Turk J Pharm Sci 2024;21(5):456-462
DOI: 10.4274/tjps.galenos.2023.78006

ORIGINAL ARTICLE

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

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ABSTRACT

Objectives: The increase in epidemic diseases and frequent use of alcoholic disinfectants, despite their side effects, prompt scientists to develop new sterilization products that do not contain alcoholic materials. The aim of this study was to develop, prepare, and evaluate a nanoemulgel skin sanitizer using essential oils (EOs) as active substances.

Materials and Methods: A microwave-based technique was used to prepare nanoemulsion. The pseudo-ternary phase plots were constructed to contain three ingredients: EOs, polyoxyethylene (80) sorbitan monooleate, and a propylene glycol mixture (1:0:75) % *(w/w)* and double distilled. Five samples of nanoemulsion (NE1-NE5) were selected for the characterization 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 at $p \leq 0.05$ as significant data.

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

Conclusion: Laboratory characterization and evaluation demonstrated the existence of a promising product for sanitizing human skin and could be a successful alternative to alcoholic products based on the growing demand for EO products.

Keywords: Essential oils, nano-emulsion, nanoemulgel, microwaves based method

INTRODUCTION

Health service providers are among the most vulnerable groups to bacterial attack, due to their reception of various and many disease cases at clinics and hospitals. In addition, 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 hand application. They are used to destroy the infection chain, making them one of the most important protocols for diminishing the burden on healthcare.[1] It is preferable to use it when soap and water are not available, or due to frequent dealing with diseases, such as those experienced by health service providers, or due to 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.[2] Non-alcoholic essential oils (EOs) hand gel is an advanced and desirable alternative for fighting various

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Received: 02.10.2023 , Accepted: 12.12.2023

germs. EOs are aromatic liquids with an oil structure that is obtained naturally from plants. Several reports show that EOs have antiseptic, antibacterial, antifungal, antiviral, antioxidant, antiparasitic, and insecticidal activities. The antimicrobial activity of EOs is achieved by destroying the cell membrane and bacterial cell wall, resulting in microbial cell disruption.[3-5] Peppermint oil and myrtle oil are medicinal EOs with many studies confirming their antimicrobial activity.[6-8] Peppermint oil and myrtle oil are hydrophobic materials that are unstable when mixed with aqueous gel components. Nanoemulsions, an oil-in-water type, represent an advanced delivery system consisting of an internal oil phase and an external aqueous phase, suitable for skin application. When a nanoemulsion combines with a gelling agent, it results in a more convenient nanosystem called a nanoemulgel that provides many advantages, such as better patient compliance, better loading capacity, better stability, and controlled release.[9,10] Various techniques are present in preparing a nanoemulsion as a part of a nanoparticulated drug delivery system but it is related to a large number of negative marks, principally more expense, invested high time and energy, and stability issues of the final dosage form. Recently developed microwave-based strategies are cheap, conservative, stable, and quickly handling on both little and enormous scopes and avoid the appearance of impurities. The radiation of microwaves is a type of electromagnetic non-ionizing radiation that has frequencies of about one meter to one millimeter with frequencies of 300 MHz to 300 GHz. Microwaves have three fundamental ascribes that work with them to be utilized in dosage form development: reflection by metal substances, absorption by substances, and ready to go through plastic, glass, paper, and comparable ingredients.[11] The aim of this research was to develop, prepare, and evaluate an antimicrobial EO nanoemulgel skin sanitizer and compare its efficacy against two bacterial strains: *Staphylococcus aureus* and *Escherichia coli*.

MATERIALS AND METHODS

Materials

The EOs of peppermint and myrtle were purchased from BAR-SUR-loup Grasse A. The 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 used in the experiments were of analytical grade.

