

# Antioxidant and Antibacterial Assay of Virgin Coconut Oil-Olive Oil Blend

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## Abstract

Virgin coconut oil (VCO) has bioactivity that benefits health, such as lowering cholesterol, anti-inflammatory and antibacterial. Antibacterial properties of VCO related to the content of lauric acid. However, VCO has a poor physicochemical resistance. One way to improve the quality of VCO is to add antioxidant compounds, such as olive oil (OO), which has high antioxidants. This study used a blend of VCO and OO with 10% OO composition (OO10). The addition of olive oil resulted in a lower IC<sub>50</sub> of the VCO-OO blend (41,666 ppm) compared to the pure VCO (125,000 ppm), thus suggesting that the VCO-OO blend has higher antioxidant capacity. However, neither VCO, OO nor OO10 blend showed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

Keywords : antibacterial, antioxidant, oil blending, olive oil, virgin coconut oil

## Abstrak

*Virgin coconut oil* (VCO) mempunyai bioaktivitas yang bermanfaat bagi kesehatan, seperti menurunkan kolesterol, anti inflamasi dan antibakteri. Sifat antibakteri VCO berkaitan dengan kandungan asam laurat. Namun VCO mempunyai ketahanan fisikokimia yang kurang baik. Salah satu cara untuk meningkatkan kualitas VCO adalah dengan menambahkan senyawa antioksidan, seperti *olive oil* (OO) yang memiliki antioksidan tinggi. Penelitian ini menggunakan campuran VCO dan OO dengan komposisi 10% OO (OO10). Penambahan OO menghasilkan IC<sub>50</sub> yang lebih rendah pada campuran VCO-OO (41.666 ppm) dibandingkan dengan VCO murni (125.000 ppm), yang menunjukkan campuran VCO-OO memiliki kapasitas antioksidan yang lebih tinggi. Namun, baik VCO, OO, maupun campuran OO10 tidak menunjukkan aktivitas antibakteri terhadap *Escherichia coli* dan *Staphylococcus aureus*.

Kata kunci : antibakteri, antioksidan, pencampuran minyak, olive oil, virgin coconut oil

## INTRODUCTION

Virgin coconut oil (VCO) is oil produced from coconuts either by heat processing or cold processing. Based on previous study, VCO has many benefits such as lowering blood glucose and cholesterol levels (Supriatna *et al.*, 2018). Hamsi *et al* (2015) stated that fresh VCO did not cause a adverse effect on blood pressure, inflammatory biomarkers and helped reduce the weight in vivo.

VCO is very rich in medium-chain fatty acids (MCFAs), especially lauric acid, which comprises almost 50% of its fatty acid content. Lauric acid is well known for its potent antimicrobial, antiviral, and antifungal

properties (Araújo de Vasconcelos *et al.*, 2023). VCO is rich in antioxidants, including tocopherols and polyphenols, which contribute to its protective effects against oxidative stress and inflammation (Mansouri *et al.*, 2024).

Pure coconut oil (VCO) has functional ingredient such as 8 % caprylic acid (C8), 10% capric acid (C10), 48% lauric acid (LA) and 17 % meristic acid. Medium chain fatty acids (MCFA) are known to be functional foods that have antibacterial potential (Zenek *et al.*, 2013). Many recent studies have shown lauric acid is a major bacterial growth inhibitor. Capric acid and caprylic acid can also inhibit bacterial growth (Tangwatcharin *et al.*, 2012; Shilling *et al* 2013; Yassen *et al.*, 2015). Lauric

acid, the main acid in VCO, has been shown to have antiviral, antibacterial, and antiprotozoal properties. In the human body, lauric acid is converted to glyceryl laurate, which has antiviral properties, and studies have shown that monolaurin has the ability to disrupt lipid-coated viruses such as HIV, herpes, cytomegalovirus, and influenza. Therefore, VCO has been recognized as a functional food due to its high nutritional and medicinal value, namely polysaturated fatty acids (MCFA), vitamins, amino acids, antioxidants, antibacterial agents, and antiviral compounds (Ghani *et al.*, 2018). The monoglyceride form of lauric acid, monolaurin, makes VCO effective in how it works against *Staphylococcus aureus* resulting in an inhibition zone of 3 mm compared to a vancomycin positive control of 4 mm. Other studies also show that lauric acid has high antibacterial activity against *S. aureus* and is good for treating atopic dermatitis caused by *S. aureus* (Silalahi *et al.*, 2014).

