American Journal of Infectious Diseases and Microbiology, 2019, Vol. 7, No. 2, 57-64
Available online at http://pubs.sciepub.com/ajidm/7/2/1
Published by Science and Education Publishing
DOI:10.12691/ajidm-7-2-1

Antibiotics Resistance Profile and Antibacterial Activities of *Combretum micranthum* and *Combretum adenogonium* Extracts on Clinical Isolated *Vibrio cholera*

Bawa Boya[1], Théodora A. Ahoyo[2], Haziz Sina[1], Victorien T. Dougnon[3], Christine N'tcha[1], Durand Dah-Nouvlessounon[1], Delphine B. M. Amoussou[2], Charisse Sossa[2], Faustine K. Houssa[2], Lamine Baba-Moussa[1,*]

[1] Laboratoire de Biologie et de Typage Moléculaire en Microbiologie (LBTMM), Département de Biochimie et de Biologie Cellulaire, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, 05 BP 1604 Cotonou, Benin
[2]Génie de Biologie Humaine, Ecole Polytechnique d'Abomey-Calavi, Benin
[3]Unité de Recherche en Microbiologie Application et Pharmacologie des substances Naturelles (URMAPha), Université d'Abomey-Calavi, Bénin.
*Corresponding author: laminesaidbm@gmail.com

Received August 17, 2019; Revised September 24, 2019; Accepted October 25, 2019

Abstract Medical care has provided for the use of chemo-selective prophylaxis for doxycycline for patients, health personnel and contacts persons who have no contraindications. Therapeutic failures and the rising costs of treating infections caused by resistant bacteria call for alternative care options. To asses the antibacterial activity of *Combretum micranthum* and *Combretum adenogonium* extracts on multi-resistant *Vibrio cholerae* strains resulting from the 2012-2019 epidemic in Benin. Strains of *Vibrio cholerae* from the 2012-2019 outbreak and three reference strains (*V. cholerae* O1 URF-ECMI U67, *V. cholerae* O1 URF-ECMI U27 and *V. cholerae* O1 URF-ECMI U14) has been re-isolated on nutrient agar. Strains were identified using standard bacteriology methods (culture, biochemistry, serogroup). The study of antibiotic sensitivity was carried out according to the Kirby-Bauer technique. Strains were sensitive to *C. micranthum* and *C. adenogonium* extracts by the agar diffusion method. A total of 84.38% of the Vibrio cholerae strains were recovered. The strains showed resistance (100%) to amoxicillin + clavulanic acid, ceftazidime and oxytetracycline while they are predominantly sensitive to gentamecine (88.46%) and ofloxacin (88.46%). Strains were resistant to Erythromycin (97.56%) and doxycycline (99%). The rate of resistance to Trimethoprim / sulfamethoxazole is 70% of the strains tested. Resistance to doxycycline is 99% while 16.67% of strains tested are resistant to all antibiotics. Strains resistant to antibiotics were sensitive to *C. micranthum* and *C. adenogonium* extracts. All are sensitive to the alcoholic extracts of C. adenogonium and 75% are sensitive to the alcoholic extract of *C. micranthum*. The minimum inhibitory concentrations of the extracts vary between 2.5 mg / ml and 10 mg / ml and the minimum bactericidal concentrations between 2.5 mg/ml and 10 m/ml. The high percentage of resistance to doxycycline and Trimethoprim-sulfamethoxazole insists on reviewing the patient care's protocol. *C. micranthum* and *C. adenogonium* will be an alternative for treating cholera in the community.

Keywords: *Resistance, Vibrio cholerae O1, antimicrobial activities, C. micranthum, C. adenogonium*

Cite This Article: Bawa Boya, Théodora A. Ahoyo, Haziz Sina, Victorien T. Dougnon, Christine N'tcha, Durand Dah-Nouvlessounon, Delphine B. M. Amoussou, Charisse Sossa, Faustine K. Houssa, and Lamine Baba-Moussa, "Antibiotics Resistance Profile and Antibacterial Activities of *Combretum micranthum* and *Combretum adenogonium* Extracts on Clinical Isolated *Vibrio cholera*." *American Journal of Infectious Diseases and Microbiology*, vol. 7, no. 2 (2019): 57-64. doi: 10.12691/ajidm-7-2-1.

1. Introduction

Cholera is an acute diarrheal infection caused by ingestion of food or water contaminated with the bacillus *Vibrio cholerae.* It is estimated that every year, there are roughly 1.3 to 4.0 million cases, and 21 000 to 143 000 deaths worldwide due to cholera [1]. Africa remains the most affected continent, with 46% of cholera-affected countries in sub-Saharan Africa [2]. Most of the Gulf of Guinea countries including Benin have an ecological potential favorable to the development of El Tor *Vibrio cholera*, responsible for this seventh pandemic according to Janny [3]. This disease of uncleanliness and poor hygiene conditions occurs in communities with a low standard of living [4]. Surveillance and response are part of the strategy for the control of epidemic-prone diseases including cholera proposed by WHO in the country [5]. In Benin, this epidemic first appeared in 1970 and then became almost annual and usually occurs during the rainy season [6]. Patient care is essentially based on parenteral

