

# Antibacterial activity of essential oil and leaf extracts from *Cleistocalyx operculatus* Roxb. Merr. et Perry (Myrtaceae)

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## ABSTRACT

**Background:** *Cleistocalyx operculatus* is a traditional medicinal plant widely consumed in Vietnam and known to contain various bioactive constituents. However, comparative data on the antibacterial activity of its leaf essential oil and solvent extracts remain limited. **Objective:** This study aimed to compare the antibacterial activity of the essential oil and different leaf extracts of *C. operculatus* against selected bacterial strains. **Materials and methods:** Dried leaves of *C. operculatus* were extracted by ultrasonic-assisted extraction using 50% ethanol, 70% ethanol, and distilled water. The total flavonoid content was determined using the UV-Vis spectrophotometric method. Essential oil was obtained from fresh leaves by steam distillation. Antibacterial activity was evaluated against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Bacillus subtilis* ATCC 6633 using the agar diffusion method. Minimum inhibitory concentration (MIC) values were determined by agar dilution. **Results:** The 70% ethanol extract showed the highest TFC (35.963 mg QE/g dry extract) and the strongest antibacterial activity, particularly against *S. aureus* (MIC 0.3125 mg/mL). The water extract exhibited moderate activity, whereas the 50% ethanol extract showed limited effects. Essential oil was active only against Gram-positive bacteria with MIC values of 0.50% against *S. aureus* and 1.00% against *B. subtilis*. **Conclusion:** The 70% ethanol extract demonstrated the greatest antibacterial potential, while the leaf essential oil exhibited selective activity against Gram-positive bacteria. These findings support the potential of *C. operculatus* leaves as a promising source of natural antibacterial agents for further investigation.

**Keywords:** *Cleistocalyx operculatus*, extract, essential oil, antibacterial activity, MIC

## 1. INTRODUCTION

Bacterial infections remain a major public health concern worldwide, particularly in the context of increasing antimicrobial resistance and the limited availability of new antibacterial agents [1]. In recent years, medicinal plants have attracted considerable interest as potential sources of bioactive compounds with antibacterial properties. Plant-derived extracts and essential oils are especially promising due to their chemical diversity and long-standing use in traditional medicine. Among them, flavonoids - a group of compounds found in high concentrations in *C. operculatus* leaves - have been shown to have many biological activities, including antibacterial and antioxidant properties [2]... showing potential applications in supporting the treatment of related diseases.

*Cleistocalyx operculatus* (Roxb.) Merr. & L.M.Perry (Myrtaceae), commonly known as "Vối" in Vietnam, is a traditional medicinal plant widely consumed as a herbal tea and used in folk medicine for digestive support, glycemic control, and cardiovascular health. Several pharmacological effects of *C. operculatus* have been reported, such as antioxidant, antiviral, anti-inflammatory, and

protective activities in different biological models [3, 4]. Previous phytochemical studies have shown that its leaves contain various biologically active constituents, including flavonoids, polyphenols, triterpenoids, and volatile compounds [5]. In particular, flavonoid-rich fractions from this plant have been suggested to contribute to its antimicrobial potential. However, most previous studies have focused on conventional extracts or flower buds, whereas comparative data on the antibacterial activity of leaf extracts obtained using different extraction systems and leaf essential oil remain limited [6].

Current usage primarily involves daily consumption in the form of aqueous decoctions or low-ethanol infusions. However, the antibacterial activities of aqueous and various ethanol extracts have not yet been systematically investigated, highlighting a gap that should be addressed to better elucidate and enhance the medicinal value of this plant. Therefore, this study aimed to comparatively evaluate the antibacterial activity of the essential oil and different leaf extracts of *C. operculatus* against selected bacterial strains, including *Staphylococcus aureus*,

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*Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, using agar diffusion and minimum inhibitory concentration (MIC) assays.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Leaves of *Cleistocalyx operculatus* (Roxb.) Merr. & L.M.Perry were collected from Dong Thap Muoi Medicinal Plant Conservation and Development Joint Stock Company (MEPHYDICA, Vietnam). The voucher specimen is deposited at the Department of Pharmacognosy - Botany, Faculty of Pharmacy, Hong Bang International University under the code DL-2025-005. The plant material was cleaned and used for the preparation of both dried leaf extracts and fresh leaf essential oil.

### 2.2. Bacterial strains

The antibacterial activity of the test samples was evaluated against four standard bacterial strains: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Bacillus subtilis* ATCC 6633. All bacterial strains were provided by the Department of Biochemistry, Microbiology and Parasitology, Binh Duong University, Vietnam.

