JCNaR
Journal of Chemical Natural Resources
Journal homepage: https://jcnar.usu.ac.id

Isolation and Identification of Flavonoid Compounds and Antibacterial Activity Test of the Canyere Badak Plant (*Bridelia glauca Blume*) (*Phyllanthaceae*)

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ARTICLE INFO

Article history:
Received 1 October 2023
Revised 31 October 2023
Accepted 31 October 2023
Available online 31 October 2023

E-ISSN: 2656-1492

How to cite:
Lamek Marpaung, Ade Theresia Ulina Lumban Toruan. Isolation and Identification of Flavonoid Compounds and Antibacterial Activity Test of the Canyere Badak Plant (*Bridelia glauca Blume*) (*Phyllanthaceae*). Journal of Chemical Natural Resources. 2023. 5(2):123-133.

https://doi.org/10.32734/jcnar.v5i1.13816

ABSTRACT

Flavonoid compounds from the stem bark of the Canyere Badak plant (*Bridelia glauca Blume*) have been isolated. 2500 g of Canyere Badak plant stem bark was macerated by methanol, and the methanol extract dissolved in distilled water. The distilled water solution was extracted by partitioning with ethyl acetate repeatedly until negative to 5% $FeCl_3$. The ethyl acetate extract was dissolved in methanol and extracted by partitioning with n-hexane until the n-hexane layer was transparent. The methanol extract was analyzed by thin layer chromatography and separated by column chromatography with a stationary phase of silica gel and a mobile phase of chloroform: methanol (90:10; 80:20; 70:30; 60:40) v/v. Fractions 148-174 were purified by preparative thin layer chromatography using benzene: acetone (80:20) v/v eluent to produce a brownish yellow paste of 10 mg at Rf value of 0,66 using chloroform: methanol (80:20) v/v as eluent. Based on the analysis of the UV-visible spectrophotometer, it had a wavelength (λ max) of 275 nm. The FT-IR spectrum shows the presence of OH, C=C aromatic, C=O ketones, C-H, C-O-C and C-O groups. The proton Nucleus Magnetic Resonance Spectrum (^{1}H-NMR) indicates the presence of H-2' & H-6' protons, H-3' & H-5' protons and methoxy protons. Based on the data analysis and interpretation, the isolated compound was a flavonoid of the flavanones group. The antibacterial activity of total flavonoids was determined by agar disk diffusion against *Staphylococcus aureus* and *Escherichia coli* bacteria. The results showed that total flavonoids strongly inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria with MIC (Minimum Inhibitory Concentration) value at a concentration of 100 mg/mL obtained a clear zone of 9.5 mm against *Staphylococcus aureus* bacteria and at a concentration of 100 mg/mL obtained a clear zone of 12.15 mm against *Escherichia coli* bacteria.

Keywords: Antibacterial Activity, Canyere Badak Plant, Flavanoid, Isolation, , Spectroscopy

ABSTRAK

Golongan senyawa flavonoida telah diisolasi dari kulit batang tumbuhan Kanyere badak (*Bridelia glauca Blume*). Sebanyak 2500 g sampel telah diekstraksi maserasi menggunakan pelarut metanol, kemudian ekstrak pekat metanol ditambahkan dengan akuades dan dipartisi dengan etil asetat berulang-ulang sampai negatif terhadap $FeCl_3$ 5%. Ekstrak pekat etil asetat dilarutkan dengan metanol dan dipartisi dengan n-heksan secara berulang-ulang sampai lapisan n-heksan berwarna bening. Ekstrak pekat metanol dianalisis menggunakan kromatografi lapis tipis dan dipisahkan dengan kromatografi kolom dengan fasa diam silika gel dan fasa gerak kloroform : metanol (90:10; 80:20; 70:30; 60:40) (v/v). Fraksi 148-174 dimurnikan dengan kromatografi lapis tipis preparatif dengan eluen benzene: aseton (80:20) v/v menghasilkan pasta berwarna kuning kecoklatan sebanyak 10 mg dengan nilai Rf 0,66 menggunakan eluen kloroform : metanol (80:20) v/v. Berdasarkan analisis Spektrofotometer UV-Visible menunjukkan panjang gelombang (λ max) 275 nm, Spektrum FT-IR menunjukkan adanya gugus OH, C=C aromatis, C=O keton, C-H, C-O-C dan C-O. Spektrum Resonansi Magnetik Inti Proton (1 H-NMR) menunjukkan adanya proton H-2' & H-6', H-3' & H-5' dan proton metoksi. Berdasarkan data analisis dan interpretasi data, maka senyawa hasil isolasi adalah

