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Original Research Paper

EVALUATION OF NUTRITIONAL CONSTITUENTS AND ERYTHROPOIETIC PROPERTIES OF *FICUS CAPENSIS* LEAVE EXTRACT IN THE TREATMENT OF ANEMIA

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ABSTRACT

Ficus capensis is used locally for haemolytic and sickle cell anaemia in south eastern Nigeria. This research ascertained the nutrient and mineral constituents, anti-microbial, erythropoietic, and anti- sickling properties of *Ficus capensis* leaf. Ethanol extraction, phyto-analysis, proximate analysis and antimicrobial screening were carried out using standard methods. Animal erythropoietic study involving 5 groups of 5 rats each was carried out. The first 3 groups were administered oral doses of 50 mg/kg, 100 mg/kg and 200 mg/kg of the extracts, while the last two groups served as negative and positive control for 14 days, with intermittent withdrawal of blood on days 0, 3, 7 and 14 from the retro-orbital veins for haematological analysis. Anti-sickling activity of the plant extract was determined by adopting the Emmel procedure. The phyto-analysis showed significant presence of flavonoids, reducing sugar, saponins, tannins, anthraquinone, starch, proteins, lipids and glycoside. The proximate and mineral analysis showed the presence of proteins, carbohydrates, iron, copper, and cobalt. The antimicrobial result showed moderate antimicrobial activity. The PCV test showed an increase with 50 mg/kg (44%. - 49%) and 100 mg/kg (45% -58%) against 45% - 49% in Positive control and 44% - 45% in Negative control, RBC 6.70±0.08 to 8.82±0.31. The Anti-sickling test shows inhibition of sickling at 32.81% and 36.9% respectively on both Sickled red blood cell samples from the old patients using concentrations of 50µg/l and 100µg/l. The result indicates significantly high erythropoietic and anti-sickling properties. This justifies its ethno-medicinal use in the treatment of anaemia and anti-sickling crisis.

Keywords: *Ficus capensis*, Erythropoietic, Antisickling, Anaemia, Antimicrobial, Nutrient.

INTRODUCTION

Erythropoiesis is the process by which red blood cells (erythrocytes) are produced. It is stimulated by decreased oxygen circulation which is detected by the kidneys, which then secretes the hormone erythropoietin.[1] This hormone stimulates proliferation and differentiation of red cell precursor, which activates increased erythropoiesis in the hemopoietic tissues, ultimately producing red blood cells. Increased level of physical activity can cause increases in erythropoiesis. The most common disease that is related to erythropoiesis is anaemia. Anaemia is medically defined as a decrease in number of red blood cells or less than the normal quantity of hemoglobin in the blood. However, it can include decreased oxygen binding ability of each hemoglobin molecule due to deformity or lack in numerical development. Anaemia, a hemolytic infection, characterized by an insufficiency in quality and quantity of the red globules, is frequent in the tropical countries.[2] In Africa, the prevalence is higher for children (30 to 40%) than among women in pregnancy or nursing. This high prevalence rate makes anaemia dreaded

among African population.[3] Plants have been an integral part of life in many indigenous communities particularly in Africa. Ethnobotanical studies show that plants are efficient in the treatment of various diseases including anaemia (and sickle cell anaemia).[4,5] In anaemia management, the drugs used are mostly multi-vitamins, which do not have direct stimulatory effect on erythropoiesis. These drugs are mostly used as management therapies and have slow to moderate actions, while the few ones that have highly stimulatory actions as well as other curative measures like bone marrow transplant and blood transfusion, are expensive. Furthermore, Africans depend on traditional plant for treatment of anaemia. Hence, it is necessary to ascertain and evaluate the actions of these plants.

Ficus capensis goes by different local names in Nigeria and some popular ones include: Opoto (Yoruba), Akakoro (Igbo), Fig tree (English). Traditionally *Ficus capensis* is used to treat anaemia and ameliorate diarrhea by decreasing gastrointestinal motility.[6] It has also been reported to have antibacterial activity.[7] Other reported pharmacological activity include: antioxidant property[8], and anti-trypanosomal activity.[9] The objectives of the research work are to ascertain and evaluate the erythropoietic effects of the leaves of *Ficus capensis* in relation to its traditional use for the treatment of anaemia. To ascertain the possible plant constituents, nutrient or minerals that may be responsible for the erythropoietic properties.

