

## RESEARCH ARTICLE

# Evaluation of Antibacterial Potentialities and *In vivo* Safety Tests of *Ocimum gratissimum* Linn. (Lamiaceae) a Leaf Vegetable for Medicinal Use in Cotonou, Republic of Benin

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## ABSTRACT

The empirical use, coupled with the toxicity of medicinal plants and the emergence of resistant bacterial strains, impose on researchers new health challenges. In Benin, *Ocimum gratissimum* is the subject of a wide range of uses in folk medicine. Initiated to promote the safe use of *O. gratissimum*, this study evaluates the antimicrobial and safety properties of this plant. The material used consists of a bacterial carrier, ethanolic extract, and female Wistar rats. The minimal inhibitory concentrations (MICs) and the reversion of the bacterial resistance were investigated by microdilution in a liquid medium. The minimal bactericidal concentrations (MBCs) were determined by tube dilution coupled with solid medium spreading and kinetics of action by spectrophotometry. The metallic trace element assays (ETMs) were measured by voltammetry with redissolution. Oral gavage was performed with an intragastric tube following Organization for Economic Co-operation and Development (OECD) guidelines, Standards 423 (acute toxicity), and 407 (subchronic toxicity), followed by blood sampling by retro-orbital sinus puncture. Hematological and biochemical analyzes were performed on the basis of standardized protocols and histological examinations by eosin staining and microscopic observation. The threshold of significance of the statistical results was set at  $p < 0.05\%$ . The *in vitro* tests reveal MICs and MBCs with a resistant, bacteriostatic, and bactericidal effect in contact with the extract, respectively, on 12.5, 25, and 62.5% of the germs tested. The bacterial load of the most sensitive seeds is reduced in the reaction medium to 72.44 or 98.49% after 32 hours. The reversal of bacterial resistance showed hypersensitive reductions in MIC and a synergistic effect for all tested germs. The plant contains negligible levels of cadmium and arsenate. *In vivo* testing did not result in any mortality or changes in behavior. The doses tested do not have a significant impact on the elements of general toxicity, the hematological and biochemical parameters. Histological examinations revealed no evidence of atypia. *O. gratissimum* with antibacterial potential requires a clinical safety of 5,000 mg.kg<sup>-1</sup> single dose or up to 1,000 mg.kg<sup>-1</sup> taken daily for 28 days.

**Keywords:** Antibacterial activities, Benin, Cotonou, *Ocimum gratissimum*, Safety.

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## INTRODUCTION

Today, bioactive products derived from medicinal plants contribute significantly to the prevention and treatment of diseases despite the recent achievements of pharmaceutical industries.<sup>1</sup> Integrating at the heart of development policies, the use and preservation of medicinal plants is a transdisciplinary theme encompassing health care, nature protection, biodiversity,

and biological control.<sup>2</sup> According to WHO statistics, 80% of the population in developing countries depend on traditional medicine based on the use of medicinal plants,<sup>3</sup> where many species are used for several pharmacological properties including antibacterial, anti-inflammatory, vasodilatory, anti-carcinogenic, anti-thrombic, anti-atherogenic, anti-pyretic, and analgesic properties.<sup>4</sup> Also about 70% of Benin's

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predominantly illiterate population use traditional medicine for primary health care,<sup>5</sup> making the pharmacopeia highly involved in the ongoing quest for the well-being of African people.<sup>6</sup> In developing countries, infectious diseases are a concern of public health because of their frequency and severity.<sup>7</sup> They are responsible for 17 million deaths a year in the world, of which more than half comes from the African continent alone.<sup>8</sup> Malaria is the leading cause of death in Africa, with 216 million cases and 438,000 deaths, 80% of which are in Africa.<sup>9</sup> Antibiotic therapy, one of the means to cope with this microbial invasion, is confronted with increasing resistance of germs.<sup>10</sup> The population most affected by these infections has low accessibility to primary health care and uses first-line traditional medicine whose effectiveness is often lacking scientific evidence.<sup>11</sup> Thus, the high cost of conventional drugs coupled with the misuse of antibiotics and the emergence of multidrug-resistant organisms is generating renewed interest in the pharmacopeia.<sup>7</sup> In light of these findings and to provide concrete and local solutions to public health problems in these countries, it is essential to direct research towards the development of traditional remedies. For this purpose, the verification of the therapeutic efficacy, the precision of the dosage, the safety study, the reduced cost pharmaceutical formulation are all parameters to be taken into account to guarantee a controlled and safe use of these remedies.<sup>12</sup>

In Benin, among the useful plants, *O. gratissimum* is listed as the most used medicinal plant.<sup>13</sup> It grows throughout the country and is strongly sought by the people who grow around houses and offer them on local markets.<sup>14</sup> The Beninese population uses *O. gratissimum* against various diseases, including vomiting, diarrhea, dystocia, pregnancy termination, digestive disorders, constipation, dysentery, hemorrhoids, headache, abdominal pain, cough, abscess,<sup>15</sup> hypertension, candidiasis,<sup>16</sup> and diabetes in pregnant women.<sup>17</sup> The essential oils of *O. gratissimum* are recognized for various pharmacological and toxicological properties. Previous studies have shown the bactericidal, virucidal, and fungicidal properties of these essential oils.<sup>18</sup> In view of the current importance of medicinal plants with antibacterial effect against the recrudescence of multi-resistant organisms and the excessive use of *O. gratissimum* in Benin, the present work aims to evaluate the biological activity and the safety of this plant.

## MATERIAL AND METHODS OF STUDY

### Study Material

#### Plant Material

The samples of *O. gratissimum* were collected in the garden of the Cotonou airport, Littoral department, southern Benin region during the last week of September 2017. They were identified and authenticated at the University of Abomey-Calavi. A specimen was deposited at the National Herbarium of Benin under the code AA 6722/HNB of September 18,

2017. The samples were dried under laboratory conditions ( $\Theta = 22 \pm 3^\circ\text{C}$ ) and then reduced to powder using an electric grinder and kept for extraction.

#### Microbial Strains

The extract was tested on microbial support consisting of six reference strains namely *Escherichia coli* (CIP 53126), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (CIP 8039), *Meticillin-resistant Staphylococcus Aureus* (MRSA), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (CIP 82118), and two hospital strains *Pseudomonas aeruginosa* and *E. coli*.

#### Animal Material

Female rats of Wistar strain, weighing between 170 and 200 g, coming from the animal center of the Teaching and Research Unit in Human Biology of the Faculty of Health Sciences was used for *in vivo* tests. Rats were divided into four groups of eight rats, including a control group for subchronic toxicity. With respect to acute toxicity, the rats were grouped into two batches of three rats, one of which was a control group. The rats were acclimated to the conditions of this animal house before any manipulation.

### Study Methods

#### Preparation of the Extract

The extraction was carried out by drawing on the methodology described previously in the work of<sup>19</sup> with small modifications. The ethanolic extract was obtained by maceration. One hundred grams of *O. gratissimum* powder was mechanically stirred in 500 mL of ethanol for 24 hours at laboratory temperature. The macerate obtained is filtered respectively on fabric, cotton fiber, and Whatman paper. Each level of filtration is repeated at least three times. The mark was taken three times after adding 500 mL of ethanol followed by sonication and successive filtrations to make extraction profitable. All the ethanol filtrate collected was concentrated under pressure using a rotary evaporator (BUCHI Rotavapor RII). The wet extract was collected in Petri dishes and placed in an oven for drying. Finally, the dry extract was scraped and stored in sterile glass spirulina and then kept cold at  $4^\circ\text{C}$ .

#### Evaluation of Antibacterial Activities

**Preparation of the culture media:** Müller Hinton agar medium (MHA) was obtained by dissolving 38 grams of the agar medium in 1-litre of distilled water ( $\text{pH} = 7.5 \pm 0.2$ ). Müller Hinton broth was obtained by dissolving 21 grams in 1 liter of distilled water. Each medium was autoclaved at a temperature of about  $121^\circ\text{C}$  for 15 minutes.

