PHYTOCHEMISTRY AND BIOLOGICAL ACTIVITIES OF LEAVES AND PULP EXTRACTS FROM *ZIZYPHUS MAURITIANA* (LAM.) COLLECTED IN MALI

ABSTRACT:

Introduction: The objectives of this work were to make a phytochemical study of the leaves and pulp of *Zizyphus mauritiana* Lam collected from the flooded and exonded sites of Niono and Sévaré in Mali on the one hand and to evaluate the biological activity of the extracts notably the antiradical activity and hemolytic activity extracts on the other hand.
Method: Phytochemical analysis and biological activities were performed at the plant biochemistry and biotechnology laboratory of the FST / USTTB. The tannins and flavonoids were extracted by maceration and assayed by spectrophotometry and the saponosides were extracted by decoction.
Results: Catechin tannins, flavonoids and abundant sugars in the leaves and pulp and saponosides are absent in the pulp of our samples.
Calcium, carbonate and chloride ions are abundant in the leaves and pulps of the excavated site of Niono and the flooded Sévaré site.
HPLC chromatograms of leaf tannin and pulp extracts showed two peaks of gallic acid.
The antiradical activity on the DPPH of the leaf extracts would be linked to the collection site Catechin tannins and flavonoids of the flooded sites have a greater antiradical activity than those of the exonded sites of Niono and Sévaré, this is not the case extracts of pulp. Their antiradical activity remains lower than that of ascorbic acid whose IC_{50} is 30 µg.
Leaf saponosides showed good hemolytic activity on red blood cells, especially those from the flooded Niono site and the Sévaré extruded site.
Conclusion: Secondary metabolites such as phenolic compounds have good antiradical activity and saponosides extracted from the leaves have hemolytic activity.

K*ey words*: *Phenolic compounds, tannins, flavonoids, saponosides, Zizyphus mauritiana Lam, biological activity*.

1. INTRODUCTION

Zizyphus mauritiana (Lam) has many nutritional, medical, artisanal and even orchard protection interests. Several previous works have shown its richness in primary and secondary metabolites [1], [2], [3] as well as its economic interests [4]. Danthu et al. Studied phytochemical composition in two wild and domestic species in Senegal [3].

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

Other studies have shown the hypoglycemic, hypertensive, anti-inflammatory, antibacterial and antioxidant activity of leaf extracts [7], [8], [9]. The antioxidant power of tannins (catechical and gallic tannins) and flavonoids would help fight against aging [7].

A comparative study of certain abundant metabolites in the leaves and pulp of the plant would make it possible to evaluate the nutritional and biological qualities according to the collection sites (site flooded, site exonde). During these works metabolites were extracted characterize and assay. Their anti-radical activities on DPPH and hemolytic on red blood cells of beef blood were evaluated

2. MATERIAL AND METHOD::

2.1. Equipment :

2.1.1. Sample collection sites:

Samplings of leaves and pulps of *Zizyphus mauritiana* (Lam.) Were collected at the following sites: In Niono flooded site: Sitan Wéré, and exonded site: Ranch. In Sévaré flooded site Dialagou and site exonded: Doundoun.

2.1.2. Plant material:

Biological material consisted of powder, leaves and pulp of *Zizyphus mauritiana* (Lam.) Sites of Niono and Sévaré, the Blood of beef has been taken at the slaughterhouse of Sabalibougou in Kati.

2.2. METHODS :

2.2.1. Characterization reactions of metabolites:

2.2.1.1. Catechetical tannins:

The catechin tannins were extracted by maceration in 100 mg of plant organ 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 3000rpm. for 10 minutes. The catechical tannins have been characterized by ferric chloride [7]

2.2.1.2. Gallic tannins:

Gallic tanins were extracted in one hundred milligram (100 mg) of organ powder delipidated with petroleum ether and then boiled in 20 mL of distilled water for 10 minutes. The dichloromethane is mixed with the filtrate to remove the pigments. Gallic tannins were extracted in the aqueous phase with ethyl acetate and evidenced by 2% ferric chloride [10], [7].

