Fabrication of *Cuminum cyminum* Essential Oil Nanoemulsion and Assessment of Its Antimicrobial Potential

Zeinab Mazarei[1,*] Somayeh Kianimehr[1]
[1] Department of Chemistry, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran
z.mazarei@scu.ac.ir

ABSTRACT

Cuminum cyminum L. (green cumin) is an aromatic medicinal herb widely recognized for its pharmacological and therapeutic properties, including antimicrobial, antioxidant, and digestive activities. The present investigation aimed to extract the essential oil from C. cyminum seeds by hydrodistillation method, formulate a physically stable nanoemulsion using high‑speed homogenization, identify and quantify the volatile components of the essential oil, and assess the antimicrobial efficacy of the obtained nanoemulsion. The chemical profile of the essential oil, analyzed by gas chromatography–mass spectrometry (GC–MS), revealed that cuminaldehyde, γ‑terpinene, and p‑cymene were the predominant constituents. The formulated nanoemulsion exhibited high physical stability with a mean droplet diameter of approximately 8.1 nm and a narrow size distribution. Antimicrobial assays demonstrated that the cumin essential oil nanoemulsion exhibited strong inhibitory effects against a broad spectrum of foodborne bacterial pathogens. These findings suggest that cumin essential oil nanoemulsion could serve as a promising natural antimicrobial agent for potential applications in food preservation and safety enhancement.

Keywords: *Cuminum cyminum L., essential oil, nanoemulsion, antimicrobial activity*

1. *INTRODUCTION*

Cuminum cyminum, commonly known as cumin, is a flowering plant in the parsley family (Apiaceae) that is widely cultivated for its aromatic seeds. Originating from the Mediterranean region and parts of Western Asia, it has become a cornerstone spice in cuisines across the globe, particularly in Indian, Middle Eastern, and Mexican cooking [1]. The plant produces small, delicate white flowers and is characterized by its slender, feathery leaves.

C. cyminum has been utilized for centuries in traditional medicine, and modern science continues to explore its diverse therapeutic potential. One of its most well-documented benefits is its effect on the digestive system; it is widely recognized for its carminative properties, meaning it helps reduce bloating, gas, and indigestion by stimulating the secretion of digestive enzymes. Furthermore, C. cyminum is a potent source of antioxidants, such as phenols and flavonoids, which help protect the body's cells from oxidative stress and reduce inflammation. Research also suggests that it may play a role in metabolic health, specifically in managing blood sugar levels and improving lipid profiles (cholesterol), which can assist in preventing metabolic syndrome. Additionally, due to its antimicrobial and antibacterial properties, cumin has been used traditionally to combat various infections [2-4].

2. *MATERIALS AND METHODS*

2.1 Essential Oil Extraction

The essential oil of Cuminum cyminum seeds was extracted using a conventional hydrodistillation method with a Clevenger-type apparatus. Initially, cleaned and dried Cuminum cyminum seeds were ground lightly to facilitate the release of volatile compounds. 100 g of the powdered seeds was mixed with distilled water in a round-bottom flask and subjected to hydrodistillation for 3 hours. During the process, steam carried the volatile constituents through the condenser, where they were cooled and collected in the graduated arm of the Clevenger apparatus. The separated essential oil layer was carefully removed, dried over anhydrous sodium sulfate to eliminate residual moisture, and stored in amber glass vials at 4 °C until further analysis. This method ensured efficient recovery of the volatile fraction while preserving the integrity of heat‑sensitive components.

2.2 Chemical Composition Analysis

The qualitative and quantitative analysis of the Cuminum cyminum essential oil was conducted using gas chromatography–mass spectrometry (GC–MS) and gas chromatography–flame ionization detection (GC–FID), respectively.

