COMPARATIVE ANALYSIS OF ANTIBACTERIAL ACTIVITY OF FATTY ACID FROM VIETNAMESE COCONUT OILS PRODUCED BY HOT-EXTRACTED AND COLD-PRESSING METHODS

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Abstract - This study investigated the antibacterial activity of fatty acids (FAs) extracted from coconut oil against *Staphylococcus aureus* and Methicillin-resistant *S. aureus* (MRSA). The FAs exhibited inhibitory effects at concentrations ≥5%, producing inhibition zones of 4 mm and above. Increasing concentration did not yield a proportional enhancement, indicating a non–dose-dependent effect. MRSA displayed slightly greater susceptibility than *S. aureus* at equivalent concentrations. Significant differences among concentrations were confirmed ($p < 0.01$). Although overall activity was comparable between cold-pressed (CCO) and hot-extracted coconut oil (HCO), FAs derived from CCO showed stronger inhibition, with minimum inhibitory concentrations (MICs) of 0.39% for both strains, compared to 0.78% for HCO against *S. aureus*. The superior efficacy of CCO-derived FAs likely reflects better preservation of medium-chain fatty acids—particularly lauric, capric, and caprylic acids—known for antimicrobial properties. These results highlight the influence of processing methods on the antibacterial efficacy of coconut oil–derived fatty acids.

Key words - fatty acid; coconut oil; antibacterial activity; *Staphylococcus aureus*; cold-pressed extraction.

1. Introduction

Coconut (*Cocos nucifera L.)* is a vital economic and cultural crop in tropical regions, particularly in Southeast Asia [1, 2]. Vietnam ranks among the world's leading coconut-producing countries, with vast plantations concentrated in the Mekong Delta [1]. For centuries, various parts of the coconut tree have been utilized in traditional medicine, cuisine, and daily life [2]. Among its many derivatives, coconut oil has gained considerable attention due to its diverse applications ranging from food and cosmetics to traditional remedies and, more recently, pharmaceutical and pharma-cosmetic innovations [2-5]. In Vietnam, coconut oil is typically obtained through two primary extraction methods: hot extraction and cold pressing [6]. The hot extraction method involves manually grating the coconut meat and applying high temperatures to break emulsions and release the oil [6]. Although simple and widely practiced, this method often leads to the degradation of heat-sensitive bioactive compounds, which may reduce the therapeutic value of the final product [6]. In contrast, cold pressing is a mechanical process performed at low temperatures and without chemical solvents [6]. This approach preserves the oil's natural composition, producing higher concentrations of antioxidants, vitamins (such as vitamin E), and biologically active fatty acids [6]. Cold-pressed virgin coconut oil (CCO) is thus considered superior quality, particularly for dermatological and therapeutic use where the integrity of active compounds is critical.

Among the bioactive constituents in coconut oil, fatty acids (FAs) - especially medium-chain fatty acids (MCFAs) such as lauric acid, capric acid, and caprylic acid - are recognized as the key contributors to its antibacterial, anti-inflammatory, antioxidant, and moisturizing properties [7-16]. Several studies have highlighted the strong antimicrobial potential of these FAs, particularly against Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutans*, *Tannerella forsythia*, *Treponema denticola* [12, 16 - 19]. However, in their natural state within coconut oil, MCFAs are primarily stored as triglycerides, where they are esterified to a glycerol backbone [5, 7]. In this bound form, their biological activity, particularly antimicrobial function, is significantly reduced [20]. To activate their full therapeutic potential, a hydrolysis step is required to cleave the ester bonds and release the fatty acids in their free form. Only as FAs can these molecules effectively interact with bacterial membranes, disrupt cellular integrity, and exert antimicrobial effects [21, 22]. Therefore, the development of an efficient FAs extraction protocol is a critical step in utilizing coconut oil as an antibacterial agent, especially for pharmacosmetic purposes.

Despite the well-documented bioactivity of coconut oil, there is a lack of comprehensive research comparing the antibacterial efficacy of FAs derived from cold-pressed versus hot-extracted coconut oil, particularly in the context of antibiotic-resistant strains. Furthermore, there is limited scientific literature specifically addressing Vietnamese coconut oil, despite the country's rich resources and long-standing expertise in coconut cultivation and processing. The present study aims to address this research gap by: (1) establishing an effective method to extract free fatty acids from both cold-pressed and hot-extracted Vietnamese coconut oil, and (2) evaluating and comparing the antibacterial activity of the extracted FAs against *S. aureus* and MRSA. The findings are expected to provide scientific insights into the impact of extraction methods on antibacterial potency and to support the development of natural, locally sourced, and effective pharmacosmetic products derived from Vietnamese coconut oil.

