Comparative Evaluation of Phytochemicals and Antisickling Activities of Extracts of Fruits of Phoenix *Dactylifera* and Leaves of *Terminalia Catappa*

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Abstract- ***Dried and blended fruits of Phoenix dactylifera_L. and leaves of Terminalia catappa L. were soaked with methanol, the water soluble extracts were obtained by decoction while the methanol soluble extracts was macerated and fractionated with n-hexane, chloroform and ethyl-acetate. The chemical screening of extracts, revealed the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins and tannins. The plants extracts were evaluated against sickled red blood cells using p-hydroxybenzoic acid and normal saline as positive and negative controls. The method involves inhibition of sodium metabisulphite induced sickling of HbSS red blood cells, collected from sickle cell patients. The methanol soluble extracts PDME and TCME (5mg/ml) showed antsickling activities of 78.2% and 82.8% at 180min of incubation respectively while the chloroform soluble extracts PDCE and TCCE (5mg/ml) demonstrated antisickling activities of 77.6% and 86.7% respectively at 180min incubation time. The n-hexane soluble extracts, PDHE and TCHE (5mg/ml) showed antisickling activities of 71.8% and 78.3% while that of the ethyl-acetate soluble extracts, PDEE and TCEE(5mg/ml) indicated antisickling activities of 70.2% and 83.5% respectively at 180min incubation time compared to p-hydroxybenzoic acid (5mg/ml) with 76.2% inhibition at 180min time of incubation and the water soluble extracts, PDWE and TCWE (5mg/ml) which showed 10.9% and 12.4% sickled red blood cells at 180min incubation time indicating antisickling activities of 89.1 and 87.6 respectively. Compared to the untreated SS suspension with 83.6% sickled red blood cells at 180mm incubation. These results showed that the fruits of date palm and leaves of Terminalia catappa are good agents for sickle cell disease therapy.***

Indexed Terms- phytochemicals, sickle cell anaemia, Antisickling activities, phoenix dactylifera, terminalia catappa.

I. INTRODUCTION

Several diseases cause millions of deaths in the world, and particularly in Africa. Among these, is the sickle cell disease (SCD) that affects more than 50 million people. Each year, approximately 100,000 children are born worldwide with this hemoglobinopathy i.e. sickle cell disease which is a genetic disorder. This disease is considered as a public health problem in many countries, but with a major burden in Africa particularly in tropical regions in west and central Africa (Buchanan, *et al*., 2004). Red blood cells (RBCs) or erythrocytes are made up of two main components, the cytoplasmic proteins and the membrane.

Haemoglobin (Hb) constitutes 97.5% (by weight) of the protein system while the other 2.5% proteins provide energy and help regulate water and ionic composition of the cell. Membrane protein are involved in active ion transport which keeps the intracellular sodium ion (Na^{+}) and calcium ion ($Ca^{2+)}$ concentration low and that of potassium ion ($K^{+)}$ and magnesium ion (Mg^{2+}) high. The proteins provide also peculiar flexibility to the RBCs membrane (Elekwa *et al.*, 2003; osuagwu and Mbeyi, 2007). In sickle cell anaemia, the shortened RBCs survival is due to increased rigidity of cells and membrane damage caused by intracellular precipitation of Hbs. Hbs aggregation (polymerization) induces a panoply of cellular and tissue injuries (substantial loss of membrane flexibility, sickle shaped e.t.c) (Buchanan *et al*., 2004).

Sickle cell anemia is a severe hereditary form of anemia in which a mutated form of hemoglobin

distorts the red blood cells into a crescent shape at low oxygen levels. Up to date, there is no affordable solution for this disorder. Several therapeutic options were tried in order to fight against SCD without appropriate solution for poor African population. All of these therapeutic approaches are either expensive or toxic and are not accessible to the populations with low incomes (Mehanna, 2001).

Various approaches have been adopted in an effort to find agents that inhibit the polymerization of haemoglobin (Hb) and hence prevent or reduce the occurrence of crisis in SCD (Iyamu *et al*., 2002). Towards this goal, oxygen, carbon monoxide and sodium nitrite were used to reduced the amount of deoxy-Hb. The above approaches did not give the much-needed beneficial effects based on the reduction of painful crisis as the criterion for successful treatment (Iyamu *et al*., 2002). Bone marrow transplantation has in recent years been found to be an efficient but practically impossible method in developing countries in controlling the scourge. The cost implications, the availability of necessary expertise and the problems of finding suitable donors, however, constitute a major setback to this approach in third world countries with weak economies like Nigeria. While genetic counseling holds a prominent position in enlightening the population about this condition and have been found to be beneficial in guiding people with respect to the choice of a mate, its role in eradicating the condition is not feasible because of balanced polymorphism and the fact that providing the right counsel does not necessarily lead to the rational choice of a mate (Moody *et al*., 2003; Akinsulie *et al*., 2005).

