# Ethnomedicine, improved traditional medicine from *Cocos nucifera* water and evaluation of antibacterial activity on four bacterial strains in Center, Cameroon

# GEORGES MAXIME LAMY LAMY<sup>1,</sup>, JULIENNE LOUISE NGO LIKENG<sup>2,3</sup>, ROSETTE CHRISTELLE NDJIB<sup>1</sup>, SANDRINE EMILIE NGO NDOUNGA<sup>2</sup>, L'AFRICAINE BLANCHE ELOMO<sup>4</sup>, SALIOH M. MBUH<sup>1</sup>, LEILA SANDRA NNANGA<sup>1</sup>, MAHAMA<sup>5</sup>, JACQUES BRUNO NGOTTA BIYON<sup>6</sup>, KARL HARMSEN<sup>7</sup>, EMMANUEL NNANGA NGA<sup>1,8</sup>, ALEMBERT TIABOU TCHINDA<sup>1</sup>

<sup>1</sup>Institute of Medical Research and Medicinal Plants Studies (IMPM). PO Box 13033 Yaoundé, Cameroon. Tel.: +237-2222-1001, \*email: geomaxlamy@gmail.com

<sup>2</sup>High Institute of Sciences and Techniques Applied to Health (ISSTAS). PO Box 33422 Yaoundé, Cameroon

<sup>3</sup>School of Health Sciences of Catholic University of Central Africa. P.O Box 11628, Yaoundé, Cameroon

<sup>4</sup>Paramedical Training Center. PO Box 25602 Yaoundé, Cameroon

<sup>5</sup>Local Co-Author, Local Counterpart for Informants who Participated by Name

<sup>6</sup>Departement of Plant Biology, Faculty of Sciences, University of Douala. PO Box 24157, Douala, Cameroon

<sup>7</sup>Marderhoek 41, NL-8223 XC Lelystad, Netherlands

<sup>8</sup>Department of Galenic Pharmacy and Pharmaceutical Legislation, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I. PO Box 812 Yaoundé, Cameroon

Manuscript received: 2 April 2024. Revision accepted: 30 May 2024.

Abstract. Lamy GML, Likeng JLN, Ndjib RC, Ndounga SEN, Elomo LB, Mbuh SM, Nnanga LS, Mahama, Biyon JBN, Harmsen K, Nga EN, Tchinda AT. 2024. Ethnomedicine, improved traditional medicine from Cocos nucifera water and evaluation of antibacterial activity on four bacterial strains in Center, Cameroon. Asian J Nat Prod Biochem 22: 27-34. In 2017, the WHO published its first list of "priority pathogens" resistant to antibiotics to combat growing antimicrobial resistance globally. These include bacteria that are multi-resistant to several antibiotics (Acinetobacter, Escherichia, etc.) and others (Salmonella, Shigella, etc.). Currently, research is focused on new antibiotics and medicinal plants are among the favored natural resources. Worldwide, Cocos nucifera water (Arecaceae) or coconut water, is traditionally reported to fight bacterial diseases. Unfortunately, information is lacking on its antibacterial potential in Center, Cameroon. The aim is to determine the traditional antibacterial uses of this water in Central, Cameroon. Then, transform this water into Improved Traditional Medicine (ITM). Finally, evaluate the antibacterial activity of ITM on 4 bacterial strains following the respective Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of 0.015 and 0.25 µg/mL. The methods were ethnomedicinal surveys, the pre-formulation protocol of medicinal syrup, and sensitivity tests in liquid and solid media. Therefore, 76 informants belonging to several ethnolinguistic groups participated. Women (55.27%) exceed men (44.73%) traditionally used coconut water more to treat digestive disorders (stomachache and constipation). An antibacterial ITM named Coco Water Cure (CWC) was manufactured. The more sensitive Salmonella enteritidis and Shigella dysenteriae bacteria with CWC had an MIC of 0.25 µL/mL, respectively. In conclusion, coconut water containing actives compounds such as lauric acid is eligible among the natural antibiotic resources from Center, Cameroon.

Keywords: Antibiotic resistance, coconut water, MIC, pathogens, traditional medicine

