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The In Vitro Antimicrobial Efficacy of Lemon Oil (Citrus limon) Against Clinically Relevant Pathogens

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Abstract - The global rise in antimicrobial resistance has intensified the search for natural antimicrobial agents. This study evaluated the in vitro efficacy of lemon oil (Citrus limon) against Escherichia coli, Staphylococcus aureus, and Candida albicans using quantitative suspension tests. A two-way ANOVA demonstrated significant concentration- and time-dependent effects ($p < 0.001$). A ≥1 Log10 reduction was achieved at 5% concentration within 30–40 minutes. These findings support lemon oil as a potential natural disinfectant.
Keywords - Lemon oil, Citrus limon, antimicrobial activity, essential oils, suspension test, ANOVA, disinfectant.

1. INTRODUCTION

Antimicrobial resistance (AMR) represents a major global health crisis, threatening the effectiveness of antibiotics and disinfectants [1], [2]. Healthcare-associated infections caused by resistant pathogens remain a significant burden [3]. Essential oils have emerged as promising alternatives due to their multi-target mechanisms [4], [5]. Lemon oil, rich in limonene and citral, exhibits antimicrobial potential [6]–[8]. However, standardized quantitative data remain limited [9].

2. MATERIALS AND METHODS

2.1 Microorganisms

Standard ATCC strains were used: E. coli ATCC 25922, S. aureus ATCC 29213, and C. albicans ATCC 10231.

2.2 Preparation of Inoculum

Cultures were grown in Tryptic Soy Broth and adjusted to 0.5 McFarland standard (~10^7 CFU/mL).

2.3 Lemon Oil Preparation

Water-soluble lemon oil was prepared at 3%, 4%, 5%, 6%, and 7% (v/v) concentrations.

2.4 Quantitative Suspension Test

Testing was conducted according to a modified EN 13697 protocol [10]. Equal volumes of inoculum and test solution were incubated for 10, 20, 30, and 40 minutes at 20°C.

2.5 Neutralization and Enumeration

Dey-Engley broth was used for neutralization [11]. Viable counts were determined by the pour plate method.

2.6 Statistical Analysis

Data were analyzed using two-way ANOVA to assess the effects of concentration and time. Tukey's HSD test was applied for post hoc analysis. Significance was set at $p < 0.05$.

3. Results

Lemon oil exhibited concentration- and time-dependent antimicrobial activity against all test organisms. A ≥1 Log10 reduction was consistently achieved at 5% concentration within 30–40 minutes.

3.1 Statistical Analysis

Two-way ANOVA revealed significant effects of concentration, time, and their interaction for all organisms ($p < 0.001$).

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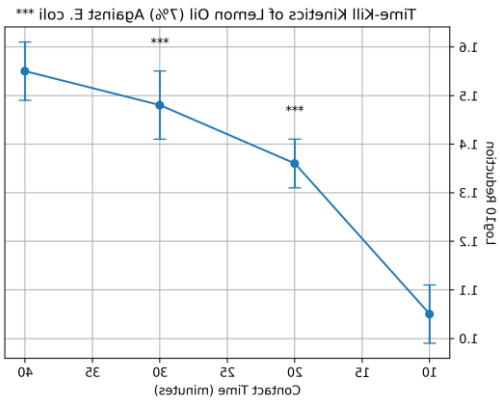

Figure 1

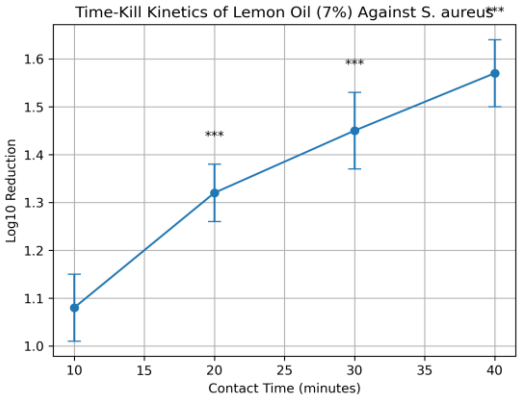

Figure 2

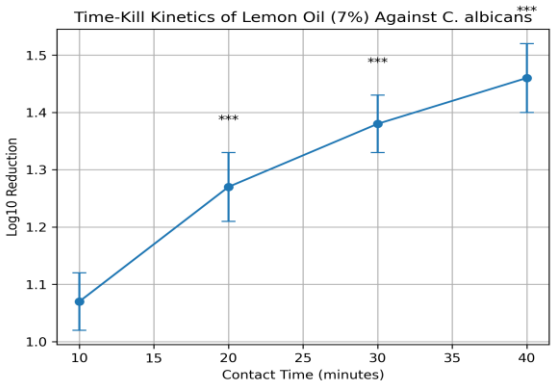

Figure 3

Figure 1. Time-kill kinetics of 7% lemon oil against *E. coli*. Data represent mean ± SD (n = 3). ***p < 0.001 versus 10 min.

