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CHEMICAL COMPONENTS AND EFFECT ON STREPTOZOTOCIN INDUCED DIABETES OF *BRIDELIA FERRUGINEA* BENTH (EUPHORBIACEAE) ROOT BARK ETHYL ACETATE FRACTION

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ABSTRACT

The root of *Bridelia ferruginea* is traditionally used as a treatment for type 2 diabetes. The objective of this study was to evaluate the effect of *Bridelia ferruginea* root bark ethyl acetate fractions (EA) on streptozotocin-induced diabetes and identify some chemical compounds. Ethyl acetate fraction (25 and 50 mg/kg/day) was administrated to streptozotocin-induced (60 mg/kg) diabetic mice for 3 weeks. Oral glucose tolerance test and biochemical parameters were estimated at the end of the study. Chemical composition of EA was analysed using HPLC/DAD/MS and ^{H}NMR. The level of fasting blood glucose, triglycerides (TG), total cholesterol (TC) insulin and glucose intolerance in streptozotocin-induced diabetic mice were significantly ($P<0.01$) reduced after daily oral administration of ethyl acetate fraction at the dose of 50mg/kg/day (EA_{50}) for 21 days compared to diabetic control mice. EA_{25} showed only a significant ($p<0.01$) reduction of TG. After chemical analysis, Epigallocatechin (EGC), Epigallocatechin gallate (EGCG) and derivates were identified from the ethyl acetate fraction of *Bridelia ferruginea*. In conclusion these catechins and derivates can be some antidiabetic compounds of this plant according to their properties in the literature.

KEYWORDS: ***Bridelia ferruginea*, Epigallocatechin, Streptozotocin, Diabetes, HPLC/MS and NMR^{H}**

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INTRODUCTION

Diabetes mellitus (DM) is one of the most severe and incurable metabolic disorders characterized by hyperglycemia as a result of a relative, or an absolute, lack of insulin secretion and/ or insulin action on its target tissue. [1] Besides hyperglycemia, several other symptoms, including hyperlipidemia, are involved in the development of microvascular complication of diabetes, which are the major causes of morbidity and death. [2] There are mainly two types of diabetes, type 1 and type 2. Type 1 diabetes is known as insulin-dependent-diabetes-mellitus (IDDM), and results from a cellular mediated autoimmune destruction of the β cells of the pancreas.[3] Type 1 Diabetes commonly occurs in child-hood and adolescence, but can occur at any age. This form of the disease may account for 5–10% of all cases of diabetes. [4] Type 2 diabetes which is responsible for more than 90% of all diabetes patients and previously referred to as non-insulin dependent diabetes mellitus (NIDDM) Nor adult-onset diabetes, is a term used for individuals who have insulin resistance and usually have relative insulin deficiency.[3] The risk of developing this form of diabetes increases with age, obesity and lack of physical activity. Obesity and type 2 diabetes are closely correlated. Most of the currently available synthetic chemical antidiabetic agents have low rates of response and remission and even severe adverse effects because the mechanism of DM is quite complex. Accordingly, it is necessary to research and develop more effective hypoglycemic agents with lower adverse effects.[5] In our previous studies, the effects of *Bridelia ferruginea* hydroethanolic extract were proven on some parameters of metabolic syndrome in type 2 diabetes, [6] there was lack of apparent toxicity, acute or sub-chronic, at doses greater than those that induce an effect in animal disease models.[7] The ethyl acetate (EtOAc) soluble fraction of the hydroethanolic extract from the roots of *Bridelia ferruginea* were found to be the most active fraction.[8] To the best of our knowledge, the active ingredients with antidiabetic activity and their probable mode of action have not been investigated so far. The present study was designed to identified some compounds of *Bridelia ferruginea* root bark ethyl acetate fraction using HPLC/DAD/MS, ^{H}NMR and its effect in streptozotocin induced type 2 diabetic mice.

MATERIALS AND METHODS

Plant material

The roots of *Bridelia ferruginea* were collected in July 2012 from Tsévié area, 35 km North East of Lomé (Togo). Botanical authentication was confirmed by the Department of Botany, University of Lomé, where a voucher specimen of *B. ferruginea* was deposited at the herbarium (TG 03068).

Animals

Male Swiss mice (BW 30-35 g) purchased from Elevage Janvier (France) were maintained under standard conditions with a 12h light/dark cycle and had free access to standard laboratory diet and water. Prior to initiation of dosing, all mice were acclimated for 7 days. After acclimatization, mice were randomized to different groups on the basis of their body weights using an electronic scale (Tefal, Ecully, Fance). Principles of laboratory animal care as described in the European Community guidelines were followed (Official Journal of European Union L197 vol. 50, July 2007). This study was approved by the ethical committee on animal experimentation of University of Bordeaux.

