Evaluation of Quality and Antimicrobial Efficacy of Locally Manufactured Ethanol-Based Hand Sanitizers Marketed in Addis Ababa, Ethiopia in the Era of COVID-19

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Abstract

Background: Coronavirus disease 2019 (COVID-19) continues to spread worldwide. Hand hygiene, via either regular handwashing with soap and water or using hand sanitizers, is among the various measures that need to be followed to control the outbreak of the disease. Alcohol-based hand sanitizers are the “gold standard” for hand disinfection because of their broad antimicrobial spectrum of activity, easy availability, better safety profile, and general acceptability to users. This study aimed at evaluating the physicochemical quality and antimicrobial efficacy of the locally manufactured Ethanol-Based Hand Sanitizers (EBHS) marketed in Addis Ababa, Ethiopia.

Methods: A cross-sectional survey was used to collect EBHS from Addis Ababa marketplaces. A total of 25 sample products were randomly selected from the different categories. The physicochemical evaluation of the products was carried out as per the United States Pharmacopoeia and WHO standards. Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella spp., and Shigella spp clinical isolates were used for the antimicrobial efficacy test.

Results: The Fourier Transform Infrared result confirmed that all the test products met the identification test for ethanol. The majority (68%) of EBHS complied with the test for ethanol content (75 – 85% v/v). However, only 3 products fulfilled the hydrogen peroxide content (0.112 - 0.137% v/v). LPC307 showed the maximum zone of inhibition of 12 mm against Escherichia coli whereas MPC204 exhibited only 3 mm. LPC101 was found to be more sensitive to Shigella and Klebsiella Spp with minimum inhibitory concentration values of 20% and 10%, respectively. The sample product LPC101 showed a minimum bactericidal concentration of 20% against Escherichia coli, Pseudomonas aeruginosa, and Klebsiella spp.

Conclusion: One-third of the tested EBHS did not comply with the WHO ethanol content limit and the majority of the products failed to meet the label claim for hydrogen peroxide content. Besides, nearly all products proved that they have activity against all the tested pathogenic microorganisms; though, they did not show 99.9% bacteriostatic or bactericidal activities as claimed. The study findings suggested regular monitoring of the quality of marketed EBHS considering the current wide use of these products

Introduction

Coronavirus disease 2019 (COVID-19) is continuing to spread around the world, with above 493 million confirmed cases and more than six million deaths affecting over 200 countries worldwide as of April 07, 2022.[1] This highly contagious viral illness is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (2). COVID-19 is emerging as the most consequential global health crisis since the era of the influenza pandemic of 1918.[2] In Ethiopia, the total number of infections and deaths due to the COVID-19 pandemic is 469,916 and 7,508, respectively as of April 07, 2022.[1]

Keeping the cleanliness of hands is among the various measures that need to be followed to control the spread of COVID-19 and other infectious diseases which can be affected via either regular handwashing

with soap and water or using hand sanitizers.[3 – 5]

Out of the various commercialized hand sanitizer products, the most popular and demanding formulations are alcohol-based hand sanitizers (ABHS) containing ethanol as an active ingredient. [3, 6] World Health Organization (WHO) strongly recommends the use of ABHS, which is regarded as the "gold standard" for hand disinfection in healthcare facilities in the community because of its broad antimicrobial spectrum of activity on various microbial strains including SARS-CoV-2, easy availability at the point of care, better safety profile, and general acceptability to users.[6 – 9] In addition, the use of ABHS for protection against bacteria (gram-positive and negative), mycobacteria, fungi, and viruses is well documented.[10 – 12]

The Centers for Disease Control and Prevention (CDC) recommended using hand sanitizers containing at least 60% v/v of alcohol. Though WHO recommended ABHS containing 60–95% alcohol as effective concentration and the standard of care for hand hygiene practice, [7, 8,13, 14] ethanol and isopropyl alcohol at percentages of 80% and 75% v/v, respectively are commonly taken as acceptable strengths.[15, 16] Hands rubbing using ABHS for 25–30 seconds is reported to kill 99.99% of microorganisms on hands.[17, 18]. Hence the strength of the formulations should be evaluated as the alcohol concentration is an active agent and a critical determinant of ABHS efficacy.[19]

Along with the increasing demand, the number of sanitizer manufacturers is booming making quality assurance and regulatory functions complicated. It is fact that the effectiveness of the ABHS is highly dependent on their quality and proper use. The high demand for such affordable products could have made them a candidate for counterfeiting.[20]

In Ethiopia, the existing pharmaceutical industries, small-scale manufacturers, and many new companies have started production and selling hand sanitizer products because of the increased demand fueled by the COVID-19 pandemic. Hand sanitizers are regulated as Over-the-Counter (OTC) drugs in many countries including the U.S.[21] Therefore, this product should satisfy the minimum requirements set by standard agencies to provide the expected result of the quality, safety, and efficacy. The main parameter to be evaluated is the ethanol content which is the active agent responsible for the antimicrobial effectiveness.[13] The desired pH, viscosity, and hydrogen peroxide content of ABHS are also the other parameters that are related to the products' functionality and acceptability by the users. Hence, there is a need to provide attention and control of the product's efficacy and safety.

The products are also required to meet minimum regulatory requirements of quality standards; which may result in health risks and misleading information if violated. However, several concerns about the quality of such products have been raised by the general public, health professionals, and regulatory experts. Falsified ABHS poses a significant public health risk-taking the importance of the products in preventing the spread of COVID-19 and other infections. The ABHS is considered falsified either when it contains ingredients not indicated in the approved list like methanol or when the alcohol content is below 60% v/v. Exposure to the falsified ABHS can result in either systemic toxicity and, in some cases, death,

due to methanol content, or vulnerability of the public to contracting and spreading COVID-19 and other infectious diseases.[22]

Because of the dire demand for the products, lack of proper understanding of the impact of quality defects, or due to business orientation by manufacturers and supply chain actors, the problem might have been pronounced calling for scientific investigation and timely taking regulatory measures.

Therefore, in this study, the locally manufactured Ethanol-Based Hand Sanitizers (EBHS) marketed in Addis Ababa, Ethiopia in the era of COVID-19 were evaluated for their physicochemical quality and antimicrobial efficacy against pathogenic bacteria according to USP and WHO standards.

Materials And Methods

Materials

Different brands of locally manufactured EBHS were collected from the marketplaces (drug retail outlets and supermarkets) in Addis Ababa and stored as recommended by their manufacturers. The details of the collected hand sanitizers are described in Table 1 (with codes representing each brand). The samples were stored in their original container under ambient conditions until analysis. All samples were within their shelf lives during analysis.