MATERIALS AND METHOD

Plant Materials

The leaves of *Ficus capensis* were collected from Anambra State and authenticated by a taxonomist, Mr. Paulinus Ugwuozor of the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra state-Nigeria. The Voucher specimen (PCG/423/A/019) is on deposit at the herbarium of faculty of pharmaceutical sciences Nnamdi Azikiwe University Awka.

Extraction

Ethanol and water were the solvents used for the various extractions**.** The dried and powdered leaves of the plant (500g) were repeatedly extracted by cold maceration with 95% ethanol for 72hrs; the aqueous extraction was by decoction with clean water, which was the usual mode of preparation. All the fractions were filtered, the ethanolic filtrate evaporated under reduced pressure using a rotary evaporator while the aqueous extract was freeze dried. Both sample fractions were labeled appropriately and stored in a refrigerator at 4 ^{0}C to prevent degradation and spoilage for subsequent use.

Biological Materials

The microorganisms used were 24 hours culture of seven clinical isolates (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli* and *Salmonella typhi* and two *fungi* which include *candida albicans* and *Aspergillus nigger*) from Bishop Shanahan hospital Nsukka, Enugu state Nigeria. Agar diffusion by cup plate method was used in the determination of sensitivity and minimum inhibitory concentration (MIC). The anti-sickling evaluation was carried out using confirmed freshly collected Sickle cell blood samples. These blood samples collected from known drepanocitary patients attending Anambra State University Teaching Hospital Amaku Awka, Anambra State of Nigeria with the ages 13 and 32 years respectively. The blood samples were found to be SS; characterization by electrophoresis on cellulose acetate gel at PH 8.5; then were finally maintained at a temperature of 0 to 4 ^{o}C in a refrigerator.

Phytochemical Screening and Proximate Analysis

Phytochemical analysis was carried out on the powdered leaf, aquous extract and 95% ethanolic extract using standard methods of Odebiyi and Sofowora[10] and Trease and Evans.[11] Moisture, ash, crude protein, fat, fiber, and nitrogen free extract was also analyzed using methods of AOAC.[12]

Erythropoietic Assay

For the erythropoietic study, 50, 100 and 200 mg/kg of the extract was administered to albino rats while 500 mg/kg of hemoglobin and 0.9% NaCl served as controls. Blood samples were collected on days 3, 7 and 14 of treatment and evaluated for PCV, HB and RBC.

Anti-sickling Assay

The blood sample was then subjected to graded concentrations of the plant extracts, and this was prepared by diluting the extract with physiological salt solution (0.9% NaCI) to avoid haemolysis of red blood cells. Emmel test procedure[13] was adopted for the evaluation. The photomicrograph and percentage inhabitation calculated and tabulated.

Mineral Assay (Using AAS)

Sample digestion was carried out for mineral analysis by weighing about 2g of the dried sample into a digestion flask and 20 ml of the acid mixture (650 ml concentrated HNO_3; 80 ml Perchloric acid; 20 ml concentrated H_2SO_4) was added. The flask was heated until a clear digest is obtained. The digest was diluted with distilled water to the 250 ml mark and appropriate dilutions were then made for each element. The sample was thoroughly mixed by shaking, and 100 ml of it was transferred into a glass beaker of 250 ml volume. The sample was aspirated into the oxidizing air-acetylene flame or nitrous oxide acetylene flame. When the aqueous sample was aspirated, the sensitivity for 1% absorption was observed.

RESULTS

Phytochemical Test

Phytochemical result for the leaf powder, the aqueous and ethanol leaf extracts are given below. However, the results of the aqueous and ethanol extracts were virtually the same.

Table 1: Phytochemical tests results of leaf powder

Aqueous Extract		Ethanol Extract	
Phytochemical Constituents	***Result***	***Phytochemical Constituents***	***Result***
Alkaloid	++	Alkaloid	++
Glycosides	++	Starch	+
Resins	+	Glycosides	++
Anthraquinones glycoside	++	Steroids	+
Saponins	++	Flavonoids	+
Tannins	+	Flavonoids	+
Proteins	++	Saponins	++
Starch	+	Fats and fixed oils	+
Reducing sugar	+	Tannins & phenolic compounds	++
Lipids, fat and oils	+		
Flavonoids	++		

+ = Present, ++ = readily present, - = absent

Proximate Analysis

The proximate analysis showed that the leaf contains 64.7%% crude fiber, 24.4% ash, 2.09, 6.8 and 1% proteins, fats and moisture respectively.

