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Triterpene Compound from Ethyl Acetate Fraction of Kesambi Bark (*Schleichera oleosa*) and Its Activity as Anti-Bacterial

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Abstract

In our earlier study, we managed to find the antibacterial activity of the ethyl acetate fraction of the kesambi bark stem. In our next study, we isolated secondary metabolite compounds from the ethyl acetate fraction of the kesambi stem bark and tested the antibacterial activity of isolates against the bacteria *Escherichia coli* and *Staphylococcus aureus*. Separation is carried out by the method of column chromatography. Analysis of stain patterns and purity was performed with thin-layer chromatography (TLC). The test of antibacterial activity was carried out by the Kirby Bauer method. Characterization of pure isolates was performed with IR, ^{1}H -NMR, ^{13}C -NMR and DEPT 135° spectroscopy. The results of the characterization of pure isolates show that the isolated compound is a triterpenoid compound with the name IUPAC compound lup-20(29)-en-3-ol. The results of antibacterial tests showed that the compound lup-20(29)-en-3-ol has antibacterial activity against *E. coli* and *S. aureus* which is relatively moderate.

Keywords: Kesambi, Schleichera oleosa, Triterpenoid, Antibacterial

INTRODUCTION

The use of traditional medicine or better known as herbal medicine in the last two decades continues to increase (Situmeang et al., 2018). The discovery of various new medicinal compounds sourced from natural materials further clarifies the significant role of secondary metabolites of plants as a source of medicinal raw materials (Nogueira et al., 2022). Starfruit seeds extract has been proven to have anti-microbial activities showing potential for natural material properties as anti-microbial (Hidayat, 2017). Information about the content of plants that have properties for health is still unknown. Like the kesambi plant (*Schleichera oleosa*), from some earlier studies it is mentioned that this plant has many benefits. The people of Bali and Madura use kesambi bark as a remarkably effective skin medicine, especially against scurvy and other skin diseases (Goswami & Singh, 2019; Sari et al., 2019; Istiqomah et al., 2021). Kesambi seeds contain about 70% oil and can be used as feedstock for biodiesel synthesis (Hendra & Wibisono, 2022; Asri et al., 2020).

Research Kumar et al., (2017) and Jose et al., (2019) reported that kesambi plant extract has antioxidant, antimicrobial, and anticancer activity, and based on phytochemical test results kesambi extract has terpenoid compounds, flavonoids, and phenolics. The antibacterial activity of the kesambi bark has been known through research conducted by Dhego et al., (2018), kesambi bark extract has an activity on healing bacterial infections *S. aureus*. Situmeang et al., (2019), conducted phytochemical screening research and antibacterial activity tests of methanol, ethyl acetate extracts, and *n*-hexane of the bark of the kesambi stem. Kesambi bark ethyl acetate extract has a strong antibacterial activity against *E. coli* and *S. aureus* (Munhoz et al., 2014; Situmeang et al., 2019).

Secondary metabolite compounds of the triterpenoid group are known to have various activities including antibacterial, antitumor, anti-inflammatory, and antidiabetic (Chuke & Deigner, 2019). Triterpenoid compounds from kesambi bark extract of Indian origin have activity as antimicrobial, antifungal, and antibacterial (Sarkar et al., 2022). The purpose of this study was to isolate and characterize triterpenoid compounds and their activity as antibacterial of the active fraction of ethyl acetate of kesambi stem bark. Previous studies conducted by Salimi et al. on 2019 shows the potential of n-hexane fraction of *Moringa Oleifera* leaf anti-bacterial activity. Therefore, this research was conducted to

research the ethyl acetate fraction of kesambi stem mainly its triterpenoid compound anti-bacterial activity as its potential has been previously proven in order to prove the fraction contains triterpenoid and to analyse the compound anti-bacterial activity.

METHODOLOGY

Materials and Instrumentals

Schleichera oleosa stem bark, ethyl acetate, *n*-hexane, distilled water, silica gel G60, H_2SO_4 concentrated, *Liebermann-Burchad*, Bacto agar, *E. coli* and *S. aureus* bacteria, laboratory glassware, analytical balance, filter paper, rotary evaporator, static pole, chromatography column, aluminum foil, chamber, capillary pipe, paper disk, cotton, stick, UV λ 254 and 65 nm lamp, silica plate GF_{254}, FT-IR and NMR.

