International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 33 No. 2 Jul. 2021, pp. 426-434

http://www.ijias.issr-journals.org/

Triphytochemistry and effects of aqueous and hydro-ethanolic extracts of Spathodea campanulata P.beauv. (Bignoniaceae) on blood-sugar level and markers of pancreatitis in type 2 diabetic rats

Koulaï Diane[1], Doumbia Idrissa[1-2], Yeo Sounta Oumar[1], M'boh Gervais Mélaine[3], Kipré Gueyraud Rolland[2], and Djaman Allico Joseph[2-3]

[1]Food and Bioproducts Processes Laboratory, Agronomic, Forestry and Environmental Engineering Department, University of Man, PO Box 20 Man, Côte d'Ivoire

[2]Biochemical Pharmacodynamic Laboratory, Biosciences Department, Felix Houphouet-Boigny University, PO Box 582, Abidjan 22, Côte d'Ivoire

[3]Biochemical Laboratory of Pasteur Institute of Ivory Coast, PO Box 490, Abidjan 01, Côte d'Ivoire

ABSTRACT: The objective of this study was to perform triphytochemistry and to evaluate the effect of aqueous (AqE) and hydroethanol (EthE) extracts of Spathodeae campanulata on blood glucose and pancreatitis markers in streptozotocin (STZ) - induced diabetic male rats. S. campanulata P. beauv, is a plant belonging to the Bignoniaceae family which is traditionally used for the treatment of diabetes, malaria and schistosomiasis. In a first step, we performed triphytochemistry of the extracts which showed that the aqueous and hydroethanolic extracts of the barks are rich in polyphenols, flavonoids, saponosides, alkaloids, sterol-polyterpenes, tannins but relatively poor in quinones. In a second phase, this study consisted in evaluating the effects on glycaemia and markers of pancreatitis of AqE and EthE of S. campanulata administered to 46 male rats of the Wistar strain divided into 9 batches of four male rats made diabetic each by intraperitoneal injection of a single dose of 60 mg/kg/bw of STZ. After 28 days of treatment with 200, 400 and 800 mg/kg/bw of S. campanulata AqE and EthE, blood glucose, alpha amylase and lipase activities were significantly decreased with both extracts.

KEYWORDS: *Spathodea campanulata*; blood glucose; pancreas marker; type 2 diabetes.

RESUME: Cette présente étude est réalisée avec *S. campanulata* P. beauv, une plante appartenant à la famille des Bignoniaceae. Elle couramment utilisée pour le traitement du diabète, du paludisme et de la schistosomiase. Cette étude vise à évaluer l'effet des extraits aqueux (EAq) et hydroéthanolique (EEth) de *Spathodea campanulata* pendant 28 jours, la glycémie et les marqueurs pancréatiques chez des rats mâles rendus diabétiques par le streptozotocine (STZ). Dans un premier temps, notre étude a porté sur un Triphytochimie de nos extraits. Elle a montré que les extraits aqueux et hydroéthanolique des écorces de la plante sont riches en polyphénols, en flavonoïdes, en saponosides, en alcaloïdes, en stéréols-polyterpens, en tanins mais relativement pauvre en quinones. Dans un second temps, cette étude consistait à démontrer les propriétés anti-hyperglycémiantes des EAq et EEth de *Spathodea campanulata* administré à 46 rats mâles de la souche Wistar répartis en 9 lots de quatre rats mâles rendus diabétiques chacun par injection intra péritonéale de 60mg/kg de poids corporel (dose unique) de la STZ. Pendant 28 jours de traitement par voie orale avec les EAq et EEth de *Spathodea campanulata* à des dose 200, 400, 800 mg/kg/pc, la glycémie et l'activité de l'alpha amylase et de la lipase ont diminué de manière significative de J0 à J28 avec les deux extraits de plante.

MOTS-CLEFS: *Spathodea campanulata;* glycémie; Marqueur du pancréas; Diabète de type 2.

Corresponding Author: Koulaï Diane

1 INTRODUCTION

Type 2 diabetes, often referred to as adult diabetes or insulin-independent diabetes, is characterized by a state of insulin resistance accompanied by a dysfunction of insulin secretion. It accounts for 85% of diabetics and has been considered in recent years as one of the scourges of the third millennium [1]. It is a disease that in recent years has continued to spread throughout the world. This is the first pandemic in the world that is not linked to an infectious disease. However, the difficulty of accessing primary health care and the limited financial means of the population lead 80% of the population to resort to medicinal plants for treatment [2]. Indeed, several herbal remedies are proposed by traditional therapists to treat type 2 diabetes.

