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In Vitro Evaluation of the Antibacterial Effect of Virgin Coconut Oil Against *Pseudomonas aeruginosa* Associated with Folliculitis

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Abstract

Virgin Coconut Oil (VCO) is a pure coconut oil rich in lauric acid, giving it potential antibacterial properties. Previous studies have successfully produced a VCO using a combined fermentation and enzymatic method with Neurospora sitophila, Lactobacillus plantarum, and papain. Nevertheless, further investigation is required to determine the antibacterial efficacy of the VCO against Pseudomonas aeruginosa, a bacterium responsible for folliculitis. This study analyzed of the antibacterial activity of VCO and Hydrolyzed Virgin Coconut Oil (HVCO) against P. aeruginosa in vitro. VCO is obtained by fermenting coconut milk using *N. sitophila*, *L. plantarum* and papain, then VCO undergoes hydrolysis with the aid of Lipozyme TL IM to yield HVCO. Inhibition zone assays were conducted to assess the antibacterial activity of 100% VCO and HVCO at varying concentrations of 25%, 50%, and 100%. The largest inhibition zone was shown by 100% HVCO (9.5 ± 0.3 mm in diameter), which was statistically significantly different from the control, ampicillin trihydrate. The data suggested that HVCO has enhanced antibacterial effectiveness against P. aeruginosa, compared to VCO. This increased activity is attributed to its higher free fatty acids and monolaurin content.

Keywords: antibacterial, folliculitis, HVCO, *Pseudomonas aeruginosa*, VCO

Introduction

Virgin Coconut Oil (VCO) is pure coconut oil obtained from the primary raw material of fresh coconut flesh and has a distinctive aroma. The production process bypasses the RBD (Refined, Bleached, and Deodorized) stage (Apriani *et al.*, 2024). VCO is commonly utilized across multiple sectors, including culinary, cosmetic, and health applications. Although not classified as a type of medicine, VCO is known to have pharmacological potential, including as an antibacterial due to its

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bioactive compound content. Fatty acids in VCO consist of approximately 90% saturated fatty acids and 10% unsaturated fatty acids (Pakpahan & Nasution, 2022). The main components are lauric acid, which makes up around 48-53% of the total fatty acids, and phenolic compounds, which are also known to have biological activity (Ghani *et al*, 2018). Lauric acid is known to inhibit bacterial growth. According to Susanti (2020), the free fatty acids (FFA) found in VCO contribute to maintaining an acidic pH on the skin, thereby preventing the proliferation of harmful bacteria that cause skin infections.

Among bacterial skin infections, folliculitis involves inflammation caused by bacteria infecting hair follicles. Folliculitis is identified by the presence of pus-containing crusts on the skin. Folliculitis is most commonly found in the head area (on children), beards, armpits, and extremities. While most cases of folliculitis are caused by gram-positive bacteria, gram-negative bacteria such as *Pseudomonas aeruginosa* can also serve as pathogens responsible for skin infections (Hazni *et al.*, 2023). *P. aeruginosa* is an obligately aerobic, rod-shaped bacterium, approximately 0.5–1.0 µm long, possessing polar flagella that facilitate its motility (Anggraeni & Triajie, 2021). This bacterium is an opportunistic pathogen, meaning it can cause infection when the host's immune system is weakened, by exploiting damage to the body's defense mechanisms to initiate infection (Haryati *et al*., 2017).

Previous research by Hati *et al*. (2020) produced VCO by fermenting coconut cream with the addition of *Neurospora sitophila, Lactobacillus plantarum*, and papain. The lauric acid content in the VCO was relatively high, at 56.73%; however, its antibacterial potential, particularly against bacteria causing folliculitis, has yet to be tested. Antibacterial activity tests were also performed using Hydrolyzed Virgin Coconut Oil (HVCO) for comparison, based on several previous studies, which stated that unhydrolyzed VCO could not show antibacterial activity against the tested bacteria because it contained too few FFA and the absence of monolaurin, but hydrolyzed VCO (HVCO) was reported to be more active in inhibiting bacterial growth (Nguyen *et al.,* 2017). This study aimed to evaluate the inhibitory effects of both VCO and HVCO against *P. aeruginosa*, one of the bacterium responsible for bacterial folliculitis.

