Glucose adsorption capacity and inhibitory potential of Pseudocedrela kotschyi (Schwein.) Harms (Meliaceae) leaf extracts against α-amylase: a comparative study over three months

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Abstract

Objective(s): The aim of this study was to evaluate the antidiabetic potential of Pseudocedrela kotschyi leaf decoction by making a comparison between extracts harvested over three months.

Design: This study is done through a few experiences. After the quantification of total polyphenols by Folin-Ciocalteu reagent method, the antioxidant activities were tested with ABTS, DPPH and FRAP essays. Then, the glucose oxidase kit was used to determine the glucose adsorption capacity of the extracts. Last, dinitrosalicylic acid method served to evaluate the α-amylase inhibitory activity of the extracts.

Results: As results the leaves extracts of Pseudocedrela kotschyi showed a polyphenol content of 39.97 ± 3.63 mg EAG/g DE for October, 74.2 ± 11.64 for November and 59.45 ± 7.024 for December. For total flavonoids, the content was 4.86 ± 0.29 mg QE/g DE (October) 5.68 ± 0.39 mg QE/g DE (November) and 5.548 ± 0.19 mg QE/g DE (December). In addition, the antioxidant activity was correlated with the phenolic content (r = -0.65 for DPPH IC50, r = -0.84 for ABTS IC50 and r = 0.97 for FRAP). Besides, the extracts exhibited a strong α-amylase inhibitory activity (IC50 = 0.272 mg DE/mL for October, 0.097 mg DE/mL for November and 0.101 mg DE/mL for December) and had a high capacity to adsorb glucose (over 219.98 ± 24.54mmol/g DE for a 30mM glucose solution for November).

Conclusion: Pseudocedrela kotschyi leaf decoction has the ability to inhibit α-amylase activity and adsorb glucose in glucose concentrated solution. So it possess a high antidiabetic potential.

Keywords: Glucose adsorption, α-amylase, Pseudocedrela kotschyi, antioxidant activity.

1. INTRODUCTION

Phytotherapy has a predominant place in the care of populations throughout the world. Indeed, starting from the knowledge of populations on medicinal plants, scientific research has been able to discover remedies for many pathologies of our world.

Diabetes is one of the most dreaded chronic diseases of our time. To date, several classes of drugs have been developed to treat it depending on their activity and site of action. Metformin and acarbose are some of the leading molecules whose effectiveness is known in diabetes care. Acarbose is a powerful inhibitor of enzymes such as α-amylase and α-glucosidase. Its action very often leads to a set of side effects including flatulence following the fermentation of non-hydrolyzed sugars due to an excess of inhibition of α-amylase. The search for molecules continues in the sense of finding compounds that are just as effective but with fewer side effects. Thus, several plants are explored for their different properties that can contribute to anti-diabetic effects.

The species Pseudocedrela kotschyi exhibits several interesting biological properties. Its leaves exhibits antidiarrheal and antimitotiy effect in vivo. Furthermore extracts of its roots exhibited antihyperglycemic activity, fertility activity and protective effect on the reproductive system against allooxan-induced diabetes-related damage. This extract also induced improvement in hematology and biochemical parameters in rats. The root bark extract showed significant anti-α-glucosidase activity. Leaf extracts of this species showed hypoglycemic activity.

Chemically the plant contains among others 3-O-rhamnoses of myricetin and quercetin and the 3-O-glucoses (or galactosides) of these aglycones.
The objectives of this study was to evaluate the antidiabetic potential of *Pseudocedrela kotschyi* leaf extract through inhibitory and antioxidant activities and glucose adsorption capacity and to make a comparison of these activities over three months.

### 2. MATERIALS AND METHODS

#### 2.1. Plant material

The plant material consisted of leaves of *Pseudocedrela kotschyi* (Schwein.) Harms. The samples were collected in the west of Burkina Faso in the classified forest of Dindéress (Bobo-Dioulasso) over three months, namely October, November and December of the year 2020. The samples were identified at the Laboratoire de Biologie et Ecologie Végétale of the Nazi BONI University and deposit under the number UNB-956 at the herbarium. They were then dried and powdered.

