Isolation, Identification, and Bioactivity Test of Secondary Metabolite Compounds from Methanol Extract from Java Bark

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Abstract

This study aims to isolate, identify, and test the bioactivity of secondary metabolites of *lannea coromandelica* stem bark methanol extract. This type of exploratory research included the stages of fractionation, purification, identification, and bioactivity testing of the methanol extract of *lannea coromandelica* stem bark. The sample used came from Tondong Bua Village, Bone District, Indonesia. The pure isolate obtained was in the form of a white powder. Identification results with TLC Three Eluent System obtained Rf 0.2, 0.53, and 0.85, respectively. FTIR spectrum data shows the presence of secondary N-H groups at wave numbers 3446.79 cm-1, C-N at wave numbers 1205.51 cm-1 to 1302.74 cm-1, C=C Aromatic at wave numbers 1577.77 cm-1 to 1643.36 cm-1 and C-H Aromatic at wave number 690.52 cm-1, melting point 156-158°C. Based on the group test results, FTIR spectral data, and GC-MS, it was concluded that the compound obtained was an alkaloid compound, namely 2,5-dimethyl pyrazine. The results of the bioactivity test on Artemia salina Leach larvae for the crude extract of the GF faction and the pure isolate showed LC50 values of 13, 15, and 36.41 ppm, respectively, indicating that the crude methanol extract obtained had higher bioactivity compared to the pure isolate. But both have high potential as anti-cancer and anti-microbial.

Keywords: isolate, bioactivity, *lannea coromandelica,* alkaloids, methanol extract.

INTRODUCTION

Indonesia is known as a country that has abundant natural wealth. This natural wealth is in the form of a diversity of plant species that can be used in traditional medicine. Traditional medicine is a herb that has the property of curing various diseases from ancient times for generations (Sadda, 2010).

Medicinal plants produce various classes of organic compounds known as secondary metabolites, which are synthesized by plants to defend themselves from pests in their environment, including flavonoids, alkaloids, phenylpropanoids, steroids, tannins, terpenoids, and others. Compounds contained in plants can be used as drugs that can potentially provide healing or disease-prevention effects for the body.

One of the many plants in Indonesia that contains secondary metabolites often used by the community as traditional medicine is the Javanese tree (Lannea Coromandelica). Traditionally the people of South Sulawesi often use Javanese wood to treat several types

of diseases, such as burns (Ismail, 2016). The people of Bambapuang Village, Enrekang Regency, also use Javanese wood plants to treat diabetes and high blood pressure (Anggreani, 2018). The classification of the Lannea Coromandelicai species belongs to the Anacardiaceae family of the Lannea genus (Wahid, 2009).

One part of the Javanese wood species that contains secondary metabolites is the bark. Based on research by Syamsurya (2016) reported that the methanol extract of Java bark has an antibacterial effect on Staphylococcus Aureus bacteria and is thought to contain the active compound 5-hydroxymethylfurfural and antibacterial 1,2,3-benzene-triol. Several pharmacological studies have been reported by researchers from India and Bangladesh that the methanol extract of Java bark has biological activities such as antibacterial, analgesic, hypotensive activity, wound healing activity, and antioxidant (Fitriyanti, 2019). Also, Sivaraj's research (2018) reported that the methanol extract of Java bark contains terpenoid phytol compounds, phenolic compounds 3,5-Bis (1,1 dimethyl ethyl) phenol, and piperazine alkaloid compounds 1-(2-adamantyl-)-4 -benzoyl (1-(2-adamantyl)-4-benzoyl-piperazine. The bark of lannea coromandelica contains several compounds belonging to the alkaloids, steroids, terpenoids, glycosides, and flavonoids (Rahman, 2016). Secondary metabolites of the alkaloid group are found in the family Anacadiceae, the stem bark of Mangifera indica, a piperidine group (Aksara, 2013).

Based on the description above, although there have been many studies on the bark of the Java tree with methanol extract, further research is needed to examine more deeply the content of secondary metabolites and bioactivity using the toxicity test method on Java stem bark from methanol extract. This research is a continuation of previous research by doing further fractionation. The methanol extract was used to explore the secondary metabolite compounds contained in Java stem bark from polar solvents.

RESEARCH METHODS

Tools and materials

The tools used in this study were TLC chamber, capillary tube, hot plate, UV lamp, tools for fractionation, including a vacuum liquid chromatography column (KKCV) and a pressure chromatography column (KKT). Then some equipment such as analytical balance, glassware, melting point Stuart type SMP11, Shimadzu Gas Chromatography type GCMS-QP2010, Shimadzu type Prestige-21FT-IR spectrophotometer.

