

# Phytochemical screening and antidiabetic effect of aqueous extract of *Abrus precatorius* Linn (Fabaceae)

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Marius Léбри<sup>1</sup>, Stéphanie Marianne Lagou<sup>2</sup>, N'guéssan Bra Yvette Fofie<sup>3</sup>, Calixte Bahi<sup>1</sup>, Guédé Noel Zirihi<sup>4</sup>, Adama Coulibaly<sup>1</sup>, Fatiha Chigr<sup>5</sup>, Mohamed Najimi<sup>5</sup>, Abderrafia Hafid<sup>6</sup> and Mostafa Khouili<sup>6</sup>

## Abstract

In less than a quarter of a century, diabetes has become a public health problem in developing countries. The purpose of this study is to valorize *Abrus precatorius* (Fabaceae), a medicinal plant of the native pharmacopoeia of Côte d'Ivoire that may have antidiabetic properties. Thus, the particular objectives of this study are to investigate the chemical composition and *in vivo* antidiabetic effect in rats of the aqueous extract of *Abrus precatorius*'s leaves (ETAAP). The aqueous extract of leaves of *Abrus precatorius* (ETAAP) was obtained by the traditional method (decoction) and the phytochemical analysis based on color reactions and / or precipitation were done. The antidiabetic effect was studied by evaluating its hypoglycemic effect in normal rats and its antihyperglycemic effect in rats previously made hyperglycemic by oral administration of glucose at 30%. Phytochemical screening of the aqueous extract of the leaves of *Abrus precatorius* revealed the presence of alkaloids, tannins, flavonoids (flavones), saponins, quinone compounds (coumarins), sterols and triterpenes and reducing compounds. The results of diabetes study showed that the extract causes a hypoglycemic dose dependent effect in normal rats after 6 hours. The experimental effective dose (ETAAP 40 mg / ml) reduced increasingly hyperglycemia every 30 minutes for 2 hours. These results show that *Abrus precatorius* (ETAAP) could be a good alternative in the treatment of diabetes

**Keywords:** *Abrus precatorius* L.; diabetes; hypoglycemia.

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<sup>1</sup>Biochemical Pharmacodynamics Laboratory, Biosciences Department, Felix Houphouët Boigny University, PO Box 582, Abidjan 22, Ivory Coast.

<sup>2</sup>Laboratory of Ethnopharmacology and Medicinal Plants, Science of Nature Department, Nangui Abrogoua University, PO Box 801, Abidjan 02, Ivory Coast.

<sup>3</sup>Laboratory of Pharmacognosy, Botany and Cryptogamie, Pharmaceutical and Biological Sciences Department, Félix Houphouët Boigny University, PO Box 747, Abidjan 22 Ivory Coast.

<sup>4</sup>Laboratory of Botany, Biosciences Department, Félix Houphouët Boigny University, PO Box 582, Abidjan 22, Ivory Coast.

<sup>5</sup>Laboratory of Biological Engineering, Sultan Moulay Slimane University, Faculty of Science and Technology, PO Box 523, 23000 Beni-Mellal, Morocco.

<sup>6</sup>Laboratoire de Chimie Organique & Analytique, Université Sultan Moulay Slimane, Faculté des Sciences et Techniques, PO Box 523, 23000 Béni-Mellal, Morocco.

\* Correspondence: lebrimarius7@gmail.com

# Criblage phytochimique et effet antidiabétique de l'extrait aqueux de *Abrus precatorius* Linn (Fabacea)

## Résumé

En moins d'un quart de siècle, le diabète est devenu un problème de santé publique dans les pays en développement. Cette étude vise à valoriser *Abrus precatorius* (Fabacea), une plante médicinale de la pharmacopée ivoirienne, susceptible d'avoir des propriétés antidiabétiques. Ainsi, les objectifs particuliers de cette étude sont d'étudier la composition chimique et l'effet antidiabétique *in vivo* chez les rats de l'extrait aqueux des feuilles de *Abrus precatorius* (ETAAP). L'extrait aqueux des feuilles de *Abrus precatorius* (ETAAP) est obtenu par la méthode traditionnelle (décoction) et l'analyse phytochimique est basée sur des réactions de couleur et / ou de précipitation. L'effet antidiabétique a été étudié en évaluant son effet hypoglycémique chez les rats normaux et chez les rats en hyperglycémies provoqués par voie orale ayant reçu 30 % de glucose. Le criblage phytochimique de l'extrait aqueux des feuilles de *Abrus precatorius* a révélé la présence des alcaloïdes, de tanins, de flavonoïdes (flavones), des saponines, des composés quinoniques (coumarines), des stérols et des triterpènes et des composés réducteurs. Les résultats de l'étude sur le diabète ont montré que l'extrait provoque un effet dose dépendant chez les rats normaux après 6 heures. La dose efficace expérimentale (ETAAP 40 mg / ml) a réduit de plus en plus l'hyperglycémie toutes les 30 minutes pendant 2 heures.

