p-ISSN: 2477-3328
e-ISSN: 2615-1588
International Summit on Science Technology and Humanity (ISETH2019)
Advancing Scientific Thought for Future Sustainable Development

Antibacterial Activity of Extract and Fraction of *Baccaurea macrocarpa* Leaf on *Escherichia coli* and *Bacillus cereus*

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Abstract

Purpose: The purpose of the present study is to investigate antibacterial activity of extract and fraction of *Baccaurea macrocarpa* leaf, and to identify secondary metabolite contents of the best activity.

Methodology: *Baccaurea macrocarpa* leaf was extracted by using maceration method with the use of methanol solvent. The extract was then fractionated by adopting *n-hexane* and *ethyl acetate* by using liquid-liquid extraction. Antibacterial activity assay was performed by using inhibition zone assay particularly Disc Diffusion Susceptibility method from methanol extract and fraction extract of ethyl acetate and n-hexane. Phytochemical screening as the best activity was performed by using by adopting Thin Layer Chromatography (TLC).

Result: Antibacterial activity was identified in the *ethyl acetate* extract of *Baccaurea macrocarpa* leaf. In addition, secondary metabolites with the best activity such as alkaloid, anthraquinone, flavonoid, and tannin were identifiable in the fraction extract of ethyl acetate of *Baccaurea macrocarpa*.

Keywords: *Antibacterial*, *Baccaurea macrocarpa, Escherichia coli*, *Bacillus cereus*

INTRODUCTION

Infection disease is one of the classic health problems and it remains the predominant health problems, although other health problems such as metabolic and degenerative diseases tend to intensify. Bacteria resistance to antibiotics has existed since the beginning of antibiotic era. Even in the last twenty years, hazardous resistant strains have emerged. The increasing resistance evolution is worsened with the existence of less effective antibiotics (Fair & Tor, 2014). The overuse and misuse of antibiotics have been identified as the significant cause of the emergence of resistance (Paterson et al., 2016). Lack of effective antibacterial cure results in failing medical procedure or becomes a high risk for patients. Diverse infections caused by resistant microbes can slow down and even complicate the cure, in addition to the increase of cure cost, and mortality risk (WHO, 2017).

South Borneo is an island with the source of wonderful biodiversity. Medicinal plants have been used mainly by the people in remote area of Borneo forest. Nature provides medicinal plants that can be developed as the new therapeutic agent, namely *tampui* or *kapul* (*Baccaurea macrocarpa*). Momand (2014) stated that *Baccaurea angulate* had antibacterial activity on human pathogenic microorganism. Another research found out that *Baccaurea angulate* had the ability to inhibit peroxidation and increase antioxidant (Mikail et al., 2016)there is an unprecedented growing interest in the exploration of the potentials of underutilized fruits as alternatives to the commercially available fruits. Baccaurea angulata is an underutilized fruit widely distributed in Borneo Island of Malaysia. The present study was conducted to investigate the effects of B. angulata whole fruit (WF. Timbrell (2001) reported antibacterial activity of *Baccaurea courtallensis*. The skin of *B. macrocarpa* fruit had antibacterial activity with the highest inhibition power on the growth of *S. aureus and E. coli* (Yunus et al., 2014). *Baccaurea spp* was identified containing secondary metabolites compound such as alkaloid, anthocyanin, flavonoid, phenol, and carotenoid. However, there has been a paucity of studies on antibacterial activity assay of *Baccaurea macrocarpa* leaf. This

p-ISSN: 2477-3328
e-ISSN: 2615-1588

research is expected to provide information about antibacterial Activity from extract and fraction of *Baccaurea macrocarpa* leaf by using Disc Diffusion Susceptibility method.

METHODOLOGY

Material

B. macrocarpa plant was obtained from Abirau Village, Karang Intan Subdistrict, Banjar Regecy, Indonesia in February 2019. The plant was determined by plant taxonomy expert from Faculty of Mathematics and Science (FMIPA) Lambung Mangkurat University, Banjarbaru, South Borneo, Indonesia.

