



Antiradical and α -amylase Inhibitory Effect of Hydroethanolic Extract of *Parinari Curatellifolia* Planch. Ex. Benth. (*Chrysobalanaceae*) and *Leptadenia Lanceolata* (Poir.) Goyder Ex *Leptadenia Hastata* Pers (Decne) (*Apocinaceae*)

ZOUNGO Daouda^{1*}, OUATTARA Lassina^{1,4}, SOMDA Martin Bienvenu^{2,3,4}, ZANTE Abdoul-Aziz¹, PODA Fabargnèrè Daniel¹, SANOU Yacouba¹, OUEDRAOGO Relwende Justin¹, KABRE Pawendé¹, KONATE Abdourasmane Kadougoudiou⁵, OUEDRAOGO Georges Anicet⁴

¹Département de Sciences Biologiques, Unité de Formation et de Recherche en Sciences de la Vie et de la terre, Université Nazi BONI, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso

²Centre International de Recherche-Développement sur l'Élevage en zone Subhumide, 01 BP 454 Bobo-Dioulasso 01, Burkina Faso

³Institut du Développement Rural, Université Nazi BONI, Bobo-Dioulasso, Burkina Faso

⁴Laboratoire de Recherche et d'Enseignement en Santé et Biotechnologies Animales, Université Nazi BONI, 01 BP 1091 Bobo-Dioulasso, Burkina Faso

⁵Institut de l'Environnement et de Recherches Agricoles, 01 BP 910 Bobo-Dioulasso, Burkina Faso

***Corresponding Author:** Daouda Zoungo, Département de Sciences Biologiques, Unité de Formation et de Recherche en Sciences de la Vie et de la terre, Université Nazi BONI, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso

Abstract: This study aimed to evaluate the antiradical and α -amylase inhibitory activity of *Parinari* (*P.*) *curatellifolia* leaves and *Leptadenia* (*L.*) *lanceolata* leafy stems in Burkina Faso. For this, extracts were prepared by cold maceration in ethanol (70%). Extracts phenolic content were determined by the Folin-Ciocalteu reagent method. Their antiradical activities were evaluated by trapping 2,2-diphenyl-2-picrylhydrazil (DPPH) stable free radical. The α -amylase inhibitory activity of the extracts was estimated by the dinitrosalicylic acid method on two Sorghum malts enzymatic extracts. From these methods, it is *P. curatellifolia* extract which presented the highest polyphenol content ($p < 0.05$). Its DPPH radical scavenging capacity ($IC_{50} = 0.0136 \pm 0.0001$ mg/mL) was approximate that of quercetin ($p > 0.05$). Positive Pearson correlation was obtained between polyphenol concentration of each extract ($r = 0.99$ for *L. lanceolata* and $r = 0.98$ for *P. curatellifolia* extract) and DPPH radical inhibition. All the hydroethanolic extracts showed α -amylase inhibitory effects without none significative difference ($p > 0.05$). But, the highest α -amylase inhibitory percentage was estimated at $76.04 \pm 2.64\%$ with *P. curatellifolia* extract. These results suggest that *P. curatellifolia* leaves and *L. lanceolata* leafy stems are an asset in the search for potentially antidiabetic substances in Burkina Faso.

Keywords: *Parinari curatellifolia*, *Leptadenia lanceolata*, DPPH, α -amylase, inhibitory activity.

1. INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder that affects human physical, mental and social health [1, 2]. It is characterized by chronic hyperglycemia that can result or be the cause of oxidative stress (glucotoxicity), or even come from an excessive activity of digestive enzymes such as α -amylase initiating the digestion of starch [3, 4, 5]. Thus, inhibition of α -amylase activity can reduce postprandial hyperglycemia and prevent the risk of developing this disease [3]. Antioxidant activity including radical scavenging property may also be an important antidiabetic property [6].

Nowadays, this disease is a public health problem reaching increasingly alarming numbers around the world [7, 8]. In Africa, this number was estimated at 19 millions of adults (20 to 79 years old) with diabetes in 2019. The problem there is more alarming because a 143% increase is expected in 2045. In Burkina Faso, the proportion of adults with diabetes has been estimated at 5.5% [8].

