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Phytochemical Content towards Weak Antioxidants Activity of Traditional Fermented VCO as the Basic Ingredient for Ear Drops

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***Abstract*— Chronic Suppurative Media Otitis is caused by pathogenic bacteria, which, if the growth is not controlled, will cause meningitis and can lead to death. Virgin Coconut Oil is an oil made through the traditional fermentation process of coconut milk and can inhibit the growth of pathogenic bacteria that cause *Chronic Suppurative Media Otitis*. So far, pathogenic bacteria can be controlled using ear drops made from artificial chemicals, which can cause resistance. Only a few have studied the manufacture of natural-based ear drops from VCO. The purpose of this study is to study the potential of VCO as a natural essential ingredient for ear drops for patients with OMSK by analyzing its antioxidant content using the DPPH method and its phytochemical content using Ultraviolet-Visible Spectroscopy (UV-Vis) and Gas Chromatography-Mass Spectrometry (GC-MS). Its antioxidants in the IC50 (ppm) region are 583.92mg/L. The types of fatty acids contained in VCO consist of Medium-Chain Fatty Acid (MCFA), Long-Chain Fatty Acid (LCFA) and Very Long Chain Fatty Acid (VLCFA), the most of which is lauric acid (MCFA), as much as 49.28%. Conventional analysis of secondary metabolites showed the presence of Alkaloids, Terpenoids, Ascorbic Acid, and Saponins. It can be concluded that the chemical content of Virgin Coconut Oil made from fermentation can be used in the health field as a primary ingredient for making ear drops for CSOM patients.**

***Keywords*— Antioxidants; traditional fermentation; phytochemistry; virgin coconut oil; UV-vis; GC-MS.**

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I. INTRODUCTION

Virgin Coconut Oil is an oil that can be made through several methods, one of which is the traditional fermentation of coconut milk [1], [2]. The advantage of this method is that it is more environmentally friendly because it does not require additional energy in the form of heat or high pressure. Another way to make Virgin Coconut Oil is by fermentation with the addition of bacterial cultures such as Lactobacillus plantarum [3], [4]. In addition, the manufacture of Virgin Coconut Oil can be done through the method of centrifugation of coconut milk [5], [6]. This method of making Virgin Coconut Oil can maintain its bioactive compounds.

Virgin coconut oil (VCO), made by traditional fermentation methods, contains lactic acid bacteria that contain bacteriocin. Bacteriocins are peptides that can inhibit the growth of pathogenic bacteria and are harmless to good bacteria [7], [8], [9]. It has been studied how Virgin Coconut oil can inhibit the growth of pathogenic bacteria that cause Chronic Suppurative Otitis Media (CSOM) [10].

Chronic Suppurative Otitis Media is an inflammation of the middle ear caused by pathogenic bacteria such as Pseudomonas aureginosa, Klebsiella, Proteus, Staphylococcus aureus, and others [11], [12], [13]. If the patient cannot be adequately treated, this infection will continue to become meningitis, which can be deadly[14], [15], [16]. Based on that, it is necessary to further study the potential of VCO as a primary ingredient for natural ear drops for OMSK patients. So far, ear drops are made with ingredients from artificial chemicals [17]. It has been learned that their use can cause resistance [18], so if ear drops can be

made from natural materials such as VCO, then OMSK sufferers can avoid death.

Not many have studied that to make ear drops from VCO, it is necessary to analyze the phytochemical and antioxidant content as a basis for determining the formula of the teleinga drops to be made. Although several studies on anti-oxidants from VCO have been studied, such as those conducted by South Sulawesi, Indonesia [19], determined the antioxidant mixture of Swietenia mahagoni and VCO, which turned out to be higher than the antioxidant of this mixture than VCO alone. In addition, the antioxidant content of VCO has also been studied to use VCO as a health supplement [20]. Likewise, in research on the physicochemistry of VCOs, some have studied the content of phenols, terpenoids, alkaloids [21], [22], saponins, and others, for the purposes of VCO benefits. Meanwhile, only a few have studied the phytochemistry of VCO as the basis for determining the formula for ear drops for patients with chronic suppurative otitis media (CSOM). So, this study aims to study the antioxidant content and check the phytochemical content of Virgin coconut oil as a basis for making a formula for ear drops for Chronic Suppurative Otitis Media sufferers in the future.

