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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 meseration 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 antibacterial activity against B. subtilis bacteria with 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

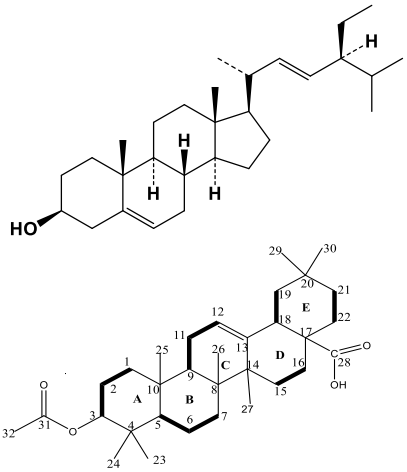
Infectious diseases caused by bacteria over time continue to increase so that the use or demand of semisynthetic antibiotic substances is also increasing. Increased use of semisynthetic antibiotics to overcome the disease caused by bacteria will cause new problems, such as chemicals used as antibiotic 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. Degrabrata 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.

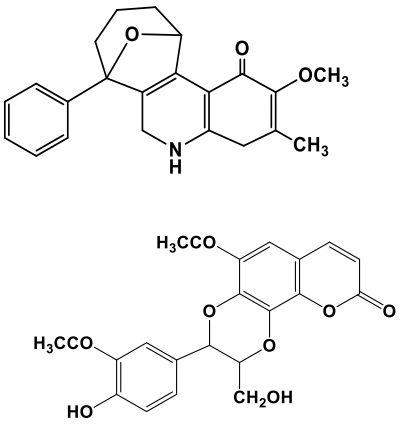
Fig. 1. Plant Melochia umbellata (Houtt) Stapf var. degbrata K

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].



Exploration of secondary metabolite compounds on the tissue bark of *M. umbellate* potentially found new compounds that have biological activities that are beneficial to humans. Therefore it is necessary to do further research to get information about secondary metabolite compound on bark of *M. umbellate* and its bioactivity. So that the use of plants as traditional medicine can be developed as a source of natural bioactive ingredients that can be used as an antibacterial drug.

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, chloroforom, 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), *Escerechia coli* (ATCC 25922), *Pseudomonas aereginosa* (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 maserate 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

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].

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 (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 ^{0} 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.

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 µml 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 control used was paper disc dyeed with dimethylsulfoxida (DMSO) 5 %. Petri dish incubated at 37 0C for 24 hours. Clearly visible zones encoded with disc paper indicate the presence of antibacterial activity. Furthermore, the clear zone formed is measured using the sliding term expressed by the size of the diameter inhibit zone.

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.

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 ^{0}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 spote and the results of melting point measurementof isolate FKa compound 115 - 117 ^{0}C, and melting point of isolate FKb compound 184 - 185 ^{0}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.

TABLE I. WEIGHT OF THE HEXANE EXTRACT AND THE THREE ISOLATES FROM THE BARK OF *M.UMBELLATE*

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	0,0 176

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. umbellate* 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. umbellate* contains alkaloids, flavonoid, phenolic, triterpenoid and saponin compounds [10]. Died Heyne (1987) reported that *M. umbellate* contains essential oil compounds, triterpenoids, alkaloids and flavonoids. Other research results from *Melochia*

corchorifolia L (Sterculiceae) 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].

TABLE II. PHYTOCHEMICAL TEST RESULTS OF HEXANE EXTRACT AND THRIRD ISOLATES FROM *M. UMBELLATE* STEM BARK.

No	Phytochemical test	Exstract and Isolate	Information
1	Alkaloids Meyer Test	- N-Hexan Extract - Isolate Compounds D - Isolate Compounds Fka	Orange precipitate is formed (+) (-) (-)
	Dragendorffs Test	- Isolate Compounds FKb - N-Hexan Extract - Isolate Compounds D	No precipitation (-) (-) (-)
	Wagner Test	- Isolate Compounds Fka - Isolate Compounds FKb - 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 / triterpenoids 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	(-) (-) (-) (-)

[a.] Information :

[b.] (+) = Positve (-) = Negative

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 inhibitory zone diameter were 13.4 mm and 11, respectively, 0 mm and showed weak activity against *P. aureginosa* bacteria with inhibition zone of 7.2 ppm.

