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Antibacterial Activities of Plant Extracts on Enterobacteriaceae Strains Resisting Hydro-Alcoholic Gels Sold in Bukavu, D.R. Congo

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Abstract: Hydro-alcoholic gel (HAG) destroys microorganisms onto a surface such as hands and contribute to fight hand-borne diseases. The COVID-19 pandemic increased the variety of HAG sold anywhere. However, the HAG quality and effectiveness to destroy bacteria after usage remain a problem. This study aimed to assess the HAG quality and their *Enterobacteriaceae* killing power, and to test the bacteria strains susceptibility face plant extracts. The *Enterobacteriaceae* strains were isolated from 43 student's hands. Subsequently an *Enterobacteriaceae* numeration after growing on Mac-Conkey Medium. The measurement of inhibition diameter after depositing disks impregnated with three plant extracts, allowed to assess their antibacterial activity (ABA). The main result showed that *Enterobacteriaceae* resisted to the HAG hands disinfection. Nevertheless, the HAG4 and HAG1 with 91.67% and 79.67% respectively, displayed an appreciable reduction power of *Enterobacteriaceae* onto the hands. Moreover, *Enterobacteriaceae* resisted to all type of plant extracts. Furthermore, the *Enterobacteriaceae* resistance increased after using HAG than before. This study confirmed the increasing of *Enterobacteriaceae* resistance power to a wide antimicrobial substance. Upcoming studies should search pathway used by *Enterobacteriaceae* to escape the ABA.

Keywords: bacterial resistance, Enterobacteriaceae, hydro-alcoholic gel, medicinal plant, Bukavu

Résumé : Les gels hydro-alcooliques (HAG) détruisent les microorganismes sur de surfaces telles que les mains et contribuent à lutter contre le portage manuel des microorganismes. La pandémie de COVID-19 a accentué l'usage des HAG. Cependant, la qualité de ces HAG et leur efficacité à éliminer les bactéries manuportées, demeure un problème. Cette étude a évalué la qualité de HAG and leur pouvoir à éliminer les entérobactéries manuportées, et tester la sensibilité de ces souches entérobactériennes face aux extraits de plantes médicinales. Les souches ont été isolées à partir de mains de 43 étudiants. Les entérobactéries ont été dénombrées après culture et croissance sur l'agar de Mac-Conkey. La mesure des diamètres d'inhibition, après le dépôt des disques imprégnés des extraits de plantes médicinales, a permis d'évaluer leur activité antibactérienne (ABA). Les résultats ont montré que les souches d'entérobactéries étaient résistantes aux HAG lors de la désinfection de mains. Néanmoins, le HAG4 et HAG1 ont montré un pouvoir appréciable de réduction du nombre d'entérobactéries manuportées, respectivement avec 91.67% et 79.67%. En outre, les entérobactéries étaient résistantes à tous les extraits de plantes. Aussi, les entérobactéries isolées après l'usage de HAG étaient plus résistantes que celle isolée avant. Cette étude a confirmé l'accroissement de la résistance des entérobactéries face à la plupart des molécules anti-microbiennes. Les futures recherches devraient se focaliser sur les mécanismes utilisés par les entérobactéries pour échapper à l'activité antibactérienne des antibiotiques.

Mots clés : résistance bactérienne, entérobactérie, gel hydro-alcoholique, plante médicinale, Bukavu.

1. Introduction

Enterobacteriaceae consist of several bacteria pathogens that cause several diseases like Salmonellosis, diarrhea and other complications outside from intestine (Madigan and Martinko, 2007). Being cosmopolites, Enterobacteriaceae are incriminated in the hand-borne diseases (Korsak et al., 2004; Kim et al., 2011; WHO, 2009). Since the end of 2019, the world has been facing the COVID-19 pandemic (Black et al., 2020; Xing et al., 2020), that leads people to adapt their protection, especially to keep safe their hands using different chemical solution, such as hydro-alcoholic gel (HAG) to regularly disinfect the hands. A good disinfection is widely depending to the disinfectant used, also the correct usage of such disinfectant (Aidara et al., 2021). It is obvious that the use of disinfectant to fight COVID-19 can likely help to reduce Enterobacteriaceae strains carriage and their circulation within population. Most Enterobacteriaceae strains become multi-drug resistant face several antibiotics (Crump, 2012; Tack et al., 2020). This resistance results from the genetic transformation and the gene transfer between species of Enterobacteriaceae, given that they usually live in the same niche (Faure et al., 2010 ; Puyvelde et al., 2019). The multi-drug resistance (MDR) is among health public concerns wide world and none country is spared from bacteria resistance and therapeutic faillure (Crump, 2012 ; Phoba et al., 2020). Nowadays, it is significant to look for novel path to contribute to fight MDR and assess the effectiveness of certain molecules used to inhibit the Enterobacteriaceae growth (Cascioferro et al., 2021). In addition, there is a lack of data on quality and antibacterial activities (ABA) of HAG sold in Bukavu.

This study aimed to assess the quality and effectiveness of different HAG sold in Bukavu; study their inhibition power after hand disinfection; and test the susceptibility of Enterobateriaceae resisting the HAG hands disinfection, face plant extracts.