2.2.1.3. Flavonoids:

In a test tube ten (10) drops of concentrated hydrochloric acid was added to 0.5mL of extract and a few milligrams of magnesium turnings. After three minutes of incubation at room temperature, specific staining of flavonoids was observed [11], [12], [7].

2.2.1.4. Coumarines:

Five milliliters (5 mL) of etheric extract (maceration for 24 hours) were evaporated in a beaker in the open and then 2 mL of hot water was added to the residue. The solution was partitioned between two tubes and 0.5 mL of 25% NH_4OH was added to the contents of one of the tubes and then mixed. Fluorescence was observed at UV at 366 nm [9], [10].

2.2.1.5. Leucoanthocyanes:

To 5 ml of infused prepared from the drug powder, 5 ml of sulfuric acid and then 5 ml of NH_4OH were added to a test tube and the appearance of leucoanthocyanin-specific staining was followed [10] .

2.2.1.6. Sterols and triterpenes:

In a test tube, one (1) gram of organ powder was added twenty (20) mL of petroleum ether. The solution was stirred and left in the refrigerator for 24 hours, then 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 we added 1 mL of acetic anhydride and 1 mL of CHCl3. 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 tube should not be shaken and the appearance of specific staining was followed.

2.2.1.7. Mucilages:

The mucilages were extracted by maceration of five grams of plant organ powder (leaves and pulp) in one hundred (100) mL of distilled water for 12 hours. Mucilages were precipitated by ethanol. Obtaining a fluffy precipitate by mixing indicates the presence of mucilage [9], [10], [11].

2.2.1.8. Reducing sugars:

The reducing sugars have been characterized by hot Fehling liquor. For this purpose, 1 milliliter of Liquor and Organ Extract Reagent was mixed to volume and then boiled at 80 ° C for 5 minutes.

2.2.2. Characterization of some mineral salts of leaves and pulps:

One gram of the organ powder was calcined in an oven at 600 ° C for 12 hours. The ash obtained is weighed and then dissolved in 10 ml of distilled water and filtered. The different ions were highlighted in the filtrate.

Table I: 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 nitrit	Needle crystal

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 spectrophotometrical. In a test tube were mixed (1 mL) of acetone extract, 5 mL of distilled water, 1 mL of ethanol and 0.5 mL of Folin reagent. After standing for 5 minutes, 1 ml of 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 [9].

2.2.3.2. Flavonoids:

To 500 µL of the extract were added 2 mL of distilled water and then 150 µL of 5% sodium nitrite ($NaNO_2$). After five minutes, 100 (100 µL) of aluminum trichloride ($AlCl_3$) at 10% (w / v) is added to the mixture. After six (6) minutes of incubation at room temperature 1 ml of 1M sodium carbonate (NaCO3) is 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 is expressed in milligram (mg) equivalent of catechical per 100 grams of dry vegetable matter (mg EC / 100g) [9], [11].

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 is poured and the operation is repeated with 2.5 mL of petroleum ether. The powder was dried at laboratory temperature. To 0.5 g of delipidated powder was added 10 mL of distilled water and the mixture was boiled with stirring for twenty (20) minutes and filtered. The decoction was used to determine saponoside content by 1/10 dilution [10].
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 drug and the foam number is 10 * 1 / 0.05 = 200.

2.2.3.4. Liquid chromatography of tannin extracts:

The standards were prepared in a 50/50 (v / v) water / methanol mixture. Several calibration ranges were used: 10 mg / ml, 20 mg / ml, 50 mg / ml to establish the calibration line.
Lyophilized tannic extracts were dissolved in the 50/50 (v / v) water / ethanol mixture. Then they were sonicated for 15min, allowed to cool to room temperature and filtered through a nylon membrane with 0.45µm pores prior to injection.[18]
LC conditions:
Mobile Phase: Water / 20mM Phosphate / Acetonitrile buffer 70: 28: 2 v / v / v
Column: C18, 4.6 x 150mm, 5µ- Zorbax- Agilent
Flow rate: 0.8ml / min, injection volume: 20µl, column temperature: 30 ° C, detection: 271nm

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 passage from the purple color

(DPPH) to the yellow color (DPPH). A negative control tube is prepared by replacing the extract with distilled water. The tubes were placed in the dark for 30min and the reading was made at 517nm. [12]
The results are expressed in% of anti-radical activity or Inhibitory in percentage (I%) according to the formula: I% = [(Abs negative control-Abs Sample) / Abs control [13],
The IC_{50} of each extract was calculated from a linear regression line established with the percentages of inhibition obtained. IC_{50} is the concentration of the extract that inhibits 50% of the activity of the radical, plus it is small plus the extract is considered a powerful antioxidant [14].