rehydration. However, some authors argue that associated antibiotic therapy would accelerate healing and break the chain of transmission [7]. The selection pressure exerted by the use of antibiotics has fostered the emergence of resistant *Vibrio cholerae* strains in several countries in Africa as well as in other countries of the world [8,9,10]. Thus, faced with therapeutic failures and the frequency of isolation of cholera strains in Benin, it becomes imperative to search for new antibacterial molecules; these must be, if possible, both effective, well tolerated and more economically accessible to the population. Plants, which have already provided major therapeutic molecules to the medicine, such as aspirin, morphine, quinine or taxol, offer a real potential for the search for molecules with antibacterial activity [11]. *Combretaceae* have several uses in Africa thanks to their metabolic activity [12], anti-inflammatory, antifungal [13], antibacterial [14], analgesic [15] and antioxidant [16]. The objective of this work is to assess the antibacterial activity of *Combretum micranthum* and *Combretum adenogonium* extracts on multi-resistant *Vibrio cholerae* strains resulting from the 2012-2019 epidemic in Benin.

2. Material and Methods

2.1. Type of Study

This is a cross-sectional study of strains of *Vibrio cholerae* isolated during the 2012 to 2019 outbreaks. The sensitivity of *Vibrio cholerae* O1 strains to conventional antibiotics and the sensitivity of strains to *C. micranthum* and *C. adenogonium* extracts was carried out on these strains.

2.2. Re-insulation of Strains of *Vibrio cholerae*

2.2.1. Resuscitation of the Strains

The re-isolation of *V. cholerae* strains was done according to the method of Rodier *et al.* [17]. To do this, three reference strains (*V. cholerae O1 URF-ECMI U67, V. cholerae O1 URF-ECMI U27 and V. cholerae O1 URF-ECMI U14*) and 64 strains were cultured in an alkaline nutrient broth (alkaline peptone water at 30g/l NaCl) for 24 h at 37 ° C. The veil developed on the surface of the medium was seeded on TCBS (Thiosulfate Citrate Bile Sucrose) medium. Thus, after 24 h of incubation at 37°C, the characteristic *V. cholerae* colonies were subcultured onto alkaline nutrient agar at 2% NaCl and incubated at 37°C to obtain pure strains. From suspicious colonies on GNA: fresh state, Gram stain, oxidase reaction, sugar fermentation and agglutination reaction were performed.

2.2.2. Purification

2.2.2.1. Elimination of *Pseudomonas spp.* and enteric bacterium

The agar slope in the Middle TSI (Triple Sugar/Iron/agar) was used to eliminate Pseudomonas spp. and some Enterobacteria. The reactions of *V. cholerae* on TSI medium which contains sucrose in addition to glucose and lactose gives type A / A reactions (alkaline slope, acid pellet), without gas, nor H_2S. Inoculate the slopes of TSI plates by pitting the pellet and streaking the surface of the medium. Incubate at 35-37 ° C and examine after 18-24 hours. The caps of each tube should not be tightly tightened during incubation, and this is especially important for TSI slopes. When the caps are too tight, anaerobic conditions appear in the TSI tube, and characteristic reactions of *V. cholerae* may not occur. This will result in an inaccurate reaction.

2.2.2.2. Oxidase Test

Place a colony on a strip of oxidase previously soaked with sterile distilled water placed on a sterile tear; after 30 seconds the positive reaction resulted in a dark purple color.

2.2.2.3. Agglutination on Blade

Serogroupage of *Vibrio cholerae* is carried out from isolated colonies on an alkaline GNA agar medium according to the principle of Bio-Rad. On one slide, 1 drop of saline water and next to a drop of antiserum. Then an öse from a pure and fresh culture of *Vibrio cholerae* was harvested. These bacteria were suspended in the drop of saline water taking care to make a homogeneous suspension by progressive addition of bacteria in the serum. Then a second step of the culture was suspended in the drop of antiserum taking the same precautions. The blade was shaken by a slight rotational movement. Observe the mixture with the naked eye over a dark surface or above a concave mirror. A positive reaction results in the appearance, with the antiserum, of agglutination in less than 30 seconds.

2.3. Preparation of Plant Extracts

2.3.1. Aqueous Extract

Fifty grams of extract powder was macerated in 500 ml of distilled water on a Bioblock Scientific Fisher Stuart shaker for 48 hours at room temperature. The homogenate obtained was filtered twice on hydrophilic cotton and once on Whatman No. 1 paper (Qualitative Circles 150 mm Cat No. 1001 150). This filtrate was then dried at 45°C in the oven and the residue thus obtained represents the total aqueous extract ready for use.

2.3.2. Successive Extraction with Ethanol

The extraction method used is an adaptation of the protocol used in the work of Sanogo et al. [18] and N'Guessan et al. [19]. It has the advantage of putting the powder correctly in contact with the solvent through continuous agitation. A mass of 100 g of plant organ powder was macerated in one liter of ethanol 96 ° with continuous stirring for 48 hours. The mixture was filtered three times on hydrophilic cotton and then once on Whatman No. 1 filter paper. The 1/5 of the filtrate obtained with ethanol was evaporated at a temperature of 40°C in an oven until a dry mass was obtained which represents the ethanolic extract.

2.4. Antibiotic and Plant Extract Sensitivity Test

The antibiogram was performed for sensitivity tests with 54 strains of *Vibrio cholerae O1* from the 2012 to 2019 epidemic in Benin. These strains were provided by

the national laboratory of the Ministry of Health of Benin. Three reference strains (*V. cholerae O1 URF-ECMI U67, V. cholerae O1 URF-ECMI U27 and V. cholerae O1 URF-EC14 U14*) were used as controls.

