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Antibacterial Properties of *Crateva adansonii* (Capparidaceae) on Strains Isolated from Chronic Wounds Diagnosed in the Commune of Ouinhi in 2021

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Abstract

Plants have always been used by people for therapeutic purposes. They are still the main source of therapeutic substances in developing countries. *Crateva adansonii*, a member of the Capparidaceae family, is a medicinal plant with antibacterial properties used in Benin. The aim of this study was to assess the efficacy of an aqueous extract of *C. adansonii* on bacterial strains isolated from chronic wounds in the Ouinhi population. To achieve this, the bacterial flora present in chronic wounds was identified using the Ikram method (2014) coupled with the API Remoel One method. The antibacterial properties of the aqueous extract of *C. adansonii* on the microbial strains isolated were then assessed by determining the Inhibition Diameters (ID), the Minimum Inhibitory Concentrations (MIC) and finally the Minimum Bactericidal Concentrations (MBC). A total of eighty (80) strains were isolated and identified on the basis of morphological, cultural and biochemical characteristics. The species *S. Aureus* species accounted for the largest proportion (67.5%). Other species such as *Listeria sp*, *Pseudomonas proteus*, *S. epidermidis* and *Bacillus cereus*, *Citrobacter freundii*, *Steno maltophila; Axin calcoaceticus*, *E. coli*, *K. pneumonia*, *Lem. richardii*, *Salmonella paratyphi A*, *Salmonella sp*, *Shigella sp* were determined in variable proportions. At a concentration of 10 mg/ml, only S. aureus was sensitive to contact with the extract. However, at 20 mg/ml, 89% of strains were sensitive and 11% very sensitive. The highly sensitive strains are *Salmonella sp* and *E. coli*. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concen-

tration (MBC) are 20 mg/ml and 40 mg/ml respectively. The MBC/MIC ratio of the aqueous mixture of *Crateva adansonii* (Capparidaceae) varied from 1.2 to 2, with a bactericidal effect on 100% of the strains tested.

Keywords

Crateva adansonii, Antimicrobial Activities, Chronic Wound Strains, Benin

1. Introduction

For centuries, people around the world have used plants to treat themselves. Today, they are still the main source of therapeutic substances in developing countries [1]. Knowledge of the virtues and risks of these medicinal plants is based on traditional knowledge specific to each culture, and has evolved empirically over the centuries. The ways in which this knowledge is developed vary from one place to another and have evolved differently in different geographical areas [2]. The knowledge acquired over time has most often been passed down orally, from generation to generation. The most complex knowledge is often held by a few scholars, recognised by the local population [1] [3]. The WHO also certifies that in certain countries in Asia, Africa and Latin America, 80% of the population still use traditional medicine, especially in rural areas, because of the proximity and accessibility of this type of care at an affordable cost, and above all because of the lack of access to modern medicine by these populations [3]. As a result, traditional medicine can be seen as an integral part of primary healthcare, to improve access to care. It is therefore essential to research the bacterial properties of certain therapeutic plants. One of the most widely used medicinal plants in Benin and elsewhere is *Crateva adansonii* (Capparidaceae). Phytochemical studies carried out on this plant have revealed that it contains the main phytochemical compounds, such as alkaloids, coumarins, anthracene derivatives, flavonoids, essential oils, lignans, anthocyanin pigments, tannins and triterpenes [4]. It also contains secondary metabolites such as total tannins, total flavonoids and total phenolics in varying proportions, and has antioxidant properties that vary according to the concentration of the extract [4] [5]. Research by [5] [6] [7] has also shown that *Crateva adansonii* has antibacterial properties against germs commonly identified in skin and digestive disorders, such as *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. In Benin, *Crateva adansonii* is used to treat arterial hypertension [6], diarrhoeal diseases [8] and diabetes in pregnant women in Cotonou [9]. Traditional therapists use *Crateva adansonii* in Togo to treat abscesses, wounds, bacterial infections and rheumatism [10]. In view of the numerous uses of *Crateva adansonii*, without any particular precautions being taken, and the enormous setbacks associated with the misuse of medicinal plants with an antibiotic effect, the present study was initiated to highlight the bacterial properties of *C. adansonii* on strains of chronic wounds diagnosed in patients in the Commune of Ouinhi

in 2021.