Methods

Preparation of EO nanoemulsions and construction of pseudoternary phase diagrams using microwave-based methostructures

Peppermint oil and myrtle oil were used to mix with hydrophilic components of double-distilled water, polyoxyethylene (80) sorbitan monooleate, and propylene glycol. Blend prepared under 1000 rpm for 5 min using a magnetic stirrer contained hydrophilic and hydrophobic phases according to the amounts described in Table 1. The mixture was inserted into a microwave device for 10-15 seconds, then a magnetic stirrer device at 1000 rpm was used for adequate time (seconds to minutes according to a final volume of dosage form) until the feature of nanoemulsion (NE1-NE5) was observed.[11] The construction process of pseudo ternary phase diagrams, which contain three components, including EO, a surfactant mixture of polyoxyethylene (80) sorbitan monooleate and propylene glycol (1:0:75) % *(w/w)*, and an aqueous phase, is developed. To determine the borderline of phases for each phase graphing, a visual inspection was performed to assess the transparency of the formulations during the magnetic stirrer process. The pseudo-ternary phase plot was drawn using triplet V4 software 4.1.2. Version. The diagramed area of the nanoemulsion is represented by the shaded area.[10]

Preparation of EO hand nanoemulgels

The carbomer 940 hydrogel was formulated by adding 0.6% *(w/w)* of the gelling agent to double-distilled water by stirring using an electric homogenizer until the mixture was 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 at a concentration of 15%, and the two were continuously and slowly stirred until a clear EO hand nanoemulsion (HN1-HN5) formulations were formed. Blank gel (HN6) was also prepared by adding a 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 obtain a clear blank gel (HN6). The EO nanoemulgel (HN1-HN5) formulations and blank gel (HN6) were stored in tightly closed containers at 25 ˚C temperatures for assessment and study.[9-11]

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

Dynamic light scattering is a technique used to determine particle size in addition to the PDI and surface charge of globules of nanoemulsion (NE1-NE5) formulations using a Horiba Instrument, Ltd. Kyoto, Japan. When a laser beam passes through a sample, a variation in scattering light intensity is observed that is time-dependent in the presence of Brownian motion of the dispersed nanoglobules in a nanosystem. This technique is highly accurate, and the measures were achieved in three trials.[11]

Atomic force microscopy (AFM)

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

Evaluation of EO hand nanoemulgel (HN1-HN5) formulations Organoleptic determination

Organoleptic tests are important for determining the physical stability of pharmaceutical preparations. The color, smell, homogeneity, and syneresis can be noticed for EO hand

nanoemulgel (HN1-HN5) formulations can be noticed at 0, 7, 14, 21, and 28 days. The data were obtained in triplicate.[12-14]

pH determination

It is an important parameter that can predict the stability of formulation and skin suitability. A digital pH meter was used to determine the pH by collecting a 10 g sample of EO hand nanoemulgel (HN1-HN5) formulations. The optimum human skin pH is in the range of 4.5-6.5. The experiment was conducted in three trials.[9,10]

Measurement of spreadability

It is a parameter related to patient compliance that leads to achieving therapeutic aims. The procedure was performed using two separated glass slides with dimensions (10 x 2.5 cm). The lower slide was tied to a wooden base containing 0.5 g of the EO 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 weight in grams and time in seconds that needed to move the second glass slide was recorded, and the spreadability parameter was determined from Equation 1:

$S = M \times L / T$ ..... Equation 1

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

Here, T = T is the time required to separate two sides. The study was conducted in three trials.[12-14]

Viscosity measurement

In addition to the analysis and development of new formulations, this parameter is crucial for pharmaceutical formulation assessment. Using a rotational digital viscometer with a spindle number (2) from Biobase Meihua Trading Co., Ltd. at 25 °C to measure the viscosity of EO hand nanoemulgel (HN1-HN5) formulations. 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.[12-14]

