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# Antioxidant and antiamylase activities of leaf and root extracts of *Ziziphus mauritiana* Lam

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## Abstract

*Ziziphus mauritiana Lam* is a plant species widely used in Mali for its medical virtues to treat various diseases including Diabetes. The present study aims to examine the antioxidant and antidiabetic activities of leaf and root extracts of Z. *mauritiana Lam* collected in Bamako. Phytochemical screening and assay were carried out using conventional methods. The DPPH test was used to evaluate the anti-radical activity, and the antidiabetic potential of the extracts was estimated via the α-amylase inhibition test. Characterization tests revealed the presence of free tannins, flavonoids, coumarins, terpenoids, triterpenes, alkaloids, saponins and anthraquinones in all our extracts. The root extracts revealed higher contents of polyphenols (561.40±22.4 to 218.00±30.2 mg EAG/g) and flavonoids (54.26±4.9 to 132.56±6, 82 mg EQ/g) whatever the solvent, with the exception of Ethyl Acetate. Ethanol extracts showed the best anti-radical potentiel. The **$IC_{50}$**was 58.13±1.85 µg/mL for roots and 85.19±0.415 µg/mL for leaves. The results provide a better understanding of the medicinal properties of *Z. mauritiana* Lam and pave the way for management strategies for diabetes and diseases associated with oxidative stress.

**Keywords:** *Ziziphus mauritiana*; Antidiabetic and antioxidant activities; α-Amylase inhibition; Hyperglycemia

## 1. Introduction

Diabetes is a chronic disease characterized by chronic hyperglycemia, resulting from impaired insulin secretion or action, or both. It constitutes a major public health problem, affecting approximately 537 million adults (20-79 years) worldwide in 2021 according to the Atlas of the International Diabetes Federation [1]and having led to the death of approximately 2 million people in 2019 according to the latest reports from the World Health Organization [2]. Its prevalence is estimated at 643 million by 2030 [1] and it is higher in low- and middle-income countries [2]. Diabetes can cause serious complications such as stroke, kidney failure, myocardial infarction, limb amputation and blindness [2]. Type 2 diabetes, which accounts for more than 90% of diabetes cases [3], is closely linked to modifiable risk factors such as obesity, a sedentary lifestyle and a diet high in sugars and saturated fats. Oxidative stress has been shown to play an important role in the development and complications of diabetes [4]. This oxidative stress results from an imbalance between the production of free radicals and the body's antioxidant defense capabilities. These free radicals can damage cells and macromolecules, contributing to insulin resistance, pancreatic beta cell dysfunction, and microvascular and macrovascular complications of diabetes. Thus, an effective way to treat the complications of Diabetes would be the use of remedies based on antioxidants, such as plant extracts.

*Ziziphus mauritiana Lam*, commonly known as jujube or sidra, is a medicinal plant traditionally used for millennia in various regions of the world for its medicinal properties: hypotensive, anti-inflammatory, antimicrobial, antioxidant,

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antitumor, hepatoprotective and hypoglycemic and also as an immunostimulant [3, 5]. It is used to treat a wide range of illnesses such as fever, diarrhea, skin infections and diabetes. Several studies have highlighted the presence of various bioactive compounds in the leaves and roots of *Z. mauritiana*, such as alkaloids, flavonoids, polysaccharides and triterpenoids, which could contribute to its antioxidant and antidiabetic properties [6, 8]. To better understand this contribution of *Z. mauritiana*, this work was undertaken to evaluate the antioxidant and antidiabetic activity of leaf and root extracts. The content of total phenolic compounds and flavonoids was determined. The antioxidant power and apha-amylase inhibitory activity of the extracts were measured to provide valuable insight into the underlying mechanisms of the effect of *Z. Mauritiana* Lam. on Diabetes and justify its use in traditional medicine.

## 2. Materials and Methods

The plant material used consisted of leaves and roots of *Z. mauritiana Lam*. collected in Bamako. The samples were dried at room temperature, pulverized and stored.

