Prof. Dr. Maulidiyah
Department of Chemistry, Faculty of Mathematics and Natural Sciences,
Universitas Halu Oleo, Kendari 93232 - Southeast Sulawesi
Phone: +6281388327118
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ISOLATION OF SECONDARY METABOLITE COMPOUNDS AND ANTIBACTERIAL ACTIVITIES TESTS FROM HEXANE EXTRACT OF STEM BARK *Melochia umbellata* (Houtt) Stapf var. degrabrata K

USMAN[1]*, MUH. AMIR M.[1], NUNUK HARIANI SOEKAMTO[2], AHYAR AHMAD[2], MAULIDIYAH MAULIDIYAH[3]**

[1] PS-MPK and Department of Chemistry Education – FKIP University of Mulawarman, Jl. Kuaro, Gn. Kelua, Samarinda, Kota Samarinda75119 – Kalimantan Timur, Indonesia

[2]Departmentof Chemistry Universitas Hasanuddin, Jl. Perintis Kemerdekaan Km. 10, Tamalanrea Indah, Tamalanrea, Tamalanrea Indah, Tamalanrea, Kota Makassar– Sulawesi Selatan 90245, Indonesia

[3]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Halu Oleo, Jl. HEA Mokodompit Kampus Baru UHO, Kendari 93232 – Southeast Sulawesi, Indonesia

Corresponding authors: *sainusman@ymail.com ; **maulid06@yahoo.com

ABSTRACT

Objectives: This research aims to determine the content of secondary metabolite compounds and antibacterial activity of stem bark extract *Melochia umbellata* (Houtt) Stapf var. degrabrata.

Methods: *M. umbellata* stem bark were extracted by meceration using methanol solvent. Separation and purification were done by partitioning, fractionation with chromatography, and recrystallization. Antibacterial activity test of hexane extract and third isolate from bark of *M. umbellata* was done by agar diffusion method against bacterium *Bacillus subtilis, Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*.

Results: Phytochemical test showed that the hexane extract of bark *M. Umbellata* containing alkaloids and triterpenoids. Isolate D is a triterpenoid group compound, while FKa, and FKb are steroid group compound. The hexane extract had the highest antibacterial activity against *B. subtilis bacteria* with inhibitory zone diameter 12.0 mm. Isolate D has a weak inhibitory

effect on all test bacteria. The highest diameters inhibition zone of isolated FKa compound against *B. subtilis* and *S. aureus* bacteria were 18.0 mm and 13.0 mm, respectively. Whereas, the highest diameter inhibition of zone FKb compound against *B. subtilis* bacteria with inhibitory zone was 12.0 mm.

Conclusion: The FKa compound from bark of *M. umbellata* 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, *Melochia umbellata*, triterpenoid, steroid.

INTRODUCTION

Plant speciesof *M. umbellata* is potential as antibacterial and belongs to the Sterculiaceae family. In Southern Sulawesi area, this plant is known by the traditional name of Paliasa. Paliasa consists of two species such as *Kleinhovia hospita* L. and *Melochia umbellata* (Houtt) Stapf and consisting of two varieties i.e. *M. umbellata* (Houtt.) Stapf var. degrabrata (Fig. 1) and *M. umbellata* (Houtt) Stapf var. Visenia. This genus comprises of herbs and shrubs distributed in the tropical and sub-tropical regions of world. About seventy species occur in India and Indonesia, some of which are used in medicine [1].These types of plants 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 is familiar with the name Wonolita used as a drug itching/scabies. Leaf powder from other species such as *Sterculia sesigara* is used as a chronic cough medicine(tuberculosis) and HIV/AIDS [3].Decoction of bark *S. setigara* is used also to treat asthma, bronchitis, diarrhea, and fever [4]. The decoction of leaves and roots of *M. corcorifolia* L. usually appliedfor treating dysentery [5].

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

The secondary metabolite compounds content in leaf tissues of *M. umbellata* is essential oils, terpenoids, alkaloids, flavonoids, steroids, and saponins [6,7]. It is also found a group of compounds; saponins, anthraquinones, and triterpenoids cycloartan [8]. Furthermore, the methanol extract of the bark of *M. umbellata* contains of alkaloids, flavonoids, terpenoids, phenolic, and saponin [9,10]. Several secondary metabolite compounds which have been isolated from the *M. umbellata* plant and have useful biological activities such as the 3-acetyl-12-oleanen-28-oat (Fig. 2a) compound has the highest inhibitory activity against the growth of bacteria *B. subtilis* and fungal *C. Albicans* [10]. Stigmasterol compounds (Fig. 2b) are potentially as an antibacterial, compounds of 9.10-epoxy melochinone are toxic to *Artemia salina*, murine leukemia P-388 cells [11], and 6,6'-dimethoxy-4,4'- dihydroxy-3',2'-furano-isoflavan.