Mineral Analysis

Mineral analysis showed the presence of calcium, iron, cobalt, copper, potassium, sodium and zinc. See Table 2.

Table 2: The mineral analysis of the leaf of *Ficus capensis*

Parameters	Mean absorbance	Wavelength (nm)	Concentrations (ppm)	Flame
Mercury	0.0005	253.7	0.0035	Air/acetylene
Iron	0.0699	248.3	0.0056	Air/acetylene
Calcium	1.7341	422.7	0.24	N_20/Acetylene
Zinc	0.2398	213.9	0.0145	Air/Acetylene
Sodium	1.3678	589.0	0.0371	Air/Acetylene
Cadmium	0.0012	357.9	0.0062	Air/Acetylene
Potassium	0.8069	766.5	0.1121	Air/Acetylene
Copper	0.0101	324.8	0.0018	Air/Acetylene
Cobalt	-0.0008	240.7	0.014	Air/Acetylene
Lead	0.0148	217.0	0.0030	Air/Acetylene

Table 3: The Minimum inhibitory concentration of aqueous extract

Conc. (mg/ml)	*S.aereus*	*E.coli*	*B.subtilis*	*P.aerugionsa*	*S.typhi*	*C.albicans*	*A.nigger*
	Inhibition zone diameters (mm)						
400	4	6	4	5	0	8	8
200	2	4	2	3	0	6	6
100	0	2	0	1	0	4	4
50	0	0	0	0	0	2	2
25	0	0	0	0	0	0	0
12.5	0	0	0	0	0	0	0

Table 4: The Minimum inhibitory concentration of ethanol extract

Conc. (mg/ml)	*S.aereus*	*E.coli*	*B.subtilis*	*P.aerugionsa*	*S.typhi*	*C.albicans*	*A.nigger*
	Inhibition zone diameters (mm)						
400	6	0	8	3	0	10	8
200	4	0	6	1	0	8	6
100	2	0	4	0	0	6	4
50	0	0	2	0	0	4	2
25	0	0	0	0	0	2	0
12.5	0	0	0	0	0	0	0

Table 5: The Minimum inhibitory concentration of Ciprofloxacin as control agent

Conc. (µg/ml)	*S.aereus*	*E.coli*	*B.subtilis*	*P.aerugionsa*	*S.typhi*
	Inhibition zone diameters (mm)				
50	8	6	10	3	0
25	6	4	8	1	0
12.5	4	2	6	0	0
6.25	2	0	4	0	0
3.125	0	0	2	0	0
1.5625	0	0	0	0	0

Table 6: The Minimum inhibitory concentration of ketoconazole as control agent

Concentration (µg/ml)	*Candida albicans*	*Aspergillus nigger*
	Inhibitory zone diameter (mm)	
2.0	8	6
1.0	6	4
0.5	4	2
0.25	2	0
0.125	0	0
0.0625	0	0

Result of *In-Vivo* Studies:

The animal study conducted to evaluate the erythropoietic actions of Ficus capensis ethanol leaf extract, gave a high level of significance for group two and three animals, which were administered 50 mg/kg, 100 mg/kg and 200 mg/kg of the extract compared to the controls. The results obtained were statistically analyzed (mean, standard deviation and analysis of variance or F test) using the SPSS software, version 16.0, and the table is shown in appendix. The bar charts and graphs plotted from the results obtained, and analysis carried out are thus displayed to compare the doses, controls and the various day intervals, with the last bar chart, showing a combination of all three doses; mean PCV, and sampling days Bar charts, showing increase in PCV against dose of extract and controls for 3day, 7day and 14day intervals with the last chart comparing and combining all three variables (Figures 1 and 2). Bar charts, showing increase in RBC against dose of extract and controls for 3day, 7day and 14day intervals with the last chart comparing and combining all three variables (Figures 3 and 4).

