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In vitro α-Amylase and α-Glucosidase Inhibitory Effects of Two Herbal Medicines extracts from Burkina Faso

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Abstract **Original Research Article**

The reducing of gastrointestinal glucose production and absorption through the inhibition of carbohydrate digesting enzymes such as α-amylase and α-glucosidase is a good antidiabetic therapeutic approach. The aim of this study was to evaluate the Chloroform, Dichloromethan, Ethyl acetate and Methanol extracts of *N. Canescens* and *H. auriculata* whole plants for their *in vitro* antidiabetic activity. Our result suggests that all extracts exhibit dose-dependent increase in percentage inhibitory activity on α-amylase and α-glucosidase enzymes. The best α-amylase IC_{50} value of 403.50 ± 4.48 µg/mL was obtained with the Chloroform extract of *H. auriculata* while the Methanol extract of *N. canescens* with an IC_{50} value of 507.50 ± 4.55 µg/mL. Regarding the α-glucosidase, the best IC_{50} value of 326,12 ± 2.86 µg/mL was obtained with *H. auriculata* Chloroform extracts followed by *N. canescens* Methanol extract with an IC_{50} value of 364.40 ± 3.48 µg/mL. Acarbose was used as a standard drug.

Keywords: *N. Canescens*, *H. auriculata*, α-amylase, α-glucosidase, inhibition.

INTRODUCTION

Nowerdays, diabetes is known to be one of the major causes of death worldwide. Diabetes, characterized by glucose dysmetabolism, is a common endocrine and metabolic disorder that can cause systemic diseases like fat and protein dysmetabolism. There are three main forms of diabetes: insulin-dependent diabetes mellitus (type 1), non-insulin-dependent diabetes mellitus (type 2) and gestational diabetes. Type 2 diabetes is known as the most common type and two digestive enzyme are more implicated for the development of the disease.

The most important digestive enzyme is pancreatic α-amylase, a calcium metalloenzyme that catalyzes the hydrolysis of the α-1, 4 glycosidic linkages of the starch, amylose, amylopectin, glycogen, and various maltodextrins and is responsible of most of starch digestion in humans. A positive correlation between human pancreatic α-amylase activity and the increase in postprandial glucose levels has been shown, demonstrating the relevance of suppressing postprandial hyperglycemia in the treatment of type 2 diabetes [1].

The second digestive enzyme, α-glucosidase or maltase catalyzes the final step of the digestive process of carbohydrates acting up on 1, 4-α bonds and giving as a result glucose.

So, the complex components of dietary carbohydrates should be broken down to monosaccharides by the α-amylase and α-glucosidase since only monosaccharides can be absorbed from intestinal lumen and transported into blood circulation [1, 2]. Therefore, one of the therapeutic approaches for decreasing of blood glucose rise after a meal is to retard the absorption glucose by inhibition of these carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase [3].

A previous study reported the hypoglycemic activity of *H. auriculata* in human subjects. Interesting, a treatment of streptozotocine-induced diabetic rats with ethanolic extracts from the aerial parts of *H. auriculata* for 3 weeks showed a significant reduction in the blood glucose levels, thiobarbituric acid reactive substances, and hydroperoxide in both liver and kidney [4]. this specie is useful in treating diabetes in many traditional system.

However, these two plants species were widely used traditionally in the treatment of diabetes in Burkina Faso, but little scientific evidence supports there use.

This paper aims to verify if the extracts of *N. Canescens* and *H. auriculata* from Burkina Faso herbal medicines can inhibit α-amylase and α-glucosidase *in vitro*.

MATERIAL AND METHODS

Plants material collection

The plant material is constituted of the whole plant of *Nelsonia canescens* (Lam) Spreng and *Hygrophila auriculate* (Schumach.) Heine. These plants were selected basing on the ethnobotanic investigation of the professor Nacoulma [5]. The plants previously identified by Prof. Millogo-Rasolodimby botanist at the Plant Biology Department of the University of Ouagadougou were collected in August 2017 at Loumbila, 15 km north of Ouagadougou (Burkina Faso). Voucher specimens with accession numbers ID 10152 and ID 10259 respectively for *Nelsonia canescens* and *Hygrophila auriculata* were deposited at the Herbarium at the OUA herbarium of the CIB (Centre d'Information sur la Biodiversité), UFR/SVT, and University of Ouagadougou. The samples were dried at room temperature and ground to fine powder for further extraction.