2. Materials and methods

2.1. Chemical agents

Traditionally hot-extracted coconut oil (HCO) was purchased from a local market in Danang, Vietnam, cold-expressed coconut oil (CCO) was supplied by Luong Quoi Coconut Co., Ltd. (Ben Tre Province, Vietnam).

The main chemicals used in this study included potassium hydroxide (KOH), hydrochloric acid (HCl), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4•2H_2O$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4•12H_2O$), absolute ethanol (C_2H_5OH), anhydrous sodium sulfate (Na_2SO_4), phenolphthalein ($C_{20}H_{14}O_4$), n-hexane, and other reagents purchased from GHTECH (China).

Bacterial strains: *Staphylococcus aureus* and Methicillin-Resistance *Staphylococcus aureus*, Gram-positive bacterial strains obtained from the Department of Biotechnology, Faculty of Chemistry Engineering, Da Nang University of Science and Technology.

2.2. Fatty Acid Extraction from coconut oil (CO)

The procedure for obtaining the FAs of CO involves several steps. Firstly, coconut oil was mixed with ethanol with ratio 2:1 (v/v) in a 250 mL three-neck flask, followed by gentle heating and stirring. To chemical hydrolysis, the solution was then heated to 80°C, and an amount of 33% NaOH was added with a ratio volumetric 1:1 with CO volume. The mixture is stirred at a speed of 550 rpm until complete dissolution of the solution. The solution is poured into a beaker containing 100mL hot saturated NaCl solution and stirred until separation into layers, with the appearance of a solid layer on the surface. The solid layer is separated and washed with distilled water before being dissolved in hot distilled water. To form the fatty acid mixture, HCl 10% is added to the solution until the pH 1. After hydrolysis, the reaction mixture was cooled to room temperature, and the hydrolyzed fatty acids were extracted using hexane. The organic layer containing free fatty acids was separated from the aqueous phase using a separatory funnel [9].

2.3. Antibacterial Activity Assessment

The antibacterial activity was evaluated using the agar diffusion method. The study was conducted on *S. aureus* and MRSA, a Gram-positive bacterial strain obtained from the Department of Biotechnology, Faculty of Chemistry Engineering, Da Nang University of Science and Technology. Bacterial suspension was prepared by dissolving bacterial colonies in Mueller-Hinton Broth (MHB) to reach a final concentration of 10^6 CFU/mL Different test samples were added to corresponding wells on the agar plates, ncluding: a negative control (DMSO 10% or n-hexan), a positive control (Ampicillin 10µg/mL) and FAs extracts from HCO and CCO at various dilutions in DMSO (100%, 50%, 20%, 10%,5%). The samples were allowed to diffuse into the medium for 2 hours at room temperature, and results were recorded after 24 hours of incubation at 37°C [9]. The antibacterial activity of the fatty acid samples using the agar diffusion method was determined using the following formula:

$$DK\ (mm) = D-d \quad (1)$$

Where, D: diameter of the sterile zone, d: diameter of the agar hole.

2.4. Determination of the Minimum Inhibitory Concentration (MIC)

The bacterial suspension was diluted in MHB to a final concentration of 10^6 CFU/mL. Each well of a 96-well plate was filled with 50 µL of the bacterial suspension and 50 µL of the 10% acid sample, along with acid at various diluted concentrations. A separate control was prepared for color change comparison by replacing the bacterial suspension with the medium. The negative control contain DMSO 10% and the bacterial suspension, while the positive control included Ampicillin 10µg/mL and the bacterial suspension. The plate was incubated at 37°C for 24 hours. After incubation, 30 µL of 0.015% resazurin was added to each well, followed by an additional 2 hours of incubation at 37°C. The color change in the wells was then observed, and the MIC value was recorded [23].

2.5. Experiment analysis

The variability of the results was expressed as means ± standard deviation (mean ± SD) based on triplicate determinations (n = 3 for replicate plates). The data were statistically analyzed using two-way ANOVA, followed by Tukey's HSD test for multiple comparisons at $p < 0.05$ (*). This analysis was performed to compare the control and treatment groups.

