

Antioxidant and Glucose Lowering Effects of Hydroethanolic Extract of *Baillonella toxisperma* Pulp

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Abstract

Hyperglycemia and oxidative stress play an important role in the pathogenesis of diabetes. Their management is a key point in the prevention and treatment of this disease which is a potential cause of mortality in the world. We evaluated the antioxidant and glucose lowering effects of hydroethanolic extract of *Baillonella toxisperma* pulp. The total polyphenol and flavonoid contents were determined and the antioxidant activity was evaluated by 3 mechanisms: scavenging by DPPH•, ABTS• and NO• radicals; reducing property by MoO₄²⁺ and Fe³⁺ reduction; metal chelation by Cu²⁺ and Fe²⁺. Glucose adsorption capacity was evaluated followed by the capacity to promote insulino-sensitivity through glucose uptake by yeast and muscle cell assays. The hydroethanolic extract of *B. toxisperma* pulp possessed high total polyphenols and flavonoids; 459.55 µg Equivalent Gallic Acid/mg and 252.15 µg of Equivalent Catechin /mg respectively. This showed their ability to scavenge DPPH•, ABTS• and NO• radicals with SC₅₀ of 3.49, 3.24 and 4.28 mg/ml respectively. The extract also reduced MoO₄²⁺ and Fe³⁺ and chelated Cu²⁺ through inhibiting their capacity to induce hemolysis with the IC₅₀ of 3.49 mg/ml. The extract showed a high glucose binding capacity with a glucose binding percentage rise of 60 %. It increased yeast cell absorption of glucose with the increasing percentage varying from 42.97 to 56.62 %. In the muscle cells, after 30 min of administration of the extract, we also noted an increased glucose absorption with the percentage glucose reducing to 22 %. We demonstrated that hydroethanolic extract of *B. toxisperma* pulp possess antiradical, reducing, metal chelating, glucose binding and insulino-sensitivity promoting properties. These mechanisms imply that *B. toxisperma* pulp is both a good antioxidant and an antihyperglycemic, thus a potential agent in the management of diabetes and its complications.

Keywords: *B. toxisperma* pulp, polyphenols, antioxidant and glucose lowering effects, and diabetes

1. Introduction

Diabetes is a metabolic disorder of carbohydrate, lipid and protein, characterized by chronic hyperglycemia resulting from a defect in the action and/or secretion of insulin. Insulin defects are responsible for the decrease in glucose uptake that leads to intracellular hypoglycemia and extracellular hyperglycemia (Asmat, Abad & Ismail, 2016). The long-term effect of hyperglycemia in diabetes, through mechanisms such as protein glycation, the polyol and protein kinase C pathways is oxidative stress (Tangvarasittichai, 2015). Oxidative stress is characterized by the excessive production of free radicals. When produced at low doses, oxidative species play a physiological role in the human body. However, excessive production causes cellular damage, disrupting certain signaling pathways (Brand, 2016; Sies, 2015) due to the deleterious effects of free radicals on proteins, lipids and nucleic acids, oxidative stress is strongly implicated in the establishment and progression of type 2 diabetes mellitus (Ayepola, Brooks & Oguntibeju, 2014).

In view of the involvement of hyperglycemia and oxidative stress in the development of type 2 diabetes mellitus, its effective management requires a focus on these two metabolic disorders. Major antihyperglycemic mechanisms are: inhibition of digestion and absorption of dietary carbohydrates, inhibition of endogenous glucose production and glucose uptake by stimulation of insulin sensitivity (Agnaniet *et al.*, 2016) while antioxidant mechanisms include: free radical scavenging, metal reduction and chelation (Meenatchi & Jeyaprakash, 2015). Through these mechanisms, numerous synthetic antihyperglycemic agents have emerged, like metformin and Vitamin E. However, synthetic compounds are associated with many side effects (hyperglycemia, gastrointestinal disorders, liver lesions) (American Diabetes Association, 2017); and despite

their heavy presence in the pharmaceutical market, diabetes continues to spread. There is therefore need to accentuate research towards new and safer molecules. Plants are important sources of bioactive molecules, which are distributed among the different parts and essentially in the fruits where one finds considerable contents of polyphenols, endowed with few side effects notably for the edible parts. The plethora of fruits in polyphenols and especially flavonoids makes them potentially useful in the effective management of diabetes (Aslam *et al.*, 2007, Rauter *et al.*, 2009).

