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Full Length Research Paper

An evaluation of the antisickling potency of *Carica papaya* dried leaf extract and fractions

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Carica papaya **dried leaves have been indicated in sickle cell anemia management by local indigenous folk and in recent scientific research. In this research, dried *C. papaya* leaves were extracted using the soxhlet extraction method with 5 different solvents to give five different fractions namely hexane, chloroform, ethyl acetate, butanol and water. This research examined the crude extract and the various leaf extract fractions of *C. papaya* L. (Caricaceae) for possible *in vitro* antisickling activities on Hb^{ss} red blood cells obtained from non-crisis state sickle cell patients involving the use of positive (p-hydroxybenzoic acid 5 □ g /ml) and negative (normal saline) controls for the antisickling experiments. Pretreatment of SS cell suspensions with *C. papaya* leaf extract and fractions all inhibited formation of sickle cells under severe hypoxia at varying degrees, with only 0 - 5% sickle cells in the crude extract at 60 min compared with untreated SS cell suspensions which had over 80% sickle cells. Analysis of two different concentrations of *C. papaya* crude extract (10 and 5 mg/ml) showed the 10 mg/ml extract as the concentration with highest antisickling effect. Butanol extract showed the highest antisickling activity at 10 mg/ml concentration, while the ethyl acetate extract had the highest antisickling activity at 5 mg/ml concentration. These results further indicate the possibility of *C. papaya* leaf extract as potential phytotherapy for sickle cell anemia.**

Key words: Antisickling, sickle cell disorder, *C. papaya*, fractionation.

INTRODUCTION

Carica papaya is a member of the Caricaceae Family, native to Nigeria and Central America. The shrub, resembling a palm, has male and female trees and it is the females that bear fruit. It is a herbaceous tree that grows very rapidly to 8 m (25 ft). It has segmented leaves, yellow flowers, and large black seeded yellow to orange fruits weighing up to 5 kg (11 lb). It is a small tree, the single stem growing from 5 to 10 m tall, with spirally arranged leaves confined to the top of the trunk; the lower trunk is conspicuously scarred, where leaves and fruits were borne (Iwu, 1993). The leaves are large, 50 - 70 cm diameter, deeply palmately lobed with 7 lobes. Papaya leaves are used as a poultice for wounds and also taken internally as vermifuge (worm expellant). Its constituents include carpaine and papain, the latter makes papaya leaves very useful as meat tenderizers.

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Recent research shows that *C. papaya* unripe fruit and dried leaves have been indicated in sickle cell anemia management. Scientists have reported that extracts of the unripe fruit and the dried leaves have definite antisickling property (Ogunyemi et al., 2008; Oduola et al., 2006; Imaga et al., 2009). *Parquetina nigrescens* root and leaf extracts (Kade et al., 2003; Imaga et al., 2010) is another plant purported to be used as a herbal therapy for Sickle Cell Disorder (SCD).

SCD is a group of hereditary illnesses affecting the red cell hemoglobin (Bunn, 1997). Various types of these disorders exist: including Sickle thalassaemia and Sickle Cell Anemia (HbSS), also known as Drepanocytosis. Sickle cell anemia is a genetic disease in which the 'SS' individual possesses an abnormal -globin gene. A single base substitution in the gene encoding the human -globin subunit results in the replacement of 6 glutamic acid by valine, which leads to the devastating clinical manifestations of sickle cell disease. This substitution causes a drastic reduction in the solubility of sickle cell

hemoglobin (HbS) when deoxygenated (Bunn, 1997). The disease is most prevalent in the black race, but it is also known in other races surrounding the Mediterranean and in India (Steinberg, 2004) . Sickle cell incidence has been closely linked to malaria incidence in tropical areas like Nigeria. These SS persons are least fit for survival in a hostile malaria environment and survival rates are particularly low in childhood (Ekeke, 2001).