Sample Preparation

A total of 1.5 kg of kesambi bark is dried at room temperature for ± 3 days. The dried kesambi bark is then cut into small pieces and ground into a fine powder.

Extraction Process

A total of 1 kg of fine powder that has been dried (simplisia) is stratified macerated using n-hexane solvents, ethyl acetate and methanol. Maceration is carried out for 3 x 24 hours, while stirring. After that, it is filtered with filter paper until *liquid* extracts of n-hexane, ethyl acetate, and methanol of the stem bark are obtained. The extract obtained was then concentrated using a *rotary evaporator* at a temperature of ± 40 °C. The concentrated extract obtained was weighed to determine the mass of the concentrated extract.

Fractionation Stage

The obtained ethyl acetate extract was analyzed using TLC with the aim of figuring out the proper solvent type on column chromatography. Fractionation was carried out using column chromatography, silica gel G60 as the stationary phase and n-hexane: ethyl acetate eluent as the mobile phase with various ratios whose polarity was improved with 10% gradient. Each fraction obtained was evaporated solvent using an *evaporator*. After the entire fraction was evaporated, stain pattern separation analysis was carried out using TLC with a ratio of n-hexane: ethyl acetate eluent. Factions that have the same stain pattern are combined.

The combined fractions obtained are further fractionated using column chromatography using isocratic separation of n-hexane: ethyl acetate eluent (7:3, v/v). The results of this process obtained one compound that has a single stain pattern observed with 254 and 365 nm UV lamps and H_2SO_4 stain imaging reagents.

Purification Process

Purification is carried out against fractions showing a single stain pattern by recrystallization to produce white solids, purity tests are carried out using TLC with 2 different eluents of polarity fractionation between n-hexane and ethyl acetate (7:3 and 8:2, v/v). Phytochemical testing is performed using *the Liebermann-Burchard reagent*. The total mass of the isolate obtained is calculated.

Characterization

The isolated compounds were characterized using NMR (^{1}H-NMR 500 MHz, ^{13}C-NMR 125 MHz, DEPT 135°).

Kirby Bauer Method of Antibacterial Activity Test

Each bacterial Ose (*E. coli* and *S. aureus*) from the stock was inoculated into a sterile test tube containing a physiological NaCl suspension of 5 mL until it reached a turbidity level of $^{1}/_{2}$ *Mac Farland*. The achievement of turbidity was carried out by comparing with the standard and then incubated for 16-18 hours at 37 °C.

Cotton swab is dipped in bacteria and then applied to the surface of the solid media until evenly distributed, then as much as 15 µL of sample, positive control, and negative control are dripped on *paper disk* then placed on solid media, incubated at 37 °C for 24 hours. After 24 hours, the diameter of the clear zone around the disk was observed and measured using calipers.

RESULTS AND DISCUSSION

Extraction and Fractionation

A total of 1 kg of simplisia was extracted by stratified maceration using n-hexane solvents, ethyl acetate and methanol. The choice of maceration method as an extraction method because it is easy to do and does not need heating, so it is unlikely that chemical compounds will be damaged or decomposed. Immersion carried out on plant samples results in the breakdown of cell walls and membranes due to pressure differences between inside and outside the cell so that the secondary metabolites contained in the cell will be dissolved in organic solvents. The use of *n-hexane* solvents aims to attract compounds that are nonpolar, ethyl acetate solvents aim to attract compounds that are semi-polar, and methanol solvents aim to attract compounds that are polar.

Furthermore, the macerated filtrate is concentrated using a *rotary evaporator* at a temperature of ± 40 °C. The concentrated extract from the n-hexane fraction obtained was 10 grams, the ethyl acetate fraction was 13 grams, and the methanol fraction was 155 grams (Table **2**). The solvent evaporation technique was carried out to obtain concentrated extracts of n-hexane, ethyl acetate, and methanol quickly and effectively. Evaporation is carried out at a temperature of ± 40 °C aimed at preventing the decomposition of the compounds contained in it.