II. Materials and Method

This experimental research was conducted in several laboratories, including the Andalas University, Padang University, Chemistry Laboratory of Padang Perintis University, and West Sumatra Provincial Health Laboratory.

A. Materials

Methanol (pa), Ethanol (pa), FeCl310% (pa), Quercetin (for standard), Concentrated Mg/HCl (pa), Water, Anhydrous Acetate/ H2SO42N, Anhydrate, Mayer. Virgin Coconut Oil (VCO), DPPH Solution (0.1 mM in methanol), Aquades.

B. Instruments

Shimadzu UV-vis Spectrophotometer Model: UV-1800; FT-IR Thermo Fisher Scientific Model: Nicolet iS50; GC-MS Shimadzu Model: GC-2010 Plus. In addition, glassware such as test tubes, Erlenmeyer (Mereck), measuring flasks, Merck glass bags, pipettes and micropipettes, Vortex mixers, and others are used.

C. Method

1) Antioxidant analysis with DPPH Method: Initially, a VCO parent solution with a concentration of 1000 ppm was prepared, then a standard solution row was made with a sample concentration (150, 300, 450, 600, 750. ppm). Add DPPH 35 ppm, homogenize, and let it sit for 30 minutes in a dark place. Measured Absorbance with UV-Vis spectrophotometer and calculated IC50 % Inhibition.

2) Phytochemical examination for determining alkaloids in VCO: It differs slightly from ordinary plant samples. VCO is a lipid-rich oil, and most of its components are non-polar. Therefore, some adjustments were made to the extraction and refining process. So, mixing VCO with polar solvents such as ethanol or methanol to help extract polar compounds is done. After mixing VCO with a polar solvent, the mixture can be partitioned with a non-polar solvent such as n-hexane to separate the lipids from the polar components. Since VCO contains many lipid components, the defatting step is essential. The non-polar layer (which includes fats and oils) will be separated from the polar layer containing polar compounds, including alkaloids (if any). After separation, a rotary evaporator can evaporate the alkaloid's polar solvent. After the extract is obtained, the qualitative test of alkaloids is continued using the special reagents that have been mentioned: Reagents and Mayer Reagents.

3) Phytochemical examination for terpenoid determination in VCO: The test used to detect terpenoids is the Liebermann-Burchard test. This reaction detects sterols, triterpenoids, and several other terpenoids in oil samples. Liebermann-Burchard reagents consist of anhydrous acetic acid and concentrated sulfuric acid. A few drops of anhydrous acetic acid are added to the test tube containing the VCO sample. After that, carefully add a few drops of concentrated sulfuric acid along the walls of the tube. The discoloration will indicate the presence of terpenoids.

4) Phytochemical examination for determining flavonoids in VCO: Since flavonoids are polar compounds, while VCOs are non-polar fat mixtures, extracting flavonoids from VCOs using more suitable solvents is an essential first step. Some solvents commonly used for flavonoid extraction are ethanol, methanol, or water. The step is to take several VCO samples. Ethanol or methanol as a polar solvent is then added to the VCO sample to extract flavonoids (if any). This mixture is then stirred or heated slightly to allow the flavonoids to dissolve in the polar solvent. After that, separation is carried out between the polar phase (containing flavonoids) and the non-polar phase (oil). To detect flavonoids is by the Shinoda test, which involves the reaction of flavonoids with the metal magnesium and hydrochloric acid. Some magnesium powder is added to the extract obtained from the extraction process (polar phase). Then, a few drops of concentrated hydrochloric acid are added to the mixture. The solution will change to pink, red, or orange if flavonoids are in the sample.

5) The working principle of the VCO phytochemical test using UV-Vis spectrophotometry: Because VCO is a non-polar compound that consists primarily of lipids (fatty acids), it is necessary to mix it with the appropriate solvent so that the target compound in VCO can be detected more easily. The polar solvent used is ethanol or methanol to help extract phytochemical compounds such as flavonoids, terpenoids, or phenolics from VCO. A blank is prepared, and a pure solvent is used to neutralize the influence of solvents in the measurement. The blank is used to calibrate the tool so that the measurement results only show the compound's absorption in the sample. The VCO solution dissolved in the solvent is inserted into the spectrophotometer cuvette. Place the cuvette containing the sample in the UV-Vis spectrophotometer. Each compound has a specific wavelength at which it absorbs the lightest, called the maximum wavelength (λ max). UV-Vis spectrophotometry for phytochemical analysis in VCO allows the detection and identification of phytochemical compounds such as flavonoids, terpenoids, or phenolics. It works on the principle of light absorption by chemical compounds in a sample at a specific wavelength. The resulting absorption peaks can

provide information about the compounds present in the VCO and their concentrations