### 2.3. Chemicals and reagents

Tryptic Soy Agar (TSA) and Mueller-Hinton Agar (MHA) were used for bacterial activation and antibacterial assays, respectively (Merck, Germany). Methanol,  $\text{AlCl}_3$ , quercetin. Ethanol, dimethyl sulfoxide (DMSO), Tween 80, and anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) were obtained from Merck (Germany). Gentamycin was used as the positive control antibiotic.

### 2.4. Methods

#### 2.4.1. Extracting the concentrated leaves

Dried leaves of *C. operculatus* were powdered and extracted using ultrasonic-assisted extraction with three solvent systems: 50% ethanol, 70% ethanol, and distilled water. Briefly, 15 g of dried leaf powder (previously dried at 50°C to a moisture content of approximately 5%) was extracted with each solvent at a solvent level sufficient to immerse the plant material by approximately 1 cm. Extraction was performed without heating for 20 min per cycle, repeated 2 - 3 times. The combined extracts were then concentrated under appropriate conditions to obtain crude extracts [7]:

$$\text{Extraction yield (\%)} = \frac{m_{\text{extracts}}}{m_{\text{materials}} - (m_{\text{materials}} - \text{moisure})} \times 100$$

Where  $m_{\text{extract}}$  is the mass of crude extract obtained

(g), and  $m_{\text{raw material}}$  is the mass of dried leaf powder used for extraction (g).

#### 2.4.2. Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) of the extract was determined using the aluminum chloride ( $\text{AlCl}_3$ ) colorimetric method with quercetin as the reference standard [9]. The absorbance of the standard and test solutions was measured using a UV-Vis spectrophotometer at 430 nm to establish the quercetin calibration curve. Based on this calibration curve, the total flavonoid content of the extracts was calculated using the following equation:

$$TFC = \frac{C \times k \times V}{1000 \times m}$$

In which:

TFC: Total flavonoid content (mg quercetin equivalent, QE/g dried extract).

C: Concentration of the test sample calculated from the quercetin calibration curve ( $\mu\text{g/mL}$ ).

k: Dilution factor.

m: Mass of the dried extract (g).

V: Volume of the sample extract used in the quantification reaction (mL).

#### 2.4.3. Essential oil extraction

Fresh leaves of *C. operculatus* were subjected to steam distillation to obtain essential oil. After distillation, the essential oil layer was separated and dried over anhydrous  $\text{Na}_2\text{SO}_4$  to remove residual moisture. The oil was then transferred to a dark glass vial and stored at 4°C until use [8]. The obtained essential oil was additionally assessed for its organoleptic and physicochemical characteristics, including color, odor, volatility, ethanol solubility, and immiscibility with water.

The essential oil content was calculated as follows:

$$X (\%) = \frac{a \times 100}{b}$$

where  $a$  is the volume of essential oil obtained (mL),  $b$  is the mass of fresh plant material used (g), and  $X$  is the essential oil content expressed as % (v/w).

#### 2.4.4. Preparation of bacterial inoculum

Each bacterial strain was subcultured on TSA and incubated at 37°C for 18 - 24 hours prior to testing. Bacterial suspensions were then prepared in sterile saline and adjusted to a turbidity equivalent to approximately 0.5 McFarland standard for use in antibacterial assays. This bacterial suspension was used for tests 2.4.4 and 2.4.5.

#### 2.4.5. Agar well diffusion assay

The antimicrobial activity of essential oils and leaf

extracts was preliminarily evaluated by the Mueller-Hinton agar (MHA) well diffusion method. Briefly, bacterial suspensions were spread evenly onto the surface of sterile MHA plates. Then, aseptic wells were made on the agar (well diameter 6 mm), and test samples at concentrations of 100 mg/ml, including 50% ethanol extract, 70% ethanol extract, and water extract, were added to the wells at a volume of 100  $\mu$ l. The plates were incubated at 37°C for 18 - 24 hours, after which the diameter of the inhibition zone was measured in millimeters (mm)[9].

The agar diffusion method was used to evaluate the antibacterial activity against the following standard bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*. DMSO 5% was used as the negative control, and gentamycin was used as the positive control.

#### 2.4.6. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the extracts and essential oils was determined by agar dilution, based on the lowest concentration capable of completely inhibiting visible bacterial growth. Crude extracts were dissolved in DMSO to a starting MIC range of 5 mg/ml, while essential oils were emulsified in distilled water containing 0.5% Tween 80 before testing to a starting MIC range of 1% (v/v). Different concentrations of each sample were prepared and mixed into sterile agar medium before freezing the plates.

