

INHIBITORY ACTIVITY AGAINST α -AMYLASE AND GLUCOSE ADSORPTION CAPACITY OF THE AQUEOUS DECOCTATE OF *CHAMAECRISTA NIGRICANS* (VAHL) GREENE

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Abstract

Diabetes management involves preventing its risk factors. Inhibition of glucosidases and adsorption of excess free glucose are approaches to the prevention of postprandial hyperglycemia. The objective of the present study was to evaluate the antioxidant activity, glucose adsorption capacity, and α -amylase inhibitory activity *in vitro* of the aqueous extract of *Chamaecrista nigricans*. Determination of phenolic compounds content was performed using the Folin-Ciocalteu reagent and the aluminum chloride method was used for total flavonoids one. The glucose oxidase peroxidase kit was used to determine the adsorption capacity of glucose while the 3,5-dinitrosalicylic acid method was used to assess the inhibitory activity against α -amylase. Levels ranging from 33.87 ± 2.48 mg GAE/100 mg dry extract (DE) for total phenolic compounds and 1.98 ± 0.51 mg QE/100 mg DE for total flavonoids were observed. The adsorption capacity was correlated with the glucose concentration of the solution ($r = 0.95$) and was up to $36.61 \mu\text{mol/g DE}$ for a glucose concentration of 30 mM. The extract from the November collection was most active against α -amylase with $\text{IC}_{50} = 0.17$ mg DE/mL. Observations confirm the traditional use of this species as a preventive measure in recipes for the treatment of diabetes. This data provides a basis for future pharmaceutical prospecting.

Keywords: Diabetes, phenolic compounds, adsorption, α -amylase, *Chamaecrista nigricans*

Introduction

The management of hyperglycemia is crucial in the therapeutic management of diabetes mellitus because it can very quickly be fatal for the patient. Postprandial hyperglycemia is partly related to gastrointestinal factors, including intestinal absorption of glucose, which derive in part from the catalytic activities of glucosidases (Paquot & Scheen 2006). Indeed, the action of these enzymes releases monosaccharides from polysaccharides rich foods that are absorbed at the intestinal level. Thus, adsorbents and intestinal glucosidase inhibitors are potential drugs sought in the management of type 2 diabetes (Puls et al. 1977). Intestinal α -glucosidase inhibitors indirectly reduce glucose absorption, thereby preventing or mitigating postprandial



hyperglycemia. On the one hand, phenolic compounds are well known to have glucosidase inhibitory activities (Tadera et al. 2006). On the other hand, polysaccharides and other secondary metabolites slow down postprandial hyperglycemia by adsorbing glucose already released by the action of enzymes and thus making it unavailable for intestinal absorption (Ahmed et al. 2011). In addition, antioxidants provide protection against metabolic disorders such as in the case of diabetes (Nimse & Pal 2015) where they are used in the management of stress. These compounds have the ability to slow down or prevent the oxidation of some molecules, most often either by the transfer of hydrogen atoms or by the transfer of electrons (Prior et al. 2005). Nowadays, plants are sources of natural compounds with multiple properties and which are often said to have fewer harmful side effects than their synthetic analogues.

Chamaecrista nigricans is a plant species in the family of Fabaceae-Cesalpinoideae. Many traditional uses of this plant have been reported and several scientific studies have mentioned a number of its very interesting properties. Thus, studies have shown its use against fungal and bacterial germs involved in dermatoses, its anti-inflammatory potential (Mogode 2005, Ayo et al. 2007, Eyang Esseng 2007), its anthelmintic properties (Oba et al. 2016) and its anti-plasmodial activity (Fatima et al. 2017). The phytochemistry of this species reveals the presence of anthraquinones such as emodin (1,6,8-trihydroxy-3-methyl-anthraquinone), chrysophanol and physcion. It also contains compounds such as 1,2-benzenedicarboxylic acid diisooctyl ester, 4-C-methyl-myo-inositol, n-hexadecanoic acid, 2-methyl-butanoic acid and octadecanoic acid. Besides coumarins, anthracenosides, flavonoids, mucilages, sterols and triterpenes, cardiogenic heterosides and leucoanthocyanins are found in its extracts. In addition to the above, other isolated molecules of this species are emic acid, luteolin (flavonoïd), hydroxyestrane acid ethyl ester, citreorosein (Ayo et al. 2007, Mogode 2005, Tangavelou et al. 2018).

