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Research Article

Antiradical and inhibitory activities of hydroethanolic extracts of *Phyllanthus amarus* and *Striga hermonthica* on α-amylase

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Abstract

Objective: The aim of this study was to evaluate the antiradical and inhibitory activity of whole plant extracts of *Phyllanthus amarus* and *Striga hermonthica* on α-amylase.

Methods: The crude 70% (v/v) hydroethanolic extracts obtained were used for the assay of polyphenols, the antiradical test with DPPH, the test for the inhibition of α-amylase with DNS (3,5-dinitrosalicylic). The enzyme extracts were obtained from sorghum malt (*Sorghum bicolor*).

Results: It appears from this study that the extract of *P. amarus* was richer in polyphenols than the extract of *S. hermonthica*. Regarding the antiradical activity, the extract of *S. hermonthica* showed an IC_{50} of 339.06 ± 1.43 µg/mL against 77.74 ±2.73 µg/mL for the extract of *P. amarus*. Regarding the anti α-amylase activity, the extract of *P. amarus* inhibited IMS127 and KMTC 274 respectively 43.64 ±0.59% and 66.28 ±1.97%. On the other hand, the extract of *S. hermonthica* presented an inhibition of 3.93 ±0.91% on IMS127 and 14.85 ±1.62% on KMTC 274. The extract of *P. amarus* presents a good antiradical activity and anti α-amylase compared to *S. hermonthica extract*. In conclusion, these results partly explain the use of these plants in traditional medicine to treat or relieve people suffering from diabetes.

Keywords : α-amylase, inhibition, polyphenols, antiradical, diabetes

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1. INTRODUCTION

Oxidative stress is defined as an imbalance between oxidants and antioxidant defenses[1]. It is involved in a large number of chronic pathologies, including diabetes [2]. Thus, diabetes mellitus is a chronic disease resulting from a lack of insulin production and/or poor use of this hormone by the body[3]. Indeed, in 2019, 463 million people were diabetic worldwide. This figure could reach 578 million by 2030 if nothing is done[4]. According to WHO estimates, it will be the seventh leading cause of death worldwide in 2030[3]. As a result, it constitutes a major public health problem[3]. Despite the efforts made by modern medicine via synthetic products such as α-amylase inhibitors (acarbose), which is a therapeutic approach aimed at reducing postprandial hyperglycaemia, mortality has continued to increase worldwide. In addition, the high cost, the unavailability of drugs and their adverse effects are also factors contributing to this increase in mortality[5]. Faced with this situation, the World Health Organization (WHO) encourages researchers to explore new avenues of control such as herbal medicine[6]. To this end, the effectiveness of this discipline is proven and its undeniable benefits for health[7] because of the active principles contained in the plants. Among these active ingredients, polyphenols are known for their antioxidant and antidiabetic properties[8,9]. Thus, *Phyllanthus (P.) amarus* Schum & Thonn. and *Striga (S.) hermonthica* are plants used in traditional medicine throughout the world, particularly by the herbalists of Bobo-Dioulasso in the management of diabetes [10,11,12]. This study has been initiated in order to investigate the free DPPH radicals scavenging and α-amylase inhibitory activities of these plants.

The whole plant of *P. amarus* and *S. hermonthica* were collected in Dinderesso (11°13'367" N 4°25'750" W), a village located about 15km from Bobo-Dioulasso, in September 2020.

2. MATERIALS AND METHODS

2.1. *Plant material*

Two varieties of sorghum (*Sorghum bicolor*) namely IMS127 and KMTC 274 from the Institute of the Environment and Agricultural Research were used as a source of α-amylase.

2.2. *Preparation and hydroethanolic extraction*

The samples were dried in the dark, pulverized and stored in suitable bags before use. After measuring the moisture using by KERN (MLS 50-3C, Germany), 10 g of each powder were macerated 100 mL ethanol-water 70% (v/v) for 48 hours at room temperature with stirring according to the method described by Souhila et al.[13] with some modifications. Then, the crude extracts were obtained following filtration using filter paper N°4 (Belle France) followed by evaporation of the solvent. Finally, these collected extracts were weighed and stored in the refrigerator in tightly closed sterile bottles to determine the antiradical and α-amylase inhibitory activity *in vitro*.

2.3. *α-amylase extract preparation*

The enzyme extracts were obtained using the protocol described by Adewale et al.[14,15] with some modifications. The protein concentration of these enzymatic extracts was determined using the bicinchoninic acid (BCA) kit (Pierce, Rockford, IL) with Bovine Serum Albumin (BSA) as standard (Sigma-Aldrich, LLC, USA). Finally, the specific activities of these extracts were also determined according to the method described by Xiao et al.[16]. The results were expressed in µg of starch consumed/minutes/mg of protein using the following regression equation ($y = 1.7\,456x + 0.0089$; $R^2 = 0.976$).

