Article

The Development of Mouth Nanospray for Black Cumin Essential Oil (*Nigella sativa* L.): Physical Properties, Stability and Antibacterial Activity

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Abstract

Halitosis is a common oral health problem that can reduce quality of life. This study aimed to evaluate the physical properties, nanoparticle characteristics, and antibacterial activity of a mouth nano spray containing black cumin essential oil (*Nigella sativa* L.). Four formulations were prepared with different essential oil concentrations: F1 (0.25%), F2 (0.5%), F3 (1.0%), and F4 (1.5%). Physical quality evaluation included organoleptic properties, pH, homogeneity, and viscosity. Nanoparticle characterization was performed using a Particle Size Analyzer (PSA), while antibacterial activity against Streptococcus mutans was assessed using the disc diffusion method, with chlorhexidine as the positive control and aquadest as the negative control. All formulations exhibited acceptable physical characteristics as clear and homogeneous liquids, with pH values ranging from 6.0 to 6.7, indicating suitability for oral application. PSA analysis was conducted on formulation F2, which was selected as a representative formulation with the best physical quality. The results showed a mean particle size of 42.7nm with a polydispersity index of 0.6, indicating a relatively broad particle size distribution. The zeta potential value of −5.6mV suggested low electrostatic stability and a potential risk of particle aggregation, indicating the need for further optimization. Antibacterial activity testing demonstrated that formulations F2, F3, and F4 exhibited strong inhibitory effects, with inhibition zone diameters of 12.62 mm, 12.53 mm, and 12.32 mm, respectively, exceeding that of the positive control (8.39mm).

Keywords: *Halitosis, mouth nanospray, Nigella sativa L., nanoemulsion, Streptococcus mutans*

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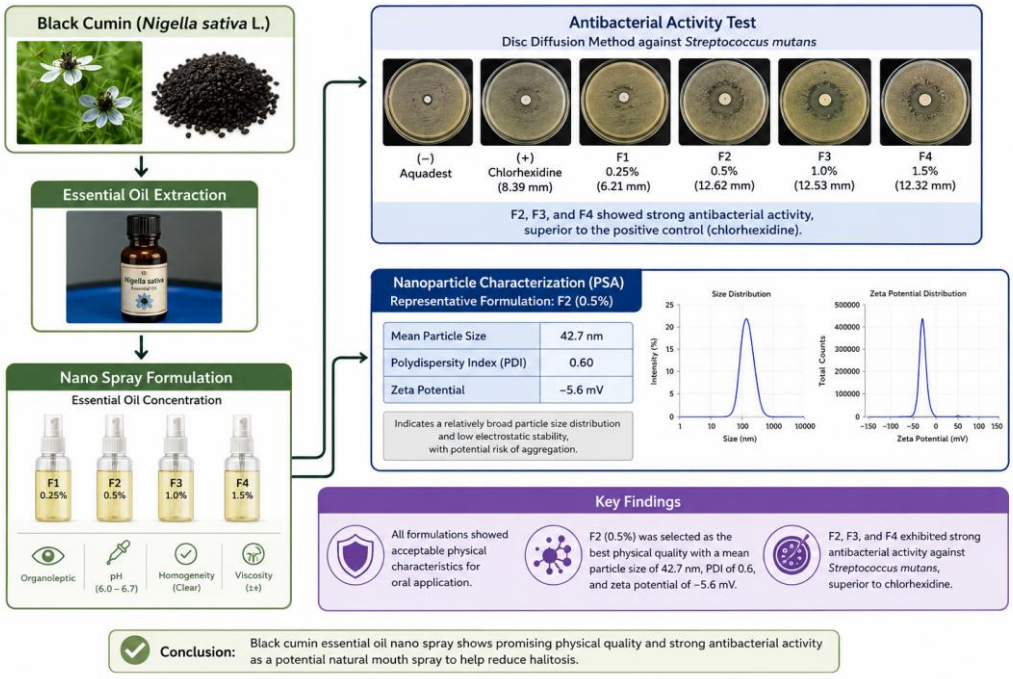
Graphical Abstract