# INTRODUCTION

The number of deaths due to antibiotic-resistant infections worldwide is worrying. To this end, the WHO published in 2017 a list of "priority pathogens" resistant to antibiotics. This led to and promoted new antibiotics research and development (Asokan et al. 2019), with 12 families of bacteria most threatening to human health; these including *Acinetobacter baumannii*, *Escherichia coli*, *Salmonella enteritidis* and *Shigella dysenteriae*. Certain antimicrobials, called conventional antibiotics, face resistance from many pathogenic microorganisms. This microbes can observe resistance during a spontaneous mutation or when new genes are transferred from another species. Several factors linked to antimicrobial resistance are, in general, microbial characteristics, selection pressure, and social and technological changes, without ignoring the abusive and uncontrolled use of antibiotics. This phenomenon, which can be described as universal, is the leading cause of infant mortality and morbidity in the world, killing around 50, 000 people per day (O'neill 2016); microbial agents are responsible for 70% of these deaths. Among antimicrobial agents, antibiotics or even antibacterial have been the subject of numerous studies (Gianluigi et al. 2015; Privalsky et al. 2021; David and Wessel 2022). Moreover, bacteria cause several diseases in humans such as cholera, diarrhea; among them responsible for these diseases are Campylobacteriosis, Salmonellosis, Shigellosis, Listeriosis, etc. These bacteria are increasingly resistant to conventional antibiotics (Maertens de Noordhout et al. 2017; Agnieszka and Katarzyna 2018). An antimicrobial evaluation obeys certain technical criteria such as the determination of the Minimum Inhibitory

Concentration (MIC), the Minimum Bactericidal Concentration (MBC), and the Susceptibility Test. Therefore, to compensate for the resistance of bacteria to antibiotics, which are high costs of modern drugs for predominantly poor populations, many hopes remain placed in the therapeutic effect of medicinal plants and the consideration of traditional medicine in Public Health systems in sub-Saharan Africa. Medicinal plants offer a varied and infinite range of secondary metabolites, making it possible to find new molecules with unprecedented antibacterial properties. Therefore, it is crucial to research new antibiotics targeting the priority pathogens on the published WHO list, and natural resources seem to be an avenue to explore in this search for new antibiotics. This is the case for medicinal plants, which are increasingly in demand; Cocos nucifera (Arecaceae) is among these medicinal plants. The water or juice of C. nucifera commonly called "coconut water," has multiple uses worldwide. More than five decades have passed since this water's use in traditional medicine was reported (Kheraro 1975). In Benin, coconut water is traditionally used against several ailments, such as colic, gastroenteritis, and abdominal pain (Dougnon et al. 2017). In Nigeria, there are numerous uses of C. nucifera water in ethnomedicine (Amujoyegbe et al. 2016). In Ivory Coast, the study of the biochemical parameters of coconut water reveals that it can be consumed (Konan et al. 2016) and that it contains soluble sugars (Assa et al. 2007). In Guadeloupe, coconut water is also used in traditional medicine (Jiounandan 2019). Unfortunately, in Central region of Cameroon, there is a lack of information on the traditional antibacterial medicinal knowledge of C. nucifera water. However, in the Center, Cameroon, such information has recently been the subject of transformation of traditional preparations into easily accessible Improved Traditional Medicine (ITM) syrups (Lamy et al. 2023). The aim is to determine the traditional antibacterial uses of this water in Central, Cameroon. Then, transform this water into Improved Traditional Medicine (ITM). Finally, evaluate the antibacterial activity of ITM on 4 bacterial strains following the respective Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of 0.015 and 0.25 µg/mL.

# MATERIALS AND METHODS

# Study area

The study took place in the Center region, Cameroon, precisely in two subdivisions (Lékié and Mfoundi) belonging to the agro-ecological zone of humid forests with bimodal rainfall (zone V) favorable to the cultivation of *C. nucifera* (IRAD 2008). Botanical samples of coconut palms (coconut, inflorescences, stipules, etc.) were collected in the study area. Then, they were brought to the National Herbarium of Cameroon (NHC). NGANSOP Eric (Botanist and Systematician) identified said samples, as *C. nucifera* (Arecaceae) compared with the specimen from herbarium collection No. 67488 HNC.

#### Ethnomedicine

The ethno-medicine part was carried out using an ethno-medicinal survey sheet. All informants voluntarily gave their informed consent by name or anonymously. In accordance with the recommendations of the Nagoya Protocol on access and benefit sharing from local knowledge, informants who agreed to participate by name were cited as co-authors (Lamy et al. 2020). The questions focused on the informant's identity, socio-economic life, nomenclature, and therapy (illness to treat, part used, method of preparation, consumption by age, duration of treatment, quantity used).

## Improved traditional medicine

## Pre-formulation of syrup based on coconut water

Pre-formulation consists of preparing 15 vials of 125 g of coconut water extract. Excipients will be associated with the active ingredients.

Generally, the preparation of the syrup is done in two stages (preparation of the simple syrup and incorporation of the medicinal and aromatic principles). In addition, the European Pharmacopoeia only recommends the dosages for 100 g of simple syrup, in particular, preservative 0.4 g and flavoring 0.03 g (Dénou et al. 2021). Because the recommended dosages (100 g, 0.4 g and 0.03 g) were insufficient for the quantity of final syrup, i.e. 1,875 g; thus, a multiplication factor named K was used to adapt the values of each ingredient to the final quantity.

