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Utilization of local natural compounds: A practical step toward hygienic society, formulation of hand sanitizers and disinfectants

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ABSTRACT. The most efficient technique to kill the germs is to use hand sanitizers and disinfectants. It is expected that there would be increasing demand for hand sanitizers, especially in light of the COVID-19 situation. In the present study, several essential oils were included in the formulation of alcohol-based hand sanitizers and disinfectants due to their antibacterial properties. The essential oil gives the hand sanitizer a pleasant scent, making it acceptable for use in public places. With the use of plant-based essential oils, antimicrobial hand sanitizers and disinfectants were effectively developed. The average amount of ethanol in hand sanitizers and disinfectants was determined to be 75.0 and 76.2 w/w %, respectively, with antimicrobial efficacy of 99.9%. The comparison of formulated and commercial hand sanitizers and disinfectants revealed that formulated hand sanitizers and disinfectants have outstanding antimycobacterial capabilities and are appropriate for commercialization.

Keywords: Hand sanitizers, Disinfectants, Antibacterial, Antimicrobial, Essential oil

INTRODUCTION

The use of green technology in environmental technology is extremely beneficial for the products that conserve natural and environmental sources while minimizing the negative impact of human activities on the environment. Cleaning procedures are generally recognized to make use of water, cleaning chemicals, and scrubbing. This cleaning procedure, however, does not eliminate fungus, viruses, bacteria, or which are collectively known as "germs". Cleaning products (hand sanitizers) and disinfectants, in theory, are substances that destroy germs. These pesticides are also known as antimicrobial/anti-virus pesticides (Leonard et al., 2013). Cleaning products are often used first, followed by disinfection products. Infectious diseases caused by germs (also known as micro bacteria or microorganisms) that enter and grow in human bodies can also have a negative impact on our health. When germs or viruses leave one body and enter another, this patient can become infected from one person (or animal) to another. Viruses are thus the most prevalent cause of illness. The virus is quite tiny and cannot generally survive for long on

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the surface (Hilburn et al., 2003). The virus causes some serious diseases such as HIV, SARS, influenza, and coronavirus (COVID-19). Just like viruses, bacteria can also cause infections. Bacteria are free to exist and multiply. Some can last on the surface for a long time. More dangerous diseases caused by bacteria include pneumonia, cough, meningitis and staph infections.

The technique that is often used to kill germs on the surface is to apply chemical disinfectants on the skin surface. This is because the surface of the skin will become a new host as soon as the patient is physically affected by hand, or sneezes or coughs. These bacteria can grow at a very rapid growth rate. The number of bacteria can be doubled in 10 min when having food and water. Germs are distributed in the body in the following circumstances: direct contact (touch), drops (when secretions come out of the human nose and mouth when sneezing, coughing, spitting, saliva, or vomiting) into the air. These germs then attach to hard surfaces or are inhaled by others, i.e., transmission through the air (when germs float in the air and attach to small droplets or dust particles and move more than 3 feet) (Doll et al., 2018). Some infections/diseases can be prevented with vaccines, but some require technology or methods to prevent germs without vaccines. To address this restriction, the World Health Organization (WHO) strongly advises the use of hand rubs, either through dispensers near the point of service or in tiny bottles, to guarantee optimal compliance with hand hygiene by making the process quicker and easier (Osei-Asare et al., 2020; WHO, 2009). The WHO's advice is supported by the fact that alcohol-based hand rubs provide evidence-based intrinsic benefits such as fast-acting and broad-spectrum microbicidal action. Similarly, when applied to hands, hand sanitizers inactivate germs or inhibit their growth, which helps to reduce disease transmission.

