**Research Article** 

DOI: 10.4274/tjps.galenos.2023.78006

Development and Evaluation Essential Oils Nanoemulgel as Human Skin Sanitizer Using Novel Method

DRAIS et al. Nanoemulgel Skin Sanitizer

Hayder Kadhim DRAIS

College of Pharmacy, Al-Mustaqbal University, Babil, Iraq

deera2020@gmail.com 09647800200325 0000-0002-2143-1817

02.10.2023 12.12.2023

#### ABSTRACT

a. **Objectives:** The increase in epidemic diseases and the frequent use of alcoholic disinfectants, despite their side effects, prompt scientists to develop new sterilization products that do not have alcoholic material. Therefore, the main purpose of this study was to develop, prepare, and evaluate a nanoemulgel skin sanitizer using essential oils as an active substance.

Materials and Methods: Microwave-based technique was used to prepare nanoemulsion. The pseudo ternary phase plots were constructed to contain three ingredients which are essential oils, polyoxyethylene (80) sorbitan monooleate, and propylene glycol mixture 1:0.75 (w/w) % and double distilled. Five samples of nanoemulsion (NE1-NE5) were selected for the characterization process and preparation of nanoemulgel (HN1- HN5). Blank gel (HN6) was also prepared to compare the antibacterial activity against HN1- HN5 formulations. Various evaluation processes were achieved for HN1- HN6 formulations. The statistical test was a one-way analysis of variance (ANOVA) at P≤0.05 as significant data.

Results: The characterization process indicates that NE1-NE5 formulations had nano-sized droplets and homogenous distribution with acceptable charge. The evaluation process for HN1- HN6 formulations indicates clear, homogenous, with distinctive essential oils odor and no phase separation, slightly acidic pH, spreadability (128.22 to 124.22 g\*cm/sec g\*cm/sec), plastic rheological flow, no skin lesion after application and conspicuous antimicrobial activity.

Conclusion: The laboratory characterization and evaluation demonstrated the existence of a promising product for sanitization of human skin and could be a successful alternative to alcoholic products, based on the growing desire for essential oil products.

Key words: Essential oils, Nanoemulsion, Nanoemulgel, Microwaves based method

#### INTRODUCTION

Health service providers are among the groups most vulnerable to bacterial attack, due to their reception of various and many disease cases in clinics and hospitals. Also, all different groups of society, from workers and employees to those sitting at home, remain vulnerable to bacterial attacks. Therefore, scientists must develop, innovate, and diversify various defensive and preventive methods against these harmful microbes. Alcohol-based hand gel is an antiseptic or hand rub, a product that removes common pathogens after its hand application. They are used to destroy the infection chain, making them one of the most important protocols for diminishing the burden on healthcare.<sup>1</sup> It is preferable to use it when soap and water are not available, or due to frequent dealing with diseases, as in health service providers, or the presence of special skin diseases such as cracks on the skin. Exposure to alcohol-based hand gel deprives the skin of water and sebum that cause skin dryness, destroy lipid barriers, and eventually cause hand eczema and dermatitis, associated symptoms like dryness, acne, wrinkles, burning, swelling, erythema, and cracking.<sup>2</sup> Nonalcoholic essential oils (EOs) hand gel is an advanced and desirable alternative for fighting various germs. Essential oils (EOs) are aromatic liquids with oil structure that was obtained naturally from plants. Several reports exhibit that EOs have antiseptic, antibacterial, antifungal, antiviral, antioxidant, anti-parasitic, and insecticidal activities. The antimicrobial activity of EOs is achieved by destroying the cell membrane and bacterial cell wall resulting in microbial cell disruption.<sup>3-5</sup> Perpermint oil and myrtle oil are medicinal essential oils with many studies that confirm their antimicrobial activity.6-<sup>8</sup> The peppermint oil and myrtle oil are hydrophobic materials that are unstable when mixed with aqueous components of gel. Nanoemulsion, an oil-in-water type, represents an advanced delivery system consisting of an internal oil phase and an external aqueous phase, suitable for skin application. When nanoemulsion combines with a gelling agent result in a more convenient nanosystem called nanoemulgel that provide many advantages such as better patient compliance, better loading capacity, better stability and controlled release.<sup>9,10</sup> Various techniques present in preparing the nanoemulsion as a part of nanoparticulated drug delivery system but it related with a large number negative marks principally more expense, invested high time and energy and stability issue of the final dosage form. The recently microwaves-based strategy is cheap, conservative, stable, quickly handling on both little and enormous scope, and nonappearance of impurities. The radiation of microwaves is a type of electromagnetic nonionizing that has frequencies about one meter to one millimeter with frequencies 300 MHz to 300 GHz. The microwaves have three fundamental ascribes that work with them to be utilized in dosage form development which are-reflection by metal substances, it is absorbed by substances, furthermore, ready to go through the plastic, glass, paper, and comparable ingredients.<sup>11</sup> Therefore, the objective of this research was to develop, prepare, and evaluate an antimicrobial essential oils nanoemulgel skin sanitizer and compare their efficacy against two bacterial strains, Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli).

