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Full Length Research Paper

Sodium metabisulphite induced polymerization of sickle cell haemoglobin incubated in extracts of three medicinal plants (*Anacardium occidentale, Psidium guajava* and *Terminalia catappa*)

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The present *in vitro* study ascertained the capacity of three medicinal plants, namely, *Anacardium occidentale, Psidium guajava* and *Terminalia catappa*, to alter polymerization of sickle cell haemoglobin (HbS). Spectrophotometric method was used to monitor the level of polymerization of haemolysate HbS molecules treated with sodium metabisulphite ($Na_2S_2O_5$) at a regular interval of 30 s for a period of 180 s in the presence of separate aqueous extracts of *A. occidentale, P. guajava* and *T. catappa*. At time intervals of 30 s, the level of polymerization was expressed as percentage of absorbance relative to the control sample at the 180th s. Although, extracts of the three medicinal plants caused significant ($p < 0.05$) reduction in polymerization of deoxyHbS molecules, the corresponding capacity in this regard diminished with increase in incubation time. Aqueous extract of *P. guajava* exhibited the highest capacity to reduced polymerization of deoxyHbS molecules. Whereas at t > 60 s, extract concentration of 400 mg% of *A. occidentale* activated polymerization of deoxyHbS molecules by 6.23 ± 1.34, 14.53 ± 1.67, 21.15 ± 1.89 and 24.42 ± 1.09%, 800 mg% of *T. catappa* at t > 30 s gave values of 2.50 ± 1.93, 5.09 ± 1.96, 10.00 ± 0.99, 15.21 ± 1.33 and 17.31 ± 0.97%. Therefore, the capacity of the three medicinal plants to interfere with polymerization of deoxyHbS molecules depended on duration of incubation and concentration of the extracts.

Key words: Polymerization, deoxyHbS, *Anacardium occidentale, Psidium guajava, Terminalia catappa*.

INTRODUCTION

Cashew *(Anacardium occidentale)* is a multipurpose tree of the Amazon and African rainforest that grows up to 15 m high. It has a thick and tortuous trunk with branches so winding that they frequently reach the ground. The cashew fruit is a rich source of vitamins, minerals and other essential nutrients (Taylor, 2005). Volatile compounds present in the fruit include esters, terpenes and carboxylic acids (Bicalho et al., 2001). The bark and leaves of cashew are a rich source of tannins, a group of plant chemicals with documented biological activity (Mota et al., 1985). The bark, leaves and shell oil around the nut are used medicinally as anti-inflammatory (Mota et al., 1985) and astringent preparations, which may be why cashew is effective in treating diarrhea (Kudi et al., 1999). Several clinical studies have shown that these biochemicals curb the darkening effect of aging by inhibiting tyrosinase activity (Kubo et al., 1994), protective against laboratory-induced diabetes (Swanston-Flatt et al., 1989; Kamtchouing et al., 1998), treatment of leishmanial ulcers due to *Leishmania* (Viannia) (Franca et al., 1996) and are toxic to certain cancer cells (Taylor, 2005). Antimicrobial properties activity of *A. occidentale* against *Escherichia coli* (Kudi et al., 1999; Akinpelu, 2001), *Pseudomonas* (Kudi et al., 1999) and *Helicobacter pylori* considered to cause acute gastritis and stomach ulcers have been reported (Ofusori et al., 2008).

Abbreviations: HbS, Sickle cell haemoglobin; **Glu,** glutamine; **Val,** valine.

Guava *(Psidium guajava)* is a tropical and semitropical plant. The branches are crooked causing opposite leaves to overlap. The flowers are white, incurved petals, 2 or 3 in the leaf axils. The fruit is small, 3 to 6 cm long, pear-shaped, reddish-yellow when ripe (Smith et al., 1992). Lectin chemicals in leaves of *P. guajava* have been shown to bind to *E. coli* (Coutino-Rodriguez et al., 2001), preventing its adhesion to the intestinal wall and thus, preventing infection (Lozoya et al., 1990). *P. guajava* fruit intake decreases blood pressure and serum high-density lipoprotein/cholesterol levels (Singh et al., 1992; Singh et al., 1993). The leaves of the guava tree in decoction are recommended for gastroenteritis (Tona et al., 1999), ulcers, vaginal and uterine problems and where an astringent remedy is needed (Taylor, 2005). Also, it has been used for spasms (Morales *et al.,* 1994), fevers, worm infections, kidney dysfuctions, epilepsy, and diabetes and even for cerebral infections (Taylor, 2005).