The material used is finely powdered bark of Java. The chemicals used are methanol, n-hexane, ethyl acetate, chloroform, acetone, distilled water. Several reagents such as iron (III) chloride reagent (FeCl3 1%) for the qualitative flavonoid test, dragendorff and Wagner reagent for the qualitative test of alkaloids, Liebermann-Buchard reagent for the qualitative test of steroids, cerium sulfate (CeSO4) for the appearance of stains. Other materials used were silica gel 60 (0.2-0.5 mm) to impregnate the sample, silica gel 60 GF254 for the vacuum column, TLC plates, aluminum foil, and Whatman filter paper number 41/125.

Work procedures

The viscous methanol extract was preliminarily tested (class test) with various reagents, including 1% FeCl3 reagent (flavonoids), Lieberman-Burchard (terpenoids), *dragendorff* and wagner (alkaloids). TLC identified the methanol extract in various eluents, and comparisons were made to determine the solvent suitable for the CCCV. The viscous extract was fractionated using the KKCV method using silica gel 60 GF254. The fractions obtained were identified using TLC, and the fractions with the same stain profile were combined. One of the combined fractions

is selected for KKT fractionation. The purpose of KKt is to separate the compounds obtained from the KKCV fraction. The fractions obtained were analyzed using TLC, and the fractions with the same stain profile were combined and then evaporated to room temperature to obtain a solid.

The solid components obtained from the combined KKF fractions are then purified (decanted) using a suitable solvent. The purity of the compounds obtained was determined by TLC using three eluent systems using chloroform: n-hexane (1:9), ethyl acetate: n-hexane (4:6), and acetone: ethyl acetate (3:7) eluents.

The isolates were group tested using 1% FeCl3 reagent to identify flavonoid compounds, *Dragendorff*, Wagner to identify alkaloid compounds, and Liebermann-Buchard to identify terpenoid compounds. Further identification using the melting point test using a device, an infrared spectrophotometer to determine the functional groups present in the combination, and GCMS to determine the type and content of the compound in the isolate.

The GF fraction and pure isolate were tested for bioactivity using the Brine Shimp Lethality Test (BSLT) method on the fry of brine shrimp (Artemia salina Leach).

RESULT AND DISCUSSION

Fractionation

The concentrated methanol extract of 9.395 grams was fractionated using the vacuum liquid column chromatography (KKCV) method. Before fractionation, thin layer chromatography (TLC) was first performed to determine the eluent type suitable for use during KKCV. Based on the TLC results, it was found that the eluent *n-hexaneethyl* acetate (3:7) showed a clear and reasonable stain separation pattern. Fractionation was carried out using a stationary phase in the form of silica gel 60 GF254 and using a mobile phase in the form of an eluent with an increased polarity gradient starting from 100% n-hexane, n-hexane: ethyl acetate, 100% ethyl acetate, ethyl acetate: acetone, 100% acetone, and acetone: methanol.

KKCV results obtained 20 fractions. The obtained fractions were identified through TLC using ethyl acetate: chloroform (9:1) as the eluent. The fractions showing the same chromatogram pattern were then combined to produce nine combined fractions. The combined fraction G with a weight of 1.6804 grams was selected. There was an isolate where the G fraction was further fractionated by KKT using the stationary phase, namely silica gel G 60 H (07734), and the mobile phase, namely eluent, whose polarity was increased gradually starting from 100% n-hexane, n-hexane: ethyl acetate, 100% ethyl acetate, 100% ethyl acetate: acetone, and 100% acetone. KKT results obtained 17 fractions. The resulting fractions of KKF were identified by TLC to see similarities in the chromatogram patterns and then combined. The fractions were evaporated at room temperature.

Purification

The 0.2071-gram GF fraction was purified (decanted) using n-hexane solvent to obtain 0.018-gram pure isolate in the form of a white powder. This purification was repeated to obtain a chromatogram with a single spot on the TLC plate. The purity test was carried out by TLC in three eluent systems using different solvent ratios as indicated by the appearance of a single stain on each TLC plate with chloroform: n-hexane (1:9), ethyl acetate: n-hexane (4:6) and acetone as eluents: ethyl acetate (3:7). Melting point test with a melting point trajectory of 156oC-158oC.

