



Effect of An Aqueous Extract of Leaves, Flowers and Pods Of *Crotalaria Retusa* (Fabaceae) On Blood Glucose Levels In Streptozotocin-Induced Diabetic Rats

Goh Bi Lané Adrien^{1*}, DJIKE Kouehiouon justin Anderson-Bel², TOTO Kouamé Noel³, ZAHOUI Ouga Stanislas³, Néné Bi Semi Anthelme³

1. Peleforo Gon Coulibaly University, Laboratory of Environment, Climate, Health, Engineering and Development, (Côte d'Ivoire), BP 1328Korhogo
2. Alassane Ouattara University, UFR Science and Technology, Laboratory of Animal Biological Sciences, Bouaké, Côte d'Ivoire
3. Felix Houphouet-Boigny University, UFR Biosciences, Laboratory of Biology and Health, Abidjan, Côte d'Ivoire

ABSTRACT

The aim of this study was to evaluate the pharmacological effects of an aqueous extract of leaves, flowers and pods of *Crotalaria retusa* (Fabaceae) on the blood glucose levels of streptozotocin-induced diabetic rats. Qualitative phytochemical screening and pharmacological tests were carried out. The animals were made diabetic by injection of a 65 mg/kg BW dose of streptozotocin followed fifteen minutes later by an injection of nicotinamide at a dose of 230 mg/kg BW. Four groups of six (6) diabetic rats and one group of six normoglycemic rats were formed. The normoglycemic rats in Group 1 and the diabetic rats in Group 2 received distilled water, while the diabetic rats in Groups 3, 4, and 5 received daily gavage for 84 days of glibenclamide at 10 mg/kg body weight (BW) and aqueous extracts of *Crotalaria retusa* leaves, flowers, and pods at 600 and 1000 mg/kg BW. Blood glucose levels were measured every two weeks. The phytochemical test revealed the presence of polyphenols, flavonoids, catechin and gallic tannins, quinonic compounds, alkaloids, sterols and polyterpenes. However, saponins were not detected in the aqueous extract of *Crotalaria retusa*. Administration of the aqueous extract of *Crotalaria retusa* at doses of 1000 and 600 mg/kg body weight resulted in a significant decrease in blood glucose levels in diabetic rats from day 28 until the end of treatment (84 days). The aqueous extract of *Crotalaria retusa* appears to have antidiabetic properties. These results would justify the use of this plant in traditional medicine for the treatment of diabetes.

Keywords: Diabetes, *Crotalaria retusa*, streptozotocin, blood glucose.

*Corresponding Author Name: Goh Bi Lané Adrien
Received 10 May 2026, Accepted 19 June 2026

INTRODUCTION

Diabetes mellitus is an endocrine and metabolic disorder that poses a public health problem. This disease is a major cause of blindness, kidney failure, heart attacks, strokes, and lower limb amputations (1). It affects approximately 2% of the world's population, according to the International Diabetes Federation (IDF). The number of deaths attributable to diabetes is 3.2 million per year and could increase by 50% in the next ten (10) years (2). Furthermore, its global prevalence is growing by 35% (3). In Côte d'Ivoire, diabetes is rapidly increasing, with a prevalence rate of 4% (4). This public health scourge has prompted widespread mobilization (government, non-governmental organizations, and associations). Consequently, localized strategies are being implemented at the national level for patient care by general practitioners, while severe cases are referred to specialized healthcare facilities (university hospitals and specialized institutes) for effective treatment (5). While global goals aimed at improving the lives of people with diabetes are a major victory, a significant proportion of those affected lack access to quality medications, technologies, and care (6). This situation motivates the search for new, low-cost, active, and effective molecules to treat this disease. Medicinal plants are emerging as a promising alternative for developing improved traditional medicines or isolating molecules with therapeutic potential. It is with this in mind that we undertook to study the effects of an aqueous extract of *Crotalaria retusa* (Fabaceae), a plant renowned for its antidiabetic properties in African pharmacopoeia.

