α -Glucosidase Inhibitory and Antiradical Properties of *Acacia macrostachya*

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ABSTRACT

In this work, the anti-diabetic activity of three extracts of *Acacia macrostachya* was investigated by following the inhibitory effect of these extracts on α -glucosidase using the in vitro model. The antiradical activity of these extracts was also determined. Methanol extracts of root and stem barks showed a very significant inhibitory effect against the enzyme activity of α -glucosidase with IC_{50} 2.487 ± 0.441 μ g/mL and 1.650 ± 0.229 μ g/mL respectively. For antiradical activity, the same extracts presented the highest scavenging of the radical DPPH $^{\bullet}$ with IC_{50} values of 9.307 ± 0.262 μ g/mL and 5.242 ± 0.068 μ g/mL respectively. With the cationic radical ABTS $^{•+}$, IC_{50} varied from 45.049 ± 0.730 μ g/mL for methanolic root barks extract to 14.136 ± 0.161 μ g/mL for methanolic extract from stem barks. Thus, the methanol extracts of the root and stem barks of *Acacia macrostachya* possess compounds with very interesting anti-diabetic and antiradical properties and could justify its traditional use.

Keywords: *Acacia macrostachya*; Antiradical Activity; Diabetes; α -Glucosidase.

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I. INTRODUCTION

Diabetes is a disease that is invading our planet and constitute a major concern for global health services. It is a scourge that is spreading both in developed and developing countries at an alarming rate. In fact, 382 million people had diabetes in 2013 and this number could reach 592 million by 2035 according to the forecasts of the International Diabetes Federation [1]. Diabetes is a chronic disease characterized by permanent hyperglycemia with fasting blood glucose levels

of 1.26 g/L on two occasions and/or greater than 2 g/L at any time of the day [2]. Two types of diabetes are commonly known: one is insulin-dependent diabetes (type 1) and the other is non-insulin-dependent diabetes (type 2) [3]. Type 1 diabetes is treated by regular injections of insulin. Type 2 diabetes is the most common and accounts for more than 90% of diabetes cases [3]. Otherwise, one of the direct complications associated with hyperglycemia has been shown to be cancer. Diabetes mellitus (type 2) is a chronic, often debilitating disease with severe complications,

including blindness, heart disease, kidney disease and refract neuropathy [4]-[6]. It is found in almost all populations and appears to be a growing problem in developing countries. It is mainly due to insulin resistance and is associated with lifestyle, obesity, malnutrition and physical inactivity. Treatment of this type of diabetes is not easy especially since it is not insulin-dependent [7]. Nevertheless, maintenance of glucose levels in healthy blood has been shown to be particularly important in the management of people with type 2 diabetes [8]. The first-line treatment for type 2 diabetes is diet, weight control and physical activity [3]. Several categories of anti-diabetic drugs are also available, including α -glucosidase inhibitors. α -Glucosidase is a key enzyme in the digestive system and catalyzes the first step in the digestion of starch, hydrolyzing the α -1,4-glucoside linkages [3]. In fact, the hydrolysis of dietary carbohydrates such as starch is the major source of glucose in the blood. Inhibition of this enzyme would significantly lower blood sugar levels and help maintain a good blood sugar level. In addition, it has also been shown that diabetic patients are under oxidative stress leading to the generation of free radicals and antioxidant deficiency that may contribute to the onset and progression of complications associated with diabetes [9]. Therefore, the use of antioxidants by diabetic patients is particularly important not only to maintain the balance between the free radicals generated and the trapping abilities of radicals but also to treat long-term complications that may occur [10]. Like other sub-Saharan countries, Burkina Faso has not been left on the sidelines of this disease. Indeed, in 2013 its prevalence rate was 4.9% among the population aged 25 to 64 years [11].