Extraction and fractionation

The air-dried and powdered root bark of *Bridelia ferruginea* (1230 g) were sliced and macerated in 9000 ml ethanol-water (80:20) three times for 72 h at room temperature. The extract was then evaporated under vacuum (40°C). The residue (172 g) was dissolved in distilled water and partitioned three times with hexane (3X400 mL), dichloromethane DCM (3X400 mL) and ethyl acetate EtOAc (4X400 mL). These three phases were evaporated under reduced pressure and total extracts of hexane (1.6 g), DCM (1.1 g), EtOAc (10.32 g) and remaining aqueous phase (157 g) were obtained. EtOAc (500 mg) was fractioned by a gel chromatography (Sephadex LH20 Merck, 5x 50 cm) using MeOH/H_2O (from 20:80 to

100:0). The solvent system yields 10 fractions which were combined into 4 main fractions A ($MeOH/H_2O$, 20:80; 800 ml ; 81 mg), B ($MeOH/H_2O$, 20:80; 200 ml ; 33 mg), C ($MeOH/H_2O$, 50:50; 500 ml ; 67 mg), D ($MeOH/H_2O$, 20:80; 500 ml ; 62 mg) after thin layer chromatography (TLC) which was performed on house TLC plates coated with silica gel 60 F254 and aluminum sheets (5×10 cm,0.2 mm thick, Merck, Germany). The four fractions were subjected to HPLC analysis performed by Heweltt Packard HP 1100 series rapid resolution LC system (USA) equipped with a vacuum degassed, auto sampler, diode array detector (DAD),a quaternary pump and a reversed-phase C18 analytical column (5 x 250 mm, 5µm). All solvents were filtered with a 0.45 µm filter disk. A gradient elution was carried out using the following solvent system: mobile phase A, water/acetic acid (97.5:2.5, v/v); mobile phase B, Methanol (100%). The gradient program was as follows: 0-15 min, 100% A; 15-45 min, 70% A; 45-65 min, 65% A; 65-70 min, 40% A;70-90 min,100% B. the Column temperature was maintained at 25°C and the injection volume was 10 µl. The DAD detector was monitored at 280, 320, 370 and 510 nm.

^{H}-NMR

After HPLC and TLC analysis, fraction B appearing to be a pure compound was subjected to ^{1H}-NMR spectra using BRUKER 300 (Germany) spectrometer operating at a temperature of 25 °C with a 5 mm probe. The solvent used was MeOD and spectra was referenced relative to internal TMS (Tetramethylsilane). Sample concentration was 5 mg/ml.

HPLC-ESI-MS analysis

Fractions C and D were subjected to HPLC-ESI-MS analysis. Samples were prepared in a mixture of MeOH/water (50/50; v/v) and filtered through a 0.22 µ MILLEX GV syringe filter (Millipore, MA, USA) prior to injecting into the HPLC system. All analysis was performed on Bruker Esquire 3000 plus HPLC system (Germany), including binary solvent manager, sample manager, column compartment and photo diode array (DAD) detector scanning from 200 to 600 nm and a C18 column (100mm × 2.1 mm i.d, 1.7 µm) was used. The column temperature was maintained at 25°C, samples were separated using a gradient mobile phase consisting of 0.1% formic acid in water (A) and acetonitrile (B). The gradient elution was: 0-60 min: 95% A, 5%B; 60-65 min: 70% A, 30% B; 65-75 min: 100%B; 75-80 min: 95%A, 5%B. The flow rate was 1mL. min^{-1} and a volume of 10 µL of sample was injected. Separation of phenolics was monitored by PDA detector at 254 and 280 nm. A mass spectrometer with an Electrospray Interface (ESI), operating in a full scan MS mode from 100 to 1500 m/z was used. Samples were analyzed in both positive and negative ion modes. ESI –MS parameters were as follows: potential of the ESI source, 3 KV; capillary temperature, 300°C. Identifications were based on chromatography and MS^2 fragmentation patterns. Data was analysed using Bruker Daltonics Data Analysis 3.2 software.

Diabetes induction and treatment

Diabetes was induced in animals with a single streptozotocin (STZ) intraperitoneal injection at dose of 60 mg/kg weight, in 0.1 M citrate buffer, pH 4.5 [9]. Group 1 only received the same volume of STZ vehicle (citrate buffer). After a week of delivering STZ, mice with blood glucose above 200 mg/dl were included in the study. Thirty five male Swiss (30–35 g) mice were randomly divided into 5 groups of 7 animals each, 24 h later animals were treated as follow: group 1 normal control (NC) and 2 or diabetic control (DC) received distilled water as vehicle of substances; group 3, 4 and 5 also diabetic rats received respectively ethyl acetate fraction (EA) 25mg/kg/day, 50mg/kg/day and metformine 50mg/kg/day . Every treatment was delivered daily by gavage for 21 days, blood glucose level was measured on the first, 7^{th}, 14^{th} and 21^{st} day after 12 hours fasted using Free style Papillon Glucometer.

