RESEARCH ARTICLE

EFFECT OF TOTAL KHAYA SENEGALENSIS (MELIACEAE) BARKS EXTRACTS ON HEPATIC LIBERATION OF GLUCOSE

Background: Increasing the use of traditional therapy is an obvious reality in some country. The bark of *Khaya senegalensis* is suspected to treat diabetes. That is what this type of work is started.
Aims & Objective: The aim of this work is to test the effect of total *Khaya senegalensis* barks extracts on the hepatic liberation of glucose in vitro.
Materials and Methods: The dried extracts obtained from barks powder had been prepared at concentrations of 0, 2, 5, 10 and 20 µg/L and applied on fragments of mice Wistar isolated liver, kept in Mack -Ewen solution. After incubation at 37°C in oven, glucose level in the solution had been measured by spectrophometer, using GOD-PAP® regent, for each extracts concentration at the times T=0, 10, 20, 30, 40, 50 and 60 minutes.
Results: Glucose level in the solution had varied in time function from one extracts concentration to another. *Khaya senegalensis* barks total extracts had reduced hepatic liberation of glucose. This effect had been comparable to the effect of insulin 2,5IU solution used like reference substance in this experimentation. The total extracts of *Khaya senegalensis* barks could actuate on enzymes implicated in the metabolism of intra hepatocytary glucose.
Conclusion: This investigation had been coupled to phytochimic analyses that showed the presence of saponoïdes, anthracitic derivers and steroids in Khaya senegalensis barks.
Key Words: Insulin; Glucose; Liver Fragments; *Khaya Senegalensis*; Hepatic Liberation Of Glucose; Mice Wistar

Takin M, Ahokpe M, Zohoun L, Assou E, Aivodji N, Agossou E, Sezan A

Laboratory of Biomembranes and Cell Signaling, Department of Animal Physiology, Faculty of Sciences and Technology (FAST), University of Abomey Calavi, Cotonou, Republic of Benin

Correspondence to: Sezan A (alphonse.sezan@fast.uac.bj)

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INTRODUCTION

Metabolic diseases such as diabetes pose enormous challenges today in global populations. Diabetes is a malfunction of the control system of blood glucose resulting from genetic and / or environmental. This condition is caused by either a deficiency in the secretion of insulin, or misuse of the said hormone in the body where the classification of diabetes into two specific groups: lean "insulin dependent diabetes" or diabetes or Type I met young patients and characterized by insulin deficiency, "the non-insulin dependent diabetes" or type II diabetes or fatty, met in subjects 40 years especially, but also increasingly in the young and obese subjects, characterized by misuse of insulin by the body tissues.[1]

Forecasts announced for 2030 a figure of 366 million diabetics in the world.[1] This increase is expected to increase, aging, obesity, too unhealthy diets and lifestyles sedentary. Diabetes is a chronic disease with a mortality rate of 2%. Modern drugs used in hospitals are too expensive for an African population with more than half live below the poverty line and poverty is considered a risk of developing chronic diseases, the continent black not less spared.[2] Indeed, diabetes is a real health problem affecting all countries, whether developed or developing, for example France has more than 3 million patients in the United States had 12 million in 1998.[3] Benin has more than 200 000 patients with diabetes according to a WHO estimate, giving a prevalence that exceeds 2% of the population. Of these patients, 90% are being treated for diabetes mellitus type II and 10% for diabetes mellitus type I. (According to WHO).[17] Without proper treatment, diabetes can cause heart disease, blindness, impotence and even amputation above the foot. In Benin, diabetes is the second leading cause of hospitalization after HIV and AIDS accounts for over 95% of consultations in internal medicine, all specialties. (According to the WHO estimate) Also, according to the World Health Organization (WHO), about 80% of the population living in the African region make use of traditional medicine for their health care needs.[1,16] Much of the Beninese population is consulted by healers, as they offer herbal extracts and cost effective.

