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PHARMACOPOEIAL STANDARDIZATION OF THREE NIGERIAN *KHAYA* SPECIES FOR ANTISICKLING PROPERTIES

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BY

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CERTIFICATION

This is to certify that the research work titled 'Pharmacopoeial Standardization of Three Nigerian *Khaya*species for AntisicklingProperties was carried out by OloladeAdesomi OYEDAPO for the award of Ph.D. in the Drug Research and Production Unit, Faculty of Pharmacy, ObafemiAwolowo University, Ile-Ife, Nigeria.

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(Co-Supervisor) Signature and date

Prof. C. O. Adewunmi _____

(Director) Signature and date

DEDICATION

ToAlmighty God and my Darling husband Barr. Olusegun Festus Oyedapo for his support, words of encouragements and for always being there for me. I thank God for you. I could not have chosen better.

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I wish to express my profound gratitude to ALMIGHTY GOD, the Alpha and the Omega who had spared my life and made it possible to start and conclude this programme. May your name be glorified oh Lord. Who is like unto thee oh Lord among other gods?

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Again I return all glory to God the creator of heaven and earth, thanks for the good health, the courage and for sparing my life out of all odds and challenges made it possible to complete the research forever Iwill worship your name. THANK YOU LORD.

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ABSTRACT

The study examined the macro and micro morphology of *Khayasenegalensis*(K.S)A.Juss (Welw), *Khayagrandifoliola*(K.G)C.Dcand*Khayaivorensis*(K.I)A.Chevof the family Meliaceae, identified any morphological differences in the three species, evaluated some pharmacognostic parameters for pharmacopoeial standardization of the three plants and compared their anti-sickling activities. This was with a view to providing information on the different species of *Khaya* and their anti-sickling potential.

The leaves and the stem bark of K. *senegalensis*A.Juss (Welw), *K. grandifoliola*C.Dc, and *K. ivorensis*A.Chev were collected, authenticated, processed and extracted separately using three different solvents:absolute ethanol, water and petroleum spirit. The extracts were concentrated *in vacuo,* freeze dried and evaluated separately for their anti-sickling inhibitory and reversal, using sodium meta-bisulphite as reducing agent, p-hydroxy benzoic acid and vanillic acid as positive controls for reversal and inhibitory activities respectively while 5% v/v Tween 80 was the negative control. The extract with the highest anti- sickling properties was subjected to thin layer chromatographic (TLC) finger printing. Comprehensive anatomical examinations, of various sections of the leaf, stem, root and stem bark were carried out using standard methods. Photomicrographs of the slides were made. Proximate analysis was carried out for the three species using simple analytical procedures, moisture content, ash values, crude fibre, total crude proteinas well as elemental analysis.

The macroscopic and microscopic study of the three species revealed that there were diagnostic features in the leaf, stem, roots and bark that can be used for distinguishing among the three*Khaya*species. The result of the proximate and elemental analysis showed that the leaves

and stem bark contained necessary rich nutrients and essential minerals. The anti-sickling study of inhibitory and reversal activities showed that both leaf and stem bark of *Khaya*species studied possessed ability to inhibit the sickling of red blood cells with KI stem bark giving highest inhibitory values of 80.71% and 71.06% for hot ethanolicSoxhlet and cold ethanolicextractions respectively while its leaf gave highest reversal activities of 74.97% and 69.97% for hot ethanolicSoxhlet and cold ethanolic extractions respectively. The inhibitory and reversal of sickled red blood cells was at higher percentages than the standard drug vanillic acid of 58.20% and para-hydroxyl benzoic acid of 46.27% . The activities are dose dependent with highest activities at 4mg/ml. The activity of the petroleum spirit extracts was very low.

The study concluded that the *Khaya* species can be developed for herbal drugs in the managementof sickle cell disease since they possessed antisickling activities.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Sickle cell disorder (SCD), is a haemoglobinopatic disease aggravated by depletion of oxygen resulting in anaemia, occlusion of blood vessels by misshapen cells, and various associated clinical consequences, including death (Platts *et al.,* 1994). It is a pathological disease of blood. Blood is a bodily fluid in animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from the same cells. Blood accounts for 7% of the human body weight (Alberts and Bruce, 2012,Elert *et al.,* 2012) with an average density of approximately 1060 kg/m^3, very close to pure water's density of 1000 kg/m^3 (Shmukler and Michael, 2004). The average adult has a blood volume of roughly 5 liters (1.3 gal) (Elert *et al.,* 2012) which are composed of plasma and several kinds of cells. These blood cells (which are also called corpuscles or "formed elements") consist of erythrocytes (red blood cells, RBCs), leukocytes (white blood cells), and thrombocytes (platelets) (Plate 1). By volume, the red blood cells constitute about 45% of whole blood, the plasma about 54.3%, and white cells about 0.7%. Blood pH is regulated to stay within the narrow range of 7.35 to 7.45, making it slightly basic (Waugh *et al.,* 2007).

