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Inhibition of α-amylase activity of *Chamaecrista nigricans* (Vahl) Greene (*Fabaceae-Caesalpinioideae*) and *Pseudocedrela kotschyi* (Schweinf.) Harms (*Meliaceae*) water extracts

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Abstract

Introduction: Antioxidants and glucosidases inhibitors are interesting in diabetes mellitus prevention and its management. However, conventional drugs have a multitude of unenviable side effects. Thus, research is turning towards herbal remedies, hence this study that was interested in two plants used in the traditional treatment of diabetes in Bobo-Dioulasso. **Objective**: The aim was to evaluate *Chamaecrista* (*C.*) *nigricans and Pseudocedrela* (*P.*) *kotschyi* roots water extracts effect on sorghum α-amylase activity.

Methodology: Total phenolic and flavonoid contents were quantified using Folin-Ciocalteu reagent and aluminum trichloride, respectively. FRAP, DPPH and ABTS methods were used to estimate the extracts antioxidant activity. The α-amylase inhibition tests were carried out using the glucose assay method with dinitrosalicylic acid.

Results: The *C. nigricans* and *P. kotschyi* extracts have shown strong antioxidant activities, 7031.52±15 and 8762.35±51 µmol AAE.g^{-1}E respectively, referring to trolox using ABTS method. These extracts have also shown an inhibitory effect of 98.57% and 97.67% respectively on α-amylase activity.

Conclusion/perspectives: These plants have *in vitro* an interesting anti-amylasic potential. Further investigations will be necessary using a pure enzyme and a wider range of concentration of the extracts in order to determine the kinetic parameters and the fractionation by chromatography to determine the most effective fraction(s). Finally, an *in vivo* evaluation of the anti-diabetic effect of the extracts could be considered.

Keywords: anti α-amylase, antioxidant, *Chamaecrista nigricans*, *Pseudocedrela kotschyi*.

1. INTRODUCTION

Carbohydrate digestion enzymes are potential targets of anti-diabetics[1,2] because their activity inhibition allow keeping the blood sugar at normal level, especially postprandial glucose level decreasing[3]. Diabetes complications are most of the time associated to oxidative stress[4]. This means that antioxidants have an interesting place in the prevention and the management of this disease. Nowadays, searching for remedies is more focused on medicinal plants[5] because of the constantly increasing side of synthetic products[6,7] on the one hand, and the fewer side effects of the medicinal plants ones[8] on the other hand.

Chamaecrista (*C.*) *nigricans* (Vahl) Greene previously known as *Cassia nigricans* Vahl[9], is a woody herb averaging 30-80 cm in height. This plant, called *duguma jala* in Dioula is widely used in traditional medicine to manage dermatoses[10] and to fight against ticks in domestic animals[11]. It is also used in the treatment of fever, diarrhea, malaria and worms[12] such as gastrointestinal nematodes in goats[13].

Pseudocedrela (*P.*) *kotschyi* (Schweinf.) Harms is a savannah tree[14] that grows mainly on heavy moist soils in valleys and reaches 20-30 m in height[15]. Known as *korugwe* in Dioula, it is of paramount importance in traditional african medicine[16], specially as an analgesic, antipyretic, anti-inflammatory and anti-diabetic[17,18,19]. This plant also enters into the treatments of hemorrhoids, leprosy, epilepsy and also oedemas[14]. It has

many other properties which justify its countless uses in traditional medicine. Some of its biological effects are antimicrobial, antioxidant, antipyretic, antihyperglycemic and anticoagulant effects. It is also an immune stimulant[20,21,22].

Since many years, *C. nigricans* and *P. kotschyi* are commonly used by local people in western part of Burkina Faso to treat diabetes and blood pressure. Thus, the objective of this study was to verify their antioxidative and α-amylase inhibitory activities.