This is the case of Spathodeae. campanulata P. beauv, a plant belonging to the family Bignoniaceae. It is commonly used for the treatment of diabetes, malaria and schistosomiasis. Many studies have described the use of different plant organs of Spathodea campanulata P.Beauv in traditional medicine. Rajesh et al. in 2010 demonstrated that the ethanolic extract of the flowers of S. campanulata was more active than that of the leaves on both Gram+ and Gram- bacteria strains [3]. He attributed this activity to the presence of flavonoids and tannins. Tanayen et al, (2016) identified the secondary metabolites and demonstrated the antidiabetic activity of methanolic extract of S. campanulata trunk barks, while Wagh and Butle in 2018 in a review article reviewed the chemical and pharmacological profile of extracts of different parts of S. campanulata and they noted the antibacterial activity of different bark extracts of this plant on many bacterial strains [4,5]. Later, Wagh et al. in 2019 demonstrated the antihelminthic activity of methanolic extract of S. campanulata leaves on adulterated earthworms [6].

The present work focuses on the effect of aqueous and ethanolic extracts of Spathodea campanulata barks on lipaemia, amylaemia and anti-hyperglycaemic activity in rats made diabetic by streptozotocin.

2 MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 PLANT MATERIAL

The plant material consisted of Spathodeae campanulata bark. These barks were harvested, cut and dried in the sun at room temperature (25-30°C) for four weeks, before being ground into a fine powder using a mechanical grinder. Then stored in glass vials in a dark and dry place for use.

2.1.2 ANIMAL MATERIAL

The in vivo study was carried out on 90-day-old male wistar rats weighing between 150 and 250 g (at the start of the experiment). The animals were housed in the vivarium of the Ecole Nationale Supérieure of Abidjan (ENS) in plastic cages with stainless steel lids (36cm ×25cm) lined with wood shavings where they had free access to water and food. The cages were equipped with a label that indicated the name of the batch, the treatment and the dates of the experiments. Prior to their use, the rats underwent a 7-day adaptation period in the animal house at a constant temperature (22 ± 2) °C and were subjected to a 12/12h light/dark cycle to respect their biological clock. They were treated in accordance with the principles and guidelines set out in the Manual on the Care and Use of Experimental Animals. Individual identification of the rats was done by tail numbering with a permanent marker.

2.2 METHODS

2.2.1 PREPARATION OF EXTRACTS

The preparation of the total aqueous, hydro-ethanolic extracts was done according to the method of Zirihi et al. in 2003 by homogenization using a blender [7]. The extracts were obtained from 100 g of powder and 1 L of solvent (distilled water and 70% ethanol) which were homogenized in a blender. After 15 min of homogenization, the homogenate obtained was collected in a white (clean) cloth square and squeezed by hand by applying strong pressure. The collected solution was filtered twice on cotton wool and then on Whatman 3 mm filter paper. Aliquots of the filtrate were placed in an drier at 40°C for 48 hours for the aqueous extract and at 50°C for 24 hours for the hydroethanol extract.

The output of the different extracts was obtained by the following formula:

$$\text{Output (\%)} = \frac{\text{Mass of the extract obtained}}{\text{Mass of initial powder}} \times 100$$

2.2.2 TRIPHYTOCHEMISTRY

Phytochemical sorting is a method of characterising the main chemical groups such as sterols, polyterpenes, alkaloids, tannins, polyphenols, flavonoids, quinones, anthocyanins, leuco-anthocyanins and saponins. These compounds were highlighted using appropriate reagents that reacted with chemical compounds to give specific colours or precipitates that attested to the presence or otherwise of the molecules in the different extracts (aqueous and hydroethanol extracts). This experiment was carried out according to the protocol of Bekro et al (2007) with some modifications [8].

SEARCH FOR STEROLS AND POLY TERPENES BY THE LIEBERANN REACTION

We evaporated to dryness without charring the residue in a capsule on the sand bath, 5 mL of each solution and dissolved the residue hot in 1 mL acetic anhydride and poured the solution into a test tube. Then we carefully poured 0.5 mL of concentrated sulphuric acid down the wall of the tube. The appearance of a purple ring at the interphase, turning blue and then green, indicates a positive reaction. We performed this test with a chloroformic control solution of cholesterol.

SEARCH FOR POLYPHENOLS BY THE FERRIC CHLORIDE REACTION

To 2 mL of each solution we added a drop of 2% alcoholic ferric chloride solution. The ferric chloride caused a blackish-blue colouration in the presence of the polyphenolic derivatives. The control was carried out with an alcoholic solution of gallic acid.