senyawa flavonoida golongan flavanon. Pada flavonoida total diuji aktivitas antibakteri terhadap bakteri *Stapylococcus aureus* dan *Escherichia coli* dengan metode difusi cakram kemudian diperoleh hasil bahwa flavonoida total kuat menghambat pertumbuhan bakteri *Stapylococcus aureus* dan *Escherichia coli* dengan nilai MIC (Minimum Inhibitory Concentration) pada konsentrasi 100 mg/mL diperoleh zona bening sebesar 9,5 mm mm terhadap bakteri *Staphylococcus aureus* dan pada konsentrasi 100 mg/mL diperoleh zona bening sebesar 12,15 mm terhadap bakteri *Escherichia coli*.

Keyword: Aktivitas Antibakteri, Flavanoid, Isolasi, Kulit Batang Tumbuhan Kanyere Badak, Spektroskopi

1. Introduction

Plant secondary metabolites are a diverse group of molecules involved in plant adaptation to its environment but are not part of the main biochemical pathway of cell growth and reproduction. Some primary or secondary plant metabolites in plants include protease inhibitors, lectins, alkaloids, nonprotein amino acids, cyanogenic glycosides, saponins, and tannins. These compounds are involved in defence against animals and pathogens, regulation of symbiosis, control of seed proliferation, and chemical inhibition of competing plant species (allelopathy), and therefore are an integral part of species interaction in plant-animal communities and plant adaptation to its environment [1].

Phenolic compounds are widely distributed secondary metabolites in high-level plants with aromatic rings with one or more hydroxyl substituents. These metabolites include flavonoids, tannins, stilbenes, kumarins, and phenolic acids. The health effects of natural phenolics have received the greatest attention among researchers in recent decades. Phenolic compounds are the largest phytochemical group showing disease prevention and health improvement effects [2].

Flavonoids are natural polyphenols that are widespread and are found in all parts of plants: roots, skins, wood, nectar, flour, flowers, fruits, and seeds [3]. The flavonoid compound is supposed to be very useful in food because it is a potential antioxidant phenolic. Therefore, foods rich in flavonoids are essential for treating cancer and heart disease [4].

Canyere (*Bridelia glauca Blume*) is a species of the family Phyllantaceae traditionally used throughout Africa and Asia to relieve pain. The plant B.glauca Blume in the world of medicine is known to have antiemetic activity, antianemia, antibacterial, antidiabetic, anti-diarrhea, anti-inflammatory, antimalarial, antiviral, hypoglycemia, treating abdominal pain, and genital diseases. Secondary metabolite compounds in leaves contain bridelionosides and brideliosides [5]. [6] have identified and evaluated bioactivity in forest plants used for medicinal purposes. One of the identified forest plants is B. glauca Blume, which indicates that a positive plant contains flavonoids, tannins, and steroids.

[7] studied the flavonoid content of sulfonate acid found in the root of the plant *Phyllanthus acidus* which is a species of the family Phylanthaceae and found that there are at least 6 sulfonic acids that contain flavonoids such as acidoflavanon, acidoauronol, 5-O-methylase auronol, acido auron, asidoisoflavon, and acido flavonol. Phyllanthus niruri Linn, one of the species of the phyllanthaceae family, contains several chemical components, including flavonoids that stimulate the immune system (thickness) of the human body to work better. The contained flavonoids are routine and quercetin flavonoids known as anti-carcinogens or cancer cell inhibitors [8].

Staphylococcus aureus is one of the gram-positive coconut bacteria that is pathogenic to humans. [9]. *Escherichia coli* (*E. coli*) bacteria is one of the causes of human health disorders. [10].The antibacterial activity test is a method to determine bacterial susceptibility to an antibacterial substance and identify a pure compound with antibiotic activity [11]. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infections. [12]. [13] Tested antioxidant activity and determined the total phenyl content of ethanol extract of the leaf of Nanamuha (*Bridelia monoica Merr*), a species of the genus Bridelia. This plant contains phenol compounds, flavonoids, alkaloids, and tannins. These active compounds have antioxidant activity.