Baillonella toxisperma is a plant of the Sapotaceae family whose fruits are edible and its wood and oil exploited for commercial purposes (Doucet & Kouadio, 2007). Its bark is traditionally used to treat abscesses, stomach disorder, infertility in men and women, rheumatism, convulsions and malaria (Nti é-Kang *et al.*, 2013). Fungo *et al.* (2015) showed that the content of polyphenols, flavonoids and proanthocyanates in the fruit of *B. toxisperma* was 686.7, 141.1 and 28µg/mg dry matter respectively. The aim of this study was to evaluate the *in vitro* antioxidant and antidiabetic properties of hydroethanolic extracts of *Baillonella toxisperma* edible fruits.

2. Methods

2.1 Chemicals

All the chemicals used were of analytical grade and were purchased from Sigma Co., Louis, MO, USA.

2.2 Plant Material

Fruits of *Baillonella toxisperma* were harvested in Ondodo (East-Cameroon). They were identified at national herbarium. After drying in an oven at 50 °C for 3 days, the pulp was separated from the kernel. The dried material was ground to obtain powder, from which their extracts were prepared.

2.3 Preparation of Extracts

The pulp (100g) was ground and extracted by maceration for 48 h with 800 ml of hydroethanol (1:1, v/v). The resulting supernatant was filtered using Whatman #1 filter paper (Whatman International Limited, Kent, England) in a funnel and concentrated to about 10% of the original volume by a rotavapor before drying in an oven at 50 °C. The hydroethanolic extract was obtained and stored in desiccators.

2.4 Quantitative Determination of Polyphenolic Compounds

The quantitative analysis of total polyphenols and flavonoids was done.

2.4.1 Determination of Total Polyphenol content

The polyphenol content was evaluated using the method described by Singleton & Rossi (1965). To 30 µL of extract (1 mg/mL) prepared in ethanol solution, 1 mL of Folin Ciocalteu (0.2 N) solution was added. Thirty minutes after incubation at 25 °C, the absorbance was read at 750 nm using a spectrophotometer. Gallic acid was used as standard. The total polyphenol content was expressed in microgram equivalence of gallic acid/g of extract.

2.4.2 Determination of Flavonoid Content

The flavonoid content was evaluated using the method described by Aiyegoro & Okoh (2010). To 1 mL of the extract (1 mg/mL), 1 mL of aluminium chloride (10 %), 1 mL of potassium acetate (1 M) and 5.6 mL of distilled water were added. The mixture was allowed to stand at 25 °C for 30 minutes. The absorbance of the reaction mixture was read at 420 nm with a spectrophotometer. Catechin was used as the standard. The flavonoid content was expressed in milligram equivalence of catechin/g of extract.

2.5 Antioxidant Potential

The antioxidant potential of the extract was evaluated through 3 mechanisms: radical scavenging , reducing properties and metal chelation.

2.5.1 Radical Scavenging

For the scavenging activity, 2 synthetic radicals (DPPH and ABTS) and one biological radical (NO) were used.

DPPH free-radical scavenging assay

The antioxidant activity of the extract (1; 2; 3; 4 and 5 mg/ml) prepared in ethanol solution was measured in terms of radical scavenging ability, according to the DPPH method (Katalini é Milos, Musi & Boban, 2004). A volume of 50 µl of extract was introduced into 1.95 ml of a methanolic solution of DPPH (0.3 mM) and kept in the dark for 30 min. Control experiments without the extract but with equivalent amounts of methanol were conducted in a similar manner. The absorbance was then spectrophotometrically read at 517 nm. Catechin was used as reference.

ABTS free-radical scavenging assay

The ABTS solution was prepared by mixing 8 mM of ABTS with 3 mM of potassium persulfate in 25 ml of distil water. The solution was maintained at room temperature in the dark for 16 hours before use (Re *et al.*, 1999). The ABTS⁺ solution was diluted 10 times with 95% ethanol. A volume of 20 µl of the extract (1; 2; 3; 4 and 5 mg/ml), was mixed with 1 mL of diluted ABTS⁺ solution and incubated for 30 min at room temperature. Control experiments without the extract but with equivalent amounts of distil water were conducted in a similar manner. The absorbance was read at 734 nm after 30 min. Catechin was used as reference.

