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Review on Formulation and Evaluation of Alcohol-Free Hand Sanitizer

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Abstract: *Hand hygiene is an essential factor to prevent or minimize the spread of infections. The ability to prepare an alcohol-free hand sanitizer (AFHS) with antimicrobial properties is crucial, especially during pandemics, when there are high demands and a low supply chain for ethanol and isopropanol. The objective of this study was to prepare AFHS gels based on natural materials that contain essential oils (Eos) that would be effective against a broad spectrum of pathogens. The results showed that the organoleptic characteristics of all prepared hand sanitizer gels were considered acceptable. The antimicrobial effectiveness test demonstrated that the prepared hand sanitizer gels had antimicrobial activities against different gram-positive and gram-negative bacteria and Candida albicans yeast. This study suggested that the prepared natural hand sanitizer gel with 1.25% (v/v) Lavender oil can be a potential alternative to commonly used alcohol-based hand sanitizers (ABHS).*

Keywords: Hand Sanitizer, Microbes, Infections, Essential Oils, Antimicrobial, Alcohol-Free, Pandemics

I. INTRODUCTION

New infections, bacterial or viral, have often raised significant threats to public health across the globe. One of these hazardous pathogens is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is renowned to cause coronavirus. The preventive protocols to cope with COVID-19 are just supportive in order to minimize the spread of this disease as the best approach. Frequent and reliable hand washing is one of the many approaches adopted to prevent the transmission of the virus.

Secondary bacterial or fungal infection can be considered as one of the most common and serious complications related to viral infections, especially in the elderly. A recent report on COVID-19 related co-infections showed that the mortality rate of 15.2% was observed for patients with pneumonia caused by antibiotic-resistant strains of Staphylococcus aureus (S. aureus) and Klebsiellapneumoniae (K. pneumoniae) .The use of an effective hand sanitizer is considered as an essential alternative to handwashing, and is one of the current protocols to prevent the spread of viral infections and related secondary infections, hence decreasing the need for intensive care administration and antibiotics use.

Following the outbreak of COVID-19, alcohol-based hand sanitizers (ABHS) have become a common alternative to conventional handwashing in healthcare and neighborhood settings as a preventative tool, causing an increased alcohol demand. Several hand sanitizers with different variations are available. It is essential to consider the types of hand sanitizers that function effectively against pathogens. ABHS recommended by the WHO are mostly composed of ethanol, isopropyl alcohols, or hydrogen peroxides in varying combinations, in which the ethanol or isopropyl alcohol concentration is mainly at a range of 60–95% .

In this study, AFHS formulations prepared from natural ingredients that include aloe vera, vitamin E, glycerin, and different essential oils (Eos) were evaluated. These ingredients are also widely available in the market, which make them easily accessible. The use of aloe vera gel as the hand sanitizer vehicle was due to its natural moisturizing and germ-retarding abilities, as well as the competence of inhibiting some bacterial strains . Vitamin E and glycerin were used for their ability to slow down rancidity (i.e., oxidation or hydrolysis of fats and oils) and to moisturize the skin. The primary active compounds of the AFHS gels are Eos, which have a wide range of antimicrobial activities . The antimicrobial activity of Eos is reported to be due to their hydrophobic nature that facilitates the partition of active components in the lipid of the bacterial cell membrane and mitochondria, hence reducing cytoplasmic membrane

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integrity . However, an optimized concentration of these compounds should be used in the preparation of AFHS, as the increased proportion could lead to dermal sensitivity and skin irritation, according to previously reported studies .

In addition, the preparation of hand sanitizer in the form of gel has several advantages over other forms of hand sanitizers, such as liquid (spray) or foam. The key desirable properties of gel formulation are the ability to create a protective layer on the site of application and the longer protection time on the skin in comparison to the other hand sanitizer forms. The retention time of the hand gel is higher than the liquid and foam hand sanitizers, and it has a preferable moisturizing feeling and adherence property on the applied skin. Therefore, hand gel was considered as a suitable hand sanitizer form for the preparation of AFHS in this study.

These formulated AFHS gels are aimed to control the spread of co-infections during pandemics. Following the preparation of hand sanitizer gels, the characterization and the evaluation of all prepared formulations were carried out in terms of organoleptic properties, pH measurement, rheological behavior, and gel spreadability. Finally, an acceptability test was conducted to assess the safety of the prepared hand gels by determining any side effects, such as skin irritation and skin redness, which may arise from their application on human skin.

II. MATERIALS AND METHODS

Aloe vera, glycerin, vitamin , clove oil, lavender oil, and tea tree oil , and three commercially available hand sanitizer gels with over 60% alcohol content:

2.1 Preparation of Hand Sanitizer Gels:

Sr. no	Ingredient	Quantity	Role/Use
1	Essential oil (Lavender oil)	2.5% v/v	Antibacterial agent
2	Aloevera gel	90%v/v	Skin moisturizer
3	Glycerine	5%v/v	Emilient
4	Vitamin E	0.05%v/v	Nourishment agent
5	Distilled water	qs	Vehicle

Several natural materials were used to prepare the hand sanitizer gels in pertinent proportions, including aloe vera, glycerin, and vitamin E, in addition to Eos. Each formulation was prepare by dispersing glycerin (5% v/v) to aloe vera gel (90% v/v) in a 250 mL beaker and mixed with gentle stirring at ambient temperature.