Introduction

Oral health problems remain a major public health concern in Indonesia, with a high prevalence reported in national surveys. According to the 2018 Basic Health Research (Riskesdas), 57.6% of the population experienced dental and oral health problems, many of which were associated with secondary conditions such as halitosis. Halitosis, or oral malodor, is often underestimated despite its significant impact on self-confidence and social interactions. This condition generally arises from microbial activity in the oral cavity, particularly the degradation of proteins by oral bacteria, resulting in the production of volatile sulfur compounds (VSCs) and organic acids that cause unpleasant odors[1]. Among oral microorganisms, *Streptococcus mutans* plays an important role in the development of oral diseases, including dental caries and plaque formation. Although halitosis is multifactorial, *S. mutans* contributes indirectly by promoting biofilm formation and creating an acidic environment that supports the growth of other odor-producing bacteria. In addition, its metabolic activity leads to the accumulation of organic acids that may exacerbate oral malodor, making it a relevant target organism in antibacterial studies related to halitosis [1,2].

Various strategies have been developed to manage halitosis, including the use of synthetic mouthwashes containing active agents such as chlorhexidine[3]. Although chlorhexidine is effective in reducing oral bacterial load, its long-term use has been associated with adverse effects, including mucosal irritation, tooth discoloration, taste disturbance, and disruption of the normal oral microbiota. These limitations have driven interest in herbal-based oral care products as safer alternatives. One such innovation is the formulation of black cumin extract mouthwash in the form of a dip bag; however, this preparation still faces challenges related to physical stability, uneven distribution of active compounds, and the potential for tooth staining [4].

Black cumin (*Nigella sativa* L.) has been widely recognized for its antibacterial properties, primarily attributed to bioactive constituents such as thymoquinone, thymohydroquinone, and thymol. These compounds have demonstrated inhibitory effects against various pathogenic microorganisms, including bacteria associated with oral infections[5]. Despite this potential, conventional herbal formulations often suffer from limited bioavailability and suboptimal delivery of active compounds within the oral cavity, thereby reducing their therapeutic effectiveness [6].

Nanotechnology offers a promising approach to overcome these limitations by enhancing the stability, bioavailability, and penetration of active compounds. Nanoemulsion-based delivery systems can improve the uniform distribution of hydrophobic essential oils and protect them from degradation. In particular, nano spray dosage forms provide additional advantages, including ease of use, improved hygiene, rapid onset of action, and more uniform dispersion of nanosized particles across the oral surfaces. Compared to conventional herbal mouthwashes, nano spray formulations are expected to offer enhanced antibacterial efficacy with reduced side effects [7].

However, studies focusing on the formulation of black cumin essential oil nano sprays for oral health applications, particularly for halitosis management, remain limited. Therefore, this study aimed to formulate and evaluate a mouth nano spray containing black cumin essential oil by assessing its physical characteristics (particle size, pH, viscosity, and spray properties) and antibacterial activity against *Streptococcus mutans*. The findings of this study are expected to provide scientific evidence supporting the development of innovative, safe, and effective nano-based herbal oral care products, as well as to contribute to further research in the fields of pharmaceutical technology and public health [8].

Materials and Methods

Materials

Black cumin essential oil (*Nigella sativa* L.) was obtained from PT. Kisbiokim Medika Laboratori, West Sumatra, Indonesia. Tween 80, Virgin Coconut Oil (VCO), polyethylene glycol 400 (PEG 400), potassium sorbate, and peppermint oil were purchased from Kimia Jaya Abadi, Semarang, Indonesia. Sterile Aquadest was obtained from Onitsuka®, Japan. Paper discs were used for antibacterial testing. *Streptococcus mutans* ATCC 25176 was employed as the test microorganism, and Mueller–Hinton Agar (MHA) medium was purchased from Himedia, Jakarta, Indonesia. Chlorhexidine 0.1% (Minosep®, Minirock Mandiri, West Java, Indonesia) was used as the positive control.

