Isolation of Secondary Metabolite Compounds and Antibacterial Activities Tests From Hexane Extract of Stem Bark *Melochia umbellate* (Houtt) Stapf var. degrabrata K

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Abstract—This research aims to determine the content of secondary metabolite compounds and antibacterial activity of stem bark extract *Melochia umbellate* (Houtt) Stapf var. degrabrata. Samples of *M. umbellate* stem bark were extracted by maceration using methanol solvent. Separation and purification is done by partitioning, fractionation with chromatography, and recrystallization. Antibacterial activity test of hexane extract and third isolate from bark of *M. umbellate* was done by agar diffusion method against bacterium *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Phytochemical test results showed that the hexane extract of bark *M. umbellate* compounds containing alkaloids and triterpenoids. Isolates of compound D is a triterpenoid group compound, while FKa and FKb compounds are steroid group compounds. The hexane extract had the highest inhibitory zone diameter 12.0 mm. Isolates of compound D have a weak inhibitory effect on all test bacteria. FKa compound isolates had the highest inhibition against *B. subtilis* and *S. aureus* bacteria with inhibitory zone diameters of 18.0 mm and 13.0 mm respectively. Whereas FKb compound isolates have the highest inhibitory effect against *B. subtilis* bacteria with inhibitory zone diameter 12.0 mm. The results of the test show that FKa compound from bark of *M. umbellate* has the potential to be antibacterial because the compound is able to inhibit bacterial growth with > 14 mm obstacle zone, especially against *B. subtilis* bacteria.

Keywords—Antibacterial; Secondary Metabolite Compound; *Melochia umbellate*

I. INTRODUCTION

Infectious diseases caused by bacteria over time continue to increase so that the use or demand of semisynthetic antibiotic substances is also increasing. Increased use of semisynthetic antibiotics to overcome the disease caused by bacteria will cause new problems, such as chemicals used as antibiotic substances are dangerous chemicals, not safe for health, and can cause resistance to pathogenic bacteria. It is a challenge for organic chemists of natural materials researchers to look for new active chemicals to serve as new antibacterials. One common source of antibacterial is plants [1].

Plant species that are potential as antibacterial is *M. umbellate*. This plant belongs to the Sterculiaceae family. In the area of southern Sulawesi this plant is known by the name Paliasa. Paliasa consists of two species namely *Kleinhovia hospita* L. and *Melochia umbellate* (Houtt) Stapf consisting of two varieties namely *M. umbellate* (Houtt.) Stapf var. Degabrata and *M. umbellate* (Houtt) Stapf var. Visenia. These three types of plants (Paliasa) have long been used by communities in South Sulawesi as traditional medicine to treat hepatitis, liver, cholesterol, diabetes, dysentery and hypertension [2]. While the people of Southeast Sulawesi region familiar with this plant with the name Wonolita and used as a drug itching / scabies [3]. Leaf powder from other species such as *Sterculia sesigara* is used as a chronic cough medicine (tuberculosis) and HIV / AIDS [4]. Decoction of bark *S. setigara* is used also to treat asthma, bronchitis, diarrhea, and fever [5]. Decoction of leaves and roots of *M. corcorifolia* L for treating dysentery [6], Fig. 1.
The content of the secondary metabolite compounds in the tissues of the leaf of M. umbellata are essential oils, terpenoids, alkaloids, flavonoids, steroids, and saponins [7]. In the leaf tissue is also found group of compounds; saponins, anthraquinones, and triterpenoids cycloartan [8]. Furthermore, the methanol extract of the bark of M. umbellate contains compounds group of alkaloids, flavonoids, terpenoids, phenolic and saponin [9].

Several secondary metabolite compounds which have been isolated from the M. umbellate plant and have useful biological activities such as; The 3-acetyl-12-oleanen-28-oat (1) compound has the highest inhibitory activity against the growth of bacteria B. subtilis and fungal C. Albicans [10]. Stigmasterol compounds (2) are potentially antibacterial, compounds of 9.10-epoxy melochinone which are toxic to Artemia salina, and murine leukemia P-388 cells, and flavonoid compounds group are 6,6'-dimethoxy-4,4'-dihydroxy-3',2'-furano-isoflavan [2].