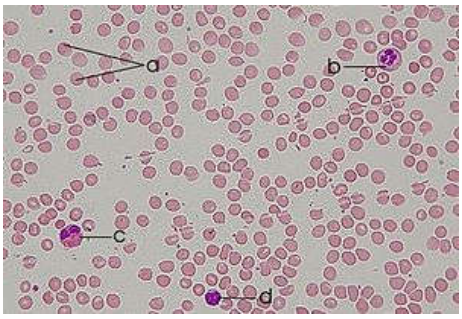


Plate 1: Human Blood Smear; Source: From Wikipedia, the free encyclopedia a – erythrocytes; b – neutrophil; c – eosinophil; d – lymphocyte.

Haemoglobin genotypes and blood groups are all inherited blood characters. The inherited disorders of haemoglobin are the most common gene disorders with 7% of the world's population being carriers (Weatheral andClegg 2001). It is on record that about 300,000 children are born with sickle cell disease (SCD) worldwide every year (Okpala *et al.,* 2002). Sickle cell disorders are found very frequently in the Afro-Caribbean populations and sporadically throughout the Mediterranean region, India and the Middle East (Weatheral *et al.,* 2001). These disorders include the heterozygous state for haemoglobin S or the sickle cell trait (AS), the homozygous state for HbS or sickle cell anaemia (SS) and the compound heterozygous state for HbS together with haemoglobin C, D, E, α-thalassemia or other structural variants.SCD is caused by mutation of the beta-globin gene. Haemoglobin S differs from haemoglobin A by the substitution of valine for glutamic acid at position 6 in the β - chain1, (Okpala *et al.,* 2002) producing haemoglobin, designated haemoglobin S that has less solubility than does of normal haemoglobin (A).

Sickle cell anaemia is hereditary. It is a disease passed down through families in which red blood cells form an abnormal crescent shape (Plate 2). (Red blood cells are normally disc shape

however sickle cell is crescent in shape Plate2) when one gene haemoglobin S, is found in association with normal gene haemoglobin A, results in the formation of haemoglobin AS. This type of red cells contains approximately 40 per cent of the abnormal haemoglobin (HbS) and 60 per cent of the normal haemoglobin (HbA), an essentially harmless state that is designated as sickle cell trait or a carrier (HbAS) are abnormal i.e. another (HbS). But if both genes are inherited, then the sickle cell disease may develop (HbSS) (Fig.1). The most common haemoglobin that interacts with sickle haemoglobin (HbS) is haemoglobin (HbC), and the β-thalassemia (beta-thalassemia) mutation also interacts with the sickle gene by restricting the formation of normal haemoglobin (Platts *et al.,* 1994).

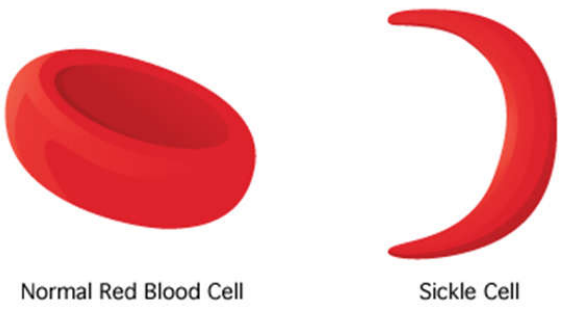
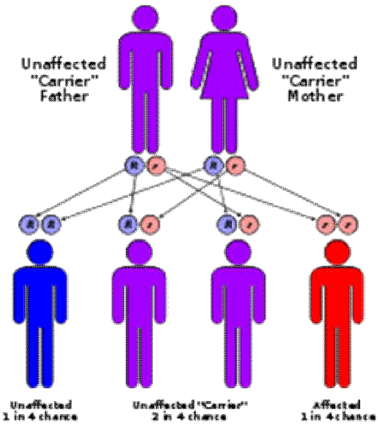


Plate 2: The Normal RBC and the Sickled RBC.

Source: http://www.nhlbi.nih.gov



Source: http://en.wikipedia.org/wiki/File

Fig 1a: Genetical result of parent carrier of sickle cell

A single dose of the sickle gene provides protection against malaria. Since malaria was a major cause of death in Africa, persons who carried the sickle gene had a survival advantage over those who did not (Wellem *et al.,* 2009). It is also a common disease in Nigeria. Some of its signs and symptoms as stated by Chrouser *et al.,* 2011 which includes anaemia (Weatherall and Clegg, 2001), pains (Geller and O'Connor, 2008), hands and foot syndrome, frequent infections (Pearson, 1977), vision problems (Elagouz *et al.,* 2010), delayed growth and puberty, fever accompanied with paleness, fatigues, yellowing of eyes and skin jaundice,