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

ABSTRACT:

Introduction: The objectives of this work were to conduct a phytochemical study of the leaves and pulp of *Ziziphus mauritiana* Lam collected from the flooded and exonded sites of Niono and Sévaré in Mali as well as the evaluation of the biological activity of the extracts, i.e. the antiradical activity and hemolytic activity.
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. The saponosides were extracted by decoction.
Results: Catechin, flavonoids and sugars were abundant in the leaves and pulp but saponosides were absent in the pulp.
Calcium carbonate and chloride ions were abundant in the leaves and pulp 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 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 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 antiradical activity and saponosides extracted from the leaves have hemolytic activity.

K*eywords*: *Phenolic compounds, tannins, flavonoids, saponosides, Ziziphus mauritiana Lam, biological activity*.

1. INTRODUCTION

Ziziphus mauritiana (Lam) has many nutritional, medical, artisanal and even orchard protection interests (Reference needed). Several 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 of *Ziziphus* 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 that leaf extracts have hypoglycemic, hypertensive, anti-inflammatory, antibacterial and antioxidant activity[7], [8], [9]. The antioxidant properties of tannins (catechin and gallic acid) and flavonoids would help fight against ageing [7].

A comparative study of certain metabolites in the leaves and pulp of *Ziziphus* sp make it possible to evaluate the nutritional and biological qualities according to the collection sites (site flooded, site exonded) (Reference needed). Metabolites were extracted, characterized and assayed. 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 pulp of *Ziziphus 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 *Ziziphus 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. Catechin 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 3000 rpm. for 10 minutes. The catechin tannins were ferified and characterized by ferric chloride [7]

2.2.1.2. Gallic acid/ tannins:

Gallic tanins were extracted fromone hundred milligrams (100 mg) of organ powder, delipidated with petroleum ether and boiled in 20 mL of distilled water for 10 minutes. 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 [10], [7].

2.2.1.3. Flavonoids:

In a test tube, ten (10) drops of concentrated hydrochloric acid were added to 0.5 mL 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 millilitres (5 mL) of etheric extract (maceration for 24 hours) were evaporated in a beaker in the open whereafter 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 mixed. Fluorescence was observed at UV at 366 nm [9], [10].

2.2.1.5. Leucoanthocyanins:

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

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 chloroform 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 appearance of specific staining was followed without shaking.

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 ethanol. Afluffy precipitate by mixing indicates the presence of mucilage [9], [10], [11].

2.2.1.8. Reducing sugars:

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

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….. The different ions were highlighted in the filtrate using colour precipitate?…..

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	The white precipitate darkens in the light
Potassium	Cobalt sodium nitrite	Needle crystal

2.2.3. Dosage of catechetical and gallic tannins, flavonoids and saponosides:

2.2.3.1. Catechetical tannins:

The content of the catechetical tannins in the extracts was determined spectrophotometrically. 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 [9]. Results were expressed as gallic acid equivalents???

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 aluminium 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 / 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 was poured and the operation was repeated with 2.5 mL of petroleum ether. The powder was dried at laboratory temperature. To 0.5 g of delipidated powder, 0 mL of distilled water was added and the mixture was boiled with stirring for twenty (20) minutes and filtered through …. Saponosides were determined on the decoction 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, then 5 mL of 1% has 0.05 g of saponoside and the foam number 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 before injection.[18].
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: 30 °C, 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 a standard antioxidant (ascorbic acid) concerning the DPPH radical was evaluated using a spectrophotometer by following the reduction of this radical, which is accompanied by its change from purple colour (DPPH) to yellow colour (DPPH). 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. [12]. The results were expressed in % of the 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 inhibitions 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 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.
Six tubes, each containing 0.5 mL of packed red blood cells, increasing volumes of 1 mg / mL or 5 mg / mL of saponosides extracted from the leaves and 2 mL of the buffered saline solution were added. The mixture was homogenized and the tubes were allowed to stand for 24 hours and centrifuged at 3500 rpm for 10 minutes.[16].
The turbidity through the red colouration 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 listed in tables II and III.

