Development and Characterization of Eucalyptus Oil-Encapsulated Nanocarriers as Antibacterial and Antioxidant Agents

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ABSTRACT

Eucalyptus oil is a natural compound that gains attention due to its antibacterial and antioxidant properties. However, the utilization of eucalyptus oil is limited by its low stability and short duration of active compound release. The development of nanoencapsulation technology is an alternative to overcome these challenges. This study aimed to develop and characterize eucalyptus oil-encapsulated nanocarriers. Four nanoparticle formulations containing different concentrations of eucalyptus oil (0%, 0.5%, 1%, and 2%) were prepared. After testing the stability over a 15-day period, the formulation containing 2% eucalyptus oil showed the highest stability. Therefore, this formulation was selected for further characterization. Dynamic Light Scattering (DLS) analysis revealed that the developed nanoparticles had an average size of 142.1 ± 2.2 nm, a polydispersity index (PI) of 0.2 ± 0.041, and a zeta potential of -73.9 ± 1.3 mV. Antibacterial activity tests using disk diffusion and agar dilution methods revealed that the nanoparticles were unable to inhibit the growth of Escherichia coli and Staphylococcus aureus in comparison to 100% pure eucalyptus oil and gentamicin, which inhibited the growth of these bacteria. Antioxidant activity test using the DPPH assay indicated that the 2% eucalyptus oil-encapsulated nanoparticles did not exhibit free radical scavenging ability, while 100% pure eucalyptus oil demonstrated a limited capacity to scavenge free radicals compared to the standard antioxidant Trolox. Furthermore, in the ABTS antioxidant assay, the nanoparticles showed no antioxidant activity, whereas 100% pure eucalyptus oil exhibited the ability to inhibit free radicals at an IC_{50} of 0.241%. However, further investigation on the optimal concentration of encapsulated eucalyptus oil is required to improve the antibacterial and antioxidant activities of the nanoparticles.

INTRODUCTION

Eucalyptus is a fast-growing tree known for its aromatic leaves, widely used in the health and cosmetic industries. Its essential oil is rich in 1,8-cineole (eucalyptol), a key compound responsible for its antibacterial and antioxidant properties. Previous studies on *Eucalyptus urophylla* clones in Thailand have shown strong antibacterial effects against Gram-positive bacteria and varying antioxidant activity linked to 1,8-cineole content. However, eucalyptus oil faces several limitations, including instability, high volatility, poor water solubility, potential skin irritation, and uncontrolled release. Nanostructured Lipid Carriers (NLCs), composing of a solid lipid core and liquid lipid phase, offer a promising solution by enhancing stability, encapsulation efficiency, controlled release, and skin penetration, making them ideal for drug delivery and cosmetic applications. Despite their advantages, scalability and long-term stability remain key challenges for future development.

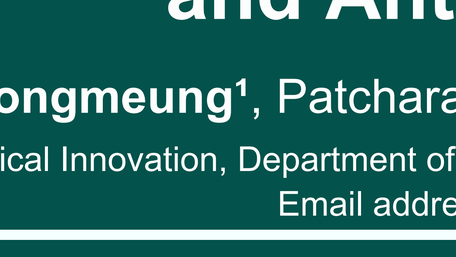


Figure 1 Chemical structure of the main biological active component of Eucalyptus

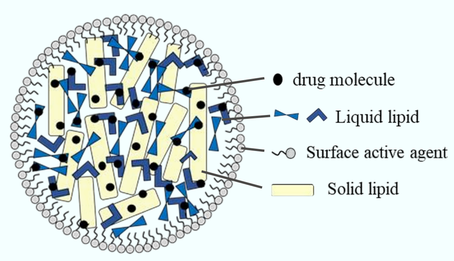


Figure 2 Nanostructured Lipid Carriers

METHODOLOGY

Formulation of Nanostructured Lipid Nanocarriers (NLCs)

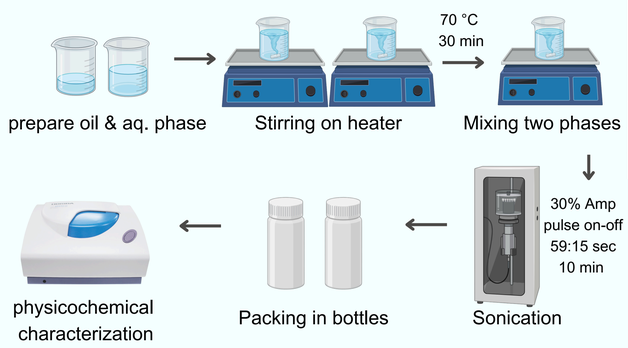


Table 1 Ingredients for each formula of oil phase

Oil phase	0% Eu-oil (g)	0.5% Eu-oil (g)	1% Eu-oil (g)	2% Eu-oil (g)
Caprylic/Capric Triglyceride	12.0	11.5	11.0	10.0
Cetearyl Glucoside	2.5	2.5	2.5	2.5
Sorbitan monooleate	3.5	3.5	3.5	3.5
Behenyl Alcohol	0.5	0.5	0.5	0.5
eucalyptus oil	0	0.5	1.0	2.0