Indian almond (*Terminalia catappa*) tree can reach a height of 35 meters (110 ft). It grows upright and forms a symmetrical crown with horizontal branches distinctively arranged in tiers. As the tree ages, the crown will become increasingly flattened and eventually take on a vase shape (Corner, 1997). Indian almond leaves contain several different flavonoids, including kaempferol and quercetin (Indian almond, 2009). Flavonoids have been shown to possess a range of biological activities that are consistent with them contributing to the protection against degenerative diseases such as cancer, diabetes, cardiovascular diseases and cataract (Block et al., 1992; Steinmetz and Potter, 1996; Knekt et al., 2002). It is also rich in various tannins; astringent, bitter plant polyphenols that either bind and precipitate or denature protein molecules (Indian almond, 2009). While juice of the leaves is used as a folk remedy against various ailments that damages the skin, such as scabies and leprosy, the leaves themselves are used to dress rheumatic joints (Indian almond leaves, 2010). The young leaves are ingested by people suffering from intestinal parasites, dysentery and are believed to help get rid of colic in babies (Tan, 2001).

The sickle cell haemoglobin (HbS) is a product of a defective genetic code of haemoglobin molecule (Mehanna, 2001). The HbS molecule is prone to deoxygenation-induced polymerization and exhibits relatively low insolubility. The reason for this phenomenon is the consequence of substitution on the beta (β) chain of HbS molecule, a polar glutamic acid residue with non-polar valine molecule thereby, generates a sticky patch (Martin, 1993; Bindon, 2003). Specifically, only this one β6 Glu→Val residue of each tetramer resides an intermolecular contact region for deoxyHbS aggregation. The hydrophobic valine side chain appears to fit into a hydrophobic pocket formed by β88 leucine and β85 phenylalanine residues on an adjacent haemglobin molecule (Ivanova et al., 2000). The normal glutamic acid would not easily fit into this pocket explaining at least part of why deoxyHbA does not polymerize. The model described in sequential steps includes - nucleation, growth and subsequent alignment of the molecule into microfibrils parallel to each other with the resultant membrane deformity and damage (Ferrone and Rotter, 2004; Rotter et al., 2005). Charateristically, the red cells normal biconcave disc appearance is distorted to a spiculated sickle shape. This aberrant cell distortion accounts for the pathophysiology in this disease, namely, haemolytic anaemia, vascular stasis, occlusion and thrombosis.

The development of chemical modification agents that reduce the tendency of deoxyHbS to aggregate represents an important chemotherapeutic goal. Whereas haemoglobin carbamylation by cyanate (Jensen et al., 1973; Manning and Acharya, 1984) is a potentially effective anti-sickling agent, methyl acetylphosphate (MAP) has been reported to bind to the 2, 3-diphosphoglycerate (2, 3-DPG) binding site of haemoglobin, where it selectively acetylates residues, resulting in increased solubility of deoxyHbS molecules (Xu et al., 1999). *In vitro* studies by Abdulmalik et al. (2005), reported that 5-hydroxymethyl-2-furfural (5HMF) forms a high-affinity Schiff-base adduct with HbS molecules and inhibits sickle cell sickling. Hydroxyurea (Charache et al., 1995) and 2-imidazolines (Chang et al., 1983) are among few developed anti-sickling agents which interfere and disrupt contact point that promotes aggregation of deoxyHbS molecules.

However, limitations of these anti-sickling agents are undesirable structural and functional changes of haemoglobin as well as toxicity resulting from modifications of other protein molecules (Jensen et al., 1973; Manning and Acharya, 1984; Xu et al., 1999; Mehanna, 2001). The expliotation and utilization of vast varieties of herbal extracts may serve as alternative measures to deter aggregation of deoxyHbS molecules. Moreover, the specimens are commonly consumed plant materials and pose little or no toxic effects. Therefore, the present study seeks to ascertain the capacity of three medicinal plants (*A. occidentale, P. guajava* and *T. catappa*) to interfere with polymerization of HbS molecules.

MATERIALS AND METHODS

Collection plant specimen

Fresh samples of *A. occidentale, P. guajava* and *T. catappa* leaves were harvested between July and August, 2010, from trees within the environment of Imo State University, Owerri, Nigeria. The plant specimen were identified and authenticated by Dr. F. N. Mbagwu at the Herbarium of the Department of Plant Science and Biothechnology. A voucher specimen was deposited at the Herbarium for reference purposes.