The concentrated extract of the ethyl acetate fraction (13 g) separated its constituent compound components using the column chromatography method with the silent phase of silica gel G60 (70-230 mesh), the *n-hexane* and ethyl acetate phases, graded 10% to the resulting 11 fractions. Purification was done using column chromatography with silica gel G60 as stationary phase and n-hexane:ethyl acetate for the mobile phase. The purification intended to separate the product from the starting material into 11 fractions. The fraction then characterized using Thin Layer Chromatography (TLC). The fraction was analyzed using UV lamp with 254 and 365 nm wavelength as seen on Figure 1.

According to the Figure 1, the compound shows single pattern proving its purity and it does not fluoresce under the said wavelength meaning no conjugated carbon group to show the characteristic of triterpenoid (Salimi et al, 2019).

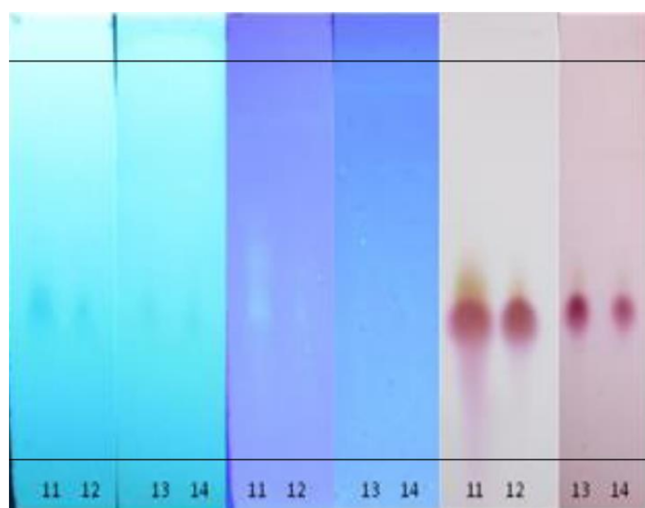

Figure 1. TLC result of fractions under UV lamp

Compound Characterization

The functional groups contained in the compound determined by using the infrared spectrum. The infrared (IR) spectrum in the KBr plate is shown in Figure 2.

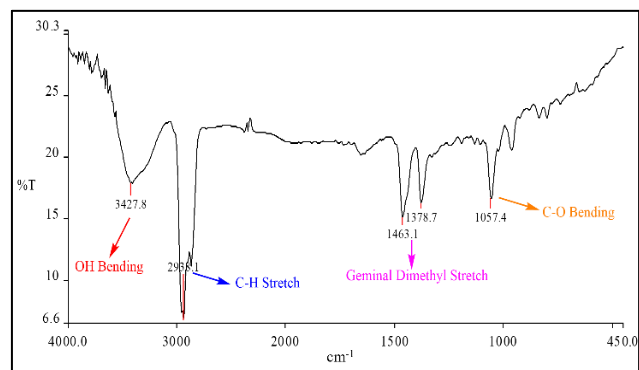

Figure 2. Infrared (IR) spectrum in KBr plates

Based on Figure 1, the IR Spectrum (KBr) of the isolated compound shows absorption bands at 3427.8 cm^{-1} which are sharp and wide enough as hydroxyl group absorption (O-H) followed by absorption at wave number 1057.4 cm^{-1} which is the extended strain of the C-OH group. At 2938.10 cm^{-1} there is a very strong uptake of the aliphatic C-H group stretch followed by absorption at 1456.32 cm^{-1} which is the C-H bend and at 1374.23 cm^{-1} which is a dimethyl gem strain (Muharni, 2010). To determine the amount and type of carbon in the isolated compound, further measurements were taken using ^{13}C-NMR. The ^{13}C-NMR spectrum is shown in Figure 3.

Based on Figure 3, Spectrum ^{13}C-NMR compound the results of the isolation showed the presence of thirty carbon signals for the triterpenoid skeleton consisting of 28 carbon atoms *sp3* and two C=C carbon atoms *sp2*. At spectrum ^{13}C-NMR It appears that there are only two signals appearing in the > region of 100 ppm indicating there is only one olefinic carbon or exocyclic double bond on the shift δ_C 109.50 and 151.15 ppm and the absence of a signal for the carbonyl C group (Muharni, 2010). Furthermore, there is also a signal on the shift δ_C 79.16 ppm, which is a typical shift from C bound to OH. The presence of a signal stack in the area under of 55 ppm indicates a carbon atom sp^3 Aliphatic which can be either a straight or cyclic chain that is thought to be derived from an aliphatic chain of the pentacyclic triterpenoid group.