### 2.1. Methods

#### *2.1.1. Preparing the extracts*

The different extracts were obtained by maceration of 10% (W/V) of powder from each organ in a solvent (70% ethanol; ethyl acetate or distilled water). The mixture was placed under magnetic stirring at room temperature for 24 h, then filtered under vacuum. The residue obtained is subjected again to the same operation. The recovered filtrates were concentrated using a rotary evaporator and stored cool for subsequent analyses.

#### *2.1.2. Determination of Phytochemical Composition*

The phytochemical screening of the extracts was carried out using qualitative characterization techniques, described by [5, 9]. The total contents of phenolic compounds and flavonoids were expressed spectrophotometrically, using the Folin-Ciocalteu and Aluminum trichloride tests respectively reported by [10]. The results are expressed in milligrams of gallic acid equivalents per gram of dry matter (mg EAG/g) for phenolic compounds and in milligrams of quercetin equivalents per gram of dry matter (mg EQ/g) for flavonoids.

#### *2.1.3. Anti-radical activity*

The 2,2-Di-Phenyl-1-Picryl-Hydrazyl (DPPH) test was used following the method described by Briand-Williams slightly modified by [5, 9]. A calibration range was established from a stock solution of 100 g/mL of the extract. Fifty (50) µL of each solution are added to 1.95 mL of DPPH solution (0.024 g/L). At the same time, a negative control was prepared by mixing 50 µL of methanol with 1.95 m of DPPH solution. The absorbance reading was taken against a blank at 515 nm after 30 min of incubation in the dark and at room temperature (30-35º C). Ascorbic acid was used as a positive control. The percentage of inhibition of the DPPH radical was calculated according to the equation below.

$$\text{DPPH inhibition rate (\%)} = [1 - \frac{DOech}{DO\ white}] \times 100$$

DO ech = Absorbance of extracts
DO white = Absorbance of methanol used as blank

#### *2.1.4. Determination of amylase inhibitory activity*

Alpha-amylase inhibitory activity was determined following the slightly modified protocol described (7,11). To 125 µL of a solution of extracts at different concentrations (20-40-80-160-320 µg/mL) are added 125 µL of a solution of α-amylase (10 µg/mL) in buffer. sodium phosphate (0.1 M pH 6.9). The reaction mixture is incubated in a water bath at 37°C for 10 minutes. After incubation, 25 µL of starch solution (1%) in the same buffer is added every 10 seconds up to a total of 125 µL. The reaction mixture is then incubated at 37 °C for 20 minutes. Then the reaction is stopped by adding 250 µL of a solution of DNS (3,5-dinitrosalicylic acid) at 1%, phenol at 0.2%, Na2SO3 at 0.05% and NaOH at 1%. Then, 250 µL of potassium - sodium tartrate solution (40%) is added to the mixtures to stabilize the color. After cooling to the room temperature, the absorbances are read at 540 nm. The acarbose, an alpha-amylase inhibitor, was used as a positive control. The results were expressed as percentage (%) of inhibition of amylase activity according to the formula below.

$$\text{Inhibition rate (\%)} = [\frac{\Delta Abs\ control - \Delta Abs\ Ech]}{\Delta Abs\ control}] \times 100$$

DAbs control = Absorbance of the tube with (Enzyme + solvent) – Absorbance of the blank

DAbs Ech = Absorbance of the tube with (Enzyme + solvent + Extract) – Absorbance of the blank

## 3. Results

### 3.1. Phytochemical screening

The phytochemical screening results are presented in Table 1.

**Table 1** Phytochemical composition of *Z. mauritiana* Lam.

| | | Leaves | | | Roots | |
|---|---|---|---|---|---|---|
| **Chemical Groups** | **Aqueous** | **Ethanol** | **Acetate** | **Aqueous** | **Ethanol** | **Acetate** |
| Alkaloids | + | + | + | + | + | + |
| Polyphenols | + | + | + | + | + | + |
| Terpenes | + | + | + | + | + | + |
| Flavonoids | + | + | + | + | + | + |
| Coumarins | + | + | + | + | + | + |
| Tannins | + | + | + | + | + | + |
| Saponines | + | + | + | + | + | + |

+ : Present ; - : Absent

The phytochemical study carried out on the macerated extracts (Aqueous; Ethanol and Ethyl Acetate) made it possible to highlight the richness of the plant in secondary metabolites. Tests revealed the presence of flavonoids, tannins, coumarins, alkaloids, Saponines, terpenes, polyphenols in all leaf and root extracts.