2. Materials and methods

2.1. Study area

This study was carried out in Bukavu city, located at 2°3’ and 28°5’ East, 1600 m of altitude, in South-Kivu province, D.R. Congo. This city is extended on 63 Km^2 of which 20 Km^2 are occupied by the Lake Kivu (Lina et al., 2015).

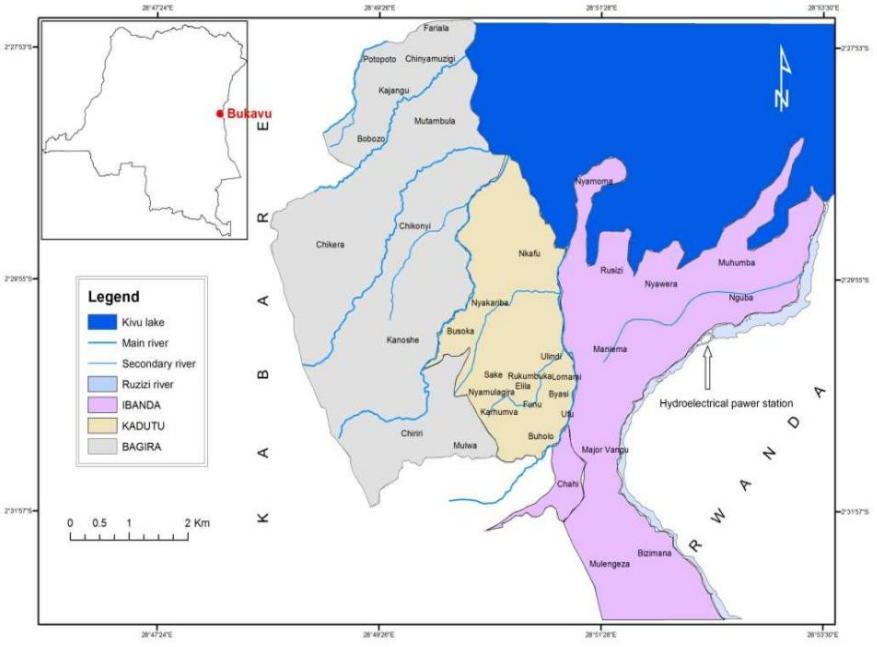


Figure 1. Map showing Bukavu city with its 3 municipalities: Bagira, Kadutu and Ibanda (Sadiki et al., 2010). It counts about 1 500 000 people. There is lack of sanitization, exposing people to all kinds of diseases, include hand-borne diseases

2.2. Sample collection

2.2.1. Brands of HAG

Four HAG brands were bought at pharmacies, after verification the expired date and lid tightness. The choice of HAG was based on their affordable price (no more than 5 US dollars) to low - income population, in order to assess the most used disinfectants. To avoid publicity, each HAG was encoded using numbering: HAG1, HAG2, HAG3 and HAG4. The bacteria killing power was 99.99% as mentioned on the bottle for each of HAG.

2.2.2. Plant extracts and discs preparation

Three medicinal plants were harvested from different sites in Bukavu (Table 1). The plant extracts have been prepared to test their inhibition power against Enterobacteriaceae that have resisted HAG, in the framework to look for novel bacteria inhibitor molecule, which can be used to fight Enterobacteriaceae resistance.

Table 1. Medicinal plants used to prepare plant extracts for testing the antibacterial activity (ABA) on Enterobacteriaceae isolates

N°	Plant	Organ	Site	Extraction solvent
1	*Bidens pilosa*	leaves	Kadutu	CCE*, Ether and Ethanol
2	*Moringa oleifera*	leaves	Bagira	CCE, Ether and Ethanol
3	*Tetradenia riparia*	leaves	Kadutu	CCE, Ether and Ethanol

*CCE : Concentrated Crude Extract

The discs of 7 mm were made with filter paper. Each of disc was labeled on the both sides according to plants and extraction solvent used (Table 1), then autoclaved at 121 °C for 15 minutes. The juice of CCE was collected after leaves pounding and squeezing, then 10 mL of juice were taken and concentrated up to 2 mL by evaporation at 50 °C. For the ethanol and ether extracts, 10 g of fresh leaves pounded, were immersed in 50 mL of each solvent. The mixture was macerated for 48 hours and filtrated using filter paper. Finally, 10 mL filtrated juice was concentrated by evaporation up to 2 mL. The concentrated extracts were mixed and absorbed in each disc (Etobo et al., 2017).

2.2.3. Collecting, packing and transportation of samples

The samples were collected before and after hands disinfection. Forty-three (43) students of "Université Officielle de Bukavu" (UOB) disinfected their hands by rubbing the palm and the back of hand during two minutes with the selected HAG according the WHO standard (WHO, 2009). Subsequently, a sterile swab was rubbed onto the disinfected hands and deposited into 5 mL of peptone water (PW). The samples were put into a cold container and transported to the Microbiology and Biotechnology Laboratory of UOB, to undergo bacteriological analyzes. The

students' choice was based on their availability at UOB during sampling activity and their consent to participate in the study.

2.3. Enterobateriaceae isolation

One mL from inoculated PW, was inoculated by mixture in Mac-Conkey agar (MCA), then incubated at 44 °C for 24 hours. The colonies counting were performed on dish after Enterobacteriaceae growth. Only the plates with less than 300 colonies were considered for numeration. Subsequently, the presumptive Enterobacteriaceae colonies have been characterized through the biochemical tests. The isolated strains have undergone the susceptibility test face plants extracts (Etobo et al., 2017).