2.5. Susceptibility of *Vibrio cholerae* Strains to Eight Antibiotics

For susceptibility testing, 3 reference strains (*V. cholerae O1 URF-ECMI U67, V. cholerae O1 URF-ECMI U27 and V. cholerae O1 URF-ECMI U14*) and 54 strains were cultured in a nutrient broth. alkaline (alkaline peptone water at 30 g/l NaCl) for 24h at 37°C. The veil developed on the surface of the medium was seeded on TCBS (Thiosulfate Citrate Bile Sucrose) medium. After incubation for 24 h at 37°C., the characteristic colonies of *Vibrio cholerae* were subcultured onto alkaline nutrient agar (GNA) at 2% NaCl and incubated at 37°C. to obtain pure strains. An antibiogram was systematically performed on these strains according to the Kirby-Bauer agar diffusion technique. The reading and the interpretation were made according to the criteria of the Antibiogram Committee of the French Society of Microbiology [20]. Eight antibiotic disks were tested: Amoxicillin / clavulanic acid (AMC, 30 µg), oxytetracycline (OT, 30 µg), sulfamethoxazole-trimethoprim (SXT, 25 µg), ceftazidime (CAZ, 30 µg), gentamicin (G, 10 µg), doxycycline, erythromycin (E, 15 µg); ofloxicin (OFX: 10 µg).

2.6. Susceptibilities of *Vibrio cholerae* Strains to Plant Extracts

In addition to conventional antibiotics, aqueous and ethanolic extracts of *C. micranthum* and *C. adenogonium* leaves were tested by the well diffusion method Bauer et al [18]. Pre-culture of *V. cholerae* was performed in Muller Hinton medium broth and incubated for 18h-24h at 37°C. One milliliter of the second decimal dilution of the 18-24 h pre-culture was flooded with a Petri dish containing the appropriate culture medium (MH). After seeding, the wells were thoroughly impregnated with 30 µl of plant extract of concentration 20 mg/ml. The impregnated cans were left for 15 to 30 min at room temperature (25°C±2°C) for pre-diffusion of the substances before being incubated at 37°C [21]. The diameters of any zones of inhibition were measured using a graduated rule after an incubation time of 24 h to 48 h. For each extract, the experiment was performed in duplicate.

2.7. Determination of the Minimal Inhibition Concentration of Plant Extracts

MICs were determined by the tube dilution method used by Dah-Nouvlessounon et al. [22]. In a series of 10 test tubes numbered T1 to T10, 1ml of an extract solution at different concentrations ranging from 20-0.039 mg / ml respectively were introduced into the tubes ranging from T1-T10. To each tube containing 1 ml of extract was added 1 ml of inoculum whose turbidity was adjusted to 0.5 Mc Farland (10^8 CFU / ml) and returned to 10^6 CFU/ml in Mueller-Hinton broth twice concentrated. After 24h incubation at 37°C, the bacterial growth in each tube, which results in turbidity was examined. The MIC of an extract against a given strain corresponds to the smallest concentration showing no visible growth in the naked eye.

2.8. Determination of Minimal Bactericidal Concentration of Plant Extracts

They were determined by the method of seeding on agar medium according to the method used by Dah-Nouvlessounon et al. [22]. Referring to the MIC results, all non-growth tubes were inoculated aseptically on MH agar medium and incubated at 37°C for 24 h. The lowest concentration of extract showing no visible growth is considered Minimal Bactericidal Concentration.

2.9. Data Analysis

The data entry and analysis was done in Excel Spreadsheet 2013. The percentage of resistance was calculated for each antibiotic by dividing the frequency of resistant bacteria by the number of bacteria tested. The data analyzes were done using the software.

3. Results

During re-isolation, 84.38% of *Vibrio cholerae* strains from the 2012-2019 outbreak were recovered.

3.1. Resistance of *Vibrio cholerae* O1 to Antibiotics

The susceptibility of *V. cholerae* strains varies with antibiotics (Figure 1). All strains showed resistance (100%) to amoxicillin + clavulanic acid, ceftazidime and oxytetracycline while they are predominantly sensitive to gentamecine (88.46%) and ofloxacin (88.46%). Strains were also resistant to sulfamethoxazole-trimethoprim (70%), erythromycin (97.56%) and doxycycline (99%).

From these observations, we can draw five different profiles (Table 1) according to their capability to resist or not to the tested antibiotics.

3.2. Antibacterial Activity of *Vibrio cholorae* in the Presence of the Extracts

Table 2 shows the diameters of inhibition of *V. cholerae* vis-à-vis strains of extracts. It appears that all extracts inhibit the proliferation of most *V. cholerae* without any persistence in time and the nature of the extract. All the reference strains are sensitive to the alcoholic extracts of *C. adenogonium* and 1/3 are sensitive to the alcoholic extract of *C. micranthum*.