RESEARCH OF FLAVONOIDS BY THE CYANIDIN REACTION

We evaporated 2 mL of each solution to dryness in a capsule. After cooling, we took the residue with 5 mL of half strength hydrochloric alcohol. We poured the solution into a test tube and added 2 magnesium chips. The pink-orange coloration was obtained. The addition of 3 drops of isoamyl alcohol, which intensified this coloration, confirmed the presence of flavonoids. The control was carried out with an alcoholic solution of quercetin. We evaporated to dryness in a 5 mL capsule of each solution, adding 15 mL of STIASNY reagent to the residue. We kept the mixture in a water bath at 80° for 30 min. After cooling, there is a precipitation in large flakes which characterises the presence of catechic tannins.

SEARCH FOR GALLIC TANNINS

We filtered the solution obtained in the search for catechic tannins, then the filtrate was collected and saturated with sodium acetate. We added 3 drops of 2% FeCl3 which caused the appearance of an intense blue-black coloration demonstrating the presence of gallic tannins.

SEARCH FOR FREE OR COMBINED QUINONES

We evaporated to dryness in a 2 mL capsule of each solution and then triturated the residue in 5 mL of 1/5 hydrochloric acid. In a test tube we heated the solution for half an hour in a boiling water bath. After cooling, we extracted the hydrolysate with 20 mL of chloroform into a test tube. Then we collected the chloroform phase in another test tube and added 0.5 mL of ammonia diluted by 1/2. The appearance of a red to purple colouration indicated the presence of quinones.

TESTING FOR ALKALOIDS

To test for alkaloids, DRAGENDORFF's reagent was used. We evaporated to dryness in a 6 mL capsule of each solution and recovered the residue with 6 mL of alcohol at 60°C. In a tube, we added 2 drops of DRAGENDORFF reagent and observed the appearance of an orange coloration indicating the presence of alkaloids.

TESTING FOR SAPONOSIDES

In a test tube of 160 mm high and 16 mm in diameter we put 15 mL of the dissolved extract. We shook vigorously for 10 s and let it stand for 10 min. The persistence of foam of more than 4 mm height indicates the presence of saponosides.

2.2.3 EVALUATION OF HYPOGLYCAEMIC ACTIVITY AND PANCREATIC MARKERS OF SPATHODEA CAMPANULATA EXTRACTS

GLUCOSE ASSAY

The evolution of blood glucose levels in the different batches of rats was monitored from the first day of treatment (D0) until the end of treatment (D28).

INDUCTION OF DIABETES

The animals were deprived of food for 16 hours, but were provided with free water. They received a single intraperitoneal dose of 60 mg/kg bw of freshly prepared streptozotocin solution. After induction, the rats were given anhydrous glucose solution (5%) overnight to overcome the hypoglycaemic shock induced by the action of streptozotocin. Seventy-two hours (72 h) after injection.

ANIMAL GROUPS

After induction of diabetes, the rats were divided into 9 batches of 4 rats and fasted for 18 hours prior to experimentation. This test was performed as follows:

Batch 1; normal controls (NC): healthy rats were given 10 mL of distilled water solution;

Batch 2; positive controls: hyperglycaemic rats treated with a reference anti-diabetic drug received 10 ml/kg bw of glibenclamide

Batch 3; negative controls: untreated hyperglycaemic rats received 10 mL of distilled water solution;

Batch 4; **800 AqE:** diabetic rats received the aqueous solution of Spathodeae campanulata administered at a dose of 800mg/kg bw respectively

Batch 5; **400 AqE:** diabetic rats received the aqueous solution of Spathodeae campanulata administered at a dose of 400 mg/kg bw respectively

Batch 6; 200 AqE: diabetic rats received the aqueous solution of Spathodeae campanulata administered at a dose of 200 mg/kg bw, respectively;

Batch 7; 800EthE: diabetic rats received the hydroethanol solution of Spathodeae campanulata administered at a dose of 800 mg/kg bw, respectively

Batch 8; 400EthE: diabetic rats received Spathodeae campanulata hydroethanol solution at a dose of 400 mg/kg bw, respectively

Batch 9; **200EthE:** diabetic rats received Spathodeae campanulata hydroethanol solution at a dose of 200 mg/kg bw, respectively

BLOOD SAMPLING IN RATS

Blood sampling was performed following the tail vein to obtain a drop of blood sufficient for blood glucose determination using an On Call Extra Active meter and test strips. Rats with blood glucose levels above 2.5 g/L were retained for further testing

The blood collected was placed in fluoridated tubes (grey tubes) for blood glucose and in dry tubes (red tubes) for pancreatic parameters such as lipase and amylase. The tubes were numbered and then centrifuged at 3000 rpm for 5 minutes to obtain the serum.

