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Research Article

Phytochemical Screening and Evaluation of Antihyperglycemic, Antiradical and Acute Oral Toxicty Activities of Aqueous Extract of Stem Bark of *Khaya senegalensis* A. Juss (Meliaceae) from Benin

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ABSTRACT

The use of medicinal herbs in traditional system of medicine is a common practice in many cultures around the world, especially in African society. Medicinal plants are a great source of bioactive molecules for treatment of differnts pathology. *Khaya senegalensis* A. Juss belongs to the family Meliaceae. *Khaya* species are highly priced in traditional medicine in Bénin. The present study aims to: (i) evaluate the phytochemical, antiradical and antihyperglycemic activities of aqueous extract of stem bark of *K. senegalensis*; (ii) determine the effect of aqueous extract of *K. senegalensis* stem bark on liver function parameters wistar rats. The screening was performed for triterpenoids, alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins, anthocyanes, leucoanthocyanes, reducing sugar, mucilage and Cyanogenic compound. The color intensity or the precipitate formation was used as analytical responses to these tests. The total antihyperglycemic capacity was evaluated by using the complex method. Antiradical activity was made by using the DPPH method. Toxicological evaluation of aqueous extract of *K. senegalensis* stem bark on some liver function parameters of wistar rats (150-200g) were critically examined. Results from phytochemical screening indicated the presence of high level of phenolic compounds and alkaloids in bark-stem of *K. senegalensis*. With a concentration of 491.11 ± 20.67 mmolEAA /mg, the plant was determined to have a high potential antioxidant activity. Moreover, an increase of concentration to 2000 mg/kg was non-toxic for the rats. No toxicity activity was observed. Blood biochemical parameters and hematological parameters remain constant after administration of the plant aqueous extract. A dosage of antihyperglycemic activity revealed a reduction of glycemia with a dose of 500 mg/kg. The important results obtained from this study justify the use of this plant in traditional medicine for treatment of diabetes. However, it will be useful to confirm a non-toxic effect of the plant extract through histological dissection of liver and kidney.

Key words: *Khaya senegalensis*, aqueous extract, antihyperglycemic activity, toxicity, *in vivo*.

INTRODUCTION

The diabetes, the sixth cause of death in the world[1], is a chronic metabolic disorder characterized by hyperglycemia resulting from a deficit of secretion of the insulin and/or an increase of its resistance[2]. This metabolic disease threat, in an increasing way, public health as well in developed countries as in developing countries[3]. According to the World Human Organization (WHO), the number of diabetics could pass from 30 millions in 2010 to the double before 2030[4]. In Benin, studies showed that prevalence of this disease increased. In 2000, its prevalence was estimated at 0.10 % and it passed to 2.9 % in 2011[5].

Ineffectiveness of modern medicine and the high cost of treatment led certain patients to use traditional medicine. Traditional medicine is used as an alternative way of treatment for the disease. It was based on the use of medicinal plant extracts obtained from leaves, stem-bark or roots. According to certain authors[6-8] medicinal plants represent a wide source of antioxidants and have very few side effects. The World Health Organization recognized the potential offered by medicinal plants in different countries[9]. For the treatment of diabetes, about 800 plants were used worldwide. An ethnobotanical study conducted in Benin on medicinal plants revealed the use of *Khaya senegalensis* for the treatment of diabetes. *K. senegalensis*,

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is a savanna tree easily recognized by its round evergreen crown of dark shining foliage pinnate leaves and characteristic round capsules. A tree of 30 m high and 3 m girth, with dense crown and short bole covered with dark grey scaly bark.

Different parts of plant were used such as leaf, bark, or root. According to some authors, this plant presented an antioxidant and antihyperglycemic properties which could justify its uses in the treatment of diabetes[10,11]. Other authors have noticed a risk of toxicity when the extracts were used for a long period. *K. senegalensis* was also used in forms of decoction for the cure of mucous diarrhea, syphilis, pyrexia and malaria fever[12]. Others properties related to antimicrobial and antiviral properties were reported. The present study aims to: (i) evaluate the phytochemical, antiradical and antihyperglycemic activities of *K. senegalensis* of aqueous extract of stem bark; (ii) determine the effect of aqueous extract of *Khaya senegalensis* stem bark on liver function parameters wistar rats.

MATERIAL AND METHODS

Sampling

Fresh stem bark from *Khaya senegalensis* was collected at Fidjrossè, situated in the coast. The material was authenticated by a botanist at the National herbarium from University of Abomey-Calavi (Benin). A voucher specimen was deposited at the herbarium under the number AA6532/HNB. The stem-bark was air-dried in the laboratory before powdering.