Nitric Oxide Scavenging Activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction (Green *et al.*, 1982). Sodium nitroprusside (2 ml, 5 mM) in standard phosphate buffer saline solution (0.025 M; pH 7.4) was incubated with 1 ml of the extract at different concentrations (1; 2; 3; 4 and 5 mg/ml) and the tubes were incubated at 25 °C for 5 hours. Control experiments without the extract but with equivalent amounts of the buffer solution were conducted in a similar manner. After 5 hours, 0.5 ml of each medium was removed and diluted with 0.5 ml of Griess' reagent (1% sulphanilamide, 2% Orthophosphoric acid and 0.1% Naphthyl Ethylene Diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with Naphthyl Ethylene Diamine was read at 546 nm. Catechin was used as reference.

For the 3 radical scavenging assays, percentage inhibition was calculated according to the following equation:

$$\text{Scavenging effect (\%)} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

Scavenging Concentration 50 (SC₅₀) parameter was used for the interpretation of the results (Brand-Williams *et al.*, 1996). The discoloration of the sample was plotted against its concentration in order to calculate the SC₅₀ value. It is defined as the amount of sample necessary to decrease the absorbance of the radical solution by 50 %.

2.5.2 Reducing Property

This activity was evaluated through the ability of the extract to reduce the MoO₄²⁻ and Fe³⁺.

Phosphomolybdenum method

The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. Total antioxidant capacity can be calculated following the method described by Prieto, Pineda & Aguilar (1999). A volume of 0.1 mL of extract (5; 6.25; 7.5; 8.75 and 10 mg/ml) solution was combined with 1 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube was capped and incubated in a boiling water bath at 95 °C for 90 min. After cooling the sample to room temperature, the absorbance of the aqueous solution was measured at 695 nm. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the sample. Catechin was used as reference and Trolox as the standard. The results were expressed as total antioxidant capacity in milligram equivalence of trolox/mg of extract.

Reducing power method

The assay was determined by assessing the ability of the extract to reduce Fe³⁺ to Fe²⁺ as described by Oyaizu (1986). A volume of 2.5 mL of extract (1 mg/ml) was mixed with 2.5 mL of sodium phosphate buffer (pH 6.6; 200 mM) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min and then 2.5 mL of trichloroacetic acid (10%) was added. This mixture was centrifuged at 650 rpm for 10 min. 5 mL of the supernatant was mixed with an equal volume of water and 1mL of ferric chloride (0.1%). Control experiments without the test compounds but equivalent amounts of distil water were conducted in identical manner. The absorbance was measured at 700 nm. Catechin was used as reference.

The results were expressed as percentages of reducing iron:

$$\text{Fe}^{3+} \text{ Reducing (\%)} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

2.5.3 Metal Chelation

This activity was evaluated using the Cu²⁺ chelating through the hemolysis assay.

Hemolysis assay

The assay was done following the method of Arbos, Ligia, Lucielly, Cid & Almeriane (2008). Blood was collected by venepuncture from wistar male rat (150 g) in heparinized tubes and centrifuged at 3,000 g for 15

min. Plasma and buffy coat were removed. Red blood cells (RBCs) were suspended in 10 volumes of NaCl (0.9 %) and centrifuged at 2,500 rpm for 5 min. The RBCs were washed three times with the same solution. During the last washing, the packed cells were resuspended in 10 volumes of NaCl (0.9 %) and utilized for the following assay. The effect of the extract on ferrous ion-induced haemolysis was evaluated. A volume of 0.2 ml of the extract (5; 6.25; 7.5; 8.75 and 10 mg/ml) was mixed with 1 mL of NaCl (0.9 %) and 0.1 ml of RBC suspension and incubated at 37 °C for 30 min. After incubation, 0.1 mL of CuSO₄ (0.1 M) was added and further incubated at 37 °C for 30 min. Haemolysis was determined by measuring absorbance of the supernatant at 540 nm. The reaction without the extract was used as control. Catechin was used as reference.

Percentage of haemolysis inhibition was calculated using the equation:

$$\text{Percentage Inhibition (\%)} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

Inhibition Concentration (IC₅₀) parameter was used for the interpretation of the results.

