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ฤทธิ์ต้านเชื้อแบคทีเรียของนาโนแคปซูลน้ำมันหอมระเหยตะไคร้ใน โพลิเมทิลเมธาไครเลท

The Antibacterial Activity of Lemongrass Essential Oil in Polymethyl Methacrylate Nanocapsule

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บทคัดย่อ

น้ำมันหอมระเหยจากตะไคร้กลั่นด้วยชุดกลั่น Clevenger ถูกห่อหุ้มด้วย polymethyl methacrylate โดยวิธีมินิอิมัลชันพอลิเมอไรเซชันได้นาโนแคปซูล polymethyl methacrylate/oil (PMMA/Oil) มีลักษณะเป็นอิมัลชันสีขุ่นขาว อนุภาคที่ได้มีความเสถียรทางคอลลอยด์สูง จากการวิเคราะห์ขนาดของอนุภาคด้วยเทคนิคการกระเจิงของแสงพบว่ามีขนาดอนุภาคเฉลี่ย 171 nm และร้อยละของการห่อหุ้มร้อยละ 91 การทดสอบฤทธิ์ทางชีวภาพโดย Disc Diffusion technique ของน้ำมันหอมระเหยจากตะไคร้ร้อยละ 1.5 โดยมวลต่อปริมาตร และ nano capsules PMMA/Oil โดยทดสอบการยับยั้งการเจริญของเชื้อแบคทีเรียได้แก่ *Bacillus subtilis* *Staphylococcus aureus* *Escherichia coli* และ *Pseudomonas aeruginosa* ในระยะเวลา 2 สัปดาห์ พบว่าน้ำมันหอมระเหยจากตะไคร้ยับยั้งการเจริญของเชื้อ *B. subtilis* *S. aureus* *E. coli* และ *P. aeruginosa* ได้เมื่อเวลาผ่านไป 12 ชั่วโมง โดยมีเส้นผ่านศูนย์การการยับยั้งการเจริญของเชื้อ 13.67 ± 0.94 mm 9.67 ± 1.31 mm 8.33 ± 0.94 mm และ 11.50 ± 0.41 mm ตามลำดับ หลังจากนั้นประสิทธิภาพในการยับยั้งการเจริญของเชื้อทั้ง 4 ชนิดได้ลดลง ในขณะที่ nano capsules PMMA/Oil สามารถยับยั้งการเจริญของเชื้อ *B. subtilis* *S. aureus* *E. coli* และ *P. aeruginosa* ได้ในเวลา 12 ชั่วโมง โดยมีเส้นผ่านศูนย์การการยับยั้งการเจริญของเชื้อ 25.67 ± 0.94 mm 21.57 ± 1.14 mm 16.50 ± 1.08 mm และ 23.67 ± 0.94 ตามลำดับ ซึ่ง nano capsules PMMA/Oil สามารถยับยั้งการเจริญของเชื้อได้มากว่าน้ำมันหอมระเหยจากตะไคร้ นอกจากนี้ nano capsules PMMA/Oil (1.52 %w/w) ยังสามารถยับยั้งการเจริญของเชื้อ *B. subtilis* และ *P. aeruginosa* ได้นานถึง 336 ชั่งโมง โดยมีเส้นผ่านศูนย์การการยับยั้งการเจริญของเชื้อ 25.83 ± 0.62 mm และ 24.83 ± 0.24 mm ตามลำดับ ในขณะที่ nano capsules PMMA/Oil สามารถยับยั้งการเจริญของเชื้อ *S. aureus* และ *E. coli* ในเวลา 336 ชั่วโมง ได้มากขึ้นโดยมีเส้นผ่านศูนย์การการยับยั้ง 50.50 ± 0.50 mm และ 27.00 ± 1.41 mm ตามลำดับซึ่งแสดงให้เห็นการปลดปล่อยน้ำมันหอมระเหยจากตะไคร้จากนาโนแคปซูล