Animal

Laboratory animals, four (4) months old male and female Wistar rats weighting 150g-200g were used in this study. Animals were obtained from Faculty of Health Sciences. Animals were lodged in the metallic cage and kept under experimental conditions. Animal room was maintained at the temperature of 25°C with an alternate cycle of 12 hours light and 12 hours dark with free access to food and water. All the experimental protocols conducted on animals were submitted to, and approved by the ethics committee of Institute of Biomedical Applied Sciences (ISBA) located at the faculty of health sciences.

Phytochemical screening

Screening was based on the determination of the main families of molecules presented in the plant after powdering. This determination was made according to the method[13] based on the differential coloring reactions. Each molecule family was then quantified after identification.

Preparation of aqueous extract

100 g of dry powder added by 500 ml of distilled water are mixed in a ball warmed with a heating skullcap. During the preparation the ball mixture was subjected to a permanent agitation for duration of 30 minutes. Then after cooling, the macerate was filtered under filter paper. The extraction was made three (03) times on the macerate. The filtrate obtained was concentrated under vacuum by using a rotary evaporator at 80°C until the obtaining of a dry extract. After extraction the yield was determined.

Quantification of some bioactive molecules

Content of the total phenolic compounds

Quantification of total phenolic compounds was made according to the standard method[14] .This determination was made in two steps. On the one hand, mixture of 125 µl of the diluted macerate solution and 625 µl of the reagent of Folin Ciocalteu was incubated during 5 minutes at room temperature. On the second hand 625 µl of Na_2CO_3 with a final concentration of 75 g/L were added to the mixture and then incubated during 2 hours. The mixture was then measured at 760 nm. The contents in phenolic compounds were determined by using a curve of calibration of gallic acid (0-100 mg / ml). The contents were expressed in equivalent milligram of gallic acid per 100mg of obtained extract.

Content of the total flavonoids

It was estimated by standard methods[15,16]. An equal volume of 0.5 ml of AlCl3 (2 %) solution was added to 0.5 ml of extract (0.1 mg / ml). Optical density (DO) was read after 10 min of incubation at a wavelength of 415 nm. Calibration was made with the rutine (0-200mg / ml).

Content of condensed Tannins

It was determined by following standard method[17,18]. 3 ml of a solution of vanillin (4 % in the methanol) and 1.5 ml of concentrated hydrochloric acid were added to 400 µl of each sample or standard. The mixture was incubated for 15 min and the absorbance read to a wavelength of 500 nm. The concentrations of the condensed tannins were deducted from the ranges of calibration established with the catechin (0-300 µg / ml), and were expressed in microgram equivalent of catechin per milligram of extract.

Content of the antiradical activity

Antiradical activity was determined by using the DPPH method[19]. 1.5 ml of each solution was mixed with 3 ml of DPPH methanolic solution and incubated at room temperature for 15 minutes. Then, absorbance was measured at the wavelength of 517 nm. Antiradical activity was determined by using the ascorbic acid calibration curve (0-10 mg/ml).

Acute oral toxicity

The essay of toxicity was done according to the guidelines of the organization of Cooperation and Economic development 423[20] (OECD, 2002) related to chemicals.

Table 1: Phytochemical analysis of stem-bark powder

Chemical compound	Aqueous extract
Alkaloids	+++
Catechin Tannins	-
Gallic Tannins	+++
Flavanoids	+++
Anthocyanes	-
Leucoanthocyanes	+++
Quinonic compound	+++
Saponosides	+++
Triterpenoids	+++
Cyanogenic compound	+++
Mucilage	-
Reducing sugar	+++
Coumarine	+

+: low color intensity ; +++ : high color intensity ; - : absence of coloration

Table 2: Total phenolic compound, flavonoids and tannins content

Aqueous extract	Total Phenolic compound [(a)]	Flavonoids [(b)]	Tannins [(c)]
Khaya senegalensis	115.62 ± 4.99	9.26 ± 0.3	29.02±0.51

[(a)]mg equivalent of gallic acid/g of extract ; [(b)]mg equivalent of rutin/g of extract; [(c)]mg equivalent of catechin/mg of extract

Table 3: Effect of extract on blood count

	HGB (g/dl)	HTE (%)	VGM (fL)	CCMH (%)	TCMH (pg)
Lot 1	11.4±0.2	32±1.0	62.33±2.52	27.93±0.40	21.8 ±0.40
Lot 2 :	11.73±0.57	33.33±2.08	60.67±1.53	27.57±0.68	21.97±0.94
P value	0.439	0.423	0.399	0.481	0.805