Oral glucose tolerance test (OGTT) in STZ induced diabetic mice

OGTT was carried out after 21 day of treatment, during which the animals were fed with normal diets. The mice were fasted over night; glucose (2 g/kg) was fed 30 min after the administration of drugs, blood was withdrawn from tail-vein at 0, 30, 60 and 120 min after glucose loading. Blood glucose

level was measured using Free style Papillon Glucometer.

Estimation of biochemical parameters

Blood was collected by heart puncture from anesthetized (pentobarbital 50mg/kg i.p.) mice in fasting condition. The samples were centrifuged at 3000 g for 15 min and the plasma obtained was aliquoted and frozen for blood glucose, plasma total cholesterol (Ch), triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT) using commercial kit (Biomerieux,Marcy l'Etoile, France). Insulin concentrations were measured from frozen plasma samples using the Rat and Mice Insulin Enzyme Immunoassay Kit (SPI-BIO, Montigny Le Bretonneux, France).

Statistical analysis

The results were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test to evaluate significant differences between groups. $P< 0.05$ was considered statistically significant. All statistical analyses were carried out using the statistical package (Graph Pad Software Inc., USA).

RESULTS

^{H}NMR: The NMR spectrum value was between 2.74 and 6.53 ppm indicating the presence of signals in characteristic zones of aliphatic protons chemical shifts: doublet of doublet at 2.76 ppm with coupling constants of 16.5 and 4.4 Hz (H4β); doublet of doublet at 2.74 ppm with coupling constant of 16.5 and 2.8 Hz (H4α); Multiplet at 4.18 ppm (H3). The values of the coupling constants of 2.8 Hz and 4.4 correspond to a proton H-3 position is a β 2,3-cis stereochemistry of the pyran ring. The stereochemistry of the analysis cannot be based on the H2 proton signal which is superimposed on that of the water signal. In the aromatic region, are observed: doublet at 5.92 coupling constant of 2.3 Hz (H8); doublet at 5.94 ppm with coupling constant of 2.3 Hz (H6); singlet at 6.53 ppm (H2 'and H6'). The two protons of the cycle A ring in the ortho position of two oxygens are more shielded than those of cycle B. The singlet integrating for two protons is characteristic of an aromatic ring of the type which comprises gallocatechol hydroxyls 3', 4' and 5'of cycle B (Table 1).
These ^{H}NMR data allow us to propose epigallocatechin as compound isolated in fraction B from the root bark of *Bridelia ferruginea.*

Table 1
^{H}NMR data of subfraction B of B. ferruginea

Proton	dppm	J Hz
H2', H6'	6.53	s
H6	5.94	d 2.3
H8	5.92	m d 2.3
H2	4.8 /H_20	m
H3	4.18	m
H4β	2.76	dd 16.5 ;4.4
H4α	2.74	dd 16.5; 2.8

Tentative identification of the chemical compounds using HPLC/MS

Representative HPLC chromatograms detected at 254 and 280 nm are shown in figure 1. The numbers written on this chromatogram (number 1 to 47) indicate component pick. The retention times (t_R), deprotonated/protonated molecules ([M–H]/[M+H]) and MS2 major fragment ions (NI/PI) are listed in Table 2. Peak identification was based on analysis of the data in the table and comparison with data in previously published literatures. The identification process was similar to that of Lin et al., [10]. All the results are listed in Table 2.