Table II: Characterization of primary and secondary metabolites

Metabolites	Feuilles				Pulps			
	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: +++ = Abundant, ++ = Not abundant, + = Traces, - = Absent

Table III: Characterization of mineral salts in leaves and pulps

Mineral salts	Leaves				Pulp			
	Niono		Sévaré		Niono		Sévaré	
	Exonded Site	Flooded site	Exonded Site	Flooded site	Exonded Site	Flooded site	Exonded Site	Flooded site
Sulfate	+	+	+	+	+	+	+	+
Calcium	++	++	++	+++	+++	+++	++	++
Carbonate	++	++	++	+	+	+	++	++
Chloride	++	++	++	+	+	+	++	++
Potassium	+	+	+	++	++	++	+	+

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

3.2. Determination of metabolites of leaves and pulp:

Table IV and Table V show the results of catechetical tannin determination in the leaves and pulp 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 was equal to 1 cm or close to the different samples of *Ziziphus mauritiana* Lam

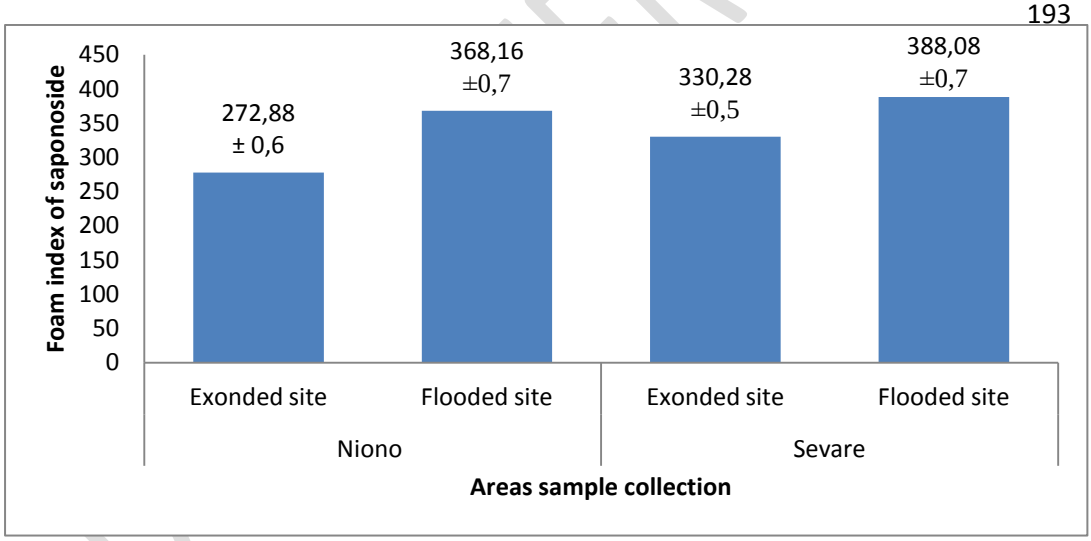

Figure 1: Saponoside content of leaf extracts from Niono and Sévaré sites

3.4. HPLC qualitative analysis of tannic extracts:

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

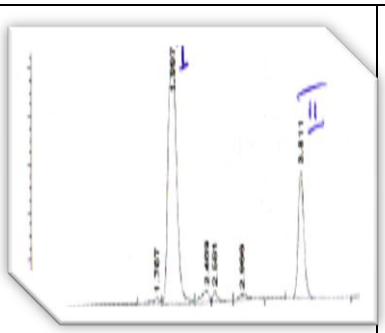

Figure 2: Chromatogram of standards

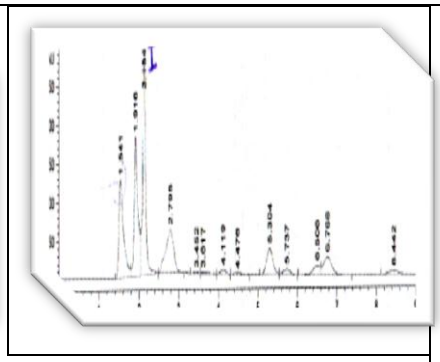

Figure 1: Chromatograms of leaf extracts

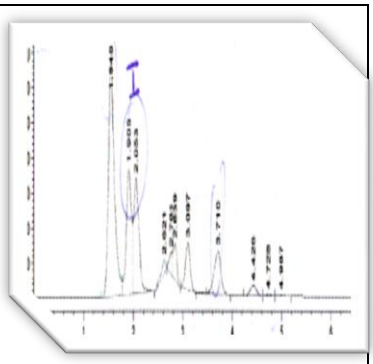

Figure 4: Chromatograms of pulp extracts:

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 per cent inhibition (% I) of *Zizyphus mauritiana* Lam pulp extracts.