Formula Mouth Nano Spray Essential Oil of Black Cumin Seeds (Nigella sativa L.)

The mouth nano spray formulation was developed using black cumin seed essential oil (*Nigella sativa* L.) as the primary antibacterial active compound. A nanoemulsion delivery system was employed to improve the formulation's physicochemical stability, solubility, and bioavailability. Tween 80 and PEG 400 were utilized as the surfactant and co-surfactant, respectively, while virgin coconut oil (VCO) served as the oil phase. Potassium sorbate was incorporated as a preservative, peppermint oil as a flavoring agent, and distilled water (aquadest) as the aqueous solvent. Four formulations (F1–F4) containing different concentrations of *Nigella sativa* essential oil were prepared to

evaluate the influence of essential oil concentration on the physicochemical characteristics and antibacterial activity of the mouth nano spray, as summarized in Table 1.

Preparation of Mouth Nanospray

The formulation of the *Nigella sativa* essential oil mouth nano spray was carried out using a nanoemulsion technique. All equipment and materials were prepared in advance, and each component was accurately weighed and measured according to the required formulation.The oil phase was prepared by mixing black cumin essential oil with Virgin Coconut Oil (VCO) until a homogeneous mixture was obtained (Mixture 1). Separately, Tween 80 (surfactant) and polyethylene glycol 400 (PEG 400, co-surfactant) were mixed until homogeneous (Mixture 2). Mixture 2 was then gradually added to Mixture 1, followed by the addition of peppermint oil. The resulting oil phase was stirred using a magnetic stirrer at 600 rpm and heated to 50 °C. This temperature was selected to reduce interfacial tension and viscosity, thereby facilitating emulsification without degrading the essential oil components. The aqueous phase was prepared by dissolving potassium sorbate (0.1 mg) in sterile distilled water, followed by heating to 50 °C to match the oil phase temperature and prevent premature phase separation.

Table 1. Formulation *of Mouth Nano Spray* Black Cumin Seed Essential Oil Preparation

Materials	Concentration (% v/v)				Function
	FI	FII	FIII	FIV	
Black cumin seed essential oil (*Nigella sativa* L.)	0.25	0.5	1	1.5	Anti-bacterial active ingredients
Tween 80	7	7	7	7	Surfactant
VCO	2	2	2	2	Oil phase
PEG 400	3	3	3	3	Co- surfactan
Potassium sorbate	0.1	0.1	0.1	0.1	Preservatives
Peppermint oil	0.25	0.25	0.25	0.25	Fresheners
Aquadest	ad 100ml	ad 100ml	ad 100ml	ad 100ml	Solvent

The emulsification process was initiated by gradually adding the oil phase into the aqueous phase under continuous magnetic stirring at 800 rpm to ensure uniform dispersion. Distilled water was subsequently added to obtain a final formulation volume of 100 mL. The resulting coarse emulsion was further homogenized using a mechanical homogenizer at 10,000 rpm for 20–30 min, followed by ultrasonication for 60 min to reduce droplet size and promote the formation of a stable nanoemulsion system. The process yielded a clear and transparent nano spray formulation, indicating successful nanoemulsion formation.

Physical Quality and Stability of Nano Spray

Physical quality and stability evaluations were conducted to assess the physical integrity of the mouth nano spray and to detect any signs of phase separation during the storage period [9]. All formulations were stored in tightly closed containers at room temperature (25 ± 2 °C) and protected from direct light for four weeks [9].

Organoleptic Evaluation. Organoleptic properties, including appearance, color, odor, and taste, were evaluated by visual and sensory observation. The assessment was performed weekly for four weeks by observing several changes in clarity, color uniformity, and odor intensity. Taste evaluation was conducted cautiously using a small amount of sample to assess acceptability and the absence of irritation [10].

pH Measurement. The pH of each formulation was measured using a calibrated digital pH meter at room temperature. Measurements were conducted weekly for four weeks. The acceptable pH range for oral preparations was set between 6.0 and 7.0 to ensure compatibility with the oral cavity and to minimize the risk of mucosal irritation [11].