Table 3 shows the inhibition diameters of the extracts with respect to *Vibrio cholerae* strains. It appears that all extracts inhibit the proliferation of most *Vibrio cholerae* without any persistence in time and the nature of the extract. It is noted that 100% of strains are sensitive to ethanolic extracts of *C. adenogonium* and 75% are sensitive to the ethanolic extract of C. *micranthum*. In addition, 95.83% that are sensitive to aqueous extracts of *C. adenogonium* and 75% are sensitive to the aqueous

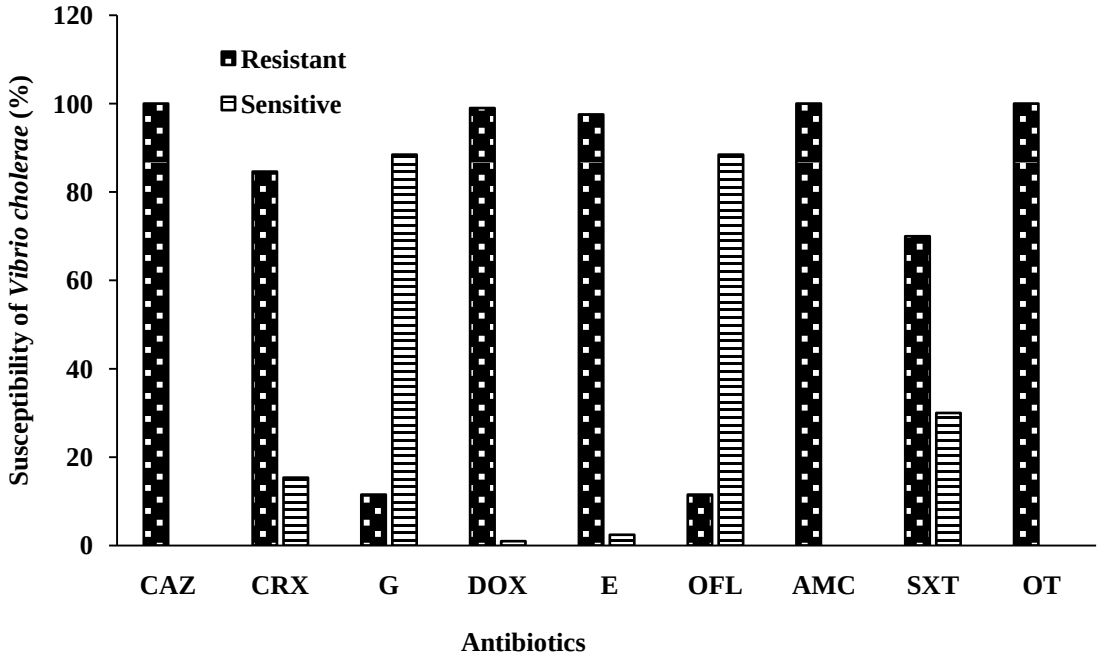
extract of *C. micranthum*. The strains of Profile 4 (W1, W5, W11, W14 etc.) are all sensitive to extracts.

3.3. Minimal Inhibitory Concentration

The Minimum Inhibitory Concentrations obtained are variable depending on the types of extract and strains (Figure 2). The minimum inhibitory concentrations of the extracts vary between 2.5 mg / ml and 10 mg / ml. There is still a greater inhibitory concentration (2.5 ± 0 mg / ml) on the strains of profile 1, 3 and 4. In addition, a greater sensitivity is observed in the presence of the strains of profiles 1, 2 and 3 regardless of the type of extract.

3.4. Minimal Bactericidal Concentration

Minimum Bactericidal Concentrations (MBC) vary with strain and type of extract (Figure 3). Minimum bactericidal concentrations between 2.5 mg / ml and 10 mg / ml. A higher sensitivity of tested *Vibrio cholerae* strains resulting from profile 1 (CMB = 2.5 mg / ml) independently of the type of extract used.



CAZ: Ceftazidime, CRX: Cefuroxime; G: Gentamicin; DOX: Doxycycline; E: Erythromycin; OFX: Ofloxicin; AMC: Amoxicillin/flunarizine acid, SXT: Sulfamethoxazole-trimethoprim, OT: Oxytetracycline

Figure 1. Susceptibility of *Vibrio cholorae* strains to antibiotics

Table 1. Distribution of resistance profiles of *Vibrio cholorae* strains

Profile of the Strains	Number of strains (%)
1-CAZ^{R} CRX^{R} G^{S} E^{R} DOX^{R} OFL^{S} AMC ^{R} SXT^{R} OT^{R}	21 (38.89)
2-CAZ^{R} CRX^{R} G^{S} E^{R} DOX^{R}OFL^{S} AMC ^{R} SXT^{S} OT^{R}	11 (20.37)
3-CAZ^{R} CRX^{S} G^{S} E^{R} DOX^{R}OFL^{S} AMC ^{R} SXT^{S} OT^{R}	4 (7.40)
4-CAZ^{R} CRX^{R} G^{R} E^{R} DOX^{R}OFL^{R} AMC ^{R} SXT^{R} OT^{R}	9 (16.67)
5-CAZ^{R} CRX^{S} G^{S} E^{R} DOX^{S}OFL^{S} AMC ^{R} SXT^{R} OT^{R}	9 (16.67)
Total	54 (100)

CAZ: Ceftazidime, CRX: Cefuroxime; G: Gentamicin; DOX: Doxycycline; E: Erythromycin; OFX: Ofloxicin; AMC: Amoxicillin/flunarizine acid, SXT: Sulfamethoxazole-trimethoprim, OT: Oxytetracycline.