2.4. Deposit of discs impregnated with plant extracts

To test the ABA of plant extracts, Enterobacteriaceae isolated before and after HAG disinfection was put into ethanol, ether and CCE extracts of *Bidens pilosa*, *Moringa oleifera* and *Tetradenia riparia*. The ABA of plant extracts was appreciated using the method of extract diffusion on Mueller – Hinton medium face Enterobacteriaceae, after incubation at 37 °C for 48 hours. The ABA displayed by plant extracts was obtained by measuring the mean inhibition diameter around discs of extracts from each of plant. The inhibition power of each extract was classified in 3 levels: *Resistant*: for an inhibition diameter under 10 mm ; *Susceptible*: ≥ 15 mm and *Intermediate*: between 10 – 14 mm (Etobo et al., 2017).

2.5. Data analysis

The non-parametric Kruskal-Wallis test was performed using R-studio software (version 4.1.1) to test the significance at 5% level.

3. Results

3.1. Numbering and killing power of Enterobacteriaceae colonies before and after HAG disinfection

The assessment of HAG killing power is summarized in Table 2 and 3.

Table 2. Number of Enterobacteriaceae colonies grew from samples taken before and after HAG hands disinfection

Hands disinfection	**Enterobacteriaceae colonies growth from samples**			
	0	**1 to 10**	**10 to < 300**	**>300**
Before	0% (0/43*)	0% (0/43)	4.65% (2/43)	95.35% (41/43)
After	18.60% (8/43)	11.63% (5/43)	67.44% (29/43)	2.33% (1/43)

*The percent compute was performed on 43 samples.

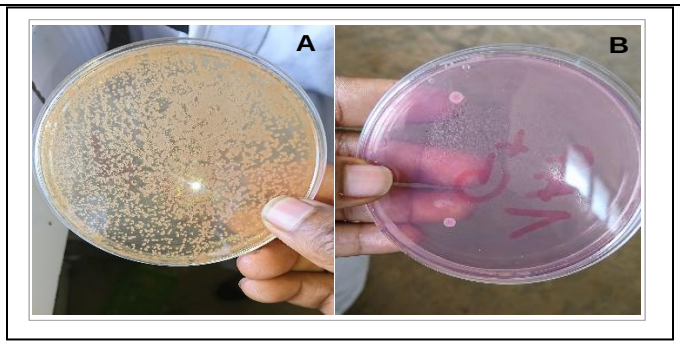


Figure 2. Enterobacteriaceae colonies grew on Mac-Conkey agar (MCA), before **(A)** and after **(B)** HAG hands disinfection

Table 3. Number of Enterobacteriaceae colonies grew on MCA before and after HAG hands disinfection

Sample	Before	after	Brand_HAG	Sample	Before	after	Brand_HAG
E1*	300	2	HAG1	E23	300	193	HAG3
E2	300	24	HAG1	E24	300	34	HAG3
E3	300	288	HAG1	E25	300	208	HAG3
E4	300	0	HAG1	E26	300	117	HAG3
E5	300	44	HAG1	E27	300	282	HAG3
E6	300	37	HAG1	E28	300	214	HAG3
E7	300	5	HAG1	E29	300	18	HAG3
E8	300	209	HAG1	E30	300	0	HAG3
E9	300	3	HAG1	E31	300	209	HAG3
E10	300	0	HAG1	E32	300	276	HAG3
E11	300	289	HAG2	E33	300	0	HAG4
E12	300	298	HAG2	E34	300	201	HAG4
E13	300	233	HAG2	E35	300	0	HAG4
E14	281	12	HAG2	E36	300	0	HAG4
E15	300	76	HAG2	E37	300	11	HAG4
E16	300	20	HAG2	E38	300	22	HAG4
E17	300	232	HAG2	E39	300	18	HAG4
E18	300	300	HAG2	E40	300	0	HAG4
E19	300	209	HAG2	E41	117	2	HAG4
E20	300	27	HAG2	E42	300	6	HAG4
E21	300	193	HAG2	E43	300	19	HAG4
E22	300	0	HAG3				

*Each of Enterobacteriaceae isolated was encoded using the letter E and the number 1 to 43; e.g. E1 means Enterobacteriaceae isolated from the first sample (first student), etc.

Computing the mean values from the colonies number grew onto each of Petri dish, the HAG4 and HAG1 demonstrated an Enterobacteriaceae reduction power, respectively from 300 to 25 and 61

colonies after hands disinfection (Figure 3), that testified an inhibiting activity of these two HAG face Enterobacteriaceae comparatively to HAG2 and HAG3.