The chemicals reagents and instruments used for the study includes ethanol absolute, ≥ 99.8% (Merck KGaA, Germany), Sulfuric acid (Merck KGaA, Germany), Primary Standard Sodium Oxalate (Alfar Aesar, Great Britain), Potassium Permanganate (Blulux Laboratories P.Ltd., India) and ultra-pure water (Anton Paar, Germany), Barium chloride dihydrate (BaCl2.2H2O), (LABKEMICAL,), MacConkey agar (Accumix, India), Mannitol Salt agar (SRL, India), Mueller Hinton agar (HIMEDIA, India), Nutrient broth (Accumix, India), Potassium hydroxide pellet 85% extra pure (LOBA Chemie, India), Salmonella Shigella agar (HIMEDIA, India), Sulfuric acid (LOBA Chemie, India), and Violet Red Glucose agar (SRL, India). pH meter, density meter, and Fourier Transform Infrared spectrophotometer. Centrifuge (DR AWELL, U.S.A), Incubator (BIOBASE, China), Spectrophotometer (OPTIZEN POP UV-VIS Smart Spectrophotometer, Korea), and Vortex Mixer (LAB STAC United Kingdom) were used for the antimicrobial efficacy study. *Escherichia coli, Klebsiella spp*., *Pseudomonas aeruginosa*, *Staphylococcus aureus, Salmonella spp.,* and *Shigella spp* were the test organisms used in the study.

Table 1

Description of locally manufactured hand sanitizer products marketed in Addis Ababa, Ethiopia, 2021

S.N	Product	Product's information		
		Expiry date (month/year)	Pack size	Source
1	LPC101	10//2022	1000 ml	DROL
2	LPC102	04//2024	1000 ml	DROL
3	LPC103	04/2022	1000 ml	DROL
4	LPC104	05/2023	500 ml	DROL
5	MPC201	Not indicated	750 ml	DROL
6	MPC202	Not indicated	1000 ml	DROL
7	MPC203	04/2024	1000 ml	DROL
8	MPC204	04/2023	1000 ml	DROL
9	MPC205	Not indicated	1000 ml	DROL
10	MPC206	Not indicated	500 ml	DROL
11	SPC301	12/2023	1000 ml	DROL
12	SPC302	05/2023	1000 ml	DROL
13	SPC303	Not indicated	1000 ml	DROL
14	SPC304	04/2023	1000 ml	Supermarket
15	SPC305	03/2022	500 ml	DROL
16	SPC306	06/2023	1000 ml	DROL
17	SPC307	Not indicated	500 ml	DROL
18	SSC401	06/2022	500 ml	Supermarket
19	SSC402	05/2022	1000 ml	DROL
20	SSC403	06/2023	1000 ml	DROL
21	SSC404	12/2022	1000 ml	Supermarket
22	SSC405	01/2022	500 ml	DROL
23	SSC406	11/2022	1000 ml	Supermarket
24	SSC407	03/2022	500 ml	DROL
25	SSC408	Not indicated	250 ml	Supermarket

DROL – Drug Retail OutLet

Methods

Study area

Addis Ababa is the political and commercial capital of Ethiopia with a population of over 5 million. The city is administratively divided into eleven sub-cities and 116 Woredas.[23] Because of the large market and access to facilities, pharmaceuticals and cosmetics manufacturing facilities, and distribution actors are largely concentrated around Addis Ababa and its outskirts.

Study design

A cross-sectional survey was used to collect EBHS from marketplaces (drug retail outlets and supermarkets).

Source and study population

The source population was all EBHS manufactured by local manufacturers and marketed to the community in Addis Ababa City. The EBHS which were manufactured by the selected local manufacturers and marketed in drug retail outlets and supermarkets in Addis Ababa and fulfilling the following eligibility criteria were included in the study population.

Eligibility criteria

The following inclusion and exclusion criteria were used to sample the hand sanitizer products from the market. Inclusion criteria:

- Manufactured by local manufacturers
- Ethanol-based solution
- Labeled with information

Sample size and sampling techniques

According to sources from the Ethiopian Food and Drug Authority (EFDA), there were 161 hand sanitizer manufacturers licensed by the authority nationwide; of which 124 were from Addis Ababa and its outskirts. The sanitizer manufacturers have different capacities and experiences in pharmaceuticals or cosmetics manufacturing. Accordingly, the manufacturers from Addis Ababa and its environs were broadly categorized into four: (i) large-scale pharmaceutical and cosmetics/chemicals manufacturers (17); (ii) medium level cosmetics and chemical manufacturers (31); (iii) small scale extemporaneous

pharmaceuticals and supplies manufacturers (34); and (iv) other small firms established following the COVID-19 pandemic (42).

Among the 124 manufacturers, considering resource constraints and sample size representativeness, a total of 25 products (20% from each category of manufacturers) were included in the study. Products from each of the four categories with reserves were randomly selected.

Sample collection procedure

Samples were purchased between October and November; 2021 from drug retail outlets and supermarkets in Addis Ababa until the required sample size was reached. A package size of 250 ml, 500 ml, and 1000 ml of the respective samples were purchased from marketplaces as found appropriate.

Physicochemical quality evaluation

Selected EBHS samples were tested for their physicochemical quality based on USP [24] and WHO standards.[16] The tests included were:

Physical examination

Physical examination was performed and recorded for colors and the presence of fragrances in sample EBHS.

Identification test for ethanol

An identification test for ethanol was performed as per USP 43 NF 38.[24] A Bruker Fourier Transform Infrared (FTIR) spectroscopy (Bruker-Tensor-II, Germany) equipped with Attenuated Total Reflectance sample compartment was used to generate the FTIR spectra of the sample EBHS in comparison with FTIR spectrum of the standard ethanol. The transmittance was measured concomitantly in the wavenumber range from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1}. Sixteen FTIR scans were performed for each sample and reference ethanol

Determination of ethanol content

The ethanol concentration (% v/v) of the EBHS samples was determined as per the USP monograph method II.[24] An oscillating transducer density meter (Anton Paar, Density Meter DMA 4200 M, Germany) that has been calibrated with standard ethanol and standard water at room temperature and atmospheric pressure was used for the ethanol content level determination.

Determination of hydrogen peroxide strength

The hydrogen peroxide content of the samples were determined as per USP 43 NF 38.[24] Each test was done in triplicate.

pH determination

The pH of EBHSs was determined using calibrated digital pH meter (HI 2550 Hanna I instruments) and it was measured in triplicate.