Preparation of Plant Extract

25 g of each plant powdered was packed in a cartridge. Then it was serially extracted into solvents of increasing polarity, Chloroform, Dichloromethan, Ethyl acetate and Methanol using a Soxhlet apparatus. The extracts obtained were stored at 4°C until use for further analysis. For stock solution, 10 mg of extract was dissolved in 1ml of DMSO.

Procedure for the *in vitro* α-amylase inhibitory assay

The extracts of *N. canescens* and *H. auriculata* were investigated for its anti-diabetic activity using α-amylase inhibitory assay according to the method described by Alara *et al.* 2018 [6]. In brief, a mixture of 0.5 mL extract or acarbose at different concentration (200, 400, 600, 800 and 1000 µg/mL), 0.5 mL of 20 mM sodium phosphate buffer (pH of 6.9) and 0.5 mL of 0.5 m M procaine pancreas α-amylase solution was pre-incubated for 10 min at room temperature. Thereafter, 0.5 mL of 1% starch dissolved in 20 mM sodium phosphate buffer solution was mixed to the mixture after pre-incubation. Dinitrosalicylic (DNS) acid colour reagent of 1.0 mL was then added to the mixture to terminate the reaction. The mixture was left to incubate for 5 min in a water bath operated at 90ºC and allowed to cool down before diluting the mixture with 5 mL of distilled water. Then, the absorbance at 540 nm was recorded using a UV-Vis Spectrophotometer. The blank was sodium phosphate buffer. All the reactions were conducted in triplicates and the α-amylase inhibitory pourcentage was calculated by comparing the readings from the sample mixture with control.

$$\alpha\text{-amylase inhibition (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}} \times 100$$

where Absorbance of Control represents α-amylase and phosphate buffer solution mixture; and Absorbance of Sample denotes α-amylase solution, phosphate buffer, and sample mixture.

In vitro determination of α-glucosidase inhibition

The method described by Kim *et al.* [7], with slight modifications was used to determine the inhibition effect of plants extracts on α-glucosidase activity. The substrate solution (5.0mM) para-nitrophenylα-D-glucopyranoside (pNPG) was prepared in 20mM phosphate buffer, pH 6.9. Using dimethyl sulphoxide (DMSO) to dissolve the extract, varying concentrations of the extract solution ranging from 200, 400, 600, 800 to 1000 µg/mL was used for the assay. Then 50 µL of α-glucosidase (1 U/ mL) was added to five tubes containing 20 µL of the different concentrations (200, 400, 600, 800 to 1000 µg/mL) of the extract or the acarbose. After which, 30 µL of 5.0 mM (pNPG) was added to start the reaction. The reaction mixture was incubated at 37˚C for 1 hour and stopped by adding 1 mL of 0.1 M sodium carbonate (Na_2CO_3). The α-glucosidase activity was determined by measuring the yellow colored para-nitrophenol released from pNPG at 400 nm using a spectrophotometer. The blank solution was prepared by adding the Na_2CO_3 to the reaction mixture before adding the enzyme. The results were expressed as a percentage of the negative control in which the extract was replaced with DMSO. All the reactions were conducted in triplicates. Percentage inhibition was calculated as:

$$\alpha\text{-glucosidase inhibition (\%)} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}} \times 100$$

Calculation of 50% Inhibitory Concentration (IC_{50})

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) was determined graphically using the concentration/inhibition plots.

STATISTICAL ANALYSIS

All of the statistical analyses were performed using GraphPad Prism 3.02 statistical. The data were expressed as mean ± SEM from three replicates of each experiment. The IC_{50} values were estimated by nonlinear curve-fitting and presented as their respective 95% confidence limits.

RESULTS

Extractive Yield (%) of Ethanobotanical Plants

The yield of the different extracts is mentioned in the Figure 1. The methanolic extracts of *H. auriculata* and *N. canescens* showed maximum extractive yield with 5.9 % and 5.2% respectively compare to the other solvent extracts using chloroform, dichloromethan and ethyl acetate. Indeed *N. canescens* showed maximum yield in chloroform, dichloromethan and ethyl acetate extracts with 2.2%, 2.6% and 3.7% respectively compare to those of *H. auriculata* with 1.8%, 2% and 3.3% respectively.