#### Yield at first pre-formulation trial

The quantity of simple syrup obtained is (100-(0.4+0.03)) = 99.57 g. For the 15 bottles of 125 mL to be packaged, we will have 15x125 g = 1,875 g (the exact quantity of syrup to prepare). For a 10% loss compensation, we have, 1/10x1875 = 185.5 g. That is a total to be prepared of 1,875+187.5 = 2,062.5 g. The multiplication factor (K), which justifies the individual quantity of simple syrup, flavoring and preservative to be obtain in a quantity of final syrup, is:

100 % — 2,062.5 g

1% - 20,625 g, So that K = 20,625

## Preparation of sugar syrup or simple syrup

The ingredients used to prepare the simple syrup were sugar (sucrose) and distilled water (Table 1). The method of preparation was decoction. The preparation was done hot, at 70°C, with purified water. The incorporation of 650 g of sugar (sucrose) was done by dissolution, followed by filtration (Dénou et al. 2021).

**Table 1.** Ingredients, their quantities (100 g and 3,500 g), and functions necessary to prepare a simple syrup

Ingredients	Quantity for 100 g or 1 L	Quantity for 3,500 g or 3.5 L	
Sugar (sucrose)	650 g	2275 g	Taste, viscosity
Distilled water	175 g	612.5 g	Solvent, excipient

## Second step

## Incorporation of medicinal and aromatic principles

The active ingredient was coconut water, collected through immature eyes coconuts using new syringes. The flavoring used was vanilla and the antimicrobial preservative sodium benzoate.

#### Examination of organoleptic characters

The sense organs (eyes, tongues, and nose) recorded some organoleptic parameters (color, flavor, smell, texture, etc.) of the syrup obtained from coconut water to evaluate the organoleptic characteristics.

# Packaging and labeling

It will be a question of producing 15 bottles with a capacity of 125 mL each, labeled with white packaging.

## **Evaluation of antibacterial activity**

## Sensitivity test in liquid media

Mueller Hinton Broth (MHB) media were prepared to perform the test in liquid media. The inoculum consisted of solutions (Mueller Hinton Broth), and the microorganisms consisted of bacterial strains (*S. enteritidis*, *E. coli*; *S. dysenteriae*, and *A. baumannii*) and 3 extracts (oil from coconut kernels or HA, Coco water cure or CWC and COVID Med) with different transplanting strains methods (reactivating the microbial strains). This subculturing is carried out in a microplate using the micro-dilution method. It is carried out in triplicate (3 times).

#### Determination of inhibition parameters (MIC, MBC)

The microdilution technique determined the extracts' inhibition parameters in a liquid medium following the CLSI protocol (2011). This is a reference method, which consists of distributing decreasing concentrations of an antimicrobial substance in the wells of a plate under the same volume, then adding, under the same volume, a culture of bacteria in the exponential growth phase. After incubation for 24 to 48 hours, microbial activity can be visible to the naked eye or by color change inside the cup. The reference antibiotic used was Ciprofloxacin.

# Determination of the Minimum Inhibitory Concentration (MIC)

The stock solutions of the two syrups and the almond oil used were prepared at a concentration of 100 mg/mL, and the stock solution of Ciprofloxacin concentrated at 20 mg/mL. The bacterial inoculum was prepared to obtain a turbidity corresponding to the 0.5 Mc Farland standard (1.5 x10<sup>8</sup> Cells/mL) and 250x 10<sup>-3</sup>  $\mu$ g/mL.

Three extracts (oil from coconut almonds, medicated syrup from coconut water and Covid Med syrup) were triplicate for a single isolate. In each well of a 96-well microplate, 100  $\mu$ L of MHB culture medium was introduced. Subsequently, 100  $\mu$ L of a stock solution of the 3 extracts or Ciprofloxacin® was introduced into the first 3 wells of column 1 (lines A, B, and C). In columns 1 to 12, successive dilutions following a geometric progression of reasons 2 were carried out (from wells A, B, and C) up to the 11th well, which should vary the concentration range of 500  $\mu$ L/mL to 0.48  $\mu$ L/mL for the 3 extracts and from 500

 $\mu$ g/mL to 0.48  $\mu$ g/mL for Ciprofloxacin. Finally, 100  $\mu$ L of bacterial inoculum was introduced into each well, thus varying the concentrations from  $250 \times 10^{-3} \mu$ L/mL to 0.24  $\mu$ L/mL for the extracts and from  $250 \times 10^{-3} \mu$ g/mL to 0.24  $\mu$ g/mL for Ciprofloxacin. All tests were carried out in triplicate.

The fourth line of the microplate was used as a negative control for the activity of our 3 extracts and MHB. The wells of column 12 were used as positive controls for bacterial growth (MHB+ inoculum). The microplate was sealed with its lid and covered with film paper, then incubated at  $37^{\circ}$ C for 18 to 24 hours.

