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Research Article

The Polarity of Organic Solvents in Coriander Seed Powder (Coriandrum Sativum) Extract is Dependent on their Content of Beta-Carotene, Total Phenolics, Flavonoids, Antioxidants, and Antibacterial Properties

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Abstract: Attempts to develop alternative antibiotics from herbal plants, especially those originating from spice plants that contain active compounds, are crucial to improve livestock health and support the growth of livestock to reach their genetic potential. This study aims to examine the effects of utilizing different solvent polarities on the solubility of coriander seed secondary metabolite chemicals, which are associated with antibacterial and antioxidant properties. Six types of bacteria used were *Escherichia coli, Salmonella SP, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis,* and *Bacillus subtilis*. Using the Step Gradient Polarity (SGP) method, 600 grams of coriander seed powder were macerated gradually using n-hexane, ethyl acetate, and methanol. The result of the maceration process from each fraction was concentrated using a rotary evaporator to obtain the weight of each fraction. A yellowish brown methanol fraction weighed 62.574 grams, a dark yellow-orange ethyl acetate fraction weighed 80.167 grams, and a dark yellow-orange n-hexane fraction weighed 96.623 grams. Oleic acid and 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester were the primary secondary metabolite components in the methanol fraction, with retention times of 43.221 and 53.961 minutes, respectively. Meanwhile, the main secondary metabolite compounds in the ethyl acetate extract were linalool, with a retention time of 13.076 minutes, and oleic acid, with a retention time of 43.221 minutes. Antioxidant activity from ethyl acetate was better than methanol fraction, and tests of its antibacterial activity revealed a comparatively strong inhibition zone for the test bacteria's development. The ability of each fraction, which can be ranked in order of strength in inhibiting bacterial growth using the minimum concentration, was ethyl acetate >methanol >n-hexane. The discovery of active fractions of coriander seeds as antioxidants and antibacterial agents can positively influence human and livestock health.

Keywords: Coriander Seed, Antioxidant, Antibacterial, Secondary Metabolite, Organic Solvent

Introduction

Antibiotic-resistant pathogenic bacteria have become a concern for the health of animals, particularly poultry. Pathogenic microorganisms' resistance to antimicrobial compounds has been widely discovered, yet it has not been confirmed whether the resistance of these pathogenic microorganisms originates from the use of synthetic antibiotics in livestock alone or is thought to be mostly caused by the overuse of antibiotics in either human or animal medicine (Marshall and Levy, 2011).

Synthetic antibiotics, which serve as growth promoters for livestock production, particularly poultry, and can raise the danger of antibiotic resistance if administered for an extended period of time, are strongly corroborated by research findings (Laxminarayan *et al*., 2013;

Thorsteinsdottir *et al*., 2010). The provision of additional feed, such as antibiotics, can cause zoonotic diseases in humans and other livestock (Fratamico *et al*., 2010). Antioxidants are commonly used as additional feed ingredients in livestock rations. Additional feed ingredients such as antibiotics and antioxidants have an imperative position not only to increase livestock production but also to improve their health. Nevertheless, the inclusion of artificial antibiotics to animal feed, especially in poultry, has been prohibited due to concerns about the residues in these livestock products, especially in carcasses. Therefore, attempts to develop alternative antibiotics from herbal plants, especially those originating from spice plants that contain active compounds, are crucial to improving livestock health and producing growth that approaches the genetic potential of livestock. Coriander seeds are one of the spice plants or herbs that can be beneficial as an additional feed in livestock rations.

Coriander seeds are a medicinal plant from the *Umbelliferae* family, widespread in the Mediterranean, Europe, Africa, and Asia (Barros *et al*., 2012). This plant is harvested for its leaves and seeds, which are used extensively in cosmetics, medicine, and cookery. Additionally, coriander seeds have ancient uses in treating rheumatism, joint discomfort, and digestive issues. (Emamghoreishi *et al*., 2005). Moreover, coriander seed extract has proven to be an antioxidant and antibacterial (Kannappan *et al*., 2011). Some researchers have demonstrated that coriander seeds' essential oil contains linalool, geraniol, and camphor (Matasyoh *et al*., 2009; Msaada *et al*., 2007). Coriander seeds are also rich in fatty acids (Sriti *et al*., 2011).

Studies on the extraction of coriander seeds conducted by isolating natural compounds with different polarities of organic solvents that can separate secondary metabolite compounds in coriander seeds (*Coriandrum sativum*) samples have never been reported. The purpose of this study was to ascertain the biological activity and capacity of each coriander seed fraction in various polarities of organic solvents concerning the amount of beta carotene, total phenolics, flavonoids, antioxidant, and antibacterial activity, to determine its suitability for use as a natural feed additive in animal feed.

Materials and Methods

The coriander seeds sample was obtained at a traditional market in Jambi City, Indonesia. Coriander seeds (*Coriandrum sativum*) were dried in an oven at 40 °C for 5x24 hours before being finely ground to obtain 600 grams of coriander seed powder. This study used three different types of organic solvents purified using the distillation method, which were then used as solvents for methanol (MeOH), ethyl acetate (EtOAc), and n-hexane extraction. The source of the solvent was PT. Bratachem in Indonesia. PT. Merck Tbk provided the gallic acid and quercetin standards used to calculate total phenolics and total flavonoids. In the meantime, sodium chloride was acquired from PT. Merck Tbk, the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), and Whatman filter sheets were gathered from PT. Sigma Aldrich.

Preparing Coriander Seeds Powder (Coriandrum Sativum) Sample

This study employed the maceration method with various organic solvents that varied in polarity, aiming at extracting all types of secondary metabolite components contained in coriander seed samples (Haroen *et al*., 2022; Lezoul *et al*., 2020). Starting with the non-polar (n-hexane), semi-polar (ethyl acetate), and polar (methanol) components, the secondary metabolite components are extracted from coriander seed powder in phases. Each maceration process with each solvent takes 3x24 hours.

