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Study on anti-sickling properties of the aqueous and methanol leaf extract of *Ficus mucuso* (Wild figs)

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Abstract
This study investigated the antisickling properties of the aqueous and methanol leaf extract of *Ficus mucuso* (wild figs) by determining its effect on haemoglobin-S (HbS) polymerization inhibition rate, haemoglobin/methaemoglobin (Fe^{2+}/Fe^{3+}) ratio and erythrocyte osmotic fragility. Preliminary phytochemical analysis of *F. mucuso* showed the presence of phenols, terpenoids, tannins, flavonoids, saponins and alkaloids. The extract significantly inhibited the rate of HbS polymerization up to 86.95±0.00%. Fe^{2+}/Fe^{3+} ratio was significantly raised from 3.83±0.82 in the control group to 23.51±0.05 and 10.55±1.00 for aqueous and methanol extracts respectively. Erythrocyte osmotic fragility was significantly reduced. The results from this study strongly suggests that the extract of *F. mucuso* possess anti-sickling properties and can be used in alternative medicine for the management of the accompanying complications of sickle cell anaemia.

Keywords: Ficus mucuso, anti-sickling, phytochemicals, sickle cell anaemia

Introduction
We live in environments surrounded by plants many of which are known as medicinal herbs; these herbs are reckoned as the foundation for health maintenance, care and preservation in all the parts of the world. Over the years, it had been discovered that these plants have in them very vital molecules known as phytochemicals with important medicinal properties for healthy living. For this reason, medicinal herbs have been very useful in the avoidance and treatment of diseases and in the overall maintenance of health all over world. The phytochemicals naturally found in medicinal plants provide defense against different types of disease-causing organisms. They show analgesic, sedative, anti-inflammatory, etc. properties [1].
Sickle cell anaemia (SCA) is better described as a disorder which is inherited and in which the red blood cells (RBCs); that are naturally spherical, are shaped in anomalous ways arising because of the inheritance of a deformed sickle cell gene from both mother and father. The condition is inherited in an autosomal recessive pattern; it develops at the point of conception and remains for life and can be passed on from one generation to another [2].
The sickle cell anaemia (SCA) condition manifest when a point mutation occurs in the β-chain of haemoglobin (the protein that carries oxygen in blood). In this mutation, glutamic acid (a relatively polar amino acid) is replaced with valine (a less polar amino acid) at the 6^{th} position of the β-chain which results in the production of haemoglobin-S (HbS), which can undergo polymerization when oxygen tension is low, resulting in distortion of the red blood cells and an inclination for them to lose their elastic characteristics. The resultant red blood cells take up a crescent shape with narrow and sharp ends [3].
The sickling process also known as sickle cell crisis can be put in motion by alterations in temperature, high altitudes, dehydration, stress, chemicals, etc. The sickling process damages blood vessels membranes which makes the blood cells to get stock in blood vessels, this obstructs the ease of flow of blood round the body. This primary effect strips the tissues and organs of oxygen and produces varying levels of organ damage and even stroke. Other damaging effects includes premature death of red blood cells, which consequently results to severe anaemia because of the presence of only a hand full of natural red blood cells in circulation.
Jaundice and severe infection also arise due to the excessive destruction of red blood cells and damage to the spleen. Normal steady growth and maturation are affected, inflammation along

joints occurs often, there is also progressive organ degeneration from long term altered circulation, severe fever, and higher risk of pains, shock, malaria, coma and even death [2]. Since the time this condition was discovered, scientists in all parts of the world have been working relentlessly and have made effort towards discovering an enduring cure to the condition. Reports shows that medical researchers working in Europe and America successfully treated a number youngsters with the SCA condition employing the technique of bone marrow transplant. This technique though successful, is however reported to be cost effective and generally not affordable by an ordinary sufferer living in underdeveloped and developing countries where the SCA condition is prevailing. The challenge of compatibility is also widely reported for many cases.

