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Original Article

Phytochemical Study and Biological Activities of Hydro-Alcoholic Extract of the Leaves of *Bridelia ferruginea* Benth and its Fractions

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ABSTRACT

Context and purpose: The roots of *B. ferruginea* are used in traditional Togo medicine in the treatment of diabetes. Studies have shown that leaves have a low antihyperglycemic effect. This study aims to improve the effect of leaves. **Methods:** The hydroalcoholic extract of the leaves underwent a fractionation (separation in cold alcohol) and an OGTT (Oral Glucose tolerance test) on healthy mice, a phytochemical screening and antioxidant tests (*in vitro*: DPPH and *ex vivo*: AAPH) were performed on the extract and its fractions. **Results**: Phytochemical screening of the extract showed the presence of phenols, flavonoids and condensed tannins as well as the absence of condensed tannins, alkaloids, saponosides and anthracene derivatives. The extract also showed antioxidant activity. The supernatant fraction showed antihyperglycemic activity at 100 mg / kg in the 30th and 60th after glucose administration. Phytochemical screening of the supernatant revealed the presence of phenols, flavonoids and condensed tannins. The supernatant also showed more pronounced antioxidant activity than the total extract. **Conclusion**: In view of these results, the most active fraction obtained by the separation technique in cold alcohol is the supernatant.

Key words: *Bridelia ferruginea*, Diabetes, OGTT, Antioxidant, Fractionation.

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INTRODUCTION

Diabetes mellitus is a metabolic disease resulting from lack of secretion and / or action of insulin. This insulin deficiency leads to chronic hyperglycemia with disruption of carbohydrate, lipid and protein metabolism.[1] According to the International Diabetes Federation (IDF), the prevalence rate of Diabetes in sub-Saharan Africa will be increased by 98% between 2010 and 2030 if urgent preventive measures are not taken to curb its incidence. In Togo, diabetes is booming because of the change of lifestyle of the population, according to the results of surveys conducted by the Ministry of Health in 2010 and 2012, the prevalence of diabetes is 2.6% in the population aged 15-64.[2,3] The treatments available for diabetes mellitus are insulin and oral antidiabetic drugs. However, these drugs do not restore carbohydrate balance but also have side effects.[4] In view of the adverse effects of these drugs and the fact that plants are more accessible to populations in developing countries, the antidiabetic properties of plants have been studied.[5] More than 400 traditional plants have been reported.[6] Among these plants is *Bridelia ferruginea*, widely studied for its antihyperglycemic properties, especially root and leaf extracts. According to our previous studies[7] leaves do not have significant antihyperglycemic activity, although they are traditionally used to treat diabetes.[8] This raises the problem of preservation of the species with the collection of roots and confirmation of the antidiabetic effect of the leaves. The purpose of our study is to evaluate the antidiabetic effect of the leaves and to improve this effect.

For this, we performed a phytochemical analysis (qualitative and quantitative) of the extract and fractions to identify and assay large chemical groups; we then carried out biological tests on healthy mice in a state of carbohydrate overload, as well as an evaluation of the antioxidant activity.

MATERIALS AND METHODS

Plant material

The leaves of *B. ferruginea* were collected at Assomé 34 km from Davié, in the prefecture of Zio between 9 am and noon, June 26, 2015. They were identified by the Laboratory of Botany and Plant Ecology of University of Lomé and a sample stored in the herbarium of this laboratory under the number TOGO15149.

Animal material

The evaluation of the antihyperglycemic effect was carried out on the ICR mouse at the Animal Physiology Departement of University of Lomé in metal cages; These animals had a weight of between 20 g

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and 30 g. For the *ex vivo* antioxidant test, the blood was taken from Wistar rats, weighing between 200 g and 300 g.

Methodology

Preparation of the total extract

For the preparation of the extract, the method used was that of Bakoma *et al.* (2012b).[9] Thus, the leaf powder (400 g) was macerated at room temperature with ethanol-water (80:20 v/v) with intermittent manual stirring for 72 h. During this operation the solvent (6 L) was renewed three times (every 24 h). The macerate obtained was filtered on hydrophilic cotton. The filtrate was concentrated under reduced pressure at 45°C using a rotary evaporator Buchi 220. The yield of the leaf extract was 20.39% (w/w) based on the weight of the initial powder. The extract was stored at 4°C in the refrigerator in a labeled plastic bottle.