**Preparation of the bacterial inoculum:** The bacterial inoculas were prepared in an aliquot of the bacterial mother solution in sterile tubes containing the nutrient agar. Bacterial suspensions were removed using a platinum loop, homogenized in 10 mL of Mueller-Hinton broth (MHB), and incubated for 18 hours at  $37^\circ\text{C}$ . Then, 0.1 mL of the pre-culture broth was taken in 10 mL of BMH. The Optical densities (OD) of the solution was

read with a spectrophotometer (UV-1600 PC) at 600 nm. The inoculum count was estimated at  $10^8$  CFU/mL when the OD value is 0.156 for *E. coli*, *P. aeruginosa*, and *E. faecalis*, but 0.3 for *Staphylococcus*.<sup>20</sup> Finally, dilution to 1/100th allowed to reduce the bacterial suspension to  $10^6$  CFU/mL used as a pure inoculum for antibacterial tests.<sup>21</sup>

**Sterility test:** Antimicrobial tests were preceded by preliminary sterility tests. The first control consisted solely of the culture medium to certify its sterility. The second control was composed of the culture medium and the extract to check the sterility of the extract. The viability of the germs used was approved by the contact of dimethyl sulfoxide (DMSO) with the inocula. The last reference was to combine the standard antibiotic with the culture medium to reveal the behavior of an active substance in the medium.

**Bacterial susceptibility tests at the extract 10 mg/mL:** The ethanolic extract of *O. gratissimum* was prepared at the mother concentration of 10 mg/mL in the acetone-water (v/v) mixture. The effect of this extract on the selected microorganisms was evaluated by the method of microdilution in liquid medium taken up by Amoussa A MO *et al.*<sup>20</sup> The Whatman sterile blotting paper discs were deposited on the agar media previously inoculated by the flood technique with an inoculum of  $10^6$  CFU concentration. Then, these disks were soaked with 10  $\mu$ L of the 10 mg/mL extract. After one hour of exposure on the bench, the time to allow a good impregnation of the discs, the Petri dishes are incubated at 37°C for 18 hours. Pre-soaked commercial antibiotic discs, namely ciprofloxacin, and ceftriaxone, were used as standards. At the end of the incubation, the diameters of inhibitions observed around the discs were measured using a vernier caliper. The strain is resistant when the diameter is less than 8 mm, sensitive if it is between 9 and 14 mm, very sensitive in the interval 15 to 20 mm, and extremely sensitive beyond 20 mm.<sup>21</sup>

**Determination of minimal inhibitory concentration (MIC):** It was determined by the microdilution method.<sup>12</sup> The inoculum used is a 24 hours culture of each bacterial strain diluted to the final concentration of  $1.10^6$  CFU/mL in BMH. A range of extract concentrations ranging from 10 to 0.078 mg/mL was used. Then, 100  $\mu$ L of inoculum were distributed in each of the 96 wells of the plates containing previously 100  $\mu$ L of extract at different concentrations. The final concentration of inoculum was 5.105 CFU/mL.<sup>20</sup> The test was performed in a triplet, and the plates were incubated at 37°C for 24 hours. An acetone-water mixture and gentamicin were used as a negative and positive control. The antibacterial activity was revealed by the colorimetric technique with the addition of 25  $\mu$ L of the aqueous solution of iodonitrotetrazolium (0.01%) in each well at the end of the incubation. Plates were then reincubated at 37°C for 30 minutes. The turning of the reaction medium from blue to red indicates the presence of living bacteria. The MIC is the smallest concentration of extract that inhibits any bacterial growth visible to the naked eye in 24 hours.

**Determination of minimal bactericidal concentration (MBC):** The technique used was dilution in liquid medium coupled with spreading on solid medium.<sup>22</sup> The solid and liquid media

used are respectively Müller Hinton agar and BMH. The inoculum used is a 24 hours culture diluted at a concentration of  $1.10^6$  CFU/mL in MHB. For each strain, a range of three extract concentrations ranging from MIC to two higher concentrations in a second-order geometric progression was prepared in sterile tubes. After this step, 10  $\mu$ L of the contents of each tube were spread on solid medium. Then, 100  $\mu$ L of the bacterial suspension at  $1.10^6$  CFU/mL was added to the different concentrations of the extract. The tubes were incubated at 37°C for 24 hours. Finally, 10  $\mu$ L of the contents of the tubes were seeded on Müller Hinton agar and reincubated for 24 hours. The MBC is the smallest concentration of extract that allows to survive at most 0.01% inoculum after 18 hours of incubation at 37°C. According to the MBC/MIC ratio, the antibacterial effect will be considered bactericidal ( $\leq 4$ ) or bacteriostatic ( $> 4$ ), according to Ouattara LH *et al.*<sup>21</sup>

**Kinetic action of the ethanolic extract of *Ocimum gratissimum*:** The protocol used for the evaluation of action kinetics is that described by<sup>23</sup> reviewed and adapted. It was performed on MRSA and *E. faecalis*. Inocula are 24-hour cultures that have been grown for seven consecutive days. The MBC was maintained for each germ. The extract was diluted in acetone-water. The bacterial cultures were diluted to  $10^6$  CFU/mL with BMH. The inoculum was mixed with the extract (v/v) and incubated at 37°C after shaking. The OD was read at regular intervals of 4 hours against a blank consisting of a mixture of Mueller Hinton (MH) and acetone-water (v/v). After each reading, the whole is reincubated until the next turn. The curves of variation of OD as a function of time have been plotted.

#### *Evaluation of the Reversion of Bacterial Resistance*

**Minimal inhibitory concentration (MIC) determination of antibiotics:** Five conventional tablet antibiotics purchased from pharmacy dispensaries, namely, amoxicillin, ciprofloxacin, cotrimoxazole, erythromycin, and ampicillin, were selected. The selection criteria are accessibility, costs, and frequency of use. MICs of conventional antibiotics were investigated by the microdilution method.<sup>12</sup> The first step is the reduction of powdered tablets, followed by their dilution with distilled water at  $1 \text{ mg.mL}^{-1}$ . This antibiotic solution obtained was sonicated, then centrifuged, and the supernatant was removed for the determination of the MIC. With regard to the germs, two hospital strains isolated from biological fluids (pus, urine), namely *E. coli* and *P. aeruginosa* were used. The inoculum used is a 24-hour culture diluted to  $1.10^6$  CFU/mL in BMH. A range of antibiotic concentrations ranging from 1 to 0.078  $\text{mg.mL}^{-1}$  was used. Each well previously containing 100  $\mu$ L of antibiotics at different concentrations received 100  $\mu$ L of inoculum. The final concentration of the inoculum is  $5.10^5$  CFU/mL.<sup>24</sup> The tests were performed in duplicate, and the plates are then incubated at 37°C for 24 hours. The antibiotic activity was detected by the colorimetric technique with the addition of 40  $\mu$ L of the aqueous solution of iodonitrotetrazolium (0.01%) in each well at the end of the incubation. Plates were reincubated at 37°C for 30 minutes. The turning of the reaction medium from blue to red indicates the presence of living germs.



**Modulation tests for antibiotic activities:** The principle of this test is based on the microdilution technique. Thus, 50  $\mu\text{L}$  of antibiotic solutions at 1  $\text{mg.mL}^{-1}$  were distributed in the first and second wells. Then, 50  $\mu\text{L}$  of BMH were deposited in all the wells, followed by the cascade dilution of the second well until the end. Finally, each well receives 50  $\mu\text{L}$  of extract at 1.25  $\text{mg.mL}^{-1}$  and 100  $\mu\text{L}$  of inoculum, respectively, and the plates are incubated at 37°C for 24 hours. The reversion of the bacterial resistance was evaluated by the colorimetric technique with the addition of 40  $\mu\text{L}$  of the aqueous solution of iodonitrotetrazolium (0.01%) in the wells, followed by reincubation of the plates at 37°C for 30 minutes. The presence of bacteria was revealed by the passage of the reaction medium from blue to red.<sup>24</sup> The modulation factor is based on the fractional inhibitory concentration (FIC) defined by the MIC [extract + antibiotic (ATB)] ratio in the MIC extract, reported by some authors.<sup>25,26</sup> Depending on the scale, the effect can be synergistic ( $\text{FIC} < 0.5$ ), additive ( $0.5 \leq \text{FIC} \leq 1$ ), indifferent ( $1 \leq \text{FIC} \leq 4$ ), and antagonist ( $\text{FIC} > 4$ ).

