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α-Glucosidase inhibition, antioxidant and cytotoxicity activities of semi-ethanolic extracts of *Bridellia ferruginea* benth. and *Ceiba pentandra* L. Gaerth from Benin

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Abstract

The use of plant extracts in the treatment of human disease requires a definition of optimal conditions. This study objective is to evaluate the inhibition capacity of α-glucosidase activity of Bridelia ferruginea benth bark and Ceiba pentandra L. Gaerth *root semi-ethanolic extracts compared to acarbose. Their antioxidant activity were also tested by three techniques: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, Ferric reducing antioxidant power (FRAP) and the Oxygen Radical Absorbance Capacity (ORAC). Results indicated that these extracts have antioxidant property and α-Glucosidase inhibitory activity (IC50 = 1.4 ± 0.04 µg / mL for B. ferruginea and 51 ± 0.7 µg / mL for C. pentandra) higher than the reference compound acarbose (IC50 = 726 ± 15 mg / mL). Bridelia ferruginea extract was the most active of the two. In vitro cytotoxicity evaluation of the extracts was done by fluorometric assay: Resazurin reduction test on human fibroblast primary culture have showed a very low toxicity. Bridelia ferruginea and Ceiba pentandra The semi-ethanolic extracts could therefore constitute a credible alternative to replace the expensive synthetic drugs in the treatment of diabetes.*

Keywords: *Bridelia ferruginea*, *Ceiba pentandra,* α-glucosidase, antioxidant, cytotoxicity.

Introduction

From immemorial time, folk medicine was the essential part of therapeutic arsenal. Plants constituted the bulk of the treatments that were available for treatment, according to formulas handed down by tradition. Nearly half of medications that are used today have their composition origin from plant and the quarter contains plant extracts or active molecules from plants directly. Thus through synthetic drugs provided as much as folk medicine, using plants in health was the most common worldwide. Indeed, plants play an important role in human disease treatment for developing countries populations, particularly in the areas where it is difficult for most to access health facility because of their remoteness from cities or their low purchasing power. The World Health Organization estimated that about 80% of Africa population (80 to 85% in Benin) use traditional medicine for their primary health care [1]. Traditional medicine was involved not only in preventing but also in the treatment of skin diseases, mental illness, digestive problems, endemic diseases, transmitted or not, as well as metabolic diseases[2]. Among metabolic diseases treated by traditional medicine, diabetes mellitus, especially type 2 diabetes represents 90% of diabetes cases[3,4]. Rated to 190 million in 2002, the number of diabetics worldwide will reach 370 million by 2030, more than 6 million annually. We can truly speak about an epidemic one [5]. Because of the prohibitive cost and toxicity of synthetic drugs used to treat diabetes, it is therefore essential to use bioactive plant extracts, which are little toxic and less expensive. *Bridelia ferruginea* and *Ceiba pentandra* are two species widely used in Africa for the treatment of many diseases such as rheumatism, diarrhea, toothache and stomach pains[6,7]. Pharmacological studies have shown some of these properties[8,9,10,11], but to our knowledge, no work in Benin was conducted on their antidiabetic property. The purpose of this study is to evaluate the antioxidant and inhibitor of α-glucosidase of semi-ethanolic extracts of *Bridelia ferruginea* barks and *Ceiba pentandra* roots in the treatment of diabetes.

Material and Methods

Plant materials. *Bridelia ferruginea* benth (Euphorbiaceae) barks were harvested in Zinvié in November 2009 and *Ceiba pentandra* L. Gaerth (Bombacaceae) roots in Lama in January 2010 in the south of the country. The two plants botanical identification was performed at the National Herbarium of Benin (HNB) respectively under a voucher number: AA6383/HNB and AA6388/HNB.

Extracts preparation: To 100 g of plant powder 1000 ml (x 3) of a mixture of ethanol: water (50%) was added, the whole subject is mechanically stirred for 3 h, at 25°C. After 1 h, the solution was filtered with paper Whatman N°1 on Buchner using a vacuum pump. The filtrates were collected and

evaporated in a rotary evaporator at 40°C. The crude extracts obtained were stored in freezer.