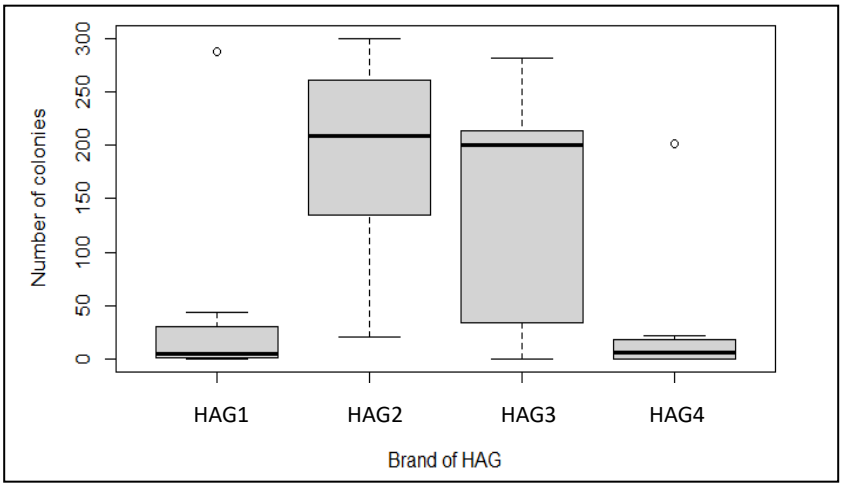


Figure 3**.** Boxplot displays the distribution of Enterobacteriaceae colonies number grew after HAG hands disinfection

The HAG4 and HAG1 demonstrated an Enterobacteriaceae killing power, respectively 91.67% and 79.67%. These percent mean that despite the Enterobacteriaceae resistance face HAG, two of them demonstrated an appreciable killing power because they reduce Enterobacteriaceae colonies from 300 to 25 and 61. Basing on the Enterobacteriaceae reduction power between the four HAG, the Kruskall-wallis test confirmed that the hand-borne Enterobacteriaceae killing power was significantly different ($p = 0.0000732$).

3.2. Susceptibility of Enterobacteriaceae face plant extracts

After three experiments, the plant extracts displayed a weaker ABA on Enterobacteriaceae strains isolated before HAG hands disinfection than those isolated after (Tables 4 and 5).

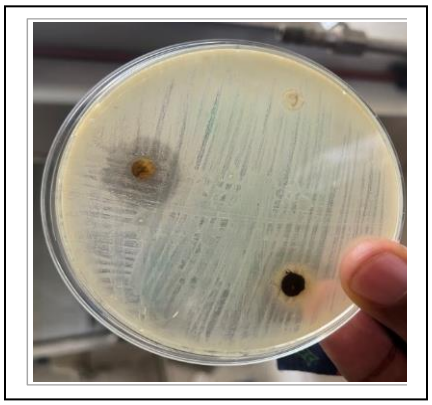


Figure 4. Inhibition around a disc impregnated of plant extract face Enterobacteriaceae strain isolated before hands disinfection

Among 43 Enterobacteriaceae strains isolated before hands disinfection, the most of Enterobacteriaceae resistance face plant extracts were observed with ethanol extracts: *Bidens pilosa* (95.34%); *Moringa oleifera* and *Tetradenia riparia (*97.67%). Ether extract: *Bidens Pilosa* 90.69%, *Moringa oleifera* (86.04%) and *Tetradenia riparia (*76.74%). Concentrated Crude Extract: *Bidens pilosa (*88.37%); *Moringa oleifera* and *Tetradenia riparia* (95.34%). However, for ABA of all extracts from each plant, the intermediate inhibitor activity displayed : *Tetradenia riparia* (27.90%), *Bidens pilosa* (25.58%) and *Moringa oleifera* (20.93%). Three Enterobacteriaceae strains E12b, E27b and E43b were a bit susceptible respectively to *Bidens pilosa* CCE, *Tetradenia riparia* CCE and *Bidens pilosa* Ether extract.

Table 4**.** Inhibition diameter (millimeter) of plant extracts on Enterobacteriaceae isolated before hands disinfection

Enterobacteriaceae isolated	**Ether Bp***	**Ethanol Bp**	**CCE Bp**	**Ether Mo**	**Ethanol Mo**	**CCE Mo**	**Ether Tr**	**Ethanol Tr**	**CCE Tr**
E1b*	3.33	4.00	4.33	5.00	4.67	3.00	3.00	2.00	0.00
E2b	2.00	5.00	3.00	1.67	3.67	2.00	13.00	3.00	4.00
E3b	8.67	4.67	4.33	5.33	4.67	3.00	1.67	3.67	2.00
E4b	2.33	4.33	9.00	8.33	5.00	3.00	2.33	8.00	2.00
E5b	3.67	4.00	4.00	7.67	7.00	9.67	10.00	3.00	1.67
E6b	2.00	3.00	2.00	7.00	3.00	4.67	1.67	5.00	2.00
E7b	4.00	2.00	3.00	5.00	1.00	4.33	1.00	6.00	4.67
E8b	5.67	3.00	3.67	6.00	5.00	4.33	3.00	8.00	3.67
E9b	2.00	13.67	7.33	4.00	3.67	5.67	2.33	4.00	2.00
E10b	3.00	4.33	8.33	13.33	7.33	10.00	11.67	8.00	4.00
E11b	3.00	6.00	6.00	6.00	3.00	6.67	2.00	1.00	1.00
E12b	12.00	7.00	15.00	14.00	1.00	6.00	4.00	4.33	8.00
E13b	3.00	5.00	6.67	4.00	4.00	6.00	6.00	3.00	7.00
E14b	6.33	6.00	3.00	3.00	2.00	4.00	8.67	4.00	5.00
E15b	1.00	1.00	2.67	5.67	5.00	3.00	1.67	3.67	2.00
E16b	2.67	4.67	4.67	3.67	7.33	2.00	1.67	2.00	8.00
E17b	3.00	2.00	4.33	8.33	7.67	4.33	2.00	5.00	4.00
E18b	7.00	3.00	2.00	2.00	1.67	7.67	8.00	1.00	2.00
E19b	2.00	3.00	4.00	2.00	3.00	3.67	7.33	8.33	6.00
E20b	6.00	2.00	7.33	2.00	1.67	2.00	4.67	3.67	2.00
E21b	8.33	8.00	10.00	14.00	8.00	9.00	10.00	4.33	9.00
E22b	7.67	3.00	5.00	4.00	4.67	4.00	4.00	4.00	4.33
E23b	7.00	5.00	3.00	1.67	3.67	2.00	13.00	3.00	4.00
E24b	5.00	6.00	3.00	6.67	6.00	3.00	3.00	2.00	6.00
E25b	6.00	8.00	8.00	6.00	10.00	3.67	2.33	3.00	2.00
E26b	4.00	4.00	8.67	2.00	5.67	7.33	11.67	13.67	4.00
E27b	13.33	8.00	14.33	7.00	4.00	8.33	6.00	4.33	15.00
E28b	4.00	4.00	3.33	2.00	3.67	4.00	11.67	3.00	5.00
E29b	4.33	2.67	4.33	4.33	4.33	4.00	3.67	4.67	2.00
E30b	5.00	4.33	9.00	8.33	5.00	3.00	2.33	8.00	2.00
E31b	4.67	4.00	4.00	7.67	7.00	9.67	10.00	3.00	1.67
E32b	3.00	6.00	5.00	4.33	3.00	3.67	2.33	4.00	7.67
E33b	3.00	4.00	7.00	2.00	8.00	3.00	5.00	6.00	8.00
E34b	2.00	2.00	5.00	5.00	4.67	5.00	7.00	3.00	1.00