#### *Evaluation of the Safety of the Ethanol Extract of O. gratissimum*

**Metallic trace element assay (ETM):** The standardized method of the HM 3000 metalyser used to quantify ETM is based on cathodic and anodic stripping voltammetry using disk electrodes. It is a sensitive electro-analytical technique for the determination of minute quantities of metals and derivatives in solution.<sup>27</sup> The indicated dose of buffer and HCl (37%) was poured into 70 mL of ethanolic extract solution of *O. gratissimum*. The whole is homogenized, followed by the selection of the current dosage and the conditioning of the electrodes for 3 minutes. Then, the method of metered additions was selected. After 3 minutes, 280  $\mu\text{L}$  of the current assay standard is added, and the whole is left to work for about 2 minutes. Finally, the result is displayed as a peak and concentration on the screen of the minicomputer connected to the metalyser.

#### *Oral Safety Studies*

**Experimental animal preparation:** Female Wistar strain rats, 12 weeks old, weighing between 170 and 180 grams were provided by the Training and Research Unit of Human Biology/Faculty of Health Sciences/University of Abomey-Calavi, (TRUHB/FHS/UAC) after clinical examination. Selected nulliparous and non-pregnant rats are fed a standard laboratory diet with free access to pellets and water. These rats are acclimatized to laboratory conditions (temperature, humidity, lighting, and darkness).

**Acute and subchronic oral safety tests:** Acute oral and intravascular subcutaneous toxicity tests using an intragastric tube are performed according to OECD guidelines, Standards 423 and 407, respectively.<sup>28,29</sup> The rats are divided into two batches of three female rats, including one control and then four batches of eight female rats, including one control for the acute and subchronic tests, respectively. Prior to administration, the rats are weighed and fasted for 18 hours with free access

to water. The treated acute-toxicity group received a single dose of 5,000  $\text{mg/kg}$  body weight of extract while the three treated subchronic-toxicity groups received doses of 500, 750, and 1,000  $\text{mg/kg}$  for 28 days, respectively, of body weight extract. After each feeding, rats have free access to water and pellets. The drinks are recorded every day, and the rats are weighed at a period of 7 days. The rats are constantly observed during the first hours after the feeding, and every day during the experiment to record deaths and clinical signs.

**Hematological and biochemical analyzes:** The hematological and biochemical examinations performed were according to standard methods. At the end of the treatment, the animals fast for 12 hours, then the blood is collected by the technique of puncture of the retro-orbital sinus under light anesthesia with ether.<sup>30</sup> Blood samples are collected in an EDTA tube (anticoagulant) and a dry tube to assay some hematological parameters [White globule (WBC), Red blood cells (RBC), Hemoglobins (Hb), Hematocrit rate (Ht), platelets (PLt), Mean corpuscular volume (MCV), average corpuscular hemoglobin concentration (MCHC), Average body hemoglobin level (MCH), and platelets] and biochemical (urea, creatinine, and transaminases) by a standard protocol as described in some works.<sup>31</sup>

**Histological examinations:** After the blood samples, the rats are sacrificed by asphyxiation. The kidneys and liver are removed, weighed immediately, and introduced into saline. These removed organs are then fixed in 10% buffered formalin for histological examinations. This fixation phase is followed by the treatment of organ samples with increasing concentrations of ethanol and then infiltrated with paraffin. Finally, the thin sections are made, stained with hematoxylin or eosin stains,<sup>32</sup> and observed under a microscope.

**Statistical analyzes:** The generated data are expressed in the mean  $\pm$  standard error of the average (SEM). Comparisons between the control values and those of the treated groups are performed by the one-way ANOVA statistical model in the Excel software. The statistical significance set at  $p < 0.05$  is analyzed using the “student” test.

## RESULTS OF STUDIES

### **Sensitivity Tests**

Sensitivity tests reveal low-spectrum inhibition zones (Table 1).

Inhibition diameters (IDs) range from 7 to 11 mm. Depending on the reading range,<sup>21</sup> 75% of the germs tested are sensitive to contact with the extract against 25% of resistant germs.

The MICs and MBCs of the microbes tested in contact with the extract are respectively between 62.5 to 125  $\mu\text{g.mL}^{-1}$  and then 125 to 5,000  $\mu\text{g.mL}^{-1}$  (Table 2). The evaluation of the antibacterial effect of the extract by the MBC ratio to the MIC allowed to record three scales of values, which are 2, 4, and 8. From the statistical point of view, 12.5, 25, and 62.5% of the tested germs were respectively resistant, bacteriostatic, and bactericidal in contact with the extract (Table 2). Bactericide

**Table 1:** Sensitivity of bacteria to the ethanolic extract of *O. gratissimum*Sample: Ethanolic extract of *O. gratissimum* at 10 mg.mL<sup>-1</sup>

Bacterial strains	Inhibition diameters (ID) in mm			Conclusions
	1st test	2nd test	3rd test	
<i>E. coli</i> isolated	09	10	09	Sensitive
<i>E. coli</i>	07	08	07	Resistant
<i>Pseudomonas aeruginosa</i> isolated	08	08	07	Sensitive
<i>Pseudomonas aeruginosa</i>	08	08	08	Sensitive
Meticillin-resistant <i>Staphylococcus aureus</i>	09	08	08	Sensitive
<i>Enterococcus faecalis</i>	10	09	08	Sensitive
<i>Staphylococcus aureus</i>	10	11	10	Sensitive
<i>Staphylococcus epidermidis</i>	08	08	07	Resistant

**Table 2:** Characterization of antibacterial effects

Bacterial strains	Ethanolic extract		Reports MBC/MIC	Antibacterial effects
	MIC (µg.mL <sup>-1</sup> )	MBC (µg.mL <sup>-1</sup> )		
<i>E. coli</i> isolated	625	5,000	8	Bacteriostatic
<i>E. coli</i> CIP53126	1,250	5,000	4	Bactericidal
<i>P. aeruginosa</i> isolated	625	5,000	8	Bacteriostatic
<i>P. aeruginosa</i> CIP82118	1,250	5,000	4	Bactericidal
Meticillin-resistant <i>Staphylococcus aureus</i>	625	2,500	4	Bactericidal
<i>E. faecalis</i> ATCC 29212	625	1,250	2	Bactericidal
<i>S. aureus</i> ATCC 6538	1,250	5,000	4	Bactericidal
<i>S. epidermidis</i> CIP 8039	Not determined			

is more prevalent for *Enterococcus faecalis* ATCC 29212 and MRSA.

### Kinetic Action of the Ethanol Extract of *O. gratissimum*

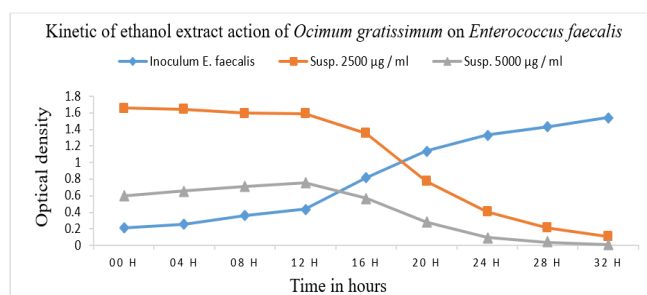
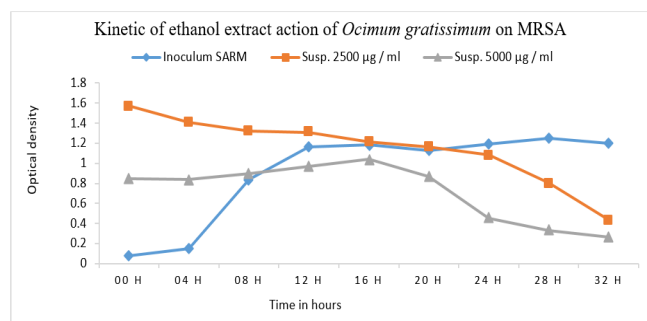
The kinetics of action is determined on *Enterococcus faecalis* and MRSA because of their frequencies and especially their increased sensitivity towards this extract. It reflects the evolution of the OD bacterial broth in contact with the extract as a function of time (Figures 1 and 2).