Until now, few studies have focused on the anti-diabetic properties of this plant. In addition, our previous work has shown that *C. nigricans* extract is a potential inhibitor of α -amylase enzymatic activity, with quite significant antioxidant activity (Sanou et al. 2021). In addition, *C. nigricans* is a potential source of phenolic compounds (Ayo 2010). These compounds are known to be involved in antioxidant activity and enzyme inhibition (Tadera et al. 2006, Kang et al. 2014). However, the bioactive compound content of a plant may very well vary depending on the season and the locality of harvest (Ouédraogo et al. 2022) and may miss out on an interesting activity if the timing of harvest is not appropriate.

The objective of the present study was to evaluate the antioxidant activity, glucose adsorption capacity and *in vitro* inhibitory activity against α -amylase of the aqueous extract of *Chamaecrista nigricans* and at the same time to make a comparative study of these activities for samples from three different months of harvest.

Materials and methods

Plant material

The plant material consisted of the leaves of *Chamaecrista nigricans* (Vahl) Greene. The samples were harvested during the months of October, November and December 2020 in the Classified Forest of Dindéresso about twenty kilometers from the city of Bobo-Dioulasso, Houet province in Burkina Faso. They have been authenticated and deposited in the herbarium of the Floristic Center of the Nazi BONI University under the number UNB-957.

Chemicals

All reagents and chemicals used were of analytical grade. Absolute methanol were procured from Chem-Lab (Belgium). Absolute anhydrous ethanol, ferric chloride, sodium carbonate, trichloroacetic, starch soluble and potassium hexacyanoferrate acid were procured from Carlo Erba (France). L-(+)-ascorbic acid, Gallic acid, 3,5-dinitrosalicylic acid, quercetin and 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonate acid, were purchased from Sigma-Aldrich-

Chemical (China). Folin-Ciocalteu reagent were obtained with MERCK KGaA, HC9059050I, Germany, and 2-2-diphenyl-1-picrylhydrazyl were purchased from Thermo Fisher, P19F002, Germany. A ELISA microplate reader (Biobase, BK-EL10C, MBY10C22040496, China) and centrifuge (Hettich Mikro 220R, Germany) were also used. A hot plate (701546-Economy hot plate, 1500 W; 230 V) were used for boiling.

Compound Extraction

The method used by Ranilla et al. (2007) was chosen to perform the extraction of the compounds according to the modifications of Sanou et al. (2023). In short, 5 g of dry matter was boiled for 30 min in 100 mL of distilled water. After filtration using Whatman No. 1 paper, the filtrate was adjusted to 100 mL with distilled water. Then, the solution was centrifuged (6530 rpm; 4°C) for 30 min. Part of the supernatant was used for the Fe³⁺ reduction test and the other part dried at 55°C for the other tests such as DPPH• Radical Sweeping Activity, ABTS⁺ discoloration, glucose adsorption capacity and α-amylase inhibition test.

Determination of total phenolic content

The total phenolic content was carried out according to the method of Kwon et al. (2006). Thus, a mixture was formed from 0.25 mL of extract, 0.25 mL of 95% ethanol and 1.25 mL of distilled water. To this mixture, 0.125 mL of Folin-Ciocalteu 50% reagent was added. After 5 min of incubation, 0.25 mL of Na₂CO₃ (5%) was added to the reaction medium and the whole was again incubated for 60 min. Optical density (OD) was read at 725 nm against a blank. The phenolic content of each sample was determined from the equation ($y = 40.798x + 0.0094$; $R^2=0.998$) of the gallic acid standard curve (0.00625 - 0.2 mg/mL).

Results were expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/100 mg DE) and the average of five readings was used.

Determination of total flavonoid content

Flavonoid testing was performed according to the protocol of Gokhan & Abdurrahman (2014). Briefly, 100 µL of 2% aluminum trichloride was added to 100 µL of extract and the mixture incubated for 15 min. The OD reading was made at 430 nm.

