**A Comparative evaluation of the Antisickling, Elemental and Proximate Analysis of the Leaves ofThree *Khaya species* found in Nigeria.**


## ABSTRACT

Sickle cell disease (SCD) is traditionally treated in Nigeria by using herbs especially by theTraditional Medical Practitioners (TMPs). One of the herbs commonly used is *Khaya*, which is widely distributed in Africa, andthree species exist in Nigeria. These are *Khaya senegalensis*A.Juss (KS), *Khayagrandifoliola*(WELW) CDC(KG) and *Khayaivorensis*A. Chev. (KI). The stem bark of *K. senegalensis* was reported to possess anti-sickling activity, hence, in this study we evaluated the anti-sickling activities ofthe leaf extracts of the three *Khaya* species leaves with a view to authenticate the ethnomedicinal claims, and assess the proximate as wellas the elemental constituents that may contribute to the properties.

Dried leaves of the *Khaya* species were extracted by cold maceration and Soxhlet methods using water, ethanol and petroleum spirit as extractives. Filtered extracts were concentrated *in vacuo* at $40^{o}$C and dried in a lyophilizer. Inhibitory and reversal models wereemployed to evaluate their antisickling effects on Hb SS red blood cells following standard procedures. Proximate and elemental analysis were determined using the simple analytical procedure as described by the Association of Official Analytical Chemistry (AOAC) of 1987, 13$^{th}$ Edition Washington DC P1094 and Dry Ashing method of AOAC 2006 respectfully.

Anti-sickling results showed that the Soxhlet ethanol extracts of the*Khaya* species demonstrated significant ($p \leq 0.05$) ability to reverse and inhibit the sickling of red blood cells *in-vitro* with KIhaving the highest inhibitory and reversal of 60.04+1.77% and 74.97+2.23% respectively at 4 mg/ml concentration.The proximate analysis revealed the richness of the*Khaya* leaves in minerals, proteins and carbohydrates. *Khaya* leaves contained calcium, magnesium, sodium, phosphorus, and other essential elements within the limits of dietary requirement.

*Khaya* species could therefore be recommended for further development into herbal remedy in the management of sickle cell disease.

**Keywords:***Khayasp*, Red Blood Cells, Sickle Cell, Anti-sickling, Physicochemical.

## INTRODUCTION

Sickle cell diseases (SCD) are a group of disorders that have in common the propensity of the red blood cells to become deformed when oxygen in the blood is loweredcausing occlusion of blood vessels by misshapen cells, anemia,and various associated clinical consequences, including death (Platt*et al*., 1994). Sickle cell disease is caused by the substitution of glutamic acid with valine at the sixth position of the beta-globin chain of hemoglobin S (HbS). This mutation causes the hemoglobin to form abnormal or long and rod-like shape within the red blood cell, because the mutated genes have low solubility in the bloodstream. Under hypoxic conditions, deoxy-HbS molecules polymerise, forming rigid, sickled cells, this in turn causes the deformation of the normal disc biconcave red blood cells(Patric*et al.*, 2000).SCD is a hereditary disease passed down through families. Inheriting one gene for hemoglobin S, together with a normal gene, results in the formation of red cells that contain approximately 40 per cent of the abnormal hemoglobin and 60 per cent of the normal hemoglobin, an essentially harmless state that is designated as sickle cell trait (Roach, 2005).However, if the gene inherited together with the sickle gene is not normal, then the sickle cell disease may develop. The most common hemoglobin that interacts with sickle hemoglobin is hemoglobin C, and the ß-thalassemia (beta-thalassemia) mutation also interacts with the sickle gene by restricting the formation of normal hemoglobin.Sickle gene, and genes that interact with it, are common in a number of different populations, but the highest gene frequencies are observed in Africa (Wellems*et al*., 2009). The gene is also found in Southern Europe, the Middle East, and India. Among African Americans, approximately 7.8 per cent are carriers of the sickle mutation, while 2.3 per cent have hemoglobin C trait and 0.8 per cent have ß-thalassemia trait (Wellems*et al.*,2009).