2.2.5. Hemolytic activity of saponosides:

The tests were performed on red blood cell pellets obtained by centrifugation at 4000 rpm for five (5). They were washed three (3) times with physiological saline buffered because of 1ml of blood dissolved in 25ml of saline.
In a series of six tubes each containing 0.5 ml of packed red blood cells were added increasing volumes of a solution at 1 mg / ml or 5 mg / ml of saponosides extracted from the leaves and 2 ml of the buffered saline solution. The mixture was homogenized and the tubes were allowed to stand for 24 hours and then centrifuged at 3500 rpm for 10 minutes. [16]
The turbidity through the red coloration observed in the tubes containing the extract and the pellet determines the haemolytic activity. The absorbance of the solutions of the tubes

3. RESULTS AND DISCUSSION:

3.1. Characterization of metabolites:

The results obtained after the characterization reactions of the metabolites are recorded in the table below.

Table II: Characterization of primary and secondary metabolites

Metabolites	Feuilles				Pulpes			
	Niono		Sévaré		Niono		Sévaré	
	exonded site	Flooded site	exonded site	Flooded site	exonded site	Flooded site	exonded site	Flooded site
Catechical tannins	+++	+++	+++	+++	+++	+++	+++	+++
Gallic tannins	++	++	+	+	++	++	+	+
flavonoids	+++	+++	+++	+++	++	++	++	++
coumarins	+	++	+	++	+	+	++	+
leucoanthocyanins	++	+	+	+++	+	+	+	++
saponosides	+++	++	++	+++	-	-	-	-
Terpenes - Sterols	++	++	++	++	+	++	+	++
mucilage	++	++	++	++	++	++	++	++
sugars	+++	+++	+++	+++	+++	+++	+++	+++

Legend: +++ = Very abundant, ++ = Not abundant, + = Traces, - = Absent

Table III: Characterization of mineral salts in leaves and pulps

	Leaves				Pulps			
	Niono		Sévaré		Niono		Sévaré	
	Exonded Site	Flooded site	Exonded Site	Flooded site	Exonded Site	Flooded site	Exonded Site	Flooded site
Sulfate	+	+	+	+	+	+	+	+
Calcium	++	++	++	+++	+++	+++	++	++
Carbonate	++	++	++	+	+	+	++	++
Chlorure	++	++	++	+	+	+	++	++
Potassium	+	+	+	++	++	++	+	+

Legend: +++ = Very abundant, ++ = Not abundant, + = Traces, - = absent

3.2. Determination of metabolite of leaves and pulp:

Table IV and table V shows the results of catechical tannin determination at the leaves and pulps of the different sites. These results are the averages of three trials.

Table IV: Content of extracts in leaves

Samples	Percentage			
	Sites de Niono		Sites de Sévaré	
	Exonded Site	Flooded site	Exonded Site	Flooded site
Content in catechical tannins	2,40 ± 0,04[a]	2,88 ± 0,03[b]	2,03 ± 0,03[a]	**3,13 ± 0,03[a]**
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 V: Content of extracts in pulp

Samples	Content			
	Niono		Sevare	
	Exonded Site	Flooded site	Exonded 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

