Study of Phytochemical, Antibacterial Activity and Toxicity on Acetone Extract Seed LeersiaHexandraSw

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Abstract. The Sayat-sayat(LeersiahexandraSw) plant is a plant used in traditional medicine by the Batak Karo people in North Sumatra. This study aims to examine the phytochemical content, antibacterial activity and cytotoxic test of acetone extract of L.hexandraSw seeds. Phytochemical tests include tests for alkaloids, flavonoids, saponins, tannins and steroids. Antibacterial tests were carried out against Propionibacterium acnes ATCC (27853), Escherichia coli ATCC 25922, Bacillus cereus ATCC 1178, Salmonella enterica ATCC 14028 with the paper disc diffusion method M02-A11 (CLSI) and continued with the determination of MIC and MBC using the microdilution method M07-A9 (CLSI). The toxicity test used the Brint Shrimp Lethality Toxicity (BSLT) method using Artemia Salina Leach shrimp larvae. The results of phytochemical screening showed that the acetone extract of the L.hexandraSw seeds contained alkaloids, flavonoids and terpenoids. The results of the antibacterial activity test showed the best activity against B. cereus ATCC 1178 and S. enterica ATCC 14028 with an inhibition zone in the range of 8.4 -8.6mm, with MIC and MBC values of 625 µg/mL. The results of the toxicity test are toxic and potentially bioactive.

Keywords: LeersiahexandraSw, phytochemicals, toxicity, antibacterial

1. Introduction
Indonesia has wealth of biodiversity, one of them can be used as traditional medicine. One of biodiversity that has long been used by the Karonese in Sumatera as traditional medicine is the Sayat-sayat plant. These herbs are used to treat toothaches [1,2].In the traditional Senegalese pharmacopoeia, L.hexandra is used for the treatment of hemoptysis in patients who experience frequent coughs. In Cameroun, an aqueous extract of L. hexandra leaves and stems is traditionally used for the treatment of hypertension [3].

L. hexandraSw is a kind of weed that grows wild in dry, watery, humid and cold areas. Therefore, most research has been carried out on L. HexandraSw plants related to the ability of these plants to absorb metals through the roots, such as detoxification in wetlands from harmful water pollutants [Cr (VI)][4]. Use of L. hexandraSw for soil phytomediation on soils contaminated with fresh and weathered oil[5]. L. hexandraSwas phytoremediation of soil and water contaminated by Cr [4,6]. Based on the results of research on existing publications, there is no information / research on L. hexandraSw that supports its use as a traditional medicine. Therefore, this paper reports on the phytochemical screening, antibacterial test and toxicity test of L.hexandraSw seeds originating from North Sumatera.
2. Materials and Methods

2.1. Plants extract preparation
The sample studied was *L. hexandra* seeds obtained from the herbal medicine shop "Sempurna" Sambu Medan. The dry seeds of *L. hexandra* are then mashed (200 grams). After that it was macerated with acetone for 3 x 24 hours. The results of maceration are concentrated by evaporation using a rotary evaporator (Heidolph) until concentrated extract is obtained.

2.2. Antibacterial activity test
Based on the standard method that recommended by Clinical and Laboratory Standards Institute [7]. Antibacterial activity test carried out on pathogen bacterial Propionibacterium acnes ATCC (27853), *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 1178, *Salmonella enterica* ATCC 14028. Solution test was prepared at concentration 1% in 10% DMSO which is equivalent to 10,000 μg/mL and a standard solution of 500 μg/mL of chloramphenicol antibiotic. Determination of the Inhibition Zone using the paper disc diffusion method (CLSI-M02-A11) and the determination of the Minimum Inhibitory Concentration (MIC) by the micro dilution method (CLSI-M07-A9). The determination of the minimum bactericidal concentration (MBC) was carried out by growing 10 μL of sample from each hole of the micro plate on the surface of the media so that the MHA MHA[8].

2.3. Cytotoxic test
The cytotoxic test was carried out using the Brine Shrimp Lethality Test (BSLT) method referring to the research of Puspitasari et al. [9]. In summary: Artemia Salina Linch larvae grown from eggs were contacted with samples of various concentrations (1000, 500, 250, 100 and 10 ppm), then left to stand for 24 hours. Then counted the number of shrimp larvae that died from each concentration. The toxicity test for each concentration was repeated 3 times. The toxicity effect was analyzed from the observed percent mortality.

\[
\text{Mortality} = \frac{\text{the number of dead larvae}}{\text{the number of test larvae}} \times 100\%
\]

If there are dead larvae in control, the percentage of mortality is determined by this formula:

\[
\text{Mortality} = \frac{\text{test} - \text{control}}{\text{control}} \times 100\%
\]

The mortality of *A. salina* larvae was obtained through the probity table and regressed linearly.

\[
Y = a + bX
\]

Information:

- Y = probity value
- a = regression concentration
- b = Slope of the regression
- X = Logarithm of 10 test concentration

2.4. Phytochemical screening
Phytochemical screening is intended to identify the flavonoids, terpenoids, saponnins, tannins steroids contained in *L. Hexandra* Sw. Phytochemical screening is done by referring to the method that has been done in previous research[10].

