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INHIBITOR EFFECT OF AQUEOUS EXTRACT OF TRUNK BARK OF SCLEROCARYA BIRREA (ANACARDIACEAE) AND ITS FRACTIONS ON α-GLUCOSIDASE AND α-AMYLASE

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ABSTRACT

Introduction: Diabetes mellitus is one of the leading causes of death worldwide. One of the therapeutic approaches for this metabolic pathology consists of inhibiting digestive enzymes. The objective of this work is to evaluate the inhibitory properties of α-amylase and α-glucosidase of the aqueous extract of trunk bark of *Sclerocarya birrea* and its organic fractions. **Methods:** In vitro, the aqueous extract, its ethanolic fraction, its cyclohexanic fraction, and its ethyl acetate fraction at concentrations of 200; 400; 600; 800; 1000; 1500 and 2000 µg/mL are preincubated with α-amylase (2%) or α-glucosidase (2%) in buffer solution for 5 min. After pre-incubation, substrate is added to each test tube and then incubated for 30 min. The enzymatic reaction is stopped by the addition of 3,5 dinitrosalicylic acid (DNS). The Test was repeated three (3) times. **Results:** The aqueous extract of trunk bark of *Sclerocarya birrea* and its different fractions at concentrations of 200; 400; 600; 800; 1000; 1500 and 2000 µg/mL inhibit digestive enzymes in a concentration-dependent manner with 50% inhibitory concentrations (IC50) of 885; 1130; 461; and 1328 µg/mL respectively for the aqueous extract, its ethanolic fraction, its cyclohexanic fraction, and its ethyl acetate fraction. The F4 fraction and the total extract presented the best inhibitory activities. **Conclusion:** The aqueous extract and its cyclohexanic fraction by inhibiting digestive enzymes can reduce the postprandial increase in blood glucose and therefore be an effective approach to manage blood glucose levels in diabetes mellitus.

KEYWORDS: Diabetes, α-glucosidase, α-amylase *Sclerocarya birrea.*

INTRODUCTION

Diabetes mellitus is one of the leading causes of death worldwide. Its prevalence is expanding in the global adult population. In sub-Saharan Africa, the number of people with diabetes is estimated at two point eight million.[1] In Ivory Coast, diabetes is the second cause of hospitalization with a mortality rate of 8.9%.[2] This metabolic pathology is often associated with various risk factors including poor eating habits rich in fat and sugar.[3] People with diabetes have chronic hyperglycemia which contributes to cardiovascular, ocular and renal complications.[4]

The management of diabetes is lifelong and expensive, requiring a combination of several therapies.[5] One therapeutic approach consists of reducing postprandial hyperglycemia by delaying glucose absorption by inhibiting enzymes that hydrolyze carbohydrates in the intestine, namely α-amylase and α-glucosidase.[6] Acarbose is the inhibitor of these enzymes used in the treatment of type 2 diabetes.[7] In search of new bioactive molecules against diabetes, previous studies have reported that certain medicinal plants are capable of inhibiting digestive enzymes.[8,9,10] In Ivory Coast, ethnobotanical surveys have revealed that there are several potentially antidiabetic plants including *Sclerocarya birrea*. The aqueous extract of the trunk bark of this plant has dose-dependent antihyperglycemic activity in the streptozotocin-induced diabetes model in rats.[11] Also, leaf decoction of the aqueous extract of *Sclerocarya birrea* has shown antidiabetic properties.[12] However, very few studies on the pharmacological mechanism of action justifying these beneficial effects of *Sclerocarya birrea* have been carried out. The objective of this work is to evaluate the inhibitory properties of α-amylase and α-glucosidase of the aqueous extract of the trunk bark of *Sclerocarya birrea and* its organic fractions (its ethanolic fraction, its cyclohexanic fraction, and its ethyl acetate fraction).

MATERIAL AND METHODS

Plant material

The plant material consists of trunk bark of *Sclerocarya birrea*. The plant was collected in the month of August 2021 in Korhogo, in the north of Côte d'Ivoire. This plant has been identified and authenticated at the National Centre of Floristics (CNF) of the University Felix Houphouët-Boigny (Abidjan, Côte d'Ivoire) by Professor ZIRIHI Guédé Noel thanks to the herbaria numbers UCJ001006. The ITIS number is 895125.

Pharmacological substances

The pharmacological substances used were 3,5 dinitrosalicylic acid, α-amylase (2%), α-glucosidase (2%), starch (1%) and sucrose (1%).