3.3. Dosage of saponosides in the leaves:

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

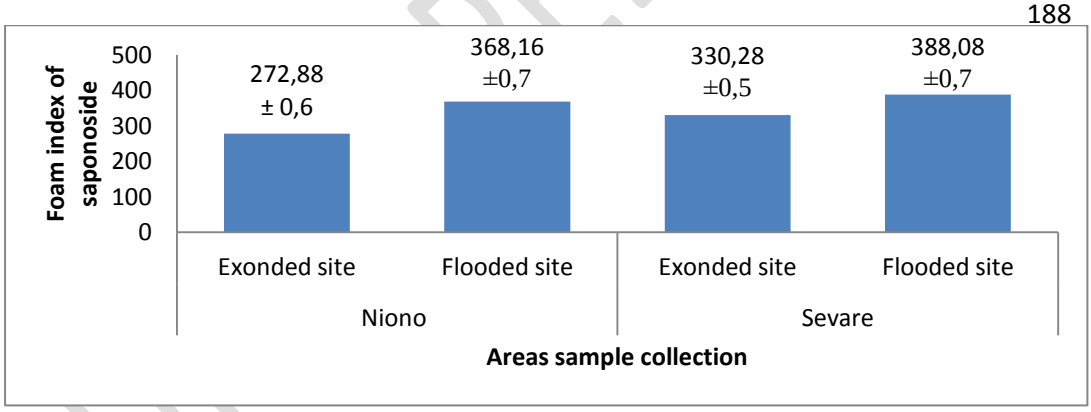


Figure 1: Saponoside content of leaf extracts from Niono and Sévaré sites

3.4. Qualitative analysis by HPLC of tannic extracts:

The following chromatograms were obtained by HPLC extracts of the leaves and pulp of the localities of Niono and Sévaré.
Gallic acid = (I): Retention time = 1.997min and Catechol = II: Retention time = 3.811min

