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Fabrication and characterization of Ceylon cinnamon bark oil-incorporated nano-emulsion for topical antifungal applications against *Trichophyton mentagrophytes*

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INTRODUCTION

Ceylon cinnamon bark oil (CCBO), obtained from *Cinnamomum zeylanicum*, possesses significant antifungal properties, but its topical pharmaceutical applications are mainly limited due to poor water solubility, skin irritation, volatility, and susceptibility to degradation. Cinnamon bark oil-incorporated oil-in-water nano-emulsion (CCBO-NE) was developed to improve the bioactivity of CCBO, while minimizing skin irritation risk in topical applications through encapsulation and CCBO dose reduction. *Trichophyton mentagrophytes* is a filamentous fungus belonging to the group of dermatophytes, which cause infections in keratinized tissues such as hair, skin, and nails. Tinea corporis (ringworm infection) is commonly caused by *T. mentagrophytes*. The development of antifungal resistance is observed as a major threat to dermatophyte infections across the globe. Cinnamaldehyde is the active constituent of cinnamon bark oil, and it is responsible for the antifungal activity.

RESEARCH AIM

To formulate and characterize a stable oil-in-water nano-emulsion incorporating Ceylon cinnamon bark oil (CCBO-NE) that:

- Enhances antifungal bioactivity of CCBO
- Minimizes skin irritation through dose reduction
- Demonstrates efficacy against a clinical isolate of *T. mentagrophytes*

METHODOLOGY

Extraction of authenticated Ceylon Cinnamon bark oil by Hydro-distillation method

Formulation of stable CCBO-NE by Phase inversion composition method (Low-energy method)

Physicochemical Characterization of CCBO-NE

Stable, transparent formulations were selected and comprehensively characterized for physicochemical and chemical properties.

- Determination of Organoleptic properties
- Determination of pH
- Determination of Droplet Size and Polydispersity Index
- Determination of Conductivity
- Determination of Dilution Test
- Determination of Dye Solubility Test
- Image Acquisition Using Light Microscopy
- Characterization Using Infrared Spectroscopy
- Characterization Using Raman Spectroscopy

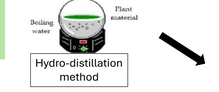
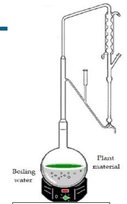


Figure 1: CCBO-NE particle size distribution

Evaluation of antifungal activity of CCBO-NE against *T. mentagrophytes* compared to CCBO by the Broth microdilution method

A serial dilution was prepared at concentrations of 50 mg/mL, 12.5 mg/mL, 6.25 mg/mL, and 3.12 mg/mL.


Microplate for the broth microdilution method



Hydro-distillation method


Extracted CCBO


Stable CCBO-NE

First reported study of CCBO-NE, demonstrating the highest significant inhibition of *T. mentagrophytes* formulated by low-energy emulsification method.

RESULTS AND DISCUSSION

Inhibition (CCBO-NE) **>80%** at all concentrations

Lowest effective [CCBO] **0.03%** w/v in CCBO-NE

CCBO content in NE **10%** w/v in formulation

Dose reduction factor **7.5×** vs. conventional range (0.1-1%)

Inhibition Comparison

% inhibition of *T. mentagrophytes*
Broth microdilution · two-fold dilution series (50→3.12 mg/mL)

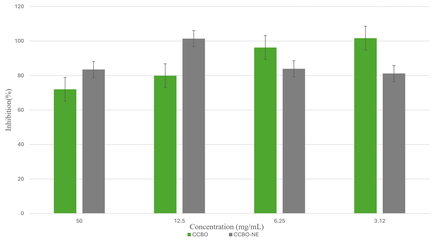


Figure 2: The percentage of inhibition of CCBO and CCBO-NE against *T. mentagrophytes*

Encapsulation, enhances antifungal activity while reducing skin irritation risk through a 7.5-fold dose reduction

Statistical significance
p < 0.0001
CCBO-NE (50 mg/mL and 12.5 mg/mL) exhibited significantly higher antifungal activity compared to crude CCBO

CONCLUSION

Potential to Enhance the Antifungal Activity
CCBO-NE enhances the antifungal activity by reducing droplet size, increasing the interfacial surface area, and improving penetration through the fungal cell membrane and improving penetration through the fungal cell membrane, demonstrating significantly higher antifungal activity against *T. mentagrophytes* compared to crude CCBO ($p < 0.0001$), with >80% inhibition across all tested concentrations.

