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Antibacterial and antifungal activities of hydroalcoholic hands products sold in Côte d'Ivoire during covid-19

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Abstract

In the midst of the COVID-19 pandemic, hydroalcoholic products are widely used for hand disinfection. Côte d'Ivoire has also opted for this barrier measure. Ivorian markets and supermarkets have been filled with new hydroalcoholic products of various compositions and colors, certified or not by the Ivorian standardization agency. It was important to verify the antimicrobial efficacy of these hydroalcoholic products. To do this, we tested 16 hydroalcoholic products collected from supermarkets in Abidjan. The microbial load on the hands of 144 volunteers was determined before and after hand rubbing. These hydroalcoholic products were also tested for antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus mirabilis*; and antifungal activity against *Aspergillus fumigatus* and *Penicillium chrysogenum*. The results of this study showed that hydroalcoholic products tested significantly reduced the microbial load on the hands after friction. The germ inhibition rate was between 94.99 and 99.97%. Of the 16 hydroalcoholic products, only 3 showed antibacterial and antifungal activity on all indicator strains. In contrast, 4 showed no antibacterial and antifungal activity on these strains. The low efficacy of hydroalcoholic products tested in vitro is an indicator of the lack of quality control of these products. Some of them could not therefore guarantee the health security of the populations.

Keywords: Hydroalcoholic products; Hand; Bacteria; Fungi; Côte d'Ivoire

1. Introduction

The emergence of novel pathogens, bacterial or viral, has always posed serious challenges to public health around the globe [1]. One of these dangerous pathogens is "severe acute respiratory syndrome coronavirus 2" or SARS-CoV-2, more commonly known for causing coronavirus disease 2019 or COVID-19, which has been declared a global pandemic by the World Health Organization in early 2020. One of the many ways implemented to prevent the spread of this virus is hand hygiene.

Hand hygiene is well known as one of the essential good practices for reducing infectious disease transmission [2]. Hand hygiene generally refers to various methods of reducing or killing microorganisms that may be present on the hands, either by washing or by disinfecting. Practicing hand hygiene with hydroalcoholic friction has become a WHO-recommended procedure for equal opportunity use of any of the hand hygiene methods: the procedure is faster, more effective, and better accepted than washing with water and antiseptic soap [3]. In addition to being useful in the absence

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of water, other advantages of using hydroalcoholic hand products are their high antimicrobial potency, shorter disinfection time, and lack of requirement for drying the hand.

In Côte d'Ivoire, as in all other countries in the world, the use of hydroalcoholic products is one of the main barrier measures decreed by the government to fight against the COVID-19 pandemic. They are now part of the daily life of the Ivorian population. Markets and supermarkets were then filled with new hydroalcoholic products, of various composition and color, certified or not by the Ivorian standardization agency (CODINORM). Among these many products, some may be ineffective against microorganisms.

This study sought to assess the antibacterial and antifungal effectiveness of hydroalcoholic products sold in Côte d'Ivoire.

2. Material and methods

2.1. Study area and design

The study was conducted at the Laboratory of Biotechnology and Food Microbiology of NANGUI ABROGOUA University. It is a cross-sectional study conducted on 48 samples consisting of 16 hydroalcoholic products codified S1 to S16 collected in supermarkets in Abidjan (3 samples per hydroalcoholic product).

2.2. Microbial Strains

Escherichia coli, *Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *Proteus mirabilis* and *Aspergillus fumigatus, Penicillium chrysogenum* were collected from Pasteur Institute of Côte d'Ivoire and Laboratory of Biotechnology and Food Microbiology of NANGUI ABROGOUA University respectively.

2.3. Volunteers

The volunteers were 144 male and female students of NANGUI ABROGOUA University without dermal lesions were included in the study (3 volunteers per sample).

2.4. *In vitro* assessment of the antibacterial effectiveness of hydroalcoholic products

2.4.1. Preparation of the microbial suspensions

The density of selected organisms was adjusted equal to that of the 0.5 McFarland standards (1.5×10^8 CFU/mL) by adding them to nutrient broth for bacteria and Sabouraud broth for molds. A 24 hold culture was used for the preparation of microbial suspension. McFarland standards were used as a reference to adjust the turbidity of microbial suspension so that the number of microorganisms would be within a given rang [4].

