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Phytochemical Screening, Antibacterial Activity And Acute Oral Toxicity Of Aqueous And Ethanolic Extracts Of Harrisonia Abyssinica (Rutaceae) Leaf: Wild Plant Used In Benin Pharmacopeia

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Abstract

Harrisonia abyssinica is a wild plant with multiple therapeutic properties used in traditional medicine in Benin. This study aimed at evaluating the preliminary phytochemical screening of large groups of

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secondary metabolites, antibacterial activity and acute oral toxicity of aqueous and ethanolic extracts of H. abyssinica leaves. Antibacterial activity evaluation was doing by microdilution method on bacterial (Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Enterococcus faecalis, Klebsiella pneumonia, Salmonella sp, Shigella sp and Vibrio cholerae). Minimum Inhibitory Concentration (MIC) was determined using Iodonitrotetrazolium salt as revelator. Wistar rats were used for acute oral toxicity test in which single dose of 2000 mg/kg body weight of ethanolic and aqueous extract were administered; the control group received distilled water. Phytochemical screening revealed the presence of gallic tannins, alkaloids, reducing sugar, coumarins, quinones, steroids, terpene and saponosides. No toxicity was observed in rats. The LD50 obtained was greater than 2000 mg / kg bw of rats. No macroscopic and histological abnormalities were seen in kidneys and liver analyzes. Ethanolic extract of H. abyssinica showed a better activity than aqueous extract with a MIC of 1.25 mg / mL compared to 5 mg / mL for aqueous extract.

Keywords: *Wistar* rats, bacterial strains, oral toxicity, Harrisonia abyssinica, Minimum Inhibitory Concentration.

Introduction

For decades, medicinal and aromatic plants have been used to treat human illnesses. Of these diseases, most microbial infections are caused by viruses, fungi, protozoa and bacteria particularly by multidrug resistant bacteria (Ahmed *et al*., 2014). Indeed, many cases of multidrug resistant bacteria have been reported in Benin (Sina *et al*., 2011). This bacterial resistance is today a tangible threat of therapeutic failure in modern medicine (Zahar and Lesprit, 2014). Nowadays, the major challenge remains the effective and lasting treatment of these conditions. Currently, modern medicine does honor to simple medicinal plants, because the effectiveness of drugs such as antibiotics decreases, while the multidrug resistance of pathogenic microorganisms, due to the abuse and inappropriate use of antibiotics increases. *Harrisonia abyssinica*, is a medicinal plant identified to treat infectious diseases (Béné *et al*., 2017) . All organs of the species are used as an herbal drug. The roots are used against swelling of the testes, intestinal worms, diarrhea and stomach problems (Damien *et al*., 2011). The leaves treated several diseases including fever, malaria, diarrhea, hemorrhoids, urinary and intestinal problems, etc. (Mubo and Osiyemi, 2012; Ogougbé *et al*., 2019). This work aimed at exploring scientifically the antibacterial

potency of extracts of *Harrisonia abyssinica* commonly evaluated on different multiresistant strains, their phytochemical and their acute toxicity.

Material and Methods

Plant material

Harrisonia abyssinica Oliv. ( )(Simaroubaceae) leaves were collected from the Pahou forest located in the agro-ecological zone (IV) of Benin, in March 2019. After three rinses, the leaves were dried in the laboratory at 22 ° C protected from the sun light for 28 days and reduced into powder with grinder.

Bacterial strains

Eight bacteria were used in this study and classified into two groups. Gram positive: bacteria strain (*Staphylococcus aureus* ATCC 25923) and isolated bacteria (*Enterococcus faecalis*) and Gram negative: bacteria strains (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) and isolated bacteria (*Klebsiella sp*, *Salmonella sp Shigella sp* and *Vibrio cholera*) responsible for intestinal infectious diseases.

Animal material

Fifteen (15) *Wistar* albino rats were used, including nine females and six males, all between 11 and 12 weeks old and weighing 180 g ± 20 g (male rats) and 225 g ± 20 g (female rats). The rats were kept in a controlled environment at room temperature (37°C) with natural and dark light cycles of 12h / 12h throughout the experiment. They were fed standard rat pellet food (Complete Food, Group Veto Services SA, Benin) and ad libitum drinking water during the experiment.

