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# Phytochemistry and Biological Activities of Leaves and Pulp Extracts from *Ziziphus mauritiana* (Lam.) Collected in Mali

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors MW and SK designed the study project, proceed the statistical analysis and the drafting of the first manuscript. Authors SK, CC, IT and ND proceeded for sampling, phytochemical analysis, biological tests, bibliographic research. Authors LBM and MW had formatted the text and conducted the bibliographic research and to the correction of the version final document. All authors read and approved the final document.*

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## ABSTRACT

**Introduction:** The objectives of this work consisted of a phytochemical study of the leaves and pulp of *Ziziphus mauritiana* Lam collected from the flooded and dried sites of Niono and Sévaré in Mali and to evaluate the biological activities of the extracts, i.e. the antiradical activity and hemolytic activity.
**Methods:** Phytochemical analysis and biological activities were performed at the plant biochemistry and biotechnology laboratory of the FST / USTTB. The tannins and flavonoids were

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extracted by maceration, dosed by spectrophotometry then analyzed by HPLC and their antiradical activity was evaluated by the DPPH method. The saponosides were extracted by decoction and their hemolytic activity was evaluated on beef blood.
**Results:** Tannins and flavonoids were abundant in the leaves and the pulp but saponosides were absent in the pulp.
Calcium ions, carbonate ions and chloride ions were abundant in the leaves and pulp of *Zizyphus mauritiana* Lam from the dried site of Niono and the flooded site of Sévaré. HPLC chromatograms showed two peaks of gallic acid in the tannins extracts.
Catechical tannins and flavonoids of the flooded sites have a greater antiradical activity than those of the dried sites. However, their antiradical activity remains lower than that of ascorbic acid whose IC50 was measured at 30 µg.
Leaf saponosides from the flooded site of Niono and the dried site of Sévaré showed hemolytic activity on red blood cells of beef.
**Conclusion:** The leaves and pulps of *Zizyphus mauritiana* Lam were rich in phenolic compounds and have interesting antiradical activity. The saponosides extracted from the leaves showed hemolytic activity.

*Keywords: Antiradical activity; flavonoids; hemolytic activity; saponosides; tannins; Ziziphus mauritiana Lam.*

## 1. INTRODUCTION

*Ziziphus mauritiana* (Lam) has many nutritional, medical, artisanal and even orchard protection interests [1,2]. Several works have shown its richness in primary and secondary metabolites [1], as well as its economic interests [2]. Khouchlaa and collaborators studied phenolic composition and evaluated *in vitro* the litholytic activity of extract of *Zizyphus lotus* L. of Moroccan origin on urolithiasis [3].

The metabolites from plant extracts have antibacterial, analgesic, astringent and anti-inflammatory properties, which can justify their use in traditional medicine [4,5]. The fruits of the plant are an important source of income for many rural families that use it for ant diabetic activity [6].

Other studies have shown that leaf extracts have hypoglycemic, hypertensive, anti-inflammatory, and antioxidant activity [7]. Aisha and collaborators were studed chemical composition and biological activities of leaves of *Zizyphus mauritiana* Lam of Pakistan [8]. The antioxidant properties of catechin tannins and gallic tannins and flavonoids which would help fight aging were studded by Roumeissa and collaborators [7].

The goal of our study were to compared the phytochemical composition of extracts from the leaves and pulps of *Zizyphus mauritiana* lam collected from different two flooded and dried sites with in Mali. The nutritional quality was évaluated and some biological activities of the extracts as the antiradical activity by DPPH and heamolytic activity to according the collection areas.

## 2. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Sample collection sites

The samples (leaves and pulps) of *Ziziphus mauritiana* (Lam.) were collected on two sites in Mali: Niono and Sévaré. For each of these two sites, a flooded and a dried area were chosen for the collection: Sitan Wéré and Ranch for Niono, and Dialagou and Doundoun for Sévaré. The blood of beef were collected at the slaughterhouse of Sabalibougou, Kati. Leaf and pulp powder samples obtained by grinding and sieving. Slaughterhouse of Sabalibougou in Kati.

### 2.2 Methods

#### 2.2.1 Characterization reactions of metabolites

*2.2.1.1 Catechin tannins*

The catechin tannins were extracted by maceration in 100 mg of plant organ fine powder in acetone diluted to 7/3 for 20 minutes with stirring. The filtrate was concentrated in a rotavapor saturated with sodium chloride and centrifuged at 3000 rpm for 10 minutes. The catechin tannins were characterized by ferric chloride [7].