So far, research on the isolation of flavonoid compounds and the testing of antibacterial activity from the skin of the stem of the Canyere Blume plant (B. glauca Blume) has not been done. Researchers have carried

out phytochemical screening tests showing that the extract of ethyl acetate powder skin of the Canyere Redhead plant showed a positive % result of flavonoids on the $FeCl_3$ reaction of 5%. From the description above, researchers were interested in isolating the contents of flavonoid compounds from the canyere redhead plant stem skin.

2. Materials and Methods

2.1. Equipment

The instruments used in the research are ^{1}H-NMR spectrophotometer, FT-IR spectrum photometer, UV-visible spectrum, chromatographic column, rotary evaporator, rotating pumpkin, UV light, analytical balance, chamber, divided pipe, beaker glass, Erlenmeyer, measuring glass, glass pipe, drop pipette, spatula, mixer rod, vial bottle, reaction tube, water pipes, capillary pipes, and clamps.

2.2. Materials

The materials used in this study are Canyere Badak plant stem, methanol, ethyl acetate, n-hexane, Silica Gel (70–230 mesh), $FeCl_3$, Mg powder, HCl, H_2SO_4, cotton, chloroform, KLT silica gel 60 F_{254} plate, KLT Preparative 60 F24 plate were purchased from E. Merck.

2.3. Sample Preparation

The skin of the stems of the plant Canyere Badak, which has been collected in the fresh condition in the blender to become a small portion – the skin is then dried up after a rough slice of the skin of a plant stem Canyere reindeer as much as 2500 g.

2.4. Preliminary Test Against Extracts

The skin of the Canyere Plant Stem is identified by performing a qualitative preliminary test with a color reaction by inserting 10 g of the skin powder of the canyere plant stem into an Erlenmeyer glass, then extracting maceration of ethyl acetate, then stacking for 15 minutes, then filtering, the filter is divided into the reaction tube and then adding a $FeCl_3$ 5% factor to produce a black colloid.

2.5. Extracting Skin of Plant Stems

The rough powder of the skin of the stem of the plant, which has been dried as much as 2500 g, is extracted and macerated with methanol of ± 20 l until all samples are completely submerged and evenly mixed. It was then filtered and fixed using a rotary evaporator to obtain a concentrated methanol extract. Then, it is applied with a water carbonizer until all the methanol solvents evaporate. Then, the non-polar compounds are separated by dissolving the methanol extract using water and then filtered, which will leave a suspected flavonoid compound. Then, the tannins are separated by extracting the water filter partition with ethyl acetate. The ethyl-acetate layer is applied until all the ethyl acid solvents are evaporated. The obtained concentrated extracts are dissolved with methanol, and the partition is extracted with n-hexane to the limestone. The methanol layer was separated from the n-hexane layer, then tested with FeCl3 5% and compressed to obtain a 13 g methanol layer concentrate extract.

2.6. Typical Layer Chromatography Analysis

The thin-layer chromatography was analyzed against the methanol extract using silica gel 60 F254 silica silica phase. This analysis was done to determine the solvent system suitable for column chromatography. The motion phase used is a chloroform solvent mixture: methanol with a 90:10, 80:20, 70:30, 60:40, 50:50 (v/v) ratio. Insert 10 ml of the chloroform motion-phase solution: metanol 90:10 (v) into the chamber, then saturate. Inject the methanol-concentrated extract of the activated KLT plate into the chamber environment containing the saturated solvent mixture, then close and dissolve until the solvent reaches the specified limit. The plates that have been diluted are removed from the chamber and then dried. Monitor the stains formed under UV rays, then scan using the $FeCl_3$ 5%. Observe the stain color resulting and calculate the Rf value obtained. The same treatment is done for chlorophorus solvents: methanol ratio 80:20, 70:30, 60:40, 50:50 (v/v).