The burden of diabetes continues to grow globally, putting considerable physical, emotional and financial pressure on individuals, families, communities and health systems [12]. Many health systems in low- and middle-income countries are least prepared to deal with this burden, resulting in thousands of deaths each year. Indeed, 87% of all diabetes related to those countries [13]. Among these countries is Burkina Faso, which is also facing the emergence of diabetes mellitus due to the precarious conditions of patient care and socio-economic indigence. The majority of the population, which does not have adequate health coverage, relies mainly on local medicinal plants for their primary health care. In these countries, more than 80% of people most often use traditional medicine for primary health care [14]. Local and international institutions such as WHO encourage and support the use of these therapeutic plants, which are often highly effective and accessible to all. This is the case of *Acacia macrostachya* (Mimosaceae), a medicinal plant widely used in Burkina Faso and widely used by therapists against certain pathologies such as inflammation, cancer and oxidative stress [15]-[17]. Some works previously done on the leaf and root extracts of this plant has revealed the presence of flavonoids, tannins, terpenoid, alkaloids, saponins and steroids [15], [17], [18]. In addition, some authors have reported in the literature that methanol and dichloromethane extracts from the plant had significant antioxidant activities [15], [17]. More interestingly, many studies have shown good correlation between antioxidant

activity and antidiabetic properties of some plant extracts. The present study aims to evaluate the antiradical and antidiabetic activities of extract of *Acacia macrostachya*.

II. MATERIAL AND METHODS

A. Vegetable material

The plant material was constituted of the root and stem barks of *Acacia macrostachya*. They were collected in February 2018 at about twenty kilometers of Ouagadougou, Burkina Faso with GPS coordinates 12°31'50,52'' N; 01°17'2,7'' W and a voucher specimen was deposited at the herbarium of University Joseph KI-ZERBO (identification number 17252). The vegetable material was, moreover, transformed into fine powder using an electric grinder after two (02) weeks of drying at the laboratory temperature and under ventilation. Then, 50 g of the powder were macerated in the appropriate solvent (dichloromethane or methanol) for 24 hours under magnetic agitation. After filtration, the macerated is evaporated to dry using a rotary evaporator (BÜCHI Rotavapor R-200). We prepared total three types of extract: dichloromethane extract from root barks (**RB1**), methanolic extract from root barks (**RB2**) and methanolic extract from stem barks (**SB3**).

B. Reagents

α -Glucosidase, p-nitrophenyl- α -D-glucopyranoside (PNPG), acarbose (antihyperglycemic reference drug) and Trolox were provided from Sigma-Aldrich. Anhydrous sodium carbonate (Na_2CO_3) solution, DPPH and ABTS reagents and all other reagents used in this work were prepared at the laboratory according to standard protocol.

C. α -Glucosidase inhibition assay

The inhibitory effect of the enzyme α -glucosidase of the extracts was assessed using the chromogenic method reported by Ranilla and al. [19] with slight modifications. The assay was carried out in 96 well plates with the maximum volume of 250 μL per well. Indeed, the reaction was initiated by mixing 20 μL of the enzyme α -glucosidase (0.5 units/mL), 10 μL sample at different concentrations and 120 μL of phosphate buffer (0.1 M; pH 6.9). After 15 minutes of pre-incubation at 37°C, the enzymatic reaction was initiated by adding 20 μL of p-nitrophenyl- α -D-glucopyranoside (5 mM prepared in a phosphate buffer 0.1 M; pH 6.9). The mixture was incubated during 15 new min, still at 37 °C. The reaction ended adding 80 μL of sodium carbonate solution (0.2 M). The inhibitory activity of α -glucosidase was determined by measuring the absorbance of p-nitrophenol released by p-nitrophenyl- α -D-glucopyranoside at 405 nm by a spectrophotometer (SPECTROstar NANO, BMG LABTECH, Ortenberg, Germany). Positive control was the reaction mixture without the inhibitory substance and the system without α -glucosidase was used as blank for correcting the background absorbance. The inhibition percentage was calculated by the following equation:

$$\% \text{ of inhibition} = \frac{\text{Abs control} - \text{Abs sple}}{\text{Abs control}} \times 100$$

