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Research Article

Assessment of the Antimicrobial Effect of *Moringa Oleifera* Seed Powder in the Treatment of Water for Consumption in Cameroon: The Case of Borehole Water in the Kribi Area

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Abstract

Background: Drinking water is a vital natural resource whose effective access remains a major concern in sub-Saharan Africa, as in Cameroon, where the majority of the population is confronted with the consumption of untreated water from boreholes or wells that pose as health risks. As a result of this, the integration of biological processes for treating water using a non-toxic natural product; *Moringa oleifera (M. oleifera*), could be a sustainable alternative for improving the quality of drinking water. The aim of this study was to assess, compared to the one in which of conventional antimicrobials, the antimicrobial effect of *M. oleifera* seed powder in the treatment of water for potable purpose in Kribi, Ocean Division in the South Region of Cameroon. *Material and Methods*: An experimental study was conducted from November 2023 to June 2024. Borehole water was collected from four different quarters (one each) in Kribi I and II and transported in a cooler to Yaounde within 24 hours, at the Microbiology Laboratory of the HIMT (Higher Institute of Medical Technology) for analyses. In the laboratory, we cultured these untreated waters, isolated and identified the microorganisms using conventional methods and API20E, and the antimicrobial susceptivity of microorganisms was determined according to disc diffusion methods for antibiotics and antifungals. The contaminated water with confirmed identified microorganisms were later treated with increasing concentrations (0.15 g, 0.25 g and 0.35 g) of *M. oleifera* seed powder. The antimicrobial activity of the Moringa seed powder was assessed again by calculating the microbiological deduction rate. *Results*: Four different types of microorganisms were isolated and identified from the borehole water samples, with frequencies of 25% for *Escherichia coli*, 25% for *Staphylococcus aureus*, 25% for *Candida albicans* and 25% for *Candida sp.* The sensitivity profile demonstrated that *E. coli* and *S. aureus* were sensitive to 13.33% and 23.08% respectively of the antibiotics tested, *C. albicans* and *Candida. sp* sensitive each to 100% of the antifungals tested. Microbiological activity rates with *M. oleifera* seed powder varied from 86.7% to 100% for *E. coli*, 83.34 to 100% for *S. aureus*, 100% each for *Candida sp* and *C. albicans. Conclusion*: Our findings revealed increasing antibacterial and antifungal activities of *M. oleifera* seed powder after a short settling time, thus demonstrating its importance in improving the quality of drinking water for human consumption and preventing health risks.

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Keywords

Drinking Water, Boreholes, Moringa Oleifera, Treatment, Antimicrobial Effect

1. Introduction

Water is one of the natural resources for humanity life and socioeconomic development of Nations and must be of a quality that poses no risk to human health if it is to be used for human consumption [1]. WHO (World Health Organization) estimated that, worldwide consumption of unsafe water that poses health risks leads to the death of 1.5 million children under the age of five every year from diarrhoeal diseases [2]. In sub-Saharan Africa, this early infant mortality remains a public health problem according to UNICEF (United Nations International Children's Emergency Fund) [3], due to the proliferation of pathogenic germs transmitted by untreated drinking water.

In Cameroon, particularly in the Kribi area, the majority of the population are confronted with the consumption of unhealthy water from various unprotected sources of water supplying such as boreholes, well, streams which may contain pathogenic germs leading to health risks and recurrent water-borne diseases. This contributes to the weakening of the already precarious living conditions of populations and to infant mortality due to the lack of appropriate water treatment methods [4, 5]. In addition to conventional chemical treatment processes, natural biological processes were implemented and integrated a few decades ago in the treatment of potable water using natural products such as *Moringa (M.) oleifera* [6]. The aim of this study was to evaluate, compared to the one in which of conventional antimicrobials, the antimicrobial activity of *M. oleifera* seed powder in the treatment of Kribi borehole water. The findings of this study will serve as a basis for improving borehole water quality at the local level and preventing of health risks.