# MATERIALS AND METHODS

#### Materials

The essential oils which are peppermint oil and myrtle oil were purchased from BAR-SUR-loup Grasse A. M Franc and Nanjing Duly Biotech Co., Ltd China respectively. Polyoxyethylene (80) sorbitan monooleate, carbopol 940, and propylene glycol were purchased from Beijing Yibai Biotechnology Co., Ltd. China. All solvents and reagents that had been in the experiment were of analytical grade. *Methods* 

Preparation of essential oil nanoemulsion and construction of pseudoternary phase diagrams using the microwaves-based method:

The peppermint oil and myrtle oil were used to mix with hydrophilic components which are double distilled water, polyoxyethylene (80) sorbitan monooleate and propylene glycol. The blend that prepared under 1000 rpm for 5 minutes of magnetic stirrer that contained hydrophilic and hydrophobic phases according to the amounts described in Table 1. The mixture was inserted in a microwave device for 10-15 seconds, then a magnetic stirrer device at 1000 rpm for adequate time (seconds to minutes according to a final volume of dosage form) until the feature of nanoemulsion (NE1-NE5) was observed.<sup>11</sup> The construction process of pseudo ternary phase diagrams, which contain three components, including essential oil, surfactant mixture which is polyoxyethylene (80) sorbitan monooleate and propylene glycol (1:0:75) % (w/w), and aqueous phase is developed. To know the borderline of phases for each phase graphing, a visual inspection was made to assess the transparent feature of formulations during the

magnetic stirrer process. The pseudo ternary phase plot was drawn by using triplot V4 software 4.1.2. Version. The diagrammed area of the nanoemulsion is represented by the shaded area.<sup>10</sup> *The preparation of essential oil hand nanoemulgel* 

The carbomer 940 hydrogel was formulated by adding 0.6% (w/w) of the gelling agent in double distilled water with stirring using an electric homogenizer until completely dissolved. A few drops of triethanolamine were added to obtain a pH of about (6.2-7.4). The previously prepared nanoemulsion (NE1-NE5) formulations were mixed with hydrogel in a concentration of 15% and the two are continuously slow stirred until a clear essential oil hand nanoemulgel (HN1-HN5) formulations is formed. Blank gel (HN6) was also prepared by adding polyoxyethylene (80) sorbitan monooleate and propylene glycol mixture to the gel base with a continuous slow stirring rate to avoid the formation of bubbles (500 rpm for 15 minutes) to get a clear blank gel (HN6). The essential oil nanoemulgel (HN1-HN5) formulations and blank gel (HN6) were stored in a tightly closed container at 25 °C temperatures for assessment and study.<sup>9-11</sup>

# *Characterization of nanoemulsion (NE1-NE5) formulations through the determination of particle size, index of polydispersity (PDI), and zeta potential (ZP)*

Dynamic light scattering (DLS) is a technique used to determine particle size in addition to the polydispersity index (PDI) and surface charge of globules of nanoemulsion (NE1-NE5) formulations using Horiba instrument, Ltd. Kyoto, Japan. When a laser beam passes through a sample result in a variation in scattering light intensity which is time-dependent in the presence of the Brownian motion of the dispersed nanoglobules in a nanosystem). This technique is highly accurate and the measures were achieved in three trials.<sup>11</sup>

# Atomic force microscopy (AFM)

The nanocarrier morphology was determined by AFM Angstrom Advanced Inc. AA3000 USA. It was scanned at a range of 100 MV/s. The study was conducted with 2-3 drops of nanoemulsion on an experimental glass slide and then measured after 3 hours.

