Antibacterial Activities of the Ethanolic Extract of *Crateva adansonii* DC. (Capparidaceae) Harvested in Dassa-Zoumè in Central Bénin

Zinsou Franck Mignanwandé[1], Roch Christian Johnson[1], Armelle Sabine Yélignan Hounkpatin[1,2]*, Gratien Boni[1], Armel Géraldo Houndeton[1], Eustache Enock Houéto[1], Wilfrid Hinnoutondji Kpètèhoto[1], Madjid Olatoundé Amoussa[3]

[1]Interfaculty Center for Training and Research in Environment for Sustainable Development (CIFRED), University of Abomey-Calavi, Abomey-Calavi, Benin
[2]Technical Higher Teacher Training School (ENSET), National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM), Abomey, Benin
[3]Faculty of Sciences and Technology, University of Abomey-Calavi, Abomey-Calavi, Benin
Email: *harmelle2011@gmail.com

Abstract

Objective: The present was initiated to study the antibacterial properties of the *Crateva adansonii* DC extract on germs commonly identified in skin and digestive infections in Benin as well as the reversion of resistance to these aforementioned germs. **Method:** The bacteria's sensitivity test to extracts was carried out by the microdilution method in liquid medium as well as the MIC and the reversion of bacterial resistance. For the determination of the MBC, this technique is used coupled with spreading on agar medium. **Results:** The results show an antibacterial activity of the extract with MICs between 0.625 - 5 mg/ml. The CMB of *Enterococcus faecalis* ATCC 29212 is 2.5 mg/ml while that of Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* CIP 8039 is 5 mg/ml. The reversion of bacterial resistance has shown a synergy of action between our extract and conventional antibiotics.

Keywords

Antibacterial Activity, *Crateva adansonii*, Ethanolic Extract, Benin

1. Introduction

Human societies across civilizations are very attached to the use of medicinal plants [1] whose effectiveness is in most cases poor of scientific evidence [2]. By way of illustration, 80% of the population of developing countries use traditional

medicine as a first line for primary health care [3] [4]. Despite this strong propensity for folk medicine coupled with recent advances in modern medicine, infectious diseases constitute a serious public health problem in view of their frequency and severity OCDE [5]. It should be emphasized that the antibiogram known as the most effective way to deal with this microbial invasion is facing a bacterial resistance [6]. *Crateva adansonii* DC, of the capparidaceae family, is a medicinal plant used by traditional healers in Benin for multiple therapeutic virtues, notably antibacterial properties. It is used in the treatment of many conditions including abscesses, sores, bacterial infections, high blood pressure, diabetes and rheumatism.

2. Materials and Methods

2.1. Material

2.1.1. Plant Material
These are exclusively samples of leafy stems of *Crateva adansonii* DC. *ssp. adansonii* harvested in Dassa-Zoumè in central Benin. This plant material was authenticated at the national herbarium of Abomey Calavi University under the number YH 269/HNB.

2.1.2. Biological Material
The microorganisms used in this study are reference strains, namely *Escherichia coli* (CIP 53126), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (CIP 8039), *Staphylococcus aureus* resistant to methicillin, *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (CIP 82118) and two hospital strains of urinary and digestive origin, *Pseudomonas aeruginosa* and *Escherichia coli* isolated from biological samples from the microbiology laboratory of the Center Hospitalier Universitaire National/Hubert Koutoukou Manga (CNHU/HKM). These germs were preserved by subculturing on an agar medium.

2.2. Methods

2.2.1. Preparation of the Ethanolic Extract
The samples dried under laboratory conditions (θ = 22˚C ± 3˚C) were reduced to powder. 100 g of powder were brought into contact with 500 ml of ethanol with mechanical stirring for 24 hours. The extract was decanted, filtered and then evaporated *in vacuo*. The extract obtained was dried in an oven at 40˚C before being stored in pill organizers at 4˚C.

2.2.2. Preparation of Culture Media
Müller Hinton Agar (MHA) was obtained by dissolving 38 g of the agar medium in 1 L of distilled water (pH = 7.5 ± 0.2). Müller Hinton broth was obtained by dissolving 21 g in 1 L of distilled water. Each medium was sterilized in an autoclave at a temperature of 121˚C for 15 min.

2.2.3. Preparation of the Bacterial Inoculum
The bacterial inoculum is prepared by introducing an aliquot of a 24-hour bacteri-

al culture into another sterile tube containing the HD broth. The optical density (OD 600 nm) of the solution is read using a spectrophotometer (UV-1600PC). The optical density of the inoculum is adjusted to 0.156 for *Escherichia. coli, P. aeruginosa* and 0.3 for the strains of Staphylococcus. These optical densities correspond to 108 CFU/ml [7]. A dilution of 1/100th of this inoculum made it possible to obtain the final inoculum (106 CFU/ml) used for the tests.