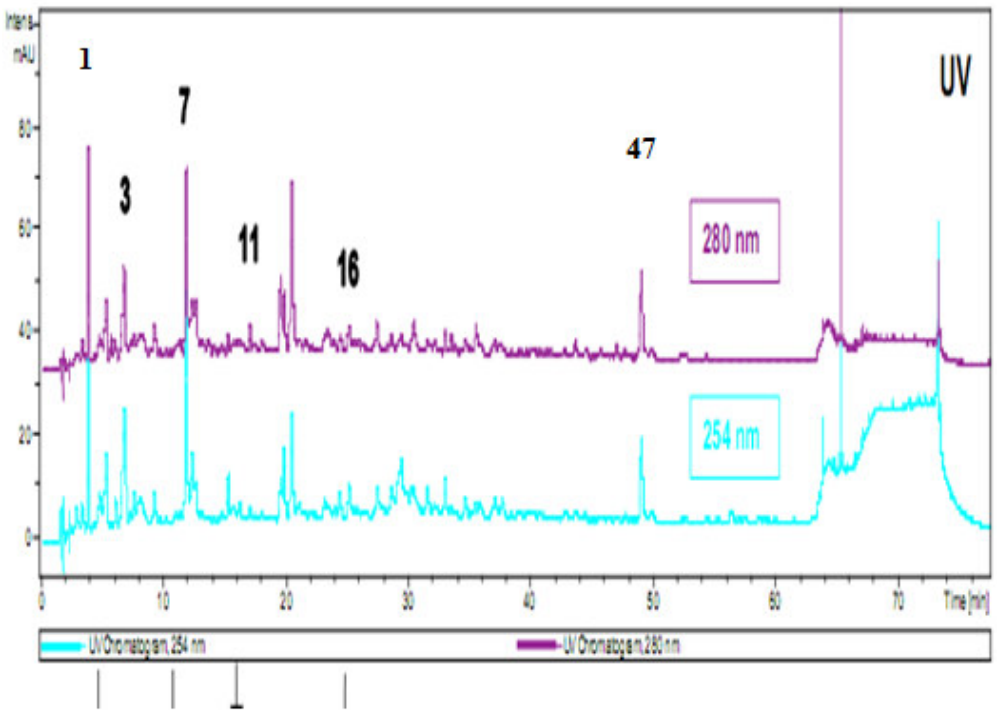

Figure 1
Total ion current chromatogram of Bridelia ferruginea obtained Using coupled HPLC and ESI-MS

Table 2
identified components in ethyl acetate fraction of Bridelia ferruginea

N°	T_R (min)	$[M-H]^-$(m/z)	$[M+H]^+$(m/z)	MM	(PI/NI) MS^2(m/z)	Components
1	2,1		611,4	610	317, 443/305, 423, 591	Dimer flavanol
2	3.4	609.2	611.2	610	317, 443/305, 423	Dimer flavanol
3	3,8	609.2	915.5	914	609, 305, 643,747/- - -	Unknown
4	4,8		611.4	610	443/305,423	Dimer Flavanol
5	6,0	304.9		306	219, 136, 125/- - -	(-) epigallocaatéchin
6	6,3	611.4	612		- - -/305	
7	7, 0	609.5	611.4	610	443, 287/305, 423, 591	Dimer flavanol
8	7,2	593,4	595.4	594	247, 427/305, 423	Kaempferol 3-Diglucosides
9	8,3		611,4	610	317, 443, 593/- - -	Dimer flavanol
10	8,7	593,4	595,2	594	291, 425/289, 425	Catechin- Gallocatechin
11	10,2	897,2	899,6	898	730/195, 437,731	Digallocatechin-catechin
12	10,2	609,3	611,4	610	287, 427, 593/305, 423	Dimer flavanol
13	10,5		595,5	594	247, 417, 577/- - -	Diglucosylapigenin
14	11,2	305,2	307,1	306	289/179, 305	gallocatechin
15	11,2	611,4	612			
16	11,6	593,4	595,4	594	- - -/305, 911	Unknown
17	12, 2	761, 1	763, 5	762	287, 425, 595/305, 623, 509	Unkwon
18	13, 1	761,4	763,4	762	305, 465, 595/423, 575	unknown
19	15,4	609,2	611,4	610	317, 425/305, 423	Dimer flavanol
20	17,3	745,4	747,5	746	427, 595/423, 575, 727	gallocatechin-catechin gallate
21	19,2		777,5	776	289, 609, 759/- - -	
22	19,4	633		634	463, 301/- - -	Strictinin
23	20	457,3	459,4	458	305,193,169,125	Epigallocatechin 3-0-gallate
24	23,6	729,5	731,5	730	635/195,446	Proanthocyanidin
25	24,7	759,5	761,5	760	299, 425, 593/423, 591	Proanthocyanidin
26	25,6	761,3	763,2	762	168, 305, 689/305, 423, 575	
27	25,7	789,2	791,5	790	316, 443, 623/169, 423, 621	Myrcetine 3-0 glucoside
28	34,8	597,4		598	- - -/ - - -	unknown
29	36,8		461,4	460	395/- - -	unknown
30	37,1	547,5			385/- - -	Unkown
31	37,2		387,1	386	219/- - -	Unknown
32	37,4		347,1	346	291/- - -	
33	39,8		593,4	592	431/- - -	
34	41,2		435,4	434	190, 361/- - -	
35	42,5	739,5	741,5	740	258, 425, 573/255, 423, 571	Kaempferol 3-O-diglucoside
36	43.0	685,6		686	639, 459/- - -	Unknown
37	44.2	555,5		556	507/- - -	Unknown
38	45,4	555,5		556	165, 541, 307/- - -	Unknown
39	46.6	591.5		592	383/- - -	Unknown
40	47,3	621,5		622	413/- - -	Unknown
41	47,5		594,5	593	247, 415/- - -	Unknown
42	48,5		577,2	578	247, 415/- - -	Unknown
43	48,9	621,6		622	413/- - -	Unknown
44	49,0		577,5	578	247, 415/- - -	Unknown
45	49,5	585,5		586	537, 371/- - -	Unknown
46	63,3	827,6		828	413/- - -	Unknown
47	63.3		247	248	- - -/- -	Unknown