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ABSTRACT

Lemongrass essential oil was encapsulated in polymethyl methacrylate (PMMA) nanocapsules using the miniemulsion polymerization method. The polymethyl methacrylate/oil (PMMA/Oil) nanocapsules exhibited high colloidal stability. The antibacterial activity of lemongrass essential oil and PMMA/Oil nanocapsules was determined using the disc diffusion technique to inhibit of *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa*, two-week period. It was found that the lemongrass essential oil inhibited the growth of *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* after 12 h, with inhibition zone diameters of 13.67 ± 0.94 mm, 9.67 ± 1.31 mm, 8.33 ± 0.94 mm, and 11.50 ± 0.41 mm, respectively. Subsequently, the efficacy in inhibiting the growth of all four strains was observed to decrease. In addition, the PMMA/Oil nanocapsules were able to inhibit the growth of *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* at 12 h, with inhibition zone diameters of 25.67 ± 0.94 mm, 21.57 ± 1.14 mm, 16.50 ± 1.08 mm, and 23.67 ± 0.94 mm, respectively. These values indicated that the PMMA/Oil nanocapsules inhibited microbial greater than those of the lemongrass essential oil alone. Furthermore, the PMMA/Oil nanocapsules were able to inhibit the growth of *B. subtilis* and *P. aeruginosa* for up to 336 h, with inhibition zone diameters of 25.83 ± 0.62 mm and 24.83 ± 0.24 mm, respectively. In contrast, the growth of *S. aureus* and *E. coli* showed larger inhibition zones the PMMA/Oil nanocapsules at 336 h, with inhibition zone diameters of 50.50 ± 0.50 mm and 27.00 ± 1.41 mm, respectively. These results indicate the release of lemongrass essential oil from the nanocapsules, resulting in enhanced and prolonged antimicrobial activity for up to 336 h.

คำสำคัญ: น้ำมันหอมระเหยตะไคร้ การห่อหุ้ม นาโนแคปซูล โพลิเมทิลเมทาไครเลต ฤทธิ์ต้านเชื้อแบคทีเรีย

Keywords: Lemongrass Essential Oil, Encapsulation, Nanocapsule, Polymethyl Methacrylate, Antibacterial Activity

INTRODUCTION

The essential oils are extracted from various medicinal plants, with lemongrass especially known for its high essential oil content. These oils consist of several chemical components, such as citral, geraniol, geranyl acetate, citronellal, citronellol, germacrene-D, and elemol. These compounds have demonstrated various biological activities, including antimicrobial, anticancer, and antioxidant properties (Zukowska and Durczynska, 2024; Mukarram *et al*., 2021; D'Agostino *et al*., 2019; Bakkali *et al*., 2008). In many countries, essential oils, stems, and leaves of lemongrass are widely used in cooking, medicine, and industrial applications. Interestingly, lemongrass essential oil has a wide spectrum of biological activities (Majewska, 2019). However, certain components within essential oils are known to be sensitive to external environmental factors, such as sunlight, oxygen, water, and heat, which causes them to volatilize and degrade rapidly. These limitations reduce the practical applications of essential oils because of their short duration of activity and limited overall efficacy. This limitation can be overcome by preparing the essential oils as polymer microcapsules, where the essential oil is encapsulated by a polymer shell to prevent direct exposure to air and other environmental factors. The polymer acts as a shell surrounding with the essential

oil core, allowing the encapsulated essential oil to exhibit prolonged activity, enhanced resistance to light and high temperatures, and controlled release at desired rates over a specific period, thereby improves the utilization efficiency (Deva, 2023; Yammine, 2023).

Nanoencapsulation is a technology used to encapsulate bioactive compounds derived from plants and herbs. This technology is utilized to increase the stability of extracts or active compounds, as well as to enhance their efficacy by enabling their targeted release in specific areas and over desired periods, which is beneficial for various products. Various techniques were employed for the preparation of nanocapsules for essential oil encapsulation, with the emulsification Method being the most widely used. In this method, polymers and surfactants were used to produce an emulsion, which is then subjected to various processes, such as solvent evaporation or precipitation, to form nanocapsules. The miniemulsion technique is of particular interest as it is considered relatively simple and efficient. It is also noted for its high encapsulation efficiency and, importantly, its high product yield percentage (Russell and Bruns, 2023; Tasker *et al*., 2016; Rakin *et al*., 2007; Luangtana-anan *et al*., 2005; Idrees *et al*., 2020; Reis *et al*., 2022).