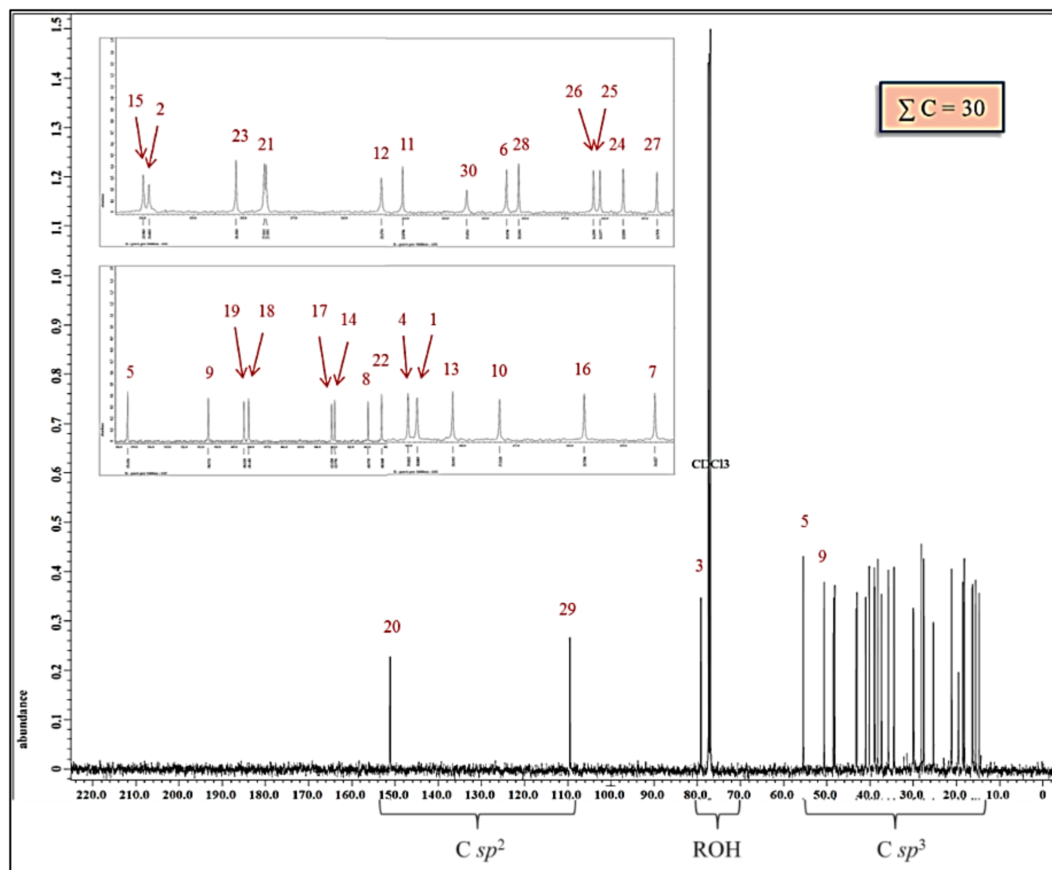

Figure 3. Spectrum of ^{13}C-NMR isolated compounds (125 MHz, $CDCl_3$)

Based on the spectrum ^{13}C-NMR it is suspected that the compounds obtained from the isolation results are triterpenoid group compounds that have one isolated double bond and one OH group. To determine the types of carbon in these compounds, a 135° DEPT spectrum measurement was carried out in Figure 4. Based on Figure 4, the analysis using the DEPT 135° technique states that methyl and methane signals will appear upwards, methylene signals will appear downwards, and quaternary carbon will not give signals. The isolated compound has thirty carbon signals consisting of seven methyl carbon atoms at δ_C 14.70; 15.55; 16.13; 16.29; 18.16; 19.47, and 28.15 ppm. Six methine sp^3 signals at δ_C 38.19; 48.14; 48.43; 50.57; 55.43, and 79.16 ppm. Eleven methylene carbon atoms consisting of ten methylene sp^3 signals at δ_C 18.47; 21.07; 25.27; 27.59; 29.88; 29.99; 34.42; 35.73; 38.84, and 40.16 ppm and one methylene sp^2 δ_C 109.50 ppm. The number of quaternary carbon atoms is six signals, five of which are sp^3 quaternary carbon at δ_C 37.31; at 39.02; 40.97; 42.97, and 43.15 ppm and one of them is sp^2 quaternary carbon δ_C 151.15 ppm.