Currently available efficient hand sanitizers and antiseptics comprise 62-90% alcohol, an active component that kills most bacteria, fungi, and viruses by denaturing their proteins (Osei-Asare et al., 2020). Since the alcohol ingredient, ethanol-based hand sanitizers are the most favoured because they are more appropriate, effective, take less time, and cause less skin inflammation than hand washing with soap, antiseptic chemicals, or water (Maryadele & Neil, 2006). It is anticipated that there are indications of increased need for hand sanitizers, particularly in light of COVID-19 condition (WHO, 2020). Washing hands with soap and plain water (not antibacterial) is the most effective ways to prevent the spread of germs but cleaning products as described earlier cannot kill germs, they easily return to the same surface (Osei-Asare et al., 2020). Some germs/viruses can live, move in the air and are not easily detected. Therefore, employing disinfectants or hand sanitizers is one of the effective methods to kill the movement of germs in the air and on the surface of the body. Hand sanitizers or disinfectants in the form of body sprays can overcome this problem very effectively. Several previous studies have shown that commercial disinfectants or hand sanitizer sprays can increase the risk of eye pain, respiratory or skin problems and various other health effects due to the presence of active chemicals (Doll et al., 2018). Adapting alternative hand sanitizer preparations based on natural plant resources might be a viable solution to the toxicity issue. Hand washing is one of the most basic and efficient techniques to eliminate germs and avoid illness. Essential oils from aromatic plants have been employed for a variety of biological purposes, including antibacterial properties (Malabadi et al., 2021). Therefore, plant essential oil-ethanol based hand sanitization has been promoted during the recent outbreak of COVID-19.

To overcome this problem, the present study engaged the incorporation of antimicrobial ingredients based on the essence of local plant essential oils such as lemongrass and lavender into disinfectant spray formulations and hand sanitizers to effectively inhibit the growth of germs/viruses. The objectives of this present study are to formulate ethanol-based hand sanitizers and disinfectants with the addition of essential oils, characterization of formulated samples to determine ethanol content and to evaluate anti-microbial efficiency, and to draw a comparison between formulated samples and commercial products in term of viscosity, pH, ethanol content and antimicrobial efficiency. In the preliminary study, the determination of ethanol content and antimicrobial efficiency is one of vital parameters to evaluate the effectiveness of formulated hand-sanitizers and disinfectants against viruses, microorganisms, and bacteria.

METHODOLOGY

Materials and chemicals

Ethanol (95% QRēC), Glycerol (99.5% QRēC), Hydrogen peroxide (30-32% QRēC), Lemongrass (100% pure), Rosemary (100% pure), Lavender (100% pure), distilled water, Dettol disinfectant spray (D1), Lysol fresh blossom disinfectant spray (D2), ScentPur disinfectant spray (D3), Dettol hand sanitizer gel (H1), Follow me hand sanitizer gel (H2), Good virtue co hand sanitizer spray (H3), Antabax hand sanitizer spray (H4), Silkygirl vanilla dream EDT (P1), Enchanteur chic adore EDT (P2), Dashing EDT (P3), Uberman EDT (P4).

Formulation of hand sanitizers

A modified WHO-recommended hand sanitizer formula is proposed for this research project. Table 1 shows the materials required by a certain amount. However, the present study followed the given steps to prepare green hand sanitizer sprays without any effect on the human body. An experimental flow diagram for the formulation of hand sanitizers is depicted in Figure 1. The preparation steps involved are as follows.

i. Ethanol for the formula to be used was poured into a large conical flask (1000 mL capacity).
ii. Hydrogen peroxide (4 mL) was added using a measuring cylinder.
iii. Glycerol was added using a measuring cylinder. Since glycerol is very viscous and attaches to the walls of the measuring cylinder, glycerol was rinsed with a little sterile distilled water and then emptied into a large conical flask.
iv. The large conical flask was then filled to 1000 mL mark with sterile distilled water then added the 3 mL of essential oil in each case (Lemongrass, rosemary, and lavender).
v. Lid cap was placed in the large conical flask as soon as possible after preparation, to prevent evaporation.
vi. The solution was mixed by gently shaking it as appropriate.
vii. Immediately we divided the solution into its final container (250 mL plastic bottles) and place the bottle in quarantine for 72 h before use. This allows time for the spores present in the ethanol or new/reused bottles to be destroyed.

Table 1. List of chemical reagents with required quantity for hand sanitizer formulation.