Table 2. Susceptibility of reference strains of *Vibrio cholerae* to *C. micranthum and C. adenogonium* extracts

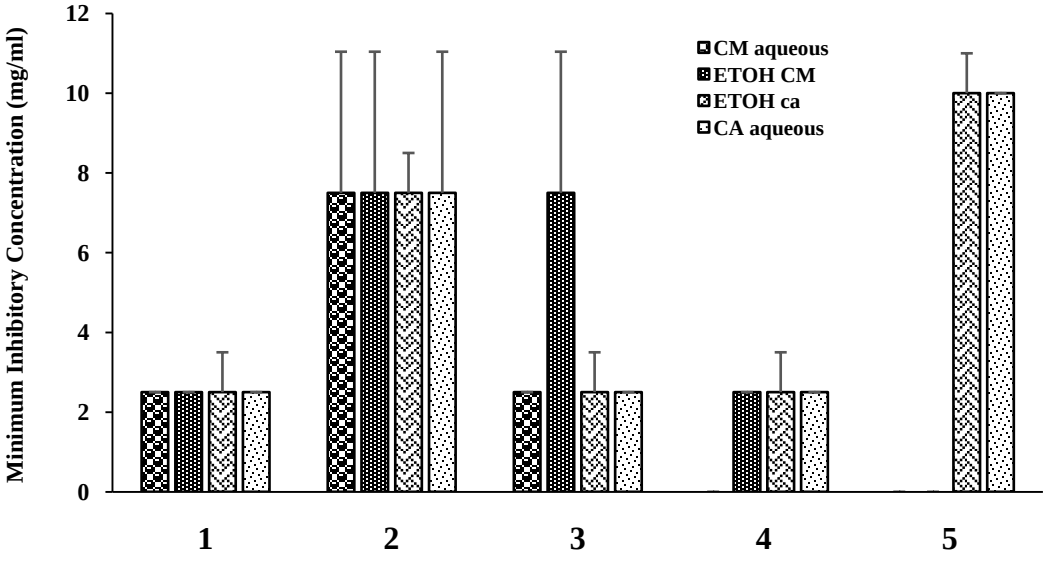
Reference Strain	Inhibition diameter (mm)							
	Aqueous CM		ETOH CM		Aqueous CA		ETOH CA	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
U14	9±0,7	10±0	13±1,4	12±0,7	12±1,4	12±0,7	12±0,7	12±0
U27	0	0	0	0	18±0,7	18±0,7	20±0,7	19±0,7
U67	0	0	0	0	14±0,7	14±0,7	12±0,7	12±0,7

Average± standard deviation; Aqueous CM: aqueous extract of *C. micranthum;* ETOH CM: ethanolic extract *C. micranthum;* Aqueous CA: aqueous extracts of *C. adenogonium*; ETOH CA: ethanolic extracts of *C. adenogonium.*

Table 3. Sensitivity of clinical strains to *C. micranthum* and *C. adenogonium* extract

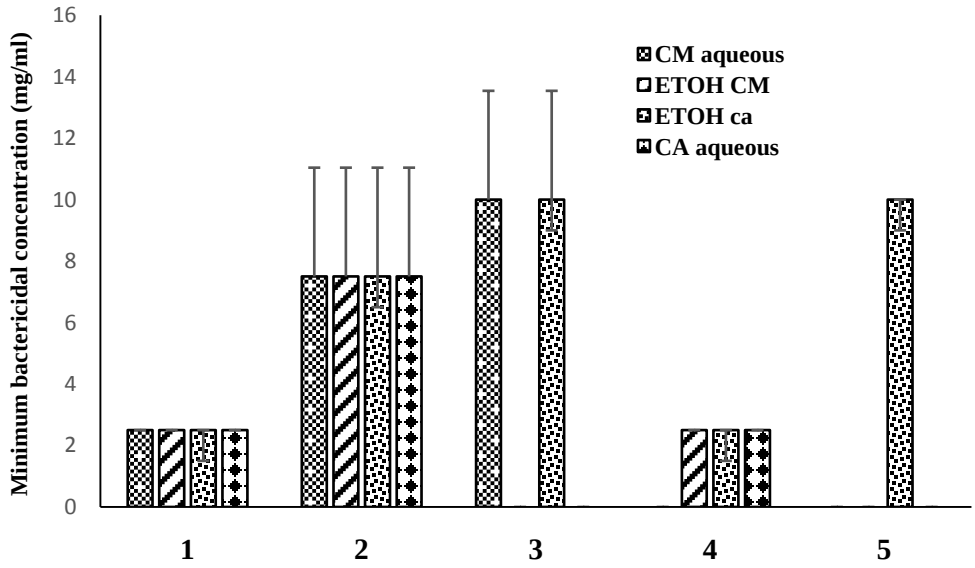
Inhibition Diameter (mm)								
Strains of *Vibrio cholerae*	CM aqueous		ETOH CM		CA aqueous		ETOH ca	
	24h	48h	24h	48h	24h	48h	24h	48h
W1	11±0.7	12±0.7	12±1.4	13±2.8	12±1.4	15±0.7	12±0.7	15±0.7
W11	10±0	10±0	10±0	9±0	11±0	13±2.8	12±0	12±0
W5	10±0.7	10±0.7	14±1.4	13±2.8	15±1.4	15±0	16±1.4	15±1.4
W17	0	0	0	0	13±2.1	15±2.1	12±2.1	15±2.1
W18	10±0	9±0	10±0	10±0	12±0	12±2.1	11±0	12±0.7
W19	10±0	9±0	10±0	10±0	12±1.4	11±0	10±0	10±0
W33	0	0	0	0	10±0	10±0	10±0	11±0
W42	0	0	12±0	12±0	15±0.7	16±2.1	14±2.1	15±1.4
W20	10±0	12±2.1	10±0	10±0	10±0	10±0	10±0	11±0.7
W21	12±1.4	12±1.4	0	0	10±0	11±0.7	12±0	10±0
W22	0	0	0	0	0	0	12±0	12±0
W2	0	9±0	13±2.8	15±0.5	13±0	15±0.7	13±1.4	15±0.6
W3	7±0.7	7±0.7	6±0.7	6±0.7	14±0.7	13±0.7	13±0.7	15±0.7
W9	11±0.7	0	12±2.8	10±0	14±2.8	12±0.7	11±2.8	10±0
W12	12±0	11±0	12±0	7±0	13±0	14±1.4	13±0	10±0
W13	0	8±0	7±0	7±0	13±0	13±0	13±0	10±0
W14	10±0	9±0	12±0	9±0.7	15±0.7	15±0.7	12±0	13±0.7
W16	6±0.7	0	10±0.7	19±2.1	15±0.7	15±0.7	13±0.7	12±0.7
W8	10±0	11±0.7	10±0	10±0	10±0	10±0	10±0	12±0.7
O1	10±0	10±0	10±0	10±0	15±0.7	15±0.7	12±0	12±0
O3	12±1.4	12±1.4	10±0	10±0	15±2.1	15±2.1	12±2.1	12±2.1
O9	10±0	10±0	12±0	12±0	12±2.1	12±2.1	13±1.4	13±1.4
O11	10±0	10±0	0	0	19±0	19±0	19±0	19±0
O12	10±0	10±0	0	0	15±0	15±0	15±0	15±0

