

# Antihyperglycemic Activity and Phytochemical Screening of *Khaya Senegalensis* (Meliaceae) in Wistar Rats

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**Abstract:** The aim of this study was to identify the chemical group, evaluate an acute toxicity study and analyze the effect of aqueous extract *Khaya senegalensis* (Meliaceae), on experimentally induced hyperglycemic condition in rats. The phytochemical study of the aqueous extract of *Khaya senegalensis* revealed the presence of active secondary metabolic compounds such as flavonoids, polyphenols, catechol tannins, quinones, saponins, sterols and terpenes. The stem bark of *Khaya senegalensis* was used for acute toxicity study by gavage in Swiss mice. The aqueous extract of *Khaya senegalensis* (AEKS) at the dose of 35±5 mg/kg administrated by gavage showed that all the mice treated were living after 24 hours. The lethal dose obtained by intraperitoneal administration with graded doses to the mice was 1778 mg/kg. This extract could be non-toxic. The study of AEKS activity on blood glucose in rats showed a significant ( $p < 0.01$ ) the concentration of glucose in the serum of the treated rats with the AEKS ( $96.2 \pm 30$  mg/dl) and Glibenclamide ( $92.4 \pm 10$  mg/dL), compared to glucose concentrations in the serum of hyperglycemic rats ( $197.1 \pm 50$  mg/dl). Thus, the study showed hypoglycemic activity of aqueous extract of *Khaya senegalensis*. In conclusion, the administration of AEKS causes hypoglycemic activity in rats given oral glucose load. Hypoglycemia this is due to the presence of active chemical groups in the extract of this plant, such as flavonoids, catechin tannins, polyphenols, quinones, sterols and terpenes. These results confirm the therapeutic indication in traditional medicine *Khaya senegalensis* in the treatment of diabetic disease.

**Keywords:** *Khaya senegalensis*, Phytochemical Screening, Toxicity, Hypoglycemic, Rat

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## 1. Introduction

The lack of health coverage in Sub-Saharan countries and the high cost of the annual management of the diabetic patient excluding complications, explain the renewed interest in the traditional [18] medicine. The antidiabetic substances research of plant begun by in vitro methods, in vivo and clinical to develop phytomedicines at affordable prices for the population is booming on all continents. Of all time, plant extracts have been used for the treatment of diabetes in Africa, America, Asia, Australia and Europe [3]. Thus, the existence of a traditional antidiabetic pharmacopeia for the

treatment of diabetic pathology is confirmed by traditional healers and medicine [18].

*Khaya senegalensis* (Meliaceae), is native to tropical Africa. It is found in an area stretching from the Atlantic Ocean to the Indian Ocean by crossing tropical West Africa.

*Khaya senegalensis* (Meliaceae), commonly known caïcédrat in West Africa, is one of the plants used in the Ivorian pharmacopeia.

The distribution of this tree covers the entire Sudano-Guinean climate area and extends slightly north of the Sahel-Sudanese climate where it must find in the soil enough moisture to compensate for the dryness of the atmosphere. It

accommodates an annual average temperature between 29°C and 40°C for the warmest month. This species is found in Côte d'Ivoire in Ferkessedougou area and Mali. The caïlcédrat shows an almost permanent leafing. The leaves fall during the dry season but are renewed at soon [11].

It is especially abundant on moist alluvium of river borders and non-flooded lowlands. It grows on moist alluvial lateritic soil and also while keeping a large diameter.

The decoction of the leafy stems or roots used in drink and bath, cures jaundice, hyperthermia and edema widespread [1, 12]. At the current Sudan, traditional healers treat malaria with the extract of *Khaya senegalensis* trunk bark. In Gambia, the fever is treated with the stem bark of this plant macerated. In Mali, the stem bark decoction is used to treat hemorrhoid bath and diabetes [1, 12].

It is also identified in the aerial parts of the plant hypothermic substances, antibacterial, antipyretic, tonic and anthelmintic [2].

The objective of this preliminary study was to identify both the active chemical groups of the extract aqueous of *Khaya senegalensis* bark and the toxicity and evaluate its effect on hyperglycemic induced in the Wistar rat.

