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Identification of Phenyl Propanoid Compound Isolated from Root Bark Datuan (*Ficus vasculosa* Wall. Ex Miq) and Antibacterial Activity Test on *Escherichia coli*

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Abstract: The purpose of this research was to identify phenyl propanoid compounds found in the root bark of Datuan (*Ficus vasculosa* Wall. Ex Miq) and to test the activity of *Escherichia coli*. Isolation was carried out by maceration method using methanol as a solvent. Then the extract was fractionated by Vacuum Liquid Chromatography and Gravity Column Chromatography methods using n-hexane, ethyl acetate, and methanol eluents. The result was obtained a yellow crystal needle orange weighing 20 mg with a melting point of 137°C - 140°C. Separation by TLC method showed that this compound had an R_f value of 0.56 with n-hexane: acetone eluent (6: 4). UV-Vis spectra showed three peaks on 310 nm, 228 nm and 200 nm. IR spectra showed that the isolated compound has unsaturated ester carbonyl groups conjugated with double bonds, aromatic substituted aromatic rings, and hydroxy groups. GC-MS analysis obtained molecular ions M⁺ = 178. The data of UV-Vis, IR and GC-MS analysis can be concluded that the isolated phenyl propanoid compound is a cinnamic derivative that is methyl p-hydroxycinnamic. The results of bioactivity tests on *E. Coli* showed that the isolated compounds did not have antibacterial activity which was marked by the absence of a clear zone around the paper disk.

1. Introduction

Indonesia is a rich country in natural resources, such as forest products, spices, marine biota, plantations, and agriculture, which are spread in almost all corners of Indonesia, both on land and sea [1]. One of the most widespread plants in Indonesia is Moraceae which is a large family of plants, consisting of 1600 species, found in the tropics and sub-tropics [2]. *Ficus* is an important genus in the family Moraceae, this group of plants usually grows in the Asian region.

Datuan (*Ficus vasculosa* Wall. Ex Miq) is a species of *Ficus*. This plant is known as a banyan plant, and usually lives in rocky areas, steep cliffs, around rivers and springs. Datuan plants are large trees and in almost all parts of the body gummy white like milk or slightly yellowish and sticky when exposed to the skin. The trunk has a smooth outer skin and green [3]. In Indonesia, some of these plant species have long been used as traditional medicine. As the root is used as a remedy for inflammation of the respiratory tract, its leaves are used as a medicine for lumbago and raw materials for herbal medicine for women [4]. Phytochemical studies of the genus *Ficus* have been carried out, but research on the species of *Ficus vasculosa* has never been done before. Reported on the genus *Ficus* the presence of secondary metabolites of flavonoids, phenyl propanoids, and steroids [4,5,6]. Kuo et al. [6] isolate compounds p-methoxycinnic acid, p-hydroxybenzoic acid, p-hydroxycinnamic trans methyl, and p-hydroxycinnamic acid, which are cinnamic-derived compounds from the stem of *Ficus septica*. In another



study, Rajab [4] isolated an isomer derivative of flavan-3-ol from methanolic extract of *Ficus deltoidea* bark ie afzelekin and epiafzelekin whose toxicity was tested against *Artemia salina* shrimp fries. Considering the presence of secondary metabolite compounds, especially phenyl propanoid, and paying attention to the use of certain *Ficus* plants in traditional medicine, it is necessary to have ongoing research on *Ficus* plants. From this research it is hoped that bioactive compounds can be obtained which can be developed into drugs that are safe to use and beneficial to the surrounding community.