Chemical reagents	Quantity (mL)
95% Ethanol	842.0
Glycerine	14.5
30% Hydrogen peroxide	4.0
Distilled water	136.5
Essential oil	3.0

Formulation of disinfectants

A modified disinfectant formula is proposed for this research project. Table 2 shows the materials required by a certain amount. However, the present study followed the steps shown below to prepare green disinfectant sprays. The experimental procedure is shown in Figure 1 and the preparation steps are as follows.

i. Ethanol for the formula to be used was poured into a large conical flask (1000 mL capacity).
ii. Hydrogen peroxide (4 mL) was added using a measuring cylinder.
iii. The large conical flask was then filled to 1000 mL mark with sterile distilled water then added the 3 mL of essential oil in each case (Lemongrass, rosemary, and lavender).
iv. Lid cap was placed in the large conical flask as soon as possible after preparation, to prevent evaporation.
v. The solution was mixed by gently shaking it as appropriate.
vi. Immediately we divided the solution into its final container (250 mL plastic bottles) and place the bottle in quarantine for 72 h before use. This allows time for the spores present in the ethanol or new/reused bottles to be destroyed.

Table 2. List of chemical reagents with required quantity for disinfectant formulation.

Chemical reagents	Quantity (mL)
95% Ethanol	900
30% Hydrogen peroxide	4
Distilled water	93
Essential oil	3