## 2. Materials and Methods

### 2.1. Plant Material and Preparation of Dosage Form

The plant material used is made of fresh bark of *Khaya senegalensis* trunk harvested in the forests of Katiola (Côte d'Ivoire) and authenticated National Floristic Center of Felix University Centre Houphouët-Boigny. The aqueous trunk aqueous extract of *Khaya senegalensis* (AEKS) was prepared from 300 g of trunk bark powder were introduced into a flask containing 3 liters of distilled water. The decoction was carried out with stirring in a water bath at 100°C for 2 hours. After cooling, the decoction was filtered through cotton wool and paper Whatman N°. 1; the filtrate obtained was concentrated in a rotary evaporator (Laborata 4000 Heidsph, France) under vacuum at a temperature of 50°C. After concentration, the filtrate was taken up with a little distilled water and then lyophilized after 48 hours of freezing.

### 2.2. Phytochemical Screening

The phytochemical from the aqueous extract of *Khaya senegalensis* is a qualitative test to characterize the different chemical groups present in this aqueous extract which present a pharmacological interest. This test identifies the chemical groups or secondary metabolite content in the plant species such as polyphenols, alkaloids, tannins, flavonoids, saponins, quinones, terpenes and sterols and cardiac glycosides. The tests were for compounds of detection methods with the indication of the reagents which are summarized in Table 1.

### 2.3. Method of Acute Toxicity Study by Gavage of the AEKS

#### 2.3.1. Determination of the Concentration at the Limit of Solubility

In some distilled water, the dry extract was gradually dissolved until the limit of the solubility to determine the concentration on the limit of solubility or maximal concentration in our study. The solution at the limit of solubility is obtained with 1g of lyophilized extract and it needed 4.29 milliliters of distilled water. Of this fact:  $C_{max} = 0.233 \pm 0.005 \text{ g/ml}$  soit  $233 \pm 5 \text{ mg/ml}$ .

#### 2.3.2. Administration of Dose on the Limit of Solubility or Maximal Dose

The experimentation consists to divide of 5 random groups of 3 mice. We determine the mortality after 24 hours according the dose during only administration to the animals experiment. Mice did not present signs of infection were put on an empty stomach during 14 hours. The administration is made by oral way in the proportions of 0.6ml for 20g [(14)] weight using a stiff with. This corresponded at the dose  $7010 \pm 30 \text{ mg/kg}$  of AEKS. The witness received only distilled water.

V: Volume administrated to mice was:  $V = 0.03 \text{ Mml}$  M: Mass of the mice

### 2.4. Animals and Induction of Oral Glucose Tolerance Test

The antihyperglycemic experiment was conducted on 32 male rats Wistar (*Rattus norvegicus*) divided into 4 groups of 8 hyperglycemic animals a lot normoglycaemic witness. The animals are kept in pet UFR - Biosciences at room temperature with a stable humidity with a day/night cycle from 12: 12h. They have free access to water and food.

The hyperglycemia was induced in rats by glucose load (4g/kg) administered 1 hour after gavage of each solution using an esophageal stainless steel.

### 2.5. Animal Treatment

32 male rats weighing on average  $185 \pm 10 \text{ g}$ , were divided into 4 groups of 8 animals rendered hyperglycemic with a normoglycemic control group that received distilled water.

These animals underwent two types of oral treatments: a single administration to rats of tested solutions (glibenclamide 0.2 mg/kg/ov; AEKS at 35 mg/kg/ov) a week for 6 weeks and repeated administration in 3 successive days solutions tested per week. Blood samples were taken before administration of extracts and glucose and subsequently at 1, 2, 3 and 4 hours after.

Blood glucose determination was produced by the enzymatic method with glucose oxidase/peroxidase using the glycemic player One Touch Basic (Lifescan, mulpital, USA) [17].

Table 1. Methods of phytochemical screening.