Homogeneity Test. Homogeneity was evaluated by visual inspection of the formulation spread on a clean glass slide under adequate lighting conditions. The presence or absence of visible coarse particles, sedimentation, or phase separation was observed at weeks 0–4. A formulation was considered homogeneous if no visible particles or separation were detected throughout the observation period.

Viscosity Test. Viscosity was determined using an Ostwald viscometer following a standard procedure. A volume of 5 mL of the formulation was introduced into the viscometer and allowed to equilibrate at 25 ± 1 °C. The flow time between the upper and lower calibration marks was recorded using a stopwatch, and viscosity values were calculated by comparison with aquadest as the reference liquid.

Characterization of Mouth Nano Spray Nanoparticles

The nanoparticle characteristics of the mouth nano spray containing black cumin (*Nigella sativa* L.) essential oil were evaluated using a Particle Size Analyzer (PSA). This analysis was conducted to determine the principal physicochemical parameters of the nanoemulsion system, including mean particle size, polydispersity index (PDI), and zeta potential. These parameters are essential for assessing particle size uniformity, dispersion homogeneity, and the colloidal stability of the developed nano spray formulation.

The mean particle size provides information regarding the nanoscale dimensions of the dispersed droplets, while the PDI value indicates the width of particle size distribution and the degree of system homogeneity. In addition, zeta potential analysis was performed to evaluate the electrostatic stability of the nanoemulsion by measuring the surface charge of the particles. Higher absolute zeta potential values generally indicate greater repulsive forces between particles, contributing to improved colloidal stability and reduced aggregation tendency.

Formulations selected for PSA characterization were based on preliminary physical evaluation, including clarity, homogeneity, absence of phase separation, and overall physical stability during storage. The selected formulation was considered the most representative system for describing the nanoparticle characteristics of the developed mouth nano spray preparation.

Particle Size Test. Particle size measurements were carried out using a Particle Size Analyzer (PSA) with the Dynamic Light Scattering (DLS) principle. The mouth nanospray sample was first diluted using distilled water as a dispersion medium until a suitable concentration was obtained for

measurement, to prevent multiple scattering. A total of ±1 mL of sample was placed into a clean cuvette that had been cleaned beforehand to avoid contamination. Measurements were carried out at a controlled temperature of 25 °C with three repetition to ensure the accuracy and reproducibility of the results. The average particle size obtained was used to confirm the formation of the nanoparticle system.

Polydispersity Index (PDI). Polydispersity index (PDI) testing was performed using a PSA instrument with the DLS principle under the same measurement conditions. The PDI value is used to evaluate the particle size distribution and the level of homogeneity of the nanoparticle system. Scientifically, a PDI value <0.3 indicates a narrow particle size distribution and a relatively homogeneous system, while a PDI value between 0.3–0.6 is still acceptable for pharmaceutical nanoparticle systems but indicates a broader size distribution. A high PDI value indicates the presence of particle size irregularities and the potential for aggregation in the system.

Zeta Potential Test. Zeta potential measurements were performed using a Particle Size Analyzer (PSA) by taking ±1 mL of sample that had been diluted using the appropriate medium. The sample was placed in a special cuvette for zeta potential measurement, then analyzed at 25 °C with three repeated measurements. The zeta potential value is used as an indicator of the colloidal stability of nanoparticles. In general, nanoparticle systems with zeta potential values greater than +30 mV or less than −30 mV are considered to have good electrostatic stability due to the presence of repulsive forces between particles that are strong enough to prevent aggregation. Zeta potential values close to zero indicate low stability and a tendency for particle clumping [12].

In Vitro Antibacterial Activity

The antibacterial activity of the mouth nano spray formulations was evaluated using the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) M07-A9 guidelines [13]. *Streptococcus mutans* ATCC 25176 was employed as the test microorganism due to its important role in oral pathogenicity and halitosis-associated conditions.