Preparation of aqueous extract of plant specimen

The samples were washed under continous current of distilled

water for 15 min and air dried at room temperature for 60 min. The separate leaves were dried for 5 h in an oven at 65°C and ground with ceramic mortar and pestle. Two grams (2 g) each, of the pulverized specimen was suspended in 100 ml of distilled water and allowed to stand for 6 h. The aqueous extracts (2 g%) of *A. occidentale, P. guajava* and *T. catappa* leaves were obtained by filteration with Whatman No.1 filter paper. The prepared extracts were kept at 4°C in a refrigerator for at least 24 h before subsequent tests. Serial dilutions of the aqueous extracts in the order of 200, 400, 600 and 800 mg% were used for polymerization analyses.

Collection of blood samples/preparation of erythrocyte haemolysate

Five milliliters (5.0 ml) of human venous blood samples of HbSS genotype were collected by venipuncture and stored in EDTA anticoagulant tubes. The blood samples were obtained between July and August, 2010, from nine (9) male volunteers (59 to 79 kg) between the age bracket of 21 to 34 years, attending clinics at the Federal Medical Center (FMC), Imo State University Teaching Hospital (IMSUTH), Orlu, St. John Clinic / Medical Diagnostic Laboratories, Avigram Medical Diagnostic Laboratories and Qualitech Medical Diagnostic Laboratories. These centers are located in Owerri, Imo State, Nigeria. The Institutional Review Board of the Department of Biochemistry, Imo State University, Owerri, Nigeria, granted approval for this study and all volunteers involved signed an informed consent form. This study was in accordance with the ethical principles that have their origins in the declaration of Helsinki.

The erythrocytes were washed by centrifugation methods as described by Tsakiris et al. (2005). Within 2 h of collection of blood samples, portions of 1.0 ml of the samples were introduced into centrifuge test tubes containing 3.0 ml of buffer solution pH = 7.4: 250 mM tris (hydroxyl methyl) amino ethane HCl(Tris HCl)/140 mM NaCl/1.0 mM $MgCl_2$/ 10 mM glucose). The erythrocytes were separated from plasma by centrifugation at 1200 x g for 10 min, washed three times by the same centrifugation method with the buffer solution. The erythrocytes were finally re-suspended in 1.0 ml of this buffer and stored at 4°C. The washed erythrocytes were lysed by freezing/thawing as described by Galbraith and Watts (1980) and Kamber et al. (1984). The erythrocyte haemolysate was used for polymerization analyses.

Polymeriztion studies

Sodium metabisulphite ($Na_2S_2O_5$) induced polymerization of HbS molecules was ascertained as described previously by Iwu et al. (1988) with minor modification according to Chikezie et al. (2010). The underlying principle is that HbS molecules undergo gelation when deprived of oxygen; $Na_2S_2O_5$ was used as a reductant. The level of polymerization was monitored by recording increasing absorbance of the assay mixture with time. A 0.1 ml of HbS hemolysate was introduced into a test tube, followed by 0.5 ml of phosphate buffered saline solution (PBS; 9 g NaCl, 1.71 g $Na_2HPO_4.2H_2O$, 2.43 g $NaH_2PO_4.2H_2PO_4.2H_2O$ per liter of distilled water, pH = 7.4) and 1.0 ml of distilled water. The mixture was transferred into a cuvette and 3.4 ml of 2 g% aqueous solution of $Na_2S_2O_5$ was added. The absorbance of the assay mixture was recorded with a spectrophotometer (SPECTRONIC 20, Labtech-Digital Blood Analyzer®) at every 30 for 180 s at λmax = 700 nm (control sample). This procedure was repeated by substituting the distilled water with 1.0 ml of corresponding four increasing concentrations of the separate extracts (test sample). Percentage polymerization was calculated according to Chikezie et al. (2010):

Percentage polymerization = $[A_{t/c}/ Ac_{180^{th}sec}] \times 100$

Where, $A_{t/c}$ =Absorbance of test/control sample at time = t (s). $Ac_{180^{th}sec}$ = Absorbance of control sample at the 180^{th} s.

Statistical analyses

The results were expressed in terms of arithmetic means (×) ± standard deviation (SD). The statistical significance of the difference between the means was evaluated by student-t-test (Saunders and Trapp, 1994).

