RESEARCH PAPER

Fabrication and Functional Characterization of Nano-Emulsions Containing Cinnamomum Tamala And Illicium Verum Essential Oils With Beeswax and Cellulose Nanocrystals for Antimicrobial, Antibiofilm, And Antioxidant Applications

Dhurga Ka, Bavya Swaminathana and Ravichandran Natesanab*

[a]*Department of Chemistry and Biosciences, Srinivasan Ramanujam Centre, SASTRA Deemed To Be University, Thanajvur, Tamil Nadu, India*
[b]*Centre For Advanced Research in Indian System of Medicine (CARISM), SASTRA Deemed To Be University, Thanajvur, tamil nadu, India*
*durgak052025@gmail.com, bavyasami@gmail.com and *ravichandran_n@scbt.sastra.ac.in*

Received: 28th Feb, 2026; Revised: 6th March 2026; Accepted: 7th April, 2026; Available Online: 20th April, 2026

ABSTRACT
The increasing prevalence of food spoilage caused by microbial pathogens such as Aspergillus and Staphylococcus necessitates the development of safe, non-toxic, and effective natural antimicrobial agents. In this study, nano-emulsions were formulated using essential oils derived from Cinnamomum tamala and Illicium verum, with beeswax serving as a stabilizing agent and cellulose nanocrystals as a functional nanomaterial. The constituents of volatile oils and beeswax were identified with the help of Gas Chromatography Mass Spectroscopy, and the nano-emulsion was characterized by particle size (176.8 nm) and zeta potential (7 mV). The study assessed the antimicrobial inhibitory power of essential oils and beeswax and nano-emulsion through the agar well method while determining the minimum inhibitory concentration through liquid culture dilution methods against Staphylococcus aureus and Aspergillus niger. The research used crystal violet staining to measure antibiofilm activity and DPPH radical scavenging assay to assess antioxidant capacity. The essential oils in the nano-emulsion showed increased antimicrobial and antibiofilm effects plus high antioxidant power because of their polyphenol and flavonoid content. These findings demonstrate that essential oil-based nano-emulsions reinforced with natural stabilizers offer a promising, eco-friendly alternative to synthetic preservatives, with potential applications in food preservation and biomedical fields.

Keywords: *Nano-emulsions, Essential oil, Beeswax, Cellulose nanocrystals, Anti-microbial activity, Anti-biofilm activity, Antioxidant activity, Food preservation*
How to cite this article: Dhurga K, Swaminathan B, Natesan R. Fabrication and Functional Characterization of Nano-Emulsions Containing Cinnamomum Tamala And Illicium Verum Essential Oils With Beeswax and Cellulose Nanocrystals for Antimicrobial, Antibiofilm, And Antioxidant Applications. Int J Drug Deliv Technol. 2026;16(34s):330-342. DOI: 10.25258/ijddt.16.34s.41
Source of support: Nil.
Conflict of interest: None

1. INTRODUCTION

Application of antimicrobial and synthetic compounds used to control the growth of food spoilage microbes. The non-scientific application of the synthetic food preserving chemicals causes the environmental and it causes the toxicity of non-target organisms and the microbes were developed resistance against the synthetic food preservatives[1].

To preserve the food the consumers are searching an alternative preservatives to avoid the side effects of the existing food preservatives[2]. The plant essential oils gaining more attention in the food industries because of their smell, taste and their potential against bacteria and fungi and also the essential oils they are not altering the organoleptic and nutritive value of the food[3]. EOs are plant secondary metabolites secreted and stored in spices and aromatic plants, these EOs are recognized as safe[4] and EOs can be used as food preservatives in combination with other food preservatives, the combined effect of EOs helps as a preservative for prolonged storage[5] The application of EOs are used as food preservative have some drawback due to their strong smell, less solubility in water, reduced stability, alteration of phsico-chemical properties of EOs due to environmental conditions[6,7]. In higher concentrations some of the essential oils are reduces the organoleptic characteristic features of the eatable products[8]. Nanoencapsulation of essential oils by using various carrier matrices helpful to solve the problems raised during the application of EOs as food preservatives and increases the utilization of EOs in food preservation[9,10].

**Author for Correspondence: ravichandran_n@scbt.sastra.ac.in*

To increase the availability and enhancement of bioactivities in moisture condition and increase the equal distribution of EOs on food surfaces there is an urgent need to encapsulate the EOs by using appropriate biopolymers[11]. Among the different encapsulation techniques, nano-emulsion one of the effective way than any other nanoencapsulation techniques and it can be applied in food industries[12]. Nanemulsion containing essential oils (EOs) is gaining interest as an alternative to synthetic preservatives, which aligns with consumer preferences for safer, eco-friendly food protection. Beeswax, obtained from honeycombs, is a complex mixture of chemical compounds with diverse industrial applications. The food industry uses it as an ingredient for glazing and coating food products which also functions as a base for food additives and as a texturing component in products like chewing gum. Viscoelastic properties of beeswax enhances resistance to water vapor and improve the hydrophobic characteristics of formulation materials, including proteins.[13] Beeswax aids in preserving active compounds through controlled release, minimizes moisture loss, reduces the rate of fruit decomposition, and lowers water vapor permeability[14]. Cellulose is the most abundant natural carbohydrate polymer.

Cellulosenanocrystals are important food additives because of their capacity in preventing the morphological and chemical changes and their repeatability in development of nano-emulsion. Hydroxyl group of the CNCs provide an amphiphilic nature to the nano-emulsion and the negative charge of the CNCs improve the stability of the nano-emulsion[15]. Several reports are indicates that the CNCs can be used as biostabilizers and EOs as biopesticides especially in food preservation[16]. CNCs-stabilised nano-emulsions nano-emulsions prepard by using EOs exhibited increased antimicrobial activities in controlling the growth of food spoiling microbes, and larvae [17].

Cinnamomum tamala (Indian bay leaf) is used as an important medicinal herb in Ayurvedic system of medicine. The essential oils of *C. tamala* isolated from its leaves and bark and stem shows multiple biological activities including antimicrobial, antioxidant, anti-inflammatory, antiparasitic and anticancer and also it is used to manage gastrointestinal ailments, haemorrhoids, and rheumatic conditions[18] *C. tamala* EO is widely utilized in food, cosmetic, and agricultural sectors as a flavouring agent, preservative, and natural pesticide. The substance demonstrates effective pathogen control through its ability to block foodborne pathogens that include *Escherichia coli* and *Staphylococcus* and *Listeria monocytogenes* and *Salmonella* spp[19].