Maceration Process of Coriander Seeds Powder Sample

The Step Gradient Polarity (SGP) method was used to macerate 600 grams of coriander seed powder using n-hexane, ethyl acetate, and methanol gradually. To determine the weight of each fraction, the maceration process result was concentrated using a rotary evaporator. The methanol fraction obtained was yellowish brown with a weight of 62.574 grams, the ethyl acetate fraction was dark yellow-orange with a weight of 80.167 grams, and the n-hexane fraction was dark yellow-orange with 96.623 grams.

Gas Chromatography-Mass Spectra Analysis

Gas chromatography analysis was carried out on a GC-MS Ultra (*Shimadzu*) connected to a QP2010 mass detector. Separation was completed by using an SH-Rxi-5Sil-MS column (30 mx 0.25 mm D, film thickness 0.25 µm) with a temperature limit of 50-250°C. The inlet temperature was set at 230°C and the detector at 250°C. Gas Chromatography was set in split mode, and the oven temperature was set to start at 50°C for 2 minutes, then increased to 250ºC at a speed of 4ºC per minute and maintained at 250ºC for 6 minutes. Helium carrier gas with a 3.0 mL/minute flow rate with a sample volume of 1 µL, was injected. Positive Electron Ionization (EI) mode with an ionization energy of 70 eV was used to operate the mass spectrophotometer. Compounds in the 50–550 m/z analytical range were examined and identified in complete analysis mode.

Determination of Total Phenolic Content

The total phenolic concentration contained in samples of coriander seeds (*Coriandrum sativum*) was quantitatively determined using a spectrophotometer (Dewanto *et al*., 2002; Widyastuti *et al*., 2021). The Folin–Ciocalteu reagent method, which was modified using the Singleton reagent, was used to test the total phenolic concentration. 0.5 mL of deionized water, 0.125 mL of Folin-Ciocalteu reagent, and 0.125 mL of coriander seed sample extract were combined to create a solution. 12.5 mL of a 7% Na_2CO_3 solution was added after the mixture had been well mixed and allowed to stand for 6 minutes. The volume of the solution was filled with 3 mL of deionized water, then stirred thoroughly and left for 90 minutes at 23°C. The spectrophotometer used to measure absorption had a wavelength of 760 nm. The device read the total phenolic concentration, which was then converted to milligrams of gallic acid equivalent per gram (mg GA/g extract) (Kumar and Goel, 2019).

Determination of Total Flavonoid Concentration

Using a spectrophotometer to measure the absorbance value, the total flavonoid concentration in the coriander seed sample was quantitatively ascertained (Khan *et al*., 2018; Stanković, 2011; Tosun *et al*., 2009). Determining the total concentration of flavonoids was accomplished with a slight modification to the method of Dewanto *et al*. (2002), where 250 µL of coriander seed extract was dissolved in 75 µL of 5% $NaNO_2$ and then left for 6 minutes. 150 µL of 10% $AlCl_3$ solution and 500 µL of 1M NaOH were added to the mixture. After adding deionized water to bring the mixture's volume up to 2.5 mL and giving it a good stir, it was allowed to stand for five minutes before its absorbance was measured. The absorbance value generated on a spectrophotometer with a wavelength of 510 nm was used to calculate the total concentration of flavonoids. A regression equation substituted the concentration read on the tool (i.e., the absorbance value) into the calculation. The total flavonoid concentration value obtained was expressed in mg, which is equivalent to quercetin per gram (mg Qu/g).

Determination of Beta-Carotene Levels

An n-hexane-acetone-calcium salt ($CaCl_2$) mixture was used to macerate a 50-gram sample of powdered coriander seeds in a 1:1:1 ratio. The extraction of beta-carotene contained in samples of coriander seeds was completed using the centrifugation technique of the extract solution, which had been separated through filtration. This centrifugation process was done at a speed of 4000 rpm for 15 minutes, after which the precipitate containing beta-carotene compounds and the filtrate were separated. The contaminant was eliminated by washing the beta-carotene-containing precipitate three times with a saturated $CaCl_2$ solution. The residual precipitate was extracted from the filtrate and dried at 40°C in a rotary evaporator (Haroen *et al*., 2022).

Determination of Antioxidant Activity

Spectrophotometry was used to assess the antioxidant capacity of coriander seed extract in binding free radicals (DPPH). Quantitative investigation of the electron donor's ability to bind free radicals was done by following research procedures according to the method suggested by Hatano *et al*. (1988) where 0.5 mL of a mixture of DPPH solution in methanol with a concentration of 0.2 mmol/L was added after 1 mg of test solution extract with different concentrations (10, 50, 100, and 200 ppm) had been dissolved in methanol. Hereafter, to achieve the ideal reaction, the test solution and DPPH mixture was well mixed and allowed to stand in a dark room for half an hour. A spectrophotometer was used to measure the absorbance value at a wavelength of 517 nm. Quercetin solution was used as a comparison solution or standard solution with varying concentrations of 5 ppm, 15 ppm, 25 ppm, and 50 ppm. The absorbance value from the measurement was entered into Equation 1 below to obtain the percentage resistance value. The result derived from the percentage inhibition graph versus sample concentration utilized for non-linear algorithm computations was used to calculate the inhibitory concentration (IC_{50}) value (Khan *et al*., 2018; Widyastuti *et al*., 2021):

$$(\%)\ \text{Inhibition} = [1-(As/A0)\times 100]$$

Antibacterial Activity Test

Samples of natural compounds can be tested for antibacterial activity using a variety of techniques, including diffusion and dilution. These two methods are widely employed in laboratories to understand the susceptibility of tested microbes, also because these two methods are straightforward, low-cost, fast, accurate, and have an easy data interpretation process (Balouiri *et al*., 2016). In this study, disc diffusion was employed to test the bacterial activity. Using this method, the interaction between the extract solution and the bacteria is more robust, thus significantly inhibiting the test bacteria's growth (Warmasari *et al*., 2020). The dilution method can be applied to a natural compound sample to ascertain its Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Utilizing a graded dilution method for antibiotics dispersed on a microtiter plate, the microdilution method was used to obtain the minimum concentration value. The Minimum Inhibitory Concentration (MIC) of coriander seed extract that has been treated can be determined visually by measuring the level of turbidity that appears on the microtiter plate (Begnami *et al*., 2010; Kowalska-Krochmal and Dudek-Wicher, 2021). The Minimum Bactericidal Concentration (MBC) was obtained by calculating the smallest concentration to kill the test bacteria. Observations were made by looking at the growth of test bacteria on agar media that had been added to a test solution with a predetermined concentration (Sarker *et al*., 2007).

