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Research article

Managing *Vibrio cholerae* with a local beverage: preparation of an affordable ethanol based hand sanitizer

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ABSTRACT

Research indicates an increased use of hand sanitizers globally, and in particular developing countries where bacterial epidemics such as cholera are common. While there is evidence of availability, high demand and use of hand sanitizers, the incidence of cholera in developing countries remains unabated. Several reasons have been put forward, and cost of hand sanitizers remains dominant. It is in response to this contradictory situation of availability but limited access, that this study was conducted to present an alternative option of formulating a high quality and cost effective ethanol-based sanitizer from a Ghanaian local beverage (akpeteshie). The concentration of ethanol in akpeteshie was determined using gas chromatography. An Ethanol based hand sanitizer (Sample C) was formulated with akpeteshie and tested against *Vibrio cholerae* using the microbial time -kill kinetics assay. Commercially available ethanol based Equi-Clean hand sanitizer (62%) (Sample D) was used as the standard. Results show that the akpeteshie contained 73.08% ethanol and formulated product (Sample C) contained 63.70% ethanol. Viscosity and pH of Sample C were; 89 rpm (1.48 cps) and 7.30 respectively whiles that of Sample D were; 80 rpm (1.33 cps) and 7.50 respectively. The formulated product (Sample C) was effective against *Vibrio cholerae* with a gradual reduction in microbial count upon exposure to the organisms at time intervals of 0, 5, 15, 30, 60 and 120 min.

1. Introduction

Poor environmental health is emerging as a major public health issue in developing countries of Africa, with cholera being a major risk factor and widespread diarrhoeal diseases outcome [1]. Current reports indicate that annually there are roughly 1.3 to 4.0 million reported cases of cholera, mostly in developing countries of Africa [10]. The high numbers of cholera cases is reflected in the increase in diarrhoeal diseases mortality. Research indicates that between 21,000 and 143,000 diarrhoeal related deaths are recorded worldwide every year [10]. Cholera is caused by the bacteria *Vibrio cholerae* transmitted by humans through faecal-oral route. Poor hygiene towards food, water and hand hygiene (HH) contribute significantly to the transmission of cholera, and easily spreads in communities with poor sanitation [9]. Given the dangers associated with cholera, in 2017, the Centres for Diseases Control and Prevention (CDC) promoted and encouraged routine hand washing to reduce the spread of diarrhoel related diseases and to improve healthy living [1]. The purpose of promoting hand washing was to emphasised global acceptability of washing of hands with soap and water as one of the effective ways to maintain good HH, which has the potential to reduce the spread of diarrhoeal diseases. In fact, official statistics show that handwashing with soap minimises the risk of diarrheal diseases by 42%–47%, and remains a fundamental approach in life saving efforts in developing countries of Africa [2].

Despite the importance of hand washing in minimising the spread of diarrhoeal diseases especially in developing countries of Africa, research has identified poor compliance as a fundamental limitation in promoting good HH, as compliance rarely exceeds 40% in situations in which hand washing is necessary [3]. In response to this limitation, the World Health Organisation (WHO) highly recommends the use of hand rubs, either through dispensers close to the point of care or in small bottles, to ensure optimal compliance with HH by making the process faster and more convenient [4]. The WHO's recommendation is underpinned by the fact that alcohol-based hand rubs have evidence-based intrinsic advantages of

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exhibiting fast-acting and broad-spectrum microbicidal activity [4]. Similarly, hand sanitizers when applied to hands inactivate microorganisms or inhibit their growth which contribute to reducing disease transmission [1]. Currently, available effective hand sanitizers antiseptics contain 62%–90% of alcohol, an active ingredient for killing most bacteria, fungi or virus via denaturing proteins of these microbes [5]. Considering the alcohol ingredient, ethanol based hand sanitisers (EBHS) are preferred as they offer convenient, effective, less time, and less skin irritation compared to hand washing with soap, antiseptic agents or water [6]. It is unsurprising that there is evidence of high demand for hand sanitizers in regions with high prevalence diarrhoeal disesases, including Sub-Saharan Africa [7].

Given the properties and potential of EBHS and the evidence of increasing demand, it is expected that the prevalence of diarrhoeal diseases globally will reduce considerably especially in Sub-Saharan Africa. However, as earlier discussed, the number of reported cases and mortalities are alarming [10]. In Ghana, for instance, the WHO reported about 591 confirmed cholera cases with five deaths only between January and May 2015. Prior to the 2015 figures, the country had recorded 28,975 cases with 243 deaths in 2013 [8]. Regardless of these alarming statistics on cholera, research shows that the increasing demand of the use of EBHS has resulted in rising prices [12], a situation that has contributed to low patronage especially among the population of low economic background. Regrettably, such people mostly live in poor sanitary conditions with low accessibility to potable water and sanitation services, making them more vulnerable to cholera. The race for alternative and affordable EBHS is urgent and tenable.