Furthermore, several current research activities are focused on identifying new drugs that are capable of preventing the loss of water from red blood cells (RBCs) or increasing the level of foetal haemoglobin, a variety of haemoglobin that prevents the sickling of RBCs. Clotrimazole, hydroxyurea and erythropoietin (a genetically engineered hormone that stimulates RBC production) were proposed in this regard. Unfortunately, these drugs are known for their serious side effects, hence, limiting their clinical use (Mehanna, 2001; Akinsulie *et al*., 2005).

Presently, first-line clinical management of sickle cell anemia include use of folic acid, amino acids (as nutritional supplements), penicillin-prophylaxis (helps prevent infection) and anti-malarial prophylaxis (helps prevent malaria attack). The faulty ‘s’ gene is not eradicated in treatment, rather the condition is managed and synthesis of red blood cells induced to stabilize the patients hemoglobin level. Further management and treatment of this disorder with compounds or techniques which directly affect the hemoglobin (Hb) molecule (e.g. hydroxyurea, bone marrow transplantation and blood transfusion) are very expensive and out of reach for the low income earners and besides may expose the patient to mutagenicity, iron overload and fatal risks (Brittain and Han, 2004).

Medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain. The use of traditional medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). Medicinal plant is defined as any plant with one or more of its organs containing substance that can be used for therapeutic purpose or which can be used as precursors for the synthesis of drugs. The bioactive ingredients that have the therapeutic activity in plants used in traditional practice are mostly unidentified and traditional healers believe in the holistic nature of their treatment. Substances found in medicinal plants, containing the healing property of plants are known as the active principle. In recent years, these active principles have been extracted and used in different forms such as infusions, syrups, concoctions, infused oils, essential oils, ointments and creams (Sofowora, 1993). The most important of these chemically active constituents of plants are; steroids, terpenoids, carotenoids, flavonoids , alkaloids tannins, saponins and glycosides. Many of these indigeneous medicinal plants are also used for medicinal purposes (Edeoga, 2005). A wide range of medicinal plant parts are used as extract for raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit and twigs exudates While some of the these raw drugs are collected in smaller quantities by folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as raw materials for many herbal industries (Uniyal *et al*., 2006).

The use of natural products in attempts at inhibiting sickling could be as old as when the sickle cell (SC) disease was discovered. Traditional history has indicated attempts made by inhabitants using plant derived recipes in parts of Nigeria to treat what they described as "fever of crises", shifting joint pains, exacerbations and "constant abnormality of the blood, though relatively few have been validated scientifically. With the associated side effects of modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions (Thomas and Ajani, 1987).

Phytochemistry and pharmacomodulation are some of the ways used to search for new drugs; research on the secondary metabolites of plants are desirable for the discovery of thier medicinal potential and to find the actual value of their therapeutic uses. Synthetic drugs are often the option for chemotherapy. Hoewever, most synthetic drugs kills not only targeted cells, but also normal cells, and most have severe side effects. There is, therefore, an urgent need for novel treatment options with improved features.

Research on the phytomedicine for the treatment of SCD has led to the development of Niprisan (a herbal based drug) which has been patented by the National institute for pharmaceutical Research and development (NIPRID), Abuja, Nigeria and produced to meet increasing global demand by sufferers of SCD. This development indicates that more of such herbal based drugs could be consequent upon scientific investigations on plants that are used in folklore medicine (Ogunyemi *et al*., 2008).

Alternative strategy in the management of SCD is now focusing on the identification of the novel antisickling agents mainly from medicinal plants. The world health organization supports the use of traditional medicine provided they are proven to be efficacious and safe (W.H.O., 1985).

Some medicinal plants have these last years, shown an antisickling activity. What indicates a new therapeutic way to the range of the poor African populations which are affected by this hemoglobinopathy. The fact that the seeds of *Cajanus cajan* accumulates phenylalanine, an aromatic amino acid known to possess sickling activity suggest that other plants parts could contain this acid or other amino acids which are known to have antisickling activity. The amino acid "tyrosine and "tryptophan", as well as small peptides containing these amino acids, have antisickling activity. Other plants reported to be used in the management of sickle cell anemia includes aqueous extracts of *Lawsonia inermis* (henna plant) which was found to inhibit sickling and increase the oxygen affinity of sickled red blood cells (Hbss) during a screen or study of substances known to bind proteins (Dean and Schechter, 1978; Ekeke and Shode, 1990). Since *Terminalia catappa* (almond) and *Phoenix dactylifera* (date palm) thrives in hostile environment, it is clear that secondary metabolites (photochemicals) play an important role in conferring a protective effect against high temperature, Uv-damage, and tolerance to drought and salinity; Proven deleterious to plant pathogens and pests. (Balandrin and Wocke, 1988).