Star anise, *"Illicium verum,"* fruits are utilized in both culinary and medicinal applications. The star anise is used as carminative, digestive, antispasmodic, expectorant, antirheumatic and anti-diuretic and also it is effective in relieving colic and is frequently incorporated into cough lozenges and veterinary formulations such as cattle sprays. The essential oil of star anise shows its ability to protect against oxidation while killing insects and serving as a fumigant and antimicrobial agent. The seed oil is used throughout the world as a treatment for various medical conditions. The essential oil contains trans-anethole as a major compound which is help in the applications of the pharmaceutical and food sectors. *I. verum* essential oil contains tannins, anethole, α-pinene and limonene and β-phellandrene, α-terpineol and farnesol[20].

2. MATERIALS AND METHODS

2.1. Study type and Design

Formulation and characterization of essential oil-based nano-emulsions followed by evaluation of antimicrobial, antibiofilm, and antioxidant activities using standard microbiological and physicochemical assays against *Staphylococcus* and *Aspergillus* species.

2.2. Materials and Reagents

The materials and reagents used in this study included essential oils extracted from Cinnamomum tamala and Illicium verum using a Clevenger-type apparatus. Beeswax and cellulose nanocrystals were employed as natural stabilizing and functional agents in nano-emulsion preparation. Surfactants such as Tween 80 and Span 80, along with hexane and distilled water, were used for formulation. Microbiological media including Mueller–Hinton agar, nutrient broth, and Sabouraud dextrose media were used for culturing *Staphylococcus* and *Aspergillus*. Additional reagents such as crystal violet, ethanol, phenol, sulfuric acid, DPPH solution, and standard antibiotics (amikacin and voriconazole) were utilized for antimicrobial, antibiofilm, EPS inhibition, and antioxidant assays.

2.3. Extraction of Essential Oils

The essential oils of *Illicium verum* and *Cinnamomum tamala* were extracted using a Clevenger-type apparatus by steam distillation. Approximately 1 kg of dried star anise and bay leaves were subjected to hydrodistillation for 4 hours separately. The extracted essential oils were then collected, stored at 4 °C, and preserved for further analysis[21]

2.4. GC-MS Analysis of Essential Oils and Bee wax

Essential oils which were obtained by steam distillation method, analysed by Gas chromatography with mass spectrometry (GC-MS). The essential oils of *Illicium verum* and *Cinnamamum tamala* were subjected to GC-MS analysis according to established testing procedures. The oils which were found by utilising the P2010 gas using the TD20 thermal desorption technology for chromatography in conjunction with mass spectroscopy (Shimadzu). The temperature program began at 80ºC and followed by linear increase to 220ºC and a final ramp to 290ºC. The injection port and GC/MS interface temperatures were set to 290ºC and 29ºC, respectively, with samples introduced via a split-mode glass injector using helium carrier gas at a flow rate of 1.2 ml/min. Percentages were calculated using peak area normalization[22,23].

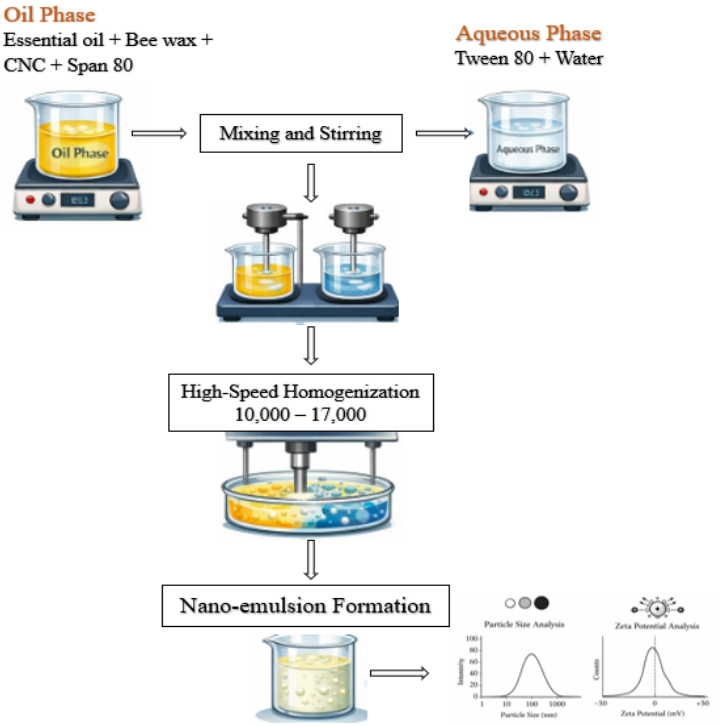


Figure 1: Formation of Nano-emulsion by Essential oil

2.5. Preparation of Nano-emulsion

The researchers created the nano-emulsion through the initial development of two distinct phases which included an oil phase and an aqueous phase. The oil phase contained essential oil and beeswax and cellulose nanocrystals (CNC) and Span 80 while the aqueous phase contained Tween 80 and distilled water. The two phases underwent magnetic stirring separately to achieve proper mixing and solubilization. The researchers combined the two phases and used continuous stirring to create a coarse emulsion. The combination underwent extreme speed mixing through high-speed homogenization which operated between 10,000 and 17,000 RPM to create smaller droplets that spread throughout the mixture. This process resulted in the formation of a stable nano-emulsion, which was further characterized using particle size analysis and zeta potential measurements to evaluate its size distribution and stability[24].

2.6. Characterization of nano-emulsions

ZETA Potential

The zeta potential which was obtained by using electrophoretic mobility technique (Malvern). The results were analysed and mentioned in millivolts (mV).

Particle size

To determine the size of the nano-emulsions through the dynamic light scattering technique zeta sizer equipment (Zetasizer®, Malvern). The results were mentioned as mean nm ± standard deviation.

2.7. pH

The p^H was tested for the nano-emulsion by using p^H meter.[25]

2.8. Antimicrobial Activity of Essential Oils and Nano-emulsion – Agar Well Diffusion Method

The researchers assessed the antimicrobial properties of essential oils and their nano-emulsions through testing on Mueller-Hinton agar plates for bacteria and potato dextrose agar plates for fungi were prepared and used for agar well diffusion method. The bacterial and fungal cultures were inoculated by controlled swabbing process which maintained sterile conditions. The researchers used a sterile borer to create wells in the agar plates that had been inoculated. Different concentration of essential oils (25, 50, 75 and 100 µL/mL) and nano-emulsion (100, 150 and 200 µL/mL) were used to determine the antibacterial activities. The cultures were maintained the plates at 37 °C for a full 24 hours for bacteria and 72 hrs for fungi. After incubation inhibition zones were measured (millimeter) and expressed in tables.