In addition, the management of the disease presents difficulties such as adverse effects (weight gain, hypoglycemia, diarrhea, etc.) related to treatment with insulin and oral pharmacological agents (sulfonyleureas, acarbose, etc.), the long-term ineffectiveness of these drugs, the high cost of treatment coupled with the lack of financial means in the countries of sub-Saharan Africa [1, 9, 10, 11, 12]. For example, in Burkina Faso, each type 1 diabetic spends an average of 180 USD for the purchase of insulin per year [13]. These reasons make necessary the search for new antidiabetic products accessible to all with fewer adverse effects. So, the use of local resources is an asset.

According to studies, there is a correlation between the content of certain plant metabolites, mainly polyphenol, and the antioxidant and α -amylase inhibitory properties [14, 15, 16, 17]. Polyphenol are mostly extracted from plants by alcoholic solvents and particularly aqueous ethanol (70%) [18]. Thus, many studies conducted elsewhere have supported the antidiabetic potentiality of alcoholic extracts of the leaves of *Parinari (P.) curatellifolia* Planch. Ex. Benth. and *Leptadenia (L.) lanceolata* (Poir.) Goyder ex *Leptadenia hastata* Pers (Decne) [19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30]. However, in Burkina Faso, these plants are locally little studied for their antidiabetic potentialities. Indeed existing studies concern, on the one hand, an ethnobotanical survey carried out on the use of plants against diabetes and hypertension in the city of Bobo-Dioulasso which reported the use of *P. curatellifolia* and *L. lanceolata* by herbalists [31]. On the other hand, Pare *et al.* [32] revealed that the leaves of *L. lanceolata* are an asset for the search for new non-toxic natural molecules against obesity. But, there is no study using pharmacological targets conducted on their antidiabetic properties.

Otherwise, existing studies elsewhere on these plants leaves hydroethanolic extracts did not report α -amylase inhibitory activity of *P. curatellifolia*. Also, according to Radušienė *et al.* [33], their results cannot be extrapolated on the plants (same species however) existing in the flora of Burkina Faso because of ecotype difference.

This is how this preliminary study aimed to evaluate the stable DPPH free radical and α -amylase inhibitory activity of aqueous ethanol (70%) extracts of the leaves of *P. curatellifolia* and leafy stems of *L. lanceolata* harvested in the flora of Burkina Faso.

2. MATERIALS AND METHODS

2.1. Collection of Plant Materials

The plant material was collected in Bobo-Dioulasso (Burkina Faso) during the period from August to September 2020. The Leaves of *P. curatellifolia* and leafy stems of *L. lanceolata* were collected respectively from Dinderesso Classified Forest (11°12'39.6'N 4°24'25.1'W) and Nasso (11°11'25.9'N 4°25'54.4'W). The materials were dried away from the sun, reduced to powder and stored in sealed bags until use. Two varieties (CMP 231 and IMS 127) of *Sorghum (S.) bicolor (L.) Moench* supplied by the Institute of Environment and Agricultural Research in Bobo-Dioulasso (INERA), was used as a source of α -amylase.

2.2. Extracts Preparation

2.2.1. Preparation of hydroethanolic extracts

The extracts were prepared by cold stirring [34]. Ten grams of dry vegetable powder was soaked in 100 mL of an ethanol/water mixture (70/30; v/v) at room temperature with periodic stirring for 48 hours. After filtration, the solvent was removed by drying in an oven at 44°C.

2.2.2. Preparation of α -amylase crude extracts

Sorghum α -amylase extracts were prepared from varieties of *S. bicolor* [35]. The protein contents of the total malt extracts were determined using a BCA kit according to the manufacturer's recommendations (BCA Protein Assay Kit, Pierce, Rockford, IL). The specific activity of each extract was evaluated according Gautam *et al.* [36].