Fractions obtaining

The separation technique used was that of Kadébé *et al.* (2015).[10] After evaporation, the hydroalcoholic extract was dissolved in an ethanol-water mixture (75:25 v/v) at the rate of 3 g of extract per 40 ml of solvent. The solution was distributed in tubes and decanted for 24 h in a refrigerator at 4°C and then centrifuged at 6000 rpm for 15 min to obtain two phases: the pellet (precipitate) and the supernatant. After evaporation, the dry fractions obtained were stored in a refrigerator at 4°C. The yield was 4.67% (w/w) for the pellet and 95.33% (w/w) for the supernatant relative to the total extract.

Evaluation of antihyperglycemic effect

This is an Orally Glucose Tolerance Test (OGTT). The method used was inspired by Bakoma *et al.* (2012a). 7Mice were fasted for 12 h, the substances were administered in a volume of 5 mL/kg; the blood glucose was measured at the tail after incision using the «One Touch Ultra 2» with test strips (One Touch).

Four groups of 5 animals were constituted:

Group 1 (Dw) /control: OGTT + distilled water;

Group 2 (Met) /control: OGTT + metformin (100 mg / kg);

Group 3 (Ext 200): OGTT + dry extract of leaves of *B. ferruginea* (200 mg / kg);

Group 4 (Ext 750): OGTT + dry extract of leaves of *B. ferruginea* (750 mg / kg);

Group 5 (S): OGTT + surnageant (100 mg/kg);

Group 6 (P): OGTT + pellet (100 mg/kg).

A measurement of blood glucose level was carried out beforehand and the mice were force-fed immediately afterwards with distilled water or extract and metformin according to the groups ($t_{0\text{-}60}$). One hour later (t_0), a measurement was performed again then mice were then force-fed immediately after with glucose at a dose of 4 g/kg of body weight. Measurement of blood glucose was performed after 30 min, 60 min and 120 min.

Phytochemical Screening on total extract and fractions

The identification tests were conducted on total extract, supernatant and pellet. This consisted of looking for large chemical groups such as: alkaloids, phenolic compounds, tannins, saponosides, flavonoids, sterols and anthracene derivated.

Determination of flavonoïds and total phenols content

The samples to be analyzed are the total extract, the supernatant and the pellet.

Determination of total phenols

The method used is that modified by Shetty *et al.* (2010)[11] with a change in proportions. The sample solution was prepared at 5 mg sample in 100 mL distilled water. In a test tube were introduced: 0.5 mL of the solution to be assayed, 2.5 mL of Folin-Ciocalteu diluted to 10th, 5 min later, 2 mL of sodium carbonate (CO_3Na_2) at 145 g/L was added. The mixture was well homogenized and the absorbance reading was made by a spectrophotometer at 765 nm after 30 min incubation at room temperature and protected from light. Four tests were performed for each sample. The standard range (gallic acid 10 to 100 mg/L) was carried out under the same conditions. The calibration curve was plotted using different concentrations of gallic acid and the results are expressed in mg of gallic acid equivalent (mg EqAG) per 100 mg of dry sample.

Determination of total flavonoïds

The method used is that of Ayoola *et al.* (2008)[12] with some modifications. A volume of 2 mL of 2% (w / v) $AlCl_3$ in pure methanol was mixed with an equal volume of 100 mg/L sample in methanol. The absorbance was read after 10 min in the dark at 415 nm using a spectrophotometer. Quercetin has been used as a reference substance. Four tests per sample were performed and the results are expressed in mg of quercetin equivalent (mg EqQ) per 100 mg of sample. The standard range (quercetin 10 to 100 mg /L) was carried out under the same conditions.

Antioxidant tests

He samples to be analyzed were the total extract, the supernatant and the pellet.

DPPH test (2,2'-diphenyl-1-picrylhydrasyl)

The measurement of the anti-radical activity of the plant extracts was carried out by the 2,2'-diphenyl-1-picrylhydrazyl test (DPPH) according to the method described by Dabire *et al.* (2011)[13] with some modifications.

A calibration curve was first established using quercetin as a reference substance. A volume of 2 mL of DPPH (19.716 mg per 500 mL of methanol) was mixed with 100 µL of quercetin (5 to 30 mg/L). The absorbance was read after 30 min in the dark at 517 nm using a spectrophotometer.