Antimicrobial efficacy test

The antimicrobial efficacy study for the EBHS was conducted in the microbiology laboratory of the Bio and Emerging Technology Institute (BETin), Addis Ababa, Ethiopia.

Test organisms

Clinical isolate bacteria like *Escherichia coli, Klebsiella spp.*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were kindly provided by the Department of Medical Laboratory, Tikur Anbessa Specialized Hospital, College of Health Sciences, Addis Ababa University whereas *Salmonella spp*. and *Shigella spp*. isolates were obtained from BETin microbiology laboratory.

Confirmation of the test organism

For the confirmation of the test organism; gram staining and biochemical identification were conducted. The test organisms were inoculated into MacConkey agar (Accumix, India), Mannitol Salt agar (SRL, India), Salmonella Shigella agar (HIMEDIA, India), and Violet Red Glucose agar (SRL, India) and were incubated at 35°C - 37°C for 24 hr. On the next day, a gram reaction was performed and followed by biochemical tests using their biochemical characteristics after overnight incubation (35°C - 37°C). The isolated test organisms were stored on storage media, kept at 2–8° C, and used when needed. Each of the test organisms was standardized using 0.5 McFarland standard.[25] This 0.5 McFarland turbidity standard was prepared from the mixture of sulfuric acid (H_2SO_4) (LOBA Chemie, India) and barium chloride dihydrate (BaC_{l2} $2H_2O$) (LABKEMICAL) solution with confirmation of the mixture absorbance (0.08 – 0.10) density accuracy through a spectrophotometer (OPTIZEN POP UV-VIS Smart Spectrophotometer, Korea) at a wavelength of 625 nm.

Antibacterial activity of the EBHS through agar well diffusion methods

Agar diffusion method was used to determine the susceptibility test of selected test organisms for each product sample. This agar diffusion method was done in triplicate for each sample. Standardized test organisms were swabbed into sterile Mueller Hinton agar (HIMEDIA, India) plates using sterile cotton swabs. After swabbing Mueller Hinton agar was dried; 5 equally spaced holes were bored in the agar plate with the blue tips. The 3 holes were filled with 100µL of the hand sanitizer at the same time while the other two holes were filled with an equal volume of sterile water and ampicillin suspension for negative and positive control purposes, respectively. The Mueller Hinton agar was incubated at 37° C for 24 hours. The zones of inhibition of the sample products to each test organism were examined with a ruler in millimeters by considering the average of two readings that were found from a triplicate of agar diffusion test for each EBHS sample.[26, 27]

Minimum Inhibition Concentration (MIC) Determination

The lowest concentration of an EBHS required to inhibit the growth of a known test organism *in vitro* was done on nutrient broth for each product sample against the selected test organisms. The minimum inhibitory concentration (MIC) was determined using broth dilution method (25) by preparing various concentrations of each product sample (10%, 20%, 30%, 40%, 50%, 60%, 70%, and 80%). Then, one milliliter from each hand sanitizer product was introduced into the tube containing equal volumes (1 mL) of nutrient broth inoculated with a standardized test organism. A tube containing nutrient broth and bacteria without sanitizer and a tube containing the sanitizer and broth without bacteria were used as a negative and positive control, respectively. Finally, the tubes were incubated for 18–24 hours and visible growth (turbidity) was assessed. When compared with the controls, the concentration of the hand sanitizers at which no visible growth was regarded as MIC.

Minimum Bactericidal Concentration (MBC) Determination

The lowest concentration of a specific hand sanitizer that can kill 99.9% of a given bacterial strain was determined from the MIC tests that showed no visible growth by taking a loopful of inoculum living test organisms from the MIC tubes by streaking on fresh Mueller Hinton agar. The streaked Mueller Hinton agar plates were incubated at 37°C for 24 hours and were observed for growth. Streaked Mueller Hinton agar plates that cannot show any growth indicates a 99.9% bactericidal effect of the sanitizer at that concentration or MBC.[25]

Quality control and data quality assurance

To maintain the quality of this project, aseptic technique was followed and all tests were performed in triplicates. Before testing, all the collected EBHS were stored as per the manufacturers' storage conditions. All the equipment used for testing were checked for their functionality. The prepared culture media were checked for sterility by incubating five percent of the prepared media overnight and observing

for the presence of any growth. The suitability of the prepared media in supporting the growth of the organisms were checked by inoculating control strains.

Ethical clearance

Before starting the research work, ethical approval was obtained from Addis Ababa University, School of Pharmacy Ethical Review Committee (ERB/SOP/307/13/2021). This study was carried out according to the Helsinki Declaration of ethical principles for research. All the information obtained from the study about EBHSs were maintained confidential by assigning codes for the products.

Data analysis and interpretation

Data were properly collected, analyzed, and presented using appropriate statistical tools. The data were interpreted and the results are presented as mean ± SD. Statistical analysis was performed using SPSS program version 25.

Results

Physicochemical quality evaluation

The FTIR spectra of the standard ethanol and the sample EBHS are demonstrated in **Figures 1 – 4**. A broad absorption band was found in the standard ethanol and all the tested sample products in the region with wavenumber ranging from 3600 to 3100 cm^{-1}, indicating the presence of a hydroxyl group (–OH). Strong absorbance peaks were also observed at 878 cm^{-1} and 1043 cm^{-1}.

The result of some physicochemical parameters evaluated for the collected EBHS showed that out of the 25 samples evaluated, 20 (80%) were found to be colorless solution whereas the remaining 20% exhibited certain specific colors (Table 2). LPC102 revealed the maximum ethanol concentration of 83.8%v/v. On the other hand, SSC408 had 54.4% v/v ethanol content which is the minimum value of all tested products. The least hydrogen peroxide content was found in SSC403 (0.03%v/v). A maximum pH value of 7.6 was recorded for product SPC301.

