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# Development of Green-Synthesized Silver Nanoparticle–Oregano Oil Nanoemulsion for Antibacterial Mouthwash: Formulation, Physicochemical Stability, and Antibacterial Evaluation

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## Abstract.

Oral hygiene is essential for maintaining health, and antimicrobial mouthwashes have important role in preventing oral microbial infections. This study developed and evaluated a natural antibacterial mouthwash formulated as a nanoemulsion, containing green-synthesized silver nanoparticles (AgNPs) and *Origanum vulgare* (oregano) essential oil. The AgNPs were synthesized with epigallocatechin gallate (EGCG) which derived from green tea, which act as both a reducing and stabilizing agent. Oregano essential oil is rich in phenolic compound such as carvacrol and thymol, which acts a natural natural antifungal and antibacterial. The effects of oregano oil concentration and nanosilver addition on the characteristics, stability, and antibacterial activity of an oregano oil-nanosilver nanoemulsion using Tween 80 as a surfactant were studied. The nanoemulsion formulation was optimized by varying oregano oil and Tween 80 concentrations. Selected formulations containing 0.5% or 1.0% oregano oil, with or without 10% AgNPs were characterized in terms of pH, particle size, polydispersity index (PDI), organoleptic properties during eight weeks of storage, and evaluated for its antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. All formulations exhibited acidic pH values ranging from 3.6 to 4.5, which remained stable throughout storage. The nanoemulsions had mean particle sizes of 20–45 nm and PDI values below 0.30, indicating a homogeneous nanoscale dispersion. The formulation containing 1.0% oregano oil and AgNPs showed particle aggregation after four weeks, whereas formulations containing 0.5% oregano oil, with or without AgNPs, remained physically stable for eight weeks. Incorporating AgNPs slightly enhanced the antibacterial activity of the oregano oil nanoemulsion against both test microorganisms compared with the corresponding formulation without nanoparticles.

These findings show that combining green-synthesized AgNPs with oregano essential oil produces a stable nanoemulsion and has potential as a sustainable, effective natural mouthwash for oral healthcare applications and aligning with SDGs 3 and 12.

**Keywords:** oregano oil, nanosilver, mouthwash, stability, antibacterial activity.

## 1. Introduction

Human health is a state of complete physical, mental, and social well-being, making disease prevention and health promotion continuous priorities. The pharmaceutical sector plays a crucial role in improving health by developing medicines and healthcare

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products that prevent, treat, and manage diseases and enhance quality of life [1]. Oral health is an integral component of general health because the oral cavity serves as the body's primary gateway. A balanced oral microbiome contributes to host defense and maintains oral homeostasis, whereas dysbiosis resulting from poor oral hygiene, excessive sugar consumption, or immune dysfunction promotes the growth of pathogenic microorganisms [2]. It also prevents infections by foreign pathogens and supports systemic functions, such as regulating blood pressure through nitrate processing. These bacteria form dental plaque, a structured biofilm that contributes to dental caries, gingivitis, periodontitis, and halitosis [3]. Therefore, controlling oral biofilms is essential for maintaining oral and systemic health [4].

Mouthwash is widely used as an adjunct to toothbrushing and flossing to reduce the microbial load in the oral cavity. In addition to freshening breath, antibacterial mouthwashes help prevent plaque buildup, dental caries, gingivitis, and other oral conditions [5]. Conventional mouthwashes commonly contain synthetic antimicrobial agents such as chlorhexidine, which effectively disrupt bacterial membranes and inhibit plaque formation. Chlorhexidine is widely used as antimicrobial mouthwash, but using it for a long time can cause side effects like tooth staining, changes in taste, and irritation of the mouth. Because of this problems, more people are looking into natural alternatives [6].

Silver nanoparticles (AgNPs) are recognized as promising antimicrobial agents due to their nanoscale dimensions, large surface area, and multiple antibacterial mechanisms. AgNPs disrupt bacterial membranes, generate reactive oxygen species (ROS), interfere with DNA replication, and inhibit essential metabolic pathways. These actions render AgNPs effective against both Gram-positive and Gram-negative bacteria and reduce the likelihood of bacterial resistance [7]. In producing AgNPs, green synthesis methods uses plant extracts such as *Aloe vera*, green tea, and neem, and become one of the promising method because it uses non-harmful reducing agents [8]. *Origanum vulgare* (oregano) essential oil, which have high levels of carvacrol and thymol, have strong antibacterial, antifungal, antioxidant, and anti-inflammatory effects [9]. Its mechanism includes disrupting bacterial membranes, prevent biofilm formation, and targets pathogens such as *Streptococcus mutans* and *Porphyromonas gingivalis*. These bacteria are often associated to dental caries and periodontal disease [10] [11]. Oregano oil also has antioxidant properties that may help reduce oxidative stress and inflammation in the mouth [12].