Skin irritation study

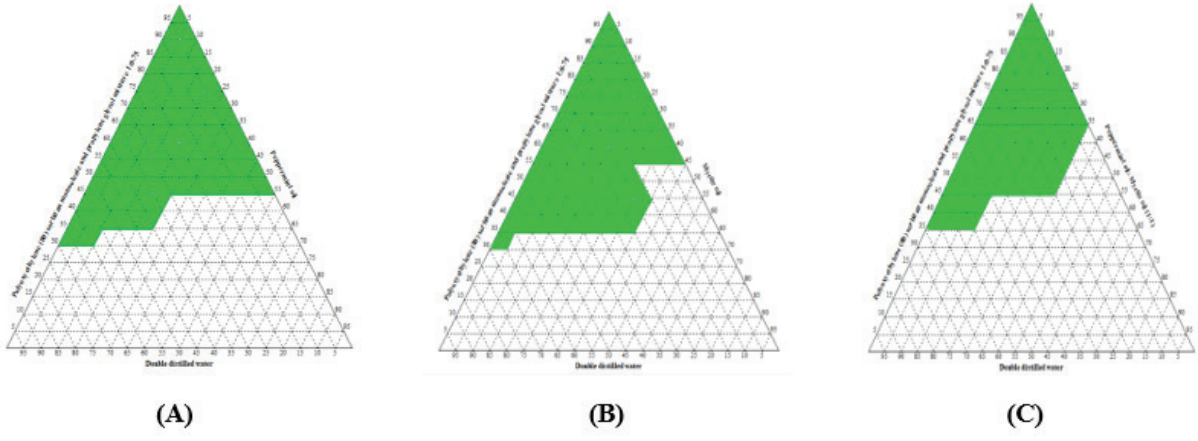
The study was conducted on 30 volunteers and was ethically approved by the Research Ethics Committee of Al-Mustaqbal University/College of Pharmacy (approval number: pHa2/2023, date: 10.02.2023). None of the volunteers had clinical signs of dermal abrasion or infection. The volunteers were asked to sign consent forms after explaining the research protocol with probable adverse effects. The evaluation was performed by applying 1 g of EO hand nanoemulgel (HN1-HN5) formulations on each intact area of the volunteer's hand skin and then allowed to wait for 10-15 min. A questionnaire was administered to the participants of the study to determine skin irritation and acceptability. The formulation was rated according to the characteristics of the EO hand nanoemulgel (HN1-HN5) formulations in terms of the formulation texture, appearance, smell, redness, and irritation or burning sensation after the product application.[12-14]

In vitro antimicrobial activity determination

The *in vitro* antimicrobial activity of the prepared EO hand nanoemulgel (HN1-HN5) formulations and blank gel (HN6) against two pathogenic bacterial species, Gram-negative *E. coli* and Gram-positive *S. aureus*, were studied using the agar disc diffusion method. The Gram-negative *E. coli* and Gram-positive *S. aureus* bacterial strains were obtained from Ali Obais Hospital, Babil Health Directorate, Ministry of Health, Iraq. All experimental conditions were performed under aseptic conditions. From each study sample, 10 µL was added to a sterile filter paper disc. Seven discs from each formula described in Table 1 were placed on each culture plate. Antibacterial activity was assessed by calculating the inhibition zone diameters in millimeters using a sliding caliper. The study was performed in triplicate.

Statistical analysis

The data of the study were obtained as the mean and standard deviation of three experimental trials. Statistical analysis was performed using Excel. One-Way analysis of variance (ANOVA) was used as a statistical test, where the level at ($p \leq 0.05$) was considered significant.[10,11]



(A) (B) (C)

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

RESULTS

Measurement of globule size, PDI, and ZP for nanoemulsion (N1-N5) formulations

The pseudo-ternary phase diagrams contained three structural components: EOs, a surfactant mixture (1:0.75) % *(w/w)*, and double-distilled water (Figure 1). A nanoemulsion of EO was prepared successfully using a microwave-based method and was characterized by ease, speed, and flexibility in preparation. The nanoemulsion is represented by a shaded area of pseudo-ternary phase diagrams, while the other area represents the emulsion. From the phase diagrams, five formulas were selected, namely, NE1, NE2, NE3, NE4, and NE5, for characterization of the globule size, PDI, and ZP. The particle size results were 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. The PDI experiment for nanoemulsion (NE1-NE5) formulations was from (0.26 to 0.385) as shown in Table 2. The outcomes of the mean absolute ZP value for nanoemulsion (NE1-NE5) formulations were (12.61 to 19.6 mV) as shown in Table 2.

