



HAL
open science

Comparative study of *Moringa stenopetala* root and leaf extracts against the bacteria *Staphylococcus aureus* strain from aquatic environment.

C.S. Metsopkeng, M.E. Nougang, P.A. Nana, A. Tamsa Arfao, P. Ngo Bahebeck, C. Lontsi Djimeli, J.S. Eheth, O.V. Noah Ewoti, L.M. Moungang, G.A. Agbor, et al.

► To cite this version:

C.S. Metsopkeng, M.E. Nougang, P.A. Nana, A. Tamsa Arfao, P. Ngo Bahebeck, et al.. Comparative study of *Moringa stenopetala* root and leaf extracts against the bacteria *Staphylococcus aureus* strain from aquatic environment.. *Scientific African*, 2020, 10, pp.e00549. 10.1016/j.sciaf.2020.e00549 . hal-03454700

HAL Id: hal-03454700

<https://uca.hal.science/hal-03454700>

Submitted on 29 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



Comparative study of *Moringa stenopetala* root and leaf extracts against the bacteria *Staphylococcus aureus* strain from aquatic environment.

C.S. Metsopkeng^a, M.E. Nougang^{a,b}, P.A. Nana^{a,c}, A. Tamsa Arfao^a,
P. Ngo Bahebeck^{a,d}, C. Lontsi Djimeli^a, J.S. Eheth^a, O.V. Noah Ewoti^a,
L.M. Mounang^a, G.A. Agbor^e, F. Perrière^f, T. Sime-Ngando^f, M. Nola^{a,*}

^aLaboratory of Hydrobiology and Environment, Faculty of Science, University of Yaounde 1, P.O. Box 812 Yaounde, Cameroon

^bZoology laboratory, Faculty of Sciences, University of Maroua, P.O.Box 814 Maroua, Cameroon

^cDepartment of Oceanography and Limnology, Institute of Fisheries and Aquatic Sciences, University of Douala, P.O.Box 7236, Douala, Cameroon

^dLaboratory of Soils, Plants, Fertilizer and Water analysis, IRAD, P.O. Box 2123 Yaounde, Cameroon

^eCenter for Research on Medicinal Plants and Traditional Medicine, Institute of Medical Research and Medicinal Plants Studies, P.O. Box 13033, Yaoundé, Cameroon

^fLaboratoire 'Microorganismes: Génome et Environnement', UMR CNRS 6023, Université Clermont Auvergne, Campus Universitaire des Cèzeaux, 1 Impasse Amélie Murat CS 60026, 63178 Aubière Cedex, France

ARTICLE INFO

Article history:

Received 25 May 2020

Revised 11 August 2020

Accepted 3 September 2020

Keywords:

Moringa stenopetala

Antibacterial activity

Water treatment

Staphylococcus aureus

ABSTRACT

The present study assessed the abiotic factors of *Moringa stenopetala* (*M. stenopetala*) extracts against *Staphylococcus aureus* in aquatic microcosm. Bacterial survival was assessed using colony forming unit (CFU) method on culture agar medium at different concentration levels (0.5 to 2 g/L). The incubation duration varied from 3 to 24 hours, and the temperatures were between 7°C and 44°C. Phytochemical screening showed that most of the constituents obtained from the extracts are the alkaloids, flavonoids and the tannins. It was observed that increase in extract concentration significantly inhibited the number of CFU obtained. Compared to the control {9.27 Log(CFU/100 mL)}, cells abundance from different concentrations of leaves extracts fluctuated between 4.74 and 0 {Log(CFU/100 mL)} after 3h incubation; 5.46 and 0 {Log(CFU/100 mL)} after 6h; 4.75 and 0 {Log(CFU/100 mL)} after 9h, and 4.85 and 0 {Log(CFU/100 mL)} after 24h incubation. As for root extracts, the abundance were between 4.80 and 0 {Log(CFU/100 mL)} under 7°C incubation; 5.03-5.23 and 0 {Log(CFU/100 mL)} under 23°C and 37°C, and 4.66 and 0 Log (CFU/100 mL) under 44°C incubation when considering the whole extract concentrations used. Incubation temperature and extracts concentration significantly impacted bacterial abundances. When the decoction extracts of roots were used, the amount of *S. aureus* cells inhibited was registered after 3-24 hours incubation. The Pearson correlation coefficient in most cases, revealed an inversely proportional relationship between the plant extracts and *S. aureus* cultured cells in a dose-dependent manner ($P \leq 0.05$). The use of *M. stenopetala* extracts could constitute an alternative in the treatment of water intended for human consumption.

© 2020 The Author(s). Published by Elsevier B.V. on behalf of African Institute of Mathematical Sciences / Next Einstein Initiative.

This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)

* Corresponding author.

E-mail address: moise.nola@yahoo.com (M. Nola).

<https://doi.org/10.1016/j.sciaf.2020.e00549>

2468-2276/© 2020 The Author(s). Published by Elsevier B.V. on behalf of African Institute of Mathematical Sciences / Next Einstein Initiative. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)

Introduction

In many parts of the world, fresh water is of bad microbial quality due to the presence of infectious agents, chemicals or radioactive substances [1]. According to the World Health Organization (WHO), drinkable water is that which can be drunk without health risk. However, 1.2 billion people do not have access to this drinkable water [2]. About 2.5 billion people worldwide lack adequate sanitation [3, 4, 5]. Several protocols have been proposed to improve water quality. These include chemical treatment of water using chlorine or other reagents, filtration, boiling, or UV sterilization. However, the residues of these chemical agents could accumulate and have negative effects on human health [6] while some of these methods could be laborious depending on the environment. Alternative methods of disinfecting water using plant extracts have been proposed [7, 8, 9]. Some plants that possess antibacterial and antifungal properties have been evaluated on microorganisms isolated from drinking water. The aqueous extracts of *Lantana camara*, *Cymbogon citratus* and *Hibiscus rosa-sinensis* have shown bactericidal effect in the aquatic environment [10]. Similarly, the extract of *Eucalyptus microcorys* possessed inhibitory effect against some pathogenic microbes in the aquatic microcosm [8].