6) VCO Component Inspection with GC-MS: Virgin Coconut Oil (VCO) phytochemical assay using GC-MS (Gas Chromatography-Mass Spectrometry) is a highly effective method for separating, identifying, and analyzing volatile and semi-volatile compounds in VCO. GC-MS combines gas chromatography (GC) to separate compound mixtures and mass spectrometry (MS) to identify molecular composition based on mass. This technique is very useful for analyzing the components in VCOs, especially fatty acids and other minor compounds such as terpenoids or sterols. With a combination of retention time from the GC chromatogram and mass spectrum from MS, the compounds in the VCO can be identified very accurately. GC-MS identifies minor compounds in VCOs, such as fatty acids, lauric acid, caprylic, caprylic, and myrithate. Sterols and terpenoids: These minor compounds can also be analyzed, albeit in smaller amounts than fatty acids.

III. Results and Discussion

A. Antioxidant Analysis with DPPH Method

Virgin Coconut Oil has the potential to be used as a natural base ingredient to make elinga drops for people with Suppative Otitis Media. For this purpose, an antioxidant analysis was carried out, whose results are shown in Table 1 below.

TABLE I
ANTIOXIDANT ACTIVITY EXAMINATION OF VCO PREPARATIONS

Concentration (ppm)	Absorbent control	Absorbent VCO + DPPH	Inhibition (%)	IC_{50} (ppm)
150 ppm	0,656	0,531	19,05 %	583,92 ppm (Very Weak)
300 ppm	0,656	0,458	30,18 %	
450 ppm	0,656	0,392	40,24 %	
600 ppm	0,656	0,317	51,67 %	
750 ppm	0,656	0,253	61,,43 %	

Remarks: 0 – 50 ppm Antioxidant Activity of the Very Strong Group

Table 1 shows the IC50 (ppm) of VCO preparations is 583.92 ppm. This means that the antioxidants are weak. IC50 (Inhibitory Concentration 50) is a parameter used to measure how effective a substance is in inhibiting specific biochemical processes, in this case, antioxidant activity. An IC50 value indicates the concentration needed to inhibit 50% of the oxidation activity. If the IC50 for VCO (Virgin Coconut Oil) is 583.92 ppm, a VCO concentration of 583.92 ppm is required to inhibit 50% of oxidation activity. The lower the IC50 value, the stronger the compound's antioxidant activity, as it only requires a small concentration to achieve a 50% inhibitory effect. In contrast, high IC50 values, such as 583.92 ppm, indicate that VCO requires relatively large concentrations to achieve the same effect, so the antioxidant activity of this VCO is considered weak. In comparison, compounds with an IC50 below 100 ppm are typically considered to have intense antioxidant activity, while IC50 values above 500 ppm tend to indicate weaker antioxidant activity. This is the same as the antioxidant test carried out [19]. Virgin coconut oil (VCO) produced through fermentation tends to have lower antioxidant levels than other methods, such as cold-press or centrifugation. The fermentation process involves the activity of microorganisms, which can affect the bioactive composition of the oil, including the content of antioxidants such as polyphenols and tocopherols. In fermentation, enzymes produced by microbes can break down some phenolic compounds, thereby reducing the concentration of antioxidants in VCOs. In addition, VCOs obtained through fermentation are usually exposed to air for longer periods and higher temperatures, which can accelerate the oxidation process, thereby lowering their antioxidant qualities. Nonetheless, fermented VCO still has significant health benefits but may not be as effective in terms of antioxidant activity compared to VCO processed through faster mechanical methods and minimal interaction with oxygen.

Meanwhile, another study obtained the antioxidant value of virgin coconut oil (VCO) [20], [21], [23], [24] had a high DPPH radical scavenging activity ranging from 7.49 to 104.52 mg/ml. Compared to refined coconut oil, VCO has a higher phenolic compound content, contributing to its antioxidant capacity.