2.4. *Determination of total polyphenols*

Polyphenol content of the extracts was evaluated by the Folin-Ciocalteu method[17]. Thus, 125 µL of each extract (100 µg/mL) was added to 625 µL of 0.2 N Folin-Ciocalteu reagent (MP Biomedicals, LLC, USA). After 5 min incubation, 500 µL of 7.5% sodium carbonate (CARLO ERBA Reagents S.A.S, France) were added to the first mixture. The whole was incubated at room temperature for 2 hours in the dark. The optical densities (OD) were read against a blank using a spectrophotometer (Spectrumlab 23A) at 760 nm. For this purpose, a calibration curve of gallic acid (Sigma- Aldrich Chemie, Steinheim, China) solution (0 to 200 mg/ L) of equation ($y = 0.0058x + 0{,}0319$; $R^2 = 0.9982$) was established and the results were expressed in mg of gallic acid equivalent per 100 mg of dry extract (GAE /100 mg) using the calibration curve.

2.5. *Assessment of antiradical activity*

DPPH free radical scaveger activity was carried out by the DPPH method (1, 1-diphenyl-2-picryl-hydrazil)[18,19]. In fact, 375 µL of each extract at different concentrations were mixed with 750 µL of DPPH (0.02 mg/mL) (Acros Organics, Spain) following by 15 minutes incubation at room temperature. Then OD was read against a blank at 517 nm. Quercetin (Sigma- Aldrich Chemie, Steinheim, China) was used as reference scavenger. The IC_{50} was determined and the results were expressed in µg/mL.

2.6. *Evaluation of the anti α-amylase activity*

Plant extracts capacity to inhib alpha-amylase was evaluated by the 3,5-dinitrosalicylic (DNS) method[20,21]. A mixture of phosphate buffer (pH=6.9; 0.02M) (Carlo Erba Reagents, France), plant extract and enzyme extract were made with the same volume (100 µL). This mixture was incubated at 37°C for 10 minutes. Then, 100 µL of 1% starch (Carlo Erba Reagents S.A, France) were added and the whole was incubated at 37°C for 15 minutes. After that, 200 µL of 1% DNS was added to stop the enzymatic reaction. The reaction mixture was incubated at 100°C for 8 minutes followed by cooling in an ice bath. Following this, was read against a blank at 540 nm. The inhibition percentage (%I) was calculated according to the formula: $\%I = \left[\frac{OD(control) - OD(test)}{OD(control)}\right] * 100$.

2.7. *Statistical analysis*

The Microsoft Excel (2016) table was used to calculate the means, standard deviations of the data and the graphs. The R version 4.0.1 software was used to compare the means by a Student's T test at the 5% threshold.

3. RESULTS

3.1. *Polyphenol content*

The average yield of the hydroethanolic extracts was respectively 12.15 ± 0.07% (w/w) and 7.77 ± 0.11% (w/w) for the extract of *P. amarus* and *S. hermonthica*. Table 1 showed that the polyphenol content of the extract of *P. amarus* is significantly higher than that of *S. hermonthica* (p = 0.02).

Table 1: Polyphenol content

Samples	*P. amarus*	*S. hermonthica*	*p-value*
Polyphenols (mg GAE/100mg DE)	32,43 ± 6,58 [a]	6,66 ± 0,85[b]	*0,02*

There is significant difference between a and b; DE: Dry Extract; GAE: Gallic Acid Equivalent

3.2. *Anti-radical activity*

The DPPH radical scavenging activity was carried out by determining the IC_{50} values of the hydroethanolic extracts. The results were obtained using the regression curve for each sample (Figure 1).

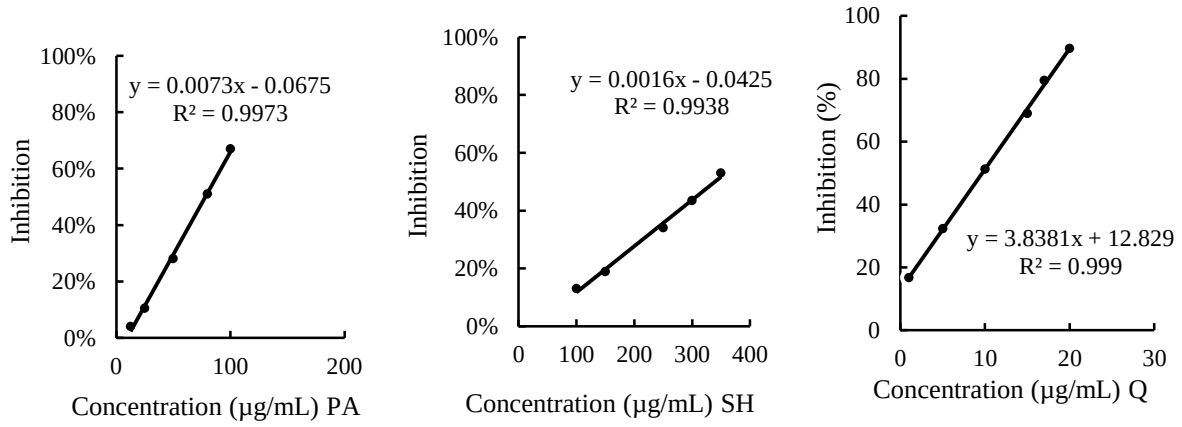

Figure 1: Regression curve of *P. amarus* (PA), *S. hermonthica* (SH) and Quercetin(Q)

Thus, the extract of *P. amarus* exhibited an IC_{50} of 77.74 ± 2.73 µg/mL versus 339.06 ± 1.43 µg/mL for *S. hermonthica*. The IC_{50} of quercetin was 9.66 ± 0.10 µg/mL (Figure 2).