AFM

The results show that the NE5 formula contains particles with regular smooth surfaces and nearly spherical shapes with nanometer-sized pores, as shown in Figure 2.

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

The organoleptic test was performed through naked-eye observations of the EO hand nanoemulgel (HN1-HN5) formulations. All HN1-HN5 formulations show clear, homogenous, with the characterized odor of EOs represented by peppermint oil and myrtle oil. No syneresis was observed.[12-14]

pH determination

pH evaluation is an important parameter that can be used to prevent unsuitable properties in nanoemulgels that are related to patient comfort. The pH values of the EO hand nanoemulgel (HN1-HN5) formulations were slightly acidic (5.4 to 5.89) as shown in Table 3.

Measurement of spreadability

The spreadability of EO hand nanoemulgel (HN1-HN5) formulations. The results were (128.22 to 124.22 g cm/sec).

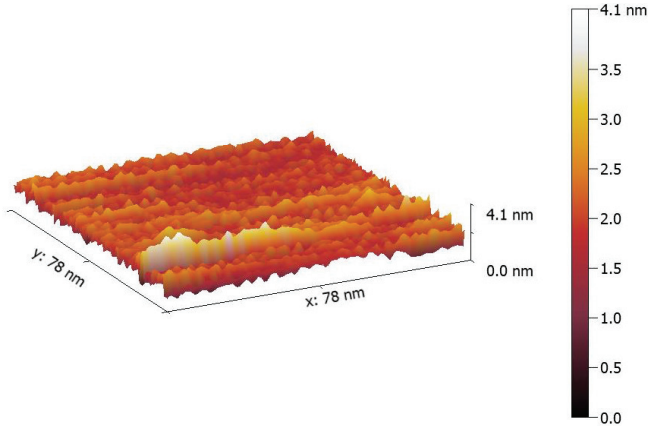


Figure 2. AFM 3D image of EO nanoemulsion (N5) formulation with a scanning area of 78 nm*78 nm

AFM: Atomic force microscopy, EO: Essential oil

Table 1. EO 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/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

EO: Essential oil

Table 2. Characterization results of nanoemulsion (NE1-NE5) formulations

Formulation code	Globule size (nm)*	PDI*	ZP*
NE1	25.83 ± 1.04	0.260 ± 0.015	12.61 ± 0.28
NE2	45.96 ± 1.00	0.368 ± 0.035	17.53 ± 0.43
NE3	29.83 ± 1.26	0.292 ± 0.006	15.69 ± 0.34
NE4	49.83 ± 0.85	0.385 ± 0.006	18.43 ± 0.23
NE5	55.86 ± 1.09	0.380 ± 0.013	19.60 ± 0.28

*Values are expressed as mean ± SD (n= 3), SD: Standard deviation, PDI: Polydispersity index, ZP: Zeta potential

Viscosity measurement

A viscometer with a spindle number (2) of a rotational digital type (Biobase Meihua Trading Co., Ltd. was exploited to measure the viscosity and study the rheology behavior of EO hand nanoemulgel (HN1-HN5) formulations as shown in Table 3.

Skin irritation study

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

In vitro antimicrobial activity determination

The experiment of *in vitro* antimicrobial activity was conducted successfully for EO hand nanoemulgel (HN1-HN5) formulations and blank gel (HN6) against Gram-negative *E. coli* and Gram-positive *S. aureus*. We found that increasing the EO concentration increased the bacterial growth inhibition for *S. aureus* and *E. coli*, as shown in Figure 3.

