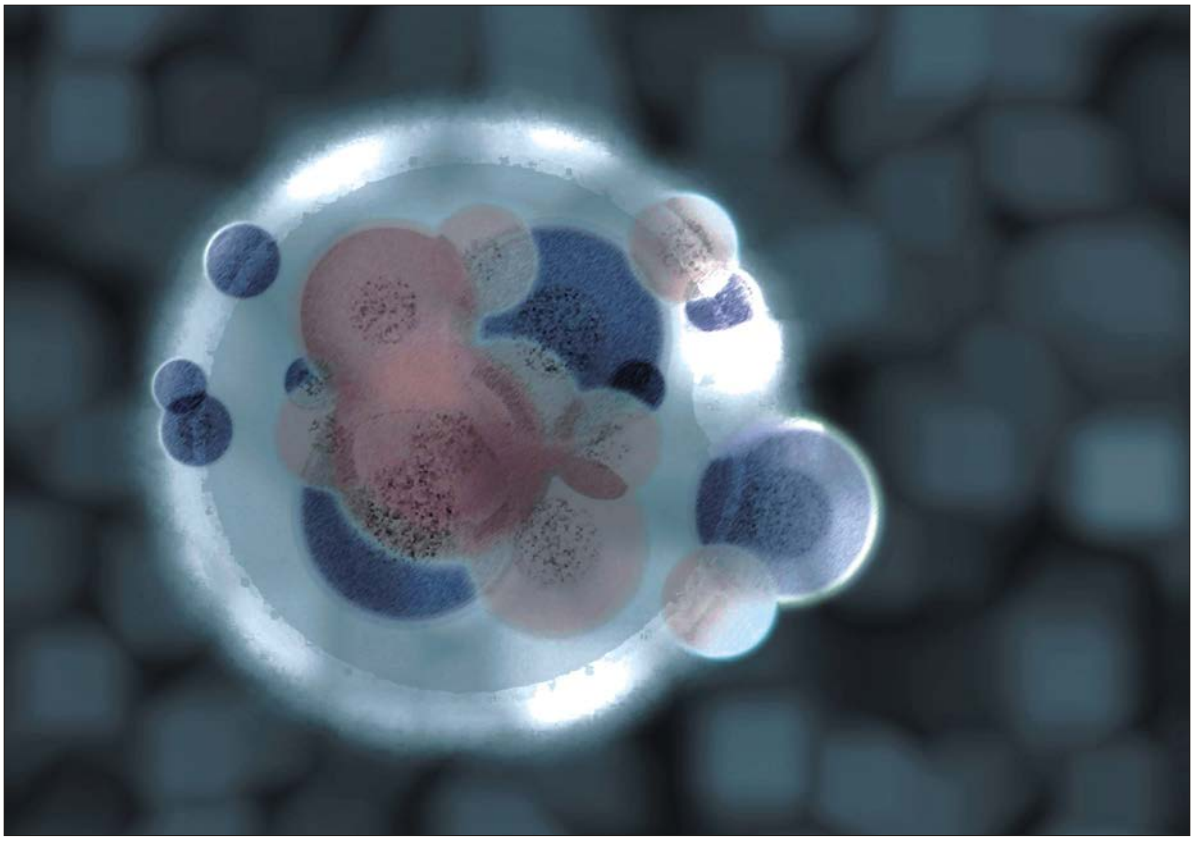

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# Research Article
# Antibacterial and Teeth Biofilm Degradation Activity of *Curcuma aeruginosa* Essential Oil