Table 2

Some physicochemical characteristics of EBHS marketed in Addis Ababa, Ethiopia, 2021

S.N	Product	Test parameters				
		Ethanol conc. (%v/v) ± SD	H_2O_2 conc. (%v/v) ± SD	pH ± SD	Color	Fragrance
1.	LPC101	78.93 ± 0.12	0.23 ± 0.00	5.40 ± 0.00	Colorless	No
1.	LPC102	83.80 ± 0.10	0.16 ± 0.00	5.87 ± 0.06	Colorless	No
1.	LPC103	78.60 ± 0.10	0.08 ± 0.02	4.90 ± 0.00	Colorless	No
1.	LPC104	80.37 ± 0.06	0.20 ± 0.01	6.67 ± 0.06	Colorless	No
1.	MPC201	80.23 ± 0.12	0.09 ± 0.01	7.13 ± 0.06	Colorless	No
1.	MPC202	78.53 ± 0.12	0.25 ± 0.01	8.97 ± 0.06	Colorless	Yes
1.	MPC203	69.60 ± 0.10	0.13 ± 0.00	6.03 ± 0.12	Colorless	No
1.	MPC204	77.70 ± 0.10	0.38 ± 0.01	7.40 ± 0.00	Colorless	No
1.	MPC205	78.83 ± 0.06	0.31 ± 0.01	5.80 ± 0.00	Colorless	No
1.	MPC206	82.33 ± 0.06	0.24 ± 0.01	5.43 ± 0.06	Colorless	No
1.	SPC301	77.03 ± 0.06	0.29 ± 0.01	7.60 ± 0.10	Colorless	No
1.	SPC302	69.67 ± 0.06	0.22 ± 0.01	6.87 ± 0.06	Light orange	No
1.	SPC303	80.73 ± 0.06	0.24 ± 0.01	6.53 ± 0.25	Light green	No
1.	SPC304	77.47 ± 0.06	0.22 ± 0.02	6.33 ± 0.06	Light red	No
1.	SPC305	78.57 ± 0.06	0.29 ± 0.01	6.77 ± 0.06	Colorless	No
1.	SPC306	72.57 ± 0.06	0.19 ± 0.01	5.77 ± 0.06	Light green	Yes
1.	SPC307	78.60 ± 0.10	0.27 ± 0.01	6.23 ± 0.06	Colorless	No
1.	SSC401	72.70 ± 0.10	0.23 ± 0.00	6.50 ± 0.00	Colorless	No

1.	SSC402	82.63 ± 0.12	0.04 ± 0.01	6.83 ± 0.12	Colorless	No
1.	SSC403	72.63 ± 0.12	0.03 ± 0.01	6.57 ± 0.06	Colorless	No
1.	SSC404	80.03 ± 0.12	0.16 ± 0.02	7.33 ± 0.06	Light yellow	No
1.	SSC405	56.80 ± 0.17	0.13 ± 0.00	8.57 ± 0.06	Colorless	No
1.	SSC406	74.40 ± 0.10	0.24 ± 0.00	6.40 ± 0.00	Colorless	No
1.	SSC407	77.33 ± 0.12	0.14 ± 0.01	5.57 ± 0.06	Colorless	No
1.	SSC408	54.43 ± 0.15	0.09 ± 0.02	8.47 ± 0.06	Green	No

Antimicrobial evaluation

All of the test organisms were confirmed for their credentials with different biochemical tests. The antimicrobial effectiveness was assessed by measuring the zone of inhibition against the specific test bacteria. Maximum inhibition was seen in LPC 103 and SSC 407 sanitizer against *Shigella spp*. and *Salmonella spp.,* respectively i.e., 15 mm. The minimum inhibition was seen in MPC 204 against *Escherichia coli* i.e., 3 mm (Table 3).

Table 3

Zones of Inhibition of EBHS against test organisms

Hand sanitizer	Zones of Inhibition (mm)					
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Salmonella spp.*	*Shigella spp.*	*Klebsiella Spp.*
LPC 101	10	12	11	10	12	6
LPC 102	6	4	5	6	12	5
LPC 103	11	11	9	13	15	6
LPC 104	4	13	6	10	10	8
MPC 201	8	8	4	4	4	4
MPC 202	10	8	6	5	6	7
MPC 203	9	9	5	5	4	5
MPC 204	3	10	10	10	14	12
MPC 205	10	12	7	10	10	8
MPC 206	9	4	4	7	5	4
SPC 301	8	11	7	8	9	6
SPC 302	10	8	5	7	5	6
SPC 303	11	10	8	8	12	5
SPC 304	10	10	5	9	4	5
SPC 305	10	5	8	6	5	8
SPC 306	9	8	5	5	6	4
SPC 307	12	9	10	4	4	6
SSC 401	10	10	10	10	9	4
SSC 402	5	11	5	4	5	5
SSC 403	4	14	9	10	12	6
SSC 404	7	5	5	5	4	5
SSC 405	5	4	9	4	5	4
SSC 406	7	13	10	9	8	9

SSC 407	10	7	12	15	5	4
SSC 408	9	13	13	10	4	7

Table 4 shows the Minimum Inhibitory Concentration (MIC) of the tested EBHS. The results revealed all hand sanitizer products displayed antibacterial activity against all of the test bacteria at a minimum concentration from 10% to 80%. Thus, LPC 101 hand sanitizer showed a 10% minimum inhibitory concentration against *E. coli*, *P. aeruginosa,* and *Klebsiella spp*. Similarly, LPC 102 and SPC 305 hand sanitizers exhibited 10% MIC against *Staphylococcus aureus.* Congruently, 10% MIC was also observed by MPC 203 hand sanitizer against *Escherichia coli.* The MIC of the majority of hand sanitizers lied 10 - 50% nearly for all of the tested bacteria.

Table 4

Minimum Inhibitory Concentration (MIC) of EBHS against test organisms

Hand sanitizer	Minimum Inhibitory Concentration (MIC (%))					
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Salmonella spp.*	*Shigella spp.*	*Klebsiella Spp.*
LPC 101	10	10	70	30	20	10
LPC 102	40	30	10	40	50	40
LPC 103	50	80	20	40	60	40
LPC 104	30	30	40	30	50	50
MPC 201	30	40	50	60	60	30
MPC 202	50	20	30	30	30	20
MPC 203	10	30	40	40	30	20
MPC 204	20	20	20	30	30	30
MPC 205	20	30	40	40	40	20
MPC 206	60	50	60	60	70	50
SPC 301	20	30	50	40	50	40
SPC 302	20	20	60	60	50	30
SPC 303	20	20	80	50	50	50
SPC 304	60	80	80	60	60	60
SPC 305	30	30	10	50	80	70
SPC 306	60	50	30	40	50	60
SPC 307	30	50	60	40	30	40
SSC 401	30	20	70	30	30	40
SSC 402	30	30	60	60	60	60
SSC 403	40	50	50	60	50	50
SSC 404	40	40	60	50	50	50
SSC 405	50	60	60	40	30	70
SSC 406	60	50	70	50	50	50

SSC 407	70	50	70	50	70	70
SSC 408	60	60	60	30	50	50

The minimum bactericidal activity of the hand sanitizers against test bacteria was found to be in the range of 20% to 80% (Table 5). From the assessed twenty-five hand sanitizers, seven of them showed 20 % bactericidal activity against test bacteria. Of which LPC 102, MPC 202, and MPC 204 hand sanitizers exhibited below 50% bactericidal activity against all of the test bacteria.