2.2.4. Sensitivity Test

The purpose of this test is to eliminate extracts having no activity at a concentration of 10 mg/ml. The bacteria sensitivity test to extracts was carried out by the micro-dilution method in liquid medium in 96-well plates [8]. 100 μl of bacterial inoculum at 106 CFU were added to 100 μl of extract prepared at 20 mg/ml. The acetone-water mixture (50:50) was used as a negative control. The plates were shaken using a mixer to make the reaction medium homogeneous and then incubated at 37˚C. After 18 h of incubation, 40 μl of a 2% aqueous solution of INT (SIGMA-ALDRICH) were added to each well. The appearance of red or pink coloring in a well indicates bacterial growth in the well.

2.2.5. Determination of the Minimum Inhibitory Concentration (MIC)

The MIC was determined by the microdilution method recommended by CLSI 2008 [9] revised and adapted to the conditions of Laboratory of Biochemistry and Natural Bioactives substances (LBSNB). Before carrying out the tests, the bacterial strains were diluted in BMH and incubated for 18 h at 37˚C. The inoculum was cultured 24 hours in advance for each bacterial species in the BMH broth, and diluted to 1.106 CFU. A range of extract concentration from 5 mg/ml to 0.039 mg/ml was used. An acetone-water mixture and gentamicin was used as a positive control. All the experiments were carried out in triplet and the micro-dillution plates are incubated at 37˚C for 24 h. Antibacterial activity was detected using a colorimetric method by adding 25 μL of 0.01% aqueous solution of Iodonitrotetrazolium (INT), to each well at the end of the incubation period. The minimum inhibitory concentration (MIC) has been defined as the lowest concentration of extract capable of inhibiting any growth visible to the naked eye in 24 hours [8]. The negative control was sterile broth (BMH).

2.2.6. Determination of Minimum Bactericidal Concentration (MBC)

The MBC is determined by spreading 10 μL of the content of each tube of concentration greater than or equal to the MIC on solid medium. Indeed, concentrations of the extract, greater than or equal to the MIC, were prepared in sterile tubes. Finally, 100 μl of the bacterial suspension at 1.106 CFU/ml in BMH were added to the extracts so as to obtain the MIC and concentrations of 2xMIC and 4xMIC. These tube media were incubated at 37˚C. After twenty-four hours, 10 μl of the contents of the tubes will be inoculated on MH agar and placed in the oven for 24 hours. From the MIC, the smallest concentration, which allows only 0.01% of the bacteria in the starting suspension to survive in 24 hours, corres-

ponds to the MBC. The antibacterial effect will be considered bactericidal or bacteriostatic depending on the ratio: MBC/MIC.

Thus, the interpretation of the results is reflected through the intervals below.

- If $1 \leq$ MBC/MIC ≤ 4, the effect is bactericidal
- If $4 <$ MBC/MIC ≤ 16, the effect is bacteriostatic [9] [10] [11].

2.2.7. Determination of the Kinetics of Action of the Extract

The kinetic index of the reaction time of the extract of *Crateva adansonii* is determined as described by Ara *et al.* [12] reviewed and adapted to the conditions of the Laboratory of Biochemistry and Natural Bioactives substances (LBSNB). The objective of this test is to know the duration of the bacteriostatic or bactericidal effect of the extract.

Principle:

The inocula used are fresh cultures of 24 hours. A 106 CFU inoculum was used for the various tests. The extracted inoculum mixture is made volume to volume in sterile tubes and placed in the oven at 37°C. The optical densities are read at T0, then at 4 hour intervals until 36h, against a blank consisting of an MH mixture and of extract diluted to the concentrations of the various tests. The curves are plotted on the basis of the variance of the turbidity during the duration of the experiment.

2.2.8. Reversion of Bacterial Resistance

The main objective of this test is to determine the synergy of action between the extract and the conventional antibiotics Amoxicillin (AMX), Ciprofloxacin (CIP), Ampicillin (AMP), Erythromycin (ERY), Erythromycin (ERY), Cotrimoxazole (SXT). *Enterococcus faecalis* and, *Pseudomonas aeruginosa* have been used because of their multidrug resistance [13]. It consisted in finding the MICs of conventional antibiotics and those of the combination of antibiotics and extract in order to calculate the fractional inhibitory concentration (CFI). According to the Antibiotic Committee of the French Microbiology Society reported by [14], there are four levels of interpretation, namely:

- synergy (CFI ≤ 0.5)
- addition ($0.5 <$ CFI ≤ 1)
- indifference ($1 <$ CFI ≤ 4)
- antagonism (CFI > 4).