Preparation of the aqueous extract

Totally, 300 g of the powder plant was used for the preparation of the aqueous extract. This quantity was divided into six different bottles at a rate of 50 g per bottle. To each bottle was added 500 ml of distilled water. The mixture was subjected to permanent agitation and incubated for 12 hours at a temperature of 40 °C. It was entirely filtered through cotton wool and Whatman # 1 filter paper. Each filtrate obtained was concentrated under vacuum using a rotary evaporator BUCHI ROTAVAPOR R II (Switzerland) at a temperature of 40 °C until the pasty extract was obtained. The pasty extract was then placed in an oven at 50 °C to obtain dry extract. The crude aqueous extracts thus obtained were stored in the refrigerator, protected from light and moisture in vials.

Preparation of the ethanolic extract

Totally, 300 g of powder was used for the preparation of the ethanolic extract. 100 g of powder was distributed in three bottles. In each bottle was added 500 ml of ethanol. The whole was homogenized manually and left at 37 °C for 24 h. The supernatant was collected in another bottle and extracted again with ethanol. The operation was repeated three times for three successive days. The process of filtration, evaporation and calculation of yield was the same to that of aqueous extraction.

Phytochemical screening

The phytochemical screening of the *H. abyssinica* extracts was performed according to the colorimetric method (tube test) describe by Houghton and Raman (1998).

Antibacterial activity

The evaluation of the sensitivity of microorganisms (*Staphylococcus aureus* ATCC 25923; *Enterococcus faecalis*; *Shigella sp*; *Vibrio cholerae*; *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 27853; *Klebsiella sp* and *Salmonella sp*) to different extracts of *Harrisonia abyssinica* was carried out by the technique of broth dilution microplate (96 wells). The test was based on the determination of minimum inhibitory and bactericidal concentrations (MIC and MBC).

Determination of the Minimum Inhibitory Concentration (MIC)

Ethanolic extract and the aqueous extract of *H. abyssinica* were prepared at 20 mg / mL in an acetone-water 60 / 40 mixture. Then, 100 µl of MH broth were taken from all the wells to which were added 100 µl of the extract at 20 mg / mL at the level of the first 8 horizontal wells, which allowed us to carry out the half dilution until the last wells of each series. The concentration series evaluated were as follows: 5,000; 2.5; 1.25; 0.625; 0.312; 0.156; 0.078 and 0.039 mg/ml. Then, 100 µl of bacterial suspension at 10^6 CFU / ml are added to all the wells except the control media. The media: MH medium (T1); MH + extract (T2) and MH + acetone (T3) were produced as control media. The media were homogenized and the plates were finally incubated at 37 °C for 18 h.

After 18 h of incubation, 40 µl of para Iodonitrotetrazolium violet (p-INT) are added to 0.2 mg/ml of distilled water at each well and the whole is incubated again at 37 °C for 30 minutes. p-INT is an indicator of bacterial growth by staining. Appearance of red color indicates bacterial growth and maintenance of extract color indicates inhibition of bacterial growth by the extract. The Minimum Inhibitory Concentration of the extracts corresponds to the smallest concentration for which the medium does not turn red (Atindéhou, 2012).

Determination of the Minimum Bactericidal Concentration (MBC)

MBC is the smallest concentration of the antibacterial substance making it possible to obtain, after 18 to 24 hours of incubation at 37 °C, 0.1% of germs, one bacterium per 1000 of the initial inoculum (Rodríguez Vaquero *et al*., 2010). A quantity of the mixture (extract, MH and bacterial broth) was inoculated on MH agar medium and incubated for 24 hours at 37 °C.

Acute toxicity test for aqueous and ethanolic *Harrisonia abyssinica* extracts on *Wistar* rats

The acute oral toxicity study was performed according to the OECD Section 4: Health effects; Test No. 423: guidelines adopted December 17, 2001 (OECD, 2001) 5 groups of rats were acclimatized for five (05) days in the laboratory. They were fasted 12 hours before the administration of *H. abyssinica* extracts. A single dose of 2000 mg / kg of each extract was administered by gavage to four batches of rats including 3 males and 3 females for each type of extract and the control batch (3 female rats) which received distilled water. The animals were observed carefully for the first four hours after administration and then daily for 14 days.

- **Food intake**

Food consumption was recorded daily during treatment. The rats received 50 g of food and the amount of food remaining was measured at the same time the next day. Food consumption was calculated by subtracting food scraps from the total food provided.

- **Clinical observations**

Observation of the physiological behavior of animals focused on changes in the coat, eyes and mucous membranes; autonomous activity (lacrimation, bristling of hair, unusual breathing); change in behavior, posture or reaction to handling; deaths; the body weight of each rat was assessed before, in the middle and at the last day.