E35b	0.00	13.67	7.33	4.00	3.67	5.67	2.33	4.00	2.00
E36b	3.00	4.33	8.33	13.33	7.33	10.00	11.67	8.00	4.00
E37b	2.00	5.00	2.33	3.67	2.00	4.00	5.67	2.00	3.00
E38b	10.00	7.00	10.00	12.00	1.67	7.00	3.00	5.67	9.67
E39b	1.00	3.00	1.67	3.67	2.00	4.67	4.33	5.33	4.67
E40b	1.67	1.00	1.00	2.00	4.67	3.00	4.00	2.00	4.33
E41b	3.67	5.00	3.00	1.67	3.67	2.00	13.00	3.33	4.33
E42b	0.00	3.67	2.33	3.00	2.00	4.00	8.67	2.00	5.67
E43b	15.00	7.33	11.67	13.67	4.00	8.00	8.00	6.00	10.00
Mean	4.57	4.81	5.63	5.72	4.42	4.95	5.77	4.44	4.43
Sd*	3.37	2.67	3.32	3.71	2.15	2.40	3.84	2.46	3.01

* E1b means Enterobacteriaceae strain isolated from the first sample before HAG hands disinfection, etc. Sd= standard deviation. Bp= *Bidens pilosa,* Mo= *Moringa oleifera,* Tr = *Tetradenia riparia.*

Before hands disinfection, the mean inhibition diameter was from 4.88 mm for *Tetradenia riparia* to 5.03 mm for *Moringa oleifera*. Kruskall-Wallis test (p = 0.98) confirmed that there is no significative difference between inhibition diameter of each plant extract. The mean diameter was ≤15 mm.

About 43 *Enterobacteriaceae* isolated after hands disinfection, all was resistant plants extracts.

Figure 5. Inhibition displayed around the discs impregnated of plant extract face Enterobacteriaceae isolated after hands disinfection

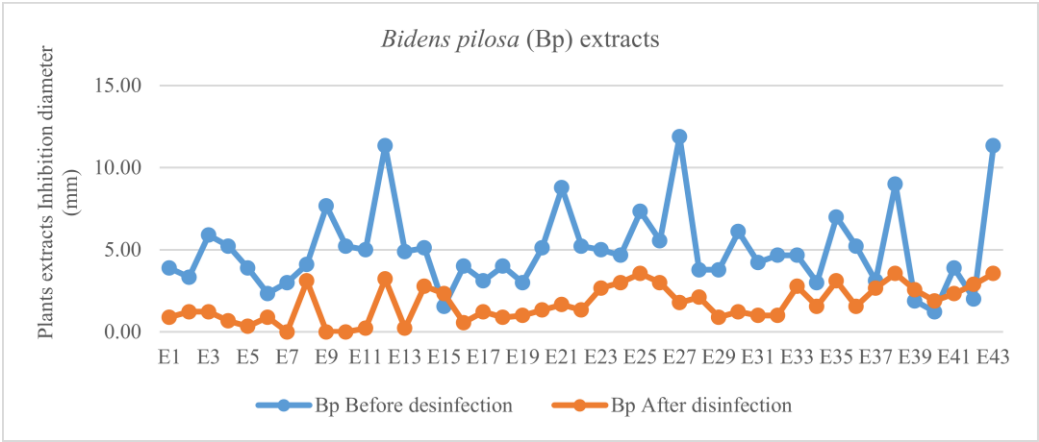
Table 5. Inhibition diameter (millimeter) of plant extracts on Enterobacteriaceae isolated after hands disinfection