Principle:

50 μL of the 1 mg antibiotic solution was dispensed into the first and second wells of the microplate. 50 μL of the HD solution was distributed into the wells from the second line. Then a cascade dilution was made. Then, each well receives 50 μL of extract and 100 μL of inoculum. After 24 hours of incubation at 37°C, the reading was made by adding 40 μL of 0.01% INT. A second incubation was carried out for 30 minutes. The presence of germs is reflected by the red coloring of the medium. Controls without plant extract, without bacterial suspension and without antibiotics were used.

3. Results

3.1. Sensitivity Test

The result of the sensitivity test by the plate microdilution method is shown in Table 1.

Of the eight germs selected for this study, seven were sensitive to the ethanolic extract of *Crateva adansonii*. *Pseudomonas aeruginosa* isolated was resistant to contact with the extract. The inhibition diameters vary from 06 - 11 mm.

3.2. Minimum Inhibitory Concentration (MIC)

The revelation of the various plates made it possible to determine the MICs of each germ in contact with the ethanolic extract of *Crateva adansonii*. Table 2 shows the MIC results specific to each germ tested.

The analysis in Table 2 reveals that the MICs determined are between 0.625 - 5 mg/ml. The MIC of isolated *Escherichia coli* is more than 5 mg/ml while that of isolated *Pseudomonas aeruginosa* is not determined.

Table 1. Result of the sensitivity test by the plate microdilution method.

Bacterial strains	Inhibition diameters in mm			Average ± Ecartype ID	Conclusions
	1st test	2nd test	3rd test		
Escherichia coli isolated	09	08	08	8.33 ± 0.58	Sensitive
Escherichia coli	10	09	09	9.33 ± 0.58	Sensitive
Pseudomonas aeruginosa isolated	07	07	06	6.67 ± 0.58	Resistant
Pseudomonas aeruginosa	11	11	10	10.67 ± 0.58	Sensitive
Methicillin-resistant Staphylococcus aureus	08	09	08	8.33 ± 0.58	Sensitive
Entérococcus faecalis	09	10	09	9.33 ± 0.58	Sensitive
Staphylococcus aureus	10	11	10	10.33 ± 0.58	Sensitive
Staphylococcus épidermidis	08	08	08	8 ± 0.0	Sensitive

Table 2. MIC result of the extract of *Crateva adansonii* on germs.

Bacterial strains	MIC values (mg·ml^{-1})
Escherichia coli isolated	>5
Escherichia coli	>5
Pseudomonas aeruginosa isolated	Not determined
Pseudomonas aeruginosa	2.5
Methicillin-resistant Staphylococcus aureus	2.5
Entérococcus faecalis	1.25
Staphylococcus aureus	1.25
Staphylococcus épidermidis	0.625

MIC = Minimal Inhibitory Concentration.

3.3. Determination of Minimum Bactericidal Concentration

Table 3 shows the results of the CMBs specific to each germ tested as well as the characterization of the antibacterial power of the extract.

Analysis of Table 3 shows that in the range of concentrations used, the CMBs of the isolated *Escherichia coli*, *Escherichia coli* CIP 53126, *Pseudomonas aeruginosa* CIP 82118 and *Staphylococcus aureus* ATCC 6538 germs are greater than 5 mg/ml. The CMB of isolated *Pseudomonas aeruginosa* is not determined. The CMB of *Enterococcus faecalis* ATCC 29212 is 2.5 mg/ml while that of *methicillin-resistant Staphylococcus aureus* and *Staphylococcus epidermidis* CIP 8039 is 5 mg/ml. As for the characterization, it reveals that the antibacterial effect of the ethanolic extract of *Crateva adansonii* is indeterminate, bacteriostatic and bactericidal respectively on 50%, 25% and 25% of the germs tested.

3.4. Determination of Antibacterial Kinetics

The result of the action kinetics of the extract for the bacterial strains used is shown in Figures 1-3 below.

Figure 2 shows the kinetics of action of the extract on *P. aeruginosa*.

Figure 3 shows the kinetics of action of the extract on *E. faecalis*.

3.5. Reversion of Bacterial Resistance

Table 4 presents the MIC results of conventional antibiotics in contact with Enterococcus faecalis and Pseudomonas aeruginosa.

Analysis of the table shows that the germs are resistant to all the antibiotics tested except amoxicillin at 1000 μg/ml and ciprofloxacin at 250 μg/ml for *Escherichia coli* and ciprofloxacin at 500 μg/ml for *Pseudomonas aeruginosa*.