Materials to search (Reagents used)	Characterization methods (Expected results)
Sterols and Polyterpenes (Liebermann-Buchard)	Evaporate 5 ml of the extract in a capsule + 1 ml of acetic anhydride + 0.5 ml sulfuric acid (Ring purple)
Flavonoids (cyanidin)	Evaporate flavonoids 2 ml of the extract into a capsule and then cooled, to the residue in 5 ml of hydrochloric alcohol, add 3 drops of isoamyl alcohol (orange to pink)
Catechic tannins (Stiany)	Evaporate 5 ml of the extract in a capsule + 15 ml mixture (formalin 30% and 30%HCl), maintain at 80°C for 30 min then cool (Rushed into large flakes)
Gallic tannins (Sodium acetate Iron trichloride)	Filter 5 ml of the extract in a capsule saturate with sodium acetate Add 3 drops of iron trichloride to 2% (Intense blue)
Quinones (Borntraeger)	Evaporate 2 ml dry extract Triturate the residue add 5 ml of HCl heated at 100°C for 30 min cool and add 2 ml of chloroform and 0.5 ml of 50% ammoniac (red).
Alkaloids (Dragendorff-Bouchardat)	Evaporate 4 ml of the extract +4 ml of alcohol at 60°C + 2 drops of Reagent-Iodo bismutate (Orange) +2 drops of Reagent Iodo-iodized (Brun)
Saponins	1 g of powder extract Add 100 ml of water to a boil for 15 minutes to cool and filter successively introduce 1ml, 2ml, 3ml....10ml of decoction, stir for 15 seconds, Let stand for 15 minutes and measure the foam height (Height of foam equal to 1 cm
Coumarines	evaporate 5 ml of the macerated extract + 2 ml of hot water + 0.5 ml of ammoniac hydroxide to 25% (Fluorescence)

## 2.6. Data Statistical Analysis

Blood glucose values were expressed as mean  $\pm$  standard deviation (error) the average. The homogeneity of blood glucose values in each group was verified by the Student test.

## 3. Results

Table 2. Groups active chemical present in the extracts of *Khaya senegalensis*.

	Extract 1 (DCM)	Extract 2 (meOH)	Extract 3 (Infusion)	Extract 4 (etOH)	Extract 5 (Decoction)
Flavonoïds	+	+	+	+	+
Alkaloids	-	-	-	-	-
Polyphenols	+	+	+	+	+
Catechic tannins	+	+	+	+	+
Gallic tannins	-	-	-	-	-
Quinones	+	+	+	+	+
saponins	-	-	-	-	-
Sterols et Terpenes	+	+	+	+	+
Coumarins	-	-	-	-	-

NB: +: Present, -: Absent

Catechin tannins and flavonoids were the most chemical groups present in the bark extracts of *Khaya senegalensis*. Alkaloids, saponins, gallic tannins and coumarins have not been highlighted in the extracts.

### 3.1. Acute Toxicity of *Khaya Senegalensis*

Table 3. Changes in the death rate of mice by dose *Khaya senegalensis* administered orally.

Groups of mice	Decreasing dose (mg/kg)	Number of deaths	Mortality (%)
1	7010 $\pm$ 30	1	20
2	3505 $\pm$ 15	1	20
3	2334 $\pm$ 10	0	0
4	1752 $\pm$ 7	0	0
5	1401 $\pm$ 21	0	0

The aqueous extract of *Khaya senegalensis* administered at a dose of 2334 mg/kg orally did not result in mortality in

The results of the blood glucose levels of the various groups were compared by the Student test. The ( $\alpha$ ) considered risk ( $p < 0.05$ ) determining the significance test, (n) is the number of experiences [10].

contrast to the dose of dilute solute  $\frac{1}{2}$ .

### 3.2. Antihyperglycemic Activity of *Khaya Senegalensis*

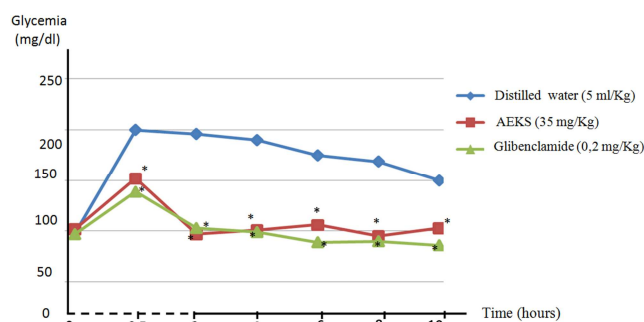


Figure 1. Administration of single tested in hyperglycemic rats.