CCBO-NE shows potential for topical antifungal applications against ringworm infections caused by *Trichophyton mentagrophytes*.
Encapsulation enabled a 7.5-fold dose reduction relative to the conventional safe topical CCBO range (0.1-1%), dramatically reducing the risk of skin irritation while improving efficacy.

Physicochemical parameters and topical application relevance

Table 1: The physicochemical characterization of CCBO-NE formulation

Parameter	Measured value	Significance
Average Droplet Size	66.01 nm	Increasing the interfacial surface area, Excellent penetration and reaches to the infected target site
Polydispersity Index	0.203	Monodisperse - stable system
Conductivity	87 ± 1 µS/cm	Confirms O/W nano-emulsion type
pH	5.63	Skin-compatible (4.5-6.5)
CCBO Content in NE	10% (w/v)	Lowest effective [CCBO] = 0.03% ; below irritation threshold
Infrared Spectroscopy	3398 cm-1	O-H stretching vibrations - due to water molecules
	1641 cm^{-1}	C=C stretching of aromatic ring of cinnamaldehyde
Raman Spectroscopy	1552 cm-1	High-intensity peak – indicating the vibrations of the main aromatic rings of cinnamaldehyde and cinnamyl acetate

Why does nano-emulsion encapsulation matter?

O/W nano-emulsion (66 nm droplets size)

Enhanced penetration and reaches the targeted infection site

How does CCBO inhibit *T. mentagrophytes*?

CCBO Cinnamaldehyde 38%

Enzyme inhibition Cytoplasmic granulation

Fungal cell membrane Ergosterol disruption

Membrane rupture Ion leakage/lysis

ROS generation Oxidative stress cascade

Growth inhibition > 80% at all concentrations

Cell death


The colony morphology of *T. mentagrophytes*

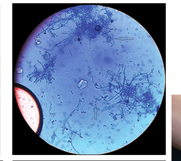

The microscopic view of *T. mentagrophytes*


Illustration of ringworm infection

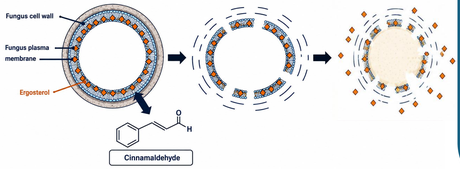


Figure 3: Illustration of Fungal cell membrane ergosterol disruption

REFERENCES

1. Nirmal, N. P.; Chunhavacharatorn, P.; Chandra Khanashyam, A.; Li, L.; Al-Asmari, F. Cinnamon Bark Oil in Water Nanoemulsion Formulation, Characterization, and Antimicrobial Activities. *LWT* **2023**, *179*, 114671. https://doi.org/10.1016/j.lwt.2023.114671.
2. Kruithoff, C.; Gamal, A.; McCormick, T. S.; Ghannoum, M. A. Dermatophyte Infections Worldwide: Increase in Incidence and Associated Antifungal Resistance. *Life* **2023**, *14* (1), 1. https://doi.org/10.3390/life14010001.
3. Makimori, R. Y.; Endo, E. H.; Makimori, J. W.; Zanqueta, E. B.; Ueda-Nakamura, T.; Leimann, F. V.; Gonçalves, O. H.; Dias Filho, B. P. Preparation, Characterization and Antidermatophytic Activity of Free- and Microencapsulated Cinnamon Essential Oil. *Journal de Mycologie Médicale* **2020**, *30* (2), 100933. https://doi.org/10.1016/j.mycmed.2020.100933.