Research into the phytotherapy of diseases is a current trend in the management of tropical and genetic disorders like sickle cell anemia, with a view to finding cheaper, alternative and less toxic therapies the poor and teeming population can have immediate access to (Sofowora, 1975; Ekeke and Shode, 1985). This research work is therefore justified since all the therapeutic approaches are either expensive or toxic and are not accessible to the populations with low incomes, coupled with the fact that very few ethnomedicinal remedies for the treatment of sickle cell anemia have been reported due to secrecy attached to the treatments of the disease. This research will therefore, go a long way in the scientific exploration of medicinal plants for the benefit of man and is likely to decrease the dependence on synthetic drugs. This study was aimed to

i. Collect and identify two plant parts; leaves of *Terminalia catappa* and fruits of *Phoenix dactylifera* .
ii. Subject the two plant materials (Almond and date palm) to different extractions.
iii. Subject the different extracts to photochemical screening.
iv. Evaluate the antisickling activities of the different extracts *in vitro* on blood samples from sickle cell patients.

v. Compare the antisickling activity of the extracts of *Phoenix dactylifera to that of Terminalia Catappa.*
vi. Find which fraction of the extracts will comparatively be most effective at inhibiting cell sickling.
vii. Create possible opportunities for alternative, cheaper and less toxic therapies for sickle cell disease management thereby decreasing the dependence on synthetic drugs.
viii. Verify claims by traditional healers and validating the ethno-medicinal use of these plant species.

II. MATERIALS AND METHODS

Samples: a. Date palm fruits (Dabino)
b. Almond leaves (Umbrella tree, Ebelebo)
c. Whole blood

Samples collection and Preparation

Plant materials
Soft date palm fruit was bought from Hausa quarter, Auchi, Edo State. Fresh leaves of *T. catappa* were collected from Faculty of Natural Sciences, Ambrose Alli University main Campus, Ekpoma. Identified and unthenticated at Pax herbal Herbarium, Ewu, Edo state.

Blood Sample (Ekeke and Shode, 1985)
Blood samples to test the antisickling activity of the plant extracts in this study were obtained from each of 6 known and confirmed sickle cell anemia patients (HbSS) not in crisis from Irrua specialist Hospital, Irrua, Edo State, Nigeria. The patients who were aged between 10-24 years and of both sexes were in reasonable good health, and were not transfused recently with HbAA blood.

III. EXTRACTIONS

The blended plant materials of *Phoenix dactylifera* and *Terminalia catappa* were subjected to various extractions as described below:

AQUEOUS (WATER) EXTRACTS
The water soluble fractions were extracted by decoction according to (sofowora *et al*, 1975, Ogunyemi *et al*., 2008).

METHANOLIC EXTRACTS

The methanolic soluble fractions were extracted, macerated and fractionated according to (Ogunyemi, et al., 2008).

PHYTOCHEMICAL SCREENING

Chemical tests were carried out on samples of plants extracts for preliminary phytochemical screening to determine the secondary metabolites using standard procedures as described by Harbone (1973); Trease and Evans (1989); Sofowora (1993); Bruneton (1999), for the presence of Alkaloids, Flavonoids, Anthraquinones, Saponins, Tannins, and Cardiac Glycosides.

ANTI-SICKLING ASSAY
Preparation of plant extracts for analysis: The procedures of Ogunyemi, *et al*., (2008) and Imaga *et al.,* (2009) were used in preparing the plant extracts for analysis of anti-sickling activity.

PROCEDURE FOR ANTI-SICKLING ACTIVITY EVALUATION
The evaluation of the different extracts for antisickling activities was carried out using modified methods of Sofowora, *et al*., (1975); Ekeke and Shode, (1985); and Imaga *et al*., (2009).

IV. RESULTS AND DISCUSSION

RESULTS
Table 1: shows the physical characteristics of the plants fractions/extracts obtained from the decoction, extraction and maceration processes.

Table 1: physical characteristics of the Extracts from fruits of *Phoenix dactylifera* and leaves of *Terminalia catappa*

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Fraction	Solvent	Colour Appearance
PDWE	Water	Brownish yellow Viscous, gummy and syrup-like

PDME	Methanol	Brownish yellow Viscous and syrup-like liquid	
PDHE	n-hexane	Yellow Gummy and pasty syrup	
PDCE	Chloroform	Greenish yellow Viscous and oil-like liquid	
PDEE	Ethyl-acetate	Yellow Syrup-like and gummy liquid	
TCWE	Water	Dark green	Dark green solid extract
TCME	Methanol	Dark green	Dark green solid extract
TCHE	n-hexame	Green	Green solid extract
TCCE	Chloroform	Greenish black	Solid extract
TCEE	Ethyl-acetate	Green	Green solid extract

KEY
PDWE = *Phoenix dactylifera* fruits water extract
PDME = *Phoenix dactylifera* fruits methanolic extract
PDHE = *Phoenix dactylifera* fruits n-hexane extract
PDCE = *Phoenix dactylifera* fruits chloroform extract
PDEE = *Phoenix dactylifera* fruits ethyl-acetate extract. TCWE = *Terminalia catappa* leaves water extract.
TCME = *Terminalia catappa* leaves methanolic extract., TCHE = *Terminalia catappa* leaves n-hexane extract.
TCCE = *Terminalia catappa* leaves chloroform extract., TCEA = *Terminalia catappa* leaves ethyl-acetate extract.

The result of phytochemical screening revealed the presence of alkaloids, saponins and other secondary metabolites as shown in table 2. below.