**Mots-clés:** *Abrus precatorius* L. ; diabète ; effet hypoglycémique.

## Background

Diabetes is a metabolic disorder characterized by a disorder in the regulation of carbohydrate metabolism leading to hyperglycemia [1]. This disorder concerns genetic and exogenic factors (viral, chemical) and damages the  $\beta$  cells of Langerhans [2]. As a result, the body becomes unable to produce insulin, a pancreatic hypoglycaemic hormone. This disorder is characterized by polyuria (frequent and abundant urine), glycosuria (presence of glucose in urines) and hyperglycaemia (glucose rate on an empty stomach higher than 1.2 g/l in plasma blood and confirmed in at least two occasions) [2]. The complications of diabetes cause severe degenerative in the heart, vessels, eyes, kidneys and nerves [3]. According to the World Health Organization, 347 million people are diabetic in the world [4]. In modern medicine, not satisfactory effective therapy is still available to cure diabetes [5]. Currently, diabetes therapy is based on the use of hypoglycaemics (sulfonamides, biguanides, insulin), on hygieno-diet measures and exercises [6]. Even if the injections of insulin or other products make it possible diabetic to remain in life, the diabetes requires a long treatment, which the patients have of the evil to support. In the search of means of fighting, man recognized and used the medicinal properties of many cultivated or wild plants and many drugs to combat this worrying affection. Among these many plants, *Abrus precatorius* (Fabaceae) is known mainly for its medicinal properties to cure various diseases. In Côte d'Ivoire, the leaves are used in a drink for the treatment of gynecological and obstetric disorders [7]. In Nigeria, leaf's decoction is used in the treatment of diabetes [8]. Pharmacological studies have shown that *Abrus precatorius* has antimicrobial [9], antidiabetic, antiasthmatic, antiepileptic, antioxidant [10] and cytotoxic activities [11]. Vadivel *et al.*, [12] showed that the methanolic extract of the seeds of *Abrus precatorius* regulates glycaemia by the inhibition of the enzymes  $\alpha$ -amylase and  $\alpha$ -glucosidase as well as by its antioxidant effects. The regulatory effect of the blood glucose level of *Abrus precatorius* has

also been highlighted by Nwanjo [13]. The author has shown that the aqueous extract of the seeds of *Abrus precatorius* exerts a dose-dependent hypoglycemic effect in diabetic rat models (Streptozotocin). The aqueous extract is not toxic by oral route to rats [14]. The present study investigated the chemical composition, *in vivo* antidiabetic effect in rats of the aqueous extract of *Abrus precatorius*'s leaves (ETAAP).

## Methods

### Collection of the plant

The leaves of *Abrus precatorius* were collected in an urban area of Abidjan (Southern Côte d'Ivoire) in month of October 2014. The plant had already been identified at the National Centre Floral of Felix Houphouët-Boigny University, Abidjan. The plant had already been identified at the National Center of Abidjan on the number: *Abrus precatorius* (Fabaceae): Aboudé-Mandéké (Coast d'Ivoire), 23 May 1990, N'guessan Koffi 165 by N'guéssan *et al.* [15]

### Extract procedure

The decoction is the method recommended in traditional medicine. In our case this decoction realized from already powder dried leaves in the laboratory condition [14].

100 g of powder of leaves were introduced into a triple-neck round-bottom of 250 mL, 100 ml of distilled water were added. A round-bottom was topped with a cooler connected to a faucet opened by pipe. The round-bottom is put down into a warm balloon (ELECTROMANTLE) maintained in a constant temperature of heating during one hour. After cooling, the mixture is filtered with cotton wool three times and the obtained filtrate was moved in the stove (SELECTA) at 55°C during 24 h. The extract was dried and the aqueous extract (ETA) was obtained. Extraction was repeated several times to obtain a sufficient quantity.