Signs and symptoms of sickle cell anemia usually commenceafter an infant is 4 months old and may include anemia which is a chronic shortage of red blood cells (Chrouser*et al.,* 2011, Weatherall and Clegg 2001). SCD is often accompanied with crisis such as Vaso-occlusive crisis, splenic sequestration crisis, aplastic, hemolytic crisis and death (Geller and O'connor 2008). Episodes of pain are one of the major symptoms of sickle cell anemia which develops when sickle-shaped red blood cells block blood flow through tiny blood vessels to the chest, abdomen and joints (Geller and O'connor 2008). These severe pains have been claiming life of children, youths and adults across the world especially among the African population.

After centuries of research by both orthodox and trado-medical researchers, there has been no other cure either traditionally or scientificallyaside bone marrow transplantation (BMT). Current therapeutic options remain limited to hydroxyurea, although more recently, L-Glutamine (Endari), Voxelotor (Oxbryta) and Crizanlizumab (Adakyeo) were approved by the US Food and Drug Administration (FDA) (Yenamandra*et al* 2020),however, the drugs are not available to the low-income countries where SCD is prevalent. Hence, researches to discover more therapeutic options are still ongoing.

Scientists have been carrying out research into ethno-medicine to confirm the potency of medicinalplants used by thetraditional medical practitioners in the treatment and management of various diseases including SCD, standardizing and formulating them into useful dosage forms. One of such medicinal plants used to manage sickle cell individuals locallyis *Khaya* Species commonly referred to as Oganwoby the Yoruba speaking people of Nigeria, and the trade name is African Mahogany.*Khaya*belongs to familyMeliaceae, native to tropical Africa and found along the Western Africa sub- region. In spite of the plants being morphologically different they are all commonly called African mahogany andare used to treat different types of diseases. Ethno-medicinally, the different parts of *Khaya*are employed in the treatment of headaches, convulsion, cough, whooping cough, stomach ache, fever, threatened abortion, as lotion for rheumatism, dermatomycosis, and malaria fever in Nigeria. When mixed with black pepper itis used to treat diarrhea and dysentery, bark decoction is used as a drink or bath for back pains, for treating syphilis, jaundice, as a laxative, treatment of mental illness, and as an aphrodisiac (WAC, 2004).Although Fall *et al.,*(1999) reported the anti-sickling activity of *K.senegalensis*stem bark, we designed this research to determine the anti-sickling of the extracted leaves of the three species (*Khaya ivorensis*A.Chev.,*K.grandifoliola*C.Dc. and *K. senegalensis* A.Juss.)found in Nigeria, evaluate the proximate and elemental constituents with a view to confirm the ethnomedicinal use and quality of the plant drug components.



## MATERIALS AND METHODS

### Plants Collection and Processing

Fresh leaves of *Khaya senegalensis* (KS), *Khaya grandifoliola* (KG) and *Khaya ivorensis*(KI) were individually collected between the month of December and January, with the geographical and altitudinal coordinates of the location recorded using the GPS device. *K. senegalensis*was collected from the Botanical Garden (7°31'20.42"N, 4°31'20.93"E) of Obafemi Awolowo University, Ile-Ife, Osun State; *K. grandifoliola* from Abata Egba village (7°19'11.18"N, 4°36'04.89"E), Osun State and *K. ivorensis* A from Oke Ado (Okomu Forest Reserves) (6°20'00"N, 5°16'00"E), in Ovia Local Government, Okada, Edo State, Nigeria.The plants were identified and authenticated by the curator at the IFE herbarium located in the Department of Botany, Obafemi Awolowo University, Ile-Ife and the voucher specimens deposited and assigned numbers as follows: 16289 IFE (KS), 16348 IFE (KG) and 16343 IFE (KI).

The leaves were first air dried at room temperature and afterwards activated in the oven at 40$^{o}$C before grinding into powder using Christy machine. The powdered leaves were stored in well-sealed amber colored bottles until ready for use.

## Extraction Procedures

### *Cold Extraction*

Powdered plant materials 250 g each of KS, KG and KI were macerated separately in water, ethanol and petroleum spirit for 72h with constant shaking using electrically operated mechanical shaker, to ensure exhaustive extraction. The extracts were filtered and concentrated *in vacuo* at 40$^{o}$C or freeze dried to achieve complete dryness.

### *Hot Extraction*

Powdered plant materials250 g each of KS, KG and KI were exhaustively extracted,using a Soxhlet extractorwith water, ethanol and petroleum spirit separately. The extracts were filtered, concentrated *in vacuo* at 40$^{o}$C and then lyophilized to achieve complete dryness.