Hydroxyurea; a drug primarily known to elevate foetal haemoglobin (Hb-F) level (that resists the sickling of RBCs) is specifically employed in managing the condition. But the drug however does not provide a lasting cure and research shows that it has mutagenic potentials [4]. Blood transfusion; another method employed in managing the condition helps to introduce normal RBCs into circulation however, there is the risk of predisposition to Hepatitis, Human Immunodeficiency Virus (HIV) and (AIDS) and other blood infections from long term usage. All these obstacles have made proper management of the condition the foremost choice for now [2].

Ficus mucuso is an infrequent indigenous coniferous tall tree that reaches up to 30m height. It belongs to the genus ficus, having about 150 different plant species widely distributed in different parts of the world. This plant adapts well to semi-deciduous and old secondary forests, its predominant habitat is however the grassland, preferably lowland rainforests. *Ficus mucuso* is a member of the Moraceae family possessing rough leaves and having a chordate base with a ring of short stiff brown hairs at each node, broad with simple leaf form, 6-17cm long. This plant is primarily a tree that produces fruits called figs that are ovoid in shape. The figs are hairy, and having a dark orange colour [5].

Ficus mucuso is ordinarily called wild figs [25]. It is locally called Odan-afomo, Obobo [7] and Ogum in Epie language. It usually has an orange-coloured bark and produces latex which immediately oxidizes to orange. Birds and chimpanzees are generally attracted to the figs of ficus and for this reason, they are normally found around the plant where they feed on the figs [25]. It is believed to be an oxygen producing plant as its evergreen leaves allows for photosynthesis. The plant is found in West Africa from Angola to Guinea, Ethiopia, Uganda, Mozambique, Tanzania and in Nigeria and named after an Angolan town called Mucuso where it was discovered for the first time. Djemgou *et al*. (2009) [25] in different studies showed that the plant is rich in terpenes and other phytochemicals like flavonoids and alkaloids. A number of species in the genus ficus, have been reported to possess vital pharmacological properties namely; antimicrobial, anti-anaemic, anti-diabetic and anticancer. Banku *et al*. (2010) [8] and Banku *et al*. (2011) [9] in separate studies found that the stem and bark of *Ficus mucuso* is highly rich in sphingolipids (mucus amide and mucusoside), isoflavone dimer derivatives (mucuso isoflavones) and other very important secondary metabolites. Slantnar *et al*. (2011) [10] and Arvaniti *et al*. (2019) [11] in different studies also reported the presence of polyphenols, monosaccharide sugar and flavonoids in the fruits of *F. mucuso.* The figs from this plant locally serves as a source of food to birds and animals, the branches are a major source of firewood in the local communities, while the tree serve as comfortable shade anywhere it grows. The timber is used in carving canoe and other wood works and the leaves of this plant is boiled and the extract is taken as a blood buster especially by women who have just put to bed.

Materials and Methods

This research received ethical approval from the university of Port-Harcourt research management and development ethics committee. Fresh red blood cell sample was obtained from haemoglobin-SS individuals not in crisis attending routine medical checkup at the Diete Koki Memorial Hospital, Opolo-Epie, Yenagoa, Nigeria.

Haemoglobin S polymerization rate

The procedure Noguchi and Schetcher (1978) as cited by [12] was adopted with mild modification. 4.4mL of 2% sodium metabisulphite ($Na_2S_2O_5$), 0.5 mL of 1% normal saline and 0.1 mL of haemolysate were put in a test-tube, the resultant solution was mixed and immediately transferred into a cuvette and the absorbance read at 700 nm every two (2) minutes for 18 minutes using a spectrophotometer (Spectrumlab 23A). This was for the standard. For the test, 0.5mL of the plant extract replace normal saline. Distilled water was used as the blank. For the control sample, 0.5 mL of 2% L-Phenylalanine was added in place of the plant extract. HbS polymerization rate was calculated as follows;

$RP = \{OD_f - OD_i /t\}$. RP= rate of polymerization, OD_f= final absorbance at time t, OD_i= initial absorbance at zero, t=time of assay in minutes.