Average± standard deviation; Aqueous CM: aqueous extract of *C. micranthum;* ETOH CM: ethanolic extract of *C. micranthum;* Aqueous CA: aqueous extracts of *C.* a*denogonium*; ETOH CA: ethanolic extracts of *C.* a*denogonium.*



CM aqueous: aqueous extract of *C. micranthum;* ETOH CM: ethanolic extract of *C. micranthum*; CA Aqueous: aqueous extracts of *C.* a*denogonium*; ETOH ca: ethanolic extracts of *C.* a*denogonium.*

Figure 2. Minimal Inhibitory Concentrations of extracts on clinical strains according to the tested profiles.



CM aqueous: aqueous extract of *C. micranthum;* ETOH CM: ethanolic extract of *C. micranthum*; CA Aqueous: aqueous extracts of *C.* a*denogonium*; ETOH ca: ethanolic extracts of *C.* a*denogonium*

Figure 3. Bactericidal Minimal Concentration of extracts on clinical strains according to the tested profiles

4. Discussion

Our objective was to evaluate the antibacterial activity of *Combretum micranthum* and *Combretum adenogonium* extracts against *Vibrio cholerae* strains from the 2012-2019 epidemic in Benin. During re-isolation, 84.38% of *V. cholerae* strains from the 2012-2019 outbreak were recovered. The death of some strains may be due to the culture medium of conservation, temperature and duration. We observed these phenomena in the strains which are from 2012 to 2014. We noted the loss of some strain of 2016. The death of these bacteria could be explained by the fact that *V. cholerae* strains were kept in skim milk. Skim milk contains 1 to 5 percent of a pure Lactobacillus acidophilus culture. These bacteria could make the environment when conditions are favorable which will cause the death of *V. cholerae* strains

Resistance rates of *Vibrio cholerae* O1 isolates varied according to conventional antibiotics (Figure 1). The resistance to doxycycline is 99% and the resistance to Trimethoprim/sulfamethoxazole is 70%. These molecules are widely used in the treatment of cholera. This could be due to the anarchic use and most often by self-medication of this molecule by the populations. Sulfametoxazole-trimethoprim resistance is lower than in other countries. It should be noted that high rates have been reported in Senegal from 2004-2006 (90.3%) [8], to Iran in 2009 (95%) 2009 [23]. In Ghana, the genotypic characterization of *V. cholerae* has revealed a transposon of SXT and a class-29 integron. These results show that this antibiotic should no longer be used in the treatment of cholera. Indeed, this molecule is often prescribed in the treatment of gastroenteritis and chemoprophylaxis in people infected with HIV. Studies have shown that 90% of subjects are asymptomatic carriers or have a common gastroenteritis. Taking this drug would participate in the selection and dissemination of emerging resistant strains in the population [10]. In addition, the use of sulfonamide-based molecules in the treatment of malaria could be the cause of cross-resistance [23]. The resistance to doxicycline observed in our study was contrary to those obtained by Manga et al [9]. In Senegal where all strains tested were sensitive to doxycline. This may be due to the chemo-selective prophylaxis for doxycycline that has been administered to all accompanying patients and to health personnel who have no contraindications in Benin. This resistance could also be explained by the inappropriate prescription of these antibiotics by health workers in health centers in health care settings without laboratory evidence. For erythromycin, resistance was 97%. Our results are superior to those obtained by Shah et al [24] in Pakistan where they found a 15% resistance to erythromycin. This could be explained by the fact that the strains they used are from 1998-1999 but our strains are from 2012-2019. The resistance of *Vibrio cholerae* O1 to oxytetracycline (100%), Amoxicillin + clavulanic acid (100) was also observed. However, *Vibrio cholerae* O1 also showed variable susceptibility to *C. micranthum and C. adenogonium* extracts. The ethanolic extracts of *C. micranthum and C. adenogoniu*m show stronger antibacterial activity than the aqueous extract (Table 2). These results corroborate those of Hamid and Aiyelaagbe [25] who showed that the Alafia barteri alcoholic extract better inhibits the in vitro growth of various bacterial strains (*S. aureus, E. coli, Pseudomonas aeruginosa and Candida albicans*) at the same concentrations ranging from 25-200mg / ml. The alcoholic extract is then more active and better concentrate the antibacterial active ingredients contained in the plant than the aqueous extracts. The antibacterial activity of this fraction is the most important of the two fractions of *C. micranthum* and *C. adenogonium* used in this study. This activity is observed both with the susceptible strains and the resistant and multi-resistant strains (Figure 1, Table 1, Table 2).