3.1. Minimal inhibitory concentration of emulsion

The minimal inhibitory concentration was evaluated using the **broth dilution method**. *Amikacin* was used as the standard antibiotic for *Staphylococcus*, and *voriconazole* as the antifungal agent for *Aspergillus*. Serial dilutions of the nano-emulsions (5–160 µL/mL % v/v) were prepared in 2 mL of nutrient broth, followed by inoculation with 200 µL of microbial suspension. Tubes without nano-emulsions served as negative controls, while tubes containing standard drugs served as positive controls. The study required all tubes to be kept at 37 degrees Celsius

for 24 hours to test bacterial growth and for 48 hours to test fungal growth. The researchers measured microbial growth after incubation by using a UV spectrophotometer which operated at 600 nanometers to detect turbidity and sediment formation in bacteria and surface mycelial growth in fungi. The study determined that the minimum inhibitory concentration (MIC) required the lowest concentration which produced no visible growth. The minimum lethal concentration (MLC) required the lowest concentration of nano-emulsion to achieve complete microbial growth suppression[26].

3.2. Antibiofilm activity of nano-emulsion

3.3. Effect of nano-emulsions on nascent biofilm using crystal violet staining

The study assessed how nano-emulsions prevent early biofilm development through crystal violet CV staining method testing at various concentration levels. The experimental setup required mixing 200 µL of bacterial *S. aureus* and fungal *A.niger* suspensions with 2 mL of nutrient broth and different nano-emulsion volumes which ranged from 22 to 220 µL representing 1 to 10 percent concentrations. The mixtures required incubation at 37 °C for 24 hours to test bacteria and 48 hours to test fungi. The researchers used a tube without nano-emulsion as their control sample. The researchers discarded the medium after incubation and stained the sticky biofilms with 0.4% crystal violet for 30 minutes. The excess stain was eliminated through three washing steps while the remaining CV was dissolved with 70% ethanol. The researchers used UV-Spectrophotometer to measure the 600 nm absorbance of CV solutions which they obtained to assess biofilm biomass[27].

3.5. Quantification of Exopolysaccharide (EPS) Reduction

The reduction of exopolysaccharides (EPS) following nano-emulsion treatment was quantified using the phenol–sulfuric acid assay by adopting a procedure[28] for both bacterial (*S.aureus*) and fungal (*A. niger*) species. Biofilm-forming cultures were incubated with nano-emulsion (1–5% concentrations) and without nano-emulsion (control) under standard conditions. The process started with the removal of non-adherent cells through washing using 0.5% NaCl solution. The researchers collected the remaining biofilm matrix and placed it into sterile tubes to apply 5% phenol before adding 0.2% hydrazine-enriched concentrated H_2SO_4 solution. The researchers established a reaction mixture that remained standing for 60 minutes to evaluate EPS content through colorimetric analysis using 490 nm absorbance measurements. The nano-emulsion achieved effective exopolysaccharide disruption through its ability to decrease absorbance values.

3.6. Antioxidant activity of emulsion

The DPPH assay which Elahe Abedi and his colleagues described in their research from 2024 was used to assess the antioxidant properties of the nano-emulsions. The researchers created different nano-emulsion concentrations which ranged from 10 to 50 micrograms per milliliter and conducted their experiments in separate test tubes while using ascorbic acid as a reference standard. The researchers added 1 mL of ethanolic DPPH solution to each tube and then completed the volume measurement by adding distilled water until they reached 2 mL. The researchers performed the reaction mixtures, which they kept in dark conditions for 30 minutes, before measuring the 515 nm absorbance.[29]

$$DPPH\ Scavenging\ Activity\ (\%) = \frac{control - test}{control} * 100$$

4.1. RESULTS AND DISCUSSION

4.2. GAS CHROMATOGRAPHY MASS SPECTROSCOPY (GC-MS)

Gas chromatography analysis shows the presence of chemical constituents in *C. tamala* and *I. verum* essential oils and beeswax. The compounds were presented in tables (1-3)

Table 1: Chemical composition of *C. tamala* essential oil identified by GC-MS

S. No	R.Time	Area%	Name
1	14.839	37.21	Eugenol
2	7.483	13.68	Thujene
3	15.626	9.04	E-Methyl isoeugenol
4	7.932	6.68	Cumene
5	17.962	4.82	Bicylogermacrene
6	8.135	3.36	Eucalyptol
7	20.031	2.81	Spatulenol
8	5.647	2.75	α-Pinene
9	16.191	2.51	Caryophyllene
10	11.828	1.27	α-Terpineol

This table 1. shows the results obtained from the GC–MS analysis of **bay leaf essential oil *(Cinnamomum tamala)*.** The analysis indicates that these compounds are present in high concentrations, confirming their abundance in bay leaf essential oil. In *C. tamala* EO eugenol is the major compound followed by thujene, e-methylisoeugenol, cumene an eucalyptol etc.

Table 2: Chemical composition of *I. verum* essential oil identified by GC-MS

S. No	R.Time	Area %	Name
1	13.710	88.50	Estragole
2	8.075	6.27	Cyclobutane
3	13.035	0.66	Aubepine
4	5.661	0.57	α-Pinene
5	11.840	0.42	α-Terpineol
6	8.149	0.39	Eucalyptol
7	7.565	0.39	3-Carene
8	11.549	0.36	Terpinen-4-ol
9	9.726	0.34	Linalool

Table 2 represent the results of the GC–MS analysis of **Star anise essential oil**. The findings indicate that the identified compounds are present in high concentrations, confirming their significant abundance in the essential oil. Estragole present as a major component and also it contains aubepine, α-pinene, α-Terpineol and eucalyptol in *I verum* EO.

Table 3 indicates the results of the GC–MS analysis of beeswax. The results indicates the compounds are present in high concentrations, confirming their significant abundance in beeswax. Saturated, unsaturated fatty acids, fatty acid esters and alkanes are major components in bees wax.

Table 3: Chemical composition of beeswax identified by GC-MS

Peak	R. Time	Area%	Name
1	34.651	0.25	n-Hexadecanoic acid
2	35.447	0.67	Hexadecanoicacid, ethylester
3	37.900	0.11	10-Octadecenoicacid,methylester
4	37.980	0.16	Heptadecane,2,6,10,15-tetramethyl
5	39.402	0.21	9-Octadecenoicacid,ethylester
6	39.541	0.06	E-11-Hexadecenoicacid,ethylester
7	42.348	2.32	Nonadecane
8	47.316	0.10	Eicosane,2,4-dimethyl-
9	49.641	1.00	Nonacos-1-ene
10	50.020	0.11	n-Pentadecanol
11	50.153	81.2	Octacosane,2-methyl-
12	50.667	0.35	Pentadecane,8-hexyl-
13	50.747	0.16	9-methylheptadecane
14	50.832	0.25	Heptadecane,7-methyl-
17	51.404	0.38	Hexacosane,9-octyl-
18	51.880	0.67	Tetratetracontane
20	53.604	12.61	Octacosane,2-methyl-