Lot 1 : Control rats ; Lot 2 : Rats which received extract of *K. senegalensis*
HGB: haemoglobin; HTE: Haematocrite; VGM: Average spherical Volume; CCMH: Corpuscular Concentration Mean in haemoglobin; TCMH: Corpuscular Content Mean in haemoglobin

The substance was tested by using a sequential process in which three females and non gravid wistar rats weighting 150 g - 200 g were used at every stage. Five (05) lots of three (03) rats were established with a total of fifteen rats. One lot used as control received only distilled water and the rest received the extract at the concentration of 2g/kg. After force-feeding, the animals were observed during the first four hours and then daily for fourteen (14) days. Animals were weighted at the beginning of the experience and afterward every seven (07) days. At the end of 14 days, the biochemical and hematological analyses were done.

Antihyperglycemic activity

It has been done according to the method[21]. Six lots of five rats were constituted with a total of thirty (30) rats. All rats were beforehand submitted to a non-hydric fast for 16 hours before the experiment. Concentrations of 500 mg/Kg and 1000 mg/Kg of aqueous extract were administered to the lot 3 and lot 4. Only distilled water with a dose of 10 ml/Kg was administered to the control. Lot 1 and lot 2 received each a dose of 5 ml/Kg of glibenclamid. Administration of D-glucose with a dose of 3 g/Kg was done after a half of hour. The glycemias were taken with a SD CHECK glucometer in a sequential way every 30 minutes from start point corresponding to T0 up to 120 minutes corresponding to T 120. Then a final measure was done at T180.

Statistical analysis

All data obtained were subjected to statistical analysis using Minitab software Version 1.0. The results were expressed as mean using Graph Pad Prism software. Differences between means were pointed out using the Kruskal-Wallis' test. Results were considered statistically significant for $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical Screening

Chemical compound searched in the powder were presented in table 1. Phytochemical analysis showed the presence of ten chemical compounds out of the thirteen (table 1). Only coumarin was present with a low level intensity. Two groups of tannins were searched among which only gallic tannin was found. The yield obtained for water extraction was evaluated to 15% of macerate and the color presented by the macerate was red.

Antiradical activity

Contents in equivalent of ascorbic acid were calculated with the use of the curve calibration of the ascorbic acid. Results obtained were presented in table 2 below. This quantification revealed that water extract had an antiradical activity evaluated to 491.11± 20.67 mmol EAA/ mg of extract. Moreover, Optical density (DO) was determined and corresponding to 0.68 ± 0.02 and 14.43.

Quantification of some bioactive molecules family

After the phytochemical screening, three chemical compounds groups such as total phenolic compounds, flavonoids and tannins were measured on the water extract. Table 2 presented values obtained and they were respectively 115.62 ± 4.99; 9.26 ± 0.3 and 29.02±0.51 for total phenolic compounds, flavonoids and tannins. The tannins content was three times content of flavonoids and represents 25% of total phenolic compounds in water extract.

Evaluation of the acute oral toxicity

During the evaluation of the acute oral toxicity, no mortality was registered within rats having received aqueous extract in single dose of 2000 mg/kg of physical weight. This result suggested that the DL50 of aqueous extract is higher than the administrated dose.

Animals were observed during 14 days and their weight was taken regularly. Figure 1 showed evolution of the rats' weight during two weeks observation. We noticed an increase of rats' weight during the two weeks of observation as well for the control as for the rats having received the extract (Figure 1). For quantification of hematological (blood count) parameters, no significant difference was observed between control and experimental rats. Data obtained were presented in table 3.

Same results were obtained for both creatin and uremia parameters for which no significant difference was observed for data (figure 2 a, figure 2b respectively). Determination of the alanine aminotranferase content and aspartate aminotransferase content on the rats which received extract showed no variation when compared to the panel of control. These results could be observed on the figure 2c and figure 2d respectively for alanine aminotransferase and aspartate amino transferase. Action of the extract on the important parameters as well the blood