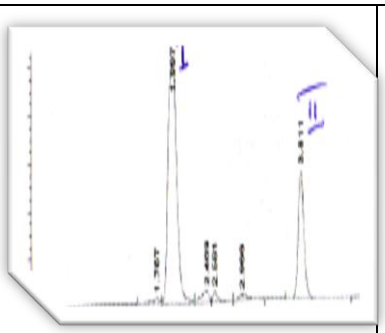


Figure 2: Chromatogram of standards

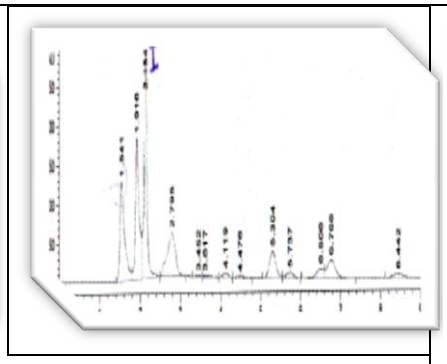


Figure 1: Chromatograms of leaf extracts

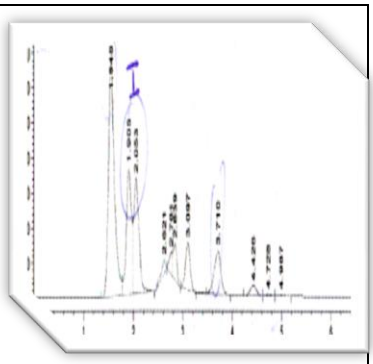


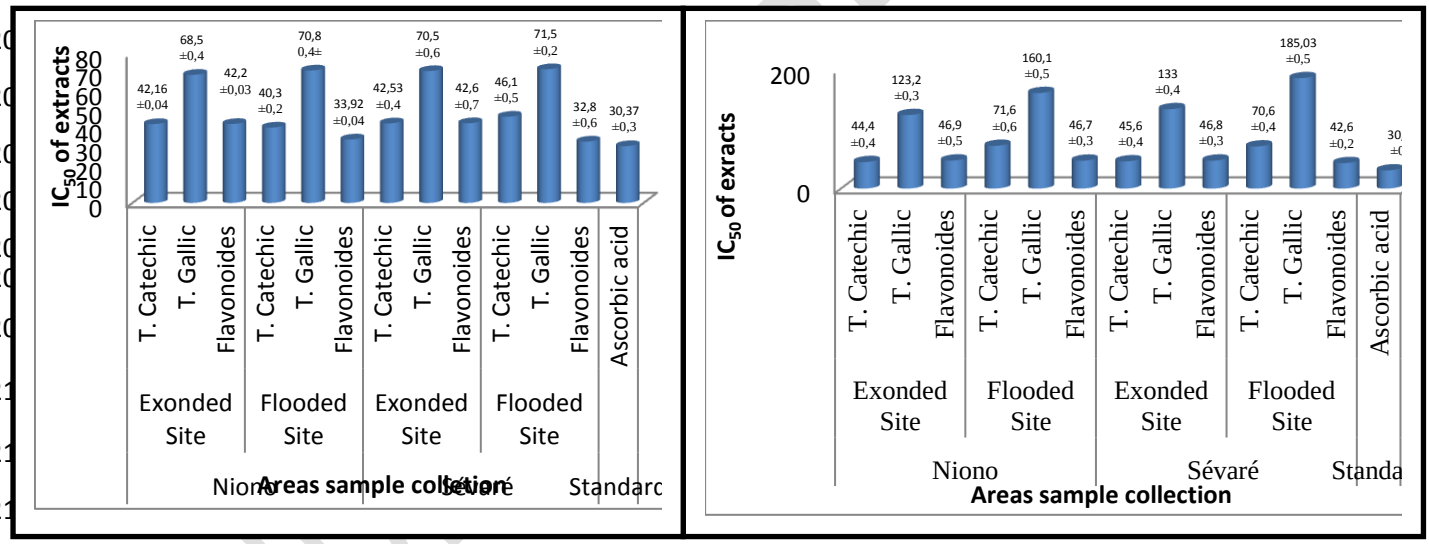
Figure 4: Chromatograms of pulp extracts:

197

198 **3.5. Biological activity**:

199 **3.5.1. Antioxidant activity of leaf and pulps extracts**:

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



213 The optical densities of the supernatants obtained after centrifugation gave the following results

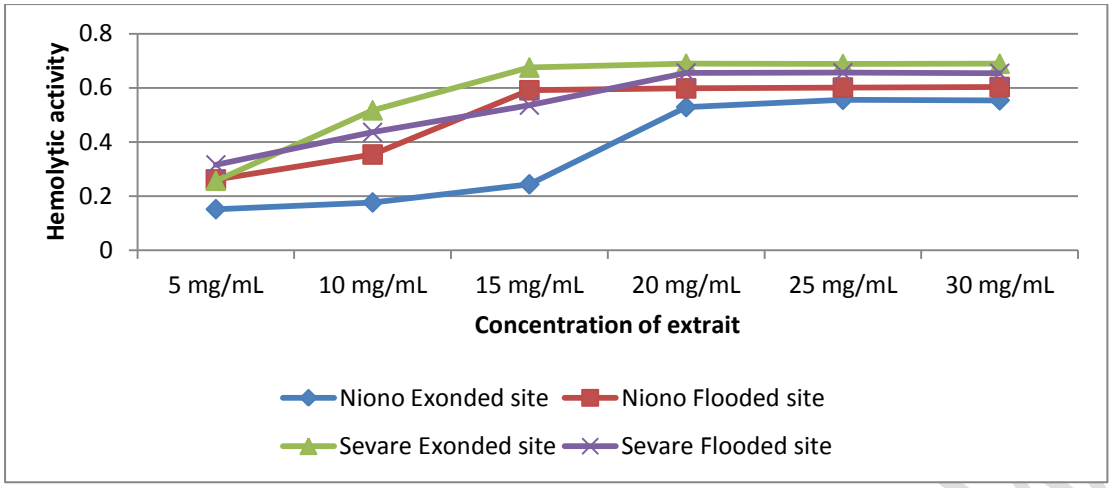


Figure 7: Curve of Hemolytic Activity of Saponoside Extracts of 5 mg / mL

Tannins, flavonoids and catechical sugars have been found abundant in the leaves and pulps of the samples, whereas The gallic tannins, coumarins, leucoanthcyanins and mucilages were less abundant. It should be noted that the saponosides are absent in the pulp of all the samples of the different sites Table II.

Sulphate ions, calcium ions, carbonate ions, chloride ions and potassium ions were found in the leaves and pulps of the no flooded and flooded sites of Niono and Sévaré Table III

The leaves of the flooded sites of Niono and Sévaré have an average rate in catechetical tannins higher than those of the exposed sites of Niono. Flavonoids and gallic tannin production is not related to the types of areas. In fact, the flooded sites of Niono and flooded with Sévaré have a higher content.