Eos (at 2.5% v/v or 1.25% v/v) were then added dropwise with constant stirring to avoid air bubble formation and to obtain uniform and homogenous gels, followed by adding vitamin E (0.05% v/v). The remainder of each formula was completed by distilled water. Control formulation was prepared using the same components of the prepared hand sanitizer gels, but with no addition of Eos. Table 1 shows the composition of all prepared hand sanitizer gels and the control gel with each ingredient's concentration.

2.2 Evaluation Parameters

A. Organoleptic Test

The prepared samples were inspected visually to check the texture, odor, and color of the gels in semisolid conditions.

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B. PH Evaluation

The pH measurement of the formulated gels was measured using a digital pH meter (Mettler Toledo pH meter, USA). The pH measurements represent the mean ± standard deviation (SD) of three replicates.

C. Viscosity (Rheological Properties)

The rheological and flowability properties of the prepared gels were determined at room temperature using a TCV 300 viscometer .A piston of a range of 1–10 cP was used, as the formulations had a texture equivalent to water, and the temperature was set to room temperature (≈24 °C). One mL from each prepared hand sanitizer was filled into the measurement chamber. The chamber was capped for 60 s until it was stable, and then the data were recorded. The results represent the mean ± SD of three replicates.

D. Gel Spreadability

The spreadability of the prepared hand sanitizers was evaluated according to the methodology described in [18]; 0.5 gm of each formulated gel was spread on pre-marked transparent glass with a 2 cm diameter. Then, another transparent glass was placed on the top, followed by adding a 500 gm weight for 5 min to disperse the content. By this method, the spreadability was measured based on slip and drag characteristics of the gels. An excess of the gel was scrapped off from the edges.

E. Antimicrobial Zone of Inhibition Test

To evaluate the antimicrobial activity of the prepared hand sanitizer gels, the zone of inhibition test against different gram-positive and gram-negative bacterial strains and a yeast was performed. Three commercially available hand sanitizers were also assessed as experimental controls. A final concentration of 1 × 106 CFU/mL inoculum was equally distributed on the surface of agar plates. A sterile microbiological disc was dipped into each hand sanitizer gel, allowed to dry for a few seconds, and then positioned on the Mueller–Hinton agar plate. All plates were incubated overnight at 37 °C. The diameter of the clear area of no growth around each disc was recorded in millimeters (mm). The results represent the mean ± SD of three replicates

F. Skin Irritation Study (Acceptability Test)

Based on the results of the previous antimicrobial effectiveness test, the most efficient gel formulation was selected to be tested in a skin irritation study. The study was carried out on 20 volunteers and ethically approved by the research ethics committee in King Abdulaziz City for Science and Technology (KACST) (IRB approval number; IRB#20007). After explaining the research protocol with possible side effects, the volunteers were asked to sign consent forms. The assessment was performed by applying 1 mL of sanitizer gel on each volunteer's palm, then allowed to stand for 5 min.

III. RESULTS AND DISCUSSION

Characterization and Evaluation of Hand Sanitizer Gel

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TEST	OBSERVATION	INFERANCE
1.Organoleptic Test	Color-green Odour-Characteristic Texture-Semisolid	Organoleptic test are present
2. PH evaluation	0.5-3.9	PH test are present
3. Viscosity	1.1-0.10	Viscosity test are present
4.Clarity	Clear bubble like apperance	Clarity test are present

IV. CONCLUSION

Hand sanitizer gel is one of the alternative options for hand hygiene. Due to the emergence of the COVID-19 pandemic, the prevention and control of bacterial or fungal co-infections using AFHS gels can be crucial, particularly when the alcohol supply chain is at risk. In this study, AFHS gels were formulated using aloe vera, glycerin, vitamin E, and

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several Eos as the active antimicrobial ingredients. It is concluded from the results that the prepared formulations have excellent organoleptic properties, pH values comparable to skin pH, and suitable viscosity and spreadability profiles.

The antimicrobial test showed varying activities of different EO-based formulations against several gram-positive and gram-negative bacteria and Candida. The results provided evidence that clove oil exhibited a profound antimicrobial activity against a broad range of microbes. The widest antimicrobial spectrum was observed with 2.5% (v/v) clove oil hand sanitizer (F1), which showed an antimicrobial activity close to the experimental ABHS controls. However, slight skin irritation sensation was observed in 20% of the volunteers. Instead, 1.25% (v/v) clove oil hand sanitizer (F2) was incorporated in the gel to prepare a superior antimicrobial product with slight or no adverse effects and higher acceptability for human skin. However, more research should be directed in future prospects to assess the efficacy against more bacterial species, yeast, and fungus. Furthermore, the antiviral activity of clove oil hand sanitizer should also be assessed to confirm its antiviral effectiveness, in order to be used as a potential and more effective alternative to ABHS during pandemics. Finally, the stability test for the hand sanitizer formulations should also be evaluated to ensure the shelf-life of this EO-based hand sanitizer.

The results of the questionnaire that was provided to the 20 volunteers to conduct the acceptability test and skin irritation study.

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