#### 2.2. Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade. Absolute anhydrous ethanol, sodium carbonate, ferric chloride, trichloroacetic, potassium hexacyanoferrate acid and starch soluble were procured from CARLO ERBA-France. L(+)-ascorbic acid, quercetin, 3,5-dinitrosalicylic acid, 2,2'-azinobis-3-ethylenbenzothiazoline-6-sulfonate acid, and Gallic acid were purchased from Sigma-Aldrich-Chemical, Steinheim, China. Absolute methanol (99.8%) were procured from CHEM-LAB, Belgium. Folin-Ciocalteu reagent were obtained with MERCK KGAA, HC90590501, Germany, and 2,2-diphenyl-1-picrylhydrazyl were purchased from Thermo Fisher, P19F002, Germany. A centrifuge (Hettich MIKRO 220R; Germany) were purchased from Thermo Fisher, P19F002, Germany. A centrifuge (Hettich MIKRO 220R; Germany) and ELISA microplate reader (Biobase, BK-EL10C, MBY10C22040496, China) were also used. A hot plate (701546-Economy hot plate, 1500 W; 230 V) were used for boiling.

#### 2.3. Extraction of compounds

The extraction followed the decoction method. Thus, 5 g of powder in 100 mL of distilled water were boiled for 30 minutes. Then, the solution was filtered through Whatman paper N°1 and the filtrate was made up to 100 mL with distilled water. This solution was centrifuged at 6530 rpm at 4°C for 30 minutes. Part of the resulting supernatant was immediately used for the ferric reduction assay and the rest dried at 55°C. The dry extract was well kept for the different analyses.

#### 2.4. Determination of total phenolic content

The determination of these compounds was done by the Folin-Ciocalteu reagent method according to the modifications of some authors. Thus, a reaction mixture was made up of 50 μL of extract, 50 μL of 95% ethanol, 250 μL of distilled water, and 25 μL of 1N Folin-Ciocalteu reagent. After 5 minutes of incubation, 50 μL of 5% Na2CO3 solution was added to the previous mixture and the whole set was again incubated in the dark for 60 minutes. Absorbance readings were taken at 725 nm against a blank with the ELISA microplate reader. The phenolic content of each sample was determined from the calibration curve equation for gallic acid (0.00625 - 0.2 mg/mL) pre-set for this purpose (y = 40.798x + 0.0094; R² = 0.9982). The average of five readings was used and the results were expressed as milligram acid equivalent per gram of dry extract (mg GAE/g DE).

#### 2.5. Determination of total flavonoids content

The determination of flavonoids was performed using the aluminum chloride method. Briefly, 100 μL of aluminum trichloride (2% in absolute methand) was added to 100 μL of extract in wells of an ELISA plate. The mixture was incubated for 15 minutes in the dark. Optical density reading was taken at 430 nm against a blank. The standard curve of a 0-50 mg/L quercetin range (y = 0.1095x + 1.2653; R² = 0.9625) was used to determine the total flavonoid content of the extracts.

#### 2.6. Evaluation of antioxidant activity

##### 2.6.1. Ferric reducing antioxidant power (FRAP)

The ability of the extracts to reduce Fe³⁺ to Fe²⁺ was evaluated according to the standard method used with some modifications. Thus, 0.25 mL of each extract was mixed with 0.625 mL of phosphate buffer (0.2 M, pH 6.6) and 0.625 mL of 1% aqueous potassium hexacyanoferrate solution. After 30 min of incubation at 50°C, 0.625 mL of 10% trichloroacetic acid was added and the mixture was centrifuged (3000 rpm; 4°C) for 10 min. Then, 0.625 mL of the supernatant was mixed with 0.625 mL of distilled water and 0.125 mL of freshly prepared 0.1% iron trichloride solution. Optical densities were read at 700 nm and ascorbic acid (0.00625 - 0.2 μg/mL) was used as a standard (y = 6.149x + 0.245; R² = 0.998). The average of five readings was expressed as micromole ascorbic acid equivalent per gram dry matter (μmol AAE/g DM).