To evaluate the antioxidant activity (AAO) of the various extracts, 100 µL of each sample are added to 2 mL of the DPPH solution. Absorbance was read after 30 min in the dark at 415 nm using a spectrophotometer.

The results determined from the equation of the calibration curve, were expressed in mg equivalent quercetin per 100 mg of extract (mg EqQ/100 mg sample).

AAPH test (hemolysis test of red blood cells)

The thermal decomposition of AAPH (2,2'-azobis (2-methylpropionamidine dihydrochloride) generates free-radicals at a constant rate that attack the membrane of red blood cells. When there are no more endogenous antioxidants, the membrane of red blood cells bursts and hemoglobin is found in the supernatant. The monitoring of hemolysis is by spectrophotometry at 540 nm. The addition in the incubation medium of molecules or extracts with antioxidant activity delays the appearance of Hemolysis. The method is calibrated against known concentrations of ascorbic acid (AA) added to the incubation medium.[14]

For this test, rat blood was collected (at the retro-orbital sinus) on EDTA and centrifuged at 1500 rpm for 10 min. The resulting red blood cell pellet was washed three times with 5 volumes of PBS (Phosphate Buffered Saline). During the last rinse, centrifugation was done at 2500 rpm for 10 min. The pellet of globules was then dissolved in 4 volumes of PBS before being contacted at 37°C with AAPH (200 mM) and the different concentrations of the sample (50 - 250 µg/mL) and ascorbic acid (50 - 250 µg/mL) according to Table 1. After 3 h of incubation at 37°C, 4 mL of PBS was added to all tubes. The tubes were then centrifuged at 1000 rpm for 10 min and the absorbance measurement was made at 540 nm.

The inhibitory concentration 50 (IC_{50}) which is the concentration of plant extract or ascorbic acid responsible for 50% inhibition of APPH

radicals, is determined on the graph representing the percent inhibition of APPH based on concentrations of the extracts and ascorbic acid. Inhibitory concentration 50 per cent determines antioxidant potency. Three trials per sample were performed.

The percent inhibition was calculated from the formula:

$$\% \text{ inhibition} = (A_{AAPH} - A_{sample}) / A_{AAPH} \times 100, \text{ with:}$$

- A_{AAPH}: Absorbance of the control
- A_{sample}: Absorbance of the sample (sample : total extract, supernatant, pellet or ascorbic acid).

Statistical analysis

We used GraphPad Prism 5.00 (USA) software to process the results. They are expressed as mean value with the standard error of the mean (M ± E.S.M., n = 5). These results are analyzed using the two-variance analysis (2way-ANOVA) followed by the Bonferroni posttest, to compare the batches. The materiality threshold is set at P <0.05.

RESULTS

Evaluation of antihyperglycemic effect

Following the oral administration of glucose at a the dose of 4 g/kg of body weight to control group mice (Dw), the blood glucose level increased from 109.40 ± 9.56 mg/dL to 304.60 ± 21, 49; 265.80 ± 20.04 and 132.60 ± 11.22 mg / dL respectively at 30 min, 60 min and 120 min. The total extract at the dose of 200 mg /kg did not induce a significant reduction in blood glucose compared to the control group (Dw); the total extract at the dose of 750 mg/kg induced a significant reduction in blood glucose at 30th min 27.71% (P<0.01) compared to the control group. The pellet at the dose of 100 mg/kg had no effect on blood glucose; the supernatant at a dose of 100 mg/kg induced a significant reduction in blood glucose; this reduction is 37.45% (P<0.01) and 25.13% (P<0.05) respectively in the 30th and 60th min compared with the control group; similarly, metformin induced in a significant reduction in blood glucose level of 51.35% (P<0.001) and 43.27% (P<0.001) in the 30th and 60th min, respectively, compared with the control group (Figure 1A).

Area under the curve shows a significant reduction in blood glucose level of all groups compared to the control (Dw) except for supernatant (P< 0.01) and metformin treated groups (P<0.001) (Figure 1B).

