Antisickling Studies of the Aqueous Leaf Extract of Detarium microcarpum (Tallow Tree)

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Abstract

Detarium microcarpum (DM) is shrub that grows wildly in Nigeria. Few studies have been conducted on the leaf extract. The plant forms part of some recipes used in the treatment of sickle cell disease. This study aims to determine the phytochemical composition and antisickling property of various concentrations of *Detarium microcarpum* aqueous leaf extract. Quantitative and qualitative phytochemical screenings were conducted using established protocols while the antisickling prevention and reversal studies were evaluated using Emmel's test. There was no significant difference between the antisickling prevention (p>0.05) and reversal (p>0.05) property of the aqueous extract compared to positive control (p-hydroxybenzoic acid). Phytochemical screening revealed high abundance of flavonoids and very little presence of terpenoids among the classes of phytochemicals screened. There was also no significant variation in the antisickling activity of the various doses (0.1 mg/ml, 1 mg/ml and 10 mg/ml) of the extract tested. The LD_{50} conducted showed that it is completely safe in mice. The findings from this study suggest that *Detarium microcarpum* aqueous extract at various concentrations possesses both sickling prevention and reversal effect which may be exploited for the management of sickle cell disease.

Keywords: *Detarium microcarpum*, Phytochemicals, Antisickling, *p*-hydroxybenzoic acid

Introduction

Sickle cell disease (SCD) is a common genetic disorder whose occurrence is highly prevalent among people with ancestral background from sub-Saharan Africa, India, Saudi Arabia and Mediterranean countries (Islam *et al*., 2021). It results from the inheritance of mutant haemoglobin genes from both parents. The mutant haemoglobin (Hbss) is highly vulnerable to low oxygen tension where deoxyhaemoglobin undergoes polymerisation and causes the cell membrane to change the morphology of the RBCs into sickle-shaped (Pecker & Lanzkron, 2021). This affects the quality of life and activities of daily living including aspects of education, employment, and psychosocial development. The management of sickle cell anaemia and its complications can exist in four main methods, namely: psychotherapy, transfusion, bone marrow transplant, and drug therapy (Osuala, 2016). Among the conventional medicines used include hydroxyurea, erythropoietin and tucaresol; these are associated with varying degrees of side effects (Iyamu *et al*., 2003; Stuart *et al*., 2004; Vadolas *et al*., 2004; Bianchi *et al*., 2007).

A number of medicinal plant productshave been used in the treatment of painful crises associated with sickle cell disease. Some of those plants include medicinal plants such as *Cajanus cajan*, *Piper guineensis*, *Pterocarpus santolinoides*, *Pterocarpa osun*, *Eugenia caryophyllala*, *Sorghum bicolor, Fagara zanthoxyloides* and *Detarium microcarpum* extracts for the treatment of sickle cell disease have been reported (Okpuzor *et al*., 2008; Gbadamosi, 2015).

Studies have shown that sesquiterpenes, anthocyanins, dihydro-stilben carboxylic acid (lunularic acid) organic acids and benzoic acid derivatives possess antisickling properties (Mpiana *et al*., 2010; Ameh *et al*., 2012;Mpiana *et al*., 2014; Ngbolua *et al*., 2015; Pierre *et al*., 2015). Other anti-sickling compounds from plant include vanillin, vanillic acid, p-hydroxy benzoic acidy (Abraham *et al*., 1991; Witting & Guinko, 1998; Moody *et al*., 2003; Adeshina, 2005).

A number of folkloric uses of *Detarium microcarpum* have been reported in literature. A decoction of the powdered bark is administered orally for relief from pain associated with backache, headache, sore throat and menstruation (Agbo *et al*., 2020). The fresh bark or leaves and poultice of the powdered seeds are applied topically to the skin to prevent or cure wound infections. The ethno-medical veterinary uses include treatment of constipation, fever and diarrhoea in cattle (Hassanin *et al*., 2019). A combination of the leaves of *Detarium microcarpum*, *Sclerocarya birrea* and *Acacia macrostachya* when pounded in milk is considered an efficient snakebites remedy. The indigenous people of Ibadan, Southwestern Nigeria use recipes containing *Detarium microcarpum* in the treatment of sickle cell crisis (Egunyomi *et al.*, 2008; Gbadamosi *et al*., 2012; Gbadamosi, 2015).

In this study, the antisickling activity of *Detarium microcarpum* leaf extract was investigated to reveal its role in the preparation of herbal recipes for the management of sickle cell crisis.

Materials and Methods

Collection and identification of plant material

The fresh leaves of were collected from Kudingi bush in Ahmadu Bello University, Samaru campus, Zaria and identified by Dr/Alhaji/Mr/Mrs US Gallah of the Herbarium Unit Department of Biological Sciences, Ahmadu Bello University, Zaria,. A voucher number (901451) was subsequently issued. The leaves for the experiment were washed under running water, shade dried and pulverised into small particles.