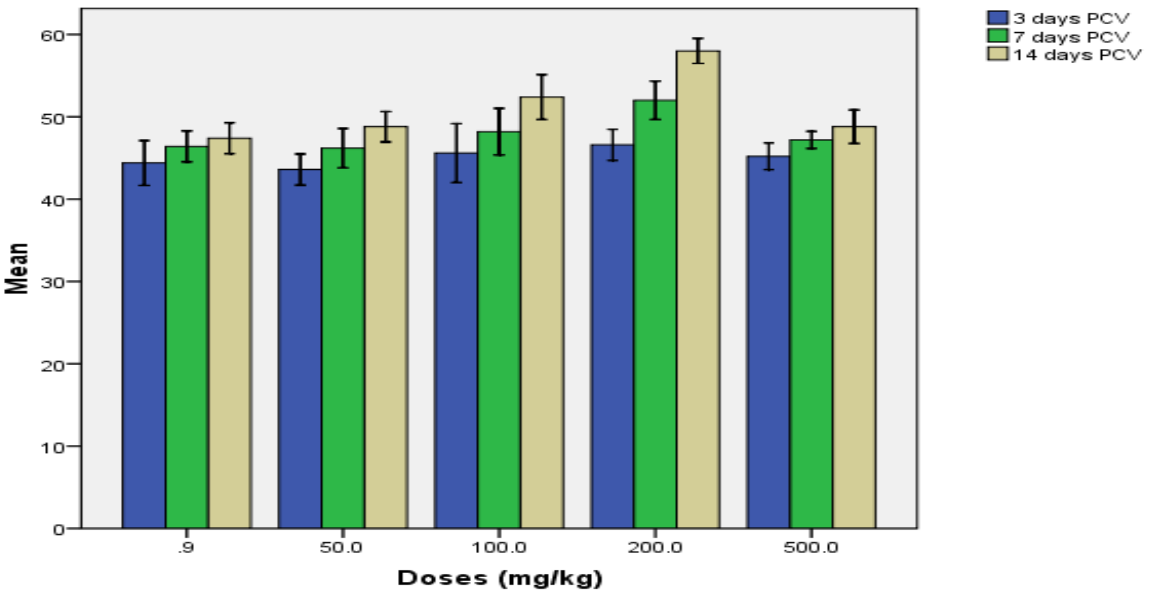
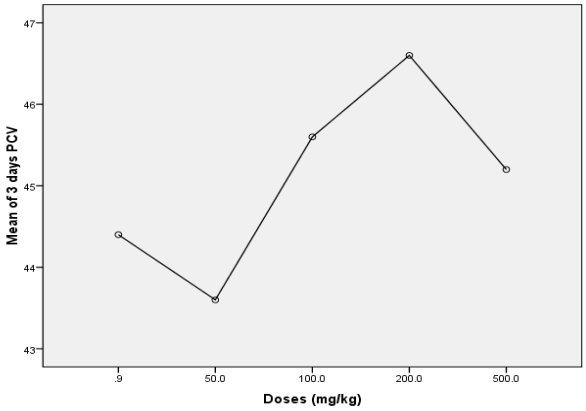
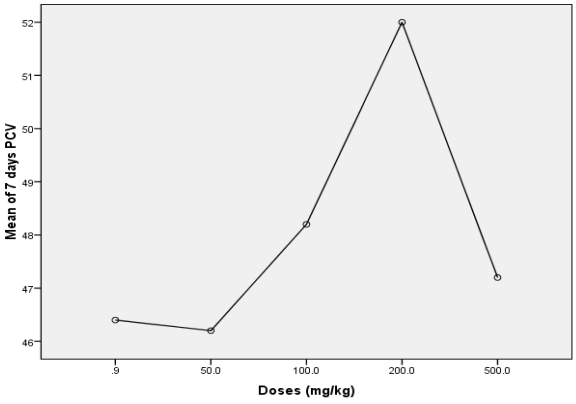


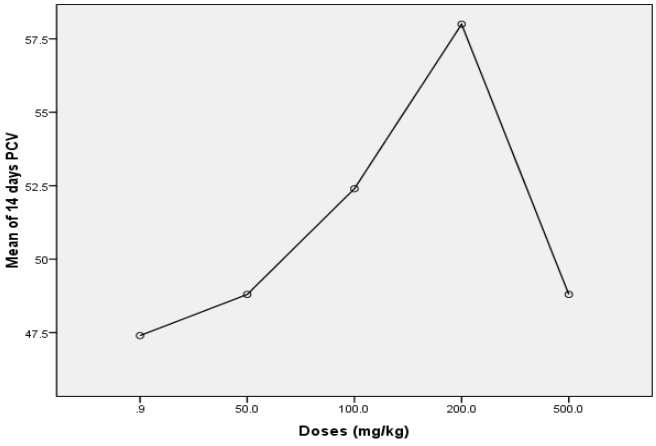
Figure 1: The effect of the extract on packed cell volume (PCV)