2.2.3. Dosage of total phenolics of hydroethanolic extracts

The determination of extract's phenolics content was done by the method using the Folin-Ciocalteu reagent [37]. To 0.125 mL of dilute hydroethanolic extract (0.1 mg/mL) obtained from a stock solution (10 mg/mL), 0.625 mL of Folin-Ciocalteu's reagent (0.2 N) was added and the resulting mixture was

kept in the dark for 5 minutes. The final mixture, obtained after adding 0.5 mL of a solution of Na₂CO₃ (75%), was incubated at room temperature for 2 hours protected from light. The absorbance was measured with a spectrophotometer at 760 nm against a blank prepared under the same conditions by replacing the extracts with an equal volume of methanol. A calibration line ($y = 0.0058x + 0.0213$; $R^2 = 0.9992$) was previously constructed with gallic acid (0 – 200 mg/L) under the same conditions as the samples to be analyzed. The results obtained were expressed in milligram gallic acid equivalent per 100 mg of dry extract (mg GAE/100 mg) using the calibration curve.

2.3. DPPH radical scavenging test

A volume of 750 μ L of methanolic solution of DPPH[•] (0.02 mg/mL; OD = 0.397 \pm 0.005) was mixed with 375 μ L of dilute solution (0.1 mg/mL) obtained from a methanolic solution of extract of plant or standard antioxidant at 10 mg/mL. Absorbance was read at 517 nm after 15 minutes incubation at room temperature. Controls without plant extract were prepared. Ascorbic acid (0 - 0.01 mg/mL) was used to establish a standard curve ($y = -0.0239x + 0.2703$; $R^2 = 0.9939$). The antioxidant capacity of the extracts was expressed in μ mol Ascorbic Acid Equivalent per g of dry extract (μ mol AAE/g) using the calibration curve produced [38]. The percentages of DPPH radical inhibiting activity were calculated from the following formula:

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

I(%): inhibition percentage; Abs_{control}: absorbance without plants extract; Abs_{test}: absorbance with extract

[Eq 1] : Determination of the DPPH radical inhibiting activity of the extracts

The concentration of extract necessary to inhibit 50% of the DPPH[•] radicals (IC₅₀) was determined from a regression line representing the percentages of inhibition of DPPH radical as a function of the concentration of the extracts (0.005-0.5 mg/mL). Gallic acid was used as a reference. The variation of DPPH radical scavenging percentage with extracts phenolic concentration was likewise studied.

2.4. α -amylase activity inhibition test

The inhibitory activities of *P. curatellifolia* and *L. lanceolata* were estimated by the method using 3,5-dinitrosalicylic acid (DNS) on the two Sorghum enzymatic extracts. The protocol adopted by Jaiswal and Kumar [39] was used. A test mixture consisting of 100 μ L of PBS (pH 6.9; 0.02 M), 100 μ L of enzyme extract and 100 μ L of aqueous solution of plant extract (10 mg/mL) was pre-incubated at 37°C for 10 minutes. Then, 100 μ L of starch paste (1%) was added and the new mixture obtained was incubated for 15 minutes at 37°C. After this incubation, the enzymatic reaction was stopped by adding 200 μ L of DNS and the mixture was placed in boiling water for 8 minutes. Finally, it was cooled after this step and diluted with distilled water. Absorbance was read at 540 nm against a blank without enzyme extract. Controls without plant extract were prepared under the same conditions and the inhibitory activity of the plant extract studied was determined as a percentage by the following formula:

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

I(%): inhibition percentage; Abs_{control}: absorbance without plants extract; Abs_{test}: absorbance with extract

[Eq 2] : Determination of the α -amylase inhibitory activity of the extracts

2.5. Statistical analyses

The Student's T, statistical test were carried out on the R software (version 4.0.1.) [40] to compare the mean total polyphenol contents and the antiradical capacities of the extracts of the plants studied with that of quercetin used as reference. Khi2 and Kruskal tests were also used respectively for the comparison of extracts α -amylase inhibitory activities and their polyphenol concentrations used for the study of DPPH radical inhibiting percentage fluctuations. The Microsoft Excel (2013) table was used to calculate the means, standard deviations of the data and the graphs. Pearson correlation between phenolic concentration and DPPH free radical inhibition's percentage for each extract was also there determined.

3. RESULTS AND DISCUSSION

3.1. Extracts DPPH free radical inhibitory activity

3.1.1. Phenolic content and DPPH radical scavenging capacity of plant extract

The total polyphenol contents of the hydroethanolic extracts were evaluated by the Folin-Ciocalteu reagent test and their antiradical activities were determined by trapping DPPH radical.