Enterobacteriaceae isolated	Ether Bp*	Ethanol Bp	CCE Bp	Ether Mo	Ethanol Mo	CCE Mo	Ether Tr	Ethanol Tr	CCE Tr
E1af*	1.67	1.00	0.00	2.67	2.67	2.67	3.33	4.67	1.00
E2af	1.00	2.67	0.00	1.00	1.67	2.33	0.33	2.67	0.00
E3af	1.67	1.33	0.67	1.00	0.67	2.33	0.33	1.67	0.00
E4af	0.33	1.67	0.00	3.33	1.33	2.33	0.00	1.33	0.00
E5af	0.33	0.67	0.00	3.33	3.00	2.33	2.33	1.67	0.67

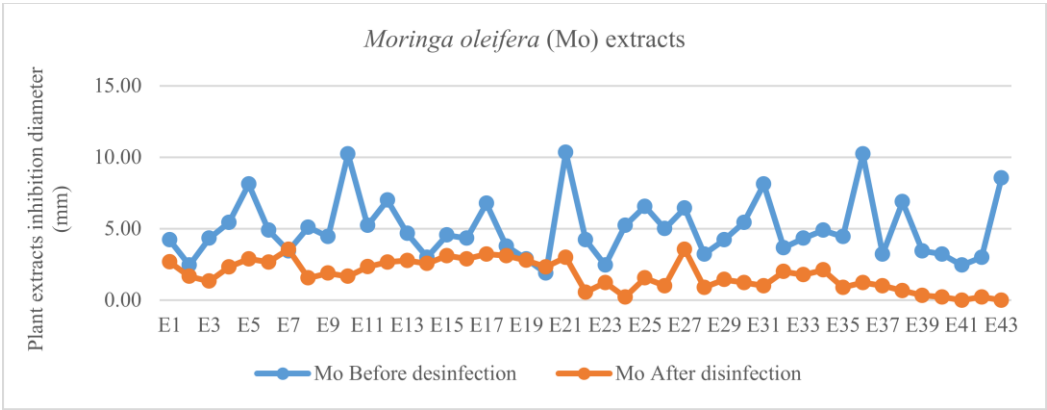
E6af	1.67	1.00	0.00	3.67	1.33	3.00	1.67	1.33	0.00
E7af	0.00	0.00	0.00	3.33	3.00	4.33	1.67	1.00	0.00
E8af	0.67	0.33	8.33	2.00	1.00	1.67	1.00	1.33	0.33
E9af	0.00	0.00	0.00	0.67	0.67	4.33	1.33	1.67	1.33
E10af	0.00	0.00	0.00	0.00	0.00	5.00	2.00	1.00	0.67
E11af	0.00	0.67	0.00	0.33	0.00	6.67	1.00	1.33	0.67
E12af	0.67	0.33	8.67	1.00	1.00	6.00	1.00	4.67	0.33
E13af	0.33	0.33	0.00	0.33	0.33	7.67	1.00	3.33	1.00
E14af	0.67	0.67	7.00	0.67	0.67	6.33	2.00	2.33	2.00
E15af	0.33	0.33	6.33	1.00	0.33	8.00	2.67	2.67	2.67
E16af	1.00	0.67	0.00	1.33	0.67	6.67	1.00	1.67	2.33
E17af	2.67	0.00	1.00	0.67	0.33	8.67	1.00	0.67	2.33
E18af	1.00	1.33	0.33	0.67	0.33	8.33	3.33	1.33	2.33
E19af	0.33	2.67	0.00	0.67	0.67	7.00	3.33	3.00	2.33
E20af	1.00	0.67	2.33	0.33	0.33	6.33	3.67	1.33	3.00
E21af	1.00	1.67	2.33	3.33	4.67	1.00	3.33	3.00	4.33
E23af	1.00	0.67	2.33	1.00	0.67	0.00	2.00	1.00	1.67
E24af	3.67	1.33	3.00	1.67	1.33	0.67	0.67	0.67	4.33
E25af	3.33	4.67	1.00	0.00	0.67	0.00	0.00	0.00	5.00
E26af	4.67	5.33	0.67	2.33	1.67	0.67	0.33	0.00	6.67
E27af	3.00	2.33	3.67	1.67	1.33	0.00	1.00	1.00	6.00
E28af	1.00	3.33	1.00	4.67	5.33	0.67	0.33	0.33	7.67
E29af	2.00	2.33	2.00	1.00	1.33	0.33	0.67	0.67	6.33
E30af	1.67	1.00	0.00	1.33	1.67	1.33	1.00	0.33	8.00
E31af	1.00	2.67	0.00	2.00	1.00	0.67	1.00	0.33	6.67
E32af	0.33	2.67	0.00	1.00	1.33	0.67	0.33	0.33	0.00
E33af	1.67	1.33	0.00	1.00	4.67	0.33	0.00	0.00	0.00
E34af	0.33	0.33	7.67	1.00	3.33	1.00	0.00	0.67	0.00
E35af	2.33	1.67	0.67	2.00	2.33	2.00	0.00	0.00	0.00
E36af	1.00	0.33	8.00	1.67	1.00	0.00	0.67	0.33	8.67
E37af	2.00	1.00	1.67	1.00	2.67	0.00	0.67	0.33	8.33
E38af	1.00	1.00	6.00	0.33	2.67	0.00	0.67	0.67	7.00
E39af	3.33	3.00	4.33	0.33	1.67	0.00	0.33	0.33	6.33
E40af	0.67	0.67	6.33	0.33	0.67	0.00	1.00	0.67	0.00
E41af	0.67	0.67	4.33	0.33	0.33	0.00	1.67	1.33	0.67
E42af	3.33	1.33	2.33	0.00	0.00	0.00	2.67	0.00	1.00
E43af	3.33	3.00	2.33	0.00	0.67	0.00	4.67	5.33	0.67
E23af	3.33	3.00	4.33	0.00	0.00	0.00	1.67	1.00	0.00
Mean	1.42	1.43	2.29	1.30	1.42	2.59	1.36	1.37	2.61
Sd*	1.20	1.25	2.77	1.16	1.31	2.87	1.17	1.31	2.86