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Antibacterial Activity Test

Antibacterial Activity Test Using the Well Dilution Method

The materials needed to test the inhibitory power of the test compound on the growth of the test bacteria were (1) Paper discs with a diameter of 5 mm, which have been treated by immersion in a test solution of 10 µL of n-hexane fraction extract, (2) Ethyl acetate, (3) Methanol standard 5000 ppm, (4) Positive control tetracycline 200 ppm, and (5) Negative control (Dimethyl Sulfoxide). Additionally, the treated paper disc was positioned on the agar surface, inoculated with the test bacteria, and then incubated for approximately 18 to 24 hours at 37°C. The clear zone was computed to assess antibacterial activity because of the chemicals in the extract's interaction with the test microorganisms surrounding the paper disc (Davis and Stout, 1971; Priyadarsini, 2014).

Test of Minimum Inhibitory Concentration (MIC)

A sterile microplate with 96 wells-eight vertical wells, A–H, and twelve horizontal wells, 1–12 was used to perform the minimal inhibitory concentration test. After adding 100 µL of the test sample to Mueller Hinton Broth (MHB) solution, well A was supplemented with tetracycline positive and negative controls. Then, dilution was carried out on the remaining wells to well H. Moreover, the prepared bacteria were diluted with sterile 0.85% physiological NaCl, and the turbidity was adjusted using standard McFarland solution or the equivalent of ±1x108 colony-forming unit (CFU/mL). 100 µL of test bacteria that had previously been treated was added to each well. Subsequently, the treated microplates were incubated at 37ºC for 18-24 hours. Calculating the minimum inhibitory concentration was done visually by measuring the smallest concentration to form a clear zone (Kowalska-Krochmal and Dudek-Wicher, 2021; Priyadarshi *et al*., 2018).

Test of Minimum Bactericidal Concentration (MBC)

The plating method on Mueller-Hinton Agar (MHA) media can be used to kill bacteria in a test sample, which is necessary to determine the lowest concentration value. After adding 5 µL of the test bacterial solution to a petri dish filled with MHA medium, the dish was incubated for 18 to 24 hours at 37ºC. The lowest concentration value of the test sample solution, which did not reveal information on bacterial growth on previously inoculated agar plates, was then used to determine the minimum bactericidal concentration of test bacteria (Priyadarshi *et al*., 2018).

Results and Discussion

Maceration-Based Extraction Results

In this study, the natural compounds found within the sample of coriander seeds were extracted using the maceration technique, using the principle of increasing solvent polarity, better known as the Step-Gradient Polarity (SGP) technique. This technique offers a better result as it dissolves according to the polarity level of the solvent used. Also, the extraction of secondary metabolites within the coriander seeds sample was maximized, minimizing emulsions' emergence during separation. This SGP technique is simultaneous because, after the maceration process, the extract results for each solvent used can immediately be obtained. The research result shows that after the maceration, the methanol turned into a yellowish brown, with the weight of the extract being 62,574g, the ethyl acetate turned into a dark yellow-orange, weighing 80.167g, and the n-hexane fraction was dark yellow-orange, weighing 96.623g.

Analysis of Gas Chromatography-Mass Spectra (GC-MS)

The primary components of secondary metabolites within the coriander seeds sample were determined using the Gas Chromatography-Mass Spectra (GC-MS) method. The main components of each coriander seed extract can be understood using the GC-MS illustrated in Fig. 1. The extract's primary and secondary metabolites, along with their molecular weights and retention durations, are listed in Table 1. From the three extracts tested using the GC-MS technique, the primary secondary metabolite components found in each macerated extract could be identified. Oleic acid, which has a retention time of 43.221 minutes, and the molecule 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester, which has a retention time of 53.961 minutes, are the primary secondary metabolite components in the methanol fraction. Meanwhile, the main secondary metabolite compounds in the ethyl acetate extract are linalool, with a retention time of 13.076 minutes, and oleic acid, with a retention time of 43.221 minutes. Lastly, the n-hexane fraction consists of linalool with a retention time of 13.079 minutes and the 9,12-Octadecadienoic acid (Z, Z) with a retention time of 43.01 minutes.

GC-MS analysis revealed that n-hexane and ethyl acetate extracts contained linalool, indicating that the distribution of this compound can be dissolved in both polar and non-polar solvents. Meanwhile, oleic acid can also dissolve well in semi-polar to polar solvents. The compounds resulting from separation using the GC-MS technique were identified using the National Research and Innovation Agency (BRIN) literature, and each compound's mass spectrum and retention time. The following is the spectrum of GC-MS (Figures 1, 2, and 3) for each extract and its retention time. Tables 2 and 3 show the main components of coriander seed methanol extract.