# Evaluation of essential oil hand nanoemulgel (HN1-HN5) formulations

#### Organoleptic determination

The organoleptic test is important to determine the physical stability of pharmaceutical preparation. The color, smell, homogeneity, and syneresis can be noticed for essential oil hand nanoemulgel (HN1-HN5) formulations at 0, 7, 14, 21, and 28 days. The data was obtained in triplicate.<sup>12-14</sup>

#### $Determination \ of \ pH$

It is an important parameter that can predict the stability of formulation and skin suitability. The digital pH meter was used to determine pH by taking a 10 g sample of essential oil hand nanoemulgel (HN1-HN5) formulations. The optimum human skin pH is in the range of 4.5 - 6.5. The experiment was done in three trials <sup>9,10</sup>

# Measurement of spreadability

It is a parameter related to patient compliance that leads to achieving therapeutic aims. It was done by two separated pieces of glass slides with dimensions ( $10 \times 2.5$  cm). The lower slide was tied to a wooden base that contained 0.5 g of essential oil hand nanoemulgel (HN1-HN5) formulations. The second piece of glass slide was tied to a weight of 25 g when applied to the first glass slide resulting in the pulling process to a distance of 7.0 cm before it detached. The data of weight in grams and time in seconds that needed to move the second glass slide was observed and the spreadability parameter can be determined from the following equation 3:

# $S = M \times L / T$ ..... Equation 3

S = Spreadability, M = Weight that tide to first slide, L = Length of slide

T = T is the time taken to separate two sides. The study occurred in three tr<sup>ials 12-14</sup>

#### Viscosity measurement

It is a crucial parameter for pharmaceutical formulation assessment in addition to the analysis and development of new formulations. Using a rotational digital viscometer with a spindle number (2) from Biobase Meihua Trading Co., Ltd. at 25°C for the viscosity of essential oil hand nanoemulgel (HN1-HN5) formulations occurred. The samples were subjected to different rotating speeds which are (0.1, 0.3, 0.6, 1.5, 3, 6, 12, 30, and 60 rpm). The experiment was performed in three trials <sup>12-14</sup>

#### Skin irritation study

The study was done on 30 volunteers and ethically approved by the research ethics committee in Al-Mustaqbal University / College of Pharmacy, ph 2/2023 in 10.2.2023. All volunteers had no clinical signs of dermal abrasion and infection. The volunteers were asked to sign consent forms after explaining the research protocol with probable side effects. The evaluation was done by applying one gram of essential oil hand nanoemulgel (HN1-HN5) formulations on each intact area of the volunteer's hand skin, then allowed to wait for 10-15 minutes. A questionnaire was given to volunteers of the study to determine the skin irritation and acceptability test. The formulation was rated according to the characteristics of the essential oil hand nanoemulgel (HN1-HN5) formulations in terms of the formulation texture, appearance, smell, redness, and irritation or burning sensation after the product application.<sup>12-14</sup>

#### In vitro antimicrobial activity determination

In vitro antimicrobial activity of the prepared essential oil hand nanoemulgel (HN1-HN5) formulations and blank gel (HN6) against two pathogenic bacterial species which were, gram-negative E. coli and gram-positive S. aureus were studied using the agar disc diffusion method. The bacterial strains which are gram-negative E. coli and gram-positive S. aureus were obtained from Ali Obais Hospital, Babil Health Directorate Ministry of Health / Iraq. All the conditions of the experiment were done under aseptic conditions. From each study sample,  $10 \,\mu$ L was added onto sterile filter paper discs. Seven discs from each formula that described in Table 1 were placed on each culture plate. Assessment of the antibacterial activity through calculating the inhibition zones diameters in millimeters using a sliding caliper. The study was done in triplicate.