There is a significant difference in blood glucose ( $p < 0.05$ )

between the control group and treated according batches time either by the aqueous extract of *Khaya senegalensis* or oral antidiabetic reference (glibenclamide).

## 4. Discussion

The preliminary assay of acute toxicity by gavage shows that the aqueous extract of *Khaya senegalensis* (AEKS) at the dose of  $2334 \pm 10$  mg/kg is not toxic at mice after 24 hours in our study condition (Table 3). Indeed, this dose obtained with the maximal concentration of the extract solubility, all the mice Swiss stayed in live. So, the AEKS could not be toxic in reduced dose to that is  $35 \pm 5$  mg/kg used by the tradipratician.

The aqueous extract of *Khaya senegalensis* at the dose 35 mg/kg/ov single dose causes a hypoglycemic action of 33.1% in a single dose in the second time only after the oral glucose load (fig 1). This observation showed that the hypoglycemic activity of AEKS on a model of hyperglycemia caused orally was short (2 hours after a peak of hyperglycemia to the 30<sup>th</sup> minute). Based on the time of action, could be called *Khaya senegalensis* of medicinal plant "quick action". Moreover, the results of phytochemical screening AEKS revealed the presence of active chemical groups such as quinones, flavonoids, catechic tannins, polyphenols, sterols and terpenes. These compounds could be causing the observed glucose lowering activity of AEKS.

Indeed, this finding is consistent with results of the work of some authors who have found these active chemicals whose quinones [9] and flavonoids, catechic tannins, polyphenols, quinones, sterols and terpenes [9, 16]. Thus, an experimental study has demonstrated hypoglycemic activity of polyphenols. In addition, intravenous administration of polyphenols (caffeic acid) is accompanied by a decrease in blood glucose after an oral administration glucose load in rats [12]. Such effects could be explained by inhibition polyphenols on glucosidases or glucose transporters at the intestinal barrier, thereby limiting the intestinal absorption of glucose.

Catechin tannins and quinones that we identified in AEKS also have hypoglycemic activity in the ethanol extract 70% *Rauvolfia vomitoria* (Apocynaceae) during a administration oral glucose load of 4g/kg/ov among wistar [13] rats. Catechic tannins and quinones exert an effect on liver enzymes stimulating gluconeogenesis [8]. Also, terpenes that we have identified are bioactive compounds found naturally in many plants with known hypoglycemic activity. The charantin, a triterpene isolated from *Momordica charantia* has a hypoglycemic effect particularly in the type 2 diabetes [5]. Andrographolide (diterpenoid lactone) isolated from *Andrographis paniculata* also exerts in vitro a significant hypoglycemic activity.

Terpenes would act by lower glucagon secretion and an action on the intake of chemical elements ( $\text{Cu}^{2+}$ ,  $\text{Mg}^{2+}$ ) for the operation of beta cells [8].

Moreover, the therapeutic role antidiabetic flavonoids is undeniable insofar as these molecules inhibit aldose

reductase [7, 15] enzyme known to catalyze the conversion of glucose to sorbitol, which participates in diabetic complications. Similarly, glycosylation (ability to set glucose) can be attributed to flavonoid glycosides, phenolic compounds that tend to bind oses [4]. The hypoglycemic activity of AEKS aqueous extract, can be explained by the action of secondary metabolites (flavonoids, polyphenols and terpenes) that stimulate the regulation and release of insulin in the pancreas in animals hyperglycemic state for a glucose uptake by muscle tissue of animal [6]. Thus, the aqueous extract of AEKS contains pharmacological substances which gave him the hypoglycemic properties. This assumes that the hypoglycemic active molecules of *Khaya senegalensis* are soluble in water and ethanol, two extraction media commonly used in traditional medicine.

## 5. Conclusion

The phytochemical screening showed in the aqueous extract of *Khaya senegalensis* the group of flavonoids, tannins catechic, polyphenols, quinones, sterols and terpenes. These active chemical groups no toxic were responsible for the hypoglycemic activity in Wistar rats induced hyperglycemia state. This property would justify its use in traditional medicine in the treatment of diabetes. Other studies are possible for isolated by thin layer chromatography, chemical subgroups active leaders of the hypoglycemic activity of the extract of bark *Khaya senegalensis*.

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