For GC–MS analysis, a GC–MS system (Agilent 7890B GC coupled with a 5977A MSD) equipped with a nonpolar capillary column (HP-5ms, 30 m × 0.25 mm i.d. × 0.25 μm film thickness) was utilized. The oven temperature was programmed to start at 60 °C for 5 minutes, followed by a ramp of 4 °C/min to 240 °C, holding for 10 minutes. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector and interface temperatures were set at 250 °C and 280 °C, respectively. The MS detector operated in electron ionization (EI) mode at 70 eV. Essential oil samples were diluted in n‑hexane (1:10 v/v) and 1 μL of the solution was injected in split mode (1:50 ratio). Identification of the individual components was achieved by comparing their retention indices and mass fragmentation patterns with those available in the NIST mass spectral database and the literature. For GC–FID analysis, the same chromatographic as those used for GC–MS were employed, but with a flame ionization detector. The detector temperature was maintained at 280 °C. The relative percentage composition of each identified compound was calculated based on the peak area normalization method. This comprehensive approach allowed for both accurate identifications of the essential oil constituents and precise quantification of their relative proportions.

2.3 Nanoemulsion Preparation

Nanoemulsions containing Cuminum cyminum essential oil were prepared using the high-energy emulsification method. Initially, the surfactant, Tween 80, was completely dissolved in deionized water at varying concentrations (2%, 4%, and 6% w/w) to form the aqueous phase. Subsequently, the oil phase, consisting of 2% (w/w) Cuminum cyminum essential oil, was added to this surfactant solution. The mixture was then subjected to homogenization using a high-speed homogenizer (XCORT). The emulsification process was carried out at a speed of 8000 rpm for 5 minutes. This procedure resulted in the formation of a uniform, opaque nanoemulsion, indicative of a stable system with small droplet sizes.

2.4 Particle Size Determination using Dynamic Light Scattering (DLS)

The particle size distribution of the prepared Cuminum cyminum essential oil nanoemulsion was determined using Dynamic Light Scattering (DLS). Prior to measurement, the nanoemulsion samples were diluted with deionized water to achieve an appropriate scattering intensity, ensuring optimal performance of the DLS instrument. The measurements were conducted at a controlled temperature (typically 25°C) to minimize thermal fluctuations. The DLS analysis provided information on the hydrodynamic diameter of the droplets and their size distribution. The mean particle size and polydispersity index (PDI) were calculated from the scattering data.

2.5 Antimicrobial Activity Evaluation

The antimicrobial properties of both the pure Cuminum cyminum essential oil and its nanoemulsion formulation were assessed against Gram-negative (Escherichia coli, ATCC 25922) and Gram-positive (Staphylococcus aureus, ATCC 29213) bacteria. The Minimum Inhibitory Concentration (MIC) and Minimum

Bactericidal Concentration (MBC) were determined using broth microdilution method according to established protocols. Serial dilutions of the essential oil and the nanoemulsion were prepared in Mueller-Hinton broth (MHB). Each dilution was then inoculated with a standardized bacterial suspension (approximately 1.5 × 10^8 CFU/mL). Following incubation at 37°C for 24 hours, the MIC was identified as the lowest concentration of the essential oil or nanoemulsion that completely inhibited visible bacterial growth. To determine the MBC, aliquots from wells showing no visible growth were sub-cultured onto Mueller-Hinton agar plates and incubated under the same conditions. The MBC was defined as the lowest concentration that resulted in a ≥99.9% reduction in viable bacterial count compared to the initial inoculum.

3.RESULTS AND DISSCUSSION

3.1 Analysis of Cuminum cyminum Essential Oil Composition

The gas chromatography-mass spectrometry (GC-MS) analysis revealed the detailed chemical composition of the Cuminum cyminum essential oil. The major constituent identified (Fig. 1) was Cuminaldehyde, accounting for 14.3% of the total oil. Other significant components include γ-Terpinene (9.6%), p-cymene (8.6%), β-pinene (6.7%), and 1,8-Cineole (6.4%). The oil also contained notable percentages of α-pinene, Terpinen-4-ol, β-Myrcene, Limonene, and Linalool. Minor components such as α-Terpinene, Caryophyllene, and Safranal were also detected. This complex mixture of volatile organic compounds, particularly the high concentration of Cuminaldehyde, is expected to contribute significantly to the characteristic aroma and potential biological activities of Cuminum cyminum essential oil.