Table 3. MIC and MBC result

Bacterial strains	MIC (mg·ml⁻¹)	MBC (mg·ml⁻¹)	MBC/MIC	Characterization
Escherichia coli isolated	>5	>5	Not determined	Not determined
Escherichia coli CIP 53126	05	>5	Not determined	Not determined
Pseudomonas aeruginosa isolated	Not determined	Not determined	Not determined	Not determined
Pseudomonas aeruginosa CIP 82118	2.5	>5	Not determined	Not determined
Methicillin-resistant Staphylococcus aureus	2.5	05	02	Bactericide
Entérococcus faecalis ATCC 29212	1.25	2,5	02	Bactericide
Staphylococcus aureus ATCC 6538	1.25	>5	>4	Bacteriostatic
Staphylococcus épidermidis CIP 8039	0.625	05	08	Bacteriostatic

MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration.

Table 5 presents the MIC results for antibiotics in combination with the ethanolic extract of *Crateva adansonii*.

Analysis of the table shows that in the presence of the extract at 1.25 mg/ml *on Enterococcus faecalis* and the MICs increased to 1000, 500, 250, and 31.2 µg/ml respectively for the antibiotics Erytromycin, Ampicillin, Amoxicillin and Ciprofloxacin. As for Pseudomonas aeruginosa at 2.5 mg/ml, the MICs in the presence of the extract increased to 1000, 500, 500, 125 µg/ml for the antibiotics Amoxicillin, Ampicillin, Erytromycin and Ciprofloxacin.

It results from Table 5, the characterization of the antibacterial power of the classic extract + antibiotic combination which is presented in Table 6 below.
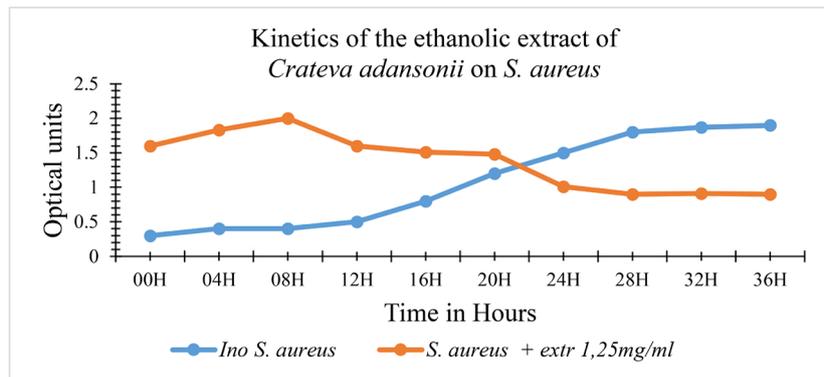

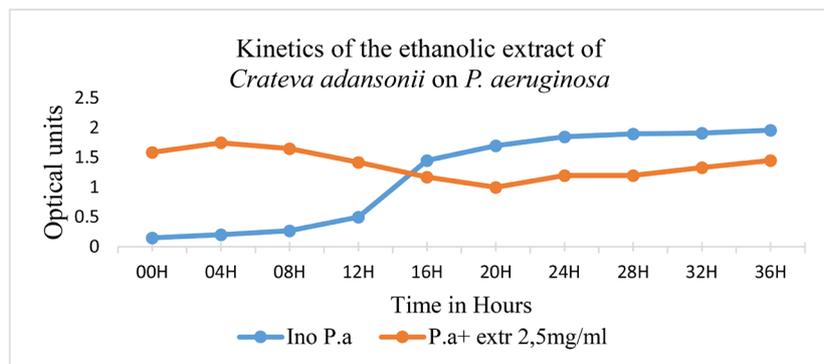**Figure 1.** Kinetics of action of the extract on *S. aureus.*

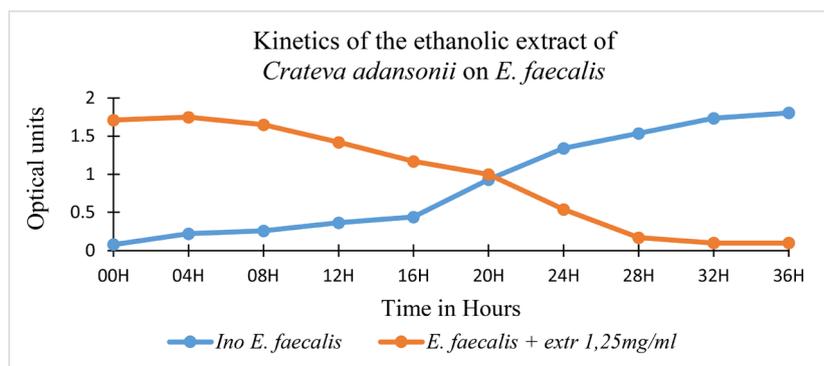**Figure 2.** Kinetics of action of the extract on *P. aeruginosa.*

Figure 3. Kinetics of action of the extract on *E. faecalis.*

Table 4. Result of the MICs of the ATBs.