The presence of methylene carbon signals and sp^2 quaternary carbon at a shift of δC of 109.50 ppm (C methylene) and 151.15 ppm (C quaternary) indicates that the double bond in the compound is a *double bond terminal*, not *an internal double bond*. This double bond contributes one degree of unsaturation.

The ^{1}H-NMR spectrum is shown in Figure 5. The ^{1}H-NMR spectrum provides information about the number of protons, the type of proton contained in a molecule, and determines the environmental properties of each proton by comparing reference data with coupling analysis of each spin. Spectrum analysis ^{1}H-NMR (Figure 5) the isolated compound shows a signal that is not separated either in the area below four which is an alicyclic proton from the basic frame of the triterpenoid and there is no visible proton signal in the aromatic region. This is a distinctive feature of the spectrum ^{1}H-NMR for compounds of the triterpenoid group (Sharma et al., 2020). Signals on the area δ_H 4.68 and 4.56 ppm are thought to be signals from methylene protons on C-29 bound to quaternary C. Shift by δ_H 4.68 and 4.56 ppm due to the influence of anisotropy fields on the alkene group, causing the chemical shift value to be more *downfield* (Mailafiya et al., 2020).

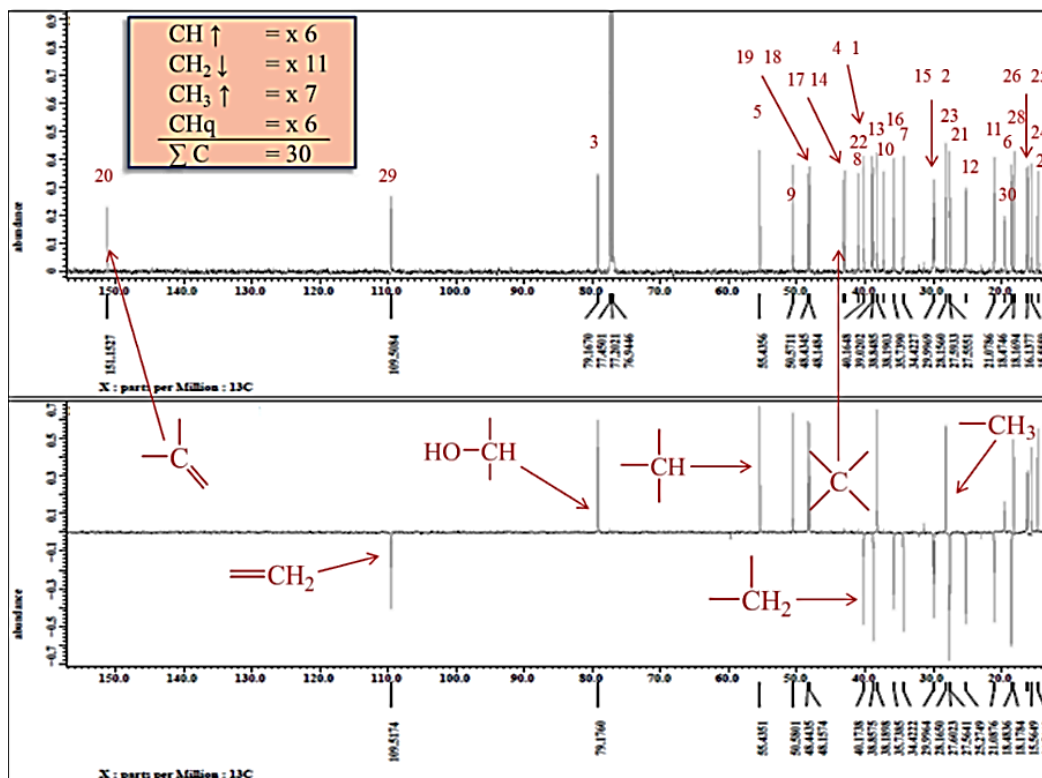

Figure 4. DEPT 135° isolated compounds (125 MHz, $CDCl_3$)