STATISTICAL ANALYSIS

Statistical analysis was performed using Graph Pad Prism 6.0 (Microsoft, USA). The Student's test was used for comparison of the mean concentrations. The ANOVA test including the Turkey multiple comparison test was used for the analysis of variances. For both tests, the significance level was set at 5% ($P < 0.05$).

3 RESULTS

The results of the aqueous and ethanolic extraction of Spathodea campanulata bark are given in Table I. The phytochemical analysis of the aqueous and hydroethanolic extracts of Spathodea campanulata revealed the presence of several secondary metabolites. Flavonoids, sterols and polyterpenes, tannins, quinones, polyphenols and alkaloids were found in both extracts (Table II).

Table 1. Raw extraction results

Solvent	Initial powder mass	Mass of extract obtained	Extraction yield	Physical appearance	Appearance of the extract
Distilled water	100g	12,5g	12,5 %	Powder	Light brown
Hydro-ethanolic	100g	14,8g	14,8 %	Viscous paste	Dark brown

Table 2. Chemical screening of aqueous and hydroethanolic extracts of the bark of Spathodea campanulata

	Extracts	Ste/ Ter	Phe	Fla	T cat	T gal	Qn	Alc D	Alc B	Sap
Spathodea campanulata	**Aqueous**	+	+	+	++	+	+	++	+	++
	Hydro-ethanolic	++	++	+	+	+	-	+	+	+

Legend:
Ste / Ter: Sterol and polyterpenes; Phe: polyphenols; Fla: flavonoids; Tcat: catechetical tannins; Tgal: gallic tannins; Qn: quinone substances; Alc D: Dragendorff alkaloids; Alc B: Bouchardat alkaloids; Sap: saponins
+: present; ++: Abundantly present; -: absent

Table 3. Activity of amylasemia and lipasemia in aqueous extracts for 28 days

	Nor Control	Neg Control	Pos Control	800 AqE	400 AqE	200 AqE
AMYL-D0	699,25± 111.55****	1164,75± 32.50	1862,50± 3.17	2845,50± 1451.13	1624,50± 178.77	1650,5± 211.36
LIP-D0	8,59± 1.92****	80.00± 0.19	72,30± 0.12	81,725± 0.81	79,42± 0.24	88,4± 0.61
AMYL-D28	699,25± 111.55****	2629.00± 145.73	136,50± 13.38****	1290,50± 43.04****	762,75± 259.86****	1105,25± 242.16****
LIP-J28	8,59± 1.92****	88,86± 0.23	8,57± 0.31****	11,20± 0.30****	9,96± 0.84****	10,4425± 1.15****

Data are expressed as mean ± SEM; n = 4
**p < 0,05; **p < 0,01; ***p < 0,001; ****p< 0,0001: significant difference between the untreated diabetic batch and the treated diabetic batches.*
***Nor Control:** non-diabetic control*
***Neg Control:** untreated diabetic control*
***Pos Control:** diabetic control treated with the reference molecule (glibenclamide)*
***800 AqE; 400 AqE; 200 AqE:** diabetic batches treated with aqueous extract at doses of 800, 400, and 200 mg / kg bw respectively*

Table 4. Activity of amylasemia and lipasemia on hydroethanolic extracts for 28 days

	Nor Control	Neg Control	Pos Control	800EthE	400EthE	200EthE
AMY-D0	699,25± 111.55****	1164,75± 32.50	1862,50± 3.17	1666± 97.65	1387,75± 92.10	1977,75± 17.90
LIP-D0	8,59± 1.92****	80.00± 0.19	72,30± 0.12	78,25± 0.10	79.00± 0.79	79,25± 0.66
AMY-D28	699,25± 111.55****	2629.00± 145.73	136,50± 13.38****	1502,5± 107.89***	1155,25± 146.47****	1460,25± 178.05****
LIP-D28	8,59± 1.92****	88,86± 0.23	8,57± 0.31****	37,01± 9.77***	25,80± 8.65****	12,42± 2.23****