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## Abstract

**Background and Objective:** *Curcuma aeruginosa* are widely used as antibacterial agent. This study was conducted to compare the potency of essential oil from rhizome, stem and leaves of *C. aeruginosa* as antibacterial toward *Streptococcus mutans* and as teeth biofilm degradation. **Materials and Methods:** The essential oils were isolated by steam distillation, meanwhile the antibacterial agent and biofilm degradation assay was carried out by micro-dilution method. **Results:** Essential oil of *C. aeruginosa* rhizome showed higher potency as antibacterial agent than stem and leaves oil with minimum inhibition and bactericidal concentration of 15.63 and 1000 μg $mL^{-1}$, respectively. Otherwise, antibacterial activity of purified fraction of rhizome oil was lower than its crude oil. Biofilm degradation activity of stem crude oil was stronger than rhizome and leaves oil. Meanwhile, purified fraction of rhizome oil revealed higher biofilm degradation activity compare to stem crude oil. Further characterization using GC-MS and NMR indicated that purified fraction of rhizome oil contain 2,2,5-trimetil-2'(H)-5',6'-dihydropyrano[3',4',9]indan-1-one. **Conclusion:** Essential oil of *C. aeruginosa* rhizome was used as natural antibacterial agent toward *S. mutans*. Otherwise, rhizome, stems and leaves oils of *C. aeruginosa* showed lower potency in in biofilm degradation. Essential oil of *C. aeruginosa* rhizome could be considered as natural antibacterial agent in mouthwash or tooth paste to prevent teeth caries.

Key words: Antibacterial, biofilm degradation, *Curcuma aeruginosa*, distillation, essential oil

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Teeth cavities and caries is the major form of mouth health problem. Approximately 36% of the populations worldwide have dental caries in their permanent teeth[1]. *Streptococcus mutans* is a pathogen bacteria associated with dental cavities and caries. The *S. mutans* forms colony on teeth surface as biofilm or dental plaque and converts extracellular polysaccharides to form lactic acid through fermentation. Eventually, this process triggers teeth caries[2]. On the other hand, biofilm formation was supported by glucan, which is produced from glucose by glucosyltransferase (GTFs)[3]. Prevention of caries could be conducted through several approaches such as inhibiting the growth of pathogen bacteria, degradation of biofilm and inhibiting glucosyltransferase (GTFs) activity.

Tooth paste and mouthwash which is used in mouth care mostly contain antibacterial agent, both natural and synthetic. Phenolic compounds such as thymol, eucalyptol, methyl salicylate and menthol are common natural antibacterial[4]. Meanwhile, prevalent synthetic antibacterial agents are cetylpyridinium chloride (CPC), chlorhexidine and domiphen bromide[5]. Considering that synthetic antibacterial agents may have side effects such as taste alteration, tongue and mucosa peeling or tooth staining when used regularly, antibacterial agent from natural resources is continuously growing.

*Curcuma aeruginosa* is an indigenous medicinal plant in Southeast Asia region[6]. In Indonesia, rhizome of *C. aeruginosa* commonly utilized as ingredient of traditional medicine to treat various diseases. The rhizome of *C. aeruginosa* was traditionally used to treat diarrhea and fungal infections[7]. Rhizome extract exhibited prospective antibacterial activity against both Gram-positive and Gram-negative bacteria[8]. The rhizome was also utilized in treating tumors, asthma and bronchitis[9]. In contrast to rhizome, utilization of stem and leaves of *C. aeruginosa* is not widely reported.

Rhizome and leaves of *C. aeruginosa* reported to contain terpenoid compounds[10-12]. Curzerenone (24.6%), 1,8-cineole (11.0%), camphor (10.6%), zedoarol (6.3%), isocurcumenol (5.8%), curcumenol (5.6%) and furanogermenone (5.5%) were the major component in essential oil of *C. aeruginosa* rhizome[10]. Meanwhile, the main constituent in leaves essential oil was 1,8-cineole (17.7%), curzerenone (10.5%), furanogermenone (7.8%), camphor (7.5%), (Z)-3-hexenol (5.8%), furanodienone (5.1%), curcumenol (4.3%), isocurcumenol (3.7%) and β-elemene (3.3%)[11]. The component of *C. aeruginosa* leaves essential oil seems similar with rhizome but its abundance were different each other. It is open the possibility that the leaves and other part of *C. aeruginosa* perhaps possess resemble activity with rhizome and could be used as substitution for rhizome. This study aims to isolate essential oil of rhizomes, stems and leaves of *C. aeruginosa* and evaluate antibacterial and biofilm degradation activity of the oil.