2. MATERIALS AND METHODS

2.1. Plant materials

The plant material consisted of the powder of the whole plant of *C. nigricans* and the root bark of *P. kotschyi*. The harvest was made in mid-October 2019 in the forest of Dindéresso (11°19'21''; - 4°22'46''; 322,6m) about 15 km from Bobo-Dioulasso. The two plants were then identified and authenticated by an expert from the Department of Biological Sciences at Nazi BONI University. The fresh plant material was then cleaned of debris by washing, reduced to small pieces for root bark, and then dried out of the sun for two weeks. Finally, it was sprayed and stored in suitable freezing plastic bag for use. A variety (*Kapelga*) of *Sorghum bicolor* (L.) Moench supplied by a local seed company, was used as a source of α-amylase.

2.2. Extract preparation

The moisture content of each sample was measured in triplicate with KERN (MLS 50-3C, Germany). The extract preparation was done according to the method used by Mahmoudi et al.[23] with some modifications. Thus, the metabolites were extracted by triplicate maceration of 40 g of powder in 400 mL of distilled water for 48 hours. Each extract was then filtered through Whatman paper N°1 (GE Healthcare companies, UK). The filtrates obtained were freeze-dried (Labconco Optix Plaskolite, INC).

2.3. Extraction of α-amylase

The *Sorghum* α-amylase was carefully extracted[24]. The protein concentration of the extract was determined using the Bicinchoninic Acid Protein Assay Kit (Pierce, Rockford, IL) with Bovine Serum Albumin as standard (Sigma-Aldrich, LLC, USA). Then, the activity of α-amylase was measured according to a method[25] using glucose as standard (Scharlau Chemie S.A, Spain).

2.4. Determination of total phenols

The determination of total phenolic content was done according to the Folin-Ciocalteu reagent method[26]. The optical densities (OD) were read at 760 nm against a blank. GENESYS 30 UV-Visible spectrophotometer (Thermo Scientific) was used for all OD readings. The total phenol contents were determined using the reference curve ($y = 0.004668x + 0.034$; $R^2 = 0.9991$) with gallic acid (0-100 mg/L). The results were expressed in mg of gallic acid equivalent per 100 mg of dry weight (mg GAE/100 mg DW).

2.5. Determination of total flavonoids

The flavonoids dosage was carried out by the aluminum chloride ($AlCl_3$) method[26]. The OD were read at 415 nm against a blank. The flavonoid levels were determined by referring to a calibration curve ($y = 0.01259x$; $R^2 = 0.9990$) preestablished under the same operating conditions mentioned above with quercetin (0-50 mg/L) as standard. The results were expressed in mg of quercetin equivalent per 100 mg of dry weight (mg QE/100 mg DW).

2.6. Antioxidant activity evaluation

2.6.1. Antioxidant activity by Ferric reducing antioxidant capacity method (FRAP).

This test was realized according to a miniaturized protocol[26]. Briefly, a mixture of 0.5 mL of the diluted extract solution (100 µg / mL), 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of potassium hexacyanoferrate [$K_3Fe(CN)_6$] (1%), were incubated for 30 minutes at 50°C. A volume of 1.25 mL of trichloroacetic acid (10%) was then added and the mixture was centrifuged at 3 000 g for 10 min. The supernatant (0.625 mL) was mixed with distilled water (0.625 mL) and 1.25 mL of a freshly prepared ferric chloride solution was added. The OD were read against a blank at 700 nm. The standard used was ascorbic acid and the results were expressed in µg of ascorbic acid equivalent per g of extract (µg AAE/gE) by referring to the calibration curve ($y = 0.003270x$; $R^2 = 0.9990$). Trolox has been used as reference for all antioxidant essays.

2.6.2. Anti-radical activity against DPPH•

The protocol[27] can be described as follows: briefly, 0.75 mL of extract diluted in methanol was added to 1.5 mL of the 2,2-Diphenyl-l-picrylhydrazyl solution (20 mg/L) and the mixture was incubated at laboratory temperature for 15 min before reading OD at 517 nm against a blank. The results were expressed in µmol of ascorbic acid equivalent per g of extract (µmol AAE/gE) with reference to the standard ($y = -0.02224x + 0.348$; $R^2 = 0.9966$).