Table 2: Phytochemical screening of the plant extracts of the fruits of *Phoenix dactylifera* and leaves of *Terminalia catappa*

Phytochemical	PDWE	PDME	PDHE	PDCE	PDEA	TCWE	TCME	TCHE	TCCE	TCEE
Alkaloids	+	+	+	-	+	+	+	-	+	-
Anthraquinones	-	+	+	+	+	-	+	+	+	+
Flavonoids	+	+	+	+	+	+	-	-	-	-
Saponins	+	+	+	+	+	+	+	-	-	+
Tannins	+	+	+	+	+	+	+	-	-	+
C. glycosides	+	+	+	+	+	+	+	+	+	+

KEY
PDWE = *Phoenix dactylifera* fruits water extract
PDME = *Phoenix dactylifera* fruits methanolic extract
PDHE = *Phoenix dactylifera* fruits n-hexane extract
PDCE = *Phoenix dactylifera* fruits chloroform extract
PDEE = *Phoenix dactylifera* fruits ethyl-acetate extract
TCWE = *Terminalia catappa* leaves water extract
TCME = *Terminalia catappa* leaves methanolic extract
TCHE = *Terminalia catappa* leaves n-hexane extract
TCCE = *Terminalia catappa* leaves chloroform extract
TCEA = *Terminalia catappa* leaves ethyl-acetate extract
+ = Present
- = Absent

ANTISICKLING ACTIVITIES OF THE DIFFERENT FRACTIONS/EXTRACTS FROM

FRUITS OF *Phoenix dactylifera* AND LEAVES OF *Terminalia catappa* USING P-HYDROXYLBENZOIC ACID AND NORMAL SALINE AS CONTROLS.

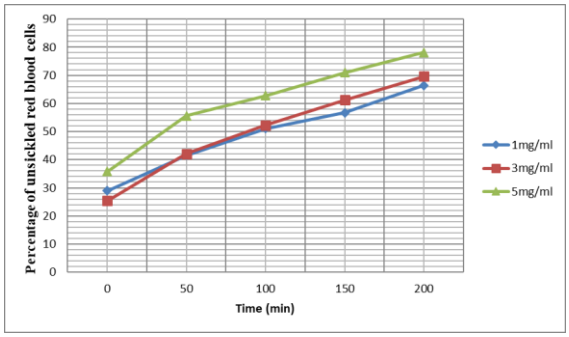

Figure 1: Effect of *Phoenix dactylifera* methanolic extract (PDME) on the sickled red blood cells at different incubation time.

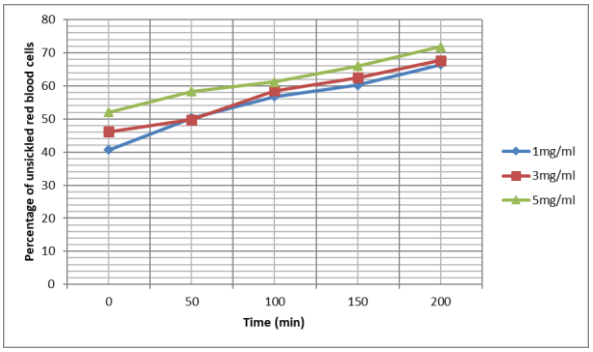

Figure 2. Effect of *Phoenix dactylifera* n-hexane extract (PDHE) on the sickled red blood cells.

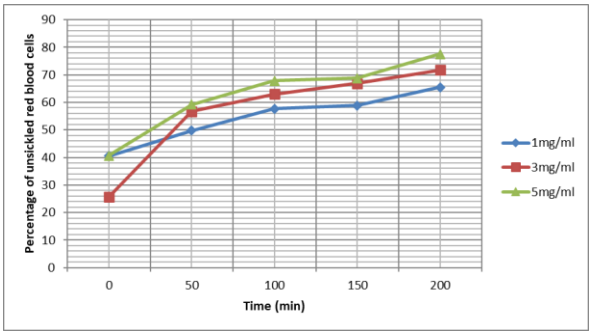

Figure 3. Effect of *Phoenix dactylifera* chloroform extract (PDCE) against the sickled red blood cells at different incubation time.

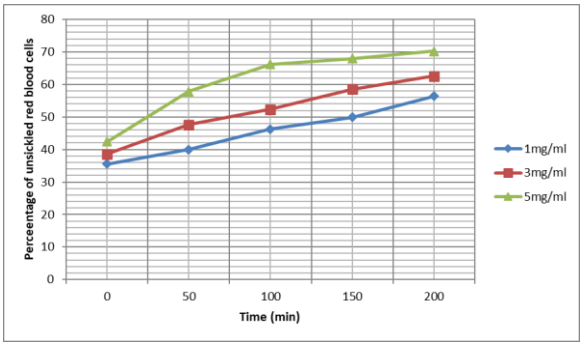

Figure 4. Effect of *Phoenix dactylifera* ethyl-acetate extract (PDEE) on the sickled red blood cells at different incubation time.