The *P. curatellifolia* sample showed the highest total polyphenol content (30.33 ± 0.26 mg GAE/100 mg) versus that of *L. lanceolata*. The difference between the total polyphenol contents of the prepared extracts could be related to the plant species and the matrix used in each case ($p < 0.05$).

The highest scavenging capacity was exhibited by quercetin (Table 1). This antiradical capacity was not significantly different ($p > 0.05$) from that of the *P. curatellifolia* leaf extract (582.76 ± 38.89 μ mol AAE/g). This could be linked to the content of that extract in total phenolics [41].

Table 1 : Phenolic content and DPPH radical scavenging capacity of the extracts

Sample	Polyphenol content (mg GAE / 100 mg)	DPPH scavenging power (μ mol AAE/g DE or quercetin)
<i>L. lanceolata</i>	$7.17 \pm 1.49^*$	$233.53 \pm 36,52^{**}$
<i>P. curatellifolia</i>	$30.33 \pm 0.26^*$	$582.76 \pm 38.89^{***}$
Quercetin	-	627.11 ± 6.86

mg GAE/100 mg DE : milligram gallic acid equivalent per 100 mg of dry extract ; * : *P. curatellifolia* versus *L. lanceolata*, $p = 0.01396$; AAE/g DE : μ mol Ascorbic Acid Equivalent per g of dry extract, $n = 3$; ** : Quercetin versus *L. lanceolata*, $p = 5.195 \times 10^{-05}$; *** : Quercetin versus *P. curatellifolia*, $p = 0.1236$

3.1.2. DPPH radical inhibiting percentage change with extracts phenolic concentration.

The figure 1 showed the DPPH radical inhibiting activity increase with the different concentration ($p < 0.05$) of total polyphenols of each plant extract. This result was consistent with Hadjadj *et al.* who reported that antiradical activity of phenolics compounds increases proportionally with their polyphenol content [41]. Positive Pearson correlation was obtained between phenolic concentration and DPPH free radical trapping activity for each extract. It was evaluated at 0.99 for *L. lanceolata* and 0.98 for *P. curatellifolia*. That suggested direct relationship between phenolic concentration and DPPH free radical scavenging [42].

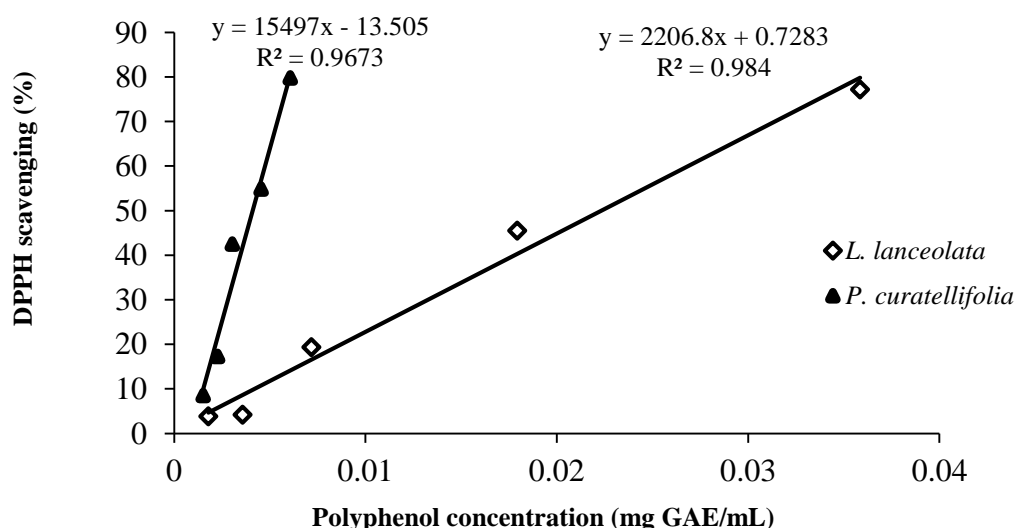


Figure1. DPPH radical trapping percentage change with extracts polyphenol concentration

3.1.3. Inhibitory concentrations of 50% of DPPH radical (IC₅₀)

The equation of the regression line used to determine the IC₅₀ of gallic acid was $y = 22112x + 27.363$; $R^2 = 0.9513$. For the extracts, the equations were

$y = 158.23x + 0.7283$; $R^2 = 0.984$ for *L. lanceolata* and $y = 4528.4x - 12.377$; $R^2 = 0.9461$ for *P. curatellifolia*.