Percentage cell polymerization inhibition (PPi)
PPi of assay = RP assay/RP control x 100/1

Haemoglobin/methaemoglobin (Fe^{2+}/Fe^{3+}) ratio: The method of Davidson and Harry (1974) as cited by [12] was adopted. 0.02 mL of 1% normal saline, 5 mL of distilled water (DH_2O) and 0.02 mL of whole blood were put into a test tube, mixed and allowed to stand at room temperature for 60 minutes and the absorbance measured at 540 nm (Fe^{2+}) and 630nm (Fe^{3+}) using a spectrophotometer. The result obtained here was for the control. 0.02 mL of the plant extract replaced 0.02 mL of normal saline for the test sample while distilled water served as blank. Calculations;

$\%Hb = (A_{540})/(A_{540}) + (A_{630}) \times 100$. $\%mHb = (A_{630})/ (A_{630}) + (A_{540}) \times 100$

Ratio= % Hb/ % mHb

% Hb= percentage haemoglobin, % mHb= percentage methaemoglobin, A_{540}= absorbance at 540nanometer and A_{630}= absorbance at 630nanometer.

Erythrocyte osmotic fragility test

The Wintrobe method as reported in Baker, F.J. and Silverton, R. E. Introduction to Medical Laboratory Technology (1976) [13] was adopted. A stock solution of sodium chloride (NaCl) osmotically equivalent to 10% was fitted out and diluted 1 in 10 to form 1% NaCl. From this, 100mL 0.85%, 0.70%, 0.65%, 0.60%, 0.55%, 0.50%, 0.45%, 0.40%, 0.35%, 0.30%, 0.20% and 0.10% were prepared. Two sets of test tube racks having 13 tubes were assembled, 4.5 mL of the corresponding graded normal saline concentrations transferred into tubes 1–12 then 5 mL of distilled water into tube 13. 0.5 mL of the plant extract of was added to tubes 1–12 and 0.02 mL of blood was also added to all the tubes, the content of the tubes was mixed by gentle inversion, kept at room temperature for up about 30 minutes, then centrifuged for 15 minutes. The supernatant from every tube was carefully put in a cuvette read at 540nm. Lysis was noted as a percentage with the reading of tube 13 as 100% lysis. The percentage of lysis was plotted against normal saline concentration to obtain a 'fragility curve'.

Percentage haemolysis = reading of test / reading of 100% x 100 / 1

Results and Discussion

Table 1: Effect of the aqueous and methanol leaf extracts of *F. mucuso* on percentage inhibition of HbS polymerization rate

Time (minutes)	Standard	L-Phe	Aqueous extract	Methanol extract
0	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]
2	0.00±0.00 [a]	66.67±0.00 [b]	67.00±0.00 [b]	0.00±0.00 [a]
4	0.00±0.00 [a]	50.00±0.13 [c]	67.00±0.51 [c]	-16.70±0.28 [b]
6	0.00±0.00 [a]	70.60±0.40 [d]	68.00±8.16 [d]	-30.00±0.00 [c]
8	0.00±0.00 [a]	69.23±1.22 [e]	69.23±0.97 [e]	-30.77±0.06 [d]
10	0.00±0.00 [a]	75.43±4.62 [f]	85.71±0.01 [f]	-35.71±0.00 [e]
12	0.00±0.00 [a]	61.11±0.01 [g]	66.67±0.03 [g]	-38.88±0.00 [f]
14	0.00±0.00 [a]	65.00±0.20 [h]	80.00±8.16 [h]	-35.00±0.00 [g]
16	0.00±0.00 [a]	68.18±1.47 [i]	77.30±0.05 [i]	-36.39±0.02 [h]
18	0.00±0.00[a]	78.26±0.00 [j]	86.95±1.00 [j]	-43.47±0.00 [i]

******* HbS activation was observed for this methanol extract.

Values in the table are Mean ± S.D from triplicate determinations.

Values with different superscript letters on the same column differ significantly at $p<0.05$.