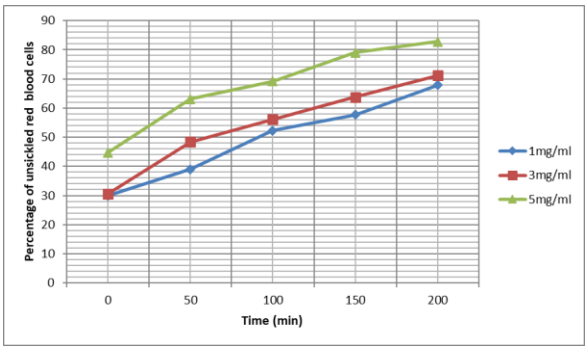

Figure 5. Effect of the *Terminalia catappa* methanolic extract (TCME) on the sickled red blood cells at different incubation time.

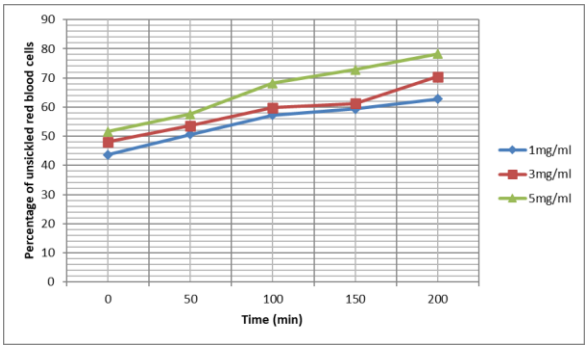

Figure 6. Effect of the *Terminalia catappa* n-hexame extract (TCHE) on the sickled red blood cells.

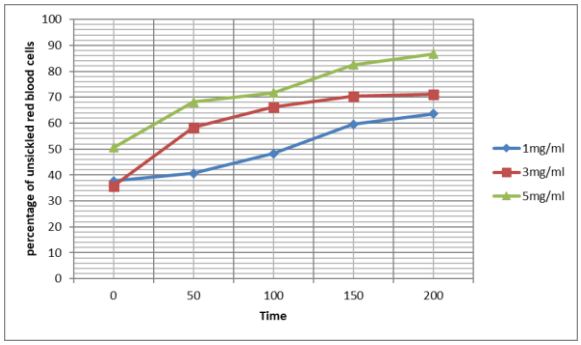

Figure 7. Effect of the *Terminalia catappa* chloroform extract (TCCE) on the sickled red blood cells.

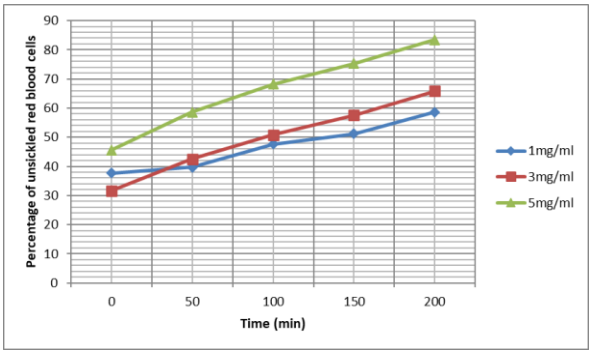

Figure 8. Effect of the *Terminalia catappa* ethyl-acetate extract (TCEE) on the sickled red blood cells.

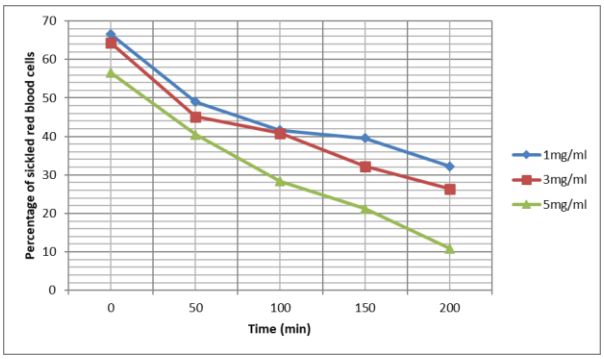

Figure 9. Effect of the *Phoenix dactylifera* water extract (PDWE) on the sickled red blood cells.

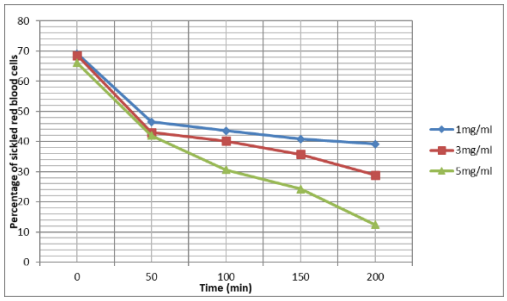

Figure 10. Effect of the *Terminalia catappa* extract (TCWE) on the sickled red blood cells.

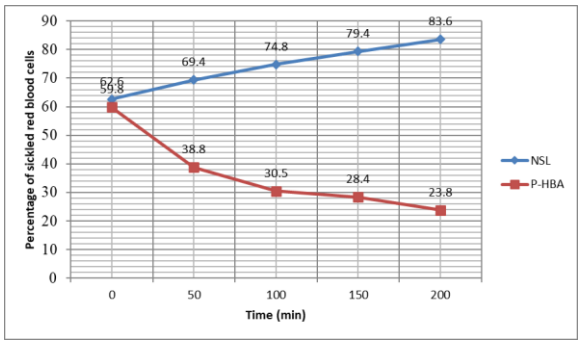

Figure 11. Effect of p-hydroxybenzoic acid (5mg/ml) and normal saline on the sickled red blood cells used as positive and negative controls.

