

Nanoparticles production of bioactive compounds from cardamom fruit ethanol extract

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ABSTRACT

Research in recent years has focused on developing nanoparticle bioactive compounds extracted from ethanol cardamom due to its potential health benefits. Cardamom (*Elettaria cardamomum*) is a spice plant commonly used in traditional medicine to treat various health problems, such as digestive disorders, headaches, and respiratory issues. This study explored the method of producing nanoparticles from bioactive compounds of cardamom ethanol extract using gas chromatography-mass spectroscopy (GC-MS) and nanoparticle techniques. The steps for producing nanoparticles through this method consist of several stages, starting with identifying bioactive compounds using GC-MS, followed by producing nanoparticles using Nanoparticle techniques. The measurement results of the prepared chromatography concentrates were performed using Shimadzu GC-MS QP2010 Ultra equipment, and the total ion chromatogram (TIC) of the measured compound molecules was obtained. This study found that the size or percentage of each peak area in the concentrate correlates with the high order of the peak, thus indicating the compound content in the concentrate

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INTRODUCTION

Research in recent years has focused on developing nanoparticles of bioactive compounds extracted from ethanol cardamom due to their potential health benefits (Zhang et al., 2016). Cardamom (*Elettaria cardamomum*) is a spice plant often used in traditional medicine to overcome various health problems, such as digestive disorders, headaches, and respiratory problems (Khadem, et al, 2019). Cardamom ethanol extract contains bioactive compounds, such as terpenoids, flavonoids, alkaloids, and other phenolic compounds (Parthasarathy, 2018), which are evidenced to have antioxidant, anti-inflammatory, anticancer, and antimicrobial activities (Kim, et al, 2017). However, its pharmacological effects are limited by solubility, stability, and biodisponibility issues (Moulai-Hacene et al., 2020; Tarfaoui et al., 2022; Yassin et al., 2022).

The use of nanoparticles has been proposed as a promising solution to solve this problem. Nanoparticles can improve the solubility, stability, and bioavailability of bioactive compounds and enhance their pharmacological effects (Chung et al., 2019; Salih et al., 2022). Therefore, the production of nanoparticles, such as bioactive cardamom ethanol extract, is carried out to improve the pharmacological effects of cardamom and increase its potential health properties (Blanco-Llamero et al., 2022; Gaucin-Delgado et al., 2022; Rivero-Montejo et al., 2021).

Cardamom (*Amomum compactum*) is a type of plant belonging to the Zingiberaceae or ginger family (Nurcholis et al., 2021). This plant has various parts of tubers, stems, leaves, and fruits that can be used as spices to flavor food (Al-Zereini et al., 2022; Alam et al., 2021; Yahyazadeh et al., 2021). In traditional medicine, cardamom fruit has long been used as an herbal therapeutic drug to treat several diseases, such as malaria, hepatitis, stomach pain, inflammation, and cancer (Cai, et al., 2021). Therapy using cardamom fruit provides a phenomenological healing effect, where patients who consume cardamom fruit experience a decrease or even recovery from the disease symptoms (Rivero-Montejo et al., 2021; Salih et al., 2022; Shaikh et al., 2022).

In order to optimally utilize the potential health benefits of cardamom fruit, a comprehensive and accurate study is required to understand the bioactive compounds contained. These compounds have an important role in the healing process of diseases (Fouda et al., 2021; Miškovská et al., 2022). Therefore, they need to be extracted, identified and studied their benefits comprehensively. The use and development of cardamom in the future can be carried out more easily and precisely, allowing for optimal utilization of its potential health benefits (da Silva Santos et al., 2019; Viegas et al., 2022).

In this study, the bioactive compounds in cardamom fruit have been extracted, analyzed, and identified. Ethanol solvents and tween 80 are used as additives in the extraction process. Gas chromatography-mass spectroscopy (GCMS) and nanoparticle techniques are employed to analyze and identify these compounds to achieve the previously mentioned research objectives. Through these combined methods, nanoparticles can be used as supports or sorbents to capture compounds from the sample. Then the analysis is performed using GC-MS. Ethanol solution reduces particle size and produces a more homogeneous particle size distribution.