3. Result and Discussion

3.1. Comparison of the antibacterial activity of FAs extracts from hot-extracted and cold-pressed coconut oil against Staphylococcus aureus

Fatty acids extract (FAs) from hot-extracted coconut oil (HCO) and cold-pressed coconut oil (CCO) at different concentrations (100%, 50%, 20%, 10%, 5%) were dissolved in 10% DMSO and used to assess antibacterial activity against *S. aureus*. The antibacterial activity was evaluated using the disc diffusion method, Ampicillin (10 µg/mL) served as the positive control and produced a clear inhibition zone of approximately 21 mm, while the negative controls (n-hexane and 10% DMSO) showed no inhibitory effect, confirming the validity of the assay (Figure 1C).

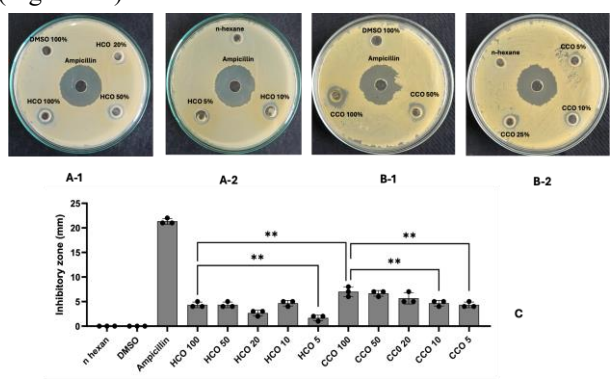

Figure 1. *Inhibitory zone of FAs extract from HCO (A-1 and A-2) and CCO (B-1 and B-2) on S. aureus at different concentrations. Control (+): Ampicillin 10µg/ml, Control (-): n-hexan or DMSO. Summary graph (C). **(p<0,01)*

According to Figure 1, although CCO and hot-extracted coconut oil HCO exhibit lower antibacterial activity compared to the commercial antibiotic (Ampicillin), both FAs from these oils initially demonstrated some level of antibacterial potential. At the 100% concentration, CCO showed significantly higher antibacterial activity than HCO. Specifically, CCO produced the largest inhibition zone (~7 mm), whereas HCO at 100% only produced a moderate inhibition zone (~4.7 mm), with a statistically significant difference ($p < 0.01$).

As the concentration decreased, the antibacterial activity of both oils also decreased, though the patterns were different. For HCO, at 20% concentration, the antibacterial activity dropped (~3.1–4.4 mm), and it continued to decrease at 5% (~1.9 mm), showing the most significant reduction in effectiveness ($p<0.05$). In contrast, CCO maintained relatively stable antibacterial activity across the intermediate concentrations (50%, 20%, and 10%), with inhibition zones of approximately 6.3 mm, 5.4 mm, and 4.8 mm, respectively. However, at 5%, CCO's activity also dropped significantly to ~5 mm, marking the first notable decline, but still higher than the 5% concentration of HCO.

These results highlight that while both CCO and HCO show weaker antibacterial activity compared to ampicillin, CCO exhibits consistently stronger activity across all concentrations tested, with a noticeable drop only at the lowest concentration (5%). This suggests that CCO maintains its antibacterial effectiveness better than HCO, particularly at intermediate concentrations, making it a promising candidate for further exploration despite its relatively low activity compared to antibiotics.

3.2. Comparison of the antibacterial activity of FAs extracts from HCO and CCO against Methicillin-Resistant Staphylococcus aureus (MRSA)

The purpose of this study was to evaluate whether fatty acids (FAs) could inhibit Methicillin-resistant *Staphylococcus aureus* (MRSA), a strain known for its resistance to antibiotics and a growing global health concern. Fatty acids extract (FAs) from hot-extracted coconut oil (HCO) and cold-pressed coconut oil (CCO) at different concentrations (100%, 50%, 20%, 10%, 5%) were dissolved in DMSO 10% and used to assess antibacterial activity against MRSA. The positive control, ampicillin, showed a large inhibition zone (~20 mm), confirming the presence of MRSA and validating the assay. The negative controls (n-hexane and DMSO) exhibited no inhibition, indicating no effect from the solvents (Figure 2C).

At 100% concentration, both HCO and CCO exhibited similar inhibition zones (Figure 2A-1 vs B-1), with no statistically significant difference between them (Figure 2C). While HCO demonstrated concentration-dependent antibacterial activity, where its effectiveness decreased as the concentration was reduced, CCO showed consistent antibacterial activity across all concentrations (100%, 50%, 20%, and 10%), with no significant variation observed (Figure 2C). However, at 5% concentration, CCO's activity decreased significantly, with an inhibition zone of approximately 5 mm, compared to around 7 mm at higher concentrations.