Methods

Preparation of the total aqueous extract

Two hundred and fifty (250) grams of *Sclerocarya birrea* bark are crushed and placed in 3 liters of distilled water. After 15 minutes of boiling (T=100°C) with magnetic stirring, the decoction obtained is filtered through hydrophilic cotton and Wattman paper (3 mm). The filtrate obtained is evaporated at 50°C in an oven (Memmert, Germany). After evaporation, we obtain a brown colored powder. This powder constituting the aqueous extract of *Sclerocarya birrea* (EAqScB) is stored at -5°C.

Fractionation of the total aqueous extract

The method used is that of N'guessan *et al.*[13] which allows the separation of chemical substances contained in the aqueous extract according to their properties as indicated in Table 1.

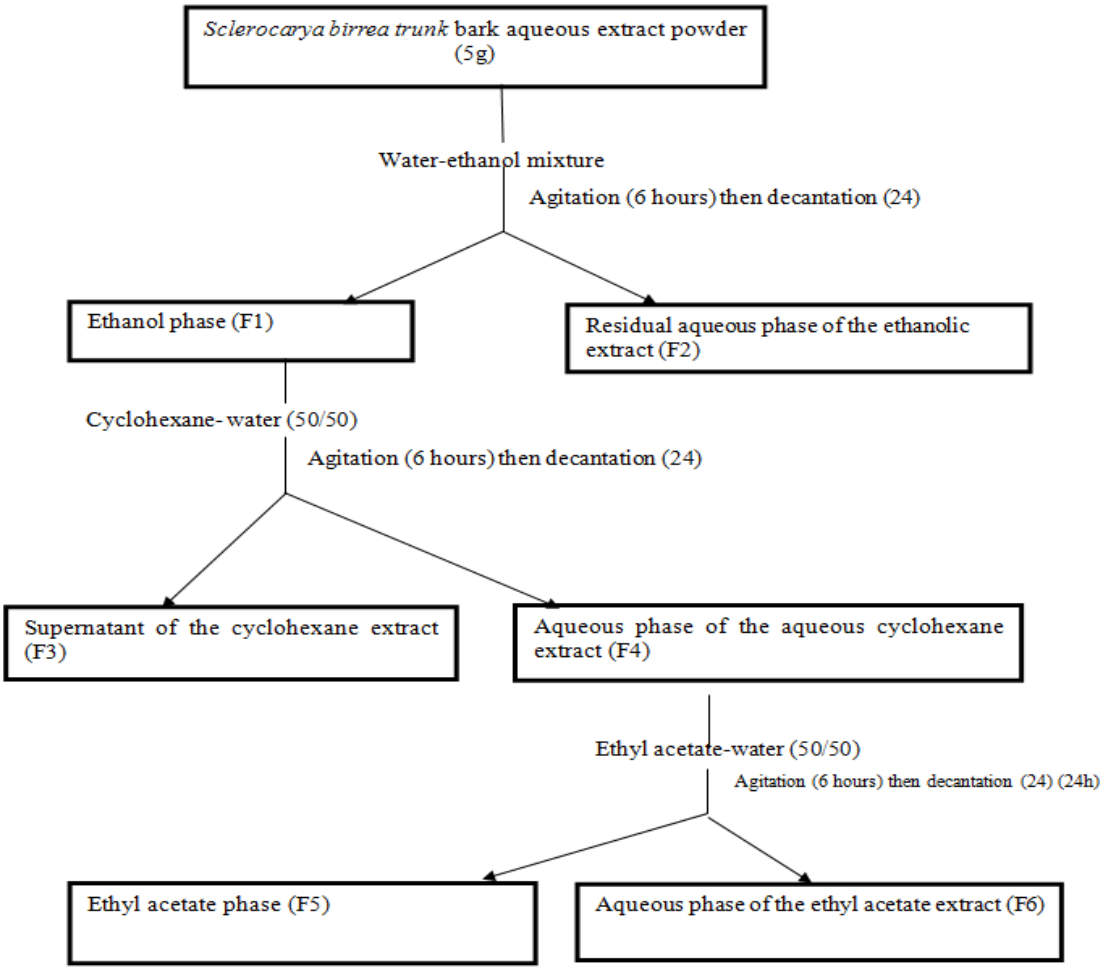

Figure 1: Synoptic diagram of the different stages of fractionation of the aqueous extract of trunk bark of *Sclerocarya birrea.*

In vitro evaluation of the effects of the aqueous extract of trunk bark of *Sclerocarya birrea* and its fractions on the enzymatic activities of α glucosidase and α-amylase

The inhibitory effect of the aqueous extract of trunk bark of *Sclerocarya birrea* and its fractions on the enzymatic activity of α-glucosidase or α-amylase was determined according to the method described by Kee *et al.*[14] with some modifications. Acarbose is used as a reference substance.