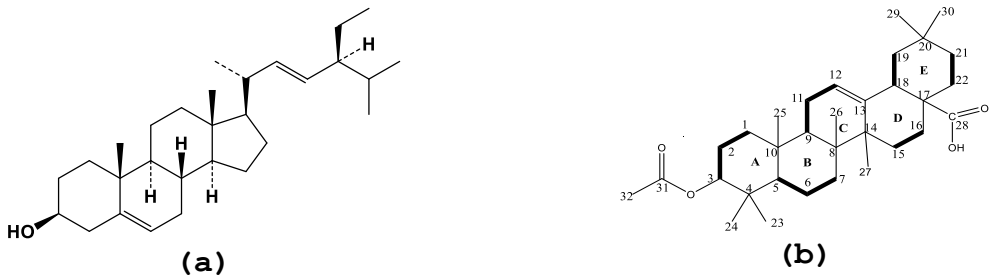

Fig.2:Structure compounds; 3-acetyl-12-oleanen-28-oat (a), stigmasterol (b)

Furthermore, two new compounds are found on the tissue stem wood of *M. umbellata*(Fig. 3a) which are highly toxic to *A. salina* and murine leukemia cells P-388 as anCleomiscosin (Fig. 3b)i.e. Walterion C and Cleomiscosin [12].

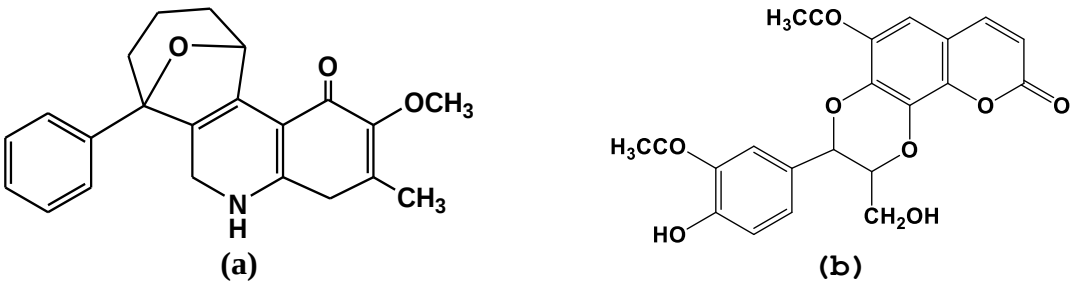

Fig.3:Structure compounds: Walterion C (a),Cleomiscosin (b)

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

EXPERIMENTAL

Equipment and materials

Equipment used are glass wares commonly used in laboratory chemistry, column vacuum chromatography (CVC), column compression chromatography (CCC), gravity column chromatography (GCC), thin layer chromatography (TLC) 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 aresamples of bark*M. umbellata* (Houtt) Stapfvar. Degrabratawith 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).

Preparation of Sample

M. umbellata bark samples used were collected from Tamalanrea Makassar, South Sulawesi. The sample is cleaned, cut into small pieces, then dried in the open air (at room temperature).

Furthermore, the bark of *M. umbellata* is milled into powder with a size of 90 mesh.

Isolation and Purification

M. umbellata bark samples of 5 Kg were macerated with methanol solvent for 3 × 24 hours. the obtained macerate 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 then weighed for rendamen determination, 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 rechromatography. The isolate purity test obtained was done by analysis of TLC of three eluent systems and melting point test using Melting Point Apparatus.

Phytochemical Test

Phytochemical test of hexane extract bark of *M. umbellata* 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_2SO_4, and 10% NaOH solution, Steroid and Triterpenoid Test using Lieberman-Burchard reagent, phenolic test using $FeCl_3$ reagent, and saponin test using hot water and 2 N HCl solution [13,14,15].

Antibacterial Activity Test

Preparation of 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 [16].