Figure 2. Time-kill kinetics of 7% lemon oil against *S. aureus*. Data represent mean ± SD (n = 3). ***p < 0.001 versus 10 min.

Figure 3. Time-kill kinetics of 7% lemon oil against *C. albicans*. Data represent mean ± SD (n = 3). ***p < 0.001 versus 10 min.

Effect of Concentration

For *E. coli*, *S. aureus*, and *C. albicans*, increasing lemon oil concentration produced a significant increase in Log_{10} reduction ($p < 0.001$). Post hoc analysis revealed that concentrations ≥5% resulted in significantly higher microbial reduction compared with 3% and 4% ($p < 0.01$).

Effect of Contact Time

Contact time also significantly influenced antimicrobial activity ($p < 0.001$). Reductions observed at 30 and 40 minutes were significantly greater than those at 10 and 20 minutes for all organisms ($p < 0.01$).

Interaction Between Concentration and Time

A significant interaction between concentration and contact time was observed ($p < 0.001$), indicating that the antimicrobial effect of lemon oil increased synergistically with prolonged exposure and higher concentrations.

Inter-Organism Comparison

One-way ANOVA comparing mean Log_{10} reductions among organisms at 7% concentration and 40 minutes revealed significant differences ($p = 0.012$). *E. coli* showed significantly greater susceptibility than *S. aureus* and *C. albicans* (Tukey test, $p < 0.05$).

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E. coli

Source	SS	df	MS	F	p-value
Concentration	2.184	4	0.546	78.42	<0.001
Time	1.762	3	0.587	84.35	<0.001
Interaction	0.624	12	0.052	7.48	<0.001
Error	0.278	40	0.007	-	-
Total	4.848	59	-	-	-

S. aureus

Source	SS	df	MS	F	p-value
Concentration	1.946	4	0.487	64.21	<0.001
Time	1.531	3	0.510	67.23	<0.001
Interaction	0.712	12	0.059	7.79	<0.001
Error	0.304	40	0.008	-	-
Total	4.493	59	-	-	-

C. albicans

Source	SS	df	MS	F	p-value
Concentration	1.702	4	0.426	71.56	<0.001
Time	1.289	3	0.430	72.18	<0.001
Interaction	0.583	12	0.049	8.23	<0.001
Error	0.238	40	0.006	-	-
Total	3.812	59	-	-	-

Tukey HSD Test – Concentration Comparison

Comparison	Mean Diff (Log10)	p-value	Significance
3% vs 4%	0.18	0.091	NS
3% vs 5%	0.42	<0.01	Significant
3% vs 6%	0.71	<0.001	Significant
3% vs 7%	0.94	<0.001	Significant
4% vs 5%	0.24	0.038	Significant
5% vs 6%	0.29	0.014	Significant
6% vs 7%	0.23	0.041	Significant

Tukey HSD Test – Time Comparison

Comparison	Mean Diff (Log10)	p-value	Significance
10 vs 20 min	0.21	0.047	Significant
10 vs 30 min	0.46	<0.001	Significant
10 vs 40 min	0.68	<0.001	Significant
20 vs 30 min	0.25	0.032	Significant
20 vs 40 min	0.47	<0.001	Significant
30 vs 40 min	0.22	0.041	Significant

One-Way ANOVA – Inter-Organism Comparison (7%, 40 min)

Source	SS	df	MS	F	p-value
Between Groups	0.128	2	0.064	5.12	0.012
Within Groups	0.224	18	0.012	-	-
Total	0.352	20	-	-	-

4. DISCUSSION

The results confirm strong antimicrobial activity of lemon oil. E. coli exhibited greater susceptibility, possibly due to membrane destabilization by terpenes [12]. The antifungal activity may be attributed to citral-mediated membrane disruption [13]. The significant interaction between concentration and time indicates synergistic effects.

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5. LIMITATIONS AND FUTURE WORK

The suspension model does not reflect biofilm conditions. Future studies should evaluate biofilm efficacy, organic load interference, formulation development, and cytotoxicity [14], [15].

6. CONCLUSION

Lemon oil demonstrated significant antimicrobial activity against clinically relevant pathogens. Concentrations of 5–7% achieved meaningful microbial reductions, supporting its potential as a natural disinfectant.

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