### Phytochemical screening

Phytochemical screening of aqueous extract was performed on a qualitative test based on color reactions and / or precipitation [14].

### Animal experiments

The animals used for all the tests are male rats *Rattus norvegicus* strain Wistar aged 8 to 9 weeks. The average weights of Wistar male rats vary between 133g and 336g according to the test. These animals come from a pet store of the Pharmaceutical and Biological Sciences Department, Félix Houphouët Boigny University (Ivory Coast) and the experiments were carried out in this pet store. The animals are divided into groups in standard cages for an acclimation period (2 weeks) before being used in the different experiments. During this period, the animals have free access to food (pellets) and water and are kept in a pet shop at constant temperature ( $22 \pm 2^\circ$  C), subjected to a light / dark cycle of 12 / 12h. The dark phase of this cycle begins at 12h and the different experiments always take place from 13h to 18h because of the nocturnal activity of the animal. Before the experiments, the animals were acclimated 2 weeks to harmonize their physiological state. The route of administration used throughout the experiment is the oral route.

## Experimental study on diabetes

### Method of reading blood glucose in rats

Blood glucose is measured using an Accus Chek blood glucose meter with a blood drop from the caudal end of the animals (venous blood). Generally glucometers consist of an absorbent layer on which the drop of blood is deposited, finely porous or covered with a membrane on its inner side, it retains the red blood cells and only allows the plasma to diffuse to the lower layers where the reagent is essentially glucose oxidase (possibly hexoxynase) associated with a chromogen. The coloration obtained is measured by reflectometry in the glucose meter [16, 17].

### Hypoglycemic effect of aqueous total extract in normal glycemic rats

The animals come from a pet store of the Pharmaceutical and Biological Sciences Department, Félix Houphouët Boigny University (Ivory Coast) and the experiments were carried out in this pet store. The extract and substances from both controls were administered to single dose animals by gavage using a probe at the recommended dose: 2 mL / 100 g b.w. [18]. The rats were put into play for 16 hours and then divided into groups of 6; a blood sample is taken in all the rats before oral administration (gavage) with the test substances. The basal blood glucose (T0) for each lot was read before feeding began. After administration of the test substances, a blood sample is taken every hour for 6 hours [17].

**Group 1 (control):** oral administration of glibenclamide at a concentration of 0.25 mg / mL.

**Group 2; 3; 4; 5:** oral administration of different concentrations of ETAAP (40; 60; 80; 100 mg / mL).

The glucose values are expressed as g / L and the variations in blood glucose are expressed as a percentage relative to the basic blood glucose (T0) according to the following formula:

$$\text{Percentage change in blood glucose (\%)} = \frac{(G_t - G_0) \times 100}{G_0}$$

$G_0$  : basic blood (T0)

$G_t$ : glucose at time t (hours)

### Hypoglycaemic effect of ETAAP in rats by hyperglycaemia caused by oral route

The experiments were carried out in this pet store of the Pharmaceutical and Biological Sciences Department, Félix Houphouët Boigny University (Ivory Coast).

Thirty (30) male Wistar rats divided into 5 lots of 6 and kept fasting for 16 hours were used. Hyperglycaemia was induced by oral administration with 30% glucose 30 minutes before administration of the test substances. A blood sample is taken at T0 and the rats receive orally 30% glucose solution. At T1 (T0 + 30 min), a blood sample was taken and hyperglycemia was observed in each batch (blood glucose > 1.50 g / L). The rats by batch received single dose oral concentrations of the total aqueous extract of the leaves of *A. precatorius* (2.5; 40; 60 mg / mL) against the negative control (distilled water) and the positive control (glibenclamide at 0.25 mL / mg). Samples are taken every 30 min for 2 hours [17].

## **Effect of effective dose of the extract on glucose tolerance in normal glycaemic rats**

The test was carried out in the animal laboratory of the Biological Engineering Laboratory Functional Pathology Team of the FST Béni Mellal Sultan Moulay Slimane University (Morocco).

The rats were divided into 3 batches of 6 and then pretreated in a single dose (2 ml / 100 g b.w.) of the total aqueous extract at the effective dose (DEF) against the controls. 30 min after the administration of the test substances, the rats of each group receive the solution of glucose 30% (2ml / 100 g b.w.) orally. The evolution of blood glucose was monitored every 30 minutes for 2 hours [17].