Methods

Plant Materials and Preparation of VCO

Fresh and ripe coconuts; a culture of *Neurospora sitophila* (obtained from the Department of Microbiology, Institut Teknologi Bandung); *Lactobacillus plantarum* InaCC B153 (obtained from the Indonesian Culture Collection, Indonesian Institute of Sciences, Bogor, Indonesia); and papain, isolated from the latex of raw papayas, were utilized in this study. The production of VCO followed the Hati *et al.* (2020) method with the optimum conditions of fermentation being 1042.4 U, 46.4°C, and 5.07 for papain activity, incubation temperature, and pH, respectively.

Enzymatic Hydrolysis of VCO

The hydrolysis process used Lipozyme TL IM (Thermomyces lanuginosus immobilized lipase), which specifically cleaves ester bonds at the sn-1 and sn-3 positions of triglycerides (sn refers to the stereospecific numbering of the glycerol backbone). A total of 30 g of VCO was placed into a 250 mL Erlenmeyer flask, followed by the addition of 30 mL distilled water, 12.5 mL of 0.05 M $CaCl_2$, 25 mL of Tris-HCl buffer solution, and 500 mg of Lipozyme TL IM. The mixture was shaken for 10 minutes at a speed of 200 rpm. Then the mixture was incubated at 55°C and was shaken every hour for 10 minutes at a speed of 200 rpm until the 14th hour (Loung *et al.*, 2014). Once hydrolyzed, the mixture was transferred to a separating funnel, acidified to pH 3.4 using 1 M HCl, and then extracted with 50 mL of n-hexane to produce two layers. The top layer (filtrate) was re-extracted with 50 mL n-hexane, then separated from the aqueous phase. As much as 50 mg of Na_2SO_4 was added to the filtrate and left to stand for 15 minutes. Subsequently, the solvent was evaporated to obtain hydrolyzed VCO (HVCO), which was then used for microbial testing (Silalahi *et al*., 2014).

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Determination of Acid Number

The acid number of the VCO and HVCO were determined during the testing. A total of 2 g of VCO/HVCO was dissolved in 3 mL of 95% ethanol. Then the solution is heated for 10 minutes on a hot plate while stirring with a magnetic stirrer. The solution was allowed to cool and then titrated with 0.1 N KOH in the presence of 1% phenolphthalein indicator until a stable pink color appeared. The result was then calculated using the following formula:

$$\textbf{\textit{Acid number}} = \frac{V\ KOH \times\ N\ KOH \times\ Mr\ KOH}{\text{weight of sample}}$$

Antibacterial Test of HVCO

The antibacterial activity test was carried out using the Kirby-Bauer inhibition zone method. The pathogenic bacteria used were *P. aeruginosa*. Bacterial isolates were prepared by inoculating the bacterial culture into physiological saline solution, followed by homogenization and adjustment of turbidity to match the McFarland standard 1 (approximately 3×10^8 cfu/mL). Then, 1 mL of this bacterial suspension was transferred into a petri dish, mixed thoroughly with 15 mL of Mueller-Hinton Agar (MHA), and left to solidify. Sterile 6 mm paper discs were soaked in the test samples—100% HVCO, 50% HVCO, 25% HVCO, 100% VCO—as well as in n-hexane (used as a negative control) and ampicillin (used as a positive control). These discs were then applied onto the agar surface and incubated for 24 hours. Then the disc paper was placed on the surface of the agar plate that had been planted with bacteria. The paper discs were placed 3 cm apart and positioned 2 cm away from the edge of the agar medium. The petri dishes were then sealed and incubated at 37ºC for 24 hours to observe the formation of inhibition zones. The antibacterial activity was assessed by measuring the diameter of these inhibition zones. Each measurement was performed in triplicate to ensure accuracy (Nguyen *et al.,* 2017; Silalahi *et al*., 2014).

Statistical Analysis

The results were presented as mean ± standard deviation. Data were analyzed statistically with analysis of variance (One-Way Anova; $\alpha = 0.05$), followed by *Tukey's Post Hoc* test to see the differences between groups more clearly. Statistical significance was established at a threshold of $p < 0.05$.