*2.2.1.2 Gallic tannins*

From one hundred milligram (100 mg) of sample powder delipidated with petroleum ether and boiled in 20 mL of distilled water for 10 minutes. The ten dichloromethane was mixed with the filtrate to remove the pigments. Gallic acid tannins were extracted in the aqueous phase with ethyl acetate and characterised/ferified by 2% ferric chloride [9,7].

*2.2.1.3 Flavonoids*

In a test tube ten (10) drops of concentrated hydrochloric acid was added to 0.5 mL of extract and a five (5) milligrams of magnesium turnings. After three minutes of incubation at room temperature, specific staining of flavonoids was observed [10,11,7].

*2.2.1.4 Coumarines*

Five (5) milliliters of etheric extract (maceration for 24 hours) were evaporated in an open beaker, then 2 mL of hot water were added to the residue. To one (1) mL of this solution 0.5 mL of 25% NH4OH was added and mixed. UV fluorescence was observed at 366 nm [12,9].

*2.2.1.5 Leucoanthocyanins*

To 5 mL of infused prepared from the drug powder, 5 mL of sulfuric acid and 5 mL of $NH_4OH$ were added to a test tube and the appearance of leucoanthocyanin-specific staining followed [9].

*2.2.1.6 Sterols and triterpenes*

In a test tube, one (1) gram of organ powder was added to twenty (20) mL of petroleum ether. The solution was stirred and left in the refrigerator for 24 hours, filtered on filter paper in a beaker and evaporated to dryness in a rotavapor. The sterols and triterpenes were extracted in the residues with 10 mL of chloroform. To 10 mL of chloroformic extract, 1 mL of acetic anhydride and 1 mL of $CHCl_3$ were added. The chloroform solution was split into two test tubes. At the bottom of one of the tubes, 2 mL of concentrated $H_2SO_4$ was deposited and the other tube was used as a reference. The the appearance of specific staining was followed without skaking.

*2.2.1.7 Mucilages*

The mucilages were extracted by maceration of five (5) grams of plant organ powder (leaves and pulp) in one hundred (100) mL of distilled water for 12 hours. Mucilages were precipitated by 10 mL ethanol. Afluffy precipitate by mixing indicates the presence of mucilage [9,10].

*2.2.1.8 Reducing sugars*

The reducing sugars were characterized by hot Fehling liquor. For this purpose, the organ extract was added to 1 mL of the Fehling's solution and the mixture was heated at 80°C for 5 minutes Fehling.

### 2.2.2 Characterization of mineral salts of leaves and pulp

One (1) gram of organ powder was calcined in an oven at 600°C for 12 hours. The ash obtained was weighed and dissolved in 10 mL of distilled water and filtered through filter paper. The different ions were highlighted in the filtrate by means of color precipitate (Table 1).

### 2.2.3 Dosage of catechical and gallic tannins, flavonoids and saponosides

*2.2.3.1 Catechetical tannins*

The content of the catechical tannins in the extracts was determined spectrophotometric method. In a test tube, 1 mL acetone extract, 5 mL distilled water, 1 mL ethanol and 0.5 mL Folin reagent were mixed. After standing for 5 minutes, 1 mL of a 5% sodium carbonate solution was added and left in the dark for 1 hour. Absorbance reading was made at 725 nm. A 1% gallic acid standard range of 10 to 100 µg was used [12]. Results were expressed as gallic acid equivalents per 100 g of powder.

*2.2.3.2 Flavonoids*

To 500 µL of the extract, 2 mL of distilled water and 50 µL of 5% sodium nitrite ($NaNO_2$) were added. After five minutes, 100 µL of aluminum trichloride ($AlCl_3$) at 10% (w / v) was added to the mixture. After six (6) minutes of stationary? incubation at room temperature, 1 mL of 1M sodium carbonate ($NaCO_3$) was added. The content was homogenized and the absorbance of the pinkish solution was determined at 510 nm against a blank. Catechin was used as a positive control. The total flavonoid content of plant extracts was expressed in milligram (mg) catechin equivalent per 100 grams of dry matter (mg CE / 100 g) [10].