At the end of the toxicity assessment, all the rats (03) in each group were sacrificed and autopsied; the liver and kidneys were removed, washed and weighed.

- **Histological sections of organs**

Histological sections of organs were processed in many steps preparation of cassettes (the organs resulting from the dissection were cut into small pieces and then placed in cassettes for fixation); Tissue fixation ; the circulation (consists in making the pieces in a series of liquids in order to give them a rigidity favorable to cutting); coating ; microtome cut and coloration. The assembly is carried out by affixing the slides on the histological slide using Eukitt glue.

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Data analysis and processing

Phytochemical screening

The yield (r) of the various extracts was expressed as a percentage (%), by the ratio between the mass of the extract (Mext) and that of the plant material (Mmat).

r (%) = (Mass of the extract (g)) X100/ (Mass of the powder (g))

Antibacterial activity

The result was encoded in the Excel spreadsheet and translated into tables. An interpretation of the images of the bactericidal or bacteriostatic action of the different extracts on the bacterial strains was made.

Acute toxicity test for aqueous and ethanolic *Harrisonia abyssinica* extracts on *Wistar* rats

The various observations on the behavior and movement of the rats were noted. The absolute and relative weights of the organs (liver and kidneys) were calculated. The absolute weight is approximate to the gross weight of the organs. The relative organ weights were calculated using the formula below:

$Relative\ weight = \frac{Organ\ weight}{Animal\ body\ weight\ on\ the\ day\ of\ sacrifice} X\ 100$ (Shendge and Belemkar, 2019)

These data were translated into a histogram. Construction of graphs was performed using Graph Pad Prism software version 6.00 (Graph Pad Prism Software, Inc., San Diego, California).

Results

Yield of extracts

For this work, the aqueous extract and the ethanolic extract of the powder from the leaves of *H. abyssinica* were used. Of the two extracts, the aqueous extract was found to have the greatest yield (19.58%). Table 1.

Table 1: Yield of the aqueous and ethanolic extract from the leaves of *H. abyssinica.*

Extracts	Mass of material (g)	Mass of extract (g)	Yield (%)
Aqueous	300	58,75	**19,58**
Ethanolic	300	54,48	**18,16**

Phytochemical screening

The results of the phytochemical analysis of these two extracts are assigned in Table 2.

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Table 2: Different secondary metabolites identified in the aqueous and ethanolic extracts of the leaves of *H. abyssinica.*

Secondary metabolites	Aqueous extract	Ethanolic extract
Tannins	+++	+++
Catechic tannins	_	_
Gallic tannins	+	+
Alkaloids	_	++
Reducing sugar	+++	+++
Coumarins	+++	+++
Quinons	++	+++
Steroids	+	++
Terpen compound	++	+++
Saponosides	+	+
Flavonoids	_	_

(—): absence of the secondary metabolite in the extract; (+) Presence of the secondary metabolite in low dose in the extract; (++) presence of the secondary metabolite in medium dose in the extract and (+++) presence of the secondary metabolite in high dose in the medium.

The results of the phytochemical test showed that both extracts from the leaves of *H. abyssinica* Oliv. (Rutaceae) (Simaroubaceae) contain phenolic compounds: tannins (gallic tannins), coumarins, anthocyanins and free quinones, and terpene compounds, to which are added saponosides and reducing sugars. However, we have found that quinones and terpene compounds are more abundant in the ethanolic extract than in the aqueous extract. Catechic tannins and flavonoids were scarce from both extracts. The presence of alkaloids was noted in the ethanolic extract.

Evaluation of the antibacterial activity of aqueous and ethanolic extracts of *Harrisonia abyssinica* on some bacterial strains

The sensitivity test results show that the ethanolic extract of *H. abyssinica* was showed important active on bacteria than the aqueous extract. All the bacteria tested were sensitive to the two *H. abyssinica* extracts except *Pseudomonas aeruginosa* and *Klebsiella spp* which were found to be resistant to the aqueous extract at different concentrations. In addition, some bacteria (*Staphylococcus aureus*; *Enterococcus faecalis*; *Shigella spp*; *Vibrio cholerae*; *Escherichia coli*) were found to be sensitive at the most optimal concentration (5,000 mg/ml) of the aqueous extract. Unlike the aqueous extract, all bacteria were sensitive to at least a concentration (2.5 mg/ml) of the ethanol extract.