2.6 Glucose Lowering Effect

The glucose lowering effect of the extract was evaluated through 2 mechanisms: glucophagic property through the glucose adsorption assay and insulin-sensitivity stimulating effects through glucose uptake by yeast and muscle cell assays.

2.6.1 Glucose Adsorption Capacity

Glucose adsorption capacity of the extract was determined according to the method of Ou, Kwok, Li & Fu (2001). Briefly, 1 ml of extract at different concentrations (5; 10; 15 and 20 mg/ml) was added to 1 mL of glucose solution of increasing concentration (12.5, 25, 37.5 and 50 mM), the mixture was well stirred, incubated in a shaking water bath at 37 °C for 1 hr, centrifuged at 4,000 g for 20 min and the glucose content in the supernatant was determined according to the method of Trinder (1959). Bound glucose was calculated using the following formula.

$$\text{Percentage bound glucose (\%)} = [(\text{Glucose}_{\text{initial}} - \text{Glucose}_{\text{final}}) / \text{Glucose}_{\text{initial}}] \times 100$$

2.6.2 Glucose Uptake by Yeast Cells

Yeast cells were prepared according to the method of Cirillo (1962). Commercial baker's yeast was washed by repeated centrifugation (4,000 g for 5 minutes) in distilled water until the supernatant was clear and a 10% (v/v) suspension prepared in distilled water. Various concentrations of extracts (2.5; 5; 7.5 and 10 mg/ml) were added to 1 mL of glucose solution (25 mmol/L) and incubated for 10 minutes at 37 °C. The reaction was started by adding 100 µL of yeast suspension, vortexed, and further incubated at 37 °C for 60 minutes. After 60 minutes, the tubes were centrifuged (3,000 g for 5 minutes) and glucose in the supernatant was determined according to the method of Trinder (1959). The reaction without the extract was used as control. The percentage increase in glucose uptake by yeast cells was calculated using the following formula.

$$\text{Percentage increase in glucose uptake (\%)} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

2.6.3 Glucose Uptake in Rat Psoas Muscle Tissue

Glucose uptake in rat psoas muscle tissue of the extract was determined according to the method of Al-Awadi, Khattar, & Gumma. (1985). Psoas muscle was isolated from two anaesthetized adult rats and placed immediately in Krebs solution containing glucose (11.1 mM). Muscle tissue was cut into pieces of equal mass, about 0.25 g, and preincubated for 5 min in CO₂ incubator as mentioned above. Triple sets including muscle tissue alone (Control), muscle tissue with insulin (50 mU/l), muscle tissue with both insulin and extract (5, 7.5 and 10 mg/ml) were incubated for 2.5 hrs in CO₂ incubator under 95% O₂ and 5% CO₂ atmosphere. Aliquots of 2 ml were removed from incubation mixture at 0, 30 and 60 min, and changes in glucose concentration were measured according to the method of Trinder (1959). Percent glucose uptake was calculated using the following formula.

$$\text{Glucose uptake (\%)} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

2.7 Statistical Analysis

All experiments were performed in triplicate. The statistical package of social sciences (SPSS) software version 20.0 (Chicago-Illinois Inc.) was used and Tukey's test was performed to compare variability amongst the groups. One-way analysis of variance (anova) significant differences were detected at 95% confidence interval. The results obtained were expressed as mean ±SD from three distinct observations. The IC₅₀ and SC₅₀ values were calculated by linear regression and Microsoft Excel was used for graphical depiction.

3. Results

3.1 Polyphenolic Compound Content

The polyphenol content of hydroethanolic extract of *B. toxisperma* pulp is shown in table 1. Polyphenols and flavonoids were indicated at 459.55 µg GAE/mg and 252.15 µg EC/mg respectively.

Table 1. Phytochemical content of extract

| Extract | Polyphenols (µg GA/mg of extract) | Flavonoids (µg CE/ mg of extract) |
|---|--------------------------------------|--------------------------------------|
| Hydroethanolic Extract of <i>B. toxisperma</i> pulp | 459.55 ± 3.79 | 252.15 ± 1.57 |

GAE: Gallic Acid Equivalence ; CE: Catechin Equivalence.