RESEARCH METHOD

This study explored the method of producing nanoparticles from cardamom ethanol extract bioactive compounds using gas chromatography-mass spectroscopy (GC-MS) and nanoparticle techniques. The steps of producing nanoparticles consisted of several stages, which started with identifying bioactive compounds using GC-MS, then producing nanoparticles through nanoparticle techniques. Extraction of Bioactive Compounds from Cardamom Fruit (Arora et al., 2017; Khan & Bhadauria, 2017; Taupik et al., 2020)

The selection and collection of cardamom fruit samples used in this study were carried out by selecting cardamom fruit that has fresh and good quality. However, in the next explanation, it was stated that the fruits used were already mature. Cardamom fruit samples were obtained from plants cultivated by the people in Parongpong District, West Bandung Regency, West Java Province. After harvesting, cardamom fruit skin was removed and cleaned using flowing water to remove any dirt adhering to its surface. After cleaning, cardamom fruits were dried in the sun by exposure to indirect sunlight. Then, the grinding process was carried out until a fine powder was formed, which aimed to facilitate the extraction process. Furthermore, the extraction process was carried out by maceration technique using ethanol solvent.

Furthermore, the extraction process was carried out using 250 mL of ethanol solvent poured into a glass flask. After preparing that, the fine powder of cardamom fruit was poured into a glass flask containing ethanol, as much as 100 g. After mixing the ethanol solvent with cardamom fine powder, continue with the stirring process using a magnetic stirrer set at 150 rpm and stir for 1 hour. This process was repeated 8 times/cycles. Then, let it stand at room temperature for 4 hours.

The resulting solution was then filtered using a Whatman No. 1 filter paper. After filtration, the results of the solution contained solvent (ethanol) and dissolved substances (bioactive compound).

Furthermore, the ethanol solvent in the filtering results was separated from the solute by evaporating them using a rotary evaporator set at 80 rpm, 0.5 atm pressure, and a temperature of 40°C for 3 hours. When the solvent has evaporated, a concentrate was obtained as a bioactive material found in the cardamom fruit. After that, the concentrate was then stored in a closed container and placed at a cold room temperature of -10°C before the identified compounds were carried out.

Identification of Cardamom Fruit Extraction Compounds using Gas Chromatography-Mass Spectroscopy (GC-MS) Method

After the extraction, which resulted in the collection of bioactive compounds from cardamom fruit, the contained compounds were identified. This identification was carried out using gas chromatography-mass spectroscopy (GC-MS) techniques and Shimadzu GC-MS QP2010 Ultra equipment (Japan). This equipment is a combined instrument system integrated between gas chromatography (GC) and mass spectroscopy (MS) when the measurement results from a gas chromatography (GC) system are obtained from the measured parameters in the form of a spectrum ratio of mass (m) to its charge (z). These parameters are used to identify specific bioactive compounds being measured.

The required volume of 1 ml concentrate was dissolved to be injected into the capillary tube of the Shimadzu GC-MS QP2010 Ultra device with a length of 30 m. The helium-carrying gas will flow into the capillary tube when the concentrate is injected. A pressure at the entry point of the capillary tube must be maintained at 42,3 kPa to produce a liquid flow rate of 0.74 mL/min in the tube. The next step is to set the temperature according to the following steps: the initial temperature setting is maintained at 80°C for 1 minute, then it was increased to 250°C at a speed of 280°C/min, then further increased at 300°C at a speed of 270°C/min, and finally increased at 320°C at a speed of 260°C/min until held for 24 minutes. As a result, the molecules of the compounds of a concentrate constituent evaporated and flew in the tube and were carried by the carrier gas. The Shimadzu GC-MS QP2010 Ultra carrying system will produce a measure of retention time (minutes) and spectrum ratio of mass (m) to charge (z) for each molecule flowing in the tube.

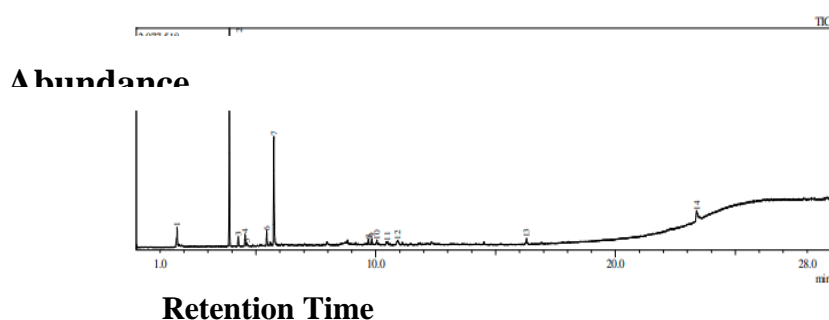


Figure 1. The results of cardamom fruit identification are concentrated bioactive compounds in cardamom fruit