Extraction and Phytochemical Screening: About 500 g of powdered leaf sample was macerated with distilled water at room temperature for 72 hours with frequent agitation. The mixture collected was then filtered in vacuo and solvent evaporated by gentle heating (50°C) over water bath for how many days?. The aqueous extract was then subjected to qualitative phytochemical screening and biological assay.

Qualitative and quantitative phytochemical screening The qualitative phytochemical screening was carried out as described by Sofowora (2008) and Evans (2009). The quantitative phytochemical assays were carried out according to Harborne (1989).

Toxicity Studies

The LD_{50} was determined using nulliparous female mice according to OECD; *Test No. 425* guidelines (OECD, 2008).

Sample preparation for anti-sickling studies

Collection of blood sample

Blood samples were collected by venipuncture from known adult sickle cell patients attending their clinic days at Murtala Muhammed Specialist Hospital,

Kano. A written informed consent form was provided for the patient to fill and sign. The blood samples were collected in sodium ethylenediamine tetraacetic acid (EDTA) bottles and Hbss status of patient confirmed by haemoglobin electrophoresis test and the remaining sample stored at 0-4 ℃ before the experiment. All research procedures were approved by the State Ethical Committee of Kano State Hospital Management Board, Kano; approval number: MOH/Off/797/TI/759

Washing of Erythrocytes

The blood sample was centrifuged to remove serum, leaving the packed erythrocytes, which was washed with normal saline, as described by Egunyomi *et al*. (2009)

Preparation of extract

A stock solution (10 mg/ml) of the plant extract was prepared by dissolving 0.1 g of dried aqueous extract in 1 ml of 10 % dimethyl sulphoxide (DMSO) in normal saline. Then, three concentrations of 0.1, 1 and 10 mg/ml in normal saline were prepared from the stock solution of plant extract by 10 fold serial dilution..

Antisickling Assay

The anti-sickling activity of the aqueous extract was evaluated *in vitro* using Emmel's test (Emmel, 1917; Elufioye *et al*., 2019).

In the sickling prevention assay, a mixture containing 100 µl of each the washed RBC 2% sodium metabisulfite ($Na_2O_5S_2$) and 0.1 mg/ml concentration of plant extract was prepared in a plane bottle and sealed with cover. The procedure was repeated to obtain mixtures containing 1 mg/ml and 10 mg/ml concentrations of the plant extract. Mixtures containing positive (p-hydroxyenoic acid; 5 mg/ml) and negative (normal saline) controls were also prepared. A drop of each of the mixtures obtained was used to prepare a thin smear on a slide (in triplicates) at 30 minutes interval over a 90 minutes incubation periodThe number of sickled and unsickled cells were counted under the oil immersion light microscope and the percentage of unsickled cells were then calculated using the formula;

{(%) unsickling = Number of unsickling cells × 100/total cells}

The above procedure was repeated for the reversal of sickling assay but the addition of samples and controls to the mixture were delayed until after 30 minutes.

All anti-sickling experiments were carried out in triplicate using fresh blood samples.

All data were expressed as mean ± SEM while One-way ANOVA statistical test using SPPS was used to determine the difference among the means. $p \leq 0.05$ was considered statistically significant. Post-hoc test was carried out using Bonferroni test.

Results

The phytochemical analysis of the extract as shown in Table 1 reveals the composition of the various classes of secondary metabolites present in the plant leaf extract.

Table 1: Phytochemical Composition of DM Aqueous Extract

Test	Inference
Alkaloid (a) Mayer's	Negative
(b) Dragendoff	Negative
(c) Wagner's	Positive
Terpenoid/Steroid	Positive
(a) **Liebermann-Burchard**	
(b) **Salkowski**	
Tannins (Ferric Chloride)	Positive
Flavonoids (a) Sodium Hydroxide	Positive
(b) **Shinoda's**	
Anthraquinones (Borntrager's)	Negative
Cardiac glycosides (Keller-Killiani)	Positive
Saponins (a) Frothing Test	Positive
(b)**Haemolysis Test**	Positive

On quantitative evaluation of the phytochemical contents, flavonoids, saponins and terpenoids were present while alkaloids were absent (Table 2).

Table 2: Quantity of some phytochemicals present in DM leaf extract

Phytochemical assay	Aqueous Extract
Total Flavonoid Content	31.3% (0.313)
Total Saponin Content	2.96 % (0.0296)
Total Alkaloid Content	No Precipitate formed
Total Terpenoid Content	16 % (0.160)

*Values in bracket indicate weight in mg/g of sample

The antisickling activity of the aqueous extract at different time intervals as compared to negative (Normal) and positive (*p*-hydroxybenzoic acid) controls are shown on Tables 3 and 4.