In this research, isolation of phenyl propanoid compounds found in the roots of Datuan plant wood and bioactivity test was carried out on *Escherichia coli* bacteria which are pathogenic bacteria in humans because it can cause diarrhea and damage the intestinal mucosa. The method of isolating phenyl propanoid compounds in this study was carried out by maceration using methanol. Purification of compounds was carried out by means of vacuum liquid chromatography and gravity column chromatography using eluents n-hexane, ethyl acetate, and methanol. The purity of the isolated compound was tested using thin layer chromatography and melting points. The isolated compound obtained has a melting point of 133°C-140°C. Identification of molecular structure is done by interpreting the spectrum of absorption of compounds using a UV-Vis, IR, and GC-MS spectrophotometer. From the results of UV-Vis, IR, and GC-MS spectroscopic analysis, it can be concluded that the isolated compound is a cinnamic-derived compound namely

2. Method

Root bark of Datuan was collected at Batunangkop village, North Sungkai, Lampung, Sumatera, Indonesia.

Extraction and Isolation

Root Bark of Datuan was cleaned with water and dried in room temperature condition. Dried root bark 3 kg macerated using methanol as a solvent for 3 x 24 hours. The methanol extract was evaporated using a vacuum rotary evaporator at temperature of 20-40°C with rotation rate of 60-70 rpm. Concentrated methanolic extract fractionated with Vacuum Liquid Chromatography with graded eluents from non-polar to polar. Column was eluted by using n-hexane, ethyl acetate, and methanol. The fractions obtained from liquid vacuum chromatography identified by TLC using a mixture of eluent n-hexane, ethyl acetate, acetone and methanol. Finally, fractions obtained from vacuum liquid chromatography eluted with gravity column chromatography using eluents based on TLC separation.

Fenil Propanoid Test

Crude methanolic extract of root bark diluted with acetone and dropped with some FeCl_3 , positive test showed by brown colour of solution.

Spectroscopy Analysis

Spectroscopy analysis was performed by UV-Vis Spectrophotometry, IR spectrophotometry and Mass spectrometry.

Antibacterial activity test

This test was carried out by using Nutrient Agar as media for bacterial growth, and chloramphenicol as **possitive** control.

3. Results and Discussion

Root bark of Datuan as much as 3 kg clean up from soil, chopped then dried room temperature and not exposed sun directly to keep the compounds from being damaged. Root bark was dried then mashed to expand the surface of sample which interacts with the solvent so that extracted compound into the solvent is maximized. The extracted method used on this study is the maceration method by immersing the sample in the appropriate Solvent. The solvent user is methanol, this Solvent was chosen because methanol has a wide range of polarity and can dissolve natural compounds which have polar to semipolar properties. Maceration used with technical methanol as much as 15 L for 3×24 hours, Cartier out 3 Times repetition. This is intended to maximize the extracted of compounds in methanol solvent.

The filtrate concentrated using a vacuum rotary evaporator at 45°C with 60 rpm, for the compounds is not damaged. Chromatogram of crude is obtained as shown on Figure 1.

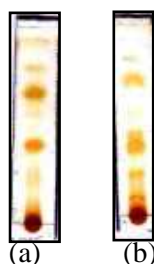


Figure 1. Chromatogram of crude sample (a) eluen n-hex ane : ethyl acetate 8 : 2
(b) eluen n-hexane : acetone 8:2

The methanol extracted separated by vacuum liquid chromatography (vlc). VLC is divided into 2 stages, the first stage 12,5 grams and the second stage 10 grams. In the first stage VLC was eluted in succession using 100% n-hexane eluent, n-hexane: ethyl acetate (10-80%), ethyl acetate 100%, ethyl acetate: acetone (20-70%), acetone (100%), acetone: methanol (20-50% and 70%), and methanol (100%) produce 20 fractions. From those 20 fractions, fractions 3,4 and 5 were taken because additional of FeCl₃, a blackish brown color was formed, which indicated phenol compounds. Then 3,4, and 5 fractions are combined and evaporator to produce one fraction named F fractions 3 grams, then separated by TLC using n-hexane eluent: ethyl acetate (8:2) and the chromatogram can be seen ini Figure 2.