The aim of this study was to evaluate the effects of an aqueous extract of *Crotalaria retusa* (Fabaceae) on blood glucose levels in rats induced by streptozotocin administration, with the aim of contributing to the development of this plant's potential in the treatment of diabetes.

MATERIALS AND METHOD

Plant Material

The plant material consists of leaves, flowers, and pods of *Crotalaria retusa* L. (Fabaceae). The plant was collected in September 2018 in Sénoufla, in the sub-prefecture of Vavoua, in the west-central region of Côte d'Ivoire. This plant was identified and authenticated at the National Floristic Center (CNF) of the Félix Houphouët-Boigny University (Abidjan, Côte d'Ivoire) by Mr. Assi Jean, using herbarium specimens numbered 15133 of February 22, 1980, 51 of February 15, 1991, 4258 of November 18, 1974, and 11022 of January 6, 1970, from the same center.

Animal Material

Male and female Wistar rats, aged 8 to 16 weeks and weighing between 140 and 150 g, were used. These animals came from the animal facility of the Pharmacy Training Unit (UFR) at Félix Houphouët-Boigny University (Abidjan, Côte d'Ivoire). The rats were reared in a room at ambient temperature with a natural light-dark cycle. They were housed in rectangular plastic cages, with 3 to 4 rats per cage, and had free access to food and water.

Preparation of the aqueous extract of leaves, flowers, and pods of *Crotalaria retusa* (EACr)

For the preparation of the aqueous extract of *Crotalaria retusa* L. (Fabaceae), the different organs (leaves, flowers, and pods) were cleaned, then dried in the shade, and subsequently finely ground in a mechanical ball mill at the laboratory of the Pharmacy Training Unit of Félix Houphouët-Boigny University (Abidjan, Côte d'Ivoire). Two hundred and fifty grams (250 g) of the ground material were placed in 5 liters of distilled water. The mixture was macerated for twenty-four (24) hours under magnetic stirring, and the macerate was filtered through absorbent cotton and Wattman number 2 filter paper. The filtrate is dried in an oven at 50°C for 72 hours. This yields a brown powder (48 g) which constitutes the aqueous extract of *Crotalaria retusa* (EACr). This extract is administered daily at different doses by gavage to animals in order to determine its pharmacological effects.

Identification of Some Secondary Metabolites of *Crotalaria retusa*

Phytochemical screening allows for the identification, through color and precipitation reactions, of the presence or absence of various families of secondary metabolites such as alkaloids, polyphenols, tannins, flavonoids, saponins, polyterpenes, and sterols in the aqueous extract of *Crotalaria retusa* (EACr). This screening was performed according to the analytical techniques described in the works of (8,9,10).

Chemical and Pharmacological Products

Streptozotocin (Sigma-Aldrich, Germany) is a diabetogen whose intraperitoneal administration destroys the beta cells of the islets of Langerhans and induces experimental diabetes. Nicotinamide (Sigma-Aldrich®, USA) is administered fifteen minutes after the streptozotocin injection to stabilize the lesions. Glibenclamide (Sanofi Aventis, France) is an antidiabetic drug (hypoglycemic sulfonylurea) that stimulates insulin secretion by the pancreas.

Technique for inducing diabetes by streptozotocin (STZ) injection in Wistar rats

Type 2 diabetes was induced according to the protocol of (11) with a slight modification. After sixteen (16) hours of fasting, each rat received an injection of freshly prepared streptozotocin (STZ) in a citrate buffer solution (pH = 4.5) between the ears at a dose of 65 mg/kg BW in a proportion of 0.1 ml/kg BW. Fifteen (15) minutes after STZ injection, all rats received an

injection of nicotinamide at a dose of 230 mg/kg body weight between the ears in the same proportions to stabilize the lesions. Five percent glucose water was then made available to the animals for twenty-four (24) hours. Seven (7) days after STZ administration, the rats' blood glucose levels were measured. Rats with blood glucose levels above 200 mg/dL and exhibiting clinical signs such as polyuria, polydipsia, and polyphagia were considered to have type 2 diabetes and were included in our study.