Despite its strong biological activity, oregano oil has limitation in the water solubility, high volatility properties and susceptible to oxidation. Therefore, it is limited in direct use for aqueous formulations [13]. Nanoemulsion technology overcome these challenges by producing nanometer-sized oil droplets with improved dispersion, stability, bioavailability, and controlled release [14]. This approach also enhance antibacterial efficacy by increasing the contact surface between bioactive compounds and bacterial cells [15]. Combination between oregano oil nanoemulsion with AgNPs offers promising stronger antibacterial effect. AgNPs has an oxidative stress and damage in bacterial membranes properties, while carvacrol and thymol from oregano oil weaken the membranes and prevent biofilm formation. Using nano-sized delivery systems also helps spread and release both active ingredients over time, which is useful for oral healthcare [16].

The safety and efficacy of oregano oil–AgNP formulations are attributed by ingredient concentration, physicochemical stability, and overall formulation composition. The synergistic effect of antibacterial from the combination permits uses at lower concentrations, which may reduce cytotoxicity and mucosal irritation risk. Excipients such as glycerin, xylitol, and *Aloe vera* may further improve formulation acceptability, while maintaining physiological pH to preserve oral tissues and enamel integrity [17]. Despite the promising antimicrobial properties of oregano oil and AgNPs, developing a stable nanoemulsion mouthwash remains challenging because phase separation risk, particle aggregation, and physicochemical instability may occur during storage [18]. Few studies have systematically evaluated how oregano oil and AgNP concentrations affect the physicochemical characteristics and stability of nanoemulsion mouthwash formulations. Therefore, this study aims to develop a nanoemulsion mouthwash containing oregano essential oil and green-synthesized silver nanoparticles, and to evaluate the effects of varying concentrations of oregano essential oil and AgNPs on organoleptic properties, particle size, polydispersity index (PDI), pH, and storage stability. The findings are expected to support the development of a safe, stable, and effective natural antibacterial mouthwash to improve oral healthcare. Furthermore, the use of green-synthesized silver nanoparticles and naturally derived oregano essential oil aligns with Sustainable Development Goal (SDG) 3, Good Health and Well-Being, by advancing preventive oral healthcare, and supports SDG 12, Responsible Consumption and Production, through sustainable, environmentally responsible formulation strategies.

## 2. Materials and Methods

### 2.1 Materials

Silver nitrate (Merck, Indonesia), oregano (*Origanum vulgare*) essential oil (Syailendra Agropreneur, Indonesia), Tween 80 and propylene glycol (PT Brataco, Indonesia), green tea extract (GTE) containing epigallocatechin gallate (EGCG) (ImaHerb Biotech, China).

### 2.2 Instrumentation

pH meter (Laqua PH2000, Horiba), Delsa Nano C particle analyzer (Beckman Coulter), UV–Visible spectrophotometer (Hitachi UH500), magnetic stirrer (Thermo Scientific), and analytical balance (Ohaus PA-2102, USA).

### 2.3 Preparation of Silver Nanoparticles

Silver nanoparticles (AgNPs) were synthesized using a green synthesis method. Silver nitrate and GTE solutions were prepared separately and then mixed under continuous magnetic stirring. The reaction pH was adjusted to 10 with 0.1 N NaOH and the mixture was stirred for 90 minutes. Formation of AgNPs was indicated by a color change from colorless to brown. The nanoparticles were characterized by UV–Visible spectrophotometry and dynamic light scattering to determine particle size and polydispersity index (PDI). The optimized AgNP formulation (1.5 mM AgNO<sub>3</sub> and 0.003% EGCG) was selected for mouthwash preparation.

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## 2.4 Optimization of Nanoemulsion Formulation

A preliminary optimization study used nine formulations prepared by varying Tween 80 and oregano oil concentrations as shown in Table 1, with 15% propylene glycol in all formulations. Distilled water was added to obtain the final volume. The optimization aimed to determine the surfactant and oregano oil concentration that produced a nanoemulsion with desirable particle size.