Despite the importance of EBHS and the alarming rate of cholera in developing countries of Africa, it remains to be demonstrated whether local ethanol products are useful in formulating or preparing EBHS that are affordable, effective, accessible and acceptable by the local people in Sub-Saharan African setting. Recent evidence suggests that a local alcoholic beverage in Ghana "Akpeteshie" (APE) which is generally distilled from palm wine, raffia palm wine, or sugarcane and generally consumed as a beverage has a formulating potential for EBHS [11]. Considering its high availability and affordability, APE is a suitable raw material for the local production of a highly effective hand sanitiser with immediate and long-term health and economic benefits. The potential benefits of this locally formulated hand sanitiser may include a marked reduction in the spread of diarhoeal diseases, reduction in government's expenditures on imported hand sanitizers, affordable and accessibility to the wider population, and improved compliance to the practice of HH. The main objective of this study was to assess and quantify ethanol present in the local beverage APE, and its usefulness for the formulation of an affordable EBHS, which could be effective against *Vibrio cholerae (V. cholerae*) and other common coliforms in Ghana, and other African countries.

2. Materials and methods

2.1. Materials and equipment

Carbopol® 940, Deionised water, glycerin, standard ethanol 99.99% v/v (Sample A), akpeteshie (APE) (Sample B), triethanolamine (TEA), *V. cholera* 01 boitype suspension, nutrient agar, sterilized nutrient agar and broth, Personal Protective Equipment (PPE), sterilized glass wares, cotton wool, 10µl micro syringe with a 26 gauge needle, aluminium foil, test tube rack, disinfectant, cotton wool, analytical balance (Mettler Toledo EL204), Magnetic stirrer (Dragon Lab MS-H-S), autoclave, incubator, pH meter, rotary viscometer (ZNN-D6), Bunsen burner, air stirrer, colony counter, flame ionization detector-gas chromatograph (FID-GC-SRI 8610CGC).

The laboratory investigation was was divided into three major parts as outlined below;

2.1.1. Part 1 (qualitative and quantitative analysis of test sample (sample B))

2.1.1.1. Collection and physical identification of ethanol in samples. Standard ethanol (99.99%), labelled as Sample A was obtained from Equatorial Healthcare Services. Three litres of APE (from palm fruit), labelled as sample B was obtained from a local brewery at Suhyen in the Eastern Region of Ghana. To confirm that the Sample B contained ethanol, the physical characteristics of the APE were compared to that of a standard ethanol (99.99%). The identification tests included Odour, Colour, Taste and Flammability [13, 14].

2.1.1.2. Qualitative identification of ethanol present in the akpeteshie. Further identification of ethanol present in the sample was done using the Gas Chromatograph (SRI-H610C GC) under the following conditions [15, 16]:

Initial temperature: 60 °C, Ramp: 10 °C, Final temperature: 150 °C, Run time: 9 min, Gas Pressures; (Carrier Gas 1, Nitrogen gas: 24psi, Carrier gas 2, Hydrogen gas: 38psi Carrier gas 3, Compressed air: 28psi). Three percent (3%) each of the Standard ethanol, 99.99% and the APE was prepared and run individually. A mixture of the two were run. Their respective Retention time (R_T) was noted and compared. The peaks obtained were investigated.

2.1.1.3. Quantification of ethanol present in the akpeteshie. In order to obtain a calibration curve of the standard Ethanol (99.9% v/v), five different concentrations (0.25%, 0.5%, 0.75%, 1.0%, and 1.25% v/v) of the standard ethanol (Sample A) were prepared and run with the GC under the already stated conditions. The Area under the Curve (AUC) of the respective concentrations was noted. A calibration curve was then plotted using the various concentrations and their resultant AUC's. A concentration of the APE (1%) was prepared and run with the GC under similar chromatographic conditions as stated earlier. The average AUC was determined and put into the equation of the graph obtained from the calibration curve to obtain the actual concentration of the APE [15, 16]. Since the obtained concentration (73.077%) of ethanol present in the APE (Sample B) was not in conformity with the desired concentration of 62% (commercial product), an adjustment was made for the concentration required.

2.1.2. Part 2: formulation of ethanol based hand sanitizer gel: (sample C)

Based on the concentration of the undiluted ethanol obtained in the test sample B, the hand sanitizer was formulated. Firstly, the obtained concentration which was not in conformity with the desired concentration (62%) was made to conform to it by calculating the amount of water needed for the adjustment. For the formulation of the hand sanitiser (sample C), Carbopol® 940 was weighed using a well calibrated analytical balance into a small beaker and then mixed gently with a stirrer in a big beaker with 10 mL of water until it was well hydrated. A carbopol-water solution was formed. A calculated amount of test sample B required to produce in situ 62% ethanol of formulation was added to carbopol-water solution and mixed until uniform. This was followed by the addition of glycerin and mixing was continued until well blended [32]. The neutralizing agent TEA was added in drops added gradually until the formulation thickened and pH adjusted to 7.3 [17,18].