2. Study Framework and Method

2.1. Study Setting

The commune of Ouinhi (Figure 1) served as the setting for this study. It is located in the south-east of the Zou department, between latitudes 06˚57' and 07˚11' north and longitudes 02˚23' and 02˚33' east. With a surface area of 483 km², Ouinhi is one of nine Communes in the department. It is bordered to the north-west by the Commune of Zagnanado, to the south-west by the Commune of Zogbodomey, to the south-east by the Commune of Bonou and to the east by the Commune of Adja-Ouèrè in the department of Ouémé. The Commune of Ouinhi comprises 28 villages spread over four arrondissements: Dasso, Sagon, Tohouè, Ouinhi [11].

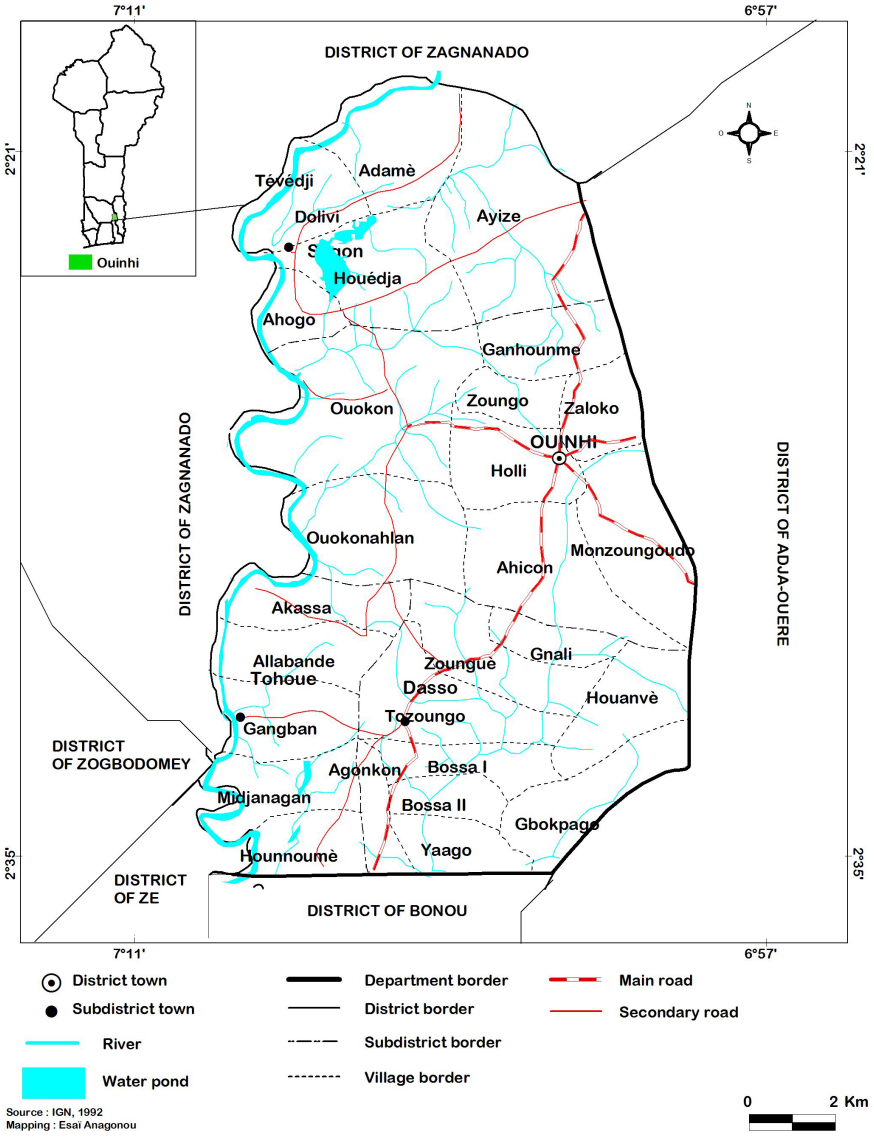

Figure 1. Graphic representation of the commune of Ouinhi. Source: IGN, Bénin.

2.2. Study Materials

The material used for this study consisted essentially of plant material (dry extract) from leafy stems of *Crateva adansonii*, technical laboratory equipment for in vitro testing, glassware, reagents and biological material consisting of bacterial strains isolated from wounds. Seventeen (17) clinical strains isolated from patients' wounds in the Ouinhi commune and two (02) reference strains were selected for sensitivity testing. Table 1 shows the different strains and the different codes assigned to these strains within the laboratory.