2.6.3. Anti-radical activity against ABTS

$ABTS^{\bullet+}$ was first generated by adding 38.4 mg of ABTS (7 mM) and 6.7 mg of potassium persulfate (2.5 mM) to 10 mL of distilled water. The mixture was incubated in the dark for 12 hours and ethanol was used to adjust the absorbance to 0.70 ± 0.02 units at 734 nm. The mixture of the extract solution at 0.1 mg/mL (10 µl) and $ABTS^{\bullet+}$ solution (990 µl) were incubated in the dark for 15 min, then the absorbance was read at 734 nm. As ascorbic acid was the standard ($y = -0.00079x + 0.709$; $R^2 = 0.9993$), the results were expressed in µmol of ascorbic acid equivalent for 1 g of extract (µmol AAE/gE)[26].

2.7. Evaluation of anti-α-amylase activity by quantitative test

The indirect colorimetric assay for glucose using 3,5-dinitrosalicylic acid was used[28]. Thus, in a final volume of 3 mL; 725 µL of sodium phosphate buffer at pH 7, 1.75 mL of 1% starch paste solution, 0.5 mL of extract and 25 µL of the enzyme extract, were mixed. The mixture was incubated at 37°C for 60 minutes. Then, 150 µL of the solution were recovered and mixed with 100 µL of 3,5-dinitrosalicylic acid (DNSA) for new incubation at 100°C for 5 min. The solution was cooled by adding 1.25 mL of distilled water. The OD were read at 540 nm and the amount of glucose produced after 60 minutes of enzymatic reaction was determined with reference calibration curve ($y = 0.0008x - 0.0104$; $R^2 = 0.9965$) carried out under the same conditions with a range of glucose concentration. The enzymatic activities were expressed in U/mg of protein. A control was prepared under the same conditions for each extract and for each of the concentration as well as a blank. The inhibition percentage (%I) was calculated according to the formula:

$$\%I = \frac{[\text{Enzyme specific activity} - \text{Enzyme specific activity in the presence of extract}]}{\text{Enzyme specific activity x100}}$$

2.8. Statistical analysis

All data was analysed by software R version 3.5.1. Results were presented as mean ± standard deviation. The Student t-test was used for the comparisons of the means of activities between the two extracts on the one hand, and between each extract compared to trolox (reference) on the other hand. P-values (p) < 0.05 were considered significant.

3. RESULTS

3.1. Determination of polyphenols

The average yield of the three times extraction was 6.15 ± 2.83% (w/w) and 3.95±0.22% (w/w) for *C. nigricans* and *P. kotschyi*, respectively.

The Table 1 showed that the two plant extracts have a relatively high content of total polyphenols. This content was higher (*p = 0.009*) for *P. kotschyi* (50.88 mg GAE/100 mg DW) compared to *C. nigricans* (27.41 mg EAG/100 mg DW). Inversely, the flavonoid content of the extract of *C. nigricans* (10.29 mg QE/100 mg DW) was higher (*p=0.0002*) than that of *P. kotschyi* (1.04 mg QE/100 mg DW).

Table 1: Phenolic and total flavonoid contents

Sample	Total phenolics (mg GAE/100 mg DW)	Total flavonoids (mg QE/100 mg DW)
CN	27.41 ± 0.45	10.29 ± 0.06
PK	50.88 ± 0.76	1.04 ± 0.01
p-value	*0.009*	*0.0002*

CN: *Chamaecrista nigricans; PK*: *Pseudocedrela kotschyi*; GAE: Gallic Acid Equivalent; QE: Quercetin equivalent; DW: Dry Weight.