2. Materials and Methods

An experimental study was conducted from November 2023 to June 2024. Boreholes water collected in four different quarters in the Kribi area were included in this study using convenience sampling. In the laboratory, 100 mL of the untreated borehole water samples were plated on cultured media agars: EMB (Eosin Methylene Blue), MSA (Mannitol salt), CLED (Cysteine Lactose Electrolyte Deficient), TCBS (Thiosulfate–Citrate–Bile Salts–Sucrose), SS (*Salmonella Shigella*) and SDA (Sabouraud Dextrose Agar) with Chloramphenicol and later incubated at 37°C for 24 hours. The positive plates with well isolated colonies were identified using conventional methods and API20E and followed by performing AST (antimicrobial susceptibility testing) according to discs diffusion methods of antibiotics and antifungals. At the end, the untreated water with positive cultures were treated with increasing concentrations (0.15 g, 0.25 g and 0.35 g) of *M. oleifera* seed powder. Moringa's antimicrobial activity was assessed by calculating the percentage of microbiological elimination after counting the colonies before and after treatment. The steps involved in the study are summarised in Figure 1:

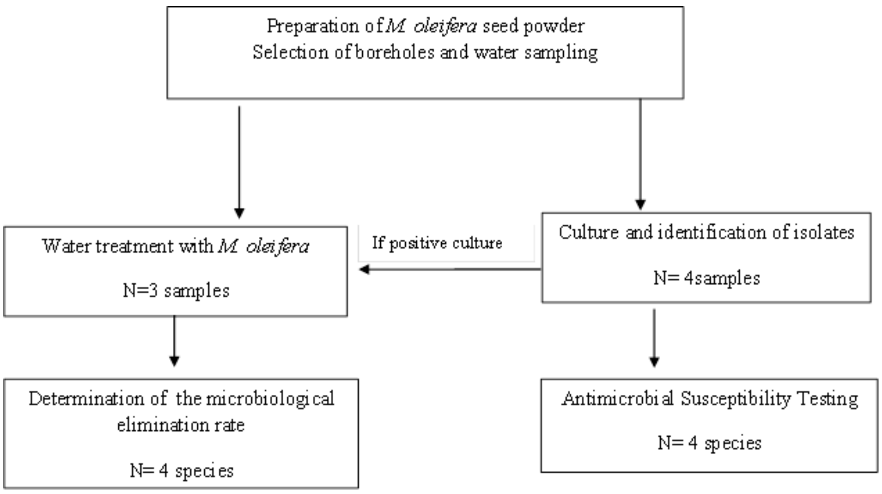

Figure 1. *Flow diagram of the study.*

2.1. Preparation of *Moringa oleifera* Seed Powder

Mature and dry *M. oleifera* seeds were obtained from a private family farm located at Bandevouri in the Ocean Department, South Region of Cameroon, which were confirmed identification at the NHC (National Herbarium of Cameroon) under number 66895 HNC.

Preparation were carried out according to following method below [7]: - clean and sort the seeds - dehull the seeds to obtain the almonds - dry the obtained almonds for two to three days in sunlight - grind or crush the dried almonds with cold press or crushing mill to obtain the powder - sift the powder to obtain fine powder - store the fine powder in sterile container (plastic bag or bottle) The fine powder obtained was weighed in order to obtain masses of 0.15 g, 0.25 g and 0.35 g for the different concentrations used to treat the water collected.

2.2. Study Sites and Sampling

Early morning borehole water samples were collected in triplicate from four different quarters in Kribi I and II, distributed in sterile bottles, stored in a icebox and were transported to the Microbiology Laboratory at the Higher Institute of Medical Technology in Yaounde within 24 hours, then stored in a cool place at -4℃ until analyses.