Abs control: absorbance control

Abs sple: absorbance sample

D. DPPH• and ABTS•⁺ radical scavenging assay

DPPH• and ABTS•⁺ are two stable free radicals marketed and widely used in the evaluation of antiradical activity of any plant extract. Faced to an antiradical substance, these two radicals initially purple and green respectively for DPPH• and ABTS•⁺ turn to yellow or fade (respectively for DPPH• and ABTS•⁺) reflecting an antiradical activity of the extract. In this work, the antiradical activity of the three extracts of *Acacia macrostachya* was assessed *in vitro* model by measuring the different optical densities of the two radicals after they were exposed to the toxic substances. For the measurement, 50 µL of each extract in methanol (different concentrations) are added 200 µL of the radical DPPH• or the cationic radical ABTS•⁺ solution in methanol. After 10 min of incubation away from the light, the absorbances of the discoloration of the reaction mixture are read at 517 nm and 700 nm respectively for the radicals DPPH• and ABTS•⁺. The Trolox was used as standard. The control contained 50 µL of methanol with DPPH• or ABTS•⁺ and the blank contained methanol without DPPH• or ABTS•⁺ radical solution. The IC₅₀, expressed in µg/mL, were determined using the equations of the calibration curves of each sample. The IC₅₀ is the value of the concentration of the extract that would trap 50% of the radicals. The lower is this value, the better is the radical activity of the extract.

E. Statistical analysis

All experiments were repeated three times (n = 3) and results are expressed as mean ± standard deviation (SD) of

the experiments. Statistical significance was evaluated by one-way analysis of variance (ANOVA) using XLSTAT version 16.0 (Addinsoft (2020) XLSTAT statistical and data analysis solution. Paris France). Values were considered statistically significant at p < 0.05. The IC₅₀ values were determined by plotting the calibration curve of the percent of inhibition according to concentrations.

III. RESULTS

A. α-Glucosidase inhibition

In this work, the inhibitory effect of α-glucosidase of three extracts of *Acacia macrostachya* was assessed. To recall, α-glucosidase is a key enzyme linked to type 2 diabetes. The obtained results are shown on the histograms below (Fig. 1). It is clear from these histograms that all extracts have an inhibitory effect on the enzymatic activity of α-glucosidase. According to the results, the activity of the extracts is dose-dependent and appears to be more pronounced with SB3 in regard to the inhibition rate depending on the concentration, which reach 94% at 2.298 µg/mL, while the other extracts, at the same concentration, have moderate inhibitory activity. To confirm this hypothesis, IC₅₀ values were determined and recorded in Table 1. It is apparent from this table that all the extracts mentioned are more active than the acarbose used as a reference anti-diabetic drug.

TABLE 1: IC₅₀ (µg/mL) VALUES OF DIFFERENT EXTRACTS AND ACARBOSE

Extracts	RB1	RB2	SB3	Acarbose
IC ₅₀ (µg/mL)	138.485 ± 5.649	2.487 ± 0.441	1.650 ± 0.229	214.830 ± 3.816

Fig. 1. Inhibitory activities of extracts of the root and stem barks of *Acacia macrostachya* and acarbose (positive control compound) against α-glucosidase activity. Values are given as the mean ± SD of three independent assays. Data were analyzed using ANOVA followed by XLSTAT. Values were considered statistically significant at p < 0.05; b: p < 0.001; c: p < 0.0001.

B. DPPH• and ABTS•⁺ radicals scavenging assay

The antiradical properties of the different extracts were evaluated according to the protocol described above. The results are reported in Table 2. It appears that all extracts showed antiradical activity, the best activities are those from methanol extracts (**RB2** and **SB3**) from the root barks and stem of *Acacia macrostachya*. Indeed, **SB3** strongly inhibited the radical activity of the two free radicals with IC₅₀ values of 5.242 ± 0.068 µg/mL and 14.136 ± 0.161 µg/mL respectively by the DPPH and the ABTS test followed by **RB2** (9.307 ± 0.262 µg/mL and 45.049 ± 0.730 µg/mL respectively). **RB1** has the lowest antiradical activity with an IC₅₀ of 539.362 ± 7.251 µg/mL. The values of antiradical activity on the radical DPPH• were found to be more important for all extracts.

TABLE 2: IC₅₀ (µg/mL) VALUES OF ANTI-RADICAL ACTIVITY OF DIFFERENT EXTRACTS AND TROLOX

Extracts	IC ₅₀ (µg/mL)	
	DPPH	ABTS
RB1	539.362 ± 7.251	ND
RB2	9.307 ± 0.262	45.049 ± 0.730
SB3	5.242 ± 0.068	14.136 ± 0.161
Trolox	4.471 ± 0.028	3.689 ± 0.033