2.1.3. Part 3 (finished product analysis (sample C))

2.1.3.1. Determination of concentration of ethanol in the final product. Another calibration curve of standard ethanol was obtained under the chromatographical conditions used earlier by using five concentrations of the standard ethanol [16]. The AUC's for the concentrations were noted and a second calibration curve was plotted. Five percent (5% of the final product was prepared and run with the G.C. The concentration of

final product was calculated by using the equation derived from the straight line.

2.1.3.2. Viscosity and pH determination of final product. The viscosity and pH of the final product, C were analysed using the ZNN S6 Rotary Viscometer at 100 rpm and the pH meter (VHS Electronic MK VI) respectively at a temperature of 25 °C. A commercially available form of ethanol based hand sanitizer (Equi Clean Hand sanitizer) from Equatorial Healthcare Services was used as the standard for comparison [18, 19]. Viscosity and pH of sample C were 89 rpm (1.48 cps) and 7.30 respectively.

2.1.3.3. Microbial test of sample C against V. cholerae (microbial time-kill kinetic assay). Microbial time-kill kinetic assay was performed on *V. cholerae* O1 biotype according to the procedure described by Appiah et al., 2017, [27] with slight modifications. A 24 h broth culture of the test organism was used with organism count of 1.0 x 10^6 CFU/mL. A volume of 3.0 mL of organism suspension was added to 3.0 mL of test sample and incubated at 37 °C. A test tube containing a 24 h broth culture of the test organism was used as the control. Aliquots of 1.0 mL of mixture (Sample C + organism) and control were taken at time intervals of 0, 5, 15, 30, 60 and 120 min. The aliquots taken were aseptically added to separate nutrient agar plates and incubated at 37 °C for 24 h. The procedure was performed in triplicates and a graph of the log CFU/mL was plotted against time. The control used did not contain any quantity of the test sample.

3. Results

3.1. Part 1 (qualitative and quantitative analysis of test sample B)

Identification and quantification of ethanol present in the akpeteshie

3% of ethanol shot eluted at 1.883 min.

3% of akpeteshie shot eluted at 2.066 min.

3% of akpeteshie +3% of ethanol shot eluted at 2.466 min and a single peak was observed confirming the presence of ethanol in the tested akpeteshie

Standard ethanol (99.99%)

RT=1.883 Concentration = 3%, Sample B. RT=2.46, Concentration= 3%

Determination of the concentration of final product

Results obtained from the GC after running 5% of the final product

pH of product

pH of the final product = 7.3

pH of standard product = 7.5

4. Discussion of results

Among the various methods of maintaining hand hygiene, the WHO affirms alcohol-based hand sanitisers as the only means for rapidly and effectively inactivating a wide array of potentially harmful microorganisms on hands. The formulation of a relatively affordable locally made hand sanitiser (from akpeteshie) also corresponds with the WHO objective of promoting improved economic benefit by reducing annual costs for hand hygiene, representing approximately 1% of extra-costs generated by Health care associated infection (HCAI) [4]. This study therefore was aimed at formulating an ethanol based hand sanitiser, effective against *V. cholerae* as well as affordable to indigens of poor communities in Ghana and beyond. This stance is based on the hypothesis that compliance with the use of hand sanitisers to attain hand hygiene will depend on subjective reactions such as safety, efficacy, personal acceptance of the product and cost [24]. In the quest to achieve the purpose of this study, akpeteshie locally brewed from palm wine was obtained, analysed, used in the formulation of the hand sanitiser and screened for antibacterial activity. The population understudy and the cost effectiveness of this research were highly considered before, during and after the laboratory work.

Table 1. Physical properties of the local gin (akpeteshie) confirming its identity.

Characteristics	Standard Ethanol	Sample (akpeteshie)
Colour	Clear	Clear
Odour	Pungent	Pungent
Taste	Burning	Burning
Flammability	Red and blue flames (lasted longer)	Red and blue flames

From the Table 1, it was observed that both the akpeteshie and the standard ethanol (99.99%) shared similar physical properties. They were both clear in colour, had a burning taste and pungent. However, the akpeteshie was observed to have a more pungent smell. To further demonstrate the presence of alcohol in the test sample B, the flammability test proved positive, confirming that the local gin contained ethanol and was therefore flammable (see Figure 1). Additionally, the flames of the standard ethanol lasted longer than that of the akpeteshie. This maybe as a result of the presence of water which was retained in the akpeteshie during its distillation process, Additionally, the flames of the standard ethanol lasted longer than that of the akpeteshie. This may be as a result of the presence of water which was retained in the akpeteshie during its distillation process, as accounted for by the binary azeotropic nature of the local gin. This characteristic phenomenon of azeotropy indicates that local gin could be a relatively safer product for convenient handling compared to the standard ethanol in terms of flammability. Nonetheless, the positive flammability response observed with local gin, though transcient, also suggests that prudence must be generally exercised with the use of ethanol based hand sanitisers (as from akpeteshie) in order to avoid any potential harm that could arise due to their flammability potential [14].