There are several compounds such as amino acids, which prevent sickling by affecting the erythrocyte membrane, causing an increase in the cell volume of the erythrocyte and thus reducing the intracellular hemoglobin concentration below its minimum gelling concentration (Abraham et al., 1982). The most popular approach to prevent or reverse sickling *in vitro* and *in vivo* is to employ compounds or techniques which directly affect the hemoglobin (Hb) molecule. Reported antisickling / antidrepanocytary agents in this group include a formulated phytomedicine – Niprisan (Nix-0699), a chemical compound 5-hydroxylmethyl -2-furfural (5HMF) and MX -1520 (a prodrug of a food additive, vanillin) which modify intracellular sickled hemoglobin and inhibit sickling of red blood cells (Abdulmalik et al., 2005; Zhang et al., 2004; Iyamu et al., 2003; Wambebe, 2001) . It is acknowledged world-wide that traditional medicine can be explored and exploited to be used along-side synthetic pharmaceutical products for enhanced health management. Due to the high mortality rate of sickle cell patients, especially in children and since chemotherapy has its adverse effects, there is need for rational drug development that must embrace not only synthetic drugs but also natural products (phytomedicines /herbal drugs), naturally occurring antisickling agents which can be obtained from our vast forest resources and can be used to effectively manage the sickle cell patient and treat the anemic condition accompanying this disorder. The antisickling activities of crude extracts of *C. papaya* L. (Caricaceae) as well as its toxicity profile have already been reported in a previous study (Imaga et al., 2009). Bioassay guided partial fractionation of the *C. papaya* dried leaf extract and analyses of the antisickling potency of each fraction were carried out in this present study to detect the fractions possessing potent antisickling action and also determine the minimum concentration of extract with maximum antisickling activity.

MATERIALS AND METHODS

Blood samples, chemicals and biochemicals

Fresh blood samples were collected with full informed consent from sickle cell individuals in the steady state of the disease aged between 18 and 20 years of both sexes, who had not taken any herbal medication for SCD during routine visits at the Sickle Cell Out Patients' Clinic of the Lagos University Teaching Hospital, Idi-araba, Lagos, Nigeria. 5 ml venous blood samples were collected in Sodium EDTA bottles and used for the antisickling tests. All chemicals used were of analytical grade obtained from Sigma Chemical Company and used without further purification.

Plant material

Leaves of *C. papaya* L. were collected from the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria and authenticated by Mr. T. K. Odewo of the Institute. A Voucher Specimen (FHI: 106994) is deposited at the herbarium in FRIN.

Extraction and fractionation of the leaves

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 extract partitioned into fractions using slightly modified methods of Ogoda et al. (2002) and Parida et al. (2010) as follows: 500 g of dark brown, smooth textured powdered sample was extracted with 1.5 l of petroleum ether using a Soxhlet extractor for six hours. The petroleum ether extract obtained was discarded. The marc was further extracted with 1.5 l of aqueous methanol (1:3 v/v) using the soxhlet extractor for another six hours. The obtained methanolic extract was then evaporated to dryness using vacuum rotary evaporator.

The methanolic extract was then dissolved in 10% sulfuric acid solution and partitioned with hexane, chloroform, ethyl acetate and n-butanol successively to give hexane fraction - 23.9 g, chloroform fraction- 5.23 g, ethyl acetate fraction - 2.68 g, n-BuOH fraction - 5.50 g and remaining aqueous fractions - 34.2 g. All extracts and fractions were encoded as: CE – crude methanol extract; HF-hexane fraction; CF-chloroform fraction; EF-ethyl acetate fraction; BF-nbutanol fraction; AF- aqueous fraction remaining after fractionation. All extract fractions were freeze dried and stored at 4°C.