With regard to the differences between the initial and final OD, the bacterial loads of MRSA in the reaction media are reduced by 68.40 and 72.44%, respectively, in contact with the suspensions of extract 5,000 and 2,500 µg.mL<sup>-1</sup> after 32 hours. By analogy, the bacterial loads of *E. faecalis* in the reaction media are reduced by 98.49 and 93%, respectively, in contact with the extract suspensions 5,000 and 2,500 µg.mL<sup>-1</sup> in 32 hours.

### Modulating Activity of the Antibiotic Resistance of the Extract

The action of conventional ATBs combined with the extract on isolated *E. coli* and isolated *P. aeruginosa* generated the following results (Table 3).

On contact with ethanolic extract of *O. gratissimum* (125 µg.mL<sup>-1</sup>), the MIC of *E. coli* isolated and *P. aeruginosa* isolated is 62.5 µg.mL<sup>-1</sup>. In the presence of the conventional ATB solutions selected, the MICs of *Escherichia coli* isolated and *Pseudomonas aeruginosa* isolated respectively vary from

**Figure 1:** Kinetic of ethanol extract action of *O. gratissimum* on *E. faecalis***Figure 2:** Kinetic of ethanol extract action of *O. gratissimum* on MRSA

125 to 1,000 µg.mL<sup>-1</sup> and 250 to 1,000 µg.mL<sup>-1</sup>. On the other hand, with an ethanolic extract mixture solution of *Ocimum gratissimum* (125 µg.mL<sup>-1</sup>) and conventional ATB, the MIC of *Escherichia coli* isolated and *Pseudomonas aeruginosa* isolated

**Table 5:** Determination of metallic trace elements

Requested ETM	Results (ppb)	Results (ppm)	WHO standards (ppm)	Reports
Arsenate (AsIII)	2, 65	2, 65.10 <sup>-3</sup>	≤ 1	≈ 377
Mercury (Hg)	Undetermined	Undetermined	≤ 0, 1	-
Lead (Pb)	4, 13	4, 13.10 <sup>-3</sup>	≤ 10	≈ 2,421
Cadmium (Cd)	Undetermined	Undetermined	≤ 0, 3	-

**Table 3:** Modulation results of antibacterial extract-antibiotic capacity

Minimal inhibitory concentrations (μg.mL <sup>-1</sup> )											
Germ	Ex.EtOH	Conventionals ATB					Ex.EtOH + Conventionals ATB				
		AMX	CIP	AMP	ERY	SXT	AMX	CIP	AMP	ERY	SXT
E.c.i	62.5	1,000	125	> 1,000	> 1,000	500	15.6	≤ 7.8	31.5	≤ 7.8	15.6
P.a.i	62.5	> 1,000	250	> 1,000	> 1,000	1,000	31.5	≤ 7.8	31.5	15.6	31.5

AMX = Amoxicillin; CIP = Ciprofloxacin; SXT = Cotrimoxazole; AMP = Ampicillin; ERY = Erythromycin; ATB = Antibiotic; Ex.EtOH = Ethanolic extract; P.a.i = *Pseudomonas aeruginosa* isolé; E.c.i = *E. coli* isolé

**Table 4:** Characterization of conventional extract-antibiotic modulation effects

Germ	Isolated <i>E. coli</i>					Isolated <i>P. aeruginosa</i>				
ATB-extract	AMX	CIP	AMP	ERY	SXT	AMX	CIP	AMP	ERY	SXT
FIC	0.02	0.06	0.03	0.01	0.03	0.03	0.03	0.03	0.02	0.03
Effect	Synergic									

vary from 7.8 to 31.5 μg.mL<sup>-1</sup>. The combined extract-ATB effect was shown to be synergistic (CFI < 0.5) for the organisms tested (Table 4).

### Dosage of Trace Metallic Elements (ETM)

The usual ETMs arsenite, mercury, lead, and cadmium are assayed (Table 5).

The determination of the ETM reveals the presence of Pb and AsIII in variable proportions. The presumptive dosage of Hg and Cd was negative (Table 5). The concentrations of Cd and AsIII are respectively 377 and 2,421 times lower than the current WHO standards.

### Evolution of the Body Mass of Rats

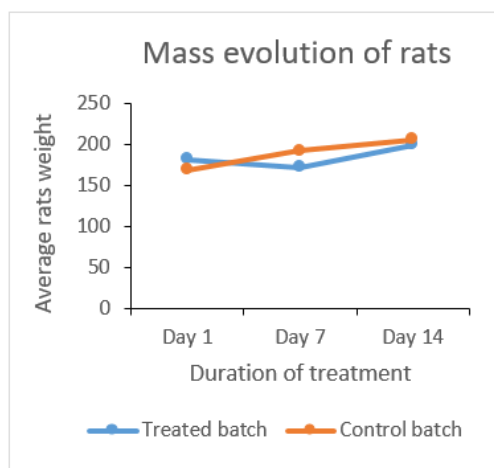
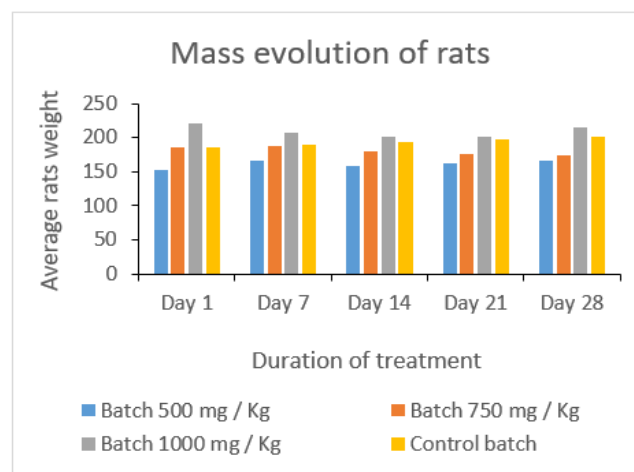
The body mass changes generated by the toxicity tests are analyzed (Figures 3 and 4).

For acute toxicity, the body mass shows a significant increase from the first week in the control group and the second week in the control group and the treated batch. By analogy, the subchronic toxicity reveals significant variations of body mass from the 1st week to the 500 and 1,000 mg/kg batches and then to the 2nd and 4th week, respectively, in the 750 and 1,000 mg/kg batches.

### From the Water Consumption of the Rats

The variation in water consumption generated by acute and subchronic toxicity is recorded (Figures 5 and 6).

Acute toxicity revealed a significant increase in water consumption at week 2 in the control and treated groups. For subchronic toxicity, a significant decrease in water consumption is noted in the 3rd week in the control group.

**Figure 3:** Evolution of masses (acute toxicity)**Figure 4:** Evolution of masses (subchronic toxicity)

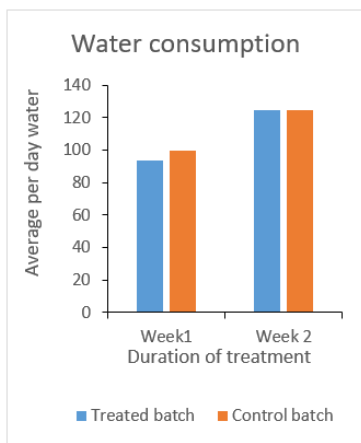


Figure 5: Consumption in water (acute toxicity)

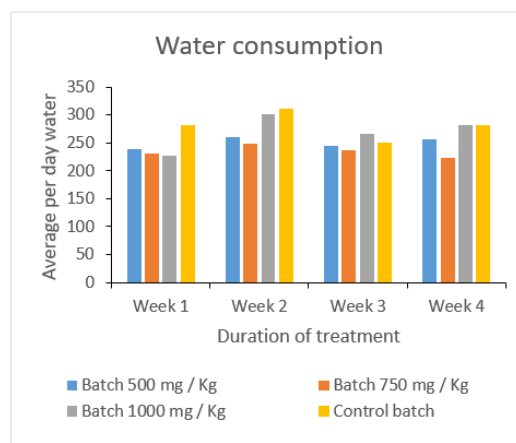


Figure 6: Consumption in water (subchronic toxicity)

