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ANTISICKLING POTENTIAL OF THE ETHANOLIC FLOWER EXTRACTS OF *COUROUPITA GUIANENSIS*

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ABSTRACT

This work was therefore aimed at investigating the Antisickling potential of the ethanol seed extract of *Couroupita guianensis* used as a traditional herbal medicine with a view of proposing an effective herbal recipe for the management of sickle cell disease. Preliminary phytochemistry, sickling inhibition test, sickling reversal test and polymerization test were carried out using standard methods. The phytochemical analysis indicated the presence of saponins, reducing sugar, carbohydrate, fats and oil, steroids, glycosides, alkaloids. The results of the antisickling test showed significantly ($p<0.05$) higher antisickling effect. The percentage sickling reversal effect was significantly higher than the control. The result of the polymerization showed that ethanolic extract significantly ($p<0.05$) increased delayed time before polymerization at 50, 25 and 12% concentrations compared to the control. From the results, the extract have shown to be therapeutically beneficial in the management of sickle cell disease and thus are strongly recommended by this study to be developed into supplements for the management of sickle cell disease.

INTRODUCTION

Sickle cell disease (SCD) is an inherited hematological disorder characterized by a banana, crescent- moon or sickle shaped human red blood cells. Sickle cell disease is one of the most prevalent hereditary disorders with prominent morbidity and mortality. While the disease may affect various ethnic groups such as the people of the Hispanic and Middle East descent, it affects those of African descent, the more. The most clinical manifestations are largely due to hemolytic processes leading to severe anemia and vaso-occlusive crises resulting in pain and organ damage. Sickling of red blood cells occur as a result of polymerization of deoxygenated Hbs. This abnormality is characterized by painful episodes, chronic anemia, enlarged spleen, serious frequent infections and damage to vital organs. Sickled red blood cells have relatively small oxygen contact area and increased blood viscosity and impede normal circulation in small blood vessels resulting in ischemia and infarction. In people with sickle cell disease, these irregular shaped red blood cells become rigid and sticky and die prematurely resulting in chronic anemia. In Nigeria, the number is believed to be up to 4 million and at least 12 million people suffer from sickle cell disease worldwide. Several therapies have been proposed and many chemical substances investigated for their possible role in the management of sickle cell disease (SCD). Among the many potential agents employed to prevent or reverse sickling include: Hydroxyurea (HU); Erythropoietin, Tucaresol, Ciklervit TM etc. Although hydroxyurea has been found, to be very effective in many patients, in others, it has yielded many pronounced side effects. In the search for effective chemotherapeutic agents with less adverse effects on sickle cell disease patients, many researchers have shown the antisickling effectiveness of most nutrients derived from plants and animals and these may provide possible and reliable option for the effective management of sickle cell

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disease. Natural plant products have been used in Nigerian folk medicine to inhibit sickling and in the management of sickle cell disease and other manifestations of the disease. Several plant parts have been reported to possess anti sickling and sickling reversal properties. However, the search continues for a more effective remedy for the ailment. *Couroupita guianesis* (Aubl) Family Lecythidaceae commonly known as Cannon ball tree, locally known as "Kailashpati" is found throughout India in plains. It is widely cultivated for its large showy flowers and reddish brown woody capsular fruits up to 20 cm in diameter. It is grown in Indian gardens as an ornamental tree. It is native to South India and Malaysia and commonly known as Nagalinga pushpam in Tamil. Traditionally, the leaves of this plant have been used in the treatment of skin disease. Native Amazoniam people used the infusion or tea obtained from leaves, flower and bark of C.guianensis to treat hypertension, tumours, pain and inflammatory processes. In Orissa decoction of flowers has been used to boost the immune system to fight number of disease. The flower extracts of this plant had been screened for larvicidal activity against vector and immunomodulatory activity. This work was therefore aimed at investigating the anti sickling potential of the ethanol flower extract in view of proposing an effective herbal recipe for the management of sickle cell disease [1,2].

MATERIALS AND METHODS

Collection and preparation of plant materials

The flowers *Couroupita guianensis* were obtained from the medicinal garden. They were authenticated by Dr. Madhava Chetty, Department of Botany, Tirupati University. The flowers were air dried under shade at room temperature and pulverized using an electrical grinding machine. The powdered material (500 g) was passed through a 40-mesh sieve and then macerated in 95% ethanol and filtered using a Whatmann filter paper 125 mm. The filtrate was concentrated to a solid matter to form the stock solution sample using a rotary evaporator. The extracts were stored in the refrigerator at 2 - 8°C.

Blood sample collection

Blood samples (5 ml) used were collected from 25 sickle cell patients of age range 10 - 20 years and of both sexes after securing consent from the individuals or accompanying parents. The blood samples were used within 24 h after collection into sample bottles containing 0.5 mg/ml of EDTA.

Phytochemical analysis

The phytochemical constituents were investigated by the method of Trease and Evans, 1996. Phytochemical tests were carried out to detect the presence of alkaloids, flavonoids, carbohydrates, reducing sugar, glycosides, saponins, tannins, fats and oil, steroids, terpenoids, acidic compounds, resins and proteins.