Identification of Cardamom Bioactive Compounds Using Nanoparticle Method

After identifying the bioactive compounds using the gas chromatography-mass spectroscopy (GC-MS) method, the study proceeded to the nanoparticle method. Method of producing cardamom fruit nanoparticles using ethanol involves extracting the active compound from cardamom fruit using ethanol as a solvent. This study uses emulsion techniques to produce nanoparticles from the metabolite compounds extracted from cardamom fruit. Four ethanol extract samples of cardamom fruit ethanol, each with concentrations of 2 ml, 1.5 ml, 1.0 ml, and 0.5 ml,

respectively, are prepared as solutions in a glass beaker as a reaction medium. After being divided into four samples, each nanoparticle sample was produced through the emulsion technique, then given additional treatment by adding tween 80 material as much as 10 ml at each sample concentration. Next, the mixture was stirred until homogeneous, and as much as 30 ml of distilled water was added using a 10 ml syringe. This stage was repeated four times to ensure homogeneity in each sample of nanoparticles produced. After the leaf extraction, the sample was evacuated and placed into a tightly closed glass bottle container, then stored in a room protected from sunlight. Furthermore, 1 ml of each sample was prepared for the size analysis size using the Horiba SZ-100 (USA) PSA (Partic Size Analyzer) equipment.

RESULTS AND DISCUSSIONS

The measurement results of chromatographic for each prepared concentrate were obtained using the Shimadzu GC-MS QP2010 Ultra equipment and a total ion chromatogram (TIC) of the molecules of the measured compound.

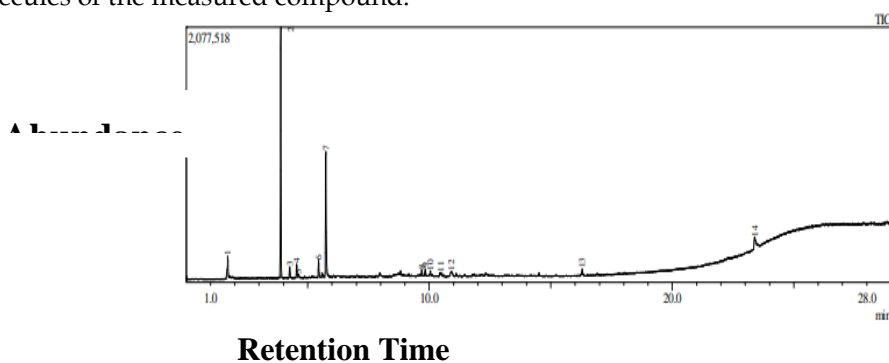
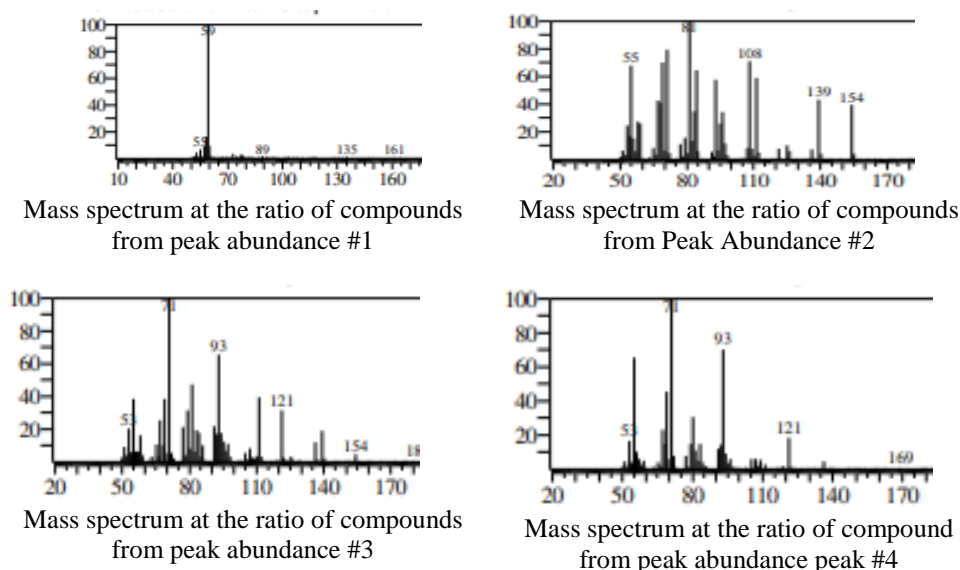
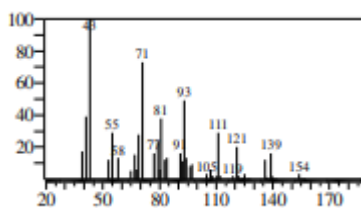