Graph 1



Graph 2



Graph 3

Figure 2: Graphs of mean PCV at various day intervals against dose of extract as well controls

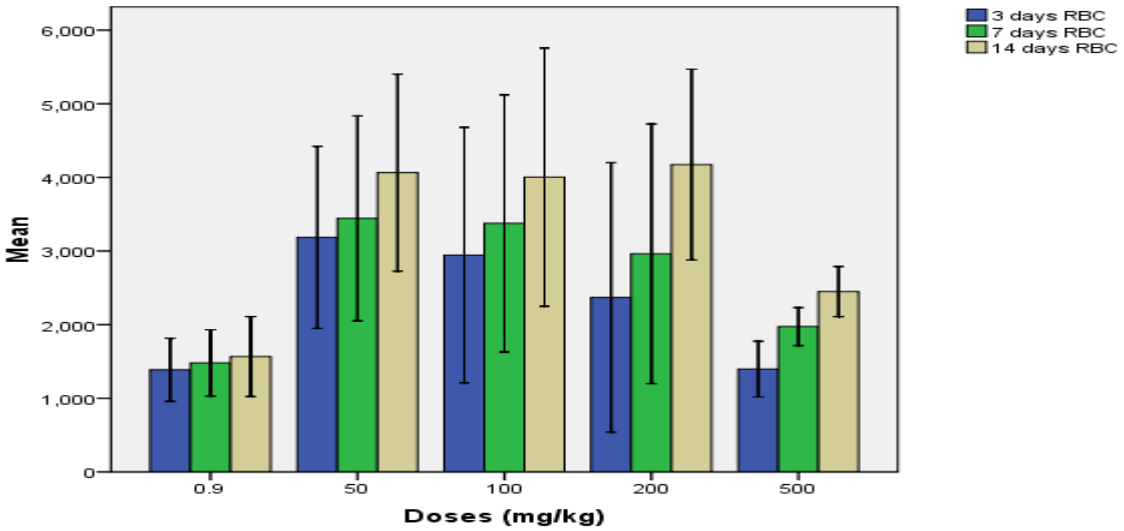
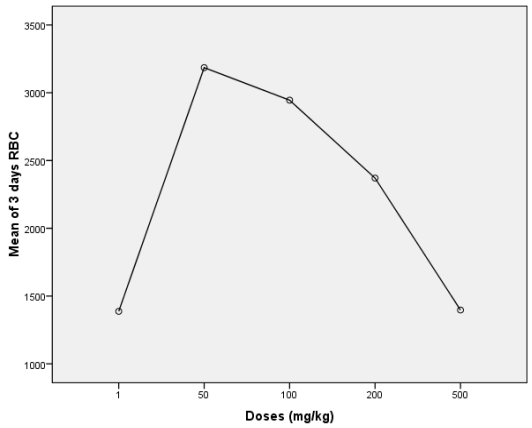
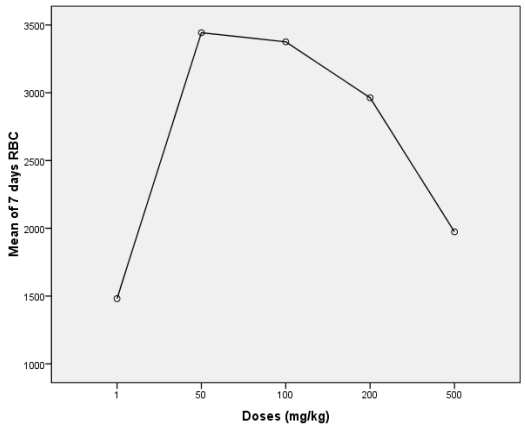


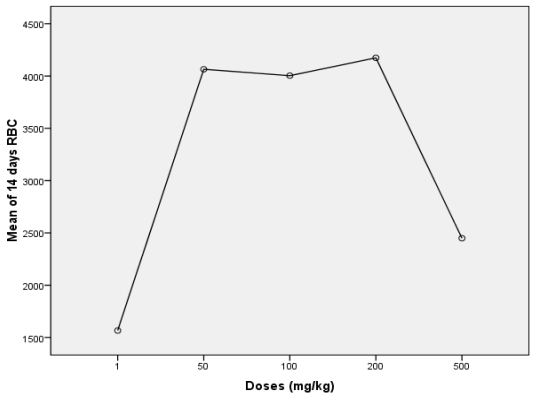
Figure 3: The effect of the extract on Red blood cell (RBC) count.