The retention times obtained in Figures 2 and 3 after running 3% of the standard ethanol (Sample A) and the akpeteshie (Sample B) individually were 1.883 and 2.066 respectively. The varying retention time values observed for each of the test samples A and B could be due to slight uncontrollable variations in conditions such oven temperature, consistency in injecting the sample and flow rate of the carrier gases. However,

Figure 1. 8Snapshot showing alcohol flammability test for Akpeteshie (ethanolic concentration of 63.70 % v/v).

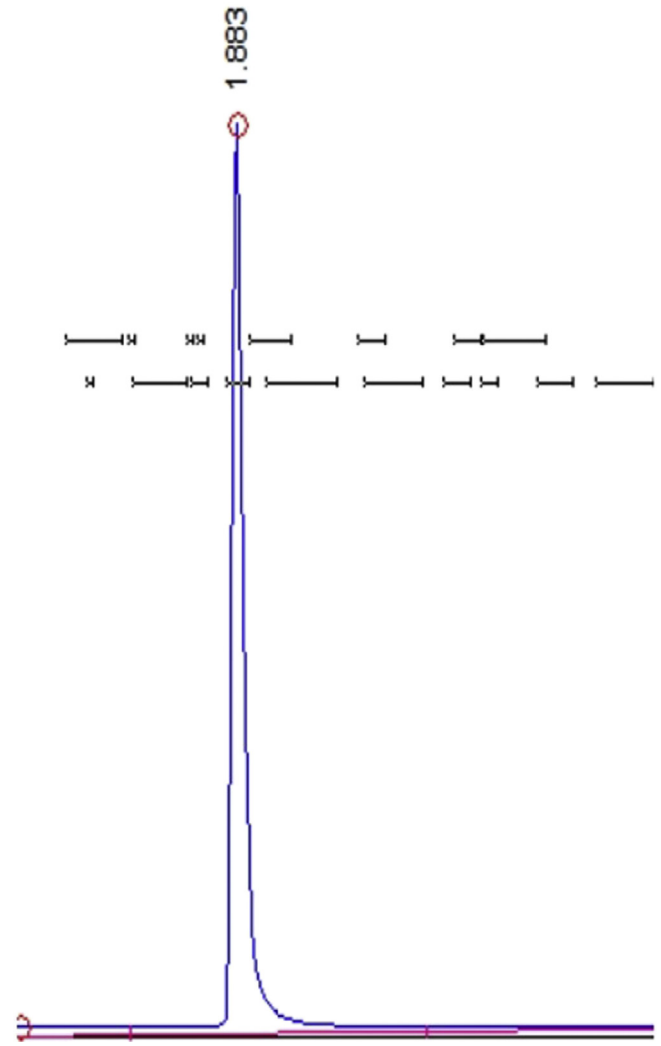

Figure 2. Represents the retention time for Sample A (Standard Ethanol 99.99%).

Figure 3. Represents the retention time for Sample B (Akpeteshie, local gin).

in order to substantiate the ethanol content of test sample B, a single peak (Figure 4) was produced with a retention time of 2.466, upon running the GC on a mixture of equal volumes of samples A and B. This single peak therefore confirmed the presence of ethanol as same component in both samples. Hence akpeteshie contained ethanol as the major ingredient [6, 16].

The concentration of ethanol present in the akpeteshie was calculated as 73.077%, using the equation of the calibration curve in Figure 5, which was obtained after running 1% of the test sample B in the gas chromatograph. The obtained R-squared value of 0.9974 indicates a 99.74% accuracy of the fitted regression line of the obtained calibration curve. This value therefore implies the extent of accuracy of the assay ethanolic content of test sample B determined by the GC run (see Figure 6 and Table 3).

From master formula Table 2, 62% ethanol based hand sanitizer was to be successfully formulated from the 73.077% akpeteshie (see Table 3), using 62% Equi-Clean hand sanitizer as the standard. The formulation required the use of various excipients: water (vehicle), ethanol (active ingredient), Carbopol 940 (thickening agent), TEA (buffering agent). Water was needful for the dilution of the alcohol to denature the protein of microorganisms [24]. Glycerin was employed as a moisturizing agent/emollient to counteract the disadvantage of alcohol in causing irritation and drying up of the skin due to its absorbent and astringent effect [19, 23, 32]. Additionally, the addition of emollients in hand sanitisers is also known to enhance their anti-bacterial activity by slowing the drying time and thus increasing the contact time of alcohol with the skin [25].