Given the expansion of these diseases whose support is high, the WHO, in its resolution AFR/RC50/R3 31 August 2000, encouraged African countries to develop regional strategies on traditional medicine to undertake research on medicinal plants and promote their optimum use in the delivery systems of health care.[1]

Our investigations are currently focused on the action of a harvested plant in Benin (*Khaya senegalensis*: Meliaceae) used by traditional healers to try to treat diabetes.[4,5] To check the effectiveness of this plant in the treatment of diabetes can we study its effect on the liver fragments to determine its involvement in the regulation of intracellular glucose.

MATERIALS AND METHODS

Harvesting and Processing of Plant Material: The bark of *Khaya senegalensis* were collected from different regions (north, center and south) of Benin and were dried in the sun for 15 days, then reduced to powder mill knife.

Preparation of Dry Extracts of *Khaya Senegalensis*: 100g of powdered bark of *Khaya senegalensis* weighed using a Sartorius ® analytical balance, were macerated in 500 ml of distilled water for 48 hours under continuous magnetic stirring water. After this time the macerate was recovered and concentrated by evaporation of the solvent at 80 ° C at the evaporator Rotavapor®. In following the extracts were dried in an oven at 45 ° C for the solids.

Preparation of Extracts at Different Concentrations: X g of solids were dissolved in Y ml of the reaction solution to achieve concentrations of extract it to 2, 5, 10 & 20 µg/L

Determining the Efficiency of Extraction: The yield R extraction was determined according to the conventional method, the extracts obtained were weighed and then following calculation rule was applied:
R= Mass of solids /Mass of powder of bark

Phytochemical Analysis: To determine the different chemical constituents of bark of *Khaya senegalensis*, the phytochemical screening was applied using the method of Houghton PJ AND RAMA A. (1998) reviewed and adapted to the conditions of the Laboratory of Pharmacognosy and Essential Oils of ISBA This technique is based on the reactions (color and precipitation) differential of the main groups of chemical compounds found in plants.

Preparation of Biological Material: A Wistar rat was dissected and the liver has been taken apart and stored in a jar containing the reaction solution (Mack-Ewen).

Preparation of the Mack-Ewen Solution: The reagents forming the Mack-Ewen solution are:

- Sodium chloride (NaCl) concentration of 100 mM,
- Potatium chloride (KCl) concentration of 7 mM,
- Calcium dichloride (CaCl2) concentration of 1.2 mM,
- Sodium dihydrogen phosphate (NaH2PO4) concentration of 25 mM,
- Sodium hydrogen phosphate (Na2HPO4) in 10 mM concentration,
- BSA (protein) concentration of 1%.

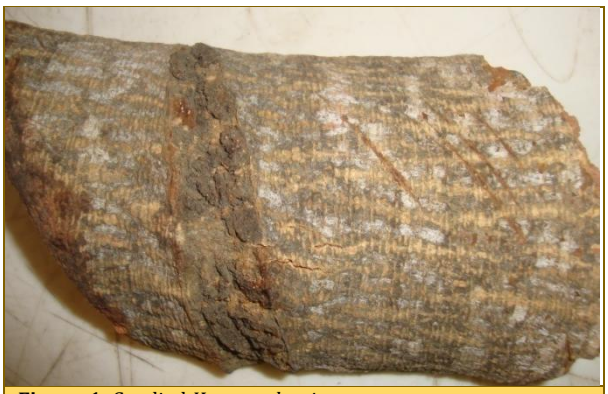

Figure-1: Candied *K. senegalensis*

Figure-2: Powdered bark of *K. senegalensis*

Figure-3: Solids *K. senegalensis*

Table-1: Assay procedure of glucose by the GOD-PAP method

tube	white	Etalon	Dosage
reagent	1mL	1Ml	1mL
distilled water	10µl	-	-
etalon	-	10µl	-
sample	-	-	10µl

Determination of Glucose: Glucose was measured for each of the concentrations of the extracts *K. senegalensis* and for each time t = 0, 10, 20, 30, 40, 50 and 60 minutes

by spectrophotometry using the GOD-PAP reagent ®. This is an enzymatic method based on the oxidation of glucose. Glucose was also assayed for insulin solution to 2.5UI and the effect was compared with extracts of *K. senegalensis* 2 and 5 µg/ L.