DISCUSSION

Particle size is a cornerstone of nanotechnology, influencing drug delivery, bioavailability, and cellular interactions. Smaller particles offer increased surface area, enhancing dissolution and absorption. Precise size control ensures targeted delivery and reduced side effects, optimizing therapeutic outcomes. In nanotechnology, mastering particle size transforms materials into powerful tools for innovation and healthcare. The results indicates the colloidal features of NE1-NE5 formulations. It was

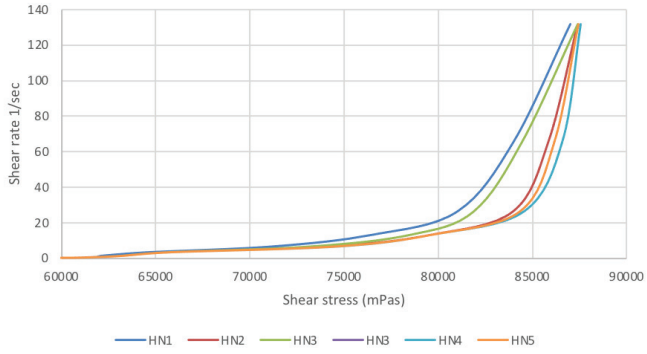


Figure 3. Shear stress against a shear rate of EO hand nanoemulgel (HN1-HN5) formulations

EO: Essential oil

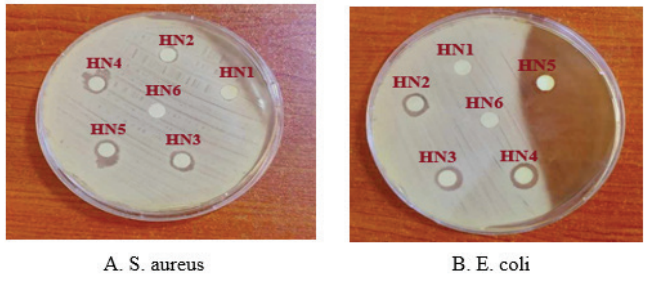


Figure 4: Inhibition zone of the prepared EO hand nanoemulgel (HN1-HN5) formulations compared to blank gel (HN6) formulation (A): *Staphylococcus aureus* and (B): *Escherichia coli*

EO: Essential oil

Table 3. Evaluation of EO hand nanoemulgel (HN1-HN5) formulations

Code	Color	Odor	Syneresis	Homogeneity	pH*	Mean spreadability (gcm/sec)*	Viscosity at 12 rpm (mP.s)*
HN1	Colorless	Aromatic smell	No	Homogeneous	5.60 ± 0.10	128.22 ± 0.12	3074.68 ± 2.54
HN2	Colorless	Aromatic smell	No	Homogeneous	5.89 ± 0.09	124.52 ± 0.09	3186.55 ± 2.50
HN3	Colorless	Aromatic smell	No	Homogeneous	5.40 ± 0.29	127.28 ± 0.14	3107.33 ± 2.08
HN4	Colorless	Aromatic smell	No	Homogeneous	5.62 ± 0.12	124.45 ± 0.12	3203.41 ± 2.53
HN5	Colorless	Aromatic smell	No	Homogeneous	5.85 ± 0.05	124.22 ± 0.11	3195.26 ± 2.40

*Values are expressed as mean ± SD (n= 3), $p \leq 0.05$. SD: Standard deviation, EO: Essential oil

Table 4. Antibacterial activity of different EO hand nanoemulgels (HN1-HN5) formulations compared with the blank gel (HN6) formulation

Formulation code	Inhibition zone of *Staphylococcus aureus* (mm)	Zone of inhibition of *Escherichia coli* (mm)
HN1	0.00 ± 0.00	0.00 ± 0.00
HN2	10.63 ± 0.15	12.33 ± 0.25
HN3	11.27 ± 0.25	12.70 ± 0.20
HN4	11.50 ± 0.20	13.76 ± 0.15
HN5	12.56 ± 0.15	37.03 ± 1.66
HN6 (BlanK)	0.00 ± 0.00	0.00 ± 0.00