In the final evaluation of the formulated product, its actual concentration obtained by calculation after running 5% of the final product was 63.70 % (see Table 4). Even though earlier research has confirmed that Ethanol based hand sanitizers considered to be effective comprise of 60%–80% ethanol as their active ingredient [19], the assay value of 63.70 % obtained closely mimics the most effective narrow range of 62%–64% cited in another study [18]. This value being less than 70% by weight has been documented by research as causing less skin drying and chemical dermatitis, with an overall lesser cost than using higher concentrations [26].

The final product had a viscosity of 89 rpm (1.48cps) and was found to be more viscous as compared to the standard (62% Equi-Clean hand sanitizer) of viscosity of 80 rpm (1.33cps). However, sample C (product)

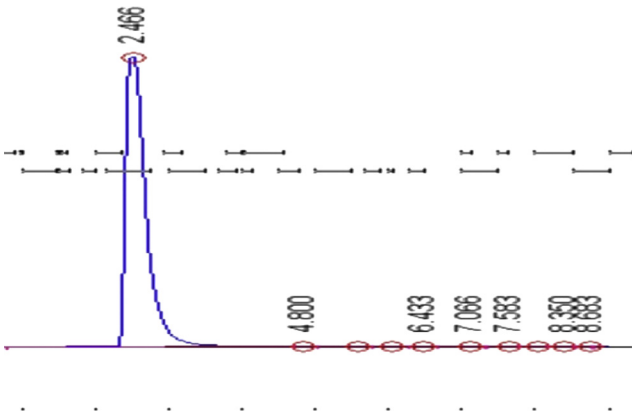

Figure 4. Represents the retention time for a mixture of Sample A (3% v/v) and Sample B (3% v/v).

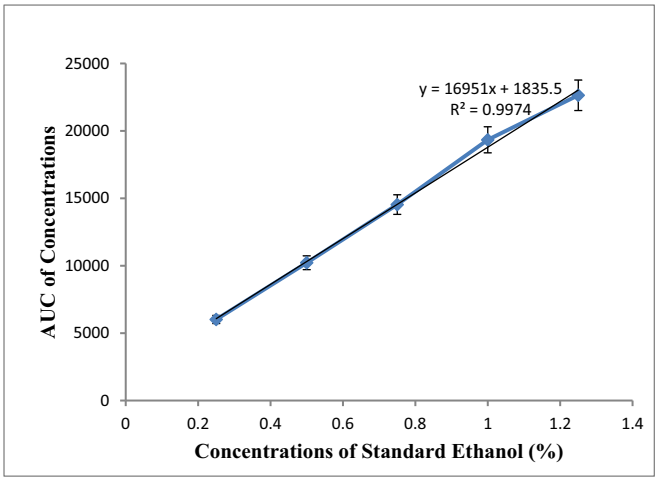

Figure 5. Calibration curve of standard ethanol (99.99% v/v).

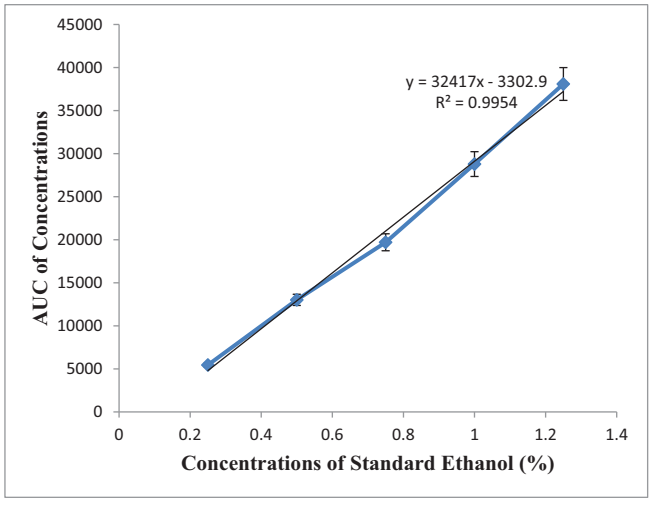

Figure 6. A Graph of Averaged AUC versus varying percentage concentrations (0.25, 0.50, 0.75, 1.0 and 1.25) of Standard Ethanol.

was as easily dispensed from container as much as the standard product irrespective of it's relatively higher viscosity. TEA was responsible for the thickness and pH of the formulation [17].

The final pH of 7.3 was obtained for the product (Sample C) whereas that of the standard product final product was 7.5. Both samples had their

Table 2. Master formula for the formulation of the ethanol based hand sanitizer [17].

Ingredients	Master Formulae (% v/v)
Water	31.70
Ethanol	62.00
Carbopol 940	0.81
Glycerin	5.10
TEA (triethanolamine)	0.39

Table 3. Concentrations of standard ethanol and their respective AUC obtained by gas chromatography for determining the concentration of akpeteshie.