RESULTS

The pattern of increase in absorbance of the assay mixture with experimental time is illustrated in Figures 1 to 3. A cursory look at Figures 1 to 3 shows that, the rate of increase in absorbance was more rapid within the time range of 0 < t < 30 s than subsequent time intervals. The control sample gave absorbance of 0.052 ± 0.05 units at t = 180 s representing 100% polymeriztion of deoxyHbS molecules. However, the test sample containing 400 mg% of *A. occidentale* gave a maximum absorbance of 0.0647 ± 0.004 units at the 180^{th} s (polymeriztion = 126.86%). Furthermore, Figure 2 shows that the test sample containing 800 mg% of *P. guajava* exhibited the lowest absorbance value of 0/0073 ± 0.01 units at t = 30 s corresponding to 14.31 ± 2.11% polymeriztion (Table 1).

The levels of polymerization of deoxyHbS at specific time intervals when compared with the control sample at the 180^{th} s are presented in Table 1. In the control sample, although the levels of polymerization of deoxyHbS molecules were higher at t = 90, 120 and 150 s, they were not significantly different when compared with the value at t = 180 s ($p < 0.05$).

Addition of 200, 600 and 800 mg% aqueous extracts of *A. occidentale* to the assay mixture caused reduction of deoxyHbS polymerization within the experimental period (t = 0-180 s). However, the capacity of the three mentioned concentrations of *A. occidentale* to inhibit polymerization of deoxyHbS molecules diminished as the experimental time progressed. Specifically, Table 2 shows that in the presence of extract concentrations of 200, 600 and 800 mg%, the capacity of *A. occidentale* to inhibit deoxyHbS polymerization fell within the ranges of 33.96 to 1.92, 45.83 to 14.81 and 34.79 to 17.88%, respectively. Extract concentration of 400 mg% of *A. occidentale* caused reduction in polymerization of deoxyHbS molecules at t = 30 s; representing 11.04 ± 1.43% inhibition (Table 2). Paradoxically, further increases in incubation time engendered activation of deoxyHbS polymerization above the control/reference values (t = 180 sec; 100% polymerization: Table 2). Furthermore, Table 2 shows at every time intervals of 30 sec, within the values of t > 60 s, the levels of activation gave 6.23 ± 1.34, 14.53 ± 1.67, 21.15 ± 1.89 and 24.42 ± 1.09%.

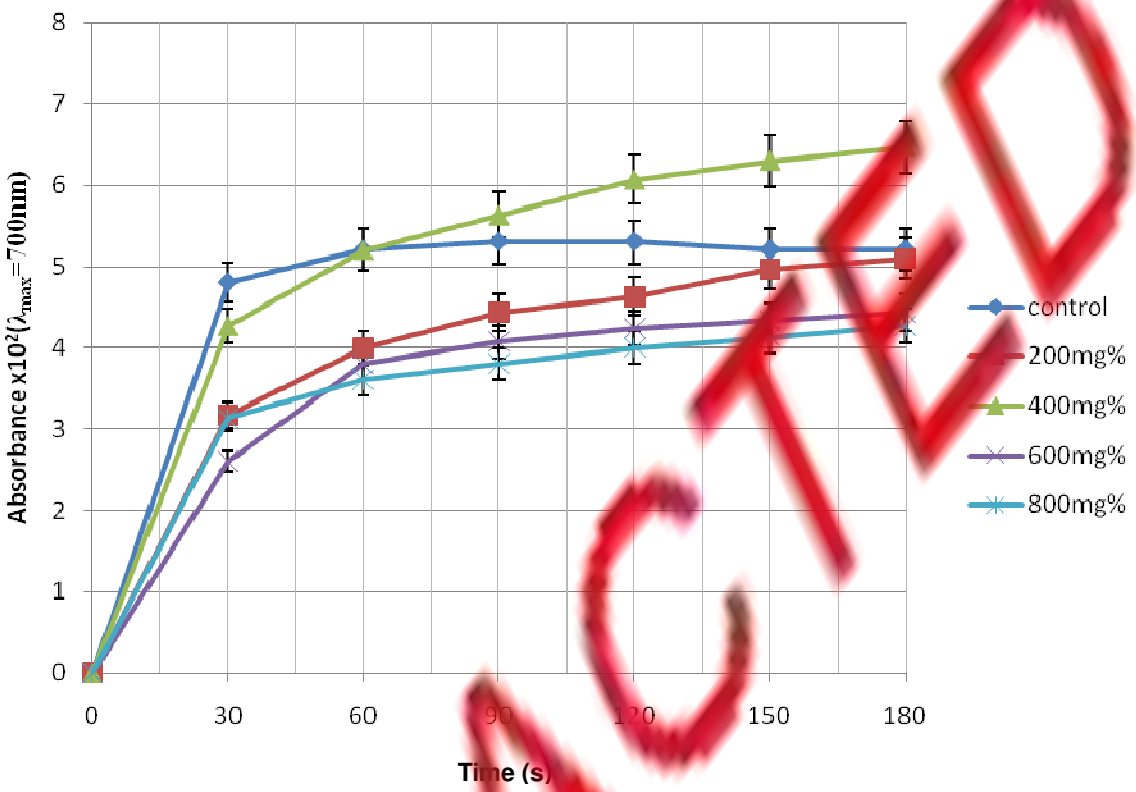

Figure 1. Change in absorbance of erythrocyte haemolysate of HbSS genotype in the presence of aqueous extract of *A. occidentale.*