**Table 1.** Composition of Tween 80 and Oregano oil for nanoemulsion optimization.

| Code | Tween 80 | Oregano oil |
|------|----------|-------------|
| A1   | 2.5 %    | 0.25 %      |
| A2   | 2.5 %    | 0.5 %       |
| A3   | 2.5 %    | 1.0 %       |
| A4   | 5.0 %    | 0.25 %      |
| A5   | 5.0 %    | 0.5 %       |
| A6   | 5.0 %    | 1.0 %       |
| A7   | 7.5 %    | 0.25 %      |
| A8   | 7.5 %    | 0.5 %       |
| A9   | 7.5 %    | 1.0 %       |

## 2.5 Preparation of Mouthwash Preparation

The mouthwash were prepared with the composition as shown in Table 2. Tween 80 and propylene glycol were mixed under magnetic stirring for 5 minutes before adding oregano oil. After homogenization, AgNPs were incorporated according to the formulation composition, followed by the gradual addition of distilled water while stirring continuously for 30 minutes to obtain a homogeneous nanoemulsion. Each formulation was prepared in triplicate and transferred to amber glass bottles for storage.

**Table 2.** The oregano oil nanoemulsion mouthwash formula, with and without nanosilver.

| Substance          | Concentration (%) |      |     |      |
|--------------------|-------------------|------|-----|------|
|                    | B1                | B2   | C1  | C2   |
| AgNPs              | 0.0               | 10.0 | 0.0 | 10.0 |
| Oregano oil        | 0.5               | 0.5  | 1.0 | 1.0  |
| Tween 80           | 5                 | 5    | 5   | 5    |
| Propylene glycol   | 15                | 15   | 15  | 15   |
| Distilled water to | 100               | 100  | 100 | 100  |

## 2.6 Evaluation of Mouthwash Preparation

The organoleptic properties were evaluated in color, appearance, odor, and evidence of phase separation. The pH was measured with a calibrated pH meter. Particle size and polydispersity index (PDI) were determined by Dynamic Light Scattering (DLS) technique using a particle size analyzer.

## 2.7 Stability Evaluation

The formulations were stored at 24°C and 45% relative humidity in the dark condition for 56 days. Physicochemical evaluations were conducted on days 1, 3, 5, 7, 14, 21, and 56. Physical stability was observed by monitoring changes in appearance, particle size, PDI, and pH during storage.

## 2.8 The Antibacterial Activity Evaluation

The antibacterial activity of the nanoemulsions was evaluated using a modified broth dilution-turbidimetric assay with *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739 as representative Gram-positive and Gram-negative bacteria, respectively. Bacterial suspensions were prepared in sterile 0.9% NaCl and standardized spectrophotometrically. An aliquot of 200 µL of the bacterial suspension was added to 19.8 mL of nutrient broth, corresponding to a 1:100 dilution, based on previously reported inoculum preparation procedures [19]. Then, 5 mL of the nanoemulsion of B1, B2, C1, and C2 was added to the bacterial suspensions, which were shaken and incubated for 30 minutes. Further, 1 mL of the suspension was added to 10 mL of fresh NB and incubated at 37 °C for 24 h. Bacterial growth was evaluated by measuring turbidity as percentage transmittance at 580 nm, following spectrophotometric approaches previously reported for bacterial inoculum standardization [20]. Similar tests were conducted using silver nanoparticles and a nanoemulsion base. An antibiotic (kanamycin) at 50 ppm was used as a positive control.

## 3 Results and Discussion

### 3.1 Optimization of Tween 80 and Oregano Oil Concentration

The optimization is conducted by varying Tween 80 concentrations (2.5%, 5%, and 7.5%) and oregano oil concentrations (0.25%, 0.50%, and 1.00%) in all formula. From table 3, distinct result was evaluated, showed that Tween 80 and oregano oil concentrations affected the particle size and appearance of the nanoemulsion mouthwash. The formulas with 2.5% Tween 80 and higher oregano oil levels (A2 and A3) had larger particle sizes (434.2 nm and 556.7 nm, respectively) with cloudy appearance, indicating inadequate emulsification. Increasing Tween 80 concentration improved clarity and reduced particle size. This is because Tween 80 that can spontaneously align at the oil–water boundary, orienting their hydrophilic polyoxyethylene heads toward the water phase and their lipophilic tails toward the oil phase, thereby directly lowering interfacial tension [21]. The A5 and A6 showed smaller particle sizes (22.6 nm and 19.9 nm, respectively) with acceptable PDI values, and were used for further formulation development.