Table 1: The main components of methanol extract in coriander seeds (*Coriandrum sativum*)

Peak number	Retention time (min)	Mass M/Z	Constituents
18	43.221	282.47 g/mol	Oleic acid
22	53.961	356.50 g/mol	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester

Table 2: The main components of the ethyl acetate extract of coriander seeds

Peak number	Retention time (min)	Mass M/Z	Constituents
7	13.076	154.25 g/mol	linalool
15	43.221	282.47 g/mol	oleic acid

Table 3: The main components of the n-hexane extract of coriander seeds (*Coriandrum sativum*)

Peak number	Retention time (min)	Mass M/Z	Constituents
15	13.079	154.250 g/mol	linalool
39	43.010	294.472 g/mol	9,12-Octadecadienoic acid (Z, Z)

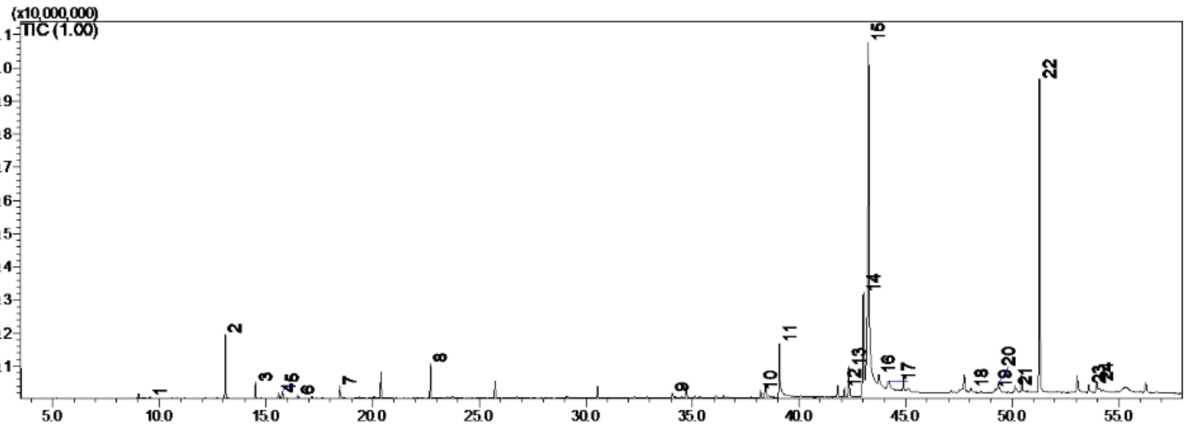


Fig. 1: Typical chromatogram from GC-MS for oleic acid and 9-Octadecenoic acid (Z)-, 2- hydroxy-1- (hydroxymethyl) ethyl ester compounds from methanol extract of coriander seeds (*Coriandrum sativum*)

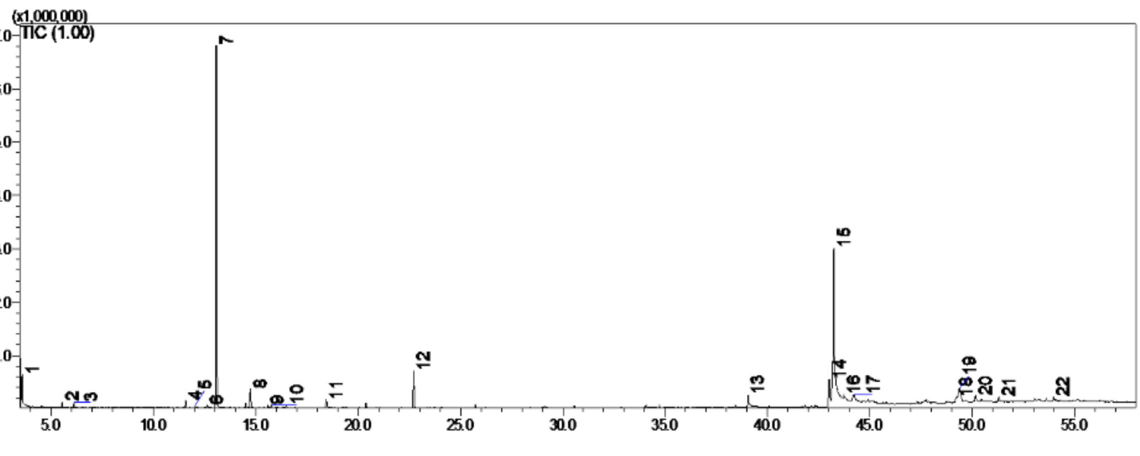


Fig. 2: Typical chromatogram from GC-MS for linalool and oleic acid from ethyl acetate extract of coriander seeds (*Coriandrum sativum*)

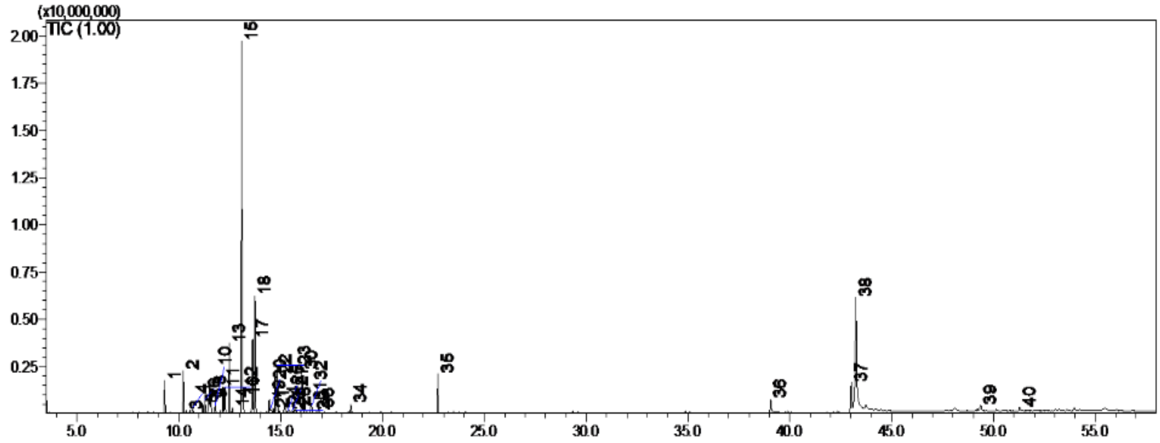


Fig. 3: Typical chromatogram from GC-MS for linalool and 9,12-Octadecadienoic acid (Z, Z) compounds from an n-hexane extract of coriander seeds

Determining Total Phenolic and Total Flavonoid Concentrations

The analysis of total phenolic and flavonoid concentrations from the samples of coriander seed powder focuses on methanol and ethyl acetate fractions. The distribution of phenolics in two fractions—methanol and ethyl acetate—was used to calculate the total phenolic and flavonoid concentrations. The presence of readily oxidized components linked to the antioxidant activity of the two test extracts is shown by the total content of phenolics and flavonoids in the coriander seed methanol and ethyl acetate fractions (Parejo *et al*., 2002). Table 4 shows the total phenolic and flavonoid findings. Total phenolics were determined to be equivalent to gallic acid in milligrams per weight of dry coriander seed sample (mg GA/g extract). In contrast, total flavonoids were determined to be equivalent to quercetin in milligrams per weight of dry coriander seed sample (mg Qu/g extract).

