Antibacterial activity of *Clidemia hirta* leaf extract against pathogenic bacteria with variation of solvents

Evi Rahayu, Ahmad Shafwan S Pulungan*
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan 20221, Medan, Indonesia
*Email: pulungan@unimed.ac.id

Received 19 April 2021, Accepted 20 May 2021

Abstract *Infectious disease is one of the health problems in society that is difficult to solve completely. One such infectious disease is diarrhea. Diarrhea is a disease that has high mortality and morbidity. Diarrhea can be caused by various types of pathogenic bacteria. One of the antibacterial potentials that can reduce the activity of pathogenic bacteria is the leaf extract of Clidemia hirta. The purpose of this study was to determine the antibacterial potential of C. hirta leaves against pathogenic bacteria with a variety of solvents. The research method used is descriptive qualitative. The solvents used were ethanol, water and n-hexane with the agar diffusion method. The extraction of the active compound was carried out using the maceration method. Measurement of antibacterial activity by looking at the minimum inhibitory concentration of C. hirta leaf extract in inhibiting the tested bacteria. The test bacteria used were Vibrio cholerae ATCC 39315 and Bacillus cereus ATCC 14574. The results showed that the minimum inhibitory concentration value for ethanol and Aqueous extract was 15,625 mg / ml for both types of bacteria, while for n-hexane solvent it was 62.5 mg/mL for V. cholerae ATCC 39315 and 250 mg/mL for B. cereus ATCC 14574. The conclusion is that ethanol extract is included in the category of very strong antibacterial activity, while Aqueous and n-hexane are categorized as strong in inhibiting the growth of pathogenic bacteria..*
[ANTIBACTERIAL ACTIVITY OF *CLIDEMIA HIRTA* LEAF EXTRACT AGAINST PATHOGENIC BACTERIA WITH VARIATION OF SOLVENTS] ***(J. Math. Nat. Sci., 1(1): 5 - 9, 2021)***

***Keywords*:** *Infectious, Activity, N-hexane, Vibrio cholerae, Bacillus cereus*

Introduction

Infensive disease is one of the health problems in society that is difficult to overcome. This type of disease affects the world's population and developing countries, including Indonesia. The term infection describes the growth or replication of microorganisms in the human body. This disease occurs when the infection produces changes in the body's normal physiology.

One of the infectious diseases is diarrhea. Diarrhea occurs frequently in developing countries such as Indonesia due to inadequate sanitation quality. Surveys show that the trend of diarrhea infections has increased every year (Otsuka et al., 2019). Diarrhea is a disease with symptoms of loose or runny bowel movements with a frequency of more than three times a day. Diarrhea can be caused by infection or non-infection (Kurokawa et al., 2018). Most of the diarrhea is diarrhea caused by infection with pathogenic microorganisms, including viruses, bacteria and parasites. Several types of bacteria are known to cause diarrhea such as *Staphylococcus aureus, Bacillus cereus, Clostridium perferingens, Escherichia coli, Vibrio cholerae, Salmonellla* sp. (Jia et al., 2018) (Yao et al., 2017) (Carroll et al., 2019). Prevention of infection can be done by using antibiotics. In line with the increasing resistance of bacteria in the world of health (Breiding, 2014), there is a need for new treatments to tackle this infectious disease, especially diarrhea. One alternative in overcoming diarrheal disease is by utilizing bioactive compounds obtained from plants. The potential of bioactive compounds from various types of plants has been widely studied such as *Centella asiatica* (Mhd Yusuf Nasution et al., 2018), *Mikania micrantha* (Muhammad Yusuf Nasution et al., 2019), taro leaves (Pulungan et al., 2018). Potential bioactive compounds are also found in the *C. hirta* plant.