Data are expressed as mean ± SEM; n = 4

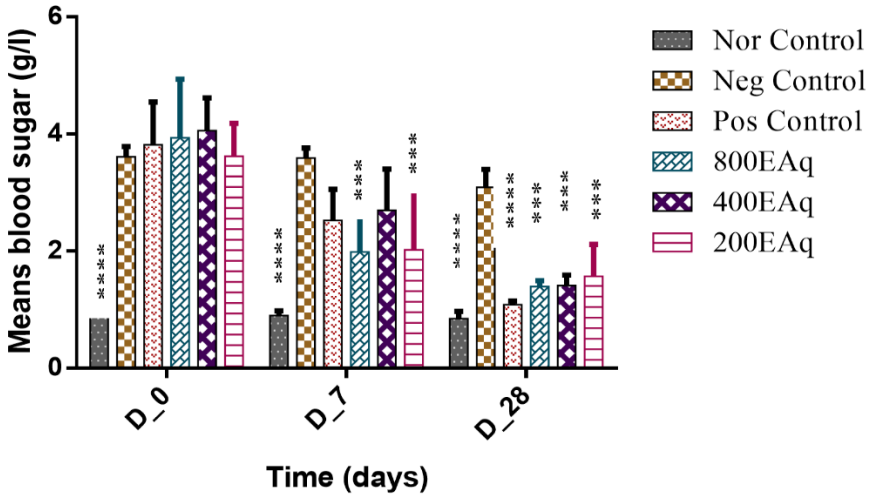
**p < 0,05; **p < 0,01; ***p < 0,001; ****p< 0,0001: significant difference between the untreated diabetic batch and the treated diabetic batches.*

Nor Control: *non-diabetic control*

Neg Control: *untreated diabetic control*

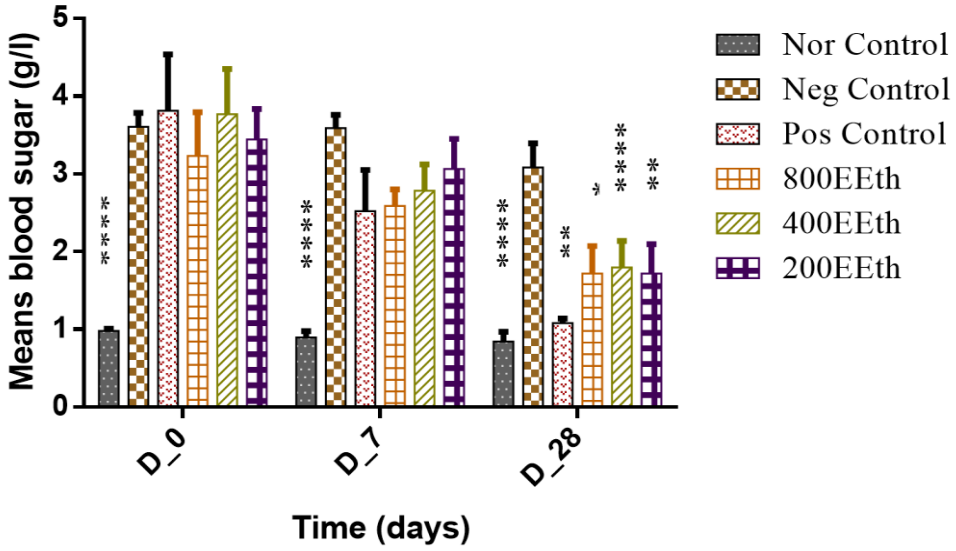
Pos Control: *diabetic control treated with the reference molecule (glibenclamide)*

800EthE; 400EthE; 200EthE: *diabetic batches treated with hydroethanolic extract at doses of 800, 400, and 200 mg / kg bw respectively*



Graphe 1: Evaluation of aqueous extracts of Spathodea campanulata on blood glucose levels over time

**p < 0,05; **p < 0,01; ***p < 0,001; ****p< 0,0001: significant difference between the untreated diabetic batch and the treated diabetic batches., Nor Control: non-diabetic control Neg Control: untreated diabetic control, Pos Control: diabetic control treated with the reference molecule (glibenclamide). 800AqE; 400AqE; 200 AqE: diabetic batches treated with aqueous extract at doses of 800, 400, and 200 mg / kg bw respectively*



Graphe 2: Evaluation of hydro-ethanolic extracts of Spathodea campanulata on blood glucose levels over time

p < 0,05; **p < 0,01; ***p < 0,001; ****p< 0,0001: significant difference between the untreated diabetic batch and the treated diabetic batches.,* ***Nor Control:* *non-diabetic control.* ***Neg Control:*** *untreated diabetic control.* ***Pos Control:*** *diabetic control treated with the reference molecule (glibenclamide).* ***800EthE; 400EthE; 200EthE:*** *diabetic batches treated with hydroethanolic extract at doses of 800, 400, and 200 mg/ kg bw respectively*