Experimental protocol

In each test tube, 100 µl of buffer, 50 µl of 2% enzymes and 100 µl of the extract are successively placed. These tubes are preincubated for five (5) min at a temperature of 37°C. After the preincubation, 100 µl of the substrate (1% starch or 1% sucrose) are added to each tube except the control tubes. Followed by incubation for 30 min at 37°C. Finally, 300 µl of 3.5 dinitrosalicylic acid (DNS) is added to each tube to end the enzymatic reaction. All tubes are heated in a water bath for 5 min. After heating, 200 µl of distilled water is added to each tube for reading the optical density (OD) at the wavelength of 540 nm. A decrease in enzymatic activity shows that there are molecules inhibiting α-glucosidase and α-amylase activity. The percentage of enzyme inhibition is calculated according to the following formula

$$I\% = \frac{DOb - DOex}{DO} \times 100$$

DOb: Optical density in the control tubes

DOex: Optical density of the different extracts

I%: Percentage of inhibition

RESULTS

Effect of the aqueous extract of trunk bark of *Sclerocarya birrea and* its fractions on the enzymatic activity of alpha glucosidase

Figure 2 A reflects the effects of EAqScB, its fractions and acarbose on the inhibition of alpha (α) glucosidase.

Acarbose, EAqScB and its different fractions inhibited α glucosidase in a concentration-dependent manner. Acarbose has the lowest fifty percent inhibitory concentration (IC50) of 8 µg/mL, followed by fraction F4 with an IC50 of 461 µg/mL then EAqScB whose IC50 is 850 µg /mL.

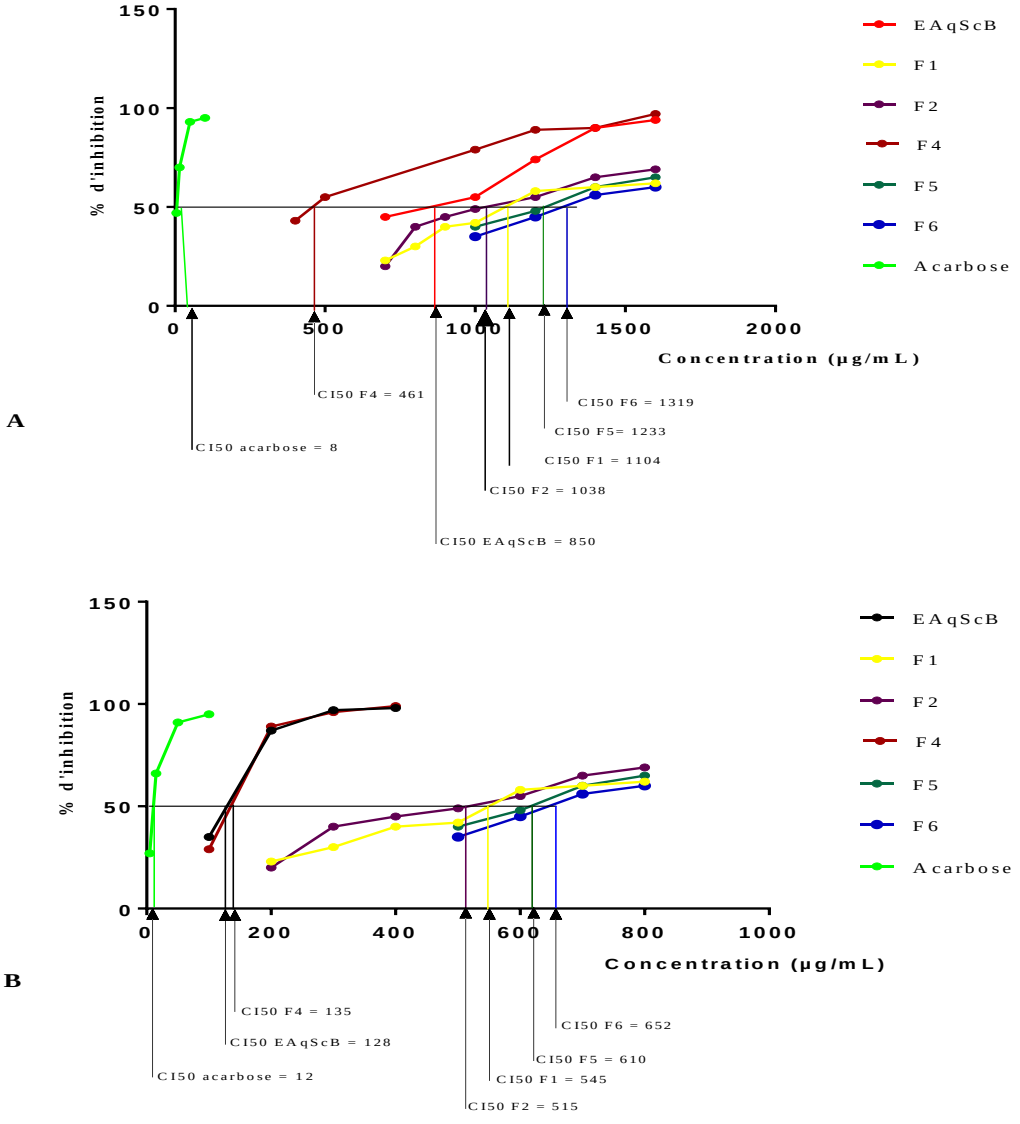

Figure 2: Inhibitory activity curves of the aqueous extract of trunk bark of S*clerocarya birrea* and its fractions on the enzymatic activities of α glucosidase and α amylase.