Furthermore, two new compounds were found on the tissue stem wood of M. umbellate is Walterion C (3) which is highly toxic to A. salina and murine leukemia cells P-388 and Cleomiscosin (4) [11].

II. METHODOLOGY

A. Tools and Materials

Equipment used are glass tools commonly used in laboratory chemistry, column vacuum chromatography equipment, column compression chromatography, gravity column chromatography, KLT plate (Kieselgel 60, F254 0,25 mm), chamber, micropipette, heater, evaporator, melting point, antibacterial and antifungal test, UV lamp.

The materials used in this research are samples of bark M. umbellate (Houtt) Stapf var. Degrabrata with BO-1912171 specimen number, organic solvent (n-hexane, chloroform, ethyl acetate, acetone and methanol), silica gel of size 60 (Brand, No. 7730, 7733, and 7734), DMSO (Brand, No. Catalog of 802912), Amoxicillin, disc paper (6 mm), pure bacterial culture of Bacillus subtilis (ATCC 6633), Staphylococcus aureus (ATCC 25923), Escherechia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853), NaCl Physiological, and phytochemical reagents (alkaloids, flavonoids, steroids, triterpenoids, phenolic, and saponins).

1) Preparation Sample (Simplicia)

M. umbellate bark samples used were collected from Tamalanrea city of Makassar, South Sulawesi. The sample is cleaned, cut into small pieces, and then dried in the open air (at room temperature). Furthermore the bark of M. umbellate is milled into powder with a size of 90 mesh.

2) Isolation and Purification

M. umbellate bark samples of 5 Kg were macerated with methanol solvent for 3 X 24 hours. The obtained maseate was combined and evaporated the solvent using a rotary evaporator at a temperature of 40 °C to obtain a condensed of methanol extract. The methanol condensed extract is further extracted by liquid-liquid partition using a solvent with an increased polarity level: hexane, chloroform, and ethyl acetate solvents. Each of the extracts obtained was evaporated again using a rotary evaporator and then weighed and determined rendamennya, phytochemical tests and antibacterial test. Isolation and purification of hexane extracts were carried out using chromatographic techniques such as vacuum column chromatography, gravity column chromatography and plash column chromatography with suitable eluents. The isolate of the obtained compound was purified by recrystallization and recromatography. The isolate purity test obtained was done by analysis of thin layer chromatography (TLC) of three eluent systems and melting point test using Melting Point Apparatus.

3) Phytochemical Test

Phytochemical test of hexane extract bark of M. umbellate was done qualitatively. Phytochemical tests performed include; alkaloid test using three types of reagents ie Meyer, Wagner and Dragendorff reagents, flavonoid test using concentrated...
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Phytochemical tests were performed to determine the presence of secondary metabolite group compounds contained in plants. The result of phytochemical test of hexane extract and the third isolate compounds from the bark of *M. umbellata* can be seen in Table II.

Based on the results of phytochemical tests as presented in Table 2 shows that the hexane extract contains alkaloid group compounds, and triterpenoids. Isolate of compound D contains triterpenoid group compound, FKa compound isolate and FKb contains steroid group compound. Flavonoids, polyphenols and saponins were not identified in the hexane extract and the third isolates of the compound. Previous research results have been reported that the methanol extract from the bark of *M. umbellata* contains alkaloids, flavonoid, phenolic, triterpenoid and saponin compounds [10]. Died Heyne (1987) reported that *M. umbellata* contains essential oil compounds, triterpenoids, alkaloids and flavonoids. Other research results from Melochia

| No. | Type Extract / Isolate Compounds | Weight (g) |
|-----|----------------------------------|------------|
| 1   | Hexane extract                   | 36,10      |
| 2   | Isolate Compounds D              | 0.0182     |
| 3   | Isolate Compounds Fka            | 0.0204     |
| 4   | Isolate Compounds FKb            | 0.0176     |