C. hirta is a plant from the melastomaceae family. Most of the plants belonging to this family have polyphenol compounds, triterpenoids, alkyl benzoquinones, flavonoids and their derivatives, phenolic acids and their derivatives and alkaloids (Breaden et al., 2012). Other studies have also shown the presence of saponins and arjunalot acid (Lopez et al., 2016). *C. hirta* is also known to have antibacterial properties against *Enterobacter cloacae, Pseudomonas flurescens, Proteus vulgaris, Micrococcus luteu, Mycobacterium phlei, M. rhodochrous, M. smegmatis* (Abdellaoui et al., 2014). Leaf extract of *C. hirta* is known to have potential as an antibacterial against *Enterococcus faecalis* and *Pseudomonas aeruginosa* (Lopez et al., 2016).

The great potential of *C. hirta* leaves encourages studies to further investigate its antibacterial potential. The antibacterial potential of *C. hirta* leaves against bacterial pathogens, especially *V. cholerae* and *B. cereus* with various variations of solvents to see the minimum inhibitory concentration possessed by each treatment.

Materials and Methods

Location and time of research. This research was conducted in the biology laboratory of the Universitas Negeri Medan. This study used two types of test bacteria representing gram-positive and gram-negative bacteria, namely *V. cholerae* ATCC 39315 and *B. cereus* ATCC 14574. The research method used was descriptive qualitative.

Sample preparation. The leaves used for *C. hirta* are leaves that are not too old, whole and the same size taken from leaves 5 to 12. This is done because leaves of the same size and color indicate that the leaves have a maximum age and level of development. The leaves used are all parts of the leaves of *C. hirta*. And then *C. hirta* was dried in open air to dry. The dried leaf sample is then crushed to form a leaf powder.

Leaf extraction. 250 g of *C. hirta* leaf powder divided into three parts, each soaked in water, ethanol and n-hexane as a solvent with a ratio of 1:10 (w / v) for 3 x 24 hours, where every 24 hours the extract is filtered with filter paper. and the residue is macerated. Return to using a new solvent. Maceration is carried out at room temperature and is occasionally induced by stirring. The macerated filtrate is combined and then concentrated by means of a rotary evaporator.

Rejuvenation of test bacteria. 2 g of NA (nutrient agar) media were dissolved in 100 ml of distilled water in Erlenmeyer then covered with aluminum foil. The suspension was heated to a boil and put into the test tube aseptically. NA media was sterilized in an autoclave at 121°C for 15 minutes at a pressure of 15 psi. After sterilization, the media is left at room temperature for one hour in a tilted position. The cultures of V. cholerae ATCC39315 and B. cereus ATCC 14574 were taken one ose then streaked on NA media slant aseptically then incubated for 24 hours at 37°C.

Antibacterial activity test. Antibacterial activity was carried out by measuring the inhibition zone formed in each growth medium. The growth medium used MHA media, namely 38 g of MHA dissolved in 1000 mL of distilled water and sterilized. Testing for antibacterial activity was carried out using the Kirby-Bauer method.

Minimum Inhibitory Concentration Test. The test solution was prepared by dissolving the viscous extract of *C. hirta* leaves with each type of solvent. The concentration used was 250 mg / mL; 125 mg / mL; 62.5 mg / mL; 31.25 mg / mL and 15.625 mg / mL.

The rejuvenated test bacteria were inoculated into a test tube containing 10 mL of physiological NaCl. The solution was then homogenized with vortex and its absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 600 nm. The absorbance values in the range 0.08-0.12 were equivalent to 0.5 McFarland (1-2 x 108 CFU / mL) as standard. The solution with the turbidity equivalent to the standard was diluted with NB media to obtain a concentration of 106 CFU / mL.

The minimum inhibitory level was determined by the smallest extract concentration in the treatment tube which had begun to inhibit bacterial growth. The lowest concentration that can inhibit bacteria is shown in the absence of turbidity after incubation.

Results

Antibacterial activity test. Observation of the antibacterial activity of ethanol, Aqueous and n-hexane extracts against the tested bacteria was indicated by the formation of an inhibition zone. Leaf extraction was tested with three types of solvents and two types of test bacteria.