**Table 1. Characterization reaction of mineral salts**

| Ions | Reagents | Results |
|---|---|---|
| Phosphate | Hot ammonium nitro-molybdate | Yellow precipitate |
| Sulfate | Barium chloride | White precipitate |
| Calcium | Ammonium oxalate | White precipitate |
| Carbonates | Acid chlorihydric on ash | Effervescence reaction |
| Chloride | Silver nitrate | White precipitate, darkens in the light |
| Potassium | Cobalt sodium nitrite | Needle crystal |

*2.2.3.3 Saponosides*

Dosage of the saponosides was done by calculation of the foam index. The extraction was done as follows. To 1 g of organ powder, 5 mL of petroleum ether was added to delipidate for 5 minutes. The supernatant was poured and the operation was repeated with 2.5 mL of petroleum ether for 10 min. The powder was dried at 35ºC To 0.5 g of delipidated powder, 10 mL of distilled water was added and the mixture was boiled with stirring for twenty (20) minutes and filtered through filter pape. Saponosides content were determined on the decoction by 1/10 dilution [9]. Each tube was shaken horizontally for 15 seconds and allowed to stand for 15 minutes. The foam index was calculated in the tube having 1 cm of foam height. That is a 1/10 dilution of the 1% decoction at a concentration of 0.1%. If the tube containing 5 mL of decoction and 5 mL of distilled water has a foam height of 1 cm, the 5 mL of 1% has 0.05 g of saponoside and the foam index is 10 * 1 / 0.05 = 200.

*2.2.3.4 High performance liquid chromatography*

The standards were prepared in a 50/50 (v / v) water / methanol mixture. Several calibration ranges were used: 10 mg / mL, 20 mg / mL, and 50 mg / mL to establish the calibration curve. Lyophilized tannic extracts were dissolved in the 50/50 (v / v) water / ethanol mixture, sonicated for 15 min, allowed to cool to room temperature and filtered through a nylon membrane filter with 0.45µm pores prior to injection [13].

HPLC conditions:

Mobile phase: Water / 20 mM phosphate / acetonitrile buffer 70: 28: 2 v / v / v

Column: C18, 4.6 x 150 mm, 5µ- Zorbax- Agilent
Flow rate: 0.8 mL / min, injection volume: 20 µL, column temperature: 30ºC, detection: 271 nm

### 2.2.4 Antioxidant activity: 1-1 diphenyl-2-pyrryl hydrazyl test (DPPH)

The antioxidant activity of the aqueous extracts of *Z. mauritiana* L. and of a standard antioxidant (ascorbic acid) with respect to the DPPH radical was evaluated using a spectrophotometer by following the reduction of this radical, which is accompanied by its change from purple color (DPPH) to yellow color (DPPH). A negative control was prepared by replacing the extract with distilled water. The tubes were placed in the dark for 30 min and the reading was made at 517 nm. [11]. The results were expressed in % of anti-radical activity or Inhibitory in percentage (I%) according to the formula: I% = (Abs negative control-Abs Sample) / Abs control [14]. The $IC_{50}$ of each extract was calculated from a linear regression line established with the percentages of inhibitions obtained. $IC_{50}$ is the concentration of the extract that inhibits 50% of the activity of the radical. The lower it is, the higher the antioxidant activity [15].

### 2.2.5 Hemolytic activity of saponosides

The tests were performed on red blood cell pellets of beef obtained by centrifugation of whole blood at 4000 rpm for five (5) min. the pellets were washed three (3) times with buffered physiological saline (1 mL of blood dissolved in 25 mL of saline).

In six tubes each containing 0.5 mL of red blood cells was added the increasing volumes of saponoside extracts of 5 mg / ml of concentration and 2 mL of the buffered saline solution. The mixture was homogenized and allowed to stand for 24 hours. The tubes were centrifuged at 3500 rpm for 10 minutes [16].

The turbidity observed through the red coloration in the tubes after centrifugation determines the hemolytic activity. The absorbance of the solutions was read absorbance at 548 nm against a blank (without extract).

## 3. RESULTS

## 3.1 Characterization of Metabolites

The results obtained after the characterization reactions of the metabolites are listed in Tables 2 and 3.