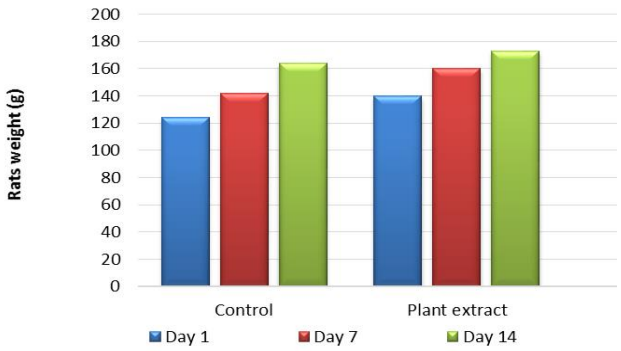

Figure 1 : Evolution of rats' weight during toxicity test

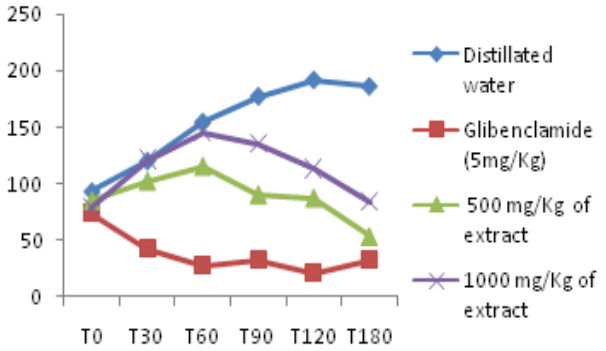

Figure 3: Evolution of the glycemia during the antihyperglycemic activity of *K. senegalensis*

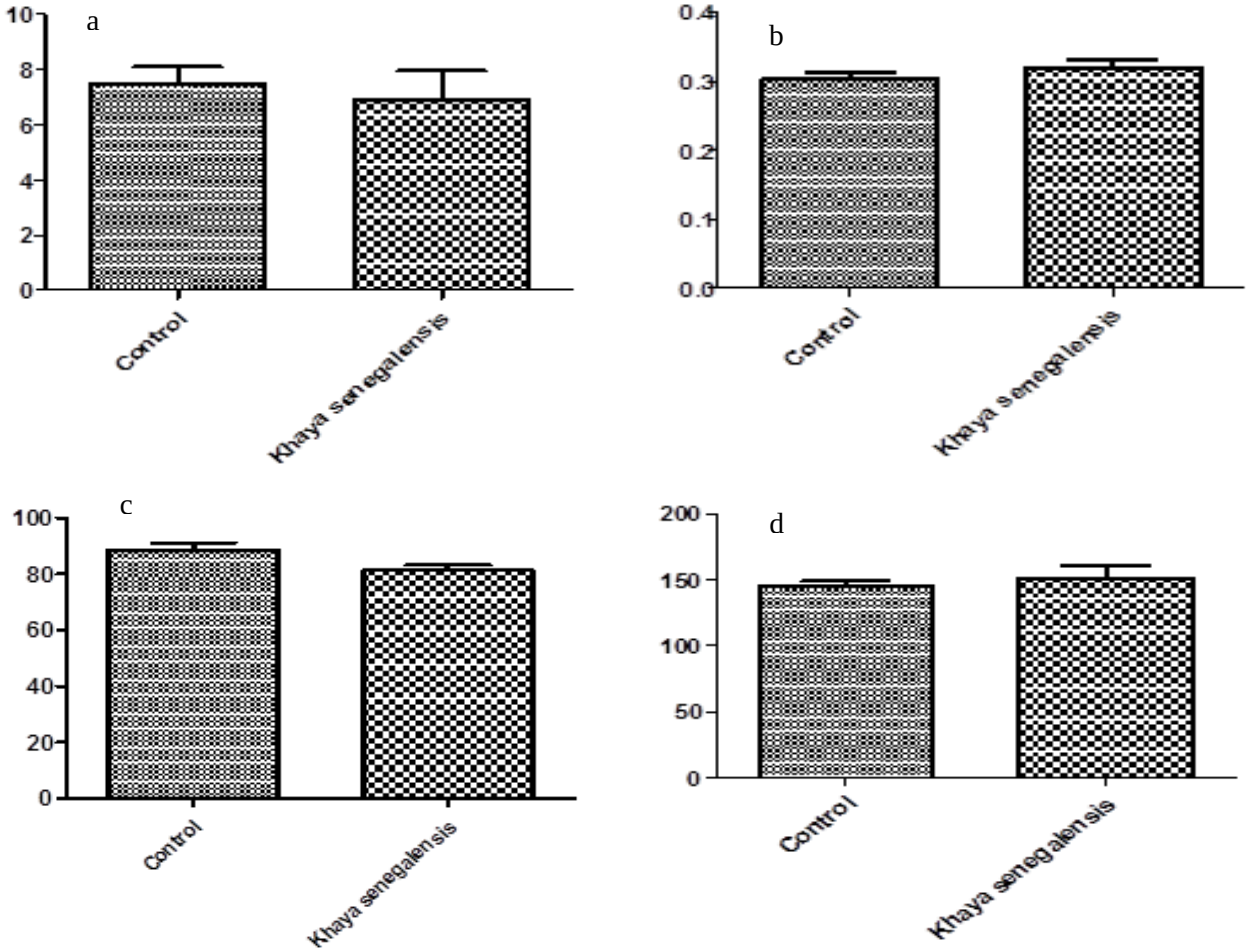

Figure 2: Determination of hematological and hepatic parameters

cell as hepatic enzymes was without damage with a dose of 2000 mg/kg.