3.2 Radical Scavenging activity

The SC₅₀ of hydroethanolic extract of *B. toxisperma* pulp are presented in table 2. The extract showed scavenging activity on DPPH•, ABTS• and NO• radicals. The SC₅₀ were 3.24, 3.49 and 4.28 respectively for ABTS•, DPPH• and NO• radicals. The extract showed a lower DPPH•, ABTS• and NO• scavenging activity than Catechin.

Table 2. Scavenging activities of extract

| EC ₅₀ of radicals | SC ₅₀ DPPH (mg/ml) | SC ₅₀ ABTS (mg/ml) | SC ₅₀ NO (mg/ml) |
|---|-------------------------------|-------------------------------|-----------------------------|
| Hydroethanolic Extract of <i>B. toxisperma</i> pulp | 3.49* | 3.24* | 4.28* |
| Catechin | 1.45 | 1.54 | 1.25 |

SC₅₀: 50% Scavenging Concentration, *p < 0.05: significantly different in comparison to catechin.

3.3 Reducing Property

The ability of the extract to reduce MoO₄²⁻ and Fe³⁺ ions is shown in table 3.

The MoO₄²⁻ reducing capacity is expressed as total antioxidant capacity (TAC). The extract TAC was proportional to its concentration. TAC varied from 2.09 to 3.51 mg TE/mg.

The Fe³⁺ reducing capacity is expressed as percentage of reduced ferric ions. The extract reduced Fe³⁺ with a reducing percentage of 15.31 %.

The extract showed the Fe³⁺ reducing capacity was lower than that of catechin.

Table 3. Reducing property of extract

| Assays | Total Antioxidant Capacity mg ET/g of sample | | | | | Fe ³⁺ (%) | Reducing |
|---------------------------------|--|----------|-----------|-----------|-----------|-------------------------|----------|
| [Sample] | 5 mg/ml | 6.25 | 7.5 mg/ml | 8.75 | 10 mg/ml | 1 mg/ml | |
| | | mg/ml | | mg/ml | | | |
| HE of <i>B. toxisperma</i> pulp | 2.09±0.02 | 2.5±0.05 | 3.07±0.01 | 3.39±0.07 | 3.51±0.01 | 31.02±1.6* | |
| Catechin | | | | | | 51.09±2.2 | |

HE: Hydroethanolic Extract; TE: Trolox Equivalence.

3.4 Metal Chelation

The ability of the extract to inhibit haemolysis by chelating Cu²⁺ is shown in table 4. The extract showed inhibitory activity on haemolysis with IC₅₀ of 3.49 mg/ml. The extract showed a lower activity than catechin.

Table 4. IC₅₀ of extract on hemolysis

| | IC ₅₀ Hemolysis (mg/ml) |
|---------------------------------|------------------------------------|
| HE of <i>B. toxisperma</i> pulp | 3.49* |
| Catechin | 2.3 |

HE: Hydroethanolic Extract; IC: Inhibitory Concentrations. *p < 0.05: significantly different in comparison to catechin.

3.5 Glucose Binding Capacity

The glucose binding capacity of the extract was evaluated through the glucose adsorption capacity and is shown in figure 1. The extract could bind glucose effectively, and the glucose binding capacity was directly proportional to the extract and glucose concentrations. The extract was effective in adsorbing glucose at both lower and higher concentrations. The percentages of glucose binding rise to 30.92, 40, 51 and 60 % respectively at extract concentrations of 5, 10, 15 and 20 mg/ml. The activity tended to stabilize at glucose concentrations up to 37.5 mM.