2.3. Study Method

2.3.1. Identification of Bacterial Flora Present in Chronic Wounds

The identification of bacterial flora from wounds was carried out using the Ikram method (2014) coupled with the API Remoel One method. It is summarised in six steps:

- Swabbing
- Inoculation of samples
- Enumeration of colonies

Table 1. Bacterial strains.

Lab code	Strain
W017/5	*Axin. calcoaceticus*
w027/1	*Citrobacter freundii*
w014/4	*E. coli*
w040/1	*Lem. richardii*
w014/3	*S. epidermidis*
w014/5	*Listéria sp*
w024/1	*Pseudomonas proteus*
w052/1	*Pseudomonas proteus*
w018/1	*S. epidermidis*
w006/3	*Salmonella sp*
w048/2	*Shigella sp*
w018/3	*Steno. maltophila*
w013/1	*S. aureus*
w055/2	*S. aureus*
w057/5	*S. aureus*
w058/1	*S. aureus*
w016/2	*S. aureus*

Source: HECOTES laboratory Benin, 2021.

The number of colonies (N) was determined using the following formula "Equation (1)":

$$N = \frac{\sum c}{V\left(n_1 + 0.1n_2\right)d}$$

where:

c: number of CFU (colony forming units);

v: volume of suspension spread on the surface of the media in ml;

n_1: number of plates retained for the first dilution (the lowest);

n_2: number of plates retained for the second (highest) dilution;

d: dilution rate corresponding to the lowest dilution retained.

- Microscopic and macroscopic examinations
- Identification on specific media
- Biochemical tests (oxidase test, catalase test, coagulase test and the Thermo Fisher Scientific Rapid One Gallery).

2.3.2. Determination of the Antibacterial Properties of the Aqueous Extract of C. Adansonii (MIC and MBC)

- **Preparation of the aqueous extract**

The solution was prepared by diluting 20 mg of dry extract in 450 µL of sterile distilled water and adding 50 µL of dimethylsulphoxide (DMSO). The mixture was then homogenised in a sonicator.

- **Preparation of culture media:**

Müller Hinton (MH) agar was obtained by dissolving 38 g of MH culture medium powder in 1 litre of distilled water (pH = 7.5 ± 0.2) and MH broth was obtained by dissolving 21 g of the powder in 1 litre of distilled water. Each medium was autoclaved at 121˚C for 15 min.

- **Preparation of the bacterial inoculum**

The bacterial inoculum is prepared by introducing an aliquot of a 24-hour bacterial culture into another sterile tube containing the MH broth. The optical density (OD 600 nm) of the solution was read using a spectrophotometer (UV-1600PC). The optical density of the inoculum was adjusted to 0.156 for *E. coli*, *P. aeruginosa* and 0.3 for *Staphylococcus* strains. These optical densities correspond to 10^8 CFU/ml [12] [13]. A 1/100th dilution of this inoculum was used to obtain the final inoculum (10^6 CFU/ml) used for the tests.

- **Sensitivity test**

Susceptibility testing was carried out using the Muller Hinton (MH) agar diffusion method to test the antibacterial activity of the extract. All cultures were grown in triplicate at incubation temperature. Extracts at 10 mg/ml, 20 mg/ml and 30 mg/ml were used with an inoculum concentration of 10^6 CFU. Sterile wattman paper was impregnated for testing purposes and commercially available antibiotic discs (Bio Mérieux) of ciprofloxacin and ceftriaxone were used as controls. The boxes were observed 18 to 24 hours later [12] [13].

- **Determination of the Minimum Inhibitory Concentration (MIC)**

The MIC was determined using the microdillution method recommended by NCCLS M07-A10 (2015), revised and adapted to laboratory conditions. Prior to testing, bacterial strains were diluted in BMH and incubated for 18 h at 37˚C.

The inoculum was grown 24 hours in advance for each bacterial species in BMH broth, and diluted to 1×10^6 CFU. A range of extract concentrations was used, from 10 mg/ml to 30 mg/ml.

An acetone-water mixture and gentamicin were used as negative and positive controls. All experiments were performed in triplicate and the microdillution trays were incubated at 37˚C for 24 h. Antibacterial activity was detected using a colorimetric method by adding 25 µL of 0.01% aqueous Iodonitrotetrazolium (INT) solution to each well at the end of the incubation period. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of extract capable of inhibiting all growth visible to the naked eye within 24 hours [14] [15].