DIFFERENT DOSES Of ATB	DOSES ATB (µg/ml)	*Enterococcus faecalis*					*Pseudomonas aeruginosa*				
		AMX	CIP	AMP	ERY	SXT	AMX	CIP	AMP	ERY	SXT
	1000	+	+	−	−	−	−	+	−	−	−
	500	−	+	−	−	−	−	+	−	−	−
	250	−	+	−	−	−	−	−	−	−	−
	125	−	−	−	−	−	−	−	−	−	−
	62.5	−	−	−	−	−	−	−	−	−	−
	31.2	−	−	−	−	−	−	−	−	−	−
	15.6	−	−	−	−	−	−	−	−	−	−
	7.8	−	−	−	−	−	−	−	−	−	−

(+): Inhibition of bacterial life, (−): No inhibition of bacterial life. ATB = Antibiotique; AMX = Amoxicilline; CIP = Ciprofloxacine; AMP = Ampicilline; ERY = Erythromycine; SXT = Cotrimoxazole.

Table 5. Result of the CMI combination ATB + Extract ATBs.

DOSES ATB + EXTRACT 1250 µg/ml	DOSES ATB (µg/ml)	*Enterococcus faecalis*					DOSES ATB + EXTRACT 2500 µg/ml	*Pseudomonas aeruginosa*				
		AMX	CIP	AMP	ERY	SXT		AMX	CIP	AMP	ERY	SXT
	1000	+	+	+	+	−		+	+	+	+	−
	500	+	+	+	−	−		−	+	+	+	−
	250	+	+	−	−	−		−	+	−	−	−
	125	−	+	−	−	−		−	+	−	−	−
	62.5	−	+	−	−	−		−	−	−	−	−
	31.2	−	+	−	−	−		−	−	−	−	−
	15.6	−	−	−	−	−		−	−	−	−	−
	7.8	−	−	−	−	−		−	−	−	−	−

(+): Inhibition of bacterial life, (−): No inhibition of bacterial life. ATB = Antibiotique; AMX = Amoxicilline; CIP = Ciprofloxacine; AMP = Ampicilline; ERY = Erythromycine; SXT = Cotrimoxazole.

Table 6. Fractional Inhibitory Concentrations (FIC).

Bacterial strains	AMX	CIP	AMP	ERY	SXT
Enterococcus faecalis	0.25	0.1248	0.5	1	-
Effect	synergistic	synergistic	additive	indifferent	Nd
Pseudomonas aeruginosa	1	0.25	0.5	0.5	-
Effect	indifferent	synergistic	additive	additive	Nd

AMX = Amoxicilline; CIP = Ciprofloxacine; AMP = Ampicilline; ERY = Erythromycine; SXT = Cotrimoxazole; Nd = not determined.

The combined extract-ATB effect has been shown to be synergistic (FIC < 0.5) for germs only for Amoxicillin, and Ciprofloxacin. As for other antibiotics, the effect is additive (0.5 ≤ FIC ≤ 1) or indifferent (1 ≤ FIC ≤ 4).

4. Discussions

The purpose of using reference strains in this manipulation is to validate our technique. To evaluate the antimicrobial activity, the ethanolic extract was chosen. This choice is justified by the fact that this solvent has no action on the growth of the germs tested [15]. It is also important to remember that the extraction solvent was evaporated and the extract dried before the start of the tests. We opted to do biological dilution tests. They have a major advantage compared to biological diffusion tests: the concentration of the test compound in the medium is defined. As a result, dilution tests are considered the method of choice for comparing MIC values [16]. The present study aims to evaluate the antibacterial activity of the ethanolic extract of *Crateva adansonii*, a plant with known medicinal value, commonly used in Benin.