**Table 3.** The particle size and PDI of nanoemulsion with different oregano oil and Tween 80 concentrations.

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| Code | Particle size (nm) | PDI   | Appearance |
|------|--------------------|-------|------------|
| A1   | 26.5               | 0.356 | Clear      |
| A2   | 434.2              | 0.124 | Cloudy     |
| A3   | 556.7              | 0.181 | Cloudy     |
| A4   | 18.2               | 0.426 | Clear      |
| A5   | 22.6               | 0.341 | Clear      |
| A6   | 19.9               | 0.400 | Clear      |
| A7   | 19.7               | 0.057 | Clear      |
| A8   | 17.5               | 0.505 | Clear      |
| A9   | 17.6               | 0.421 | Clear      |

Although the formula containing 7.5% Tween 80 (A7–A9) had smaller particle sizes, this higher surfactant concentration was avoided because it can affect mouthfeel and cause bitterness. The results indicate that an appropriate balance between oregano oil concentration and surfactant level is essential for a stable nanoemulsion. Increasing oregano oil concentration may raise viscosity and decrease Tween 80's effectiveness in stabilizing oil droplets, which may lead to droplet aggregation. In contrast, adequate Tween 80 levels provide steric stabilization by forming a protective layer around oil droplets, producing smaller and more stable particles. [15].

### 3.2 Evaluation of Oregano Oil–Nanosilver Nanoemulsion Mouthwash

**Table 4.** The characteristics of oregano oil–nanosilver nanoemulsion mouthwash.

| Code                    | B1                   | B2                   | C1                   | C2                   |
|-------------------------|----------------------|----------------------|----------------------|----------------------|
| Clarity                 | Clear                | Clear                | Clear                | Clear                |
| Colour                  | Slight yellowish     | Slight yellowish     | Slight yellowish     | Slight yellowish     |
| Odour                   | Slight oregano smell | Slight oregano smell | Strong oregano smell | Strong oregano smell |
| pH ± SD                 | 3.53 ± 0.02          | 4.31 ± 0.01          | 3.65 ± 0.03          | 4.46 ± 0.01          |
| Particle size ± SD (nm) | 31.0 ± 2.95          | 27.8 ± 2.75          | 25.3 ± 4.20          | 33.9 ± 6.71          |
| PDI ± SD                | 0.278 ± 0.03         | 0.304 ± 0.02         | 0.336 ± 0.07         | 0.209 ± 0.06         |

Organoleptic and physicochemical evaluations of the nanoemulsion B1, B2, C1, and C2 showed that all formulas were optically clear with a slight yellowish tint, where B1 and B2 had a mild oregano aroma, and C1 and C2 had a strong oregano aroma. The pH values of the nanoemulsions ranged from 3.53 ± 0.02 to 4.46 ± 0.01. This variation indicated that changes in phase composition ratios or surfactant-co-surfactant adjustments influence overall acidity. Mouthwash formulations generally require a lower pH range (3.5–6) to be effective against microbes. Although the results show a slightly acidic pH, short rinsing durations allow essential oil to retain their bioactive

properties and prevent significant changes to the tooth enamel surface morphology [22]. Particle size measurements confirmed the formation of nanosized droplets, with mean diameters ranging from  $25.3 \pm 4.20$  nm to  $33.9 \pm 6.71$  nm.

Formula C1 yielded the smallest mean droplet size ( $25.3 \pm 4.20$  nm), followed by B2 ( $27.8 \pm 2.75$  nm), B1 ( $31.0 \pm 2.95$  nm), and C2 ( $33.9 \pm 6.71$  nm). The PDI values ranged from  $0.209 \pm 0.06$  (C2) to  $0.336 \pm 0.07$  (C1), with B1 and B2 recording  $0.278 \pm 0.03$  and  $0.304 \pm 0.02$ , respectively. PDI values between 0.1 and 0.3 suggest a narrow and monodisperse size distribution, reflecting desirable kinetic stability against droplet coalescence and Ostwald ripening [23].

### 3.3 Stability Evaluation

Four optimized formulas (B1, B2, C1, and C2) were evaluated during 8-week. On day 1 evaluation all formulas appeared clear with a slightly yellowish color. Formulas containing 0.5% oregano oil (B1 and B2) exhibited a mild oregano odor, while formulas containing 1% oregano oil (C1 and C2) showed a stronger oregano aroma. The particle sizes ranged from 25.3–33.9 nm, with PDI values between 0.209–0.336, indicating acceptable nanoemulsion characteristics.