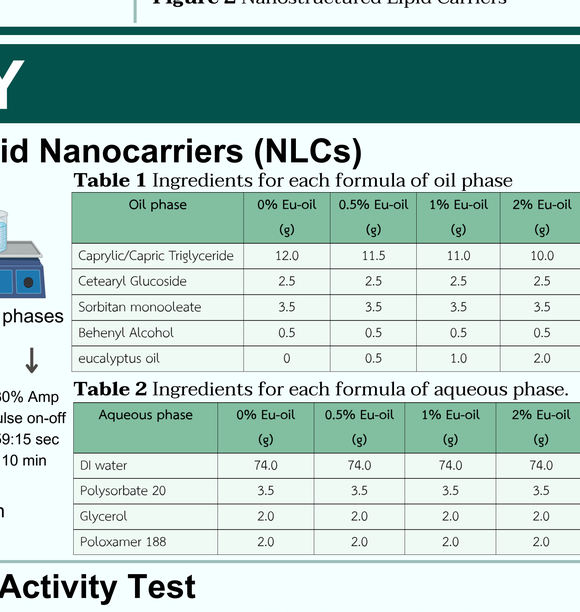
Table 2 Ingredients for each formula of aqueous phase.

Aqueous phase	0% Eu-oil (g)	0.5% Eu-oil (g)	1% Eu-oil (g)	2% Eu-oil (g)
DI water	74.0	74.0	74.0	74.0
Polysorbate 20	3.5	3.5	3.5	3.5
Glycerol	2.0	2.0	2.0	2.0
Poloxamer 188	2.0	2.0	2.0	2.0

Antibacterial Activity Test

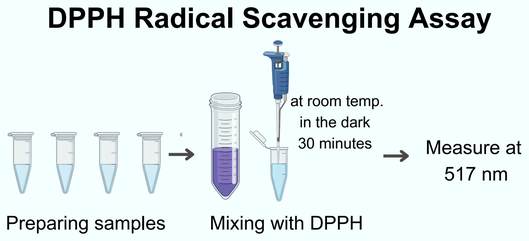
Disk Diffusion Method

Agar dilution method

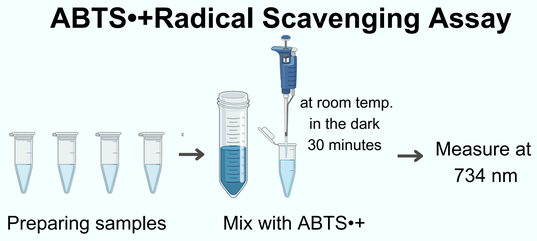


Antioxidant Activity Test

DPPH Radical Scavenging Assay



ABTS•+Radical Scavenging Assay



RESULTS & DISCUSSION

Physiochemical Properties of NLCs

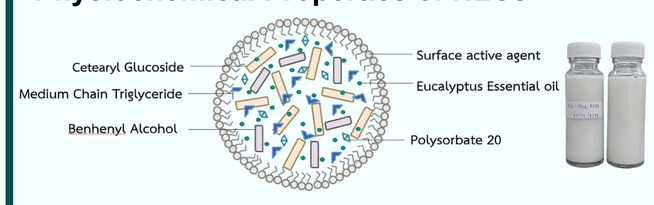


Figure 3 Eucalyptus Essential Oil Encapsulated in NLCs

The EU-NLCs are structured with hydrophobic components inside the core and hydrophilic components on the outer layer. The essential oil is encapsulated in the core for stability. The 1% and 2% Eucalyptus Oil formulations remained stable for 15 days, while the 0% and 0.5% formulations showed phase separation within 7 days.

Table 3 Comparison of particle size, polydispersity index (PI), and zeta potential of eucalyptus oil-loaded NLCs at different concentrations.

Sample name	Particle size (nm)	PI	Zeta potential (mV)
0% Eucalyptus Oil (control)	238.4 ± 4.7	0.395 ± 0.019	-59.9 ± 6.7
0.5% Eucalyptus Oil (0.5% EU-NLCs)	230.9 ± 12.3	0.5 ± 0.068	-47.5 ± 1.1
1% Eucalyptus Oil (1% EU-NLCs)	143.6 ± 2.2	0.3 ± 0.035	-74.1 ± 2.1
2% Eucalyptus Oil (2% EU-NLCs)	142.1 ± 2.2	0.2 ± 0.041	-73.9 ± 1.3

The 2% Eucalyptus Oil formulation exhibited the most optimal properties for NLC development, with the smallest particle size, narrowest size distribution, and highest zeta potential. The small particle size improves dispersion and reduces sedimentation, while a narrow size distribution ensures uniformity, minimizing instability. Additionally, the high zeta potential enhances electrostatic repulsion, preventing aggregation and maintaining colloidal stability. These properties make the 2% formulation the most stable and suitable for further applications.