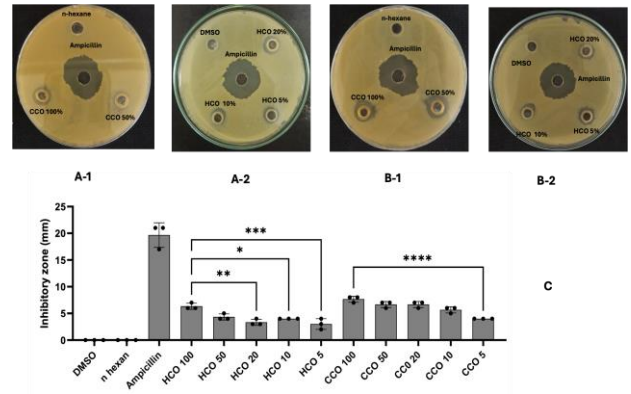

Figure 2. *Inhibitory zone of FAs extract from HCO (A-1 and A-2) and CCO (B-1 and B-2) on MRSA at different concentrations. Control (+): Ampicillin 10µg/ml, Control (-): n-hexan or DMSO. Summary graph (C). *(p<0,05); **(p<0,01); ***(p<0,001), ****(p<0,0001)*

The results showed the stable antibacterial activity of fatty acids (FAs) extracted from CCO within the concentration range of 10% to 50% against MRSA suggesting that the antimicrobial effect is not strictly concentration-dependent. One plausible explanation is the saturation of interaction sites on the bacterial membrane. Medium-chain fatty acids (MCFAs), such as lauric and capric acid, exert their antibacterial effects primarily by integrating into the lipid bilayer, disrupting membrane integrity, increasing permeability, and causing leakage of intracellular contents, ultimately leading to cell lysis [20]. However, once a critical concentration is reached - where all susceptible membrane regions are already occupied - further increases in FA concentration do not result in greater damage. This saturation effect is conceptually similar to enzyme kinetics, where the reaction rate plateaus once all enzyme active sites are occupied. Additionally, at higher concentrations, FAs tend to self-aggregate into micelles or other supramolecular structures, reducing the availability of free FA molecules that can interact with bacterial membranes effectively [24]. Moreover, it is possible that concentrations as low as 10–20% are already sufficient to achieve a maximum bactericidal effect**,** and increasing the concentration beyond this point does not lead to a larger inhibition zone or further reduction in viable bacterial count. To validate these hypotheses, future experiments could include measuring leakage of intracellular contents (e.g., proteins, ions), using electron microscopy to visualize membrane disruption, and determining the critical micelle concentration (CMC) through techniques such as dynamic light scattering (DLS). Such data would provide deeper insight into the concentration-effect relationship of FAs and help optimize their effective dosage in antimicrobial formulations - maximizing efficacy while minimizing waste, cost, and potential side effects.

3.3. Comparison of MIC of FAs extract from HCO CCO against S. aureus and MRSA

Additionally, to confirm the antibacterial concentration, the Minimal Inhibitory Concentration (MIC) test was also conducted. This assay determines the lowest concentration of the FA extract that effectively

inhibits visible bacterial growth, providing a more precise evaluation of its antimicrobial potency.

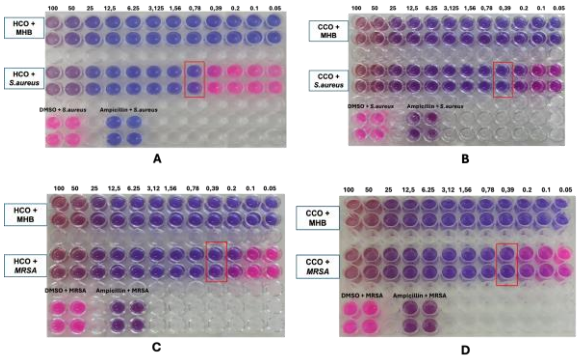

Figure 3. *Resazurin-based 96-well plate microdilution method to determine MIC value of FAs extract from HCO (A, C) and CCO (B, D) against S. aureus (A, B) or MRSA (C, D)*

The results presented in Figure 3 highlight a notable difference in the antibacterial efficacy of FAs extracted from coconut oil produced by two common processing techniques - hot pressing and cold pressing - against *S. aureus* and MRSA. Specifically, the MIC required to inhibit *S. aureus* was 0.78% for the HCO extract (Figure 3A), while it was only 0.39% for the CCO (Figure 3B). In the case of MRSA, both extracts (HCO and VCO) exhibited an MIC of 0.39%, indicating similar potency (Figure 3C and 3D).