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Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

- **Minimum Inhibitory Concentration (MIC)**

The lowest concentration of extracts inhibiting any visible growth of bacterial strains after an incubation time of 18 to 24 hours is considered here as the Minimum Inhibitory Concentrations of extracts against the various bacterial strains (Table 3).

Table 3: Minimum inhibitory concentration (MIC) of the different bacterial strains.

Bacteria	Aqueous extract (MIC)	Ethanolic extract (MIC)
Staphylococcus aureus ATCC *25923*	>5mg/ml	1.25 mg/ml
Enterococcus faecalis	5 mg/ml	1.25 mg/ml
Shigella sp	5 mg/ml	1.25 mg/ml
Vibrio cholerae	5 mg/ml	2.5 mg/ml
Escherichia coli ATCC *25922*	>5mg/ml	1.25 mg/ml
Pseudomonas aeruginosa ATCC *27853*	>5mg/ml	2.5 mg/ml
Klebsiella sp	>5mg/ml	2.5 mg/ml
Salmonella sp	5mg/ml	2.5 mg/ml

The Minimum Inhibitory Concentration (MIC) varied between 1.25 mg/ml and greater than 5 mg/ml. In fact, the inhibition is greater for the ethanol-based extract with a MIC varying from 1.25 to 2.5 mg/ml compared to that of the water-based extract whose low MIC is 5 mg/ml. Furthermore, the ethanol-based extract exhibited interesting inhibitory activity against *Staphylococcus aureus*; *Enterococcus faecalis* and *Shigella sp* with a MIC of 1.25 mg/ml and an average activity on *Vibrio cholerae*; *Pseudomonas aeruginosa*; *Klebsiella sp* and *Salmonella sp* with a MIC of 2.5 mg/ml.

- **Determination of the Minimum Bactericidal Concentration (MBC)**

No Minimum Bactericidal Concentration was obtained for concentrations varying from 1.25 to 10 mg/ml. Therefore the extracts probably have bacteriostatic activity on the strains of bacteria studied.

Oral acute toxicity test of aqueous and ethanolic extracts of *Harrisonia abyssinica* on *Wistar* rats

- **Clinical observations and survival of rats**

No mortality was observed during the first 4 hours of simultaneous observation and also after 24 hours. No lethal effects was also observed after administration of the *H. abyssinica* extract for the experimental period of 14 days and the morphological characteristics appeared normal. No salivation,

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diarrhea or unusual behavior. The breathing was also normal. This indicates that the two extracts of *H. abyssinica* leaves at the dose of 2000 mg/kg of body weight are physically safe. The control group that also received distilled water showed no toxic effects or mortality during the study period. As there was no recorded mortality for this dose, it can be assumed that the LD_{50} value is greater than the limit test dose of 2000 mg/kg body weight.

- **Effect of ethanolic and aqueous extracts of the leaves of *Harrisonia abyssinica* on the change in body weight (bw) of the *Wistar* rats during the test**

In male rats, those that received the extracts already during the first eight days gained weight unlike the controls. From the eighth day, those that received the aqueous extract and the controls increased slightly in weight unlike those that received the ethanolic extract and tend to keep their weight (Figure 1). As for the female rats, the weight of those that received the ethanolic extract dropped slightly after the eighth day as well as those that received the aqueous extract unlike the controls that received distilled water and gained weight (Figure 2).

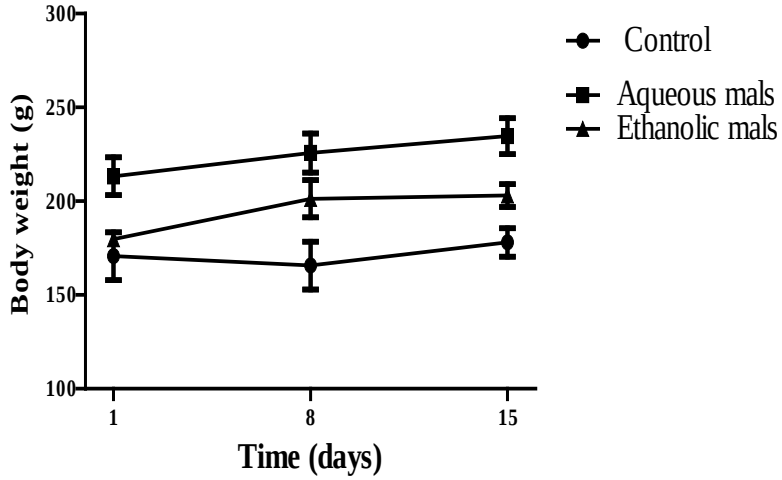

Figure 1: Weight of male rats in the time.