## MATERIALS AND METHODS

*Curcuma aeruginosa* was collected from cultivation unit of Tropical Biopharmaca Research Center, Bogor Agriculture University, located at Bogor, Indonesia. The *C. aeruginosa* was identified at Research Center for Biology, Indonesian Institute of Science prior to use. The *S. mutans* was collected from Microbiology Laboratory, Faculty of Medicine, University of Indonesia (ATCC® 35668™).

**Isolation of *C. aeruginosa*'s essential oil:** Each 5 kg of fresh rhizomes, stems and leaves of *C. aeruginosa* was distilled by steam distillation for 6 h. Distillation was carried out at 100-105°C. Yield of distillation process was calculated based on weight of each sample[13].

**Antibacterial assay:** *Streptococcus mutans* was cultured in Tryptic Soy Broth (TSB) at 37°C. The potency of essential oil of *C. aeruginosa* as antibacterial toward *S. mutans* was analyzed by micro-dilution method at 96 well plates. One hundred microliters samples with concentration of 15.63-2000 μg $mL^{-1}$ were prepared in 96 well plates. One hundred microliters of TSB and 20 μL of *S. mutans* inoculants at the concentration of $10^{-2}$ CFU $mL^{-1}$ were added to each well of 96 well plates. The aliquot was incubated at 37°C for 24 h. The extract concentration at which there was no visually detectable bacterial growth was described as the Minimum Inhibitory Concentration (MIC). Further, 100 μL of aliquot with no visually detectable bacterial growth was inoculated in new medium and incubated at 37°C for 24 h. The extract concentration at which there was no visually detectable bacterial growth was described as the Minimum Bactericidal Concentration (MBC). The DMSO 20% was used as control-negative and tetracycline was used as control-positive[14].

**Biofilm degradation assay:** Biofilm degradation activity of *C. aeruginosa* essential oil was analyzed by micro-dilution method in 96 well plates[15]. Biofilm was formed from 100 μL synthetic saliva (Mc Dougall solution) with TSB medium, 3% glucose and bacterial inoculants. The mixture was incubated for 24 h at 37°C. Once a biofilm is formed, the remaining medium is discarded. Essential oils are added at a concentration of 16-2000 mg $mL^{-1}$ and then incubated 24 h at temperature of 37°C. Biofilms attached to the wall of the

wells is washed using phosphate buffer. Crystal violet 1% was added to the wells and left for 15 min. Well rinsed with sterile water 3 times and 95% ethanol was added. The suspension was incubated for 45 min and the solution was transferred to a new micro-plate. Suspension absorbance of each well was measured using a micro-plate reader at a wavelength of 595 nm to determine the percentage degradation. Chlorhexidine was used as positive control and 20% DMSO as a negative control. Degradation (%) is given as in Eq. 1:

$$\text{Degradation } (\%) = \left(1 - \left(\frac{A_{\text{corrected sample}}}{A_{\text{corrected blank}}}\right)\right) \times 100 \quad (1)$$

where, $A_{\text{corrected sample}}$ is absorbance (Essential oil or chlorhexidine+inoculants *S. mutans*) and $A_{\text{corrected blank}}$ is absorbance (DMSO 20%+inoculants *S. mutans*).

**Identification of chemical constituents of *C. aeruginosa*'s essential oil:** Component of *C. aeruginosa*'s essential oils was identified using gas chromatography-mass spectroscopy (GC-MS) agilent 5873. The HP-5 MS (30 m × 0.25 mm × 250 µm) and helium gas at flow rate of 20 mL $min^{-1}$ were used as stationary and mobile phase system in GC. Injector and detector temperature were set at 80 and 250°C, respectively. The separation temperature started at 80°C for 5 min and gradually increased in rate of 10°C $min^{-1}$ to reach 250°C, the temperature keep at 250°C for 45 min. The electron impact ionization (EI-MS) was carried at 70 eV. The MS data were collected and compare with library index MS. Component of *C. aeruginosa*'s rhizome essential oils were further identified by Nuclear Magnetic Resonance (NMR) JOEL ECA-600, $CDCl_3$ was used as solvent.

