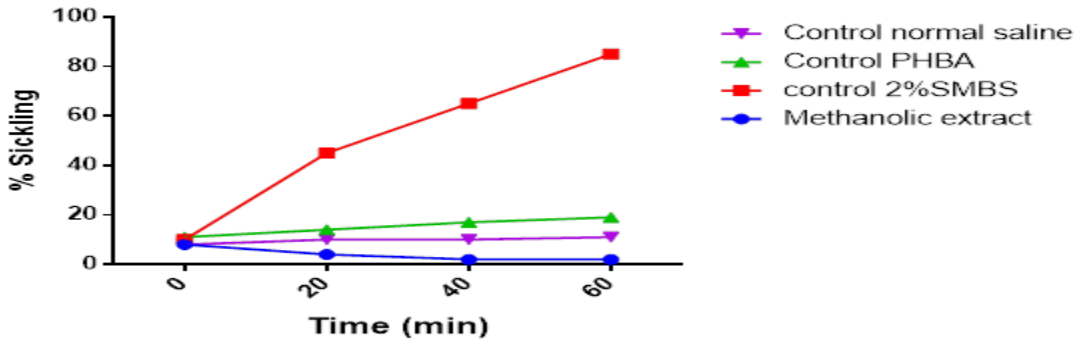

Fig.10: *In vitro* antisickling activity: % sickling of 10 mg/ml concentrations of *unripe papaya fruit* Methanol extract.

In vitro studies reveal that at concentration of 10mg/ml (fig.10) the antisickling effect of the methanolic extract of *C. papaya* unripe fruit had higher potency of 2% sickling at 60minutes compared to that of 5mg/ml concentration (fig.9) which had 6% sickling at 60minutes.

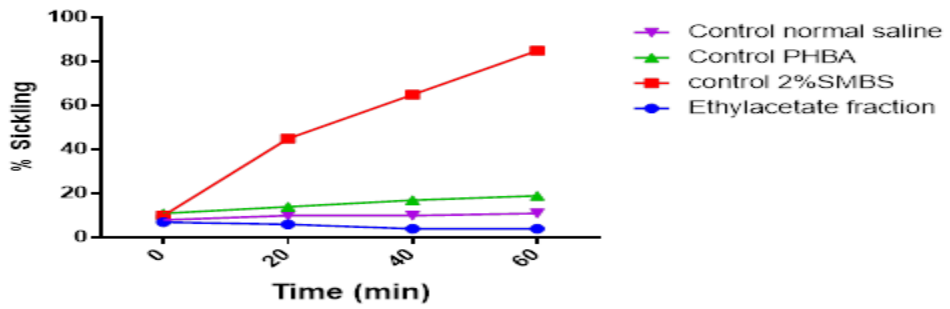

Fig. 11: *In vitro* antisickling activity: % sickling of 5 mg/ml concentrations of *unripe papaya fruit* ethylacetate fraction.

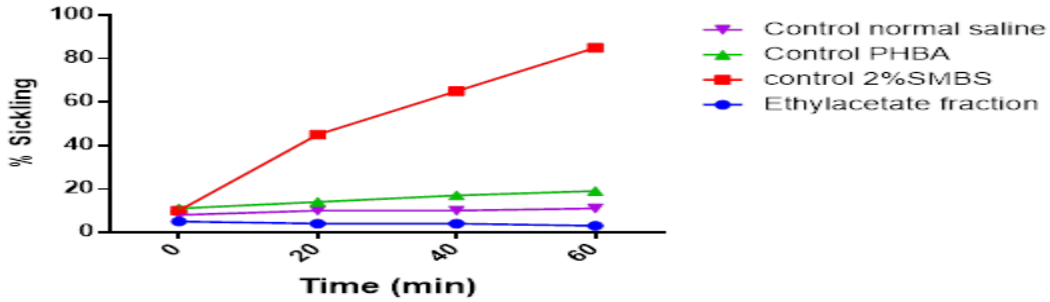

Fig.12: *In vitro* antisickling activity: % sickling of 10mg/ml concentrations of *unripe papaya fruit* ethylacetate fraction.

Comparing the sickling potential of *C. papaya* unripe fruit at concentrations of 10mg/ml and 5mg/ml (fig.11 and 12) results show that at 10mg/ml the ethylacetate fraction possess higher antisickling potential of 3% sickling at 60minutes compared to 5mg/ml which had 4% sickling at 60minutes.