Table 4: Concentration of total phenolics and total flavonoids found in coriander seed samples

Fractions	Total Phenolic (mg GA/g extract)	Total Flavonoid (mg Qu/g extract)
Ethyl acetate	56.37±0.29	56.46±0.62
Methanol	45.45±0.95	48.50±0.01

Based on the results of measuring the quantities of total phenolic and total flavonoid in samples of powdered coriander seed, it was observed that the ethyl acetate fraction contained the majority of the phenolic compounds, or those with OH (hydroxy) groups. The phenolic and flavonoid groups in the fraction were shown to be semi-polar. Additionally, this study shows that the distribution of secondary metabolite chemicals in the natural resource sample is strongly influenced by the extraction method. The absorbance value generated by a spectrophotometer was used to determine the total phenolic concentration in the coriander seed powder sample. This absorbance value was then substituted into the regression equation ($y = 0.0459x-0.1803$, $R^2 = 0.9987$) to obtain a total phenolic concentration of 56.37±0.29 mg GA/g extract for the ethyl acetate and 45.45±0.95 mg GA/g extract for the methanol fraction. The same calculation was also carried out to determine the total concentration of flavonoids from both fractions (ethyl acetate and methanol). With the following regression equation $y = 0.0098x+0.0011$ $R^2 = 0.9995$, the flavonoid concentration can be determined as follows: 56.46±0.62 mg Qu/g extract for the ethyl acetate, and 48.50±0.01 mg Qu/g extract for the methanol. The ethyl acetate fraction has a larger phenolic and flavonoid concentration than the methanol fraction, according to the computation. One explanation for this is that phenolic and flavonoids belong to the group of phenolic compounds and their semi-polar derivatives (Chebil *et al*., 2007; Demir and Korukluoglu, 2020; Khan *et al*., 2018).

Total Antioxidant Activity

Antioxidant activity is valued as IC_{50} (mg/mL), which is the concentration of the test solution consisting of the methanol and ethyl acetate fractions required to obtain 50% of the concentration value of the test solution required for radical scavenging ability. The IC_{50} value for the DPPH test obtained was 95.79 mg/mL for the methanol and 26.08 mg/m for the ethyl acetate. The value of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability of the two test fractions of the coriander seed extract is presented in Table 5. The high levels of phenolic and flavonoid chemicals in the ethyl acetate fraction are closely correlated with the IC_{50} value that the fraction produces (Mandal and Mandal, 2015; Shan *et al*., 2005; Tosun *et al*., 2009).

Table 5: Coriander seed (*Coriandrum sativum*) ethyl acetate and methanol antioxidant activity

Fraction	Concentration	Absorbance	% Inhibition	IC50
Ethyl acetate	200	0.1846	90.7044	26.08
	100	0.4708	76.2928	
	50	0.8088	59.2753	
	10	1.1972	39.7149	
Methanol	200	0.6093	69.3187	95.79
	100	0.8709	56.1483	
	50	1.1322	42.9906	
	10	1.4583	26.5673	

Note**:** In 2023, the BRIN Chemical Research Center's Chemistry Laboratory investigated the antioxidant properties of coriander seed ethyl acetate

When the ethyl acetate and methanol fractions were subjected to an antioxidant activity test, the results indicated that ethyl acetate was more active than methanol. The comparatively high concentration of phenolic and flavonoid chemicals in the ethyl acetate fraction is strongly associated with its capacity to absorb radicals generated by DPPH (Stanković, 2011). The ability of phenolic and flavonoid compounds to stabilize free radicals from DPPH is influenced by free electron pairs in the hydroxyl group (-OH), which function as a stabilizer or binder for free radicals. In other words, during a chemical reaction, this class of phenolic and flavonoid chemicals acts as a shield against free radicals (El Guiche *et al*., 2015; Sepahpour *et al*., 2018). Table 5 shows the antioxidant activity levels of the two fractions (methanol and ethyl acetate). Figures 4 and 5 show the connection between the percentage of inhibition and the ethyl acetate and methanol concentrations.

In Figure 4, the IC_{50} value acquired through computation was 26.08 mg/mL for the ethyl acetate portion by substituting the value of the regression equation derived from the graph showing the correlation between the percentage of inhibition and the concentration of ethyl acetate (Y = 0.257x+43.363, R2 = 0.9195). The ethyl acetate fraction from coriander seeds is highly effective at reducing the concentration of free radicals contributed to this study, as demonstrated by the calculation's comparatively low IC_{50} value (i.e., less than 50 mg/mL). This research result found that the ethyl acetate fraction of coriander seeds is a good source of natural free radicals (Parejo *et al*., 2002).

In Figure 5, by substituting the value of the regression equation obtained from the graph above (Y = 0.2149x+29.413, R^2 = 0.9303), the IC_{50} value of methanol fraction obtained is 95.79 mg/mL. This result denotes that the methanol of coriander seeds has less vigorous antioxidant activity. It takes at least 95.79 mg/mL of methanol fraction extract to inhibit 50% of the free radicals used in this study. Hence, it can be inferred that the methanol fraction of coriander seeds had comparatively less antioxidant activity than the ethyl acetate fraction. This outcome shows a strong correlation with the previously reported levels of total phenolic and flavonoid concentrations, where fractions containing high amounts of phenolics and flavonoids will provide suitable barriers to antioxidant activity.