## RESULTS

Moisture content and yield of essential oil from fresh rhizomes, stems and leaves of *C. aeruginosa* were presented in Fig. 1. The moisture content of fresh *C. aeruginosa* was in range of 84-95%. The highest yield of essential oil was obtained from rhizome distillation (1.99 %), meanwhile the yield of essential oil derived from leaves and stems distillation was a 10th and 20th smaller than rhizome, respectively. The color of essential oil obtained from rhizome, stems and leaves of *C. aeruginosa* were similar, dark brown.

The essential oil from rhizomes of *C. aeruginosa* showed good antibacterial activity toward *S. mutans.* The MIC value of rhizome oil was 15.63 µg $mL^{-1}$. The MIC value of rhizome oil was same with the MIC of synthetic antibacterial tetracycline (Table 1). This result indicated that rhizome oil of *C. aeruginosa* was potential as antibacterial agent. On the contrary, the antibacterial potency of stems and leaves oil were smaller than rhizome oil.

Biofilm degradation activity of stems oil was higher than rhizomes and leaves oil, the $IC_{50}$ value was 1347.27 µg $mL^{-1}$ (Table 1). Otherwise, fractions from column chromatography of rhizome oil exhibited higher biofilm degradation activity than stem oil ($IC_{50}$ value was in range of 228-508 µg $mL^{-1}$). Meanwhile, the biofilm degradation activity of all crude oil and purified fractions were lower than positive control, chlorhexidine (Table 1).

Rhizomes, stems and leaves oil of *C. aeruginosa* were identified using GC-MS. The results showed that 2,2,5-trimethyl-2'(H)-5',6'-dihydropyrano[3',4',9]indan-1-one (40%) and 1,8-cineol (13%) were the major compounds in rhizome oil (Table 2). The major compounds in stem and leave oil almost similar, namely β-elemene, caryophyllene and epicurzerenone. Trans-6-ethenyl-4,5,6,7-tetrahydro-3, 6-dimethyl-5-isopropenyl was identified in stem oil at higher amount (7%) than in leaves oil.

Fraction 1 from rhizome oil indicated the potency as antibacterial toward *S. mutans.* Its biofilm degradation activity also stronger than stem oil. The GC-MS analysis of fraction 1 showed that 2,2,5-trimethyl-2'(H)-5', 6'-dihydropyrano[3',4',9]indan-1-one is the major component with relative abundance more than 50%. Further fractionation

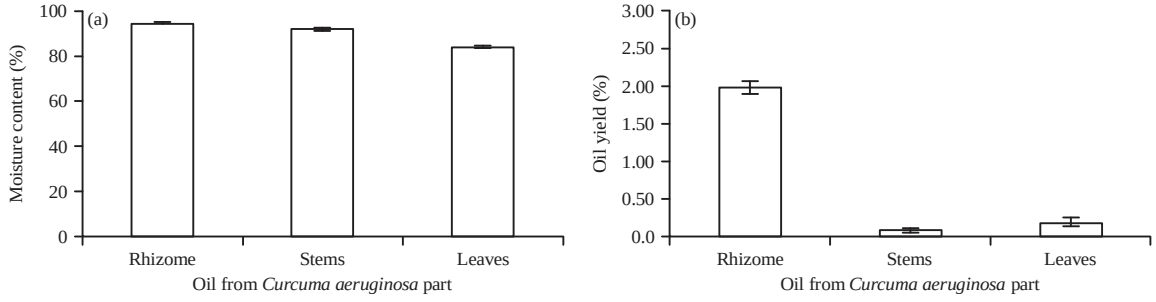


Fig. 1(a-b): (a) Moisture content and (b) Yield of essential oil of *C. aeruginosa* rhizomes, stems and leaves