Procedure: In each of the three (03) tanks labeled respectively White Stallion and Dosage, we 1mL of reagent GOD-PAP ® using a micropipette Ependhorff® P1000. Then we add respectively, using pipettes Ependhorff® P10, 10µL distilled water, 10µL of standard glucose and 10µL of the sample to be assayed. (Table 1) Mix well and keep in the dark for 10 minutes at 37 ° C or 20 minutes at room temperature. Soon realize reading optical densities (OD) spectrophotometer BIOLAB the wavelength of 540 nm.

RESULTS

Extraction Efficience: The mass of solids is obtained 22.62 g, corresponding to a yield,
R= (22.62 g /100 g) X 100 = ***22.62%***

Results of Phytochemical Analysis: Extracts from the bark of *Khaya senegalensis* contain abundant polyphenolic compounds such as tannins, catechin tannins and leuco-anthocyanins, and abundant saponins such as sanosides with a foam index of 4. Other polyphenolic compounds such as anthocyanins as anthracene derivatives such as 0-glycosides reduced aglucones are scarce in these extracts unlike steroids which are there in small quantities. (See Table 2)

Glucose Determination (measuring the variation in optical density versus the concentration of the total extracts of *Khaya senegalensis*): The figure 4 shows the change in optical density as a function of different concentrations of total extracts of *Khaya senegalensis*. Changes in optical density obtained for the determination of glucose are shown in Figure 4 for concentrations 2 µg/L, 5 µg/L, 10 µg/L, 20 µg/L of total extracts of *Khaya senegalensis* to time T = 0, 10, 20, 30, 40 and 60 min. Changes in optical density in the control linear and evolve from the bottom up in time. Changes in optical density for total extracts of *Khaya senegalensis* to different concentrations are in general lower than the control in the same time. Changes in optical density obtained for the determination of glucose with total extracts of *Khaya senegalensis* to a concentration of 10 µg/L were maintained above those obtained with the control at times T = 0, 10, 20, 30 and 40 (mn). At time t = 60 min extracts at different concentrations were maintained lower than that of the control for the same time optical density. The extracts at concentrations below 10 µg / L seem to record the lowest optical densities. The following figure shows the comparison between the curves of variation of the optical density of the control and the optical density of the extract at 10 µg/L.

Table-2: Results of phytochemical analysis

Chemical Group	Subgroup	Observation
Polyphenolic Compounds	Tannins	+++
	Cate classy tannins	+++
	Anthocyannes 2	++
	Leuco anthocyanin	+++
Saponins	Sanosides (index foam is 4)	+++
Steroids	Steroids	+
Anthracene Derivatives	O-glycosides with reduced aglucones	++

+++: abundant; ++: little; +: low

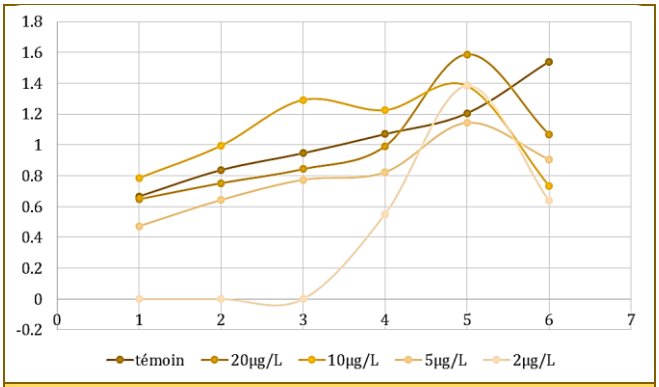

Figure-4: Variation of the optical density as a function of different concentrations of total extracts of *Khaya senegalensis*

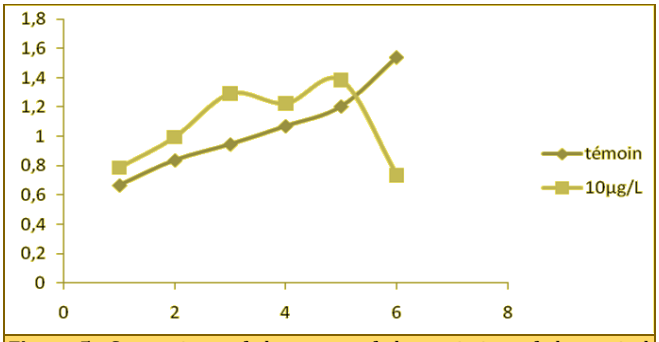

Figure-5: Comparison of the curve of the variation of the optical density of the control and extract to a concentration of 10 µg/ L