4.3. ZETA Potential of Nano-emulsion

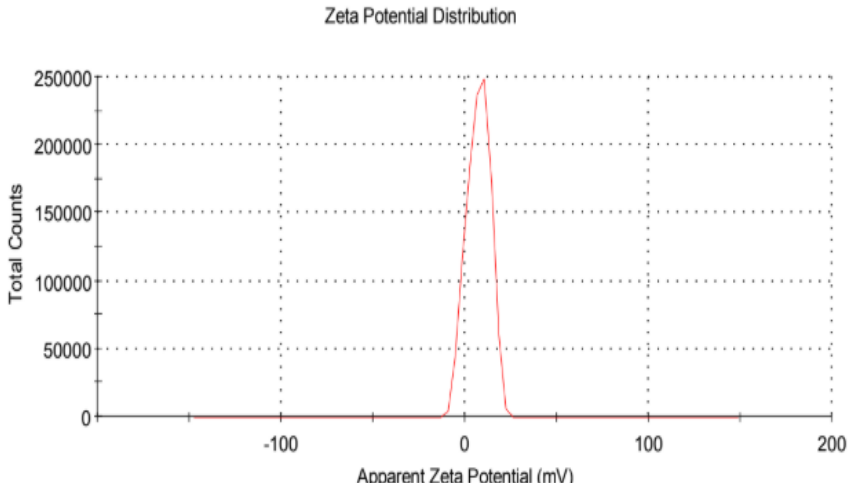


Figure 2 ZETA potential of Nano-emulsion of essential oil

Figure 30 shows the zeta potential of nano-emulsions. Zeta potential, a stability analysis was recorded on a MALVERN zeta sizer. An emulsion which is stability was attained at 20Mv that might positive charged and it sustain stability. In this study the zeta potential of nano-emulsionsare 7 mV.

4.4. Particle size for Nano-emulsion

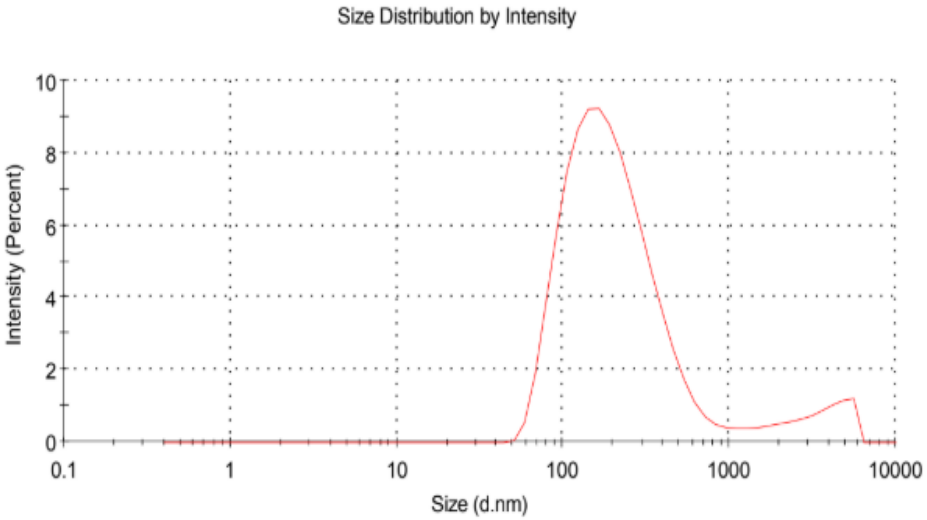


Figure 3 Particle Size of Nano-emulsion of essential oil

Figure 2 represents the size of the emulsion. The size of the emulsions in the range of 179.6 nm which is correlated with Yan Cao *et al.,* (2021). The average size of the nano-emulsions was observed to be 179.6 nm.

4.5. pH

The pH of the nano-emulsions was analyzed by p^H meter as 5.0 ± 0.5

4.6. Isolation of Organism

The gram-positive bacteria (*Staphylococcus)* and fungi (*Aspergillus)* was isolated and identified by biochemical test and slide culture method.

Table 4: Biochemical test for bacteria

Test	Observation
Gram stain	Gram positive
Colony shape	Round, convex
Colony opacity	Opaque
Nitrate	+
Triple sugar test	+
Citrate	+
Methyl red	+
Voges-proskauer	-
Motility	Non-motile
Catalase	+
Oxidase	-

4.7. Antimicrobial Activity of Essential Oils and Emulsion

The antimicrobial activity of star anise and bay leaf essential oils, along with their nano-emulsions, was evaluated using the agar well diffusion method against *S. aureus* and *A. niger.* The *C. tamala* EO showed maximum zones of inhibition of 18mm at 100µL/mL against *S. aureus* and 15mm at 75 µL/mL against *A. niger*. But the *I verum* EO exhibited lesser antibacterial and antifungal activity than *C. tamala*. It shows the antibacterial activity against *S. aureus* 8mm at 50 µL/mL and 10 mm at 100 µL/mL. The nano-emulsion exhibited a synergistic effect, producing inhibition zones of 17 mm against *S. aureus* at 200µL/mL and 19 mm against *A. niger* at 100 µL/mL. These results indicate enhanced and broad-spectrum antimicrobial activity of the nano-emulsion.

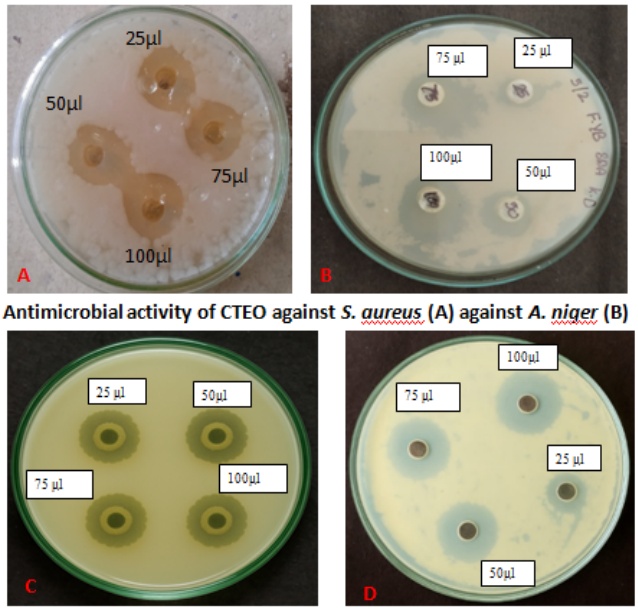
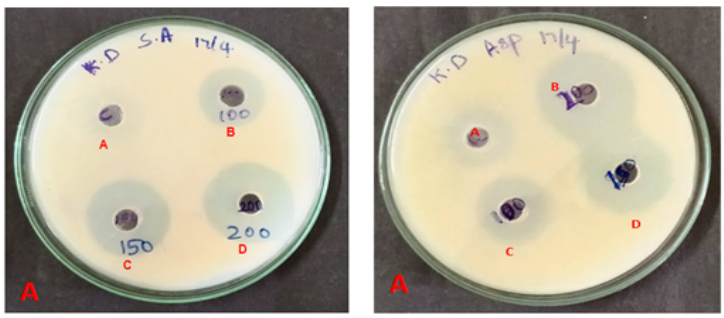