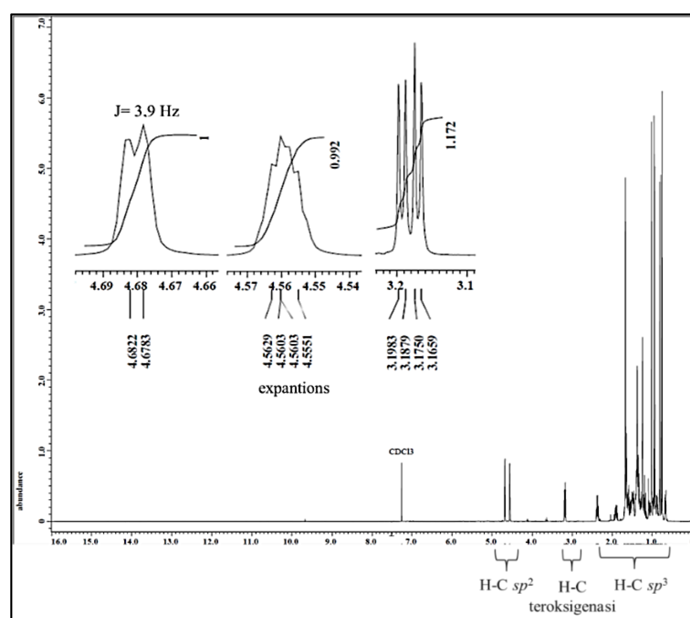

Figure 5. Spectrum of ^{1}H-NMR isolated compounds (500 MHz, $CDCl_3$)

Furthermore, it is seen that there is a signal for methine protons in the δ_H 3.17 ppm (1H, *q*) which is thought to be a methane proton bound to C that binds to OH that is coupled with its two neighboring protons and is also coupled with protons from the OH group so that it appears as the peak of the quartet. This signal is typical for triterpenoids that bind OH at position C-3. Signals on δ_H 2.36 ppm (1H, *m*) is a signal of an alicyclic ring methine proton of the triterpenoid frame. On the spectrum ^{1}H-NMR Also seen are seven signals typical of methyl protons for triterpenoids in δ_H 0.75 (3H), 0.78 (3H), 0.82 (3H), 0.94 (3H), 0.96 (3H), 1.02 (3H), and 1.67 (3H) ppm. After analysis of the spectrum ^{1}H-NMR, ^{13}C-NMR, DEPT 135°, HMQC, ^{1}H-^{1}H COSY, and the HMBC spectrum then processed the complete data of its

chemical shifts shown in Table 1. Based on the analysis of NMR two-dimensional data, fragments and alleged structures of the isolated compounds shown in Figure 6 were obtained. Based on the merger of fragments of isolated compounds, the alleged structure of these compounds was obtained, namely the triterpenoid compound pentacyclic lup-20(29)-en-3-ol. A distinctive characteristic of this compound is the presence of a dimethyl gem and a double bond that is outside of the cyclic skeleton located in C-20 and C-29.

Such *dimethyl gem clusters* are located on carbon numbers 23 and 24. This compound has the molecular formula $C_{30}H_{50}O$. The IUPAC name of this compound is lup-20(29)-en-3-ol with the trivial name lupeol (Muharni, 2010). Based on the alleged molecular formula, *a double bond equivalent* (DBE) value is obtained with the following formula (Equation 1).

$$DBE = \Sigma Catom - \frac{\Sigma \text{ atom H}}{2} - \frac{\Sigma \text{ Halogen}}{2} + \frac{\Sigma \text{ atom N}}{2} + 1 \quad (1)$$

Based on the calculation of the DBE formula, it is known that the DBE value of the compound is 6, meaning that the compound has 5 cyclic and 1 double bond.