Graph 4



Graph 5



Graph 6

Figure 4: Graphs of mean RBC against dose of extracts as well as controls

Anti-Sickling Test Result

anti-sickling test shows moderate inhibition of sickling by the extract which showed about 32.81% and 36.9% mean inhibition by the ethanol extract on both SS red blood cell samples from the 13years old, 26years old and 32years old patients using concentrations of 50µg/l and 100µg/l as shown in figure 5-7: picture of cell morphology and table 5: percentage inhibition for individual samples.

Table 5: Antisickling effect of the extract

Blood Samples From Patients	Sickle Cells Inhibition (%)	
	100µg/I	50µg/1
13 years old	32.33	28.24
26 years old	35.96	33.25
32 years old	42.69	36.95
Mean % inhibition	32.81	36.93

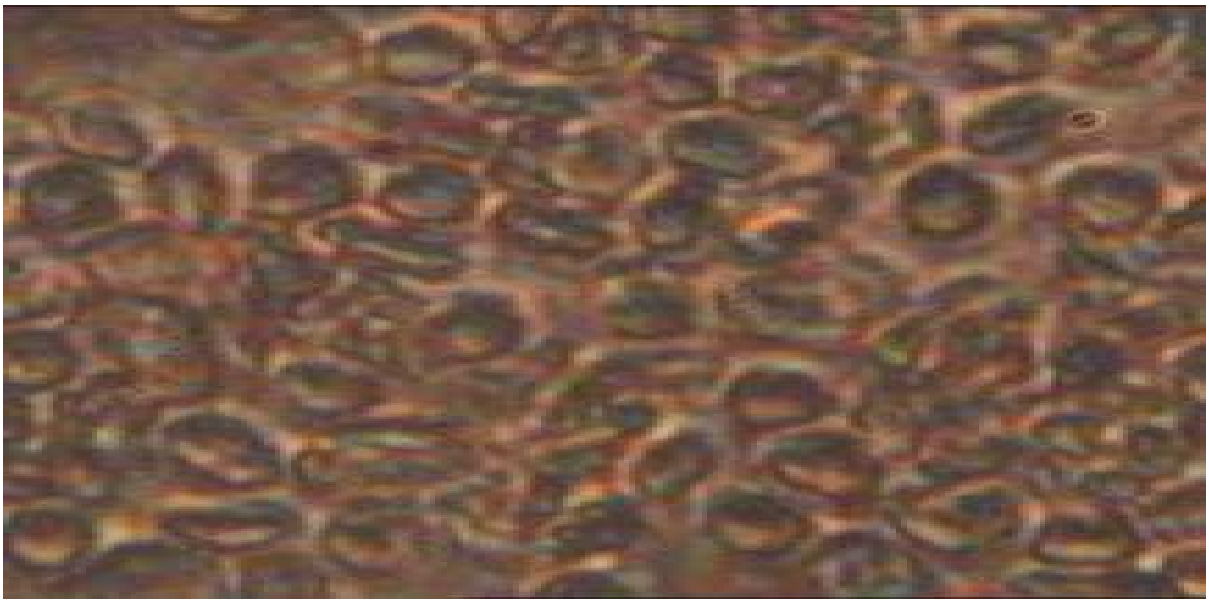


Figure 5: Photomicrograph showing the sickle cell morphology and the effect of the extract on sickle cell morphology: Non treated sickle cell blood