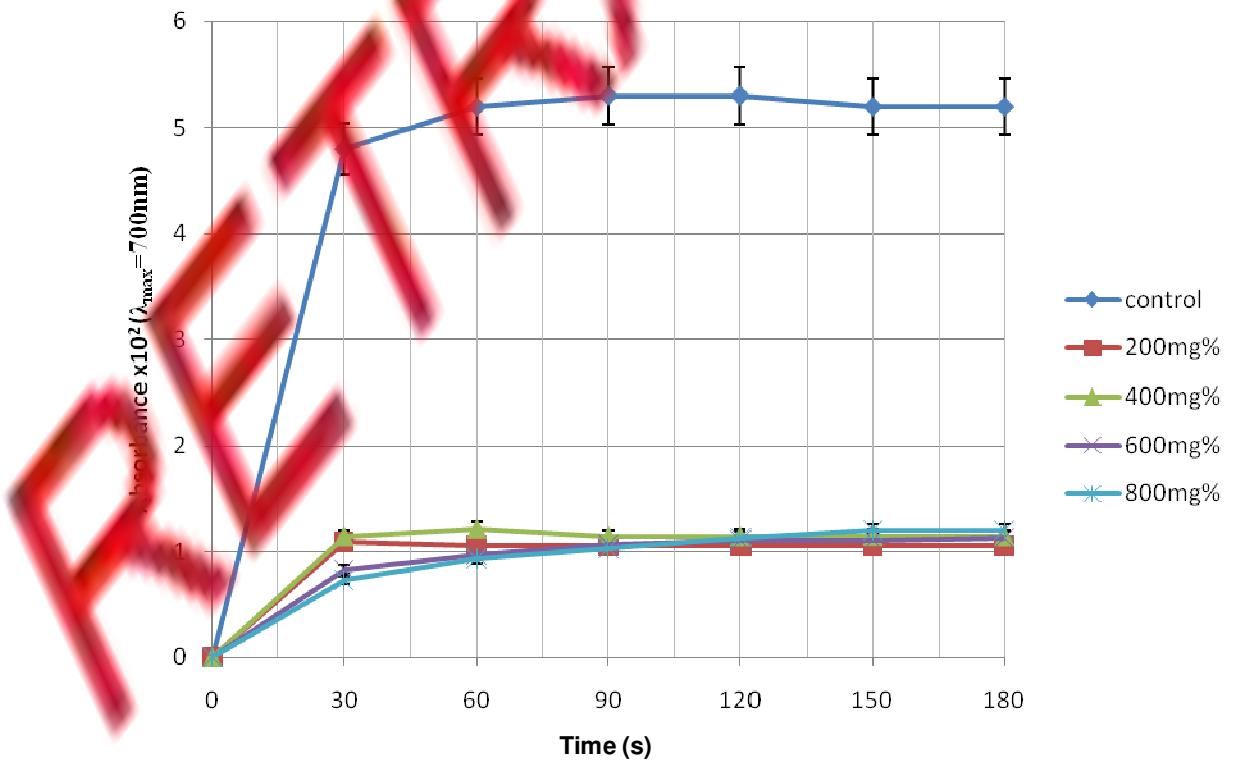

Figure 2. Change in absorbance of erythrocyte haemolysate of HbSS genotype in the presence of aqueous extract of *P. guajava.*

Table 1. Comparative levels of polymerization of deoxyHbS (t = 180th s) in the presence of aqueous extracts of *A. occdentale, P. guajava* and *T. catappa*.