Evaluation of the effects of the aqueous extract of *Crotalaria retusa* leaves, flowers, and pods on blood glucose levels in diabetic rats

Blood glucose measurement

The blood glucose levels of the diabetic rats were measured using an Accu-Chek Active glucometer and test strips (Roche Diagnostics, Germany). In this study, the rats were fasted for 16 hours prior to the experiments. The substances to be tested were administered orally.

Experimental Protocol

For this study, twenty (20) Wistar rats were divided into 5 groups. The average weight of each group was determined. The rats were divided as follows:

- Group 1 consisted of normoglycemic control rats that received distilled water.
- Group 2 consisted of diabetic control rats that received distilled water.
- Group 3 consisted of diabetic rats treated with glibenclamide at a dose of 10 mg/kg BW.
- Groups 4 and 5 consisted of diabetic rats treated with 600 mg/kg BW and 1000 mg/kg BW of EACr, respectively.

The rats' blood glucose levels were measured every two weeks for eighty-four (84) days.

Statistical Analysis Methods and Data Processing

Data analysis was performed using GraphPadInstat software (San Diego, CA, USA). Results are presented as the mean followed by the standard error of the mean ($M \pm ESM$). The difference between two values was determined using the Student-Newman-Keuls test and was considered statistically significant for $p < 0.05$. GraphPadPrism software (San Diego, CA, USA) was used to plot the graphs.

RESULTS AND DISCUSSION

Phytochemical Compounds of an Aqueous Extract of *Crotalaria retusa* L. (Fabaceae)

The phytochemical screening revealed several groups of chemical compounds in the aqueous extract of *Crotalaria retusa* L. (EACr). Specifically, this study detected polyphenols, flavonoids, catechin and gallic tannins, quinone compounds, alkaloids, sterols, and polyterpenes. However,

saponins were not detected in the aqueous extract of *Crotalaria retusa*. The results of this study are presented in Table I.

Table 1: Phytochemical compounds of the aqueous extract of *Crotalaria retusa* (EACr)

Compounds of interest	Reactions/Reagents	Results
Polyphenols	Reaction to ferric chloride	+
Alkaloids	Dragendorff and Bouchardat reagent	+
Flavonoids	Cyanidin reaction	+
Saponins	Vigorous agitation	-
Catechiol tannins	Stiasny reagent	+
Gallic tannins	Hydrochloric acid reagent	+
Quinonic compounds	Borntraeger's reaction	+
Sterols and Polyterpenes	Liebermann-Bouchard's reaction	+

+ Presence of the target compound

- Absence of the target compound

Effects of the aqueous extract of *Crotalaria retusa* (EACr) on blood glucose levels in diabetic rats.

The mean blood glucose level in each group was determined before the start of the experiment. It was 80 ± 1.47 mg/dL in untreated healthy control rats and 288 ± 5.05 mg/dL in all other animals in the other groups, in which diabetes was induced by streptozotocin. After eighty-four (84) days of the experiment, blood glucose levels in normoglycemic control rats and positive control rats (untreated diabetic rats) did not change significantly ($p > 0.05$). EACr, administered at doses of 600 and 1000 mg/kg body weight, modifies blood glucose levels in diabetic rats. For both doses, EACr lowers ($p > 0.05$) blood glucose levels from day 14 of treatment compared to that of untreated diabetic rats, and this decrease becomes significant ($p < 0.01$) from day 28 until the end of the experiment, where blood glucose levels decrease from 281.8 ± 10.85 to 94.75 ± 3.25 mg/dl and from 257 ± 19.93 to 82 ± 4.98 mg/dl, respectively, for the 600 and 1000 mg/kg body weight doses, with respective reduction rates of 68.72% and 71.52%.

In this reduction, the 1000 mg/kg body weight dose of EACr allowed treated rats to recover baseline blood glucose levels comparable to those of normoglycemic control rats. Similarly, glibenclamide administration resulted in a significant decrease ($p < 0.01$) in blood glucose levels in treated diabetic rats compared to untreated diabetic rats. After 12 weeks of treatment, blood glucose levels in these animals were only 78.75 ± 6.21 mg/dL, representing a 73.06% reduction. During this experiment, the effect of the reference substance was slightly more pronounced than that of the aqueous extract of *Crotalaria retusa* (Figure 1).