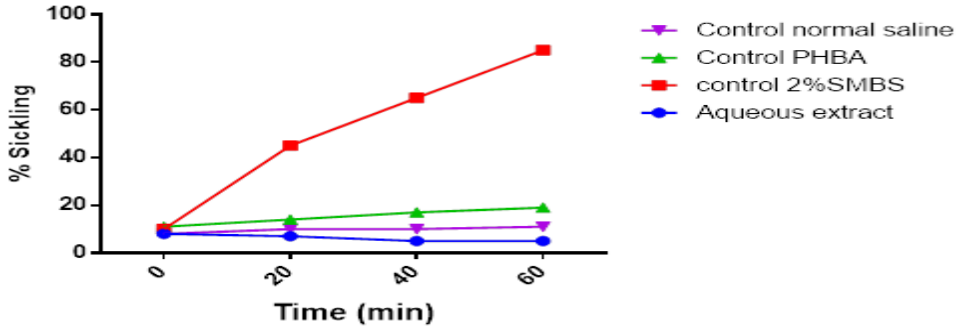

Fig. 13: *In vitro* antisickling activity: % sickling of5mg/ml concentration of aqueous extract

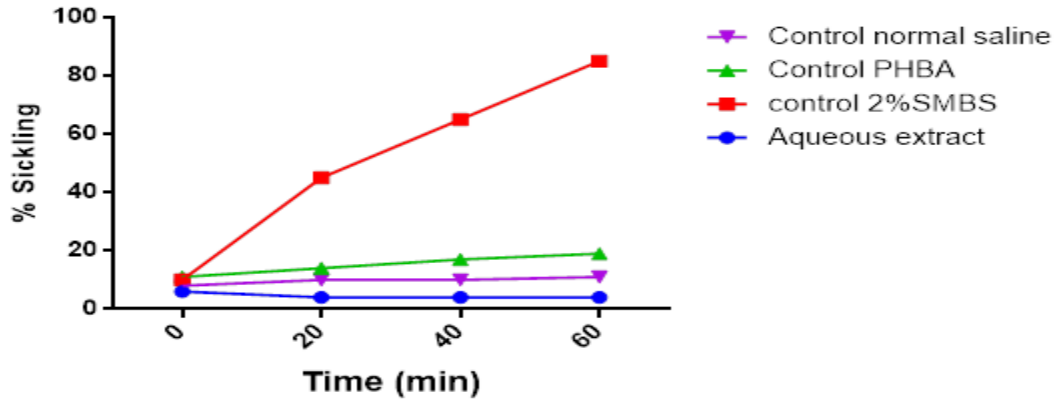

Fig. 14: *In vitro* antisickling activity: % sickling of5mg/ml concentration of aqueous extract

From the graph (fig.13 and 14), 10mg/ml concentration *C. papaya* unripe fruit aqueous fraction has higher antisickling efficacy of 3% sickling at 60minutes compared to that at 5mg/ml which had 4% sickling.

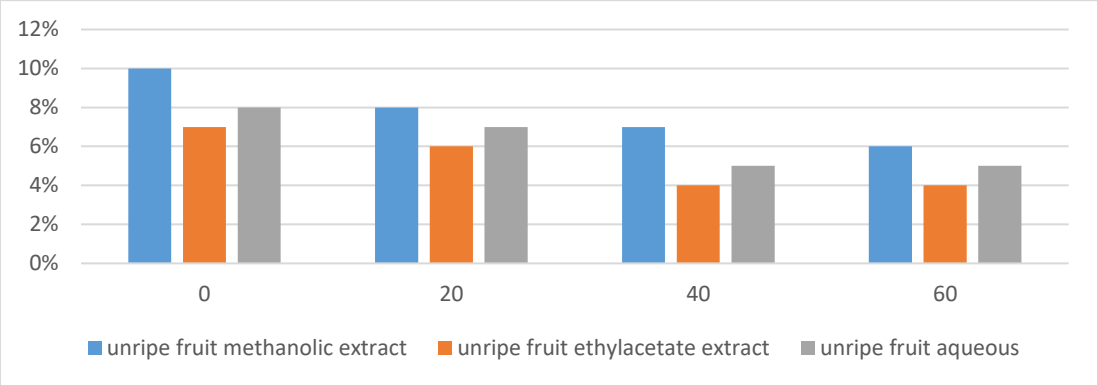

Fig 15: Percentage sickling of 5mg/ml unripe fruit extract for all fractions.