2.4.2. Antimicrobial susceptibility test

The National Committee for Clinical Laboratory Standards was the reference in determining antimicrobial susceptibility through the Well Diffusion Method. The hydroalcoholic products samples were tested using standard Mueller Hinton II plates to detect the antimicrobial activity of these testing solutions [5]. The plates with inoculated microorganisms that had been adjusted to the 0.5 Marcland standard were provided with 80 µL aliquots in 6 mm diameter punched wells with the testing solutions. The surface of the agar plate was streaked over the entire sterile agar surface rotating the plate to ensure an even distribution of inoculum with a final swab around the rim. All of the plates were placed in the incubator set at a temperature of 37°C/ 24h. The antibacterial and antifungal activities of the hydroalcoholic products were measured using a ruler by determining the zone of inhibition. This was carried out in at least an average of three parallel independent trials. A substance is said to be ineffective if the diameter of inhibition is less than 8 mm while it is said to be effective if the diameter is between 9 and 14 mm. It is considered very effective when the diameter is between 15 and 19 mm and then extremely effective if the diameter is greater than 20 mm.

2.4.3. Determination of the minimum volume of inhibition

The determination of the minimum volume of inhibition was carried out with a series of volumes of the various hydroalcoholic products: 80 µL, 70 µL, 60 µL, 50 µL. These volumes were placed in the wells of the Muller-Hinton agar already containing the tested microorganisms inoculated into the agar. Petri dishes were incubated for 24 h at 37°C. The minimum volume of was obtained from the smallest volume of hydroalcoholic product for which no microbial colony was obtained.

2.5. Determination of the effectiveness of hydroalcoholic products on the hands

Using a sterile swab soaked in physiological water the hands of each of the volunteers were removed before and after rubbing with the hydroalcoholic products tested [6]. Then the swabs were suspended in 9 mL of EPT and successive decimal dilutions were performed. The total aerobic flora was counted on the PCA agar at 30°C / 72h according to the ISO 4833: 2003 standard. This count was done by counting the colonies of each Petri dish before and after friction. The dishes containing colonies of between 30 and 300 were selected for the enumeration of the bacterial loads.

2.6. Calculation of the percentage inhibition of hydroalcoholic products

The percentage inhibition of hydroalcoholic products was calculated according to the following mathematical formula:

Percentage inhibition (%) = [(microbial load beforehand rubbing - microbial load after hand rubbing)/ microbial load beforehand rubbing] X 100

2.7. Statistical analysis

The one-way analysis of variances (ANOVA) was carried out with the XLSTAT software to compare the variables measured on the different hydroalcoholic products. This software was used to calculate the means and standard deviations of the analysis parameters.

3. Results and discussion

3.1. Antimicrobial activity of hydroalcoholic products on organisms tested

Table 1 shows the diameter of inhibition of hydroalcoholic products on organisms tested including *Escherichia coli, Proteus mirabilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Aspergillus fumigatus* and *Penicillium chrysogenum*. Out of sixteen (16) hydroalcoholic products tested during this study, only three (3) hydroalcoholic products (S3, S6 and S7) showed antibacterial and antifungal activity on all the microorganisms tested with diameters between 10 and 26 mm. The hydroalcoholic products S1, S9, S12 and S13 had no bactericidal and fungical effect on all the microorganisms tested. The difference in effectiveness of hydroalcoholic hand products could result from the type and actual percentage of alcohol present in these products. For most hydroalcoholic products, alcoholic components are the main active ingredients. Indeed, the hydroalcoholic products tested during this study are all prepared from ethanol according to the composition mentioned on the packaging. Nine samples (S4; S5; S11; S14; S15; S16; S2; S10; S8) had a bactericidal effect on some microorganisms tested and four samples (S1; S9; S12; S13) had no microbial activity on all microorganisms tested.

Table 2 shows the minimum volumes of inhibition of the hydroalcoholic products tested. The three samples S3, S6 and S7 had minimal inhibition volumes between 50 and 60 µL.