(PI/NI) : Positive Ion / Negative Ion

Effect of substances on STZ-induced diabetic mice blood glucose level during the experiment

The anti-hyperglycemic effect of ethyl acetate fraction was evaluated in STZ-induced diabetic rats. Blood glucose level was measured in normal and experimental mice on day 0, 7, 14, 21 of drug treatment. Administration of STZ (60 mg/kg) led to over 2.8 fold elevation of blood glucose level in a time-dependent manner (p<0.001) compared to normal mice. STZ-induced diabetic mice treated with ethyl acetate fraction (50mg/kg/day) or metformin (50mg/kg/day) orally for 3 weeks showed a significant (p<0.01) decrease in blood glucose level compared to diabetic control group. But ethyl acetate fraction at the dose of 25mg/kg/day did not reduce significantly blood glucose level at the 14^{th} and 21^{st} day. Normal control mice did not exhibit any significant alteration in their glucose levels through the duration of the experiment (Table 3).

Table 3
Effect of Ethyl acetate fraction on blood glucose level during the experiment

Groups	Blood glucose (mg/dl)			
	Day 0	Day 7	Day 14	Day 21
NC	81±3.1	86±2.8	88±3.6	85±3.1
DC	226±21	241±17###	282± 28###	317±25###
EA_{50}	213±12	222±24	210±13***	225±26**
EA_{25}	247±26	249±35	257±19*	271±23
MET	250±24	218±13	208±9***	182±14***

*NC: Normal Control; DC: Diabetic Control; EA_{50}: Diabetic animal treated with Ethyl acetate Fraction at the dose of 50 mg/Kg/day; EA_{25} : Diabetic animal treated with Ethyl acetate Fraction at the dose of 25 mg/Kg/day; Met: Diabetic animal treated with Metformin 50mg/Kg/day. The data were expressed as mean±S.E.M. (n = 7) and evaluated by ANOVA followed by Tukey's test at 5% * P < 0.05 ; ** p < 0.01; ***P<0,001 (vs DC); ## P < 0.01 ; ### P < 0.001 (vs NC).*

Oral glucose tolerance test

Administration of glucose (2 g/kg,) produced significant (p <0.001) increased in blood glucose level in diabetic control mice compared to normal control mice during 120 min. Treatment with ethyl acetate fraction at the dose of 50mg/kg/day (EA_{50}) and metformin significantly (p<0.001) reduced blood glucose level at 30 min, 60 min and 120 min after glucose load compared to diabetic control mice. Respectively 321±34, 291±26, 278±9 mg/dl for EA_{50}; 318±13, 249±9, 190±11(mg/dl) for MET and 441±17, 382±8, 412±10 mg/dl for DC. EA_{25} showed a significant (P< 0.05) reduction of blood glucose level at 120 min only (Table 4).

Table 4
Effect of Ethyl acetate fraction on oral glucose tolerance test in STZ-induced diabetic and treated mice

Groups	Blood glucose concentration (mg/dl)			
	0 min	30 min	60 min	120 min
NC	85±3.1	146±2.8	181±4.5	162±5
DC	317±25	441±17###	382±8###	412±10###
EA_{50}	225±26	321±34**	291±26 **	278±9
EA_{25}	221±13	399±25	365±15	327±18*
MET	182±24	318±13**	249±9**	190±11**

*NC: Normal Control; DC: Diabetic Control; EA_{50}: Diabetic animal treated with Ethyl acetate Fraction at the dose of 50 mg/Kg/day; EA_{25}: Diabetic animal treated with Ethyl acetate Fraction at the dose of 25 mg/Kg/day; Met: Diabetic animal treated with Metformin 50mg/Kg/day. The data were expressed as mean±S.E.M. (n = 7) and evaluated by ANOVA followed by Tukey's test at 5% * P < 0.05 ; ** p < 0.01; ***P<0,001 (vs DC); ## P < 0.01 ; ### P < 0.001 (vs NC).*

Effect of substances on plasma biochemical parameters in control, STZ-induced diabetic and treated mice