Result and Discussion

Production of VCO via Enzymatic Fermentation

In this study, VCO was produced following preliminary findings by Hati *et al*. (2020) through enzymatic fermentation utilizing a mix of *Neurospora sitophila, Lactobacillus plantarum*, and papain enzyme. Fermenting 300 mL of coconut cream resulted in a VCO yield of 45.06%.

Figure 1. VCO obtained from the fermentation of coconut cream using a combination of N. sitophila, L. plantarum, and papain enzyme. The resulting VCO is characterized by its clear appearance and distinct coconut aroma.

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Figure 1 shows the produced VCO, which is noted for its clear appearance and characteristic coconut scent. The VCO meets the quality criteria set by the Asian and Pacific Coconut Community (APCC) in 2009.

Hydrolysis and Antibacterial Activity of VCO

After hydrolysis, the triglycerides in VCO would break down into two FFA and 2-monoglycerides because Lypozyme TL IM cuts specific triglycerides at sn-1 and sn-3 positions (Siregar *et al*., 2021). The parameters for the success of the hydrolysis of VCO were tested by looking at the acid numbers of HVCO and comparing them with those of VCO. The acid number indicates the quantity of FFA in the oil; a higher acid number suggests a more successful hydrolysis process. Table 1 compares the FFA/acid numbers of VCO and HVCO, showing that VCO contains only a small amount of FFA at 0.30 mg KOH/g oil, whereas HVCO has a significantly higher value of 139.38 mg KOH/g oil.

Table 1. Comparison of the value of acid numbers in VCO and HVCO.

Sample	The acid number (mg KOH/g oil)
VCO	0.30 ± 0.06
HVCO	139.38 ± 0.34

According to Elysa *et al.* (2014), the enzymatic hydrolysis of VCO produced 2-monoglycerides and two FFA which are dominated by monolaurin and lauric acid (C12) which are known to be the most active as antimicrobials compared to other medium chain fatty acids such as caprylic acid (C8), capric acid (C10) or myristic acid (C14). Monolaurin and lauric acid have been shown to have antibacterial activity, as Nitbani *et al*. (2022) reported. Lauric acid and monolaurin can destroy gram-positive bacteria, especially *Staphylococcus aureus*, fungi such as *Candida albicans*, and viruses. In addition, a study by Nguyen-Son & Nguyen-Minh (2022) demonstrated that VCO is also capable of inhibiting the growth of Gram-negative bacteria such as *Escherichia coli, Salmonella spp.,* and *Shigella spp*.

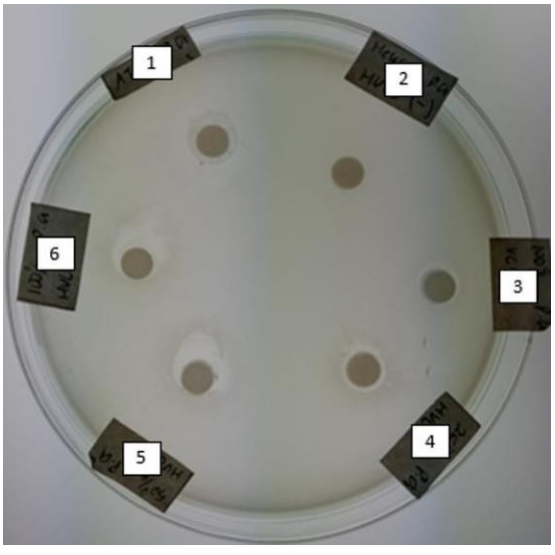

Figure 2. The zone of inhibition of VCO and HVCO against P. aeruginosa with ampicillin trihydrate (5 mg/mL) used as positive control (1), n-hexane as a blanko (2), VCO 100% (3) and HVCO concentration variation of 25% (4), 50% (5), dan 100% (6). The diameter of the disc paper was about 6 mm.