Table 1 Diameter of inhibition of hydroalcoholic products in millimeters (mm)

	Escherichia coli	***Proteus mirabilis***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Aspergillus fumigatus***	***Penicillium Chrysogenum***
S1	00	00	00	00	00	00	00
S2	4	00	00	00	12	00	00
S3	14	15	14	12	13	19	15
S4	00	00	4	8	8	00	00
S5	16	00	00	6	17	00	00
S6	16	20	12	10	26	16	18
S7	13	12	14	16	12	20	13
S8	8	00	8	6	8	00	00
S9	00	00	00	00	00	00	00
S10	00	10	8	00	00	00	00

S11	7	8	00	6	00	00	00
S12	00	00	00	00	00	00	00
S13	00	00	00	00	00	00	00
S14	00	6	12	00	6	00	00
S15	00	10	9	00	8	00	00
S16	00	14	6	8	00	00	00

Table 2 Minimum volume of inhibition of hydroalcoholic products in µL

	Escherichia coli	***Proteus mirabilis***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Aspergillus fumigatus***	***Penicillium Chrysogenum***
S2	80	-	-	-	60	-	-
S3	50	50	50	60	60	50	50
S4	-	-	80	70	70	-	-
S5	60	-	-	80	50	-	-
S6	50	50	60	60	50	50	60
S7	60	60	50	60	50	60	60
S8	70	6	70	80	70	-	-
S10	-	70	70	-	-	-	-
S11	70	80	-	80	-	-	-
S14	-	70	60	-	70	-	-
S15	-	70	80	-	80	-	-
S16	-	60	70	70	-	-	-

(-): no antibacterial activity; minimum volume zero inhibition

3.2. Hand-carried flora of the hands before and after rubbing hydroalcoholic products

Table 3 indicates a considerable decrease in the microbial load (CFU / cm^2) after hand rubbing compared to that beforehand rubbing. This is observed for each sample of PHA tested and per volunteer. Table 4 shows the averages of the percentages of inhibition of each hydroalcoholic product after rubbing the hands. These percentages of inhibition are between 96 and 99.85% showing no significant threshold difference of 5%. The use of the hydroalcoholic products tested in this study significantly reduced the microbial load on the hands after rubbing. The percent inhibition of aerobic mesophilic organisms (GAM) ranged from 96 to 99.85%. The results obtained during this study are close to the optimal inhibition rate (99.99%) written on the packaging of each hydroalcoholic product according to European standards for hand hygiene NF EN 1499 (Antiseptics and chemical disinfectants - Hygienic hand washing), NF EN 1500 (Hygienic treatment of hands by friction), NF EN 12 791 [NF T 72.503] (surgical disinfectants for hand washing) and NF EN 1275 T 72 202 (basic fungicidal activity of antiseptics and chemical disinfectants). Hand hygiene is an essential part of the fight against microorganisms, especially with the advent of several diseases such as Ebola and COVID-19 [7]. The good performance of the alcoholic hand sanitizers mainly depends on the percentage and type of the alcohol, but also on the amount applied on hands and contact time [8]. Indeed, prolonged storage can cause an increase in temperature leading to denaturation of the active ingredients [4]. A reduction of the microbial load on the hands after rubbing a hydroalcoholic product in the USA was also observed [9]. Other authors such as [10] have shown by multiple in vitro and in vivo experiments, a considerably better antimicrobial destruction with hydroalcoholic products than with hand washing. Lower percentages of inhibition (73.8% to 89.9%) were determined on hydroalcoholic products sold in Benin [4]. The concentration of alcohol used in the preparation of hydroalcoholic products plays a very important role in its ability to inhibit microorganisms in the hands [11, 12]. All hydroalcoholic products must contain alcohol such as ethanol, isopropanol, n-propanol or a combination of these [13, 14]. Tolerance on the skin is better with ethanol than with n-propanol or isopropanol. Ethanol is therefore often the alcohol of choice in the preparations of hydroalcoholic products [15, 16]. However, for the general public, the Centers for Disease Control and Prevention suggests washing their hands with soap and water, rather than using hydroalcoholic products, as soon as possible. Indeed, hand washing can virtually eliminate all types of pathogens, while hydroalcoholic hand product can effectively kill 99.9% of microorganisms but is less effective on pathogenic microorganisms [17].