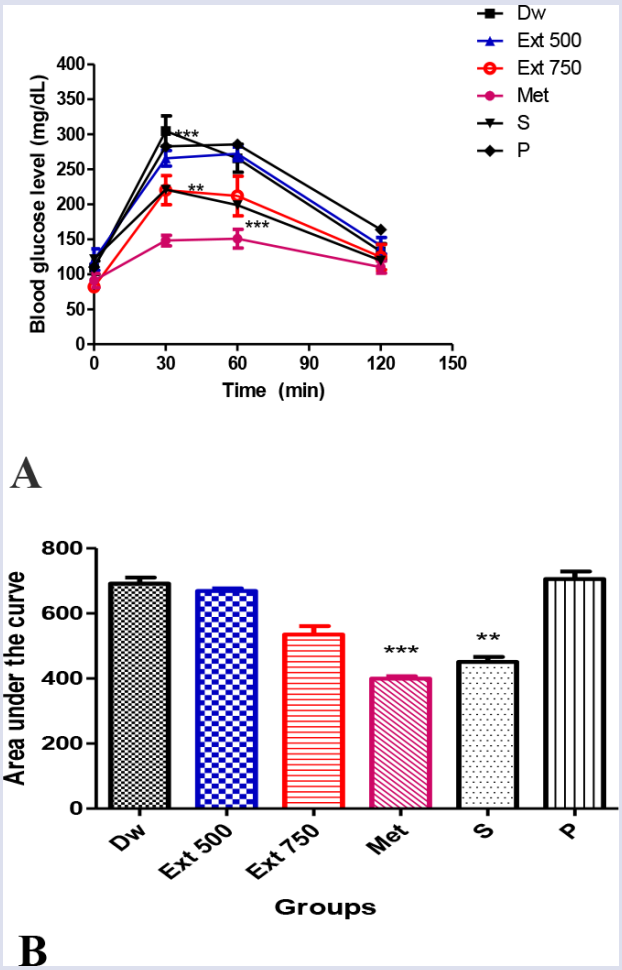

Figure 1: Effect of the total extract and fractions of *B. ferruginea* on the blood glucose of mice subjected to temporary hyperglycemia test. A: evolution of blood glucose; B: areas under the curve.
** P<0.01; *** P<0.001 vs ED; ED: distilled water; Ext 200: total extract of *B. ferruginea* leaves (200mg/kg); Ext 750: total extract of leaves of *B. ferruginea* (750mg / kg); Met: metformin (100mg/kg), S: supernatant fraction, P: pellet.

Phytochemical Screening

Phytochemical analysis of the leaf extract of *B. ferruginea* (Table 1) revealed the presence of tannins, diorthophenolic groups (phenols) and flavonoids in the extract; while alkaloids, saponosides, sterols and anthracene derivatives are absent in our experimental conditions. Fraction analysis revealed the presence of phenols, flavonoids and tannins in the supernatant while alkaloids, saponosides and anthracene derivatives were absent. In the pellet, the presence of phenols and flavonoids was revealed while tannins, alkaloids, saponosides and anthracene derivatives were absent in our experimental conditions (Table 1).

Determination of total phenols and flavonoids content

Total phenol and flavonoid concentrations were determined from the linear regression curves for phenols and flavonoids respectively (y = 0.0121x + 0.0323, R^2 = 0.9896) and (y = 0.0189x +). 0.0133; R^2 = 0.8528). The total phenol levels obtained are 56.40 ± 0.44 mg EqAG / 100 mg for the total extract, 32.42 ± 0.54 mg EqAG / 100 mg for the supernatant and 18.84 ± 2.62 mg EqAG/100 mg for the pellet. Flavonoids level obtained are 9.47 ± 2.15 mg Eq / 100 mg for the total extract, 6.47 ± 087 mg Eq /100 mg for the pellet and 14.69 ± 0.84 mg Eq/ 100 mg for the supernatant (Table 2).

Antioxidant tests

DPPH test

The standard range was used to plot a linear regression line of equation: y = -0.0092x + 1.0575; R^2 = 0.9956. The antioxidant activity of total extract was equivalent to 40.99 ± 2.23 mg of quercetin per 100 mg of extract (mg Eq / 100 mg). That of the supernatant and pellet were equivalent to 40.13 ± 0.55 and 16.80 ± 0.83 mg quercetin, respectively, per 100 mg of sample (Table 3).