**Table 1.** Extraction yield and moisture content of *C. operculatus* leaf extracts

Extraction solvent	The extract mass (g)	Moisture (%)	Extraction efficiency (%)
Ethanol 50%	2.33 $\pm$ 0.012	10.87 $\pm$ 0.210	17.4 $\pm$ 1.003
Ethanol 70%	2.36 $\pm$ 0.015	12.2 $\pm$ 0.231	17.9 $\pm$ 1.012
Distilled water	1.49 $\pm$ 0.043	19.01 $\pm$ 0.960	12.3 $\pm$ 0.974

### 3.2. Determination of Total Flavonoid Content (TFC)

#### 3.2.1. Construction of the calibration curve

After measuring the absorbance at 430 nm, the

After solidification, each plate was inoculated with the corresponding bacterial suspension and incubated at 37°C for 24 hours. Following incubation, bacterial growth was visually assessed, and the MIC was recorded as the lowest concentration at which no visible bacterial growth was observed.

All experiments were performed in triplicate, and the results were expressed as mean  $\pm$  standard deviation (SD)[10].

#### 2.4.7. Statistical analysis

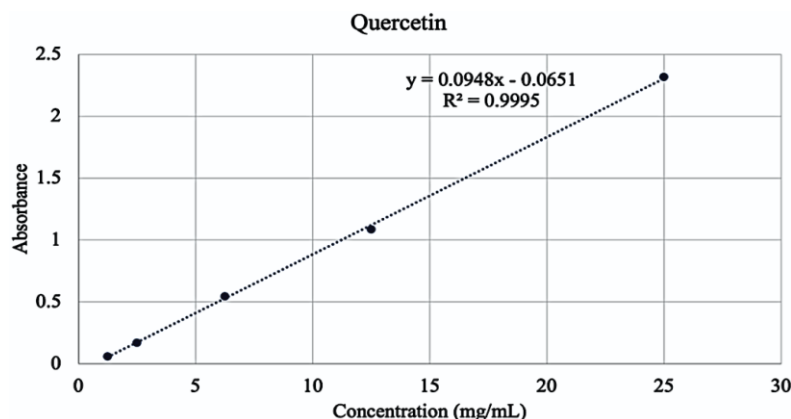
Data were summarized descriptively as mean  $\pm$  SD. Comparative statistical analysis was not performed in the present study, and the antibacterial activities were interpreted based on inhibition zone diameters and MIC values obtained under identical experimental conditions.

## 3. RESULTS

### 3.1. Extracting the concentrated leaves

The extraction yields obtained using different solvent systems are presented in Table 1. Among the tested solvents, 70% ethanol produced the highest extraction yield (17.9  $\pm$  1.012%), corresponding to 2.36  $\pm$  0.015 g of crude extract. The 50% ethanol extract showed a comparable extraction yield (17.4  $\pm$  1.003%) with 2.33  $\pm$  0.012 g of crude extract. In contrast, extraction with distilled water resulted in the lowest crude extract mass (1.49  $\pm$  0.043 g) and the lowest extraction yield (12.3  $\pm$  0.974%). The moisture contents of the obtained extracts ranged from 10.87  $\pm$  0.210% to 19.01  $\pm$  0.960%.

calibration regression equation was established from the concentration-absorbance data of quercetin, as illustrated in Figure 1.



**Figure 1.** Quercetin standard calibration curve

Comment: The absorbance and concentration of the quercetin standard showed a strong linear correlation, with a correlation coefficient of  $R^2 = 0.9995$ . The linear regression equation was  $y = 0.0948x - 0.0651$ .

Where x: the concentration of the solution (mg/mL)

and y: the absorbance ( $\mu\text{g/mL}$ ).

### 3.2.2. Determination of Total Flavonoid Content of *C. operculatus* Leaves

Based on the calibration curve established above, the absorbance values of the test sample was measured in Table 2.

**Table 2.** Results of the tested samples at 430 nm - Quercetin

Test sample	Concentration ( $\mu\text{g/mL}$ )	Absorbance	TFC (mg QE/g dried extract)
Water extract	1.00	$0.256 \pm 0.200$	$13.737 \pm 0.002$
70% extract	1.00	$0.402 \pm 0.009$	$35.963 \pm 0.001$
50% extract	1.00	$0.334 \pm 0.019$	$30.181 \pm 0.065$

Comment: The results showed that the 70% ethanol extract exhibited the highest flavonoid content ( $35.963 \text{ mg QE/g dried extract}$ ), followed by the 50% and water extracts. These findings suggest that solvents with intermediate polarity exhibit a greater ability to extract flavonoids, reflecting the characteristic properties of this group of compounds.

### 3.3. Extraction and quantitative analysis of leaf essential oil

A total of 700 g of fresh *C. operculatus* leaves was subjected to steam distillation using a plant material-water ratio of 1:5 (70 g of plant material/

350 mL of water) for a duration of 3 hours. The procedure yielded 2 mL of essential oil, which was subsequently dried over anhydrous  $\text{Na}_2\text{SO}_4$ . The essential oil content of the leaves was determined to be 0.286% (v/w).