Mueller–Hinton Agar (MHA) medium was prepared by dissolving 38 g of MHA powder in 1000 mL of distilled water, followed by heating until completely dissolved. The prepared medium was sterilized in an autoclave at 121 °C under 1–2 atm pressure for 15 min After sterilization, the medium was cooled to approximately 40–45 °C and aseptically poured into sterile Petri dishes.

The bacterial culture was rejuvenated on MHA plates and incubated at 37 °C for 24 h. A standardized bacterial suspension was then prepared by transferring 2–3 well-isolated colonies into sterile 0.9% NaCl solution. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL [14], to ensure uniform bacterial inoculum density during antibacterial testing.

The agar surface was inoculated evenly using a sterile cotton swab and allowed to dry for 3–5 min. Sterile paper discs (6 mm diameter) were impregnated with 20 µL of each mouth nano spray formulation and placed on the agar surface. Chlorhexidine 0.1% (Minosep®) and sterile distilled water were used as positive and negative controls, respectively. All tests were performed in triplicate [15]. The plates were incubated at 37 °C for 24 hours, after which the diameters of inhibition zones were measured using a digital caliper and expressed in millimeters

as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA followed by a post hoc test, with a significance level set at $p < 0.05$.

Result and Discussion

Physical Properties of the Nano Spray

Organoleptics. Organoleptic tests are carried out to assess the physical characteristics of the preparation including appearance, color, aroma, and taste. The results of observations show that the F1 0.5% formula is liquid, clear in color, transparent, and has a minty taste and aroma. At F2 1% it is liquid, clear in color, transparent, has a minty taste and aroma; F3 1.5% is liquid, white in color, has a minty flavor and aroma. While F4 1.5% is liquid, slightly brownish white (the typical color of black cumin seed essential oil) due to the highest concentration of essential oils used in the formulation, has a minty flavor and aroma. The formulas that are in accordance with the characteristics of mouth spray preparations are F1 and F2 (Table 2). In terms of color, F1 and F2, show good clarity and appear transparent, indicating that there is no precipitation or color change that can reduce the physical stability of the product. While F3 and F4 show that there is an effect of the concentration of active ingredients on the physical appearance of the prepared produced. In terms of aroma, the entire formula shows a distinctive mint smell that gives a fresh sensation. This is due to the volatile content of essential oils or mint flavors. The difference in aroma intensity in each formula can be affected by variations in the concentration of additives (e.g. *flavoring agent*) used. Meanwhile, in terms of taste, all formulas show a distinctive mint taste. The fresh taste produced is one of the important factors in increasing acceptability. Differences in flavor intensity between formulas are possible due to differences in concentration *flavoring agent* as well as the interaction of active ingredients with additives.

Table 2. Organoleptic data of Nano Spray

Formula	Organoleptics			
	Appearance	**Color**	**Aroma**	**Taste**
F1	Liquid	Clear	Mint	Mint
F2	Liquid	Transparent	Mint	Mint
F3	Liquid	White	Mint	Mint
F4	Liquid	Brownish White	Mint	Mint

Overall, the organoleptic evaluation demonstrated that all formulations fulfilled the essential quality requirements for mouth spray preparations, including a liquid dosage form, clear appearance, fresh aroma, and mint flavor. Differences among formulations were primarily observed in the intensity of aroma and taste, which were influenced by the concentration of black cumin essential oil incorporated into the formulation. These sensory variations may serve as important considerations in determining the formulation with the highest consumer acceptability and overall preference [16].

pH of Nano Spray

The pH evaluation was conducted to ensure that the mouth nano spray formulations were within the acceptable pH range for safe oral application (Table 3). An ideal pH range for oral spray preparations generally falls between 5.0 and 7.0, as this range minimizes the risk of irritation to the oral mucosa while

maintaining the stability of active compounds, particularly black cumin (*Nigella sativa* L.) essential oil.