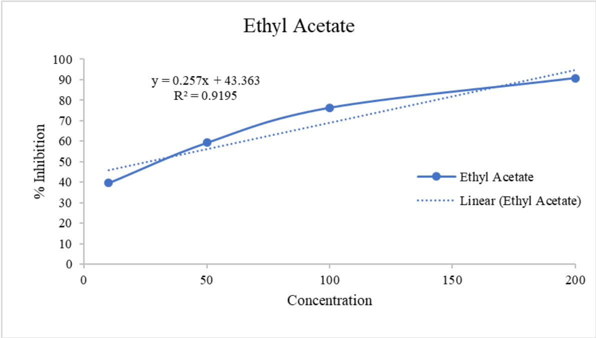


Fig. 4: The correlation between inhibition percentage and ethyl acetate fraction concentration of coriander seeds

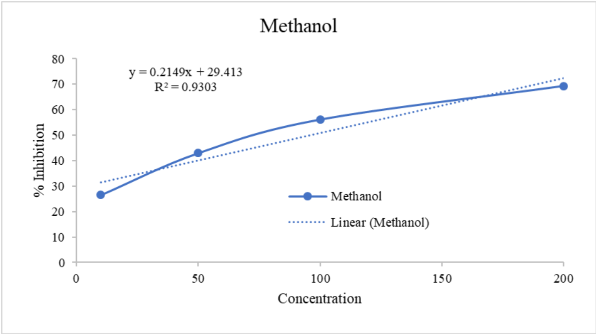


Fig. 5: The correlation between inhibition percentage and methanol fraction concentration of coriander seeds

Determining Beta-Carotene Levels

The beta-carotene concentration found in coriander seeds (*Coriandrum sativum*) has been successfully determined using qualitative methods. The beta-carotene in coriander seeds is required in the livestock sector as it functions as a source of vitamin A to improve the quality of poultry eggs; beta-carotene greatly influences the brightness of egg yolk. When beta-carotene was isolated from coriander seed samples, the result was a vibrant yellow-orange solid with a weight of 78.3 mg. The extraction procedure significantly influenced the isolation process and the determination of beta-carotene levels from coriander seed samples. In other words, the group of beta-carotene compounds in the sample can be perfectly extracted by using the right concentration of the acetone, calcium chloride, and n-hexane mixture, and the results of the isolated compounds are optimized. In other words, the correct concentration of the acetone, calcium chloride, and n-hexane mixture means that the group of beta-carotene compounds contained in the sample can be extracted perfectly. Thus, the results of the isolated compounds are more optimal. This research also determined the speed and time of centrifugation so that it could separate the precipitate (beta-carotene) and supernatant (impurity solution) (Fikselová *et al*., 2008; Haroen *et al*., 2022).

Well Dilution Method for Antibacterial Activity Test

Testing the three fractions produced during the maceration process with three distinct solvent polarity types allowed for the determination of the antibacterial activity of coriander seed extract. Antibacterial activity was tested on the three test fractions using six different kinds of bacteria, consisting of 2 groups of test bacteria, namely, gram-positive and gram-negative. The six different kinds of bacteria used in this study consisted of *Escherichia coli* (ATCC 11725), *Salmonella SP* (ATCC 22504), *Pseudomonas aeruginosa* (ATCC 10574), *Staphylococcus aureus* (ATCC 11526), *Enterococcus faecalis* (ATCC 12458), and *Bacillus subtilis* (ATCC 11626). In this work, a tetracycline antibiotic control solution with a 200 ppm concentration and a negative control solution—100% ethanol—were employed. By serving as comparison solutions, these two control solutions made it simpler to observe and compute the findings of the conducted research. This research aims to observe the inhibition zone generated by the test solution on the growth of test bacteria that have been inoculated on agar media (Balouiri *et al*., 2016; Davis and Stout, 1971). In addition, this research used the well dilution method because, with this method, the ability of antibacterial activity can be determined both qualitatively and quantitatively (Greenwood *et al*., 2003).

Table 6 displays the findings of the coriander seed antibacterial activity test. Ethyl acetate was determined to be the active fraction in the antibacterial activity test based on the calculation of the clear zone or inhibition test generated from the test solution of the three coriander seed fractions against the test bacterium. From strongest to weakest, the three fractions' inhibitory test activity is as follows: Ethyl acetate fraction > methanol fraction > n-hexane fraction. The kind of secondary metabolites present in the ethyl acetate fraction is correlated with its ability. Given the ethyl acetate fraction's higher concentration of phenolic and flavonoids than the other two fractions, it's probable that some derivative molecules from these groups have antibacterial properties (Farhadi *et al*., 2019; Rusli *et al*., 2019).

Table 6: Test of the antibacterial activity of coriander seed fractions

The research results of anti-bacterial tests on coriander seed extract						
Sample	Clear zone diameter (mm)					
	Eschericia coli	*Salmonella SP*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*	*Enterococcus faecallis*
MeOH	11.25	9.25	10.25	10.25	10.50	9.75
	10.50	9.50	10.50	10.50	10.25	9.50
	10.88	9.38	10.38	10.38	10.38	9.63
	0.53	0.18	0.18	0.18	0.18	0.18
Sample	Clear zone diameter (mm)					
	Eschericia coli	*Salmonella SP*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*	*Enterococcus faecallis*
EtOAc	11.75	10.25	12.25	11.50	11.50	10.25
	11.50	10.50	12.00	11.75	11.75	10.50
	11.63	10.38	12.13	11.63	11.63	10.38
	0.18	0.18	0.18	0.18	0.18	0.18
Sample	Clear zone diameter (mm)					
	Eschericia coli	*Salmonella SP*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*	*Enterococcus faecallis*
n-hexane	9.25	9.50	9.25	9.25	9.50	8.75
	9.25	9.75	9.00	9.50	9.75	8.50
	9.25	9.63	9.13	9.38	9.63	8.63
	0.00	0.18	0.18	0.18	0.18	0.18

Note: The Vaccine and drug research center's microbiology laboratory, BRIN, conducted an antibacterial activity test in 2023

The coriander seed extract used as a sample in this study was discovered to contain compounds that are active as antibacterials, and their activity can be seen in Figures 6, 7, and 8.