3. Results and Discussion

3.1. Antibacterial test
The paper disc diffusion test is a preliminary test to see the inhibition of the sample against pathogenic bacteria. Antibacterial activity was measured from the clear zone diameter (mm). The acetone extract of *L. Hexandra* Sw seeds showed activity against gram-positive bacteria as summarized in Table 1.
Table 1. Data of average value of inhibition zone diameter of acetone extract of L. Hexandra Sw

|                      | Gram - | Gram + |
|----------------------|--------|--------|
| E. coli              | 22.4   | 22.2   |
| S. enterica          | 30.7   | 7.5    |
| P. acne              | 22.2   | 8.4    |
| B. cereus            | 25.9   | 8.4    |

Chloramphenicol

Aceton L. Hexandra Sw seeds extract

The zone diameter is categorized as weak if it is smaller or equal to 5 mm, moderate category if the inhibition zone is 5-10 mm, strong if the inhibition zone is 10-19 mm and is said to be very strong if the inhibition zone is greater than or equal to 20 mm [11]. Based on these criteria, the acetone extract of L. Hexandra Sw seeds showed moderate category against S. enterica ATCC 14028, P. acne ATCC (27853) and B. cereus ATCC 1178.

When compared with the standard chloramphenicol, the percentage effectiveness of the inhibition zone diameter of L. hexandra Sw acetone extract against S. enterica bacteria was 28%, the effectiveness against P. acne bacteria was 33%, and against B. cereus bacteria was 32%. Determination of MIC value to determine the minimum inhibitory concentration against bacteria. The MIC value of the seed extract of L. Hexandra Sw is as shown in Table 2.

Table 2. Data value of MIC acetone L. Hexandra Sw seeds extract

|                      | MIC (μg/mL) |
|----------------------|-------------|
|                      | Bacterial   |
| Chloramphenicol      | Pa Ec Se Bc |
| Acetone L.Hexandra Sw seeds extract | 0.49 0.97 0.48 0.48 |
|                      | 5000 ND 625 625 |

Note ND: Is not done

The activity of an extract is categorized as active when the MIC value is below 100 μg / mL, moderate if the MIC value is around 100 <MIC <625 μg / mL and weak if the MIC value is > 625 μg / mL [12]. Thus, the acetone extract of L.HexandraSw seeds inhibited the "medium" category of bacteria S. enterica and B. cereus, and the "low" category of P. acne. Determination of MBC was intended to see whether the acetone extract of L. HexandraSw seeds had bacteriostaticor bactericidal properties. The MBC value data is summarized in Table 3.

Table 3. Data value of MBC acetone L. Hexandra Sw seeds extract

|                      | MIC (μg/mL) |
|----------------------|-------------|
|                      | Bacterial   |
| Chloramphenicol      | Pa Ec Se Bc |
| Acetone L.HexandraSw seeds extract | 31.5 31.3 7.8 1.95 |
|                      | >5000 ND 625 625 |

Based on the data in Table 3, the acetone extract of L.hexandra Sw seeds is bactericidal against S.enterica and B.cereus with a concentration of 625 μg / mL and to kill P.acne requires a concentration greater than 5000 μg / mL.

3.2. Toxicity test

Toxicity test is a test to detect the toxic effect of a substance on a biological system and to obtain typical dose-response data from the test preparation. There is a positive correlation between the BSLT method and the cytotoxic test on cancer cell culture, so the method is often used as a preliminary test.
to determine whether a compound has potential or not as an anticancer [13]. The sample toxicity test was determined based on the value of the LC$_{50}$ which can kill $A$. salina by up to 50%. Furthermore, statistical calculations are carried out using probit analysis (probability). The results of the cytotoxy test on $A$.salina Leach are summarized in Table 4.