The results showed that giving variations of *C. hirta* leaf solvent to the two types of tested bacteria showed antibacterial activity. The ethanol extract produced the largest zone of inhibition, then the aqueous and n-hexane

solvent. The average diameter of the inhibitory power produced by ethanol extract in *V. cholerae* ATCC 39315 was 20.83 mm and in *B. cereus* ATCC 14574 was 23.96 mm. Comparison of antibacterial activity based on the formed inhibition zone value showed that the ethanol extract of *C. hirta* leaves had the highest ability of the two other types of solvents.

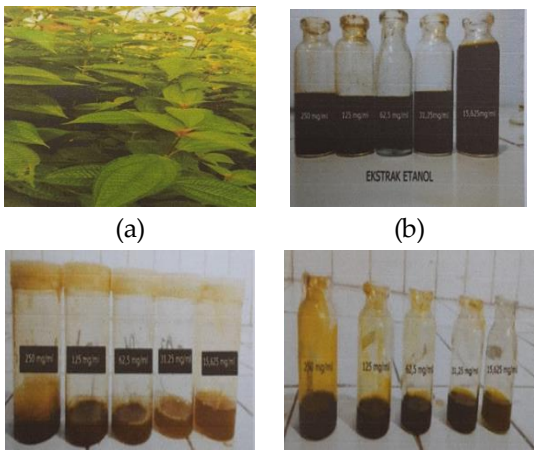

Figure 1. (a) *Clidemia hirta* leaf; (b) ethanol extract *Clidemia hirta*; (c) Aqueous extract *Clidemia hirta*; (d) n-hexane extract *Clidemia hirta*.

Minimum Inhibitor Concentration (MIC). MIC measurements were carried out on the three types of extracts, namely ethanol extract, water extract and n-hexane extract against the two tested bacteria. As a negative control, a tube containing Nutrient Broth (NB) media and bacterial suspension was prepared, while for positive control a tube containing chloramphenic antibiotic and NB media was prepared. Based on the test results, the difference in absorbance values was obtained.

Table 1. Antibacterial activity test of *Clidemia hirta* leaf extract.

Bacterial	Average Diameter of Inhibition Zone Based on Solvent			
	Ethanol extract	Aqueous extract	n-hexane extratct	Positif control
Vibrio cholerae ATCC 39315	20.83 ± 1.5	16.90 ± 0.7	11.90 ± 0.3	29
Bacillus cereus ATCC 14574	23.96 ± 0.4	17.90 ± 0.3	12.30 ± 0.4	30.1

Table 2. Minimum Inhibitor Concentration Test Results.

Microorganism	Minimum Inhibitory Concentration (mg/mL)		
	Ethanolic Extract	Aqueous Exctract	n-hexane Extract
Vibrio cholerae ATCC 39315	15.625	15.625	62.5
Bacillus cereus ATCC 14574	15.625	15.625	250

