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Preparation of Ethanol-Free Hand Sanitizers Gels and Studying its Sterile Efficacy

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Abstract. Due to the spread of the Corona pandemic, the demand for hand sanitizers has increased dramatically. This led to a global scarcity of sterilizers. Therefore, this study was performed to produce and evaluate the effectiveness of new commercially available, cheap and ethanol-free hand sanitizers from various sources. The new hand sanitizers were prepared by mixing (glycerin) as a moisturizer and (carbomer) as thickening agents to form the gel with (tri-ethanolamine base) as pH neutralizer and (Tea tree oil, Aloe vera extract or Povidone 10%) respectively as antiseptic agents. All prepared hand sanitizers were examined with four kinds of bacteria (Enterococcus faecalis, E. coli, Pseudomonas aeruginosa and Staphylococcus aureus) And two kinds of fungi (Candida albicans and Aspergillus flavus). The prepared hand sanitizer gels showed good sterilization efficacy and similar to commercially prepared hand sanitizers (70% ethanol).

1. Introduction

Due to the spread of the Corona pandemic in the world beginning with the Chinese city of Wuhan at the end of 2019, the global demand for many preventive medical products increased, causing an acute shortage of these products. Among these items is hand sanitizer. In fact, there are two types of hand sanitizer gels. The first type is based on alcohols as an active sterilization substance, such as ethanol and isopropanol. But this type of sterilizer causes many health problems for the user, such as skin irritation. As for the second type of sterilizers, it does not depend on alcohol, but rather on other materials such as (benzene alkonium chloride and quaternary ammonium chloride) [1-2].

As part of contributing to the global efforts to confront this pandemic and stope the virus, our aim is to find alternative ethanol-free hand sanitizers that can be used as commercial, safe, inexpensive and widely available such as tea tree oil, aloe vera and povidone solution.

Aloe vera is a succulent herb belonging to the Aloe family [3]. It is an evergreen perennial that hails from the Arabian Peninsula but thrives in tropical, semitropical, and arid climates all over the world. It is grown for both agricultural and medicinal purposes such as anti-ulcer, anti-hypercholesterolemic, antioxidant, antibacterial activity, antiviral activity, antifungal activity, anti-acne, nutraceutical,

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humectant, skin protection against UV-A and UV-B, wound healing properties, the prevention of type II diabetes and cancer [4].

Tea tree oil is the common name for the essential oil extracted from Melaleuca alternifolia trees' leaves and terminal branches. Tea tree oil is extracted by blowing steam through the fresh leaves and terminal roots. The oil extracts from the water after cooling and can be collected. Melaleuca alternifolia essential oil is a mixture of aldehydes, terpenes, alcohols, esters, and other chemical molecules, and therefore has been used in many medical applications for its antioxidant, antimicrobial and digestive stimulant properties [5-6].

2. Experimental

The raw materials (Carbomer, Sodium methylparaben, Glycerine, Propylene glycol, and Triethanolamine, povidone solution 10%) were purchased from chemical centres, Baghdad city. While, tea tree oil from herbs centres, Najaf city. All solvents in the experiment were supplied by Fluke, Merck, and BDH company.

2.1. Chemical Methods

2.1.1. Preparation of Povidone hand sanitizers gels (A)

The first brown product A, Ethanol-free, was prepared by using (2%) of Povidone solution 10% as disinfectant material. (0.3%) of Carbomer material 940 type as a thickening agent was mixed with the amount of water in a beaker for one day. The preservative (Sodium methyl paraben) (0.2%) was dissolved in another beaker with a small amount of water. The povidone and preservative were added to the carbomer mixture, After the components are stabilized, (0.1%) of triethanolamine base as pH neutralizer was added to the mixture in droplets to form the gel.

2.1.2. Preparation of tea tree oil hand sanitizers gels (B)

As in the first product, but here tea tree oil is used. The blue second product B. was prepared by using (0.07%) of tea tree oil as disinfectant material was added to the mixture of (0.3%) Carbomer material 940, (99%) of D. water, (0.2%) Sodium methyl paraben as preservative material and (0.05%) of Glycerine. After (24 hrs), (0.1%) of triethanolamine base was added to the mixture drop by drop to manufacture the gel [7].

2.1.3. Extraction of aloe vera extract

Aloe Vera extract was extracted from mixing (25 %) of aloe Vera plant after removing the leaves with (75%) of distilled water. After mixing and faltering, (60%) of the aloe Vera juice was treated with (30%) isopropanol and (10%) glycerin. After two weeks, the product is purified and used to prepare hand sanitizer gel [7].

2.1.4. Preparation of Aloe Vera hand sanitizers gels (C)

The last yellow product C was prepared using (2%) of aloe Vera extract, (0.05%) of Glycerine and (0.2%) of Sodium methyl paraben, the carbomer (0.3%) and triethanolamine (0.1%) were responsible for forming the yellow gel [7], Table 1.