**Table I. Weight of the Hexane Extract and the Three Isolates from the Bark of *M. umbellata***

**C. Testing of antibacterial activity**

Antibacterial activity test by the method of diffusion agar or Kirby-Bauer. As many as 1 mL of test bacterial suspension was inoculated into 200 mL Erlenmeyer flask that contain 100 mL of media NA. The mixture is homogenized by using a shaker so that suspension is well blended and then poured into a petri dish and let stand until the suspension mixture of the test bacteria in the petri dish solidifies. Furthermore, prepared a paper disc (6 mm) and dregs hexane extract samples and the third isolates with variation concentrations of 250 μg/mL, 500 μg/mL, 750 μg/mL, and 1000 μg/mL and then stand for 15 minutes. The aseptic paper cakram is placed on the surface of a petri dish containing the test bacteria. Positive controls used were paper discs with chloramphenicol (0.2 mg/ml), Negative controls used was paper disc dyeed with dimethylsulfoxida (DMSO) 5 %. Petri dish incubated at 37 °C for 24 hours. Then the bacteria test suspended by means of growing the bacteria test in physiological NaCl molten then incubated at 37 °C for 24 hours while shaked using a water bath rocked with a speed of 100 rpm [14].

3) Preparation of sample solution

The concentration of the sample solution (hexane extract and third isolates) were used to test the antibacterial activity was 250 μg/mL, 500 μg/mL, 750 μg/mL, and 1000 μg/mL.

**B. Antibacterial Activity Test**

1) Manufacture media

A total of 23 grams of nutrient agar powder (NA) in Erlenmeyer flask was dissolved in 1 liter of distilled water sterile then heated to a complete dissolution. Furthermore, the medium nutrient agar in Erlenmeyer is clogged with cotton and covered with aluminum foil and sterilized in an autoclave at 120 °C for 20 minutes [14].

2) Manufacture of bacterial suspension test

Test bacteria (*B. subtilis, S. aureus, E. coli,* and P. aeruginosa) were cultured on growth medium nutrient agar (NA) tilted. Rejuvenation is done by transferring one ose of the test bacteria into the media NA tilted then incubated at 37 °C for 24 hours. Then the bacteria test suspended by means of growing the bacteria test in physiological NaCl molten then incubated at 37 °C for 24 hours while shaked using a water bath rocked with a speed of 100 rpm [14].

The purity test of the isolate D compound was carried out in a general way i.e., TLC analysis using three eluent systems and the determination of the melting point. The TLC analysis showed one spot after being sprayed with serum sulfate and heated over the hot plate. The result of measurement of melting point of compound D is 149 - 150 °C. Then the phytochemical test of isolate D compound using Liberman-Bucher reagent gave brownish red color after addition of concentrated sulfuric acid and acetic anhydride indicating that the isolate of compound D is a triterpenoid group compound.

The purity test of FKa and FKb isolate compounds was carried out in the same way as purity test of compound D. The result of TLC analysis of FKa and FKb isolate compounds by using a comparison eluent hexane: ethyl acetate (7: 3), showing single spot and the results of melting point measurement of isolate FKa compound 115 - 117 °C, and melting point of isolate FKb compound 184 - 185 °C. Based on phytochemical test results of isolates Fka and FKb compound by using Liberman-Bucher reagent give the color of turquoise blue. This shows that both isolates are steroid group compounds. The weight of the hexane extract and the third isolates obtained from the bark of *M. umbellata* are presented in Table I.

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HCL reagents with Mg metal, concentrated H 2 SO 4, and 10% NaOH solution, Steroid and Triterpenoid Test using Lieberman-Burchard reagent, phenolic test using FeCl3 reagent, and saponin test using hot water and 2 N HCl solution [8, 12, 13].

**III. RESULTS AND DISCUSSION**

The result of extraction (5 kg sample / simplicia) by means of maceration (solid-liquid extraction) using methanol solvent obtained methanol extract reddish brown as much as 396,5 g. Then 300 g of methanol extract was partitioned (Liquid-liquid extraction) using hexane solvent and obtained hexane extract of yellowish green as much as 36,10 g. Furthermore, 30 g of hexane extract were separated by using vacuum column chromatography using eluent with ratio hexane: ethyl acetate (9: 1) obtained 57 fractions. Based on the results of TLC analysis fractions that have similar stain profiles combined to obtain 16 main fractions. The combined fraction is further fractionated by compression column chromatography and gravitation column chromatography with eluent; hexane, hexane: ethyl acetate ratio, ethyl acetate, and methanol. The fractionation results obtained by white crystals from the D fraction and two other white crystals of the F fraction i.e., FKa and FKb compounds.