### 3.2. Total polyphenol and flavonoid contents

The Table 2 presents the results relating to the determination of total polyphenols and flavonoids

**Table 2** Contents of total polyphenols and flavonoids

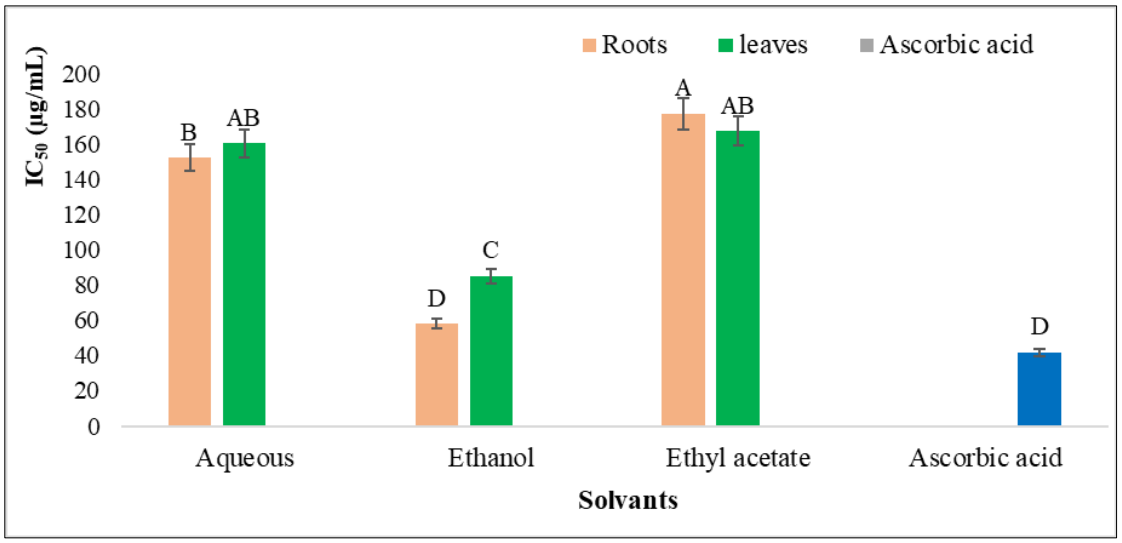
| **Organs** | **Solvents** | **Total polyphenols (mg EAG/g)** | **Flavonoids (mg EQ/g)** |
|---|---|---|---|
| | Aqueous | 171,70±17,9$^{C}$ | 43,41±2,29$^{E}$ |
| leaves | Ethanol | 247,20±12,71$^{B}$ | 87,24±7,59$^{B}$ |
| | Ethyl acetate | 102,00±14,99$^{D}$ | 73,62±2,13$^{C}$ |
| | Aqueous | 218,00±30,2$^{B}$ | 55,03±1,706$^{D}$ |
| Roots | Ethanol | 561,40±22,4$^{A}$ | 132,56±6,82$^{A}$ |
| | Ethyl acetate | 250,21±23,9$^{B}$ | 54,26±4,9$^{D}$ |

NB: For each compound, the means not sharing any letter are significantly different (p-value < 0.05).

The results in Table 2 shows that *Z. mauritiana* is very rich in polyphenols and flavonoids. These contents are respectively higher in the roots (561.40±22.4 mg EAG/g and 132.56±6.82 mg EQ/g) than in the leaves (247.20±12.71 mg EAG/g and 87.24±7.59B mg EQ/g). The results also show that ethanol is the most effective solvent for the extraction of polyphenols and flavonoids from both leaves.

### 3.2. Antioxidant potential

The results obtained with the DPPH test are translated into extract concentrations ( µg/mL) necessary to reduce the DPPH Absorbance by 50% and are presented in Fig. 1.