Figure 2. TLC chromatogram of F fraction using n-hexane : ethyl acetate (8 : 2) eluent

The F fraction is then separated by KCV using eluents n-hexane: polarity of ethyl acetate which is increased its yielding 28 fractions. Based on TLC analysis, the main fraction was obtained, namely the Fa fraction (fractions 8-13), the Fb fraction (fractions 14-21). The Fa and Fb fractions were then in a gravitational column, but the results of the gravity columns of the two fractions were not carried out further because the stains formed showed a large mixture of compounds while the sample supply was very small.

In the next analysis, the second stage of VLC. In the second stage KCV weighing 10 grams of sample were eluted in succession using n-hexane eluent: ethyl acetate, ethyl acetate, ethyl acetate: acetone, and methanol produced 14 fractions. From the 14 factions, 3,4 and 5 fractions were taken, because these three fractions indicate the presence of phenol (flavonoid) compounds. This is evidenced by the addition of FeCl₃, forming a blackish brown color. Then the three fractions were merged and evaporated to produce one fraction named the fraction H weighing 1 gram. The extract is then separated by TLC using an eluent n-hexane: ethyl acetate (8: 2) whose chromatogram can be seen in Figure 3.



Figure 3. TLC chromatogram of H fraction using n-hexane eluent: ethyl acetate (8: 2)

The H fraction is then separated by KCV using eluents n-hexane: ethyl acetate enhanced polarity produces 43 fractions, then each fraction in TLC uses the n-hexane: ethyl acetate (7: 3) eluent to see the pattern of separation, and the chromatogram can be seen in Figure 4.

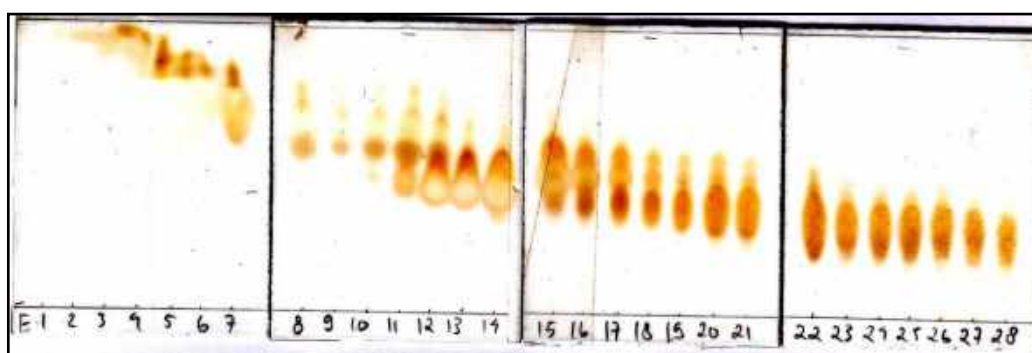


Figure 4. TLC chromatogram results from VLC of H fraction using eluents n-hexane : ethyl acetate (7: 3)

Then the fractions that have the same separation pattern are recombined and obtained 6 main fractions c3 (fractions 12-14), d3 (fractions 15), e3 (fractions 16 and 17), f3 (fractions 18-21), g3 (fractions 22), and h3 (fractions 23-28). The six combined fractions are then in TLC again using the eluent n-hexane: ethyl acetate (7: 3) and the chromatogram can be seen in Figure 5.

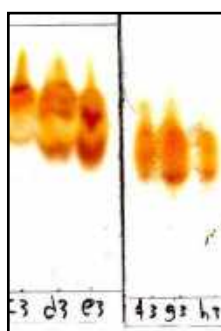


Figure 5. TLC chromatogram of combined H fractions using eluents n-hexane: ethyl acetate (7: 3)

At fraction c3 there are 20 mg yellow needle-shaped crystals, then the crystals are separated from the filtrate and then washed using n-hexane. In the process of washing crystals, n-hexane is used because the crystals do not dissolve in n-hexane. The melting point test shows that the crystal has a melting point of 137°C - 140°C. From the range of melting points, it can be ascertained that the isolated compound is not pure.