Table 5

Minimum Bactericidal Concentration (MBC) of EBHS against test organisms

Hand sanitizer	Minimum Bactericidal Concentration (MBC (%))					
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Salmonella spp.*	*Shigella spp.*	*Klebsiella Spp.*
LPC 101	20	20	80	40	30	20
LPC 102	30	40	30	30	40	30
LPC 103	40	70	30	50	50	50
LPC 104	40	40	50	40	60	60
MPC 201	40	50	60	50	50	40
MPC 202	40	30	40	20	40	30
MPC 203	20	40	50	50	40	30
MPC 204	30	30	30	40	40	40
MPC 205	30	40	50	50	50	30
MPC 206	50	40	70	50	60	40
SPC 301	30	40	60	50	60	50
SPC 302	30	30	70	50	40	40
SPC 303	30	30	70	40	40	40
SPC 304	50	70	70	50	50	50
SPC 305	40	40	20	40	70	60
SPC 306	70	40	40	50	60	70
SPC 307	40	40	70	50	20	30
SSC 401	40	30	60	40	20	50
SSC 402	20	20	50	50	50	50
SSC 403	30	40	60	50	60	60
SSC 404	30	30	50	40	40	40
SSC 405	40	50	70	50	40	60
SSC 406	50	40	60	40	40	40

SSC 407	60	40	60	40	60	60
SSC 408	50	50	50	20	40	40

Discussion

Promoting good hygiene in healthcare facilities and communities is important to avoid pathogenic diseases. [28] Hand hygiene practice is an essential part of daily life which is the simplest and least expensive measure proven to be effective in preventing COVID-19 and other infections to keep humans healthy.[6, 7] Among the range of strategies proposed for the promotion and improvement of hand hygiene, use of hand sanitizers is well advocated as it offers a convenient, effective, and relatively low-cost alternative, especially for developing countries.[29 – 31]

Center for Disease Control and Prevention (CDC) recommends hand sanitizer that contains at least 60% alcohol as an important step that one can take to avoid getting sick and spreading germs to others (32). On the other hand, WHO stated the use of ABHS with ethyl alcohol at a concentration of 80% v/v for optimal antimicrobial efficacy.[10]

If failed to meet minimum quality standards, hand sanitizer can be ineffective (misleading users due to perceived effectiveness and aggravating the spread of COVID-19 and other infections) and also cause public health risks. There are also many risks associated with low quality hand sanitizers which include harm to healthcare providers, patients, and the general public. Unless these quality issues are addressed and managed appropriately, the risks outweigh the benefits of these products.

The current study attempted to evaluate the physicochemical and antimicrobial efficacy of sample EBHS marketed in Addis Ababa sourced from different local manufacturers following the outbreak of the current pandemic COVID 19. All the test products were formulated as per WHO formulation 1[16]that contains ethanol, glycerol, and hydrogen peroxide ingredients with an anticipated concentration of 80% v/v, 1.45% v/v, and 0.125 v/v%, respectively in the final product. The reliability of products labelling information was checked with physicochemical analysis,

The FTIR spectra of all the tested sample products showed the presence of ethanol in the formulations as the characteristic peaks of ethanol are indicated in the figures (Figures 1 – 4). The appearance of strong absorbance peaks at 878 cm^{-1} (C–C–O symmetric stretch) and 1043 cm^{-1} (C–O stretch of primary alcohol) could serve as the signature FTIR characteristics for ethanol.[20, 24] The result revealed the matching of alcohol type indicated in the labels with the analysis outcome. It's essential to check the presence of the claimed alcohol in the formulation since hand sanitizers devoid of the labeled ingredient may be circulated in the market due to their current high demand. A study conducted in Nairobi showed about 14.9% of the tested 74 samples had methanol, instead of ethanol, as the main component of alcohol-based hand sanitizers.[33] Another study in Johannesburg area revealed that 3 of the 94 different hand sanitizer products were found to contain no alcohol.[34] Such circulation of falsified hand sanitizer

products in the market compromises the control of infection transmission and may expose users to the undesired effects.

Considering the physical appearance of the tested products, all samples were in solution form as stated on their label and 20% exhibited distinct colors such as light green, light yellow, and red whereas the remaining 80% were found to be colorless. Moreover, two products contained fragrance in the formulations, as indicated on their labels. Fragrances and coloring agents are commonly incorporated in formulations to increase the acceptability of the product and for product identification. But, it is clearly indicated that such addition of fragrances and colorants is not recommended due to the potential risk of allergic reactions and might increase the risk of ingestion by children.[13, 16, 35, 36] In addition to this, the antimicrobial effectiveness of the products may be compromised by these agents which otherwise their influence should be justified with tests. [16, 20] It is also indicated that such addition of other ingredients may affect the overall product efficacy, safety, and long-term utility.[4, 37]

Ethanol is the main active agent in the formulation that is responsible for the lethality of microorganisms. As the efficacy of alcohol is dependent on its concentration, the accurate determination of alcohol content of EBHS may act as a surrogate for efficacy.[33, 37] The limit of ethanol content to comply with the requirement is stated to be within ±5% variation (75 % – 85 %) from the claimed potency (80 % v/v).[16] Density measurement was explored as an approach for estimation of the ethanol content.[38] The current evaluation result depicted that those 8 products (32%) failed to meet the requirements and all were found to contain lower content for ethanol (< 75% v/v). The maximum variation was noted for SSC408 with only 54% v/v ethanol content. Five out of the eight products that failed the test for ethanol content were from the small-scale manufacturer's category (i.e. SSC) which indicates the need for close control of such companies by the regulatory body.