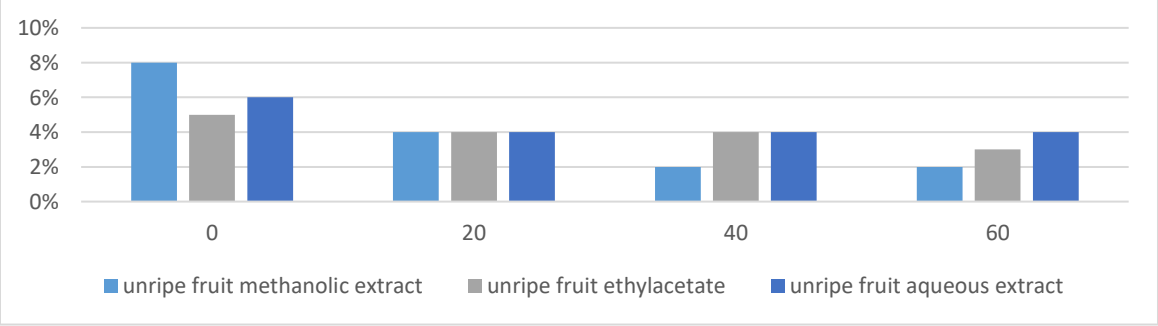

Fig 16: Percentage sickling of 10mg/ml leaf extract for all fractions.

Figure15 and figure 16 above reveal that the 10 mg/ml concentration of *C. papaya* unripe fruit fractions were more effective in inhibiting sickling than the 5 mg/ml concentrations at longer incubation periods of 60 minutes.

4.0 DISCUSSION

C. papaya dried leaves and unripe fruits have been shown in the management of drepanocytosis by indigenous people and logical research. Research has demonstrated the antisickling properties of the leaves and unripe fruit concentrates (Oduola*et al*., 2006; Imaga and Adepoju, 2010). In this investigation, *C. papaya* leaf extract was found to have a considerable strong antisickling action and extraordinarily influenced the time course for sickling in a dose-dependent way, the best dosages being 5 and 10 mg/ml concentrations of the extract and fractions. Antisickling compounds have been accounted for to lengthen the delay time of red blood cell polymerization as a component of the process for its antisickling activity (Iyamu *et al*., 2002). *C. papaya* leaf extract and fractions were not found to extend the delay time in this work yet unquestionably repressed sickle cell polymerization demonstrating that the extract may apply an objective strike on HbSS polymerization in enervating SS cell sickling. This could likewise demonstrate that the impact of the extract and fractions is presumably at the biomembrane level and not unswerving connection with sickle cell molecules dissimilar to other agents (Abdulmalik *et al*., 2005; Iyamu *et al*., 2003), whose antisickling activities are prefaced on the exchange with HbS molecules.

The discoveries in this investigation acclimate with the prior report of Oduola *et al*. (2006) which detailed that the antisickling factor in the extract of unripe pawpaw fruit is resident in the ethyl acetate part as this fraction averted sickling of HbSS red cells and switched sickled red cells in 2% sodium metabisulphite.

Ogunyemi *et al*. (2008) revealed that the aqueous extract of *C. papaya* unripe fruit has a 93% inhibitory and 87% reversal antisickling activities which aren't in concurrence with the report of this study. The inhibitory and reversal antisickling activities of the methanol extract of *C. papaya* unripe fruit gave 64% and 55% separately which is in concurrence with this report.

Thomas and Ajani (1987), suggested that the active compound(s) in the fermented *C. papaya* unripe fruit averting and reversing sickling could be organic acids delivered after the hydrolysis of the relating esters in the group.

Imaga and Adepoju (2010), in an investigation, noticed that 10mg/ml concentration of the crude leaf methanol extract has a higher antisickling impact in examination with the 5mg/ml concentration. The ethyl acetate extract of this plant has the most noteworthy antisickling activity at 5mg/ml which doesn't corroborate the result of this investigation that 10mg/ml of the ethyl acetate concentrate has higher antisickling action.

Parahydroxybenzoic acid and 3, 4-dihydroxybenzoic acid (Protocatechuic acid), 4-hydroxy-3-methoxybenzoic acid (vanillic acid) have been recently identified from Mulberry (*Morusalba*), *Lobelia sessilifolia*, which makes the sickle cell reversal activities of the unripe *C. papaya* extract obvious. These recently isolated and identified compounds frames the core of the confined compound (caricapinoside) (Oduola *et al*., 2012).

5.0 CONCLUSION

It can be deduced that the unripe fruit has more potent antisickling activities when compared with the leaf extracts. These indigenous extracts could be utilized in amalgamation with different nourishments in the handling and prophylactic control of sickle cell anaemia and other pathophysiological impediments of this and other related disorders.