- **Determination of the BMC**

The MBC was determined by spreading 10 µL of the contents of each tube with a concentration greater than or equal to the MIC on solid medium. Concentrations of extract equal to or greater than the MIC were prepared in sterile tubes. Finally, 100 µl of the bacterial suspension at 1.10^6 CFU/ml in BMH was added to the extract to obtain the MIC and concentrations of 2× MIC and 4× MIC. These media in tubes were incubated at 37˚C. After 24 hours, 10 µl of the tube contents were inoculated onto MH agar and incubated for 24 hours.

From the MIC, the smallest concentration that allows no more than 0.01% of the bacteria in the starting suspension to survive in 24 hours corresponds to the BMC.

The antibacterial effect was judged to be bactericidal or bacteriostatic according to the ratio: BMC/MIC.

- If $1 \leq$ CMB/CMI ≤ 4, the effect is bactericidal.
- If $4 <$ CMB/CMI ≤ 16, the effect is bacteriostatic [16] [17] [18].

3. Results

3.1. Identification of the Bacterial Flora Present in Chronic Wounds

3.1.1. Microbiological Profile

Table 2 presents the microbiological profile of the germs identified. A total of eighty (80) strains were isolated and then identified on the basis of morphological, cultural and biochemical characteristics. The species *S. aureus* accounted for the highest proportion, at 67.5%. Species such as *Listeria sp*, *Pseudomonas proteus and S. epidermidis* on the one hand and *Bacillus cereus*, *Citrobacter freundii* and *Steno maltophila* on the other were isolated in equal proportions (5% and 3.75% respectively). The low proportion of germs (1.25%) identified were *Axin calcoaceticus*, *E. coli*, *K. pneumonia*, *Lem. richardii*, *Salmonella paratyphi A*, *Salmonella sp*, *Shigella sp*, and others.

Table 2. Breakdown of germs identified by species.

Strains	Number	Percentage (%)
Staphylococcus aureus	54	67.5
S. epidermidis	3	3.75
Cocci à Gram positif	**57**	**71.25**
Listéria sp	3	3.75
Bacillus cereus	2	2.5
Gram-positive bacilli	**5**	**6.25**
Total Gram-positive bacteria	**62**	**77.5**
Escherichia coli	1	1.25
Klebsiella pneumoniae	1	1.25
Citrobacter freundii	2	2.5
Lem. rachidii	1	1.25
Salmonella paratyphi A	1	1.25
Salmonella sp	1	1.25
Shigella sp	1	1.25
Steno. maltophila	2	2.5
Pseudomonas proteus	3	3.75
Pseudomonas sp	4	5
Enterobacteriaceae	**17**	**21.25**
Acinetobacter calcoaceticus	1	1.25
Non-fermenting Gram-negative bacilli	**1**	**1.25**
Total Gram-negative bacteria	**18**	**22.5**
Grand total	**80**	**100**

3.1.2. Distribution of Germs Identified

Figure 2 below shows the results of microbial profiles of wounds diagnosed in the commune of Ouinhi in 2021 for patients with wounds lasting more than four weeks.

3.1.3. Proportion of Germs Isolated According to the Microbial Profile of Wounds

Figure 3 shows the proportions of germs isolated according to the microbial profile of chronic wounds. Of the 63 subjects sampled, 1.6% (1/63) were sterile samples; monomicrobial wounds accounted for 74.6% (47/63) of the samples and polymicrobial wounds for 23.8% (15/63) of the cultures.

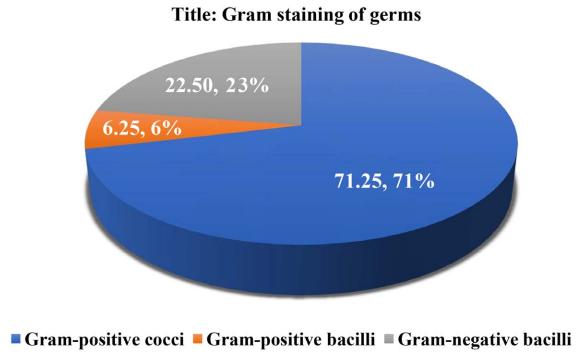

Figure 2. Distribution of germs identified.