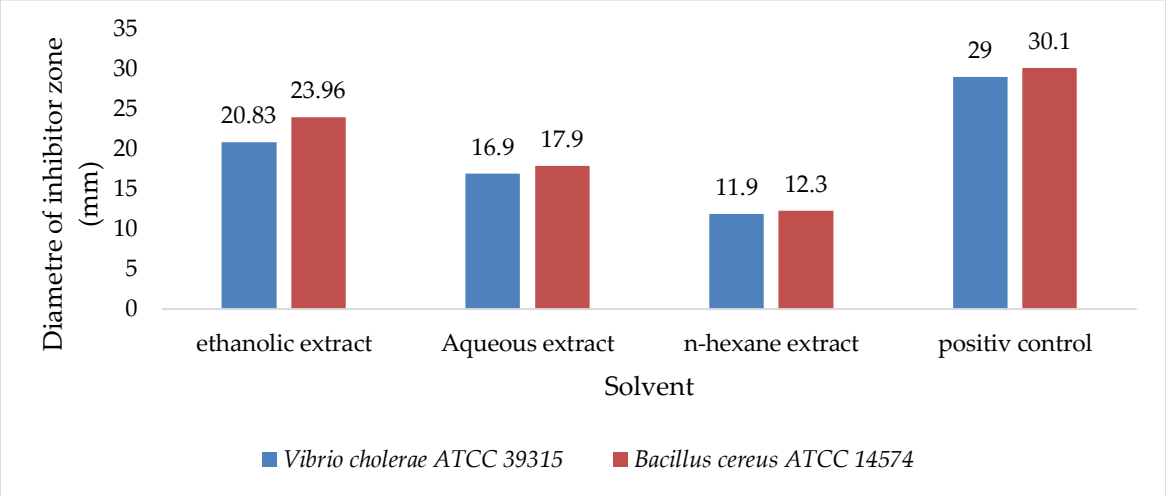

Figure 2. Graph of the average inhibition zone diameter comparison of each solvent to the tested bacteria.

Discussion

The minimum inhibitory concentration is the lowest concentration of an antibacterial required to inhibit bacteria. In this study it was shown that the three extracts showed antibacterial activity respectively 15,625 mg / mL for ethanol extract and water extract and 62.5 mg / mL for n-hexane extract for the type of bacteria *V. cholerae* ATCC 39315 and 250 mg / mL for *B. cereus* ATCC. 14574.

The antibacterial activity test showed that the ethanol extract produced the greatest inhibition zone. This is possible because in the ethanol extract more active compounds are dissolved than the water extract. Meanwhile, n-hexane extract has the least inhibitory power compared to the other two solvents because there are fewer metabolites dissolved.

The higher antibacterial activity of the ethanol extract compared to the aqueous extract could be attributed to the presence of a higher amount of polyphenols in the ethanol extract compared to the aqueous extract. Ethanol is easier to penetrate the cell membrane to extract intracellular material from plant material. Methanol is more polar than ethanol, but because of its toxicity, it is not suitable for extraction.

In the ethanol extract the dissolved compounds are flavonoids, saponins, tannins, and polyphenols. Water extract contains alkaloid compounds, saponins and tannins (Dirar et al., 2019). Meanwhile, the n-hexane extract can be dissolved, namely terpenoid and steroid compounds (Namdar et al., 2019). Each solute has a mechanism to inhibit bacterial growth. Flavonoids can inhibit bacterial growth by inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism (Farhadi et al., 2019). Saponins are able to inhibit bacterial growth by interacting with membrane sterols (Dong et al., 2020). The main effect of saponins on bacteria is the release of proteins and enzymes from the cell.

Conclusion

The ethanol extract produced a very strong inhibition zone, aqueous extract and n-hexane produced a strong inhibition zone. Ethanol extract and aqueous extract of *C. hirta* leaves showed Minimum Inhibitory Concentration (MIC) against *V. cholerae* ATCC 39315 and *B. cereus* ATCC 14574 at a concentration of 15.625 mg / mL. The n-hexane extract of *C. hirta* leaves showed Minimum Inhibitory Concentration (MIC) against *V. cholerae* ATCC 39315 at a concentration of 62.5 mg / mL and *B. cereus* ATCC 14574 at a concentration of 250 mg / mL.

References

Abdellaoui, S.E.l., Destandau, E., Krolikiewicz-Renimel, I., Cancellieri, P., Toribio, A., Jeronimo-Monteiro, V., Landemarre, L., André, P., Elfakir, C. (2014) Centrifugal partition chromatography for antibacterial bio-guided fractionation of *Clidemia hirta* roots. *Separat. Purif. Technol.*, 123: 221–228

Breiding, M.J. (2014) 肌肉作为内分泌和旁分泌器官 HHS Public Access. *Physiol. Behav.*, 63(8): 1–18

Carroll, L.M., Wiedmann, M., Mukherjee, M., Nicholas, D.C., Mingle, L.A., Dumas, N.B., Cole, J.A., Kovac, J. (2019) Characterization of emetic and diarrheal *Bacillus cereus* strains from a 2016 foodborne outbreak using whole-genome sequencing: Addressing the microbiological, epidemiological, and bioinformatic challenges. *Frontiers Microbiol.*, 10