Extraction and Fractionation

A total of 20 kilograms of *B. macrocarpa* leaf were obtained and extracted by using maceration method in methanol solvent for 15 days. The extraction was conducted in three times by using the same solvent in room temperature condition, and it was filtered. The obtained 120,48 g of methanol extracts were then parted by adopting liquid-liquid extraction method using distinct solvents namely *ethyl acetate* and *n-hexane*. The partition produced 72,16 g extract fraction of *ethyl acetate* and 48,42 g extract fraction of *n-hexane*.

Antibacterial activity assay

Antibacterial activity assay on methanol extract and fraction adopted Disc Diffusion Susceptibility method. The second bacterial strains used as antibacterial were *E.coli* and *B. cereus*. Twenty milliliters of NA solvents were poured into each sterile petri dish. Bacteria were planted on NA media. They were incubated at the temperature of 35-37^{0}C in 24 hours. 6-mm-disc Whatman filter paper and antibiotic disc were prepared to be put into glass petri dish, and sterilized in autoclave at 1 atm, 121^{0}C, in 15-20 minutes. The disc was soaked using methanol solvent and extract fraction of ethyl acetate and n-hexane, which were dissolved in DMSO. Then, petri dish was incubated for 24 hours at the temperature of 35-37^{0}C before reading the diameter of inhibition zone. The diameter of inhibition zone around the disc reflected the tested extract activity. Clindamicyn antibiotic was used for positive control of *B. cereus* and ciprofloxacin was for *E. coli*.

Thin Layer Chromatography

Separation by Thin Layer Chromatography (TLC) was carried out several times using several eluents with different levels of polarity to obtain solvents that we're able to provide good separation and good dye stains. Determination of the class of compounds in the TLC test was done by spraying the TLC plate with several reagents. Chemical components that are evaluated from extracts that are active against bacteria include assays of alkaloids, tannins, terpenoids, anthraquinones, and flavonoids.

RESULTS AND DISCUSSION

In vitro antibacterial activity of methanol extract, and extract fractions of *ethyl acetate* and *n-hexane* of *B. macrocarpa* leaf produced the value of diameter zone as depicted in Table 1. Phytochemical screening assay on fraction extract of ethyl acetate of *B. macrocarpa* leaf, which adopted thin layer chromatography method, contained of several metabolite compounds (Table 3).

Table 1. The merits of antibacterial from extract and fraction of *B. macrocarpa* leaf.

Microorganisms	Ethanol Extract	Ethyl Acetate Fraction Extract	N-Hexane Fraction Extract
E. coli	-	+	-
B. cereus	-	+	+

Table 2. 100% of antibacterial activity from extract and fraction of *B. macrocarpa* leaf with disc diffusion method.

Microorganisms	Ethanol Extract	*Ethyl Acetate* Fraction Extract	*N-Hexane* Fraction Extract	Antibiotic Standard
Microbial / antibiotic	Diameter of inhibition zone in mm (mean)			
E. coli / ciprofloxacin	0	8,33 ± 0,577	0	31,67 ± 0,577
B. cereus / clindamycin	0	7,33 ± 1,528	7,00 ± 0,000	17,00 ± 0,000

Results were presented as mean±SD

Table 3. Metabolite compound of *ethyl acetate* fraction extracts of *B. macrocarpa* leaf

Compound	Reagent	Positive Reaction	Observation Result	Conclusion
Alkaloid	Dragendorf	Orange color produced	Orange color detected	+
Anthraquinone	KOH	Orange and green colors produced	Orange and green colors detected	+
Flavonoid	$AlCl_3$	Yellow color formed	Yellow color detected	+
Tannin	$FeCl_3$	Black sediment produced	Black color detected	+
Terpenoid	Lieberman Buchard	Brown ring formed	No detection	-

Fraction extracts of *ethyl acetate* and *n-hexane* of *B. macrocarpa* were able to produce antibacterial activity. However, *ethyl acetate* fraction extract was highly active in producing antibacterial activity on *E. coli* and *B. cereus*. On the other hand, fraction of N-hexane extract was found active in positive gram bacteria namely *B. cereus*. Compounds in ethyl acetate fraction extract possibly contained of high flavonoid and phenolic acid compound (Li HF, 2012). The second positive controls, ciprofloxacin for *E. coli*, clindamycin for *B. cereus,* showed the biggest inhibition activity on all the tested bacteria. Meanwhile, DMSO control did not show inhibition zone, which means that pure inhibition zone was from *ethyl acetate* fraction extract.