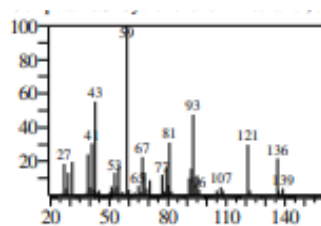
Figure 1. Chromatogram with total ion abundance (total ion chromatogram, TIC) from bioactive compounds contained in the concentrate of cardamom fruit extract

Peaks in total ion abundance occurred at different retention times. The pattern of total ion abundances in all measurable compounds of the three types of concentrates above is shown for each. The area and percentage of each peak abundance recorded in each chromatogram are summarized in the following figure

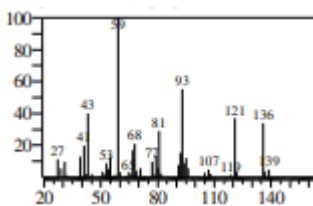




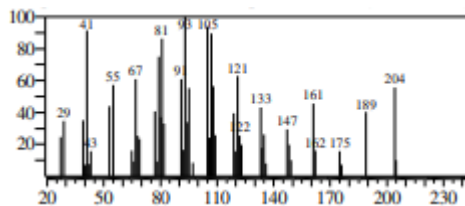
Mass spectrum at the ratio of compounds from peak abundance #3



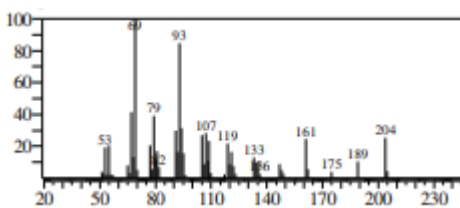
Mass spectrum at the ratio of compounds from peak abundance #4



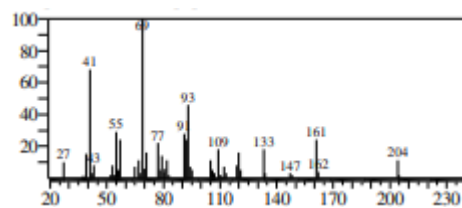
Mass spectrum at compound ratio of peak abundance #7



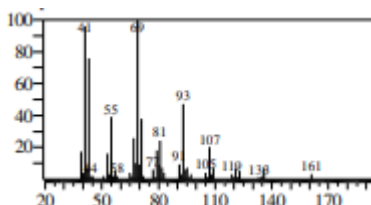
Mass spectrum at the ratio of compounds from peak abundance #8



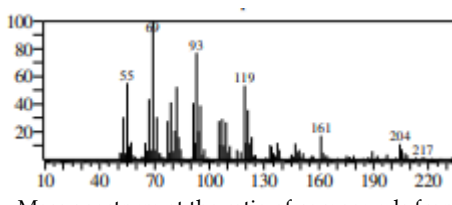
Mass spectrum at the ratio of compounds from peak abundance #9



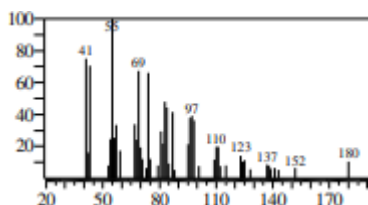
Mass spectrum at the ratio of compounds from peak abundance #10



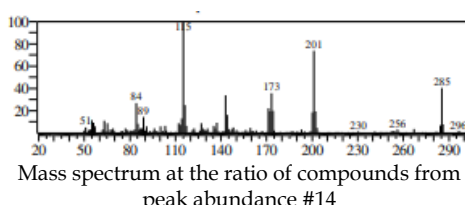
Mass spectrum at the ratio of compounds from peak abundance #11