## Anti-sickling Assay

### *Collection of Blood Samples*

Whole blood sample (5ml) from sickle cell anemia patients in steady state between the ages of 12 and 23 years (both sexes) collected into an EDTA (Ethylene Diamino Tetra-acetic Acid) bottle, by vein puncture was used. This exercise was carried out by the hematologists from confirmed sickle cell patients attending the regular hematology out-patient clinic at Obafemi Awolowo University Teaching Hospital Complex (OAUTHC) after securing Ethical Clearance from the OAUTHC Ethical Committee.

***Inhibitory and Reversal Anti-sickling Test***

The anti-sickling assays were carried out using modified methods of Egunyomi*et al*. 2009. Vanillic acid solution,4 mg/ml, was used in place of the extract as positive control while 0.2ml of phosphate buffered saline (pH 7.0) was used as negative control. All experiments were carried out in triplicates.

Using a light microscope, sickled and un-sickled cells were counted to calculate the percentage inhibitory or reversal activity according to Cyril-Olutayo *et al.,* 2015.

**Proximate Analysis and Elemental Analysis Procedures**

***ProximateAnalysis***

Simpleanalyticalprocedureforproximateanalysiswasusedasdescribedby the Official methodsof Analysisof the Associationof OfficialAnalyticalChemistry (AOAC,1987),$13^{th}$editionWashingtonD.C.P1094.UnitedStatesofAmericaandOfficialmethodsof Analysis of the AssociationofOfficial AnalyticalChemistry (AOAC, 2006),revised edition Washington D.C. UnitedStates of America. The MoistureContent, TotalAsh, TotalCrudeFibre,EtherExtract(%Fat, %Oilor% Lipid) and TotalCrudeProtein were determined according to AOAC, 2006 methods.

**Elemental Analysis Procedure**

***Determination of digestion for the determination of Minerals (Dry AshingMethod).***

Samples of powdered*Khaya* leaves(2g) were placed inaceramic crucible, heated ina mufflefurnaceof hot air ovenat600$^{0}$Cfor 3h and thereafter allowedtocoolinadesiccator.5mlof6M HClwas added into each container and allowed to stand for30minsfor proper digestion.After digestion the contents were filteredwithWhatman No 1filterpaper intoa50mlconical flask. Thefiltrate wasmadeuptomark(50ml)withdistilledwater, shakenvigorously and the elemental content of each of the three species were read with the aid of Atomic Absorption Spectrophotometer (AAS)(AOAC 2006).

**Statistical Analysis**

All experiments were carried out in triplicate and result presented as mean ± SEM. One-way ANOVA was used to detect significant differences and the mean significant value was set at $p \leq 0.05$.

**RESULTS**

**Anti-Sickling Activities**

The result showed concentration dependent inhibitory and reversal properties.The Soxhlet extracts of the three extraction solventsexhibited higher anti-sickling properties than the extracts of cold maceration. For the cold extracts, ethanol extracts of KI gave the highest inhibitory and reversal activities (Figures1 and 2). Constituents of the petroleum spirit extracts were not active in preventing nor reversing sickling of red blood cells.

For the Soxhletextracts, ethanol extract of KIgave the highest inhibitionof 60.04±1.77% followed by KG(49.71±1.14%) and KS with the least activity (44.88±1.95) at 4 mg/ml concentration (Figure 3). The same trend was observed with the reversal activity. At 4 mg/ml concentration, KI reversed Hb SS red blood cellsby 74.97±2.23% followed by KG (59.96±3.10%) and lastly KS with 51.62±1.83% reversalactivity (Figure 4). The study generally revealed that reversal activities were dose dependent as activities increased with increase in concentration.