##### 2.6.2. DPPH radical scavenging activity

The DPPH* radical scavenging ability of the extracts was evaluated according to the known method. Thus, for each extract a concentration range (5-200 μg/mL) was prepared in ethanol (96%). Then 375 μL of this solution was mixed with 750 μL of DPPH* solution whose initial optical density was adjusted to OD0 = 0.78 ± 0.03). This mixture was incubated in the dark for 15 minutes at room temperature. The ODs were then read at 517 nm using a spectrophotometer (1100A). A control prepared under the same conditions without extract was used. The tests were performed in triplicate. The percent inhibition (%) of the DPPH* radical was calculated from the formula below:

\[
\text{%I} = \left(\frac{\text{ODcontrol} - \text{ODtest}}{\text{ODcontrol}}\right) \times 100
\]

ODcontrol: optical density of the control at the wavelength of 517 nm

ODtest: optical density of the sample at the wavelength of 517 nm

IC50s which correspond to the extract concentrations that caused the loss of 50% of DPPH* free radicals were determined using GraphPad Prism 9.2.0.332x64 software.

##### 2.6.3. ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonate) radical discoloration assay

The modified standard method was used. First, a 0.7 mmol solution of ABTS in PBS (20mM; pH 6.6) was activated with a purified extract of peroxidase extracted from *Ipomea batatas* peels and *H.2* (0.3%). The absorbance was adjusted to 0.85 ± 0.02 at 405 nm. Then, 10 μL of each extract was reacted with 990 μL of fresh ABTS* solution and incubated in the dark for 15 min. Using the ELISA plate reader, the average of three readings of this reaction mixture was retained. Thus, the concentration that decolorized the ABTS* radical solution by half was determined using an extract concentration range from the DPPH IC50 Value to 1 mg/mL.

#### 2.7. Glucose adsorption capacity

Glucose adsorption capacity was evaluated based on previous work by other authors. Thus, a weight of each extract was dissolved in glucose solutions at concentrations of 5; 10; 15; 20 and 30 mM to give a final extract concentration corresponding to the IC50 of the DPPH assay for each extract. As a control each extract was prepared under the same conditions without the glucose. The glucose concentration of
the different mixtures was determined at zero time and the different solutions were then incubated at room temperature (29 ± 1°C) for 6 hours. After incubation, each solution was centrifuged at 4800 rpm for 10 minutes. Then, the GOD method was used to determine the glucose concentration of the supernatant according to the manufacturer’s instructions using a spectrophotometer (Mindray BA, 88A). The tests were performed in triplicate. The average amount of adsorbed glucose ± standard deviation was calculated and reported in mmol/g DE using the formula below:

\[
\text{Bound glucose} = \frac{(G0 - G6) \times \text{Volume of the solution}}{\text{Extract weight}}
\]

G0: concentration of glucose at the initial time
G6: concentration of glucose after 6 hours of incubation

2.8. Amylase inhibitory activity

2.8.1. Extraction of α-amylase

α-amylase was extracted from a tablet of Megamylase® 30000UCIEP (Inpharmasci, Lot 22002). The tablet was first carefully removed from its thin colored shell. It was then reduced to powder. This powder was dissolved in 10 mL of PBS buffer (20 mM; pH 6.9) to obtain a 300U enzyme solution to which 1mL of calcium chloride (0.1%) was added. After centrifugation, the supernatant was aliquoted and stored at 3±1°C for immediate use.

2.8.2. Inhibitory activity against α-amylase

From the previously formed solution, a 2U enzyme extract solution was prepared for quantitative inhibition test. But before that, a qualitative test of the enzyme solution was performed using 1% starch and lugol.

For the quantitative test, the modified 3,5-dinitrosalicylic acid (DNSA) indirect colorimetric method was used. Thus, to 7 mL of 1% starch, 2 mL of PBS buffer pH = 6.9 and 1 mL of extract at different concentrations and 100 µL of enzyme solution were added. After incubation at room temperature (29±1°C) for 60 min, 1mL of DNSA was added to 1.5 mL of reaction mixture and incubated for 5 min at 100°C. This mixture was then cooled by adding 7 mL of fresh distilled water. The ODs reading was finally taken at 540 nm with the ELISA plate reader (BIOBASE BIODUSTRY (SHANDONG) CO., LTD, BK-L10C) against a blank for each concentration where the enzyme extract was replaced by buffer. Also in the control, the volume of extract was replaced by buffer. The results were expressed as inhibition rates and, in addition to the inhibition rate caused by DPPH* IC50s, the concentration that inhibited the activity of the enzyme extract by 50% was determined.