2.2. Biological activity test [8-9]

Studies of antibacterial and antifungal activity were performed using a disc diffusion technique with (Muller Hinton) Agar plates for bacteria and (potato dextrose) agar plates for fungi. In testing the products against bacteria and fungi, the bacteria and fungi kinds (*Enterococcus feacalis, E. coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus*) and (*Candida albicans* and *Aspergillus flavus*) were spread and activated on the Muller Hinton agar and potato dextrose agar respectively. The thin samples (1:1, water: gel), heavy samples, in addition to the standard product were injected for each disc.

The bacterial and fungal plates were incubated at 27°C and 24°C for 24 and 72 hrs. respectively. The results were recorded based on the diameter of the inhibition zone in mm.

No	Ingredients	(Stander)	Α	В	С
1	Ethyl Alcohol	70%	-	-	-
2	Povidone 10%	-	2%	-	-
3	Tea tree oil	-	-	0.07%	-
4	Aloe Vera extract	-	-	-	2%
5	Glycerin	0.7%	0.05%	0.05%	0.05%
6	Propylene glycol	0.2%	-	-	-
7	Carbomer	0.47%	0.3%	0.3%	0.3%
8	Triethanolamine	0.33%	0.1%	0.1%	0.1%
9	D.water	28.77%	97.3%	99.229%	97.299
10	Sodium methyl paraben	-	0.2%	0.2%	0.2%
11	Color	-	-	0.001%	0.001%
12	Perfume	-	0.05%	0.05%	0.05%

Table 1	Percentages	of prepared	hand	sanitizers	oels
	reicemages	of prepared	nanu	Samuzers	geis

3. Results and Discussion

Industrial companies and factories resorted to the manufacture of sterile products profusely, which led to an increase in the prices of raw materials and their lack in non-industrialized countries. The most important of these raw materials are ethanol and isopropanol and Hydrogen peroxide. That's why, we've prepared a group of hand sanitizers gels using alternative materials for ethanol material such as povidone 10%, tea tree oil, and aloe Vera extract.

The commercially colorless product (S) was prepared as a stander product to compare it with new products in terms of effectiveness against germs. The product was prepared using 70% of ethyl alcohol; Ethanol has been used as an effective, preservative and deadly substance for all kinds of bacteria and viruses as (COVID-19) according to recent studies [10-12].

The three new hand sanitizers gels (A, B, and C) were prepared by using povidone 10%, tea tree oil (Melaleuca alternifolia), and aloe Vera extract as sterile raw materials[13-15]. Glycerin was used as an emollient agent for the hands [16], while sodium methylparapene or (Sodium 4-(methoxycarbonyl) phenolate) was used as a preservative for the product. The acid-based polymer (Polyacrylic acid or carbomer) was used as a chelating agent for the gel [17]. Triethanolamine as the final step was added to neutralize the acidity and form the gel. It is a high safety compound that is widely used in many products such as thickeners, emulsifiers, detergents, wetting agents, shampoos, and cosmetics [18], Figure 1.

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Figure 1. Prepared hand sanitizer gels

The physical stability of the three prepared products (A, B and C) was examined after 10 weeks of storage, the color, odor and homogeneity were stable at 25 ° C and 10 ° C. The pH value as chemical property was stable at the mentioned temperatures for the three products, the pH value was within the normal values of the stander hand sanitizer gel to avoid health problems for skin such as itching, irritation, and scaling. The pH was ranged between (6.2-6.6), Table 2.

No	Properties	After 10 w	(Stander)	А	B	С
1	State	Gel	Clear	Turbid	Semi turbid	Clear
2	Color	Stable	Silvery	Brown	Blue	Yellow
3	Odor	Stable	Alcohol	Pine	Pine	Pine
4	Homogeneity	Stable	Homogeneou	Homogeneou	Homogeneou	Homogeneou
			S	S	S	S
5	pН	Stable	6.5	6.2	6.4	6.6

Table 2. Some physical and chemical properties of prepared products

As for the gel volatilization time with rubbing hands, the time was calculated in seconds, the first product (A) remained on the skin for (13) seconds, the second product (B) remained for (15) seconds, the third product (C) was stayed for (13) seconds, while the standard product (S) was volatilized for (7) seconds. The reason is that the products contain a high percentage of water (97.299- 99.229%) and that the products do not contain a fast volatile substance such as ethanol as in the standard product. The gel volatilization time in the prepared compounds is approximately twice that of the standard gel, the gel staying time on the hands for a longer time, gives a good result in sterilization, Figure 2.