In the phytochemical test by adding a few drops of FeCl_3 , green causes the solution. According to [7] the reaction with FeCl_3 has been used extensively to identify phenol compounds. To reinforce these allegations, followed by analysis using a spectrophotometer UV-Vis, GC-MS, and IR.

Crystal purity was carried out by TLC analysis using 5 eluent variations and different concentrations produced a single stain with R_f 0.5 (n-hexane: ethyl acetate (8: 2)); R_f 0.22 (n-hexane: acetone (8: 2)); R_f 0.56 (n-hexane: acetone (6: 4)); R_f 0.7 (chloroform: acetone (8: 2)); R_f 0.79 (ethyl acetate: chloroform (7: 3)), and the chromatogram can be seen in Figure 6.

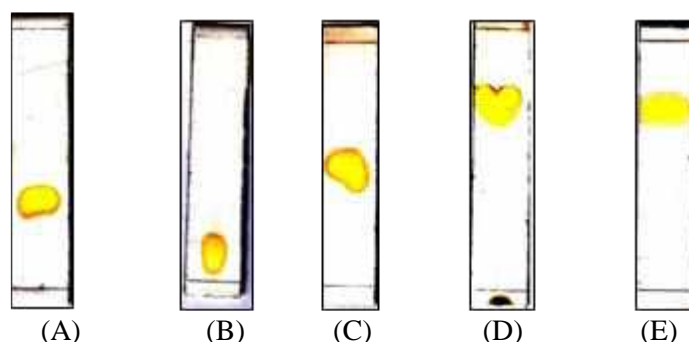


Figure 6. Chromatogram of isolated compounds using 5 eluent systems.

(A) n-hexane: ethyl acetate (8: 2), (B) n-hexane: acetone (8: 2), (C) n-hexane: acetone (6: 4), (D) chloroform: acetone (8: 2), (E) ethyl acetate: chloroform (7: 3)

The fractions c3, d3 and e3 are then combined to produce the main fraction I weighing 35 mg. This fraction I was then fractionated again by means of gravity column chromatography using eluent n-hexane: ethyl acetate which was enhanced in polarity and produced 30 fractions. This fraction was then on KLT to see the pattern of its separation. Based on TLC analysis, the fractions were not carried out further because the stains formed showed that there was still a large mixture of compounds while the sample supply was very small.

Spectrophotometric Analysis

Analysis with UV-Vis spectrophotometer

All phenol compounds are aromatic compounds so all of them show strong absorption in the UV spectrum region. In addition, phenol compounds typically exhibit a bathochromic shift in their spectrum when bases are added [7]. The UV spectrum of compounds isolated from the roots of datuans (*Ficus vasculosa* wall. ex Miq) provide maximum absorption information at λ_{maks}^{MeOH} 310.5 nm ; 228.5 nm ; dan 200 nm. The resulting spectrum provides information that the isolated compound contains conjugated and / or aromatic double bonds, the presence of this conjugated double bond is also reinforced by the existence of a bathochromic shift when adding NaOH shear reagents.

The UV spectrum of the addition of NaOH shear reagents provides maximum absorption information at $\lambda_{maks}^{MeOH+NaOH}$ 357.5 nm ; 235.5 nm ; 199.5 nm, s shown in Figure 7. The maximum absorption at wavelength 310.5 nm undergoes a bathochromic shift to a wavelength of 357.5 nm with the addition of NaOH in the UV spectrum of 47 nm. This indicates that the isolate has a conjugated phenolic chromophore.