Moringa stenopetala (*M. stenopetala*) is a multipurpose plant in traditional medicine used as nutrients source and also against human gastrointestinal infections. The different parts of *M. stenopetala* are widely used in traditional medicine for a varied range of diseases such as diabetes, hypertension, stomach ache, malaria, leprosy, epilepsy, diarrhea, asthma, colds and anthelmintic properties [11]. Numerous studies have shown that the extract has antileishmanial activity and the bark and root extract have been shown to be active against *Trypanosoma brucei* trypomastigotes, which indicates anti-trypanosomal activity [12]. Likewise, extracts and fractions obtained from various parts of the plant have shown antimicrobial activity [13].

Little information is available on the impact of the aqueous and ethanolic extracts, of the different parts of *M. stenopetala* on bacterial growth. In addition, the impact of various temperature ranges on the activity of these plant extracts against microorganisms is less documented. *Staphylococcus aureus* is one of the pathogenic species for humans, the best known among the coagulase-positive staphylococci found in ground and surface waters. Its pathogenicity results also from the penetration of the microbe into the organism generally after rupture of the cutaneous barrier (wound, burn) or at the level of the hair follicle, among others. It can also occur after a viral infection such as the flu and measles virus, or can also occur after a host immunodeficiency [14]. This pathogenicity also results from the consumption of poor-quality water containing germs. Biological treatment of water using this extract from different parts of *M. stenopetala* could help improve its quality.

The aim of this study was to assess the bioactivity of different extracts of this plant organs against *Staphylococcus aureus* strain isolated from aquatic environment.

Materials and methods

Plant material

The plant material consisted of the leaves and roots of *M. stenopetala*. They were collected from an adult tree in a house garden in Yaounde (3° 52' north latitude and 11° 32' east longitude, at an average altitude of 759 m) (Cameroon) [15]. The plant was identified in the National Herbarium of Cameroon on N ° 8241/SRF/Cam (Yaounde).

Preparation of extracts

The leaves and roots were dried at room temperature ($25 \pm 2^\circ\text{C}$) for 4 to 6 days; then crushed to obtain a powder according to the technique described by Folkard and Sutherland [16]. For each part of the plant, three types of extracts, namely water maceration extract (WME), water decoction extract (WDE) and alcohol maceration extract (AME) were prepared from 100 g of powder. All the extracts were prepared according to Ciulei [17].

For the AME, 100 g of plant powder were added to 1000 mL of 70 % ethanol. The whole solution was left in the dark with stirring for 48 hours. The solution was then filtered, first on Whatman paper (0.47 μm of porosity) and then on sterile cotton. The filtrate obtained constitutes the ethanol extract. This filtrate was then dried in an oven at a temperature of 45–50°C. The residues obtained were kept in the refrigerator (4–6°C) for further experiments.

For the WDE, 100 g of plant powder was added to 1000 mL of boiling water at 100°C. The boiling was maintained for 15 minutes. After cooling, the solution was filtered and dried in an oven at a temperature of 45–50°C. The residue obtained was kept in the refrigerator as above, until it was used.

For the WME, 100 g of plant powder was added to 1000 mL of water. The mixed solution was left at room temperature (22–25°C) and in the dark for 48 hours. The solution was then filtered as indicated above. The filtrate obtained was then dried in an oven at a temperature of 45–50°C. The residue obtained was also kept in the refrigerator until used.

The percentage yield (Rd) of extracts were calculated according to Xue Zhaohi [18] as follow:

$$\text{Rd} = \left(\frac{m_E}{m_P} \right) \times 100$$

where m_E is the mass of the dried residue obtained and m_P the mass of the plant powder used.

Choice and isolation of the bacterial strain

The bacteria used was *Staphylococcus aureus*. It was selected because of its importance in hygiene and public health. It is the best known among the coagulase-positive staphylococci found in ground and surface waters and is used as one of the indicators of the microbiological quality of water for consumption [19, 20]. This bacterial strain was isolated from groundwater in Yaounde using membrane filtration method (cellulose nitrate membrane, porosity 0.45 μ m) and cultured on the Chapman Mannitol culture medium. The bacterial cells were then identified using biochemical criteria [21] and then stored in glycerol at 0°C for later use.

Preparation of the different concentration of extracts

After extracts filtration through sterile whatman cellulose acetate membranes (0.45 μ m porosity) [8, 22], as indicated above, the concentration ranges prepared were 0; 0.5; 1; 1.5 and 2 g/L. This was done by adding the dried residues obtained above in different volumes of sterilized NaCl solution (8.5 g/L) contained in the Erlenmayer glass bottles. After, the homogenized solution was filtered successively through Whatman paper, then sterile cotton, and finally through a cellulose nitrate filter membrane (0.45 μ m) [8, 22].

Phytochemical screening of the different extracts

The colorimetric protocols described by Trease and Evans [23]; Parekh and Chanda methods [24] were used to highlight the different classes of substances (secondary metabolites) present in the different plant extracts. For this qualitative analysis, the absence of color reflects the absence of the desired compound or metabolite. Likewise, high staining intensity reflects a high concentration of the desired compound or metabolite whereas a low intensity reflects its low concentration.

Preparation of bacterial suspensions

The bacteria were activated on nutrient agar (OXOID, Thermo Fisher Scientific Inc. UK.) for the preparation of bacterial inocula. The bacterial suspensions were carried out on cultures aged 18 to 24 hours, introduced into a sterile NaCl solution (8.5 g/L) and compared to 0.5 McFarland standard (0.5 mL of 0.48 mol/L BaCl₂, 2H₂O at 1.175 % w/v) added with 99.5 mL of 0.18 mol/L H₂ SO₄ (1 % v/v) [25].