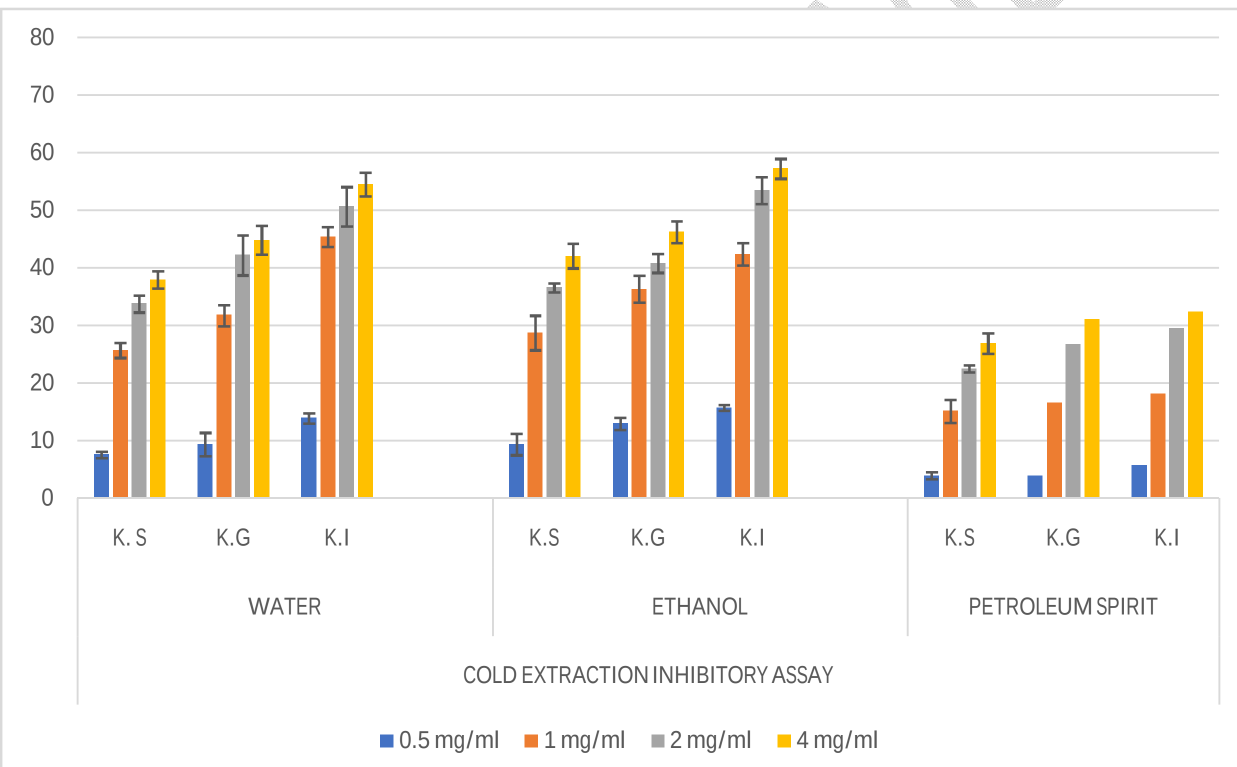


**Figure 1: Inhibitory properties of the macerated aqueous, ethanol and petroleum spirit extracts of KS, KG and KI at various concentrations.**