2.9. Statistical analysis

The data were analyzed with the GraphPad_Prism_9.2.0.332x64 software. Analysis of variance (ANOVA) of the different parameters and Tukey’s multiple comparison test at the 5% significance level were performed and a Pearson correlation coefficient was calculated between the different parameters.

3. RESULTS

3.1. Polyphenols compounds content

The results of the determination of total polyphenol compounds extracted from the leaves of the three months showed that the content varies according to the harvest period. Thus, the content of the November sample is significantly higher than that of October (p = 0.005). However, there is no significant difference between the contents of December and October (p = 0.059). The observation was the same between November and December extracts (p = 0.145). As for the total polyphenol content, there is a significant difference between the total flavonoid content of the November extract and that of the October extract (p = 0.36), which is not the case between the November and December extracts on the one hand (p = 0.857) and between the October and December extracts on the other hand (p = 0.070). Interestingly, the results showed that the richer the extract is in total polyphenols, the more it is also in total flavonoids, as shown by the Pearson correlation coefficient (r = 0.96) between these two parameters.

![Figure 1: Total polyphenol and flavonoid content of P. kotschyi leaf extracts over three months](image)

3.2. Antioxidant activity

3.2.1. DPPH radical essay

The inhibitory concentrations at 50% were determined for these three extracts. The result showed an interesting DPPH radical scavenging activity. The November extract showed the highest activity (figure 2) versus the lowest for the October extract. In fact, the activity against this radical followed the content of total polyphenol compounds in the extract. This is justified by the negative correlation coefficient (r = -0.65) since it is a 50% inhibitory concentration, inversely proportional to the content of polyphenol compounds. However, the difference in the antiradical activity of the extracts of the different months is not significant (p = 0.0772).
3.2.2. ABTS radical scavenging activity.

Similar to the DPPH* radical scavenging activity, the result of ABTS* radical decolorization activity showed a negative correlation coefficient ($r = -0.84$) with the phenolic content of extracts (Figure 3). There is a highly significant difference between the activity of extracts from October and November ($p < 0.0001$) on the one hand and between October and December on the other ($p < 0.0001$), although this is not the case between November and December ($p = 0.2115$).

3.2.3. Reduction of iron III

The extracts showed a good activity of reduction of iron III to iron II. As for DPPH, this activity is strongly correlated to the content of total polyphenols ($r = 0.97$) and flavonoids ($r = 0.999$) in the leaf extracts (figure 4).
3.3. Glucose adsorption capacity

The in vitro ability of *P. kotschyi* leaf extracts to adsorb glucose was evaluated by the GOD method. Overall and for the range of concentrations evaluated the results showed that *P. kotschyi* leaf extracts are able to adsorb glucose. This ability is globally a function of glucose concentration. The November extract showed the highest glucose adsorption capacity over the entire glucose concentration range evaluated (44.23 mmol/g DE against 5.73 for October and 8.09 for December for glucose concentration of 5 mM, 92.31 mmol/g DE against 15.42 for October and 21.95 for December for glucose concentration of 10 mM, 164.80 mmol/g DE against 113.23 for October and 55.51 for December for glucose concentration of 15 mM, 158.02 mmol/g DE against 45.75 for October and 37.08 for December for 20 mM and 219.98 mmol/g DE against 112.18 for October and 181.73 for December for 30 mM).

![Figure 4: Polyphenol content and iron III reduction activity of *P. kotschyi* leaf extracts over three months](image)

### 3.4. Inhibitory activity against α-amylase

Leaf extracts of *Pseudocedrela kotschyi* exhibited a fairly high inhibitory activity against the megamylase enzyme extract. A very substantial negative correlation was displayed between flavonoid content and inhibitory activity (IC50) of α-amylase ($r = -0.99$). The November leaf extract showed the highest inhibitory activity, as evidenced by the inhibition rate given by its IC50 against the DPPH radical. With IC50s of 0.025, 0.021, and 0.027 mg EAG/mL against DPPH for leaf extract of October, November, and December, respectively, inhibition rates of 4.85%, 5.048%, and 31.03% were obtained in this order. However, the IC50 of this November extract (0.097 mg/mL) and that of the December extract (0.101 mg/mL) against the enzyme remain statistically the same ($p = 0.97$). The IC50 of the October extract (0.272 mg/mL) was significantly different from that of the other two months ($p = 0.0002$).