In vitro susceptibility test

Hundred milliliters (100 mL) of the extracts concentrations (0.5; 1; 1.5 and 2 g/L) earlier prepared, were introduced into sterile 350 mL bottles. The bottle free of extract (0 g/L) served as the negative control. In accordance with the selected extract concentrations, bottles were divided into 4 series A, B, C and D. Each series was further subdivided into 12 ranges, for example, bottles of the A series were subdivided into A_{3a}, A_{3b}, A_{3c}, A_{6a}, A_{6b}, A_{6c}, A_{9a}, A_{9b}, A_{9c}, A_{24a}, A_{24b} and A_{24c}. Each of the extract concentration was done in triplicate. The same subdivision was done for bottles of B, C and D series. In each bottle 1 mL of bacterial suspension with an initial concentration of 9.27 units {Log(CFU/100 mL)} was added, homogenized and incubated at different temperatures. Bottles of series A were incubated in the refrigerator adjusted at 7 \pm 1°C. Those of series B were incubated at room temperature 23 \pm 2°C. Those of series C and D were incubated in the oven adjusted at 37 \pm 1°C and 44 \pm 1°C, respectively. In addition, bottles coded A_{3a}, A_{3b}, A_{3c} and A_{6a}, A_{6b}, A_{6c} and those coded A_{9a}, A_{9b}, A_{9c} and A_{24a}, A_{24b}, A_{24c} were incubated for 3, 6, 9 and 24 hours, respectively. Bottles of series B, C and D were also incubated according to the same subdivisions.

After each incubation period, 100 μ L of each extract contained in the triplet of bottles a, b and c was analyzed on Chapman mannitol culture medium using surface spreading method. For example, after 3 hours incubation, the content of the 3 bottles A_{3a}, A_{3b}, A_{3c} were analyzed. Petri dishes were then incubated at 37°C for 24 hours and the colony forming units (CFU) counted [26, 27].

This protocol was used for the two parts of the plant (leaves and roots) and three types of extracts (WME, WDE, AME). The bacterial abundances were expressed in units {log(CFU/100 mL)}.

Data analysis

The comparisons ($P \leq 0.05$) of the average of cells abundance were carried out using the analysis of variance test (ANOVA). Relationships between cells abundance and different concentrations of each type of *M. stenopetala* extracts, different incubation temperature and different incubation duration were assessed using Pearson correlation tests at $P < 0.01$ and $P \leq 0.05$. These analysis were carried out using Statistical Package for the Social Sciences (SPSS) 18.0 software.

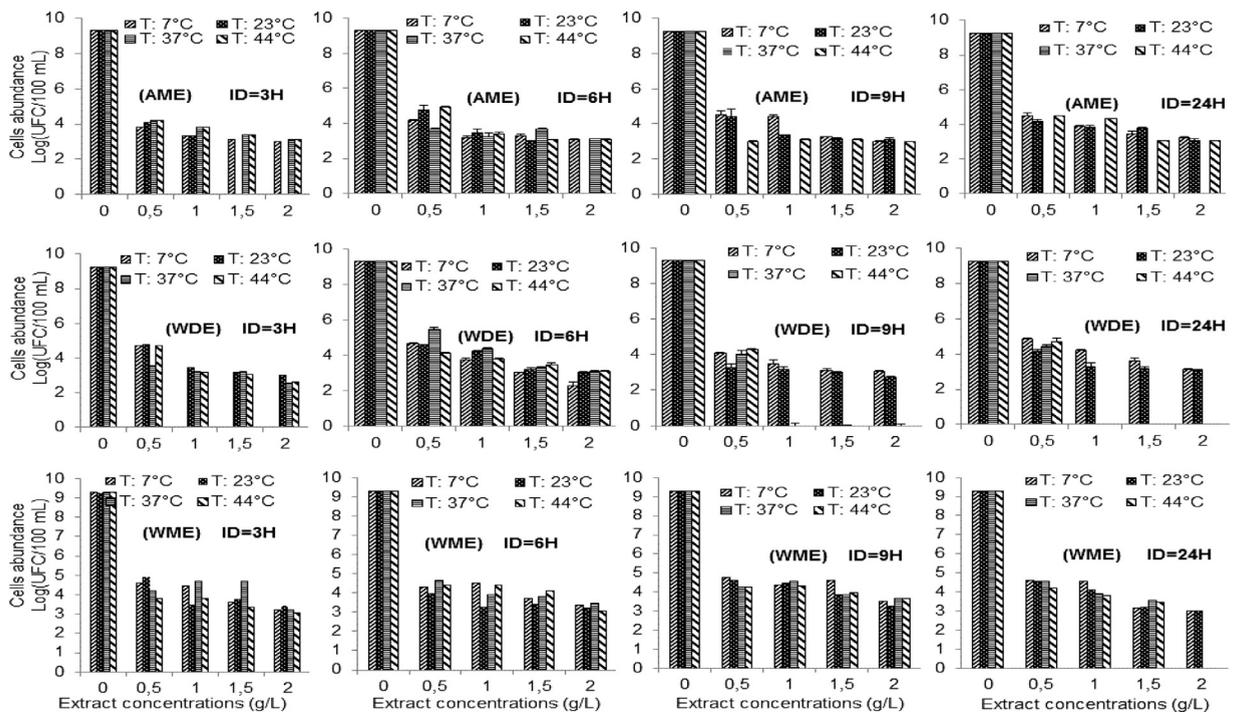


Figure 1. Variation in the abundance of *Staphylococcus aureus* cells with respect to the concentration of alcohol maceration extracts (AME); water decoction (WDE) and water maceration (WME) of *Moringa stenopetala* leaves under different incubation temperatures (7°C, 23°C, 37°C and 44°C) and during different incubation durations (ID) (3H, 6H, 9H, and 24H)

Results

Extraction yield

From leaves the percentage yield of the WME, WDE and AME was 49.06, 50.03 and 39.47 % respectively. For their aspects, WME was gluey; that of WDE was solid and the AME was pasty. For the WME, WDE and AME from roots, the extract percentage yield was 12.77, 31.13 and 17.2 % respectively. For their aspects, WME and WDE were solid and AME was gluey. The extract percentage yield thus was between 12.77 and 50.3 %. Whatever part of the plant used (leaf and root), it was highest with the water decoction extract (WDE). In most cases, the lowest yield was obtained with the roots. The extracts have a solid, sticky or pasty appearance regardless of the part of the plant.