### 3.4. Antibacterial activity of leaf extracts and essential oil

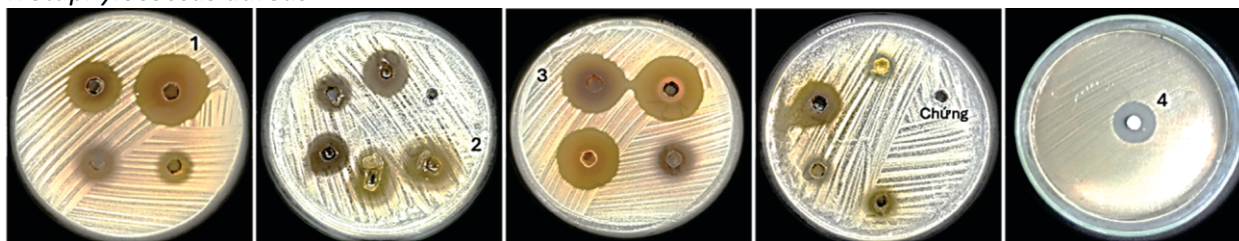
#### 3.4.1. Agar well diffusion assay

The antibacterial activity of the *C. operculatus* leaf extracts was initially assessed using the agar well diffusion assay, and the results are shown in Table 3 and Figures 2 - 5.

**Table 3.** Inhibition zone diameters of *C. operculatus* leaf extracts against tested bacterial strains

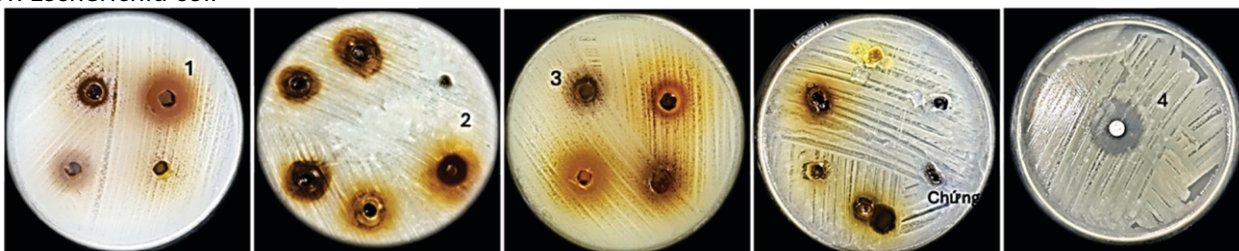
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>
	ATCC 25923	ATCC 25922	ATCC 27853	ATCC 6633
70% ethanol extract	$27.00 \pm 0.58$	$16.00 \pm 0.58$	$10.00 \pm 0.58$	$18.00 \pm 0.58$
50% ethanol extract	$10.00 \pm 0.58$	$12.00 \pm 0.58$	$17.00 \pm 0.29$	-
Water extract	$24.00 \pm 0.58$	-	-	-
Gentamycin	21.00	20.00	18.00	19.00

On *Staphylococcus aureus*



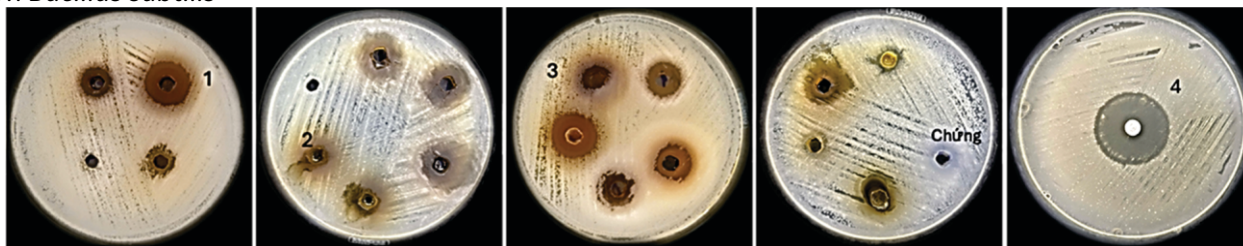
**Figure 2.** Representative agar diffusion assay results of *C. operculatus* extracts against *Staphylococcus aureus*  
1. 70% extract; 2. 50% extract; 3. Water extract; 4. Gentamycin