Preparation of bacterial suspension test

Test bacteria (bacteria *B. subtilis, S. aureus, E. coli,* and P. aeruginosa) were cultured on growth NAmedia 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 shaken using a water bath rocked with a speed of 100 rpm [17].

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.

Testing of antibacterial activity

Antibacterial activity was tested 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 contains 100 mL of media NA. The mixture was 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, a paper disc was prepared, dregs hexane extract samples and the third isolates by variation concentrations of 250 µg/mL, 500 µg/mL, 750 µg/mL, and 1000 µmL and then stand for 15 minutes. The aseptic paper discwas placed on the surface of a petri dish containing the test bacteria. Positive controls used were paper discs with chloramphenicol (0.2 mg/mL), the negative control used was paper disc dyed with dimethylsulfoxide (DMSO) 5%. Petri dish was incubated at 37°C for 24 hours. Clearly visible zones encoded with disc paper was indicated the presence of antibacterial activity. Furthermore, the clear zone formed was measured using the sliding term expressed by the size of the diameter inhibit zone.

RESULTS AND DISCUSSION

The result of extraction (5 kg sample) by means of maceration (solid-liquid extraction) using methanol obtained extract reddish brown as much as 396.5 g. The extract 300 g was partitioned (Liquid-liquid extraction) using hexane solvent and obtained hexane extract of yellowish green as much as 36.10 g. Subsequently, 30 g of hexane extract were separated by using CVC 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 were combined to obtain 16 main fractions. The combined fraction is further fractionated by CCC and GCC with eluent; hexane, hexane : ethyl acetate ratio, ethyl acetate, and methanol. The fractionation was obtained by white

crystals from D fraction and two other white crystals of F fraction i.e. FKa and FKb compounds. The purity test of isolate D compound was carried out in a general way i.e. TLC analysis using three eluent systems and the determination of melting point. TLC analysis showed one spot after being sprayed with serum sulfate and heated over the hot plate. The result of melting point measurement 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 were carried out in the same way as purity test of compound D. The result of TLC analysis of FKa and FKb isolate compounds were by using eluent of hexane: ethyl acetate (7: 3), showing single spot. The melting point measurement of isolate FKa and FKb compounds were 115 – 117°C and 184 – 185°C, respectively. Phytochemical test of isolates FKa and FKb compoundswere prepared by using Liberman-Bucher reagent with 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 1.

Table 1. Weight of the hexane extract and the three isolates from the bark of *M.umbellata*

No.	Type Extract / Isolate Compounds	Weight (g)
1	Hexane extract	36.1000
2	IsolateCompounds D	0.0182
3	IsolateCompounds FKa	0.0204
4	IsolateCompounds FKb	0.0176

Phytochemical tests were performed to determine the presence of secondary metabolite group compounds contained in plants. The phytochemical test result of hexane extract and the three isolate compounds from *M. umbellata* bark can be seen in Table 2.

Table 2. Phytochemical test results of hexane extract and three isolates from *M. umbellata* stem bark.

No	Phytochemical test	Extract and Isolate	Information
1	Alkaloids • Meyer Test	- N-Hexan Extract	Orange precipitate is formed (+)

		- solateCompounds D	(-)
		- Isolate Compounds FKa	(-)
		- Isolate Compounds FKb	(-)
	• Dragendorffs Test	- N-Hexan Extract	No precipitation (-)
		- Isolate Compounds D	(-)
		- Isolate Compounds FKa	(-)
		- Isolate Compounds FKb	(-)
	• Wagner Test	- N-Hexan Extract	White precipitate is formed (+)
		- Isolate Compounds D	(-)
		- Isolate Compounds FKa	(-)
		- Isolate Compounds FKb	(-)
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	(-)

Information :

(+) = Positve (-) = Negative

Based on the phytochemical tests results (Table 2) as presented that the hexane extract contains alkaloid and triterpenoids group compounds. The isolate of compound D contains triterpenoid. FKa and FKb contain steroid group compound. Flavonoids, polyphenols, and saponins were not identified in the hexane extract and the third isolates of the compound. Previous researchhas been reported that the methanol extract from the bark of *M. umbellata* contains alkaloids, flavonoid, phenolic, triterpenoid and saponin compounds [10]. Uddin et al. reported that *M. umbellata* contains essential oil compounds,

triterpenoids, alkaloids, and flavonoids [7].Other research from *Melochia corchorifolia* L (Sterculiceae) is known to contain alkaloid group compounds, terpenoids, steroids, phenolic compounds, flavonoids, and glycosides [18]. Then the stemwood tissue of *Kleinhovia hospita* (Sterculiaceae) is known to contain triterpenoid group compounds.