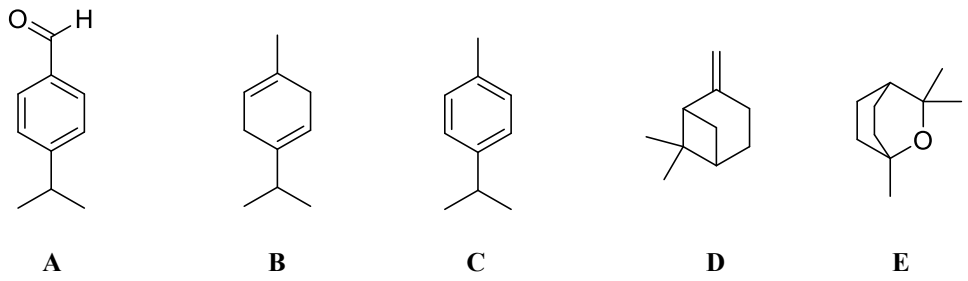

Fig. 1. The main chemical constituents of *Cuminum cyminum* essential oil, **A**: Cuminaldehyde, **B**: γ-Terpinene, **C**: p-cymene, **D**: β-pinene, **E**: 1,8-Cineole

Table 1. The essential oil composition of *Cuminum cyminum*

	Compound name	%
1	Cuminaldehyde	14.3
2	γ-Terpinene	9.6
3	p-cymene	8.6
4	β-pinene	6.7
5	1,8-Cineole	6.4
6	α-pinene	4.1
7	Terpinen-4-ol	3.8
8	β-Myrcene	3.2
9	Limonene	3.6

10	Linalool	3.6
11	α-Terpinene	2.8
12	Caryophyllene	1.5
13	Safranal	0.7

3.2 Nanoemulsion Formulation and Characterization

The effect of surfactant concentration on the properties of Cuminum cyminum essential oil nanoemulsions was investigated (Table 2), maintaining a constant essential oil concentration of 2% across all formulations. Three different concentrations of Tween 80 (2%, 4%, and 6%) were used as the surfactant. The results indicate a significant influence of surfactant concentration on the mean particle size.

In Formulation 1, with 2% surfactant, the mean particle size of the nanoemulsion is relatively large, measuring 113.4 nm (with a standard deviation of 14.2). By increasing the surfactant concentration to 4% in Formulation 2, the mean particle size significantly decreased to 20.3 nm (with a standard deviation of 6.7). This reduction suggests improved stability and the formation of smaller droplets. In Formulation 3, utilizing the highest surfactant concentration of 6%, the smallest mean particle size was achieved, measuring 8.1 nm (with a standard deviation of 3.5). This indicates that increasing the surfactant concentration to this level has aided in the formation of the finest droplets and likely the most stable nanoemulsion.

In summary, this table demonstrates that as the surfactant concentration increases, the particle size of the cumin essential oil nanoemulsion decreases, with the formulation containing 6% surfactant yielding the best results in terms of achieving nano-sized particles and potentially better stability.

Table 2. The characteristics of *Cuminum cyminum* essential oil nanoemulsions

Formulation	Essential oil %	Surfactant %	Mean particle size (nm)
1	2	2	113.4 ± 14.2
2	2	4	20.3 ± 6.7
3	2	6	8.1 ± 3.5

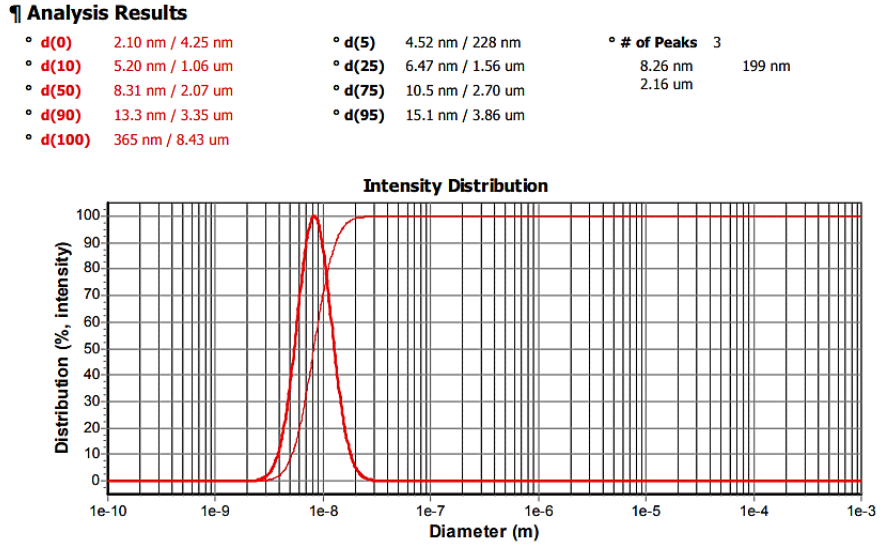

Fig. 2. The particle size distribution diagram of *Cuminum cyminum* essential oil nanoemulsions