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

No	Extract / Isolate	Const. (ppm)	Inhibition zone diameter (mm)			
			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 D	1000	9,0	NI	NI	NI
		500	8,0	NI	NI	NI
		250	8,5	NI	NI	NI
3	Isolate Compounds 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 FKb	1000	13,0	11,5	8,0	7,0
		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

PC (positive control) = Chloramphenicol

[c.] Information: N.I = Not Inhibit
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. aureginosa 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-oleanene-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 teapixic 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. aureginosa* 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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References

[1] M. T. Madigam, J. M. Martinko, Stahl, and D. P. Clark, "Biology of Microorganism 13th ed.," ed. San Fransisco: Pearson, 2012, p. 34.

[2] A. Ridhay, A. Noor, N. H. Soekamto, and T. Harlim, "A Stigmasterol Glycoside from the Root Wood of Melochia umbellata (Houtt) stapf var. degrabrata K.," Indonesian Journal of Chemistry, vol. 12, 1, pp. 100-103, 2012.

[3] F. Windadri, M. Rahayu, T. Uji, and H. Rustiani, "Uses of Plants as Medicine by Muna Peuple, Sub District Wakarumba, District Muna, Province of Southest Sulawesi," Biodiversitas, vol. 7 (4), pp. 333 – 339, 2006.

[4] I. B. Babalola, E. A. Adelakun, Y. Wang, and F. O. Shode, "Anti-Tuberculosis Activity of Sterculia setigera Del., Leaves (Sterculiaceae)," Journal of Pharmacognosy and Phytochemistry, vol. 1 No. 3 2012., 2012.

[5] S. Shanmugam, M. Kalaiselvan, P. Selvalumar, K. Suresh, and K. Rajendran, "Ethnomedicinal Plants Used to Cure Diarrhoea and Dysenteryin Shivangangai District of Tamil Nadu, India," International Journal of Research in Ayyuveda And Pharmacy, vol. 2 (3), pp. 991 – 994, 2011.

[6] S. Wulur, Firdaus, H. Natsir, and N. H. Soekamto, "Study of Compounds from Extract of Melochia umbellate (Houtt) Stapf. Degrabrata K (Paliasa) Leaves That Has Potential As Antibacterial," Journal Indonesia Chemical, vol. 2015. Jun. 8 (1), 2015.

[7] N. Joshi, S. Bhatt, S. Dhayani, and J. Nain, "Phytochemical Screening of Secondary Metabolites of Argemone mexicana Linn. Flowers," Int J Pharm Bio Sci, vol. 2013 March ; 5 (2), pp. 144 – 147, 2013.

[8] Usman, N. H. Soekamto, H. Usman, and A. Ahmad, "The Potential of Secondary Metabolites Compound From Methanol Extract of Stem Bark Melochia umbellate (Houtt) Stapf var Degrabrata As Antibacterial," in International Conference On Mathematics, Sciences, Technology, Education And Their Aplication (ICMSTEA). 2014, pp. 1- 178.

[9] Usman, N. H. Soekamto, H. Usman, and A. Ahmad, "Toxicity and Antimicrobial Activity from Extract and Oleanan Derivative Compounds of The Bark Melochia umbellate (Houtt) Stapf var. Degrabrata K," Int J Pharm Bio Sci, vol. July ; 5 (3), pp. 231 – 238, 2014.

[10] A. N. Erwin, N. H. Soekamto, I. V. Altena, and Y. M. Syah, "Waltherione C and Cleomiscosin from Melochia umbellate var. degrabrata K. (Malvaceae)," Biosynthetic and Chemotaxonomic Significance361., vol. 55, pp. 358-361, 2014.

[11] H. O. Edeoga, D. E. Okwu, and B. O. Mbaebie, "Phytochemical Constituents of Some Nigerian Medicinal Plants," African Journal of Biotechnology, vol. 4(7), pp. 685-688, 2005.

[12] M. B. Patil and P. A. Khan, "Primary Phytochemical Studies of Catunaregam spinosa (Thunb) Triven For Secondary Metabolites," Int J Pharm Bio Sci, vol. 2017 Apr. ; 8 (2), pp. 320 – 323, 2017.

[13] S. S. Gowri and K. Vasantha, "Solvent Based Effectiveness of Antibacterial and Phytochemical Derivatized from The Seeds of Harpullia arbarea (Blanco) Radlk (Sapindaceae)," 2009.

[14] V. Harini, M. Vijayalakshmi, C. Sivaraj, and P. Arumugam, "Antioxidant and Anticancer Activities of Methanol Extract of Melochia corchorifolia L," International Journal of Science and Research (IJSR), vol. 2017 Jan. 6(1), pp. 1310–1316, 2017.

[15] G. Bonjar, A. Nik, and S. Aghighi, "Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran," J Biol Sci, vol. 4, pp. 405-412, 2004.

[16] J. R. Wattimena. (1991). Farmako dinami dan Terapi Antibiotik.