After incubation, bacterial growth was demonstrated by adding 20  $\mu$ L of Blue Alamar at a concentration of 0.4 mg/Ml in two (2) of the three (3) wells of the test lines; the wells of the third line will used for determining the MBC. The MIC was defined as the lowest concentration of our extracts and Ciprofloxacin, for which no bacterial growth was visible to the naked eye (CLSI 2011).

## Determination of Minimum Bactericidal Concentration (MBC)

A volume of 50  $\mu$ L from the wells of the third line, whose concentrations of extracts and Ciprofloxacin® are greater than or equal to the MIC, was transferred into a microplate containing 150  $\mu$ L of sterile culture broth. The plate was incubated under optimal conditions. After incubation, 20  $\mu$ L of Blue Alamar was added to the wells at concentration of 0.4 mg/mL and left to act for 30 minutes. The smallest dilution of the extracts where no color change was observed corresponds to its CMB.

#### Data processing and analysis

The data were processed with Word and Excel 2013 software. Data analysis was done using STATGRAPHICS Plus 5.0 software.

# **RESULTS AND DISCUSSION**

## Ethnomedicine

## Uses of coconut water in traditional medicine

Regardless of the subdivisions, constipation and stomachaches have the greatest incidence (Figure 1). These results reflect that coconut water is mainly used in the study area to treat digestive disorders (stomachaches and constipation). The pharmacological activities of coconut water could explain these results. Indeed, previous studies have demonstrated that coconut water has antibacterial and antifungal activities, as in Benin (Dougnon et al. 2017).

## **Characteristics of informants**

Moreover, 76 informants participated in the study, i.e. 35 people in the Lékié Department and 41 in Mfoundi (Table 2). Regardless of the department, women were in the majority, with a total of 42 or 55.27%, than men, with 34 or 44.73%. These results reflect that women in the study area have more knowledge than men about the coconut water's antibacterial uses in traditional medicine. These results could be explained by the fact that, in rural areas, women being closer to the sick use coconut water for

primary health care as practiced from generation to generation. Using coconut water as an antibacterial in traditional medicine for primary health care has already reported in Ivory Coast (Assa et al. 2007).

# Improve traditional medicine

## Pre-formulation of syrup based on coconut water

The results show that the pre-formulation of coconut water as medicated syrup is real (Table 3). These results could be explained by the meticulous follow-up of the observed syrup preparation protocol, which the European Pharmacopoeia recommends. According to Ouedraogo et al. (2021), herbal medicines can be packaged in liquid forms, including syrup, thanks to the introduction of modern technology in the commercial production of herbal products.

# Packaging and labeling

Figure 2 shows that the final syrup was packaged in bottles with a label filled in as follows: name of the syrup (Coco Water Cure); active ingredient (coconut water); simple syrup (99.5%); preservative (Sodium Benzoate); aroma (0.03%); place of manufacture (Galenics and Pharmaceutical Legislation Laboratory); date of manufacture (2022.09.16); expiration date (2026.09.15) and bundle number (001). These results are consistent with drug packaging and label presentation (Begert 2015). From the manufacturing and expiration dates on the label, we see that coconut water can be stored for 4 years. This long-term conservation was made possible thanks to the preservative. However, the shelf life of coconut water is generally one year. Recently, the use of sodium benzoate as a preservative has made it possible to preserve a traditional preparation based on medicinal plants in the long term (Lamy et al. 2023).

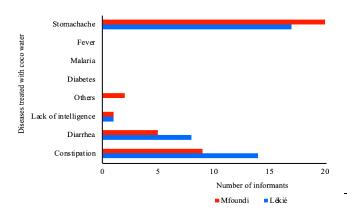


Figure 1. Diseases treated by coconut water depend on the number of people

 Table 2.
 Socio-economic characteristics of coconut water informants in the Center region, Cameroon

Characteristics	Lékié	Mfoundi
	LCKIC	Wiloului
Gender		
Women	20	22
Men	15	19
Ages groups (years)		
15-35	15	16
36-55	12	13
55-75	8	12
Religions		
Christians	34	37
Muslims	2	4
Others	0	0
Matrimonial status		
Single	15	17
Divorcee	3	10
Married	12	8
Windwer/ Window	5	6
Educational levels		
Illiterate	0	4
Primary	8	6
Secondary	12	20
Superior	15	11
Number of individuals per family		
1-5	6	8
6-10	25	22
11-20	4	7
20	0	4
Income per day (FCFA)		
655-1300	5	0
1300-2000	13	11
2000-5000	12	19
Others	5	11
Ethnolinguistics groups		
English-speaker	0	0
Bamileke	8	4
Ewondo	6	13
Fufulde	2	3
Sawa	1	5
Bulu	1	8
Eton	11	5
Others	6	3
Note: NTFPs: Non Timber Forest	Products,	1 Dollar = $500$