**Table 2. Characterization of primary and secondary metabolites**

| Metabolites | Leaves | | | | Pulps | | | |
|---|---|---|---|---|---|---|---|---|
| | Niono | | Sévaré | | Niono | | Sévaré | |
| | Dried site | Flooded site | Dried site | Flooded site | Dried site | Flooded site | Dried site | Flooded site |
| Catechical tannins | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| Gallic tannins | ++ | ++ | + | + | ++ | ++ | + | + |
| Flavonoids | +++ | +++ | +++ | +++ | ++ | ++ | ++ | ++ |
| Coumarins | + | ++ | + | ++ | + | + | ++ | + |
| Leucoanthocyanins | ++ | + | + | +++ | + | + | + | ++ |
| Saponosides | +++ | ++ | ++ | +++ | - | - | - | - |
| Terpenes - Sterols | ++ | ++ | ++ | ++ | + | ++ | + | ++ |
| Mucilage | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| Reducing sugars | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |

*Legend: +++ = Abundant, ++ = Not abundant, + = Traces, - = Absent*

**Table 3. Characterization of mineral salts in leaves and pulps**

| Mineral salts | Leaves | | | | Pulps | | | |
|---|---|---|---|---|---|---|---|---|
| | Niono | | Sévaré | | Niono | | Sévaré | |
| | Dried site | Flooded site | Dried site | Flooded site | Dried site | Flooded site | Dried site | Flooded site |
| Sulfate | + | + | + | + | + | + | + | + |
| Calcium | ++ | ++ | ++ | +++ | +++ | +++ | ++ | ++ |
| Carbonate | ++ | ++ | ++ | + | + | + | ++ | ++ |
| Chloride | ++ | ++ | ++ | + | + | + | ++ | ++ |
| Potassium | + | + | + | ++ | ++ | ++ | + | + |

*Legend: +++ = Abundant, ++ = Not abundant, + = Traces, - = absent*

## 3.2 Dosage of Metabolites of Leaves and Pulp

Tables 4 and 5 shows the results of catechical tannin determination in the leaves and pulp of the different sites. These results are the averages of three trials.

## 3.3 Dosage of Saponosides in the Leaves

The foam index of the saponosides was calculated in the tube whose foam height was equal to 1 cm or close to the different samples of *Ziziphus mauritiana* Lam.

## 3.4 HPLC Qualitative Analysis of Tannin Extracts

The following HPLC chromatograms of the leaves and pulp of the localities of Niono and Sévaré were obtained:

Acid galic pics I: 1,905, and 2,035

## 3.5 Biological Activity

### 3.5.1 Antioxidant activity of leaf and pulp extracts

The antiradical activity of leaf extracts from Niono and Sévaré sites was evaluated by their concentration which inhibits 50% of the radical $IC_{50}$ from equations of the linear regression line of the percentages of inhibition (% I).

### 3.5.2 Antiradical activity of pulp extracts from different sources

The $IC_{50}$ was calculated from the equations of the regression line of percent inhibition (% I) of *Ziziphus mauritiana* Lam pulp extracts (Fig. 5).

### 3.5.3 Hemolytic activity of leaves saponins

The hemolytic activity was carried out on red blood globular pellets obtained by centrifugation at 4000 rpm and washed three (3) times with physiological buffered water. To 0.5 ml of red

blood cell pellet in different tubes with increasing volumes of saponins 2 ml of the buffered saline solution were added. Then the tubes were left to stand for 24 hours and centrifuged. The turbidity through the red coloration observed in the tubes containing the extract determines the hemolytic activity. The absorbance of the tubes was assessed against a blank (without extract) at 548 nm. The control tube contained 2 mL of buffered solution and 0.5 mL of red cell pellet.

**Table 4. Content of extracts in leaves**

| Samples | Percentage | | | |
|---|---|---|---|---|
| | Niono | | Sévaré | |
| | Dried site | Flooded site | Dried site | Flooded site |
| Content in catechical tannins | 2,40 ± 0,04 | 2,88 ± 0,03 | 2,03 ± 0,03 | 3,13 ± 0,03 |
| Content in gallic tannins | 2,32± 0,04 | 0,60 ± 0,02 | 0,52 ± 0,03 | 1,07 ± 0,03 |
| Flavonoids content | 1,11 ± 0,3 | 0,83 ±0,2 | 0,35 ±0,3 | 1,17 ± 0,2 |