#### Statistical analysis

The data of the study were observed as the mean and standard deviation (SD) of three experimental trials. The statistical analysis was achieved using the Excel program. The one-way analysis of variance (ANOVA) was a statistical test, where the level at (P $\leq$ 0.05) was kept as significant.<sup>10,11</sup>

#### **RESULTS AND DISCUSSION**

# *Measurement of globule size, polydispersity index (PDI), and zeta potential (ZP) for nanoemulsion (N1-N5) formulations*

The pseudo-ternary phase diagrams were constructed that contain three structural components which are essential oils, surfactant mixture (1:0.75) % (w/w), and double distilled water. The nanoemulsion of essential oil was prepared successfully by microwaves-based method which was characterized by ease, speed and flexibility in preparation. The nanoemulsion was represented by a shaded area of pseudoternary phase diagrams while the other area was emulsion. From phase diagrams, five formulas were selected which are NE1, NE2, NE3, NE4, and NE5 for characterization of globule size, PDI, and ZP. The result of particle size was NE1=25.83 nm, NE2=45.96 nm, NE3=29.83 nm, NE4=49.83 nm, and NE5=55.86 nm as shown in Table 2. This indicates the colloidal features of NE1-NE5 formulations. It was found that as the concentration of essential oils increases leads to an increase in globule size at a constant of surfactant:co-surfactant blend concentration. The particle size values have the following ascending order for NE1 < NE2 in formulations that contain peppermint oil while NE3 < NE4 in formulations that contain myrtle oil. In comparison between the similar quantities of essential oil for different types, it had the following ascending order for NE2 < NE4 < NE5. The globule size increases as lipid content quantity increases due to an increase in the colloidal dispersion viscosity that made dispersed globules more resistant to breakdown of large droplets into smaller ones during the emulsification process.<sup>9,11</sup> PDI experiment for nanoemulsion (NE1-NE5) formulations was from (0.26 to 0.385) as shown in Table 2. This indicates high homogeneous and constricted size distribution for nanosystems.<sup>9-11</sup> The outcomes of the mean ZP absolute value for nanoemulsion (NE1-NE5) formulations were (12.61 to 19.6 mV) as shown in Table 2 which gave the stability of nanoemulsions. There should be a higher electrical charge on the surface particles of nanoemulsion to preclude aggregation of the nanoemulsions in the solutions due to the strong resistance violence among particles. Globular surface charge values according to the thumb rule are: the range-5 mV to mV shows fast aggregation, about 20 mV supplies only short-term stability, above 30 mV offers good stability, and above 60 mV excellent stability. The thumb rule can apply for ionic stabilizers, but not for large or great molecular weight surfactants such as tween 80 which are nonionic stabilizers that provide steric stability.<sup>9,11</sup> The analysis of variance confirmed and accepted the alternative hypothesis and rejected the null hypothesis due to there being a

significant relationship between oil content and particle size as a dependent variable where the P-value  $\leq$  0.5.

### Atomic force microscopy

The result shows that the NE5 formula contains particles with regular smooth surfaces and nearly spherical shapes with nanometers in size as shown in Figure 2. There was no particle aggregation which indicates the physical stability of the preparation.

Evaluation of essential oil hand nanoemulgel (HN1-HN5) formulations Organoleptic assay

The organoleptic test was done through naked-eye observations of the essential oil hand nanoemulgel (HN1-HN5) formulations. All HN1-HN5 formulations show clear, homogenous, with the characterized odor of essential oil represented by peppermint oil and myrtle oil. There was no syneresis, which indicates high physical stability.<sup>12-14</sup>

# Determination of pH

The pH evaluation is an important parameter that can be used in the prevention of unsuitable properties in nanoemulgel that are related to patient comfort. The pH values of essential oil hand nanoemulgel (HN1-HN5) formulations were slightly acidic (5.4 to 5.89) as shown in Table 3. It was found that an increment in essential oil concentration led to a slight increase in the pH. The outcomes provide suitable pH that guarantees the patient comfort and avoids skin allergic reactions and dermatitis.<sup>12-14</sup> The analysis of variance showed a significant relationship between the dependent factor which is pH and quantity of essential oil at the level (P $\leq$ 0.05).