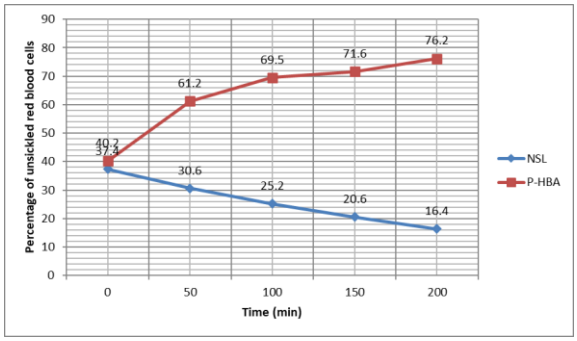

Figure 12. Effect of p-hydroxybenzoic acid (5mg/ml) and normal saline on the sickled red blood cells used as positive and negative controls.

DISCUSSION

The main goal of this work was to investigate the potential antisickling properties of the fruits of *Phoenix dactylifera* and leaves of *Terminalia catappa* and their chemical constituents comparatively, providing scientific validation for their use in traditional medicine. Five extracts each from the plants species were screened for their potential antisickling properties against sickle cell blood. The extracts were prepared by extracting the plants material with water, methanol, n-hexane, chloroform and ethyl-acetate.

The plants fractions/extracts showed different physical appearances. The water extract of *P. dactylifera* was found to be sticky, gummy and highly viscous yellowish syrup-like liquid while that of the organic solvents (methanol, n-hexane, chloroform and ethyl-acetate), though gummy and viscous oil-like liquid, were less viscous than that of the water extract. All

extracts of *T. catappa* were found to be solid with green colouration. (Table 4.1.1).

The results of the phytochemical screening of the fruits and leaves extracts of *Phoenix dactylifera* and *T. catappa* respectively, in various solvents for secondary metabolite revealed the presence of alkaloids, anthraquinones, flavonoids, saponins, tannins and cardiac glycosides. Alkaloids were present in both plants extracts. The result shows the presence of alkaloids in the aqueous, methanolic, n-hexane and ethyl-acetate solvent extracts of *P. dactylifera* and aqueous, methanolic and chloroform solvent extracts of *T. catappa* but absent in chloroform extract of *P. dactylifera* and n-hexane and ethyl-acetate solvent extracts of *T. catappa* respectively. Anthraquinones were present in all the extracts except the equeous extract of both plants parts. Flavonoids were present in all the solvent extracts of *P. dactylifera* and the water extract of *T. catappa* (TCWE) but absent in all other extracts of *T. catappa*. Saponins were present in all extracts except for n-hexane and chloroform extracts of *T. catappa.*

The PDWE, PDME,PDHE, PDCE and PDEE all gave a positive test for tannins as well as the TCWE, TCME, and TCCE whereas the n-hexane and chloroform extracts of *T. catapp*a indicated a negative test for tannins. Cardiac glycosides were present in all the solvent extracts of both plants (Table 2).

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases such as in management of sickle cell disorders, gonorrhea, edema, cancer, rheumatism, and sexual dysfunction e.t.c. For example, alkaloids protect against chronic diseases, saponins protect against hypercholesterolemia and antibiotic properties, steroids show analgesic properties. The steroids and saponins were responsible for central nervous system activities. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported.

Alkaloids were nerve stimulants, convulsants and muscle relaxant. The presence of alkaloids in the extracts is an indication that they may be useful in alleviating some of the symptoms associated with pains. Anthraquinones act on the gastro-intestinal tract to increase peristalsis action. The presence of anthraquinones in the extracts is an indication that the plants may be useful as mild laxative especially in cases where sickle cell patients complain of constipation. Tannins are phenol derivatives and are non-nitrogeneous plant constituents with astringent properties on mucous membranes. The tannins present make the plants useful in bathing or cleansing the surface of the skin ulcers that develop as a result of sickle cell disease. The presence of cardiac glycosides may be potent in curing cardiac insufficient, cough and circulatory problems. Also, they may act as good sedatives and have antispasmodic properties (Evans, 1989).

Figures 1-12 shows the effect of both plant extracts in inhibiting red blood cells sickling. The aqueous extract of *P. dactylifera* was more active against the sickle cells with maximum inhibition of 89.1% at 180min incubation compared to that of *T. catappa* which showed 87.6% inhibition also at 180min incubation. However, the organic solvents (methanol, n-hexane, chloroform and ethyl-acetate) extracts of *T. catappa* demonstrated more sickling inhibition than that of the organic solvents extracst of *P. dactylifera* with the chloroform extract showing the highest degree of inhibition (86.7%) at 180min incubation compared with the highest degree of 78.2% inhibition exhibited by the methanolic extract of *P.* dactylifera.