Research on virgin coconut oil (VCO) processing technologies has been extensively conducted, primarily focusing on improving yield and maintaining its natural bioactive compounds. Nevertheless, further studies are required to enhance the functional properties and health benefits of VCO, particularly its antioxidant and antimicrobial activities. One promising approach is physical blending, which allows modification of oil characteristics without altering their chemical structures. Several studies have reported that physical blending of VCO with other edible oils, such as palm oil (Sonwai *et al.*, 2013; Motamedzadegan *et al.*, 2020) and fish oil (Patil and Benjakul, 2019), successfully improves the physical stability and quality attributes of the resulting blends. Similar blending strategies have also been applied to coconut oil combined with peanut oil, groundnut oil (Sura *et al.*, 2020), sunflower oil (Suliman *et al.*, 2018; Ramos *et al.*, 2019), and linseed oil (Grover *et al.*, 2021).

Despite these extensive studies, limited attention has been given to the physical blending of VCO with olive oil (OO), particularly in relation to their combined bioactive properties. Olive oil is well known for its high content of natural antioxidants and antimicrobial compounds, especially phenolic compounds such as oleuropein, hydroxytyrosol, tyrosol, gallic acid, and luteolin, which contribute to its strong radical scavenging and antimicrobial activities. Previous studies have demonstrated that methanolic extracts of olive oil by-products effectively inhibit both Gram-positive bacteria (*Listeria monocytogenes* and *Staphylococcus*

*aureus*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*), with enhanced antibacterial effects observed at lower temperatures (Janakat *et al.*, 2015).

The selection of olive oil (OO) for blending with virgin coconut oil (VCO) is scientifically justified by the complementary nature of their bioactive components. VCO is rich in medium-chain fatty acids, particularly lauric acid, which has been associated with antibacterial activity, while olive oil contains abundant phenolic compounds that contribute to strong antioxidant capacity and oxidative stability (Lanza and Ninfali, 2020). Previous studies have shown that blending oils rich in phenolics with saturated or medium-chain lipid matrices can enhance antioxidant performance and improve the functional stability of the oil system through synergistic interactions (Hashempour *et al.*, 2016).

However, comprehensive studies evaluating the synergistic antioxidant and antibacterial activities of VCO–OO blends remain limited. Most existing research focuses on the individual bioactivity of VCO or olive oil, or on blending VCO with other vegetable oils mainly to improve physicochemical properties. Therefore, a research gap exists regarding the biofunctional synergy of VCO–OO blends. This study aims to address this gap by investigating the antioxidant and antibacterial activities of VCO–OO blends prepared through physical blending.

## MATERIALS AND METHOD

### Materials

The materials used in this study were virgin coconut oil and olive oil purchased from local supplies, 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma Aldrich), and methanol (Merck). Culture of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* were obtained from the laboratory collection. Microbial medium nutrient agar and potato dextrose agar were purchased from local supplies.

### Physical Blending of VCO–OO

The VCO–olive oil blend (OO10) was prepared at a VCO:OO ratio of 9:1 (v/v). This ratio was selected to maintain VCO as the dominant component while allowing the incorporation of olive oil, as commonly applied in oil blending studies (Hashempour *et al.*, 2016). Homogenization was performed using a shaker at 28 °C and 250 rpm for 5 min to ensure uniform mixing and physical stability of the blend.

### Antioxidant Assay

The antioxidant assay of VCO, OO, and OO10 was carried out based on radical

scavenging method (Gulcin *et al.*, 2023). Sample was added with DPPH solution and incubated at 37°C for 30 minutes and measured using a UV-Vis spectrophotometer at 517 nm. All tests were conducted in duplicate.