Figure 4: Antimicrobial activity of essential oils. *Cinnamomum tamala* essential oil (CTEO), *Illicium verum* essential oil (IVEO)



Antimicrobial activity of nanoemulsion against *S. aureus* (A) and against *A. niger* (B) (A. control; B-100µl/ml, C-150 µl/ml and D- 200µl/ml)

Figure 5 Antimicrobial activity of nano-emulsion

Table 5: Antimicrobial activity of Essential oil

S.NO	NAME	Concentration µl/mL	Organism	Zone of inhibition (mm)
1.	***C. tamala* E0**	75	*A. niger*	15 ± 0.5
2.		100	*S. aureus*	18 ± 0.5
3.	***I. verum* EO**	100	*A. niger*	10 ± 0.5
4.		50	*S. aureus*	8 ± 0.5

Table 6: Antimicrobial activity of nano-emulsion

S.NO	NAME	ORGANISM	50 µl	100 µl	150 µl	200 µl
1.	NANO-EMULSION	*A. niger*	13 ± 0.5	19 ± 0.5	16 ± 0.5	12± 0.5
2.		*S. aureus*	7 ± 0.5	13 ± 0.5	14 ± 0.5	17 ± 0.5

4.8. Minimal Inhibitory Concentration of Nano-Emulsion

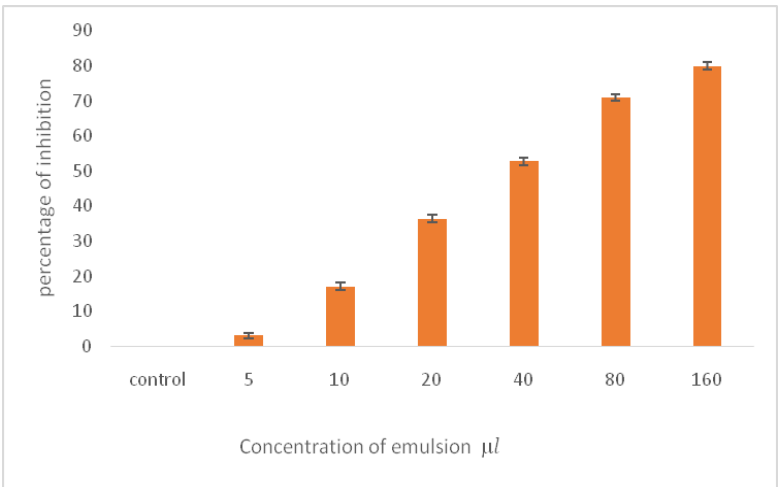


Figure 6 Minimal inhibitory concentration of Nano-emulsion against *S. aureus*

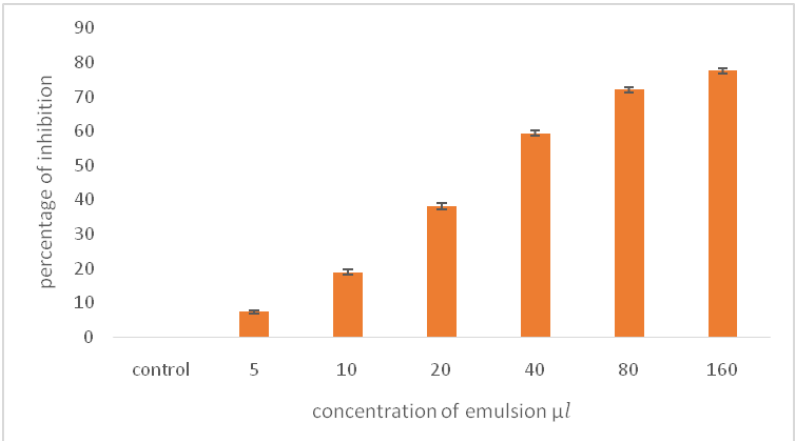


Figure 7 Minimal Inhibitory Concentration against *A. niger*

The minimal inhibitory concentration (MIC) of nano-emulsions against *S. aureus* and *A. niger* were analysed by using 5 to 160µL/mL. The results indicates that the MIC was 40 µL/mL for both *S. aureus* (Fig. 6) and *A. niger* (Fig. 7).