The fraction PDME (5mg/ml) at 180min of incubation showed maximum inhibition of 78.2% and the fraction PDHE (5mg/ml) gave a maximum inhibition of 71.8% at 180min time of incubation while the remaining fractions of PDCE and PDEE demonstrated a maximum inhibition of 77.6% and 70.2% respectively. Comparatively, the fraction TCME (5mg/ml) at 180min of incubation gave a maximum inhibition of 82.8% and the fraction TCHE (5mg/ml) has maximum inhibition of 78.3% at 180min time of incubation while the other two fractions of TCCE and TCEE demonstrated a maximum inhibition of 86.7% and 83.5% respectively (Figures 1-8).

When compared with the standard; p-hydroxybenzoic acid (5mg/ml) with maximum inhibition of 76.2% at

180min incubation, the fractions, PDME, PDCE, PDWE, TCME, TCHE, TCCE, TCEE and TCWE were found to be very active against the sickle cells. The fractions PDWE (89.1%), TCWE (87.6%), TCCE (86.7%) all at 5mg/ml were found to be more active against the sickle cells while the fractions PDEE (70.2%) and PDHE (71.8%) at 5mg/ml concentrations were found to be least active.

The negative control (normal saline), showed relatively no inhibition and so majority of the cells still remained sickled after incubation.

The results obtained in this study shows that the fruits extracts of *P. dactylifera* and leaves extracts of *T. catappa* exhibited substantial antisickling activity. The extracts of these plants, showed significant inhibitory effect on sodium metabisulphite induced sickling. Thus, PDWE, TCWE, TCME, TCHE, TCCE, TCEE, PDME and PDCE could be a better remedy for sicklers than the standard p-hydroxybenzoic acid.

Also, from the results, aqueous extraction (decoction by water) seems to be the best mode of extraction for *P. dactylifera*. This sugest that the active principles in *P. dactylifera* may be more soluble in water than organic solvents. In comparism, *T. catappa* seems to be more favoured by organic solvent extraction. Inorganic (water) and organic solvents where used for the extractions because the majority of traditional healers use water to extract active compounds from plants, this is due to the fact that water is not harmful to humans and is generally cheap and easily acquired. However, successful isolation of compounds from plant materials is largely dependent on the type of solvent use in the extraction process (Masoko *et al*, 2008). Use of water alone leads to difficulties in isolating non-polar active compounds hence, the use of both water and organic solvents.

Furthermore, from the results, the reversal of sickling is concentration dependent. This may be as a result of the synergestic effect of all the fractions in the plants as all fractions in a plant must work together for maximum therapeutic action. The hemoglobin molecule is believe to have a high affinity for most substrates that reverse the sickling phenomenon. The active constituents must be actively transported across the membrane barrier of the erythrocytes before interacting with the haemoglobin (Hb) molecule hence, the incubation of the extracts with the sickled red blood cells for interaction and treatment.

Therefore, it seems from all twelve (12) graphs that the inhibition followed a dose dependent pattern. This results agrees with the statement made by Ekeke and Shode (1985) that the efficacy of an antisickling agent, whether *in vitro* must be assessed by a set of reproducible criteria. It must act effectively and rapidly, especially in cases of severe crisis. Antisicckling activity appeared to have also increased with incubation period.

Alkaloids are formed as metabolic by-products and have been reported to be responsible for antibacterial activity. Glycosides serves as defence mechanisms against predation by many microorganism, insects and herbivores. The optimal effectiveness of a medicinal plant may not be due to the one main active constituent, but may be due to the combined action of different compounds originally in the plant. The phenolic compound such as flavonoids and tannins are considered to be major contributors to the antioxidants capacity of plants. The diverse biological activities may be related to their antioxidant activity (Chung and Huang, 1998). Phytochemicals such as saponins, flavonoids, tannins and alkaloids have anti-inflammatory effects. Tannins play a major role as antihaemorrhage agent and has been shown to have immense significance as antihyper cholesterol, hypotensive and posses cardiac depressant properties. It has been reported that flavonoids, holds great promise as a chemopreventive agent for a variety of cancers and exhibits significant activity against skin cancer. It also inhibits the growth of a variety of human cancer cells including leukemia, breast, colon, skin, thyroid and prostate cancers. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants of free radical scavengers (Evans, 1989).

Tannins is a group of phenolic compounds found to form irreversible complexes with proline rich protein resulting in the inhibition of cell protein synthesis. Tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that

have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and desentery. Alkaloids exhibit marked physiological effects when administered to animals and hence their wide use in medicine for the development of drugs (Harbone, 1983). Natural antioxidants mainly come from plants in the form of phenolic such as flavonoid. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganism *in vitro*. Their activity is probably due to their ability to complex with extra cellular and soluble proteins and to complex with bacterial cell wall. They also are effective antioxidant and show strong anticancer activities. The present of alkaloids and flavonoids could also act as an adjuvant that enhances the activity of the components actually responsible in maintaining the integrity of red blood cells and subsequently improving the quality of life in individuals with sickle cell anemia (Evans, 1989).

Results of the antisickling assay of the fruits of *P. dactylifera* and leaves of *T. catappa* established their abilities to inhibit sickling under hypoxic condition, thus justifying their use in folklore medicine for SCD management. The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle.