AAPH test (hemolysis test of red blood cells)

The IC_{50} (inhibitory concentration 50) were determined from the linear regression curves of equation y = 0.2301x + 3.786 (R^2 = 0.9287); y = 0.2444x + 9.877 (R^2 = 0.9264); y = 0.1075x - 0.4298 (R^2 = 0.9780) respectively for the total extract, the supernatant and the pellet. The hemolysis inhibitory concentration of 50 percent of red blood cells for ascorbic acid was 126.74 ± 3.30 µg/ml, the total extract, the supernatant and the pellet were respectively 200.84 ± 12, 47 µg/ml, 134.16 ± 6.00 µg / ml 469.11 ± 18.85 µg/ml (Table 4).

Table 1: Phytochemical Screening.

	Supernatant	Pellet Total extract
Alcaloïds	-	- -
DiorthophénolicGroupements	+	+ +
condensed Tannins	+	- +
Saponins	-	- -
Flavonoïds	+	+ +
Anthracenic derivated	-	- -

- : absence ; + : presence

Table 2: Total phenol and flavonoïds content.

	Total extract	Supernatant	Pellet
Total phenol (mgEqAG/100mg)	56,40±0,44	32,42±0,54	18,84±2,62
flavonoïds (mgEqQ/100mg)	9,47±2,15	14,69±0,84	6,47±0,87

Each value represents the mean ± ESM (n = 4) for 100mg of sample; AG = gallic acid; Q = quercetin.

Table 3: Antioxidant activity of the hydroalcoholic extract of *B. ferruginea* and its fractions.

	Total extract	Supernatant	Pellet
AAO en mg EqQ/100 mg	40,99 ± 2,23	40,13 ± 0,55	16,80 ± 0,83

AAO: antioxidant activity in mg of quercetin equivalent per 100 mg of sample (EqQ/100). Values expressed on average ± ESM (n = 4).

Table 4: Summary of IC_{50} (inhibitory concentration 50) of the extract and its fractions.

	Ascorbic Acid	Total extract	Supernatant	Pellet
CI_{50} (µg/mL d'échantillon)	126,74 ± 3,30	200,84 ± 12,47	134,16 ± 6,00	469,11 ± 18,85

Values expressed as mean ± ESM (standard error of mean).

DISCUSSION

Studies have already reported the antihyperglycaemic activity of the roots of *B. ferruginea* while that of the leaves used in traditional medicine has yet to be proven.[7,15] Indeed, the previous work[7] showed that the root extract induced a significant reduction in glycemia at the dose of 250 mg / kg of body weight in rats in a state of carbohydrate overload. The extract leaves at the dose of 750 mg/kg induced a non significant reduction in blood glucose under the same experimental conditions. These results have been confirmed in part by our work, since the evaluation of the antihyperglycemic effect that we made with leaf extract of *B. ferruginea* showed that at the dose of 200 mg /kg, the leaf extract had no effect and at 750 mg /kg, a reduction in blood glucose is achieved only at the 30th min. These results show the existence of compounds with low concentration antihyperglycemic activity in the leaf extract or the existence of other compounds which would inhibit their action.

The collection of plant roots endangering the survival of the species, we set ourselves the goal of improving the antihyperglycemic effect of leaf extract. To achieve this goal, we conducted a phytochemical screening and assayed some secondary metabolites and then proceeded to a fractionation. The qualitative test revealed the presence of tannins, phenols and flavonoids known for their antihyperglycemic activity.[16-18] Fractionation of the extract by the separation technique in cold alcohol yielded two fractions, the supernatant and the pellet. An evaluation of their antihyperglycemic effect has shown that the supernatant has an antihyperglycaemic effect while the pellet does not have any. Indeed the supernatant had a significant reducing effect on the blood glucose of mice and this reduction is significant in the 30th and 60th min. This effect would have its origin in the presence of phenolic compounds since the supernatant is richer in phenols like flavonoids. In addition, flavonoids are part of the larger family of phenols including tannins, stilbenes, lignans and coumestans, phytosterols and phytostanols.[19]

These results show the interest of the separation technique in cold alcohol. Nevertheless, phytochemical screening showed the presence of phenols and flavonoids in both fractions. These results imply that compounds with antihyperglycemic activity are concentrated in the supernatant fraction.