In Benin, the decoction or infusion of leaves, sometimes with the bark of these plants, are simply absorbed as

beverages for treatment in fever and malaria. They are also used in the steam bath. Decoction of stem and root powder are easily treated. It is noted that antibiotic resistant strains are susceptible to the extract. All resistant strains are sensitive to the ethanoid extract of *Combretum adenogonium*. Therefore, these plant extracts were a healthy and sustainable alternative to overpriced conventional antibiotics and the misuse of antimicrobial resistance. In addition, according to Guevara *et al* [26] in Peru, the decoction of *Malus sativa* and *Cydenia oblonga* showed a bactericidal effect for its acidity and avocado stone (*Persea gratissima*), a late bactericidal effect. The infusion of tea and the decoction of *Punica granatum* bark showed the best bactericidal effect. Thakurta *et al* [27] also tested the susceptibility of *V. cholerae* to plant extracts. They found that serotypes O1, O139, non-O1 and non-O139 were inhibited by extracts of neem (*Azadirachta indica*). In addition, Akinsinde and Olukoya [28] observed the effects of vibrios from medicinal plants in Nigeria and, in one study, the bactericidal in vitro of 14 plant species on *V. cholerae*, Guevara et al [26] proposed Alternate of *Punke granatum*, on the Alternate for Chopera.

5. Conclusion

Since effective management of cholera is based primarily on replacement of fluid loss and antibiotic therapy, the circulation of multidrug resistant *Vibrio cholerae* strains can compromise the efficacy of probabilistic treatments and explain treatment failures. Therefore, it is important and urgent to set up community and prescriber awareness actions on the reasoned use of antimicrobials in order to mitigate the expansion of resistant strains in Benin. The aqueous and organic extracts of these plants had variable antimicrobial activities against the in vitro growth of clinical *Vibrio cholerae* strains tested. Our results will contribute to improving the living conditions of our populations and set up a validation of the use of *C. adenogonium*, *C. micranthum*, in traditional medicine.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Ali, M., Nelson, A.R., Lopez, A.L., and Sack, D, "Updated Global Burden of Cholera in Endemic Countries". *PLoS Neglected Tropical Diseases*, 2015; 9(6): e0003832.

[2] Mengel, M.A., Delrieu, I., Heyerdahl, L., and Gessner, B.D, "Cholera outbreaks in Africa". *Current Topics in Microbiology and Immunology*. 2014; 379: 117-144.

[3] Yatala, T.N, "Evaluation of the knowledge level of women in the area of health of Kituku on the pathways of contamination of diarrheal diseases". *In* Revue University without Borders for an Open Society of the University Production Remote House. 2014, 2: 128-132.

[4] Dao, S., Konaté, I., Alassane, O.A., Sacko, M., Maiga, I., Toure, K. et al., **"**The epidemics of cholera in Mali from 1995 to 2004. *Public Health*. 2009, 21: 263-269.

[5] WHO-AFRO. "Regional Framework for the implementation of the global strategy in the fight against cholera 2018-2030". 2018*/AFR/RC68/7*.

[6] Makoutode, M., Diallo, F., Mongbo, V., Guevart E., Bazira, L, "The response to the cholera epidemic of 2008 in Cotonou." *Public Health.* 2010, 22: 425-435.

[7] Fournier, J.M., "Cholera Medical Encyclopedia surgery." *Infectious Diseases*, 8-026-F-10, 1996, 5p.

[8] Adabi, M., Bakhski, B., Goudarzi, H., Zahraei, S.M., Pourshafi, M.R., "Distribution of class 1 integron and sulfamethoxazole trimethoprim constin in *Vibrio cholerae* isolated from patients in Iran." *Microbiology and Drug Resistance.* 2009; 15(3): 179-184.