Overall, the findings suggest that extraction methods play an essential role in determining the antioxidant potential of coconut oil, with heat application being particularly beneficial [25], [26]. Virgin Coconut Oil extracted by fermentation has lower antioxidant levels.

B. Phytochemical Analysis by Organic Analysis Method or Qualitative Test

The phytochemical content of virgin coconut oil is analyzed conventionally or qualitatively and using tools. The results of the qualitative phytochemical analysis are shown in Table 2.

TABLE II
VCO PHYTOCHEMICAL SCREENING TEST RESULTS

Chemical content	Reagen	Results in theory	Examination results	Information
Fenolik	$FeCl_3$10%	Blue/blackish green	Not formed	-
Flavonoid	Mg/HCl Concentrated	Yellow-Red	Not formed	-
Saponin	Air	Permanent foam (± 15 menit)	Not formed	+
Terpenoid	Anhidratasetat/ $H_2SO_4$2N	Red	Red color formed	+
Steroid	Anhidratasetat/ H_2SO_4	Blue/green	Not formed	-
Alkaloid	Mayer	White haze/white blotch	Not formed	+

Information:
(+) : Presence of the compound being tested
(-) : Absence of the compound tested.

Table 2 shows virgin coconut oil analyzed for its phytochemical content containing Saponins, Terpenoids, and Alkaloids. It does not contain phenolics, flavonoids, or steroids. This is due to its low antioxidant examination [19] where the absence of phenolic compounds can cause low antioxidants. Also by [25], [4] found that the phytochemical content of Virgin Coconut Oil, especially those processed by fermented coconut milk, does not contain phenolics because the fermentation process is exposed to air for a longer time and can last up to one night.

C. VCO Phytochemical Examination with UV-Vis

The phytochemical content was analyzed using a UV-vis spectrophotometer. In Figure 1, the analysis uses 2 VCO samples: VCO A and VCO B. The difference is in the water used to extract it. Ordinary water is used in VCO A, and VCO B uses its own coconut water.

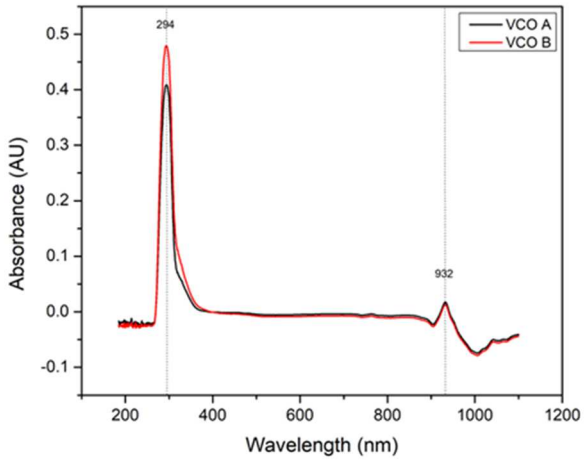


Fig.1. UV-Vis VCO spectrum, namely VCO A and VCO B

Two samples were analyzed using UV-Vis to reveal their chemical composition and optical characteristics. UV-Vis spectroscopy is a tool for identifying the presence of various compounds in oils, specifically those that can absorb light in the ultraviolet and visible wavelength ranges.

D. Analysis of Absorbance Peaks at 294 nm

Figure 1 shows a peak at the wavelength of 294 nm, which is most likely related to the presence of aromatic compounds or unsaturated fatty acids that have conjugated double bonds. It can also be said or associated with compounds that have chromophore groups, such as phenol groups, or organic compounds that have conjugated double bonds. This suggests that VCO contains phenolic compounds, also known as antioxidants, as VCO includes components such as phenolic acids and flavonoids. Phenol groups and conjugated double bonds in organic compounds typically absorb at 280-300 nm wavelengths, which supports spectral yields at 294 nm.

Absorbance at 294 nm likely indicates the presence of phenolic compounds in VCO, which is also related to its antioxidant properties. Unsaturated fatty acids, especially those with double bonds, can absorb at 200-300 nm wavelengths. A wavelength of 294 nm may indicate the presence of double bonds in the compound, which may be derived from unsaturated fatty acids in small amounts in VCOs. VCO contains a mixture of fatty acids, especially lauric acid (a saturated fatty acid). Still, there may also be small amounts of unsaturated fatty acids, such as oleic and linoleic. This can indicate a small amount of unsaturated fatty acids with a conjugated double bond in the VCO.