To this end, the extracts were tested on six reference bacterial strains, namely *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* CIP 8039, *Enterococcus faecalis* ATCC 292012, *Escherichia coli* CIP 53126, *Pseudomonas aeruginosa*, *Methicillin-resistant Staphylococcus aureus* CIP 82118 and two hospital strains *Pseudomonas aeruginosa* and *Escherichia coli* isolated in urine and pus. According to the study antimicrobial properties and according to the degree of sensitivity of acuity, the sensitive germs in the presence of the ethanolic extract of *Crateva adansonii* are in the order *Staphylococcus epidermidis* CIP 8039 (0.625 mg/ml) *Staphylococcus aureus* ATCC 6538 (1.25 mg/ml), *Enterococcus faecalis* ATCC 292012 (1.25 mg/ml), *Pseudomonas aeruginosa* CIP 82118 (2.5 mg/ml) and *Methicillin-resistant Staphylococcus aureus* (5 mg/ml). In a comparative logic, these results agree with those of [17] [18] then of [19] who respectively indicated ranges of MIC varying from 1, 25 - 2.5 mg/ml, 6.25 - 12.5 mg/ml and 1.5 - 5 mg/ml with different types of *Crateva adansonii* extracts. This difference in result could be explained by the diversity of the extracts used but also by the diversity of the origins of the bacterial strains used. In contrast, Agboke *et al.* [18] obtained higher concentrations on the germs: *E. coli* (MIC: 12.5 mg·ml^{-1}), *S. aureus* (12.5 mg·ml^{-1}) and *K. pneumoniae* (25 mg·ml^{-1}) with the methanolic extract of *Crateva adansonii* leaves. This is probably due to the difference in solvent used.

The action of the extract on the germs is done according to a certain dynamic that is interesting to know. The general analysis of the graphs (**Figures 1-3**) of the evaluation of the action kinetics of the extract shows: Suspension curves over three phases. They are characterized by a phase of slight growth in contact with the extract. For another *Momordica charantia* plant, inhibition is observed after approximately 4 hours [20]. This is explained by the fact that this plant has a high antibacterial power because the MIC of the various germs exposed to the

effect of its ethanolic extract vary between 125 and 250 μg while that of *Crateva adansonii* is largely above (1250 μg/ml). This confirms the works of Adounkpe [21]. Nounagnon *et al.* [22]; Lagnika *et al.* [17]; and of Agbankpe *et al.* [19] who underlined the inhibitory antibacterial power of *Crateva adansonii*. The relative effectiveness of activity of the ethanolic extract of *Crateva adansonii* on the various germs under study, pushes for more research, by associating the use of plants with that of conventional antibiotics for the assessment of a possible reversion of bacterial resistance. From the analysis of the table it follows that in the presence of the extract at 1.25 mg/ml on *Enterococcus faecalis* and the MICs increased to 1000, 500, 250, and 31.2 μg/ml respectively for the antibiotics Erytromycin, Ampicillin, Amoxicillin and Ciprofloxacin. As for *Pseudomonas aeruginosa* at 2.5 mg/ml, the MICs in the presence of the extract increased to 1000, 500, 500, 125 μg/ml for the antibiotics Amoxicillin, Ampicillin, Erytromycin and Ciprofloxacin. In the presence of the extract, the sensitivities on Enterococcus faecalis went from 1000 to 250 μg/ml for amoxicillin, from 250 to 31.2 μg/ml for ciprofloxacin. It became sensitive to 500 μg/ml for ampicillin and 1000 μg for erythromycin. As for *Pseudomonas aeruginosa*, the MIC increased from 500 to 125 μg/ml for ciprofloxacin. It became sensitive to 1000 μg/ml amoxicillin and 500 μg/ml respectively for erytromycin and ampicillin. These results confirm those of Houéto *et al.* [20] who showed similar results with the ethanolic extract of *Momordica charantia* in contact with two germs *Pseudomonas aeruginosa* and *E. coli*. This explains the principle of the use of the extract + ATB observed in populations but increases the risk of intoxication in the absence of knowledge of the dosages and of the pharmacodynamic properties of these extracts. Many studies have also shown a significant reduction in the MIC of antibiotics, when combined with plant extracts [23] [24] [25]. It is therefore important to conduct studies on the toxicity of this plant in order to guarantee to the population the fearless use of this plant with antibacterial properties.

5. Conclusion

This study revealed that *Crateva adansonii*, a medicinal plant commonly used by the population, has antibacterial activity on the germs responsible for skin and digestive disorders, in particular *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Methicillin-resistant Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus feacalis*. In addition, the ingenious initiative to combine the ethanolic extract of *Crateva adansonii* with conventional antibiotics has revealed a reversal of the bacterial resistance of many germs.