![Figure 5: Glucose adsorption capacity of *Pseudocedrela kotschyi* leaf extracts harvested in October, November and December at the Dindéresso forest](image)
DISCUSSION

The determination of bioactive compounds such as total phenolic is very often used as a backbone on which the explanation of biological activities is based. Flavonoids are one of the subclasses of phenolic compounds near tannins, flavanols, stilbenes,... Although it is not self-evident, there may be a proportional relationship between the flavonoid content of a plant extract and its total phenolic content as in this study. Phenolic compounds in general and flavonoids in particular have remarkable antioxidant properties. These compounds have the ability to scavenge several types of radicals such as DPPH*, ABTS*, and also to reduce iron III to iron II. This constitutes a means of control for plants permanently under biotic or abiotic stress in their living environment. In part, the antioxidant activity of the extracts in the present study is attributable to soluble phenolic compounds. These potentially antioxidant compounds have a real beneficial effect on human health, especially against certain metabolic products such as free radicals. A positive proportionality is often observed between the content of phenolic compounds in medicinal plant extracts and their antioxidant activity. Some of these compounds may even appear to have no direct antioxidant activity, but may be able to raise the level of antioxidant enzymes.

Blood glucose is a very crucial parameter in the management of diabetes. The digestion of carbohydrates, and therefore the activity of the enzymes involved in this digestion, has a strong and direct influence on postprandial blood glucose levels. The root extract of P. kotschyi has already shown an inhibition of α-amylase in previous work. The result of the in vitro tests in the present study showed that the extract is able to bind glucose over 219 mmol/g DE in a 30 mM glucose solution. That to say 1 g DE is capable of adsorbing the whole glucose of about 7 litres of a 30 mM glucose solution. This adsorption combined with the inhibitory effect of the extracts on carbohydrate digestion can significantly decrease the availability of glucose for transport through the intestinal wall. This can result in a slowing of glucose absorption and thus help to avoid postprandial hyperglycemia peaks which are often the cause of fatal crises in diabetics. The adsorption activity has been attributed to dietary fiber in many studies. However, it could result from other metabolites such as phenolic compounds as it was more dependent on the content of these compounds in the extracts. The extracts of Pseudocedrela kotschyi leaves also exhibited significant inhibitory activity of the α-amylase extract. Phenolic compounds such as flavonoids are potential enzyme inhibitors. The structure of these compounds allows them to interact either with the active site of the enzyme or with the substrate which can disrupt the catalytic activity of the enzyme. In addition to the inhibition of digestive enzymes such as α-amylase and α-glucosidase, polyphenols are capable of interacting with starch, thus modifying the arrangement of the molecules. This state of affairs could explain its slow digestion and thus glucose release. P. kotschyi, in view of the properties of its leaf extracts constitutes a valid source of compounds of interest with obvious therapeutic value in different fields such as diabetes care.

About the different months of harvesting, the sample of November was interestingly stronger for most of the activities evaluated in accordance of its phenolic content followed by that of December. It is known that the component content of plant extracts can vary with the season because of the raining conditions and relative humidity and even the areas of harvesting due to soil parameters. Thus, what is the most interesting is the relationship between phenolic content and most of the activities for which the extract were here explored.

CONCLUSION

The study showed that the leaf extract of P. kotschyi has several interesting properties. The extracts of December and mainly November were stronger than that of October for the activities or parameters which have been here assayed. The activity of the extracts was generally correlated with the flavonoid and total phenolic content. The glucose adsorption capacity combined with the antioxidant activity and the inhibitory activity of Pseudocedrela kotschyi leaf extracts against α-amylase make this species a promising source of compounds with antidiabetic activities. It is therefore necessary to evaluate a set of other parameters such as the inhibitory potential of these extracts towards α-glucosidase activity, their influence on glucose diffusion and also their capacity to promote glycogenesis. These results constitute a good basis for our very next study on the extracts of this plant whose toxicity would be interesting to evaluate before continuing other sides.

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