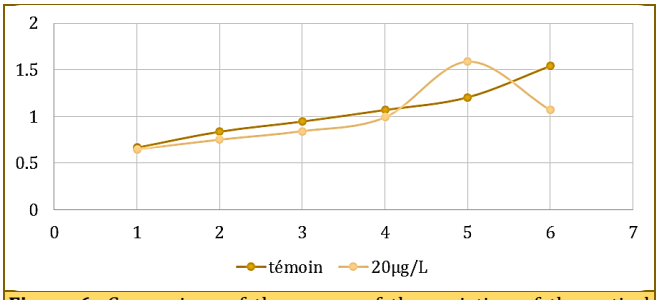

Figure-6: Comparison of the curves of the variation of the optical density of the Witness and the extract concentration of 20 µg / L

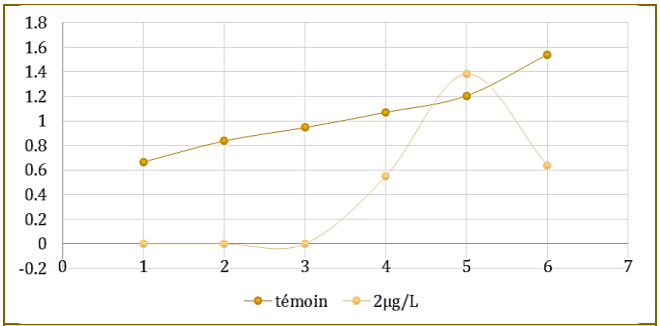

Figure-7: Comparison of the curves of the change in optical density of the control and the extract at the concentration of 2 µg/L

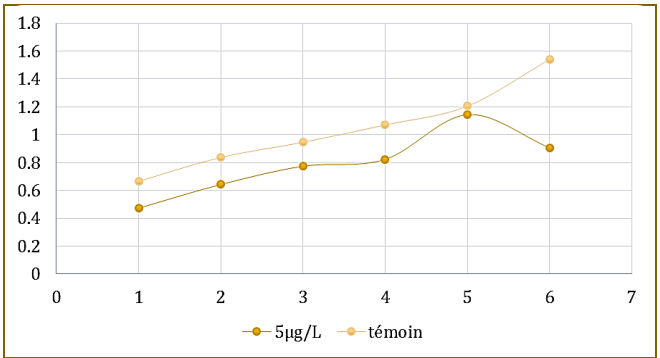

Figure-8: Comparison of the curve of variation of the optical density of the control and that of the extract to the concentration 5 µg/L

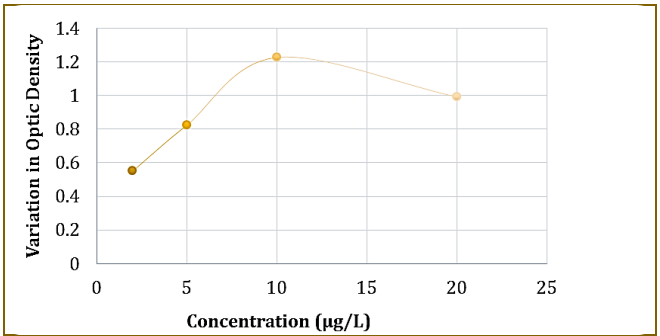

Figure-9: Dose response curve

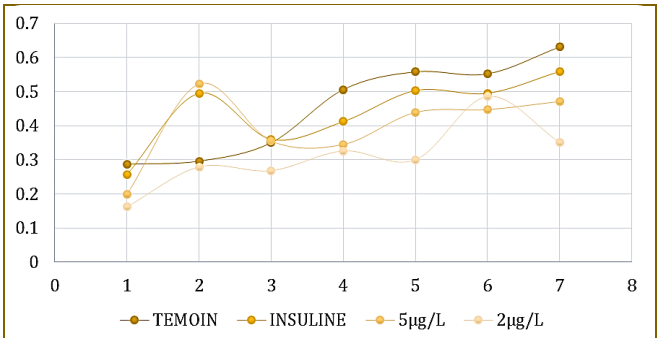

Figure-10: Changes in optical density due to the solution of 2.5 IU insulin and extracted 2 µg/L and 5 µg/L