4 DISCUSSION

In this work, maceration was carried out with the powdered bark of Spathodea campanulata P. Beauv in two solvents namely distilled water and ethanol (70%) + water (30%) mixture. The value of the extraction output was calculated in relation to the initial mass of the plant bark powder. It was found that the hydro-ethanol mixture had the highest extraction yield of 14.8% compared to 12.5% for the aqueous extract. The variations in extraction yields can be attributed to the difference in solubility of the phenolic compounds in the extraction solvents. Besides the extraction method, other factors can influence the efficiency of the extraction of the compounds. It depends on several parameters such as: the nature and volume of the solvent used, pH, temperature, extraction time and sample composition, sample collection [10]

Similarly, Triphytochemistry carried out with aqueous and hydro-ethanolic extracts of Spathodea campanulata barks revealed the presence of important secondary metabolites which are: alkaloids, catechic tannins, flavonoids, polyphenols, sterols, terpenes, saponins, gallic tannins but relatively poor in quinones. All responsible for therapeutic effects. Our results are similar to those of [11] who showed that the aqueous extract of Spathodea campanulata bark (collected in Nigeria) contained alkaloids, flavonoids, saponins, quinones, polyphenols and terpenoids. However, tests for the characterization of tannins (gallic and catechins) were negative. This difference could be due to the place of collection, the drying and storage conditions of the bark of this plant. Studies have shown the benefits of compounds found in Spathodea campanulata bark; for example, polyphenols, tannins, and flavonoids have antioxidant properties. They favor tissue regeneration, decrease the permeability of blood capillaries and increase their resistance to haemolysis [12]. The presence of tannins suggests the ability of our plant to play a major role as an antimicrobial and antioxidant agent [13]. On the other hand, the anti-diabetic action of tannins is observed by its action on diabetes itself at the cellular level, by promoting the action of insulin (by reducing insulin resistance) and on the complications of diabetes through their antioxidant and anti-enzymatic power, neutralising the effect of free radicals and limiting the inflammatory reaction in the various tissues [14]. Flavonoids are endowed with hypoglycaemic and antidiabetic properties according to the results of several studies [15,16,17]. According to these authors [18,19,20] flavonoids prevent diabetes by inhibiting alkalosis reductase. Other studies have also shown that the consumption of flavonoid-rich foods is inversely correlated with the risk of developing cardiovascular disease [21,22]. Alkaloids, sterols and terpenes are compounds that exhibit various activities in plants and have beneficial effects on humans and animals [23]. Alkaloids have several pharmaceutical applications in humans. These applications have been clinically proven [24,25]. These alkaloids have a discrete hypoglycaemic effect, lowering blood glucose levels and reducing glycosuria [26]. According to a study, the significant and long-lasting total acetone hypoglycaemic effect may well be related to the presence of both flavonoid and alkaloid compounds acting probably synergistically [27].

In addition, the anti-diabetic activity was carried out during the 28 days by administering the aqueous and hydro-ethanolic extracts at doses of 800, 400 and 200 mg/kg body weight (bw) per treatment. A significant decrease in blood glucose levels was observed from D0 to D28 in both extracts. For the aqueous extract at 800 mg/kg bw the blood glucose level decreased from 3.93±0.50 to 1.39±0.05 and from 4.05±0.28 to 1.41±0.08 at 400 mg/kg bw. Similarly for the ethanolic extract, there was also a decrease in blood glucose from 3.23±0.58 to 1.71±0.34 at 800 mg/kg/bw; from 3.77±0.58 to 1.79±0.34 for 400EthE and from 3.44±0.44 to 1.71±0.38 for 200 mg/kg/bw.

In addition, a significant decrease was observed in rats treated with glibenclamide. The effect of these two extracts on the reduction of blood glucose could be related to the presence of flavonoids as it has been pointed out by some authors [28]. Flavonoids act by improving the sensitivity of the body's cells to insulin, thus reducing the incidence of type 2 diabetes [29]. In addition to flavonoids, the presence of saponins has been noted and the anti-hyperglycaemic effect of saponins has been shown. It would be difficult to relate the mode of action of the extracts to that of glibenclamide because of the mixture of compounds that may interfere or have a synergistic action in these extracts. This synergy or interference could explain the difference in action of the extracts.