2.3. Microbiologic Analyses of Borehole Water

In the laboratory, each of the borehole water sample was first subjected to a presumptive microbiology test, consisting of aseptic culture by flooding in EMB, SS agar, CLED, MSA, TCBS and SDA-chloramphenicol media, then incubated at 37℃ for 24 hours. After incubation, Gram staining was performed on all positive culture agars and isolates from EMB, MSA, SDA-chloramphenicol positive culture agars were then identified by identification microbiology test using conventional methods and the Api 20E gallery followed by antimicrobial susceptibility test (Figure 2):

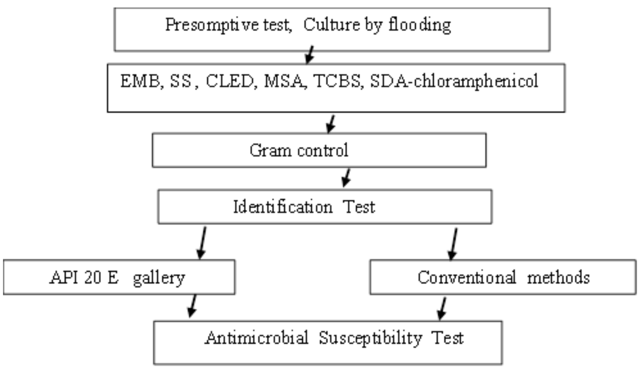

Figure 2. *Steps of microbiologic analysis.*

2.4. Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing of the microorganisms isolated and identified after positive culture of the untreated borehole water was carried out using the discs diffusion methods with Oxoid and Liofilchem antibiotics and antifungals discs on solid media. This in accordance with the recommendations of the CASFM *(Comité d'Antibiogramme de la Société Française de Microbiologie)* and the EUCAST (*European Committee on Antimicrobial Susceptibility Testing*) of 2023 for the disc diffusion method of antibiotics (Kirby-Bauer) [8] and the recommendations of the *Clinical Laboratory Standards Institute* of 2016 (CLSI, 2016) for the disc diffusion method of antifungals [9].

2.5. Treatment and Microbiological Analyses of Culture-positive Water with *M. oleifera*

The culture-positive borehole water samples with isolates from EMB, MSA and SDA chloramphenicol agars were treated in parallel with *M. oleifera* seed powder in triplicate according to following method below [10]:

1) 100ml of each cultured positive water samples were transferred into three sterile bottles - followed by the addition of 0.15g, 0.25g and 0.35g of *M. oleifera* seed powder to obtain concentrations of 1.5g/L, 2.5g/L and 3.5g/L respectively,
2) the mixture was homogenised rapidly for 1min using a sterile stirrer, then slowly for 5 minutes - followed by decantation for 2 hours and 24 hours, then by filtration through sterile filter paper, - followed by culture and incubation at 37℃ for 24 hours.

After incubation, the colonies were counted and the microbiological elimination rates (A), which is defined as the difference in bacterial or parasite concentrations between that of the untreated water (Eb) and that of the treated water (Em), were calculated using the Halmaidi and *al*. formula [11]: $A = (Eb – Em/Eb) \times 100$. An elimination rate of 100% represents the total elimination of microorganisms in water intended for human consumption [1].

2.6. Statistical Analysis

Statistical analysis was carried out using SPSS (Social Package for Statistical Science) version 26 and Excel 2016. Means were compared using the ANOVA test, with a statistically significant threshold $p < 0.05$.

3. Results

3.1. Frequency of Microorganisms Isolated from Borehole Water

Isolation and identification of isolates from the borehole water collected in the four different quarters were *E. coli* (25%) in the Makawum-Kribi II site, *S. aureus* (25%) in the Londji 1-Kribi II site, *Candida sp* (25%) in the Ngoe wami é-Kribi I site and *C. albicans* (25%) in the Londji 1-Kribi II site. No microorganism was isolated from the borehole at the Mpangou-Kribi I site (Table 1).