Antisickling activity

In vitro screening of the herbal extracts for antisickling properties with blood samples collected from confirmed non-crisis sickle cell individuals was carried out using earlier described methods of Imaga et al. (2009). Blood samples were washed thrice in 0.15 M saline phosphate buffer, pH 7.4 to obtain the red cells which were then re-suspended in normal saline and used for the analysis. The blood cell suspensions were then pre-incubated with 5 – 10 mg/ml concentrations of the crude extracts and various fractions separately in the presence of 2% Sodium metabisulphite solution and then microscopically analyzed for the time course for sickling of erythrocytes and percentage inhibition of hemoglobin S polymerization compared with parahydroxybenzoic acid as control. A plot of percentage sickling inhibition against extract concentration was analyzed for possible explanation of the observed antisickling effect.

RESULTS

Data from *in vitro* studies on the antisickling activity of the herbal extract carried out on blood samples collected from confirmed non-crisis sickle cell individuals, show that pretreatment of SS cell suspensions with *C. papaya* leaf extract and fractions inhibited formation of sickle cells under severe hypoxia at different rates and different concentrations.

The crude methanol extract inhibited formation of sickle

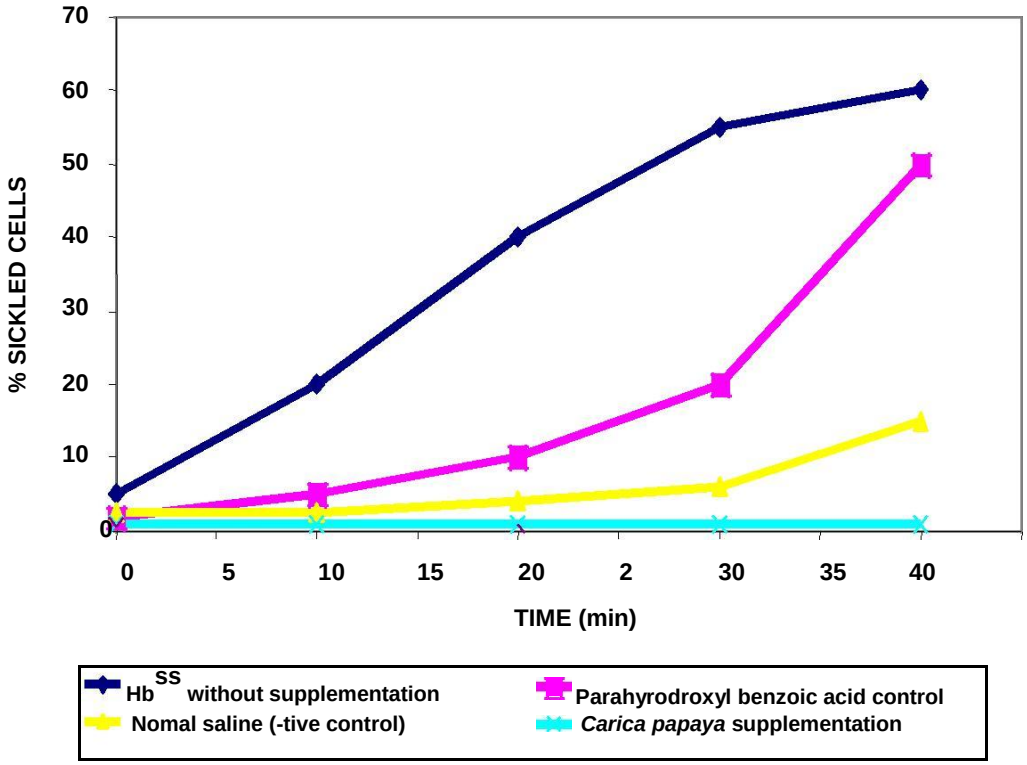

Figure 1. *In vitro* antisickling activity of *C. papaya* crude methanol extract. This represents data obtained from triplicate studies using blood samples from SS patients. Sickle cell suspensions were preincubated with extracts prior to exposure to 2% sodium metabisulphite solution: as shown, the time course for 80% sickling was 60 min for the control (blood without supplementation) but *C. papaya* {5 mg/ml} reduced sickling to 0% as shown.

cells, with only 0 - 2% sickle cells at 60 min compared with untreated SS cell suspensions which had over 80% sickle cells compared with controls(Figure 1). The aqueous crude extract fraction reduced the degree of sickle cell formation in a dose-dependent manner (1, 3, 5 – 10 mg/ml), with the highest dose exhibiting a more effective antisickling activity compared to the crude methanol extract concentrations and the HbSS-sodium metabisulphite control (Figure 2). After 2 h, in the presence of 5 and 10 mg/ml extract concentrations and under the view of the microscope, the original discoidal shape was retained in nearly all cells, unlike the control where over 80% of the SS cells had assumed a sickle shape.