## **Statistical Analysis**

The software used for this study is Graph Pad Prism (version 5.01, Software, USA).

The results are presented as mean  $\pm$  standard deviation (SD). Analysis of variance (ANOVA) with repeated measures was employed to compare the results according to the administered doses and times of treatment. Analysis of variance was considered significant when the level of probability (p) was  $<0.05$ ; if  $p < 0.01$ , this difference is considered as very significant; if highly significant  $P < 0.001$ .

## **Resultats**

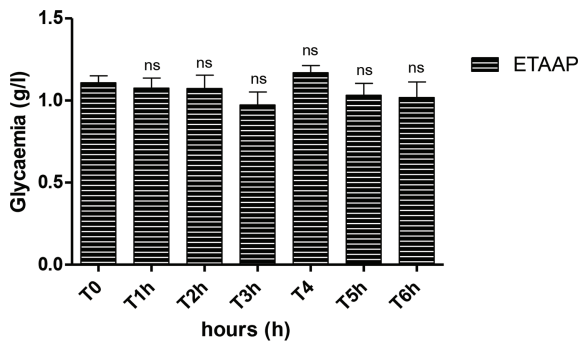
### **Phytochemical screening**

The results revealed that the aqueous extract contained 9 groups of chemical compounds: alkaloids, tannins, flavonoids (flavones), saponins, quinone compounds (coumarins), sterols and triterpenes and reducing compounds [14].

### **Experimental study on diabetes**

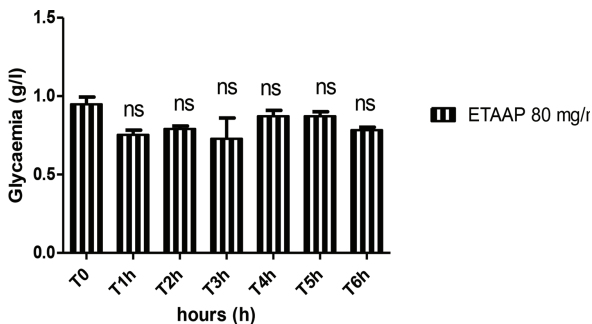
#### **Decrease of the basic glycemia**

The extract (40 mg / ml ETAAP) did not cause a significant decrease ( $P > 0.05$ ) of basal blood glucose during the 6-hour period, from  $1.10 \pm 0.10$  to  $1.02 \pm 0.23$  is a decrease of 7.27% (figure 1). In the rats treated with the extract (ETAAP 60 mg / mL), at the end of the 6-hour period, a very significant decrease ( $P < 0.001$ ) of the order of 27% is recorded, the blood glucose level goes from  $0.93 \pm 0.13$  of  $0.68 \pm 0.05$  g / L (figure 2). The results show that in rats treated at a single dose of the extract (ETAAP 80 mg / ml), the basal glucose level decreases insignificantly ( $P > 0.05$ ) at the end of the 6 hours, it goes from  $0.95 \pm 0.11$  to  $0.78 \pm 0.04$  g / l or a 17.89% (figure 3). In the extract-treated rats (100 mg / mL ETAAP), basal blood glucose significantly decreased ( $P < 0.01$ ) from 2 hours and remained constant at the end of the 6-hour period. a blood glucose level of  $1.31 \pm 0.16$   $0.74 \pm 0.34$  g / L, a decrease of 43.51% after 6 hours (figure 4; 5; 6).



**Figure 1 :** Evolution of glycaemia during 6 hours in normal glycaemic rats after treatment of extract (ETAAP 40 mg / ml)

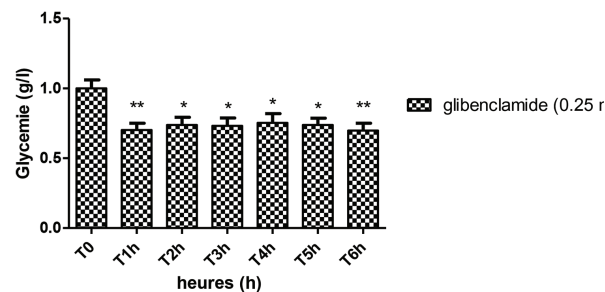
Dunett Multiple Comparison Test ; ns  $P > 0.05$ : not significant versus basic glycaemia at T0 (n = 6)



**Figure 3 :** Evolution of glycaemia during 6 hours in normal glycaemic rats after treatment of extract (ETAAP 80 mg/ml).