Furthermore, our results indicate an inhibition of α-amylase and lipase by Spathodea campanulata extracts which presents a beneficial effect against hyperglycaemia in diabetics. Insulin increases amylase biosynthesis in diabetic rats only. The anti-diabetic activity of the plants may depend on several mechanisms: inhibition of α-amylase [29] which reduces the degradation of starch and oligosaccharides; therefore, it acts by reducing the absorption of glucose at the intestinal level.

According to the results obtained, we recorded a steady gain in body weight of normal control rats, the average body weight values increase from 265g at the beginning of the experiment to 285g at the end of this study.

5 CONCLUSION

At the end of our study, the aqueous and ethanolic extracts of Spathodea campanulata barks showed a good hypoglycaemic activity in rats made diabetic by streptozotocin by decreasing the serum concentration of glucose, lipase and amylase

All our work has highlighted the beneficial effects of the administration of these plant extracts due to their richness in compounds such as: tannins, total phenols, alkaloids and flavonoids responsible for the decrease of glycaemia, lipase and amylase. The present study validates the use of Spathodea campanulata bark in traditional medicine in Côte d'Ivoire and presents promising perspectives in the management of diabetes, pancreatitis and their possible complications. However, further studies are needed to identify the biologically active molecules in order to give an accurate picture of the molecular mechanism (s) responsible for these effects.

REFERENCES

[1] Drouin P., Blickle. J.F., Charbonnel B., Eschwege E., Guillausseau P.J., Plouin P.F., Daninos J.M., Balarac N., et Sauvanet J.P. Diagnostic et classification du diabète sucre les nouveaux critères, Diabetes & amp; Metabolism (Paris), 1999; 25: 72-83.

[2] Ngbolua K.N., Rafatro H., Rakotoarimanana H., Ratsimamanga Urverg S., Mudogo V., Mpiana P.T. Tshibangu D.S.T. Pharmacological screening of some traditionally used antimalarial plants from the Democratic Republic of Congo compared to its ecological taxonomic equivalence in Madagascar. International Journal Biological and Chemical. Sciences, 2011; 5 (5): 1997-1804.

[3] Rajesh Kowti, Harsha R, Mohammed GA, Hareesh AR, Thammanna Gowda SS, Dinesha R, Satish Kumar BP, Irfan Ali M,. Antimicrobial activity of leaf and flower of Spathodea campanulata P. Beauv. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2010; 1 (3): 8.

[4] Tanayen JK, Ajayi AM, Oloro J, Ezeonwumelu JOC, Tanayen GG, Adzu B, Arthur van Aerschot, Gert L, Agaba AG,. Phytochemical and Antidiabetic Evaluation of the Methanolic Stem Bark Extract of Spathodea campanulata (P. Beauv.) Bignoniaceae. Pharmacognosy Journal, 2016; 8: 243-249. DOI: 10.5530/pj.2016.3.12.

[5] Wagh AS et Butle SR. Plant profile, phytochemistry and pharmacology of Spathodea campanulata P. Beauvais (african tulip tree): a review. International Journal of Pharmacy and Pharmaceutical Sciences. 201810 (5): 1-6.

[6] Wagh A, Butle S, Telang P. In Vitro Anthelmintic Efficacy of Spathodea campanulata P. Beauv. (Bignoniaceae) Against Pheretima posthuma. Asian Journal of Pharmacognosy. 2019; 3 (1): 32-38.

[7] Zirihi G., Kra A.K.M., & Guede-Guina F. Évaluation de l'activité antifongique de Microglossa pyrifolia (Lamarck) O. Kantze (Astéracée) « PYMI » sur la croissance in vitro de Candida albicans, Revue de Médecine et pharmacie Afrique, 2003; 17: 11-18.