Activity Test for Minimum Inhibitory Concentration (MIC)

The findings of the study indicate that the ethyl acetate fraction is more effective than the methanol and n-hexane fractions at enhancing the antibacterial impact. The susceptibility of bacteria to each test fraction solution is determined through the Minimum Inhibitory Concentration (MIC) test. Using the minimum concentration of the test extract can inhibit or even kill the test bacteria (Begnami *et al*., 2010; Revathi and Malathy, 2013). Furthermore, the Minimum Inhibitory Concentration (MIC) activity test was carried out to see a straight relationship between the initial test using the well dilution method, where the ethyl acetate fraction from coriander seeds was the active fraction in the antibacterial activity test. It offers a comparatively strong inhibitory zone value against the test bacteria's growth. In the Minimum Inhibitory Concentration (MIC) activity test, which uses the lowest concentration of ethyl acetate to prevent the growth of the test bacteria, the ethyl acetate fraction of coriander seeds also demonstrated a similar outcome, as evidenced by the absence of turbidity on the microtiter plate. Furthermore, the ability of the ethyl acetate fraction to suppress the development of the test bacteria—both gram-positive and gram-negative—was compared to that of the methanol and n-hexane fractions. Observations were completed on all microtiter plates that had been treated by placing a certain concentration of each test extract after 24 hours of experimentation using the turbidity test method, produced by the test extract solution in each microtiter plate.

The ethyl acetate fraction was indicated by the Minimum Inhibitory Concentration (MIC) test to be highly reactive to the development of *Escherichia coli*, *Salmonella SP,* and *Pseudomonas aeruginosa* bacteria. A small concentration of the ethyl acetate fraction was able to inhibit the growth of the three gram-positive bacteria (3.125 mg/mL, 4.687 mg/mL, 6.250 mg/mL). The gram-negative bacteria in the ethyl acetate fraction also required a small amount of concentration to inhibit the growth of the test bacteria of *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis*; that was 6.250 mg/mL, 9.375 mg/mL, and 9.375 mg/mL, respectively. The ethyl acetate fraction was found to be an appropriate fraction for assessing antibacterial activity following a series of tests that measured the inhibition zone and determined the minimal concentration needed to prevent bacterial growth (Priyadarshi *et al*., 2018; Rezaei *et al*., 2015).

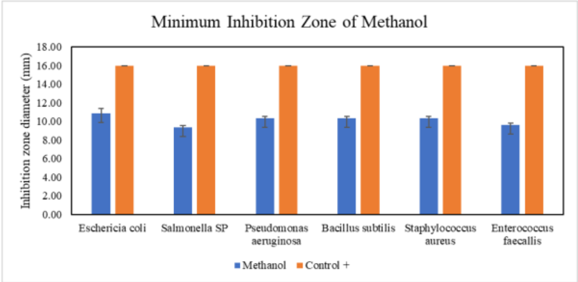


Fig. 6: Results of tests on the methanol fraction of coriander seeds' (Coriandrum sativum) antibacterial activity

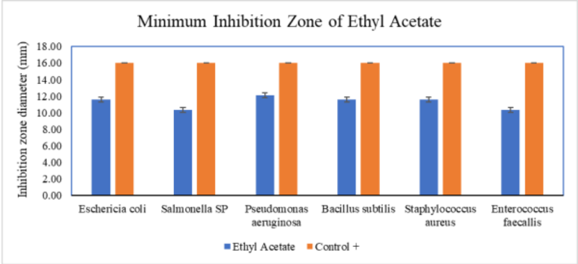


Fig. 7: Results of tests on the ethyl acetate fraction of coriander seeds' (Coriandrum sativum) antibacterial activity

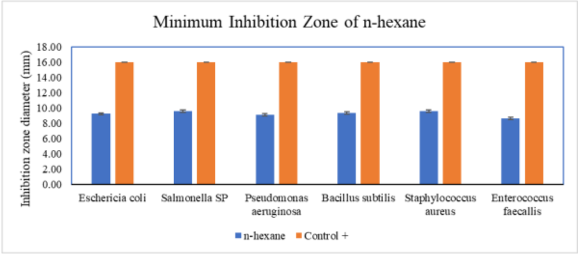


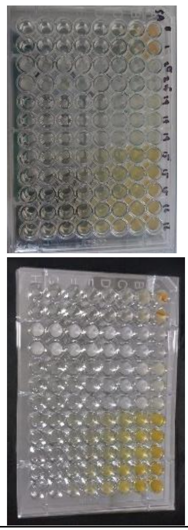
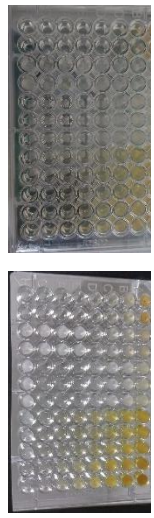
Fig. 8: Results of tests on the hexane fraction of coriander seeds' (Coriandrum sativum) antibacterial activity

The Minimum Inhibitory Concentration (MIC) activity values for each fraction of coriander seeds are summarized in Table 7. Each of the coriander seed fractions used in this study can be graded according to how well it inhibits bacterial growth at the lowest concentration possible: fraction of ethyl acetate > fraction of methanol > fraction of n-hexane.

Activity Test for Minimum Bactericidal Concentration (MBC)

The findings of the activity test for Minimum Bactericidal Concentration (MBC) of the three fractions of coriander seed show that the ethyl acetate fraction is more active than the methanol and n-hexane fractions. The Minimum Bactericidal Concentration (MBC) activity of the ethyl acetate fraction produced was between 3.125 mg/mL to 9.375 mg/mL for the six types of test bacteria used. Meanwhile, for the methanol fraction, the MBC activity was in the range of 6.250 to 12.50 mg/mL for the six types of test bacteria used. However, because the lowest concentration required to kill the test bacteria was rather high, between 78.12 mg/mL and 90.62 mg/mL, the n-hexane fraction did not exhibit much activity in the MBC activity test. The kind of secondary metabolites present in the ethyl acetate fraction is directly linked to its satisfactory capacity or activity to eradicate test microorganisms. Small quantities can affect the development of the test bacteria in this study because the group of phenolic and flavonoid chemicals found in the ethyl acetate and methanol fractions is what increases the antibacterial activity of the MBC (Hamudeng and Serliawati, 2019; Rezaei *et al*., 2015). Table 8 shows the Minimum Bactericidal Concentration (MBC) activity value for each coriander seed test fraction.