A similar study done in Johannesburg resulted in 37 (41%) products containing less than 60% v/v alcohol. [34] Additional literature have also reported the circulation of substandard ABHSs in various market places.[34, 39 – 42] The concentration of ethanol beyond the specified limit is associated with the low effectiveness of the product for its antimicrobial role and promoting of hand hygiene.[16]

Due to the increasing consumer demand, these products could become easy targets of fraud or counterfeiting by bulking the preparation by diluting the alcohol content with water or cheaper substitutes like methanol which end up with a less functional product. [20]

Moreover, the influence of other formulation ingredients on ABHS efficacy, safety, and usage should be taken into consideration. Hydrogen peroxide is among the ingredients which are added to avoiding spore-forming organisms in the product.[13] Spore forming organisms may result from the raw materials such as water and the packaging bottles or during the production process. The limit of acceptance, according to USP specification, to hydrogen peroxide topical solution is found to be in the range of 90 -110% of the claimed potency. Only three products (MPC203, SSC405, and SSC407) gave a satisfactory result for the hydrogen peroxide content test (0.112 – 0.137% v/v).[24] The maximum and minimum concentration of

hydrogen peroxide was found to be 0.38% v/v and 0.03% v/v, respectively. The availability of this ingredient beyond the required limit affects either the performance of the product or creates discomfort to the users. The reason for the failures of most products this test should be investigated by the manufacturers themselves as well as by the regulatory body.

The optimum pH value of hand sanitizers is important for the effectiveness of the product as well as for its comfortability/suitability during application on hands. The incorporation of ingredients beyond the defined concentration or other ingredients (such as colorants) may affect the pH of the final product. The tested products showed a pH range between 4.90% and 8.97%. Normally, skin pH range between 5.4 and 5.9, [43, 44] and this neutral pH is generally accepted for cosmetic products. Only 6 products (24%) lay in this pH range and the majority (72%) had higher pH values. Such high pH levels might be resulted from the nature of ingredients incorporated in the formulation of EBHS. It is important to consider skin pH during the formulation of dermatological products like hand sanitizers so that the product will not cause skin dryness or irritation and brings soft and smooth skin. Overall, considering the tests outlined in Table 2, only one product (SSC407) complied with all the tests.

Many studies have been conducted to assess the quality and antimicrobial effectiveness of hand sanitizers elsewhere and failure to meet the quality standard has been reported for some products. [19, 40, 45, 46] Following the public health emergency due to COVID-19, the Ethiopian Food and Drug Administration (EFDA) has licensed more than 100 manufacturers for the production of ABHRs to meet the growing demand for this product in the country. Even though these products are considered as drugs,[21] interested companies without the required professionals are allowed to engage in manufacturing the sanitizers to address the supply shortage. In addition, some beverage firms have reconfigured their operations to produce hand sanitizer products. Such involvement of individuals without adequate knowledge and experience for similar products may contribute to the poor quality of products and there are regulatory requirements to be known.

Because of lack of manufacturers' understanding or due to business orientation, quality defects are often reported by regulatory authorities and individual users which may endanger users' safety. Moreover, there are several hand sanitizers sold to the Ethiopian market with labels on their package that claim that the hand rub can kill 99.9% of germs without generating evidence. This problem may be further intensified in light of a limited regulatory capacity to conduct regular inspection and quality surveillance.

Despite the claims of efficacy and 99.9% bacterial reduction by hand sanitizer manufacturers, there still exists a need for verification of these claims. The present study also evaluated the sample products for their antibacterial efficacy. All the EBHS displayed bactericidal activity against all the selected test organisms at a concentration of range from 20% to 80%. Subsequently, the highest bactericidal effect was observed against *S. aureus* with 80% activities. This is in line with the findings of a similar study conducted by. [47] contrarily [48, 49] studies showed that efficacy on *E.coli* was higher compared to the other pathogens.

LPC 101 EBHS had the highest bactericidal activity against *S. aureus*. Subsequently, SPC 304 EBHS was the most effective hand sanitizer against all the tested bacteria with a range of 50 - 70 % bactericidal activities. Consistently, research finding has shown hand sanitizers to have antimicrobial effects against bacteria such as *S. aureus*, *E. coli*, *Pseudomonas spp*., and *Klebsiella Spp.*[50]

The minimum bactericidal activity was observed in some hand sanitizers. Correspondingly, MPC 202 exhibited the lowest bactericidal activity (20 to 40 %) against all of the test bacteria. In line with this, a study conducted by Otokunefor & Princewill [47] revealed that 25% was the minimum concentration of bacterial inhibition in which below 25% was the minimum bactericidal concentration. In contrast to the current study, hand sanitizers were found to be not efficacious against test bacteria in another study.[49] Minimum bactericidal activities could be due to the relatively decreased concentration of ethanol in hand sanitizer as the efficacy of alcohol-based hand sanitizer is affected mainly by the type and content of alcohol used. Moreover, the minimum bactericidal effect could be due to poor or extended storage of the hand sanitizer which could lead to increased temperature causing evaporation of the active ingredient. Furthermore, the minimum bactericidal effect could be due to the resistance development of test bacterial strains. Due to this, not all sanitizers are equally effective in eliminating all microorganisms. [51, 52] Provided that there is rational use of quality ABHS available in health facilities and the communities, a decrease in the incidence of multidrug-resistant bacterial and viral isolates and patient colonization will be observed. [10]

Considering the pandemic COVID-19 and other infections, consumers shall be vigilant about which hand sanitizers they use. The findings of the current study revealed the spectrum/status of locally manufactured EBHS quality and antimicrobial efficacy which can help various stakeholders to implement timely interventional strategies on parameters in which defects were observed through proper public education, and engagement of key stakeholders. It also provides the regulatory body (EFDA) with objective evidence to take appropriate regulatory measures.

Limitations of the study

This study has some limitations. The antimicrobial efficacy test was determined only for bacteria through the EBHS is also known for its effect on enveloped viruses like SARS-CoV-2 laboratory setup constraint. In addition, the study is limited to hand sanitizers manufactured as per the WHO formulation 1 (i.e. solution form). The study also failed to determine the methanol limit for the EBHS due to the unavailability of a validated method and gas chromatography for methanol content determination. However, to the best of our knowledge, this study is the first to comprehensively evaluate the physicochemical quality and efficacy of EBHS in the market obtained from local manufacturers.

Conclusion

Quality problems of the EBHS in the market were observed especially for the hydrogen peroxide and ethanol content. About one-third of the tested products failed to satisfy the WHO requirement for the ethanol content. Moreover, the majority of the products showed higher pH values than the recommended range.

Nearly all EBHS exhibited good activities against the tested bacteria though the products did not show 99.9% bacteriostatic or bactericidal activities as claimed.

Hand hygiene is recognized as the best and most cost-effective way to prevent the spread of infectious diseases, and this study contributes to the implementation of appropriate actions by the concerned stakeholders regarding the quality and efficacy of EBHS circulating in the Addis Ababa market.