[8] Bekro Y.-A., Mamyrbekova J., Boua B.B., Bi F.T. Ehile E.E. Étude ethnobotanique et screening phytochimique de Caesalpinia benthamiana (Baill.) Herend. Et Zarucchi (Caesalpiniaceae). Sciences & Nature, 2007; 4 (2): 217-225.
[9] Mangambu M, Mushagalusa K F. & Kadima N.J. Contribution à l'étude phytochimique de quelques plantes medicinales antidiabétiques de la ville de Bukavu et ses environs (Sud Kivu- RD Congo). Journal of applied Biosciences, 2014; 75: 6211- 6220.
[10] Mawa T, Doumbia I, Yeo S O, Yapi H F, N'guessan J D and Djaman A J, phytochemical studies and evaluation of the hypoglycemic activity of the methanolic extract of tetrapleura tetraptera sheets ((schumach & thonn.) taub.1891), world journal of pharmacy and pharmaceutical sciences, 2019; 12 (8) 1189-1201.
[11] Bruneton J. Tanin, Pharmacognosie: phytochimie, plantes medicinales. Technique et Documentation. Lavoisier, Paris, France, 1999; pp. 370-404.
[12] Tepe B., Sokmen M., Akpulat HA., Sokmen A. Screening of the antioxidant potentials of six Salvia species from Turkey" Food Chemistry, 2006; Volume (95), 200p.
[13] Punitha ISR, Rajendran K, Shirwaikar A, Shirwaikar A. Alcoholic stem extract of Coscinium fenestratum regulates carbohydrate metabolism and improves antioxidant status in Streptozotocine nicotinamide induced diabetic rats. CAM, 2005; 2 (3): 375-381.
[14] Kim SH., Hyun SH, Choung SY. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. Journal of Ethnopharmacology, 2006; 104 (1–2): 119–123.
[15] Kebieche M. Activité biochimique des extraits flavonoïdiques de la plante Ranunculus repens L: effet sur le diabète expérimental et l'hépatotoxicité induite par l'Epirubicine, Thèse de Doctorat, Université Mentouri Constantine Faculté des Sciences de la Nature et de la Vie Département de Biochimie –Microbiologie, Alger, Algérie, 2009; 143p.
[16] Tringali C. Bioactive Compounds from Natural Sources: Isolation Characterization and Biological Properties. Taylor & Francis, 2001; 36: 339-367.
[17] Huang DJ, Lin CD, Chen HJ, Lin YH. Antioxidant and antiproliferative activities of sweet potato (Ipomoea batatas [L.] Lam 'Tainong 57') constituents. Bot. Bull. Acad. Sin. 2014; 45: 179-186.
[18] Raccah D. Epidémiologie et physiopathologie des complications dégénératives du diabète sucré. EMC-Endocrinologie, 2004; 1 (1): 29-42.
[19] Pietta P. Flavonoids as Antioxidants. Journal of Natural Products, 2000; 63 (7): 1035-1042.
[20] Hollman P. H. Evidence for health benefits of plant phenols: local or systemic effects? Journal of the Science of Food and Agriculture, 2001; 81 (9): 842-852.
[21] Bouic P.J. et Lamprecht J.H. Plant sterols and sterolins: à review of their immune modulating properties. Alternative Medicine Review, 1999; 4: 170-177.
[22] Mc Calley D.V. Analysis of the cinchona alkaloids by high-performance liquid chromatography and other separation technical. Review Journal of Chromatography, 2002; 967: 119-122.
[23] Silvestrini A., Pasqua G., Botta B., Monacelli B., Heijden R., Von D., Verpoorte R. Effects of alkaloid precursor feeding on a camptotheca acumisate cell line. Plant Physiol. Biochem, 2002; 40: 749-753.
[24] Pamplona RG. Guide des plantes médicinales. Vol.1, Bibliothèque, éducation et santé, Madrid, Paris, 2001; 232-298.
[25] Sy Gy, Faye FS, Wele A, Gueye PM, Gueye CD, Cissé A, Diaye AM, Bassene E, Faye B. Activité antihyperglycémiante de la fraction F2 de l'extrait total acétonique des feuilles de Vernonia colorata (Composeae). Pharmacopée et Médecine Traditionnelle Africaines, 2008; 15: 6- 10.
[26] Olagbende-Dada S.O., Ogbonnia S.O., Coker H. A. B., & Ukpo G.E. Blood glucose lowering effect of Aqueous extract Of Gratophyllum pictum (Linn) Griff on alloxan induced diabetic rats and its acute toxicity in mice. African Journal Of Biotechnology, 2011; 10 (6): 1039-1043.
[27] Lean Me., Moroozu M., Kelly I., Burns J., Taluar D., Sattar N., Crozier A. Dietary flavonols protect diabetic human lymphocytes against oxidative damage to DNA. Diabetes, 1999; 48: 176 181.
[28] Kambouche N., Merah B., Derdour A., Bellahouel S., Bouayed J., Dicko A., Younos C. et Soulimani R. Hypoglycemic and antihyperglycemic effects of Anabasis articulata (Forssk) Moq (Chenopodiaceae), an Algerian medicinal plant. African Journal of Biotechnology, 2009; 8 (20): 5589-5594.
[29] Jarald E, Balakrishnan S.J, Chandra J.D. Diabetes and Herbal Medicines. Iranian journal of pharmacology and therapeutics. 2008; 97-106.