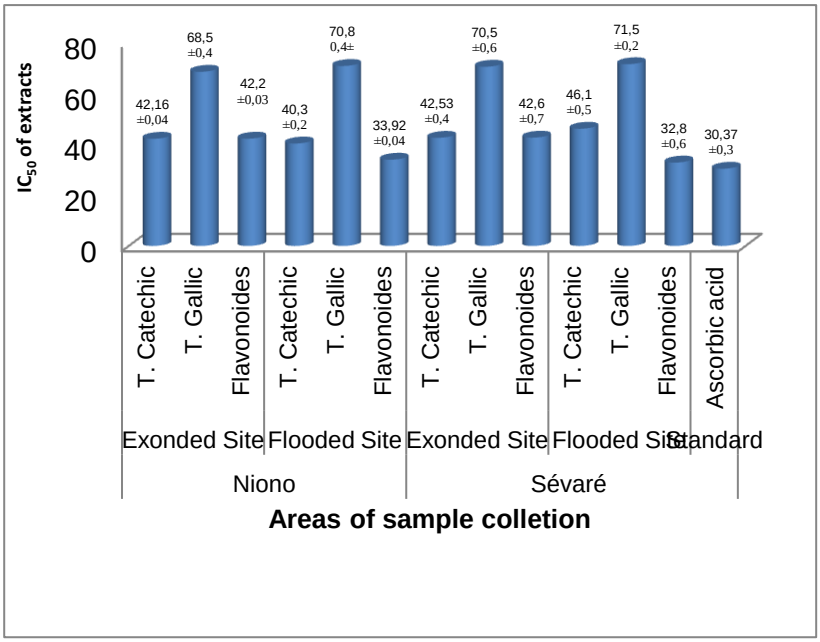

Figure 5: IC_{50} histogram of leaf extracts

Figure 6: Histogram of IC_{50} of pulp extracts

3.5.4. Hemolytic activity of leaf saponosides:

Figure 7 depicts the optical densities of the supernatants obtained after centrifugation

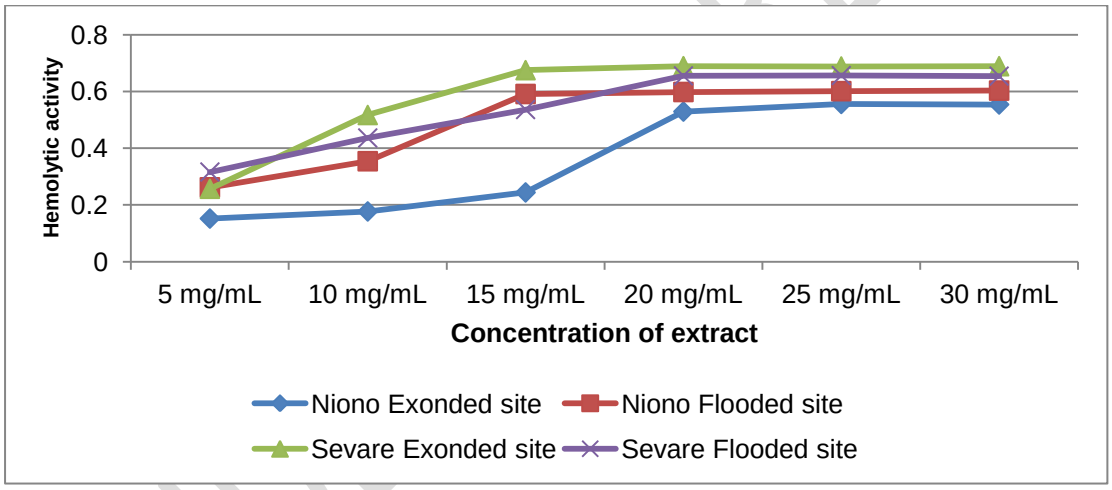

Figure 7: Curve of hemolytic activity of saponoside Eetracts

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 all pulp samples of the different sites (Table II).