The results demonstrated that all formulations exhibited pH values within the acceptable range of 5.0–7.0, indicating their suitability for application in the oral cavity. None of the formulations were excessively acidic or alkaline, thereby reducing the potential risk of irritation, discomfort, or damage to oral tissues during use. Overall, all formulations showed good pH stability, suggesting that the developed mouth nano spray possesses favorable physicochemical characteristics for oral administration and may effectively contribute to halitosis management without causing adverse effects on the oral mucosa.

Table 3. pH Test Results

Formula	pH value	Acceptable Range
F1	6.0	5.0-7.0
F2	6.0	5.0-7.0
F3	6.0	5.0-7.0
F4	6.0	5.0-7.0

Homogeneity of Nano Spray

On the homogeneity test of the preparation, it was obtained that all formulations (F1 0.25%; F2 0.5%; F3 1%; F4 1.5%) shows a homogeneous result. This indicates that all materials used can be mixed evenly with a liquid, transparent, and clear form. Homogeneous *nano spray mouths* are important because they are able to distribute the active substances evenly, thereby supporting the stability of the preparation while increasing the comfort of use and clinical effectiveness in overcoming halitosis.

Table 4. Homogenity result

Formula	Result
F1	Homogeneous
F2	Homogeneous
F3	Homogeneous
F4	Homogeneous

The main objective of homogeneity testing is to ensure that each component of the ingredients in the preparation is thoroughly mixed without any phase separation or accumulated particles. Based on the results shown in Table 4, no particles were found to clump on the glass of the object, so it can be concluded that the preparation meets the homogeneity requirements with an even arrangement and is free of particle clumps.

Viscosity of Nano Spray

Based on the viscosity test results, the four oral nano spray formulations showed a decrease in viscosity after storage (Table 5). The F1 formulation decreased from 1.76 cP to 1.07 cP, F2 from 1.55 cP to 0.87 cP, F3 from 1.53 cP to 0.82 cP, and F4 from 1.76 cP to 1.20 cP. This decrease indicates the possibility of physical or chemical changes occurring in the dosage form, such as degradation of thickening agents, changes in solution structure, or interactions between formulation components during storage.

Table 5. Viscosity Test Results

Formula	Viscosity Value (cP)		Standard
	Before	After	
F1	1.76	1.07	1.0
F2	1.55	0.87	1.0
F3	1.53	0.82	1.0
F4	1.76	1.20	1.0

Among the four formulations, F4 showed the smallest decrease in viscosity, thus showing better physical stability compared to other formulations. However, the overall viscosity value after storage is still within the appropriate range for solution-based oral sprays, namely around 0.8 to 1.2 cP, thus still

supporting the spray characteristics and comfort of use.

Investigation Particle Size of Nano Spray

The particle characteristics test for the Mouth Nano Spray preparation was carried out on a formula that was considered the best and was physically stable and had good inhibitory power. The formula chosen is F2 which has a concentration of 0.5%. The tests carried out were PSA test, Polydispersity index test and Zeta potential test (Table 7). In this study, nanoemulsion testing was carried out using *a Particle Size Analyzer* (PSA). Based on the measurement results, the black cumin seed essential oil nanoemulsion preparation has a particle size at the nanometer scale with a good polydispersibility index value. The test data obtained a particle size of 42.7 nm with a polydispersity index value of 0.6. The particle size is in accordance with the nanoemulsion criteria, which is in the range of 1–100 nm. The obtained polydispersity index (PDI) value of 0.6 indicates a relatively broad particle size distribution; however, this value remains within the acceptable range for nanoemulsion systems, suggesting moderate homogeneity while indicating the need for further formulation optimization [17].

Table 7. PSA (*Particle Size Analyzer*) Test Results

Formula	Uji PSA		
	Droplet Size	**PdI *(Polydispersity Index*)**	**Potential Zeta**
F2	42.7 nm	0.6	-5.6 µV
Standard	1-100 nm	<0.7	> 30 µV

However, the potential zeta value of black cumin seed essential oil nanoemulsion preparations was recorded lower than the stability standard, which was –5.6 µV. The relatively low zeta potential value suggests limited electrostatic stabilization; however, physical stability of the system may also be supported by steric stabilization provided by the nonionic surfactant Tween 80 present in the formulation. The range of potential zeta values can be used as an indicator of the stability of the stockpile during storage. A potential zeta value of more than 30 mV indicates that the preparation has good electrostatic stability. If it is in the range of 5–15 mV, it indicates limited flocculation, while in the range of 3–5 mV, it indicates maximum flocculation[18]. The low value of this potential zeta reduces the repulsive force between particles, making it easier for particles to flocculate. This flocculation condition can trigger the separation of the oil phase and the water phase which ultimately affects the stability of the nanoemulsion [19].