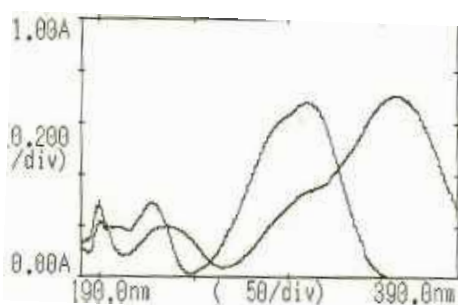


Figure 7. UV spectra of isolated compounds. (a) in MeOH, (b) in MeOH + NaOH

Indharto [8] reported that ethyl p-methoxycinnamic in ethanol solvents showed maximum absorption at 310 nm, 228 nm, and 212 nm. The maximum absorption value is almost similar to the maximum absorption value in the compound which is isolated. From this data it can be estimated that the isolated compound is a cinnamic derivative compound which includes phenyl propanoid compound, to ensure this data then an analysis using an infrared spectrophotometer and a mass spectrophotometer is performed.

Structure analysis with infrared spectrophotometer

Analysis using IR spectrophotometer provides more complete information about the functional groups contained in the structure of the isolated compound. Based on the IR spectrum of the isolated compound (Figure 8) shows that the absorption band widens with the absorption peak at 3379.1 cm⁻¹ is a stretching vibration for the hydroxyl group (-OH). The aliphatic C-H uptake contained in this compound is shown by the absorption peaks of 3047.3 cm⁻¹ and 2947.0 cm⁻¹. The uptake at 1689.5 cm⁻¹ shows the existence of stretching vibrations for the carbonyl group (C = O). Absorption at 1635.5 cm⁻¹; 1596.9 cm⁻¹; 1512.1 cm⁻¹; and 1434.9 cm⁻¹ shows the absorption of C = C aromatic bonds. Uptake in the fingerprint region with peaks of 1326.9 cm⁻¹ and 1280.6 cm⁻¹ and 1195.8 cm⁻¹ is estimated to have vibration stretching C-O-C groups. At 987.5 cm⁻¹ and 947.2 cm⁻¹ shows flexural vibrations outside the O-H area, while the absorption band at 833.2 cm⁻¹ indicates flexural vibrations outside the aromatic ring C-H area.

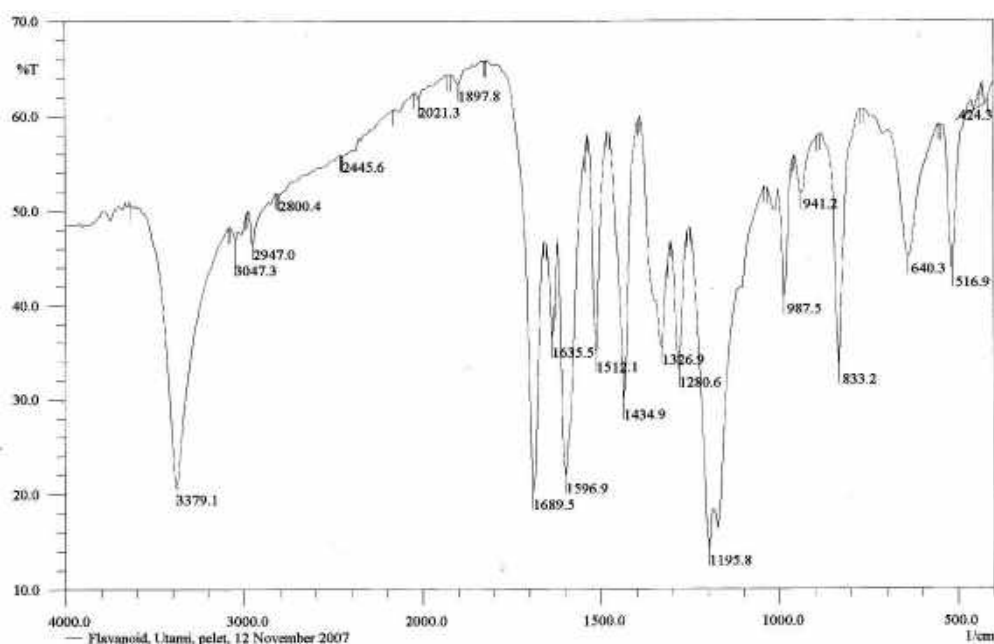


Figure 8. IR spectra of methyl p-hydroxycinnamic.