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 and above. For both10 and 5 mg/ml concentrations, the ethyl acetate fraction showed the highest antisickling activity followed by the butanol fraction. The hexane and chloroform extracts showed lower antisickling activities with the hexane fraction being the least effective compared to the other extract fractions (Figures 3 and 4). In general, the order of activity of all the extracts and fractions were: - CE > EF > BF > CF. HF had little or no antisickling activity.

DISCUSSION

Research into phytotherapy of diseases is a current trend in the management of tropical diseases and genetic disorders like sickle cell anemia, with a view to finding cheaper, alternative medicines that the wide populace can have immediate access to. Recent studies show that papaya leaf crude extract has antisickling activity (Imaga et al., 2009). In this study *C. papaya* leaf extract fractions were also found to have appreciable potent antisickling activity and greatly affected the time course for sickling in a dose-dependent manner.

C. papaya leaf crude extracts showed the highest antisickling activity after 40 min of incubation compared to the other extract fractions and chemical standards used. This finding is comparable to previous studies which have found crude extracts of a plant extract to be more effective than its various fractions (Parida et al., 2010). The antisickling properties of the leaf could be concentrated in the polar and phenolic constituents of the *C. papaya* plant as evidenced by the potent antisickling activities of the ethyl acetate, butanol fractions and crude