Poly (methyl methacrylate) (PMMA) exhibits several outstanding properties, including high chemical stability, good biocompatibility, low toxicity, and favorable mechanical properties, such as a high Young's modulus and low elongation at break. Owing to these characteristics, PMMA has been widely considered a versatile and suitable shell material for the fabrication of micro- and nanocapsules in various applications. In the case of nanocapsules, they can be prepared using the miniemulsion polymerization method, which is similar to conventional emulsion polymerization. A typical miniemulsion system consists of oil droplets dispersed in an aqueous phase, with droplet sizes ranging from approximately 50 to 500 nm. Miniemulsion polymerization is a simple and effective technique for the preparation of PMMA nanocapsules encapsulating various substances, such as liquid crystals, thermal energy storage materials, hydrophobic drugs, and hydrophobic fragrance compounds (Wang *et al*., 2025).

The disc diffusion technique is widely used for determining the tolerance of bacteria to antibiotics. In this method, a Petri dish containing Mueller-Hinton agar was used to cultivate bacteria effectively within the plate. Subsequently, antibiotic-impregnated discs were placed onto the plate, the plates are then incubated under suitable conditions. The diameter of the zone of inhibition was measured and interpreted according to the standards of the Clinical and Laboratory Standards Institute (CLSI). In addition to its application, this method is also widely modified for various uses, such as screening for multidrug-resistant bacteria, studying the efficacy of novel antibiotics, evaluating the antimicrobial activity of natural extracts, being used in epidemiological research to monitor the spread of resistant strains, and serving as a reference method for comparison with other testing approaches (Hudzicki, 2009; Patel *et al*., 2015).

The aim of this research is to compare the efficacy of nanoencapsulated lemongrass essential oil with non-encapsulated essential oil in inhibiting the growth of both Gram-positive and Gram-negative microorganisms.

MATERIALS AND METHODS

Lemongrass essential oil was extracted from dried lemongrass leaves and pseudostems by hydrodistillation using a Clevenger-type apparatus. The dried plant material was distilled for 4 - 6 h, after which the essential oil was collected, separated from the aqueous phase, dried over anhydrous sodium sulfate, and stored in sealed amber glass bottles at 4ºC until further use.

Preparation of PMMA/Oil nanocapsules by Miniemulsion Polymerization method

PMMA nanocapsules were prepared by the miniemulsion polymerization method. Briefly, 3.52 g of methyl methacrylate (MMA) monomer and 0.28 g of azobisisobutyronitrile (AIBN) as the polymerization initiator were mixed to prepare the organic phase. The MMA monomer and AIBN were dissolved until a homogeneous solution was obtained. Subsequently, 1.52 g of lemongrass essential oil was added and mixed thoroughly to form the organic phase. The organic phase was then slowly added to the aqueous phase, which consisted of 90.00 g of deionized water containing 0.90 g of myristyltrimethylammonium bromide (TTAB) as the surfactant.

The monomer emulsion was prepared ultrasonication. The synthesis was carried out at 80ºC for 3 h, followed by an increase in temperature to 90ºC for an additional 5 h. PMMA nanocapsules encapsulating lemongrass essential oil were obtained. The procedure for the preparation of the oil loaded nanocapsules via the miniemulsion polymerization method is shown in Figure 1.

