Original Articles

Effect of Lemongrass Essential Oil on *Candida albicans*-infected Raw 264.7 Macrophages: An *In Vitro* Study

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Abstract

Macrophages are key components of the innate immune system by eliminating pathogens through phagocytosis. Their activity can be influenced by various agents including plant-derived essential oil. Lemongrass essential oil (LG-EO) exhibits potent antifungal properties, however, its immunomodulatory effects on macrophage function remain unclear. This study aimed to evaluate the effect of LG-EO on the phagocytic activity of RAW 264.7 macrophages infected with *C. albicans.* The cytotoxicity of LG-EO was assessed using the MTT reduction method. Three concentrations--0.003% (1/20 MIC), 0.006% (1/10 MIC), and 0.03% (1/2 MIC) (v/v)--were selected to evaluate their effects on the phagocytosis of *C. albicans*-infected RAW 264.7 cells. Infected macrophages were treated with various concentrations of LG-EO for four, six, and eight hours, after which surviving fungal cells were quantified using a cultivation method. LG-EO exhibited dose-dependent cytotoxicity, with fewer than 5% of RAW 264.7 cells surviving at concentrations ≥ 0.03% (v/v). In the untreated control, fungal recovery (log10 CFU/mL) was 2.08 (1.84-2.08), 2.48 (2.42-2.52), and 2.56 (1.93-2.66) after four, six, and eight hours of incubation, respectively. Treatment with 0.003% and 0.006% LG-EO showed no significant enhancement in phagocytosis, with fungal counts comparable or slightly higher than controls. In contrast, 0.03% LG-EO reduced fungal recovery to 1.48 (1.39-1.54), 1.7 (1.59-1.85), and 1.7 (1.5-1.83) at the respective time points, likely due to its cytotoxicity rather than enhanced phagocytic activity. At sub-cytotoxic concentrations, LG-EO did not significantly modulate the phagocytic activity of *C. albicans*-infected RAW 264.7 macrophages. These findings imply that LG-EO has limited immunomodulatory effects on macrophage mediated fungal clearance.

Keywords: *Candida albicans,* Cytotoxicity, Lemongrass essential oil (LG-EO), RAW 264.7 macrophages

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Introduction

Lemongrass essential oil (LG-EO), a natural extract from *Cymbopogon citratus*, has been investigated for various medicinal properties, particularly its antimicrobial activity. LG-EO has demonstrated potent antifungal effects, especially against *Candida albicans*, an opportunistic pathogen that primarily causes superficial or invasive candidiasis in immunocompromised individuals.[1-4] Citral, a key active compound in LG-EO, plays a crucial role in inhibiting the growth of planktonic yeast cells and their biofilm counterparts by targeting the fungal cell wall,

inhibiting enzymes essential for fungal growth, and suppressing hyphal formation.[5-7] Consequently, LG-EO has been incorporated or applied as a coating on the surfaces of dental materials, including tissue conditioners, denture acrylic resin, and silicone elastomers, to reduce the risk of fungal infections.[8-11] However, other medicinal properties of LG-EO—particularly its effects on immune responses--have not yet been extensively investigated.

The innate immune response is the first line of defense in the immune system, activated upon exposure to microorganisms, particularly pathogens. Phagocyt es such as macrophages, neutrophils, and dendritic cells play a major role in pathogen clearance and immune signaling. The clearance of *C. albicans* involves phagocytosis by macrophages, infiltrating monocytes, and neutrophils.[12] However, the hyphal form of the fungus produces candidalysin, a hypha-associated cytolytic peptide toxin that damage the membrane of immune cells and enables the fungus to escape from host defense mechanism.[13] Consequently, this study aimed to investigate the effect of LG-EO on macrophage phagocytosis of *C. albicans.* The findings may contribute valuable data for further studies on the role of LG-EO in modulating immune responses.