**Table 5. Content of extracts in pulp**

| Samples | Content | | | |
|---|---|---|---|---|
| | Niono | | Sevare | |
| | Dried site | Flooded site | Dried site | Flooded site |
| Content in catechical tannins | 3,00 ± 0,02 | 3,25 ± 0,05 | 4,02 ± 0,05 | 2,39 ± 0,04 |
| Content in gallic tannins, | 2,62 ± 0,04 | 1,90 ± 0,04 | 1,19 ± 0,04 | 2,12 ± 0,04 |
| Flavonoids content | 1,70 ± 0,58 | 2,14 ± 0,5 | 3,55 ± 0,5 | 3,28 ± 0,6 |

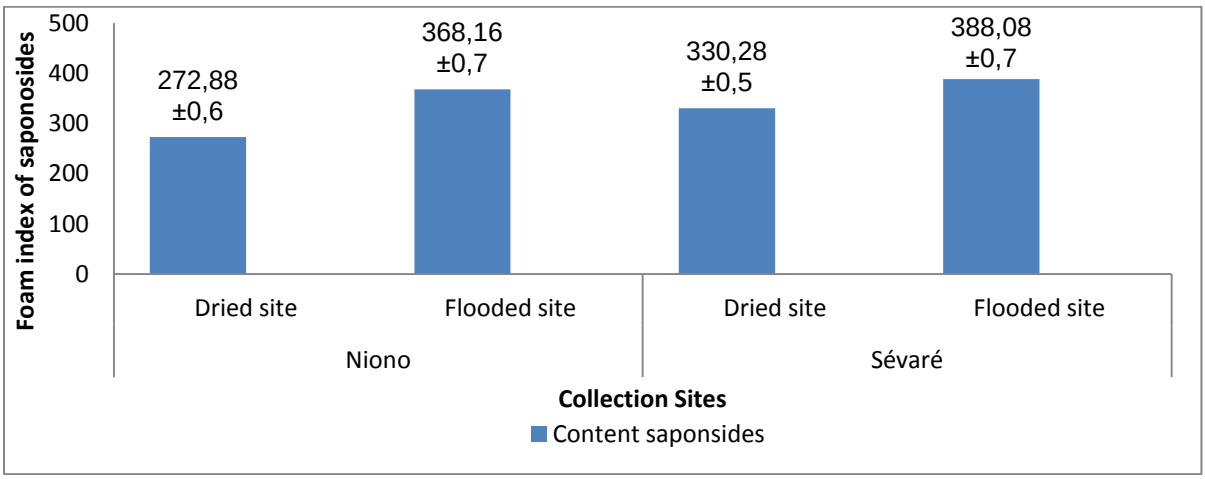


**Fig. 1. Saponoside content of leaf extracts from Niono and Sévaré sites**

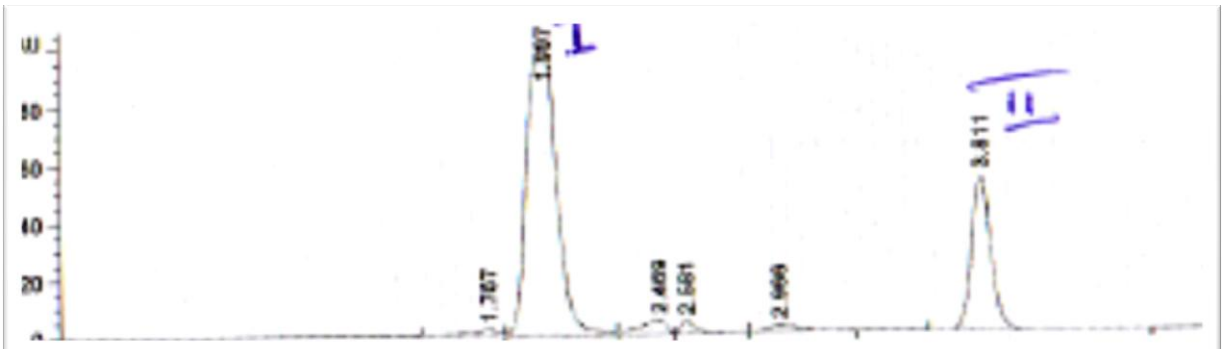


**Fig. 2. Chromatogram of standards Pic I=Acid gallic, Pic II= Catechol**

## 4. DISCUSSION

Tannins, flavonoids and sugars have been found abundant in the leaves and pulp samples, whereas gallic tannins, coumarins, leucoanthocyanins and mucilages were less abundant. It should be noted that the saponosides were absent in the pulp of this plant (Table 2).

