Isolation And Characterization Of Flavonoid From Leaves Of Bauhinia kockiana Lour And Antibacterial Activity

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Abstract. The Isolation and purification of flavonoid compounds from the leaves of Bauhinia kockiana Lour had been done. Concentrated methanol extract was hydrolyzed with HCl 6%, partitioned with chloroform and it was separated by column chromatography using n-hexane: ethyl acetate (60:40 v/v). Pure yellow-crystalline amorphous compound; mass = 33.8 mg, m.p = 155 °C and Rf = 0.29, was then characterized by spectroscopy UV-Visible, FT-IR and 1H-NMR. Interpretation of Spectrofotometer of compound compared with data standard showed the compound is flavonoid Flavonol. The antibacterial activity test toward methanol, hexane and ethyl acetate extracts respectively was conducted using diffusion method which inoculated bacteria were dipped into Mueller Hinton Agar. Results showed that inhibitory effect of bacterial growth was better with effective inhibition zone againsts Escherichia coli at concentration 500 mg/ml with diameter zone 17.8 mm

1. Introduction

Bauhinia kockiana Lour is a very spineless tropical plant which has yellowish leaves color.[1] It was used traditionally by local people in Malaya Peninsula and Indonesia as a medicine to cure wounds, burns, besides it also consumed as a meal of salad for vegetarian people.[2] Some of the previous researchers had been reported on antioxidant activity of the plant extracts, and by using IC50 it was found the total of phenolic compounds 27.0 ± 5.0 μg/mL.[2] The capacity of the antioxidant was measured using diphenyl-2,4,6-trinitrophenyl iminoazanium (DPPH) with bacteria of Methicillin Resistant Staphylococcus Aureus (MRSA) gave MID value ±100 μg / disc. This results showed the antioxidant and antibacterial activity of Bauhinia kockiana Lour are very effective to prevent wounds.[3] Flavonoids are a major groups of natural phenol compound which abundantly found in plants and foods. They are polyphenolic molecules containing 15 carbon atoms which soluble in water.[4] Flavonoids consist of chalcone, flavonone, isoflavon, flavanon, flavonol, and antocyanidin.[5] It is approximately 2 % of all carbons photosynthesized by plants were produced flavonoids and its derivates. It was reported that flavonoids in plants was used as body protections against free radicals, reduced cancer and infections.[6] Flavonoids also can play a role to provide color to attract pollinator in flower, fragrance, fruits taste, flowers and seeds.[7] In leaves, flavonoids protect plant from UV radiation and pathogenic
fungi. Literature study on isolation of flavonoids in this plant has not been reported yet and therefore it is our interests in studying of Bauhinia kockiana Lour. We have conducted chemical investigation of the compound and its structural characterization that displayed its bioactivity and report herein a flavonoid compound and its antibacterial activity.

2. Materials and Methods

2.1. Plant Material

Fresh leaves of Bauhinia kockiana Lour were collected from Tanjung Morawa area in Deli Serdang Sumatera Utara Indonesia (3° 31’ 0”, 98° 49’ 0” E) and kept frozen until work up. Identification of plant taxonomy was done by Herbarium Medanense USU Medan. Specimens are maintained in the Department of Biology at Universitas Sumatera Utara Medan.

2.2. Extraction and Isolation of Bauhinia kockiana Lour

The Bauhinia kockiana Lour (1200 g dried-fine powder) was extracted five times with technical grade of Methanol, CH₃OH to yield 55 g methanol extract, then partitioned with n-hexane after had dissolved with ethyl acetate. The concentrated methanol was then partitioned with chloroform, CHCl₃ to attract all phenolic/flavonoids compounds in extract. The chloroform extract was subjected to silica gel coloum chromatograpy to yield four fractions. Each of the fractions that has the same Rf value was collected and subjected to be purified by Thin Layer Chromatography (TLC) preparative to obtain pure crystal compound (33.8 mg ).

2.3. Spectroscopy analysis

A Shimadzu UV-Vis spectrometer was used to analyze ultraviolet-visible spectra. Infrared spectrum was recorded as KBr pellets using Shimadzu FT-IR spectrometer. ¹H-NMR spectra were obtained on Jeol/Delta2 NMR operating at 500 MHz. All this instrumentations were operated in LIPI Serpong Tangerang Jawa Barat Indonesia.

2.4. Preparation of Plant extract for bioactivity

Methanol extract of Bauhinia kockiana Lour was prepared according to Larson et.al.¹⁰ 1000 g of fresh leaves were put in air to be dried at constant weight and grinded them using electrical grinder to 500 g of fine powder and dissolved in methanol for 48 hours at room temperature. This was then filtered using Whatman’s No.1 filter paper. The combined filtrates were concentrated using rotary evaporator. This was repeated three times to give 350 g extract which appears as semi-solid greenish paste. The same procedure was applied to the obtained extract for ethyl acetate and hexane.