### 3.4 Organoleptic Properties

The organoleptic properties of the formulas were evaluated over 56 days of storage and are shown in Figure 1. Formulas B1, B2, and C1 maintained clear appearance, stable color, and consistent odor throughout the study period. However, C2 became cloudy after Day 21 and developed a slightly pungent odor, indicating physical instability. This instability may be associated with the high concentrations of both oregano oil and nanosilver, which may promote interactions between active components and destabilize the nanoemulsion system. Finding the appropriate oil concentration ensures nano-encapsulation, protecting active components such as carvacrol and linalool from degradation and preventing phase separation [24].

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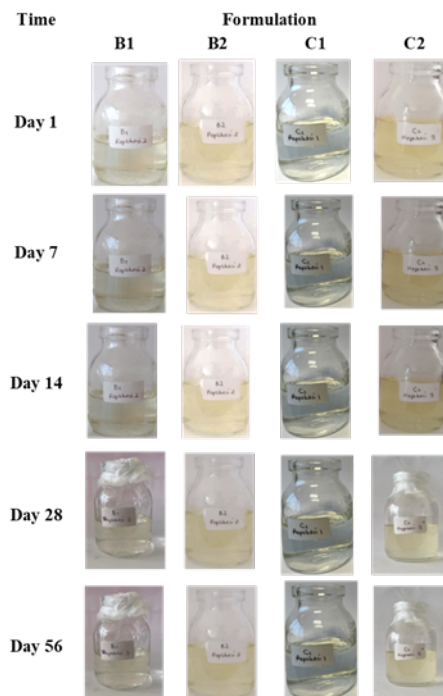


**Fig.1.** The visual of Oregano Oil–Nanosilver Nanoemulsion Mouthwash during a 56-day stability test.

### 3.5 pH Evaluation

All formulas maintained a slightly acidic pH of approximately 4.0 to 4.5 throughout the stability study. Nanosilver slightly increased the pH values of B2 and C2 compared with formulations without nanosilver. The pH values remained relatively constant, indicating good formulation stability. Slightly acidic conditions favor reduced microbial growth, but pH below 5.5 can initiate enamel erosion [25]. Increasing the nanoemulsion's pH to 6.0-7.0 is suitable for mouthwash.

### 3.6 Particle Size Analysis

Over the 56-day storage period, formulations B1, B2, and C1 maintained particle sizes below 100 nm, indicating good physical stability. The particle size from C1 formulation, ranged from approximately 23.3–31.2 nm during storage, suggesting improved dispersion and stability. In contrast, formulation C2 showed a high increase in particle size after day 21, from 74.6 nm to 239.4 nm during prolonged storage.

**Table 5.** The particle size of oregano oil–nanosilver nanoemulsion mouthwash during a 56-day stability test.

| Particle size (nm) $\pm$ SD |
|-----------------------------|
|-----------------------------|

| Time<br>(day) | B1          | B2          | C1         | C2           |
|---------------|-------------|-------------|------------|--------------|
| 1             | 31.0 ± 2.9  | 27.8 ± 2.74 | 25.3 ± 4.2 | 33.9 ± 6.7   |
| 5             | 30.7 ± 5.4  | 60.5 ± 24.5 | 31.2 ± 5.3 | 47.8 ± 10.5  |
| 7             | 34.2 ± 11.4 | 43.8 ± 11.9 | 25.3 ± 2.7 | 27.4 ± 2.5   |
| 14            | 29.7 ± 7.1  | 34.7 ± 5.5  | 24.8 ± 3.1 | 30.5 ± 3.6   |
| 21            | 64.5 ± 43.8 | 54.9 ± 32.6 | 25.2 ± 3.3 | 74.6 ± 42.2  |
| 56            | 55.4 ± 50.8 | 70.8 ± 3.9  | 23.3 ± 7.2 | 239.4 ± 24.3 |

This increase may be attributed to aggregation resulting from interactions between nanosilver particles and oregano oil components. Phenolic compounds such as carvacrol and thymol may interact with nanoparticle surfaces, disrupting colloidal stability and leading to particle aggregation [23].