Determination of anti sickling potentials of the extract

The anti sickling potential of the extracts was carried out by the method of Barbara, 1980 as modified by Elekwa et al as described below. A drop of Hbss blood, a drop of freshly prepared 2% sodium metabisulphite and a drop of the extract were mixed on a clean slide and covered.

The cover slit was gently pressed to remove excess mixture and the edges of the cover slip sealed with Vaseline to avoid air from going in. the slides were incubated at 37°C for 30 min and then observed under a microscope using ×10 and ×40 magnification to determine the effect of the extracts on the sickling of the Hbs erythrocytes.

Determination of sickling reversal test

The sickling reversal test was done by the method of Barbara, 1980 as modified by Elekwa et al. (2005) as outlined. Two drops of Hbss blood were mixed with 2 drops of freshly prepared 2% metabisulphite and covered tightly to avoid air from going in. This was incubated for 30 min during which time sickling was induced. Two drops of the buffered extract were added to the mixture. A drop was placed on a clean slide and covered. This was incubated for another 30 min and then observed at ×40 magnification.

Sickle hemoglobin polymerization inhibition test

Polymerization test was carried out using the method described by Noguchi and Schechter (1985). Five microlitres of normal saline (0.9%v/v) was added into different test tubes containing various dilutions of the extracts. Freshly prepared 2% sodium metabisulphite (4.4 ml) and Hbss erythrocytes (0.1 ml) were added into the test tubes simultaneously and mixed.

After standardizing with blank (distilled water), the absorbance of the mixtures were read using a spectrophotometer (model, 700D) at 700 nm taken at 5 min intervals for 35 min. Appropriate control experiment was set up excluding the extract [3-5].

Statistical analysis

Data entry and analysis were done using SPSS version 15.0 and values were represented as mean ± SD. Significant differences were established using independent t-test.

RESULTS

Phytochemical analysis

The results of the phytochemical analysis reveals the presence of saponins, carbohydrate, fats and oil, glycosides, alkaloids, reducing sugar and protein.

The anti-sickling test

The anti-sickling effect of extract was significantly ($p<0.05$) higher than that of the Hbss control with CG showing a higher anti sickling potential (Table 1).

Reversal of sickling test

The reversal of sickling for CG was significantly ($p<0.05$) higher than the Hbss control as shown on Table 1, could potentially be used to reverse sickling in cases where sickling has occurred.

Polymerization of Hbss erythrocytes

The effect of extract on the polymerization of Hbss erythrocyte showed significantly increased ($p<0.05$) delay time before polymerization at 50, 25 and 12% concentrations compared to the parallel control (Table 2).

Table 1. The anti-sickling test and Reversal of sickling test

	50%	25%	12%	Control
Anti sickling	80.33±1.15	66.66±1.15	42.33±2.51	25.00±2.0
reversal of sickling effect	54.60±5.03	45.33±1.15	33.22±1.15	25.30±1.15

Table 4. The effect of CG on the polymerization of Hbss erythrocytes measured at 5 min interval for 35 min.

Time	50%	25%	12%	Control
5 min	0.134±0.002	0.160±0.021	0.031±0.013	0.027±0.001
10 min	0.028±0.001	0.044±0.012	0.060±0.002	0.026±0.002
15 min	0.025±0.002	0.041±0.001	0.047±0.002	0.039±0.001
20 min	0.060±0.011	0.070±0.002	0.011±0.002	0.178±0.002
25 min	0.126±0.002	0.030±0.001	0.068±0.009	0.131±0.001
30 min	0.825±0.001	0.615±0.002	0.715±0.013	0.730±0.012
35 min	0.915±0.032	0.630±0.001	0.775±0.004	0.748±0.001

DISCUSSION

The anti sickling and sickling reversal effect of CG showed marked improvement upon incubation with compared to the normal saline control. An erythrocyte was considered to be unsickled if the cell was not in the characteristic sickle shape or in created holly leaf pattern. The effects of the extract were significant at the different concentrations used but were dose and tie dependent. This result agrees with the report that sickling activity of the drug tellurite, thiocyanate and hydroxyurea were dose and time dependent. The extracts used in this study achieved a significant decrease ($p<0.05$) in percent irreversible sickle cell upon incubation with sodium metabisulphite pre – sickled erythrocytes thus indicating positive effect in maintaining me mbrane integrity. The use of sodium metabisulphite to induce sickling is probably a more drastic approach than what actually happens in the vascular system of humans. It is therefore expected that the extracts may achieve more efficient sickle inhibition *invivo*. The polymerization of Hbss erythrocyte is a major event in the pathophysiology of sickle cell disease. Measuring delay time has been suggested to be the most reliable tool in assessing the effectiveness of a potential anti sickling agent. The extract used in this study significantly ($p<0.05$) increased delay time of sickle hemoglobin polymerization at all the three concentrations used with respect to the parallel control.

From the result of this study, we sincerely conclude that courapitia guianaenaia have proven to be therapeutically beneficial and thus can be developed into a supplement for the management of sickle cell disease.

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