3.3 Antibacterial Activity of Cuminum cyminum Essential Oil and its Nanoemulsion

The antibacterial efficacy of Cuminum cyminum essential oil, both in its free form and encapsulated within a nanoemulsion, was evaluated against two significant bacterial strains: Escherichia coli and Staphylococcus aureus. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were determined.

Against Escherichia coli, the free essential oil exhibited an MIC of 8 mg/mL and an MBC of 8 mg/mL, indicating moderate inhibitory and bactericidal activity. However, when formulated as a nanoemulsion, the antibacterial potency significantly increased, with an MIC of 4 mg/mL and an MBC of 4 mg/mL. This four-fold increase in efficacy suggests that the nanoemulsion formulation enhances the accessibility and activity of the essential oil against E. coli. A similar trend was observed against Staphylococcus aureus. The free essential oil showed an MIC of 4 mg/mL and an MBC of 8 mg/mL. The nanoemulsion formulation demonstrated a remarkable enhancement in activity, achieving an MIC of 1 mg/mL and an MBC of 2 mg/mL. This represents an approximately four-fold increase in inhibitory activity and a two-fold increase in bactericidal activity compared to the free essential oil.

Overall, these results highlight the superior antibacterial performance of the cumin essential oil nanoemulsion compared to the free essential oil against both Gram-negative (E. coli) and Gram-positive (S. aureus) bacteria. The nanoencapsulation appears to potentiate the antimicrobial action, likely due to improved dispersion, increased surface area, and enhanced cellular uptake of the essential oil components.

Table 3. Antibacterial activity of *Cuminum cyminum* essential oil and nanoemulsion against selected bacteria

	Escherichia coli		*Staphylococcus aureus*	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
essential oil	8	8	4	8
nanoemulsion	4	4	1	2

The potent antimicrobial activity observed for both free Cuminum cyminum essential oil and its nanoemulsion can be attributed to the synergistic action of its primary chemical constituents. Analysis has revealed that compounds such as Cuminaldehyde, γ-Terpinene, p-cymene, α-pinene, β-pinene, and 1,8-Cineole are present in significant percentages. Cuminaldehyde, the most abundant compound at 14.3%, is widely recognized for its strong antimicrobial and antioxidant properties. It is believed to disrupt bacterial cell membranes, inhibit essential enzyme activities, and interfere with cellular processes, thereby exerting its inhibitory and bactericidal effects [5-6]. Terpenes like γ-Terpinene (9.6%), p-cymene (8.6%), α-pinene (4.1%), and β-pinene (6.7%) also contribute significantly to the oil's antimicrobial profile. These monoterpenes are known to possess varying degrees of antibacterial activity, often by increasing cell membrane permeability and leading to leakage of intracellular components [7-8]. For instance, p-cymene has been shown to inhibit bacterial growth by affecting cell division and DNA replication. Furthermore, 1,8-Cineole (6.4%), known for its antiseptic properties, can penetrate bacterial cell walls and disrupt their function [9]. Other identified compounds like Terpinen-4-ol (3.8%), Linalool (3.6%), and Limonene (3.6%) also contribute to the overall antimicrobial spectrum, exhibiting varying mechanisms such as membrane disruption and inhibition of metabolic pathways [10-11].
The presence of these diverse compounds, particularly Cuminaldehyde and the various terpenes, creates a multifaceted approach to combating microbial growth. The enhanced activity observed with the nanoemulsion formulation likely stems from improved delivery and bioavailability of these active components, allowing them to more effectively interact with and damage bacterial cells.