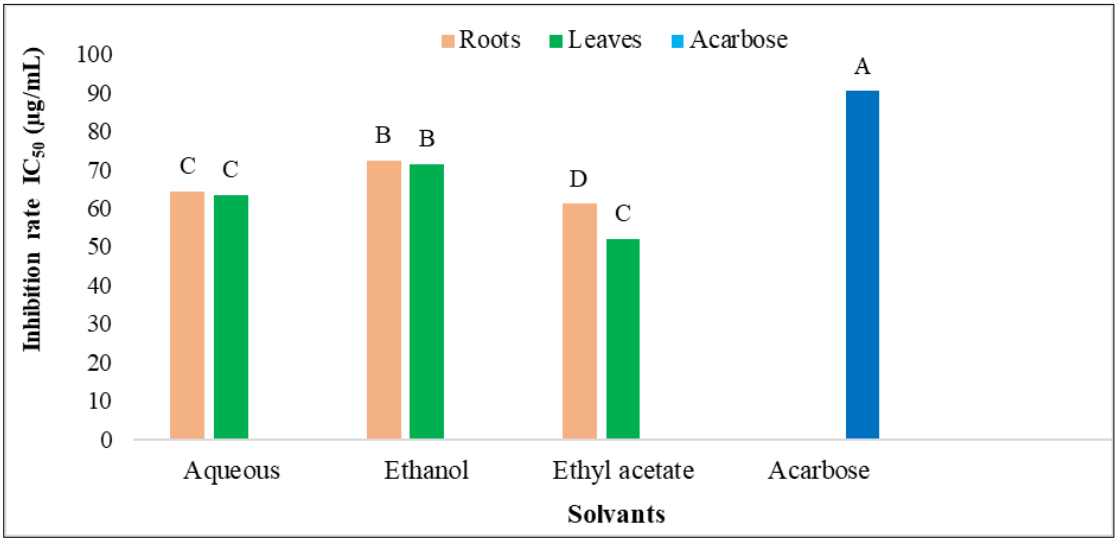

*For each value, the means sharing no letters are significantly different (p-value < 0.05). Ascorbic Acid was used as a reference.

**Figure 1** Ability to reduce DPPH radicals by 50% by the extracts ($IC_{50}$)

The **Fig. 1** showed that the greatest anti-radical activity was obtained with the ethanolic extract of the roots (58.13±1.85 µg/ mL), statistically equivalent to that of ascorbic acid.

### 3.4. Inhibitory activity of alpha-amylase

The rate of inhibition of α-amylase activity by leaf and root extracts are summarized in the **Fig.2.**




*For each value, the means sharing no letters are significantly different (p-value < 0.05). Acarbose was used as a reference.

**Figure 2** Inhibition rate of α-amylase activity

The **Fig. 2** showed that the leaf and root extracts have a greater alpha-amylase inhibition power than the reference, Acarbose. The greatest inhibitory activity was obtained with Ethyl acetate extracts.

## 4. Discussion

This study aimed to study the phytochemical composition of leaf and root extracts of *Z. mauritiana* Lam., widely used by traditional medicine to treat various ailments including diabetes. The contents of polyphenols and flavonoids, known for their antioxidant properties, were determined as well as their anti-radical and anti-amylase activities. The results of the phytochemical screening revealed the presence of tannins, coumarins, flavonoids, alkaloids, polyphenols, terpenes,