Sulphate, calcium, carbonate, chloride and potassium ions were present in the leaves and pulp of the non-flooded and flooded sites of Niono and Sévaré (Table III)
The leaves of the flooded sites of Niono and Sévaré had an average rate in catechetical tannins higher than those of the exposed sites of Niono. Flavonoids and gallic tannin production are not related to the types of areas. The flooded sites of Niono and flooded with Sévaré have higher content.
The samples from non-flooded 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 et al. (2013), obtained in the bracts of *Cynara scolymus* L by maceration in the water at 2.39% in 2.15% ethanol, in acetone and 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 methanol 2.05% [11].

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

Flavonoids of the flooded sites had 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 respectively. The catechetical tannins extracted from the Niono sites with IC_{50} of 42.16 µg and 40.2±0,3 µg had greater antiradical activity than extracts from the Sévaré sites. Gallic tannins had less antiradical activity.

The flavonoids in the pulp of the flooded site of Sévaré had more activity, followed catechetical tannins of the exonerated 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, i.e. 30.37±0,3 µg. These results are similar to those of Nabila (2011) who obtained 90% [13] for bile tannins, the percentage was between 55.5% and 67.4%. Flavonoid percentage inhibition was between 36.1% and 59.4%.
The total haemolytic activity was from 15 mg of saponoside from the flooded Niono site and the exonded Sévaré site,., whereas the Sévaré flooded site and the non- flooded site of Niono, the total haemolytic activity was from 20 mg of saponoside. The hemolytic activity could not be linked to collection sites. These results are similar to those of Ouedraogo et al. (2009), 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]. Results obtained in this study are different from those of Haddouchi et al. (2016), 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 exposed and flooded sites of the Niono and Sévaré sites.
Leaf and pulp samples of *Ziziphus mauritiana* Lam from the flooded and exposed sites of Niono and Sévaré are rich in 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, I .e.catechin and gallic tannins, flavonoids, coumarins, leucoanthocyanins, terpenes and sterols, mucilages and saponosides. Catechin tannins are more abundant in the leaves than gallic tannins.

Extracts of catechin tannins and flavonoids from leaves and pulp showed 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 which showed greater antiradical activity than extracts from the excavated sites. tThe antiradical activity in pulp could not be linked to the site. The three collection sites have almost the same IC_{50} 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. The hemolytic activity could not be related to the collection site.

Competing interests:

Authors have declared that no competing interests exist.

References:

1. Preeti and Shalini Tripathi (2014): *Ziziphus jujuba*: a phytopharmacological Review. International Journal of Research and Development in Pharmacy and Life Sciences. Available online at http//www.ijrdpl.com, Vol. 3, No.3, pp 959-966.

2. Singou Keita, Mamadou Wélé, Cheickna Cisse, Nouhoum Diarra, Laura Kirkman and Lamine Baba-Moussa (2018): Antibacterial and Antiplasmodial Activities of Tannins Extracted from *Ziziphus mauritiana* in Mali. International Journal of Biochemistry Research & Review 24 (2): 1-8

3. Danthu Pascal, Pierre Soloviev, Anne Totté, Emmanuel Tine, Nicolas Ayessou, Abibou Gaye, Thierno (2002): Caractères physico-chimiques et organoleptiques comparés de jujubes sauvages et des fruits de la variété Gola introduite au Sénégal. Fruits, vol. 57 (3) 173-182.

4. Hassan Mahmood, Mueen Ahmad Chaudhry, Zeeshan Masood, Muhammad Asad Saeed and Sherjeel Adnan (2017): A mechanistic evaluation of the traditional uses of *Nepeta ruderalis* in gastrointestinal and airway disorders, Pharmaceutical Biology, 55:1, 1017-1021,

5. Niamat Rabia, Mir Ajab Khan, Kiran Yasmin Khan, Mushtaq Ahmed, Paras Mazari, Barkat Ali, Mazhar Mustafa and Muhammad Zafar (2012): A Review on *Ziziphusm mauritiana* Lam as Antidiabetic. Journal of Applied Pharmaceutical Science 02 (03); 2012: pp. 177-179

6. Diallo Drissa Innocent Pierre Guissou Mahamane Haïdara Coumbo Tall Ossy MJ Kasilo (2010) : Recherche Sur la Médecine Traditionnelle Africaine: HyPertension. pp63-74.