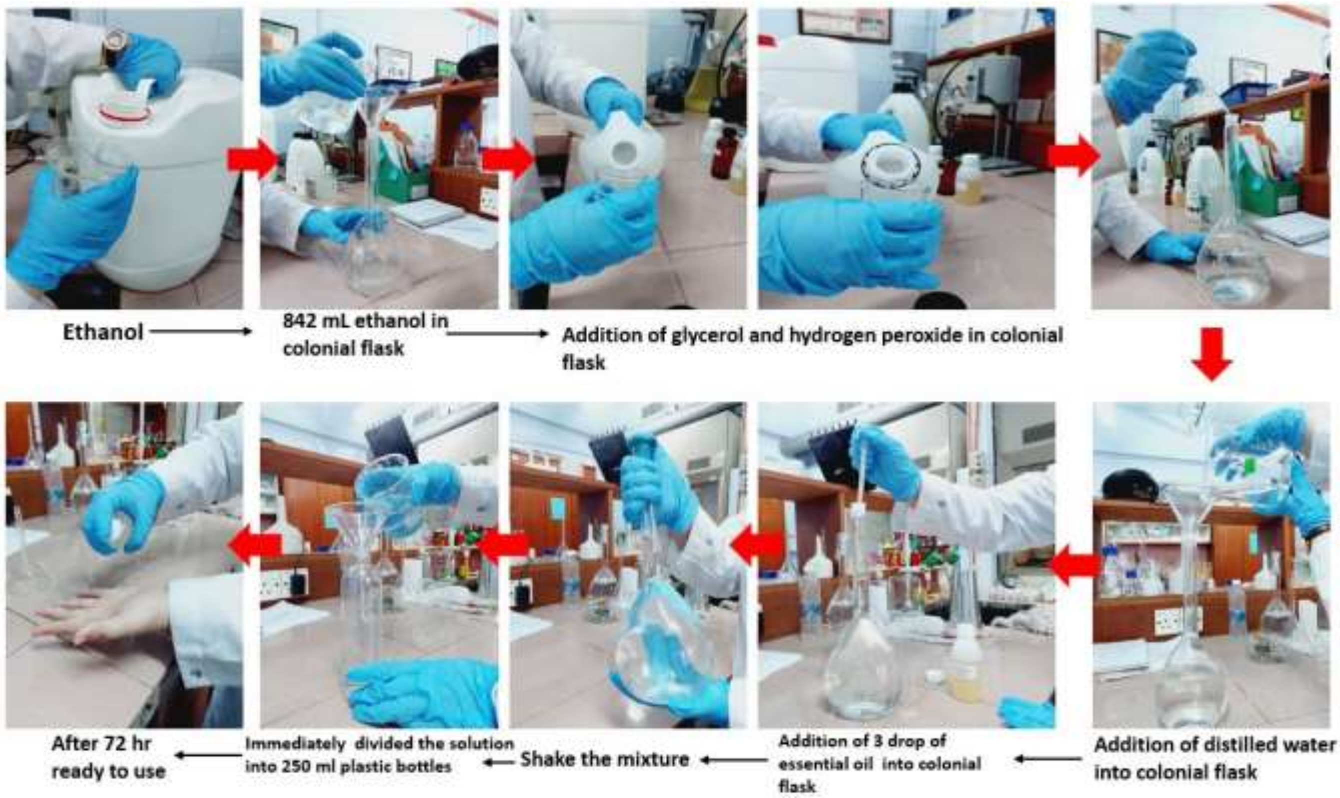

Figure 1. Formulation steps of hand sanitizers and disinfectants.

Determination of viscosity and pH

The viscosity and pH of formulated samples and commercial products were determined using Brookfield Model DV-II viscometer at 100 rpm (spindle S05) and microprocessor pH meter (Hanna instruments pH 211) at room temperature (25 °C), respectively.

Microbial test of commercial and formulated products against microorganisms

Antimicrobial analysis was conducted to assess the efficacy of antimicrobial properties of hand sanitizers and disinfectants against microorganisms. Several microorganisms were used in this analysis which are *Escherichia coli*, *Bacillus subtilis, Klebsiella* sp., *Salmonella* sp., *Pseudomonas* sp., and *Staphylococcus aureus*. The procedure is stated below:

i. The surface of nutrient agar plates was inoculated with the stock culture of each of the tested microorganisms, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella* sp., *Salmonella* sp., *Pseudomonas* sp., and *Staphylococcus aureus*.
ii. 9 mL of the test product (disinfectant or hand sanitizer or perfume) was transferred into the universal bottle.
iii. Each universal bottle was inoculated with 1 mL of suspension of the test microorganism.
iv. The test microorganism was exposed to the test product for 30 sec, 1 min and 5 min.
v. Serial dilution was carried out using sterile peptone water.
vi. 100 µL of sample was removed from each container and the number of viable microorganisms was determined

by spread plate count method in triplicate. Nutrient agar was used for all the tested microorganisms.

vii. Then all the plates were incubated at 37 ºC for 24 h for all microorganisms.

viii. The percentage change in the count of each microorganism concentration was calculated.

Quantification of alcohol content of commercial products and samples

Ethanol content was analyzed *via* Gas Chromatography (GC-FID model Agilent 7890 B). The conditions of GC-FID were set as follows: injection volume: 1 uL, running time: 21 min, injector temperature: 250 °C, detector temperature: 280 °C and carrier gas flow rate at 1 mL/min. The oven was programmed at 40 °C for 4 min then ramp to the temperature 180 °C with the rate of 15 °C/min with holding time of 5 min. The column used is ZB-Wax.

RESULTS AND DISCUSSION

Determination of viscosity and pH

The viscosity and pH of commercial products and formulated products are summarized in Table 3. At 100 rpm, the viscosity of commercial products and formulated products were 10.0-12.0 cP. pH value indicates the chemical stability of the product, which shows the suitability of the products to the skin. For instance, hand sanitizer should possess appropriate pH value to prevent irritation to the skin. Previous study revealed that, an acceptable pH range for skin is 4.5-6.5 (Ningsih et al., 2017). The average pH of commercial hand sanitizers, disinfectants and fragrances were pH 7.04, pH 9.26 and pH 8.27, respectively. Meanwhile, for formulated products, the average pH of hand sanitizers and disinfectants were pH 5.47 and pH 5.20, respectively. Obtaining low pH values for formulated products was due to the addition of H_2O_2 as a preservative to prevent growth of microbes. According to a past study, antimicrobial properties are not highly affected by pH value (Osei-Asare et al., 2020). The viscosity and pH value of formulated products fall within the acceptable range and is suitable to be commercialized.

Table 3. Viscosity and pH values of commercial and formulated products.