At the end of the study, fasting blood glucose level of STZ-diabetic control (271±16mg/dl) was significantly (p< 0.001) higher compared to normal control group (129±34mg/dl). Ethyl acetate fraction and metformin treated groups showed significant (p< 0.01) decrease in blood glucose level over the 21 days of treatment compared to STZ-diabetic control. Plasma triglycerides and total cholesterol levels at the end of the study were significantly (p< 0.001) higher in the diabetic control group (167±13; 131±4.9 mg/dl) than in normal control group (121±5.6; 117±3.9). Diabetic treated groups showed a significant (p< 0.05) reduction of plasma cholesterol and triglycerides level compared to diabetic control group. Only ethyl acetate fraction at the dose of 50 mg/kg (EA_{50}) showed a significant (p< 0.05) reduction of plasma ALT level (48±7 UI/L) compared to diabetic control group (71±6.1 UI/L). AST level was significantly (p <0.001) higher in diabetic control group (286±42 UI/L) compared to normal control group (114±7.3 UI/L); treated groups showed a significant (p <0.001) reduction of plasma AST, neither ethyl acetate fraction at dose of 25 mg/kg/day treated group (EA_{25}). Plasma insulin concentrations were significantly lower in diabetic control group (0.47±0.05 ng/ml) compared to normal control group (0.85±0.2 ng/ml) but only Ethyl acetate fraction at the dose of 50 mg/kg and metformin treated groups showed significant (p <0.05) increase of plasma insulin concentrations compared to diabetic control group (Table 5).

Table 5

Effect of Ethyl acetate fraction on plasma biochemical markers, insulin index in control, diabetic and treated mice

Parameters	NC	DC	EA_{50}	EA_{25}	Met
Plasma glucose (mg/dl)	129±34	271±16$^{\#\#\#}$	228±26**	257±18*	144±28***
Insulin (ng/ml)	0.85±0.1	0.47±0.05$^{\#\#}$	0.65±0.1*	0.48±0.08	0.56±0.06*
AST (UI/L)	114±7.3	286±42$^{\#\#\#}$	155±15**	238±41	166±21**
ALT (UI/L)	64±6.8	71 ±6.1	48±7*	65±10	63±7.7
TG (mg/dl)	121±5.6	157±13$^{\#\#}$	118±9**	137±24*	93±5**
Ch (mg/dl)	117±3.9	131±4.9$^{\#\#}$	130±8.7**	134±10*	104±10**

NC: Normal Control; DC: Diabetic Control; EA_{50}: Diabetic animal treated with Ethyl acetate Fraction at the dose of 50 mg/Kg/day; EA_{25} : Diabetic animal treated with Ethyl acetate Fraction at the dose of 25 mg/Kg/day; Met: Diabetic animal treated with Metformin 50mg/Kg/day. The data were expressed as mean±S.E.M. (n = 7) and evaluated by ANOVA followed by Tukey's test at 5% * P < 0.05 ; ** p < 0.01; ***P<0,001 (vs DC); $^{\#\#}$ P < 0.01 ; $^{\#\#\#}$ P < 0.001 (vs NC).

DISCUSSION

Ethyl acetate fraction was tested in diabetic mice. To induced diabetes *in vivo*, we used Streptozotocin (STZ), a nitrosourea analogue produced by Streptomyces achromogenes, it is a broad-spectrum antibiotic with diabetogenic properties [11]. In this study, a single high-dose of STZ (60 mg/kg) significantly induced hyperglycemia accompanied by hypoinsulinemia. Oral administration of ethyl acetate fraction, and metformin for 21 days exhibited a marked anti-hyperglycemic activity in STZ-induced-diabetic mice by lowering the blood glucose levels and showed significant improvement in glucose tolerance. This effect may be attributed in part to a decrease in the rate of intestinal glucose absorption, stimulation of peripheral glucose utilization or enhancing glycolytic and/or glycogenic process. STZ