Evaluation of the antihyperglycemic activity of the aqueous extract

Evolution of the antihyperglycemic activity during three (03) hours was presented of the figure 3.

After administration of glucose to the control panel which previously received distilled water, we noticed an increase of glycemia rate until T180. Rats which received extract at the concentration of 500 mg/kg presented an increase of glycemia rate after sixty minutes (T60) and then this rate decreases significantly until time T180 minutes. With the concentration of 1000 mg/kg, effect of extract was lower than that obtained with the concentration of 500 mg/kg. The activity of the reference drug is better for the first two hours. After two hours of action, the glycemia raises. An increase of glycemia was not observed with rats which received the extract.

DISCUSSION

Previous studies showed that water is a better solvent used for extraction of phenolic compounds and having a better antioxidant activity[22,23]. It allowed us to obtain a yield of 15.38% after extraction using 100g of *K. senegalensis*

powder. For the same plant, other authors obtained respectely 8.5% from 300g of powder[24] and 22.62% from 100g of powder[25]. The difference observed could be resulted probably from the method used by each author. Besides, yield obtained from 100g of powder was higher than which from 300 g of product. This remark was pointed out as well by our result as the results[25]. Phytochemical analysis confirmed the presence of important phenolic compounds such as tannins and flavonoids which were known to be natural bioactives substances and antioxydant properties. These results confirmed which already have been published by other authors[10,25,26]. Contrary to our results, these authors didn't reveal the presence of alkaloids. This difference could be explained by humidity, soil and season during which plant was collected, and the extraction method, which are factors that influenced highly the presence or not and the concentration of phytochemical compounds in plant[27,28]. Results from antioxidant activity using DPPH' method revealed a high potential antioxidant activity of *K. senegalensis* with a concentration of 491.11±20.67 mmolEAA /mg. This result differs from which obtained by other authors[10,29] for the same plant. Differences could be explained as well by the standard (Trolox) and the method used[10] as the content of phenolic compounds. Indeed, phenolic compounds such as phenolic acid, flavonoids, and tannins contribute highly to the antioxidant activity. Polyphenol has capacity to absorb free radical. Moreover, the content of compounds in plant extract depends on soil type, part of plant, and environmental conditions related to the plant growth.

With a dose of 2000 mg/kg, aqueous extract showed a non toxicity effect on rats. Some authors reported that a DL_{50} of aqueous extract obtained from leaf was higher than 3000 mg/kg and which from stem extract was higher than 5000 mg/kg in the case of subchronic toxicity[30,31]. No significant difference was observed as well on biochemical parameters as on hematological parameters. This result could suggest the non destructive effect of this plant on liver and kidney. Nevertheless, some authors[32,33,34] reported the hepato propective and nephroto protectives properties of *K.senegalensis*. This result could be confirmed by histological dissection of the liver and kidney.

A search of an antihyperglycemic activity showed a reduction of glycemia rate with a dose of 500 mg/kg. However, no difference was observed with the rats which received distilled water and the lot which received 1000 mg/kg. Our results are different from which obtained other author[24] who noticed a significant reduction of 30% of glycemia after 2 hours of treatment with a single dose of 35±5 mg/Kg and then 40% after 4 hours.

The lot of rats treated with the reference medicine (glibenclamide 5 mg/kg) showed a significant reduction of glycemia from T30 to T120.

The antihyperglycemic effect of *K. senegalensis* could be explained by an increase of insulin secretion through its anti oxidant activity as it has been described for terpen and flavonoids[10]. Likewise, phenolic compounds could also contribute to the antihyperglycemic effect as this could inhibit alpha amylase activity, and inhibition of alpha glucosidase enzyme could resulted from alkaloids[35,37].

CONCLUSION

The results of the present research contributed to better understand efficiency of *K. senegalensis* in the treatment of diabetes. An *in vivo* action of the aqueous extract showed no toxicity on the rats. Moreover, there is no modification in the blood parameters. The high level of the antioxidant in *K. senegalensis* is a major factor which contributed to its antihyperglycemic activity of that plant.

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