Concentration of Standard Ethanol (% v/v)	Averaged AUC
0.25	6012.325 ± 0.708
0.50	10218.832 ± 0.605
0.75	14533.768 ± 0.707
1.00	19337.283 ± 0.834
1.25	22641.914 ± 0.906

Table 4. Concentrations of standard ethanol and their respective AUC for determining the concentration of the final product C.

Concentration of Standard Ethanol (% v/v)	Averaged AUC
0.25	5453.238 ± 0.846
0.5	13016.454 ± 0.957
0.75	19703.336 ± 0.889
1.00	28786.162 ± 1.455
1.25	38089.399 ± 0.997

Table 5. Microbial test against *Vibrio cholerae* (The table below shows the log reduction of *Vibrio cholerae* over specific times).

	Time/min					
CFU (Control)	**0.00**	**5.00**	**15.00**	**30.00**	**60.00**	**120.00**
In 0.1 mL	1637	1600	1660	1728	1680	1550
In 1 mL	16370	16000	16600	17280	16800	15500
Log CFU/mL	4.2140	4.2041	4.2201	4.2375	4.2253	4.1903
CFU (Test)	**0.00**	**5.00**	**15.00**	**30.00**	**60.00**	**120.00**
In 0.1 mL	1637	178	100	48	20	21
In 1 mL	16370	1780	1000	480	200	210
Log CFU/mL	4.2140	3.2500	3.0000	2.6810	2.3010	2.3222

*CFU = colony forming units.

pH falling within the acceptable range of 7–7.7 as cited in another study [18, 19]. However, according to Padsalg et al., 2014, antibacterial effect is not greatly affected by pH as compared to substantial increase in temperature and prolonged exposure times [19].

Since the FDA monograph does not specify performance criteria for time-kill testing of health-care antiseptic drug products, antiseptic hand wash and hand rub products but requires a demonstration in the reduction in viable counts of test organisms [22], the time kill test method was employed in this study to determine the antimicrobial effect of sample C on *V. cholerae* (see Table 5). Time Kill Test is a basic microbiology method used for assessing antimicrobial activity of an antimicrobial test material or disinfectant. The Kill Time Test is done basically to figure out the microbial reduction by a disinfectant against selected bacteria or fungi [21].

In Figure 7, an antibacterial logarithmic reduction effect of test Sample C against *V. cholerae* was observed upon plotting a graph of log CFU/mL of organism against time frames of zero, five, fifteen, thirty, sixty and one hundred and twenty minutes. The red and blue lines

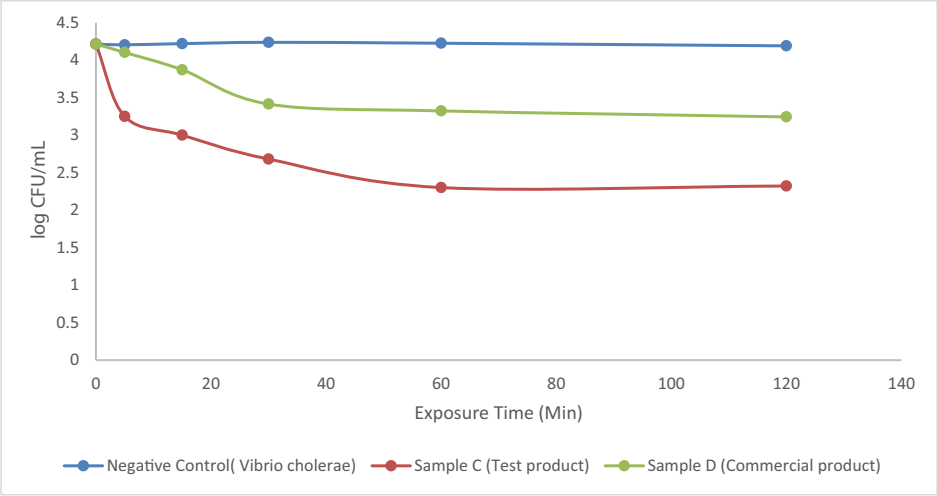

Figure 7. Log CFU/mL of *Vibrio cholerae* against exposure time (min) of samples C and D.

represent microbial load of organisms in the presence and absence of test sample C respectively. Contrary to the blue line (Control) in which bacterial growth was steady and continual, the microbial load of organisms with the test sample showed a gradual decline. In effect, the log reduction CFU of the *V. cholerae* after exposure to the test sample C establishes the fact that, the 63.7 % ethanol based hand sanitizer produced would be effective against *V. cholerae*. Its effectiveness during the extended activity of 2 h in this study reiterates findings in another research in which bacterial counts on alcohol-scrubbed hands continued to drop for several hours even after gloving probably due to continued deaths of damaged organisms [19, 24]. Results in another study revealed that in as little as 15 s, alcohol applications have been proven effective in preventing hand transmission of gram negative bacteria [20]. In general a vast decrease in microbial load was observed upon comparing results obtained in the presence of sample C to those obtained in its absence with regards to the corresponding time frames. In effect, the formulated sample C, could be considered effective against *V. cholerae*.