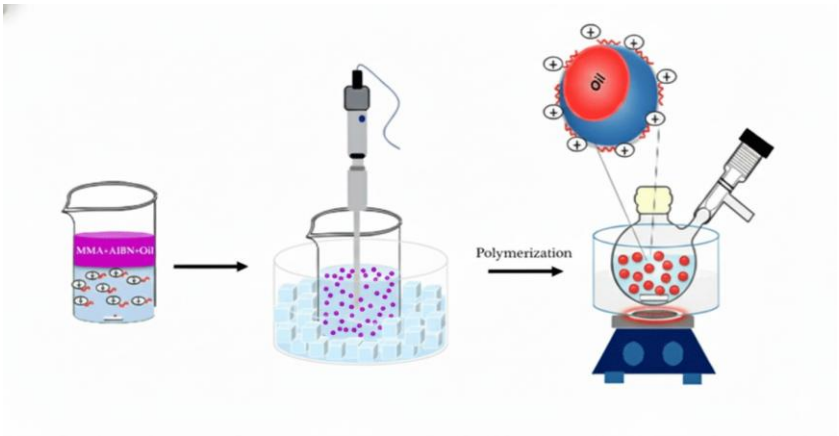


Figure 1 Schematic illustration of the preparation of PMMA nanocapsules by the miniminiemulsion polymerization method.

The nanocapsules encapsulated were then analyzed for particle size using Dynamic Light Scattering (DLS; Delsa Nano-C, Beckman Coulter, USA) at an angle of 165° in room temperature. Subsequently, the loading experiment percentage of the essential oil within the nanocapsules was determined using a Thermogravimetric Analyzer (TGA). The TGA analysis was performed over a temperature range of 50 - 500ºC at a scanning rate of 10ºC/min.

Determination of antimicrobial activity of PMMA/Oil nanocapsules by Disc Diffusion technique

The bacterial strains used in this study were *Pseudomonas aeruginosa* TISTR 2370, *Escherichia coli* FD, *Staphylococcus aureus* TISTR 746, and Bacillus subtilis TISTR 1930. Each strain was inoculated into nutrient broth (NB) and incubated at 37ºC with shaking at 150 rpm for 24 h using an incubator shaker. After 24 h of incubation, the bacterial suspensions were transferred to sterile test tubes, and their turbidity was adjusted to a 0.5 McFarland standard, corresponding to an absorbance of 0.08 - 0.13 at 625 nm, using sterile 0.85% (w/v) sodium chloride solution. The standardized bacterial suspension was then used for the agar disc diffusion assay to determine the inhibition zone.

The cotton swabs were dipped into the 0.5 McFarland microbial solutions and were spread evenly over the surface of prepared nutrient agar (NA) plates. Subsequently, anti-microbial testing was carried out by using 6.0 mm diameter paper discs containing 1.5% w/v lemongrass essential oil extract (with Dimethyl sulfoxide (DMSO) as the solvent), PMMA/Oil nanocapsules, and encapsulation material onto the agar plates at a volume of 20 microliters of each is applied to the discs. These plates were incubated in the incubator at 37ºC for 2 weeks.

RESULTS AND DISCUSSION

Preparation of PMMA/Oil nanocapsules by Miniminiemulsion Polymerization method

The lemongrass essential oil was encapsulated with polymethyl methacrylate via the miniemulsion polymerization method. A white, opaque miniemulsion of polymethyl methacrylate/oil (PMMA/Oil) nanocapsules is obtained. Figure 2 shows that the obtained PMMA/Oil nanocapsules exhibited high colloidal stability and are not found to coagulate. Figure 3 shows the particle size analysis obtained using a light scattering technique. The average particle size of PMMA was 275 nm, whereas the PMMA/Oil nanocapsules exhibited a reduced average particle size of 171 nm. The decrease in particle size after encapsulation is mainly due to the formation of a stable miniemulsion system, where surfactants reduce interfacial tension and generate smaller droplets. These droplets act as templates for nanocapsule formation, while improved stabilization prevents aggregation, resulting in smaller and more uniform particles (Wang *et al*., 2025; Ahangaran *et al*., 2019). The experimental encapsulation percentage was found to be 41%, which means 41% of the initial monomer was successfully converted into polymethyl methacrylate. This value is considered to be close to the calculated encapsulation percentage of 45%. In addition, a relatively high encapsulation efficiency of 91% was observed (Table 1), indicating that the majority of the core material was successfully entrapped within the PMMA shell. This high efficiency suggests that the miniminiemulsion system was stable and effective in preventing leakage or diffusion of the encapsulated substance into the continuous phase.