Note: NTFPs: Non Timber Forest Products, 1 Dollar = 500 FCFA; 1 Euro = 650 FCFA

Table 3. Ingredients, their quantities and roles that made it possible to obtain the final syrup

Ingredients	Quantities per 100 g	Quantities per 2,062.5 g for 100g x K	Functions (roles)
Simple syrup	99.57 g	2,053.63g	Taste, viscosity
Vanilla	0.03 g	0.618g	Flavoring
Sodium benzoate	0.4 g	8.25g	Antimicrobial reservative
Coconut water	175 g	612.5 g	Active ingredient

# **Evaluation of antibacterial activity**

Sensitivity tests in solid media

Regardless of the bacterial strain, it is observed that the reference antibiotic (CIP) shows the largest zone of inhibition (36 mm) (Table 4). Regarding the extracts used, CWC (Coco water cure) does not present any zone of inhibition (00 mm) with three strains (E. coli, S. dysenteriae and S. enteritidis). In comparison, it presents a zone of inhibition with the bacterial strain A. baumannii with a value of 10 mm. According to Asif et al. (2019), the scientific community pays particular attention to A. baumannii because of its resistance to the latest wave of antimicrobials. It is a Gram-negative bacteria is multiresistant to several antibiotics and causes nosocomial infections (Reina et al. 2022). The results obtained could be explained as follows. Coconut water (CWC) consists of: simple syrup (95.25 mL), flavoring (0.03 mg), preservative (0.4 mg) and active ingredient (4.32 mL). The active compounds present in this medicinal syrup (lauric acid, etc.) can explain its action on A. baumannii which would not have been the case with the three other strains. It should be remembered that lauric acid is a fatty acid. Fatty acids (oleic acid, etc.) inhibit A. baumannii (Khadke et al. 2021). The impact of fatty acids on the physiology of A. baumannii has been reported (Zang et al. 2022). Escherichia coli and S. dysenteriae are enterobacteria similar in terms of biochemical characteristics (gramnegative bacillus, mobile or immobile, aerobic-anaerobic). According to Ragupathi et al. (2018), molecular studies are needed to differentiate between these two bacteria. S. enteritidis, also an enterobacterium, differs from the two previous strains regarding antigenic characteristics. The genus Salmonella has antigens. This difference in their structure and biological compounds may explain why only A. baumannii reacted and not the other three strains. The active compounds in coconut water, particular lauric acid, have an antimicrobial effect on A. baumannii by disrupting its cell membrane (Marion et al. 2018). Lauric acid has a lipid structure that can penetrate the bacteria's cell membrane and disrupt its stability, leading to a leak of essential substances inside the bacteria, which leads to its death. Also, lauric acid can interfere with the bacteria's metabolic pathways, disrupting its function and survival. However, it should be noted that the effectiveness of lauric acid may vary depending on various factors, such as concentration, exposure time, individual resistance of the bacteria, etc.

**Table 4.** Values of the inhibition zones of bacterial strainsdepending on the reference antibiotic (CIP) and the 3 extracts(CWC, COV Med and HA)

Bacterial strains	Reference antibiotic	Extracts used			
strams	CIP	CWC	COV Med	HA	
E. coli	36 mm	0	0	0	
	32 mm	0	0	0	
	32 mm	0	0	0	
S. dysenteriae	36 mm	0	0	0	
	30 mm	0	0	0	
	32 mm	0	0	0	
S. enteritidis	30 mm	0	0	0	
	30 mm	0	0	0	
	26 mm	0	0	0	
A. baumannii	26 mm	0	0	0	
	30 mm	0	0	0	
	30 mm	10mm	0	0	

Note: E.C: *Escherichia coli*, CIP: Ciprofloxacin, CWC: Coco Water Cure, COV Med: COVID medicinal syrup, HA: Almond oil, 0: no inhibition zones observed



Figure 2. Syrup bottles after labeling

#### Table 5. MIC and CMB of the antibiotic and the three extracts on the four strains used

		Bacterial strains tested							
	Priority pathogens	S. dysenteriae		S. enteritidis		A. baumannii		E. coli	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Extracts used	H.A (µL/mL)	-		-		-		-	
	Cov Med ( $\mu$ L/mL)	-		-		-		-	
	CWC ( $\mu$ L/mL)	0.25	0.25	0.25	0.25	-		-	
Reference antibiotic	$CIP (\mu g/mL)$	3.96	3.96	3.96	3.96	3.96	3.96	3.96	3.96

Note: Indeterminate: (-), MIC: Minimum Inhibitory Concentration, CMB: Minimum Bactericidal Concentration, µL: microliter, mL: milliliter, HA: Almond Oil, CWC: Coconut water cure