A. Curves of the inhibitory activity of the aqueous extract of trunk bark of *Sclerocarya birrea* and its fractions on the enzymatic activity of α glucosidase
B. Curves of the inhibitory activity of the aqueous extract of trunk bark of *Sclerocarya birrea* and its fractions on the enzymatic activity of α amylase.

Acarbose presents the best inhibitory activity on the activities of α glucosidase and α amylase with respective IC50s of 12 and 8 µg/mL. The F4 fraction presents an inhibitory activity on the activated α glucosidase better than that of EAqScB with an IC50 of 461 µg/mL. EAqScB: Aqueous extract of trunk bark of Sclerocarya birrea F_1: Ethanol fraction;
F_2: Residual aqueous fraction of the ethanolic extract.
F_3: Cyclohexanique fraction;
F_4: Residual aqueous fraction of the cyclohexane extract;
F_5: Ethyl acetate fraction;
F_6: Residual aqueous fraction of ethyl acetate extract, CI50: Inhibitory concentration 50.

Effect of the aqueous extract of trunk bark of *Sclerocarya birrea* and its fractions on the enzymatic activity of alpha amylase

Figure 2 B reflects the effects of EAqScB, its fractions and acarbose on the inhibition of α amylase. EAqScB, its different fractions and acarbose inhibited α amylase in a concentration-dependent manner. Acarbose presented the best alpha amylase inhibitory activity with an IC50 of 12 µg/mL, followed by EAqScB with an IC50 equal to 128 µg/mL then the F4 fraction whose IC50 is 135 µg/mL. mL.

DISCUSSION

Inhibition of the activity of α amylase and α glucosidase would lead to a reduction in the bioavailability of glucose.[15] Our results show that the total extract and its fractions as well as acarbose inhibited the activity of these enzymes in a concentration-dependent manner. As expected, acarbose presented the best inhibitory activity on the enzymatic activity of α amylase and α glucosidase compared to EAqScB and its F4 fraction with inhibitory concentrations 50 (IC50) of 8 ± 0 .03 µg/mL and 12 ± 0.09 µg/mL on α glucosidase and α amylase. The F4 fraction presented an inhibitory activity on α glucosidase better than that of EAqScB with an IC50 of 461 ± 0.07 µg/mL as for EAqScB it presented an inhibitory activity on α amylase better than that of the fraction F4 with a IC50 of 128 ± 0.06 µg/mL. Our results are similar to those of Oboh et al.[16] who showed an inhibitory activity of the methanolic extract of the leaves of *Persea americana* (Lauraceae) with an IC50 of 219 ± 0.012µg/mL for α amylase and an IC50 of 67 ± 0.001 mg/mL for α glucosidase. Dédou,[17] found that the inhibitory activity of the aqueous extract of the fruits of *Bauhinia thonningii* (Fabaceae), presents an inhibitory activity on α amylase better than that of acarbose with an IC50 of 1159 ± 0, 4 µg/mL unlike its effects on α glucosidase with an IC50 of 3130 ± 0.1 µg/mL. *Sclerocarya birrea* therefore seems less effective than *Bauhinia thonningii*. This difference in results could be explained according to Weinman et al.[18] by experimental conditions and parameters such as incubation duration, temperature and PH. Indeed, enzyme inhibitors can act according to various mechanisms, by combining either with the enzyme, with the enzyme-substrate complex, or with the substrate itself. Also this difference could also be explained by the too high concentration of flavonoids in our extracts which is 59.33 ± 1.88 in the aqueous extract and 74 ± 1.63 in the cyclohexanic fraction. Indeed, Hanhivea *et al*.[19] proved that flavonoids, phenolic acids and tannins prevent the activity of enzymes such as α-amylase and α-glucosidase which are the key enzymes in carbohydrate digestion.

CONCLUSION

This study revealed an interesting concentration-dependent inhibitory effect of the aqueous extract of *Sclerocarya birrea* trunk bark and its cyclohexanic fraction on the activity of α-amylase and α-glucosidase. *Sclerocarya birrea* could reduce postprandial hyperglycemia and therefore be an effective approach for the treatment of diabetes mellitus.

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