α-Amylase and α-Glucosidase Inhibitory Effect of Plant Extracts

The α-amylase and α-glucosidase inhibitory activity of various extracts of the two Burkina Faso herbal medicines are presented in tables 1 to 4. The Chloroform, Dichloromethan, Ethyl acetate and Methanol extracts of Both medicinal plants exhibited signicant inhibition of α-amylase and α-glucosidase activity in a dose-dependent manner. The α-amylase inhibitory activities for *N. canescens* and *H. auriculata* are repectively ranged between 13-75% and 17-86% using Chloroform extracts, 10-55% and 13-45% using Dichloromethan extracts, 14-45% and 16-65% using Ethyl Acetate extracts, 26-71% and 36-75% using Methanol extracts. Concerning the α-glucosidase inhibitory activities, the *N. canescens* and *H. auriculata* inhibition pourcentage are repectively ranged between 25-83% and 34-92% using Chloroform extracts, 26-80% and 22-75% using Dichloromethan extracts, 23-64% and 30-71% using Ethyl Acetate extracts, 30-79% and 31-83% using Methanol extracts.

The best α-amylase IC_{50} value of 507.50 ± 4.55 µg/mL was obtained with *N. canescens* Methanol extract while the Chloroform extract of *H. auriculata* was presented the best IC_{50} value of 403.50 ± 4.48 µg/mL. Regarding the α-glucosidase, the best IC_{50} value of 326,12 ± 2.86 µg/mL was obtained with *H. auriculata* Chloroform extracts followed by *N. canescens* Methanol extract with an IC_{50} value of 364.40 ± 3.48 µg/mL.

Although these IC_{50} values of *H. auriculata* chloroformic extract and *N. canescens* methanolic extract are comparatively lower than the referee compound (Acarbose) with IC_{50} values of 268.95 ± 2.76 µg/mL for α-amylase and 235.10 ± 1.88 µg/mL for α-glucosidase, we note in this study that the good results obtained with these two medicinal plants could justified their strong use in folk medicine of Burkina Faso for the treatment of type 2 diabetes.

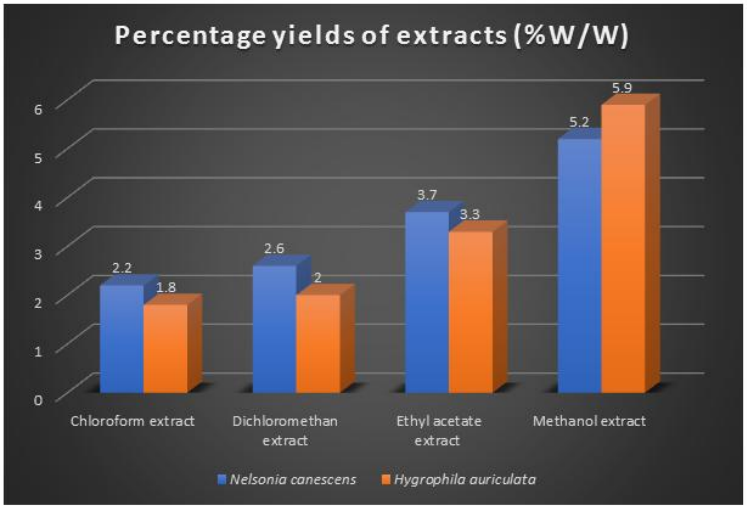

Fig-1: Percentage yields of extracts (%W/W)

Table-1: Enzymes inhibition by *N. canescens* and *H. auriculata* Chloroform extracts