* E1af means Enterobacteriaceae strains isolated from the first sample after HAG hands disinfection, etc. Sd= standard deviation.

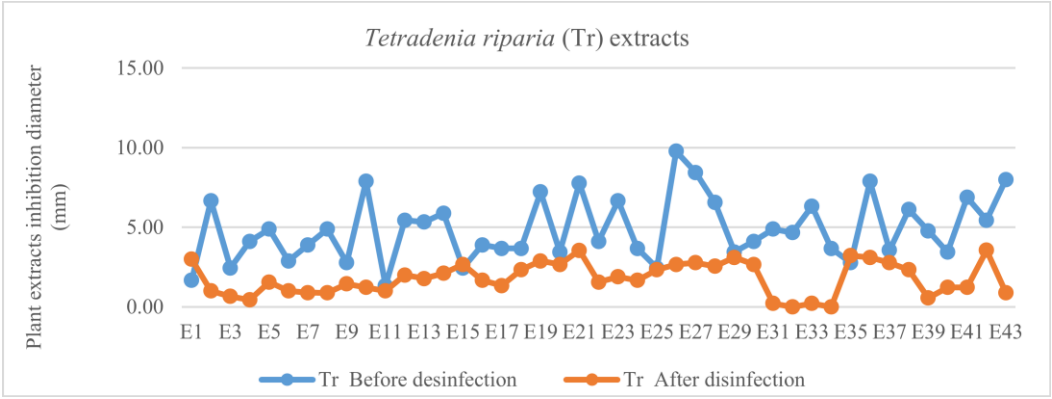
After hands disinfection, the mean inhibition diameter was from 1.71 mm for *Bidens pilosa* to 1.78 for *Tetradenia riparia* (Table 5) on Enterobacteriaceae isolated.


Bidens pilosa (Bp) extracts
Plants extracts Inhibition diameter (mm)
15.00
10.00
5.00
0.00
E1 E3 E5 E7 E9 E11 E13 E15 E17 E19 E21 E23 E25 E27 E29 E31 E33 E35 E37 E39 E41 E43
Bp Before desinfection
Bp After disinfection

(A)


Moringa oleifera (Mo) extracts
Plant extracts inhibition diameter (mm)
15.00
10.00
5.00
0.00
E1 E3 E5 E7 E9 E11 E13 E15 E17 E19 E21 E23 E25 E27 E29 E31 E33 E35 E37 E39 E41 E43
Mo Before desinfection
Mo After disinfection

(B)


Tetradenia riparia (Tr) extracts
Plant extracts inhibition diameter (mm)
15.00
10.00
5.00
0.00
E1 E3 E5 E7 E9 E11 E13 E15 E17 E19 E21 E23 E25 E27 E29 E31 E33 E35 E37 E39 E41 E43
Tr Before desinfection
Tr After disinfection

(C)

Figure 6. The inhibition diameter of plants extracts on Enterobacteriaceae isolated before and after hands disinfection. *Bidens pilosa* extracts (A), *Moringa oleifera* extracts (B) and *Tetradenia riparia* extracts (C). The figures A, B and C display that none of plant extracts reached 15 mm of inhibition diameter.

Plant extracts did not display an inhibition power face Enterobacteriaceae isolated before and after HAG hands disinfection. This means the Enterobacteriaceae isolated before and after HAG hands disinfection, resisted to plant extracts. Moreover, the Enterobacteriaceae isolate released their resistance after contact with HAG.

4. Discussion

This study aimed to assess the HAG quality, its Enterobacteriaceae effectiveness power and to test the susceptibility of Enterobacteriaceae face plant extracts. Among the four HAG assessed, it appears that the HAG4 and HAG1 had an appreciable Enterobacteriaceae killing power after hands disinfection, comparatively to HAG2 and HAG3. Therefore, the HAG4 and HAG1 were of good quality. However, the statistical test showed that the colonies number of Enterobacteriaceae isolated before and after hands disinfection, remained the same. This means, overall, the difference observed on Enterobacteriaceae elimination power between the HAGs can be due to the hazard effect, either it depends to the number of Enterobacteriaceae isolated onto the hands, or to the Enterobacteriaceae species submitted to HAGs. All HAGs assessed, were ineffective on Enterobacteriaceae isolated from hands. These results proved that the Enterobacteriaceae killing power after disinfection with different HAG was significantly different with the notice on each HAG brand, which highlights 99.99% of bacteria killing power after usage. Beyond all, this study encourages people to use the HAG4 and HAG1 because they showed a somewhat Enterobacteriaceae killing power that can help keep hands safe. Therefore, the four HAG brands did not display an ABA able to inhibit or kill significantly the hand-borne Enterobacteriaceae.