Absorbance at 294 nm can also indicate the presence of oxidation products from coconut oil. VCO can oxidize during storage or processing, forming peroxide or aldehyde compounds that absorb 250-300 nm. Minor components in VCOs, such as vitamin E (tocopherol) or other antioxidant components, can also be absorbed in the 200-300 nm range. The wavelength of 294 nm may be related to the absorbance of these minor compounds. Tocopherol is one of the natural antioxidant components present in coconut oil and absorbs UV radiation around this range. Tocopherol is one of the natural antioxidant components present in coconut oil and absorbs UV radiation around this range.

E. VCO Component Inspection with GC-MS

The VCO sample was analyzed for its chemical component content using a Gas Chromatography-Mass-Spectrometry (GC-MS) spectrometer. The results of the chromatogram data show that 15 peaks are read. This indicates the presence of 15 chemical compounds contained in the VCO sample. The fifteen peaks read on this chromatogram are compared to the data in the National Institute of Standards and Technologies (NIST) database, which has a Similarity index (SI) value or an index of similarity close to the compounds contained in the sample. From the data obtained from the GC-MS results, compounds that give a Similarity index (SI) value of 91-97% were selected. The data from the chromatogram data of the VCO chemical component is shown in Figure 2.

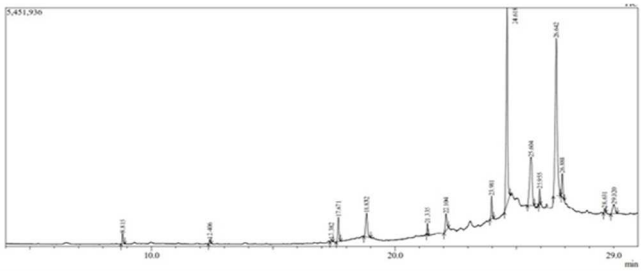


Fig. 1 GC-MS chromatogram of VCO sample

Figure 1 shows that the higher the peak of a chromatogram, the greater the % area value of a compound. The % area value shows the percentage of a compound's abundance in a sample, so the larger the % area of a chromatogram peak, the higher the peak is indicated as the main compound in a sample [27]. Table 3 shows the results of identifying chemical components, the percentage area, and the similarity index of compounds contained in VC3.

Based on the data in Table 3, it is known that the 15 chemical components contained in the VCO sample are dominated by fatty acid compounds and 1 group of ascorbic acid derivative compounds (Vitamin C). The results of secondary metabolite tests show the absence of a class of terpenoid compounds. The types of fatty acids contained in VCO consist of medium-chain fatty acid (MCFA), long-chain fatty acid (LCFA), and very-long chain fatty acid (VLCFA). The number (%) of the content of each compound group and some examples of structure are shown in Table 4.

The chemical components contained in the VCO sample are different, including compounds with 0-10%, as many as 12 compounds, compounds with 10-20%, as much as 1 compound, and 30-35% as much as two compounds. Based on the % area value of each compound, it was determined that there were three dominant compounds (≥ 10%), namely Methyl behenate (12.95%), L-Ascorbic acid dihexadecanoate (30.10%) and 6-Octadecenoic acid (33.58%). The structure of these main compounds is shown in Table 5.