Recommendations

Hand sanitizers have become an essential product in hospitals and communities in day-to-day life. They have gained much popularity and have become a highly accepted form of personal hygiene because of their effectiveness and ease of use. Assuring the quality of these products will enhance the compliance of healthcare providers and other individuals with these products and contributes to the containment of COVID-19 and other infections.

The quality of ABHRs in the local market should be given attention and addressed carefully even after the end of the current pandemic, COVID-19.

As the hand sanitizer products are considered over-the-counter (OTC) drugs, periodical inspection/evaluation of these products should be in place by the responsible organizations such as regulatory authority (EFDA), research, and academic institutions to make sure that quality products are reaching the market. The regulatory body should take the leading role in controlling the ABHS products at every stage of their lifecycle, including manufacturing and distribution, to ensure that the products are safe and effective. Moreover, the presence of methanol in the ABHSs and their compliance with the specifications have to be assessed to protect the users from unwanted effects.

Despite the claims of efficacy and 99.9% bacterial reduction by hand sanitizer manufacturers, there still exists a need for verification of these claims.

Declarations

Data Sharing Statement

The datasets used for this publication can be obtained from the corresponding author on reasonable request.

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Disclosure

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Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Authors' contributions

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; MS took part in designing and drafting the manuscript, BMH participated in designing and revising the article, TM carried out the sample collection and Physicochemical analysis of the samples, MB participated in the anti-microbial analysis and critical review of the manuscript, TG carried out the anti-microbial analysis and drafting the manuscript, SG participated in the spectral analysis and interpretation of the manuscript, AA take part in the Physicochemical analysis and spectral interpretation of the manuscript, MM conducted the antimicrobial analysis, culture selection, and growth of the microorganisms, MM participated in critical evaluation and editing of the manuscript, GB conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors agreed to submit it to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Figures

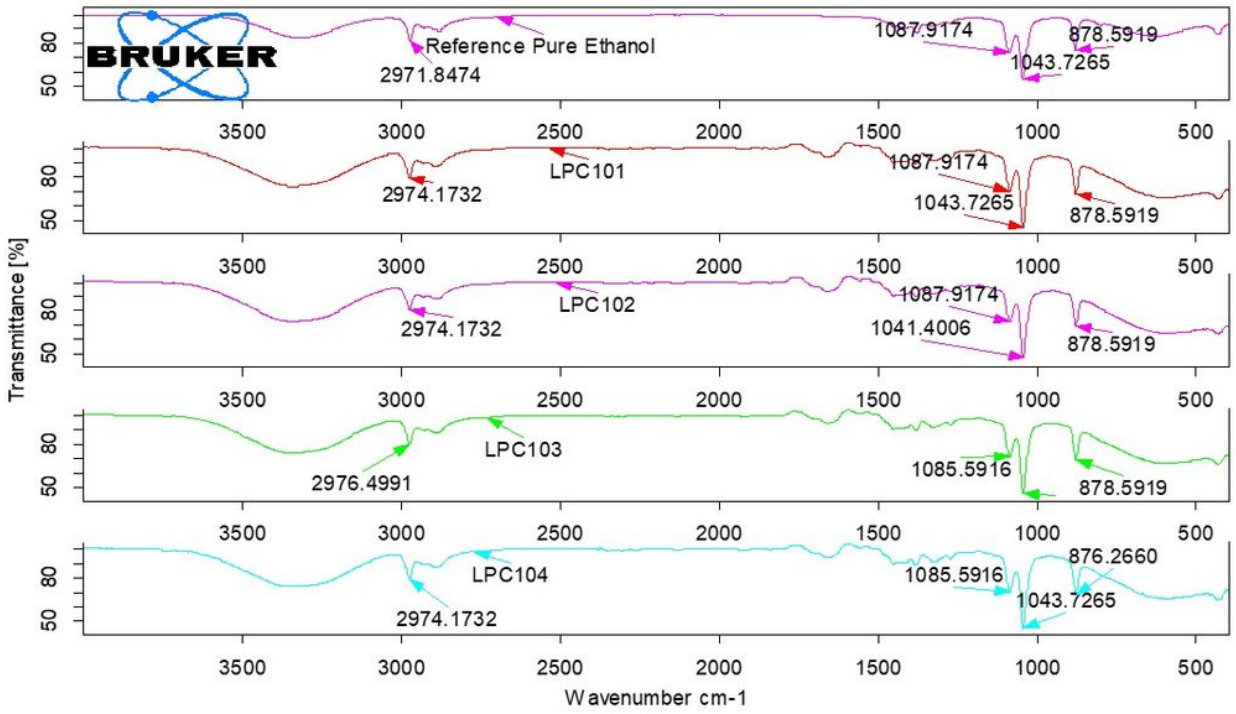

Figure 1

The FTIR spectrum of ethanol reference standard and tested hand sanitizers (LPC101, LPC102, LPC103, and LPC104).

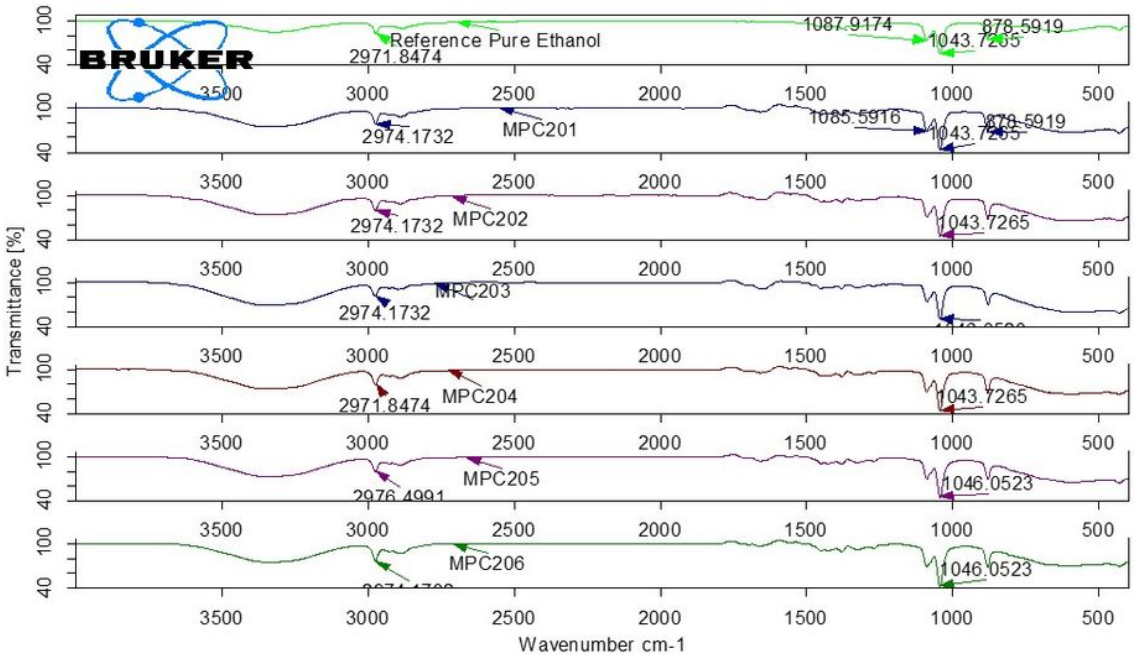

Figure 2

The FTIR spectrum of ethanol reference standard and tested hand sanitizers (MPC201, MPC202, MPC203, MPC204, MPC205, and MPC206).