Time (s)	Polymerization (%)						
	0	30	60	90	120	150	180
Control (n = 9)	0.00 ± 0.00	93.53 ± 1.06	100.00 ± 1.14	101.96 ± 1.13	101.96 ± 2.14	101.37 ± 1.02	100.00 ± 0.65
***A. occidentale*; n = 9**							
200 mg%	0.00 ± 0.00	62.16 ± 1.03	78.43 ± 1.02	86.86 ± 1.13	90.78 ± 0.95	97.45 ± 0.99	100.00 ± 1.33
400 mg%	0.00 ± 0.00	83.73 ± 0.97	101.96 ± 1.00	119.65 ± 1.07	110.39 ± 0.95	123.53 ± 0.56	126.86 ± 1.78
600 mg%	50.98 ± 1.06	0.00 ± 0.00	74.51 ± 0.98	79.98 ± 0.9[illegible]	82.94 ± 0.56	84.90 ± 1.22	86.86 ± 1.11
800 mg%	61.37 ± 0.96	0.00 ± 0.00	70.59 ± 1.00	74.51 ± 1.66	78.43 ± 1.88	80.98 ± 0.96	83.73 ± 1.33
***P. guajava*; n = 9**							
200 mg%	0.00 ± 0.00	21.37 ± 1.34	20.59 ± 2.32	20.61 ± 1.88	20.59 ± 2.11	20.62 ± 2.00	20.63 ± 2.22
400mg%	0.00 ± 0.00	22.55 ± 0.77	23.92 ± 0.99	22.56 ± 1.34	22.55 ± 1.23	22.57 ± 1.77	22.55 ± 1.00
600 mg%	0.00 ± 0.00	16.27 ± 0.88	19.02 ± 1.54	20.98 ± 1.43	21.57 ± 0.97	21.76 ± 0.98	22.16 ± 1.34
800 mg%	0.00 ± 0.00	14.31 ± 2.11	18.24 ± 1.78	20.20 ± 1.56	22.16 ± 2.00	23.53 ± 1.32	23.53 ± 1.43
***T. catappa*; n = 9**							
200 mg%	0.00 ± 0.00	75.10 ± 1.34	79.02 ± 2.34	81.76 ± 2.43	82.35 ± 1.09	84.31 ± 1.22	84.31 ± 1.89
400 mg%	0.00 ± 0.00	83.73 ± 1.43	92.75 ± 1.54	94.41 ± 1.43	101.96 ± 1.32	103.92 ± 0.97	105.88 ± 0.76
600 mg%	62.75 ± 1.[illegible]	0.00 ± 0.00	71.18 ± 1.43	75.10 ± 1.09	80.39±0.98	82.94 ± 0.89	84.31 ± 1.00
800 mg%	0.00 ± 0.00	92.[illegible] ± 1.23	104.51 ± 0.85	109.22 ± 1.33	114.31 ± 1.11	117.64 ± 1.37	119.61 ± 1.20

The results are means (X) ±S.D of nine (n = 9) determinations.

The four concentrations of *P. guajava* reduced polymerization of deoxyHbS molecules throughout the experimental time (Table1). Specifically, aqueous concentration of 600 mg% caused maximum inhibition of 84.75% at the 30th s (Table 2), which corresponded to 14.31% polymerization when compared with the control sample at t = 180 s (Table 1). Generally, between t = 30 to 180 s, whereas, the diminishing capacity of *P. guajava* extract to inhibit polymerization of deoxyHbS molecules were as follows: 600 mg (4.44) and 800 mg% (7.87%), inhibition was sustained and increased by 200 mg (2.48) and 400 mg% (1.84%).

Although, 800 mg% extract concentration of *T. catappa* hindered polymerization of deoxyHbS by 1.46% at t =30 s, further increases in experimental time (t > 60 s), engendered activation of the polymerization process. The levels of polymerization were 2.50, 5.09, 10.00, 15.38 and 17.31% greater than the control sample at corresponding time intervals of 30 s (Table 2). Also, between the 150th and 180th s, extract concentration of 400 mg% caused activation of polymerization of deoxyHbS molecules by 1.89 and 3.85%, respectively (Table 2). The maximum capacity of aqueous extract of *T. catappa* to inhibit polymerization of deoxyHbS molecules occurred at t = 30 s, in the presence of extract concentration of 600 mg%. However, the level of inhibition attenuated as experimental time progressed.