The values of the variation of the optical density recorded at time T = 0, 10, 20, 30, 40, 50minutes and under the effect of the extracts concentration 10 µg/L were higher than the control. At time t = 60 minutes the change of the optical density of the current is below that of the control (Figure 5).

The figure 6 shows the comparison between the curves of variation of the optical density of the control and the optical density of the extract at 20 µg/L. The values of the variation in optical density recorded under the effect of the extracts on concentration 20 micrograms / L as a whole are lower than the control at times T = 0, T = 10, T = 20, T = 30, 4 T = 0, T = 60 (minutes). Driven extracts the concentration of 20 micrograms / L, a value of the change in higher than the control at time t = 50 min optical density is observed.

The figure 7 shows the comparison between the curves of variation of the optical density of the control and the optical density of the extract at 2 µg/L. The values of the variation in optical density recorded under the effect of the extracts to 2µg/L as a whole are lower than the control at times T = 0, T = 10, T = 20, T = 30, T 4 = 0, T = 60 (minutes). Driven extracts the concentration of 2 µg/L, a value of the change in higher than the control at time t = 50 min optical density is observed.

The figure 8 shows the comparison between the curves of variation of the optical density of the control and the extract at a concentration of 5 µg/L a variation value of higher than the control at the time T = 50 min optical density. The values of the variation in optical density recorded under the effect of the extracts on concentration 5 µg/L is lower than the control at different times. The following figure shows the dose response effect of the extract.

The curve the figure 9 takes the form of a graph expressing the ideal relationship between the concentration (dose) of a drug and the magnitude of the response produced. The aqueous extracts of *Khaya senegalensis* to concentrations 2 µg/L and 5 µg/L recorded the lowest values of the change in optical density. The change in optical density appears to increase gradually as we increase the concentration of the extract to a peak OD = 1.2 with the extract concentration to 10 µg/L. At concentrations greater than 10 µg/L extract appears to reduce the value of the optical density without reaching the values of the optical density recorded for the extract concentration to 2 µg/L and 5 µg/L.

Comparison with Insulin (changes in optical density due to the solution of 2.5 IU insulin and extracted 2 µg/L and 5 µg/L): Changes in optical density obtained with insulin concentration 2.5 IU as those obtained with total extracts of *Khaya senegalensis* concentrations 2 micrograms / L and 5 g / L T = 0, T = 10, T = 20, T = 30, T

= 40, T = 50, T = 60 min are below the OD of the control. The shape of the curves of total extracts of *Khaya senegalensis* concentrations 2 µg /L and 5 µg/L is similar to the curve of insulin. Changes in optical density for insulin are above the optical density variations in extracts at concentrations 2 µg/L and 5 µg/L (figure 10).

DISCUSSION

Movement of glucose through the membrane of hepatocytes: The graph for the determination of glucose in the control tube is a straight line upward over time. This would explain hepatic glucose release in the reaction solution. This release is probably due to a mobilization of glucose in liver cells. Several ways of mobilization may be causing this. You could think of a priori simple diffusion of glucose (movement of glucose following a concentration gradient), but this is much more complex. It could involve a group such as glycogen synthase, glycogen phosphorylase and phosphatase enzymes, and molecules such as ATP and protein type PEPCK. When glucose levels drop significantly in the environment of hepatocytes, glycogen synthase phosphatase as glycogen are inactivated, resulting in the activation of glycogen phosphorylase by phosphorylation (contribution by ATP phosphatase group or other form of cellular energy reserves) which phosphorylate glycogen and will hydrolyze to glucose. The pyruvtate phosphoenol (PEPCK), the enzyme which catalyzes the gluconeogenesis (the conversion reaction of intermediate non-carbohydrate such as oxaloacetate glucose) could also be activated, thereby increasing the flux of glucose to the outside environment of the hepatocytes.[6] Then we could justify the release of glucose by the proper functioning of enzymes isolated from rat liver, which would be kept in conditions or very close physiological offered the reaction solution. This would also make a good preparation of said solution.