Iron III Reduction Activity

The ability of extracts to reduce iron was evaluated according to the method used by Ouédraogo et al. (2022). Thus, 0.25 mL of extract was mixed with 0.625 mL of phosphate buffer (0.2 M, pH 6.6) and 0.625 mL of aqueous solution of potassium hexacyanoferrate [K₃Fe(CN)₆] 1%. An incubation was made for 30 min at 50°C. Then, 0.625 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm and 4°C for 10 min. Next, 0.625 mL of the supernatant was mixed with 0.625 mL of distilled water and 0.125 mL of a freshly prepared 0.1% iron trichloride solution. Optical density were read at 700 nm and ascorbic acid (0.00625 - 0.2 µg/mL) was used as a standard ($y = 6.149x + 0.245$; $R^2 = 0.99$). The average of five readings was expressed as µmol ascorbic acid equivalent per gram dry matter (µmol AAE)/g DM).

DPPH radical scavenging activity

The DPPH• radical scanning ability of the extracts was evaluated according to the method used by Kwon *et al.* (2006). Thus, a range of extract concentration (5 - 100 µg/mL) was prepared in ethanol. Then, 375 µL of extract was mixed with 750 µL of the DPPH• solution. The initial optical density (OD₀) of DPPH• solution was 0.78 ± 0.03. The mixture was incubated in the dark for 15 min at laboratory temperature. The optical density was then read using a spectrophotometer (1100A) at 517 nm against a control. The tests were carried out three times. The percent inhibition (%I) of the DPPH• radical was calculated from the formula below:

$$\%I = (OD_{control} - OD_{test}) \times 100 / OD_{control}$$

Where: OD_{control}: Control optical density at 517 nm wavelength

OD_{test} : optical density of the sample at the wavelength of 517 nm

The IC_{50} s corresponding to the value of the extracts that neutralized 50% of the initial DPPH[•] free radicals were determined with the software GraphPad_Prisme_9.2.0.332x64.

ABTS^{•+} radical discoloration Test

The ability of the extracts to remove ABTS^{•+} radicals was evaluated by the standard method of discoloring the ABTS^{•+} solution. To do this, 10 μ L of extract at 1 mg/mL was added to 990 μ L of ABTS^{•+} solution and incubated in the dark for 15 min. Optical density was read at 405 nm against a blank and with control. The experiment was performed in triplicata and the percentage of inhibition (%I) of ABTS^{•+} radicals was calculated from the following formula:

$$\%I = (OD_{control} - OD_{test}) \times 100 / OD_{control}$$

With:

$OD_{control}$: Control absorbance at 405 nm wavelength

OD_{test} : Absorbance of the sample at the wavelength of 405 nm

Glucose Adsorption Capacity

Glucose adsorption capacity was assessed using the glucose oxidase peroxidase kit method (Rehman *et al.*, 2018) with modifications. Thus, each extract was tested on glucose solutions at 5 ; 10 ; 15 ; 20 and 30 mM. To make control, each extract was prepared under the same conditions without glucose. The different mixtures were then incubated for 6 h at laboratory temperature ($29 \pm 0.5^\circ\text{C}$). After incubation, each solution was centrifuged (4800 rpm, 10 min). The GOD kit was used to determine the glucose concentration of the supernatant at time zero and at the end of incubation using a Mindray BA-88A UV-Visible spectrophotometer. The average of four readings was used to calculate the amount of adsorbed glucose expressed as micromoles of glucose per gram of dry extract ($\mu\text{mol/g DE}$) using the formula below:

$$\text{Glucose bound} = \frac{(G_1 - G_6) * \text{Volume of the solution}}{\text{Dry extract mass}}$$

G_1 : concentration of glucose at the initial time

G_6 : glucose concentration after 6 h of incubation

Inhibitory activity against α -amylase

The enzyme was extracted from a Megamylase[®] tablet (Sanou et al. 2023). Next, a 2U α -amylase extract solution was prepared for the quantitative inhibition assay. But, a qualitative test of the α -amylase solution was performed before on 1% starch using lugol.