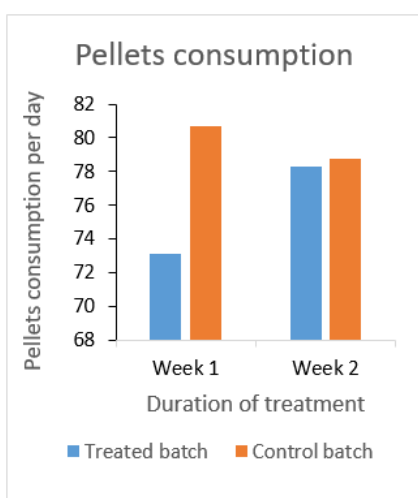


Figure 7: Pellets consumption of rats (acute toxicity)

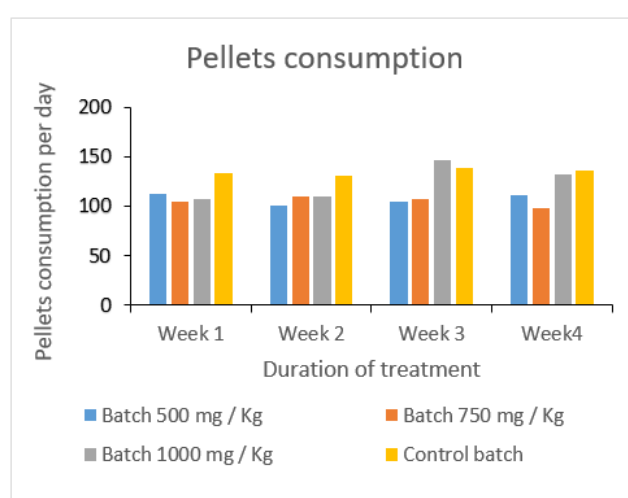


Figure 8: Pellets consumption of rats (subchronic toxicity)

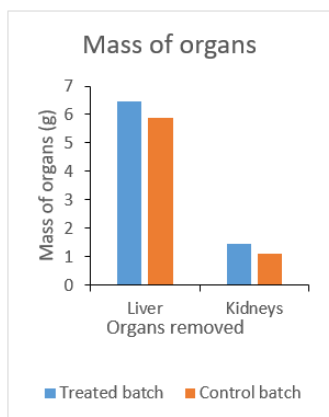


Figure 9: Masses of organs (acute toxicity)

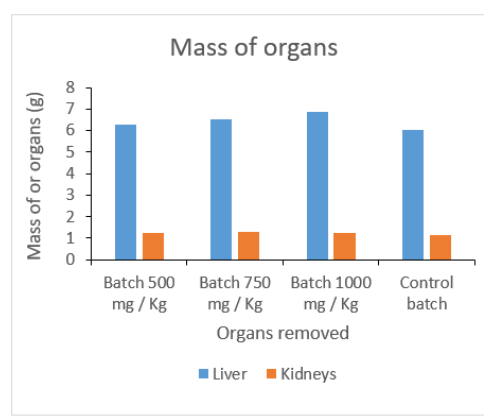


Figure 10: Masses of organs (subchronic toxicity)

### From the Consumption of Granules of Rats

The consumption of the pellets caused slight fluctuations (Figures 7 and 8).

The consumption of granules is not significant except at the 3rd week of the subchronic toxicity to the 1,000 mg/kg batch, or we noted a significant increase ( $p < 0.05$ ).

### Weight Gain of Organs Removed

The organs (liver, kidneys) very sensitive in terms of toxicity are removed, and the masses of the batch treated are compared with those of the control group (Figures 9 and 10).

In a comparative approach to the control lot, only the liver masses of the lots treated 500 and 750 mg/kg of subchronic

toxicity showed a significant increase ( $p < 0.05$ ).

### Biochemical Examinations

The biochemical parameters (urea, creatinine, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT)) are assayed at the end of the treatments, and the results of the treated batches are compared with the control batches (Tables 6 and 7).

Acute toxicity showed a significant increase in the urea concentration of the treated lot. On the other hand, the subchronic toxicity revealed a significant increase in the urea

concentration of the three treated lots and a significant decrease in the creatinine concentrations of the 500 and 750 mg/kg batches.

### Hematological Examinations

For acute toxicity, the MCH and MCV increased significantly while the PLt and RBC concentrations significantly decreased (Table 8). In the case of subchronic toxicity, the Ht and the MCH showed, respectively, a decrease and a significant increase in the three treated lots while the PLt and RBC concentrations decreased significantly in batches 750 and

**Table 6:** Determination of biochemical parameters at the end of acute toxicity

Parameters	Control	Single dose: 5,000 mg/kg
Urea (g/L)	0.35 ± 0.046	0.677 ± 0.049*
Creatinine (mg/L)	6.37 ± 0.456	5.73 ± 0.611
ASAT (UI/L)	188.57 ± 21.09	150.53 ± 16.259
ALAT (UI/L)	100.20 ± 11.39	89.93 ± 11.737

**Table 7:** Determination of biochemical parameters at the end of subchronic toxicity

Parameters	Control	Different batches of rats		
		500 mg/kg	750 mg/kg	1,000 mg/kg
Urea (g/L)	0.335 ± 0.03	0.558 ± 0.1*	0.666 ± 0.1*	0.558 ± 0.1*
Creatinine (mg/L)	6.27 ± 0.31	5.9 ± 1.0*	4.5 ± 1.3*	5.9 ± 1.0
ASAT (UI/L)	175.85 ± 17.5	150.7 ± 32.2	192.013 ± 35.9	150.7 ± 32.2
ALAT (UI/L)	89.01 ± 13.6	95.963 ± 16.1	109.3 ± 30.682	95.963 ± 16.1

\* = significant difference ( $p < 0.05$ ); WBC : White globule ; RBC : Red blood cells ; Hb : Hemoglobins ; Ht : Hematocrit rate ; MCV : Mean corpuscular volume ; MCHC : Average corpuscular hemoglobin concentration ; MCH : Average body hemoglobin level ; PLt : Platelets

**Table 8:** Determination of hematological parameters after acute toxicity

Hematological parameters	Control	Single dose: 5,000 mg/kg
WBC ( $10^3/\text{mm}^3$ )	9.47 ± 0.775	8.467 ± 1.193
RBC ( $10^8/\text{mm}^3$ )	7.56 ± 0.375	6.413 ± 0.627*
Hb (g/dL)	12.92 ± 1.662	13 ± 0.954
Ht (%)	42.70 ± 2.3	37.967 ± 3.743
MCV ( $\mu\text{m}^3$ )	54.57 ± 2.04	59.33 ± 0.577*
MCHC (g/dL)	32.45 ± 2.21	34.2 ± 1.25
MCH (pg)	17.61 ± 1.37	20.27 ± 0.81*
PLt ( $10^3/\text{mm}^3$ )	748.50 ± 193.90	383 ± 146.369*

\* = significant difference ( $p < 0.05$ ); WBC : White globule ; RBC : Red blood cells ; Hb : Hemoglobins ; Ht : Hematocrit rate ; MCV : Mean corpuscular volume ; MCHC : Average corpuscular hemoglobin concentration ; MCH : Average body hemoglobin level ; PLt : Platelets

**Table 9:** Determination of hematological parameters at the end of subchronic toxicity

Hematological parameters	Control	Different batches of rats		
		500 mg/kg	750 mg/kg	1,000 mg/kg
WBC ( $10^3/\text{mm}^3$ )	9.325 ± 0.85	5.963 ± 1.696*	8.45 ± 2.668	8.313 ± 1.923
RBC ( $10^8/\text{mm}^3$ )	7.64 ± 0.47	7.09 ± 0.557	6.676 ± 0.616*	6.933 ± 0.332*
Hb (g/dL)	13.43 ± 1.62	13.638 ± 1.077	12.725 ± 1.221	13.338 ± 0.607
Ht (%)	42.563 ± 2.07	39.75 ± 2.971*	37.4 ± 3.575*	39.713 ± 1.129*
MCV ( $\mu\text{m}^3$ )	55.775 ± 2.70	56.625 ± 1.768	56.375 ± 1.302	57 ± 1.195
MCHC (g/dL)	31.35 ± 3.21	34.175 ± 0.59*	33.925 ± 1.325*	33.4625 ± 0.956
MCH (pg)	17.58 ± 1.89	19.2 ± 0.83*	19.1875 ± 0.42*	19.2375 ± 0.65*
PLt ( $10^3/\text{mm}^3$ )	726.75 ± 182	596.13 ± 91.94	512.63 ± 89.77*	499.5 ± 122.6*