2.7. Separation of Flavonoid Compounds

The separation of flavonoid compounds by column chromatography is done against the concentrated extracts of the obtained methanol. The quiet phase used is silica gel 60 (70-230 mesh), and the motion phase is chloroform: methanol with a ratio of 90:10, 80:20, 70:30, 60:40, and 50:50 (v/v)—calculated column chromatography device. First, silica gel 60 (70-230 mesh) was dissolved using chloroform, mixed to

homogeneous, inserted into a chromatographic column, and then diluted using 100% chloroform to silica gel and homogeneous. Melt 6 g of methanol concentrated extract with silica gel with methanol solvent, insert it into a chromatographic column containing silica gel powder, then slowly add the chloroform motion phase: 90:10 (v/v) methanol. Improved irradiation by adding the chloroform motion phase: methanol with a ratio of 80:20, 70:30, and 60:40 (v/v). The result is stored in a vial every 10 ml, then in the KLT, combined fractions with the same Rf value, and tested with $FeCl_5$%. Then, apply until a paste is formed.

2.8. Purification of Compounds Resulting from Insulation

The pasta obtained from column chromatography separation is re-solved with methanol and then analyzed by the KLT to see if the compound is already pure or not at the same time, looking for a suitable phase of money movement for the preparatory KLT. Benzene: 80:20 acetone (v/v) is the motion phase that indicates the best separation for further use in saturating the preparatory KLT veins. The dissolved pasta is smoothed slowly and evenly along the bottom edge of the KLT plate. The plate is inserted into a pot containing saturated solvents and then closed. After the dilution, the plates are removed from the vein and dried, and the result is observed under UV light. Each zone is marked and diluted with methanol: ethyl acetate 1:1 (v/v). Then, the result of the elusion is applied so that the yellow-brown paste is obtained.

2.9. Test Purity of Isolation Results with Thin-Layer Chromatography

The solid purity test was carried out with thin-layer chromatography using silica gel 60 F_{254} still phase with chloroform motion phase: methanol 80:20 (v/v). Insert 10 mL of the motion phase solution into a thin layer chromatography chamber, then saturate. Bottle the previously dissolved pasta with methanol on a KLT plate. Insert the KLT plates into a thin layer chromatography chamber that has been saturated. After the movement phase solvent rises to the upper limit, the KLT plate is removed from the chamber, dried, tested under UV light, and scanned using a $FeCl_3$ 5% reaction to produce a black stain that indicates the presence of the flavonoid compound and calculates the Rf value obtained.

2.10. Antimicrobial Test Procedure

Sterilization of the instrument.

The instrument used is washed until clean and dried and then closed tightly with parchment paper. Then, it was inserted into an autoclave and closed closely. It was sterilized for 15 minutes at 121°C.

Manufacture of Mueller Hinton Media

To a maximum of 38 g of Mueler Hinton powder To be inserted in an Erlenmeyer, then dissolved in 1000 mL of equates and mixed while mixing until dissolving, covered with cotton, then sterilized within an autoclave for 15 minutes at a temperature of 121°C.

Making a bacterial inoculum

A total of 5 mL of Mueller Hinton is inserted into the reaction tube and then the bacteria colony. *Staphylococcus aureus* was taken from a culture stock using a sterile oxygen needle, then suspended into a Mueller Hinton medium to be sterile in a reaction tube and incubated at 36°C, then compared to the standard Mc. Farland hardness. The same thing is done for the bacterial colony of *Escherichia coli*.

Flavonoid Antibacterial Activity Test.

As much as 0.1 mL of *Staphylococcus aureus* inoculum is inserted into a sterile petri cup, after which the Mueller Hinton media To 10 ml at a temperature of 45-50°C is homogenized until the media and bacteria are mixed evenly, then left until the medium is saturated. Insert disc paper that has been soaked with total flavonoid extract with various concentration variations of Petri cups that have contained bacteria, then incubate in an incubator at 36°C for 18-24 hours. Next, measure the diameter of the barrier zone around the disc paper with the length of the slope. It's done similarly against the *Staphylococcus aureus* and *Escherichia coli* bacteria.

3. Results and Discussion

3.1. Phytochemical Screening Tests of Skin Extracts

Stem bark powder of the Canyere Badak plant obtained as much as 2500 g in a test with reaction. Phytochemical screening of ethyl acetate extract of the stem bark of the Canyere badak plant using $FeCl_3$ 5% produces black colloid because the ethyl acetate extract contains flavonoid compounds.