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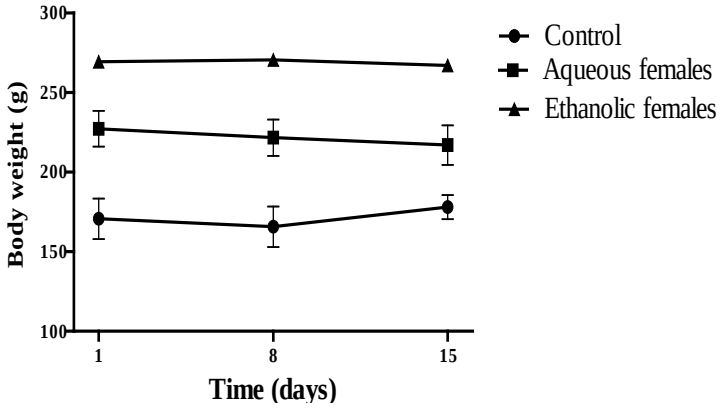
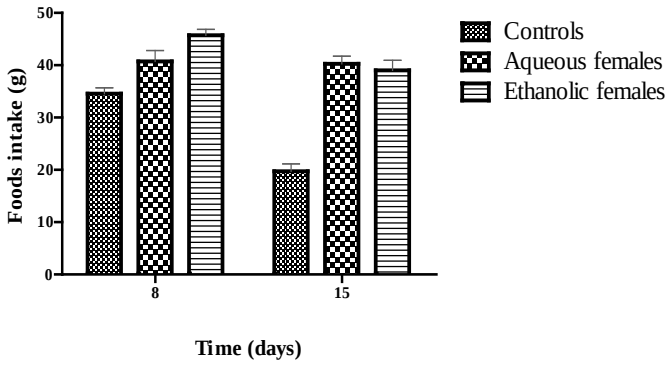


Figure 2: Weight of female rats in the time.

- **Effect of *H. abyssinica* leaf extracts on food consumption by rats during the test period**

The results obtained in female rats show from the first to the fifteenth day an increase in food intake both at the level of those that received *H. abyssinica* aqueous extract and the ethanolic extracts. From the eighth to fifteenth day, we observed a drop in the considerable quantity of food taken by the control rats, a constant in those that received the aqueous extract and a slight drop for those that received the ethanolic extract (Figure 3). Figure (4) show an increase in food in the male rats that received the extracts compared to the controls. From the eighth to the fifteenth day we have a considerable drop in the amount of food taken by the controls while for those that received the aqueous extract a slight increase unlike those that received the ethanolic extract a slight drop

Figure 3: Variation of food consumption in female rats as a function of time.



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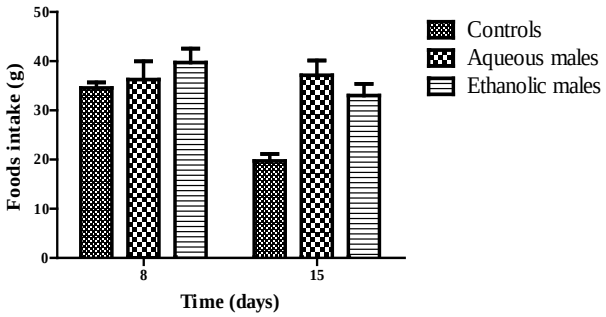

Figure 4: Variation of food consumption in male rats as a function of time

- **Effect of *Harrisonia abyssinica* extracts on the liver and kidney organs of Wistar rats**

The acute effect of *H. abyssinica* leaf extracts on absolute and relative organ weight in rats is shown in Figures 5 and 6. Absolute weights of liver and kidneys in male rats treated with single 2000 mg / kg bw of *H. abyssinica* leaf extracts (Figure 5) was significantly altered ($p \leq 0.05$) for the kidneys in those given the aqueous extract and the liver was highly significant ($p \leq 0.001$), while the change in relative liver and kidney weights was not significant. The relative liver and kidney weights in female rats treated with 2000 mg / kg bw of extract also had no change but only the change in absolute liver weight in those given the aqueous extract which exhibited a significance ($p \leq 0.05$) (Figure 6). The Macroscopic observation showed no effect on liver and kidney of rats (Figure 7, 8).