Phytochemical screening of the different extracts

Phytochemical screening made it possible to highlight the different classes of secondary metabolites. The terpenoids, flavonoids, coumarins and anthraquinones were present in the 6 kinds of extracts used. Terpenoids and anthraquinones were in relatively higher concentrations in WME than AME and WDE. The flavonoids were in lower concentrations in leaves AME and in high concentrations in other extracts. The coumarins were in lower concentration in the 6 kinds of extracts. The steroids were present only in the leaves AME, phenols only in leaves WME, AME and WDE. The alkaloids were absent in roots WME and WDE, and were in higher concentration in leaves WME and AME. They were in lower concentration in roots AME and leaves WDE. The saponins were absent in leaves WME and WDE and were present in other extracts, although in low concentrations. The tanins were absent in roots AME and WDE whereas in higher concentrations in other extracts. The glucosides and anthocyanins were absent in all extracts analyzed.

Effect of *Moringa stenopetala* leaf extracts and temperature variation on the *S. aureus* cells abundance

Variation in the abundance of *Staphylococcus aureus* cells with respect to the concentration of alcohol maceration extracts (AME); water decoction (WDE) and water maceration (WME) of *Moringa stenopetala* leaves under different incubation temperatures (7°C, 23°C, 37°C and 44°C) and during different incubation durations (3 h, 6 h, 9 h and 24 h) are shown in [Figure 1](#). When different types of crude extract were used, a decrease in the cells abundance was noted and the magnitude depends on the extract concentrations.

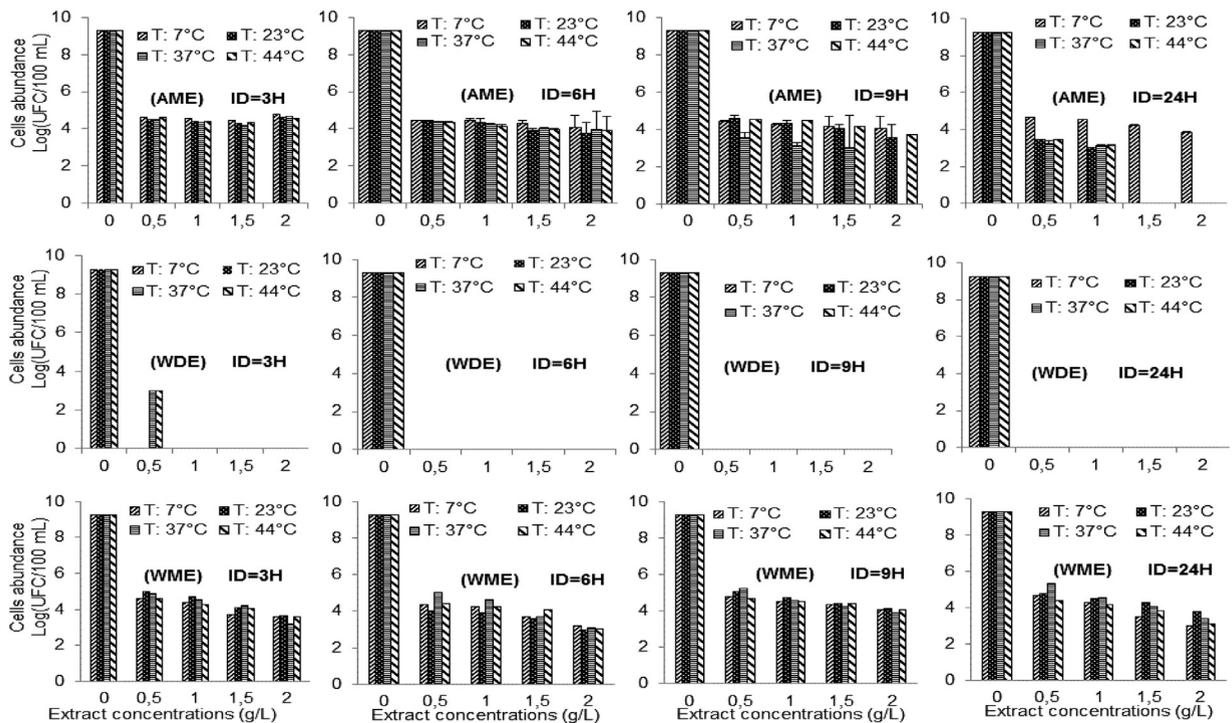


Figure 2. Variation in the abundance of *Staphylococcus aureus* cells with respect to the concentration of alcohol maceration extracts (AME); water decoction (WDE) and water maceration (WME) of *Moringa stenopetala* root bark at different incubation temperatures (7°C, 23°C, 37°C and 44°C) and during different incubation durations (ID) (3H, 6H, 9H, and 24H)

In the presence of AME extract of leaves, after 3 h incubation the highest cells abundance was 4.20 units {Log(CFU/100 mL)}. After 6 h, 9 h and 24 h incubation, the cells abundance were 4.90, 4.53 and 4.48 units {Log(CFU/100 mL)} respectively. However, when the AME was used, no cells CFU was registered after 3 h and 6 h, at 23°C incubation temperature for 1.5 to 2 g/L concentrations (Figure 1). After 9 h and 24 h incubation in the same extract, total cells inhibition was observed under 37°C incubation temperature, for all the extracts concentrations used.