Type	Sample	Viscosity (cP)	pH
Commercial products	Hand sanitizer:		
	H3	12.0	7.38
	H4	12.0	6.70
	Disinfectant:		
	D1	12.0	9.38
	D2	12.0	9.23
	D3	12.0	9.17
	Fragrance:		
	P1	12.0	8.23
	P2	12.0	8.30
	P3	12.0	8.10
	P4	12.0	8.45
Formulated products	Hand sanitizer:		
	H-lavender	12.0	5.83
	H-rosemary	12.0	5.25
	H-lemongrass	12.0	5.03
	H-yalang	10.0	5.40
	H-bidara	10.0	5.71
	H-kaffir lime	10.0	5.60
	Disinfectant:		
	D- lavendar	12.0	5.12
	D-rosemary	12.0	5.45
	D-lemongrass	12.0	5.03
	D-yalang	10.0	5.20
	D-bidara	10.0	5.25
	D-kaffir lime	10.0	5.10

Microbial test of commercial products and samples against microorganisms

The time kill method was employed to evaluate the effectiveness of bactericides (hand sanitizers and disinfectants) against microorganisms. It is a basic microbiology method to study the antimicrobial activity of bactericides over time. Table 4 summarizes the average antimicrobial efficiency of commercial products and formulated products. Antimicrobial efficiency is defined as percentage of microorganisms reduced by bactericides over the exposure time of 30 sec, 1 min and 5 min. Analysis revealed that commercial and formulated bactericides were 99.9 % efficient to kill microorganisms. In terms of efficacy over time, formulated bactericides showed excellent protection in shorter exposure time as compared to commercial products. For formulated bactericides, 99.9 % of microorganisms were killed after 30 sec been exposed to the test agent. This refers to the denaturation of protein of microbes upon its contact with alcohol. Previous study confirmed that the formulation that contain 62 to 90 % of alcohol are highly effective for killing most of bacteria, fungi or virus (Dixit et al., 2014). The addition of essential oils also contributes to the enhancement of the antimicrobial activities of formulated hand sanitizers and disinfectants. For instance, lemongrass essential oil possessed strong antimicrobial properties due to its bioactivity of geraniol and citral isomers (Soltanzadeh et al., 2021). Also, limonene content in kaffir lime was found to be very effective to rupture the membrane integrity of bacteria/microorganism leading to the inactivation of bacteria activity and inhibition the subsequent growth of microorganism (Liew et al., 2020).

Meanwhile for commercial hand sanitizers and disinfectants, 99.9 % of microorganisms were killed after 30 sec to 1 min of exposure time. Based on formulation (refer to Table 1 and Table 2), hand sanitizers and disinfectants were formulated with 90 % alcohol as an active ingredient. Alcohol has a high tendency in causing irritation and drying up of the skin. However, it can be overcome by the addition of emollient (glycerine), which is greatly effective in reducing the absorbent and astringent effect of alcohol. Moreover, past studies revealed that emollient can enhance antimicrobial activity of hand sanitizer by its moisturizing effect thus increasing the contact time of alcohol with skin (Kampf & Hollingsworth, 2008; Lauharanta et al., 1991). Hence, it can be attested that the formulated bactericides have comparable antimicrobial properties with the products available in the market.

Table 4. Antimicrobial efficiency of commercial and formulated products against microorganisms.

Type	**Sample**	**Antimicrobial efficiency (%)**
Commercial products	Hand sanitizer:	
	H1	99.9 (after 1 min exposure)
	H2	99.9 (after 1 min exposure)
	H3	99.9 (after 30 sec exposure)
	H4	99.9 (after 30 sec exposure)
	Disinfectant:	
	D1	99.9 (after 30 sec exposure)
	D2	99.9 (after 30 sec exposure)
	D3	99.9 (after 30 sec exposure)
	Fragrance:	
	P1	99.9 (after 1 min exposure)
	P2	99.9 (after 1 min exposure)
	P3	99.9 (after 1 min exposure)
	P4	99.9 (after 1 min exposure)