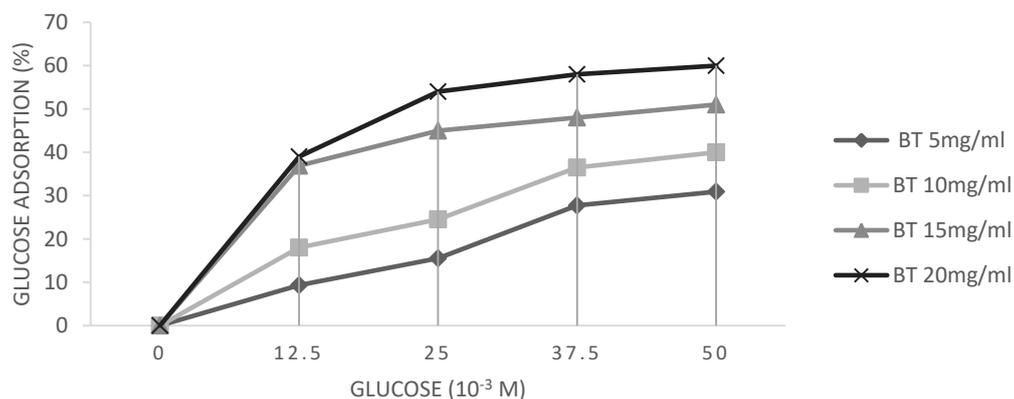


Figure 1. Glucose binding capacity of extract

BT: *B. toxisperma*

3.6 Insulino-sensitivity Stimulating Properties

The insulino-sensitivity stimulating properties of the extract was evaluated through glucose uptake by yeast cells as shown in table 5 and by muscles tissues as shown in table 6.

The extract promoted glucose uptake by yeast cells in a manner proportional to their concentrations. The increase of glucose uptake varied from 42.97 to 56.62 %.

The extract also promoted the effects of insulin on glucose uptake by muscle tissues. Insulin causes a glucose uptake of 11.89 % and in the presence of extract, the increase in glucose uptake was approximately 22% in 30 min, but it decreased to approximately 12% in 60 min. The extract ameliorated glucose uptake in the presence of insulin to approximately 10 %.

Table 5. Increase of glucose uptake by yeast cells

| HE of <i>B. toxisperma</i> pulp | 2.5 mg/ml | 5 mg/ml | 7.5 mg/ml | 10 mg/ml |
|---------------------------------|------------|------------|------------|------------|
| Increase of glucose uptake (%) | 42.97±1.05 | 48.62±2.54 | 51.36±3.11 | 56.62±1.49 |

HE: Hydroethanolic Extract

Table 6. Increase of glucose uptake by muscle tissues

| Time | Glucose uptake (%) | | |
|------------------------------------|--------------------|-------------|------------|
| | 0 min | 30 min | 60 min |
| MT | 0 | 0* | 0* |
| MT + Insulin | 0 | 11.89±0.75 | 11.82±0.07 |
| MT + Insulin + Extract (5 mg/ml) | 0 | 21.94±2.03* | 11.95±0.76 |
| MT + Insulin + Extract (7.5 mg/ml) | 0 | 22.06±0.89* | 12.03±1.04 |
| MT + Insulin + Extract (10 mg/ml) | 0 | 22.39±2.69* | 12.22±1.1 |

MT: Muscle Tissue; *p < 0.05 : significantly different in comparison to MT + Insulin.

4. Discussion

The objective of this study was to evaluate the antioxidant and hypoglycaemic properties of hydroethanolic extract of *B. toxisperma* pulp. Specifically, it systematically had to quantify the main bioactive compounds that

can decrease or prevent oxidative stress and reduce the high glucose level. The main target compounds were polyphenols, including flavonoids (Kim, Keogh & Clifton, 2016; Brewer, 2011). The extract had high levels of total polyphenols and flavonoids (Table 1). In the first part of this work, we evaluated the mechanisms by which these compounds exert their possible antioxidant activity: radical scavenging, reducing agent and metal chelating capacity (Liyana-Pathirana & Shahidi, 2006).

Concerning the antiradical power, we first evaluated the effect of the extract on 2 synthetic radicals: DPPH• and ABTS•, which was confirmed by means of a biological radical: NO•. ABTS• and DPPH• are synthetic radicals widely used to study the anti-radical potential of antioxidants (Joginder, Akansha, Pardeep, Pooja & Surekha, 2015). On the other hand, NO• is a free radical produced in mammalian cells which intervenes in many physiological processes but once in excess in the organism, it can initiate the development of numerous pathological processes (Prety & Surech, 2012). The extract trapped the DPPH•, ABTS• and NO• radicals with high SC₅₀ values (Table 2). These results can be explained by the content of polyphenols and in particular of flavonoids in the extract which, due to the redox potential of their OH groups, would be capable of yielding a proton and/or an electron thus trapping the ABTS• and DPPH• radicals to give the stable compounds ABTS and DPPH-H. Indeed, the low redox potential of flavonoids (FLOH) makes them thermodynamically capable of reducing free radicals (R•) by the transfer of a hydrogen atom or of electrons from hydroxyl groups (Procházková & Bousová & Wilhelmová 2011). The ability of phenolic compounds to inhibit the interaction of NO• could also be noted. With oxygen generating nitrite which is a highly oxidizing species. This could account for the antiradical capacity against NO• (Parul, Kundu & Saha, 2013).