*Values are expressed as mean ± SD (n= 3), $p \leq 0.05$. SD: Standard deviation, EO: Essential oil

found that as the concentration of EOs increased, the globule size increased at a constant surfactant: co-surfactant blend concentration. The particle size values have the following ascending order for NE1 < NE2 in formulations containing peppermint oil while NE3 < NE4 in formulations containing myrtle oil. In comparison with similar quantities of EO of different types, it had the following ascending order for NE2 < NE4 < NE5. The globule size increased as the lipid content increased due to an increase in the colloidal dispersion viscosity that made the dispersed globules more resistant to the breakdown of large droplets into smaller droplets during the emulsification process.[9,11] The polydispersity index reflects particle size uniformity, ensuring stability and consistent performance in nanotechnology applications. Low PDI enhances reliability, enabling precise drug delivery, controlled release, and reproducible therapeutic outcomes in advanced formulations. The outcom of PDI experiment indicates a high homogeneous and constricted size distribution for nanosystems.[9-11] Zeta potential determines nanocarrier stability by indicating surface charge, preventing aggregation. High zeta potential enhances colloidal stability, prolongs shelf life, and ensures efficient drug delivery through improved bioavailability and targeted interactions. The outcomes of the mean absolute ZP value indicates the stability of the nanoemulsions. There should be a higher electrical charge on the surface particles of the nanoemulsions to prevent their aggregation in the solutions due to the strong resistance violence among the 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 to ionic stabilizers, but not for large or large molecular weight surfactants such as tween 80, which are nonionic stabilizers that provide steric stability.[9,11] The ANOVA 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 ≤ 0.05. AFM enables precise characterization of nanocarriers, revealing surface morphology, size, and mechanical properties. Its high-resolution imaging ensures quality control, advancing nanocarrier development for effective drug delivery and targeted therapies. The results show that the NE5 formula contains particles with regular smooth surfaces and nearly spherical shapes with nanometer-sized pores, as shown in Figure 2. There was no particle aggregation, indicating the physical stability of the preparation. Organoleptic assays assess nanoemulgel's sensory attributes like color, odor, and texture, ensuring patient acceptance. These evaluations enhance product appeal and compliance, crucial for the success of innovative pharmaceutical and cosmetic formulations. It was performed that HN1-HN5 formulations show clear, homogenous, with the characterized odor of EOs represented by peppermint oil and myrtle oil. No syneresis was observed, which indicates high physical stability.[12-14] The pH is critical in nanoemulgel formulations, ensuring stability, compatibility with skin, and therapeutic effectiveness. Proper pH balance minimizes irritation, enhances drug delivery, and preserves the formulation's integrity during storage and application. It was found that an increment in the EO concentration slightly increased the pH. The outcomes provided suitable pH that guaranteed patient comfort and prevented skin allergic reactions and dermatitis.[12-14] The ANOVA showed a significant relationship between the dependent factor, which was pH, and the quantity of EO at the level ($p \leq 0.05$). Spreadability measures the ease of application of a nanoemulgel, ensuring uniform distribution on the skin. Optimal spreadability enhances user comfort, drug penetration, and overall efficacy of the therapeutic formulation. 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 because 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 decreased spreadability parameter due to increased viscosity of EO hand nanoemulgel (HN1-HN5) formulations. Generally, the outcome indicates low spreadability time for all EO hand nanoemulgel (HN1-HN5) formulations that enhance patient compliance upon application on the skin.[12-14] The ANOVA indicates a significant relationship between the spreadability and experimental oil (peppermint and myrtle oil) at the level ($p \leq 0.05$). Viscosity determines the nanoemulgel's consistency, impacting application, stability, and drug release. An optimal viscosity ensures smooth spreading, prevents phase separation, and enhances the formulation's performance, making it suitable for therapeutic and cosmetic applications. The obtained data include the shear rate, shear stress, and viscosity. The outcome of viscosity as shown in Table 3 indicates that the quantity of EO 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 12 rpm in (mP.s) units were HN1 = 3074.68, HN2 = 3186.55, HN3 = 3107.33, HN4 = 3203.41, and HN5 = 3195.26. This is due to the increase in the volume concentration of nanoglobules, which makes the colloidal dispersion system more resistant to flow. In addition, decreasing the aqueous phase volume will reduce the continuous phase volume and make the nanosystem more viscous. The rheogram chart was obtained by plotting the shear rate (1/sec) against the shear stress (mP.s), as shown in Figure 3. All EO hand nanoemulgel (HN1-HN5) formulations show plastic flow, which is a non-Newtonian flowing system because there is no gel flowing related to shear stress until it reaches a specific transition point. This plastic flow made formulations easier to wipe on infected skin or membranes and provided additional stability to EO hand nanoemulgel (HN1-HN5) formulations. ANOVA confirmed a significant relationship ($p \leq 0.05$) between viscosity and EO.[12-14] Skin irritation studies are essential in nanoemulgel formulation to ensure safety and patient comfort. By assessing potential skin reactions, these studies help optimize ingredients, prevent adverse effects, and guarantee that the formulation is suitable for sensitive skin. Ultimately, they enhance the therapeutic effectiveness and