The results of the VCO and HVCO antibacterial activity test at concentrations of 25, 50, and 100% against *P. aeruginosa* by the inhibition zone method are shown in Figure 2. The observations focused

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on the presence or absence of a clear zone formed around the disc paper. The clear zone formed showed the inhibition zone for bacterial growth. The zone of inhibition could be classified into three categories based on the diameter of the formed zone, namely: very active (> 11 mm), active/medium (6-11 mm) and inactive (<6 mm) (Silalahi *et al.,* 2014). Based on the inhibition zone grouping criteria, VCO and HVCO concentration variations in this study were included in the active medium category because they have an inhibition zone ranging from 6 to 11 mm. Besides that, the higher the HVCO concentration the higher the antibacterial activity. Based on these results, VCO and HVCO in this study can be said to have reasonably good antibacterial activity in inhibiting the growth of *P. aeruginosa*.

Table 2. The average of the inhibition zone of VCO and HVCO concentration variation against *P.aeruginosa* after incubation for 24 hours at a temperature of 37°C.

Sample	Concentration	Mean ± SD Inhibition zone (mm) n=3
Ampicillin trihydrate (control +)	5 mg/mL	$8.4 \pm 0.9^{a,b}$
n-hexane (control -)	-	6.0^{c}
VCO	100% (v/v)	7.8 ± 0.2^{b}
HVCO	25% (v/v)	$7.3 \pm 0.3^{b,c}$
	50% (v/v)	$8.2 \pm 0.3^{a,b}$
	100% (v/v)	9.5 ± 0.3^{a}

Note: a,b,c,d and e value was grouped in analytical statistics one-way ANOVA and post-hoc Tukey with $p < 0.05$. Different values showed a significant difference statistically.

Based on Table 2, it is known that the antibacterial activity of VCO and HVCO 100% against *P. aeruginosa* that caused folliculitis showed a significant difference in treatment (one-way Anova and Post-hoc Tukey, $p<0.05$), which means that HVCO had more influence in inhibiting the growth of *P. aeruginosa*. This may be due to the influence of lauric acid, monolaurin and bioactive compounds in HVCO which also contribute as antibacterial agents. Previous studies by Margata *et al*. (2019) reported that 50% hydrolyzed virgin coconut oil (HVCO) exhibited significant antibacterial activity against *Bacillus subtilis*, as indicated by an inhibition zone diameter of 10.53 ± 0.10 mm. Lauric acid exhibits more potent antibacterial activity compared than other medium-chain fatty acids (MCFAs) such as caprylic, capric, and myristic acid. Generally, fatty acids and monoglycerides exert their antibacterial effects by disrupting the bacterial plasma membrane (lipid bilayer) (Silalahi *et al*., 2014). Lauric acid and monolaurin, which are derived from the hydrolysis of virgin coconut oil (VCO), are effective against various bacteria, viruses, and fungi. Their antibacterial mechanism involves lipid-protected bacterial cell membranes' physical and chemical disruption and interference with cellular processes such as signal transduction and transcription (Dayrit, 2014).

Conclusion

VCO and HVCO produced by enzymatic fermentation using *N. sitophila, L. plantarum*, and papain have antibacterial activity against folliculitis-causing bacteria; *P. aeruginosa*. HVCO

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concentration 100% had the best antibacterial activity with the inhibition zone diameter against *P. aeruginosa* is 9.5 ± 0.3 mm due to the higher free fatty acids and monolaurin content.

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Reference

Anggraeni, A., & Triajie, H. (2021). Uji Kemampuan Bakteri (*Pseudomonas aeruginosa*) Dalam Proses Biodegradasi Pencemaran Logam Berat Timbal (Pb), Di Perairan Timur Kamal Kabupaten Bangkalan. *Juvenil*, 176-185.

Apriani, A., Muna, T., Riski, M., & Azhari, S. (2024). Penyuluhan Pembuatan Virgin Coconut Oil (VCO) di Dusun Surabaya Kelurahan Ateuk Pahlawan Kecamatan Baiturrahman. *Jurnal Pengabdian Masyarakat*, 172-177.

Dayrit, F.M. (2014). The properties of lauric acid and their significance in coconut oil. *Journal of the American Oil Chemists' Society.* **92**(1), 1-15.

Elysa, Harahap, U. & Silalahi, J. (2014). Antibacterial activity of enzymatic hydrolysis of virgin coconut oil against *Salmonella. International Journal of PharmTech Research*. **6**(2), 589-599.