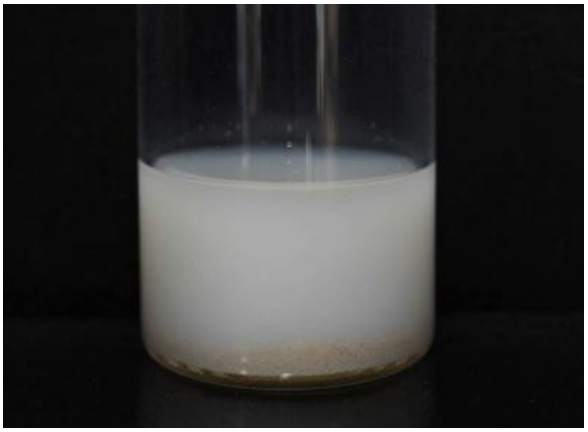


Figure 2 Nanocapsules of PMMA/Oil

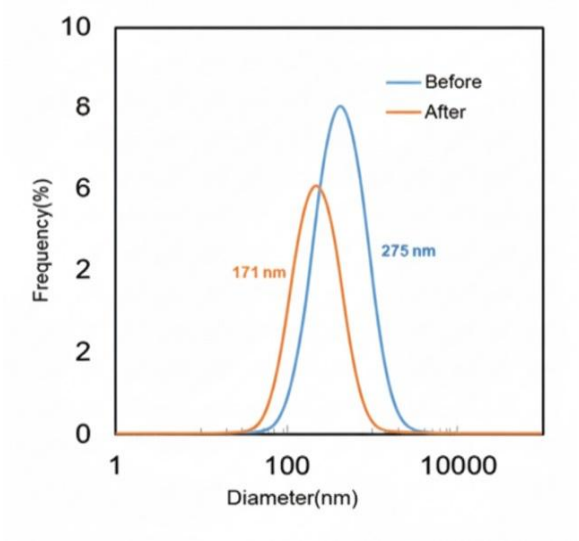


Figure 3 Particle size of PMMA/Oil nanocapsules (After) and polymethyl methacrylate (Before) from DLS.

Table 1 Percentage of loading and percentage of encapsulation efficiency of encapsulated PMMAOil nanocapsules

	Loading (wt%)		Encapsulation (wt%)
	experiment[a]	calculation[b]	
PMMA/Oil nanocapsules	41	45	91

[a]TGA

[b]Calculation using the following equation: %loading = [A/{A+(%conversion*B)/100}]*100

A = weight of essential oil B = weight of MMA

Antimicrobial activity of PMMA/Oil nanocapsules by disc diffusion technique

Lemongrass essential oil (1.5% w/v), obtained by water extraction, and PMMA/Oil nanocapsules were evaluated for their inhibitory activity against Gram-positive bacteria, including *B. subtilis* and *S. aureus*, and Gram-negative bacteria, including *E. coli* and *P. aeruginosa*. The disc diffusion technique was employed to investigate antibacterial activity over a period of 336 h (2 weeks). The experimental results, as presented

in Figures 4 - 7, showed that the lemongrass essential oil was able to inhibit the growth of all four microorganisms after 12 h. The inhibition zone diameters were 13.67 ± 0.94 mm for *B. subtilis*, 9.67 ± 1.31 mm for *S. aureus*, 8.33 ± 0.94 mm for *E. coli*, and 11.50 ± 0.41 mm for *P. aeruginosa*. However, the inhibitory efficacy decreased over time, and after 24 h, no inhibition of microbial growth was observed. This loss of activity can be attributed to environmental factors such as sunlight, oxygen, moisture, and heat, which accelerate the degradation of the essential oil components, thereby reducing its antimicrobial effectiveness.