saponines in all the extracts (Table 1). These results obtained are consistent with those of [6,7,12] who reported the presence of these major phytochemical groups in *Z. mauritiana* extracts. The determination of the contents showed that the extracts studied are very rich in polyphenol compounds and flavonoids (Table 2). These contents are respectively higher in the roots (561.40±22.4 mg EAG/g and 132.56±6.82 mg EQ/g) than the leaves (247.20±12.71 mg EAG/g and 87.24±7.59B mg EQ/g). With acetone extracts, (13)obtained much higher total polyphenol contents with the fruits of *Z. mauritiana* (6035.38 mg EAG/100g). On the other hand, [14] reported much lower polyphenol contents (13.99±0.50 mg EAG/g) with hydroalcoholic extracts of *Z. mauritiana fruits.* Other previous works reported higher contents of polyphenols (42.84 and 94.70 mg EAG/g) and total flavonoids (47.02 and 427.33 mg EQ/g) with acetate fractions of ethyl and n-butanol from *Ziziphus fruits* [3, 6]. These differences in contents may be due to the organs used, the extraction method and harvesting areas which differ depending on the climate and varieties (9). Many studies have shown that several of these chemical groups are involved in the antioxidant activities of plant extracts [14, 16]. The work of [3] reported that the leaves and roots of this species presented hepatoprotective and hypoglycemic properties. Flavonoids have been shown to promote tissue regeneration and reduce blood capillary permeability. To better understand the activities biological of *Z. mauritiana*, the antiradical capacity was measured by the DPPH test. All the extracts showed an interesting effect on the trapping of the free radical DPPH with inhibitory concentrations ($\mathbf{IC_{50}}$) varying from 58.13±1.85 to 177.45±7.15 µg/mL. Our values ranged within values indicated by (6) with different extracts (aqueous, methanolic and acetate) in flavonoids from *Ziziphus fruits*. The $\mathbf{IC_{50}}$being inversely proportional to the anti-radical power, the greatest anti-radical activity was recorded at the roots ($\mathbf{IC_{50}}$= 58.13±1.85 µg/ mL) like that of ascorbic acid used as a reference. This result is close to those of [13] who also reported anti-radical activity with acetone extracts of *Ziziphus fruits*. It is higher than those of [14, 15]. Likewise, the differences in antioxidant potential between the extracts could be partially attributed to the quantitative and qualitative variations of the phenolic compounds present in these different extracts [9]. These results show that the extracts studied have good anti-radical activity. Since free radical scavenging activity is a good indicator of antioxidant potential, the extracts could be a potential source of natural antioxidants such as polyphenols and flavonoids. To better understand the link of the use of this plant with the treatment of Diabetes, the inhibitory activity of the extracts was evaluated in the presence of alpha-amylase, an important enzyme in the degradation of starch into glucose [8]. The result of α-amylase activity revealed that *Z. mauritiana* extracts possess the capacity to inhibit the enzyme and consequently an anti-hyperglycemic potential [7, 8]. The statistical tests reveal that this potential inhibitor of α-amylase activity varied significantly ($\rho<0.05$) depending on the extraction solvent and the organ. The strongest inhibitory activities were observed with the ethanolic extracts of the roots (72.48 µg/mL) and leaves (71.51 µg/mL), more favorable than the standard used, Acarbose. These results are very promising because [7, 11] an d respectively obtained 82.12±1.81 and an $\mathbf{IC_{50}}$value=18.34 (18.07–18.61) µg/mL with extracts of different species of Ziziphus. Other studies had also highlighted the hypoglycemic potential of *Z. mauritiana fruits* with inhibition rates up to 91.77±2.00% of amylase activity [8]. This hypoglycemic potential observed with these extracts could be linked to their richness in secondary metabolites in particular polyphenols and flavonoids which are strongly incriminated (R2 = 0.94) in the inhibition of this enzyme [14]. By reducing the activity of this key enzyme in sugar metabolism, these extracts could in turn help to reduce blood sugar levels in diabetic people. These results support those of [17] who reported in an ethnobotanical survey the use of this species in the traditional treatment of Diabetes in Mali. However, more precise enzymatic tests as well as toxicity studies must be carried out to link the anti-amylase activity of the extracts and the composition of the phytochemical groups.

## 5. Conclusion

The data from the present study show that extracts from the leaves and roots of *Z. mauritiana* are rich in bioactive substances. Among the solvents tested, ethanol was the best solvent for extracting phenolic compounds. As for the organs, the roots have been shown to be the richest in phenolic compounds and to have the best antioxidant and anti-amylase potential. With this inhibitory potential recorded against the amylase, the extracts of the leaves and roots of *Z. mauritiana* could be used to help diabetic people regulate their blood sugar and manage their oxidative stress.

## Compliance with ethical standards

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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