Mass spectrum at the ratio of compounds from peak abundance #12



Mass spectrum at the ratio of compounds from peak abundance #13



Mass spectrum at the ratio of compounds from peak abundance #14

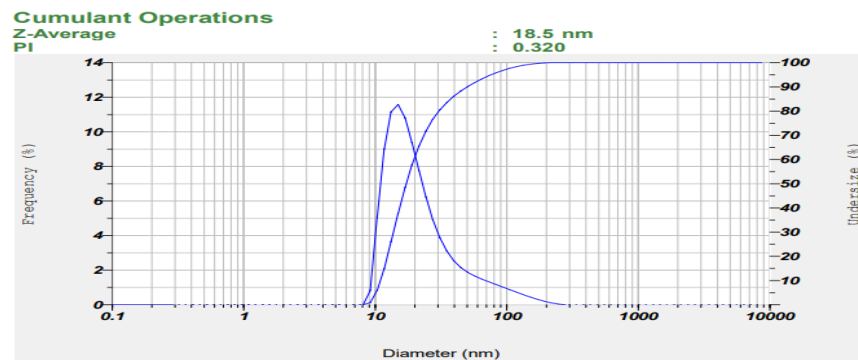


Figure 4. The results of nanoparticles on the bioactive compounds of cardamom fruit ethanol extract by synthesizing in concentrate treatment: ethanol solution of 4 mg: 100 ml. The average Nanoparticle size is 18.5 nm with a polydispersity index of 0.320

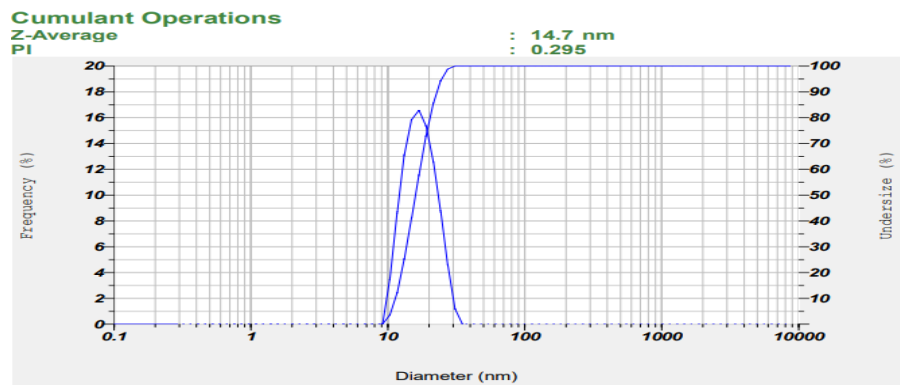


Figure 5. The results of nanoparticles on bioactive compounds of cardamom fruit ethanol extract by synthesizing in concentrate treatment: ethanol solution of 6 mg: 100 ml. The average nanoparticle size is 14.7 nm with a polydispersity index of 0.295

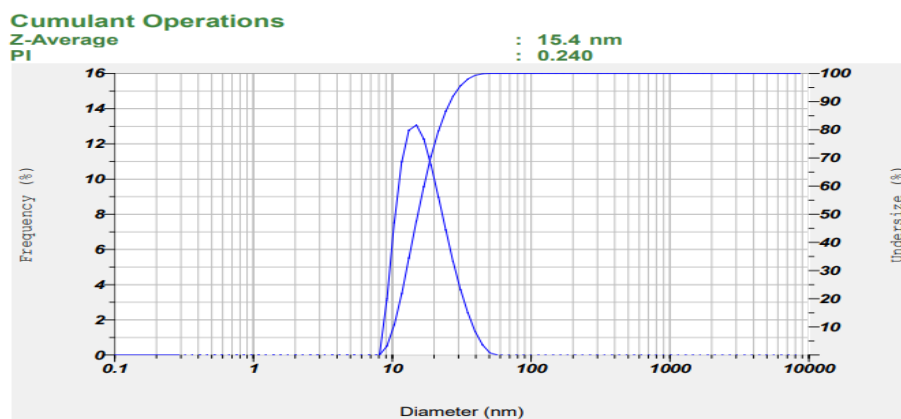


Figure 6. The resultsof raticle nanopa on the bioactive compound of cardamom fruit ethanol extract by synthesizing in concentrate treatment: ethanol solution of 8 mg: 100 ml. The average nanoparticle size is 15.4 nm with a polydispersity index of 0.240