#### Measurement of spreadability

The spreadability study was achieved for essential oil hand nanoemulgel (HN1-HN5) formulations. The results were (128.22 to 124.22 g X cm/sec). It was found that formulations containing peppermint oil had greater spreadability than formulations that contained myrtle oil for a similar quantity of oil at a constant concentration of surfactant mixture (1:0.75), this is due to the that peppermint oil that has been used in the experiment was less viscous than myrtle oil. Also, it was found that the quantity of peppermint oil, and myrtle oil increased at the constant quantity of polyoxyethylene (80) sorbitan monooleate, propylene glycol, and carbomer 940, leading to decrease spreadability parameter due to increased viscosity of essential oil hand nanoemulgel (HN1-HN5) formulations. Generally, the outcome indicates low spreadability time for all essential oil hand nanoemulgel (HN1-HN5) formulations that enhance patient compliance upon application on the skin.<sup>12-14</sup> The analysis of variance indicates a significant relationship between the spreadability factor and experimental oil (peppermint and myrtle oil) as independent factors at the level (P≤0.05).

# Viscosity measurement

The viscometer with a spindle number (2) of rotational digital type, Biobase Meihua Trading Co., Ltd was exploited to measure the viscosity and study rheology behavior of essential oil hand nanoemulgel (HN1-HN5) formulations. The data obtained are shear rate, shear stress and viscosity.. The outcome of viscosity as shown in Table 3 indicates as the quantity of essential oil increase leads to increase viscosity at a constant concentration of polyoxyethylene (80) sorbitan monooleate and propylene glycol mixture 1:0.75 (w/w) %, therefore it was found the value of viscosity at 12rpm in (mP.s) unit are HN1=3074.68, HN2=3186.55, HN3=3107.33, HN4=3203.41, and HN5=3195.26. This is due to the increase in volume concentration of nanoglobules that make the colloidal dispersion system more resistant to flow in addition decreasing aqueous phase volume will reduce continuous phase volume and make the nanosystem more viscous. The rheogram chart was obtained by plotting the shear rate(1/sec) against shear stress (m P.s) as shown in Figure 3. All essential oil hand nanoemulgel (HN1-HN5) formulations show plastic flow which is a non-Newtonian flowing system due to there being no gel flowing related to shear stress until reaches a specific transition point. This plastic flow made formulations easier to wipe on the infected skin or membranes and provided additional stability to essential oil hand nanoemulgel (HN1-HN5) formulations. The ANOVA confirmed a significant relationship (P≤0.05) between viscosity as a dependent factor and essential oil as an independent factor.<sup>12-14</sup>

#### Skin irritation study

A skin irritation experiment was conducted by using 30 volunteers for essential oil hand nanoemulgel (HN1-HN5) formulations. It was found that all formulations did not produce a sense of skin itching, irritation signs, or any skin painful effect after the application of gel to the participants in the experiment.

This indicates that all essential oil hand nanoemulgel (HN1-HN5) formulations a comfortable, well-tolerated.

#### In vitro antimicrobial activity determination

The experiment of in vitro antimicrobial activity was conducted successfully for essential oil hand nanoemulgel (HN1-HN5) formulations and blank gel (HN6) against microbial species which are, Gramnegative E. coli and Gram-positive S. aureus. It was found that increasing essential oil concentration led to increased bacterial growth inhibition for S. aureus and E. coli, as shown in Figure 3. There was no S. aureus activity for HN1 and blank gel (HN6) formulation. The comparability profile of the bacterial susceptibility for S. aureus had the following ascending order: HN2< HN3< HN4 < HN5. The profile of bacterial susceptibility was significantly higher (P value < 0.05) in microbial susceptibility for HN5 and was significantly lower (P value < 0.05) in microbial susceptibility for HN2 as shown in Table 4. It was found that S. aureus was relatively higher sensitive for formulations that contained myrtle oil as HN3, and HN4 in comparison to the formulations containing peppermint oil as HN1, and HN2 at the same concentrations. It was found that essential oil hand nanoemulgel (HN1-HN5) formulations had higher microbial activity against E. coli in comparison to S. aureus as shown in Figure 4. There was no E. coli activity for HN1 and blank gel (HN6) formulation. The comparability profile of the bacterial susceptibility for E. coli had the following ascending order: HN2< HN3< HN4 < HN5. The bacterial susceptibility profile was significantly higher (P≤0.05) in microbial growth inhibition for HN5 and was significantly lower (P≤0.05) in microbial growth inhibition for HN2 as shown in Table 4. It was found that a mixture of essential oils increased activity against microbial growth as HN5 formulation was significantly higher (P $\leq$ 0.05) in microbial susceptibility for S. aureus and E. coli.