Table 1: Antibacterial activities toward *S. mutans* and biofilm degradation of essential oil of *C. aeruginosa* rhizomes, stems and leaves

| Essential oil | Antibacterial activities (μg mL$^{-1}$) | | Degradation biofilm activities |
|---|---|---|---|
| *C. aeruginosa* | MIC | MBC | $IC_{50}$ (μg mL$^{-1}$) |
| Rhizome (RE) | 15.63 | 1000.00 | * |
| Stem | 125.00 | 2000.00 | 1347.27 |
| Leaves | 500.00 | * | * |
| Tetracycline | 15.63 | 15.63 | * |
| Chlorhexidine | * | * | 2.58 |
| F1 RE | 500.00 | 500.00 | 508.95 |
| F2 RE | 1000.00 | 1000.00 | 375.71 |
| F3 RE | 250.00 | 1000.00 | 228.53 |
| F4 RE | 2000.00 | 2000.00 | * |
| F5 RE | * | * | * |
| F6 RE | * | * | * |
| F1.1 RE | 2000.00 | * | * |
| F1.2 RE | 500.00 | 2000.00 | * |
| F1.3 RE | * | * | * |

*>2000 μg mL$^{-1}$

Table 2: Component of *C. aeruginosa* essential oil analyzed by GC-MS

| | | Abundance (%) | | | |
|---|---|---|---|---|---|
| Classification | Compound name | Leaves | Stems | Rhizomes | F1 RE |
| Monoterpenoids | Camphene | - | - | + | - |
| | Camphor | + | - | - | + |
| | β-Pinene | + | - | + | - |
| | Isoborneol | | - | + | - |
| | Limonene | + | - | - | - |
| | 1.8-cineol | + | - | 13.23 | + |
| | Alcanfor | - | - | + | - |
| | α-terpinene | + | + | - | - |
| Sesquiterpenoids | β-elemene | 7.72 | 10.01 | + | + |
| | β-eudesma | - | - | - | + |
| | Humulene | + | + | - | - |
| | Alloaromadendrene | - | + | - | - |
| | Caryophyllene | 8.35 | 6.59 | - | + |
| | Germacrene-D | + | + | - | - |
| | Trans-6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl benzofuran | + | 7.03 | + | - |
| | Epicurzerenone | 13.98 | 18.80 | - | - |
| | Isocurcumenol | - | - | - | + |
| | 2,2,5-trimethyl-2'(H)-5',6'-dihydropyrano[3',4',9]indan-1-one | - | - | 40.11 | 54.21 |

-: Unidentified, +: Identified at abundance of 1-6%

of fraction 1 of rhizome oil produced three fractions, namely F1.1, F1.2 and F1.3. Fraction F1.2 gave highest antibacterial activity with MIC value of 500 μg mL$^{-1}$. The GC-MS analysis of fraction F1.2 showed single dominant peak with molecular weight of 230 g mol$^{-1}$ (Fig. 2). The $^{13}$C NMR spectrum revealed 15 carbon atoms with chemical shift of 195.0, 145.6, 141.1, 139.6, 119.3, 115.7, 113.0, 64.1 and 50.8 ppm. Meanwhile, $^{1}$H NMR spectrum showed 18 hydrogen atoms with various chemical shifts (Table 3).

## DISCUSSION

Distillation is a suitable technique for separation of volatile compound and essential oil from sample. Yield of distillation process will depend on type of distillation, time and temperature operation. Yield of essential oil from rhizome of *C. aeruginosa* was 1.99%, higher than yield of *C. aeruginosa* rhizome reported by Kamazeri *et al.*[16]. The difference could be due to the separation of oil using dichloromethane after