Dunett Multiple Comparison Test

ns  $P > 0.05$ : not significant versus basic glycaemia at T0 (n = 6)

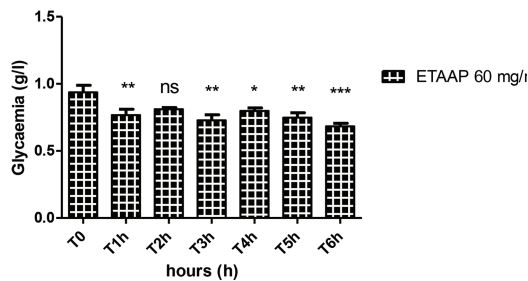


**Figure 5 :** Evolution of glycaemia during 6 hours in normal glycaemic rats, after treatment with the reference product Glibenclamide (0.25 mg/ml)

Dunett Multiple Comparison Test

\*  $P < 0.05$ : significant versus basic glycaemia at T0 (n = 6)

\*\*  $P < 0.01$ : significant versus basic glycaemia at T0

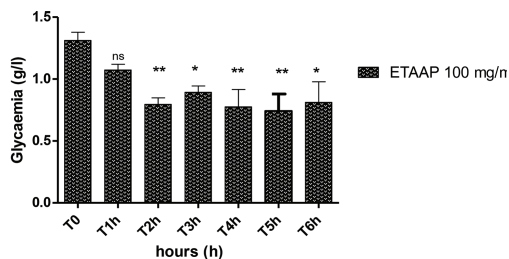


**Figure 2 :** Evolution of glycaemia during 6 hours in normal glycaemic rats after treatment of extract (ETAAP 60 mg/ml)

Dunett Multiple Comparison Test

ns  $P > 0.05$ : not significant versus basic glycaemia at T0 (n = 6)

\*  $P < 0.05$ : significant versus basic glycaemia at T0



**Figure 4 :** Evolution of glycaemia during 6 hours in normal glycaemic rats after treatment of extract (ETAAP 100 mg/ml)

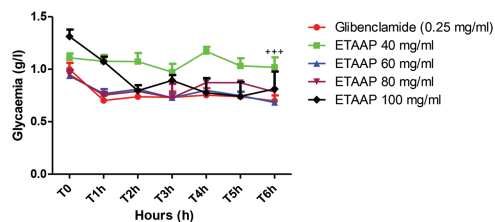
Dunett Multiple Comparison Test

ns  $P > 0.05$ : not significant versus basic glycaemia at T0 (n = 6)

\*  $P < 0.05$ : significant versus basic glycaemia at T0

\*\*  $P < 0.01$ : significant versus basic glycaemia at T0

\*\*\*  $P < 0.001$ : significant versus basic glycaemia at T0



**Figure 6 :** Comparative evolution of the glycaemia test groups at different concentrations of aqueous extract compared to the reference product (glibenclamide at 0.25 mg / ml)

Dunett multiple comparison Test

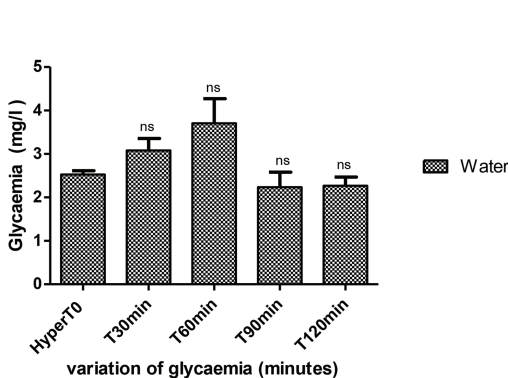
+++  $P < 0.001$  significant versus to reference product (glibenclamide 0.25 mg/ml)

ns  $P > 0.05$  not significant versus to de reference product (glibenclamide 0.25 mg/ml)