**Keys: KS: *Khaya senegalensis;* KG*: Khaya grandifoliola;* KI: *Khaya ivorensis***

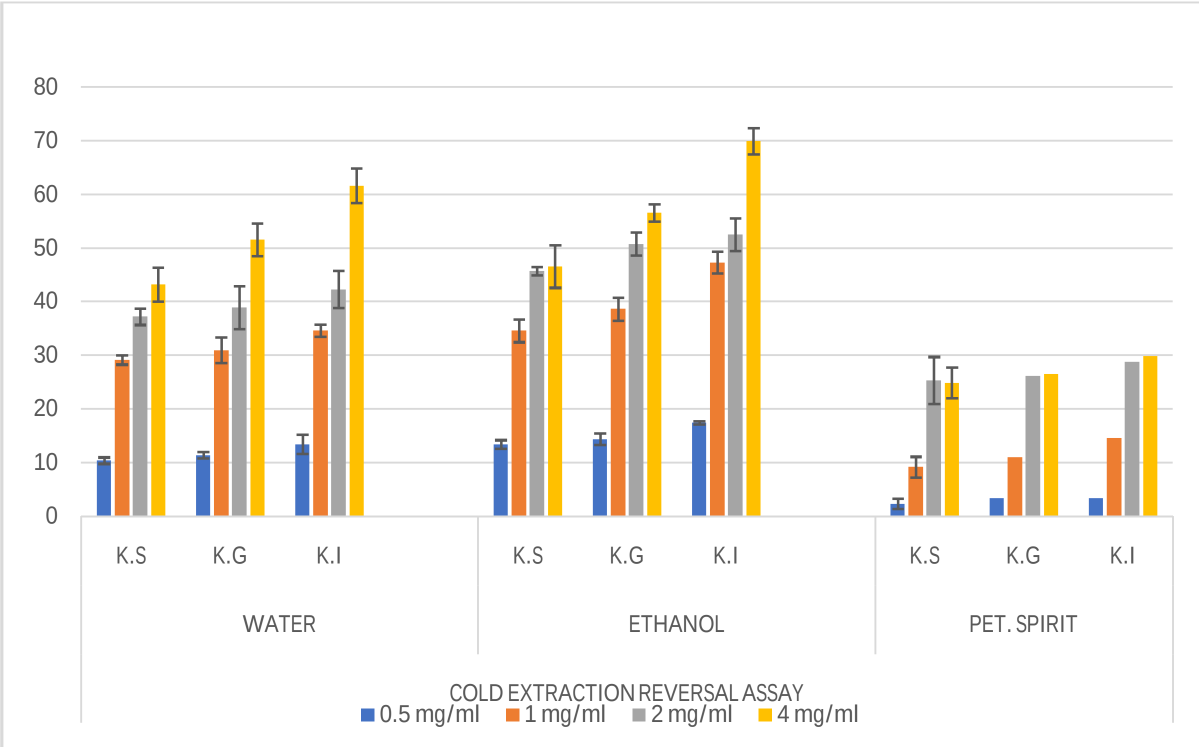


**Figure 2: Reversal properties of the macerated aqueous, ethanol and petroleum spirit extracts of KS, KG and KI at various concentrations.**