Materials and methods

Chemical reagents

LG-EO was purchased from Thai-China Flavours and Fragrances Industry Co., Ltd. (TCFF), Thailand. Dulbecco's Modified Eagle Medium (DMEM) and Triton X-100 were purchased from Sigma-Aldrich, USA, whereas L-glutamine, and fetal bovine serum (FBS) were purchased from Gibco labs, USA, and Cytiva, USA, respectively. Sabouraud dextrose agar and broth were purchased from Becton, Dickenson and company, NJ, USA.

LG-EO cytotoxicity assessment

RAW 264.7, a murine macrophage cell line, (Department of Oral Microbiology, Faculty of Dentistry, Mahidol University) was cultured in DMEM supplemented with 10% FBS and 1% L-glutamine (complete DMEM, CDMEM). Cells were maintained at 37°C in a humidified incubator (NuAire, Air-jacketed automatic CO_2 incubator, Model NU-5810E) with 5% CO_2 and subcultured every two days. RAW 264.7 cells ($1.75x10^5$ cells) were seeded into each well of a 96-well plate and incubated for 24 hours under the conditions described above. The cells were then treated with various concentrations of LG-EO--2%, 1%, 0.5%, 0.25%, 0.125%, 0.06%, 0.03%, 0.015%, 0.007%, and 0.003% (v/v) in CDMEM. After 18 hours of LG-EO exposure, cell viability was assessed using the MTT assay. Briefly, MTT solution [3-(4,5-dimethylthaizol-2-yl)- 2,5, Diphenyltetrazolium Bromide] was added to each well and incubated for two hours before the enzymatic reaction product was measured at 590 nm using microplate reader (BioTek ELx800, BioTek Instruments. Inc., USA). RAW 264.7 cells cultured in CDMEM without treatment and those treated with 1.0% Triton X-100 were used as negative and positive controls, respectively. Macrophage viability was calculated as follows.

$$\% \text{ Cell viability} = \frac{(\text{Mean OD of treated cells})}{(\text{Mean OD of control})} \times 100$$

Effect of LG-EO on *Candida albicans*-infected macrophages

C. albicans ATCC 10231 (Department of Oral Microbiology, Faculty of Dentistry, Mahidol University) was cultured in Sabouraud dextrose broth and incubated overnight at 37°C. The yeast suspension was confirmed by Gram staining (Figure 1) and adjusted to an optical density (OD) of 1.0 at 600 nm using a cell density meter (WPA CO 8000, Biochrom Ltd., UK). Subsequently, RAW 264.7 cells ($5x10^5$ cells) were seeded into 6-well plates and incubated overnight under the conditions described above. The cells were then infected with *C. albicans* at a multiplicity of infection (MOI) of 2. After an hour of fungal exposure, various concentrations of LG-EO—0.003%, 0.006%, and 0.03% (v/v)—were added to the cells, followed by incubation under the previously described conditions for four, six, and eight hours. After LG-EO treatment, the cells were harvested, washed three times with phosphate-buffered saline (PBS), and lysed using 0.1% Triton X-100 for 5 min. A 100 µL aliquot of the lysate was then plated onto Sabouraud dextrose agar and incubated at 37°C for 48 hours. Fungal survival was quantified and expressed as Log_{10} colony-forming units per millimeter (CFU/mL). RAW 264.7 cells cultured in CDMEM

were used as the negative control, while *C. albicans*-infected macrophages without treatment served as baseline for the phagocytic process.

All experiments were performed in triplicate for three different time periods.

Statistical analysis

Statistical analysis was performed using descriptive statistics, the Shapiro-Wilk test to assess data normality, and the Kruskal-Wallis test to evaluate differences between groups. A *P* value of <0.05 was considered statistically significant.