Table 2: Effect of the aqueous and methanol leaf extracts of *F. mucuso* on haemoglobin/methaemoglobin (Fe^{2+}/Fe^{3+}) ratio

Proportion (mL)	Aqueous extract			Methanol extract		
	%Hb	%mHb	Fe^{2+}/Fe^{3+} ratio	%Hb	%mHb	Fe^{2+}/Fe^{3+} ratio
1.00	77.30±0.01[h]	22.71±0.00[h]	3.40±0.00	75.66±0.09[h]	24.32±0.00[h]	3.11±0.10
0.10	87.16±0.09[g]	12.83±0.01[g]	6.79±0.00	86.78±0.05[g]	13.21±0.10[g]	6.57±0.00
0.08	88.55±0.00[f]	11.45±0.09[f]	7.73±0.01	69.05±0.04[f]	30.94±0.01[f]	2.23±0.00
0.06	90.24±0.00[e]	9.73±0.00[e]	9.25±0.05	91.34±0.15[e]	8.66±0.00[e]	10.55±1.00
0.04	73.43±0.07[d]	26.57±0.03[d]	2.76±0.000	77.68±0.05[d]	22.32±0.09[d]	3.50±0.01
0.02	94.54±0.00[c]	5.45±0.08[c]	17.35±0.01	80.45±0.03[c]	19.55±0.10[c]	4.15±0.07
0.01	95.92±0.00[b]	4.08±0.05[b]	23.51±0.05	82.67±0.00[b]	17.33±0.00[b]	4.77±0.05
Control	79.28±0.20[a]	20.72±09[a]	3.83±0.82	79.28±0.20[a]	20.72±0.09[a]	3.83±0.82

Values in the table are mean ± S.D from triplicate determinations.

Values with different superscript letters on the same column differ significantly at $p<0.05$.

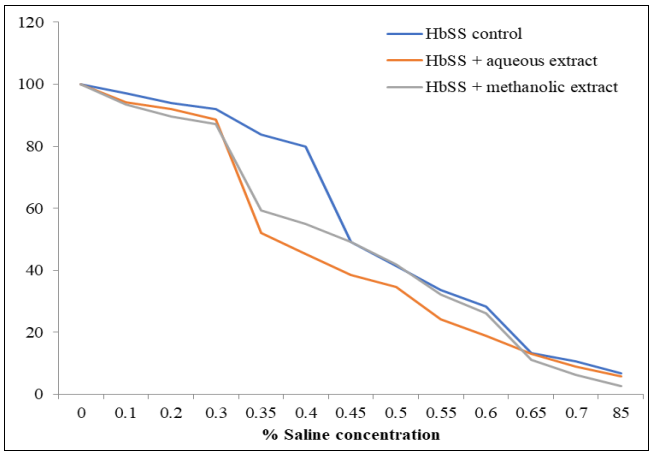

Fig 1: showing effect of the aqueous and methanol leaf extracts of *Ficus mucuso* on erythrocyte osmotic fragility.

The lookout for medicinal herbs possessing viable potential for use as drugs in the management and treatment of diseases is in progress. Pharmacognosists, phytochemists, biochemists, microbiologists, botanists, etc. are working relentlessly in concert to accomplish this feat. Alternative medicine holds a pivotal position in the foundational health care system especially in our part of the world. This is largely due to its proven efficacy and cost effectiveness a larger percentage of the populace is seen to be dependent on alternative medicine in many ways [14]. From the results of this study, the aqueous extract of *F. mucuso* was able to effectively inhibit HbS polymerization rate with time. This is not unconnected with the high presence of phytochemicals in the medicinal herb which are known to adhere to and protect the points of assembly of HbS monomers necessary for the polymerization process to take place [15] which then stamped down the whole process. The inhibition of polymerization must have been achieved due to the *in vitro* reversal of sickle erythrocytes possibly mediated by the phytochemicals in the extract, an increased gelling period for the altered erythrocytes with specific impact on the in vitro sickling process [16]; and a general improvement in oxygen binding affinity. The anti-sickling characteristics shown by the aqueous leaf extract of *F. mucuso* is in agreement with those reported for *Aloe vera* [17], *Eugenia carryophylla*, *Sorghum bicolor* [18], *Carica papaya* [19], *Telferia occidentalis*, *Allium cepa*, *Allium sativum* [15], and the leguminous seed, *Cayanus cajan* [20], etc.