Moreover, based on the established working principle that hand sanitisers offer a faster, transcient, effective and convenient anti infective activity, the expected evaporation of their volatile ethanolic content will limit the residual antibacterial activity of Sample C, causing possible reinfection tendencies. However, since the intended purpose of this formulation is for use in local settings, where shorter residual activity wouldn't be as harmful as in a clinical setting [28], it can be safely presumed that it will be a suitable product for use to maximize compliance to hand hygiene in minimising the spread of bacterial diseases such as *V. Cholerae*.

Coupled with the emergence of alcohol tolerant microorganisms and bacterial adaptation in multi drug resistant strains such as *E. faecium*, infection control is becoming more complicated in clinical settings, where persistent antimicrobial activity is vital. Therefore alternative antibacterials such as Benzalkonium Chloride could be considered more useful under such settings by providing a longer residual antibacterial effect to minimise transmission of pathogens among patients and healthcare workers [29, 31].

Furthermore, a handful of studies have demonstated that antimicrobial efficacy of ethanolic hand sanitisers can be maximized with reduced reinfection tendencies by ensuring rigorous adherence to a multifaceted set of hand hygiene protocols [28, 30, 31]. Sample C, therefore, with an effective concentration of 63.7% (falling between the recommended concentration of 60%–80%), with applied volume above 0.3 mL but preferably between 3 to 5 mL and with a rubbing duration of 15–30 s will provide the adequate contact time, widespread covering of all hand surfaces and the biocidal concentration required for the denaturation of proteins and cell lysis of most viable bacteria.

5. Conclusion

The study demonstrated that, akpeteshie contains ethanol. The formulated Ethanol based hand sanitizer (EBHS), 63.70% made from akpeteshie is effective against *V. cholerae*. The formulation promises to be relatively affordable to all people, particularly those of lower economic status considering the relative low cost of local gin and low cost of preparation method employed. High patronage and rational use of the formulated product should consequently contribute to enervating the spread of cholera in Ghana and beyond.

The findings from this study suggest the need for further research in optimising the use of local gin as a relatively affordable local raw material for the manufacture of other antiseptic cleaning agents such as rubbing alcohols, hand scrubs and in the base preparation of antibacterial shower gels and wipes. Comparison of new formulations of hand sanitiser from local gin using varying but higher concentrations of local gin can be done to decide on the optimal concentration with highest efficacy against pathogens.

Declarations

Author contribution statement

Christina Osei-Asare: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Esther Eshun Oppong: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

John Antwi Apenteng: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ofosua Adi-Dako, Doris Kumado: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ansah Akyeampomaah Akosua: Performed the experiments; Analyzed and interpreted the data.

Kwasi Adomako Ohemeng: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] Centres For Disease Control and Prevention Foundation. CDC foundation. Centres for disease control and prevention Web site.[Cited: February, 8th, 2017] Http://www.cdc.gov/handwashing/index.htmL.

[2] V. Curtis, S. Cairncross, Effect of washing hands with soap on diarrhoea risk in the community: a systematic review, Lancet Infect. Dis. 3 (5) (2003) 275–281.

[3] P. Mathur, Hand hygiene: back to the basics of infection control, Indian J. Med. Res. 134 (5) (2011) 611.

[4] WHO Guidelines on Hand Hygiene in Health Care: First Global Patient Safety Challenge Clean Care Is Safer Care, World Health Organization, Geneva, 2009, p. 12. WHO-recommended handrub formulations. Available from: https://www.ncbi.nlm.nih.gov/books/NBK144054.

[5] Alcohol Based Hand Sanitizer: Assurance and Apprehensive Revised, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2014, pp. 558–560.

[6] M.J. O'Neil, The Merck Index-An Encyclopedia of Chemical, Drugs and Biologicals, Merck and Co., Inc., Whitehouse, N, 2006, p. 645.

[7] Doe, Hand Sanitizers and Wipes on High Demand. Accra, Ghana: the General Telegraph, 2014, 2014.

[8] Dowuona Samuel, 591 Cholera Cases,five Deaths Recorded in 5 Months - WHO. Accra, Ghana, Adom News, Ghana, 2015.

[9] Y. Ali, M.J. Dolan, E.J. Fendler, E.L. Larson, Alcohols, in: SS Block (Ed.), Sanitization, Disinfection and Sterilization. 5th. Philadelphia, 2001, pp. 229–253.

[10] M. Ali, A.R. Nelson, A.L. Lopez, D. Sack, Updated global burden of cholera in endemic countries, Plos Negl Dis 9 (6) (2015) e0003832.