When the WDE was used, cells abundance after 6 h, 9 h and 24 h incubation was 5.46, 4.85 and 4.29 units {Log(CFU/100 mL)} respectively (Figure 1). The WDE inclusion in the medium reduced the cells abundance at 3 h, 6 h, 9 h and 24 h incubation to a range between 2.25 and 0 units {Log(CFU/100 mL)}. Regarding WDE (ID = 3 h, 9 h and 24 h), total cells inhibition was observed at 1 g/L after 3 h and from 1.5 to 2 g/L after 9 and 24 h incubation at 37°C and 44°C respectively.

With the WME the highest cells abundance after 3 h was 4.93 units {Log(CFU/100 mL)} (Figure 1). After 6 h, 9 h and 24 h incubation the cells abundance were 4.63, 4.75 and 4.61 units {Log(CFU/100 mL)} respectively. The WME reduced the cells abundance after 3 h, 6 h, 9 h and 24 h incubation to 3.06, 3.06, 3.28 and 0 units {Log(CFU/100 mL)}, respectively. However with WME of leaves, total cells inhibition was not observed except at 2 g/L after 24 h at 37°C and 44°C (Figure 1).

Effect of *Moringa stenopetala* root bark extracts and temperature variation on the *S. aureus* cells abundance

Figure 2 presents the effect of root bark extracts and temperature variation on the accumulation of *S. aureus* cells. In general the root bark extract presented a remarkable effect in the reduction of *S. aureus* cells abundance. Cells abundance in the presence of AME varied between 4.63 to 3.61, 4.97 to 0, 4.87 to 0 and 4.60 to 0 units {Log(CFU/100 mL)} under 7°C, 23°C, 37°C and 44°C incubation, respectively (Figure 2). At 37°C total cells inhibition was observed at 2 g/L after 9 h of incubation. Total cells inhibition was also observed, in the presence of AME (ID = 24 h) at the concentrations 1.5 and 2 g/L at 23°C, 37°C and 44°C.

When using the WDE, the cells abundance ranged between 3 and 0 units {Log(CFU/100 mL)}, under temperatures 7°C, 23°C, 37°C and 44°C (Figure 2). Total cells inhibition was observed in all the concentrations except at 37°C and 44°C after 3h incubation culture conditions, with the WDE concentration of 0.5 g/L.

With the WME, the cells abundance varied between 4.80 and 3, 5.03 and 3, 5.23 and 3.10, and between 4.66 and 3.02 units {Log(CFU/100 mL)}, respectively at temperatures 7°C, 23°C, 37°C and 44°C. However, in the presence of the WME very little inhibitory effect was noted at all incubation temperatures and extract concentrations (Figure 2).

Table 1

Pearson correlation coefficients “r” between the abundances of *Staphylococcus aureus* and the concentrations of the extract of the different parts of *Moringa stenopetala* for each type of extract and incubation time.

Parts of plant	Types of extracts	Correlation coefficients after each incubation duration			
		3h	6h	9h	24h
Leaves	AME	-0.352**	-0.336**	-0.269*	-0.326*
	WDE	-0.344**	-0.354**	-0.281*	-0.223
	WME	-0.334**	-0.326*	-0.292*	-0.320*
Roots	AME	-0.356**	-0.364**	-0.279*	-0.328*
	WDE	-0.317*	-0.317*	-0.326*	-0.336**
	WME	-0.311*	-0.313*	-0.293*	-0.263*

** P < 0.01; * P ≤ 0.05; df: 59

WME: Water Maceration Extract; WDE: Water decoction Extract; AME: Alcohol Maceration Extract

Table 2

Pearson correlation coefficients “r” between the abundances of *Staphylococcus aureus* and the concentrations of the extract of the different parts of *Moringa stenopetala* at each type of extract and incubation temperature.

Parts of plant	Types of extracts	Correlation coefficients under each incubation temperature			
		7°C	23°C	37°C	44°C
Leaves	AME	-0.662**	-0.670**	-0.579**	-0.703**
	WDE	-0.638**	-0.572**	-0.656**	-0.634**
	WME	-0.755**	-0.561**	-0.607**	-0.681**
Roots	AME	-0.659**	-0.655**	-0.584**	-0.694**
	WDE	-0.637**	-0.598**	-0.684**	-0.673**
	WME	-0.753**	-0.706**	-0.707**	-0.821**

** significant correlation to P < 0.01, * significant correlation to P ≤ 0.05, df: 59

WME: Water Maceration Extract; WDE: Water decoction Extract; AME: Alcohol Maceration Extract

Correlations between abundance of *S. aureus* and concentrations of extracts of *M. stenopetala* at each incubation duration

The Pearson correlation coefficients “r” between cell abundance and concentrations of *M. stenopetala* extracts at each incubation period are presented in **Table 1**. It appears that in the most cases (for all parts of the plant and the types of extracts), the increase in the concentration of the extract under all the incubation durations considered significantly favors (P < 0.01) the decrease in the abundance of cultivable *S. aureus* cells. The exception was noted only for cells in the presence of WDE of leaves after 6 h, 9 h and 24 h of incubation (**Table 1**).

Correlations between abundance of *S. aureus* and concentrations of the extracts at each incubation temperature

Table 2 presents the Pearson correlation coefficients “r” between the bacterial abundance and the concentrations of *M. stenopetala* extracts at each incubation temperature. It appears that for all the parts of the plant tested and the 3 types of extracts, the increase in the concentration of the extract under the considered incubation temperatures significantly favors (P < 0.01), the decrease in abundance of cultivable *S. aureus* cells.

Comparison between the average abundance of cultivable planktonic *S. aureus* recorded under various incubation temperatures and concentrations of *M. stenopetala* extract

The average abundance of cultivable planktonic bacteria recorded in different concentrations of *M. stenopetala* extract and at each incubation temperature were compared with each other. The values of the significant coefficients are presented in **Table 3**. It appears that for leaf extracts; the P values in most cases are not significant compared with those obtained with the roots extracts. The degree of significance (P < 0.05) also varies from one type of extract to another depending on the concentrations of the extracts (**Table 3**).