ND: Not Determined

IV. DISCUSSION

The results of the inhibitory effect show that all the extracts significantly inhibit the enzymatic activity of α -glucosidase and are more active than the acarbose. This has been observed by other authors [3],[20]. The best activity of the extracts compared to the acarbose could be explained by a synergy of action of several molecules while acarbose intervenes through a single type molecule. Also, the molecules of the extracts would easily access the site attack of the enzyme unlike acarbose due steric hindrance. The methanol extracts (**RB2** and **SB3**) showed a highest inhibitory effect against the enzyme α -glucosidase with IC₅₀ values of 2.487 ± 0.441 µg/mL and 1.650 ± 0.229 µg/mL respectively. **RB1** showed also a significant inhibitory activity against the enzyme with an IC₅₀ of 138.485 ± 5.649 µg/mL compared to the reference substance (acarbose). There are several reports on the anti-diabetic activity of methanol extracts from medicinal plants. Indeed, Peyman and al. 2013 showed that the methanolic extracts of *Darchin*, *Chay-e-makki*, *Tameshk* (*R. fruticosus*), *Sorkhevalik*, *Qareqat* and *Toot* (*M. alba*) strongly inhibited the activity of α -glucosidase with IC₅₀ values of 0.5 ± 0.2 , 0.7 ± 0.4 , 1.1 ± 0.6 , 1.5 ± 0.5 , 3.9 ± 0.9 and 8.9 ± 1.0 µg/mL respectively [3]. Phytochemical screening revealed the presence of certain chemical groups including flavonoids, tannins, terpenoids/steroids and alkaloids in the extracts investigated [15]. The presence of these chemical groups could justify the results obtained on the inhibitory activity of α -glucosidase. Indeed, phenolic compound (flavonoids and tannins) have been pointed out to be positively correlated with α -glucosidase inhibitory activity [3]. These extracts with very interesting antidiabetic properties could therefore have a

particular interest in prevention of the risk of developing cancer in diabetics. The relationship between diabetes and cancer is well established [21]. Indeed, epidemiological data have recently shown that the existence of type 2 diabetes promotes carcinogenesis [21], [22]. Specific management in diabetic patients is therefore necessary to avoid many complications with significant repercussions in terms of morbidity and mortality. One of the most common complications of diabetes is the onset of cancer. Numerous recent studies and meta-analyses have shown an association between diabetes and the occurrence of several types of cancer, including hyperglycemia [21], [23], [24]. So, the bioactive molecules contained in these extracts would be preventive molecules of the risk of excess mortality of diabetic patients by malignant tumors. They would also prevent the risk of several types of cancers (pancreatic, liver, colorectal, leukemia, lymphoma, etc.) in hyperglycemic patients. It has been demonstrated some oral antidiabetics, especially metformin, play a dual role: promotes patient management and reduces the risk of cancer [25]. In summary, current evidence suggests that diabetes increases the risk of several types of cancer.

DPPH• and ABTS•⁺ radicals scavenging assay reveals that all extracts mentioned contain interesting antiradical properties. Ours previous studies have shown that the same extracts were rich in antioxidants with contents of 646.063 ± 3.454 ; 422.748 ± 6.045 and 8.176 ± 0.188 µg Trolox equivalent per gram of extract respectively for **SB3**, **RB2** and **RB1**. Then we notice that the radical scavenging effect of all samples was found to be stronger depending on the antioxidant content in the extract. It has been reported in the literature that antiradical activities are correlated with antioxidant levels in plant extracts as shown in Table 2 and 3. Other authors have shown a positive correlation between phenolic compounds and antioxidant activity of a plant extract on the one hand [3],[26],[27],[28] and between antioxidant activity and inhibition activity of α -glucosidase on the other hand [3],[20]. Nguyen and al. 2019 had shown that extracts from the barks of *Canarium tramdenum* were more active on the radical DPPH• than the radical ABTS•⁺ with IC₅₀ of 12.33 µg/mL and 47.87 µg/mL respectively [20]. The low antiradical activity on the cationic radical ABTS•⁺ may be due to the presence of certain compounds in extracts that would absorb at the same wavelength as the radical. The literature tells us about the need to reduce the harmful effects of free radicals in the body. Indeed, reactive oxygen species such as the hydroxyl radical OH•, the superoxide anion O₂⁻ and the nitrous oxide NO• generate many pathophysiological disorders such as arthritis, inflammation, cancer and diabetes [29]. It has also been shown that diabetic patients are under oxidative stress. So an increased generation of free radicals and a lack of antioxidants can contribute to the beginning and progression of complications associated with diabetes [9]. One of the direct complications associated with diabetes is the onset of cancer. However, the use of antioxidants by diabetic patients is particularly important to prevent long-term complications that may occur. Based on the above, methanol extracts of *Acacia macrostachya* contain

pharmacological molecules that could be used to prevent the occurrence of cancer in diabetics.