On *Escherichia coli*



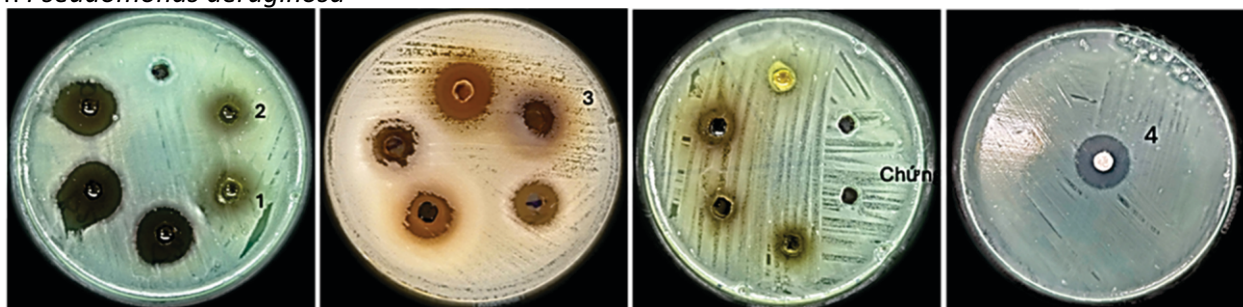
**Figure 3.** Representative agar diffusion assay results of *C. operculatus* extracts against *Escherichia coli*  
1. 70% extract; 2. 50% extract; 3. Water extract; 4. Gentamycin

On *Bacillus subtilis*



**Figure 4.** Representative agar diffusion assay results of *C. operculatus* extracts against *Bacillus subtilis* 1. 70% extract; 2. 50% extract; 3. Water extract; 4. Gentamycin

On *Pseudomonas aeruginosa*



**Figure 5.** Representative agar diffusion assay results of *C. operculatus* extracts against *Pseudomonas aeruginosa* 1. 70% extract; 2. 50% extract; 3. Water extract; 4. Gentamycin

Overall, the 70% ethanol extract exhibited the broadest and strongest antibacterial activity among the tested extracts, showing inhibitory effects against all four bacterial strains. The largest inhibition zone was observed against *S. aureus* ATCC 25923 (27.00 ± 0.58 mm), followed by *B. subtilis* ATCC 6633 (18.00 ± 0.58 mm), *E. coli* ATCC 25922 (16.00 ± 0.58 mm), and *P. aeruginosa* ATCC 27853 (10.00 ± 0.58 mm).

The 50% ethanol extract showed weaker antibacterial activity and inhibited only three of the four tested strains, with inhibition zones of 10.00 ± 0.58 mm

against *S. aureus*, 12.00 ± 0.58 mm against *E. coli*, and 17.00 ± 0.29 mm against *P. aeruginosa*. No inhibition zone was observed against *B. subtilis*. The water extract demonstrated inhibitory activity only against *S. aureus*, with an inhibition zone of 24.00 ± 0.58 mm. In comparison, gentamycin, used as the positive control, produced inhibition zones of 21.00, 20.00, 18.00, and 19.00 mm against *S. aureus*, *E. coli*, *P. aeruginosa*, and *B. subtilis*, respectively.

**3.4.2. Minimum inhibitory concentration (MIC)**

The MIC values of the *C. operculatus* leaf extracts and essential oil are presented in Table 4 and Figure 6.