The results from this study did not show the similarity with those obtained by Bakli et al. (2020), who found that the HAG had a microorganism inhibition power. In addition, our results were different from those found by Aidara et al. (2021), where 80% of HAG brand demonstrated a microorganism inhibition power from 58.3 to 100%. The effectiveness of ethanol face Enterobacteriaceae could be explained by the ability of this solvent to reach the membrane of the target strains. The use of HAG containing 60-80% of ethanol reduces many virus and bacteria on an infected surface (WHO, 2009; Rouzic et al., 2011), such as hands. Even in vitro conditions, the HAG at 80% is able to inhibit many bacteria and virus isolated from hands (Grayson et al., 2009).

On other hand, the findings were similar to those reported by Aidara et al. (2021). They found two HAG brand (E2 and E5), which did not demonstrate any ABA. The Enterobacteriaceae resistance releasing might due to the bad HAG physic-chemical quality, because HAG effectiveness on a microorganism depends to its physic-chemical quality (Aiello et al., 2008). The HAG sold and used in Bukavu are almost ineffective to kill significantly the hand-borne Enterobacteriaceae. This means also, Enterobacteriaceae are among bacteria which are highly MDR face most HAG sold in Bukavu. Further studies should be conducted to evaluate physic-chemical qualities and effectiveness of HAG brand sold in Bukavu.

Testing Enterobacteriaceae isolated before hands disinfection, *Tetradenia riparia* CCE displayed a weak ABA on Enterobacteriaceae. There were some Enterobacteriaceae inhibitor activities for *Tetradenia riparia* extracts. Also, 2 Enterobacteriaceae strains were susceptible to *Bidens pilosa* and one to *Tetradenia riparia*.
However, the statistical test results showed that all plant extracts had the same inhibition power face Enterobacteriaceae isolated before HAG disinfection. Moreover, the inhibition diameter average of plant extracts did not reach 15 mm. Also, all Enterobacteriaceae isolated after HAG disinfection were resistant, comparatively to those isolated before HAG disinfection. This study observed that Enterobacteriaceae resisted entirely after their contact with HAG.

These results were different to those obtained by Gnonsoro *et al.* (2011), who observed that plant extracts displayed an ABA of 19 and 16 mm of inhibition diameter respectively testing *Moringa oleifera* and *Bidens pilosa* extracts on *Escherichia coli*; while these both plant extracts did not display any ABA on Enterobacteriaceae resisting to HAG. The difference between these both results might be due to the difference of maceration method. Falowo et al. (2016) macerated 200 g into 800 mL (dilution 1/5) under agitation, while in the reporting study 10 g were macerated into 50 mL (dilution 1/6) without agitation. For the upcoming research, it could be better to take a much quantity of plant material to macerate under permanent agitation to obtain a strong concentrated dilution.

In general, the CCE did not display any ABA on Enterobacteriaceae isolated before as after HAG disinfection. Tsuchiya et al. (2016), demonstrated that the presence of active ingredient can be combined with soluble extra-cell proteins in bacteria wall. This mixture leads the decreasing of active ingredient effectiveness face bacteria. Also, without an extraction solvent, the CCE does not contain a high rate of flavonoids or phenol allowing bacteria inhibition (Falowo et al. 2017). That is why the Enterobacteriaceae can become MDR. The Enterobacteriaceae inhibition requires that the active ingredient concentration reaches the required rate to express its effectiveness. Also, the active ingredient concentration in a plant is different in each organ, depends the plant harvesting time and the solvent used to extract the active ingredient.

Our results are different from those found by Thilza et al. (2010), who found that the *Moringa oleifera* water extract with 1000 mg/mL, 700 mg/mL, 400 mg/mL and 200 mg/mL of dilution, displayed a higher ABA on *Escherichia coli* and *Enterobacter aerogenes* than face *Staphylococcus* species. The difference with Thilza et al. (2010), might result from the type of solvent used in the both study. Moreover, water extract displayed a better extraction of *Moringa oleifera* active ingredient than ether and ethanol. Moyo et al. (2012), found that a concentration of 5 mg/mL of *Moringa oleifera* acetone extract showed an ABA on *Escherichia coli* and *Enterobacter cloace*. This finding show that Enterobacteriaceae remain among bacteria multi - drug resistant (MDR) with using many path face plant extracts. The upcoming research need to diversify solvents to extract active ingredient from more medicinal plants.

Conclusion

This study showed that the quality of HAG selling and using in Bukavu is generally ineffective to kill significantly the hand-borne Enterobacteriaceae. The Enterobacteriaceae strains isolated before and after HAG hands disinfection were highly resistant. Furthermore, the Enterobacteriaceae

resistance increased after their contact with HAG. This study also observed the increasing of Enterobacteriaceae power to escape ABA of medicinal plants. The upcoming studies should be conducted to determine all strategies or the escape pathway by Enterobacteriaceae to resist antimicrobial molecules. It will be important to look for whether HAG usage can release the Enterobacteriaceae resistance. The physic-chemical parameters of HAG should be evaluated to isolate and identify. Further studies should include other medicinal plant species in order to isolate and identify their active molecular having an antibacterial activity, especially face the bacteria strains multi-drug resistant.

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