Phytochemical Screening: This analysis were used to reveal the family compounds in extract: Saponins by the foam index technics, flavonoids by Shibata test and reducing compounds with Fehling's test, Tannins were revealed by $FeCl_3$ and Stiasny reactif, and Dragendorff's Test for alkaloids[12]. Total phenolic content was determined using Folin-Denis' reagent[13]. Gallic acid is used as reference and for the calibration curve; results were expressed as gram of gallic acid equivalent/gram of dry weight (mg GAE/g DW). The total flavonoid content was determined using aluminum trichloride[14]. The flavonoïd content was calculated from a quercetin standard curve, the results are expressed as mg quercetin equivalent per gram of dry weight (mg QE/g DW). Condensed tannins were determined using solution of vanillin[15]. The tannins content was calculated from catechin standard curves, and the results are expressed as mg Catechin per gram DW. (mg CE/g DW).

α-glucosidase inhibitory activity: the slightly modified method of Tiwari *et al.*[16], was adopted for the determination of the α-glucosidase inhibitory activity. Briefly, in a 96-well microplate 100 µl of a sample of different concentrations was incubated with 50 µL α-glucosidase (1.0 U/ml) (from *Saccharomyces cerevisiae*) in phosphate buffer (0.1 M, pH 6.8) for 10 min at 37°C. The reaction was initiated by addition of 50 µL of substrate: 5 mM, *p*-nitrophenyl- α-D glucopyranoside in a 0.1 M phosphate buffer of pH, 6.8. Kinetics of release of *p*-nitrophenol was measured spectrophotometrically with a microplate reader Multiskan MS, Labsystems (Minneapolis, USA) for 5 min at the intervals of 30 seconds at 405 nm. Acarbose was used as reference. The IC50 (concentration required to decrease the reaction rate to 50%) was then determined from the curve of concentration-dependence.

Antioxidant activity: 2, 2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging assay*:* free radical scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazil (DPPH) [17]. Ascorbic acid was used as positive control. IC50 value of each sample was calculated from the linear regression curve.

Ferric reducing/antioxidant power (FRAP) assay: the ferric reducing property of the extract was determined according to the technique described by Piljac-Zegarac *et al*[18]. Results are expressed in milligrams equivalent of iron II per gram of dry weight (µmoL Fe II / g DW)

Oxygen Radical Absorbance Capacity (ORAC) assay: ORAC assay was carried out using fluorescein [19]. ORAC values were expressed in µmol Trolox equivalents per gram of dry weight (µmol Trolox / g DW).

Cytotoxicity activity: In vitro cytotoxicity was performed by a fluorometric assay: Resazurin reduction test (RRT) on human fibroblast primary culture [20].

Statistical analysis: All experiments were performed at least in triplicate, and results are expressed as mean ± SEM. Statistical analysis was performed using the statistical software XLSTAT (version 2012. 1. 01, Addinsoft, Paris, France). The results were analyzed by the univariate ANOVA test followed by Dunnet test / Tukey for multiple comparisons and determination of significance level. Group means were considered to be significantly different at $P < 0.05$.

Results and Discussion

Phytochemical Screening*:* The preliminary phytochemical screening of ethanol extract hydro-alcoholic (50%) showed the presence of flavonoids, tannins, saponins and reducing compounds (table-1). The spectrophotometric results indicated that the two extracts contain polyphenolic compounds, flavonoids and condensed tannins excepted *C. pentandra* extract in which flavonoids were not be detected (table-2). There was a significant difference between the two extracts content ($P < 0.05$) with higher concentration in the extract of *B. ferruginea*.

Table- 1
Total chemical composition of the studied extracts

Chemical composition	*B .ferruginea*	*C. pentandra*
Flavonoids	+++	+
Tannins	+++	+
Alkaloids	+	-
Saponins	+	+
Reducing compounds	+++	-

- = Negative, + = Positive, +++ = Abundant

Table- 2
Total phenolics, flavonoids and condensed tannins contents

Family coumpunds	*B. ferruginea*	*C. pentandra*
Total polyphenolic (mg GAE/gDW)	86 ± 2^a	$6.4 \pm 0,6^b$
Flavonoids (mg QE/g DW)	1.1 ± 0,4	nd
Condensed tannins (mg CE/g DW)	1277 ± 72^a	10 ± 2^b

nd = not detected.
Means not sharing a common letter in the same row denote a significant difference at $P < 0.05$

α-glucosidase inhibitory activity: α-glucosidase inhibitors (AGIs) are among the available glucose-lowering medications. The glucosidase enzyme is located in the brush border of the small intestine and is required for the breakdown of carbohydrates and absorption of monosaccharids. The AGIs delay, but do not prevent, the absorption of ingested carbohydrates, reducing the postprandial glucose and insulin peaks[21]. Testing the α-glucosidase inhibitory effect of those two plants, contribute to the understanding of their mechanisms of action. Figure-1 showed that the extracts displayed strong α-glucosidase inhibitory activity in a dose dependent manner.