### Antibacterial Activity Assay

The antibacterial activity of VCO, olive oil, and OO10 was evaluated using the agar well diffusion method, adapted from the M100-22 Clinical and Laboratory Standards Institute (CLSI, 2012). The assay was performed against *E. coli* and *S. aureus* bacteria. Bacterial suspensions were evenly spread on agar plates, and 6 mm wells were aseptically prepared. Each well was filled with 50 µL of sample, followed by incubation at 37 °C for 24 h. The diameter of the inhibition zone was measured in millimeters, with zones >9.0 mm considered indicative of antibacterial activity (Almeida *et al.*, 2006). Oxytetracycline hydrochloride (50 mg/mL) and 0.9% NaCl were used as positive and negative controls, respectively. All tests were conducted in duplicate.

## RESULTS AND DISCUSSION

### Physical Blending of VCO-OO

Physical blending represents the simplest method for modifying oil properties without involving chemical reactions. Each type of oil contributes its own physicochemical and sensory attributes to the final mixture (Hashempour *et al.*, 2016). The blending of virgin coconut oil (VCO) and olive oil (OO) at a ratio of 9:1 resulted in a homogeneous mixture, as presented in Figure 1 and Table 1. Visually, the blend exhibited a clear, uniform appearance without phase separation, indicating good miscibility between the two oils. This suggests that at the selected ratio, the fatty acid composition and polarity differences between VCO and OO were sufficiently compatible to maintain phase stability.



Figure 1. (a) VCO, (b) OO, (c) OO10 (VCO-OO blend with 10% olive oil)

Table 1. Comparison between VCO, OO, and OO10

Attributes	Samples		
	VCO	OO	OO10
Color	Transparent	Darker Yellow	Yellow
Homogeneity	Homogeneous	Homogeneous	Homogeneous
Aroma	Coconut (mild)	Olive (strong)	Olive (mild), with hint of coconut
Taste	Distinct coconut flavor	Slightly bitter (strong sensation)	Slightly bitter
Mouthfeel	Greasy	Greasy	Greasy

n = 15 panelists

Sensory descriptive analysis indicated that the blended oil maintained the characteristic aroma of VCO but exhibited a complex flavor profile. The addition of olive oil contributed to a strong sensation compared to pure VCO. The panelists also perceived the blend OO10 as smoother and less greasy, which could be attributed to the higher proportion of unsaturated fatty acids from olive oil.

### Antioxidant Activity of VCO-OO

The antioxidant activity of virgin coconut oil (VCO), olive oil (OO), and their blend (OO10) was evaluated using the DPPH radical scavenging assay, with ascorbic acid as a positive control (Figure 2). Based on the classification proposed by Molyneux (2004), antioxidant activity can be categorized

according to IC<sub>50</sub> values as very strong (<50 ppm), strong (50–100 ppm), moderate (101–150 ppm), weak (151–200 ppm), and very weak (>200 ppm). According to this classification, all samples tested in this study exhibited very weak antioxidant activity. Ascorbic acid showed the lowest IC<sub>50</sub> value (950.36 ppm), followed by olive oil (9098 ppm), the VCO-OO blend (OO10; 41,666 ppm), and pure VCO (125,000 ppm).

Although the overall antioxidant activity remained low, olive oil demonstrated higher radical scavenging capacity than VCO, which is consistent with its phenolic and tocopherol content. Notably, physical blending of VCO with 10% olive oil significantly improved the antioxidant activity of VCO, resulting in approximately a fourfold reduction in IC<sub>50</sub> value compared to pure VCO. This

enhancement, although still classified as very weak, indicates a relative improvement attributable to the contribution of olive oil phenolic compounds. As reported by Lanza and Ninfali (2020), olive oil contains potent antioxidants such as lignans, flavones, and phenolic acids, which may contribute to the observed increase in radical scavenging activity. These findings suggest that physical blending with a small proportion of olive oil can enhance the antioxidant performance of VCO, even though the overall activity remains limited under the DPPH assay conditions.