The modified indirect colorimetric method of 3,5-dinitrosalicylic acid (DNA) (Adewale et al. 2006) was used to carry out the quantitative test. Thus, a mixture was constituted with 7 mL of 1% starch, 2 mL of PBS pH = 6.9, 1 mL of extract and 100 μ L of enzymatic solution. After incubation at room temperature ($29 \pm 0.5^\circ\text{C}$) for 1 h, 1.5 mL was removed from the reaction mixture and 1 mL of DNA was added to. That solution was incubated for 5 min at 100°C and then cooled down by adding 7 mL of distilled water. OD readings was made at 540 nm with an ELISA plate reader (Biobase Biodustry (Shandong) CO., LTD, BK-L10C) against one blank where the α -amylase extract was replaced by buffer. For the control, the extract volume was replaced by buffer. The results were expressed in inhibition rates calculated as follows:

$$\%I = (Abs_{control} - Abs_{test}) \times 100 / Abs_{control}$$

Where:

$Abs_{control}$: Control absorbance at 540 nm

Ab_{test}: Absorbance of the sample at 540 nm

Statistical analysis

The data were processed and analysed with the software GraphPad_Prisme_9.2.0.332x64. The analysis of variance of the different parameters and the multiple comparison test of Tukey at the 5% threshold were performed. A Pearson correlation coefficient was calculated between the parameters. The charts were generated using Microsoft Excel.

Results

Total phenolic content and antioxidant activity

The phenolic content of the extracts shows a variation depending on the month of harvest of the sample. Indeed, the highest content with is about $33,87 \pm 2,48$ mg GAE/100 mg DE was observed with the extract from the December collection (CNDD) and the lowest with the November extract (CNDN). This last content was about $23,88 \pm 2,80$ mg GAE/100 mg DE (Table 1). A correlation of $r = 0.76$ was observed between the total phenolic and total flavonoid content of the three extracts (Table 2).

The inhibition concentration of half of the DPPH• radicals was determined for all extracts. The results showed that the extract activity of the October collection is the highest. The lowest activity was observed with the extract from the December collection (Table 1). These values were $24,72 \pm 2,17$ µg GAE/mL and $51,05 \pm 4,13$ µg GAE/mL respectively for CNDO and CNDD. The correlation study between total phenolic content and the activity against free radical yielded a coefficient of 0.85 (Table 2).

The results of the discoloration activity of ABTS radicals are presented as inhibition rate. Of the three extracts, none was able to discolor ABTS⁺• by 50% with an initial control absorbance of 0.89 ± 0.01 at the evaluated concentration (1 mg ES/mL). However, the extract from the October collection showed the highest percentage of inhibition (45.91%) compared to 12.66% for the extract from the November collection (Table 1). A strong correlation was observed between this activity against ABTS⁺• and flavonoid content ($r = 0.98$) (Table 2).

Iron reduction power was evaluated with liquid extracts immediately after extraction. The extract from the November collection (CNDN) showed the greatest metal iron reduction activity, about $2444 \pm 74,44$ µmol AAE/g DM despite the lower total flavonoid content in contrast to the other two extracts (Table 1). This observation is supported by the negative correlation coefficient ($r = -0.92$) found between iron reduction activity and flavonoid content (Table 2).

Table 1. Total phenolic content and antioxidant activity of aqueous extracts from *C. nigricans* leaves

Compounds/activity	CNDO	CNDN	CNDD	p-value
Total phenolic content (mg GAE/100mg DE)	$27,15 \pm 4,31^{ab}$	$23,88 \pm 2,80^a$	$33,87 \pm 2,48^b$	0,0257
Total flavonoid content (mg QE/100 mgES)	$1,95 \pm 0,15^a$	$0,66 \pm 0,31^b$	$1,98 \pm 0,51^a$	0,006
DPPH• IC₅₀ (µg GAE/mL)	$24,72 \pm 2,17^a$	$30,92 \pm 1,39^a$	$51,05 \pm 4,13^b$	<0,0001
ABTS⁺• inhibition rate (%)	$45,91 \pm 1,69^a$	$12,66 \pm 1,07^b$	$39,88 \pm 1,33^c$	<0,0001
FRAP (µmol AAE/g DM)	$1544 \pm 29,39^a$	$2444 \pm 74,44^b$	$1894 \pm 72,95^c$	<0,0001

Extract from the October (CNDO) November (CNDN) and December (CNDD) collection; DE: dry extract; QE: quercetin equivalent; GAE: gallic acid equivalent; AAE: Ascorbic Acid Equivalent. Values that have the same letter in the same line are not significantly different.