**Table 4.** Minimum inhibitory concentrations (MICs) of *C. operculatus* leaf extracts and essential oil

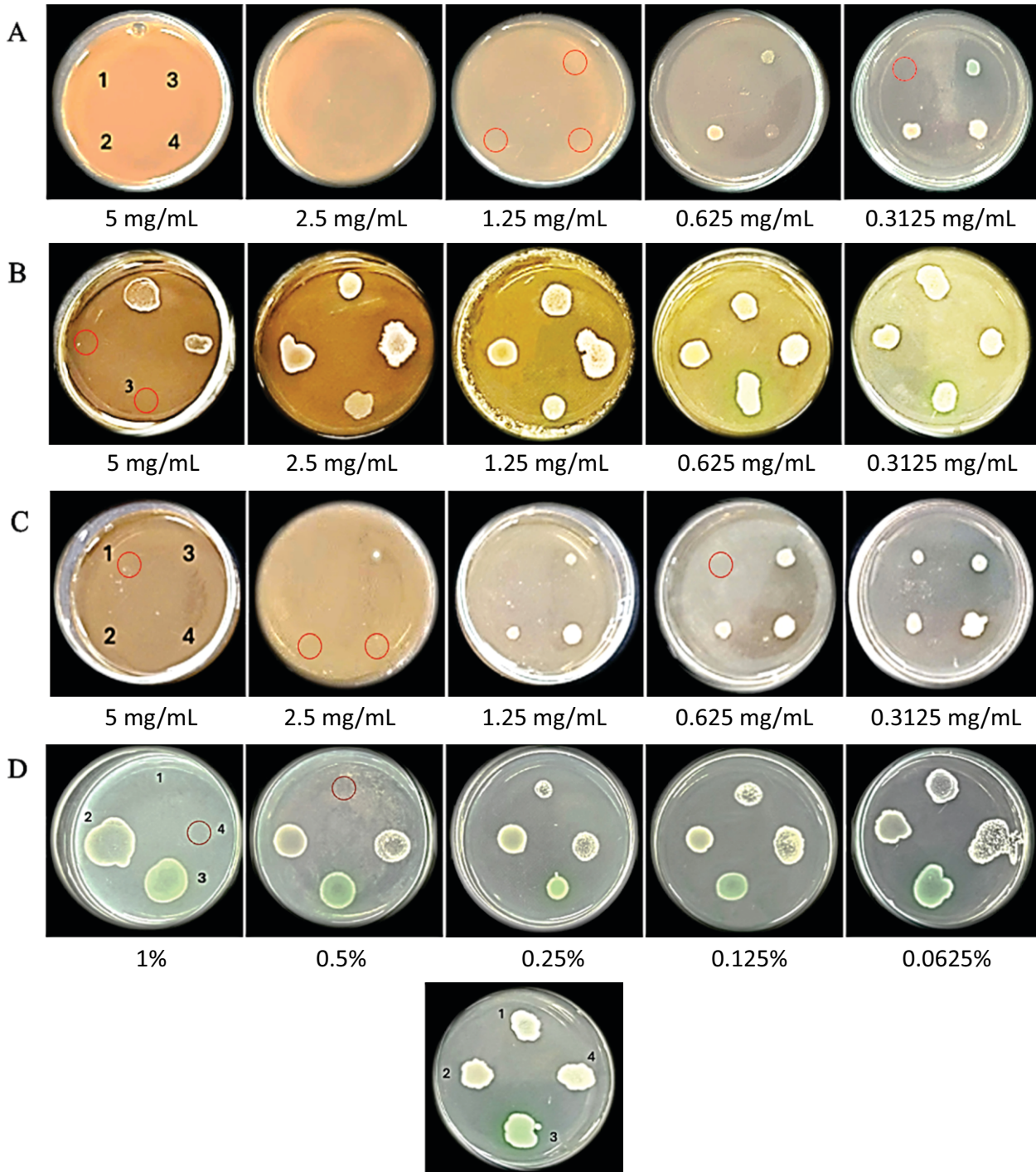
	70 extract (mg/mL)	50 extract (mg/mL)	Water extract (mg/mL)	Essential oil (%)
<i>Staphylococcus aureus</i> ATCC 25923	0.31 ± 0.01	-	0.63 ± 0.02	0.50 ± 0.05
<i>Escherichia coli</i> ATCC 25922	1.25 ± 0.05	-	2.50 ± 0.10	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	1.25 ± 0.05	5.00 ± 0.10	5.00 ± 0.10	-
<i>Bacillus subtilis</i> ATCC 6633	1.25 ± 0.05	5.00 ± 0.10	2.50 ± 0.10	1.00 ± 0.05

Consistent with the agar diffusion results, the 70% ethanol extract showed the strongest antibacterial potency, with measurable inhibitory activity against all four tested bacterial strains. The lowest MIC was recorded against *S. aureus* ATCC 25923 (0.31 ± 0.01 mg/mL), followed by *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 (both 1.25 ± 0.05 mg/mL), and *B. subtilis* ATCC 6633 (1.25 ± 0.05 mg/mL).

The water extract also showed antibacterial activity against all four strains, although with lower potency than the 70% ethanol extract. Its MIC values were 0.63 ± 0.02 mg/mL against *S. aureus*, 2.50 ± 0.10 mg/mL against *E. coli*, 5.00 ± 0.10 mg/mL against *P. aeruginosa*, and 2.50 ± 0.10 mg/mL against *B. subtilis*. In contrast, the 50% ethanol extract exhibited limited antibacterial activity and was active only against *P. aeruginosa*

and *B. subtilis*, with MIC values of  $5.00 \pm 0.10$  mg/mL for both strains. The essential oil showed selective antibacterial activity and inhibited only Gram-positive bacteria.

The MIC value was  $0.50 \pm 0.05\%$  against *S. aureus* and  $1.00 \pm 0.05\%$  against *B. subtilis*, while no inhibitory activity was observed against *E. coli* or *P. aeruginosa* within the tested concentration range.