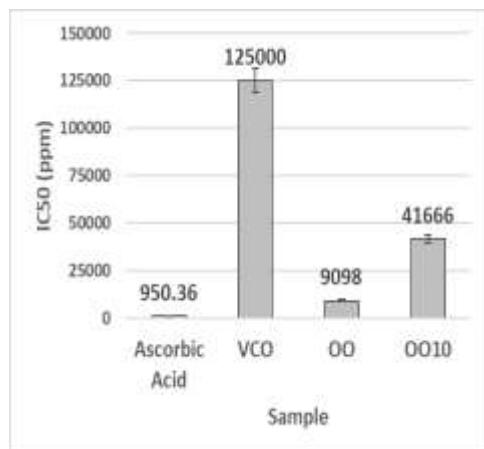


Figure 2. Comparison of IC<sub>50</sub> values

### Antibacterial Activity of VCO-OO

The antibacterial activity of virgin coconut oil (VCO), olive oil (OO), and their blend (OO10) was evaluated against *Escherichia coli* and *Staphylococcus aureus* using the agar diffusion method (Figure 3). None of the samples exhibited inhibitory zones against either the Gram-negative (*E. coli*) or Gram-positive (*S. aureus*) bacteria. These findings indicate that the tested oils did not demonstrate measurable antibacterial effects under the present experimental conditions. Similar results have been reported by Tangwatcharin *et al.* (2012) and Silalahi *et al.* (2014), who found that while lauric acid and its monoglyceride derivative, monolaurin, effectively inhibit *S. aureus*, the unmodified VCO does not exhibit direct antibacterial activity. It should be noted that the agar diffusion method may underestimate the antibacterial activity of hydrophobic substances such as oils due to limited diffusion in the agar matrix. The lack of inhibition observed in this study may be related to the nonpolar nature of the oils, which limits the diffusion of active components through the aqueous agar medium. Therefore, alternative methods such as broth microdilution assays are recommended for future studies.

Furthermore, antibacterial compounds such as phenolics in olive oil are often present in bound or lipophilic forms, reducing their availability for interaction with bacterial cell membranes in solid media. Although olive oil has previously been reported to inhibit *S. aureus* (Heidari *et al.*, 2017), differences in extraction methods, oil composition, or testing concentration could explain the absence of activity in the present work. Consequently, physical blending of VCO with OO (OO10) did not enhance antibacterial performance, suggesting that blending alone is insufficient to release or activate antibacterial constituents.

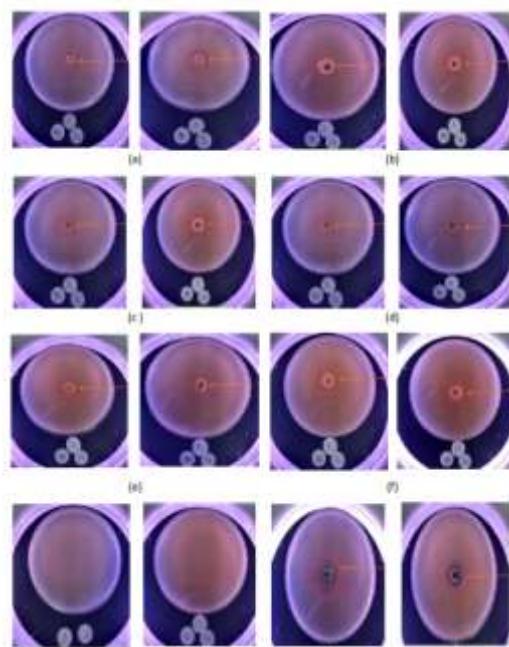


Figure 3. Inhibition zone of antibacterial test : (a) VCO against *E.coli*; (b) VCO against *S.aureus*; (c) OO against *E.coli* (d) OO against *S.aureus* (e) OO10 against *E. coli*; (f) OO10 against *S.aureus*; (g) Negative control of *E.coli*; (h) Negative control of *S. aureus*; (i) Positive control of *E.coli*; (j) Positive control of *S. aureus*

### CONCLUSION

The physical blending of virgin coconut oil (VCO) with 10% olive oil (OO) produced a homogeneous mixture, indicating good physical compatibility between the two oils. Based on the DPPH assay results, the VCO-OO blend (OO10) exhibited a lower IC<sub>50</sub> value (41,666 ppm) compared to pure VCO (125,000 ppm), demonstrating an improvement in antioxidant activity following

the blending process. However, none of the tested samples, including VCO, OO, and OO10, showed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* under the experimental conditions applied in this study. These results indicate that physical blending with olive oil can enhance the antioxidant performance of VCO but does not improve its antibacterial activity. From a practical perspective, the OO10 blend shows potential for application as an oil-based product with improved antioxidant properties, particularly for functional food formulations where oxidative stability is of interest.

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