Table 2. Pearson's correlation coefficient between phenolic content and antioxidant activity

Parameters	Total phenolics	Total flavonoids
Flavonoids content	0,76	-
DPPH• (CI ₅₀)	0,85	0,31
ABTS ⁺ •	0,63	0,98
FRAP	-0,44	-0,92

Glucose adsorption

The October collection sample showed the highest *in vitro* adsorption capacity for almost all glucose test concentrations (figure 1). Its adsorption capacity is strongly correlated with glucose concentration ($r = 0.95$). Similarly, the extract from the November collection come in second place with a Pearson correlation coefficient of 0.97 (Table 3). The analysis showed that there is a high positive correlation between the DPPH anti-free radical activity of the extracts and their adsorption capacity at high glucose concentrations (15 mM, 20 mM, 30 mM) in contrast to the activity against ABTS⁺• (Table 4).

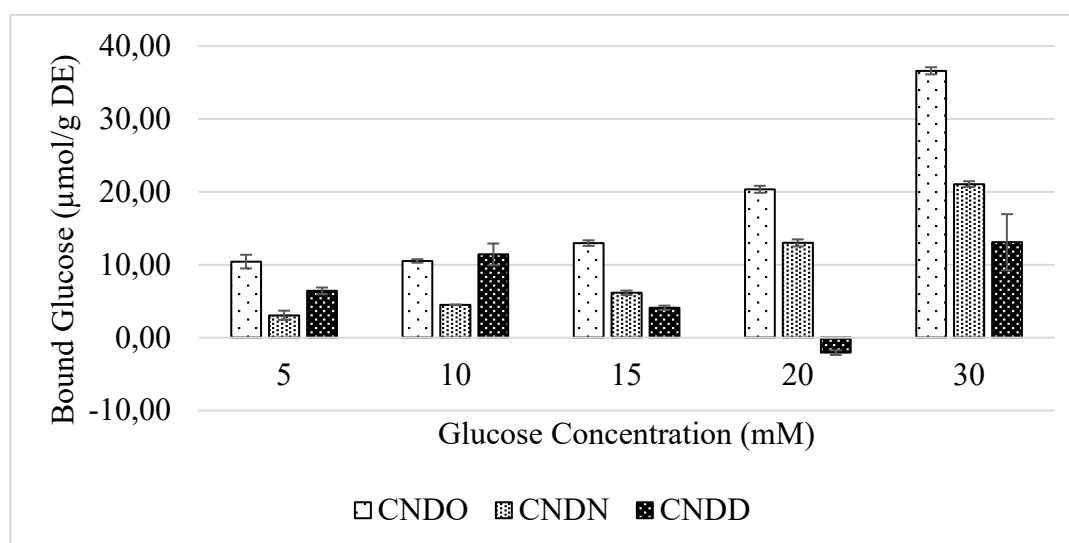


Figure 1. Glucose adsorption capacity of *Chamaecrista nigricans* extracts collected in October (CNDO), November (CNDN) and December (CNDD) at the Dindéresso site.

Table 3. Pearson's correlation coefficient between the change in the concentration of glucose solution and the adsorption capacity of aqueous extracts of *C. nigricans* leaves

Extract	Correlation coefficient
CNDO	0,95
CNDN	0,97
CNDD	0,14

CNDO: excerpt from the October collection; CNDN: excerpt from the November collection; CNDD: excerpt from the December collection

Table 4. Pearson correlation coefficient between glucose adsorption capacity and antioxidant activity of extracts from the October, November and December collection of *Chamaecrista nigricans* as a function of changes in the glucose concentration of the solution

Glucose solution concentration (mM)	5	10	15	20	30
r(C150 DPPH•)	-0,27	0,40	-0,83	-0,99	-0,89
r(ABTS+•)	0,92	0,96	0,45	-0,02	0,35
r(FRAP)	-0,98	-0,87	-0,64	-0,20	-0,55

Inhibitory activity against α -amylase

Chamaecrista nigricans extracts showed different levels of inhibition of α -amylase activity depending on the month of collection. The extracts from the October and November collections were more active compared to the December one with IC₅₀s of 0.23 and 0.17 mg DE/mL, respectively (Table 5). The December collection extract, exhibited stimulatory rather than inhibitory activity with negative inhibition rates for all concentrations tested (Figure 2).