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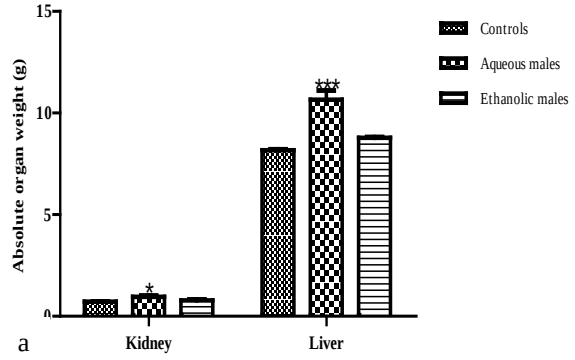

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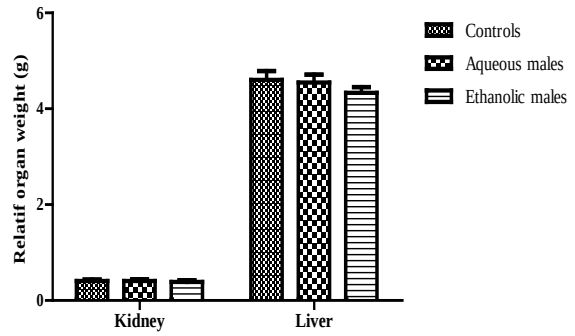

Figure 5: Absolute (a) and relative (b) organ weights (liver and kidneys) in male rats.

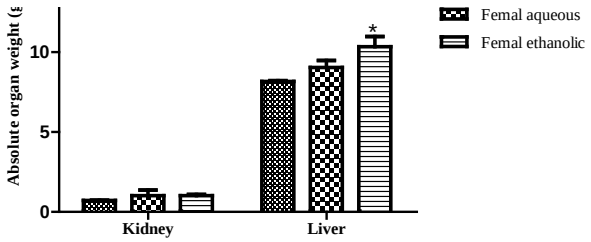

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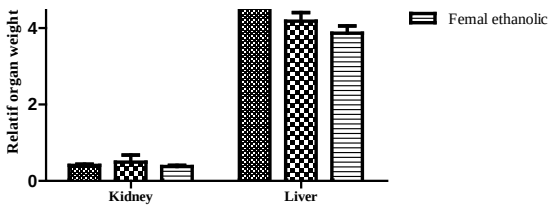
Figure 6: Absolute (a) and relative (b) organ weights (liver and kidneys) in female's rats.



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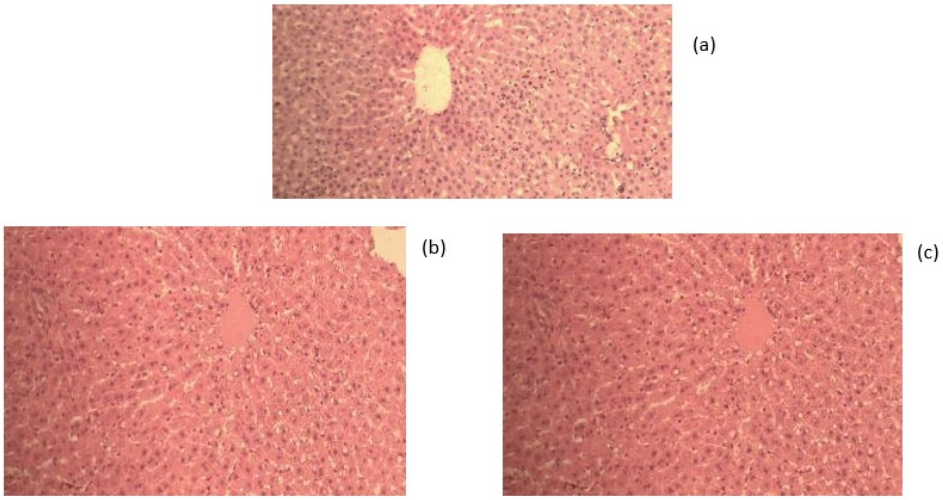

Figure 7: Photometry of liver of the rats: (a) "control group"; (b) "rats was received ethanolic extract of *H. abyssinica*" ; (c) "rats was received aqueous extract of *H. abyssinica*".