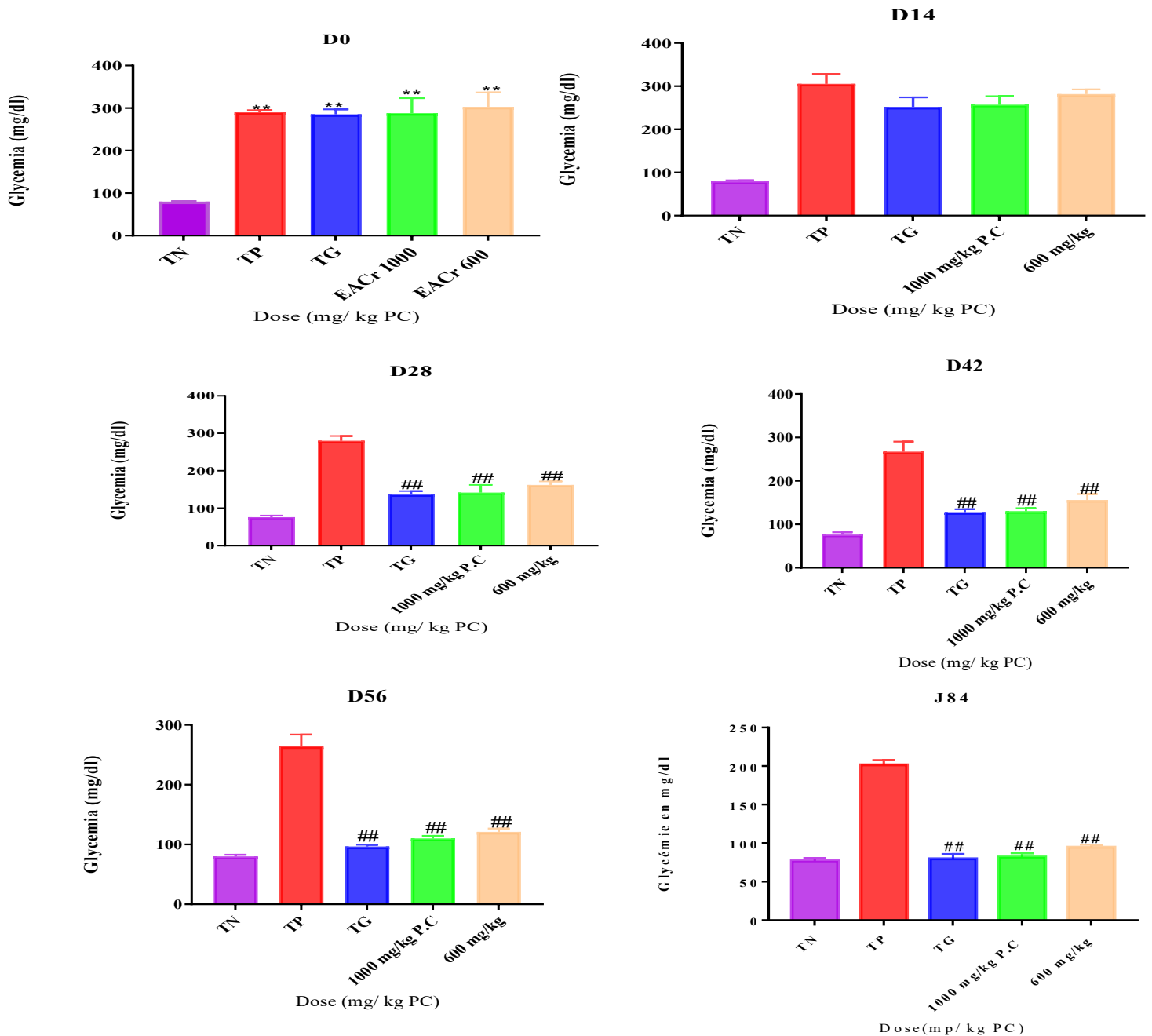


Figure 1: Effects of aqueous extract of *Crotalaria retusa*, at doses of 600 and 1000 mg/kg bw, and of Glibenclamide, on blood glucose levels in diabetic rats.