However, the PMMA/Oil nanocapsules exhibited inhibitory activity against *B. subtilis* and *S. aureus* after 12 h, with inhibition zone diameters of 25.67 ± 0.94 mm and 21.57 ± 1.14 mm, respectively. Notably, the inhibition of these bacteria was sustained, with the inhibition zone diameters remaining relatively constant up to 336 h. Furthermore, the growth of *E. coli* and *S. aureus* was also inhibited by the PMMA/Oil nanocapsules after 12 h, with inhibition zone diameters of 16.50 ± 1.08 mm and 23.17 ± 0.25 mm, respectively. The inhibition zone of *E. coli* was maintained up to 72 h and increased to 27.00 ± 1.41 mm at 336 h. Similarly, the inhibition zone of *S. aureus* reached 50.50 ± 0.50 mm. An increase in the inhibition zone diameter under the “extended inhibition (encapsulated)” condition was observed, as shown in Figures 5 - 6. In contrast, the encapsulating material without lemongrass essential oil showed no inhibitory activity against any of the tested microorganisms.

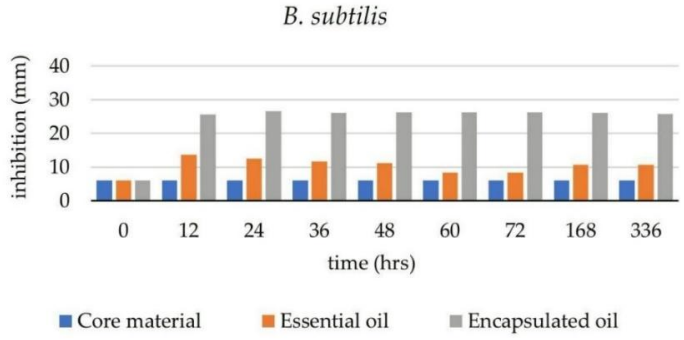


Figure 4 Inhibition zone diameter of *B. subtilis* by the essential oil sample over 336 h.
(■Core material, ■ Essential oil, ■ Encapsulated oil)

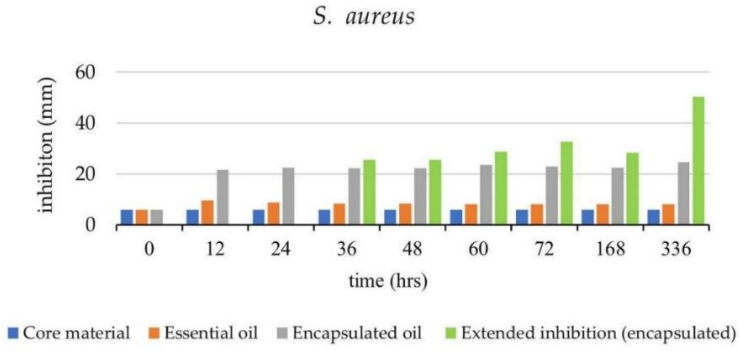


Figure 5 Inhibition zone diameter of *S. aureus* by the essential oil sample over 336 h.
(■Core material, ■ Essential oil, ■ Encapsulated oil, ■ Extended inhibition (encapsulated)

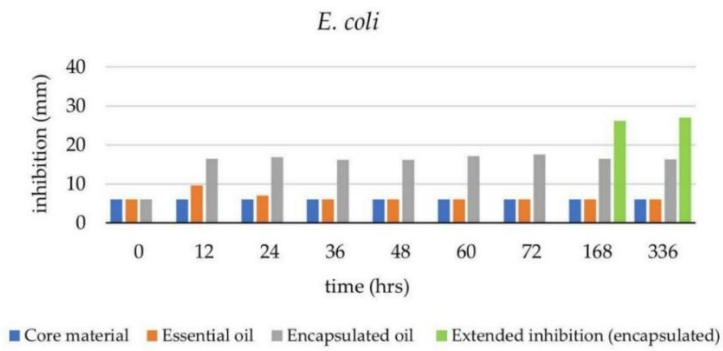


Figure 6 Inhibition zone diameter of *E. coli* by the essential oil sample over 336 h.
(■ Core material, ■ Essential oil, ■ Encapsulated oil, ■ Extended inhibition (encapsulated)