Table 1. NMR data of isolated compounds
(500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR, *chloroform-$CDCl_3$*)

C Position	^{13}C-NMR d_C (ppm)	DEPT 135°	^{1}H –NMR d_H (Int., mult., J=Hz)
1	38,84	CH_2	1,19 (1H; s) & 1,43 (1H; m)
2	29,88	CH_2	1,65 (2H; m)
3	79,16	CH	3.17 (1H; q)
4	39,02	Cq	-
5	55,43	CH	0,83 (1H; s)
6	18,47	CH_2	1,42 (1H; m) & 1,57 (1H; m)
7	34,42	CH_2	1,55 (2H; m)
8	40,97	Cq	-
9	50,57	CH	1,38 (1H; m)
10	37,31	Cq	-
11	21,07	CH_2	1,62 (2H; m)
12	25,27	CH_2	1,65 (2H; m)
13	38,19	CH	0,75 (1H; m)
14	42,97	Cq	-
15	29,99	CH_2	1,22 (2H; m)
16	35,73	CH_2	1,3 (1H; m) & 1,9 (1H; m)
17	43,15	Cq	-
18	48,43	CH	0,96 (1H; m)
19	48,14	CH	2.36 (1H; (*m*)
20	151,15	Cq	-
21	27,59	CH_2	0,79 (1H; s) & 2,3 (1H; dt)
22	40,16	CH_2	1,48 (2H; m)
23	28,15	CH_3	0,96 (3H; s)
24	15,55	CH_3	0,75 (3H; s)
25	16,13	CH_3	0.82 (3H; s)
26	16,29	CH_3	1.02 (3H; s)
27	14,70	CH_3	0,94 (3H; s)
28	18,16	CH_3	0,78 (3H; s)
29	109,50	CH_2	4,68 (1H; dd; 3,9 Hz) 4.56 (1H; *d*)
30	19,47	CH_3	1,67 (3H; s)

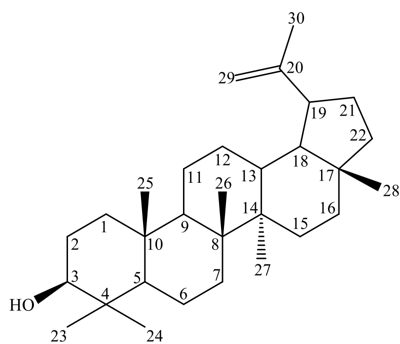

Figure 6. Lup-20(29)-en-3-ol compounds

Antibacterial Activity Test

Results of antibacterial tests of triterpenoid compounds (lupeol) against *E. coli* and *S. aureus.* According to the result obtained as shown on Table 2, lupeol has moderate anti-bacterial activity towards *E.coli* and *S. aureus* based on inhibition test. The result is moderate inhibition for value between 10-20 mm and high inhibition for value of >20 mm.

Table 2. Data on antibacterial test results

Bacteria	(-) control	(+) control	600 ppm	800 ppm	1000 ppm
E. coli	0	21	16,8	16,9	17,4
S. aureus	0	22	18,8	20,2	21,5

E. coli = amoxicillin
S. aureus = ciprofloxacin

CONCLUSION

Based on the research that has been done, it can be concluded that the triterpenoid compound obtained from the ethyl acetate fraction of the kesambi stem bark *(Schleichera oleosa)* is a lup-20(29)-en-3-ol compound. The results of antibacterial tests showed that the compound lup-20(29)-en-3-ol has antibacterial activity against *E. coli* and *S. aureus* which is relatively moderate.

REFERENCES

Asri, N. P., Yuniati, Y., Hindarso, H., Suprapto, & Yogaswara, R. R. (2020). Biodiesel Production from Kesambi (Schleichera oleosa) Oil Using Multi-Walled Carbon Nanotubes Supported Zinc Oxide as a Solid Acid Catalyst. *IOP Conference Series: Earth and Environmental Science*, *456*(1).

Csuk, R., & Deigner, H. P. (2019). The potential of Click Reactions for The Synthesis of Bioactive Triterpenes. *Bioorganic and Medicinal Chemistry Letters*, *29*(8), 949–958.

Dhego, A. P., Susanti, L., & Wibawa, D. A. A. (2018). Antibacterial Activity Test of Kesambi Bark Extract Ointment (Schleichera oleosa Merr) against Infected *Staphylococcus aureus* ATCC 25923 in Rabbits. *Biomedics*, 10(2), 11–17.

Goswami, S., & Singh, R. P. (2019). Antidiabetic Potential and HPTLC Fingerprinting of Schleichera oleosa (Lour) Oken. *Pharmacognosy Journal*, *11*(3), 469–474.