**Figure 6.** Representative MIC assay results of *C. operculatus* leaf extracts and essential oil  
 A. 70% extract; B. 50% extract; C. Water extract; D. Essential oil; E. Gentamycin  
 1. *Staphylococcus aureus* ATCC 25923; 2. *Escherichia coli* ATCC 25922;  
 3. *Pseudomonas aeruginosa* ATCC 27853; 4. *Bacillus subtilis* ATCC 6633

**4. DISCUSSION**

The present study demonstrated that both the leaf extracts and essential oil of *Cleistocalyx*

*operculatus* possess measurable antibacterial activity, although the magnitude and spectrum of activity varied considerably depending on the

extraction system and the tested bacterial strain. Among the investigated samples, the 70% ethanol extract consistently exhibited the strongest and broadest antibacterial activity in both agar diffusion and MIC assays, whereas the essential oil showed selective activity against Gram-positive bacteria only. After extraction, the research team obtained an essential oil content of 0.286% (v/w). The essential oil yield obtained in this study was higher than that reported for *C. operculatus* leaves collected in Ho Chi Minh City (0.1%). The chemical composition of essential oil reported in previous studies includes compounds, including 6-camphenol, isopinocarveol, p-cymen-8-ol, (-)-myrtenol, l-verbenone, cis-carveol, ethaneperoxoic acid (1-cyano-4,4-dimethyl-1-phenylpentyl ethaneperoxate), (+)-carotol, caryophyllene oxide, (-)-globulol, 2-(4a,8-dimethyl-2,3,4,4a,5,6-hexahydronaphthalen-2-yl)propan-1-ol, and longipinocarvone. These constituents indicate a complex chemical profile that may contribute to the observed biological activities [11]. These findings suggest that the antibacterial constituents of *C. operculatus* leaves are differentially recovered depending on solvent polarity and extraction type. Some components present in the leaves of *C. operculatus* include carotenoids, essential oils, and free triterpenoids in the less polar fractions, while the medium to highly polar fractions reveal groups of compounds including flavonoids, tannins, saponins, organic acids, reducing agents, and polyurethane compounds [16].

The superior activity of the 70% ethanol extract may be attributed to the ability of hydroethanolic solvents to extract a broader range of bioactive phytochemicals with intermediate polarity. Compared with absolute water or lower-strength ethanol systems, 70% ethanol is often more effective for recovering phenolic compounds, flavonoids, tannins, and other semi-polar constituents that are frequently associated with antibacterial activity. This interpretation is consistent with previous reports indicating that *C. operculatus* leaves are rich in flavonoids and polyphenolic compounds, which are considered major contributors to their biological activity. In the present study, the 70% ethanol extract not only showed the highest extraction yield but also exhibited the most pronounced antibacterial effect, suggesting that this solvent system may be more suitable for concentrating antibacterial constituents from the leaves. In addition, this

extract exhibited the highest total flavonoid content among the investigated extracts, which may partly explain its superior antibacterial activity.

A notable finding of this study was the particularly strong susceptibility of *Staphylococcus aureus* to the tested samples, especially the 70% ethanol extract and the water extract. This pattern is biologically plausible because Gram-positive bacteria are generally more susceptible to plant-derived antibacterial compounds than Gram-negative bacteria. The outer membrane of Gram-negative bacteria, which contains lipopolysaccharides, serves as an additional permeability barrier that can reduce the penetration of many hydrophobic and moderately polar phytochemicals. In contrast, Gram-positive bacteria lack this outer membrane, making their peptidoglycan-rich cell wall relatively more accessible to antimicrobial agents. This structural difference may partly explain why *S. aureus* showed the greatest susceptibility, whereas *Pseudomonas aeruginosa*, a species well known for its intrinsic resistance and low membrane permeability, exhibited comparatively weaker susceptibility.

The antibacterial profile of the essential oil further supports this interpretation. In the present study, the essential oil inhibited only the Gram-positive strains, namely *S. aureus* and *B. subtilis*. Essential oils are typically rich in volatile lipophilic compounds such as monoterpenes and sesquiterpenes, which can disrupt bacterial membranes, alter permeability, and interfere with essential cellular functions. However, these compounds are often less effective against Gram-negative bacteria because the outer membrane limits their diffusion. Therefore, the selective activity of *C. operculatus* leaf essential oil observed in this study is consistent with the general antibacterial behavior reported for many plant essential oils.

An important observation in this study is that the antibacterial patterns obtained by agar diffusion and MIC assays were not entirely identical, particularly for the water extract. While the water extract showed a clear inhibition zone only against *S. aureus* in the diffusion assay, it exhibited measurable MIC values against all four tested strains. This discrepancy is not uncommon in plant extract studies and may be explained by methodological differences between the two assays. Agar diffusion depends not only on antibacterial potency but also on the ability of active constituents to diffuse

through the agar matrix. In contrast, the MIC assay reflects bacterial growth inhibition under direct contact conditions. Therefore, a sample may exhibit weak diffusion-based inhibition despite retaining inhibitory activity in dilution-based testing, especially when its active compounds are relatively large, polar, or poorly diffusible in solid media.