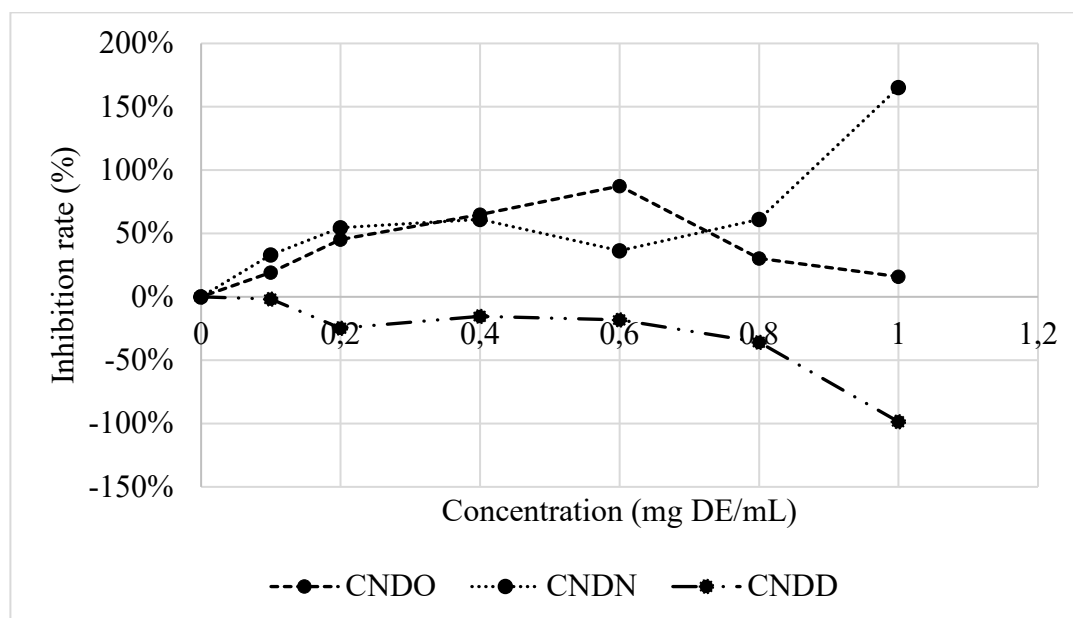


Figure 2. Effect of *Chamaecrista nigricans* leaf extracts on the activity of α -amylase enzyme extract at 2 U/mL.

CNDO: extract from the October collection; CNDN: extract from the November collection; CNDD: extract from the December collection; DE: Dry extract.

Table 5. IC₅₀ value of the inhibitory activity of *Chamaecrista nigricans* leaf extracts against α -amylase (2 U/mL)

Extract	IC ₅₀ α -amylase (mg DE/mL)
CNDO	0,23
CNDN	0,17
CNDD	ND

CNDO: extract from the October collection; CNDN: extract from the November collection; CNDD: extract from the December collection; DE: dry extract; ND: Not determined