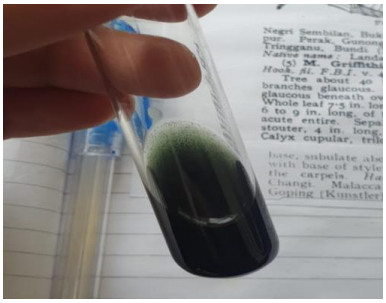

Figure 1. Phytochemical screening results.

3.2. Separation and Purification of Compounds.

Results of the isolation of flavonoid compounds from 2500 g of skin powder stem of the Canyere Badak plant begin the maceration process with 300 g of methanol. Subsequently, it dissolved with water to separate the non-polar compound by laying fat and obtained as much as 10 litres of water extract. Then, the partition is extracted using an ethyl acetate solvent. In this case, the water and ethylacetate are not perfectly mixed, and the tannins are not soluble in ethyl Acetate. Diperoleh ekstrak pekat etil asetat sebanyak 20 g. Ekstrak pekat etil asetat dilarutkan dengan metanol kemudian dipartisi kembali dengan nheksan untuk memisahkan senyawa yang bersifat non polar sehingga diperoleh ekstrak pekat metanol sebanyak 13,23 g.

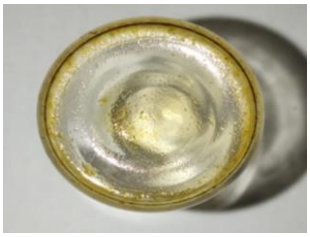

Figure 2: Compounds resulting from insulation

From the analysis of thin-plated chromatography before column chromatograph, it was found that a good solvent comparison for isolating flavonoid compounds from the skin of the plant stem of the Canyere Redhead is chlorophorus: methanol (80:20) (v/v) which indicates better separation of the resulting stains. After separation with column chromatography, an excellent solvent comparison for the isolation of flavonoid compounds from the skin of the stems of the plant Canyere Snake is chloroform: methanol (80:20) (v/v), which shows a better separation of the resulting stain (fracture). After the separation by column chromography, a KLT analysis is performed for the combination of fractional results using eluent chloroform: methanol (80:20/v), where the stain is produced on the thin stain chromatographic plate in the fraction 148-174 with the $FeCL_3$ 5% per action is the best separation for the two stains, 1608 mg. Then, the KLT preparation is done with eluent benzene fraction 80:20 (v) and observed with a lamp. The desired UV dioxide is then compared with the dilution value of methyl ethanol (0.66 mg/v1) so that the stains are yellow and white. (Figure 3).

Figure 3: KLT Compounds Isolation Results

3.3. UV-Visible Spectrum Compounds Results Isolation

The UV-visible compound spectrum resulting from the isolation by dissolving methanol is shown in Figure 4.

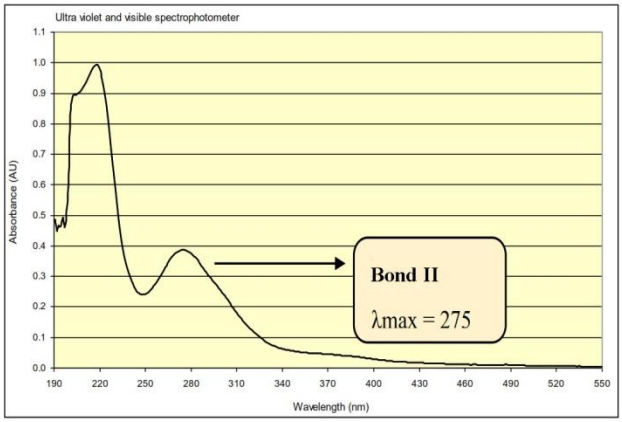

Figure 4. UV-visible Spectrum of Compound Isolation Results.

From the results of analysis using UV-vis spectroscopies, two maximum wavelength absorptions can be seen in Table 1 below:

Table 1. UV-visible wavelength insulation result

Bond	Wavelength (nm)	Absorbance
I	275	0.67156
II	217	0.61910

The results of interpreting the UV-visible spectrum with the methanol solvent (Figure 4) give the absorption tape with the wavelength (λ max) of the compound isolation result with a value of 275 nm. On the UV -Vis spectrum occurs a hypochromic effect due to the absence of a hydroxyl group on C-5, which causes a decrease in the intensity of light absorptions and usually also occurs due to changes in the solvent.