Sulphate, calcium, carbonate, chloride and potassium ions were present in the leaves and pulp of the dried and flooded sites of Niono and Sévaré (Table 3).

The leaves of the flooded sites of Niono and Sévaré had an average rate in catechetical tannins higher than those of the dried sites of Niono. Flavonoids and gallic tannin production was not related to the types of areas. In fact, the flooded sites of Niono and Sévaré showed the highest content.

The samples from dried site of Niono (Ranch) and the flooded Sévaré (Dialagou) site had the highest flavonoid levels at 1.11 ± 0.3 and 1.17 ± 0.2, respectively.

These results are similar to those of Souhila and collaborators who have obtained in the bracts of *Cynara scolymus* L by maceration in water at 2.39% in 2.15% ethanol, in acetone and 2.82% in 1.99% methanol. They found the content in flowers by maceration in water 3.53%, in ethanol 3.75%, in acetone 2.74% and in methanol 2.05% [10].

Saponosides were abundant in the leaves of the Niono and Sévaré samples. The highest foam index was that of the flooded site of Sevare (Dialagou) with 388 and dried site of Niono with the lowest index with 239.

Flavonoids of the flooded sites had a greater antiradical activity on DPPH than those of the dried sites of Niono and Sevare with $IC_{50}$ of 33.92 ± 0,04 µg and 32.8 ± 0,6 µg respectively. The catechical tannins extracted from the Niono sites with $IC_{50}$ of 42.16 µg and 40.2±0,3 µg had a greater antiradical activity than extracts from the Sévaré sites. Gallic tannins had less antiradical activity.