\* = significant difference ( $p < 0.05$ )

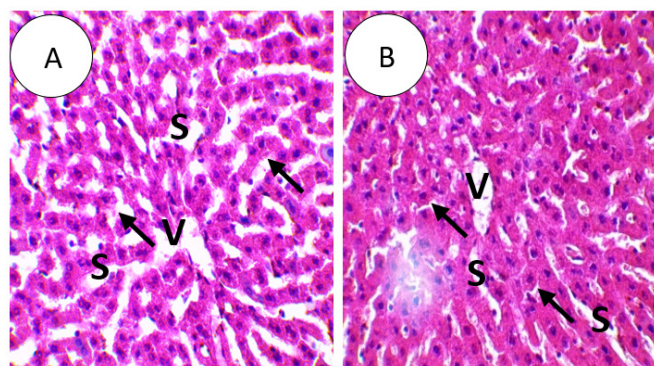


1,000 mg/kg (Table 9). Concentrations of MCHC increased significantly in batches 500 and 750 mg/kg with a significant decrease in the WBC concentration in the 500 mg/kg lot (Table 9). The parameters WBC, Hb, MCHC, and Ht generated by the acute toxicity against Hb and MCV of the subchronic toxicity show insignificant variations.

### Results of Histological Sections of Liver and Kidneys of Acute Toxicity

The results of the histological sections of the liver and kidney generated by the acute toxicity are shown in Figures 11A, B, 12A, and B.

The liver and kidney of the rats fed ethanolic extract of *O. gratissimum* 5,000 mg.kg<sup>-1</sup> bodyweights did not show any visible atypia compared to the control lot.



**Figure 11:** Hepatic histology of the rats of the control batch A and B treated with the ethanolic extract of *O. gratissimum* 5,000 mg.kg<sup>-1</sup> of body weight (Gr = 400X); V = Centrilobular vein; arrows = Hepatocytes; S = venous sinusoid

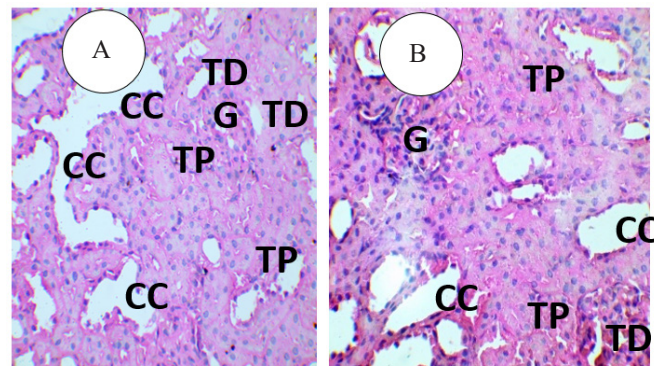
### Results of Histological Liver Sections of Subchronic Toxicity

The results of reading slides of histological sections of the liver at the end of the subchronic toxicity are summarized in Figures 13A to D.

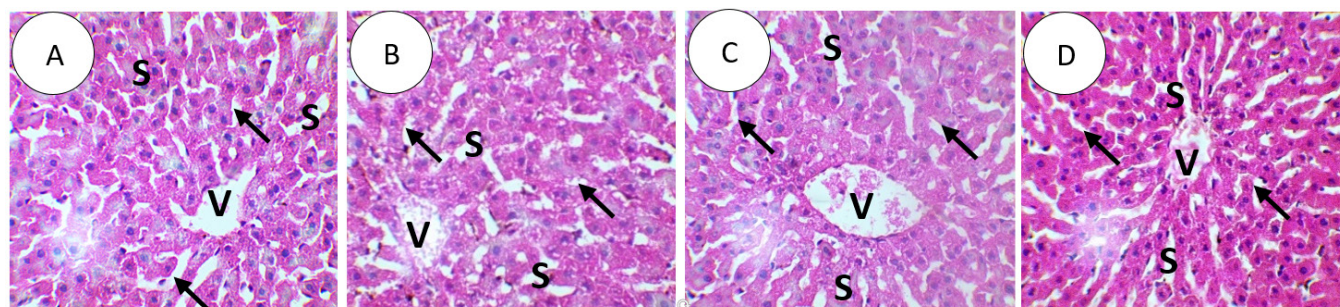
The liver of the rats gaved with the ethanolic extract of *O. gratissimum* at 500 mg.kg<sup>-1</sup> (Figure 13B), 750 mg.kg<sup>-1</sup> (Figure 13C), and 1,000 mg.kg<sup>-1</sup> (Figure 13D) did not show any form of visible atypia compared to the control lot (Figure 13A).

### Results of Histological Sections of Kidneys of Subchronic Toxicity

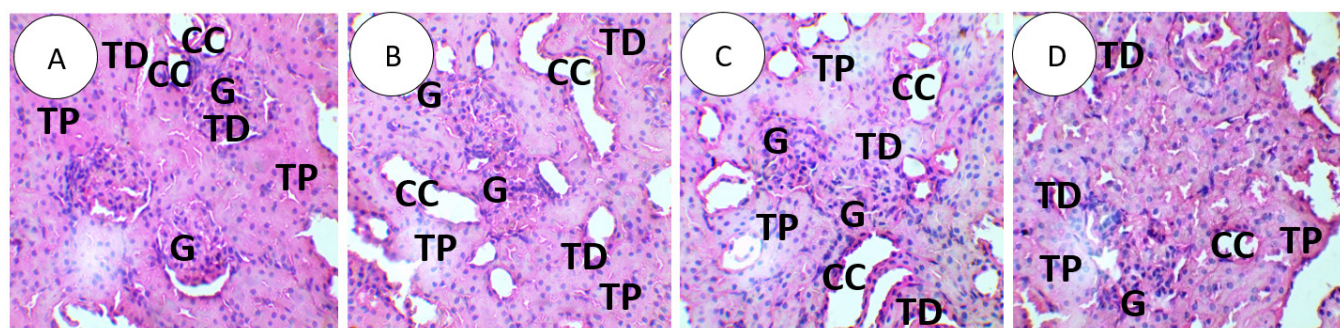
Figures 14A to D show the result of reading the slides of the histological sections of the kidneys at the end of the subchronic toxicity.



**Figure 12:** Renal histology of the rats of control batch (A) and treated batch (B) with ethanolic extract of *O. gratissimum* 5000 mg.kg<sup>-1</sup> body weight (Gr = 400X); G = glomeruli; PT = proximal tubes; TD = distal tubes; CC = collector channels



**Figure 13:** Hepatic histology of the rats of the control batch A gaved; B (500 mg.kg<sup>-1</sup>); C (750 mg.kg<sup>-1</sup>); D (1,000 mg.kg<sup>-1</sup>) at the same time ethanolic extract of *O. gratissimum* (Gr = 400X); arrow = hepatocyte; V = centrilobular vein; S = venous sinusoid



**Figure 14:** Renal histology rats in the control lot (A) gaved rats; lot B (500 mg.kg<sup>-1</sup>); lot C (750 mg.kg<sup>-1</sup>); lot D (1,000 mg.kg<sup>-1</sup>) to the ethanolic extract of *O. gratissimum* (Gr = 400X); G = glomeruli; PT = proximal tubes; TD = distal tubes; CC = collector channels



The architecture of the rats gaved with *Ocimum gratissimum* extract at 500 mg.kg<sup>-1</sup> (Figure 14B), 750 mg.kg<sup>-1</sup> (Figure 14C), and 1,000 mg.kg<sup>-1</sup> (Figure 14D) is normal as observed in control lot rats (Figure 14A).