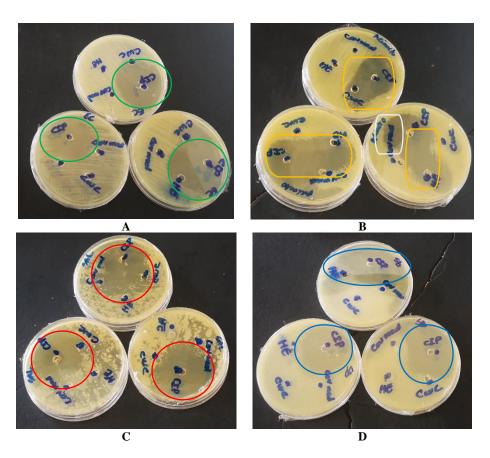


Figure 3. Three respective replicates for A. E. coli, B. A. baumanii, C. S. enteritidis and D. S. dysenteriae strains. Note: Circle/rectangular: Halo zone. White rectangular: Delimitation of CWC extract inhibition zone

Figure 3 shows the inhibition zones formed by the different bacterial strains in contact with the extracts and the reference antibiotic. As for the behavior of the E. coli bacterial strain (Figure 3.A), regardless of the petri dishes used (repetition), only the reference antibiotic (CIP) presents a zone of inhibition delimited by the box green in color because no zone of inhibition is observed with the extracts used (CWC, Cov Med and HA). Regarding the behavior of the bacterial strain A. baumanii (Figure 3.B), whatever the petri dishes used (repetition), the reference antibiotic (CIP) presents an inhibition zone delimited by the yellow box. However, with the extracts used, only the CWC extract presents a zone of inhibition delimited by the white box. Concerning the bacterial strain S. enteritidis (Figure 3.C), whatever the petri dishes used (repetition), only the reference antibiotic (CIP) presents a zone of inhibition delimited by the red box. Therefore, no zone of inhibition is observed with the extracts used (CWC, COV Med and HA). Regarding the behavior of the bacterial strain S. dysenteriae (Figure 3.D), regardless of the petri dishes used (repetition) only the reference antibiotic (CIP) presents a zone of inhibition delimited by the blue box because no zone of inhibition is observed with the extracts used (CWC, COV Med and HA).

## Sensitivity tests in liquid media

Table 5 on the 3 (CWC, COV Med and HA) and reference antibiotic (CIP) show that of the four (4) bacterial

strains tested, only two (2) of them (S. enteritidis and S. dysenteriae) were sensitive. Indeed, S. enteritidis and S. dysenteriae, respectively, show a MIC of 0.25 µL/mL, were much more sensitive to the CWC extract. These results reflect that in CWC extracts  $\leq 0.25 \ \mu L/mL$ concentration, the bacteria S. enteritidis and S. dysenteriae are sensitive and not sensitive to the other bacterial strains (A. baumannii and E. coli). Indeed, these CWC extract compounds would act on S. enteritidis and S. dysenteriae due to more biochemical and bacteriological similarities. Unlike A. baumannii and E. coli, which are similar in constitution and biochemistry (El-Housseiny et al. 2017). Specifically for S. dysenteriae, fatty acids, including lauric acid, interact with certain proteins to abolish their transcription and promotion activities (Trirocco et al. 2023). In addition, all the bacterial strains tested on the reference antibiotic have an identical MIC and CMB value (3.96 µg/mL). These results reflect that all the bacterial strains tested are sensitive for a value  $\leq 3.96 \ \mu g/mL$  of extract. MIC values for ciprofloxacin may vary depending on the type of bacteria targeted. Generally, the MIC of ciprofloxacin for Gram-negative bacteria is around 0.015 to 0.25 µg/mL, while for Gram-positive bacteria, it can be around 0.015 to 0.5 µg/mL. However, these values may vary depending on the bacterial strain and the testing method. When a bacteria is inhibited or killed at ciprofloxacin MICs greater than 1 µg/mL, this indicates that this bacteria is resistant to ciprofloxacin MICs less

than 1  $\mu$ g/mL (Grillon et al. 2016). In other words, the bacteria requires a higher concentration of ciprofloxacin to be inhibited or killed, indicating that ciprofloxacin treatment is less effective against that specific bacterial strain. Additionally, those four bacterial strains tested are in the list of "priority pathogens" made public by the WHO in 2017. The results of the antibacterial ITM from coconut water of Center, Cameroon, on these strains, indicate that this water is eligible for research into new antibiotics.