Structure analysis with a mass spectrophotometer

Analysis with a mass spectrophotometer of the isolated compound which is suspected to be a cinnamic derivative gives a mass spectrum (Figure 9) with a molecular ion peak $M^+ = 178$ and as a base peak is $m/e = 147$. The other ion fragment peaks appear at $m/e = 160$, $m/e = 133$, $m/e = 119$, $m/e = 107$, $m/e = 91$, $m/e = 77$, $m/e = 65$, $m/e = 51$ and $m/e = 39$.

From the infrared spectrum data it is known that the isolated compound has an unsaturated ester carbonyl group conjugated with a double bond, a para-substituted aromatic ring, and a hydroxy group. From this data, it can be estimated that the isolated compound is methyl p-hydroxycinnamic.

R.Time:11.142

Base Peak: 147.00

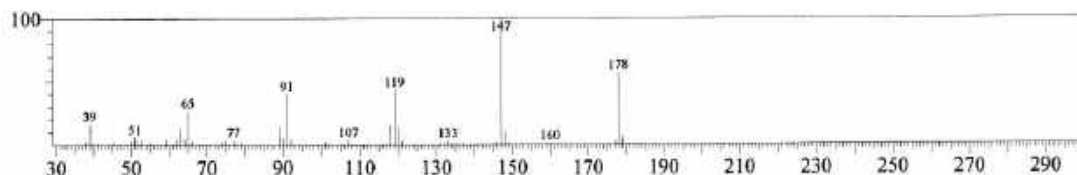


Figure 9. Mass spectra of isolated compounds

Based on this spectrum, the details of the fragmentation patterns of isolated compounds are as follows. The molecular ion peak at $m/e = 178$ is the molecular weight of methyl p-hydroxycinnamic. The ion at $m/e = 147$ is the p-hydroxycinnamoylium ion, which is produced by the release of methoxy radicals (OCH_3) from the molecular ion M^+ ($m/e = 178$). Ions with $m/e = 119$ are the result of fragmentation of ions p-hydroxycinnamoylium ($m/e = 147$) through the release of CO molecules. Peak $m/e = 77$ and 91 indicate that there are benzene aromatic groups in the structure, this reinforces the interpretation of aromatic uptake in the IR spectrum.

Thus it is clearly seen that the resulting fragmentation pattern corresponds to the structure of methyl p-hydroxycinnamat ($\text{C}_{10}\text{H}_{10}\text{O}_3$). To show the double bond and circumference structure of the compound resulting from isolation, a DBE (Double Bond Equivalent) calculation is performed as follows:

$$\begin{aligned} \text{DBE} &= \text{Number of atoms C} - (\text{Number of atoms H} : 2) - 1 \\ &= 10 - (10 : 2) - 1 \\ &= 6 \end{aligned}$$

The DBE calculation results are in accordance with the methyl p-hydroxycinnamic compound, which are three for the structure of benzene, one for double bonds, one circumference of benzene, and one for carbon carbonyl. Approximate of this methyl p-hydroxycinnamic fragmentation pattern are shown in Figure 10.