Table 1. *Frequency of microorganisms isolated from borehole water.*

	E. coli n (%)	S. aureus n (%)	C. albicans n (%)	Candida Sp n (%)	Total n (%)
Mpangou-Kribi I	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Makawum Kribi II	1 (25%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)
Ngoe wami ékribi I	0 (0%)	0 (0%)	0 (0%)	1 (25%)	1 (25%)
Londji 1-Kribi II	0 (0%)	1 (25%)	1 (25%)	0 (0%)	2 (50%)
Total	1	1	1	1	4 (100%)

3.2. Susceptibility and Resistance Profile of Isolates from Borehole Water to Antimicrobials

The sensitivity profile of isolates to antimicrobials as seen in Table 2 revealed that *E. coli* was sensitive to two of the fifteen antibiotics tested (13.33%) and *S. aureus* to three of the thirteen antibiotics tested (23.08%) according to CASFM/EUCAST, 2023 [8], *C. albicans* and *Candida sp* to five of the five antifungals tested (100%) each according to CLSI, 2016 [9].

Table 2. *Frequency of susceptibility of isolated microorganisms to antimicrobials.*

	Frequency of active antimicrobials n (%)	Antimicrobial names
E. coli	2 (13,33%)	Amikacin (Aminosids class), Fosfomycin (Others classes)
S. aureus	3 (23,08%)	Nitofurantoin, Rifampicin (Others classes), Clindamycin (Macrolids class)
C. albicans	5 (100%)	Amphotéricin B (Polyens class), Econazole, Clotrimazole, Miconazole, Kétoconazole (Azoles class)
Candida sp	5 (100%)	Amphotéricin B (Polyens class), Econazole, Clotrimazole, Miconazole, Kétoconazole (Azoles class)

The resistance profile of isolates to antimicrobials revealed that:

1) *E.coli* was resistant at 86,67% (13/15) of the antibiotics tested: to ampicillin, amoxicillin-clavulanic acid, piperacillin, piperacillin-tazobactam (within Penicillin class), to cefoxitin, ceftazidin, cefotaxime, cefepim (within Cephalosporins class), to azotreonam (within Monobactams class), to ciprofloxacin (within Fluoquinolons class), to gentamicin, tobramycin (within Aminosids class) and to trimethopin-sulfamethoxazol (within others classes);
2) *S. aureus* was resistant at 76,92% (10/13) of the antibiotics tested: to cefoxitin (within Cephalosporins class), to gentamicin, kanamycin, tobramycin (within Aminosids class), to tetracyclin (within Cyclins class), to ciprofloxacin, norfloxacin (within Fluoroquinolons class), to erythromycin (within Macrolids class), to fusidic acid, trimethopin-sulfamethoxazol (within others classes);
3) *C. albicans* and *Candida sp* were resistant at 0% (0/5) of the antifungals tested (within Polyens and Azoles classes).

3.3. Antimicrobial Activities of *M. oleifera* in Water

The microorganisms isolated and identified were counted

(CFU/100ml) per site of untreated and treated water according to the increasing concentrations of the *M. oleifera* seeds powder. The number of microorganisms isolated from samples ranged between 50 to 100 CFU/ml. After the treatment with *M. oleifera* and after two hours of decantation, the quantity of microorganisms decreated significantly (Table 3):

Table 3. *Number of microorganisms isolated and identified from water in each site.*

Sample sites	Microorganisms	Number of colonies (CFU/100ml) isolated in water before and after treatment with *Moringa oleifera*									
		Untreated water	0,15g of *M. oleifera*			0,25g of *M. oleifera*			0,35g of *M. oleifera*		
S1: Mpangou-Kribi I	-	00		*00*			*00*			*00*	
S2: Makawum-Kribi II	*Escherichia coli*	80	18	14	0	6	0	0	0	0	0
S3 Ngoe wami é-kribi I	*Candida sp*	50	0	0	0	0	0	0	0	0	0
S4: Londji 1-Kribi II	*Staphylococcus aureus*	100	30	20	0	8	0	0	0	0	0
S4: Londji 1-Kribi II	*Candida albicans*	80	0	0	0	0	0	0	0	0	0