DISCUSSION

An overview of the present study showed increasing level of polymerization of deoxyHbS molecules with time that is consistent with the pattern described elsewhere (Nwaoguikpe and Uwakwe, 2005; Chikezie, 2006; Chikezie et al., 2010). *In vitro* deoxygenation of haemolysate HbS molecules by sodium metabisulphite caused

Table 2. Relative levels of inhibition/activation of DeoxyHbs polymerization (t = 30 s intervals) in the presence of aqueous extracts of *A. occdentale, P. guajava* and *T. catappa.*

	Polymerization inhibition/activation (%)					
Time (s)	**30**	**60**	**90**	**120**	**150**	**180**
***A. occidentale*; n = 9**						
200 mg%	33.96 ± 1.54	23.08 ± 1.09	12.64 ± 1.34	16.42 ± 0.98	4.42 ± 1.11	1.92 ± 1.87
400 mg%	11.04 ± 1.43	0.01 ± 0.11	14.53* ± 1.67	6.23* ± 1.34	1.15* ± 1.89	24.42* ± 1.09
600 mg%	45.83 ± 1.34	26.92 ± 1.76	20.19 ± 1.12	23.21 ± 1.09	16.73 ± 1.66	14.8 ± 2.09
800 mg%	34.79 ± 1.32	30.77 ± 0.99	28.30 ± 0.67	24.53 ± 0.89	20.58 ± 1.67	17.38 ± 1.77
***P. guajava*; n = 9**						
200 mg%	77.29 ± 1.01	79.81 ± 1.34	80.19 ± 1.78	80.7 ± 1.99	79.78 ± 1.0	79.77 ± 1.37
400 mg%	76.04 ± 1.43	76.54 ± 1.32	78.30 ± 1.11	78.29 ± 1.09	77.8 ± 1.28	77.88 ± 0.99
600 mg%	82.71 ± 1.00	81.35 ± 1.95	79.25 ± 0.90	79.81 ± 1.45	78.65 ± 0.69	78.27 ±1.56
800 mg%	84.79 ± 1.99	82.12 ± 1.98	80.57 ± 0.67	78.68± 1.07	76.92 ± 0.97	76.92 ± 1.43
***T. catappa*; n = 9**						
200 mg%	20.21 ± 1.77	22.50 ± 1.27	21.32 ± 1.34	20.75 ± 2.04	17.31 ± 1.09	17.31 ± 1.04
400 mg%	11.04 ± 1.54	9.04 ± 1.00	1.89 ± 1.90	4.34 ± 1.71	1.89* ± 1.00	3.85 ± 1.43
600 mg%	37.47 ± 1.48	30.19 ± 1.00	27.74 ± 1.07	22.64 ± 1.45	18.65 ± 1.04	17.31 ± 1.07
800 mg%	1.46 ± 1.66	2.50* ± 1.93	5.09* ± 1.96	10.00* ± 0.99	15.38* ± 1.33	17.31* ± 0.97

The results are means (X) ±S.D of nine (n = 9) determinations. *Activation (%).