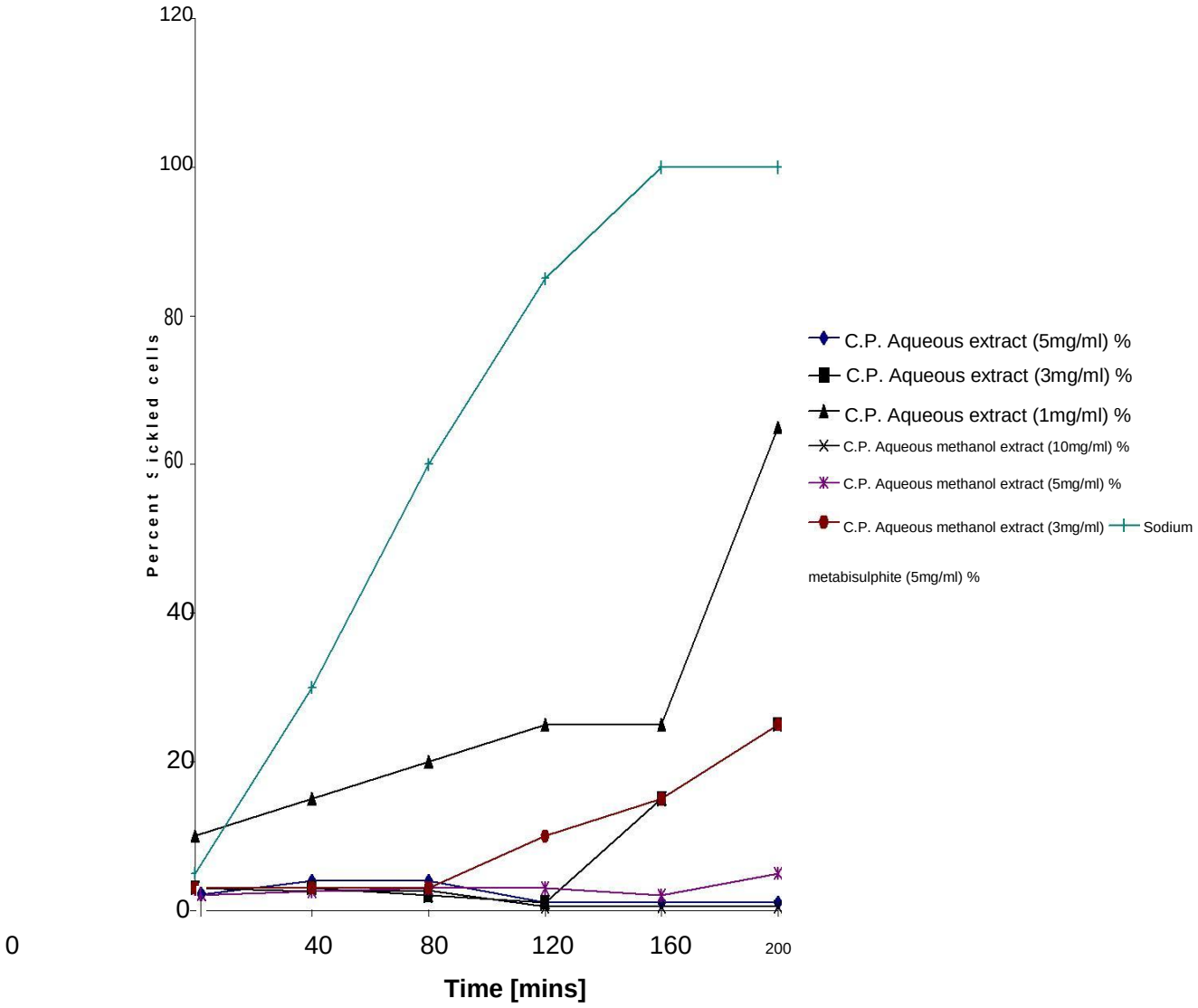

Figure 2. *In vitro* antisickling activity of different concentrations of *C. papaya* aqueous and methanol extracts. This represents data on the time course of sickling by *C. papaya* crude extracts. Key: *C.P = Carica papaya*

crude aqueous methanol extracts.

This present study also showed that all the extracts reflected the same delay time for HbSS polymerization. So *C. papaya* did not prolong the delay time of Hb polymerization but affected the time course for sickling appreciably (the most effective dose range being 5- 10 mg/ml aqueous and crude methanol extracts) compared with para-hydroxybenzoic acid. Antisickling agents have been reported to prolong delay time of Hb polymerization as part of the mechanisms for its antisickling action as reported in a previous study (Iyamu et al., 2002). However, papaya leaf extract and fractions were not found to prolong the delay time in this study but definitely inhibited hemoglobin polymerization in the red blood cell suspensions and thus inhibited the time course for sickling of HbSS cells. This indicates that the extract may apply a target hit on HbS polymerization in attenuating SS cell sickling. Patients given this herb will benefit from its total inhibition of the sickling phenomenon at 10 mg/ml dose concentrations. From the results obtained in this study, it was observed that the crude methanol and aqueous extracts showed the highest antisickling activity over time followed closely by the ethyl acetate fraction and then the butanol fraction. It was also observed that the 10 mg/ml concentration of *C. papaya* extracts and fractions were more effective than the 5 mg/ml concentrations at longer incubation periods.

Conclusion

This study provides further evidence that *C. papaya* possesses antisickling activity in a dose-dependent manner and a decrease in concentration is associated with decrease in antisickling activity of extract.