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corchorifolia L (Sterculiaceae) are known to contain alkaloid group compounds, terpenoids, steroids, phenolic compounds, flavonoids, and glycosides [15]. Then the stemwood tissue of Kleinhovia hospita (Sterculiaceae) is known to contain triterpenoid group compounds [7].

Phytochemical content such as alkaloids, flavonoids, tannins, phenols, saponins, and some other aromatic compounds are secondary metabolite compounds of plants that play an important role in the defense mechanism of microorganisms against insect and other herbivorous disorders. The presence of class compounds such as phenols, alkaloids, flavonoids, tannins, saponins, and steroids in the extract may act as an antimicrobial [16].

Antibacterial activity can be determined by looking at the presence or absence of the inhibit zone (clear zone) on the growth of test bacteria shown by the extract and the three isolates encapsulated in the solid paper. The results of this study showed hexane extract and the three isolates from the bark of M. umbellate able to inhibit the growth of test bacteria. The mean inhibitory zone diameter which is a test of antibacterial activity can be seen in Table III.

Based on the results of antibacterial activity test in Table III, it showed that hexane extract at concentration of 1000 ppm showed inhibitory effect on bacterial growth of B. subtilis and S. aureus with inhibitory zone diameter were 12.0 and 10.4 mm, respectively. Resistance to the growth of E. coli bacteria is relatively weak with a diameter of 8.0 mm inhibition zone. Isolate D compound showed only inhibitory to growth of B. subtilis bacteria with 9.0 mm inhibitory zone diameter at 1000 ppm concentration and included in weak category. At concentration of 1000 ppm FKa compound isolates had the highest activity against B. subtilis bacteria with 18.6 mm inhibition zone diameter and moderate activity against S. aureus and E. coli bacteria with inhibition zone diameter were 13.4 mm and 11, respectively, 0 mm and showed weak activity against P. aeruginosa bacteria with inhibition zone of 7.2 ppm.

TABLE II. PHYTOCHEMICAL TEST RESULTS OF HEXANE EXTRACT AND THIRD ISOLATES FROM M. UMBELLATA STEM BARK.

| No | Phytochemical test | Extract and Isolate | Information |
|----|--------------------|---------------------|-------------|
| 1  | Alkaloids Meyer Test | - N-Hexan Extract - Isolate - Compounds D - Isolate - Compounds Fka - Isolate - Compounds Fkb | Orange precipitate is formed (✓) No precipitation (-) |
|    | Dragendorf's Test | - N-Hexan Extract - Isolate - Compounds D - Isolate - Compounds Fka - Isolate - Compounds Fkb | (✓) (-) (✓) (✓) (✓) (✓) |
|    | Wagner Test | - N-Hexan Extract - Isolate - Compounds D - Isolate - Compounds Fka - Isolate - Compounds Fkb | White precipitate is formed (+) (✓) (✓) (✓) (✓) (✓) |
| 2  | Flavonoids | - N-Hexan Extract - Isolate - Compounds D - Isolate - Compounds Fka - Isolate - Compounds Fkb | (✓) (✓) (✓) (✓) (✓) |
| 3  | Steroids/terpenoids LB | - N-Hexan Extract - Isolate - Compounds D - Isolate - Compounds Fka - Isolate - Compounds Fkb | (+) (+) (+) (+) (+) (+) |
| 4  | Phenolic | - N-Hexan Extract - Isolate - Compounds D - Isolate - Compounds Fka - Isolate - Compounds Fkb | (-) (-) (-) (-) (-) (-) |
| 5  | Saponin | - N-Hexan Extract - Isolate - Compounds D - Isolate - Compounds Fka - Isolate - Compounds Fkb | (-) (-) (-) (-) (-) |

TABLE III. RESULTS OF ANTIBACTERIAL ACTIVITY TEST OF HEXANE EXTRACT AND THE THREE ISOLATE COMPOUNDS FROM STEM BARK OF M. UMBELLATA (HOUTT) STAPF VAR. DEGRABBRE.