Antibacterial Activity against Streptococcus mutans

The antibacterial activity of the formulated *mouth nano spray* was evaluated against *Streptococcus mutans*, a primary bacterium associated with dental caries, using the agar diffusion method. This assay was conducted to determine the inhibitory effect of each formulation by measuring the diameter of the inhibition zone formed around the sample. The detailed inhibition zone diameters and their corresponding activity categories are presented in the Table 8.

Table 8. Inhibition Zone Diameter Result

Formula	Diameter of the barrier zone (mm)	Category
F1	0.00 ± 0.00	None
F2	12.62 ± 0.79	Strong
F3	12.53 ± 0.99	Strong
F4	12.32 ± 0,74	Strong
Positive Control	7.21 ± 1.47	Medium
Negative Control	0.00 ± 0.00	None

Based on the results of antibacterial inhibition testing, it can be seen that the F2, F3, and F4 formulas are able to produce inhibition zones with diameters of 12.62 mm, 12.53 mm, and 12.32 mm, respectively. All three formulas showed a clear antibacterial activity, even higher than positive controls (Minoseps containing *Chlorhexidine* 0.2%) which only results in an inhibition zone of 8.39 mm. Meanwhile, the F1 formula and negative control (*Aquadest*) does not show the presence of an inhibitory zone, so it can be concluded that neither has antibacterial activity. These results show that differences in composition in the formula affect the ability of the preparation to inhibit bacterial growth, where F2 provides the greatest yield compared to other formulas.[20] The antibacterial activity demonstrated by formulations F2–F4 showed promising inhibitory effects and was comparable to the positive control, indicating the potential of the developed nano spray as an alternative herbal-based oral antibacterial preparation [21].

Conclusions

Based on the results of the study, it can be concluded that the entire formula *mouth nano spray* Black cumin essential oil (F1–F4) has good physical quality, indicated by its liquid, clear, homogeneous form, and pH suitable for oral use. Characterization of the nanoparticles showed that the preparations had particle sizes at the nanometer scale with a narrow size distribution, although the potential zeta values were still low and the electrostatic stability was less than optimal. Antibacterial activity tests show that F2, F3, and F4 are able to inhibit growth *Streptococcus mutans* with a larger diameter of the inhibition zone than the positive control, whereas F1 shows no activity. Of all the formulas, the formula with the best physical properties and stability is F2 which contains 0.5% black cumin essential oil, because it has the clearest appearance, is stable without phase separation, and has the appropriate pH and viscosity, and also good nanoparticle characteristics for use in the oral cavity. Formula F2 also has strong inhibitory power against the bacteria Streptococcus mutans which causes halitosis.

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Author Contributions

Conceptualization, Milda Rahayu and Septi Hariyani; Methodology, Septi Hariyani; Software, Milda Rahayu; Validation, Milda Rahayu and Septi Hariyani; Formal Analysis, Milda Rahayu and Septi Hariyani; Investigation, Milda Rahayu and Septi Hariyani; Resources,Septi Hariyani ; Data Curation, Uce Lestari ; Writing – Original Draft Preparation, Uce Lestari and Milda Rahayu; Writing – Review & Editing, Milda

Rahayu ; Visualization, Milda Rahayu ; Supervision, Uce Lestari; Project Administration, Uce Lestari; Funding Acquisition, BELMAWA Kemdikbud Ristek.

Conflict of Interest

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Ethical Standards

This article does not contain any studies involving human or animal subjects.

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