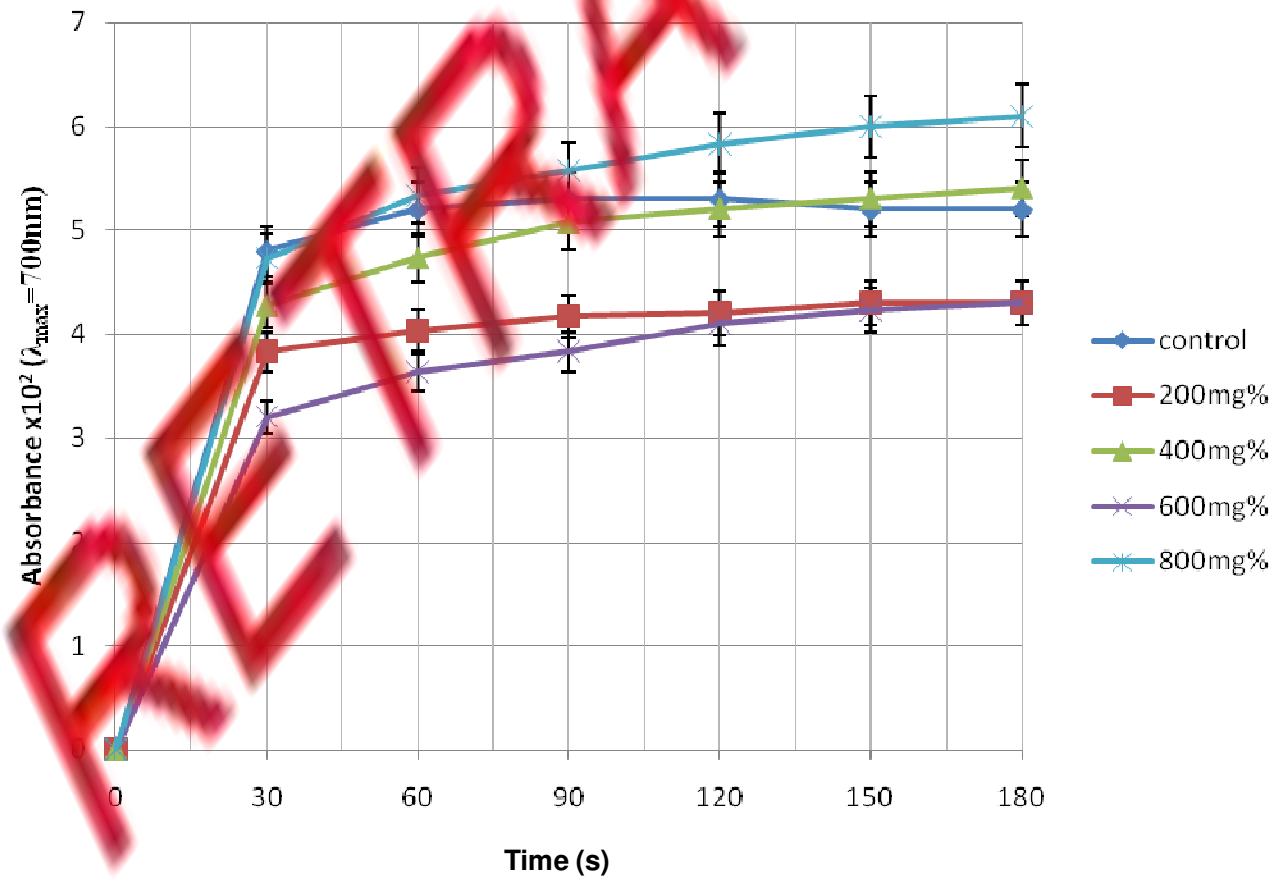

Figure 3. Change in absorbance of erythrocyte haemolysate of HbSS genotype in the presence of aqueous extract of *T. catappa.*

aggregation and polymeriztion of the individual haemoglobin molecules. This process of gelation (polymerization) of haemoglobin molecules resulted in increasing absorbance of the assay solution.

Although, after 60 s extract concentrations of 400 mg% of *A. occidentale* and 800 mg% of *T. catappa* activated polymerization of deoxyHbS molecules, the present study showed that, aqueous extracts of the three medicinal plants (*A. occidentale, P. guajava* and *T. catappa*) exhibited variable capacity to hinder polymerization of deoxyHbS molecules. In conformity with earlier reports of Oyewole et al. (2008), a measure of inhibition of deoxyHbS polymerization by these extracts was found to be dose and time dependent. Previous reports have proposed the use of herbal preparations as candidate for management of sickle cell disease (Ekeke and Shode, 1985; Kade et al., 2003; Chikezie, 2006; Okpuzor et al., 2008; Imaga et al., 2009; Imaga et al., 2010). Their proposals were drawn from the fact that these plant extracts, from *in vitro* studies, exhibited anti-sickling/ polymeriztion property. The findings of this research are comparable to those previous reports. Research findings have established that the capability of a biomolecule to impede *in vitro* polymerization depends on one or combinations of the following options: (a) The tendency and efficiency to bind to the complimentary contact region/site of deoxyHbS monomers (Chang et al., 1983; Charache et al., 1995; Abdulmalik et al., 2005); (b) modification of amino acid residues that contribute to the three dimensional structures of HbS contact region and other critical sites (Oyewole et al., 2008; Manning and Acharya, 1984; Xu et al., 1999); (c) stabilization of the R (relaxed) state of HbS molecule (Oyewole et al., 2008; Kark et al., 1978; Manning and Acharya, 1984; Stuart et al., 1994).

The diminishing capacity of the three plant extracts to inhibit polymerization of deoxyHbS molecule with progression of experimental time suggest that the constituents of the extracts did not covalently modify the amino acid residues unlike other reported compounds (Jensen et al., 1973; Manning and Acharya, 1984; Charache, et al., 1995; Xu et al., 1999; Mehanna, 2001; Chang et al., 1983; Abdulmalik et al., 2005). Rather, the anti-polymerization principles of the plant extracts may have formed a relatively weaker hydrophobic interaction with the contact regions of HbS molecules that temporarily reduced polymerization of HbS monomers. Furthermore, the protein/ligand associations may have transiently stabilized the R-state conformation, but were subsequently displaced by more thermodynamically favourable interactions that engendered and promoted haemoglobin polymerization. Therefore, the capacity of the three extracts to inhibit HbS polymerization was not sustained with the progress of experimental time.

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