**Keys: KS: *Khaya senegalensis;* KG*: Khaya grandifoliola;* KI: *Khaya ivorensis***

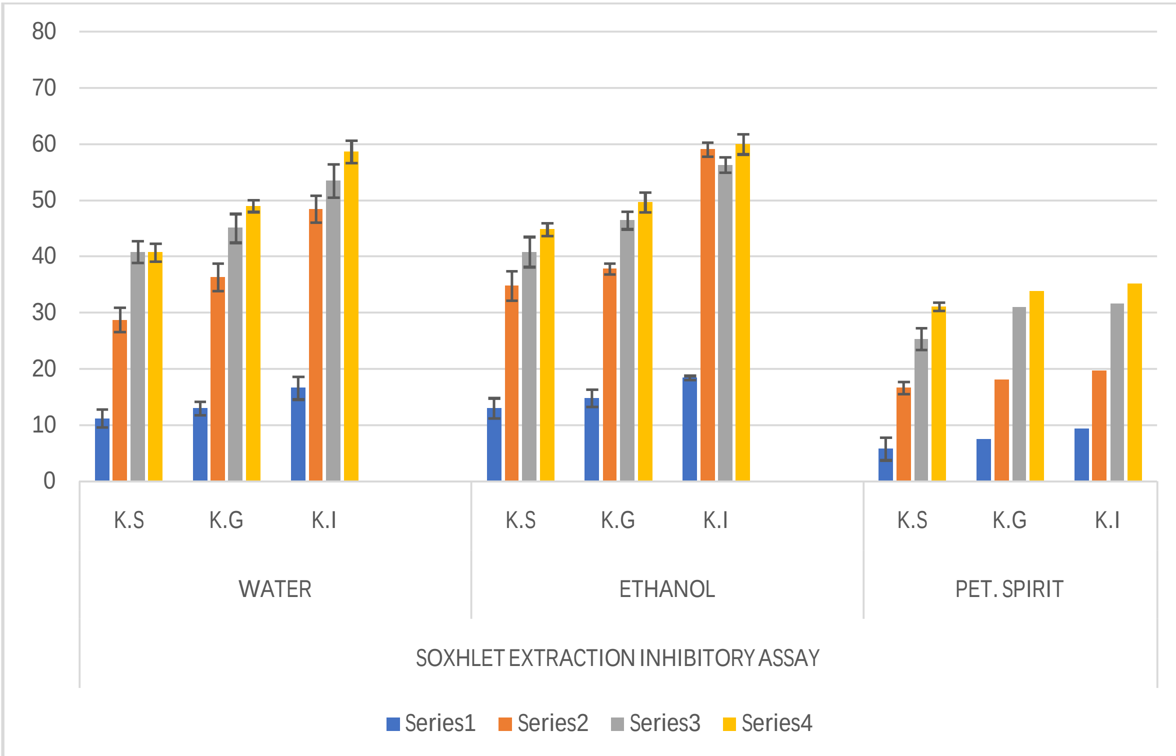


**Figure 3: Inhibitory properties of the aqueous, ethanol and petroleum spirit Soxhlet extracts of KS, KG and KI at various concentrations.**

**Keys: KS: *Khaya senegalensis;* KG*: Khaya grandifoliola;* KI: *Khaya ivorensis***

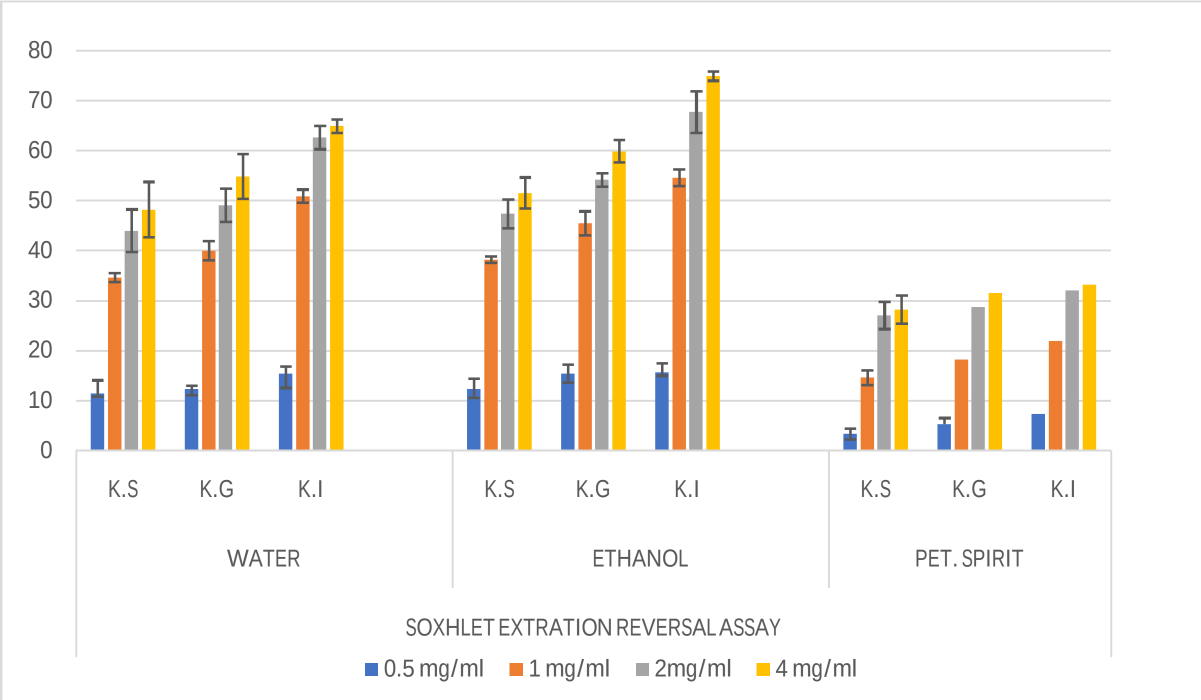


**Figure 4: Reversal properties of the aqueous, ethanol and petroleum spirit Soxhlet extracts of KS, KG and KI at various concentrations.**

**Keys: KS: *Khaya senegalensis;* KG: *Khaya grandifoliola;* KI: *Khaya ivorensis***

**Proximate Composition**

**Table 1: Proximate Composition of the three *Khaya* species leaves.**

| Sample Description | % Moisture | % Total Ash | % Crude Fibre | % Ether Extract | % Crude Protein | % Nitrogen Free Extract (NFE) | % Total Carbohydrate |
|---|---|---|---|---|---|---|---|
| KS | 10.55 | 10.09 | 15.26 | 3.18 | 23.63 | 37.29 | 52.55 |
| KG | 10.35 | 7.72 | 24.89 | 3.02 | 20.56 | 33.46 | 58.35 |
| KI | 9.32 | 7.22 | 17.29 | 6.71 | 18.38 | 41.08 | 58.37 |