Early researches (Brugnara, 2002) stated that in treatment of SCD, it is required that one focuses on the ways of inhibiting sickle cell haemoglobin polymerization, prevention or repair of red blood cell dehydration and interrupting the interaction of sickle cells with the endothelium.

Dennis and Roberts (1990) attempted an explanation of the antisickling of plant species on sickled erythrocytes. They were of the view that it may be due to inhibition of Ca^{2+} activated K^+ channel. Activation of this channel results in K^+ and water loss from sickled erythrocytes with subsequent dehydration which brings about increase in intracellular concentration of HbS leading to polymerization of deoxy HbS with its associated painful episodes. Inhibition of this pathway increases K^+ content, rehydration of red blood cells and an increase in haemoglobin level. This approach results in cell swelling, decreased HbS concentration and decreased sickling.

Results of this study, indicates that *in vitro* action of the extracts of these plants is rapid and probably helps in the inhibition of the sickling pathway such that potassium cell content is increased and rehydration also increased. More than 40-60% of sickled erythrocytes were reverted at 180 minutes using both plants extracts. If this action can be reproduced *in vitro*, then the plants extracts may well hold a lot of promises in the treatment of the disease. Depending on its half life and maximum inhibition, it would reduce both the frequency and duration of crisis.

It then becomes clear that each constituent of the plants for the management of SCD have a peculiar role it performs. This corroborates the opinion of Gillete *et al*. (2004) that all components of a therapeutic mixture of plants are necessary.

This study has demonstrated that the extracts from fruit of *P. dactylifera* and leaves of *T. catappa* could significantly inhibit sickling in the presence of sodium metabisulphite. The use of sodium metabisulphite in sickling induction is probably a more drastic approach than what actually happens in the vascular system of humans. In that, the extracts may perform its antisickling action more efficiently under *in vivo* conditions that has hitherto been demonstrated (Egunyomi *et al.,* 2009).

CONCLUSION

Extracts/fractions of *P. dactylifera* and *T. catappa* shows the presence of key phytochemicals and exhibited anti-sickling activity to different degrees. The findings of this study suggests that this plants could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or ameliorating sickle cell associated oxidative stress related degenerative diseases.

The results revealed the presence of medicinally important constituents in the plants studied. Active constituents of medicinal plants and naturally occurring compounds, known as antisickling agents,

which improve the health of sickle cell individuals are rich in aromatic amino acids, phenolic compounds and antioxidant nutrients (Evans, 1989), these and other phytochemicals are thought to be responsible for their observed antisickling action. From reported findings, one can appreciate the antioxidant properties of these phytomedicines and their role in maintaining the integrity of red blood cells and subsequently improving the quality of life in individuals with sickle cell anemia. Characterization and isolation of the active chemical components possessed by these traditional plants for further study may lead to the development of a potential drug that may treat various kinds of infections and may lead to full utilization by the local community. The results of this study may also be of commercial interest to research institutes and pharmaceutical industries in the development of new drugs.

Little or no work has been carried out on the antisicking property of *P. dactylifera* and *T. catappa* and hence, extensive investigation especially on the clinical efficacy is needed to exploit the therapeutic utility of different species. As the global interest towards traditional medicines over the conventional treatment is increasing due to safe and well tolerated remedies provided by them for the chronic illness without or lesser side effects, this research confirm *P. dactylifera* and *T. catappa* probably as a good source for a potentially safe and effective plants that has important medicinal values and benefits. The study also showed the economic and medicinal benefits arising from the fruits and leaves of *P. dactylifera* and *T. catappa* respectively. The plants contains therapeutically active compounds which impart excellent medicinal properties. This phytochemicals could be used against damaging effects of free radicals and can inhibit degenerate/oxidative disorders, neurodegenerative, Carcinogenesis, delay aging microbial and sickle cell related diseases. From literature review, the use of date palms and *terminalia catappa* have positive effects on human health and results of these findings suggests that they can also be a useful commercial drug after identification and isolation of active components that will assist in the treatment of cardiac, gastric and neuronal diseases. The current treatment approach for sickle cell diseases on synthetic drugs is expensive, shows unwanted adverse effects, alter the the genetic and metabolic pathways. Thus, a safe, effective and affordable approach is needed to control the disease development and progression. Leaves of *T. catappa* and date palm fruits will be a good remedy for sickle cell disease as they are inexpensive, effective and easy to access. Earliar findings showed therapeutic effect of *T. catappa* and *P. dactylifera* in diseases management via antioxidants, anti-inflammatory and anti-tumur properties. The use of these plants parts in the control of diseases create optimism towards the novel therapeutic strategy, keeping all informations in hand as antioxidant, anti-inflammatory and anti-tumur. Due to their low cost and abundance, the plants remain very important with incredible potential and innumerable possibilities for further investigation.

This research work will no doubt enhance the management of sickle cell disease while creating opportunites for cheaper, less toxic and alternative means for treating sickle cell disease, thereby reducing dependency on synthetic drugs and its side effects.

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