Effect of total extracts of *Khaya senegalensis* glucose: The change in optical density obtained in this experiment by spectrophotometry is a quantity that provides information on the amount of glucose present in the medium. Is directly proportional to the glucose concentration in the medium. The phenomenon we observe in the absence of total extracts of *Khaya senegalensis* is a gradual release of glucose from hepatocytes into the external environment. When we apply the total extracts of *Khaya senegalensis* to different concentrations 2 µg/L, 5 µg/L, 10 µg/L, 20 µg/L in time T = 0, 10, 20, 30, 40 and 60 minutes, we see so overall decrease in the rate of glucose in the medium. Two possible events could justify these results:

- *A glucose degradation in the environment under the effect of the extracts:* All times should be noted that pyruvate would not in this case part of the degradation products of glucose degradation product that would give a reading of optical density variation in the experimental wave length of 500 nm, which is to add the variations of the optical density recorded under the effect of the extracts, which may then erroneously increase the level of glucose in the medium, the assay itself is based on oxidation of glucose to pyruvate. , We would have had values of optical density change significantly above the values of optical density change of control, as a result of total extracts of *Khaya senegalensis* to different concentrations, which is not the case that we already record the determination of glucose values of the change in optical density which are below those of the variations in optical density of the control. This would lead us to reject the hypothesis of a possible oxidation of glucose to pyruvate in the medium under the effect of extracts of *Khaya senegalensis*.

- *Oxidative degradation of glucose into hepatocytes:* There would be an oxidation of glucose to pyruvate by the activation or induction of pyruvate kinase. The pyruvate could deteriorate to acetyl coenzyme A, which enters the tricarboxylic acid cycle (Krebs cycle) which is a preparatory step in the respiratory chain. The final product is ATP, which is a form of energy storage needed for cellular metabolism.[10-15] This hypothesis would have two consequences on the one hand, degradation of glycogen phosphorylase activation or induction of glycogen as it is explained above, this reaction remained upstream of the cascade of biochemical reactions that lead to a complete glycolysis with the results in the formation of ATP, the other possible entry of glucose into hepatocytes through activation of glycogen synthase. If we extrapolate our experimental in vitro phenomenon of glycemic control, the breakdown of glucose within hepatocytes to form ATP would result in a decrease of PEPCK therefore glucose by glucose enters the liver, and hepatocytes seeking their physiological balance, and glycogen are in dynamic equilibrium with the blood glucose.

- For concentrations 10 µg/L and 20 µg/L it would have produced a saturation of the enzymes involved in the

degradation of intracellular glucose to ATP. Indeed extracts of *Khaya senegalensis* to concentrations below 10 µg/L seem to reported changes in the optical density lower than concentrations of the extracts to 10 µg/L and 20 µg/L. Would occur for concentrations 10 µg/L and 20 µg/L excess of substrate (glucose) for the enzyme pyruvate kinase (which degrades glucose to pyruvate to borrow the Krebs cycle and the process of breathing with the formation energy ATP), may be this is due to saturation of receptors and therefore, part of the glucose escape hepatocytes experimentation by the mechanism of facilitated transport of glucose across the biological membrane to its action on PEPCK level explained above kernel this would explain the values of the variation of the high optical density of the extracts at concentrations 10 µg/L and 20 µg/L in contrast to those obtained for concentrations 2 µg/L and 5 µg/L.

- The effect of the extracts concentration 5µg / L of glucose kinetics show an interest in this because the glucose concentration was kept almost constant 30 min after application.