Overall, the standardized research results on the antibacterial activity of *C. operculatus* leaves extracts and essential oil are consistent with previous research results [12]. The present findings are generally in agreement with previous studies reporting antibacterial activity of *C. operculatus* extracts and supporting the importance of extraction conditions in determining biological efficacy. The relatively favorable antibacterial performance observed for the 70% ethanol extract in this study reinforces the view that extraction optimization is a critical step in evaluating medicinal plant potential [13, 14]. Furthermore, essential oil also showed higher antibacterial activity than in previous research [15] with MIC of 0.50% on *S. aureus* and 1.00% on *B. Subtilis*, the observed activity of the leaf essential oil is noteworthy because this fraction remains less studied than conventional extracts and may represent an underexplored source of antibacterial constituents.

Despite these promising results, several limitations should be acknowledged. First, the study focused only on in vitro antibacterial screening against four standard bacterial strains. Second, the chemical composition of the extracts and essential oil was not characterized, so the specific compounds responsible for the observed antibacterial effects could not be identified. Third, bactericidal activity, cytotoxicity, and mechanism-of-action studies were not performed. Therefore, further research should include phytochemical profiling, isolation of active constituents, expanded antibacterial testing, and safety evaluation to better assess the pharmaceutical potential of *C. operculatus* leaf-derived products.

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## 5. CONCLUSION

The present study demonstrates that preparations derived from the leaves of *C. operculatus* possess *in vitro* antibacterial activity, with the degree of inhibition strongly dependent on the extraction system and sample type. Among the tested samples, the 70% ethanol leaf extract exhibited the strongest and broadest-spectrum antibacterial activity against all four bacterial strains, as evidenced by both agar diffusion and minimum inhibitory concentration (MIC) assays. The aqueous extract also showed measurable antibacterial activity, whereas the 50% ethanol extract displayed more limited effects. In contrast, the essential oil demonstrated selective activity, primarily against Gram-positive bacteria.

From a scientific perspective, these findings contribute to elucidating the relationship between extraction solvent systems and bioactive chemical constituents, thereby providing a basis for optimizing extraction conditions in pharmacognostic research. From a practical standpoint, the use of 70% ethanol, 50% ethanol, and water - safe, accessible, and cost-effective solvents - highlights the potential for developing effective antibacterial herbal preparations suitable for community use and pharmaceutical production.

Overall, the results provide a scientific basis supporting the traditional use of *C. operculatus* leaves, while also serving as a foundation for further investigations into their mechanisms of action and safety profiles.

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## Chiết xuất và đánh giá hoạt tính kháng khuẩn của tinh dầu và cao chiết từ lá Vối *Cleistocalyx operculatus* Roxb. Merr. et Perry (Myrtaceae)

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### TÓM TẮT

Đặt vấn đề: Cây Vối (*Cleistocalyx operculatus* Myrtaceae) là dược liệu truyền thống phổ biến ở Việt Nam, chứa nhiều hợp chất có hoạt tính sinh học. Tuy nhiên, dữ liệu so sánh về hoạt tính kháng khuẩn giữa tinh dầu

và các cao chiết từ lá vẫn còn hạn chế. Mục tiêu nghiên cứu: So sánh hoạt tính kháng khuẩn của tinh dầu và các cao chiết từ lá Vối trên một số chủng vi khuẩn chọn lọc. Đối tượng và phương pháp nghiên cứu: Bột lá Vối khô được chiết siêu âm với ethanol 50%, ethanol 70% và nước cất. Hàm lượng flavonoid toàn phần được xác định bằng phương pháp đo quang UV-Vis. Tinh dầu được thu từ lá tươi bằng phương pháp cất kéo lôi cuốn hơi nước. Hoạt tính kháng khuẩn được đánh giá trên các chủng *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 và *Bacillus subtilis* ATCC 6633 bằng phương pháp khuếch tán trên đĩa thạch. Giá trị nồng độ ức chế tối thiểu (MIC) được xác định bằng phương pháp pha loãng trong thạch. Kết quả: Cao chiết ethanol 70% có hàm lượng flavonoid toàn phần cao nhất (35.96 mg QE/g cao khô) và thể hiện hoạt tính kháng khuẩn mạnh nhất trên cả bốn chủng nổi bật trên *S. aureus*, (MIC 0.3125 mg/mL). Cao chiết nước có hoạt tính trung bình và cao chiết ethanol 50% cho thấy hoạt tính hạn chế. Tinh dầu thể hiện hoạt tính kháng khuẩn chọn lọc trên vi khuẩn Gram dương, với MIC 0.50% với *S. aureus* và 1.00% với *B. subtilis*. Kết luận: Những kết quả này gợi ý tiềm năng ứng dụng của lá Vối như một nguồn chất kháng khuẩn tự nhiên cho các nghiên cứu tiếp theo.

**Từ khoá:** *Cleistocalyx operculatus*, chiết xuất, tinh dầu, kháng khuẩn, MIC

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