induced diabetic mice showed also an hypoinsulinemia, EA treatment lead to a significant increase of plasma insulin level suggesting that there were more functional cells in the EA treated group. This may explain the higher level of insulin that was found. Hypercholesteremia and hypertriglyceridemia are primary factors involved in the development of atherosclerosis and coronary heart disease which are the secondary complications of diabetes [12]. Ethyl acetate fraction and metformin significantly reduced serum triglycerides and total cholesterol in STZ-diabetic mice, suggesting that EA modulate blood lipid abnormalities. Liver is the vital organ of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites. ALT, AST and are reliable markers of liver function [13]. An increase in the activities of ALT, AST in plasma might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream which gives an indication of hepatotoxic effect of STZ Ramesh et al., [14]. Treated diabetic mice showed a reduction of these enzymes activities in plasma compared to the diabetic untreated mice and consequently alleviated liver damage caused by STZ-induced diabetes. Significant reductions in the activities of these enzymes in treated diabetic mice indicated the hepatoprotective role in preventing diabetic complications. EA_{50} was very active than EA [25], this suggest that ethyl acetate fraction of *B. ferruginea* act in dose dependent manner. The ethyl acetate fraction was subjected to a LPLC on Sephadex LH20 gel holder and 10 subfractions were obtained. The subfractions after analysis by TLC and HPLC according to Cai et al., [15] proved the presence of flavanol structure. Subfraction B was pure and subjected to NMR^H which data compared to those obtained by Davis et al., [16] in similar conditions indicate that this subfraction was epigallocatechin. 33 mg of epigallocatechin was obtained after 500 mg of ethyl acetate fraction deposited on the column. This indicate that epigallocatechin can be one of major compounds of ethyl acetate fraction of *Bridelia ferruginea* root bark. According to the literature, it is the first report of isolating epigallocatechin from the root of *Bridelia ferruginea*. HPLC coupled to mass spectrometry was conducted to identify the presence of other molecules. The analysis of mass spectra obtained based on some works [10, 17-19] allow to suggest the presence of catechins derivates and flavonoids dihexoside. Indeed, the spectra we obtained with compounds 5, 8, 10, 11, 14, 20, 23 and 27 are respectively similar to those of gallocatechin, kaempferol 3O-diglucoside, gallocatechin-catechin, digallocatechin-catechin, epigallocatechin, gallocatechin- catechingallate, epigallocatechin3-0-gallate and myrcetine3-0 glucoside. Spectra obtained with the compounds 22 and 35 respectively, have also been attributed to those of strictinin and kaempferol3-O-diglucoside by some authors [20, 21]. Catechins are known for their hypoglycemic properties and act to control diabetes and are powerful antioxidants [22-25], they increase the sensitivity of cells to insulin, prevent glucose intolerance [26], inhibit the lipogenic enzymes (acetyl CoA carboxylase, AG synthetase), appetite and inhibit fat absorption [27-32]. These data suggest that catechins can be responsible for the activity of the ethyl acetate fraction and indicate the mechanism by which this fraction can be effective in the treatment of type 2 diabetes.

CONCLUSION

Ethyl acetate fraction of *B. ferruginea* root bark improves STZ induced diabetes in mice. Phytochemical study allowed us to identify predominant molecules as epigallocatechin and derivatives. These molecules have antidiabetic properties by well-known mechanisms, suggesting that the antidiabetic activity of *B. ferruginea* root bark can be due to the presence of these compounds. This study provided scientific basis for its use in folk medicine to treat diabetes.

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CONFLICT OF INTEREST

Conflict of interest declared none

REFERENCES

1. Hahm SW, Park J, Son YS. . Opuntia humifusa stems lower blood glucose and cholesterol levels in streptozotocin-induced diabetic rats. Nutr Res, 31: 479–487, (2011)
2. Taskinen R**.** Diabetic dyslipidaemia: from basic research to clinical practice. Diabetologia, 46:733–749, (2003)
3. Gavin JR, Alberti, K, Davidson, M. B, DeFronzo, R. A, Drash, A, Gabbe, S G. et al. Report of the expert committee on diagnosis and classification of diabetes mellitus. Diab Care, 20: 1183-1197, (1997)
4. Stumvoll M, Goldstein BJ, & van Haeften, TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet, 365: 1333–1346, (2005)
5. Sun JE, Ao ZH, Lu ZM, Xu HY, Zhang XM, Dou, WF, Xu H. Antihyperglycemic and antilipid peroxidative effects of dry matter of culture broth of Inonotus obliquus in submerged culture on normal and alloxan-diabetes mice. J Ethnopharmacol,118: 7–13, (2008)
6. Bakoma B, Eklu-Gadegbeku K, Agbonon A, Aklikokou K, Bassene E, Gbeassor M. Preventive effect of *Bridelia ferruginea* Benth against high-fructose diet induced glucose intolerance, oxidative stress and hyperlipidemia in male Wistar rats. J Pharmacol Toxicol, 3: 249–257, (2011)
7. Bakoma B, Eklu-Gadegbeku K, Agbonon A, Aklikokou K, Bassene E, Gbeassor M, et al.. Acute and sub-chronic (28 days) oral toxicity evaluation of hydroethanolic extracts of *Bridelia ferruginea* Benth root bark in male rodent animals Food Chem. Toxicol, 53: 176-179, (2013)
8. Bakoma B, Berké B, Eklu-Gadegbeku K, Aklikokou K, Bassene E, Gbeassor M. Effect of *Bridelia ferruginea* Benth (Euphorbiaceae) ethyl acetate and acetone fractions on insulin resistance in fructose drinking mice. J. ethnopharmacol, 153: 896- 899, (2014)
9. Gupta R K, Kuma D, Kumar A, Mukesh C, Maithan R. Antidiabetic activity of *Passiflora incarnata* Linn. In streptozotocin-induced diabetes in mice. J. ethnopharmacol, 139: 801-806, (2012)
10. Lin LZ, Chen P, & Harnly J M. New phenolic components and chromatographic profiles of green and fermented teas. J Agri Food Chem, 56(17): 8130–8140, (2008)
11. Like, AA, Rossini AA.. Streptozotocin-induced pancreatic insulitis: new model of diabetes melitus. Sci, 193: 415–417, (1976)
12. Ananthan R, Latha M , Ramkumar K , Pari L , Baskar C , Bai V. Effect of Gymnema montanum leaves on serum and tissue lipids in alloxan diabetic rats. Exp. Diab. Res, 4: 183–189, (2003)
13. Ohaeri OC. Effect of garlic oil on the levels of various enzymes in the serum and tissue of streptozotocin diabetic rats. Biosci, Rep, 21:19–24, (2001)
14. Ramesh BK, Maddirala DR, Vinay KK, Shaik SF, Tiruvenkata KEG, Swapn, S., et al.. Antihyperglycemic and antihyperlipidemic activities of methanol:water (4:1) fraction isolated from aqueous extract of *Syzygium alternifolium* seeds in streptozotocin induced diabetic rats. Food Chem Toxicol, 48: 1078–1084, (2010)
15. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chiniese medicinal plants associated with anticancer. Life Sci, 74: 2157-2184, (2004)
16. Davis AL, Cai Y, Alan PD, Lewis J. R. ^{H} and ^{13C} NMR Assignments of Some Green Tea Polyphenols. Magn Reson Chem,; 34: 887-890, (1996)
17. Callemien D and Collin S. Use of RP-HPLC-ESI (–)-MS/MS to Differentiate Various Proanthocyanidin Isomers in Lager Beer Extracts. American Society of Brewing Chemists, 0215-01, (2008)
18. Zhao Y, Chen P, Lin L, Harnly JM, Liangli Y, Li Z. Tentative identification, quantitation,and principal component analysis of green puerh, green, and white teas using UPLC/DAD/MS. Food Chem, 126: 1269-1277, (2011)
19. Wang D, Lua J, Miaob A, Xie Z, Yanga D. HPLC-DAD-ESI-MS/MS analysis of

