



Free Fatty Acids in VCO Nanoemulsion Formulation: Nutraceuticals for Colon Cancer

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Abstract: Colon cancer ranks as the second leading cause of cancer-related deaths globally. Conventional treatments often cause numerous side effects and lack selectivity. Therefore, developing anticancer agents derived from virgin coconut oil (VCO) nanoparticles has become a promising approach in cancer drug discovery. This study investigates the anticancer potential of VCO nanoemulsion (nVCO) for colon cancer by analyzing its free fatty acid composition. Characterization of nVCO was performed using Gas Chromatography–Mass Spectrometry (GC-MS). The findings revealed that the nVCO formulation met the criteria for a nanoemulsion. The free fatty acid content of nVCO was 0.9%, and GC-MS analysis identified lauric acid at 4.29%. Understanding the anticancer mechanism of VCO is crucial, given that colon cancer cells exhibit diverse characteristics. This research offers scientific support for the development of VCO nanoparticles as a safe, natural nutraceutical innovation for colon cancer therapy based on free fatty acid.

Keywords: nanoemulsion, nVCO, colon cancer, free fatty acid

1. INTRODUCTION

Colon cancer ranks as the second leading cause of cancer-related deaths worldwide and remains a significant health concern in Indonesia (Andinata et al., 2023). Lifestyle and dietary habits of the modern millennial generation are among the major factors contributing to its development. These factors can induce inflammation, metabolic disorders, and oxidative stress, which collectively promote cancer formation (Mahmod et al., 2022). Given the growing trend of consuming diverse yet unhealthy foods and beverages, maintaining a balanced intake of nutraceuticals is essential to support colon health (Castro-Espin & Agudo, 2022). Such nutraceuticals are expected to help reduce or prevent the incidence of colon cancer. Virgin Coconut Oil (VCO) has been recognized as a potential nutraceutical for cancer therapy (Wallace, 2019). It is known to counteract oxidative stress through its rich content of bioactive compounds, including tocopherols, tocotrienols, phytosterols, phytostenols, flavonoids, vitamin E, and phenolic substances, which contribute to its antioxidant activity (Rao et al., 2024). The intake of VCO during chemotherapy has been shown to enhance the functional status and overall quality of life of breast cancer patients, as well as alleviate symptoms related to chemotherapy side effects (Law et al., 2014). Moreover, VCO has demonstrated roles in cancer prevention and the induction of apoptosis in cancer cells (Ramya et al., 2022). Therefore, it is hypothesized that VCO may also possess potential as a nutraceutical agent against colon cancer.

Currently, cancer treatment strategies are increasingly focused on the application of nanoparticle technology (Cho et al., 2008; Heydari & Rashidipour, 2015; Paramasivam et al., 2023). Nanoparticles in drug delivery systems enable active compounds to target specific sites of action with greater precision and efficiency (Davis et al., 2008). Previous studies have shown that incorporating methotrexate into a coconut oil nanoemulsion enhances its antiproliferative effects and reduces oxidative stress in lung cancer (A549) cells (Alkhatib et al., 2020). However, research exploring pure VCO nanoparticles as nutraceuticals for colon cancer remains scarce. This study is expected to contribute to advancements in the medical application of VCO nanoparticles, particularly in colon cancer therapy. Since free fatty acids are known to play a role in colon cancer through mechanisms involving inhibition and apoptosis induction, this research focuses on determining their levels (Ramya et al., 2022).

2. MATERIALS AND METHODS

2.1 Equipment and Materials

The materials used in this research were coconut flesh, tween 80 (Sigma-Aldrich), PEG 400 (Sigma-Aldrich), Aquadestilata, NaOH (Merck), methanol pro analysis (Merck). The equipment used in this research were a steering bar, measuring cup (Pyrex®), beaker (Pyrex®), Gas Chromatography–Mass Spectrometry (GC-MS) (Perkin Elmer Clarus 500 (USA), volume pipettes, pH meter, drop pipettes, buret, magnetic stirrer, Hot plate (Heidloph).