In conclusion, the initial hypothesis confirms that coconut water has traditional antibacterial uses in Center, Cameroon, and improved traditional antibacterial medicine (ITM) has been made from this water. The evaluation of the antibacterial activity of this ITM on four bacterial strains (*A. baumannii*, *E. coli*, *S. enteritidis* and *S. dysenteriae*) shows an effect on two of them. Only the bacteria *S. enteritidis* and *S. dysenteriae* were observable in a MIC and MBC of 0.25  $\mu$ g/mL with the antibacterial ITM extract; therefore, *C. nucifera* water of Center, Cameroon is eligible for research into new antibiotics. In perspective, it would be interesting to extend the evaluation of the antibacterial activity of this ITM on other bacterial strains. Other methods of evaluating antibacterial activity should also be explored.

# **ACKNOWLEDGEMENTS**

The authors would like to thank the populations of Center, Cameroon for sharing its local knowledge. They thank the Laboratory of Botany and Traditional Medicine of the Institute of Medical Research and Medicinal Plants Studies (IMPM), Cameroon. As well as the laboratory of Galenic Pharmacy and Pharmaceutical Legislation (PGLP) from the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I, Cameroon for allowing them to handle easily.

## REFERENCES

- Agnieszka C, Katarzyna S. 2018. Campylobacteriosis, Salmonellosis, Yersiniosis, and Listeriosis as Zoonotic Foodborne Diseases: A review. Intl J Environ Res Public Health 15: 863. DOI: 10.3390/ijerph15050863.
- Amujoyegbe OO, Idu M, Agbedahunsi JM, Erhabor JO. 2016. Ethnomedicinal survey of medicinal plants used in the management of sickle cell disorder in Southern Nigeria. J Ethnopharmacol 185: 347-360. DOI: 10.1016/j.jep.2016.03042.
- Asif M, Alvi IA, Rehman SU. 2019. Insight into Acinetobacter baumannii: Pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infect Drug Resist 11: 1249-1260. DOI: 102147/IDR.S166750.
- Asokan GV, Ramadhan T, Ahmed E, Sanad H. 2019. WHO global priority pathogens list: A bibliometric analysis of medline-PubMed for knowledge mobilization to infection prevention and control practices in Bahraim. Omam Med J 34 (3): 184-193. DOI: 10.5001/omj.2019.37.
- Assa RR, Konan JL, Agbo N, Prades A, Nemlin J. 2007. Caractéristiques physico-chimiques de l'eau des fruits de quatre cultivars de cocotier (*Cocos nucifera* L.) en Côte d'Ivoire. Agron Afr 19 (1): 41-51. DOI: 10.4314/aga.v19i1.1701.
- Begert L. 2015. Le conditionnement des médicaments: Un élément essentiel de protection des patients. Sci Pharm hal-01731903.