Table 7: Assessment of each fraction's MIC activity values in relation to the six test bacteria

Minimum Inhibitory Concentration (mg/mL)						
Bacteria/ Concentration	Escherichia coli	Salmonella, SP	Pseudomonas aeruginosa	Staphylococcus aureus	Bacillus subtilis	Enterococcus faecalis
Fraction of MeOH	6.250	12.50	9.375	6.250	9.375	12.50
Fraction of EtOAc	3.125	4.687	6.250	6.250	9.375	9.375
Fraction of n-hexane	78.12	90.62	87.495	81.245	87.495	84.37
Control +	0.39	0.39	0.39	0.39	0.39	0.39
Control -	100	100	100	100	100	100
Documentation						

Note: MIC activity test was completed in the Microbiology Laboratory, Vaccine and Drug Research Center, *BRIN* 2023

Table 8: The three extracts of coriander seed fractions' Minimum Bactericidal Concentration (MBC) activity values against test bacteria

Minimum Bactericidal Concentration (mg/mL)						
Bacteria/ Concentration	Escherichia coli	Salmonella, SP	Pseudomonas aeruginosa	Staphylococcus aureus	Bacillus subtilis	Enterococcus faecalis
Fraction of MeOH	>6.250	>12.50	>9.375	>6.250	>9.375	>12.500
Fraction of EtOAc	>3.125	>4.687	>6.250	>6.250	>9.375	>9.375
Fraction of n-hexane	>78.120	>90.620	>87.495	>81.245	>87.495	>84.370
Control +	≥0.39	≥0.39	≥0.39	≥0.39	≥0.39	≥0.39
Control -	≥100	≥100	≥100	≥100	≥100	≥100

Note: MBC activity test was completed in the microbiology laboratory, vaccine and drug research center, *BRIN* 2023

The growth of test bacteria in media that had been incubated at 37°C for 18 to 24 hours was then assessed for Minimum Bactericidal Concentration (MBC) activity. By comparing the identical concentration utilized for the Minimum Bactericidal Concentration (MBC) test with the lowest concentration found in the Minimum Inhibitory Concentration (MIC) activity test, the test bacteria were plated once more. The objective of this phase is to compare the test extract's capacity to stop the test bacterium's growth with its strength or killing power against the test bacteria. Analysis was carried out on the media that had been given the above treatment on the two lowest concentrations taken from the MBC results. The results showed growth of the test bacteria in the media, so it was concluded that the MBC>MIC value.

Conclusion

The ethyl acetate fraction was found to include the majority of phenolic compounds or those with OH (hydroxy) groups, according to the results of measuring the amounts of total phenolic and total flavonoid in samples of coriander seed powder. The total phenolic concentration was 56.37±0.29 mg GA/g extract for ethyl acetate and 45.45±0.95 mg GA/g extract for methanol. Meanwhile, the total concentration of flavonoids from both fractions was 56.46±0.62 mg Qu/g extract for ethyl acetate and 48.50±0.01 mg Qu/g extract for methanol. Ethyl acetate had better activity than methanol identified from the antioxidant activity test. The antioxidant activity of coriander seed powder, determined as IC_{50} (mg/mL) by the DPPH test, was 95.79 mg/mL for methanol and 26.08 mg/mL for ethyl acetate. When beta-carotene was extracted from coriander seed samples, the resulting solid was yellow to bright orange in color and weighed 78.3 mg. The ethyl acetate fraction is more effective than the methanol and n-hexane fractions in terms of Minimum Inhibitory Concentration (MIC). The ethyl acetate fraction was very reactive against the growth of *Escherichia coli*, *Salmonella SP*, and *Pseudomonas aeruginosa* bacteria. The three gram-positive bacteria were inhibited from growing at concentrations of 3.125, 4.687, and 6.250 mg/mL of ethyl acetate. In the meantime, 6.250, 9.375, and 9.375 mg/mL were found for the test bacteria *Staphylococcus aureus, Bacillus subtilis,* and *Enterococcus faecalis*. The ethyl acetate fraction was the fraction that could be used in an antibacterial activity test.

The ethyl acetate fraction is also more active than the methanol and n-hexane fractions in terms of Minimum Bactericidal Concentration (MBC). For each of the six test bacterial species, the ethyl acetate fraction's Minimum Bacterial Concentration (MBC) activity ranged from 3.125 mg/mL to 9.375 mg/mL. In the MBC activity test, n-hexane was not really active, with a range of 78.12 to 90.62 mg/mL, but the MBC activity for methanol ranged from 6.250 mg/mL to 12.50 mg/mL for the six types of bacteria examined.

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Authors Contributions

Syafwan: Conceptualization, methodology, writing original draft, writing – review, and editing of the manuscript.

Ucop Haroen: Conceptualization, methodology, project administration, and funding acquisition.

Agus Budiansyah: Conceptualization, methodology, writing original draft, writing – review, and editing of the manuscript.

Kiki Kurniawan: Formal analysis, investigation, data curation, interpretation of the data, and writing original draft of the manuscript.

Ethics

This article is original, has no ethical issues, and was not published elsewhere. Although this study tested antibacterial, it was not applied to animals and humans. So, it does not require an ethical statement from the ethics commission at the testing laboratory institution. The corresponding author confirmed that all authors have read and approved the final manuscript.

Conflict of Interest

The authors declared that they have no conflict of interest during the research and during writing of the manuscript.

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