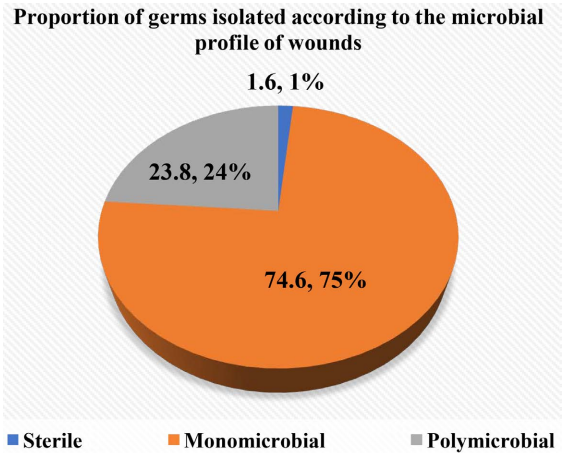

Figure 3. Proportion of germs isolated according to the microbial profile of wounds.

3.2. Antibacterial Properties of the Aqueous Extract of *Crateva adansonii*

3.2.1. Sensitivity of Bacterial Strains to Aqueous Extract of *Crateva adansonii*

Table 3 below shows the results of the sensitivity of germs to the aqueous extract of *Crateva adansonii* at concentrations of 5 mg/ml, 10 mg/ml and 20 mg/ml.

Analysis of this table reveals that at 5 mg/ml no germs were sensitive to the aqueous extract of *C. adansonii*. At 10 mg/ml only *Pseudomonas proteus* showed sensitivity with an inhibition diameter of 08 mm. All the other germs present in the study were sensitive to the 20 mg/ml concentration.

3.2.2. Antimicrobial Activity of the Aqueous Extract of *Crateva adansonii* (Capparidaceae)

Table 4 shows the results of the antimicrobial effects of the aqueous extract of *Crateva adansonii* on the strains tested.

Analysis of Table 4 shows that the MICs and BMCs of the aqueous mixture of *Crateva adansonii* (Capparidaceae) range from 20 to 25 mg/ml and 20 to 40 mg/ml respectively. The BMC/MIC ratio of the aqueous mixture of *Crateva adansonii* (Capparidaceae) varied from 1.25 to 2, so the effect of this mixture was bactericidal on all the strains tested.

Table 3. Results of sensitivity tests.

Lab code	Strain	Inhibition diameter in mm		
		5 mg/ml	10 mg/ml	20 mg/ml
w017/5	*Axin. calcoaceticus*	0	0	12
w027/1	*Citrobacter freundii*	0	0	14
w014/4	*E. coli*	0	0	15
w040/1	*Lem. richardii*	0	0	10
w014/3	*S. epidermidis*	0	0	12
w014/5	*Listéria sp*	0	0	13
w024/1	*Pseudomonas proteus*	0	0	11
w052/1	***Pseudomonas proteus***	**0**	**8**	**9**
w018/1	*S. epidermidis*	0	0	11
w006/3	*Salmonella sp*	0	0	15
w048/2	*Shigella sp*	0	0	12
w018/3	*Steno. maltophila*	0	0	11
w013/1	*S. aureus*	0	0	12
w055/2	*S. aureus*	0	0	12
w057/5	*S. aureus*	0	0	14
w058/1	*S. aureus*	0	0	12
w016/2	*S. aureus*	0	0	13
***E coli* ATCC**		**0**	**10**	**12**
Salmonelle ATCC 1428		0	0	13

Table 4. Results of sensitivity tests.

Strain	MIC (in mg/ml)	BMC (in mg/ml)	MBC/MIC	Result and interpretation
Axin. calcoaceticus	25	40	1.6	Bactericidal
Citrobacter freundii	20	30	1.5	Bactericidal
E. coli	20	30	1.5	Bactericidal
Lem. richardii	20	30	1.5	Bactericidal
S. epidermidis	20	30	1.5	Bactericidal
Listéria sp	20	40	2	Bactericidal
Pseudomonas proteus	20	20	1	Bactericidal
Pseudomonas proteus	20	20	1	Bactericidal
S. epidermidis	20	30	1.5	Bactericidal
Salmonella sp	20	20	1	Bactericidal

Continued

Shigella sp	20	20	1	Bactericidal
Steno. maltophila	20	30	1	Bactericidal
S. aureus	25	40	1.6	Bactericidal
S. aureus	25	40	1.6	Bactericidal
S. aureus	20	40	2	Bactericidal
S. aureus	20	30	1.5	Bactericidal
S. aureus	20	30	1.5	Bactericidal
E. coli ATCC	20	30	1.5	Bactericidal
Salmonelle ATCC 1428	20	40	2	Bactericidal