4. CONCLUSION

This study successfully investigated the chemical composition, nanoemulsion formulation, and antibacterial efficacy of Cuminum cyminum (cumin) essential oil. Gas chromatography-mass spectrometry (GC-MS) analysis revealed a rich chemical profile, with Cuminaldehyde (14.3%) as the predominant compound, alongside significant concentrations of γ-Terpinene, p-cymene, β-pinene, and 1,8-Cineole. These compounds are recognized for their aromatic properties and are known contributors to the biological activities of essential oils. The formulation of nanoemulsions demonstrated that surfactant concentration critically influences droplet size. Increasing the concentration of Tween 80 from 2% to 6% resulted in a significant reduction in mean particle size, from 113.4 nm to as low as 8.1 nm. The formulation containing 6% Tween 80 yielded the smallest particle size, indicating the formation of a highly stable and monodisperse nanoemulsion system. Crucially, the nanoemulsion formulation significantly enhanced the antibacterial activity of cumin essential oil against both Escherichia coli and Staphylococcus aureus. The nanoemulsified oil exhibited lower Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values compared to the free essential oil for both bacterial strains. Notably, against S. aureus, the nanoemulsion demonstrated a four-fold increase in inhibitory and a two-fold increase in bactericidal activity. This potentiation is attributed to the nanoencapsulation, which likely improves the dispersion, increases the surface area, and facilitates enhanced cellular uptake of the active antimicrobial compounds.

In conclusion, Cuminum cyminum essential oil possesses a complex chemical composition that contributes to its inherent antimicrobial properties. Its formulation into a stable nanoemulsion effectively enhances its antibacterial efficacy, suggesting that nanoencapsulation is a promising strategy for developing more potent antimicrobial agents from natural sources for various applications. Future research could explore the efficacy of these nanoemulsions against a broader spectrum of microorganisms and in different matrices.

REFERENCES

[1] Singh N, Yadav SS, Kumar S, Narashiman B. A review on traditional uses, phytochemistry, pharmacology, and clinical research of dietary spice Cuminum cyminum L. Phytotherapy Research. 2021;35(9):5007-30.

[2] Koohsari S, Sheikholeslami MA, Parvardeh S, Ghafghazi S, Samadi S, Poul YK, et al. Antinociceptive and antineuropathic effects of cuminaldehyde, the major constituent of Cuminum cyminum seeds: Possible mechanisms of action. Journal of ethnopharmacology. 2020;255:112786.
[3] Gohari AR, Saeidnia S. A review on phytochemistry of Cuminum cyminum seeds and its standards from field to market. Pharmacognosy Journal. 2011;3(25):1-5.
[4] Ebada ME. Cuminaldehyde: A potential drug candidate. J Pharmacol Clin Res. 2017;2(2):1-4.
[5] Monteiro-Neto V, de Souza CD, Gonzaga LF, da Silveira BC, Sousa NC, Pontes JP, et al. Cuminaldehyde potentiates the antimicrobial actions of ciprofloxacin against Staphylococcus aureus and Escherichia coli. PLoS One. 2020;15(5):e0232987.
[6] Li H, Zhang M, Addo KA, Yu Y, Xiao X. Action mode of cuminaldehyde against Staphylococcus aureus and its application in sauced beef. LWT. 2022;155:112924.
[7] Baginska S, Gologńko A, Swislocka R, Lewandowski W. Monoterpenes as medicinal agents: Exploring the pharmaceutical potential of p-cymene, p-cymenene, and γ-terpinene. Acta Pol Pharm—Drug Res. 2023;80:879-92.
[8] da Silva Rivas AC, Lopes PM, de Azevedo Barros MM, Costa Machado DC, Alviano CS, Alviano DS. Biological activities of α-pinene and β-pinene enantiomers. Molecules. 2012;17(6):6305-16.
[9] Merghni A, Belmamoun AR, Urcan AC, Bobiş O, Lassoued MA. 1, 8-cineol (eucalyptol) disrupts membrane integrity and induces oxidative stress in methicillin-resistant Staphylococcus aureus. Antioxidants. 2023; 12(7):1388.
[10] Liu X, Cai J, Chen H, Zhong Q, Hou Y, Chen W, Chen W. Antibacterial activity and mechanism of linalool against Pseudomonas aeruginosa. Microbial Pathogenesis. 2020; 141:103980.
[11] Han Y, Sun Z, Chen W. Antimicrobial susceptibility and antibacterial mechanism of limonene against Listeria monocytogenes. Molecules. 2019; 25(1):33.