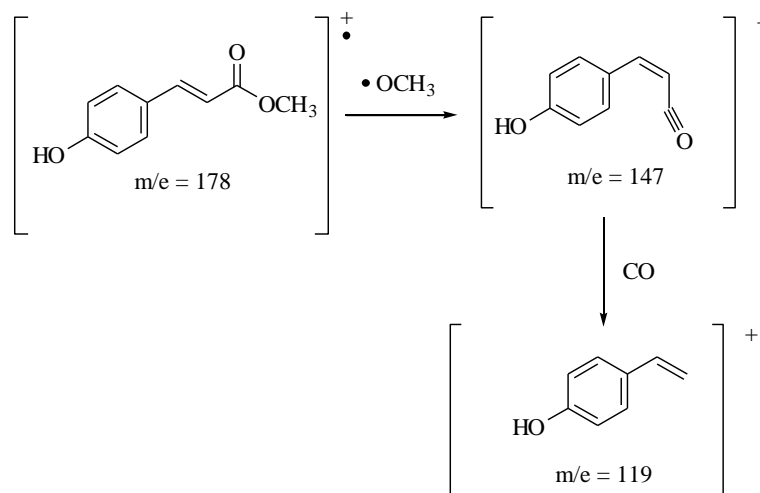


Figure 10. Approximate pattern of methyl p-hydroxycinnamic fragmentation

This methyl p-hydroxycinnamic compound is also found in *Ficus septica* species, with the structure as shown in Figure 11.

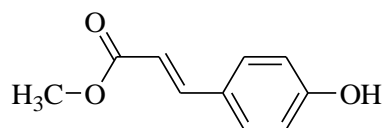


Figure 11. Structure of methyl p-hydroxycinnamic

Antibacterial Bioactivity Test

In the antibacterial bioactivity test, *Escherichia coli* is used because its growth is very fast and easy to handle. In this antibacterial bioactivity test 5 paper disks were used, namely positive control (chloramphenicol), negative control (methanol), methanol extract, fraction of the first KCV results, and compounds resulting from isolation. In the chloramphenicol there is a clear zone but in the other four paper disks there is no clear zone after 3 repetitions, as shown in Figure 12.

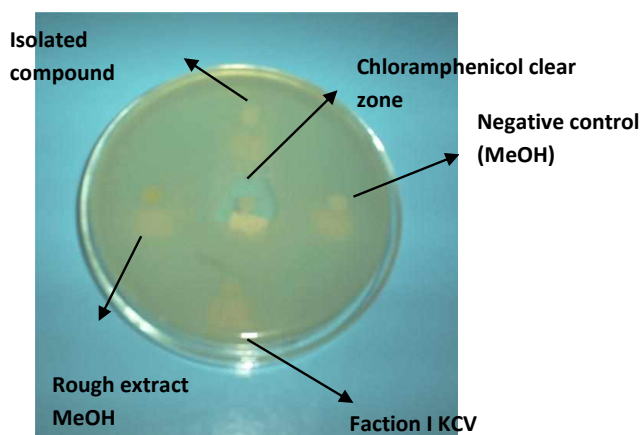


Figure 12. Results of antibacterial bioactivity tests

Clear zone expresses sample activity against test bacteria. This reaction is an inhibition by antimicrobial chemical compounds which can be in the form of inhibition of enzyme function, interference with the composition of the constituents of cell walls, inhibition of protein synthesis, or changes in membrane function [9]. Classification of responses to bacterial growth inhibition can be seen in Table 1.

Table 1. Classification of responses to bacterial growth inhibition

inhibition zone diameter	response to growth inhibition
> 20 mm	Strong
16 - 20 mm	Medium
10 – 15 mm	Weak
<10 mm	No response

Sources: Volk and Wheeler, 1990

Based on the information in Table 1, the antibacterial activity test on the isolated compound can be said to have no growth inhibition response, because the clear zone diameter is not formed.

4. Conclusions

The isolated compound was obtained in the form of a 20 mg yellow orange crystal needle with a melting point of 137^oC - 140^oC. Spectroscopic analysis of UV-Vis, IR, GC-MS shows that the isolated compound is a methyl p-hydroxycinamic compound with M⁺ = 178 and in its structure there is an unsaturated ester carbonyl group conjugated with a double bond, a para-substituted aromatic ring, and a group of aromatic rings and hydroxy. The results of the bioactivity test of samples against *Escherichia coli* bacteria showed no activity, which was marked by the absence of a clear zone around the paper disk.

Conflicts of interest

“There are no conflicts to declare.”

Acknowledgments

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