Dirar, A.I., Alsaadi, D.H.M., Wada, M., Mohamed, M.A., Watanabe, T., Devkota, H.P. (2019) Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. *South Afr. J. Botan.*, 120: 261–267

Dong, S., Yang, X., Zhao, L., Zhang, F., Hou, Z., Xue, P. (2020) Antibacterial activity and mechanism of action saponins from *Chenopodium quinoa* Willd. husks against foodborne pathogenic bacteria. *Indust. Crops Prod.*, 149: 112350

Farhadi, F., Khameneh, B., Iranshahi, M., Iranshahy, M. (2019) Antibacterial activity of flavonoids and their structure–activity relationship: An update review. *Phytother. Res.*, 33(1): 13–40

Jia, Z., Chen, A., Bao, F., He, M., Gao, S., Xu, J., Zhang, X., Niu, P., Wang, C. (2018) Effect of nisin on microbiome-brain-gut axis neurochemicals by *Escherichia coli*-induced diarrhea in mice. *Micr. Pathog.*, 119: 65–71

Kurokawa, S., Kishimoto, T., Mizuno, S., Masaoka, T., Naganuma, M., Liang, K. ching, Kitazawa, M., Nakashima, M., Shindo, C., Suda, W., Hattori, M., Kanai, T., Mimura, M. (2018) The effect of fecal microbiota transplantation on psychiatric symptoms among patients with irritable bowel syndrome, functional diarrhea and functional constipation: An open-label observational study. *J. Affect. Disord.*, 235: 506–512

Lopez, T., Corbin, C., Falguieres, A., Doussot, J., Montguillon, J., Hagège, D., Hano, C., Lainé, É. (2016) Influence de la composition du milieu de culture sur la production de métabolites secondaires et les activités antioxydantes et antibactériennes des extraits produits í partir de cultures in vitro de

Clidemia hirta L. *Compt. Rend. Chimie*, 19(9): 1071–1076

Namdar, D., Voet, H., Ajjampura, V., Nadarajan, S., Mayzlish-Gati, E., Mazuz, M., Shalev, N., Koltai, H. (2019) Terpenoids and phytocannabinoids co-produced in *Cannabis sativa* strains show specific interaction for ell cytotoxic activity. *Molecules*, 24(17)

Nasution, M.Y., Restuati, M., Pulungan, A.S.S., Pratiwi, N., Diningrat, D.S. (2018) Antimicrobial activities of *Centella asiatica* leaf and root extracts on selected pathogenic micro-organisms. *J. Med. Sci.*, 18(4): 198–204

Nasution, M.Y., Restuati, M., Syahputra, R.A., Pulungan, A.S.S. (2019) Antibacterial activity of Mandailing traditional plant leaves ethanol extract of *Mikania micrantha*. *J. Biosci. Res.*, 16(01): 793–797

Otsuka, Y., Agestika, L., Widyarani, W., Sintawardani, N., Yamauchi, T. (2019) Risk factors for undernutrition and diarrhea prevalence in an urban slum in Indonesia: Focus on water, sanitation, and hygiene. *American J. Trop. Med. Hyg.*, 100(3), 727–732

Pulungan, A.S.S., Wuyung, W., Brata, W., Gultom, R. (2018) Antibacterial activity of the Taro ethanol extract against pathogenic bacteria. *Sci. Stu. Res. Biol.*, 31–34

Breaden, R.C., Brooks, S.J., Murphy, H.T. (2012) The biology of Australia weeds. *Plant Protec. Quart.*, 27(1): 2012

Yao, D.W., Yu, Z.Z., Li, N., Hou, Y.N., Xu, J.R., Yang, D.J. (2017) Copper-modified palygorskite is effective in preventing and treating diarrhea caused by *Salmonella typhimurium*. *J. Zhejiang Univ.: Sci. B*, 18(6): 474–480