Class test

Identification using Wegner's and Dragendroff's reagent showed that the GF

isolate was alkaloid positive, which was indicated by a change in color from clear to reddish brown, and there was a precipitate.

IR spectroscopy

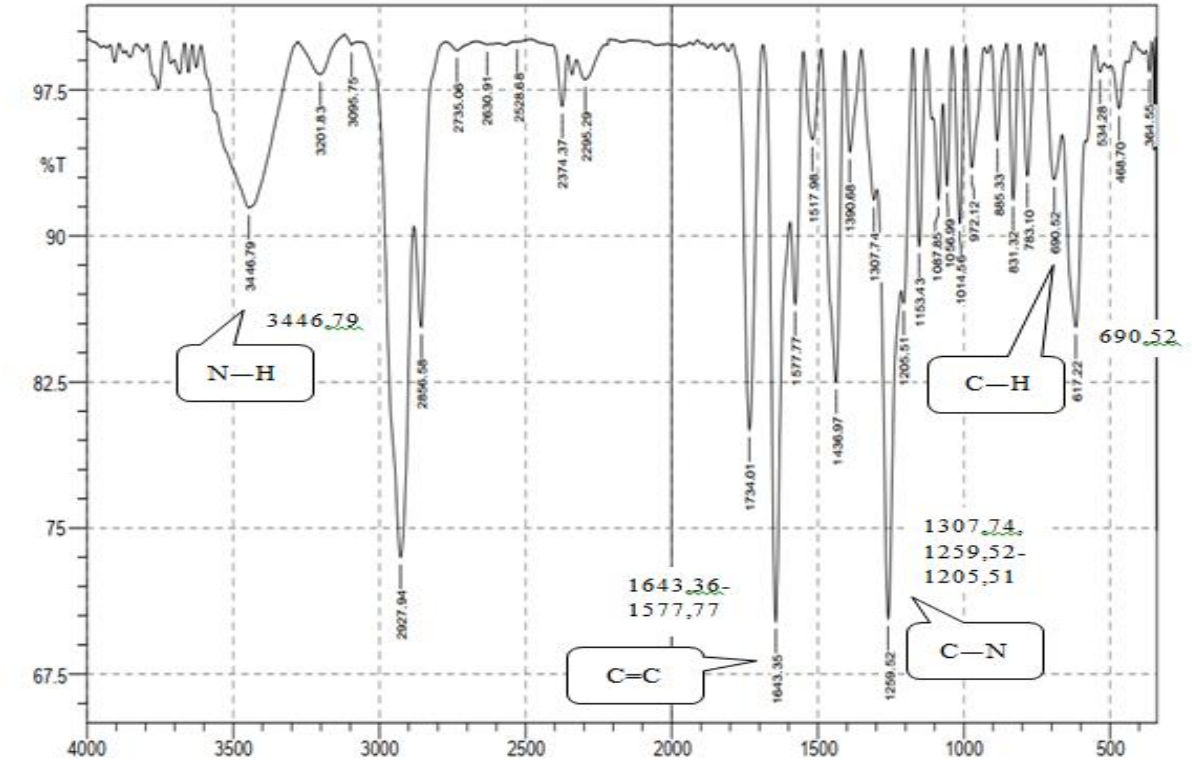
Identification of isolates was carried out by IR spectroscopy analysis; there were several functional groups. Moderate absorption with a sharp band at 3446.79 cm-1, which is identified as a secondary N-H stretching vibration which is supported by absorption with solid intensity at wave numbers 1302.79 cm-1, 1239.52 cm-1 and 1205 .51 cm-1, which was identified as a C-N stretching vibration. Absorption at strong intensities with sharp bands in the region of 1643.36 cm-1 and 1577.77 cm-1 identified the presence of aromatic C=C stretching vibrations as well as, and absorption at the medium intensity with sharp bands in the region of 690.52 cm-1 identified as aromatic C-H stretching vibrations can be seen in Table 1 and Figure 1.

Table 1. Interpretation of Pure GF Isolates

Wave number (cm-1) of GF isolates	Ribbon shape	Absorption Band (cm-1) FTIR	Functional groups	Intensity
3446,79	Sharp	3100- 3500*	Secondary N—H vibration	Currently
1577,77 dan 1643,36	Sharp	1475-1667*	Aromatic C=C vibration	Strong
1205,51, 1239,52 dan 1302,74	Sharp	1000- 1350*	C—N vibration	Strong
690,52	Sharp	690-900*	Aromatic C—H vibration	Currently

Source: Results of data processing

Figure 1. Infrared Spectrum of GF Isolates

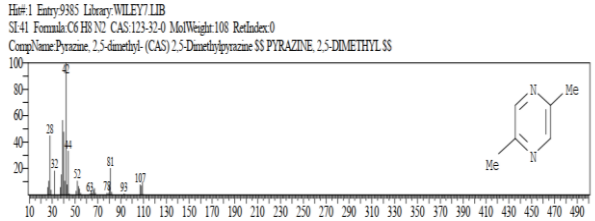


Based on the interpretation of the IR spectrum data above, pure isolate from the methanol extract of Java bark is an alkaloid.