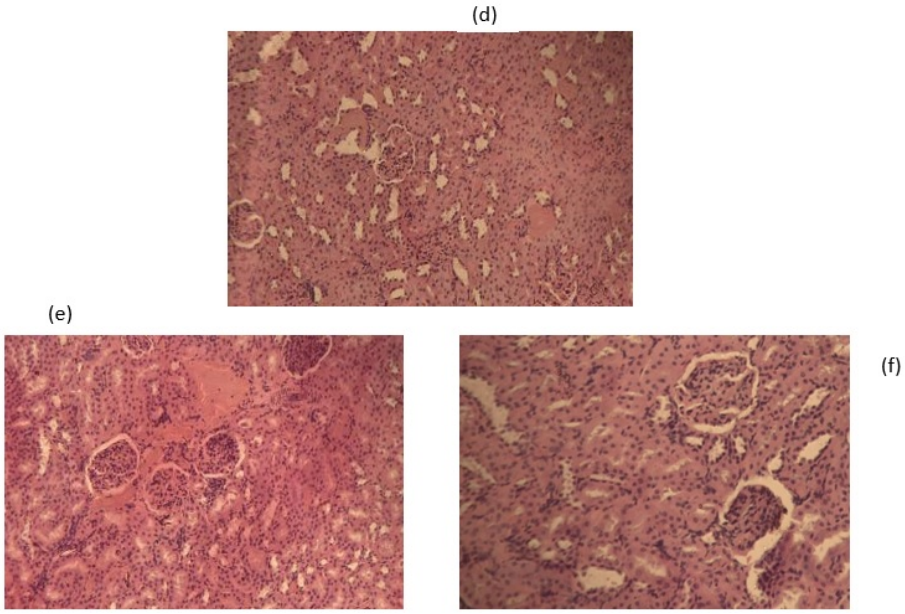

Figure 8: Photometry of kdneyof the rats : d "control group"; e " rats was received ethanolic extract of *H. abyssinica*" ; f "rats was received aqueous extract of *H. abyssinica*".

Discussion

The ethanolic and aqueous extracts contain almost the same active ingredients except for the alkaloids contained in the ethanolic extract and absent in the aqueous extract and moreover the difference in concentration of the latter (Tannins; Gallic tannins; Alkaloids; Reducing sugars; Coumarins; Quinones; Steroids; Terpene compounds and Saponosides). In their studies, Bene *et al*. (2015) found the same phytochemical compositions except for the terpene compounds found in this study and also the difference in concentration for certain metabolites can be justified by the extraction methods, the time place of harvest or place of harvest of the species.

Moreover, the antimicrobial and antiviral properties of coumarins have been reported (Kong *et al*., 2010). They appears to be inhibitors of Gram positive and Gram negative bacteria (Kong *et al*., 2010). Based on a study on the structure-activity relationship of coumarins, they concluded that the extra "furo" cycle increases the antimicrobial activity of coumarins (Kuete *et al*., 2007). Therefore, three-cycle coumarins have better antibacterial activity than two-cycle coumarins (Kuete *et al*., 2007). The antibacterial activity observed with our study species at the highest concentration studied could therefore be linked to the high concentration of two-ring coumarins in our extracts. Plants rich in tannins have an astringent nature and are used in the treatment of intestinal disorders such as diarrhea and dysentery, there by exhibiting antimicrobial activity. Except from the phenolic compounds that are more involved in the antibacterial activity, we also have the alkaloids. Alkaloids were identified in the ethanolic extract and not in the aqueous extract. They are generally insoluble in water, but soluble in certain organic solvents such as alcohol, ether, chloroform, etc. This could justify its absence in the aqueous extract. Several studies have demonstrated the antibacterial effect of alkaloids (Delso *et al*., 2010; Nenaah, 2010; Yu *et al*., 2010). Orhan *et al*. (2007) reported that all types of alkaloids appear to be more active against Gram negative bacteria than Gram positive bacteria. Terpenoids are also known to have antibacterial properties (Cowan, 1999).