Hendra, D., Wibowo, S., & Wibisono, H.S. (2022). *Biodiesel Biodiesel from Several Types of Forest Crops*. Bogor: Forest Products R&D Center.

Hidayat, H. H. (2017). Biochemical Test and Isolation of Antimicrobial Activity from Seeds Starfruit Extract (Averrhoa carambola.L). *Indonesian Journal of Chemical Research*, *3*(1), 6–19.

Istiqomah, Yahdi, & Dewi, Y. K. (2021). SPIN Based on World Health Data Based on Ni Made's Research. *Chemistry & Chemistry Education*, *3*(1), 22–31.

Kumar, A., Kataria, D., & Chahal, K. K. (2017). A Review on Phytochemistry and Pharmacological Activities of Cyperus scariosus. *Journal of Pharmacognosy and Phytochemistry*, *6*(1), 510–517.

Mailafiya, M. M., Pateh, U. U., Hassan, H. S., Sule, M. I., Bila, A. H., Musa, T. L., … Achika, J. I. (2020). Isolation of Lupeol from The Stem Bark of *Leptadenia Hastata* (Pers.) Decne. *Journal of Applied Sciences and Environmental Management*, *24*(10), 1835–1838.

Muharni. (2010). Triterpenoid lupeol of Forest Mangosteen (Garcinia bancana Miq.). *Journal of Science Research*, *13*(3), 40–45.

Munhoz, V. M., Longhini, R., Souza, J. R. P., Zequi, J. A. C., Mello, E. V. S. L., Lopes, G. C., & Mello, J. C. P. (2014). Extraction of Flavonoids From Tagetes Patula: Process Optimization and Screening for Biological Activity. *Revista Brasileira de Farmacognosia*, *24*(5), 576–583.

Nogueira, L. A., Figueiredo, Y. G., Ramos, A. L. C. C., Correia, V. T. da V., Nunes, B. V., Ribeiro, L. V., … Melo, J. O. F. (2022). The Presence of Flavonoids in Some Products and Fruits of the Genus Eugenia: An Integrative Review. *Frontiers in Food Science and Technology*, 2(May), 1–12.

Salimi, Y., Bialangi, N., Abdulkadir, W., & Situmeang, B. (2019). Triterpenoid Compound From n-Hexane Extract of Kelor Leaves (Moringa oleifera Lamk.) And Antibacterial Activity Test Against Staphylococus aureus and Escherichia coli, *Indonesian Journal of Chemical*

Research, 7(1), 32-40.

Sari, P., Sugita, P., & Santoso, A. (2019). Antioxidant, Antibacterial, and Toxicity Activity of Kesambi Tree Bark Extract (Schleichera oleosa (Lour) Oken). *Indonesian Journal of Herbal Medicine*, *4*(3), 112–118.

Sarkar, P. K., Sinha, A., Das, B., Dhakar, M. K., Shinde, R., Chakrabarti, A., … Bhatt, B. P. (2022). Kusum (Schleichera oleosa (Lour.) Oken): A potential multipurpose tree species, it's future perspective and the way forward. *Acta Ecologica Sinica*, *42*(6), 565–571.

Sharma, N., Palia, P., Chaudhary, A., Shalini, , Verma, K., & Kumar, I. (2020). A Review on Pharmacological Activities of Lupeol and its Triterpene Derivatives. *Journal of Drug Delivery and Therapeutics*, *10*(5), 325–332.

Situmeang, B., Malik Ibrahim, A., Bialangi, N., J.A Musa, W., & Silaban, S. (2019). Antibacterial activity and phytochemical screening of Kesambi (Sapindaceae) against Eschericia coli and Staphylococcus aureus. *Jurnal Pendidikan Kimia*, *11*(1), 14–17.

Situmeang, B., Suparman, A. R., Kadarusman, M., Parumbak, S., & Herlina, T. (2018). Isolation of Triterpenoid Compounds from Ethyl Acetate Extract of Pirdot (Saurauia vulcani. Kurth) Isolation of Triterpenoid Compound from Ethyl Acetate Extract of Pirdot, *Jurnal Kimia VALENSI, 4*(November), 93–97.