TABLE III
THE CHEMICAL COMPONENTS OF THE VCO SAMPLE

No	RT (Minutes)	Compound Name	Molecular Formula	Area (%)	Similarity Index (%)
1	8.815	*Methyl octanoate*	$C_9H_{18}O_2$	1,06	97
2	12.405	*Methyl decanoate*	$C_{11}H_{22}O_2$	0,54	96
3	17.380	*Methyl dodecanoate*	$C_{13}H_{26}O_2$	0,30	93
4	17.670	*Methyl tridecanoate*	$C_{14}H_{28}O_2$	3,18	92
5	18.830	*Lauric Acid*	$C_{12}H_{24}O_2$	4,51	96
6	21.335	*Methyl tetradecanoate*	$C_{15}H_{30}O_2$	0,94	96
7	22.105	*Myristic Acid*	$C_{14}H_{28}O_2$	2,74	96
8	23.980	*Methyl palmitate*	$C_{17}H_{34}O_2$	2,45	95
9	24.615	*L-Ascorbic acid dihexadecanoate*	$C_{38}H_{68}O_8$	30,10	92
10	25.605	*Methyl behenate*	$C_{23}H_{46}O_2$	12,95	92
11	25.955	*Methyl oleate*	$C_{19}H_{36}O_2$	1,39	95
12	26.640	*6-Octadecenoic acid*	$C_{18}H_{34}O_2$	33,58	93
13	26.890	*Stearic Acid*	$C_{18}H_{36}O_2$	3,15	91
14	28.630	*Oxiran-2-ylmethyl tetradecanoate*	$C_{17}H_{32}O_3$	0,63	91
15	29.020	*Methyl tricosanoate*	$C_{24}H_{48}O_2$	2,49	93

TABLE IV
VCO'S CHEMICAL CONTENT AND ITS STRUCTURE

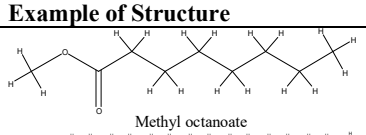
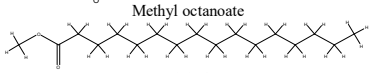
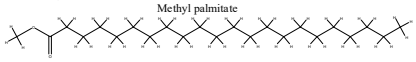
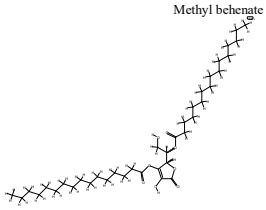
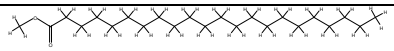
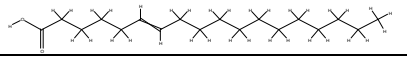
No	Type of Fatty Acids / compounds	Number of Compounds	Percentage (%)	Example of Structure
1	Medium-Chain Fatty Acid (MCFA)	3	20	 Methyl octanoate
2	Long-Chain Fatty Acid (LCFA).	9	60	 Methyl palmitate
4	Very Long Chain Fatty Acid (VLCFA)	2	13.33	 Methyl behenate
5	Senyawa Lain	1	6.67	 L-Ascorbic acid dihexadecanoate

TABLE V
STRUCTURE OF THE MAIN COMPOUNDS OF THE VCO SAMPLE

No	Name of the Compound	% Area	Structure
1	*Methyl behenate*	12.95	
2	*L-Ascorbic acid dihexadecanoate*	30.10	
3	*6-Octadecenoic acid*	33.58	

In previous research [28], it has also been reported that the content of VCO chemical components is dominated by fatty acid groups consisting of Caproic acid, Caprylic acid, Capric acid, Lauric acid, Myristic acid, Palmitic acid, Stearic acid, Oleic acid, and Arachidic acid. This component has several similarities with the content of chemical components carried out in this study. The components of the VCO sample compounds that have similarities with this study include Lauric Acid, Myristic Acid, and Stearic Acid. Some of the differences in chemical components in the VCO sample in this study are due to the influence of environmental conditions where they grow such as temperature, CO2, lighting, ozone, altitude, groundwater, salinity, soil fertility, and several other factors that have a significant impact on the physiological response of plants that can produce different chemical compound components [29], [30], [31].

IV. CONCLUSION

Virgin Coconut Oil (VCO) produced through the traditional fermentation process contains phytochemical components such as saponins and alkaloids and is rich in saturated fatty acids such as lauric acid, meristic acid, and stearic acid. The analysis used UV-Vis, and GC-MS revealed that VCO has optical characteristics similar to the UV-Vis spectrum, which shows the content of unsaturated compounds that contribute to the antioxidant properties of this oil.. In contrast, GC-MS identified 15 major chemical compounds, including Methyl behenate, L-Ascorbic acid dihexadecanoate, and 6-Octadecenoic acid as the dominant compounds. The VCO of the traditional fermentation process has acceptable qualities with potential applications in the nutraceutical, pharmaceutical, and cosmetic industries. The

antioxidant content and anti-inflammatory properties found in this VCO support its use in various health applications. Furthermore, this research provides a foundation for further exploration of the potential applications of VCO in the medical and pharmaceutical fields, as well as to improve more efficient production methods.

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