The IC_{50} is inversely proportional to the antiradical activity [43]. The lower its value, the greater the antiradical activity of the extract. Thus, gallic acid exhibited the highest DPPH radical scavenging activity. That could be due to its purity [44]. It was followed by *P. curatellifolia* extract (Figure 2).

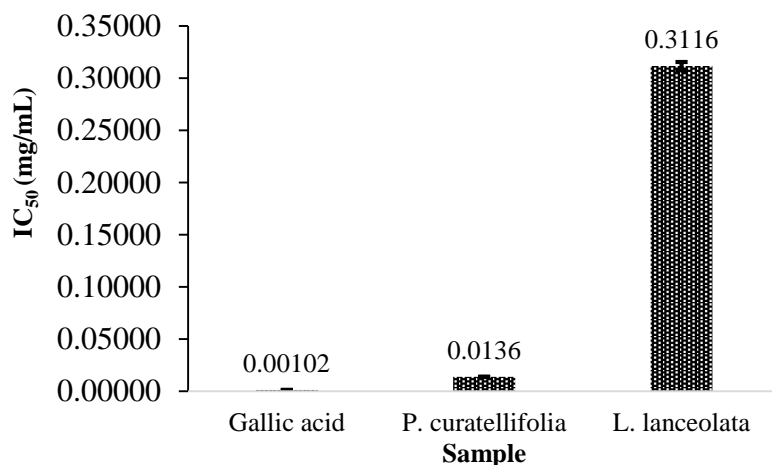


Figure 2: IC_{50} of extracts and gallic acid

3.2. α -amylase inhibitory activities of hydroethanolic extracts

The inhibitory activity of the hydroethanolic extracts at 10 mg/mL on the two enzymatic extracts of sorghum (IMS 127 and CMP 231) was evaluated by the dinitrosalicylic acid test.

All the hydroethanolic extracts showed an inhibitory effect on α -amylase activity of *Sorghum* malts enzymatic extracts without none significant difference ($p > 0.05$). That could be explained by their polyphenol contents. Indeed, according to Ozdal *et al.*, polyphenol can precipitate proteins and affect their functional properties [45]. Highest α -amylase inhibition was evaluated at $76.04 \pm 2.64\%$ with the extract of *P. curatellifolia* (Table 2).

Table 2. α -amylase inhibitory activities of extracts on IMS 127 and CMP 231

<i>Sorghum</i> variety	Total protein (mg BSAE/mL)	α -amylase specific Activity (μ g amidon/min /mg)	Inhibitory activity of <i>L. lanceolata</i> (%)	Inhibitory activity of <i>P. curatellifolia</i> (%)
IMS 127	9.98 ± 0.00	164.77 ± 3.22	$55.04 \pm 11.3^*$	$67.29 \pm 7.38^*$
CMP 231	10.81 ± 0.07	161.78 ± 5.73	$74.56 \pm 2.91^{**}$	$76.04 \pm 2.64^{**}$

mg BSAE/mL: milligram bovine serum albumin equivalent per mL of *Sorghum* malts extracts; *: *P. curatellifolia* versus *L. lanceolata* inhibitory effects on ISM 127 extract, $p = 0.268$; **: *P. curatellifolia* versus *L. lanceolata* inhibitory effects on CMP 231 extract, $p = 0.904$

4. CONCLUSION

This study showed that the hydroethanolic extracts of the leaves of *P. curatellifolia* and leafy stems of *L. lanceolata* contain polyphenols which could be at the origin of their scavenging activities for DPPH radicals. They also have inhibitory effects on α -amylase activity. This suggests that the prepared extracts could be beneficial for search for substance to fight against oxidative stress and the reduction of postprandial blood sugar. Thus, these crude extracts deserve to be considered in subsequent studies which will aim to research the different bioactive chemical groups they contain and determine their

toxicity and effects *in vitro* and *in vivo* against the main physiopathological states favoring the development of diabetes.

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ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

DECLARATION OF ORIGINALITY

This work is original and has not submitted or published elsewhere with other publisher.

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