[9] Manga, N.M., Ndour, C.T., Diop, I.T.S., Dia, N.M., Ka-Sall, R., Diop, B.M., Sow A., Sow P.S. **"**Cholera in Senegal from 2004 to 2006: lessons learned from the successive outbreaks. *Medecine Tropicale. 2008; 68(6): 589-92.*

[10] Opintan, J.A., Newman, M.J., Nsiah-Poodoh, O.A., Okeke, I.N, **"***Vibrio cholera* O1 from Accra, Ghana carrying a class 2 integron and the SXT element". *Journal of Antimicrobial and Chemotherapy*. 2008; 62(5):929-933.

[11] Barchan, A., Bakkali, M., Arakrak, A., Laglaoui H.A.S., "Antibacterial effect and anti-biofilm of three species of Mentha: *Mentha spicata*, *Mentha pulegium* and *Mentha piperita*." *Herbal Medicine. 2015, 9.*

[12] de Morais, L.G.R., Sales, I.R., Caldas, F.M.R., Jesus, N.Z., De Sousa, F.H., Barbosa-Filho, J.M., et al., "Bioactivities of the genus *Combretum* (Combretaceae): *a review". Molecules, 2012, 17(8): 142-206.*

[13] Eloff JN., Katerere dr., McGaw, Lj. "The biological activity and chemistry of the Southern African Combretaceae". I *Ethnopharmacol*, 2008, 119 (3): 686-699.

[14] Nounagnon, S.M., N'tcha, C., Sina, H., Noumavo, A.P., Dah-Nouvlessounon, D., Assogba, M.R.F, et al., Antimicrobial activities of *Combretum micranthum* extracts on *Staphylococcus aureus* strains isolated from skin infections and some reference strains. *Asian Journal of Plant Science and Research*, 2016, 6(4): 40-47.

[15] Ojewole, J.A., "Analgesic and antiinflammatory effects of mollic acid glucoside, has 1 alpha-hydroxycycloartenoid extractive saponin from soft Combretum R. Br. ex G. Don (Combretaceae) leaf. *Phototherapy Research*, 2008; 22 (1): 30-35.

[16] Touré, A., Xu, X., Michel, T., Bangoura, M., "*In vitro* Antioxidant and radical scavenging of Guinean kinkeliba leaf (*Combretum micranthum* G. Don) extracts". *Natural Product Research*, 2011, 25 (11): 1025-1036.

[17] Rodier, J., Legube B., Merlet, N., "L'Analyse de l'eau, " *9ème édition DUNOD, 2009, 749-775.*

[18] Sanogo, R., Diallo, D., Diarra, S., Ekoumou, C., and Bougoudogo, A, "Antibacterial activity and anodyne of two traditional recipes used in the treatment of urinary tract infections and cystitis in Mali." *Medical MALI.* 2006, 21: 18-24.

[19] N'Guessan K., Kadja B., Zirihi G. N., Traoré D. and Aké-Assi L. "Phytochemical screening of some medicinal plants in Côte d'Ivoire used to Country Krobou (Agboville, Ivory Coast)." *Science and Nature*, 2009, 6, 1-15.

[20] CA-SFM (Commission de l'Antibiotique de la Société Française de Microbiologie). "Recommandations 2018 du Comité de l'Antibiogramme de la Société Française de Microbiologie". 2018; 144 p.

[21] Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M., "Antibiotic susceptibility testing by a standardized single disk method." *American Journal of Clinical Pathology, 1996, 45, 493-496.*

[22] Dah-Nouvlessounon, D., Adoukonou-Sagbadja, H., Diarrassouba, N., Sina, H., Adjonohoun , A., Inoussa M.R., et al. "Phytochemical analysis and biological activities of *Cola nitida* bark." Biochemistry Research International. 2015, 1-12.

[23] Mandomando, I., Espasa, M., Vallès, X., Sacarlal, J., Sigaupe, B., Ruiz, I., Alonso, P, "Antimicrobial resistance of *Vibrio cholerae* O1 serotype ogawa isolated in Manhiça District hospital, southern Mozambique." Journal of *Antimicrobial and Chemotherapy.* 2007, 60(3): 662-664.

[24] Shah, R., Parveen, G., Shoukat, M., Khalid , S., Hameed, A.**,** "Isolation, identification, characterization and antibiotic susceptibility of Vibrio cholera during 1998-99. *Interantional Journal of Bioscience. 2017, 11(3), 135-147.*

[25] Aiyelaagbe, O.O, Hamid, A.A, Fattorusso, E., Taglialatela-Scafati, O., Schröder, H.C., and Müller, W.E., "Cytotoxic activity of crude extracts as well as of pure components from jatropha species, plants used extensively in African Traditional Medicine." *Evidence-Based Complementary and Alternative Medicine, 2011, Article ID 134954, 7 page.*

[26] Guevara, J.M, Chumpitaz, J., Valencia, E., "The in vitro action of plants we *Vibrio cholerae*. *Revista de Gastroenterologia del Peru.* 1994; 14:27-31.

[27] Thakurta, P., Bhowmik, P., Mukherjee, S., Hajra, T.K., Patra, H.A.S, Bag, P.K., "Antibacterial, antisecretory and antihemorrhagic activity of *Azadirachta indica* used to treat cholera and diarrhea in India". *Journal of Ethnopharmacology.* 2007; 111: 607-12.

[28] Akinsinde, K.A., Olukoya D.K., "Vibriocidal activities of some local herbs". *Journal of Diarrheal Disease Research. 1995;* 13: 127-129.