Comparison between the average abundance of cultivable *S. aureus* cells recorded under various incubation periods and concentrations of *M. stenopetala* extracts

The average values of the abundance of cultivable *Staphylococcus aureus* recorded in different concentrations of *M. stenopetala* extract and at each incubation period were compared with each other using ANOVA. The P values are presented

Table 3

Comparison of the average cells abundance of *Staphylococcus aureus*, between the incubation temperatures at each concentration of extract of the different parts of *Moringa stenopetala*.

Parts of plant	Types of extracts	Average cells abundance of <i>S. aureus</i>				
		0 g/L	0.5 g/L	1 g/L	1.5 g/L	2 g/L
Leaves	AME	P=0.004*	P=0.055	P=0.092	P=0.465	P=0.007*
	WDE	P=0.342	P=0.205	P=0.373	P=0.477	P=0.025*
	WME	P=0.447	P=0.016*	P=0.056	P=0.135	P=0.767
Roots	AME	P=0.005*	P=0.014*	P=0.001*	P=0.002*	P=0.033*
	WDE	P=0.007*	P=0.077	P=0.001*	P=0.001*	P=0.001*
	WME	P=0.001*	P=0.001*	P=0.194	P=0.481	P=0.008

* Significant correlation to $P < 0.05$

Table 4

Comparison of average cells abundance of *Staphylococcus aureus*, between incubation periods at each concentration of *Moringa stenopetala* extract.

Parts of plant	Types of extracts	Average cells abundance of <i>S. aureus</i>				
		0 g/L	0.5 g/L	1 g/L	1.5 g/L	2 g/L
Leaves	AME	P=0.004*	P=0.055	P=0.092	P=0.465	P=0.007*
	WDE	P=0.342	P=0.205	P=0.373	P=0.477	P=0.025*
	WME	P=0.447	P=0.016*	P=0.056	P=0.135	P=0.767
Roots	AME	P=0.004*	P=0.013*	P=0.001*	P=0.002*	P=0.033*
	WDE	P=0.007*	P=0.077	P=0.001*	P=0.001*	P=0.001*
	WME	P=0.447	P=0.001*	P=0.001*	P=0.194	P=0.481

WME: Water Maceration Extract; WDE: Water decoction Extract; AME: Alcohol Maceration Extract;

* $P \leq 0.05$

in **Table 4**. It is noted that whatever the type and concentration of extract, the plankton cells abundance differ significantly ($P \leq 0.05$) with the incubation periods and extracts when using the leaves and roots (**Table 4**).

Discussion

Phytochemical screening

The results obtained highlighted antibacterial properties of the aqueous and ethanolic extracts of leaves and roots (*Moringa stenopetala*) tested against the bacterium *S. aureus*. Extraction yields varied significantly from part of the plant to the type of extract. These yields are higher than those recorded by Soma [28] and Sadou Nassirou *et al* [29] on the same plant, and that of Dzotam *et al* [30] on *Moringa oleifera* which is a related specie. This difference could be explained by the geographic origin on the one hand and internal factors related to the nature of the plant species on the other.

Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins which are compounds cited by earlier authors as having a significant bactericidal effect in aquatic environment [31]. Anthraquinones were present in all the parts of *Moringa stenopetala* studied. They are known to be excellent singlet oxygen generators [32]. In the presence of light, these compounds are capable of generating singlet oxygen which attacks and damages most of the microorganisms present in the environment [33]. In addition, volatile substances such as terpenes which were also present, at high dose, in the different parts of the species have been well documented for their antimicrobial effects [34].

Effect of extracts from different parts of the plant on *S. aureus* cells

The variation in the abundance of the cells depended on the concentration of the extract, on the types of the extracts, the different parts of the plant, and also on related factors such as the temperature and the incubation time. In general, the direct impact of aqueous and ethanolic extracts of *Moringa stenopetala* resulted in a decrease in the abundance of *S. aureus* cells. Cells abundance vary differently depending on the incubation temperature. *S. aureus* is a mesophilic bacterium whose growth temperature is between 20 and 40°C with a thermal growth optimum between 30 and 37°C [35]. Total inhibitions of 0 units {Log(CFU/100 mL)} of the sample, was recorded with the root decoction extract, at after incubation duration periods of 3 to 24 hours respectively at temperatures 7-44°C (**Figure 1**). These results would be linked to the nature of its Gram-positive cell wall, its mesophilic nature, with temperature ranged from 25 to 35°C and optimal temperature of 37°C [36]. It has also been noted that the average abundance of cultivable *S. aureus* cells differ significantly ($P \leq 0.05$), from one extract concentration to another and from one type of extract to another, from one plant part to another. The same observation was noted for each temperature and incubation time.

This study also showed that the aqueous and ethanolic extracts of *Moringa stenopetala* have an antibacterial effect in aquatic environment. Similar data have been reported by Sunda *et al* [37] who showed that the aqueous extracts of *Lantana*

camara, *Cymbogon citratus* and *Hibiscus rosa-sinensis* have a bactericidal effect in aquatic environment. Lutgen and Michels [38] have shown that by adding *Artemisia annua* tea to water contaminated with bacterial cells, there was a considerable reduction in the load of bacterial contaminants compared to that obtained after boiling water. There is also a negative and significant correlation ($P < 0.01$) between the incubation temperature and cells abundance on one hand, and between the concentration of extract on the other. In addition, a significant and negative correlation ($P \leq 0.05$) between cells abundance, types of extracts and the two plant parts has been registered. In most cases, a progressive decrease in the abundance of cultured cells was observed during the exposure duration in each experimental condition, which indicates a progressive increase in cellular inhibition. This inhibition seems to be significant when the incubation duration or residence time of the bacteria in the solutions of plant extracts increases.

Importance of the incubation temperature in the inhibition of planktonic cells of S. aureus, in the presence of aqueous and ethanolic Moringa stenopetala extracts

The results obtained showed that the abundance of cultivable cells relatively vary with respect to the incubation temperature. In most cases, it decreases with increase in incubation temperatures (Figures 1 and 2). The total inhibition of planktonic cells with roots decoction extract was noted during the different incubation temperature, particularly under 7 and 23°C.