## DISCUSSION

The clinical trials revealed the susceptibility of the tested organisms to contact with the ethanolic extract of *O. gratissimum* except for *S. epidermidis* and *E. coli* standard. This explains the use of this plant for therapeutic purposes in single preparation or mixture. The small variation of the ID (7 to 11 mm) reflects a low sensitivity of the germs, which is manifested by their resistance. This result gives the opportunity to think about a reversion of bacterial resistance. Wagura AG *et al.*<sup>33</sup> followed by Krishnamurthy V *et al.*<sup>34</sup> reported similar IDs ranging from 3 to 11 mm and 6 to 15 mm, respectively. On the contrary, Junaid SA *et al.*<sup>35</sup> and Stanley MC *et al.*<sup>36</sup> in this order obtained larger ID variations ranging from 5 to 18 mm and 6 to 32 mm. For Krishnamurthy V *et al.*<sup>34</sup> apart from *S. epidermidis* (CIP 8039), all organisms with low ID ( $\leq 7$  mm) are gram (-). Thus, the difference in sensitivity can be explained by the presence of a lipid layer in the wall of gram (-) bacteria making them less permeable and more resistant.<sup>12</sup> This comparative exercise reveals that the level of sensitivity of germs defined by DIs is under the control of the type of extract, nature of the germs, concentrations, and the origin of the samples.

The antibacterial properties of the extract result in MICs and MBCs ranging respectively from 625 to 1,250  $\mu\text{g.mL}^{-1}$  and 1,250 to 5,000  $\mu\text{g.mL}^{-1}$ . Referring to the categorization scale reported by Ouattara LH *et al.*,<sup>21</sup> the antibacterial activity is bacteriostatic for *E. coli* isolated and *Pseudomonas aeruginosa* isolated, but bactericidal for the rest of the germs tested at high concentrations in both cases. However, the characterization range of the antibacterial power proposed by Bashige CV *et al.*<sup>11</sup> is evidence of the low antibacterial activity (MIC  $> 325 \mu\text{g.mL}^{-1}$ ) of the ethanolic extract of *O. gratissimum* Junaid SA *et al.*<sup>35</sup> reported ultra-higher concentrations of MIC between 12,500 to 150,000  $\mu\text{g.mL}^{-1}$  and MBC ranging from 3,130 to 100,000  $\mu\text{g.mL}^{-1}$  Kpodékon MT *et al.*<sup>14</sup> obtained relatively low MICs 8 to 18  $\mu\text{g.mL}^{-1}$  with MBCs ranging from 16 to 144  $\mu\text{g.mL}^{-1}$  Kporou KE *et al.*<sup>18</sup> reported MICs in the range of 12.50 to 100  $\mu\text{g.mL}^{-1}$  induced by the essential oil of *O. gratissimum*. The pharmacological properties of different types of plant extract have also been highlighted by Gallé J-B *et al.*<sup>37</sup> and Krishnamurthy V *et al.*<sup>34</sup> on a wide range of bacterial media. This work confirms very interesting antibacterial potentialities of *O. gratissimum*, whose effectiveness is determined by the type of extract, the concentration, the nature of the species of germs and their virulences. This is the basis of clinical trials on the action kinetics of the extract developed in this work. The results of these examinations reveal a reduction of bacterial loads of *Escherichia faecalis* of 98.49 and 93.50%, respectively, in contact with suspensions of extract 5,000 and 2,500  $\mu\text{g.mL}^{-1}$  after 32 hours. Similarly, the bacterial loads

of MRSA are reduced by 68.40 and 72.44%, respectively, in contact with suspensions of extract 5,000 and 2,500  $\mu\text{g.mL}^{-1}$  in 32 hours. The germicidal capacity of this extract is not proportional. Nevertheless, these results raise a wedge of sail on three essential factors for good use of the plant to know the type of germ, the concentration, and the duration of action. However, these results are contrary to the endogenous practices of the traditional pharmacopoeial populations that daily consume *O. gratissimum* leaves in various forms of drug and food over long periods.

These results challenge the optimization of the antibiotic effects of *O. gratissimum* for therapeutic purposes. Previously, the modulation capacity of antibiotic resistance of several plant species has been reported.<sup>24</sup> This bacterial resistance reversion test allowed the determination of MICs of selected ATBs coupled with the extract-ATB coupled action evaluation. These tests showed a drastic reduction in the MICs of colonies of *E. coli* isolated and *Pseudomonas aeruginosa* isolated in contact with the extract-ATB mixture solution compared to the singular action extracted or ATB conventional (Table 3). This significant decrease in MICs recorded with this conventional binary system is the scientific proof of the reversal of bacterial resistance. In addition, the combined extract-ATB effect on all the tested microorganisms is of the synergistic type (Table 4). Thus, to prove the ability of *O. gratissimum* to modulate ATB activity against resistant bacteria, this plant could be used in combination with some conventional ATB to fight against bacterial resistance. These results are comparable with those of some works,<sup>24,38,39</sup> which showed synergistic effects extract-ATB. This explains the principle of extract-ATB dosing observed in the populations but gives rise to fears of poisoning in the absence of knowledge of the assays and the pharmacodynamic properties of the extracts. This explains the research on the safety of *O. gratissimum* developed in this study.

From the foregoing, the positive dosage of as of type AsIII ( $2.65 \times 10^{-3}$  ppm) and Pb ( $2.65 \times 10^{-3}$  ppm) is obvious evidence of contamination of the plant by external factors. It should be noted, however, that the AsIII and Pb levels, respectively 377 and 2,421 times lower than the WHO standards, are negligible. From a qualitative and quantitative point of view, these results do not reflect those of Kpètèhoto HW *et al.*<sup>13</sup> who reported higher Pb<sup>2+</sup> and Cd<sup>2+</sup> levels in *O. gratissimum* samples. Before these authors Montcho S,<sup>40</sup> reported Pb and Cd contamination of five antimalarial plants in Cotonou. In the same vein Chidikofan GF<sup>41</sup> and Dougnon TV,<sup>42</sup> have focused on the pollution of Cotonou market garden sites, causes, and consequences. For Slatni I,<sup>43</sup> the differences observed could be explained by the origin of the samples, the physicochemical composition of the soils, the cultivation techniques, the duration of exposure, the dosing techniques, the age of samples, and climatic variations. Plants in general and *O. gratissimum*, in particular, contain sites for attachment to Metallic traces element (MTEs). In toxicology, the first test on a compound is acute toxicity<sup>44</sup> generally coupled with subchronic

toxicity. These evaluations involve the surveillance of general behaviors, the enumeration of death cases, and the taking of certain constants, which are the first signs of toxicity.<sup>45</sup> Thus, oral gavage of ethanolic extract from *O. gratissimum* single-dose (5,000 mg/kg) and daily (500, 750, and 1,000 mg/kg) revealed no significant change in animal behavior. In addition, no deaths were counted during the two trials. This result could indicate the safety of the ethanolic extract of *O. gratissimum* at these tested doses. Similar results were obtained by Ojo OA *et al.*<sup>46</sup> and Ajayi AM *et al.*<sup>47</sup> in acute or subacute toxicity (250, 1,500, 1,600, 2,000, and 5,000 mg/kg) of ethanolic or methanolic extract of *O. gratissimum*.

With regard to the body mass of the rats, the tests revealed some variations with significant differences recorded in lot 500 (1st week), lot 750 (2nd week), lot 1,000 (1st and 4th week), and lot 5,000 (2nd week). Subchronic toxicity would indicate a decrease in body mass due to the ethanolic extract of *O. gratissimum* above 750 mg/kg. This finding is inconsistent with the results of acute toxicity, which did not induce any decrease in body weight in rats. Ethanolic extract from *O. gratissimum* up to 1,000 mg/kg once daily and 5,000 mg/kg once-once has no direct impact on the body mass evolution of Wistar strain rats. These results partially reflect those of Okon UA *et al.*<sup>48</sup> then Ajayi AM *et al.*,<sup>47</sup> who found only non-significant changes in the mass of the rats treated with methanolic *O. gratissimum* extract. The acute test shows significant variations in water consumption in the control group and batch treated at the 2nd week. For the subchronic test, a decrease and a significant increase are recorded respectively for the water consumption (control group) and granules (lot 1,000) in the 3rd week. From these analyzes, the consumption of granules and water of the rats is neither a function of the type of toxicity nor of the dependent doses. As a comparison, work on *Ocimum basilicum* and *Ocimum suave* Wild, two closely related species respectively by Rasekh HR *et al.*<sup>44</sup> then Tan PV *et al.*,<sup>49</sup> revealed only insignificant changes in feed consumption or feeding behavior. Significant regressive differences would be due to experimental conditions and the internal metabolism of the animal body. On the other hand, the progressive differences could be related to a stimulation of the appetite of the animals by the extract and which would result in an increase in their consumption. These interpretations corroborate those of Okon UA *et al.*<sup>48</sup> and Ghedjati N.,<sup>45</sup> who note that in toxicology the significant differences in food consumption can be explained by the effect of the extracts plants and factors related to the organism.