Managing SCA with this aqueous leaf extract will possibly show a duplicate impact of lowering the pathological issues and alleviating the corresponding anaemia condition as well as the overall advancement of health status of the individual. This is in conformity with the report of [16] stating that native traditional therapists not having adequate technological and scientific knowledge have for many years efficiently managed the sickle cell anaemia challenge employing various herbs and herbal preparations. In this study, the methanol extract could not suppress the pace of haemoglobin S polymerization; this could be attributable to be due to the effect of alcohol on sickle cell anaemia. The extracts viably raised haemoglobin/ methaemoglobin (Fe^{2+}/Fe^{3+}) ratio clearly showing an extract induced furtherance in the comprehensive oxidant status of the erythrocyte cells. Haemoglobin (Hb); a protein in red blood cells responsible for oxygen transport has iron (Fe) constantly maintained in the bivalent +2 oxidation state (Fe^{2+}) to properly attach to and move oxygen (O_2) within the body. Methaemoglobin; is a variable form of haemoglobin where the iron (Fe) is in the ferric state (Fe^{3+}) that is, the +3-

oxidation state with an impaired oxygen binding and transport ability [21]. The extracts significantly (p<0.05) elevated the ratio of haemoglobin to methaemoglobin from 3.83 ± 0.83 in the control group to 10.55 ± 1.00 and 23.51 + 0.05 for methanol and aqueous extracts respectively. The extracts must have through the help of the phytochemicals pulled a positive effect on the cytochrome b_5 reductase enzyme complex (NADH methaemoglobin reductase) that is responsible for catalyzing the reduction of iron (Fe) to ferrous state with the attendant restoration of the haemoglobin molecules to their natural functional state. The extracts displayed erythrocyte membrane preservative characteristics against lysis mediated by variable saline concentrations. The cell's osmotic fragility is an indication of the strength by which it takes up water without lysis and this strength for a normal healthy human erythrocyte cell is mainly rested on its original spherical conformation which allows the cell to accomplish greater volume up to 70% before the surface tissue layer is stretched [15]. In one study, [22] reports that erythrocytes exposed to osmotic stress show reduced resistance giving rise to increased lysis. The erythrocytes showed significant resistance to osmotic disturbance on treatment with the extracts. Anosike *et al*., (2019) [23] reports that extract preparations with membrane stabilizing activities achieve that by adhering on the erythrocyte membranes where they effect a change on the charges, they bear with the resultant effect being in preventing material interactions with the component parts coming together or the separation by correlative repulsion of the charges actively involved in the haemolysis of the red blood cells. The extracts clearly demonstrated membrane stabilizing activities by lowering osmotic fragility. This is attributable to the action of the relevant phytochemicals. The vital medicinal components of the extracts must have exerted a positive outcome on the conformation of the erythrocytes by elevating their volume and returning the altered erythrocytes to their natural spherical form thereby retaining their stability. Kang and Benjamin (1975) [24] had earlier stated this mechanism for homoserine known to inhibit in vitro sickling of red blood cells in hypotonic preparations. The results obtained from this study is in agreement with their findings and buttress the claim of efficacy and potency of these extracts to manage sickle cell anaemia.