Sample	Concentration (µg/mL)	α-amylase inhibition		α-glucosidase inhibition	
		Inhibition (%)	*IC_{50} (µg/mL)*	*Inhibition (%)*	*IC_{50} (µg/mL)*
Nelsonia canescens	200	13.21 ± 0.13	590.62 ± 6.52	25.24 ± 0.05	398.54 ± 3.68
	400	39.01 ± 0.31		50.12 ± 0.38	
	600	50.53 ± 0.28		56.34 ± 0.19	
	800	68.54 ± 0.33		76.55 ± 0.71	
	1000	75.39 ± 0.76		83.86 ± 0.07	
Hygrophila auriculata	200	17.58 ± 0.30	403.50 ± 4.48	34.57 ± 0.90	326,12 ± 2.86
	400	49.65 ± 0.27		57.69 ± 0.49	
	600	57.54 ± 0.88		71.00 ± 0.18	
	800	74.61 ± 0.59		86.07 ± 0.04	
	1000	86.76 ± 0.91		92.03 ± 0.25	
Acarbose (standard)	200	38.37 ± 0.09	268.95 ± 2.76	45.01 ± 0.53	235.10 ± 1.88
	400	69.03 ± 0.84		71.77 ± 0.09	
	600	72.22 ± 0.46		76.28 ± 0.98	
	800	81.98 ± 0.61		87.65 ± 0.52	
	1000	96.11 ± 0.55		98.09 ± 0.37	

Table-2: Enzymes inhibition by *N. canescens* and *H. auriculata* Dichloromethan extracts

Sample	Concentration (µg/mL)	α-amylase inhibition		α-glucosidase inhibition	
		Inhibition (%)	*IC_{50} (µg/mL)*	*Inhibition (%)*	*IC_{50} (µg/mL)*
Nelsonia canescens	200	10.09 ± 0.22	785.30 ± 3.98	26.69 ± 0.17	428.51 ± 5.01
	400	24.52 ± 0.16		48.29 ± 0.06	
	600	30.77 ± 0.51		55.02 ± 0.33	
	800	51.00 ± 0.10		63.34 ± 0.18	
	1000	55.08 ± 0.67		80.14 ± 0.73	
Hygrophila auriculata	200	13.21 ± 0.35	> 1000	22.76 ± 0.89	571.04 ± 4.56
	400	24.08 ± 0.62		43.09 ± 0.57	
	600	29.98 ± 0.11		51.36 ± 0.52	
	800	38.30 ± 0.73		64.96 ± 0.00	
	1000	45.59 ± 0.73		75.22 ± 0.54	
Acarbose (standard)	200	38.37 ± 0.09	268.95 ± 2.76	45.01 ± 0.53	235.10 ± 1.88
	400	69.03 ± 0.84		71.77 ± 0.09	
	600	72.22 ± 0.46		76.28 ± 0.98	
	800	81.98 ± 0.61		87.65 ± 0.52	
	1000	96.11 ± 0.55		98.09 ± 0.37	

Table-3: Enzymes inhibition by *N. canescens* and *H. auriculata* Ethyl Acetate extracts

Sample	Concentrations (µg/mL)	α-amylase inhibition		α-glucosidase inhibition	
		Inhibition (%)	*IC_{50} (µg/mL)*	*Inhibition (%)*	*IC_{50} (µg/mL)*
Nelsonia canescens	200	14.55 ± 0.97	> 1000	23.09 ± 0.99	807.98 ± 6.25
	400	20.79 ± 0.44		28.35 ± 0.79	
	600	31.70 ± 0.67		35.01 ± 0.87	
	800	39.46 ± 0.71		49.40 ± 0.60	
	1000	45.00 ± 0.35		64.77 ± 0.72	
Hygrophila auriculata	200	16.50 ± 0.64	604.57 ± 4.09	30.93 ± 0.08	415.00 ± 3.34
	400	40.48 ± 0.98		49.00 ± 0.71	
	600	49.81 ± 0.09		58.09 ± 0.34	
	800	58.00 ± 0.39		66.11 ± 0.89	
	1000	65.14 ± 0.52		71.46 ± 0.40	
Acarbose (standard)	200	38.37 ± 0.09	268.95 ± 2.76	45.01 ± 0.53	235.10 ± 1.88
	400	69.03 ± 0.84		71.77 ± 0.09	
	600	72.22 ± 0.46		76.28 ± 0.98	
	800	81.98 ± 0.61		87.65 ± 0.52	
	1000	96.11 ± 0.55		98.09 ± 0.37	

Table-4: Enzymes inhibition by *N. canescens* and *H. auriculata* Methanol extracts