REFERENCES

Abdulmalik, O.O., Safo, M.K., Chen, Q., Yang, J., Burguara, C., Ohene-Frempong, K., Abraham, D.J., Asakura, T. (2005). 5-hydroxylmethyl – 2-furfural modifies intracellular sickle hemoglobin and inhibits sickling ofred blood cells. *British J. Hematol*., **128**: 552-561.

Acquaye, C.T.A., Young, J.D., Ellory, J.C., Gorecki, M., Wilcher, M. (1982). Mode of transport and possible mechanism of action of LPhenylalaminebenzylester as an antisickling agent. *BiochimicaetBiophysicaActa*. **693**: 407-416

Ekeke, G.I., andShode, F.O. (1985). The Reversion of Sickled cells by *Cajanuscajan*. *Plantamedica*, **6**: 504-507.

Ekeke, G.I., Shode, F.O., (1990). Phenylalanine is the predominant Antisickling agent in *Cajanuscajan*seed. *PlantaMedica*, **56(1)**: 41-43.

Elekwa, I., Monanu, M.O., Anosike, E.O. (2005). Effects of aqueous extractsof *Zanthoxylum macrophylla* roots on membrane stability of humanerythrocytes of different genotypes. *Biokemistri*, **17(1)**: 7-12.

Elujoba, A.A., (2001): Traditional medicine practice experience. (Personal Communication).

Imaga, N. O. A., Shaire, E. A., Ogbeide, S., and Samuel K. Akindele, S.K. (2011). *In vitro* biochemical investigations of the effects of*C. papaya*and *Fagarazanthoxyloides*onantioxidant status and sickle erythrocytes. *African Journal of Biochemistry Research*, **5(8)**, 226-236,

Imaga, N.A., and Adepoju, O.A. (2010). Analyses of antisickling potency of *C. papaya* dried leaf extract and fractions. *Journal of Pharmacognosy and Phytotherapy*, **2(7)**: 97-102.

Imaga, N.O.A., Gbenle, G.O., Okochi, V.I, Akanbi, S.O., Edeoghon, S.O,Oigbochie, V., Kehinde, M.O., and Bamiro, S.B. (2009). Antisickling Property of*C. papaya*leaf extract. *Afr. J. Biochem. Res*., **3(4)**: 102-106.

Iyamu, E.W., Turner, E.A., Asakura, T. (2002). *In vitro* effects of Niprisan(Nix–0699): A naturally occurring, potent antisickling agent. *Br. J.Hematol.*, **118:** 337-343.

Iyamu, E.W., Turner, E.A., Asakura, T. (2003). Niprisan (Nix–0699) improvesthe survival rates of transgenic sickle cell mice under acute severehypoxic conditions. *Br. J. Hematol.*, **122**: 1001-1008.

Khadem, S., Marles, R.J. (2010). Monocyclic Phenolic Acids; Hydroxy- and Parahydroxybenzoic acids: occurrence and recent bioactivity studies. *Molecules*, **15**: 7985-8005.

Mehanna, A.S. (2001). Sickle cell anemia and antisickling Agents Then and Now. *Curr. Med. Chem*., **8(2)**: 79-88.

Oduola, T., Adeniyi, F.A.A., Ogunyemi, E.O., Bello, I.S., Idowu, T.O. (2006).Antisickling agent in an extract of unripe pawpaw (*C. papaya*): Isit real? *Afr. J. Biotech*., **5(20)**: 1947-1949.

Oduola, T., Idowu, T.O., Bello I.S., Adeniyi F.A., Ogunyemi E.O. (2012). Haematological response to intake of unripe *C. papaya* fruit extract and the isolation and characterization of *caricapinoside*: a new antisickling agent from the extract. Asian journal of pharmaceutical and clinical research, **5(3)**: 76 - 81

Ogoda, O.J., Akubue, P.I., Okide, G.B. (2002). The Kinetics of Reversal ofPre-sickled Erythrocytes by the Aqueous Extract of *Cajanuscajan*seeds. *Phytother. Res.*, **16:** 1-3.

Ogunyemi, C.M., Elujoba, A.A., Durosinmi, M.A. (2008). Antisickling properties of *C. papaya* Linn. *Journal of Natural Products*,**1**: 56-66.

Sofowora, E.A., Issacs-Sodeye, N.A., Ogunkoya, L.O. (1975). Antisickling properties of Fagara. *Lloydia*, 38: 169-171.

Thomas, K.D., Ajani, B. (1987). Antisickling agent in an extract of unripe pawpaw fruit. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81**:510-511.

Thomas, K.D., Ajani, B., (1987). Antisickling agent in an extract of unripe pawpaw fruit. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81**:510-511.