Subsequently, the ability to yield electrons was demonstrated in the second mechanism, which consisted of evaluating the reducing power through the capacity of the extract to reduce the MoO₄²⁻ and Fe³⁺ ions. The extract reduced phosphomolybdenum VI to phosphomolybdenum V and ferric iron to ferrous iron (Table 3). These results can still be linked to the flavonoid content of the extract as confirmed by their ability to yield electrons. Khan, Khan, Sahreen & Ahmed (2012) had already demonstrated the ability of flavonoids to reduce phosphomolybdenum by electron transfer.

The third mechanism consisted of evaluating the metal chelating capacity of the extract, using Cu²⁺ and to evaluating their effects on biological processes including the oxidation of membrane lipids in general on the one hand, and on the other hand, the oxidation of lipids of the erythrocyte membrane more precisely. Our extract chelated Cu²⁺ thus inhibiting haemolysis respectively (Table 4). These observations could be due to the phenolic compounds contained in this extract, that chelate Cu²⁺, blocking the reactive oxygen species thus preventing their oxidative action on the membrane lipids of erythrocytes (Giuseppina, 2012). Indeed, flavonoids are renowned for their ability to chelate Cu²⁺, thus preventing lipid peroxidation (Rahal *et al.*, 2014).

On the other hand, Djiokeng *et al.* (2014) already revealed the same 3 antioxidant mechanisms with the hydroethanolic extract of *B. toxisperma* bark. Numerous fruits of the same family as that of *B. toxisperma* (Sapotaceae) have also proved their radicals scavenging, reducing and metals chelating properties: *Manilkara hexandra* (Parikh & Patel, 2017), *Mimusops elengi* (Valvi, Rathod & Yesane, 2011).

The second part of the study focused on the antidiabetic potential of the hydroethanolic extract of *B. toxisperma* pulp through glucose binding ability and insulin-sensitivity stimulating properties. The extract demonstrated the ability to engulf glucose (Figure 1). This activity is attributed to the high flavonoid content. Flavonoids through condensation reactions complex with glucose molecules through hydroxyl groups forming glycosyl-flavonoids containing osidic bonds (Li *et al.*, 2014). This activity could also be attributed to the fiber content (5.41%) of *B. toxisperma* as reported by Fungo *et al.* (2015). In effect, insoluble fibers are renowned for their ability to form complexes with glucose, thus rendering them unavailable, which contributes to the reduction in intestinal glucose absorption (McRonie & McKeown, 2017). The antihyperglycemic effect was evaluated *ex vivo*, by studying the ability of the extract to stimulate glucose uptake, which is greatly reduced in case of insulin resistance (Anuradha, Malini & Jyoti, 2015). The extract stimulated the transport of glucose across the yeast cell membrane (Table 5), it was also noted that 30 minutes after administration, the extract increased the insulin-mediated glucose uptake (Table 6). This would reflect the ability of polyphenols contained in the extract to stimulate cellular uptake of glucose and GLUT translocation, hence the reduction of glucose in the reaction medium. Gaikwad, Krishna & Sandhya (2014) had previously noted the ability of polyphenols to reduce glucose uptake due to an increase in insulin sensitivity. It has also been proven *in vitro* that polyphenols especially flavonoids stimulate insulin-mediated glucose uptake (Ueda-Wakagi, Mukai, Fuse, Mizushina & Ashida, 2015; Kurimoto *et al.*, 2013). This may be due to the ability of the extract to stimulate insulin binding to its receptors at the muscle level or to increase the number of insulin receptors (Gupta, Kesari, Watal, Murthy & Chandra, 2005).

5. Conclusion

The hydroethanolic extract of *B. toxisperma* pulp are potent antioxidant and hypoglycemic, acting through different mechanisms of action and may be potential tools in the control of metabolic disorders related to diabetes that represent a burden for the society, both in terms of the increasing mortality and the costs allocated healthcare.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TNGR carried out the study and wrote the manuscript; NJL contributed to conception, design and analysis of data, and OJE assisted with and supervised the manuscript writing. All authors have read and approved the final manuscript.

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