2.5. Determination of antibacteria activity of Plant extract

The inoculated bacteria was dipped into sterilized petridishes (9 cm diameter) that were filled with solid fine Media Hinton Agar (MHA) with temperature 45 °C-50 °C.¹⁰ Into MHA media added the suspended bacteria S. aureus which was already dissolved in sterlized distilled water by scratching it using ose needle. A disk paper soaked with methanol, ethyl acetate and hexane extract respectively was transferred into petridishes (9 cm diameter) and pour plated in dichloran glycerol 18 % agar (DG18 agar) with different concentrations ( 500 mg of sample was diluted in 1 ml DMSO). The suspension was homogenized using shaker (Gallenkamp, orbital shaker SG92, England) 100 rpm for 2 minutes. Five concentrations 500,400,300,200,100 mg were made, 1 ml was transfered onto petridish (diameter 9 cm) and pour plated in DG18 agar. The plates were incubated at ambient temperature (30±2 °C) for 6 days in (Zenith Lab Digital Incubator Dnp 9052). Bioactivity of the extract against the bacteria was measured based on the diameter distances of clear zone around the disk paper using Vernier calliper.
3. Results

3.1. A sample of pure crystal compound dissolved in methanol was run using Shimadzu UV-Vis spectrometer to measure ultraviolet-visible spectra. The spectrum of the compound showed 2 absorptions band of wavelength; band I and band II as seen on Figure 1.

![UV-Vis spectrum of crystal compound](image1)

The absorptions of each band versus intensity of absorbancy were described on Table 3.1.

| NO | Wave length, \( \lambda \) (nm) | Absorbancy |
|----|--------------------------------|------------|
| 1. | 269.00                         | 2.622      |
| 2. | 369.00                         | 0.678      |

3.2. Infrared spectra were recorded in KBr pellets using Shimadzu FT-IR spectrometer and the spectrum of the compound can be seen on Figure 3.2.

![Spectrum FT-IR of crystal compound](image2)

Based on FT-IR spectrum, it can be inferred a major functional and its supporting group of the compound which were related to wavenumber and typical vibrations (Table 3.2).
| Wave number (cm\(^{-1}\)) | Intensity   | Functional group                             |
|---------------------------|------------|----------------------------------------------|
| 3323.35 – 3296.35         | Medium-broad | Stretching Vibration –OH                     |
| 3062.25                   | Medium     | Stretching Vibration C-H Aromatics           |
| 3012.81 – 2954.95         | Medium     | Stretching Vibration C-H Aliphatics          |
| 1697.36                   | Sharp      | Stretching Vibration C=O                     |
| 1608.63                   | Medium     | Stretching Vibration C=C Aromatics           |
| 1319.31                   | Medium     | Bending Vibration C-H Aromatics              |
| 1035.77 – 1001.06         | Sharp      | Stretching Vibration C-O-C Symetric          |
| 921.97                    | Medium     | Stretching Vibration C-O-C Unsymetric        |

3.3. \(^1\)H-NMR spectra were obtained on Jeol/Delta2 NMR operating at 500 MHz. The crystal compound was dissolved in Aceton d-6 NMR solvent and TMS as an internal standard gave rises' chemical shift (\(\delta\)) peaks of proton compound as seen on Figure 3.3. The spectrum was run up to lower energy frequency 16 ppm.

Figure 3.3. Spectrum \(^1\)H-NMR of crystal compound

\(^1\)H-NMR spectrum of proton-proton that appeared as peaks on a specific chemical shift was characteristic of chemical structure of the compound which was described on Table 3.3.
| Atomic H       | δ H crystal compound            |
|---------------|--------------------------------|
| H-2’ & H-6’   | 7.3833 - 7.3755 (d)            |
| H-5’          | 6.7127 – 6.6959 (d)            |
| H-8           | 6.5233 (s)                    |
| H from OCH3-5 | 3.8915 (s)                    |

The antibacterial activity test with agar diffusion method in media of methanol extract with 3 types of bacteria is as described on Table 3.4.

| Inhibition zone Bacteria | Concentration of Methanolic extract (mg/ml) |
|--------------------------|---------------------------------------------|
|                          | 100  | 200  | 300  | 400  | 500  |
| *Staphylococcus aureus*  | 0    | 10.3 | 10.9 | 12.4 | 13.5 | 16.6 |
| *Salmonella typhi*       | 0    | 10.5 | 11.2 | 12.9 | 14.2 | 16.9 |
| *Escherichia coli*       | 0    | 11.2 | 12.3 | 13.5 | 15.0 | 17.2 |

Note:

* = Blank (disk paper soaked with DMSO)

Based on concentration of clear zone on Table 3.4, the inhibition zone of bacteria *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* from methanolic extract can be seen on Figure 3.4.1, 3.4.2, and 3.4.3 belows:

**Figure 3.4.1.** Inhibition zone of *Staphylococcus aureus* from methanolic extract
4. Discussion

4.1. Spectral Data
UV-Vis spectrum gave 2 absorptions wave length, \( \lambda \) (nm) 369 nm and 269 nm. This results showed the range absorptions of flavanol flavonoid group of isolated compound with –OH substituted in ring structure. The infrared spectrum described all major functional groups and their typical vibrations were present in flavanol structure such as hydroxy -OH , Keton C=O, Eter C-O-C and aromatic C=C group. The \(^1\)H-NMR showed no peaks of protons in C-2 and C-3 either singlet nor doublet-doublet at \( \delta \) 2.5 - 3.0 ppm, therefore this was the key point that the structure belongs to flavanol group (data also compared to standard).

4.2. Antibacterial activity test
The antibacterial activity of methanolic extract showed the inhibition zone against the growth of Staphylococcus aureus, Salmonella typhi, Escherichia coli. Diameter clear zone was formed and measured around petridishes. The test also showed that the inhibitory effect of bacterial growth was better with inhibition zone against Escherichia coli at concentration 500 mg/ml with diameter inhibitory zone 17.2 nm.

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