3.4. Spectrum FT-IR Compounds Isolation Results

The spectrum of FT-IR compounds isolated using KBr pellets can be seen in Figure 5.

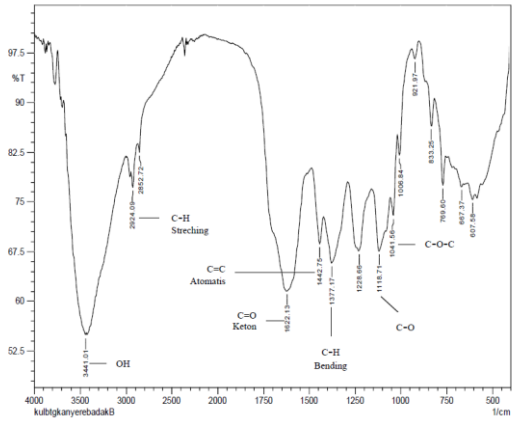

Figure 5. Inframerah Spectrum (FT-IR)

The analysis of the amorphous solidity FT-IR spectroscopic photometer results from the isolation resulting in absorption bands in the area of the number of waves that can be seen in Table 2 below:

Table 2. Result Analysis Spectrum FT-IR

Wavenumbers of isolated compounds (cm^{-1})	Wavenumbers (cm^{-1})	Functional Groups	Vibration	Intensity
3441.01	3650-3200	O-H	Stretching	Strong
2924.09	3000-2850	C-H	Stretching	Strong
1622.13	1750-1630	C=O ketones	Stretching	Strong
1442.75	1600-1450	C=C Aromatic	Stretching	Medium
1377.17	1450-1365	C-H	Bending	Medium
1228.66	1300-1000	C-O	Stretching	Strong
1118.17	1150-1070	C-O-C	Stretching	Medium

The results of the analysis of the spectrum FT-IR showed the number of waves 3442,94 cm^{-1} peak indicating the presence of the stretching vibration of O-H, at the number waves 2924,09 cm^{-1} Peak is showing the presence of the C-H Aromatic stretching vibrating, a number of the waves 1622,13 cm-1 sharp peak indicates the C - O stretching Vibrating in the ketone system, at the count of the wavelengths 1442,75 cm-1 indicates C-C stretching vibration in the aromatic system. The wave number 1377,17 cm-1 shows C-H curved vibration. In the numbers of those waves, 1228,66 cm^{-1} acute peak suggests C-O curvation, at a number of these waves, 1118,71 cm^{-1} indicating C-O-C curve vibration following the comparison data of the Infrared Spectrum [14], [15]. According to [16], the number of waves in the C=O group of ketones does not correspond to the literature because C=C bonds are adjacent to C=O, thus resulting in the relocation of the electron π and increasing the single bonding properties of the C-O group which causes the absorption to occur at smaller wave numbers.

3.5. ^{1}H-NMR Compound Isolation Result Results Analysis Results

Magnetic Resonance Spectrum of Proton Core (^{1}H-NMR) compounds isolated using methanol and TMS solvents as internal standards can be seen in Figure 6 below:

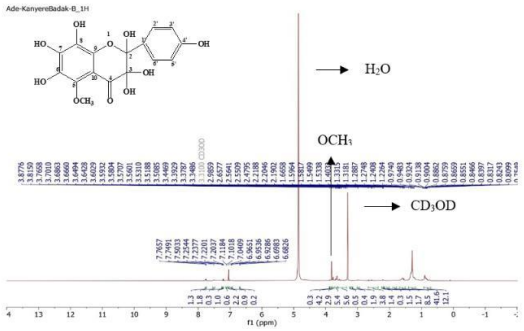

Figure 6. ^{1}H-NMR Spectrum Compound Isolation Outcome

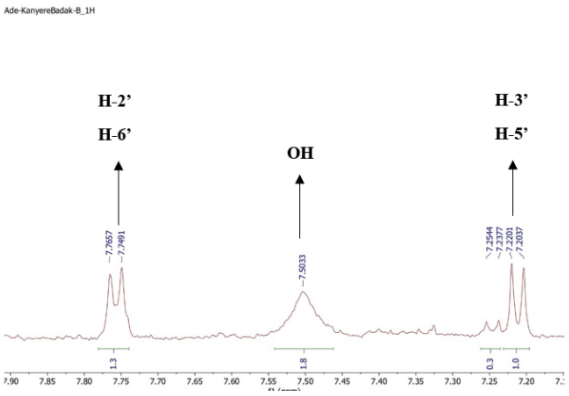

Figure 7. Spectrum ^{1}H-NMR Compound Isolation Outcome at = 7.10-7.90

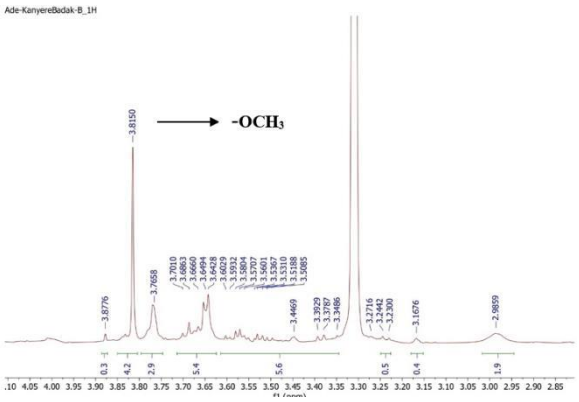

Figure 8. Spectrum ^{1}H-NMR Compound Isolation result at = 2.85-4.10

Results of analysis of Proton Core Magnetic Resonance Spectrophotometer (^{1}H-NMR) compound isolation results can be seen in Table 3 below:

Table 3: Results of ^{1}H-NMR Spectrum Analysis Compounds Isolation Results

H	σ H (ppm)	Peak type
H – 2' & H – 6'	7.7657;7.7491	Doublet
H – 3' & H – 5'	7.2201; 7.2037	Doublet
H of OCH_3	3.8150	Singlet

The ^{1}H-NMR spectrum analysis using methanol solvents in TMS standard obtained the presence of chemical shifts in the area = 3.8150 ppm singlet peak indicating proton at -OCH_3 bound with carbon 5 in the ring A. The spectrum ^{1}H -NM R ring A at the chemical shift of 5-6 ppm has no peak, meaning no proton in Ring A following the comparison structure. Furthermore, in the chemicals shift area 6-7 ppm, there is a peak doublet-doublet indicating a peak on the carbon 2',3',5', and 6' in rings B. The shift in chemical areas = 7.7657; 7.7491 ppm peak is a double proton indicating H–2' and H–6' in aromatic rings, and the chemistry shift of the area in ≥ 7.2201; 7.20 ppm is the peak of the double ppm indicating the proton of H' – 3' – H – 5' and. The C ring can be determined by UV-visible spectroscopy analysis to determine the group based on the approximate λ max of the flavanon group. The NMR spectrum of the flavonoid C ring was not found, so there was no proton in the ring, so it was filled with substituent -OH to supplement the binding on the carbon ring C. Chemical shifts in the H-NMR also showed the presence of a signal -OH in the chemical shift of the area = 7.5033, but the researchers have not yet been able to interpret -OH is bound to the C atom so that additional analysis is required with the MS and 13C-NR spectrophotometers [17].

Based on the analysis of the three spectroscopies, it can be concluded that the compound isolated from the skin of the stem of the plant Canyere Blume (*Bridelia glauca Blume*) is suspected to be a flavonoid compound with the flavanone group as in the following picture:

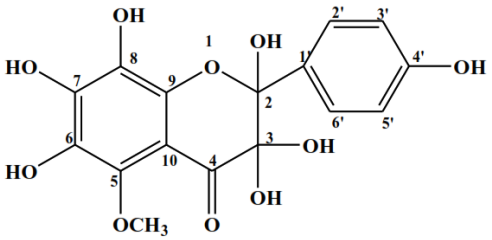

Figure 9: Isolation Compound Structure

3.5.1 Analysis of Antibacterial Activity Test Results

On total flavonoid extracts, antimicrobial tests were conducted using gram-positive and gram-negative bacteria. There is a barrier zone in the growth of bacteria against *Staphylococcus aureus* and *Escherichia coli*, as shown in the following picture:

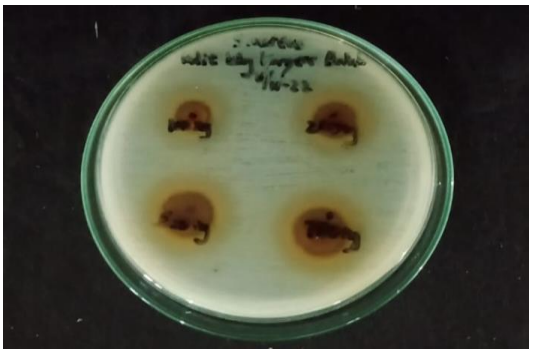

Figure 10: Growth inhibition zone on *S. Aureus* bacteria

Figure 11. Growth inhibition zone on *E. coli* bacteria

The measurement of the diameter of the lymphatic zone of antibacterial activity of the total flavonoid extract of the skin of the stem of the plant Canyere Horns against the bacteria *Staphylococcus aureus* and *Escherichia coli* can be seen in the following table:

Table 4: Antibacterial Activity Lymph Zone Diameter Measurement Results

Isolate	Concentration (mg/mL)	Inhibition zone diameter (mm)	Antimicrobial index
Staphylococcus aureus	100	9.5	0.583
	250	12.1	1.017
	500	13.4	1.233
	750	13.7	1.283
Escherichia coli	100	12.15	1.008
	250	15.05	1.508
	500	16	1.776
	750	16.1	1.683

Based on these criteria, the total antibacterial flavonoid strength of the skin of the Canyere Rabbit in Staphylococcus aureus bacteria with extract concentrations of 100 mg/mL (9,5mm) is classified as moderate. The extraction concentrations of 250 mg/ml (12.1mm), 500 mg/mol (13.4mm), and 750 mg/ML (13.7) are strong. The concentration of the total flavonoid extract is strong enough to create a large barrier zone.

The results of the antibacterial activity test of the total flavonoid of the skin of the plant *Bridelia glauca Blume* showed that concentrations of 100 mg/mL indicated a 9.5 mm barrier zone diameter against *Staphylococcus aureus* bacteria and at a concentration of 100 mL showed a 12.15 mm lymphatic zone against *Escherichia coli* bacteria. It can be concluded that the MIC value (Minimum Inhibitory Concentration) of the total flavonoids of the skin stems of the Canyere Blume plant (*Bridelia glauca Blume*) at a concentration of 100 mg/mL against the *Staphylococcus aureus* and *Escherichia coli* bacteria.

The activity of the total flavonoid extracts of the skin of the Canyere raven in inhibiting the growth of Gram-negative bacteria *Escherichia coli* is more sensitive when compared to Gram-positive bacteria *Staphylococcus aureus*. According to [18], this is due to differences in cell wall structure between the two types of bacteria. The Gram-positive bacterial cell walls consist of several peptidoglycan layers that form a thick, rigid structure and contain a cell wall substance called teichoic acid. In contrast, the Gram-negative bacteria cell wall consists of one or more thin layers of peptidoglycans so that the Gram-negative bacteria's cell wall is more susceptible to physical shocks, such as the administration of antibiotics or other antibacterial substances. Besides, the differences in cell wall structure cause the two types of bacteria to respond to Gram colouring.

4. Conclusion

The compound isolated from the skin of the stem of the Canyere Badak plant (*Bridelia glauca Blume*) of 2,500 g produced a brown-yellow paste of 10 mg with Rf 0.66 with chloroform: methanol (80:20) (v/v). Analysis with the UV-visible Spectrophotometer, the Infrared Spectral Photometer (FTIR), and the Proton Magnetic Resonance Spectrometer (^{1}H-NMR) showed that the isolated compound is suspected to be a flavonoid of the flavonone group. Next, the antibacterial tests have shown that the total flavonoid inhibits strong bacterial growth against *Staphylococcus aureus* and *Escherichia coli*. It has been demonstrated by the barrier zones produced against the bacterium Staphylococcus with 9.5 mm, 12.1 mm, 13.4 mm, and 13.7 mm.

5. Acknowledgements

The authors thank the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, for providing the facilities.

6. Conflict of Interest

The authors declare no conflicts of interest**.**

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