References

1. Atanasor AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrim P, *et al*. Discovery and resupply of pharmacologically active plant derived natural products; A review. Biotechnology Advances. 2015;33(8):1582-1614.
2. Ekeke GI. Sickle cell anaemia, basic understanding and management. Harrisco Press, Port-Harcourt; c2001. p. 1-62.
3. Rees DC, Williams TN, Gladwin TN. Sickle-cell disease. Lancet. 2010;376(9757):2018-2031.
4. Buchana GR, Debaun MR, Quinn CT, Steinberg MH. Sickle cell disease Haematology; c2004. p. 35.
5. Berg CC. Reproduction and Evolution in ficus (Moracae). Traits connected with adequate rearing of pollinators. Memoirs of the New York Botanical Garden. 1990;55:169-185.
6. Khan WA. *Ficus palmata* a nutraceutical under took advantage of the product. Int. J Agric. Nutr. 2021;3(2):14-18. DOI: 10.33545/26646064.2021.v3.i2a.67
7. Tolu O. Traditional medicine. The arts, ways and practice. outlines and pictures of medicinal plants from Nigeria, University of Lagos Press; c2008. p. 13, 117, 131, 148.
8. Banku JJ, Mustafa SA, Gojayev AS, Tchamo ND, Lenta BN, Ngouela SA, *et al*. Ceramide and Cerebroside from the stem of *Ficus mucuso* (Moracae). Chemical and Pharmaceutical Bulletin. 2010;58(12):1661-1665.
9. Banku JJ, Khayala R, Lenta BN, Noungue DT, Ngouela SA, Mustafa SA, *et al*. Isoflavonone figs of Ficus mucuso. Journal of Natural Products. 2011;74(6):1370-1378.
10. Slantnar A, Klancar U, Stampar F, Veberic R. Effect of drying of figs on the contents of sugars, organic acids, and phenolic compounds. Journal of Agricultural and Food Chemistry. 2011;59(21):11696-11702.
11. Arvaniti OS, Samaras Y, Gatidou G, Thomaidis NS, Stasinakis AS. Review on the fresh and dried figs: Chemical analysis and occurrence of Phytochemical compounds, antioxidant capacity and health effects. Food Research International. 2019;119:244-267.
12. Uwakwe AA, Nwaoguikpe RN. In vitro anti-sickling effects of xylopia aethiopica and Mondara myristica. Journal of Medicinal Plant Research. 2008;2(6):119-124.
13. Baker FJ, Silverton RE. Haematology. Introduction to Medical Laboratory Techniques. 5th ed. Butterworth and Co. publishers Limited, London; c1976. p. 549-602.
14. Shrestha PM, Dhillion SS. Medicinal Plant diversity and use in the hills of Dolakha district, Nepal. Journal of Ethnopharmacology. 2003;86(1):81-96.
15. Nwaoguikpe BN. The anti-sickling effect of some edible vegetables. International Journal of Biological and Chemical Sciences. 2009;3(5):1005-1012.
16. Dash BP, Archana Y, Nibarana S, Soumendra KN. Search for anti-sickling agents from plants. Pharmacognosy Reviews. 2013;7:53-60.
17. Ejele AV, Njoku PC. Anti-sickling potential of Aloe vera extract. Journal of the Science of Food & Agriculture. 2008;88:1482-1485.
18. Mechanna AS. Sickle cell anaemia and anti-sickling agents then and now. Current Medicinal Chemistry. 2001;8:79-88.
19. Imaga NOA, Gbenle GO, Okochi VI. Anti-sickling property of Carica papaya leaf extract. African Journal of Research. 2009;3(4):102-106.
20. Ekeke GI, Uwakwe AA, Nwaoguikpe RN. Edible legumes as beneficiary anti-sickling agent. Nigerian Journal of Biochemistry and Molecular Biology. 2000;16:200-203.
21. Norbert WT. Biochemical aspect of haematology. In: Carl, B. & Edward, R.A. (eds). Tiez Fundamentals of Clinical Chemistry, 4th ed. W.B. Saunders Company, Philadelphia, London; c1996. p. 704-719.
22. Chikezie PC, Uwakwe AA, Monago CC. Comparative osmotic fragility of three erythrocyte genotypes. (HbAA; HbAS & HbSS) of male participants administered with five antimalarial drugs. African Journal of Biochemistry Research. 2010;4(3):57-64.
23. Anosike CA, Igboegwu ON, Nwodo OFC. Antioxidant properties and membrane stabilization effects of methanol extract of Mucuna pruriens leaves on normal and sickle erythrocytes. Journal of Traditional and Complementary Medicine. 2019;9(4):278-284.
24. Kang SS, Benjamin G. Homoserine inhibition of in vitro sickling of erythrocytes. Experimental & Molecular Pathology. 1975;20:220-225.
25. Djemgou PC, Ngandeu F, Hegazy MEF, Nkanwem ER, Neguim G, Chonsson E, *et al*. GC – MS analysis of terpenes from Ficus mucuso. Pharmacognosy Research. 2009;01:197-201.