Although both CCO and HCO exhibited modest antibacterial activity compared to the conventional antibiotic ampicillin, they demonstrated clear inhibitory effects against both S.aureus and MRSA. Notably, MRSA showed greater sensitivity to CCO than SA, despite its well-documented resistance to β-lactam antibiotics. This observation can be attributed to several factors, particularly differences in extraction methods that significantly influence the composition and bioactivity of fatty acids (FAs) present in coconut oil. CCO, produced at lower temperatures, preserves a higher proportion of medium-chain free fatty acids - especially lauric acid (C12:0), which typically constitutes 45–53% of total fatty acids - along with minor bioactives such as tocopherols and polyphenols [7, 25]. These compounds exhibit well-established membrane-disruptive antibacterial effects, especially against Gram-positive bacteria [20, 16]. In contrast, thermal processing in HCO may cause partial degradation or modification of these components, thereby reducing its antimicrobial potential. Furthermore, the enhanced susceptibility of MRSA to these fatty acids - despite its resistance to antibiotics like ampicillin - suggests that the mechanism of FA action bypasses conventional resistance pathways by directly targeting bacterial membranes. Resistance-associated modifications in MRSA, such as altered surface proteins or membrane fluidity, may paradoxically increase its vulnerability to amphiphilic agents like lauric acid [18, 21]. Supporting this, Ogbolu *et al.* [26] and Verallo-Rowell *et al.* [21] reported that lauric acid and virgin coconut oil were effective against both SA and MRSA, with MRSA showing notable sensitivity. While FAs from CCO and HCO are not potent enough to substitute antibiotics, their intrinsic antibacterial properties - especially from CCO - could be harnessed through optimized extraction or formulation strategies.

These findings highlight the potential of fatty acids (FAs) derived from CCO as natural antimicrobial agents, particularly against antibiotic-resistant pathogens such as MRSA. While their in vitro activity is promising, further studies are needed to characterize the specific FA profiles - preferably using techniques like gas chromatography–mass spectrometry (GC-MS) - and to validate their efficacy in vivo. In addition, future research should explore the molecular interactions between FAs and bacterial membranes to better understand their mechanism of action and assess whether MRSA's apparent susceptibility to membrane-targeting compounds can be strategically exploited for adjunctive or preventive therapeutic approaches.

4. Conclusion

These findings underscore the potential of cold-pressed coconut oil-derived fatty acids as natural, preliminary candidates with promising baseline antibacterial activity that warrant further investigation and optimization. Beyond their antimicrobial capacity, the use of ethanol and NaCl in the extraction process further supports a more biocompatible and environmentally friendly methodology, eliminating the need for toxic solvents such as methanol or toluene, commonly employed in traditional extraction. When compared to prior studies, such as those by Verallo-Rowell et al [16], which explored the antimicrobial properties of virgin coconut oil and lauric acid against *P. acnes* and *S. aureus*, our findings are consistent with the general conclusion that medium-chain fatty acids (MCFAs) possess strong antibacterial effects [21, 22]. However, our work expands upon this by providing a comparative analysis of processing techniques, which has been largely underexplored in the literature. Additionally, while many studies have emphasized the role of lauric acid as the main bioactive component, our work suggests that the full mixture of FAs - including capric and caprylic acids - contributes synergistically to the observed activity.

Despite the encouraging findings, this study has several limitations that should be addressed in future research. Firstly, the investigation was limited to in vitro antimicrobial assays; further studies are needed to explore the anti-inflammatory and immunomodulatory properties, which are essential for clinical and cosmeceutical applications. Additionally, the study did not isolate or quantify individual fatty acids extracts from HCO and CCO. This limits the understanding of which specific components contribute most significantly to the observed biological activities. Future work should focus on isolating and characterizing individual fatty acids to better elucidate their distinct and synergistic effects, thereby providing deeper mechanistic insights into the formulation's efficacy. Ultimately, these findings serve as a foundational step toward the development of safe, natural antimicrobial products, with significant potential for application in dermatology, cosmeceuticals, and public health,

particularly in the context of antibiotic-resistant pathogens. The integration of these high-value natural products into global markets has the potential to contribute to national GDP growth and rural economic development.

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