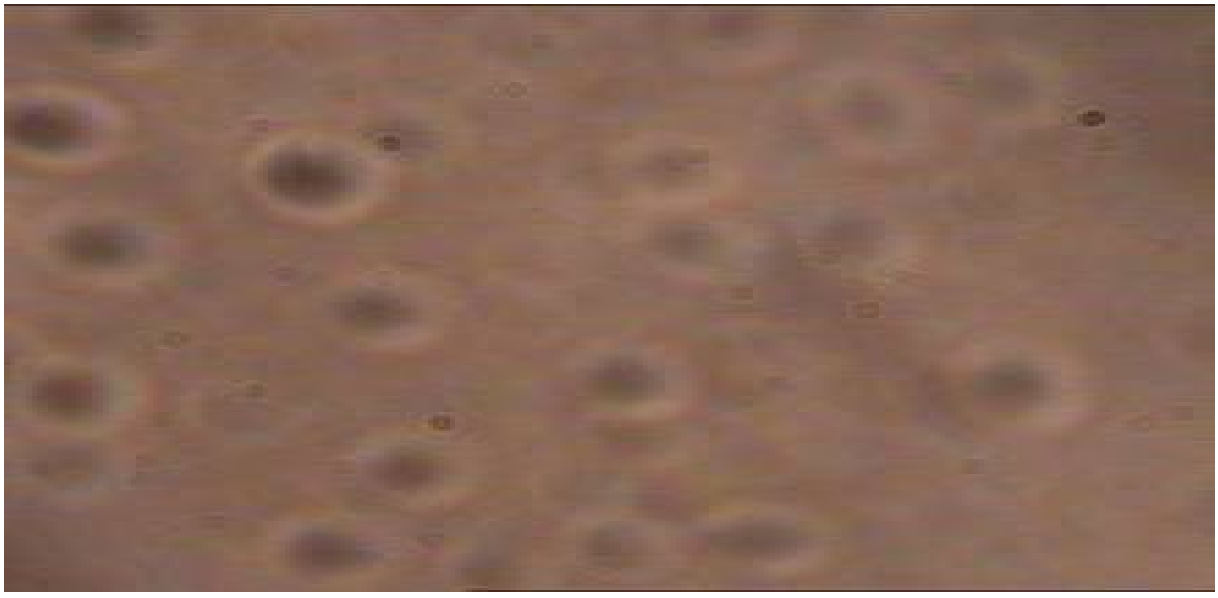


Figure 6: Photomicrograph showing the sickle cell morphology and the effect of the extract on sickle cell morphology: Effect of 50 µg/ml of extract