Comparing the effect of total extracts of *Khaya senegalensis* the effect of insulin: We note that the curve of the effect of extracts of *Khaya senegalensis* to the concentration of 5 µg / L and the effect of insulin seem to have the same kinetic (see figure 10), one could think of a similarity between the mode of action of insulin and extracts, the role of insulin in regulating blood glucose is accelerated by the breakdown of glucose to form ATP or stimulating the entry and storage of glucose as glycogen in the liver.[7-9] Curve insulin concentration 2.5 IU/ml is above the curve extracts of *Khaya senegalensis* the concentration 5 µg/L, this would not mean that extracts of *Khaya senegalensis* have greater activity than insulin, it cannot be said for the present study did not address this aspect.

At this stage, our experiments do not allow us to clarify the mechanism of action of *Khaya senegalensis*. We will continue this work to explore mechanisms of action.

CONCLUSION

The total extracts of *Khaya senegalensis* caused the decline in the rate of hepatic glucose release, this could be explained by a blockade of cellular glucose metabolic pathways that would lead to a decrease in hepatic glucose output. The anti-hyperglycemic effect reported by some traditional healers would thus be justified. The mode of action of total extracts of *Khaya senegalensis* is comparable to that of human insulin.

REFERENCES

1. Adjotin P. Médecines traditionnelles et médecine moderne au Bénin. Collaborations ou conflits ? (Mémoire de DES) Genève : Institut universitaire d'études du développement (IUED), 1996. p. 57.
2. Lehninger AL, Nelson DL. Principios de Bioquimica. 2da Edicion. Spain : Omega 1999. p. 453-6.
3. Grimaldi A, Jacqueminet S, Heurtier A, Bosquet F, Collectif. Guide pratique du diabète. 3e édition. Médiguides (Paris): Elsevier Masson. 2005. p. 271.
4. Juss A. CTFT Khaya semgalensis (Desr.). Bois et Forêts des Tropiques, no 218, 4è trimestre; 1988 : 43-55.
5. Christine O. Contribution à la conservation du Khaya senegalensis (Desr). A. JUSS: importance socio-économique, structure et dynamiques des peuplements naturels et productivités des plantations d'essai au Bénin. 2000.
6. Koukoui ASI. Omédine. étude des signaux calciques hépatocytaires induits par différents agonistes modulateurs de la sécrétion biliaire et de la production hépatique du glucose : acides biliaires, prostaglandines et glucagon. Thèse de doctorat en signalisation cellulaire, endocrinologie, reproduction/science de la vie.2005. p. 169.
7. Boullard B. Dictionnaire plantes médicinales du monde. Réalité et croyance. Ed ESTEM 7, paris: De Boeck Secundair, 2001. p. 636.
8. Yaro B. Contribution à l'étude du traitement traditionnel du diabète au mali. thèse de pharmacie. 1992. p. 133.
9. Coulibaly B. Contribution à l'étude des remèdes traditionnels utilisés dans le traitement du diabète au mali. Thèse de pharmacie. 1988. p.113.
10. Gbenou JD, Oyeniran C. Contribution à l'étude des propriétés hypoglycémiantes de l'huile essentielle d'Eucalyptus canaldulensis. Mémoire de Maîtrise en physiologie. Cotonou ; Bénin 2004;33-45.
11. Keita A, Mariko E. Etude de l'activité hypoglycémiante des feuilles de Sclerarya birrea, revue Médecine Traditionnelle Africaine 1998; 10 :16-25
12. Lawson-evi P, Eklu-Gadegbeku. Activité hypoglycémiante de quelques plantes médicinales. Revue Médecine traditionnelle Africaine 1997;1(9):60-69.
13. Oga ASS, Tebi A, Aka J, Adouéni KV, Malan KA, Kouadio LP, Lokrou. Le diabète sucré diagnostiqué en Côte d'Ivoire: des particularités épidémiologiques. Med 2006;66:241-6.
14. Rao BK, Rao CH. Hypoglycemic and antihyperglycemic activity of , syzygium alternifolium (wt) Walp. Seed extracts in normal and diabetic rat. Phytomedecine 2001;8:88-93.
15. Goodman, G. Gilman, G. Bases Farmacológicas de la Terapéutica. 7th Edición. México: McGraw- Hill. 1994. p. 1459-70.
16. World Health Organization. Rapport de l'OMS. Press Release WHO/38. 16 May 2002.

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