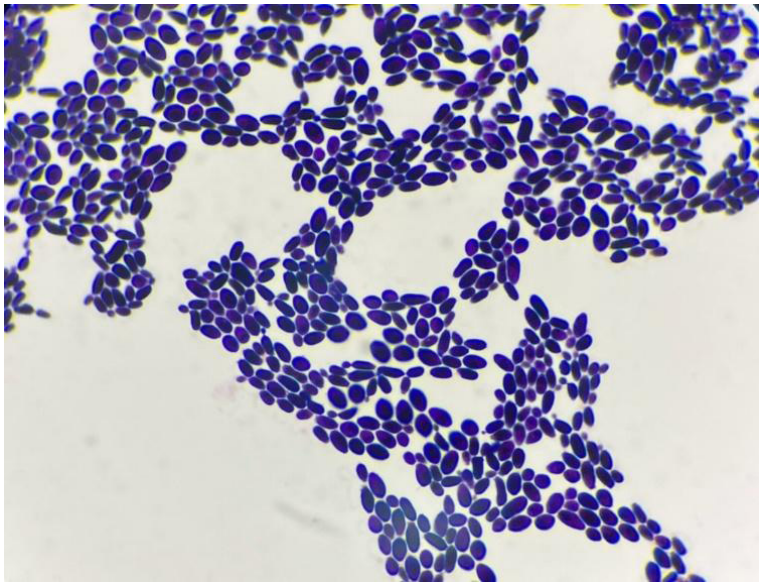

Figure 1 *Gram staining of Candida albicans suspension prepared for evaluating phagocytosis by RAW 264.7 cells (1000x)*

Results

LG-EO cytotoxicity assessment

The viability of RAW 264.7 cells treated with various concentrations of LG-EO—2%, 1%, 0.5%, 0.125%, 0.06%, 0.03%, and 0.015% (v/v)--was significantly reduced, ranging from 15.5%±8.5% to 4.65%±0.7%, compared to untreated cells (control), which exhibited 100% viability. In contrast, cell viability was 81%±10.5% and 51%±5% when the cells were treated with 0.003% and 0.007% (v/v) LG-EO, respectively, as shown in Figure 2.

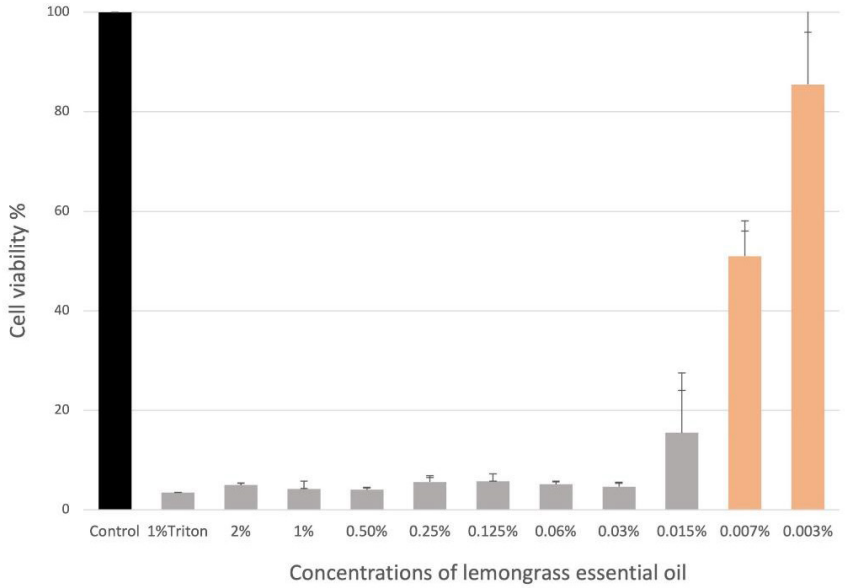

Figure 2 *Cytotoxic effect of lemongrass essential oil (LG-EO) on RAW 264.7 cells. Data are expressed as the percentage of viable cells (mean ± SEM) (n = 9 per group)*