**Table 4.** BSLT (Brine shrimp lethality test) of acetone extracts $L$. hexandra$Sw$Seeds

| Treatment | Number of Mortality of Artemia Salina Leach |
|-----------|-----------------------------------------------|
|           | Concentration (ppm)                           |
|           | 10   | 100 | 500 | 1000 |
| 1         | 6    | 8   | 10  | 10   |
| 2         | 7    | 8   | 9   | 10   |
| 3         | 8    | 9   | 10  | 10   |
| Number of Mortality | 21 | 25 | 29 | 30 |
| Average   | 7    | 8.3 | 9.6 | 10   |
| % Mortality | 70% | 83% | 96% | 100% |

Based on the data in Table 4, this study shows that the higher the concentration of the test solution, the more $A$.salina larvae will die. Determination of the LC$_{50}$ value using the probit method which is summarized in Table 5.

**Table 5.** Death of larvae on acetone extracts $L$. hexandra$Sw$Seeds

| Concentration | Concentration log(x) | % Mortality | Probit Value |
|---------------|----------------------|-------------|--------------|
| 1000          | 3                    | 100%        | 7.33         |
| 500           | 2.69                 | 96%         | 6.75         |
| 100           | 2                    | 83%         | 5.92         |
| 10            | 1                    | 70%         | 5.52         |

From the data in Table 5, it is obtained the straight line equation $y = 0.8744x + 4.4787$ as shown in Figure 1.

**Figure 1.** LC$_{50}$ of the acetone seed extract

Based on the regression equation, the value of LD$_{50} = 3.9445$ ppm is obtained. Determination of bioactive potential is carried out by comparing the LC$_{50}$ value of a sample extract with the following criteria: LC$_{50}$ ≤ 30 ppm very toxic, 31 ppm ≤ LC$_{50}$ ≤ 1000 toxic, LC$_{50}$> 1000 ppm is not toxic. Thus,
the acetone extract of *L. Hexandra* Sw seeds is categorized as very toxic and has the potential as an anticancer.

3.3. Phytochemical screening

Phytochemical screening is one way to determine the active ingredients which are secondary metabolites contained in plant samples. The results of phytochemical screening on *L. Hexandra* Sw seeds are shown in Table 6.

Based on the results of phytochemical screening, the acetone extract of *L. Hexandra* Sw seeds contained secondary metabolites of the alkaloids, flavonoids and terpenoids, and tannins. Thus the antibacterial activity is caused by the presence of these secondary metabolites. Alkaloids can interfere with the peptidoglycan components of bacterial cells so that the cell wall layer is not formed completely and causes cell death [14]. Flavonoids cause damage to cell wall permeability [15]. Terpenoids cause cell membrane damage [16,17]. Whereas tannins cause cells to become lysed, besides that they have the ability to activate bacterial enzymes and disrupt the passage of proteins in the inner layers of cells [18]. Likewise, the death of larvae is related to the function of active compounds that can inhibit larvae. The way these compounds work is by acting as stomach poisoning, therefore when the secondary metabolites enter the body of the larvae, it will disturb the digestive tract and inhibit the taste receptors in the mouth area of the larvae [19].

| Phytochemical Test | Test Result |
|--------------------|-------------|
| Alkaloids          | +           |
| Flavanoids         | +           |
| Terpenoids         | +           |
| Steroids           | -           |
| Saponins           | -           |
| Tannins            | +           |

Information:

(+) There is a chemical content

(-) There is no chemical content

4. Conclusion

The acetone extract of the seeds contains secondary metabolites of alkaloids, flavonoids, terpenoids, and tannins. The best activity was shown against *B. cereus* ATCC 1178 and *S. enterica* ATCC 14028 with the inhibition zone in the range of 8.4 - 8.6 mm, with MIC and MBC values of 625 µg/mL. The results of the toxicity test were very toxic with an LD$_{50}$ value of 3.9445 ppm.

References

[1] Sembiring R, Utomo B and Batubara R 2013 Keanekaragaman vegetasi tanaman obat di hutan pendidikan Universitas Sumatera Utara kawasan taman hutan raya tongkoh kabupaten Karo Sumatera UtaraPeronema Forestry Science Journal219-22.

[2] Silalahi M, Nisyawati, Walujo E B, Supriatna J and Mangun wardoyo W 2015 The local knowledge of medicinal plants trader and diversity of medicinal plants in the Kabanjahe traditional market, North Sumatra, Indonesia Journal of Ethnopharmacology175432-443.

[3] Bilanda D C, Tcheutchoua Y C, Djomeni Dzefiet P D, Fokou D L D, Fouda Y B, Dimo T and Kamchouing P 2019 Antihypertensive activity of leersiahexandra Sw.(poaceae) Aqueous extract on Ethanol-Induced hypertension in wistar rat Evidence-Based Complementary and Alternative Medicine20191–9.