#### CONCLUSION

The new method, which is based on microwaves, proved the success of the task in preparing nanoemulsions NE1-NE5, that were used in essential oil nanoemulgel HN1-HN5 formulations that makes it the most high-level technique for the nanocarriers preparation. The great trend towards the use of skin antiseptics and the presence of some side effects of alcoholic antiseptics enhances the status of vegetable essential oils and their use as successful alternatives in the process of cleansing the skin and combating germs for various groups of human society. This study proved the effectiveness of vegetable essential oils in combating germs, and that mixing between essential oils gives additional strength and motivation towards combating germs, as in the HN5 formulation that contains peppermint oil: myrtle oil (1:1) % w/w that shows greater antimicrobial activity toward S. aureus and E. coli.

#### REFERENCES

1. Golin AP, Choi D, Ghahary A. Hand sanitizers: a review of ingredients, mechanisms of action, modes of delivery, and efficacy against coronaviruses. Am J Infect Control. 2020;48(9):1062–7.

2. Prajapati, Parixit, Heli Desai and Chandni Chandarana. "Hand sanitizers as a preventive measure in COVID-19 pandemic, its characteristics, and harmful effects: a review." Journal of the Egyptian Public Health Association. 2022; 97: 1-9.

3. Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives. Medicines (Basel). 2017;4(3):58.

4. Vostinaru O, Heghes SC, Filip L. Safety profile of essential oils. In Essential OilsBioactive Compounds, New Perspectives and Applications. Intech Open. 2020; 27:1-13.

5. Booq, Rayan Y., Abdullah A. Alshehri, Fahad A. Almughem, Nada M Zaidan, Walaa S Aburayan, Abrar A. Bakr, Sarah Kabli, Hassa A. Alshaya, Mohammed S. Alsuabeyl, Essam J. Alyamani and Essam A. Tawfik. Formulation and Evaluation of Alcohol-Free Hand Sanitizer Gels to Prevent the Spread of Infections during Pandemics. International Journal of Environmental Research and Public Health.2021;18:14 – 26.

6. Mohamadi, Y, Takia Lograda, Messaoud Ramdani, Gilles Figuérédo and Pierre Chalard. Chemical composition and antimicrobial activity of Myrtus communis essential oils from Algeria. Biodiversitas.2021; 22: 933-946

7. Tiwari, Ritesh Kumar, Devika Tripathi, Lalit Singh, Vijay Kumar Sharma and Shashikant Kumar Verma. Development and antibacterial characterization of mint handwash. International Journal of

Research Publications. 2018;9(1): 25 - 32.

8. El-Sheikh, M A and Aisha El-Sharary. Preparation, characterization, and antibacterial efficacy of green aromatic soap. Egyptian Journal of Chemistry.2022: 385–393.

9. Drais, Hayder Kadhim and Ahmed Abbas Hussein.Formulation and characterization of carvedilol nanoemulsion oral liquid dosage form. International Journal of Pharmacy and Pharmaceutical Sciences. 2015;7: 209-216.

 Drais, Hayder Kadhim and Ahmad A. Hussein. Formulation Characterization and Evaluation of Meloxicam Nanoemulgel to be Used Topically. Iraqi Journal of Pharmaceutical Sciences. 2017; 26: 9-16.
Drais HK, Ahmed AH. Lipid-polymer hybrid nanocarriers for oral delivery of felodipine: formulation, characterization and ex-vivo Evaluation. Advanced Pharmaceutical Bulletin. 2022;12(4):791-800.
Eid, Ahmad M., Linda Issa, Omamah Al-Khartoum, Raghad Jaber, and Fatima Hreash. "Development of coriandrum sativum oil nanoemulgel and Evaluation of Its Antimicrobial and Anticancer Activity." BioMed Research International. 2021: 1-10.

13. Booq, Rayan Y., Abdullah A. Alshehri, Fahad A. Almughem, Nada M Zaidan, Walaa S Aburayan, Abrar A. Bakr, Sarah Kabli, Hassa A. Alshaya, Mohammed S. Alsuabeyl, Essam J. Alyamani and Essam A. Tawfik. Formulation and Evaluation of Alcohol-Free Hand Sanitizer Gels to Prevent the Spread of Infections during Pandemics. International Journal of Environmental Research and Public Health. 2021; 18: 14 – 26.