Table 3 Microbial load before and after hand rubbing

Hydroalcoholic products	Volunteers	microbial load beforehand rubbing (CFU/ cm^2)	microbial load after hand rubbing (CFU/ cm^2)
S1	1	$1.5\ 10^4$	$8\ 10^2$
	2	$1.1\ 10^4$	$6\ 10^2$
	3	$2.2\ 10^{15}$	$5\ 10^2$
S2	1	$3\ 10^4$	$3\ 10^2$
	2	$3\ 10^5$	$1.4\ 10^3$
	3	$3\ 10^5$	$7\ 10^2$
S3	1	10^3	0
	2	$5.8\ 10^4$	$3\ 10^2$
	3	$4\ 10^5$	$2\ 10^2$
S4	1	$5\ 10^3$	$2\ 10^2$
	2	$4.5\ 10^4$	10^2
	3	$1.5\ 10^5$	10^2
S5	1	$2.2\ 10^5$	$3\ 10^2$
	2	$4.8\ 10^4$	$2.2\ 10^3$
	3	$1.8\ 10^4$	10^3
S6	1	$3\ 10^4$	10^2
	2	$8\ 10^3$	0
	3	$3\ 10^5$	$3\ 10^2$
S7	1	$4\ 10^3$	0
	2	$3\ 10^4$	$3\ 10^2$
	3	$5\ 10^4$	$3\ 10^2$
S8	1	$2.6\ 10^5$	$3\ 10^2$
	2	$1.2\ 10^4$	$2\ 10^2$
	3	$7\ 10^3$	$2\ 10^2$
S9	1	$3\ 10^5$	$2\ 10^2$
	2	$3\ 10^3$	$2\ 10^2$
	3	$5\ 10^3$	$2\ 10^2$
S10	1	$1.5\ 10^4$	$5\ 10^2$
	2	$5\ 10^3$	10^2
	3	$6\ 10^4$	10^2
S11	1	$2.1\ 10^4$	$8\ 10^2$

	2	9 10^3	3 10^2
	3	2,1 10^5	7 10^2
S12	1	2 10^4	9 10^2
	2	4 10^4	10^2
	3	2.9 10^4	1.6 10^3
S13	1	5 10^3	10^2
	2	4 10^3	2 10^2
	3	4 10^3	2 10^2
S14	1	9 10^3	5 10^2
	2	5 10^3	10^2
	3	6.2 10^4	3 10^2
S15	1	3 10^5	5 10^2
	2	3 10^5	3 10^2
	3	2 10^3	10^2
S16	1	6.8 10^4	10^3
	2	5 10^3	3 10^2
	3	5 10^3	0

Table 4 Percentage inhibition of hydroalcoholic products

Hydroalcoholic products	**Test**	**Percent inhibition (%)**	**Average Percent Inhibition (%)**
S1	1	94.66	96.32 ± 2.98^a
	2	94.54	
	3	99.77	
S2	1	99	99.43 ± 0.38^a
	2	99.53	
	3	99.76	
S3	1	100	99.81 ± 0.28^a
	2	99.48	
	3	99.95	
S4	1	96	98.56 ± 2.22^a
	2	99.77	
	3	99.93	
S5	1	99.86	96.57 ± 2.89^a
	2	95.41	
	3	94.44	
S6	1	99.66	99.85 ± 0.17^a

	2	100	
	3	99.9	
S7	1	100	99.46 ± 0.50[a]
	2	99	
	3	99.40	
S8	1	99.88	98.45 ± 1.37[a]
	2	98.33	
	3	97.14	
S9	1	99.93	96.42 ± 3.31[a]
	2	93.33	
	3	96	
S10	1	96.66	99.16 ± 1.01[a]
	2	98	
	3	99.83	
S11	1	96.19	97.50 ± 3.12[a]
	2	96.66	
	3	99.66	
S12	1	95.5	96.57 ± 2.79[a]
	2	99.75	
	3	94.48	
S13	1	98	96.00 ± 1.73[a]
	2	95	
	3	95	
S14	1	94.44	97.31 ± 2.60[a]
	2	98	
	3	99.51	
S15	1	99.83	98.24 ± 2.80[a]
	2	99.9	
	3	95	
S16	1	98.52	97.50 ± 3.12[a]
	2	94	
	3	100	

In column, the averages affected by the same letter are not significantly different at the 5% threshold according to the Newmann-Keuls test.

The low efficacy of the hydroalcoholic products tested in this study shows the problem of quality control of hydroalcoholic products sold in Côte d'Ivoire. Indeed, the formulations recommended by the World Health Organization (WHO) for the manufacture of hydroalcoholic products may not be observed. Also, these results could be due to poor storage conditions or the long shelf life of these products. The majority of these products could also be counterfeit and could not guarantee the safety of the users.

4. Conclusion

The hands are the most common mode of transmission of pathogens. Good hand hygiene practice and the best way to prevent the spread of infectious diseases. The low efficacy of the tested PHAs revealed by the present study therefore poses the problem of controlling the quality of these products. Some of these products may not guarantee user safety. Regular national surveys are envisaged by collecting samples from outlets and manufacturing plants.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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