4. Discussion

In the present study, strains isolated from wounds were predominantly monomicrobial (74.6%) and polymicrobial (23.8%). These results are similar to those reported by [19] and [20]. In contrast, [21] and [22] showed the predominance of polymicrobial cultures in chronic wound infections. The bacteria identified were predominantly gram-positive, with an isolation rate of 78%. Gram-negative bacteria accounted for 22% of germs detected. The same is true of the work by [20] [23] on the identification of germs, which obtained proportions of 60% and 66% respectively of Gram + bacteria in the wounds diagnosed. In contrast to our study, [24] [25] [26] obtained higher levels of Gram-negative bacterial isolation, with proportions of 58% for the first three and 55% for the last. The dominant species was *Staphylococcus aureus*, which accounted for 67.5% of isolates. Several studies show that *Staphylococcus aureus* is the most frequently isolated pathogen in chronic wound infections [20] [27] [28]. Studies by [20] [23] [26] showed similar results to those reported in this study. In contrast to our study, [24] [25] had *Pseudomonas aeruginosa* and *Escherichia coli* as the most frequently isolated strains, respectively. Among gram-negative bacteria, enterobacteriaceae predominated, accounting for 21.25% of isolates. *Pseudomonas sp* was the species most frequently found among gram-negative bacteria and the second most frequently found among all germs. It accounted for 5% of isolates. The same applies to the work of [19], with the difference that *Escherichia coli* was the second most frequently isolated pathogen, after *Staphylococcus aureus*, with an isolation rate of 18% [21].

The bacterial sensitivity results showed inhibition diameters of between 8 and 15 mm. These results are higher than those obtained by (5, 25), which recorded inhibition diameters of 9 - 13 mm and 06 - 11 m respectively, and are lower than those of [5], whose inhibition diameters ranged from 3 - 17 mm. The inhibition diameters of *Escherichia coli* (9 mm), *Staphylococcus aureus* (11 mm) and *Staphylococcus epidermidis* (08 mm) obtained by [29] are smaller than those obtained in our work with the same plant. With regard to the distribution of inhi-

bition diameters, at a concentration of 20 mg/ml all the strains tested were sensitive to the aqueous mixture of *Crateva adansonii*. These results are contrary to those of [5] which obtained 8.75% sensitive strains and 91.25% resistant strains. They are close to those of (27), which obtained 12.5% resistant strains and 87.5% susceptible strains.

Antibacterial activity showed MICs of 20 and 25 mg/ml respectively. These results are superior to those obtained by [29] who recorded MICs ranging from 0.31 to 10 mg/ml with *Crateva religiosa* leaves. Similarly, these results are higher than those obtained by [9] who recorded the same MIC for *E. coli* ATCC and *S. aureus* with the ethanolic extract of *Crateva adansonii* leaves. The BMCs ranged from 20 to 40 mg/ml. These results are superior to those obtained by [10] who recorded BMCs of between 0.31 and 10 mg/ml with *Crateva religiosa* leaves. Similarly, these results are similar to those obtained by [9] who recorded the same BMC for *E. coli* ATCC and *S. aureus* with the metanolic extract of *Crateva adansonii* leaves. Characterisation of the antibacterial effect of *Crateva adansonii* (Capparidaceae) revealed that the aqueous mixture had a bactericidal effect on all the strains tested. This result differs from those obtained by [7] who, using the same plant, had indeterminate, bacteriostatic and bactericidal effects respectively on 50%, 25% and 25% of the germs tested.

5. Conclusion

Measurement of the antibacterial activity of the aqueous extract of *Crateva adansonii* on germs isolated from chronic wounds diagnosed in the commune of Ouinhi showed that *Crateva adansonii* possesses antimicrobial properties that depend on the concentration used. In the concentration range used (10 mg/ml to 20 mg/ml), the best sensitivities were obtained at 20 mg/ml. It should also be noted that the aqueous extract of *C. adansonii* showed variable sensitivities to reference strains and clinical strains. The MBC/MIC ratio of the aqueous extract of *Crateva adansonii* (Capparidaceae) showed a bactericidal effect on all the strains tested. This confirms the therapeutic usefulness of the *Crateva adansonii* plant.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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