3.2. Antioxidant activity

The results of the antioxidant activity of the two plant extracts are shown in Table 2. The two extracts showed quite interesting activities against the $ABTS^{\bullet+}$radical. The activity against the $ABTS^{\bullet+}$ radical of *P. kotschyi* extract (8 762.35 µmol AAE.g^{-1}E) was higher (*p = 0.03*) than trolox (8 005.11 µmol AAE.g^{-1}E), and the activity of *C. nigricans* (7 031.52 µmol AAE.g^{-1}E) is not significantly different (*p = 0.07*) comparing to the reference (trolox).

$DPPH^{\bullet}$ radical scavenging activities of the aqueous extract of *C. nigricans* (454.5 ± 32 µmol AAE.g^{-1}E) and the root bark of *P. kotschyi* (584.72 ± 8 µmol AAE.g^{-1}E) were not significantly different (*p = 0.996).* They are quite high but remain lower than the reference (*p = 0.003* for *C. nigricans* and *p = 0.0006* for *P. kotschyi*).

However, the ability of the two extracts to reduce the ferric ions were not different (*p = 0.2*) and were 1 840.77 µmol AAE.g^{-1}E and 2 629.78 µmol AAE.g^{-1}E for *C. nigricans* and *P. kotschyi*, respectively. Moreover, *P. kotschyi* roots extract was presented a higher activity (*p = 0.04*) than trolox (2 231.52 µmol AAE.g^{-1}E).

Table 2: Results of the antioxidant activity of the two plant extracts

Sample	ABTS (µmol AAE.g^{-1}E)	DPPH (µmol AAE.g^{-1}E)	FRAP (µmol AAE.g^{-1}E)
CN	7 031.52 ± 15[a]	454.5 ± 32[a]	1 840.77±392.94[a]
PK	8 762.35± 51[b]	584.72 ± 8[a]	2 629.78 ± 35.21[a]
Trolox	8 005.11 ± 0[a]	765.99 ± 18[b]	2 231.52 ± 12.29[a]

CN: *Chamaecrista nigricans*; *PK*: *Pseudocedrela. kotschyi*; FRAP: Ferric reducing antioxidant power; ABTS: 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic) acid ; DPPH: 2,2-diphenyl 1-picrylhydrazyl; AAE: ascorbic acid equivalent. Values with the same letter in the same column are not significantly different.

3.3. Anti-α-amylase activity

The specific activity of the enzyme was 3.03 U/mg protein with a protein concentration of 5.03 mg/mL. The results of the inhibition test showed a good inhibitory effect for both extracts (Tables 3 and 4). The xtracts of *C. nigricans* and roots of *P. kotschyi* at 10 mg/L, inhibited α-amylase at levels of 98.57% and 97.67%, respectively.

Table 3: Inhibition rate of α-amylase in different concentrations of *Chamaecrista nigricans* extract

Concentration (mg/mL)	Esa (U/mgp)	Inhibition rate (%)
10	0.04	98.57
0.31	0.69	77.02
0.08	2.55	16.07
0.02	2.75	9.67

Esa: enzyme specific activity in the presence of extract; U/mgp: microgram of glucose per minute and per milligram of protein.

Table 4: Inhibition rate of α-amylase in different concentrations of *Pseudocedrela kotschyi* extract

Concentration (mg/mL)	Esa (U/mgp)	Inhibition rate (%)
10	0.07	97.67
0.31	1.32	56.24
0.08	2.56	15.66
0.02	2.67	12.32

Esa: enzyme specific activity in the presence of extract; U/mgp: microgram of glucose per minute and per milligram of protein.