4.9. Antibiofilm assay by crystal violet method against *S. aureus* and *A. niger*

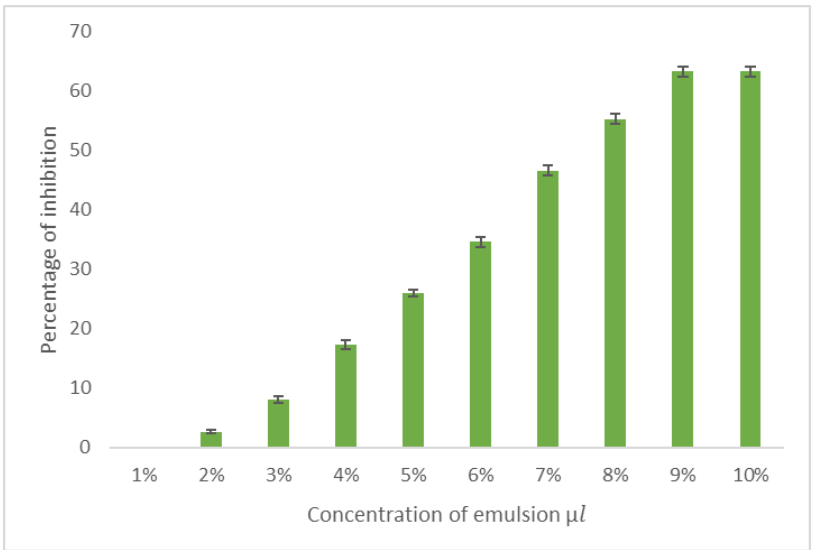


Figure 8 Antibiofilm activity against *S. aureus* (Nascent

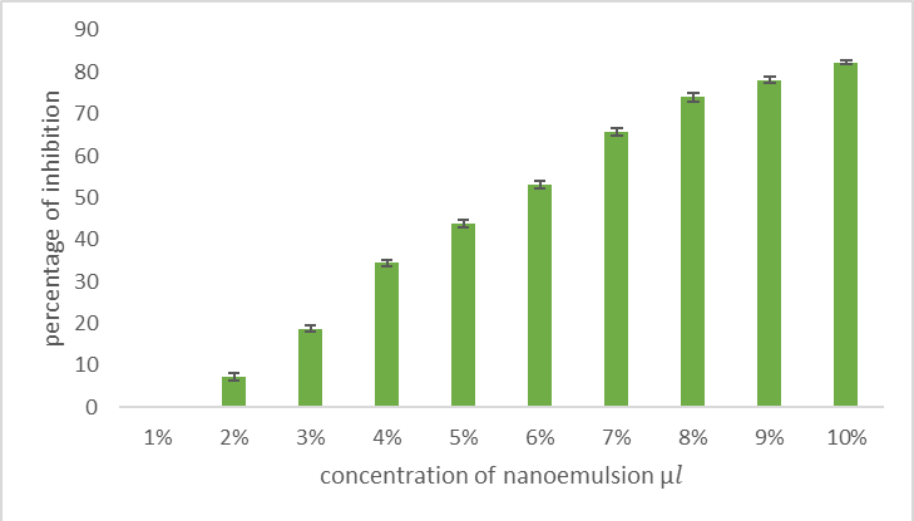


Figure 9 Antibiofilm activity against *S. aureus* (Mature)

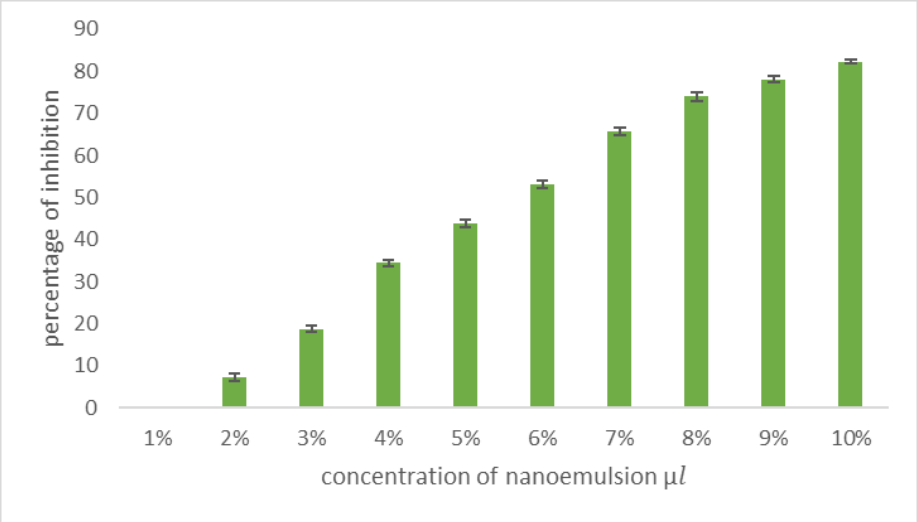


Figure 10 Antibiofilm activity against *A. niger* (Nascent)

The antibiofilm activity of nano-emulsions against nascent *S. aureus* was *evaluated with* different concentration of nano-emulsion (1% to 10%). The inhibition of biofilm formation was dose dependent, the anti-biofilm activity was increasing with increasing the concentration of test solution. The maximum inhibition of biofilm formation was observed observed at 10% (65% inhibition). Qualitative antibiofilm activity of nano-emulsions against mature *S. aureus* using crystal violet tube method was used with various concentration of nano-emulsion (1–10%).

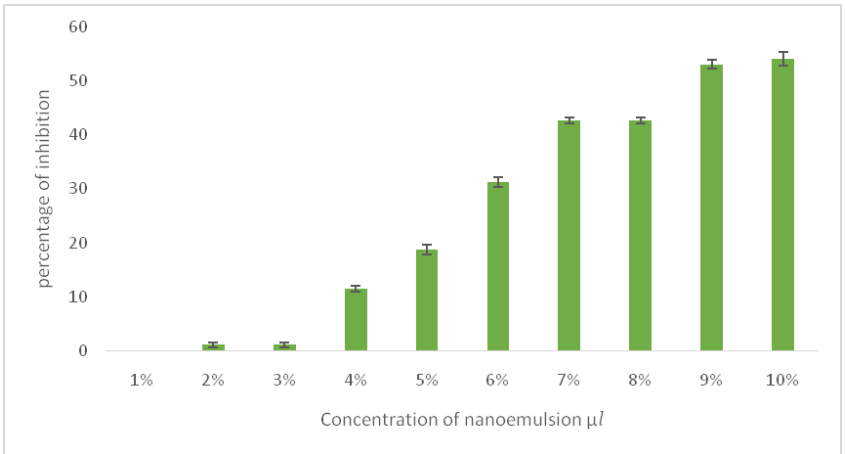


Figure 11 Antibiofilm Activity against *A. niger* (Mature)

Decreasing the intensity of purple colour was noticed with increasing concentration, this result indicates that reduced biofilm formation. Dense biofilm formation was observed at the concentrations of 1–3%. At higher concentration the biofilm formation was significantly reduced. At 7–10%, the violet colour becomes faint or nil, which indicates the inhibition of anti-biofilm formation of nano-emulsion. The results shows the nano-emulsions effectively inhibited mature biofilms (Fig. 9).

Anti-biofilm Activity of nano-emulsion against *A. niger* (Mature)

The anti-biofilm activity of nano-emulsion against *A. niger* mature biofilm through quantitative antibiofilm activity was determined with 1-10% of nano-emulsion. The Anti-biofilm activity against *A. niger* was increasing while increasing the concentration. The higher concentration of nano-emulsion shows the highest anti-biofilm activity 80-85% was observed at 7–10%. These results indicate that nano-emulsions effectively disrupt mature fungal biofilms, with enhanced activity at higher concentrations. The qualitative anti-biofilm activity against *A. niger* was carried out by using crystal violet staining techniques. At higher concentrations (7–10%), staining becomes faint, which indicates the strong inhibition of biofilm formation (Fig. 10).