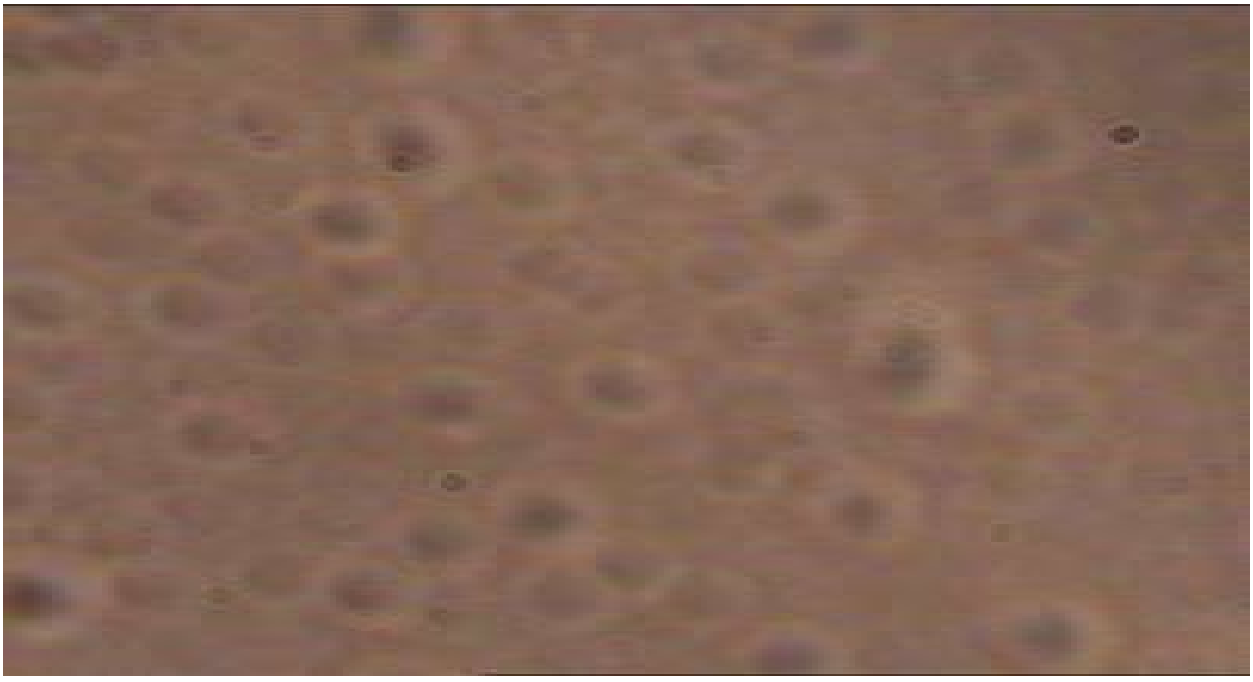


Figure 7: Photomicrograph showing the sickle cell morphology and the effect of the extract on sickle cell morphology: Effect of 100 µg/ml of extract

DISCUSSION

The antimicrobial result showed that the plant extract has moderate antimicrobial activity against test organisms in comparison with the standard control drug. Table 3 and 4 showed the effect of the aqueous and methanolic extract against tested organisms of bacteria and fungi respectively, the two fractions showed better activity against *Candida albicans* with the inhibition zone diameter of 8 and 10 mm against the highest concentration of 400mg/ml each and MIC of 50 and 25mg/ml respectively, while *Aspergillus nigger* was recorded with the 8 mm as against highest concentration as thesame above with the MIC of 50mg/ml each while the control standard of ketoconazole in table 6 against fungi used showed similar inhibition zone diameter of 8 and 6 mm against *C.albicans* and *A.nigger* respectively with the plant extracts but with minimal concentration in microgramme of 2µ/ml as highest concentration used with the MIC of 0.25 and 0.5µg/ml respectively. The results of the extracts against bacteria tested reveal that the two fractions showed more effective against Gramm positive than Gramm negative organism when consider their respective MIC's against the bacteria cells used in comparism with the positive control drug. The proximate and mineral analysis, further gives an insight on the indirect mechanism of stimulation of erythropoiesis by the extract since the nutrients and minerals such as proteins, carbohydrates, iron, copper, and cobalt, present in the plant leaf, helps to provide the body with the required "materials" for the synthesis of haemoglobin and red blood cells. However, this does not account for the significant erythropoietic effect of the leaf extract ($P< 0.05$ for group two and three animals), compared to the positive control as displayed in the comparative bar charts and means plot, which showed an increase in packed cell volume (PCV) and red blood cell count, as the dose of the extract was increased, with increase in time (days). Increase in PCV indicates an increase in haemoglobin concentration since haemoglobin concentration can be calculated by dividing the PCV by 3.[14] Furthermore, the red blood cell count (RBC), given by the 100mg/kg and 200mg/kg doses of the extract was lower than that given by the 50mg/kg dose on day 3. However, after days 7 and 14, the RBC given by the 100mg/kg and 200mg/kg doses exceeded that given by the 50mg/kg dose. This shows that at higher doses, there is a greater stimulation of erythropoiesis by the extract, given rise to a larger amount of circulating proerythroblast (immature red blood cells) in the blood; hence taking a longer time for

all of them to mature unlike when the proerythroblasts are smaller in number and requires a smaller synthesis of haemoglobin and other blood cell forming factors for their maturation. Thus, lower doses of the extract stimulate lower production of red blood cells but with faster maturation; while higher doses stimulate a higher production of red blood cells, but this will result to a slower or longer maturation time.

Hence it can be said that there are other mechanism(s) of stimulation of erythropoiesis by the *Ficus capensis* ethanol leaf extract, not explained by the mineral and nutrient content of the extract. The moderate anti-sickling activity of the leaf extract further gives an explanation on the traditional use of the plant for the management of sickle cell anaemia as the result of the anti-sickling experiment (Emmel's test), proves that the leaf extract can be effective in managing patients suffering from mild to moderate sickle cell crisis. But the management of severe crisis is questionable and requires further research. However, an activity guided fractionation and isolation work is on going on this plant part; in order to ascertain the compounds responsible for this significant activity.

CONCLUSION

The ethanol leaf extract of *Ficus capensis,* can stimulate erythropoiesis to a significant level and also has moderate anti-sickling action on the red blood cells of sickle cell patient, thereby justifying the use of the plant leaves for the treatment of anaemia in local African communities.

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