Table 3. Phytochemical positive test results of hexane extract and three isolates from *M. umbellata*stem bark

No	Phytochemical test	Exstract and Isolate	Information
1	Alkaloids		
	• Meyer Test	- N-Hexan Extract	Orange precipitate is formed (+)
	• Wagner Test	- N-Hexan Extract	White precipitate is formed (+)

Table 4. Phytochemical positive and negative test results of hexane extract and three isolates from *M. umbellata* stem bark

No	Phytochemical test	Exstract and Isolate	Information
1	Steroids / triterpenoids LB	- N-Hexan Extract	**(-/+)**
		- Isolate Compounds FKa	**(+/-)**
		- Isolate Compounds FKb	**(+/-)**

Table 5. Phytochemical positive and negative test results of hexane extract and three isolates from *M. umbellata* stem bark

No	Phytochemical test	Exstract and Isolate	Information
1	Alkaloids	- Isolate Compounds D	(-)
	• Meyer Test	- Isolate Compounds FKa	(-)
		- Isolate Compounds FKb	(-)
	• Dragendorffs Test	- N-Hexan Extract	No precipitation
			(-)
		- Isolate Compounds D	(-)
		- Isolate Compounds FKa	(-)
		- Isolate Compounds FKb	(-)
	• Wagner Test	- Isolate Compounds D	(-)
		- Isolate Compounds FKa	(-)
		- Isolate Compounds FKb	(-)
2	Flavonoids	- N-Hexan Extract	(-)
		- Isolate Compounds D	(-)
		- Isolate Compounds FKa	(-)
		- Isolate Compounds FKb	(-)
3	Steroids / triterpenoids LB	- Isolate Compounds D	(-)

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	(-)

Tables 3, 4, and 5 shows that the statistical analysis results of hexane extract and three isolates from *M. umbellata* stem bark. In Table 3 explained that the alkaloids compound by using Meyer and Wagner tests give the information of N-Hexane extract is a positive result (one extract). Table 4 shows that the phytochemical test steroids/triterpenoids LB compounds in N-hexane, Isolate Fka, and FKb (Three extracts) give the positive and negative responses. Meanwhile, Table 5 gives the negative responses in 5 photochemical tests (alkaloids, flavonoids, steroids/triterpenoids LB, phenolic, and saponin) where the N-hexane extract, isolate compounds D, compounds FKa, and compounds FKb. In shortly, the positive, positive and negative, then negative tests results explain that one extract, three extracts, and four extracts, respectively.

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 [19].

Antibacterial activity can be determined by detecting the inhibit zone (clear zone) on the bacteria growth test. It is shown by the extract and the three isolates encapsulated in the solid paper. The results showed hexane extract and three isolates from the *M. umbellata* bark were able to inhibit the bacteria growth test. The mean inhibitory zone diameter which is a test of antibacterial activity can be seen in Table 3.

Based on Table 3 showed that hexane extract at concentration of 1000 ppm has an inhibitory effect on the 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 was 13.4 mm and 11 mm, 0 mm and showed weak activity against *P. aureginosa* bacteria with inhibition zone of 7.2 ppm,respectively.

Table 6. 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

Information :
PC (positive control) = Chloramphenicol 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 ppm 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 isolate from these plants showed the inhibitory effect against *B. subtilis, S. aureus,*and *E.coli* bacteria at a 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. umbellata* 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 extract has the highest inhibitory on the growth of *S. dysenteriae* bacteria with a diameter of the inhibitory zone of 17.70 mm [20]. 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 [11].

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 [21].

According to Wattimena that theact as an antimicrobial if such compound provides an average of inhibition zone > 14 mm. Based on the results of antibacterial test, it can be concluded that FKa compound isolates from *M. umbellata* 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* [22].

CONCLUSION

The hexane extract of the stem bark (*M. umbellata*) contains alkaloid and triterpenoids group compounds, Isolate D compound contains triterpenoid group compounds, as well as isolates FKa and FKb compound containing steroid group compounds. IsolateFka compound has 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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Author contributions

The contributions of author for this paper to describe the traditional plant (*M. umbellata*)from South Sulawesi-Indonesia was potentially for medicinal drug (antibacterial).

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