consumer acceptability of the product. It was found that all formulations did not produce a sense of skin itching, irritation signs, or any painful skin effect after the application of gel to the participants in the experiment. This indicates that all EO hand nanoemulgel (HN1-HN5) formulations are comfortable and well-tolerated. *In vitro* antimicrobial activity determination is crucial in nanoemulgel formulations to evaluate their effectiveness against pathogens. This testing ensures the formulation's therapeutic potential, supporting its use in treating infections and promoting skin health. It was found no *S. aureus* activity in the HN1 and blank gel (HN6) formulations. The comparability profile of bacterial susceptibility for *S. aureus* was in the following ascending order: HN2 < HN3 < HN4 < HN5. The bacterial susceptibility profiles were significantly higher ($p < 0.05$) for HN5 and significantly lower ($p < 0.05$) for HN2, as shown in Table 4. It was found that *S. aureus* was relatively more sensitive to formulations containing myrtle oil as HN3 and HN4 than those containing peppermint oil as HN1 and HN2 at the same concentrations. It was found that EO hand nanoemulgel (HN1-HN5) formulations had higher microbial activity against *E. coli* than *S. aureus*, as shown in Figure 4. There was no *E. coli* activity in the HN1 and blank gel (HN6) formulations. The comparability profile of bacterial susceptibility for *E. coli* was in the following ascending order: HN2 < HN3 < HN4 < HN5. The bacterial susceptibility profile was significantly higher in microbial growth inhibition for HN5 and significantly lower ($p \leq 0.05$) in microbial growth inhibition for HN2, as shown in Table 4. It was found that a mixture of EOs increased activity against microbial growth, as shown by the 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,which were used in EO nanoemulgel HN1-HN5 formulations that makes it the most high-level technique for nanocarrier preparation. The great trend toward the use of skin antiseptics and the presence of some side effects of alcoholic antiseptics enhances the status of vegetable EOs 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 EOs in combating germs, and that mixing these EOs gives additional strength and motivation toward combating germs, as in the HN5 formulation that contains peppermint oil: myrtle oil (1:1) % *w/w*, which shows greater antimicrobial activity against *S. aureus* and *E. coli*.

Acknowledgements

The authors would like to thank Prof. Dr. Hasan Shakir Majdi, President of Al-Mustaqbal University for scientific research support.

Ethics

Ethics Committee Approval: The study was ethically approved by the Research Ethics Committee of Al-Mustaqbal University/ College of Pharmacy (approval number: pHa2/2023, date: 10.02.2023).

Informed Consent: Informed consent was obtained.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: This project was made possible with financial support from Al-Mustaqbal University (grant number: MUC-M-0222).

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