Conflicts of Interest

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

References

[1] Hacini, N. and Djelloul, R. (2017) Study of the Antibacterial and Antifungal Activi-

ties of Oils of *Pistacia lentiscus l. International Journal of Applied Environmental Sciences*, **12**, 133-143. http://www.ripublication.com

[2] Balakrishna, K., Rath, A., Praveenkumarreddy, Y., Guruge, K.S. and Subedi, B. (2017) A Review of the Occurrence of Pharmaceuticals and Personal Care Products in Indian Water Bodies. *Ecotoxicology and Environmental Safety*, **137**, 113-120. https://www.ncbi.nlm.nih.gov/pubmed/27915141

[3] WHO (2002) Rapport sur la Santé dans le Monde 2002: Réduire les risques et promouvoir une vie saine. https://www.who.int/whr/2002/fr/.

[4] Olou, B.A., Bio, A., Deleke, E.I.K., Djego, G.J. and Sinsin, A.B. (2018) Connaissances Ethnobotaniques et Valorisation de Deux Plantes Antihypertensives (Carissa edulis L. et *Crateva adansonii* DC) au Sud et au Centre du Bénin (Afrique de l'Ouest). *International Journal of Biological and Chemical Sciences*, **12**, 2602-2614. https://www.ajol.info/index.php/ijbcs/article/view/183837

[5] OCDE (2003) Biotechnologie et durabilité: la lutte contre les maladies infectieuses. https://www.oecd.org/fr/sti/tech-emergentes/2713735.pdf

[6] N'tcha, C., Sina, H., Pierre, A., Kayodé, P., Gbénou, J.D. and Baba-Moussa, L. (2017) Antimicrobial Activity and Chemical Composition of Kpètè-Kpètè. A Starter of Benin Traditional Beer Tchoukoutou. *Biomed Research International*, **6**, 10-17. https://doi.org/10.1155/2017/6582038

[7] Reddy, K.S., Kumar, A.S. and Ganapaty, S. (2011) Evaluation of Hypoglycemic and Wound Healing Activities of Lantana Wightiana Wall Exgamble Leaves. *International Research Journal of Pharmacy*, **2**, 264-266. https://irjponline.com/admin/php/uploads/772_pdf.pdf

[8] Amoussa, A.M.O., Sanni, A. and Lagnika, L. (2015) Antioxidant Activity and Total Phenolic, Flavonoid and Flavonol Contents of the Bark Extracts of *Acacia ataxacantha. Journal of Pharmacognosy and Phytochemistry*, **4**, 172-178. http://www.phytojournal.com/vol4Issue2/Issue_Jul_2015/4-2-35.1.pdf

[9] Mamadou, R.S., Moussa, I., Sessou, P., Yèhouénou, B., Agbangnan, P.D.C., Illagouma, A.T., Abdoulaye, A., Sohounhloué, D.C.K. and Ikhiri, K. (2014) Etude phytochimique, activités antiradicalaire, antibactérienne et antifongique d'extraits de Sebastiania chamaelea (L.) Müll. *Journal de la Société Ouest-Africaine de Chimie*, **37**, 10-17. http://soachim.org/files/volume_37/2-%20R%20S%20%20Mamadou%20et%20al%20Vol%20037%202014%2010-%2017.pdf

[10] Traoré, Y., Ouattara, K., Yéo, D. and Doumbia, I. (2012) Recherche des activités antifongique et antibactérienne des feuilles d'Annona senegalensis Pers. (Annonaceae). *Journal of Applied Biosciences*, **58**, 4234-4242. http://www.m.elewa.org/JABS/2012/58/3.pdf

[11] Riwom, E.S.H., Nyegue, M.A., Foe, N.C., Silihe, K.K., Tamo, B.S.P. and Etoa, F.X. (2015) Antibacterial and Antioxidant Activities of Hydro-Ehanol Extracts of Barks, Leaves and Stems of *Annona muricata. American Journal of Pharmaceutical Sciences*, **3**, 126-131.

[12] Ara, C. and Asmatullah, A. (2011). Eratogenic and Embryotoxic Effects of Clomiphene Citrate in Developing Mice. *Asian-Australasian Journal of Animal Sciences*, **24**, 1053-1059. https://doi.org/10.5713/ajas.2011.10392

[13] Li, M., Zhang, H., Wang, J., and Pan, Y. (2012) A New Essential Protein Discovery Method Based on the Integration of Protein-Protein Interaction and Gene Expression Data. *BMC Systems Biology*, **6**, 6-15. https://bmcsystbiol.biomedcentral.com/track/pdf/10.1186/1752-0509-6-15

[14] Bassolé, I.H.N. and Juliani, H.R. (2012) Essential Oils in Combination and Their

Antimicrobial Properties. *Molecules*, **17**, 3989-4006.
https://doi.org/10.3390/molecules17043989