Day 0: Start of the experiment on diabetic rats; Day 14: After two weeks of treatment; Day 28: After four weeks of treatment; Day 42: After six weeks of treatment; Day 56: After eight weeks of treatment; Day 84: After twelve weeks of treatment. Results are expressed as mean \pm SEM (n = 5).

DISCUSSION

The qualitative characterization of the aqueous extract of *Crotalaria retusa* L. (Fabaceae) revealed the presence of certain chemical compounds such as alkaloids, sterols and polyterpenes, flavonoids, quinones, polyphenols, and catecholic and gallic tannins in this plant. However, saponins were not detected in this extract. These results are consistent with those obtained by (12), but with a slight difference.

Indeed, the results of phytochemical screening performed on the same plant by this author revealed the presence of saponins. This difference could be explained by the natural ecological environments in which the plant was harvested and also by the equipment used for the test. According to (13), a variation in secondary metabolites could be observed in the same plant due to the different habitats of that plant. Streptozotocin (STZ) is currently the most widely used diabetogenic agent in animal research for new antidiabetic drugs (14). Its injection into test rats induced fasting hyperglycemia, thus characterizing their diabetic state. This hyperglycemia, approximately 288 ± 5.05 mg/dL, persisted, indicating the development of experimental diabetes following pancreatic beta cell necrosis. This hyperglycemia persisted throughout the eighty-four (84) days of the experiment in untreated diabetic rats. However, when the diabetic rats were treated with EACr (at doses of 600 and 1000 mg/kg BW) or with glibenclamide, the hyperglycemia decreased significantly, and blood glucose levels tended to return to normal. This observed hyperglycemia gradually decreased and became highly significant from the 4th week onward compared to untreated diabetic rats, stabilizing until the end of the experiment, at which point baseline blood glucose levels were restored in rats treated with 1000 mg/kg BW of EACr and glibenclamide (10^{-2} g/kg BW). The same effect was observed by (15) and (16). These authors reported that aqueous extracts of *Sclerocarya birrea* and *Pseudarthria hookeri*, respectively, decreased blood glucose levels with a significant increase in insulin levels in diabetic rats. We can deduce that EACr, like glibenclamide, possesses antidiabetic properties due to its chemical composition. The presence of these compounds in EACr could explain the favorable pharmacological profile of this plant species. Indeed, flavonoids prevent diabetes by inhibiting alkalose reductase, according to the work of (17) and (18). As for tannins, their antidiabetic effect is manifested at the cellular level, by promoting insulin action, and on diabetic complications through their antioxidant and anti-enzymatic properties. They neutralize the effect of free radicals and limit the inflammatory response in various tissues (19).

CONCLUSION

The aqueous extract of *Crotalaria retusa* leaves, flowers, and pods contains several chemical compounds with pharmacological properties. It significantly lowers hyperglycemia in streptozotocin-induced diabetic rats. This property makes the aqueous extract of *Crotalaria retusa* a potentially active substance in the treatment of diabetes.