Finally, according to the work of Bakoma *et al.*[7] the antihyperglycaemic effect of the *B. ferruginea* root is partly due to its antioxidant effect, this is also shown in the supernatant. In fact, the *in vitro* test of the antioxidant activity of *B. ferruginea* leaf extract and its fractions accounts for the radical activity of the various samples tested. The results showed that the supernatant has better antiradical activity than the pellet. The *in vitro* tests being insufficient to assert an *ex vivo* antioxidant activity, we carried out a hemolysis test of red blood cells (AAPH) in order to observe the behavior of the extract and fractions in the living medium. The AAPH test revealed that the supernatant, which already possessed better antihyperglycaemic activity, induced better protection of the red cell membrane than total extract and pellet. The supernatant thus had a better antioxidant activity than the total extract and the pellet under our experimental conditions; a very strong oxidizing power since the IC50 of the supernatant is very close to that of ascorbic acid, which is a reference antioxidant.[20]

CONCLUSION

The aim of this work is to improve the effect of leaf extract by fractionation with several objectives to evaluate the antihyperglycaemic activity of the total extract of leaves of *B. ferruginea* and its fractions on healthy mice in state carbohydrate overload as well as antioxidant activity *in vitro* after a phytochemical study and a determination of the chemical components. Our results showed that the total extract (200 mg /kg body weight) has no effect on OGTT despite antioxidant activity. These results confirm those of previous authors who showed that the total leaf extract did not have antihyperglycemic activity. By contrast, one of the fractions of the total extract obtained by separation technique in cold alcohol, the supernatant, administered at a dose of 100 mg/kg of body weight showed a significant antihyperglycemic effect on OGTT; this fraction is the richest in total phenol, flavonoids and has a stronger antioxidant activity. These results can justify the use of *B. ferruginea* leaves in the treatment of diabetes in traditional medicine.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

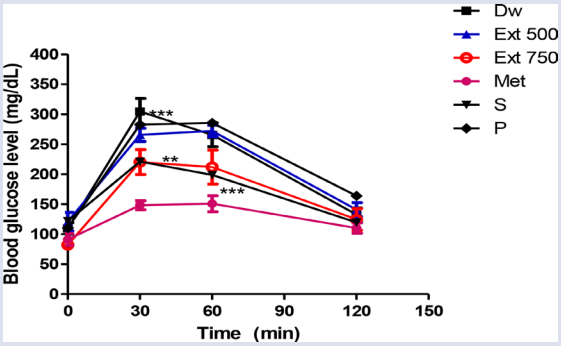
ABBREVIATIONS

OGTT: Oral glucose tolerance test; **DPPH:** 2,2'-diphenyl-1-picrylhydrasyl; **AAPH:** 2,2'-azobis (2-methylpropionamidine dihydrochloride; **PBS:** Phosphate Buffered Saline.