The environmental temperature is one of the main important factors because it acts directly on the microbial development. Biochemical reactions of the cell metabolism depends on the activities of enzymes, which are themselves largely influenced by the temperature of the environment [39]. Some authors have shown that under psychropilic conditions, bacteria show resistance to extracts from plant [40]. An increase in temperature would be related to an increase in the speed of metabolic and biochemical reactions. Hence, strong inhibitions observed at 37°C and 44°C. The analysis of the variances test showed that there is overall a significant difference ($P < 0.05$) between the average abundance of *S. aureus* cells under each temperature, at different concentrations, types of extracts and parts of plant *M. stenopetala*.

The results presented in Table 3 shows that there is a significant difference between abundance of *S. aureus* cells at all extract concentrations and parts of the plant at the threshold ($P \leq 0.05$), except at the level of WDE and WME the leaves in the control solution. The difference in cells susceptibility towards the *M. stenopetala* extract provides information on a possible membrane effect of these extracts. It has been noted that there is no significant difference between the leaves, the abundance of *S. aureus* at concentrations 1 and 1.5 g/L, with all the three types of extracts (Table 4). This shows a resistance of these germs against the leaves extract at the concentrations 1 and 1.5 g/L. The two parts of *M. stenopetala* tested, showed antibacterial activities of various magnitude on *S. aureus*. This can be interpreted that the active component of the plant is more abundant in the root bark than the leaves and this plant part is also more effective on *S. aureus*.

Conclusion

The presence of *Moringa stenopetala* extract significantly induces the total and sometimes the partial inhibition of *S. aureus* cells in water at the 1% and 5% threshold. The incubation temperature and the concentrations of the different types of extract significantly influenced the bacterial survival. Significant difference between the leaves and roots extracts of *M. stenopetala* has been noted. Future work on ecotoxicology will show if this plant could constitute an alternative in the treatment of water intended for human consumption.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Supplementary material

Not yet

Declaration of Competing Interest

The author declares that there is no conflict of interest whatsoever.

References

- [1] WHO, the World Intellectual Property Organization Quality guidelines for drinking water fourth ed. incorporating the first Geneva additive, World Health Organization, 2017 CC BY-NC-SA 3.0 IGO <http://www.who.int/about/licensing>.
- [2] WHO, global water, sanitation and hygiene: annual report 2018. World Health Organization. License: CC BY-NC-SA 3.0 IGO; (2019) <https://apps.who.int/iris/handle/10665/327118>.
- [3] M. Pritchard, T. Mkandawire, A. Edmondson, J.G O'Neill, G. Kululunga, Potential of using plant extracts for purification of Shallow well water in Malawi, *Phys. Chem. Earth* 34 (2009) 799–805.
- [4] UNICEF, Control of diarrheal diseases (CDD) adapted from facts of life, watsan health education unit. 2nd ed. Lagos: Edproprint (1993).
- [5] J. Zhang, F. Zhang, L. Yand, H. Yang, A preliminary study on cactus as coagulant in water treatment, *Process Biochem.* 41 (3) (2006) 730–733.