Table 3: Sickling Reversal Activity of Aqueous DM Extract

SAMPLE	TIME			
	T0	T30	T60	T90
Normal saline	42.83±7.48	25.80±5.86*	45.00±0.84*	18.23±1.79*
0.1mg Aqueous	32.27±2.31	92.56±3.02	97.55±1.53	97.06±0.85
1mg Aqueous	44.72±0.83	95.77±1.29	100.00±0.00	97.06±1.77
10mg Aqueous	43.90±1.47	100.00±0.00	100.00±0.00	99.38±0.62
PHBA (5mg/ml)	39.59±3.50	99.17±0.83	100.00±0.00	100.00±0.00

Values expressed as mean ±SEM, * $p \leq 0.05$

Table 4: Sickle Cell Prevention Activity of Aqueous DM Extract

SAMPLE	TIME			
	T30	T60	T90	T120
Normal saline	20.75±2.05*	29.11±4.04*	24.36±1.86*	21.38±5.34*
0.1mg Aqueous	79.55±3.67	92.29±1.36	87.63±4.77	94.58±2.92
1mg Aqueous	87.68±1.72	93.33±3.33	93.46±1.64	96.13±1.26
10mg Aqueous	88.25±2.95	88.48±0.52	95.39±0.53	93.56±0.51
PHBA (5mg/ml)	98.75±0.09	97.83±2.17	97.00±1.51	99.36±0.64

Values expressed as mean ±SEM, * $p \leq 0.05$

The LD_{50} was recorded to be greater than 5000mg/kg.

Discussion

A syrupy aqueous filtrate was obtained after maceration which may be due to presence of free sugars or glycosides as indicated by the preliminary phytochemical screening. Other phytochemicals present in the extract include terpenoids, saponins, tannins, and deoxy-sugars (indicating the presence of cardiac glycosides). Another study has shown the presence of anthraquinones and steroids in the aqueous extract of the plant in addition to the phytochemicals reported in this study (Iful, 2008). This may be due to the difference in habitat of the plant or the season of collection. In agreement with this study, Ugokwe *et al.* (2017) has also reported the presence of tannins, flavonoids, saponins and steroids in methanol extract of the leaf. Variations have been observed in the phytochemical content of the leaf of *Detarium microcarpum* depending on the solvent of extraction (Jimoh *et al*., 2021; Abdullahi *et al*., 2020).

The quantitative phytochemical assay revealed the presence of 31.3 mg/g flavonoids in the extract. Due to the antioxidant activity of flavonoids, studies have shown that they play a significant role in the prevention and management of many chronic diseases including sickle cell diseases (John *et al*., 2018; Dermane *et al*., 2018). Thus, the observed antisickling activity may be due to the abundant flavonoids present in the extract. Diterpenes

have been isolated from the fruit and stem bark of DM (Cavin *et al*., 2006). The considerable amount (16%) of terpenoids present in the sample may also produce anticikling effect (Mammo, 2018).

Management of sickle cell anaemia involves crises prevention measures as well as crisis and complications treatment.. The prevention of sickling assay tests the ability of drug candidates to prevent sickle cell crises while the reversal assay investigates ability to manage crisis and complications. The antisickling studies revealed no significant difference($p \leq 0.05$) between the antisickling effects produced by the different doses of the extract There was also no significant difference between the activities produced by extract and the positive control. Thus, the activity can be said to be independent of the doses used in this study. However, the difference between the antisickling activities of the three concentrations of the extract as well as the positive control were significantly different ($p \leq 0.05$) from that of the negative control. The time variation at which readings of the antisickling effects were measured did not show a time-dependent activity. In a recipe containing DM stem bark, a 63.4 % inhibition was recorded which was lower than 95.39 % reported in this study (Egunyomi *et al*., 2009). An in silico studies using using Autodock Vina algorithm has shown that the diterpenes, oxokolavenic acid, 5α, 8α -2-oxokalavenic acid and Copalic acid isolated from DM possess the ability to prevent the occurrence sickle cell crisis (Ayevbuomwan *et al*., 2021).

While many drug requirements involved in sickle cell treatment are targeted towards the management of associated or secondary diseases, the most important treatment mechanism seem to be that which will reverse the sickled red blood cell (Salinas & Thein, 2020). The plant extract has in addition to preventing sickling of red blood cells, also possess the ability to reverse sickle cell crisis. The plant's extract ability to reverse the sickled red blood cells is also not dose or time-dependent. The LD_{50} recorded in this study was greater than 5000 mg/kg. A study has also shown that no histopathological presentations were observed in animal organs within the period of an acute toxicity studies (David *et al.,* 2017). Hepatotoxic, haematotoxic and nephrotoxic effects of the leaf extract of the plant on some liver enzymes, kidney function tests and haematological indices respectively showed mild to no adverse effects after oral administration (Abdullahi *et al.*, 2020). These studies show that the plant extract is safe for consumption

Conclusion

The aqueous extract of the leaf has revealed the presence of several phytochemical constituents that could be of medicinal value. The extract ha good safety profile and also possesses high antisickling activity at the various doses tested. The activities between the doses and the positive control were not significantly different. Increasing the treatment duration does not increase antisickling activity.

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