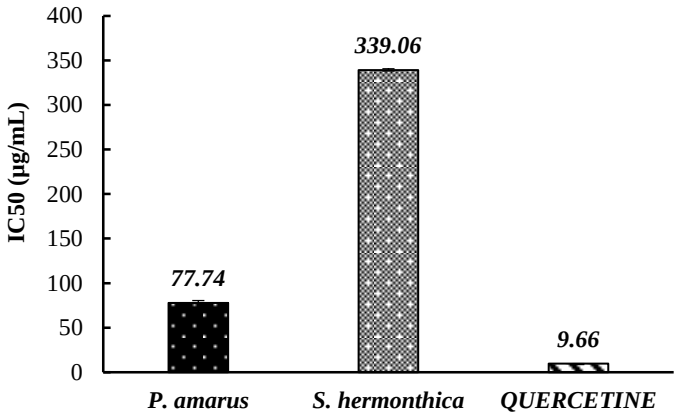

Figure 2: IC_{50} of *P. amarus*, *S. hermonthica* and Quercetin

3.3. *Anti α-amylase activity*

The inhibitory activities of the hydroethanolic extracts at 10 mg/mL on IMS 127 and KMTC 274 were evaluated. Thus, the extract of *P. amarus* presented the strongest inhibition whatever the malt extract used against a weak inhibition for the extract of *S. hermonthica* (Table 2).

Table 2: Inhibition of *P. amarus extract* and *S. hermonthica* on both malt extracts

Malt extract	Pc (mg /mL)	As (U*/mg of protein)	Inhibition (%) *P. amarus*	Inhibition (%) *S. hermonthica*
IMS 127	9.98	170.36	43.64 ± 0.59	3.93 ± 0.91
KMTC 274	10.18	158.05	66.28 ± 1.97	14.85 ± 1.62

Pc: Protein concentration; As: Specific activity U in* µg of starch consumed/minutes

4. DISCUSSION

The present study reported high polyphenol content for *P. amarus* extract unlike *S. hermonthica extract* which showed low content (p = 0.02). Other studies have shown the presence of polyphenols in these plants[22,23]. Thus, the use of hydroethanolic solvent could explain this presence of these compounds because it has the advantage of solubilizing these compounds[24]. The antiradical activity was evaluated using the DPPH test, most often used for the screening of molecules endowed with antioxidant activities present in plant extracts because of its rapidity[25]. This activity made it possible to determine the IC_{50} which is the concentration required to inhibit the DPPH radicals by half. In fact, the lower this concentration (IC_{50}), the higher the antioxidant activity[26]. From the study, the extract of *P. amarus* exhibited a lower IC_{50} than that of *S. hermonthica.* Therefore, the *P. amarus* extract was significantly more antiradical than the *S. hermonthica extract* (p = 2.2e-14). These results could be justified by their respective polyphenol content. Other studies have confirmed that these compounds are endowed with such activity[8,9]. In short, the two extracts having this ability to trap DPPH free radicals are then potential antioxidants. Beyond these antiradical properties, polyphenols are also involved in the inhibition of α-amylase[27]. During this study, both extracts exhibited an anti α-amylase effect on both varieties of sorghum malt. *P. amarus* extract showed higher inhibition compared to *S. hermonthica* extract which showed weak inhibition. This difference would be due to their respective content of polyphenolic compounds. Other studies have reported this inhibition of α-amylase par *P. amarus* [28,29]. In addition, this plant appears to be involved in lowering blood sugar levels in rats rendered diabetic by Alloxan [30]. All these results could support the use of these plants by the herbalists of Bobo-Dioulasso in the management of diabetes.

5. CONCLUSION

The aim of this study was to evaluate the antiradical and α-amylase inhibitory potential of extracts of *S. hermonthica* and *P. amarus.* The results of this study have highlighted the presence of polyphenols in the hydroethanolic extracts. The highest polyphenol content was obtained with *P. amarus* extract. In addition, all extracts showed both antiradical properties and inhibitory effects on α-amylase. These results partly explain the use of these plants in traditional medicine to treat or relieve people suffering from diabetes. For future investigation, it will be interesting to explore not only other complementary methods of antioxidant evaluation such as 2,2-Azinobis 3-ethyl-BenzoThyazoline 6-Sulphonate (ABTS) and Ferric Reducing Antioxidant Power (FRAP) but also to use a pure enzyme to determine the kinetic parameters in order to better appreciate the antidiabetic activity.

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Conflicts of interest

The authors declare that they have no competing interests.

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