5.1. EPS Inhibition Assay of Nano-emulsion

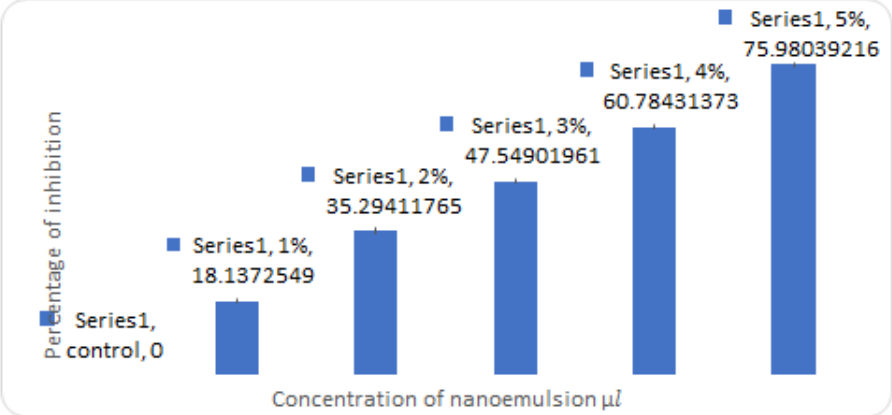


Figure 12 EPS Inhibition in *S. aureus*

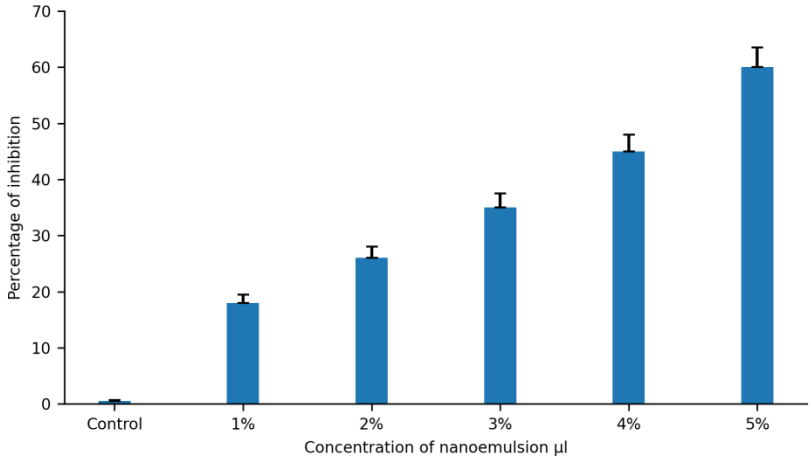


Figure 13 EPS Inhibition in *A. niger*

Extracellular polymeric substances are biopolymers produced by microorganisms that provide a protective and functional environment for the microbial community. The results indicate that the *S. aureus* exhibited 75% EPS inhibition at 5% of nano-emulsion (Fig. 11), while *A. niger* shows 60% EPS inhibition at 5% of nano-emulsion (Fig. 12). These findings demonstrate that nano-emulsions at a concentration of 5% effectively inhibit EPS production

5.2. Antioxidant Activity of Emulsion

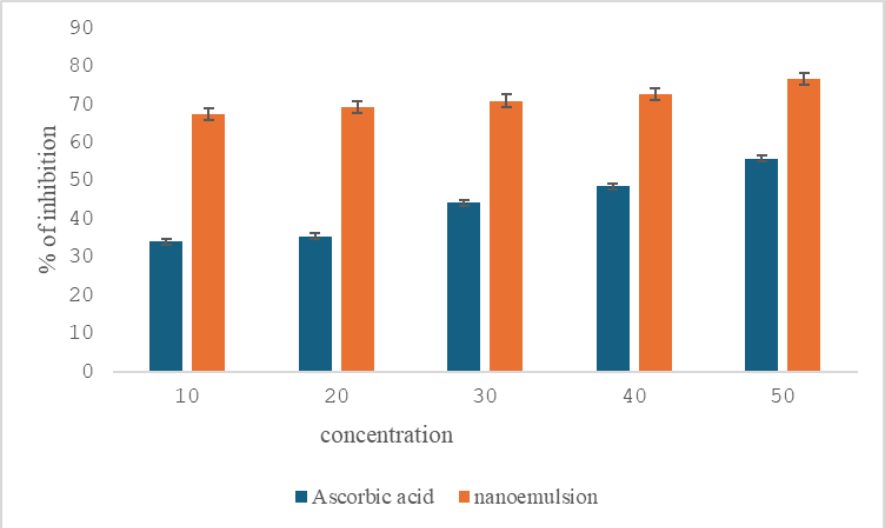


Figure 14 DPPH Antioxidant activity of nano-emulsion

Figure 14 The DPPH assay performed to evaluate the antioxidant capacity of the nano-emulsions based on their free radical scavenging activity. The researchers selected ascorbic acid as the standard for their study. The results demonstrated that the nano-emulsions exhibited highest antioxidant potential (76.7%) at 50µL/mL. The result indicates that the nano-emulsion has increased capacity to scavenge free radicals.

6.1. CONCLUSION

The nano-emulsion produced by using *Cinnamomum tamala* and *Illicium verum* essential oils beeswax and cellulose naocrystals. The study used GC-MS analysis to identify the chemical components found in the essential oils and beeswax. The nano-emulsion has stable smaller uniform droplets with low zeta potential. Nano-emulsion showed an average diameter of 50.498nm. The researchers used GC-MS to identify chemical compounds present in EOS and beeswax, estragole, α-pinene, aubepine, eucalyptol, eugenol, methyl isoeugenol, thujene, β-cymene, spatulenol, bicylogermacrene, caryophyllene and terpenol were present in EOs. In beeswax hexadecanoic, octadecenoicacid, methylester acid, nonadecane, Octacosane,2-methyl and octacosane were identified. The study results show that both tested bacterial strains were effectively inhibited by both essential oils and nano-emulsion, the antimicrobial activities due to the presence of secondary metabolites present in the essential oils. The cellulose nanocrystals nano-emulsion prepared by using *I. verum, C. tamala* and beeswax showed strong antibacterial and antifungal activities against food spoiling microbes especially *Staphylococcus aureus* and *Aspergillus niger*. The further researches are required to improve the physical characters and to increase the stability and activities of the nano-emulsion.

7. FUNDING SOURCES

This research did not receive any specific funding from external grant agencies.

8. ACKNOWLEDGMENT

The authors thank the Department of Chemistry and Bioscience, SASTRA Deemed to be University, Srinivasa Ramanujan Centre, Kumbakonam.

9. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

10. AUTHOR CONTRIBUTIONS

Dhurga R – Writing original Manuscript, **Bavya S S** – Editing, Grammar check and Plagiarism,

Ravichandran Natesan* - Formatting, Review and Resources

REFERENCES

1. Calvo H, Marco P, Blanco D, Coria R, Venturini ME. Potential of a new strain of *Bacillus amyloliquefaciens* BUZ-14 as a biocontrol agent of postharvest fruit diseases. **Food Microbiol.** 2017;63:101–110. doi:10.1016/j.fm.2016.11.004.
2. Das S, Singh VK, Dwivedy AK, Chaudhari AK, Dubey NK. Insecticidal and fungicidal efficacy of essential oils and nanoencapsulation approaches for development of next-generation ecofriendly green preservatives for management of stored food commodities: an overview. **Int J Pest Manag.** 2021:1–32.
3. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. **Int J Food Microbiol.** 2004;94:223–253.
4. Ju J, Xu X, Xie Y, Guo Y, Cheng Y, Qian H, et al. Inhibitory effects of cinnamon and clove essential oils on mold growth on baked foods. **Food Chem.** 2018;240:850–855.
5. Jiang Z, Akhtar Y, Bradbury R, Zhang X, Isman MB. Comparative toxicity of essential oils of *Litsea*

pungens and *Litsea cubeba* and blends of their major constituents against the cabbage looper, *Trichoplusia ni*. **J Agric Food Chem.** 2009;57:4833–4837.