14. M. Diviyagahage C., S. Thuvaragan, J. Gnanakarunyan T. Srikaran R. Formulation and characterization of essential oils based antibacterial hand sanitizer gels. The Pharmaceutical Journal of Sri Lanka. 2021; 11(1): 14 – 26.

Table 1. Essential oil hand nanoemulgel (HN1-HN5) formulations and blank gel (HN6) for optimization						
Code	Peppermint oil (w/w) %	Myrtle oil (w/w) %	Peppermint oil: myrtle oil (1:1) (w/w) %	Polyoxyethylene (80) sorbitan monooleate and propylene glycol mixture 1:0.75 (w/w) %	Carbopol 940 (w/w) %	Double distilled water Up to (w/w) %
HN1	5			4	0.6	100
HN2	10			4	0.6	100
HN3		5		4	0.6	100
HN4		10		4	0.6	100
HN5			10	4	0.6	100
HN6				4	0.6	100

formulations				
Formulation	Globule size	PDI*	Zeta potential*	
Code	(nm)*			
NE1	25.83±1.04	0.26±0.015	12.61±0.281	
NE2	45.96±1.001	$0.368 \pm 0.035$	17.53±0.427	
NE3	29.83±1.258	0.292±0.006	15.69±0.340	
NE4	49.83±0.85	$0.385 \pm 0.006$	18.43±0.235	
NE5	55.86±1.096	0.38±0.013	19.6±0.28	
*Values are expressed as mean $\pm$ SD (n=3)				

						R	of
Table 3.	Evaluation r	esults of es	sential oil	hand nanoemu	lgel (HN1-	HN5) formula	ations
Code	Color	Odor	Syneres	Homogeneit	pH*	Mean	Viscosity at
			is	у		spreadabil	12rpm
						ity	(mP.s)
						(g*cm/sec	
						)	
HN1	Colorless	Aromati	No	Homogeneo	5.6±0.1	128.22±0.	3074.68±2.539
		c smell	$\langle 2 \rangle$	us		12	
HN2	Colorless	Aromati	No	Homogeneo	5.89±0.0	124.52±0.	3186.55±2.501
		c smell		us	9	091	
HN3	Colorless	Aromati	No	Homogeneo	5.4±0.29	127.28±0.	3107.33±2.081
		c smell		us	8	14	
HN4	Colorless	Aromati	No	Homogeneo	5.62±0.1	124.45±0.	3203.41±2.526
		c smell		us	15	125	
HN5	Colorless	Aromati	No	Homogeneo	5.85±0.0	124.22±0.	3195.26±2.4
		c smell		us	5	11	
*Values	*Values are expressed as mean $\pm$ SD (n=3)						

<b>Table 4.</b> Antibacterial activity of different essential oil hand nanoemulgel (HN1-HN5) formulations     compared to blank gel (HN6) formulation				
Formulation code	Inhibition zone of	Inhibition zone of Escherichia		
	Staphylococcus aureus (mm)	coli (mm)		
HN1	0±0	0±0		
HN2	10.63±0.152	12.33±0.251		
HN3	11.266±0.251	12.7±0.2		
HN4	11.5±0.2	13.76±0.152		
HN5	12.56±0.152	37.03±1.66		
HN6 (BlanK)	0±0	0±0		
*Values are expressed as mean $\pm$ SD (n=3)				



**Figure 1.** Pseudoternary phase diagrams contain polyoxyethylene (80) sorbitan monooleate: propylene glycol mixture 1:0.75 (w/w) % double distilled water and oil component which are peppermint oil, myrtle oil, peppermint oil: myrtle oil (1:1) mixture in plots (A), (B) and (C) respectively





**Figure 2.** AFM 3D image of essential oil nanoemulsion (N5) formulation where scanning area is 78 nm \* 78 nm

Figure 3. Shear stress against a shear rate of essential oil hand nanoemulgel (HN1-HN5) formulations



**Figure 4.** Inhibition zone of the prepared essential oil hand nanoemulgel (HN1-HN5) formulations compared to blank gel (HN6) formulation (A): S. aureus and (B): E. coli