### 3.7 Polydispersity Index (PDI)

The PDI evaluation during stability can be seen in Figure 2. All formulations showed acceptable PDI values during the stability study, ranging from approximately 0.2 to 0.35. The formulation exhibited a relatively uniform particle size distribution with moderate polydispersity (PDI 0.30–0.50), indicating an acceptable dispersion [26]. Formulation C2 maintained acceptable PDI values despite increased particle size, suggesting that particle growth occurred uniformly rather than through extensive aggregation. A narrow size distribution (low PDI) indicates uniform droplet sizes. High polydispersity promotes physical instability because droplets with different radii have different interfacial curvature and Laplace pressure, creating a chemical potential gradient that drives molecular diffusion from smaller droplets to larger droplets [27].

10

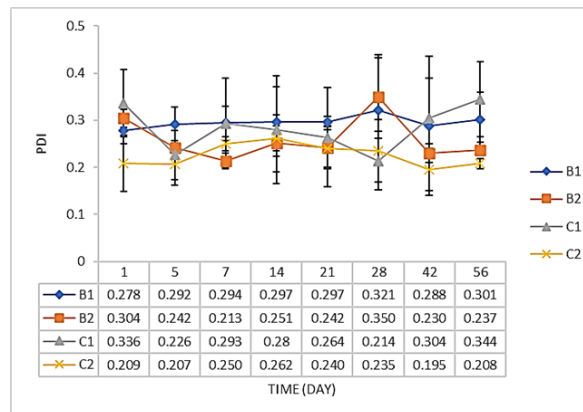


**Fig. 2.** The PDI of oregano oil–nanosilver nanoemulsion mouthwash during a 56-day stability test.

The formulations B1, B2, and C1 showed good stability based on organoleptic properties, pH, particle size, and PDI measurements. Among these formulations, C1 has maintained stability in term of particle size, pH, and physical appearance. Conversely, C2 was considered unstable because particle size increased and it became cloudy after 21 days, suggesting that excessive concentrations of oregano oil and nanosilver may affect nanoemulsion stability.

The findings demonstrate that the concentration of active ingredients plays a key role in determining the physicochemical properties and stability of nanoemulsion mouthwash formulations. Therefore, optimizing the ratios of oregano oil, nanosilver, and surfactant is essential for developing a stable and acceptable antibacterial mouthwash formulation.

### 3.8 The Antibacterial Activity

The antibacterial activity test showed that the combination of oregano oil and nanosilver slightly increased activity against the Gram-positive bacteria (*Staphylococcus aureus*) compared with the Gram-negative bacteria (*Escherichia coli*). Increasing the oregano oil concentration in the formula, with and without silver nanoparticles, results in higher activity against both bacteria.

**Table 6.** The results of the antibacterial activity test using a dilution-turbidimetry test of the positive control, silver nanoparticles (AgNPs), nanoemulsion base, and oregano oil–nanosilver nanoemulsion mouthwash

| Sample           | Transmission $\pm$ SD (%) |                              |
|------------------|---------------------------|------------------------------|
|                  | <i>Escherichia coli</i>   | <i>Staphylococcus aureus</i> |
| Positive control | 97.5 $\pm$ 0.05           | 97.6 $\pm$ 0.05              |
| Ag NP            | 40.8 $\pm$ 1.84           | 40.5 $\pm$ 0.99              |

|                   |             |             |
|-------------------|-------------|-------------|
| Nanoemulsion base | 47.0 ± 0.85 | 61.5 ± 0.68 |
| B1                | 81.5 ± 2.71 | 75.7 ± 1.98 |
| B2                | 81.5 ± 4.77 | 80.0 ± 2.19 |
| C1                | 91.3 ± 1.67 | 85.3 ± 3.91 |
| C2                | 91.7 ± 1.12 | 87.9 ± 0.41 |

## 4 Conclusion

This study showed that oregano oil and nanosilver concentrations influence the physicochemical characteristics and stability of nanoemulsion-based antibacterial mouthwash formulations. Varying the oregano oil concentration primarily affected particle size, whereas incorporating nanosilver altered particle growth and stability during 56 days of storage. Formulations B1, B2, and C1 remained stable throughout the 56-day stability study in term of organoleptic properties, pH, and particle size. C1 formula showed the most favorable properties, attributed to its small particle size, physicochemical properties, and antibacterial activity, but it needs to adjust the pH that is suitable for mouthwash formulation. In contrast, formulation C2, which contained higher concentrations of both oregano oil and nanosilver, exhibited instability, characterized by increased particle size and cloudiness after prolonged storage. These results showed the importance of optimizing active-ingredient concentrations to achieve a stable nanoemulsion mouthwash with desirable physicochemical properties and antibacterial activity.

## Acknowledgement

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