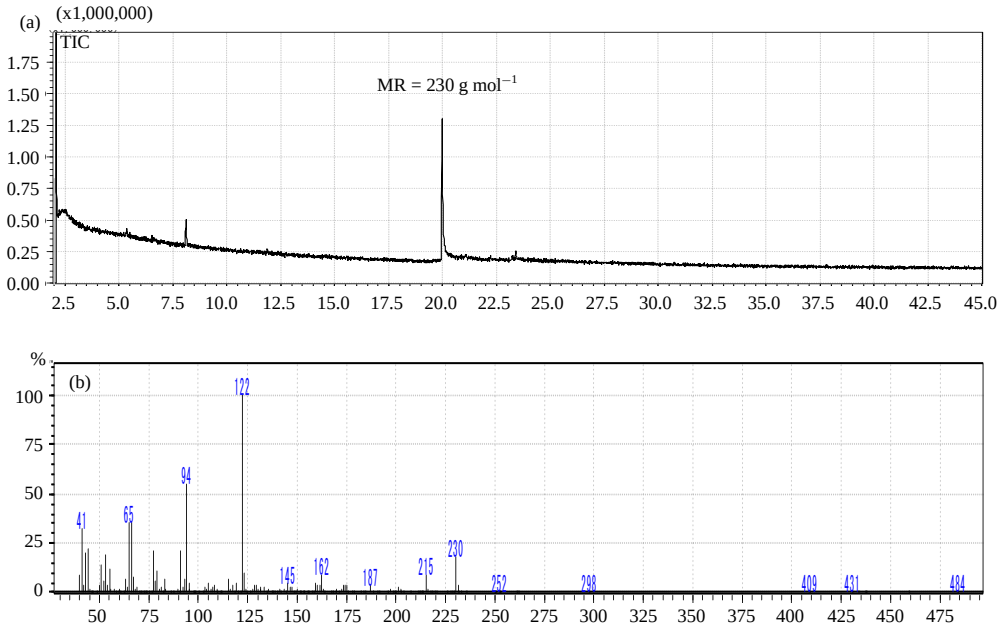


Fig. 2(a-b): (a) Chromatogram and (b) MS spectrum of fraction F1.2

Table 3: Chemical shift of $^{1}H$ and $^{13}C$ NMR of F1.2 MR (solvent: $CDCl_3$)

| Atom C/H | $\delta_H$ 600 MHz (ppm) (multiplicities, ∑H) | $\delta_C$ 150 MHz (ppm) |
|---|---|---|
| 1 | - | 195.0 |
| 2 | - | 42.9 |
| 3 | 2.2 (s, 2H) | 33.6 |
| 4 | 7.1 (s, 1H) | 113.3 |
| 5 | - | 115.7 |
| 2' | 4.7 (s, 2H) | 50.8 |
| 3' | - | 141.1 |
| 4' | - | 139.6 |
| 5' | 2.9 (t, 2H) | 24.9 |
| 6' | 5.0 (t, 2H) | 64.1 |
| 1" and 2" | 1.5 (s, 6H) | 9.0 |
| 3" | - | 119.3 |
| 4" | 1.8 (s, 3H) | 24.9 |
| 5" | - | 145.6 |

distillation by Kamazeri *et al.*[16] method. In this study separation step using dichloromethane did not conducted since the oil was easily separated from water using separation funnel attached to distillation apparatus. Yield of rhizome oil distillation was a 10 and 20 times higher than leaves and stem oil, denoted that oil content in rhizome was higher than leaves and stem oil. The color of essential of rhizome, leaves and stem are similar (dark brown) indicated possibility of similarity constituent in each oil.

The essential oil from rhizomes of *C. aeruginosa* exhibited strong antibacterial activity toward *S. mutans*. The MIC value of rhizome oil was equal with tetracycline (Table 1). Kamazeri *et al.*[16] reported that rhizome oil of *C. aeruginosa* possess antibacterial activity toward Gram-positive and Gram-negative bacteria and also fungi. The constituent of rhizome oil predicted to be act as antibacterial agent through one or combination of the following mechanisms: Ruin the cell wall of bacteria, hamper the protein synthesis and obstruct the DNA and RNA replication. Trombetta *et al.*[17] reported that mechanism of antibacterial action of monoterpene compounds was through perturbation of the lipid fraction of microorganism plasma membrane, changing membrane permeability and leaking intracellular materials. Furthermore, the drugs might interacting with intracellular sites critical for antibacterial activity after cross the cell membranes and penetrating into the interior of the cell[17].

*Streptococcus mutans* produce exopolysaccharides and use it to form biofilm as their habitat on teeth[2,3]. Degradation of biofilm correlated with the destruction of *S. mutans* habitat and expected to inhibit its growth. Stem oil of *C. aeruginosa* exhibited weak activity in biofilm degradation, meanwhile the fraction of rhizome oil indicated stronger activity in biofilm degradation. On the other hand, leaves oil denoted no activity in biofilm degradation. Batubara *et al.*[18] reported that leaves oil of other *Curcuma* plants showed biofilm degradation activity with $IC_{50}$ in range of 289 and 689 μg $mL^{-1}$.