Effect of LG-EO on *Candida albicans*-infected macrophages

Three concentrations of LG-EO—0.003%, 0.006%, and 0.03% (v/v)--were tested to evaluate their effects on the phagocytosis of *C. albicans*-infected RAW 264.7 cells. In the untreated control group, the number of *C. albicans* recovered from macrophages was expressed as the median of log10 CFU/mL with interquartile range and measured at 2.08 (1.84-2.08), 2.48 (2.42-2.52), and 2.56 (1.93-2.66) after four, six, and eight hours of incubation, respectively. Following treatment with 0.003% LG-EO, fungal recovery was 2.18 (2.02-2.21), 2.28 (2.28-2.33), and 3.1 (2.81-3.16) at the same time points, while treatment with 0.006% LG-EO resulted in values of 2.26 (2.25-2.48), 2.87 (2.87-2.97), 2.82 (2.59-2.96), respectively. In contrast, treatment with 0.03% LG-EO led to a reduction in fungal recovery, with values of 1.48 (1.39-1.54), 1.7 (1.59-1.85), and 1.7 (1.5-1.83) at four, six, and eight hours, respectively. However, none of the differences observed between LG-EO-treated and untreated groups were statistically significant. The effects of LG-EO on phagocytosis of *C. albicans*-infected RAW 246.7 cells are summarized in Figure 3.

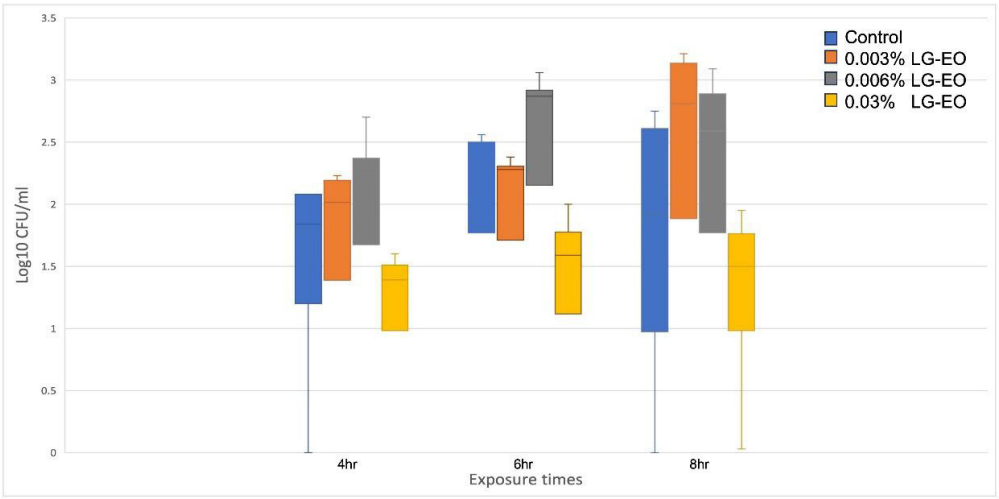

Figure 3 *Effect of lemongrass essential oil (LG-EO) on the phagocytosis of Candida albicans by RAW 264.7 macrophages. Data are expressed as the median (log10 CFU/mL) and interquartile range (n = 9 per group)*

Discussion

LG-EO, which contains citral as its major active component (>70%), has demonstrated potent antifungal activity, with a MIC ranging from 0.04% to 0.06% (v/v)[8, 10] It is of interest to evaluate whether LG-EO, at MIC and sub-MIC concentrations, affects the phagocytic activity of RAW 264.7 cells infected with *C. albicans*. Determining the cytotoxicity effect of LG-EO on RAW 264.7 cells is essential, as macrophage viability directly influences their phagocytic function. This study found that RAW 264.7 cells were highly sensitive to LG-EO, with fewer than 5% of the cell surviving after exposure to concentrations ≥ 0.03% (v/v). In contrast, approximately 80% of RAW 264.7 cells remained viable when exposed to 0.003% (v/v) LG-EO, which corresponds to a 20-fold dilution of its minimum inhibitory concentration (MIC) against *C. albicans*.[8] These findings differ markedly from those of a previous study, which reported that RAW 264.7 cells remained viable after exposure to 2.5% (w/v) LG-EO.[14] The discrepancy may be attributed to differences in testing procedures, the batch of LG-EO used, or the cell viability assay employed.[15] Cytotoxicity of LG-EO on RAW 264.7 cells was dose-dependent. Consequently, three concentrations of LG-EO—0.003% (1/20 MIC), 0.006% (1/10 MIC), 0.03% (v/v) (1/2 MIC)--were selected for subsequent evaluation of their effects on phagocytosis of *C. albicans*-infected RAW 264.7 cells.