## Reduction of induced hyperglycaemia

The results obtained show a reduction in induced hyperglycaemia as a function of the doses extract (2.5; 40; 60 mg / ml). This effect on hyperglycemia is different from that of water (figure 7). At the lowest concentration of the extract (ETAAP 2.5 mg / ml), a no significant reduction ( $P > 0.05$ ) in hyperglycaemia was recorded one hour after the induction of hyperglycaemia. (figure 8). Basic blood glucose pass from  $0.95 \pm 0,05$  to  $2.70 \pm 1$  g/l 30 minutes after induction of hyperglycaemia. From 60 minutes, hyperglycaemia decreases insignificantly ( $P > 0.05$ ) every 30 minutes during the experimental period. At the end of 2 hours, the basic hyperglycaemia increases to  $1.85 \pm 0.55$  g / l, a non significant reduction of 31.48%. In the batch treated with the extract (ETAAP 40 mg / ml), the basal blood glucose level increases from  $0.80 \pm 0.16$  to  $1.57 \pm 0.25$  g / l 30 minutes after induction of hyperglycaemia. (figure 9). No significant reduction ( $P > 0.05$ ) of basic hyperglycaemia at T0 is recorded every 30 minutes for 1 hour. After 2 hours, the reduction becomes significant ( $P < 0.001$ ) with a blood glucose level of  $1.22 \pm 0.008$ , a significant decrease of 22.29%. These results show that the total extract (ETAAP 40 mg / ml) causes a no significant reduction in hyperglycaemia induced 30 minutes after oral administration to rats and this variation is maintained every minute during 1 hour. In a test batch (ETAAP 60 mg / ml), the basic blood glucose level is  $0.88 \pm 0.11$  to  $1.57 \pm 0.25$  g / l, 30 minutes after oral 30% glucose administration (figure 10). No significant ( $P > 0.05$ ) increase in induced hyperglycemia is recorded every 30 minutes for 1 hour after oral administration of extract (ETAAP 60 mg / ml). At the end of the 2 hours, a not significant increase ( $P > 0.05$ ) of the hyperglycaemia is recorded, it passes to  $1.73 \pm 0.20$  or an increase of 10% compared to the basic hyperglycaemia T0. The extract (ETAAP 40 mg / ml) showed an action on induced hyperglycaemia comparable to glibenclamide at 0.25 mg / ml (figure 11 and figure 12).

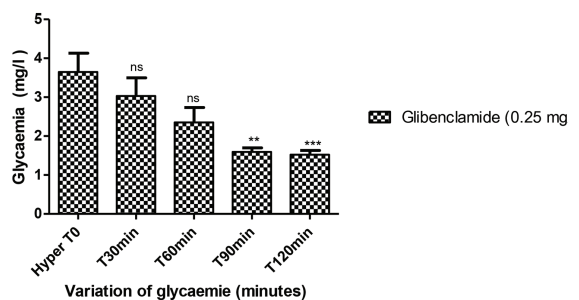
The 40 mg / ml extract was considered as the effective dose of aqueous extract of *Abrus precatorius*'s leaves which could promote good regulation of blood glucose levels in both normal and normal rats in hyperglycemia induced by oral.



**Figure 7 :** Evolution of hyperglycemia induced in normal glycaemic rats after administration of the vehicle (water)

Dunett Multiple Comparison Test

ns  $P > 0.05$  not significant versus to basic hyperglycaemia at T0 ( $n = 6$ )



**Figure 8 :** Evolution of hyperglycemia induced in normal glycaemic rats after administration of Glibenclamide 0.25 mg/ml

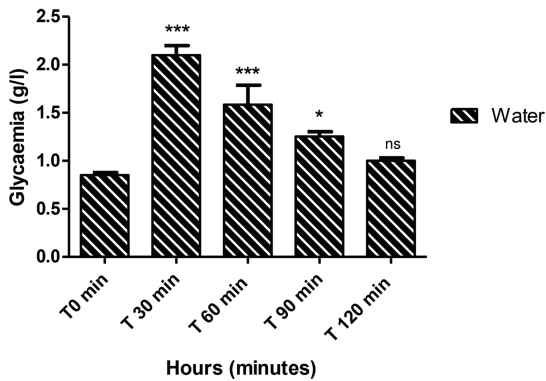
Dunett Multiple Comparison Test

ns  $P > 0.05$  not significant versus to basic hyperglycaemia at T0 ( $n = 6$ )