7. Roumeissa Lehout and Laib Maya (2015) : Master: Comparaison de trois méthodes d'extraction des composés phénoliques et des flavonoïdes à partir de la plante médicinale : *Artemisia herba alba Asso*. Université des Frères Mentouri Constantine. Faculté des Sciences de la Nature et de la Vie P. 1-76.

8. Abdoulaye Sereme, Millogo Rasolodimby, Guinko andNacro (2008): Propriétés thérapeutiques des plantes à tannins au Burkina faso. Université 03 BP 7021 Ouagadougou 03 Burkina Faso (Afrique de l'ouest). pp 41- 49.

9. Nouhoum Diarra, Adama Dénou, Issiaka Togola Cheickna Daou Nagnouma Doumbia and Mamadou A Konaré (2019): Composition physico-chimique et biochimique des tubercules de *plectranthus rotundifolius* (poir.) spreng, (lamiaceae) utilisée en alimentation au Mal. American Journal of Innovative Research and Applied Sciences. ISSN 2429-5396.

10. Dohou N., Yamni K., Tahrouch S., Idrissi Hassani L.M., Badoc A., N. Gmira (2003): Screening phytochimique d'une endémique Ibéro-marocaine, *Thymelaea lythroides*. Bull. Soc. Pharm. Bordeaux, 142, 61-78.

11. Souhila Mahamoudi, Khali Moustapha and Mahmoudi Nacéra (2013): Étude de l'extraction des composés phénoliques des différentes parties de la fleur de l'Artichaut (*Cynara scolymus*). pp 1-6.

12. Karima and Meziani Amal (2015) : Magister: Étude de l'activité biologique de l'extrait Aqueux des feuilles du *Zizyphus lotus* L. Université des Frères Mentouri Constantine Faculté des Sciences de la Nature et de la Vie. pp1-98

13. Bougandoura Nabila (2011): Magistère : Pouvoir antioxydant et antimicrobien des extraits d'espèces végétales *Saturejacalaminthasspnepta* (nabta) et *Ajugaiva L*. (chendgoura) de l'ouest d'Algérie. Université Abou BakrBelkaid-Tlemcen. Faculté des Sciences de la Nature et de la Vie et Sciences de la terre P:1-97.

14. Logopho Hyacinthe Ouattara, Guy Roger Mida Kabran, Amani Brice Kadja, Mamyrbekova-Bekro, and Yves-Alain Bekro (2016) : Étude phytochimique, activité anti-oxydante d'extraits de plantes de Cote d'Ivoire utilisées dans le traitement traditionnel des hémorroïdes. International Journa of Innovation and Applied Studies ISSN 2028-9324 Vol 15 NO. 4 May 2016, pp. 881-893.

15. Ouedraogo Y., Nacoui.ma O. Guissou L.P., Guede Gulna F·J (2009): Evaluation *in vivo* et *in vitro* de la toxicité des extraits aqueux d'écorces de tige et de racines de *Mitragyna inermis* (wilid).o.ktz (rubiaceae). Pharm. Méd. Trad. Alr 2001, VoU1, pp. 13-29.

16. Haddouchi F, Chaouche T.M., Halla N.(2016): Screening phytochimique, activités antioxydantes et pouvoir hémolytique de quatre plantes sahariennes d'Algérie. Lavoisier SAS 2016. Phytothérapie DOI 10.1007/s10298-016-1086-

17. Hanifa Djelili (2013): Effets Pharmacologiques Pulmonaires des Flavonoïdes : Caractérisation *in Vitro* des effets de la quercétine et de la génistéine**.** Université Ferhar Abbas Serif pp :1-160

18. Matthew W. Giese, Mark A. Lewis, Laura Giese, and Kevin M. Smith (2015): Development and Validation of a Reliable and Robust Method for the Analysis of Cannabinoids and Terpenes in Cannabis Napro Research, Westlake Village, CA 91362. Giese et al.: Journal of AOAC International Vol. 98, No. 6, 2015 1503