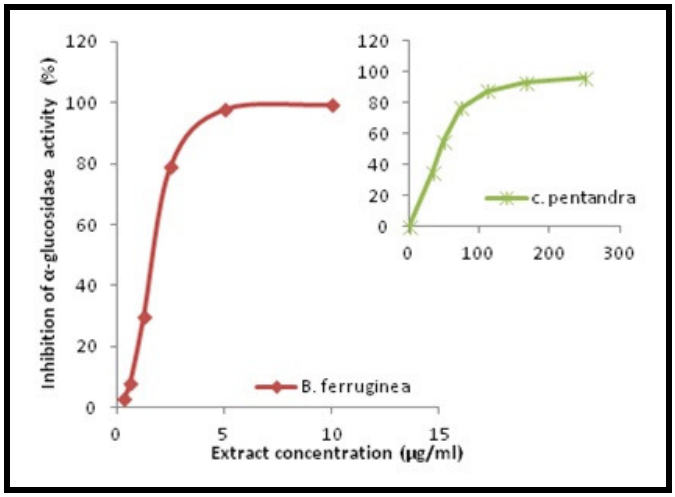

Figure- 1
α-glucosidase inhibitory activity by the two extracts

The concentration required to decrease the reaction rate to 50% (IC50) were respectively : 1.4 ± 0,04 µg/ml for *B. ferruginea* and 51 ± 0,7 µg/mL for *C. pentandra* extract. With a significant difference in the inhibitory activity between the two extracts ($p < 0.0001$). The tested extracts showed higher α-glucoxidase inhibitory activity than that (IC50 726 ± 15 µg/mL) (figure-2) of the reference compound acarbose. Only a few articles had discussed it in details[22-25] and where acarbose was found to have little inhibition on α- glucosidase activity and authors justified this by the nature of α-glucosidase enzyme: mammalian (rat intestine), bacterial (*Bacillus stearothermophilus*), yeast (*Saccharomyces cerevisiae*, baker's yeast) sources. This could justify why all the studied extracts were more active against this enzyme than acarbose. Furthermore, the nature of some extract constituents (phenolics, flavonoids and their glycosides) was in accordance with some work[26-28] as being effective inhibitors of α-glucosidases.

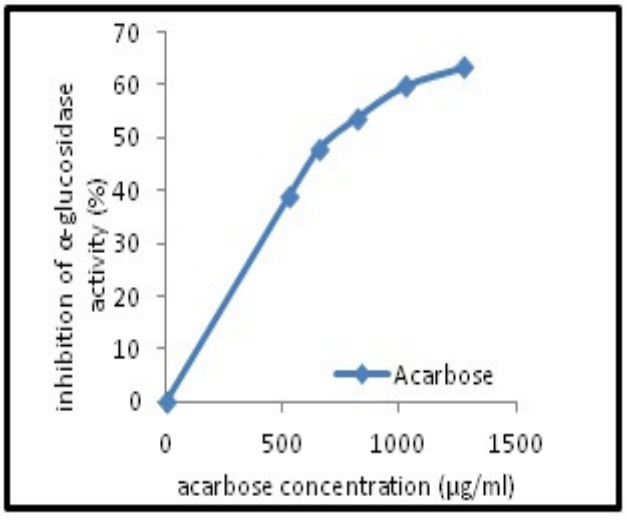

Figure- 2
α-glucosidase inhibitory activity by acarbose

Antioxidant activity: several antioxidant assays are frequently used to estimate antioxidant capacities in fresh fruits and vegetables. These assays could roughly be classified into two types: assays based on hydrogen atom transfer (HAT) reactions and assays based on electron transfer (ET) [29]. In this work, we tested two types of ET assay: 2,2- diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) and one HAT assay: Oxygen Radical Absorption Capacity (ORAC).