2.2 Plant material and nanoemulsion preparation

The sample used was 120 grams of coconut flesh. The sample was extracted using a fat extractor. The solvent used was n-hexane. Determination of the sample was carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, University of Lampung. This research was conducted experimentally by formulating nanoemulsion using Tween 80 and PEG 400 base with a ratio of 2:1 (36 mL and 18 mL). VCO used was 5 mL. Nanoemulsion was made by mixing the oil phase, namely tween 80 and the active ingredient of VCO using a magnetic stirrer for 10 minutes at a speed of 5000 rpm, then PEG 400 was added and homogenized again using a magnetic stirrer for 120 minutes at a speed of 5000 rpm. Distilled water was added little by little until the volume of the nanoemulsion reached 100 mL while stirring. Emulsion making was carried out at a temperature of 40°C (Lasanudin et al., 2024).

Percentage of transmittance was carried out using a UV-Vis spectrophotometer by dissolving 100 µL of nanoemulsion into 5 mL of distilled water which was homogenized with the help of a vortex mixer for 1 minute and the absorbance was measured at a wavelength of 650 nm with a distilled start at 35°C, hold for 2 min, then ramp at 20°C/min to 300°C and hold for 5 min. The helium carrier gas was set to a flow rate of 2 mL/min (constant flow mode). The injection temperature used was 250°C (Olasehinde et al., 2022).

2.4 Determination of free fatty acids (FFA)

Free fatty acids are formed during the decomposition of glycerides in oil. The FFA value is the number of milligrams of NaOH required to neutralize 1 g of oil. A 5 g oil sample was weighed. %FFA = (Titration value x N x 20.03 (lauric acid)) / (Weight of sample) (Okene E.O., 2014).

3. RESULTS

3.1 Formulation of Nanoemulsion

The nanoemulsion formulation (nVCO) made has a bright yellow color and a distinctive odor of VCO oil. The formulation tends to be stable and does not show phase separation at room temperature for 2 months. This indicates that the ratio of VCO, surfactant, and cosurfactant affects the stability of the nanoemulsion. The absorbance value of the VCO nanoemulsion was also obtained at 0,028, resulting in a transmittance value of 86,19%, a pH of 7,5; and a viscosity of 36 cP. The percentage of transmittance of the formula is close to 100%, indicating that the size of the dispersion

water blank. An absorbance value close to 100% indicates that the size of the dispersion droplets produced by the nanoemulsion has reached the nanometer size, which is visually visible from the transparency of the system formed). pH testing is carried out using a digital pH meter test equipment that has been calibrated first and the reading taken 3 times. To test the viscosity and flow type of nanoemulsion preparations, a Brookfield viscometer with a 63 mm Concentric Spindle cylinders were used at a speed of 100 rpm (Lasanudin et al., 2024). Observation of nVCO particles was carried out using a scanning electron microscope (SEM) at the Integrated Laboratory Academic Service Unit, University of Lampung, Indonesia.

2.3 GC-MS Analysis

GC-MS analysis was performed using a Perkin Elmer Clarus 500 gas chromatograph (USA), connected to a mass spectrometer (GC-MS SQ 8S), equipped with a straight, deactivated 2 mm direct injector liner, and a Perkin Elmer Elite-5ms Capillary Column (-30 m x 0.25 mm I.D. x 0.25 µm). Separate injections were used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to droplets produced by the nanoemulsion has reached nanometer size. The pH value of nVCO also meets the pH range set by the Indonesian National Standard (SNI) 1996, namely 4,5 to 8 and nanoemulsions with a pH range of 6,5-9,0 have good dispersibility and transparency without flocculation, coagulation, segregation and also show good stability. In addition, it can be seen that the viscosity value of the formula is in the range of nanoemulsion viscosity, namely 10-2000 cP. It can be concluded that nVCO meets the requirements of nanoemulsion.

3.2 Free Fatty Acid

The free fatty acid content of nVCO was found to be 0,9%. The free fatty acid content of nVCO tends to be low. However, it is thought that free fatty acid content play a role in the biological activity of nVCO.

3.3 GC-MS test

The GC-MS results showed that VCO had predicted compounds of isopropyl myristate; dodecanoic acid; 2,3 dihydroxypropyl ester; octanoic acid; hexadecyl ester; hexanoic acid; and 2 ethyl hexadecyl ester). The free fatty acid content is thought to play a role in influencing the activity of nVCO.