REFERENCES

1. Kumar S, Kumar V, Prakash O. Antidiabetic and hypolipidemic activities of *Dillenia indica* extract in diabetic rats. Journal of Chinese Integrative Medicine. 2011;9(5):570-4.
2. Karou SD, Tchacondo T, Tchibozo DMA, Abdoul-Rahaman S, Anani K, Koudouvo K, *et al*. Ethnobotanical study of medicinal plants used in the management of diabetes mellitus and hypertension in the Central Region of Togo. Pharm Biol. 2011;49(12):1286-97.
3. Tchacondo T, Karou SD, Batawila K, Agban A, Ouro-Bang'na K, Anani KT, *et al*. Herbal remedies and their adverse effects in Tem tribe traditional medicine in Togo. Afr J Tradit Complement Altern Med. 2011;8(1):45-60.
4. Bandawane D, Juvekar A, Juvekar M. Antidiabetic and antihyperlipidemic effect of *Alstonia scholaris* Linn bark in streptozotocin induced diabetic rats. Indian J Pharm Educ Res. 2011;45(2):114-20.
5. Kumar S, Malhotra R, Kumar D. Antidiabetic and free radicals scavenging potential of *Euphorbia hirta* flower extract. Indian J Pharm Sci. 2010;72(4):531-3.
6. Ramachandran V, Mandal D, Payyavala U, Sangai PD, Muthureddy NS, Shanish A, *et al*. Hypoglycemic, antioxidant and hypolipidemic activity of *Asparagus racemosuson* in streptozotocin induced diabetic in rats. Adv Appl Sci Res. 2011;2(3):179-85.
7. Bakoma B, Diallo A, Eklu-Gadegbeku K, Agbonon A, Yoro SY, Aklikokou K, *et al*. Evaluation de l'activité antihyperglycémiante de *Holarrhena africana*, *Strophantus hispidus* et *Bridelia ferruginea*, plantes utilisées dans le traitement du diabète en médecine traditionnelle au Togo. J Rech Sci Univ Lome. 2012;14(1):147-56.
8. Cimanga K, DeBruyne T, Apers S, Dieters L, Totte J, Kambu K, *et al*. Complement-inhibiting constituents of *Bridelia ferruginea* stem bark. Plant Med. 1999;65(3):213-7.
9. Bakoma B, Berké B, Eklu-Gadegbeku K, Agbonon A, Aklikokou K, Gbeassor M, *et al*. Acute and sub-chronic (28 days) oral toxicity evaluation of hydroethanolic extract of *Bridelia ferruginea* Benth root bark in male rodent animals. Food Chem Toxicol. 2013;52:176-9.
10. Kadébé ZT, Metowogo K, Bakoma B, Lawson-Evi SP, Eklu-Gadegbeku K, Aklikokou K, *et al*. Antidiabetic Activity of *Plumeria Alba* Linn (*Apocynaceae*) Root Extract and Fractions in Streptozotocin-Induced Diabetic Rats. Trop J Pharm Res. 2016;15(1):87-94.
11. Shetty K, Curtis OF, Levin RE, Wikowsky R, Ang W. Prevention of verification associated with *in vitro* shoot culture of oregano (*Oreganum vulgare*) by *Pseudomonas* spp. J Plant Physiol. 1995;147(3-4):447-51.
12. Ayoola GA, Ipav SS, Solidiya MO, Adepoju-Bello AA, Coker HAB, Odugbemi O. Phytochemical screening and free radical scavenging activities of the fruits and leaves of *Allanblackia floribunda* oliv (*Guttiferae*). Inter J health Res. 2008;1(2):81-93.
13. Dabire C, Nebie RHC, Belanger A, Nacro M, Sib FS. Effet du séchage de la matière végétale sur la composition chimique de l'huile essentielle et l'activité antioxydante d'extraits d'*Ocimum basilicum* L. Inter J Biol Chem Sci. 2011;5(3):1082-96.
14. Yang Y, Wan-Shun L, Bao-Qin H, Hai-Zhou S. Antioxidative properties of a newly synthesized 2-glucosamine-thiazolidine-4(R)-carboxylic acid (GlcNH2-Cys) in mice. Nutr Res. 2006;26(7):369-77.
15. Thomford KP, Yeboah R, Thomford AK, Edoh DA, Mensah MLK, Appiah AA. Inter J Herb Med. 2015;2(5):43-5.
16. Diatewa M, Badila SC, Coffi HAT, Abena AA. Hypoglycemic and antihyperglycemic effects of diethyl ether fraction isolated from the aqueous extract of the leaves of *Cogniauxia podoleana* (Baillon) in normal and alloxan induced diabetic rats. J Ethnopharmacol. 2004;92(2-3):229-32.
17. Suba V, Murugesan T, Bhaskara RR, Lopamudra G, Pal M, Subhash CM, *et al*. Antidiabetic potentiel of *Barheria lupulinaextract* in rats. Fitoterapia. 2004;75(1):1-4.
18. Kamiya K, Hamabe W, Harada S, Murakami R, Tokuyama S, Satake T. Chemical constituents of *Morinda citrifolia* roots exhibit hypoglycemic effects in streptozotocin-induced diabetic mice. Biol Pharma Bull. 2008;31(5):935-8.
19. Dacosta E. Les phytonutriments bioactifs. Yves Dacosta (Ed). Paris. 2003;317.
20. Popovici C, Saykova I, Tylkowski B. Evaluation de l'activité antioxydante des composés phénoliques par la réactivité avec le radical libre DPPH. Rev Génie Industr. 2010;4:25-39.

GRAPHICAL ABSTRACT



SUMMARY

- These results confirm those of previous authors who showed that the total leaf extract did not have antihyperglycemic activity. By contrast, one of the fractions of the total extract obtained by separation technique in cold alcohol, the supernatant, administered at a dose of 100 mg/kg of body weight showed a significant antihyperglycemic effect on OGTT. Phytochemical screening of the supernatant revealed the presence of phenols, flavonoids and condensed tannins. The supernatant also showed more pronounced antioxidant activity than the total extract.

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