- **Antibacterial activity of the two extracts**

The antibacterial activity of plant extracts is due to the various chemical agents present in these extracts, flavonoids and triterpenoids as well as other compounds of a phenolic nature having antibacterial properties (Morel *et al*., 2005; Daglia, 2012). According to Dorman and Deans (2000), the biological activity of a plant extract is related to its chemical composition, the functional groups of the major compounds and their synergistic effects. The activity of a plant substance depends on several factors, including the method of extraction

and the concentration of active ingredients (Onzo *et al*., 2016). The sensitivity of the different bacteria to extracts justifies the use of the leaf in the treatment of microbial infections and it is explained by its richness in secondary metabolites (tannins and coumarin) classified as very active antibiotic compounds (Rojas *et al.*, 1992). The variation observed with respect to the activities on the strains is therefore explained by the difference in the composition in secondary metabolites of the extracts. Also, the difference in MICs at the level of the two extracts may be justified by the fact that the ethanol solvent would be able to extract other secondary metabolites that water cannot extract. According to Essawi and Srour (2000), the effectiveness of an extract may not be due to a single active constituent but to the synergistic effect of two or more. Also, the active ingredient composition of an extract relates to its activity, but we can also report the concentration of these active ingredients in extracts (Bolou *et al*., 2011). The effectiveness of the ethanolic extract is therefore justified by its composition and concentration of active ingredients. According to Bolou *et al* (2011), the preliminary phytochemical screening on *Terminalia glaucescens* Planch shows that the fraction most active on bacteria contains terpenoids, phenolic derivatives and alkaloids. The bacteriostatic action of the extracts justifies the dosage chosen by the population during the ethnobotanical survey.

- **Acute toxicity of ethanolic and aqueous extracts of leaves of *H. abyssinica* on *Wistars* rats**

The toxicity study showed that the aqueous and ethanolic extracts of *H. abyssinica* at a dose of 2000 mg/kg body weight did not cause any mortality, nor any morphological changes and also a change in behavior. *Harrisonia abyssinica* was showed to be non-toxic and the lethal dose would be greater than 2000mg/kg. This result corroborates those of several authors on different species of medicinal plants use (Etame-Loe *et al*., 2018).The change in body weight is used as an indicator of the adverse effects of chemical compounds (Hilaly *et al*., 2004) . Weight loss is correlated with the physiological state of the animal. These results may also explain a certain compatibility of the organism with the different modes of use and the dose taken of this plant for weight loss. Other studies have also demonstrated a reduction in the weight of rats after oral administration of the extract of *Stryphnodendron adstringens* (Rebecca *et al*., 2002). The decrease in body weight over the 14 days of daily treatment suggests that the dose 2000 mg/kg was administered once to each animal have effects on the development of Albino *Wistar* rats. As for the consumption of food, a certain increase was noted at the level of the two sexes and of the two extracts though if it was not significant even at the end of the

fifteenth day. This increase in food intake may be justifying the use of this plant as an aperitif. The insignificance of the effect of the two aqueous and ethanolic extracts of *H. abyssinica* leaves on the relative weights of the organs (kidneys and livers) in the two sexes may be due to the non-toxic effect of the plant. This during the increase in absolute liver weight may be linked to congestion by reserving blood in the liver (Rasekh *et al*., 2008). However, these results require further analysis to know the effect of the extract on these organs.

Conclusion

Medicinal plants are a source of new molecules with economical antibacterial activity available to deal with the emergence of resistance phenomenon of germs to antibiotics. The study species in this case is *H. abyssinica*. Based on the results obtained, we can retain that the biological extracts of *H. abyssinica* present a bactericidal effect on the strains used but at a dependent dose. The results help to understand the use of the plant in traditional medicine. As for the recommendation on the use of the plant, more studies are needed, especially clinical studies.

Table caption

Table 1: Yield of the aqueous and ethanolic extract from the leaves of *H. abyssinica.*

Table 2: Different secondary metabolites identified in the aqueous and ethanolic extracts of the leaves of *H. abyssinica.*

Table 3 : Minimum inhibitory concentration (MIC) of the different bacterial strains.

Figure caption

Figure 1: Weight of male rats in the time.

Figure 2: Weight of female rats in the time.

Figure 3: Variation of food consumption in female rats as a function of time.

Figure 4: Variation of food consumption in male rats as a function of time.

Figure 5: Absolute (a) and relative (b) organ weights (liver and kidneys) in male rats.

Figure 6: Absolute (a) and relative (b) organ weights (liver and kidneys) in female's rats.

Figure 7: Photometry of liver of the rats: (a) "control group"; (b) "rats was received ethanolic extract of *H. abyssinica*" ; (c) "rats was received aqueous extract of *H. abyssinica*".

Figure 8: Photometry of kdneyof the rats : d “control group”; e “ rats was received ethanolic extract of *H. abyssinica*” ; f “rats was received aqueous extract of *H. abyssinica*”.

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Competing interest

The authors declare they have no competing interests.

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