The liver and kidneys, which regulate metabolism and excretion, are primarily sensitive to toxic agents.<sup>50</sup> Taking the masses of these organs is a better way to understand the effect of a drug. For the most part, the affected organs have abnormal atrophy compared to the control group.<sup>51</sup> Liver masses show significant differences at doses of 500 and 1,000 mg/kg. Changes in kidney masses were not significant for acute and subchronic toxicity. The effects (gain or loss) on the mass of the organs are partial and would not have been attributed to

the extract. The assays of the extract and types of toxicities are, therefore, not determining factors in the evolution of the mass of organs. Similarly, *in vivo* tests of *Ocimum basilicum* and *Ocimum suave* Wild extracts indicated no significant changes in organ mass.<sup>44,49</sup> This again supposes the safety of this extract at the studied doses.

The usual renal (urea, creatinine) and hepatic (ASAT, ALAT) markers are evaluated to assess the effects of the extract on these organs. These biochemical parameters are the main indicators of hepatorenal pathologies.<sup>52</sup> Acute toxicity showed a significant increase of the urea concentration coupled with an insignificant decrease in creatinine concentration of the rats in the treated batch compared with the control group. On the other hand, the subchronic toxicity revealed a significant increase of the urea concentration in the three treated batches and a significant regression of the creatinine concentration of the 500 and 750 mg/kg batches. This uremia manifested is not dependent dose and would probably be related to the richness of this plant in secondary metabolites, especially proteins. Thus, these concentrations of urea and creatinine testify to the sensitivity of the renal cells to this extract but are not likely to jeopardize the proper functioning of the kidneys. In a comparative approach,<sup>44</sup> also resulted in a significant decrease in creatinine levels in female rats treated with the hydroalcoholic extract of *Ocimum basilicum*. Changes in ASAT Glutamo Oxaloacetate Transferase (GOT), and ALAT Glutamo Pyruvate Transferase (GPT) concentrations showed no significant difference for both types of toxicity. These transaminases are enzymes that have a high metabolic activity inside cytosols and mitochondria, which are essential in certain energy reactions, and whose serum level gives information on hepatic or cardiac cell damage.<sup>30</sup> According to Ojo OA *et al.*,<sup>46</sup> the ALAT concentration of the treated lot above the control group for subchronic toxicity could be due to a synthesis of new enzymes or an intensification of metabolic activities of the liver response to the extract administered. On the other hand, the reduction in ALAT after acute administration can be explained by a decrease in hepatic enzyme synthesis or an enzyme leak in the blood by modification of the membrane permeability. In addition, according to Ojo OA *et al.*,<sup>46</sup> the combined decrease in ALAT and ASAT concentrations following acute toxicity confers on this extract a protective role for liver tissues. For comparison, these results are contrary to those of Effraim KD *et al.*<sup>53</sup> who reported a significant decrease in rabbit concentrations of ALAT and ASAT as a function of aqueous extract concentrations of *O. gratissimum*. On the contrary, these results are similar to those of Ojo OA *et al.*<sup>46</sup> apart from the significant values of transaminase recorded by these authors. This is explained by the nature of the extracts (ethanolic, aqueous) and the origin of the samples. Differences in ALAT and ASAT concentrations are not significant and indicate that the liver of the treated rats is functioning well and that *O. gratissimum* is safe for this organ in traditional use at the doses studied. These results corroborate with those of Fandohan P *et al.*<sup>54</sup> who report a dose-dependent effect

without adverse effects on the liver of *O. gratissimum* oil at 1,500 mg/kg.

Blood is the most important tissue in which metabolic processes occur whose abnormal changes in hematological parameters are the reliable indicator of the toxic effects of xenobiotics.<sup>55</sup> The hematological examinations show significant variations of certain figured elements of the blood. The significant regression of WBCs in the 500 mg/kg lot coupled with the non-sensitive decrease in the other lots would indicate that the observed leukopenia is not dose-dependent. The rats under investigation being considered healthy at the beginning this leucopenia would be linked to the stress generated by the gavages, the experimental, and environmental conditions. In addition, the significant regression of RBCs at the 750, 1,000, and 5,000 mg/kg batches, respectively, are thought to be related to spontaneous regulation of the rat organism. This significant regression of WBCs and RBCs was confirmed by Jimoh OR *et al.*<sup>56</sup> and Ojo OA *et al.*<sup>57</sup>

The Ht results show significant decreases at the 500, 750, and 1,000 mg/kg batches but a non-significant decrease at the 5,000 mg/kg lot. This reduction in Ht and WBC levels would indicate a possible effect on red blood cell integrity, according to Rasekh HR *et al.*<sup>44</sup> The MCV obtained has small variations with a lot of significance of 5,000 mg/kg. Significant results from MCHC are recorded at batches 500 and 750 mg/kg. MCH is significant in all treated lots. Counting PLt in treated lots showed a significant decrease at 750, 1,000, and 5,000 mg/kg batches. The resulting thrombocytopenia may be due to a slowdown in the production or destruction of PLt. The likely mechanism for reducing the number of PLt would be bone marrow suppression, a condition that could also lead to the suppression of precursor granulocytes.<sup>44</sup> These results are not compatible with those of Offem OE *et al.*<sup>58</sup> who found no significant change in the levels of MCHC and MCH, but recorded some significant increases in the number of PLts with the ethanolic extract of *O. gratissimum* in strain rats Wistar.

Confirmation of this integrity of the internal environment following the gave (different doses) of the rats with the ethanolic extract of *O. gratissimum* resulted in histological examinations. The acute and subchronic oral toxicity tests conducted in this study at the above-indicated doses revealed no liver or kidney atypia. For hepatic toxicity, the liver architecture of the rats fed the extract *O. gratissimum* is normal, comparable to that observed in control group rats. Hepatocytes (arrows) of normal appearance are arranged in radial cords around the centrilobular vein (V), and venous sinusoids (S) appear clearly visible. In the case of renal toxicity, careful observation revealed in the rats gaved with the *O. gratissimum* extract the presence of normal-looking renal parenchyma. The glomeruli (G), the proximal tubes (TP), the distal tubes (TD), and the collecting ducts (CC) of the treated batches showed no visible atypia compared to the control batches.

## CONCLUSION

The *in vitro* tests revealed real antimicrobial potential in the samples of *O. gratissimum* as a pure fraction. This biological

potency is strongly modulated by the conventional extract-ATB combination. However, the plant contains negligible levels of cadmium and arsenate.

*In vivo* tests showed no significant mortality or major changes in the general behavior of the rats. The doses studied do not have a significant impact on diet, body mass, and target organs (liver and kidneys). The extract contains negligible levels of cadmium and arsenate. Hematological and biochemical investigations revealed a disparate disturbance of the blood parameters and, consequently, of the hematopoietic system. Histological sections of the liver and kidneys examinations revealed no evidence of atypia. The use of *O. gratissimum* in a single dose in traditional pharmacopeia requires preclinical safety in the range of concentrations studied. Depending on the duration of use, the ethanol extract of *O. gratissimum* is a potential disruptive factor in the hematopoietic system of Wistar strain rats. Its use in continuous high dose is strongly discouraged.

## CONTRIBUTION OF THE AUTHORS

Kpètèhoto HW, Johnson RC, Amoussa AMO, ensured the conception, the realization, and the writing of the study. Houéto EEM, Mignanwandé FMZ. contributed to the writing and proofreading of the study. Loko F, Lagnika L supervised the work.

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