[11] D.H. Tambekar, S.D. Shirsat, S.B. Suradkar, P.N. Rajankar, Y.S. Banginwar, Prevention of transmission of infectious disease: studies on hand hygiene in health-care among students, Cont. J. Biomed. Sci. 1 (2007) 6–10.

[12] Gregory Mankiw, Law of Demand. Principles of Economics, S.l. South-Western Cengage Learning Press, 2007, p. 450.

[13] D.R. Lide (Ed.), CRC Handbook of Chemistry and Physics, 81st ed., CRC press, 2000.

[14] Samuel Almengor, W. Patrick Monaghan, F.L. Jacksonville, Up in flames: a flammability assessment of alcohol-based hand sanitizers on common perioperative materials, Int. J. Adv. Nurs. Stud. 4 (1) (Jan. 2015) 13–15. ISSN 2227-488X.

[15] Chemistry Department of Physical Sciences, Cape Coast University, GC analysis of methanol in locally distilled beverage "akpeteshie in Ghana, Cape Coast Int. J. Pure Appl. Chem. (2007) 259–261.

[16] Q. Wang, D.Z. An, L.L. Wang, Y. Shi, Y. Zhang, H.J. QI, R.B. Sun, C.F. Zhang, X.L. Liu, L.Q. Jin, Identity and content determination of alcohol compounds in hand disinfectant, Chin. J. Disinfect. 6 (2010) 8.

[17] N.S. Wani, A.K. Bhalerao, V.P. Ranaware, R. Zanje, Formulation and evaluation of herbal sanitizer, Int. J. PharmTech. Res. 5 (1) (2013) 40–43.

[18] I. Vilka, L. Skudra, Development and Analysis of the Hand Alcohol Disinfectant, FOODBALT-2008, 2008, p. 36.

[19] A. Padsalg, D. Jain, S. Bidkar, D. Harinarayana, V. Jadhav, Preparation and evaluation of hand rub disinfectant, Asian J. Pharm. (AJP) 2 (1) (2014). Free full text articles from Asian J Pharm.

[20] G. Kampf, A. Hollingsworth, Comprehensive bactericidal activity of an ethanol-based hand gel in 15 seconds, Ann. Clin. Microbiol. Antimicrob. 7 (1) (2008) 2.

[21] Time kill study. Accugen Lab.com [Cited: December 19, 2017], http://www.accugenlab. Online com/timekillstudy.html.

[22] Food and drug administration, Topical antimicrobial drug products for over-the-counterhuman use: tentative final monograph forhealth care antiseptic drug products- proposed rule.federal register 59 (June 17, 1994) 1441–1452, 31.

[23] J. Lauharanta, J. Ojajärvi, S. Sarna, P. Mäkelä, Prevention of dryness and eczema of the hands of hospital staff by emulsion cleansing instead of washing with soap, J. Hosp. Infect. 17 (1991) 207–215.

[24] E. Larson, APIC guideline for handwshing and hand antisepsis in health care settings, Am. J. Infect. Contr. 23 (1995) 251–269.

[25] E.L. Larson, P.I. Eke, B.E. Laughon, Efficacy of alcohol-based hand rinses under frequent use conditions, Antimicrob. Agents Chemother. 30 (1986) 542–544.

[26] E.L. Larson, H.E. Morton, Alcohols, in: S.S. Block (Ed.), Disinfection, Sterilization and preservation.4th edition.Philadelphia: Lea and Febiger, 1991, pp. 191–203.

[27] T. Appiah, Y.D. boakye, C. Agyare, Antimicrobial activity and time-kill kinetics of extracts of selected ghanaian mushrooms, Evid. Based Compl. Altern. Med. (2017).

[28] M. Suchomel, M. Rotter, Ethanol in pre-surgical hand rubs: concentration and duration of application for achieving European Norm EN 12791, J. Hosp. Infect. 77 (3) (2011) 263–266.

[29] S.W. Bondurant, C.M. Duley, J.W. Harbell, Demonstrating the persistent antibacterial efficacy of a hand sanitizer containing benzalkonium chloride on human skin at 1, 2, and 4 hours after application, Am. J. Infect. Contr. 47 (8) (2019) 928–932.

[30] G. Kampf, S. Ruselack, S. Eggerstedt, N. Nowak, M. Bashir, Less and less–influence of volume on hand coverage and bactericidal efficacy in hand disinfection, BMC Infect. Dis. 13 (1) (2013) 472.

[31] S.J. Pidot, W. Gao, A.H. Buultjens, I.R. Monk, R. Guerillot, G.P. Carter, A.A. Mahony, Increasing tolerance of hospital Enterococcus faecium to handwash alcohols, Sci. Transl. Med. 10 (452) (2018).

[32] Guide to local production: WHO recommended Hand-rub. https://www.who.int/gpsc/5may/Guide_to_Local_Production.pdf.