REFERENCES

1. Coulibaly O. Etudier la dyslipidémie au cours du diabète de type 2 au laboratoire d'analyse biomédicale de l'hôpital de Sikasso Thèse de Doctorat d'Etat en Pharmacie, Université des Sciences, des techniques et des Technologies de Bamako (Mali). 2020 ; 76 p.
2. Benm'hamed abdelhalim. Pharmaco-épidémiologie du diabète sucre chez les bénéficiaires de la caisse nationale des assurances sociales des travailleurs salariés «cnas» durant la période allant de 2010 à 2014. Thèse de Doctorat en Science médicale, Université Saad Dahled Blida faculté de médecine Algérie 2018 167p.
3. Deruelle P. Obésité et grossesse. Gynécologie obstétrique & fertilité. 2011 ; 39(2) : 100-105.
4. Abodo J, Wafo M, Koffi-Dago P, Yao A, Hué A, Danho J, Lokrou A. Caractéristiques gérontologiques des patients diabétiques hospitalisés en Côte d'Ivoire. Médecine des Maladies Métaboliques. 2019 ; 13(7) : 632-636.
5. Adoueni KV, Ankotche A, Azoh A, Sibailly P, Derbe A, Sanogo M, Kouakou E. P88 Prise en charge du patient diabétique en Côte d'Ivoire sur le mode de la décentralisation. Diabetes & Metabolis. 2012 ; 38 : A52.
6. Van der Sluijs MT, Schroer-Joosten DP, Fid-Fourkour A, Vrijenhoek M P, Debyser I, Moulin V, De Smit AJ. Transplacental transmission of bluetongue virus serotype 1 and serotype 8 in sheep: virological and pathological findings. PLoS ONE. 2013 ; 8(12) : e81429.
7. Bagre I, Bahi C, Gnahoue G, Djaman AJ, Guede GF. Composition phytochimique et évaluation in vitro de l'activité antifongique des extraits des feuilles de *Morinda morindoides* (Baker) *milne-redhead* (Rubiaceae) sur *Aspergillus fumigatus* et *Candida albicans*. Journal of Pharmaceutical and Biological Sciences. 2007 ; 8 (1) : 15-23.
8. Néné Bi SA, Traore F, Zahoui OS, Soro TY. Composition chimique d'un extrait aqueux de *Bridelia ferruginea benth.* (Euphorbiaceae) et études de ses effets toxicologiques et pharmacologiques chez les mammifères. Afrique Science. 2008 ; 04 (2) : 287-305.
9. ABO KJC. De la plante à la molécule : toxicité, effets pharmacologiques et mécanisme d'action de *Justicia secunda* (Acanthaceae), plante antihypertensive, sur le système cardio-

- vasculaire de Mammifères. Thèse de Doctorat d'Etat ès Sciences Naturelles, Université Félix Houphouët-Boigny (Abidjan, Côte d'Ivoire). 2013 ; 351 p.
10. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, Novelli M, Ribes G. Development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes*. 1998; 47: 224-229.
 11. Mibindzou MA. Screening phytochimique de deux espèces de plantes : *Crotalaria retusa* L (Papilionaceae) *Ethallea ciliata* Aubrev & *Pellegr* (Rubiaceae) récoltées au Gabon. Thèse de Doctorat de Faculté de Médecine de Pharmacie et d'Odonto-Stomatolog, Université de Bamako, Mali. 2004 ; 54 p.
 12. Sofowora A. Plantes médicinales et médecine traditionnelle d'Afrique. Académique Suisse des Sciences Naturelles et édition Kharthala, co-édit., Berne Paris, France. 1996 ; 378 p.
 13. Qinna NA, Badwan AA. Impact of streptozotocin on altering normal glucose homeostasis during insulin testing in diabetic rats compared to normoglycemic rats. *Drug Design, Development and Therapy*. (2015); 5(9): 2515-2525.
 14. Djike KJAB, Koffi C, Zaoui OS, Néné Bi SA, Traore F. Évaluation des propriétés antidiabétiques de l'extrait aqueux d'écorces de tronc de *Sclerocarya birrea* (anacardiaceae) et de sa fraction cyclohexanique chez des rats wistar diabétiques de type 2. *International Journal of Advanced and Research*. 2022; 10(06): 712-721.
 15. Kahou BGP, Abo KJ C, Irié BJS. Effet d'un extrait aqueux de *Pseudarthria hookeri* Wight & Arn. (Fabaceae) sur la glycémie et sur la libération et le stockage du glucose hépatique de rats diabétiques. *European Scientific Journal*. 2016; 12 (6): pp: 1857 – 7881.
 16. Pietta P. Flavonoids as Antioxidants. *Journal of Natural Products*. 2000; 63 (7): 1035-1042.
 17. Hollman PCH. Evidence for health benefits of plant phenols: local or systemic effects *Journal of the Science of Food and Agriculture*. 2001, 81 (9): 842-852.
 18. Mc Calley DV. Analysis of the cinchona alkaloids by high-performance liquid chromatography and other separation technical. *Review Journal of Chromatography*. 2002; 967: 1-19.