Essential oil from leaves, stems and rhizome of *C. aeruginosa* contained some terpenoid compounds. Rhizome oil contained 1,8-cineol (13%), higher than reported by Sirat *et al.*[10]. Beside terpoid, essential oil from rhizome also contained nonterpenoid compounds as the main component, namely 2,2,5-trimethyl-2'(H)-5',6'-dihydropyrano[3',4',9]indan-

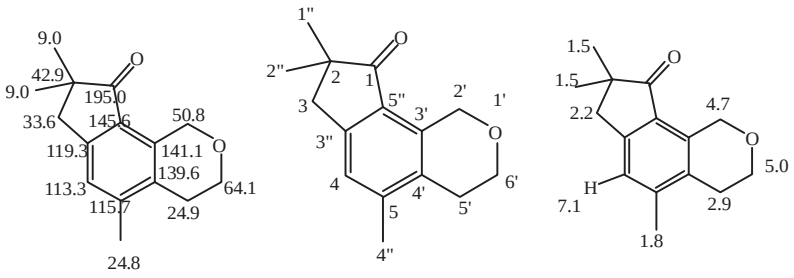


Fig. 3: Structure of 2,2,5-trimethyl-2'(H)-5',6'-dihydropyrano[3',4',9]indan-1-one (δ ppm $^{13}C$ NMR and $^{1}H$ NMR)

1-one (40%). Sirat *et al.*[10] reported that essential oil of *C. aeruginosa* rhizome from Malaya contained curzerenone (24.6%), 1,8-cineole (11.0%), camphor (10.6%), zedoarol (6.3%), isocurcumenol (5.8%), curcumenol (5.6%) and filranogermenone (5.5%). Stem and leaves oil of *C. aeruginosa* possessed similar major terpenoid compounds such as β-elemene, caryophyllene and epicurzerenone.

The NMR spectrum indicated pointed a shift at 195 ppm indicated carbonyl group of conjugated ketone. Another chemical shift was monitored at 145.6, 141.1, 139.6, 119.3, 115.7 and 113.0 ppm related with substituted benzene. The NMR data referred that the compound in F1.2 suggested to be 2,2,5-trimethyl-2'(H)-5',6'-dihydropyrano[3',4',9]indan-1-one ($C_{15}H_{18}O_2$) (Fig. 3).

## CONCLUSION

Rhizome of *C. aeruginosa* provided the highest yield of essential oil (1.99%) compare with stems and leaves. The rhizome essential oil showed good antibacterial activity toward *S. mutans* with the same MIC value with synthetic antibacterial tetracycline. 2,2,5-trimethyl-2'(H)-5',6'-dihydropyrano[3',4',9]indan-1-one and 1,8-cineol were the major compounds in rhizome oil. Biofilm degradation activity of stems oil was higher than rhizomes and leaves oil. Otherwise, fractionation of rhizome oil produced fractions with higher biofilm degradation activity than stems oil. This fraction also could maintain its antibacterial activity. Further purification toward the selected fraction yielded F1.2. According to MS and NMR spectrum, the identity of compound in F1.2 was 2,2,5-trimethyl-2'(H)-5',6'-dihydropyrano[3',4',9]indan-1-one.

## SIGNIFICANT STATEMENT

- Oil of *C. aeruginosa* rhizome was able to inhibit the growth of oral bacteria which cause teeth caries, namely *S. mutans*
- Inhibition power of *C. aeruginosa* rhizome oil toward *S. mutans* was similar to synthetic antibacterial agent, tetracycline
- Separation of *C. aeruginosa* rhizome oil yield fractions with lower inhibition power than crude oil
- Degradation of bacteria colony on teeth (biofilm degradation) activity of stems oil was higher than rhizome and leaves oil
- Fractions of *C. aeruginosa* rhizome oil exhibited higher biofilm degradation activity than stems oil

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