Figure-3 shown IC_{50} of the two extracts on DPPH radical scavenging activities: *C. pentandra* (50 ± 2 µg/ml) and *B. ferruginea* (5 ± 0.3 µg/ml), the IC_{50} of the positive control L-ascorbic acid was 1.25 ± 0.07 µg /ml.

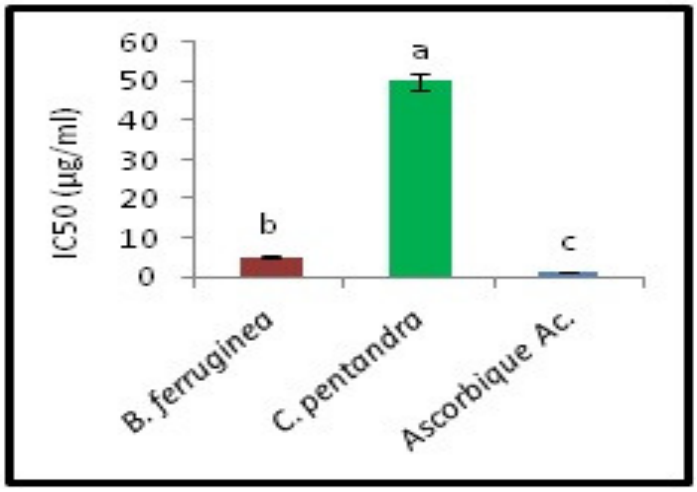

Figure- 3
DPPH radical scavenging of extracts

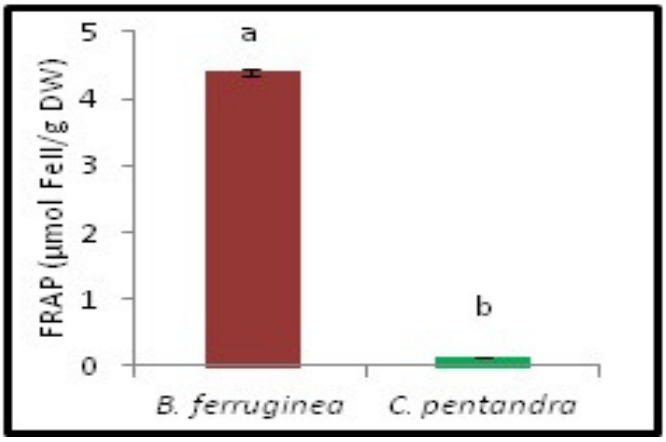

Figure- 4
FRAP values of extracts

The FRAP assay measures the reducing potential of an antioxidant that reacts with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to produce a colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ). The FRAP values of the two extracts were in the following order:, *B. ferruginea* (4.4 ± 0.06 µmol Fe II / g DW),

C. Pentandra (0.14 ± 0.01 µmol Fe II / g DW). ORAC assay is widely employed to determine antioxidant content of foods using fluorescein as a probe for oxidation by peroxyl radical. Figure-5 shows the ORAC values : C. *Pentandra* (917 ± 139 µmol Trolox / g DW and *B. ferruginea* (5133 ± 161 µmol Trolox / g DW). For the three essays, there was a statistically significant difference between the tested extract ($P < 0.0001$) and were consistent with each other: when the extract showed low IC50 value using DPPH test, it showed in the same time a high FRAP and ORAC value. This has been observed with the two extracts. About their capacity to transfer a hydrogen atom by the ORAC test, may be due to the presence of phenolic compounds in the extract[30]. This is especially true that root extracts of *C. pentandra* were poor in polyphenols and showed low ORAC value than *B. ferruginea* extract. Generally, the reducing properties were associated with the presence of compounds, which exerted their action by breaking the free radical chain through the donation of an hydrogen atom[31].