**Keys: KS: *Khaya senegalensis;* KG: *Khaya grandifoliola;* KI: *Khaya ivorensis***

**Table 2: Elemental Analysis of the three *Khaya* species leaves**

| Extracts | **Cu (Copper)** | **Ni (Nickel)** | **Fe (Iron)** | **Zn (Zinc)** | **Mn (Manganese)** | **K (Potassium)** | **Cd (Cadmium)** | **Na (Sodium)** | **Cr (Chromium)** | Ca (Calcium) | Mg (Magnesium) | Pb (Lead) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **KS** | 0.237 ± 0.004 | 0.160 ± 0.007 | 12.68 ± 0.008 | 0.842 ± 0.002 | 1.005 ± 0.008 | 10.27± 0.091 | 0.033 ± 0.001 | 0.036 ± 0.001 | - | 8.384 ± 0.069 | 0.714 ± 0.002 | 0.108 ±0.013 |
| **KG** | 0.991 ± 0.001 | 0.038 ± 0.007 | 9.139 ± 0.016 | 1.016 ± 0.005 | 1.267 ± 0.002 | 10.16 ± 0.312 | 0.022 ±0.001 | 0.323 ± 0.002 | 0.416 ± 0.041 | 4.322 ± 0.019 | 0.824 ± 0.002 | 0.053 ± 0.007 |
| **KI** | 0.440± 0.001 | 0.063 ± 0.002 | 11.34 ± 0.020 | 0.713 ± 0.002 | 0.943 ± 0.002 | 9.051 ± 0.134 | 0.018 ± 0.001 | 0.059± 0.001 | 0.491 ± 0.010 | 5.422± 0.032 | 0.832 ± 0.000 | 0.045 ± 0.027 |

**Keys: KS: *Khaya senegalensis;* KG*: Khaya grandifoliola;* KI: *Khaya ivorensis***

## Discussion

The inhibitory activities exhibited by the aqueous and ethanol extracts of the *Khaya* species at 4 mg/ml were significantly comparable to that ofVanillic acid (58.20 ± 0.92%) while the reversal activities were significantly higher (Figures1-4).

The antisickling activities of KS, KG and KI were dose dependent as the highest activities were recorded at 4mg/ml (Figures 1-4). This was observed across the three-plant species regardless of their mode and solvent of extraction. We employed water, ethanol and petroleum spirit as the extraction solvents to determine the class of putative compounds responsible for the activities, and also used cold and hot extraction methods to know whether active compounds are thermolabile or not.

Across all the tested plant extracts, the ethanol extracts gave the highest inhibitory properties followed by the aqueous and the non-polar petroleum spirit extracts. Although flammable, alcohol is recognized as non-toxic and has less handling risks (Rittner, 1992). Due to the organic nature of ethanol it has a better extractive capability of plant metabolites, the putative compounds, which are often organic in nature. The putative active compounds responsible for inhibiting and reversing the sickling of red blood cells in the *Khaya* species were polar compounds. These compounds were also not thermolabile as their activities were not hindered by the exertion of heat during extraction.

The general trend of the anti-sickling activities of the three plants was KI > KG > KS in all the tested concentrations, irrespective of hot or cold extraction methods.

The percentagemoisture contents (MC) of KS, KG and KI were at acceptable levelsas specified by the British pharmacopoeia which stated that the general requirement of MC in crude drug should not be more than 14% (British Pharmacopoeia, 1980) and the value obtained in this study is within the acceptable range of 9.32%-10.55% (Table 1) implying a long storage period with less chances of microbial degradation of the drugs(Ajazuddin and Shailendra, 2010).