[15] Gbogbo, K. A., Amégninou, A., Yawo, A. W., Eyana, K. A., Hoekou, P. Y., Hoekou, P. Y., Batawila, K., Koumaglo, K. and Akpagana, K. (2013) Evaluation de l'activité antimicrobienne de Momordica charantia (cucurbitaceae), Psidium guajava (myrtaceae) et Pteleopsis suberosa (combretaceae). *European Scientific Journal*, **9**, 411-421. https://eujournal.org/index.php/esj/article/view/2240

[16] Rios, J.L., Recio, M.C. and Villar, A. (2006) Screening Methods for Natural Products with Antimicrobial Activity: A Review of the Literature. *Journal of Ethnopharmacology*, **23**, 127-149.

[17] Lagnika, L., Anago, E., Atindehou, M., Adjahoutonon, B. and Dramane, K. (2011) Antimicrobial Activity of *Crataeva religiosa* Forst against Bacteria Isolated from Thryonomys swinderianus Temminck. *African Journal of Biotechnology* **10**: 10034-10039. https://www.ajol.info/index.php/ajb/article/view/95870

[18] Agboke, A.A., Attama, A.A. and Momoh M.A. (2011) Evaluation of the Antimicrobial Activities of Crude Extract of *Cryptolepis sanguinolenta* and *Crateva adansonii* Leaves and Their Interactions. *Journal of Applied Pharmaceutical Science*, **1**, 85-89. https://www.japsonline.com/abstract.php?article_id=303

[19] Agbankpe, A.J., Dougnon, T.V., Bankole, S.H., Houngbegnon, O., Dahnouvlessounon, D. and Baba-Moussa, L. (2016) In Vitro Antibacterial Effects of *Crateva adansonii*, *Vernonia amygdalina* and *Sesamum radiatum* Used for the Treatment of Infectious Diarrhoeas in Benin. *Journal of Infectious Diseases and Therapy*, **4**, 1-7. https://www.omicsonline.org/open-access/in-vitro-antibacterial-effects-of-crateva-adansonii-vernonia-amygdalina-and-sesamum-radiatum-used-for-the-treatment-of-infectious-2332-0877-1000281.php?aid=74280

[20] Houéto, E.E.M., Johnson, R.C., Amoussa, A.M.O., Kpètèhoto, H.W., Mignanwandé, F.M.Z., Loko, F. and Lagnika, L. (2019) Antimicrobial Potency and Reversion of the Bacterial Resistance of Ethanolic Extract of *Momordica charantia linn*. *International Journal of Biology and Biotechnology*, **16**, 79-93.

[21] Adounkpe, M.F., Medehouenou, T.C.M., Klotoe, J.R. and Dougnon, V.T. (2018) Antibacterial Pharmacochemical Activity "*in Vitro*" of Total Alkaloid Extracts of *Crateva religiosa* G. forst. (Capparidaceae) versus Amoxicillin + Clavulanic Acid on Germs Responsible of Human Common Affections. *Journal of Medicinal Plants Studies*, **6**, 175-179.

[22] Nounagnon, M.S., Dah-Nouvlessounon, D., N'tcha, C., Legba, B., Baba-Moussa, F., Adjanohoun, A. and Baba-Moussa, L. (2018) Phytochemistry and Biological Activities of *Crateva adansonii* Extracts. *International Journal of Pharmacy and Pharmaceutical Sciences*, **10**, 62-67. http://dx.doi.org/10.22159/ijpps.2018v10i9.27197

[23] Emami, S., Shojapour, S., Faramarzi, M.A., Samadi, N. and Irannejad, H. (2013) Synthesis, *in Vitro* Antifungal Activity and *in Silico* Study of 3-(1,2,4-triazol-1-yl)flavanones. *European Journal of Medicinal Chemistry*, **66**, 480-488. https://www.sciencedirect.com/science/article/abs/pii/S0223523413003760?via%3Dihub

[24] Betoni, J.E., Mantovani, R.P., Barbosa, L.N., Di Stasi, L.C. and Fernandes, J.A. (2006) Synergism between Plant Extract and Antimicrobial Drugs Used on *Staphylococcus aureus* Diseases. *Memórias do Instituto Oswaldo Cruz*, **101**, 387-390. https://doi.org/10.1590/S0074-02762006000400007

[25] Atteia, H.G. and Hussein, E.A.M. (2014) *In Vivo* Antibacterial and Synergitic Effect of Some Plant EXTRACTS against *Staphylococcus aureus* and *Klebsiella pneumonia*. *Journal of Antimicrobials*, **129**, 338-346.