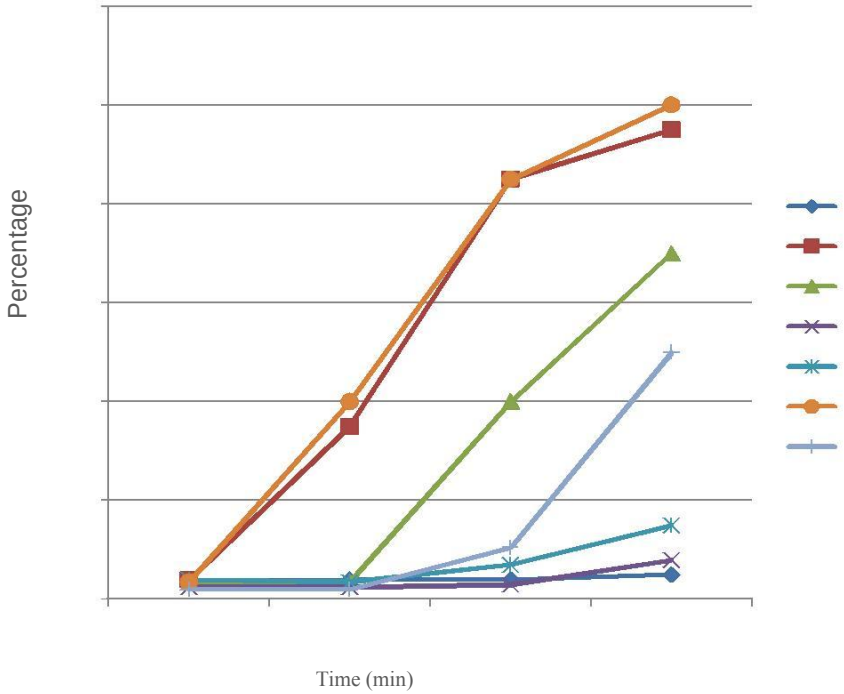

Figure 3. *In vitro* antisickling activity: % sickling of 10 mg/ml concentrations of *Carica papaya* leaf fractions. Key: CE= crude extract; EF= ethyl acetate fraction; BF = butanol fraction; HF = hexane fraction; CF = chloroform fraction; SMBS =Sodium Metabisulphite; PHBA = Parahydroxybenzoic acid.

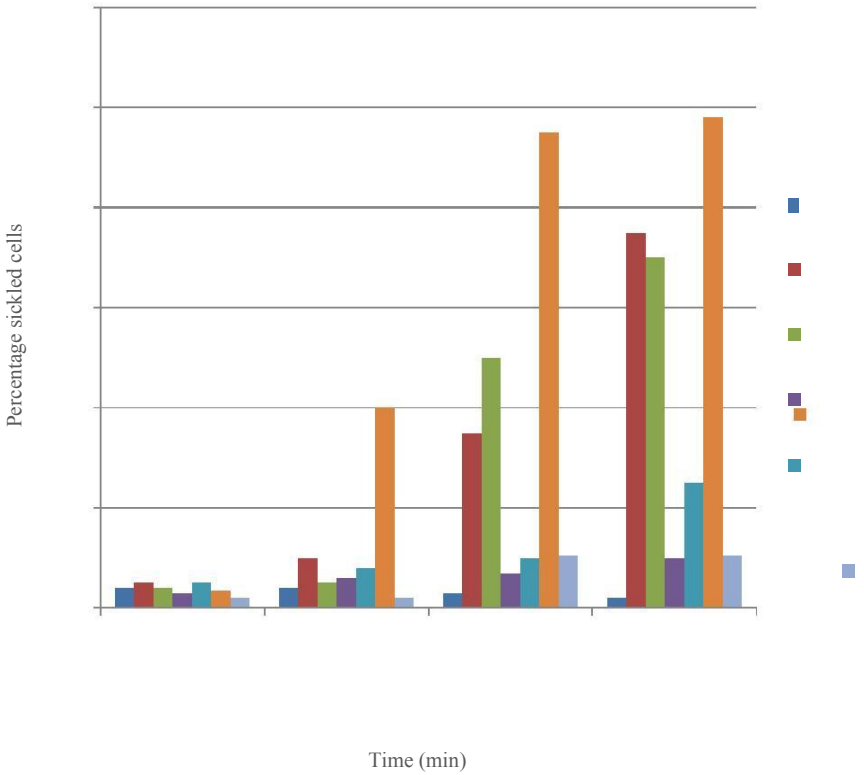

Figure 4. *In vitro* antisickling activity: % sickling of 5 mg/ml concentrations of *Carica papaya* leaf fractions. Key: CE= crude extract; EF= ethyl acetate fraction; BF = butanol fraction; HF = hexane fraction; CF = chloroform fraction, SMBS = Sodium Metabisulphite; PHBA = Parahydroxybenzoic acid.