\*\*  $P < 0.01$ : significant versus to basic hyperglycaemia at T0

\*\*\*  $P < 0.001$ : significant versus basic hyperglycaemia at T0





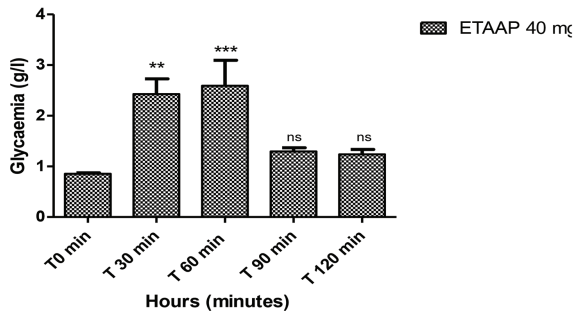
**Figure 13 :** Evolution of glycaemia every 30 minutes for 2 hours after induction of oral hyperglycaemia in normal rats pretreated by control (water)

Dunett Multiple Comparison Test

ns  $P > 0,05$ : not significant versus basic glycaemia at T0 (n = 6)

\* $P < 0,05$ : significant versus basic glycaemia at T0

\*\*\* $P < 0,001$ : significant versus basic glycaemia at T0



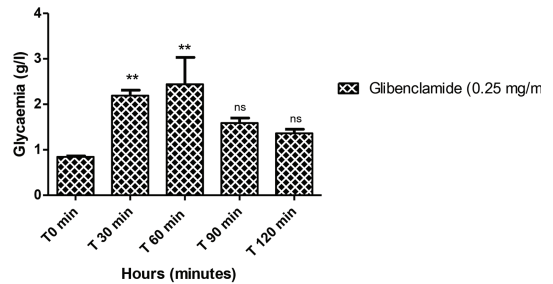
**Figure 15 :** Evolution of glycaemia every 30 minutes for 2 hours after induction of oral hyperglycaemia in normal rats pretreated by extract (ETAAP 40 mg/ml)

Dunett Multiple Comparison Test

ns  $P > 0,05$ : not significant versus basic glycaemia at T0 (n = 6)

\* $P < 0,01$ : significance versus basic glycaemia at T0

\*\*\* $P < 0,001$ : significance versus basic glycaemia at T0



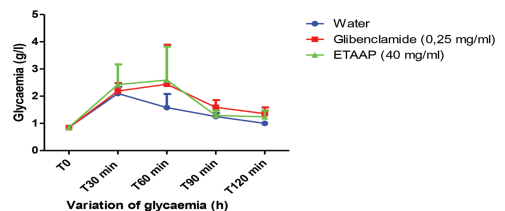
**Figure 14 :** Evolution of glycaemia every 30 minutes for 2 hours after induction of oral hyperglycaemia in normal rats pretreated by glibenclamide 0.25 mg/ml)

Dunett Multiple Comparison Test

ns  $P > 0,05$ : not significant versus basic glycaemia at T0 (n = 6)

\*\* $P < 0,01$ : significance versus basic glycaemia at T0

\*\*\* $P < 0,001$ : significance versus basic glycaemia at T0



**Figure 16 :** Comparative evolution of glycaemia every 30 minutes for 2 hours in normal glycaemic rats pretreated by gavage according to the test solutions after induction of oral hyperglycemia (HPGO)

Test comparaison multiple des groupes par pair de Newman-Keuls ( $\alpha = 0,05$ )

## Discussion

Phytochemical analysis of the total aqueous extract of the leaves of *Abrus precatorius* revealed the presence of saponosides, quinone compounds (coumarin), sterols, triterpenes, tannins and alkaloids. The presence of these chemical groups in the total aqueous extract of the leaves of *Abrus precatorius* may be responsible for its proven antidiabetic effect.

These same compounds have been found in the aqueous extracts of leaves of some plants used in traditional medicine in the treatment of diabetes. Indeed, saponosides, quinone compounds and alkaloids could be responsible for the antidiabetic properties attributed to the plant *Mitragyna inermis* [19]. Alkaloids, sterols or triterpenes may be responsible for the antidiabetic properties attributed to the plant *Terminalia catappa* [2; 20]. Investigation of the hypoglycaemic effect of

the total aqueous extract of the leaves of *Abrus precatorius* at different concentrations in normal short-term oral glucose-producing rats (6 hours) showed a dose dependent decrease in blood glucose levels. This dose-dependent decrease is probably due to the low or strong presence of natural chemical compounds in the range of prepared concentrations.