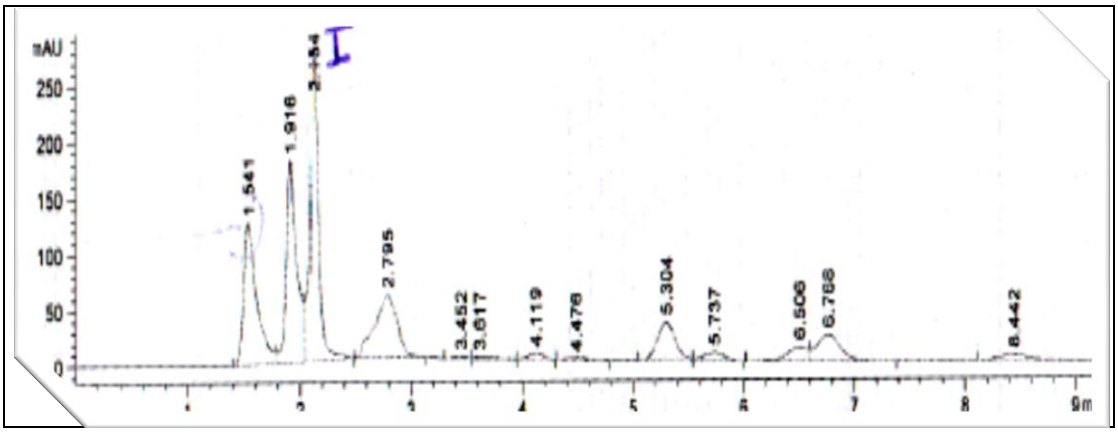


**Fig. 3. Chromatograms of leaf extracts**

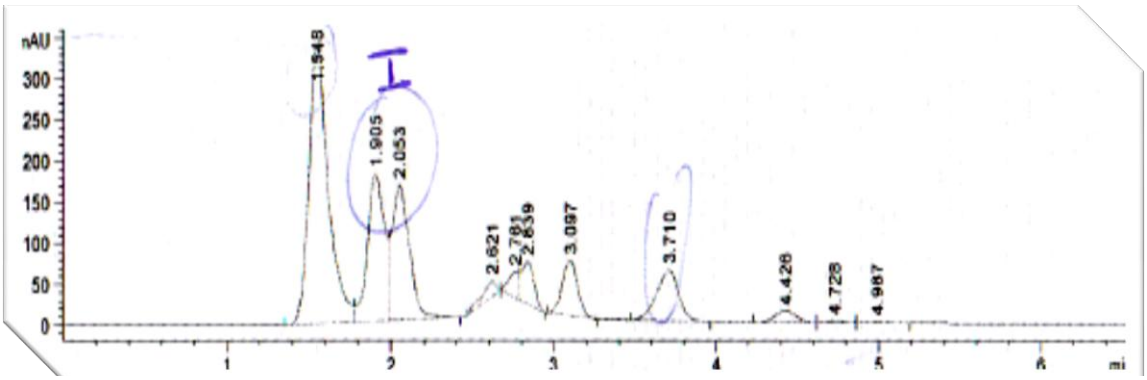


**Fig. 4. Chromatograms of pulp extracts**

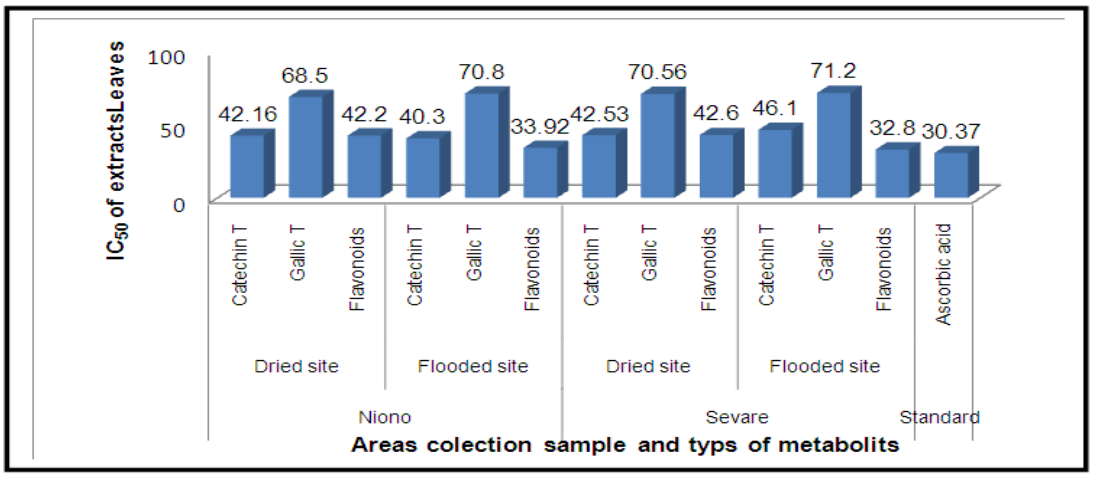


**Fig. 5. $IC_{50}$ histogram of leaves extracts**

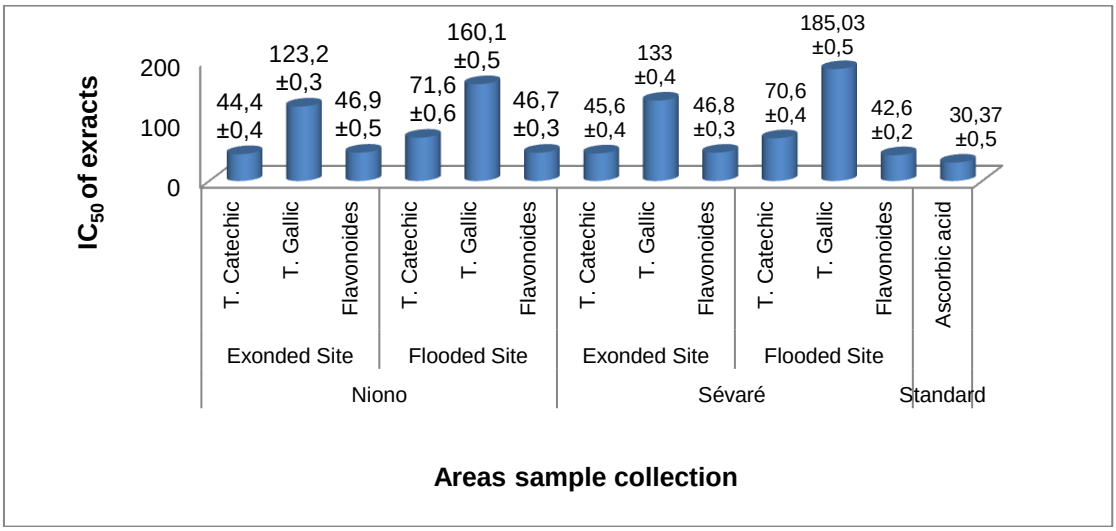


**Fig. 6. Histogram of $IC_{50}$ of pulp extracts**

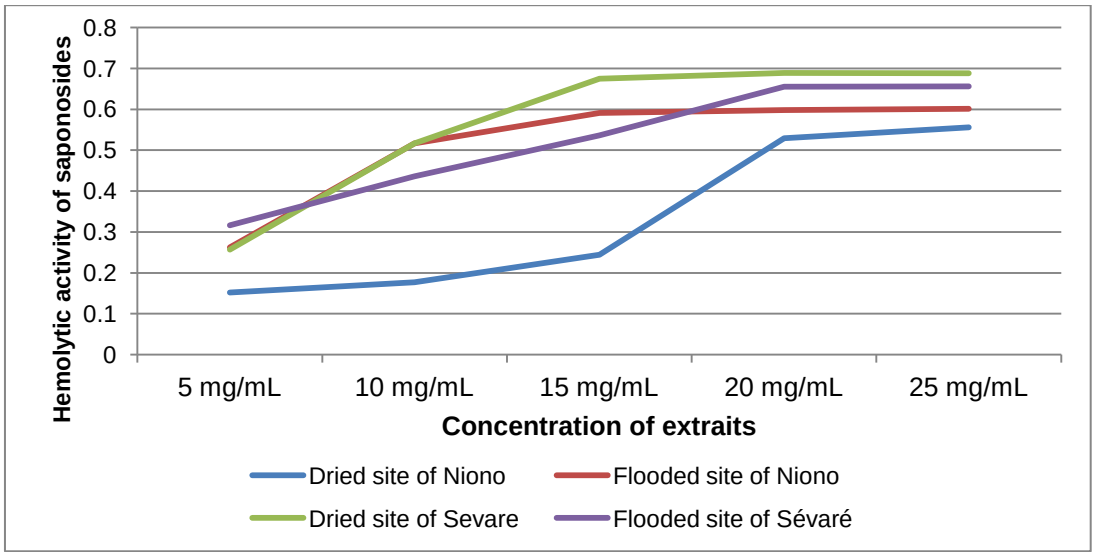


**Fig. 7. Curve of hemolytic activity of saponoside extracts**

The flavonoids in the pulp of the flooded site of Sévaré had more activity, followed by catechical tannins of the dried site of Niono with $IC_{50}$ of 42.6± 0,2 µg and 44.4± 0,04 µg. The $IC_{50}$ values were close to those of ascorbic acid. 30.37±0,3 µg. These results were similar to those of Nabila et al. (2011) who obtained 90% [14] for bile tannins, the percentage was between 55.5% and 67.4%. Flavonoid percentage inhibition was between 36.1% and 59.4%. The total hemolytic activity was 15 mg of saponoside for the flooded Niono site and the Sévaré site, whereas the Sévaré flooded site and the dried site of Niono, the total haemolytic activity was from 20 mg of saponoside. Hemolytic activity could not be linked to collection sites. These results were similar to those of Ouedraogo and collaborators, who achieved total hemolytic activity with 21 mg of stem extracts and 15 mg of Mitragyna inermis root extract [15]. Najiba and collaborators obtained a 54.21% haemolytic activity with total alkaloid extracts at 5 mg/mL of *Berberis vulgaris* L. [11]. Results obtained in this study were different from those of Haddouchi and collaborators.whose haemolysis test showed that four species had a weak hemolytic effect [16].

## 5. CONCLUSION

This study allowed us to compare the content and activities of metabolites (sugar flavonoid tannins, coumarins, leucoanthocyans, etc…) of extracts from the leaves and pulps of *Zizyphus mauritiana* Lam according the dried and flooded sites of Niono and Sévaré. Excepted for flavonoids antiradical activity which was more greater for flooded site of Niono than the dried sites, the other content and activities were similar for the both sites. The leaf and pulp extracts displayed presence of calcium, phosphate, and chloride. The saponosides which were only present on the leaves, displayed interesting hemolytic activity on the blood of beef suggesting attention to the consumption of this part by livestock. Other in vivo studies could confirm these hypotheses.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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