M. oleifera exhibited antibacterial and antifungal activities on the microorganisms isolated and identified in the culture-positive treated water after 2 hours of decantation, with microbiological elimination rates of 86.7%, 97.5% and 100% for *E. coli* at concentrations of 1.5g/L, 2.5g/L and 3.5g/L respectively, 83.34%, 97.34% and 100% for *S. aureus* at concentrations of 1.5g/L, 2.5g/L and 3.5g/L respectively and 100% for *C. albicans* and *Candida sp* each at the various concentrations tested as presented in Figure 3:

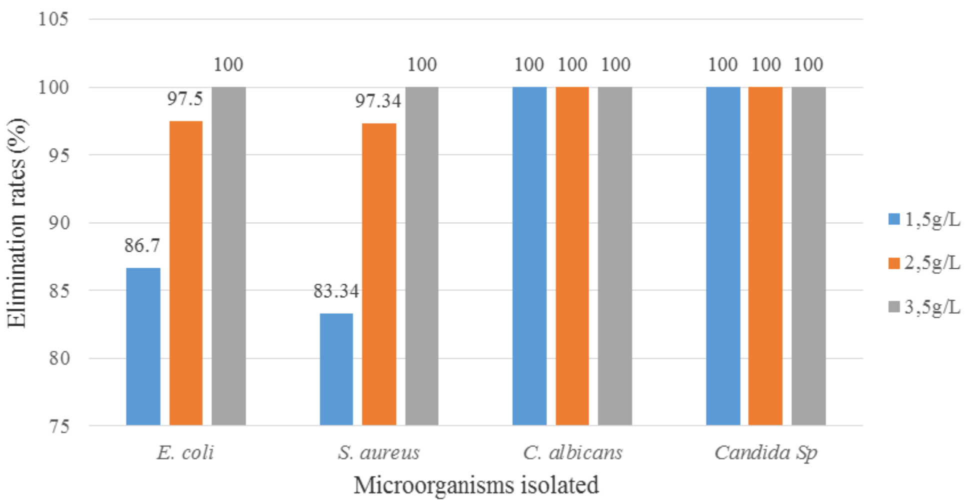

Figure 3. *Elimination rate of Moringa oleifera after 2 hours of decantation.*

However, the isolation of microorganisms after 24 hours of decantation was characterised by an invasion of the agar plates due to bacterial proliferation.

4. Discussion

Water cuts due to the distribution system and the constant need of the population force them to seek several different options of sources of water supply. One of the most popular sources of portable water is the borehole. However, the development of agriculture with the use of pesticides, disasters linked to climate change and the proximity of households are factors that encourage the pollution of boreholes. In order to improve the microbiological quality of drinking water, we assessed the antimicrobial activity of *M.*

oleifera seeds in borehole water consumed by the population of Kribi town.

In this study, 75% of boreholes water were contaminated by *E. coli* (80 CFU/100mL), *S. aureus* (100 CFU/100mL), *C. albicans* (80 CFU/100mL) and *Candida sp* (50 CFU/100mL). The isolation and identification of these microorganisms in the borehole water intended for human consumption demonstrates a health risk to the population, since it revealed microbial load higher than the standards recommended by the WHO (<10 CFU/100mL) at 37 ℃ [12]. These results may be explained by recurrent flooding, the construction of boreholes that do not comply with standards, and the absence of a functional system for monitoring the microbiological quality and for treating of borehole water in Cameroon. Unlike our study, bacterial groups such as Coliforms and *Streptococcus* have been isolated from water in other studies [13].