Macrophages are key components of the innate immune system, serving as a first line defense by rapidly eliminating pathogens through phagocytosis--the process by which macrophages engulf and kill microbes. This study demonstrated that LG-EO at concentrations of 0.003% and

0.006% (v/v) has no significant effect on the killing activity of RAW 246.7 cells against *C. albicans*. These findings are consistent with a previous study, which reported that LG-EO did not directly enhance the killing activity of RAW 246.7 cells against *C. albicans*, likely due to its inhibitory effect on nitric oxide (NO) production.[14] To further clarify the impact of LG-EO on the killing activity of *C. albicans*-infected RAW 264.7 cells, extended incubation times such as 12, 24 and 48 hours should be considered, as the yeast cells are typically destroyed after at least 6 hours of phagocytosis.[16] Moreover, under culture conditions using CDMEM supplemented with FBS, *C. albicans* may transition to its hyphal form, make it more resistant to phagocytic clearance.[17] Since the number of viable and functional macrophages plays a crucial role in phagocytosis against microorganisms, it is important to assess the viability of RAW 264.7 cells after exposure to LG-EO for varying durations (four, six, and eight hours).

Phagocytic endocytosis, or engulfment--the process by which macrophages internalize pathogens--is a critical initial step in their clearance. Therefore, a higher rate of endocytosis generally correlates with more effective pathogen elimination. In this study, treatment with 0.003% and 0.006% (v/v) LG-EO did not significantly affect the endocytosis rate of RAW 264.7 cells infected with *C. albicans*, compared to untreated *C. albicans*-infected cells. This may be due to limitations of the cultivation method used to assess surviving fungi, which does not directly reflect the endocytosis process. Interestingly, the fungus-infected RAW 264.7 cells treated with these LG-EO concentrations appeared to exhibit increased endocytosis, as suggested by the higher numbers of surviving fungi in the raw CFU/mL data. To verify this observation, further experiment should be conducted using LG-EO treatment durations of 0.5, one, two, and three hours, followed by the quantification of internalized fungi using fluorescent-labeled or stained *Candida* cells, rather than relying solely on cultivation assay. Consequently, further studies using shorter exposure times and alternative methods for assessing phagocytosis—such as fluorescent labeling—are warranted to clarify the impact of LG-EO on the early stages of fungal uptake and clearance.

Conclusion

This study demonstrated that RAW 264.7 macrophages were highly sensitive to LG-EO, with significant cytotoxicity observed at concentrations above 0.03% (v/v). At sub-cytotoxic concentrations [0.003% and 0.006% (v/v)], LG-EO did not significantly enhance the phagocytic or killing activity of macrophages against *C. albicans*. These findings suggest that, despite its strong direct antifungal effects, LG-EO does not promote macrophage-mediated antifungal immunity at concentrations that are non-toxic to innate immune cells.

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References

1. Baumgardner DJ. Oral fungal microbiota: To thrush and beyond. ***J Patient Cent Res Rev*** 2019;28;6(4):252–61.

2. Sahu SR, Bose S, Singh M, Kumari P, Dutta A, Utkalaja BG, *et al.* Vaccines against candidiasis: Status, challenges and emerging opportunity. ***Front Cell Infect Microbiol*** 2022;18:12:1002406.