- Clinical and Laboratory Standards Institute (CLSI). 2011. Performance Standards for Antimicrobial Susceptibility Testing, 21st Informational Supplement. CLSI Document M100-S21. Clinical and Laboratory Standards Institute, Wayne.
- David T, Wessel C. 2022. The State of Innovation in Antibacterial Therapeutics. BIO Industry Analysis, Washington DC, USA.
- Dénou A, Diallo D, Koumaré M. 2021. Mise au point d'une technique d'amélioration de la conservation des sirops d'extraits de *Guiera* senegalensis J.F. Gmel (Combretaceae). J Appl Biosci 165: 17110-17119. DOI: 10.35759/JABs.165.7.
- Dougnon TV, Déguénon E, Fah L, Lègba B, Hounmanou YMG, Agbankpè J, Amadou A, Koudokpon H, Fabiyi K, Aniambossou A, Assogba P, Hounsa E, de Souza M, Dougnon TJ, Gbaguidi F, Boko M, Bankolé HS, Baba-Moussa L. 2017. Traditional treatment of human and animal salmonelloses in Southern Benin: Knowledge of farmers and traditherapsis. Vet World 10 (6): 580-592. DOI: 10.14202/vetworld.2017.580-592.
- El-Housseiny GS. 2017. Antibacterial effects of lauric acid against Acinetobacter baumanii. J Adv Med Pharm Sci 13 (2): 1-9.
- Gianluigi F, Annarita F, Stefania G, Luciana P, Mahendra R, Giancarlo M, Massimiliano G. 2015. Silver nanoparticles as potential antibacterial agents. Molecules 20: 8856-8874. DOI: 10.3390/molecules20058856.
- Grillon A, Schramm F, Kleinberg M, Jehl F. 2016. Comparision activity of Ciprofloxacin, Levofloxacin and Moxifloxacin against *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* assessed by minmum inhibitory concentration and timekill studies. PloS ONE 11 (6): e0156690. DOI: 10.1371/journal.pone.0156690.
- IRAD. 2008. Deuxième rapport sur l'état des ressources phytogénétiques pour l'alimentation et l'agriculture au Cameroun. Institut de Recherche Agricole pour le Développement. Yaoundé, Cameroun.
- Jiounandan LL. 2019. Les plantes médicinales utilisées par les descendants d'engagés indiens en Guadeloupe: étude bibliographique et enquête de terrain. Sciences du Vivant [q-bio].
- Khadke SK, Lee JH, Kim YG, Raj V, Lee J. 2021. Assessment of antibiofilm potencies of nervonic and oleic acid against *Acinetobacter baumannii* using in vitro and computational approaches. Biomedicines 9: 1133. DOI: 10.3390/biomedicines9091133.
- Kheraro J. 1975. La médecine et la pharmacopée traditionnelles sénégalaises. ORSTOM, France.
- Konan BR, Agnememel AB, Akely PMT, Assa RR, Konan KJL, Amani NG. 2016. Variation des paramètres biochimiques de l'eau de coco (*Cocos nucifera* L.) issu de la culture in vitro pendant la période de stockage. Intl J Biol Chem Sci 10 (3): 957-965. DOI: 10.4314/ijbcs.v10i3.4.
- Lamy LGM, Meli KP, Talba D, Amougou AC, Dona A, Zambou ZL, Ndjib R, Fawa G, Nzweundji GJ, Donfagsiteli TN, Agbor AG, Wadjiri PBJV, Wackilou. 2020. Reciprocity in ethnobotanical research: Case of a study carried out in the Mbe plain of Adamawa, Cameroon. Ethnobot Res Appl 20 (38): 1-11. DOI: 1032859/era.20.38.1-11.
- Lamy LGM., Nnanga LS, Mahama, Ossongo AF, Elomo LB, Tagne SR, Mbo AJ, Likeng NLJ, Nnanga NE, Harmsen K. 2023. Medicinal syrup for children from the association of significant parts of anti-COVID-19 medicinal plants from the Centre, Cameroon. Asian J Nat Prod Biochem 21 (2): 58-66. DOI: 10.13057/biofar/f2110202.
- Maertens de Noordhout C, Devleesschauwer B, Haagsma JA, Havelaar AH, Bertrand S, Vandenberg O, Quoilin S, Brandt PT, Speybroeck N. 2017. Burden of salmonellosis, campylobacteriosis and listeriosis: A time series analysis, Belgium, 2012 to 2020. Euro Surveill 22 (38): 30615. DOI: 10.2807/1560-7917.ES.2017.22.38.30615.
- Marion N, Luizet AB, Skogman M, Jouene T, Salcedo SP, Dé E. 2018. Unsaturated fatty acids affect quorum sensing communication system and inhibit motility and biofilm formation of *Acinetobacter baumannii*. Intl J Mol Sci 19 (214): 1-10. Doi: 10.3390/ijms19010214
- O'neill J. 2016. The Review on Antimicrobial Resistance. Tracking Drug-Resistant Infections Globally: An Overview of Our Work. Wellcome Trust, UK.
- Ouedraogo S, Yoda J, Traoré KT, Nitiema M, Sombie BC, Diawara ZH, Yameogo BG, Djande A, Belemnaba L, Kini FB, Ouédraogo S, Semde R. 2021. Production de matières premières et fabrication des médicaments à base de plantes médicinales. Intl J Biol Chem Sci 15 (2): 750-772. DOI: 10.4314/ijbcs.v15i2.28.
- Privalsky TM, Alexander MS, Jinhua W, Christopher TW, Gerard DW, Eric MG, Nathanael SG, Chaitan K. 2021. Prospects for antibacterial

discovery & development. J Am Chem Soc 143 (50): 21127-21142. DOI: 10.1021/jacs.1c10200.

- Ragupathi NKD, Sethuvel DPM, Inbanathan FY, Veeraraghavan B. 2018. Accurate differentiation for *E. coli* and *Shigella* serogroups: Challenges and Strategies. New Microb New Infect 21: 58-62. DOI: 10.1016/j.nmni.2017.09.003.
  Reina R, Léon-Moya C, Garnacho-Montero J. 2022. Treatment of
- Reina R, Léon-Moya C, Garnacho-Montero J. 2022. Treatment of Acinetobacter baumannii severe infections. Med Intensiva 46: 700-710. DOI: 10.1016/j.medine.2022.08.007.
- Trirocco R, Pasqua M, Tramonti A, Grossi M, Colonna B, Paiardini A, Prosseda G. 2023. Fatty acids abolish Shigella virulence by inhibiting its master regulator, VirF. Microbiol Spectr 11 (3): e0077823. DOI: 10.1128/spectrum.00778-23.
- Zang M, Adams FG, Hassan KA, Eijkelkamp BA, 2022. The impact of omega-3 acids on the evolution of Acinetobacter baumannii drug resistance. Microbiol Spectr 9: e0145521. DOI: 10.1128/Spectrum.01455-21.