M. oleifera seed powder had antibacterial activity against *E. coli* and *S. aureus* and antifungal activity against *C. albicans* and *Candida sp*. These results are similar to previous studies who reported 100% elimination of *E. coli*, *Staphylococcus* and *C. albicans* respectively by *M. oleifera* seeds [14-16]. Previous studies have revealed that *M. oleifera* seeds possess active proteins that attract particles suspended in water and to which microorganisms are attached. Some of these proteins, known from molecular characterisation, exert antibacterial activity [17, 18] and antifungal activity [19], making it possible to improve the quality of drinking water. However, the bacterial proliferation observed after 24 h decantation in the presence of *M. oleifera* could explain the bacteriostatic and fungistatic activities of this plant's seeds. Studies carried out by Jahn *and al*. in Indonesia have revealed that, despite the antimicrobial activities of *M. oleifera*, the organic matter provided by Moringa seeds can promote bacterial growth in treated water in the long term [20], hence the need for a relatively short or reduced storage time to avoid the proliferation of microorganisms.

In our study, 13.33% and 23.08% of the conventional antibiotics tested were active on *E. coli* and *S. aureus* respectively, and 100% of the antifungals tested were active on *C. albicans* and *Candida sp* each. The multi-resistance observed on *E. coli* (86.67%) and *S. aureus* (76.92%) to antibiotics demonstrated the population's exposure to multi-resistant bacteria, putting patients vital prognosis at risk.

While conventional antimicrobials (antibiotics and antifungals) each acted specifically on their specific bacterial or fungal isolates, Moringa seed powder acted simultaneously as an antibacterial and antifungal agent, with effective antimicrobial activity on both bacterial and antifungal isolates, depending on its concentration and decantation time.

This study revealed the antimicrobial potential of *M. oleifera* seed powder on several bacterial and fungal species, demonstrating its usefulness in treating borehole water intended for human consumption.

However, there are a number of limitations, in particular the small sample size and the absence of physico-chemical water parameters and inhibition parameters. It would be necessary to extend the study to several boreholes and in other towns, and to determine the physico-chemical parameters of water and inhibition parameters of *M. oleifera* seeds.

5. Conclusion

Potable water is a major health issue for populations, particularly in developing countries such as Cameroon. Simple, low-cost, biological water treatment methods are essential criteria for the sustainability of processes in these countries. Biological water treatment using *M. oleifera* revealed antibacterial and antifungal activities for microbiological elimination after two hours of decantation. These results provide a clear indication that *M. oleifera* could be used to treat water intended for human consumption in areas where access to drinking water is difficult to improve drinking water quality and prevent health risks.

Abbreviations

API	Analytic Profile Index
CFU	Colony-Forming Unit

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Author Contributions

J-RM conceived and designed the intellectual content, collected the data and wrote the original manuscript; conducted the primary analysis, HGK reviewed and edited the manuscript, WBA reviewed and edited the manuscript, YNT reviewed and edited the manuscript, TNN reviewed the manuscript, DN coordinated research activities and reviewed the manuscript. All authors have approved the final manuscript and agreed to be personally accountable for the author's own contributions.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] WHO: Guidelines for drinking water quality (4e édition), Genève, 2011, p. 531.

[2] OMS : Les effets de l'environnement sur la santé de la mère et l'enfant, aide-mémoire, Genève, 2005, p. 285.

[3] UNICEF: La situation des enfants dans le monde, l'égalité des chances pour chaque enfant. Soduc, 2016, French Last, pdf.

[4] Assako A. R. J., Djilo C. A., Tomneu et Bley D.: Risques sanitaires et gestion des eaux usées et déchets à Kribi: Sociétés, Environnement, Santé, IRD Editions, Cameroun 2010.

[5] Kamgho T. B. M.: L'accès à l'eau potable et à l'assainissement au Cameroun: situation actuelle, contraintes, enjeux et défis pour l'atteinte de l'OMD 7, Cameroun. *Revue d'Economie Et De Management*, 2008, *9*(1), 111–124.

[6] Pise C. P. Halkude S. A: A new technique for purification of water using natural coagulant. International Journal of Engineering and Technology, 2015, 6, 2564-2572.