Sample	Concentrations (µg/mL)	α-amylase inhibition		α-glucosidase inhibition	
		Inhibition (%)	*IC_{50} (µg/mL)*	*Inhibition (%)*	*IC_{50} (µg/mL)*
Nelsonia canescens	200	28.06 ± 0.59	507.50 ± 4.55	30.34 ± 0.79	364.40 ± 3.48
	400	40.01 ± 0.62		53.47 ± 0.03	
	600	57.77 ± 0.94		66.02 ± 0.43	
	800	64.07 ± 0.30		72.54 ± 0.86	
	1000	71.76 ± 0.37		79.79 ± 0.27	
Hygrophila auriculata	200	36.47 ± 0.08	414.16 ± 5.10	31.94 ± 0.90	284.52 ± 3.04
	400	49.34 ± 0.09		70.22 ± 0.46	
	600	54.36 ± 0.57		72.08 ± 0.01	
	800	68.41 ± 0.84		80.61 ± 0.55	
	1000	75.08 ± 0.44		83.12 ± 0.37	
Acarbose (standard)	200	38.37 ± 0.09	268.95 ± 2.76	45.01 ± 0.53	235.10 ± 1.88
	400	69.03 ± 0.84		71.77 ± 0.09	
	600	72.22 ± 0.46		76.28 ± 0.98	
	800	81.98 ± 0.61		87.65 ± 0.52	
	1000	96.11 ± 0.55		98.09 ± 0.37	

DISCUSSION

The recent advances in understanding the activity of intestinal enzymes such as α-amylase and α-glucosidase, two important enzymes in carbohydrate digestion and glucose absorption have lead to the development of newer pharmacological agents [8]. But, the available oral chemotherapeutic drugs available come with a range of uncomfortable side effects such as flatulence, abdominal pain, renal tumors, hepatic injury, acute hepatitis, abdominal fullness and diarrhea [9, 10]. As a consequence the use of medicinal plants to treat diabetes requires a growing craze. Moreover, several plants have been reported to have α-amylase and α-glucosidase inhibitory effect. This research was focused on *H. auriculata* and *N. canescens* who have shown their potency in the management of diabetes in folk medicine in Burkina Faso. The ability to inhibit carbohydrate digesting enzymes is one of the major characteristics of drugs used in the management of Type 2 diabetes [11].

It is a general opinion that medicinal plants inhibit α-amylase activity due to the presence of several possible factors / mechanisms, such as, fiber concentration, presence of inhibitors on fibers, encapsulation of starch and enzyme by the fibers present in the sample, thereby reducing accessibility of starch to the enzyme, and direct adsorption of the enzyme on fibers, leading to decreased amylase activity [12, 13]. The α-glucosidase inhibitors are known to block the actions of the enzyme in the small intestine, which is rate-limiting in the conversion of oligosaccharides and disaccharides to monosaccharides, necessary for gastrointestinal absorption. So, postprandial glucose peaks may be attenuated by delayed glucose absorption [13].

Phenolic compound are known to interact with proteins and inhibit enzymatic activity [14]. Numerous studies have reported a positive correlation between the effects of flavonoids and polyphenols content on inhibitory potentials of α-amylase and α-glucosidase [15]. Regarding our two medicine herbals, Previous research demonstrated the rich polyphenols, flavonoids, flavonols and tannins content of *H. auriculata* and *N. canescens* [16]. In addition, a previous phytochemical investigations by HPLC-MS using methanol extract of *N. canescens*, revealed the presence of five phenol acids (p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid and gentisic acid) and three flavonoids (apigenin, luteolin, Quercetol) [17]. Also, a large number of studies have been carried out into the antidiabetic activity of terpenoids of plant origin [18].

The finding in this study may be attributed to the presence of these several interfering substances in the extracts. Further compound purification and identification in the active extracts is necessary to known the mode of enzymes inhibition.

CONCLUSION

The results obtained from this study suggest that both *H. auriculata* and *N. canescens* chloroform and methanol extracts exerts good inhibitory effect on α-amylase and α-glucosidase. Further, the data revealed that both extracts could be a good candidate for anti-diabetes evaluations in experimental animals. In addition, these results support the traditional use of *H. auriculata* and *N. canescens* in the management of diabetes in Burkina Faso.

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