These results further indicate the possibility of Carica papaya as a potential phytomedicine for SCD therapy. Identification and purification analytical studies on the active phytochemicals and biochemicals present in the potent antisickling extracts and fractions is an on-going research by the authors.

REFERENCES

Abdulmalik OO, Safo MK, Chen Q, Yang J, Burguara C, Ohene-Frempong K, Abraham DJ, Asakura T (2005). 5-hydroxylmethyl – 2-furfural modifies intracellular sickle hemoglobin and inhibits sickling of red blood cells. Br. J. Hematol., 128: 552-556.

Abraham DJ, Mehanna AS, William FL (1982). Design, synthesis and testing of potential antisickling agents 1: Halogenated benzyloxy and phenoxy acids. J. Med. Chem., 25: 1015-1017.

Bunn FH (1997). Pathogenesis and Treatment of Sickle Cell Disease. The New Eng. J. Med., 337: 762-769.

Ekeke GI (2001). Sickle Cell Anaemia: Basic Understanding and Management. P. 78. Harrisco Press, Port-Harcourt, Rivers State, Nigeria.

Imaga NOA, Gbenle GO, Okochi VI, Adenekan SO, Edeoghon SO, Kehinde MO, Bamiro SB, Ajiboye A, Obinna A (2010). Antisickling and Toxicological Profile of Parquetina nigrescens. J. Med. Plants Res., 4(8): 639-643.

Imaga NOA, Gbenle GO, Okochi VI, Akanbi SO, Edeoghon SO, Oigbochie V, Kehinde MO, Bamiro SB (2009). Antisickling Property of Carica papaya leaf extract. Afr. J. Biochem. Res., 3(4): 102-106.

Iwu MM (1993). Handbook of African Medicinal Plants, CRC Press, USA, pp. 141-142.

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

Iyamu EW, Turner EA, Asakura T (2003). Niprisan (Nix–0699) improves the survival rates of transgenic sickle cell mice under acute severe hypoxic conditions. Br. J. Hematol., 122: 1001-1008.

Kade IJ, Kotila OO, Ayeleso AO, Olaleye AA, Olawoye TL (2003). Antisickling properties of Parquetina nigrescens. Biomed. Res. (Alligarh), 14: 185-188.

Oduola T, Adeniyi FAA, Ogunyemi EO, Bello IS, Idowu TO (2006) Antisickling agent in an extract of unripe pawpaw (Carica papaya): Is it real? Afr. J. Biotech., 5(20): 1947-1949.

Ogoda OJ, Akubue PI, Okide GB (2002). The Kinetics of Reversal of Pre-sickled Erythrocytes by the Aqueous Extract of Cajanus Cajan seeds. Phytother. Res., 16:1-3.

Ogunyemi CM, Elujoba AA, Durosinmi MA (2008). Antisickling properties of Carica papaya, Linn, J. Nat. Prod., 1: 56-66.

Parida S, Patro VJ, Mishra US, Mohapatra L, Sannigrahi S (2010). Anthelmintic Potential of Crude Extracts and Its Various Fractions of Different Parts of Pterospermum Acerifolium Linn. Int. J. Pharm. Sci. Rev. Res., 1(2): 107-111.

Steinberg MH (2004). Sickle Cell Disease. Hematol., 1: 35.

Wambebe C (2001) Double-blind, placebo-controlled, randomized cross-over clinical trial of Niprisan in patients with Sickle cell disorder. Phytomed., 8: 252-261.

Zhang C, Li X, Lian L, Chen Q, Abdulmalik O, Vassiter V, Lai C, Asakura T (2004). Anti-sickling effect of MX -1520, a prodrug of vanillin: an *in vivo* study using rodents. Br. J. Hematol., 125: 788-795.