Formulated products	Hand sanitizer:	
	H-lavender	99.9 (after 30 sec exposure)
	H-rosemary	99.9(after 30 sec exposure)
	H-lemongrass	99.9 (after 30 sec exposure)
	H-bidara	99.9 (after 30 sec exposure)
	H-kaffir lime	99.9 (after 30 sec exposure)
	H-yalang	99.9 (after 30 sec exposure)
	Disinfectant:	
	D-lavendar	99.9 (after 30 sec exposure)
	D-rosemary	99.9 (after 30 sec exposure)
	D-lemongrass	99.9 (after 30 sec exposure)
	D-bidara	99.9 (after 30 sec exposure)
	D-kaffir lime	99.9 (after 30 sec exposure)
	D-yalang	99.9 (after 30 sec exposure)

Quantification of alcohol content of commercial products and samples

Ethanol content is quantified by using gas chromatography (GC). The determination of ethanol percentage is important in hand sanitizer and disinfectant because it is an active ingredient for bactericides. The percentage of ethanol for commercial products and formulated samples were tabulated in Table 5. GC analysis revealed that hand sanitizer formulated with addition of rosemary and bidara essential oil contain 72.0 and 77.9 w/w % of ethanol. Meanwhile, 70.1 and 82.3 w/w % of ethanol was attained from disinfectant formulation of lemongrass and bidara, respectively. For commercial hand sanitizers and disinfectants, the ethanol contents were 70.3, 55.0, 73.5 and 53.1 w/w % for H1, H4, D1, and D2, respectively. Besides, the ethanol content in commercial fragrances were 72.5 and 64.8 w/w % for P1 and P4, respectively. It can be affirmed that the alcohol content of formulated hand sanitizers and disinfectants falls within the acceptable range (62-90 %) as reported in other studies (Alghamdi, 2021; Osei-Asare et al., 2020; Su et al., 2021). Due to the vast spread of Covid-19 virus, the Centre for Disease Control and Prevention (CDC) strongly recommend using alcohol-based hand sanitizers and disinfectants to reduce infection rates in the community. In terms of toxicity concern, American Association of Poison Control, highlighted the risk of alcohol poisoning among the children which can cause confusion, nausea, and lethargy (America's Poison Centers, 2024). In this present study, bactericides were formulated with plant-based essential oil. The addition of plant-based essential oils in hand sanitizers and disinfectants was reported to be greatly efficient to solve the toxicity problem (Saad et al., 2011). Moreover, plant-based essential oils also can function as antimicrobial and antiviral agents.

Table 5. Percentage of ethanol in commercial and formulated products.

Type	Sample	Percentage of ethanol (w/w %)
Commercial products	Hand sanitizer:	
	H1	70.3
	H4	55.0
	Disinfectant:	
	D1	73.5
	D2	53.1
	Fragrance:	
	P1	72.5
	P4	64.8
Formulated products	Hand sanitizer:	
	H- rosemary	72.0
	H-bidara	77.9
	Disinfectant:	
	D-lemongrass	70.1
	D-bidara	82.3

CONCLUSION

Antimicrobial hand sanitizers and disinfectants were successfully formulated with modification by the addition of plant-based essential oils. Formulated hand sanitizers and disinfectants were found to contain average values of ethanol of 75.0 and 76.2 w/w %, respectively with antimicrobial efficiency of 99.9 %. The comparison between formulated products and commercial products proved that formulated hand sanitizers and disinfectants have excellent antimycobacterial properties and are ready to be commercialized.

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AUTHOR CONTRIBUTIONS

Asim Ali Yaqoob: Data collection, data analysis, writing original draft. **Nur Fatin Silmi Mohd Azani:** Data collection, writing original draft. **Showkat Ahmad Bhawani:** Data analysis, writing-review and editing. **Mohamad Nasir Mohamad Ibrahim:** Supervision, project administration, resources. **Ahmad Faiz Abdul Latip:** Project administration, supervision. **Nasehir Khan E.M. Yahaya:** Data analysis, writing-review and editing. **Kamarul Zaman Bin Zarkasi:** Investigation, data curation. **Muhammad Azroie Bin Mohamed Yusoff:** Data curation. **Tuan Sherwyn Hamidon:** Writing-review and editing. **M. Hazwan Hussin:** Conceptualization, project administration, methodology, supervision, resources, writing-review and editing.

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COMPETING INTEREST

The authors declare that there are no competing interests.

COMPLIANCE OF ETHICAL STANDARDS

Not applicable.

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