| No | Extract / Isolate | Const. (ppm) | B. subtilis | S. aureus | E. coli | P. aeruginosa |
|----|-------------------|-------------|------------|----------|--------|-------------|
| 1  | Hexane Extract    | 1000        | 12.0       | 10.4     | 8.0    | NI          |
|    |                   | 500         | 9.8        | 8.3      | 7.5    | NI          |
|    |                   | 250         | 7.0        | 8.0      | 8.0    | NI          |
| 2  | Isolate Compounds | 1000        | 9.0        | NI       | NI     | NI          |
|    | D                 | 500         | 8.0        | NI       | NI     | NI          |
|    |                   | 250         | 8.3        | NI       | NI     | NI          |
| 3  | Isolate Fka       | 1000        | 18.6       | 13.4     | 11.0   | 7.2         |
|    |                   | 500         | 15.8       | 11.2     | 9.4    | 7.0         |
|    |                   | 250         | 11.4       | 9.3      | 8.8    | 7.0         |
| 4  | Isolate Compounds | 1000        | 13.0       | 11.5     | 8.0    | 7.0         |
|    | Fkb               | 500         | 11.2       | 9.5      | 7.8    | 7.2         |
|    |                   | 250         | 9.8        | 10.0     | 7.0    | 7.2         |
| 5  | PC (+)            | 25          | 25.0       | 23.7     | 20.3   | 21.2        |
| 6  | NC (-)            | NI          | NI         | NI       | NI     | NI          |

PC (positive control) = Chlormphenicol
NC (negative control) = DMSO

While Fkb compound isolates have moderate activity against B. subtilis and S. aureus bacteria with 13.0 and 11.5 ppm inhibition zone at a concentration of 1000 ppm, and have low activity against E. coli bacteria and P. aeruginosa bacteria.
In general, hexane extract, FKa, and FKb compound isolates from these plants showed inhibitory effect against B. subtilis, S. aureus, and E. coli bacteria at concentration of 1000 ppm. At concentrations below 1000 ppm the inhibitory power is demonstrated by the hexane extract, and the two isolates of the compound on the growth of test bacteria are getting weaker or even showing no inhibitory or inactivity. The results of this study are supported by other research showing that hexane extract from M. umbellate leaves has the highest inhibition of growth of S. aureus bacteria with a diameter of the inhibitory zone of 11.45 mm at a concentration of 2500 ppm, while the ethyl acetate ethyl acetate extract has the highest inhibitory on the growth of S. dysenteriae bacteria with a diameter of the inhibitory zone of 17.70 mm [7]. Other research reported that at a concentration of 1000 μg/mL, hexane extract, methanol and 3-acetyl-12-oleane-28-oic acid compound showed the highest inhibition of B. subtilis bacteria and Candida albicans fungi. While ethyl acetate extract showed the highest inhibitory resistance to S. aureus bacteria and A. niger fungi with each inhibition zone > 14 mm [10].

Each type of bacteria has a different sensitivity to antibacterial substances, because each bacterium has a different cell wall structure so that the antibacterial effect on bacteria is also different. Gram-positive bacteria such as S. aureus and B. subtilis have only one layer containing peptidoglycan, thin-filmed teapxic acid and theuric acid while Gram-negative bacteria have layers outside the cell wall containing 5-10 % peptidoglycan, in addition to proteins, lipopolysaccharides and lipoproteins. Gram-negative bacteria such as E. coli and P. aeruginosa bacteria have two layers of lipid (lipid bilayer) called lipopolysaccharide layer (LPS), so that antimicrobial substances more difficult to penetrate into the cell wall of bacteria Gram negative bacteria [1].

According to [17], a compound is said to act as an antimicrobial if such compound provide an average of inhibition zone > 14 mm. Based on the results of antibacterial test, it can be concluded that FKa compound isolate from M. umbellate plant has potential as antibacterial because the compound is able to inhibit bacterial growth with diameter of inhibit zone > 14 mm, especially against bacterium B. subtilis.

IV. CONCLUSION

The hexane extract of the stem bark (M. umbellate) contains alkaloid group compounds, and triterpenoids have been explored. Isolates D compound contains triterpenoid group compounds, as well as FKa and FKb compound isolates containing steroid group compounds. Then, Fka compound isolates have a strong inhibitory effect on the growth of B. subtilis bacteria with 18.6 mm inhibition zone diameter and potentially as an antibacterial compound.

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