polyphenols and purine alkaloids in leaves of 22 tea cultivars in China. J Food Compos Anal, 21: 361–369 (2008)
20. Dou JP, Lee VSY, Tzen JTC, Lee MR. Identification and comparison of phenolic compounds in the preparation of oolong tea manufactured by semi fermentation and drying processes. J Agric Food Chem, 55: 7462–7468, (2007)
21. Mizukami Y, Sawai Y, Yamaguchi Y. Simultaneous analysis of catechins, gallic acid, strictinin, and purine alkaloids in green tea by using catechol as an internal standard. J. Agric Food Chem, 55: 4957-4964, (2007)
22. Kao Y H, Hiipakka RA, Liao S. Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. Endocrinology, 141: 980–987, (2000)
23. Mai TT, Chuyen, NV. Anti-hyperglycemic activity of an aqueous extract from flower buds of *Cleistocalyx operculatus* (Roxb.) Merr and Perry. Biosci. Biotechnol Biochem, 71: 69–76, (2007)
24. Yun SY, Kim S P, Song DK. Effects of (-)-epigallocatechin-3-gallate on pancreatic beta-cell damage in streptozotocin-induced diabetic rats. Eur J Pharmacol, (10) 541:115-121, (2006)
25. Fu Z, Zhen W, Yuskavage J, Liu D.. Epigallocatechin gallate delays the onset of type 1 diabetes in spontaneous non-obese diabetic mice. Br J Nutr, 105(8):1218-25, (2011)
26. Ortsäter H, Grankvist N, Wolfram S, Kuehn N, Sjöholm A, Diet supplementation with green tea extract epigallocatechin gallate prevents progression to glucose intolerance in db/db mice. Nutr Metab (Lond), 14:9-11, (2012)
27. Thielecke F. and Boschmann M. The potential role of green tea catechins in the prevention of the metabolic syndrome. Phytochemistry, (70): 11–24, (2009)
28. Roghani M. and Tourandokht B. Hypoglycemic and hypolipidemic effect and antioxidant activity of chronic epigallocatechin-gallate instreptozotocin-diabetic rats. Pathophysiol,17:55-59,2010
29. Cherniack EP. Polyphenols: Planting the seeds of treatment for the metabolic syndrome. Nutr, 27 :617–623, (2011)
30. Rains TM, Sanjiv A, Kevin M C. Antiobesity effects of green tea catechins. *J Nutr Biochem,*; 22: 1-7, (2011)
31. Sae-tan S, Grove A, Joshua, DL. Weight control and prevention of metabolic syndrome by green tea. Pharmacol Res, 64: 146-154, (2011)
32. Jibu T and Gavin T, Effect of catechin rich green tea Camellia sinensis extracts on obesity triggered hepatic steatosis in rats fed with HFCS. Int J Pharm Bio Sci 4(4):n525–532,(2013).