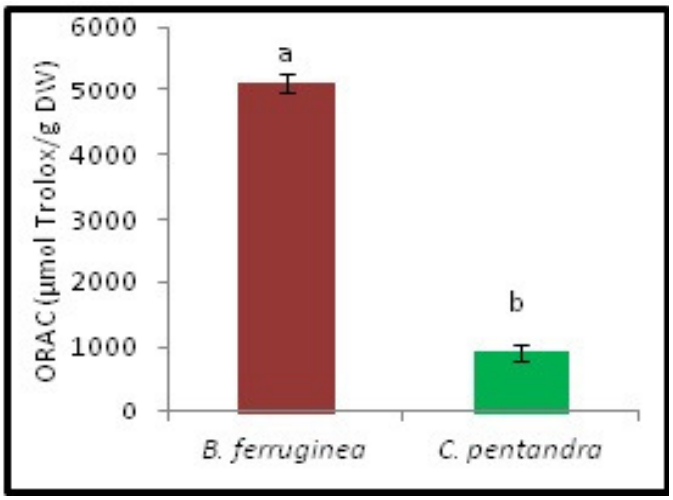

Figure- 5
ORAC values of extracts

Cytotoxicity: The IC50, defined as the drug concentration required to inhibit cell proliferation by 50%, was calculated from the curve of concentration-dependent survival percentage, defined as fluorescence in experimental wells compared with fluorescence in control wells, after subtraction of the blank values.

In this work, the maximum concentration of extract tested was 25 µg/ml. The two extracts had no cytotoxic activity on human fibroblast primary culture. The criterion of cytotoxic activity for the crude extracts, as established by the National Cancer Institute (NCI), was an IC50 of less than 30 µg/mL in the preliminary[32]. But *B. ferruginea* extract reduced around 40 % (figure-6) of the initial number of the fibroblast cell compared to *C. pentandra* (figure-7). This could be due to the presence of derivate of podophyllotoxin[33]. Podophyllotoxin which is a natural molecule at the base of the synthesis of two antiproliferative molecules etoposide and teniposide used in the clinical[34].

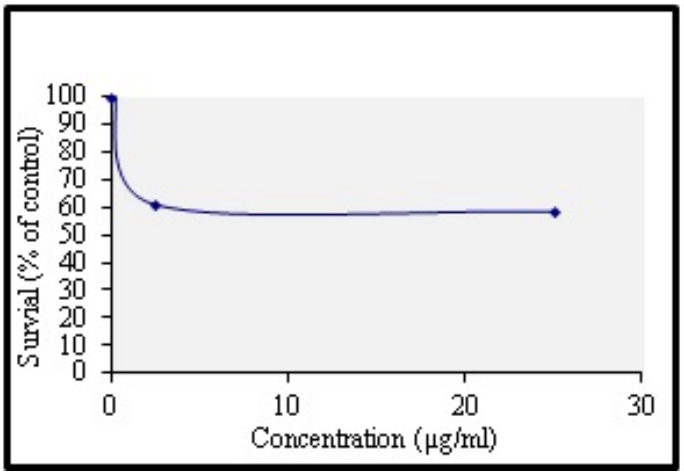

Figure- 6
Growth of human fibroblast primary culture in the presence *B. ferruginea* extract

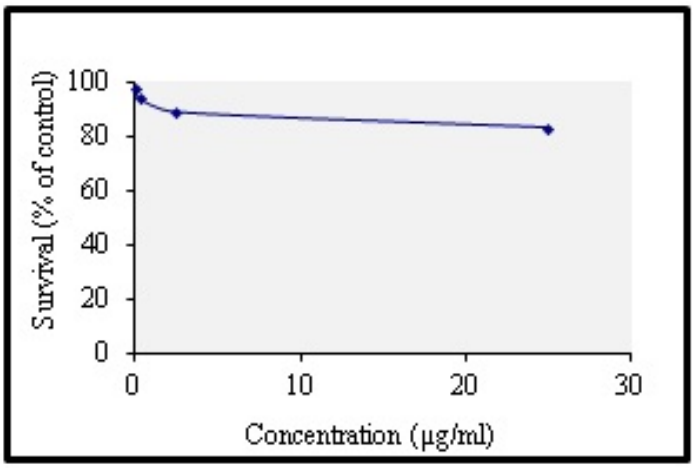

Figure- 7
Growth of human fibroblast primary culture in the presence *C. pentandra* extract

Conclusion

The results obtained in this study supported the already use of *B. ferruginea* (stem bark), *C. pentandra* (root), traditional medicinal plants in Benin against some diseases such hyperglycemia and stress oxidant disease, because of their phytonutriments content and their very low cytotocity. Therefore, in vitro assay protocol cannot be used alone to test all relevant parameters. A complementary method used to evaluate the antioxidant activities of fruits and vegetables extracts directly in live mammalian cells could be useful.

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