Ghani, N., Channip, A., H.H.P., C., Ja'afar, F., Yasin, H., & Usman, A. (2018). Physicochemical Properties, Antioxidant Capacities, And Metal Contents Of Virgin Coconut Oil Produced By Wet And Dry Processes. *Food Science & Nutrition*, 1298–1306.

Hati, F.K., Ishmayana, S., Soedjanaatmadja, U.M.S. (2020). Determination of significant parameters and optimization production of virgin coconut oil using *Neurospora sitophila, Lactobacillus plantarum*, and papain. *International Journal of Research in Pharmaceutical Sciences.* **11**(4), 6123-6135.

Haryati, S., Darmawati, S., & Wilson, W. (2017). *Perbandingan Efek Ekstrak Buah Alpukat (Persea Americana Mill) Terhadap Pertumbuhan Bakteri Pseudomonas aeruginosa Dengan Metode Disk Dan Sumuran.* Semarang: Universitas Muhammadiyah Semarang.

Hazni, K., Suhartono, E., & Hasudungan, J. (2023). *Klasifikasi Penyakit Folikulitis Berdasarkan Citra Digital Dengan Metode Fraktal dan KNearest Neighbor.* Bandung: Telkom University.

Jansen, S., & Darmareja, R. (2023). Literature Review Protokol Pemberian Virgin Coconut Oil (VCO) pada Anak dengan Ruam Popok. *Jurnal Keperawatan Widya Gantari Indonesia*, 194-202.

Loung, F.S., Silalahi, J. & Suryanto, D. (2014). Antibacterial activity of enzymatic hydrolyzed of virgin coconut oil and palm kernel oil against *Staphylococcus aureus, Salmonella thypi* and *Escherichia coli. International Journal of PharmTech Research.* **6**(2), 628-633.

Margata, L., Silalahi, J., Harahap, U., Suryanto, D. & Satria, D. (2019). The antibacterial effect of enzymatic hydrolyzed virgin coconut oil on *Propionibacterium acne, Bacillus subtilis, Staphylococcus epidermidis,* and methicillin-resistant *Staphylococcus aureus. Rasayan Journal of Chemistry*. **12**(2), 987-993.

Nguyen, V.T.A., Le, T.D., Phan, H.N. & Tran, L.B. (2017). Antibacterial activity of free fatty acids from hydrolyzed virgin coconut oil using lipase from *Candida rugosa*. *Journal of Lipids.* 1-7.

Nguyen-Son, T. & Nguyen-Minh, T. (2022). Production of the virgin coconut oil from induced fermentation with lactic acid bacteria. *European Onliine Journal of Natural and Social Sciences,* 11(4), 57-70.

 E-ISSN: 2303-0720

Nitbani, F. O., Tjitda, P. J. P., Nitti, F., Jumina, & Detha, A. I. R. (2022). Antimicrobial Properties of Lauric Acid and Monolaurin in Virgin Coconut Oil: A Review. *ChemBioEng Reviews*, *9*(5), 442–461.

Pakpahan, R., & Nasution, E. (2022). Sifat Fisika Virgin Coconut Oil (VCO) yang Dibuat Dengan Metode Pengadukan. *Jurnal ESTUPRO*, 17-20.

Silalahi, J., Permata, Y.M. & Putra, E.D.L. (2014). Antibacterial activity of hydrolyzed virgin coconut oil. *Asian Journal of Pharmaceutical and Clinical Research.* **7**(2), 90-94.

Siregar, E., Silalahi, J., & Suryanto, D. (2021). Application of partial hydrolysis of Virgin Coconut Oil (VCO) on carrageenan-based edible coating as *Sphyraena barracuda* fishball preservative during room storage. *Journal of Food and Pharmaceutical Sciences*, *9*(1), 1–8.

Susanti, E. (2020). Upaya Penyembuhan Ruam Popok (Diaper Rash) Menggunakan VCO (Virgin Coconut Oil) Di Pulau Mandangin Tengah Wilayah Kerja Puskesmas Banyuanyar Kabupaten Sampang. *Jurnal Ilmiah Obsgin: Jurnal Ilmiah Kebidanan dan Kandungan*, 1-11.