6. Maurya A, Prasad J, Das S, Dwivedy AK. Essential oils and their application in food safety. **Front Sustain Food Syst.** 2021;5:133.
7. Singh BK, Tiwari S, Dubey NK. Essential oils and their nanoformulations as green preservatives in boosting food safety against mycotoxin contamination of food commodities: a review. **J Sci Food Agric.** 2021;101:4879–4890.
8. Olatunde OO, Benjakul S. Natural preservatives for extending the shelf-life of seafood: a revisit. **Compr Rev Food Sci Food Saf.** 2018;17:1595–1612.
9. Salvia-Trujillo L, Rojas-Graü A, Soliva-Fortuny R, Martín-Belloso O. Physicochemical characterization and antimicrobial activity of food-grade emulsions and nanoemulsions incorporating essential oils. **Food Hydrocoll.** 2015;43:547–556.
10. Chaudhari AK, Singh VK, Kedia A, Das S, Dubey NK. Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: prospects and retrospects. **Environ Sci Pollut Res.** 2021;28:18918–18940.
11. Das AK, Nanda PK, Bandyopadhyay S, Banerjee R, Biswas S, McClements DJ. Application of nanoemulsion-based approaches for improving the quality and safety of muscle foods: a comprehensive review. **Compr Rev Food Sci Food Saf.** 2020;19:2677–2700.
12. Anwer MK, Jamil S, Ibnouf EO, Shakeel F. Enhanced antibacterial effects of clove essential oil by nanoemulsion. **J Oleo Sci.** 2014;63:347–354.
13. Ledjanac S, Hoxha F, Jasnić N, Tasić A, Jovanović M, Blagojević S, et al. The influence of the chemical composition of beeswax foundation sheets on their acceptability by the bee's colony. **Molecules.** 2024;29(23):5489. doi:10.3390/molecules29235489.
14. Kaur J, Jawandha SK, Gill PS, et al. Composite coatings of beeswax + naphthalene acetic acid preserved fruit quality and antioxidants in stored lemon fruits. **Food Sci Biotechnol.** 2024;33:589–598. doi:10.1007/s10068-023-01364-4.
15. Dong H, Ding Q, Jiang Y, Li X, Han W. Pickering emulsions stabilized by spherical cellulose nanocrystals. **Carbohydr Polym.** 2021;265:118101.
16. Mikulcová V, Bordes R, Kašpárková V. On the preparation and antibacterial activity of emulsions stabilized with nanocellulose particles. **Food Hydrocoll.** 2016;61:780–792.
17. Zhou Y, Sun S, Bei W, Zahi MR, Yuan Q, Liang H. Preparation and antimicrobial activity of oregano essential oil Pickering emulsion stabilized by cellulose nanocrystals. **Int J Biol Macromol.** 2018;112:7–13.
18. Liu Y, Liang Y, Yuhong J, Xin P, Han JL, Du Y, et al. Advances in nanotechnology for enhancing the solubility and bioavailability of poorly soluble drugs. **Drug Des Devel Ther.** 2024;18:1469–1495. doi:10.2147/DDDT.S447496.
19. Bolgen UMG, Kayiran SD, Ozogul Y, Ozogul F. Essential oil-based nanoemulsions with current knowledge: formulation, characterization, and applications in food and pharmaceuticals. **Ind Crops Prod.** 2025;233:121411. doi:10.1016/j.indcrop.2025.121411.
20. Noumi E, Ahmad I, Adnan M, Patel H, Merghni A, Haddaji N, et al. *Illicium verum* L. (star anise) essential oil: GC/MS profile, molecular docking, in silico ADME profiling, quorum sensing, and biofilm-inhibiting effect on foodborne bacteria. **Molecules.** 2023;28(23):7691. doi:10.3390/molecules28237691.
21. Pradhan S, Paudel HR, Maharjan R, Sharma K. Essential oils from six aromatic plants of Langtang National Park: insights on their chemical constituents via GC-MS analysis. **Separations.** 2023;10(1):52. doi:10.3390/separations10010052.
22. Amawi RM, Ashmawy NS. GC-MS-based metabolic profiling of essential oils from *Citrus paradisi*, *Lawsonia inermis*, and *Ruta graveolens* and assessment of their acetylcholinesterase inhibitory potential. **Front Chem.** 2026;13:1761973. doi:10.3389/fchem.2025.1761973.
23. Raza MF, Hyder M, Zhao C, Li W. GC-MS analysis and evaluation of essential oils as volatile biopesticides: assessing their acaricidal potential against *Varroa destructor*. **Agriculture.** 2024;14(6):940. doi:10.3390/agriculture14060940.
24. Quatrin PM, Verdi CM, de Souza ME, de Godoi SN, Klein B, Gundel A, et al. Antimicrobial and antibiofilm activities of nanoemulsions containing *Eucalyptus globulus* oil against *Pseudomonas aeruginosa* and *Candida* spp. **Microb Pathog.** 2017;112:230–242. doi:10.1016/j.micpath.2017.09.062.
25. Liana D, Chatwichien J, Phanumartwiwath A. Enhanced anti-inflammatory and skin barrier repair effects of nanoemulsions supplemented with *Boesenbergia rotunda* for atopic dermatitis. **ACS Nanoscience Au.** 2024;5(1):37–51. doi:10.1021/acsnanoscienceau.4c00053.
26. Mitsagga C, Petrotos K, Giavasis I. Antimicrobial properties of lyophilized extracts of olive fruit, pomegranate and orange peel extracts against foodborne pathogenic and spoilage bacteria and fungi in vitro and in food matrices. **Molecules.** 2021;26(22):7038. doi:10.3390/molecules26227038.

27. Kamimura R, Kanematsu H, Ogawa A, Kogo T, Miura H, Kawai R, et al. Quantitative analyses of biofilm by using crystal violet staining and optical reflection. **Materials (Basel).** 2022;15(19):6727. doi:10.3390/ma15196727.

28. Durairajan R, Farisa Banu S, Nisha P, Murugan R, Thamotharan S, Percino MJ, et al. Essential oils from unexplored aromatic plants quench biofilm formation and virulence of methicillin-resistant *Staphylococcus aureus*. **Microb Pathog.** 2018;122:162–173. doi:10.1016/j.micpath.2018.06.028.

29. Wang S, Cheng Y, Wang J, Ding M, Fan Z. Antioxidant activity, formulation, optimization and characterization of an oil-in-water nanoemulsion loaded with lingonberry (*Vaccinium vitis-idaea* L.) leaves polyphenol extract. **Foods.** 2023;12(23):4256. doi:10.3390/foods12234256.