[4] Liu J, Zhang X H, You S H, Wu Q X, Chen S M and Zhou K N 2014 Cr (VI) removal and detoxification in constructed wetlands planted with Leersiahexandra Swartz Ecological engineering7136–40.

[5] Arias-Trinidad A, Rivera-Cruz M D C, Roldán-Garrigós A, Aceves-Navarro L A, Quintero-
Lizaola R and Hernández-Guzmán J 2017 Uso de Leersia hexandra (Poaceae) en la fitorremediación de suelos contaminados con petróleo fresco e intemperizado Revista de Biología Tropical65 21-30.

[6] Zhang X H, Liu J, Huang H T, Chen J, Zhu Y N and Wang D Q 2007 Chromium accumulation by the hyperaccumulator plant Leersia hexandra Schwartz Chemosphere67 1138-1143.

[7] Clinical and Laboratory Standards Institute 2012 Performance standards for antimicrobial disk susceptibility tests; Approved standard-eleventh edition CLSI document M02-A11, Clinical and Laboratory Standards Institute, USA.

[8] Juwitaningsih T, Jahro I S, Dumariris I, Hermawati E and Rukayadi Y 2020 Phytochemical, antibacterial, antioxidant and anticancer activity study of M. candidum leaf acetone extract Actualites Permanentes En Microbiologie Clinique2 13-20.

[9] Puspitasari E, Rozirwan R and Handri M 2018 Uji toksisitas dengan menggunakan metode brine shrimp lethality test (bslt) pada ekstrak mangrove (Avicennia marina, Rhizophoramucronata, Sonneratia alba dan Xylocarpusgranatum) yang berasal dari Banyuasin, Sumatera Selatan Jurnal Biologi Tropis18 91-103.

[10] Juwitaningsih T, Jahro I S, Sari S A and Rukayadi Y 2020 Antibacterial activity of various medicinal plants in North Sumatera against common human pathogens Research Journal of Chemistry and Environment24 99-105.

[11] David W W and Stout T R 1971 Disc plate method of microbiological antibiotic assay Microbiology22 659-665.

[12] Djeussi D E, Sandjo L P, Noumedem J A, Omosa L K, Ngadjui B T and Kuete V 2015 Antibacterial activities of the methanol extracts and compounds from Erythrina sigmoidea against Gram-negative multi-drug resistant phenotypes BMC complementary and alternative medicine15 453.

[13] Carballo J L, Hernández-Inda Z L, Pérez P and García-Grávalos M D 2002 A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products BMC biotechnology2 17.

[14] Darsana I G O, Besung I N K and Mahatmi H 2012 Potensi daun binahong (Anredera cordifolia (Tenore) Steenis) dalam menghambat pertumbuhan bakteri Escherichia coli secara In vitro Indonesia Medicus Veterinus1 337-351.

[15] Cushnie T T and Lamb A J 2005 Antimicrobial activity of flavonoids International journal of Antimicrobial Agents26 343-356.

[16] Simorangkir M, Nainggolan B, Doloksaribu J F and Silaban S 2020 Effect of Sarang Banua (Cl erodendrum fragrans) leaves extract on serum globulin levels of rabbit (Oryctolagus cuniculus) Journal of Physics: Conferences Series1485 012016.

[17] Simorangkir M, Nainggolan B and Silaban S 2019 Antioxidant activity of vacuum column chromatography fractions of ethanol extract of sarangbanua (Cl erodendrum fragrans) leaves Journal of Physics: Conference Series1374 012016.

[18] Ngajow M, Abidjulu J and Kamu V S 2013 Pengaruh antibakteri ekstrak kulit batang matao (Pometia pinnata) terhadap bakteri Staphylococcus aureus secara in vitro Journal Mipa2 128-132.

[19] Rusdi M, Ayu K, Noer S F and Bariun H 2018 Uji toksisitas akut ekstrak partisi akar parang romang (Boehmeriavigata (Forst) Guill) terhadap larva artemiasalina leach dengan metode Brine Shrimps Lethality test JF FIK UINAM5 166-173.

Acknowledgements
Thanks are conveyed to the Directorate General of Research and Development Strengthening, Ministry of Research, Technology and Higher Education, Republic of Indonesia, who has funded this research through "Higher Education Leading Basic Research (PDUPT)" with research contract No. 12/UN.33.8/PL-DRPM / 2020.