Antibacterial Activity Test

Disk Diffusion Method

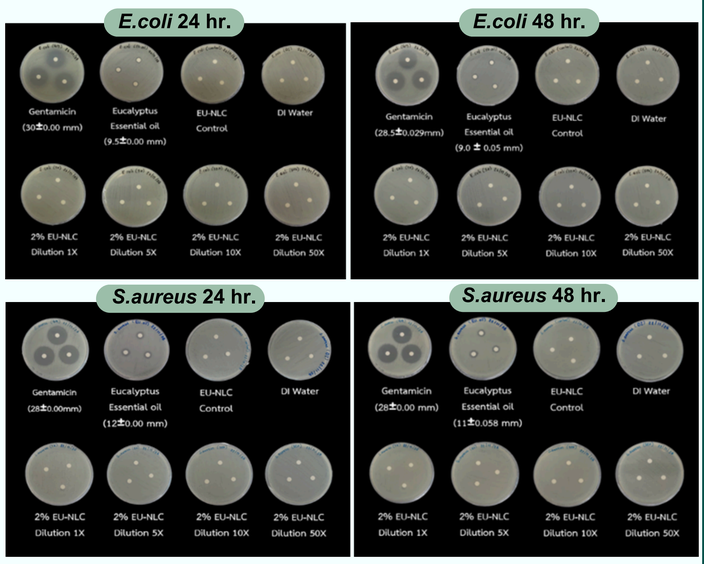


Figure 4 The antibacterial efficacy against *E. coli* and *S. aureus* after 24 and 48 hours of incubation.

Agar dilution method

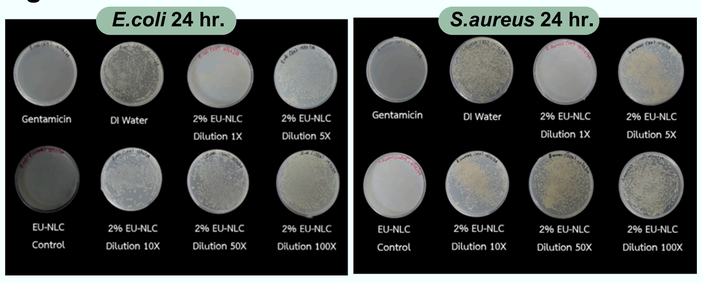


Figure 5 The antibacterial efficacy against *E. coli* and *S. aureus* after 24 hours of incubation.

Gentamicin and Pure eucalyptus oil effectively inhibited *E.coli* and *S.aureus*, but 2% EU-NLCs showed no antibacterial activity, possibly due to testing limitations or insufficient EU-Oil concentration. The agar dilution method confirmed this, indicating the need for formulation optimization or increased EU concentration.

Antioxidant Activity Test

DPPH & ABTS•+Radical Scavenging Assay

Table 4 Antioxidant activity of 2% EU-NLCs and pure eucalyptus oil using DPPH and ABTS·+ radical scavenging assay.

Sample name	IC_{50}	
	DPPH assay	ABTS assay
Trolox	24.22 ± 0.01 µg/ml	18.58 ± 0.18 µg/ml
Pure Eu-Oil	N/A	0.241 ± 0.001%
0% EU-NLCs	N/A	N/A
2% EU-NLCs	N/A	N/A

2% EU-NLCs failed to inhibit free radicals in both DPPH and ABTS methods, possibly due to incomplete release of antioxidants or insufficient concentration in the nanoencapsulation. In contrast, pure eucalyptus oil demonstrated better free radical inhibition in the ABTS method with an IC_{50} value of 0.241%, indicating superior antioxidant activity compared to standard trolox.

CONCLUSIONS

- The optimized formulation contained 2% eucalyptus oil.
- NLCs had a size of 140–240 nm, a polydispersity index of 0.2–0.395, and a zeta potential ranging from -77.0 to -46.6 mV.
- Pure eucalyptus oil exhibited antimicrobial activity against *E.coli* and *S.aureus*, while its NLC formulation showed no inhibition, likely due to insufficient oil concentration.
- Pure eucalyptus oil had slight antioxidant activity, whereas the NLC formulation showed no antioxidant activity, possibly due to unsuitable testing conditions and insufficient active compound concentration.

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