The marked decrease in blood glucose recorded with this reference product (Glibenclamide 0.25 mg / mL) is close to that obtained with the extract at high concentrations (60, 80, 100 mg / mL). The lower concentration of the extract (40 mg / ml ETAAP) causes a small decrease in blood glucose. This variation is different from that recorded by glibenclamide 0.25 mg / ml. These results are in agreement with those of Ndomou *et al.*, [20], whose work has shown that the methanolic, hexane and acetal extracts of the leaves of *Gnetum africanum* and *Gnetum bulchozianum* induce hypoglycemia in normal glycemic rats (180 mg). / Kg pc, per os). The reduction of hyperglycemia induced at different concentrations of the aqueous extract of leaves of *A. precatorius* is the probable result of antihyperglycaemic effect of the active ingredients contained in the plant, in particular flavonoids, sterols and glycosides (reducing compound). In the literature, these compounds present in figs are recognized for their antidiabetic effect [16]. However, the time taken by the extract to act on the hyperglycemia induced may be due to the level of presence of the active ingredients in the extract as a function of the concentrations prepared. The 40 mg / mL extract showed an action on induced hyperglycemia comparable to Glibenclamide at 0.25 mg / mL. The results obtained are in agreement with those of N'Guessan *et al.*, [2], who showed that the aqueous extract of *Terminalia catappa* leaves at 40 mg / ml reduces the oral hyperglycemia caused in rabbits. normal in HPGO compared to Glibenclamide 0.25 mg / ml. For these authors, Glibenclamide 0.25 mg / ml causes significant hypoglycemia in normal glycemic rabbits in contrast to the 40 mg / ml extract. The same results were observed with the total aqueous extract of the leaves of *Abrus precatorius* at 40 mg / ml on the glycemia of normal oral glycemic rats. Thus, the 40 mg / ml extract was considered as the effective dose of the total aqueous extract of *A. precatorius* leaves which could promote good regulation of blood glucose levels in both normal and normal HPGO rats. Indeed, the aqueous extract of the seeds of *Abrus precatorius* exerts a dose-dependent hypoglycemic effect in models of diabetic rats [21]. Vadivel *et al.*, [12] showed that the methanolic extract of the seeds of *Abrus precatorius* regulates glycaemia by the inhibition of the enzymes  $\alpha$ -amylase and  $\alpha$ -glucosidase as well as by its antioxidant effects. Inhibitors of  $\alpha$ -amylase enzymes that are used to modify or control the activity of this enzyme have medical applications such as the regulation of blood glucose [22]. According to the results obtained with the total aqueous extract of the leaves of *Abrus precatorius* and the bibliographic data on the plant, its use in the treatment of diabetes in traditional medicine seems to be justified.

## Conclusion

The total aqueous extract of the leaves of *Abrus precatorius* (ETAAP) has a potential *in vivo* oral antidiabetic effect in laboratory rats. This effect is dose dependent and also depends on the chemical composition of the extract. The effective dose (40 mg / ml) determined proved to be a good regulator of blood glucose. All these results show that the *Abrus precatorius* plant could be a good alternative in the treatment of diabetes. However, additional studies must be carried out on this plant in order to know the mechanism of action of its antidiabetic effect. Also identify and isolate the molecules that would be responsible for this effect in order to set up a very effective phytomedicine for the treatment of diabetes.

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## Availability of data and materials

All data and materials of this work are available from the corresponding author on request.

### Authors' contributions

ML performed study design under the supervision of NBYF; CB; GNZ and AC. He carried out the collection of the medicinal plant in collaboration with SML. ML performed the extraction and the phytochemical screening under the supervision of MK and AH. ML performed the antidiabetic under the supervision of FCH and MN, carried out collection and interpretation of the data, literature search and wrote the manuscript.

## Ethics approval and consent to participate

### Animal experimentation

During this period, animals have free access to food (pellets) and water and are kept in a pet shop at constant temperature ( $22 \pm 2$  °C), subjected to a light / dark cycle of 12 / 12h. The dark phase of this cycle begins at 12h and the different experiments always take place from 13h to 18h because of the nocturnal activity of the animal (active phase). Before the experiments, the animals were acclimatized 2 weeks in the pet shops to harmonize their physiological state.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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