Discussions

The purpose of this study was to investigate potential preventive ingredients for type 2 diabetes. To this end, we evaluated the antioxidant activity, the *in vitro* glucose adsorption capacity of extracts of this species and their effect on α -amylase activity. The determination of secondary metabolite compounds in plant extracts often largely explains their biological activities. Phenolic compounds in general and flavonoids in particular are renowned for their antioxidant potential. The structure of these natural compounds and their reactivity with free radicals give them a real power to neutralize or stabilize these radicals, thus limiting their damage to the body (Prior et al. 2005). Several types of radicals are generated during metabolism and also in response to any kind of aggression or stress. Thus, some radicals can be stabilized by proton and/or electron transfer such as DPPH[•], ABTS^{•+} (Prior et al. 2005). This electron transfer also reduces some oxidants in redox reactions which are very important in metabolism. As a result, *C. nigricans* leaf extracts exhibited notable antioxidant activity through the DPPH, ABTS, and iron reduction methods. However, this activity did not correlate positively with the total flavonoid content of the different months extracts. This shows a difference in trend from studies of some authors (Kwon et al. 2006, Laoufi et al. 2017, Sanou et al. 2023) who found an apparent positive correlation between antioxidant activity and phenolic content in medicinal plant extracts. Nevertheless, this state of affairs is understandable, especially since the structure of certain flavonoid molecules, and therefore the flavonoid profile of an extract, is likely to influence its antioxidant activity. Indeed, the number of hydroxyl groups and their position influences the antioxidant activity of a molecule. Indeed, the plant contains compounds with therapeutic potential such as anthraquinone derivatives, including 1,6,8-trihydroxy-3-methyl anthraquinone, also known as emodin, emic acid, citreorosein and liteolin (Ayo 2010). Other classes of secondary metabolites present in crude extracts have good antioxidant potential and are therefore thought to be responsible for the different activities of the extract (Skerget et al. 2005). These compounds are also a factor in the response to the antioxidant activity of these plants in their living environment (Skerget et al. 2005).

In addition, the extract from the December collection with the highest phenolic content was found to be less active on α -amylase than those from the October and November collection. Phenolic compound, and particularly flavonoids, have α -amylase inhibitory activity that depends on their profile (Tadera et al. 2006, Kwon et al. 2008, Kang et al. 2014). Flavonoids are likely to establish hydrogen bonds with the functional amino acid groups of the enzyme's active site. These interactions can inhibit reactions between α -amylase and starch, thereby inhibiting starch digestion (Ng et al. 2015). As with phenolic compound in general, the structure of flavonoids and the number and position of their hydroxyl groups are determining factors for enzymatic inhibition. Thus, the inhibitory activity increases with the number of these groups in the B cycle (Tadera et al. 2006).

Besides, the aqueous decocts of *C. nigricans* leaf extracts showed an interesting *in vitro* glucose adsorption capacity. The ability of *C. nigricans* extracts to adsorb glucose is a property that could be very beneficial in the therapeutic management of postprandial hyperglycemia and therefore diabetes mellitus. The season is one of the factors of variability in the bioactive composition of samples, and therefore in activity (Ouédraogo et al. 2022). Among the extracts from the three harvesting months, the one from the October collection is the most active for *in vitro* glucose adsorption. Dietary fibres, such as non-starch polysaccharides, are known to have glucose adsorbent properties (Ahmed et al. 2011). This potential presented by these extracts can therefore be explained by the probable presence of these polysaccharides, as reported in the extracts of Cassia species (Ayo 2010). In addition, other compounds such as phenolic compounds may well have this ability because of the possible interactions between them and

simple sugars. A concerted property of partial inhibition of carbohydrate degradation by α -amylase supported by adsorption of residual monosaccharides could induce a considerable reduction in postprandial hyperglycemia. Still and all, at the intestinal level, it is α -glucosidase that releases most of the monosaccharides that can be absorbed by the body. Thus, a study of the inhibitory activity of this species extracts against this α -glucosidase will be an asset in the search for natural antidiabetic drugs from *C. nigricans*.

Conclusions

In order to find potential therapeutic agents for type 2 diabetes, research has turned to medicinal plants which remain a valid source of bioactive compounds. During the present study, we were interested in studying the glucose adsorption capacity and the inhibitory activity of aqueous extracts from different months of *Chamaecrista nigricans* in order to highlight its antidiabetic potential. The results obtained showed a potential for glucose adsorption and significant inhibition against α -amylase. These activities may truly have a concerted action for the reduction of postprandial hyperglycemia. This work is therefore very encouraging. To complete this study, we are considering further *in vitro* and *in vivo* investigations. Also, splitting will be necessary to determine the most active stable fractions.

Acknowledgment: No applicable

Conflict of Interest: No conflicts of interest have been reported by the authors of this manuscript

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