[7] Lea M.: Bioremediation of Turbid Surface Water using seed extract from *Moringa oleifera* Lam (Drumstick), tree clearing house: low-cost water treatment technologies for developing countries, emerging technologies, current protocols in Microbiology, 2010, 1-14. https://doi.org/10.1002/9780471729259.mc01g02s16

[8] CASFM/EUCAST: Recommandations 2023, 1.0. 2023, 1-177.

[9] CLSI: Method of antifungals disks diffusion susceptibility testing of yeasts; approved guideline-Second edition. 2009, 29(17), 1-13.

[10] Folkard G. The development of the *Moringa oleifera* and *stenopetala* tree to provide valuable products coagulant for water/wastewater treatment and vegetable oil. Rapport à la Commission Européenne, 1998, 1-5.

[11] Hamaidi-Chergui F, Zoubiri A. F, Debid A, Hamaidi M. S, Kais H.: Evaluation de la charge en pathogenèse et de la microfaune dans les eaux de l'effluent brute et traité, rejeté dans un milieu récepteur: cas de la station d'épuration de Médéa, Larhyss Journal, 2016, 13(2), 183-208.

[12] OMS: L'utilisation des eaux usées en agriculture et en aquaculture: recommandations à visées sanitaires. Rapport d'un groupe scientifique de l'OMS, Genève, 1989.

[13] Behnas Sadrine, Benabdelkader Safia: Evaluation de l'activité antibactérienne des graines de *Moringa oleifera* dans la dépollution des eaux usées, Université de Constantine, Algérie 2021. 1-71.

[14] Kaboré A, Savadogo B, Rosillon F, Traore AS, Dianou D: Optimisation de l'efficacité des graines de *Moringa oleifera* dans le traitement des eaux de consommation en Afrique sub-saharienne: cas des eaux du Burkina-Faso. Revue des sciences de l'eau, 2013. 209-220.

[15] Magaji U. F, Sahali D. M, Abubakar M. K et Muhamad A. B: Biocoagulation activity of *Moringa oleifera* seeds for water treatment. The International Journal of Engineering and Science (UES), Nigéria, 2015, 4(2), 19-26.

[16] Neto JXS, Pereira ML, Oliveira1 TA, Rocha-Bezerra LCB, Lopes TDP, Costa HPS et al.: A chitin-brinding protein purified from *Moringa oleifera* seeds presents anticandidal activity by increasing cell membrane permeability and reactive oxygen species production. Frontiers in microbiology, 2017, 8: 980. 1-12. https://doi.org/10.3389/fmicb.2017.00980

[17] Suarez M., Haenni M., Canarelli SP., Fisch F., Chodanowski P., Servis C. et al.: Function characterization and optimization of a plant-derived antibacterial peptide. Antibacterial agents and chemotherapy, 2005, 49(9): 3847-3857. https://doi.org/10.1128/AAC.49.9.3847-3857.2005

[18] Moulin M., Mossou E., Signor I., Kieffer-Jaquinod S., Kwaambwa HM., Nermark F. et al.: Towards a molecular understanding of the water purification properties of Moringa seed proteins. Journal of colloid and interface science, 2019, 554, 296-304. https://doi.org/ 10.1016/j.jcis.2019.06.071

[19] Freire JE., Vasconcelos IM., Moreno FB., Batista AB., Lobo MD., Pereira ML. et al.: Mo-CBP3, an antifungal chitin-binding protein from *Moringa oleifera* seeds is a member of the 2S albumin family. Plos One, 2015, 10(3), 1-24. https://doi.org/10.1371/journal.pone.0119871

[20] Jahn, Samia AL., Azaria H. D: Différents rôles des coagulants naturels dans la clarification de l'eau, dans les technologies appropriées à usage domestique et dans les installations communales d'épuration. Dans: *Proceedings of International Seminar or the Use of national coagulants for water treatment, Yogyakarta,* Indonesisa, 1989, 1-11.