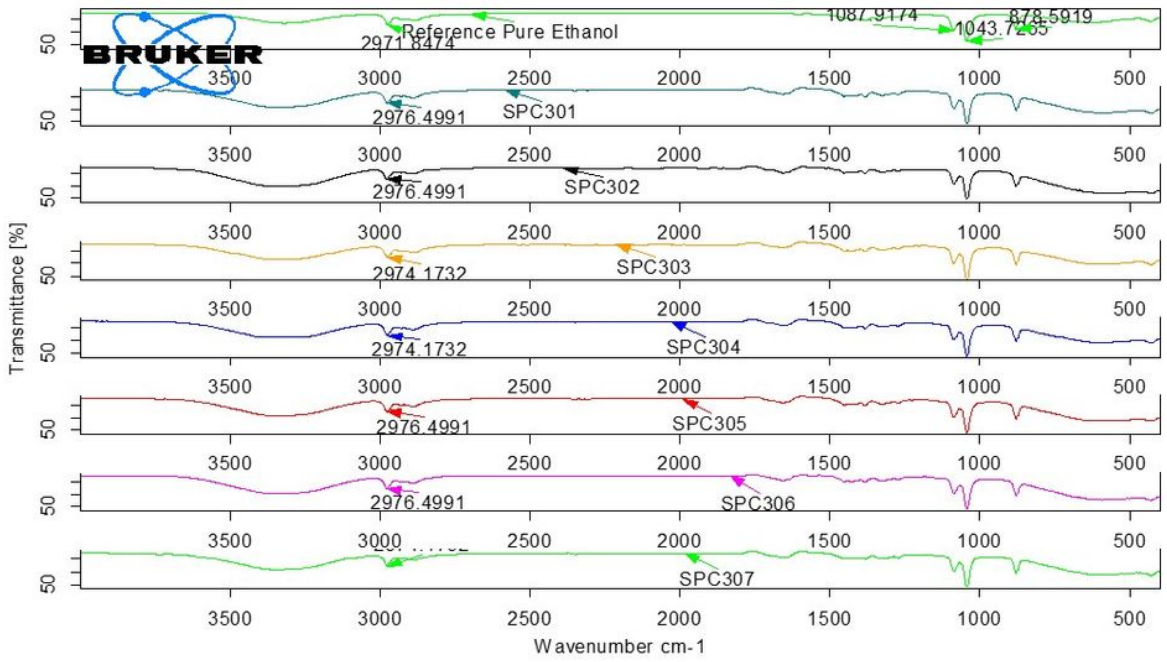

Figure 3

The FTIR spectrum of ethanol reference standard and tested hand sanitizers (SPC301, SPC302, SPC303, SPC304, SPC305. SPC306, and SPC307).

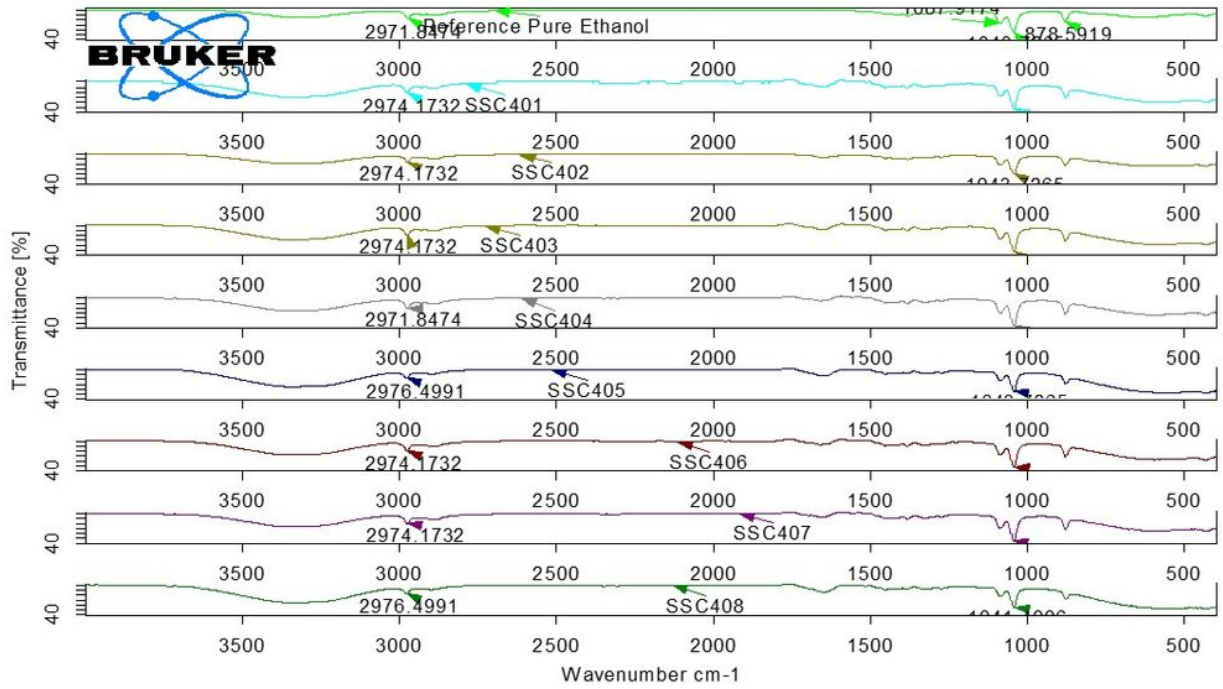

Figure 4

The FTIR spectrum of ethanol reference standard and tested hand sanitizers (SSC401, SSC402, SSC403, SSC404, SSC405, SSC406, SSC407, and SSC408)