3. Talapko J, Juzbasic M, Matijevic T, Pustijanac E, Bakic S, Kotris I, *et al. Candida albicans*- The Virulence Factors and Clinical Manifestations of Infection. ***J Fungi (Basel)*** 2021;22;7(2):79.

4. Chen CY, Cheng A, Tien FM, Lee PC, Tien HF, Sheng WH, *et al.* Chronic disseminated candidiasis manifesting as hepatosplenic abscesses among patients with hematological malignancies. ***BMC Infect Dis*** 2019:19:635.

5. Mukarram M, Choudhary S, Khan MA, Poltronier P, Khan MMA, Ali J, *et al.* Lemongrass essential oil components with antimicrobial and anticancer activities. ***Antioxidants (Basel)*** 2021;22:11(1):20.

6. Paiva LFDE, Teixeira-Loyola ABA, Schnaider TB, Souza ACDE, Lima LMZ, Dias DR. Association of the essential oil of Cymbopogon citratus (DC) Stapf with nystatin against oral cavity yeasts. ***An Acad Bras Cienc*** 2022;94(1):e20200681.

7. Miranda-Cadena K, Marcos-Arias C, Perez-Rodriguez A, Cabello-Beitia I, Mateo E, Sevillano E, *et al. In vitro* and *in vivo* anti-*Candida* activity of citral in combination with fluconazole. ***J Oral Microbiol*** 2022;14(1):2045813.

8. Amornvit P, Choonharuangdej S, Srithavaj T. Lemongrass-incorporated tissue conditioner against *Candida albicans* culture. ***J Clin Diagn Res*** 2014;8(7):ZC50-2.

9. Choonharuangdej S, Srithavaj T, Chantanawilas P. Lemongrass-incorporated tissue conditioner with adjustable inhibitory effect against Candida albicans: An *in vitro* study. ***Int J Prosthodont*** 2022;35(3):338–42.

10. Choonharuangdej S, Srithavaj T, Thummawanit S. Fungicidal and inhibitory efficacy of cinnamon and lemongrass essential oils on *Candida albicans* biofilm established on acrylic resin: An *in vitro* study. ***J Prosthet Dent*** 2021;125(4):707.e1-707.e6.

11. Mat-Rani S, Chotprasert N, Srimaneekarn N, Choonharuangdej S. Fungicidal effect of lemongrass essential oil on *Candida albicans* biofilm pre-established on maxillofacial silicone specimens. ***J Int Soc Prev Community Dent*** 2021;11(5):525–30.

12. Rudkin FM, Bain JM, Walls C, Lewis LE, Gow NAR, Erwig LP. Altered dynamics of Candida albicans phagocytosis by macrophages and PMNs when both phagocyte subsets are present. ***mBio*** 2013; 4(6):e00810-13.

13. Konig A, Hube B, Kasper L. The dual function of the fungal toxin candidalysin during Candida albicans-macrophage interaction and virulence. ***Toxins (Basel)*** 2020;12(8):469.

14. Wisidsri N, Thungmungmee S, Khobjai W. Nitric oxide inhibitory and cytotoxic activities of spice essential oils. ***CMU J Nat Sci*** 2019; 18(3):373-92.

15. Madorran E, Ambroz M, Knez J, Sobocan M. An overview of the current state of cell viability assessment methods using OECD classification. ***Int J Mol Sci*** 2025;26(1):220.

16. Zhang Y, Tang C, Zhang Z, Li S, Zhao Y, Weng L, *et al.* Deletion of the ATP2 gene in *Candida albicans* blocks its escape from macrophage clearance. ***Front Cell Infect Microbiol*** 2021; 16:11:643121.

17. Chen H, Zhou X, Ren B, Cheng L. The regulation of hyphae growth in *Candida albicans.* ***Virulence*** 2020; 11(1):337–348.

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