The samples from no flooded of Niono (Ranch) and the flooded Sévaré (Dialagou) site show the highest flavonoid levels at 1.11 ± 0.3 and 1.17 ± 0.2, respectively.

These results are similar to those of Souhila et al., Obtained in the bracts of Cynara scolymus L by maceration in water at 2.39%, in 2.15% ethanol, in acetone. 2.82% in 1.99% methanol. They found in flowers by maceration in water 3.53%, in ethanol 3.75%, in acetone 2.74% and in methanol 2.05% [11].

Saponosides are abundant in the leaves of the Niono and Sévaré samples and absent in the pulps. The highest foam index is that of the flooded site of Sevare (Dialagou) with 388 and non-flooded site of Niono have the lowest index with 239.

The flavonoids of the flooded sites have a greater antiradical activity on DPPH than those of the non-flooded sites of Niono and Sevare with IC_{50} of 33.92 ±0,04 µg and 32.8 ± 0,6 µg respectivement. The catechical tannins extracted from the Niono sites with IC_{50} of 42.16 µg and 40.2±0,3 µg have a greater antiradical activity than extracts from the Sévaré sites. Gallic tannins have less activity activity antiradicalaire.

At the level of the pulp the flavonoids of the flooded site of Sévaré have more important activity followed catechical tannins of the exonated site of Niono with IC_{50} of 42.6± 0,,2 µg and 44.4± 0,04 µg.

The IC_{50} values are very close to those of ascorbic acid, 30.37±0,3 µg. The IC_{50} values are very close to those of ascorbic acid, 30.37 µg graphe These results are close to those of Nabila who obtained 90% [13] for bile tannins, the% is between 55.5% and 67.4%.and different for flavonoids whose the percentage inhibition is between 36.1% and 59.4%

At the level of saponoside extracts from the flooded Niono site and the exonded Sévaré site, total haemolytic activity was observed from 15 mg of saponins. Whereas at the level of the Sévaré flooded site and the non- flooded site of Niono, the total haemolytic activity was obtained from 20 mg of saponoside. Hemolytic activity would not be linked to collection sites.

These results are similar to those of Ouedraogo et al., Who achieved total hemolytic activity with 21 mg of stem extracts and 15 mg of Mitragyna inermis root extract [17]. Najiba obtained a 54.21% haemolytic activity with total alkaloid extracts at 5 mg / ml of Berberis Vulgaris L. [13]. Our results are different from those of Haddouchi et al., Whose haemolysis test showed that four species had a weak haemolytic effect [18]

4. CONCLUSION:

Tannins, flavonoids and saponosides were extracted characterized and assayed in the samples collected on the exonated and flooded sites of the Niono and Sévaré sites. . Their biological activities have been assessed through

In conclusion, leaf and pulp samples of Zizyphus mauritiana Lam from the flooded and exposed sites of Niono and Sévaré are rich in mineral salts such as phosphates, sulphates, calcium, carbonates and potassium. Their production in the leaves or pulp is not related to the collection site. Secondary metabolites have been found in the leaves as well as in the pulp such as catechic and gallic tannins, flavonoids, coumarins, leucoanthocyanins, terpenes and sterols, mucilages and saponosides. Catechin tannins are more abundant in the leaves than gallic tannins.

We note that extracts of catechic tannins and flavonoids from leaves and pulps showed good anti-radical activity on DPPH. This activity would be linked to the collection site at the leaf level extracts from flooded sites of Niono and Sévaré showed a greater antiradical activity than extracts from the excavated sites. At the pulp level, the antiradical activity would not be linked to the site. The three collection sites have almost the same IC50 except that of the flooded site of Sévaré, which is inferior to the others and therefore more active. Their antiradical activity remains lower than that of ascorbic acid.

Leaf saponosides showed good haemolytic activity on red blood cells, especially those from the flooded Niono site and the Sévaré extruded site. Hemolytic activity would not be related to the collection site.

Competing interests::

Authors have declared that no competing interests exist.

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