**Table 1.** Free fatty acid content of nVCO formula

IUPAC name	Trivial name	Percentage (%)
isopropyl miristate	asam tetradekanoat	8,29
dodecanoic acid	asam laurat	4,29
2,3 dihydroxypropyl ester	gliserol monostearat	nd
octanoic acid	asam kaprilat	34,2
hexadecyl ester	setil palmitat	nd
hexanoic acid	asam kaproat	19,51
2 ethyl hexadecyl ester	setil 2- etilheksanoat	nd

*IUPAC: International Union of Pure and Applied Chemistry, nd: not detected. Caprylic acid is the highest content of free fatty acids in nVCO.

4. DISCUSSION

Nanoemulsions are a flexible delivery system platform for drug delivery in various therapeutic areas because they can coat both hydrophilic and hydrophobic drugs (Preeti et al., 2023). This study proves that nanoemulsions play a role in coating hydrophobic VCO. Associated with colon cancer, it is estimated that the strength or weakness of the cytotoxic effect is influenced by several factors, namely active compound content and the amount of free fatty acids.

A long-standing problem with chemotherapy is the lack of tumor-specific treatments. In addition to their effects on tumor cells, a major limitation of chemotherapy drugs is their severe side effects on normal cells and tissues. This leads to high systemic toxicity and prevents the use of high drug doses needed to effectively kill cancer cells, thus limiting antitumor efficacy. Therefore, exploring various drug delivery protocols and systems is a promising approach to improving the therapeutic efficacy of chemotherapy. Nutritional and epidemiological studies have shown that cancer development is correlated with fatty acid consumption, but the exact mechanism remains unknown (Jóźwiak et al., 2020). This study found that nVCO contains several fatty acids (isopropyl myristate; dodecanoic acid; 2,3-dihydroxypropyl ester; octanoic acid; hexadecyl ester; hexanoic acid). Dominasi kandungan asam lemak nVCO yaitu asam kaprilat, asam laurat, asam tetradekanoa, dan asam kaproat (Table 1). The

free fatty acid content of nVCO was also found to be 0.9%. This fatty acid content is thought to contribute to nVCO's cytotoxic effects.

VCO itself is reported to contain many fatty acids such as caprylic acid (0.7%), caproic acid (0.3%), lauric acid (38,4%), myristic acid (20,2%), palmitic acid (13,5%), stearic acid, oleic acid (15,5%), and linoleic acid (6.1%). VCO also provides antioxidant activity of 6.17 -10.17 $\mu\text{mol TE/g}$ (de Azevedo et al., 2020). Another study revealed that in the SH-SY5Y cell test (human neuroblastoma cells), there were several morphological changes in cells triggered by VCO such as chromatin condensation, nuclear fragmentation, and disintegration of membrane integrity. The morphological changes observed in target cells indicate that VCO kills cancer cells through the process of apoptosis. The results showed that lauric acid in VCO increases ROS production, induces oxidative stress, decreases mitochondrial membrane potential, and causes cell death in neuroblastoma cells (Ramya et al., 2022). It is suspected that the fatty acid content in nVCO (especially lauric acid) causes cytotoxic effects in colon cancer cells through the same mechanism.

The effect of fatty acids on carcinogenesis may occur through various mechanisms. Several studies have shown that dietary intake of n-3 polyunsaturated fatty acids (PUFAs) may play a role in preventing the development of various types of cancer and have elucidated the mechanisms underlying their action. Various mechanisms related to cell metabolism or chemosensitization have been proposed and recently investigated, including changes in gene regulation or cell signaling, modulation of cell proliferation, differentiation, or induction of apoptosis. PUFAs can also enhance drug transport across cell membranes, altering membrane fluidity by incorporating phospholipids into the cell membrane (Jóźwiak et al., 2020). It is estimated that part of the mechanism of nVCO in inhibiting the growth of colon cancer cells is also through this phospholipid membrane interaction.

5. CONCLUSION

VCO nanoemulsion contains free fatty acids which can play a role in inhibiting colon cancer. Further investigations are required to refine the VCO extraction process in order to yield a higher and more optimal concentration of free fatty acids. This research indicates that nVCO has the



potential to serve as a natural nutraceutical candidate for the prevention and adjunct treatment of colon cancer.

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