The physicochemical data would help in determining the richness and usefulness of the *Khaya* species in combating SCD and for the identification of the drug from its substitute or adulterants. The total ash contents of*Khaya*leavesranged between 7.22%-10.09% (Table 2). In all, the highest % ash content was found in KS leaf 10.09%. The high ash content in *Khaya* species showed that it contained high mineral contents and may contain inorganic radicals like carbonates, phosphates, and silicates of sodium, potassium, calcium, magnesium (Oduntan*et al., 2012*)which may be the reason for their effect in inhibiting and reversal of sickled red blood cells (Table 1). Values are comparable to other leafy vegetables reported such as *Amaranthus hybridus*, and *Curcubitapepo* (Iheanacho *et al.*, 2009). The quantitative evaluation is an important parameter in setting standard of crude drugs and the physical constant parameters could be useful in detecting any adulterant in the drug.The crude fibers of the *Khaya* species studied were within 15.26-24.89%, which is within the allowed values of 8.50 - 20.90% for some Nigerian vegetables (Isong and Idiong, 1997).Crude fiber analysis is an effective tool to determine the nutritional value of animal feed and some plant-based foods. The effects of dietary fiber on health outcomes include improving digestion, reducing cholesterol levels, weight management, and preventing chronic illnesses like heart disease and diabetes (Asaolu*et al.*, 2012, Shaikh *et al* 2019).The crude protein content ranged from 18.38 - 23.63%. KS leaves has the highest with 23.63%, KG with 20.56%, and KI with 18.38% protein content. The result showed that the crude proteins were generally high in the leaves of the three species studied (Table 1). The crude protein content is higher than what was reported for some other green leafy vegetables such as *Momordicabalsamina* (11.29%), *Moringa oleifera* (20.72%) and *Leptadeniahastane* (19.10%) (Sena *et al*., 1998, Lockettt*et al*, 2000, Hassan and Umar, 2006). Plant foods that provide more than 12% of their calorific values from protein has been shown to be good sources of protein (Ali, 2009). Besides this, the high protein content will help in replacing worn out cell/tissues of SCD patients thereby supporting their well-being and growth. Although the crude protein content of KS was the highest it did not correlate to a higher anti-sickling property.

Qualitative and quantitativeelemental analysis of KI, KG and KS are presented in Table 2.Calcium, iron, manganese, zinc and copper are of great importance and essence to our daily body due to their physiological and biological roles and functions (Garcia-Rio *et al*., 2007).Manganese (Mn) is a vital mineral needed for normal growth and skeletal formation. It helps to break down fats, carbohydrates and proteins, and serves as co-factor for enzymes (Gomez *et al.,* 2004). Mn deficiency causes diabetes, nervous instability, disorder of bony cartilaginous growths ininfant children and rheumatic arthritis in adults (Underwood, 1971); and since rheumatic arthritis is a symptom in SCD patient then Mn supplement in medicinal plants like *Khaya* can alleviate this. Zinc is an intercellular cation present in all body tissues and fluids next to Iron. It is the second abundant trace elements in humans. It is important for enzymatic functions. It takes part in synthesis of DNA, protein, insulin. It is also essential for normal functioning of the cells including protein synthesis, carbohydrates metabolism, cell growth and cell division (Garcio-Rio *et al.,*2007, Gomez *et al.,* 2004). *Khaya* leaves contain significant amount of zinc implying its importance in replacement of worn-out tissues which is important to SCD patients.*Khaya*species can supply substantial portion of Mn, Zn and Cu requirement of adults in the tropics who cannot afford other sources of these minerals such as milk, cheese and eggs. Since SCD has effect on blood content and quality, the use of *Khaya* will go a long away in fighting the anemic state frequently presented by SCD patients in crisis.Calcium is another important mineral present in the leaves of the three *Khaya* species. Patients with SCD are always also presented with bone problems therefore calcium in the leaves will help in formation and maintenance of strong bones and teeth if developed into remedy.Sodium and potassium are also important and are present in the leaves of these species; they have important intracellular and intercellular activities respectively. Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction (Akpanyung, 2005).Heavy metals present in the leaves of *Khaya* species are cadmium, nickel,lead and chromiumwithin permissible limit (Table

2) and implies the three *Khaya* are safe for consumption if developed into herbal remedy for management of SCD.

## CONCLUSION

The three species of *Khaya* leaves showed a concentration and thermal dependent anti-sickling activities.The proximate analysis also reveals that *Khaya* leaves are very rich in nutrients like minerals, protein, carbohydrates in amounts required by the body and a moisture content that will allow storage if eventually developed into herbal remedy or drugs. In addition, the elemental analysis also revealed that the leaves of the three *Khaya* species studied supports healthy living because they contain elements within permissible limit for the body.

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