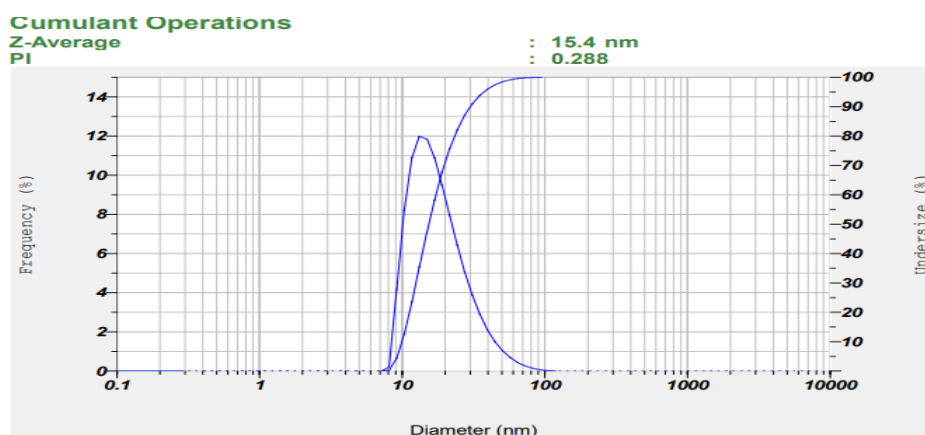


Figure 7. The results of nanoparticles on bioactive compounds of cardamom fruit ethanol extract by synthesizing in concentrate treatment: ethanol solution of 10 mg: 100 ml. The average nanoparticle size is 15.4 nm with a polydispersity index of 0.288

Previous findings have shown that the size of the area (and therefore a large percent area) indirectly indicates the compound content in the concentrate. The total peak abundance of ions in a provided compound is produced at varying retention times. The magnitude of the area value or percentage area of the peak can indirectly indicate the relative percentage of each compound contained in the mixture. The identification results of the extraction concentrate using ethanol solvent showed the presence of 13 types of compounds, including 3-pentanol, 1,8-cineole, cissabinene hydrate, l-linalool, trans sabinene hydrate, alpha-terpineol, beta-selinene, beta bisabolene, beta-sesquiphellandrene, nerolidol B (cis or trans), d-ledol, oleic acid methyl ester, and 1-piperoylpiperidine.

Traditional communities have long used cardamom fruit as an herbal therapy ingredient for the treatment of several types of diseases. Scientists have conducted several studies to study the benefits of compounds in cardamom fruit as herbal therapeutic medicinal ingredients. In the study of Cai et al. (2021), the compound of 1,8-cineole in cardamom fruit is known to have anti-inflammation and antioxidant properties. It was found that this compound can inhibit the production of implanted cytokines and reactive oxygen species (ROS). This indicates the potential of cardamom fruit in treating diseases related to inflammation and oxidative damage.

Based on the research results, 1,8-cineole has been shown to have potential to treat several types of diseases, such as cardiovascular disease, gastrointestinal disease, Alzheimer's disease, and respiratory disease. The alpha-terpineol compound has been found to have the ability to inhibit tumor cell growth. Therefore, this compound can be a therapeutic drug to help overcome cancer. In addition, alpha-terpineol also has the ability to treat bronchitis. This compound can be used as a therapeutic drug to help treat an inflammatory lung disease called COPD. The disease is characterized by shortness of breath and prolonged cough with phlegm. Alpha-terpineol also has antimicrobial effects that can help prevent infection from periodontopathic and carcinogenic bacteria that can cause lung inflammation.

CONCLUSION

This study found that the area or percent area of each peak in the concentrate correlated with the order of the peak, thus indicating the compound content in the concentrate. The identification results in the extraction concentrate using ethanol solvent showed the presence of 13 types of compounds, including 1,8-cineole and alpha-terpineol compounds. The compound of 1,8-cineole in cardamom fruit has anti-inflammation and antioxidant properties, which shows the potential of cardamom fruit in treating diseases related to inflammation and oxidative damage. In addition,

alpha-terpineol has been proven to potentially treat several types of diseases, such as cancer and respiratory diseases, and possess an antimicrobial effect that can prevent infections. Therefore, cardamom fruit and alpha-terpineol compounds can be used as potential herbal therapeutic ingredients. Future research is expected to conduct research with the addition of variables in order to deepen the problem and find new things.

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