4. DISCUSSION

Both plant extracts studied contain phenolic compounds. The presence of these compounds in various extracts of *C. nigricans* and *P. kotschyi* has been reported by several studies[18,29]. However, these studies were generally limited at the characterization, which did not provide an idea of the content of the phenolic compounds in these plant extracts. Here we measured the polyphenols and flavonoids in the aqueous extracts of two plants. Although *C. nigricans* extract had the lower content of phenolics, it had the higher one concerning flavonoids compared with *P. kotschyi*. In the case of flavonoids, the result for *P. kotschyi* extract was slightly similar with a study carried out in Bénin[18]. These authors were found 0.24 ± 0.01 mg QE /g DW, the equivalent of 0.024 mg QE/100 mg DW, as the flavonoid content in a hydro-alcoholic of *P. kotschyi* roots. In the present study, these results could be explained by the chemical compositions of plant extracts can vary widely with the age and the growth conditions of the plant and even with the geographic area[30].

The phenolic compounds are excellent inhibitors of enzymes[31,32] and often have a high antioxidant activity, so the richness of the aqueous extracts of these two plants in these compounds could be interesting in the control of diabetes. The *P. kotschyi* extract which seemed to be less rich in flavonoids, exhibited greater antioxidant activity than that of *C. nigricans*. The antioxidant activity of *P. kotschyi* was comparable with the reference in the case of ABTS and even higher in FRAP case. This result might suggest on the one

hand that the compounds responsible for these activities, even if they can be polyphenols, are not necessarily of the only flavonoid class. Indeed, phenolic compounds include other equally interesting classes besides flavonoids such as stilbenes, phenolic acids, etc., which also have antioxidant properties like all polyphenols[33]. On the other hand, our plant extracts have a significant antioxidant activity. Particularly for the roots of *P. kotschyi*, Bothon et al.[18] were evaluated the antioxidant activity of this extract through the method based on the oxidation of 2',7'-dichlorofluorescin-diacetate (DCFH-DA). These authors were estimated the IC_{50} at 6.3 µg GAE/mL. This means that this plant therefore has a proven antioxidant activity and can have beneficial effects in the treatment of diabetes and the prevention of its complications by reducing oxydative stress.

Natural α-amylase inhibitors in particular have fewer or no side effects[8] and authors have shown the inhibitory effect of plant extracts on carbohydrates digestive enzymes activity[18,34,35]. The extracts of *C. nigricans* and *P. kotschyi* were shown anti-α-amylase activity with a rather interesting inhibition rate of more than 97%. Furthermore, Bothon et al.[18] who used a hydro-alcoholic solvent probably less polar than ours, showed that the extract of *P. kotschyi* had an inhibitory activity (IC_{50} = 5.0 ± 0.2 µg / mL) on α-glucosidase. According to the same authors, polyphenolic compounds in plants have long been recognized to inhibit the activities of digestive enzymes because of their ability to bind with proteins. Studies had shown that plant extracts rich in phenolic compounds could have an inhibition on α-amylase activity[35,36]. The inhibitory action of the two extracts on α-amylase activity may therefore be due to their polyphenol content. Polyphenols are involved in the regulation of glucose metabolism in part by various mechanisms such as the inhibition of digestive enzymes and glucose transporters. These compounds activate enzymes with antioxidant activity and also have many beneficial effects on endothelial function and vascular homeostasis[37]. Based on the above, the extracts of *P. kotschyi* and *C. nigricans* could have beneficial effects in the treatment of diabetes.

5. CONCLUSIONS

The aqueous extracts of the two plants were quite rich in phenolic compounds and had a non-negligible antioxidant activity, which proves the presence of various molecules with different biological activities. These extracts had an interesting inhibition rate ranging to more than 97% of the activity of *Sorghum* α-amylase. This could partly justify the traditional use of these plants in the treatment of diabetes. Further investigations will be necessary using of a pure enzyme and a wider range of concentration of the extracts in order to determine the kinetic parameters and the fractionation by chromatography to determine the most effective fraction (s). Finally, an *in vivo* evaluation of the anti-diabetic effect of the extracts could be considered.

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Declaration of interest statement

The authors of this manuscript declared no conflict of interest.

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