V. CONCLUSION

This study was focused on anti-diabetic and antiradical activities of *Acacia macrostachya*, traditionally used as medicinal plant in Burkina Faso against inflammation, cancer and oxidative stress. The obtained results highlighted the highly significant inhibitory effect against α -glucosidase and antiradical activity that its root and stem barks possess. All these two properties are implicated in cancer prevention in diabetics. In our future investigations, we will focus on the bio-guided isolation of the anti-diabetic and antioxidant molecules of this plant.

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editor of “Sciences et Techniques” and Academic Editor of “International Research Journal of Pure and Applied Chemistry”. The main topic of his research is the evaluation of biological properties of natural products as anticancer, anti-inflammatory and antiepileptic drugs. He did his first postdoctoral position (2008-2009) at University of Paris-sud XI, in the laboratory of pharmacognosy (Faculty of pharmacy) under grant from “Agence Universitaire de la Francophonie”. From 2011 to 2013 he was a postdoctoral researcher at “Laboratoire de Biologie Moléculaire et Cellulaire du Cancer (LBMCC)” in Luxembourg under grant from “FNR-Luxembourg” and “Marie-Curie Actions” of European Union. From 01/2019 to 10/2019 he was a visiting scholar of American Fulbright program at the University of Chicago, School of Medicine. He evaluated the anticancer, anti-inflammatory, analgesic, antipyretic, antioxidant and antiepileptic effects of many plant extracts, fractions or isolated compounds and he published 36 papers (impact factor between 1 to 4.7). He has got two Prizes in 2004, one from the President of Burkina Faso and the other from the Ministry of Health of Burkina Faso for the impact of his research results to solve a public health problem in this nation. From 2013 to 2018, he was the Head of the Unit of applied Biology at Institute of health sciences research and from 2014 - 2015, he was the General Director sectoral studies and statistics at Ministry of scientific research and innovation, Ouagadougou, Burkina Faso. Since 2015 he is the inspector general for the ministry of higher education, scientific research and innovation of Burkina Faso.

His publications:

[1] Sawadogo WR, Luo Y, Elkington B, He TC, Wang CZ, Yuan CS. Cytotoxicity and Preliminary Analysis of the Pro-apoptotic and Cell

Cycle Arrest Effects of *Lantana ukambensis* Against colorectal Cancer Cells. International Journal of Applied Biology and Pharmaceutical. 2020; 11 (3): 170-187.

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Dr. Issa TAPSOBA was born in Ouagadougou, Burkina Faso (24 January 1974). He received his PhD in organic electrochemistry from the faculty of sciences of Tunis El Manar University, Tunisia in 2004. He was in postdoctoral position from 2004 to 2007 at the “Ecole Normale Supérieure ULM de Paris”, France under grant from “Agence Universitaire de la Francophonie (AUF) and a contract with LVMH”. on the design of microfluidic devices for the continuous measurement of oxidative

stress and monitoring of the differentiation of stem cells in microsystems. He has been received as visiting researcher by the Professor LO Gorton at the Lund University, Sweden where he worked on the design of biosensors based on plant's enzyme immobilization for H₂O₂ and NO measurement. Since 2007, he was recruited as Lecture at the University Joseph KI-ZERBO, Burkina Faso in the department of chemistry where he is actually serving as Associate Professor. His main research activities deal with the isolation and identification of bioactive products from local plants and the development of analytical tools for the detection of pollutants. From 2013 to 2019, he was the Director general of the scientific research and innovation at the Ministry of Higher Education, Scientific Research and Innovation of Burkina Faso. Currently he is the Director general of the Polytechnic School of Ouagadougou, the Executive Secretary of the African Network of Electroanalytical Chemists (ANEC) and the Head of the Analytical and environmental chemistry team in the “Laboratoire de Chimie Analytique Environnementale et Bio-Organique (LCAEBiO)”, University Joseph KI-ZERBO.

His publications:

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