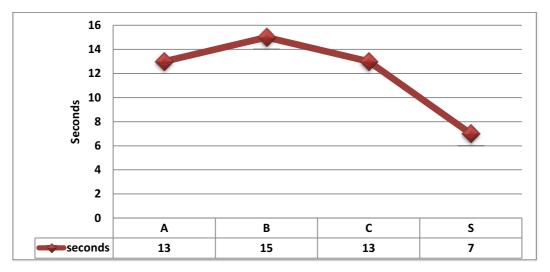


Figure 2. Gel volatilization time of products

The second part of the research is to study the microbial activity of the prepared products and compare the results with the prepared standard product. The prepared products were examined in the Central Laboratory /Biology Science / University of Baghdad. By using Muller Hinton Agar medium for bacteria and Potato Dextrose agar for Fungal, the prepared products (A, B, and C) were examined against four kinds (Enterococcus feacalis, E. coli, Pseudomonas aeruginosa, and Staphylococcus aureus) with two kinds of fungal (Candida albicans and Aspergillus flavus). In Table 3 & Figures 3.4, All prepared products gave similar results to the standard product in the thin and heavy products, the product (C) exhibited high antibacterial activity against all bacteria types. The prepared compounds and the standard gel in the thin state gave inactive results against bacteria (E. Coli and P. aeruginosa).

Table 5. Antibacterial activity for heavy products								
Sample	Inhibition zone diameter (mm\mg sample)							
	E. fea	E. feacalis E. Coli P. aeruginosa S.aureus						
	Heavy	Thin	Heavy	Thin	Heavy	Thin	Heavy	Thin
Α	13	11	11	R	13	R	12	11
В	12	11	11	R	12	R	13	12
С	14	11	12	R	13	R	16	12
S	12	11	11	R	12	R	11	11

Table 3 Antibacterial activity for heavy products

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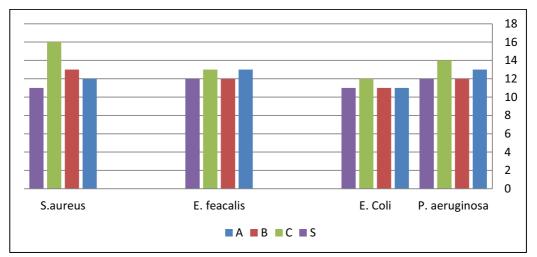


Figure 3. Antibacterial activity for heavy products



Figure 4. Antibacterial activity of heavy products against all bacteria

In the antifungal activity, the prepared products (A, B, and C) were tested in the Central Laboratory /Biology Science / University of Kufa. The products were also tested against two kinds of fungal (Candida albicans and Aspergillus flavus). The results of the fungi test differed significantly from the bacterial test. The thin and heavy prepared products gave high activity against fungi. In Candida albicans, the results ranged between (30-25) mm\mg in heavy products, whereas in the thin products between (20-10) mm\mg, as the C product gave highly active compared with other products (A and B) and similar with standard product. While in Aspergillus flavus, the prepared product A was showed high activity compared with other products (B and C) and nearer than standard product, Table 4 & Figure 5,6.

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Sample	Inhibition zone diameter (mm\mg sample)					
	C. alb	C. albicans A. flavus				
	Heavy	Thin	Heavy	Thin		
Α	25	20	28	10		
В	28	15	20	15		
С	30	10	10	10		
S	30	20	30	20		

Table 4. Antifungal activity for heavy and thin products

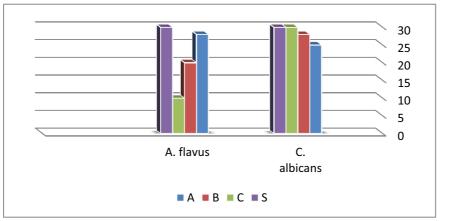


Figure 5. Antifungal activity for heavy products

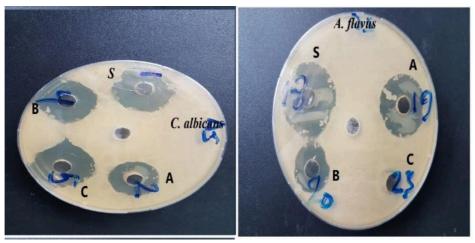


Figure 6. Antifungal activity of heavy products against C. albicans and A. flavus

4. Conclusion

Using active raw materials instead of ethanol 70%, such as povidone 10%, tea tree oil, and aloe Vera extract, and studying some of the physical and chemical properties of prepared products, as well as studying the biological activity of hand sanitizers gels products against four kinds of bacteria (Enterococcus faecalis, E. coli, Pseudomonas aeruginosa and Staphylococcus aureus) and two kinds of

fungi (Candida albicans and Aspergillus flavus) and comparing them with the standard product (70% hand sanitizer gel). All these previous steps were a summary of the research. The heavy prepared hand sanitizer gels gave high activity or efficacy against types of bacteria and fungi and Similar effectiveness to a commercial standard product.

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