- [6] O.C. Eneh, A.N. Eneh, Potable water access and management in Africa: implications for poverty, hunger and health, *Technol. Sci. Rev.* 5 (1-2) (2014) 3–15.
- [7] O. Mobili, C. Lontsi, A. Tamsa, M.M. Nougang, O. Allahdin, O. Noah, et al; Synergistic effect of light, pH and *Artemisia annua* extract on *Enterococcus faecalis* in aquatic microcosms, *Int. J. Appl. Microbiol. Biotechnol. Res.* 3 (2015) 62–72.
- [8] A. Tamsa, C. Lontsi, O. Noah, M. Djaouda, A. Yaouba, R. Tchikoua, et al; Use of the aqueous extract of *Eucalyptus microcorys* for the treatment in microcosm, of water containing *Enterococcus faecalis*: hierarchisation of cells' inhibition factors, *H2 Open. J.* 1 (1) (2018) 47–56, doi:10.2166/h2oj.2018.002.
- [9] C.S. Metsopkeng, C. Lontsi, O.V. Noah Ewoti, L.M. Mounang, P.A. Nana, A. Tamsa, et al; *Moringa oleifera* seeds extract activity on Enteropathogenic *Escherichia coli* and *Aeromonas hydrophila* cells in aquatic microcosm, *J. Appl. Biotechnol.* 7 (2) (2019) 13–30, doi:10.5296/jab.v7i2.14917.
- [10] B. Adriana, A.N.M. Almodovar, C.T. Pereira, T.A. Mariangla, Antimicrobial efficacy of *Curcuma zedoaria* extract assessed by linear regression compared with commercial mouth rinses, *Brazilian J. Microbiol.* 38 (2007) 440–445, doi:10.1590/S1517-83822007000300011.
- [11] S. Habtemariam, The African Moringa is to change the lives of millions in Ethiopia and far beyond, *Asian Pac. J. Trop. Biomed.* 6 (2016) 355–356.
- [12] Y. Mekonnen, V. Yardley, P. Rock, S. Croft, In vitro antitrypanosomal activity of *Moringa stenopetala* leaves and roots, *Phytother. Res.* 13 (6) (1999) 538–539.
- [13] B. Chekesa, Y. Mekonnen, Antibacterial activity of *Moringa stenopetala* against some human pathogenic bacterial strains, *Sci. Technol. Arts Res. J.* 4 (2) (2015) 190–198.
- [14] L. Le Minor, M. Véron, *Medical Bacteriology*. Flammarion ed. Paris, 1989.
- [15] F. Kengne, The overuse of intra and peri-urban space in Cameroon: the case of the cities of Douala and Yaounde, Communication presented at the 2nd Congress of African Geographers, colloquia in Rabat and Agadir, 1993.
- [16] G. Folkard, J. Sutherland, Development of robust water treatment systems incorporating natural coagulants, Field study report, January-March, Thyolo, Malawi, 1992.
- [17] I. Ciulei, Practical manuals on the industrial utilization of chemical and aromatic plants, Methodology for analysis of vegetable drugs, Ministry of chemical industry ed., Bucharest, 1982.
- [18] X. Zhao, Structure Identification of Triterpene from the Mushroom *Pleurotus eryngii* with inhibitory effects against Breast Cancer, *Plant Foods Hum. Nutr.* 70 (3) (2015) 1–6 29, doi:10.1007/s11130-015-0492-7.
- [19] D. Lacasse, Introduction to food microbiology. ed. Québec: St-Martin, 2004.
- [20] WHO Hardness in drinking-water, Background document for preparation of WHO Guidelines for drinking water quality, World Health Organization, Geneva, 2011 (WHO/HSE/WSH/10.01/10/Rev/1).
- [21] J. Holt, N. Krieg, P. Sneath, J. Staley, S. Williams, *Bergey's manual of determinative bacteriology*, 9 ed, Lippincott William & Wilkings, Philadelphia, 2000.
- [22] A. Tamsa, C. Lontsi, O. Noah, G. Bricheux, M. Nola, T. Sime-Ngando, Detachment of adhered enteropathogenic *Escherichia coli* cells from polythene fragments immersed in aquatic microcosm using *Eucalyptus microcorys* extract (Myrtaceae), *Curr. Res. Microbiol. Biotechnol.* 4 (3) (2016) 847–857.
- [23] G.E. Trease, W.C. Evans, Textbook of pharmacognosy. 12th ed., Tindall and Co, London, 1983.
- [24] J. Parekh S. Chanda, In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plant, *Turk. J. Bio.* 31 (2007) 53–58.
- [25] J. McFarland, Nephelometer an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines, *J. Am. Med. Assoc.* 14 (117) (1907) 6–8.
- [26] N. Marchal, J. Bourdon, D. Richard, Culture media for the isolation and identification of bacteria. 3rd ed., Dion, Paris, 1991.
- [27] APHA, (American Public Health Association). Standard methods for the examination of water and wastewater; 1998.
- [28] O.B. Soma, Antibacterial activity of extracts of *Euphorbia hirta* (Linn), a plant traditionally used in the treatment of urinary tract infections, State Doctorate thesis, University of Ouagadougou, Burkina Faso, 2002.
- [29] R. Sadou Nassirou, M.L. Ibrahim, I. Moussa, B. Mahamadou, A. Ilagouma, A. Abdoulaye, et al; In vitro evaluation of the antiplasmodial activity of plant extracts from the traditional pharmacopoeia of Niger: *Sebastiania chamaelea* (L.) Müll. Arg., *Euphorbia hirta* L., *Cassia occidentalis* L. and *Cassia nigricans* (Vahl) Greene, *Int. J. Innov. Appl. Stud.* 2 (2015) 498–505.
- [30] J.K. Dzatam, K.F. Touani, V. Kuete, Antibacterial and antibiotic-modifying activities of three food plants (*Xanthosoma mafaffa* Lam., *Moringa oleifera* (L.) Schott and *Passiflora edulis* Sims) against multidrug-resistant (MDR) Gram-negative bacteria, *BioMed Central Complem. Altern. Med.* 16 (9) (2016) 2–8.
- [31] A. Tamsa, O.V. Noah Ewoti, F.M. Onana, C. Lontsi Djimeli, S. Tchakonté, Djafsia Boursou, et al., The combined effect of *Eucalyptus microcorys* plant extract and light on enteropathogenic *Escherichia coli* survival in aquatic microcosm, *Journal of Microbiology, Biotechnology and Food Sciences* 9 (5) (2020) 1003–1008, doi:10.15414/jmbfs.2020.9.5.1003-1008.
- [32] C. Fufezan, C.M. Gross, M. Sjodin, Influence of the potential of the primary quinone electron acceptor on photoinhibition in photosystem II, *J. Biol. Chem.* 282 (2007) 12492–12502.
- [33] A. Cavalcante, G. Martinez, P. Mascio, C. Merck, L. Agnez, Cytotoxicity and mutagenesis induced by singlet oxygen in wild type and DNA repair deficient *Escherichia coli* strains, *DNA Repair* 1 (2002) 1051–1056.
- [34] M.S. Rakotonirainy, B. Lavédrine, Screening for antifungal activity of essential oils and related compounds to control the biocontamination in libraries and archives storage areas, *Inter Biodeter Biodegra* 55 (2) (2005) 141–147.
- [35] A. Meyer, J. Deiana, H. Leclerc, Course in general microbiology, New ed. Paris, Dion, 1994.
- [36] A. Aguilar-Galvez, R. Dubois-Dauphin, J. Destain, D. Campos, P. Thonart, Enterococci: advantages and disadvantages in biotechnology (bibliographic synthesis), *Biotechnol. Agronom. Socia. Environ* 1 (16) (2012) 67–76.
- [37] M. Sunda, F. Rosillon, K. Taba, Contribution to the study of water disinfection by photosensitization with plant extracts, *Eur. J. Water quality* 39 (2) (2008) 199–209.
- [38] P. Lutgen, B. Michels, Bactericidal properties of *Artemisia annua* tea and dosimetry of artemisinin in water by fluorescence under UV light. Proceedings of the International Conference "Tropical diseases, humanitarian and economic aspects", Luxembourg, June, (2008) 3–4.
- [39] J. Regnault, Elements of microbiology and immunology. 1st ed., Decarie, Canada, 2002.
- [40] A. Tamsa, Effect of the aqueous extract of the leaves of *Eucalyptus microcorys* Müller, 1860 on the cultivability of some bacteria of health importance, Doctoral thesis Ph.D, University of Yaounde 1, Cameroon, 2017.