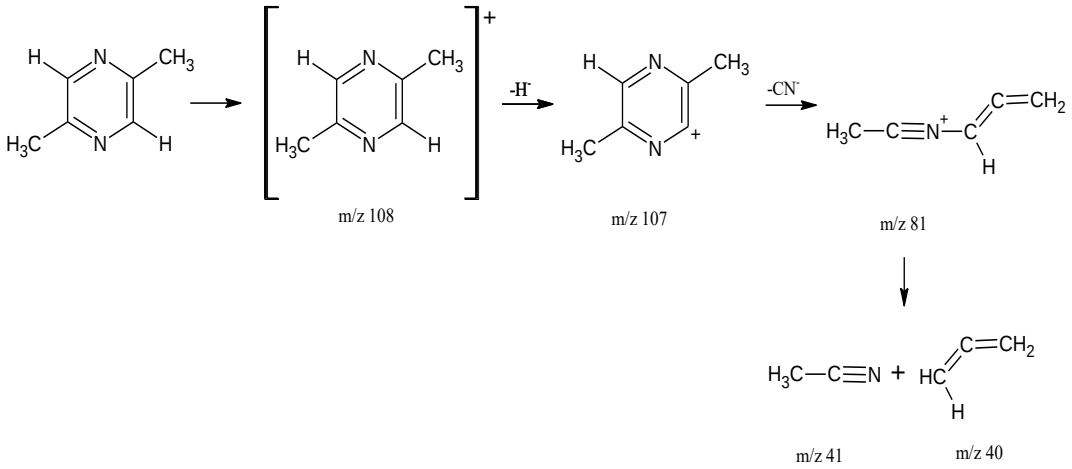
GC-MS spectroscopy

Identification GC-MS analysis detected secondary metabolites suspected of being a pyrazine-derived alkaloid group compound, with a retention time of 13.108 with the molecular formula C10H13N3 which has a mass spectrum with a molecular ion m/z of 108 thought to be a 2,5-dimethyl pyrazine compound can be seen in Figure 2.

Figure 2. MS Isolate GF Spectrum



Based on the mass spectrum, the 2,5-dimethyl pyrazine compound gives the ion peak M+ 108, which is the molecular weight of C6H8N2. -H release produces [C6H7N2]+ fragment with m/z 107. –CN release produces [C5H7N1]+ fragment with m/z 81. C=C-CH3 release produces [C2H4N1] + fragment with m/z 42. Fragmentation pattern of the 2,5-dimethyl pyrazine compound based on the mass spectrum, namely:



Bioactivity assay

Toxicity tests were carried out on the GF fraction and pure isolates with various concentrations of 1 ppm, ten ppm, and 100 ppm, which showed that the LC50 value for the GF fraction was 13.15 ppm, while the LC50 for pure isolate increased by 36.41 ppm. This indicates that the undiluted GF fraction is more active than the pure isolate. The decrease in activity in pure compounds is due to the loss of other compounds that support each other in pure compounds. The bioactivity potential of a combination if it has an LC50 below 1,000 ppm (Meyer, 1982). Based on this, both the extract (GF fraction) and its pure isolate have a high potential for bioactivity as an anti-cancer and anti-microbial.

CONCLUSION

Pure isolate from methanol extract of *lannae coromandelica* stem bark in the form of white powder, identification results with three eluent system TLC, Wagner and *dragendorff* reagent tests, IR spectra, and GC-MS spectra showed alkaloid group compounds with a melting point trajectory of 156°C-158° C. Based on the results of the bioactivity test; the GF isolate before purification had higher bioactivity compared to the pure isolate. However, both still have very high anti-cancer and anti-microbial potential. Based on the IR and GC-MS spectroscopy tests, the purified compound found was 2,5-dimethyl-pyrazine. Further research is needed on other fractions from other methanol extracts. It is necessary to identify using NMR to ensure the structure of the isolated compounds obtained.

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