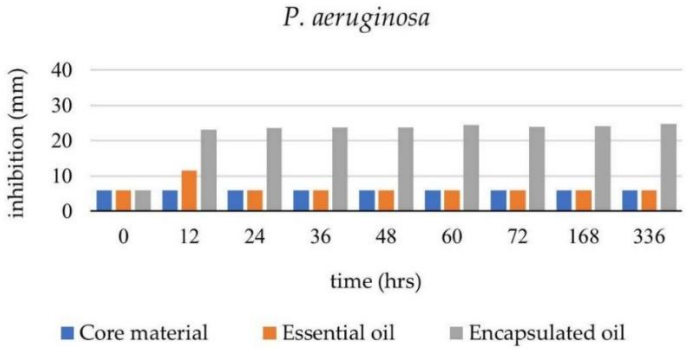
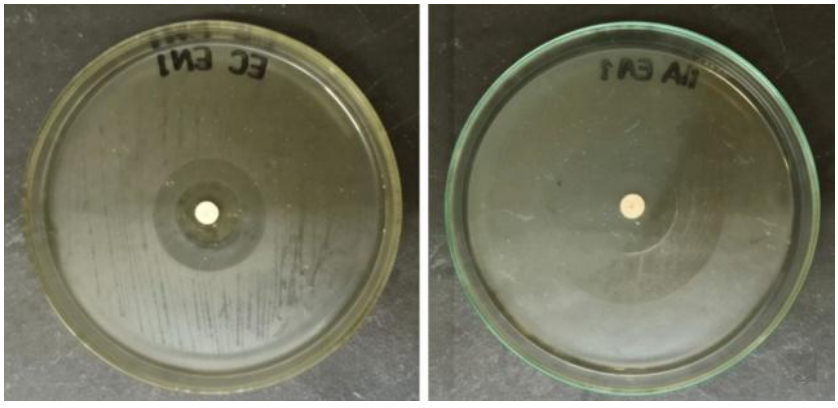


Figure 7 Inhibition zone diameter of P. aeruginosa by the essential oil sample over 336 h.
(■ Core material, ■ Essential oil, ■ Encapsulated oil)



(a) (b)

Figure 8 Inhibition zone of PMMA/Oil nanocapsules over 336 h; *E. coli* (a) and *S. aureus* (b).

Figure 8 shows the inhibition zones of PMMA/Oil nanocapsules after 336 h against E. *coli* and S. *aureus.* The sustained release of lemongrass essential oil from the nanocapsules led to an expanded inhibition zone of microbial growth. The release behavior of essential oils from PMMA nanocapsules is mainly governed by a diffusion-controlled mechanism, where the encapsulated oil gradually diffuses

through the polymeric shell. The PMMA matrix acts as an effective barrier, reducing the evaporation rate and enhancing the stability of volatile compounds. Previous studies have demonstrated that encapsulation significantly decreases the evaporation rate of aromatic oils compared to their free form (Lee *et al*., 2007). In addition, the high thermal stability and dense structure of PMMA further contribute to the sustained release behavior. The release rate is also influenced by the shell thickness and morphology, which control the diffusion path of the encapsulated compounds. Therefore, PMMA-based micro- and nanocapsules are highly suitable for controlled release applications, particularly for volatile substances such as essential oils (Ouyang *et al*., 2021).

CONCLUSIONS

The antimicrobial efficacy of lemongrass essential oil decreased over time. Based on the experimental results, no inhibition of microbial growth was observed after 24 h. This loss of activity can be attributed to environmental factors such as sunlight, oxygen, moisture, and heat, which accelerate the degradation of the essential oil components, thereby reducing its antimicrobial effectiveness. In contrast, PMMA/Oil nanocapsules demonstrate that encapsulation can enhance antimicrobial efficacy for up to 336 h by protecting the essential oil from environmental degradation factors such as oxidation, light, and heat. In addition, it enables a sustained release of active compounds, facilitating continuous interaction between the active agents and microbial cells. As a result, the antimicrobial activity is improved and prolonged compared to that of free essential oil, highlighting its potential for applications in antimicrobial formulations, cosmetics, and biomedical fields.

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