Confirmation of secondary metabolite compound contained in ethyl acetate extract was done by using thin layer chromatography assay. Analysis using TLC was performed by separating chemical components based on adsorption principle and partition determined by stationery phase and mobile phase. Chemical compound will intensify following mobile phase since the absorption of adsorbent on chemical components were dissimilar, therefore chemical components can move with different distances based on the polarity level (Stahl, 2013). TLC assay to identify alkaloid compound was performed with mobile phase of ethyl acetate: eluent comparison of methanol was 5:5 with the dragendorf reagent to obtain orange color. TLC assay to identify anthraquinone was performed with mobile phase of n-hexane: eluent comparison of ethyl acetate was 3:7 with injection reagent of KOH of 10%, obtaining brown and green colors. TLC assay to identify Flavonoid was performed

p-ISSN: 2477-3328
e-ISSN: 2615-1588

with mobile phase of n-hexane: eluent comparison of ethyl acetate was 7:3 with injection reagent of AlCl3, obtaining yellow color. KLT assay to identify tannin was performed with mobile phase of comparison of methanol solvent: eluent comparison of water was 6:4 with injection reagent of $FeCl_3$, obtaining black color. KLT assay to identify terpenoid compound was done with mobile phase of n-hexane: eluent comparison of ethyl acetate was 9.5:0.5.

Antibacterial activity in ethyl acetate fraction extract of *B. macrocarpa* was presumed due to the presence of secondary metabolite compounds containing of antibacterial activity such as anthraquinone, alkaloid, flavonoid, and tannin. This is found by Endra et al. (2013) and Fadzelly et al. (2014) revealing that *Bacaurea spp* contained of secondary metabolite compounds namely anthraquinone, alkaloid, flavonoid, saponin, phenol, and caretonoid. Flavonoid is a group of phenol compound, acid alcohol commonly known as carbolic acid. Cell wall of bacteria were made of peptidoglycan and mucopeptide, lipopolysaccharide, and lipoprotein. Phenol has the ability to denaturize protein and damage cell membrane. Phenol bonds with protein through hydrogen bond, which damages protein structure. Cell membrane damage hampered nutrition transport through cell membrane, therefore microbial cells were lack of nutrition needed for its growth (Volk et al., 1992). Flavonoid played role as antibacterial since it could damage permeability of bacteria cell wall, as well as inhibit mortality of bacteria (Darsana, 2013). Permatasari (2013) said that alkaloid had antibacterial ability by hampering the components of peptidoglycan on the cell of bacteria, therefore cell wall layer was not wholly formed, and caused mortality of the cell. Tannin could damage polypeptide on the cell wall, so the damage was on the cell wall (Ji Ys, 2012).

The second antibiotic standards in this research were determined based on antibacterial activity on positive gram, negative gram, and fungi. Ciprofloxacin belongs to fluoroquinolone that inhibits topoisomerase II (DNA gyrase) and topoisomerase IV that are needed by bacteria to replicate DNA (Raini, 2017; Pratiwi, 2017). Jahir & Naveen (2011) stated that ciprofloxacin antibiotic hampered topoisomerase II and IV enzymes as the formation of DNA. In addition, according to Cushnie & Lamb (2005) secondary metabolite compound of flavonoid hampered the formation of DNA gyrase.

Clindamycin belongs to macrolide antibiotic with the mechanism of protein synthesis of bacteria with bactericidal and bacteriostatic effects by hampering synthesis of protein without hampering normal cells and inhibiting steps of protein synthesis (Pratiwi, 2017). The mechanism of flavonoid inhibiting the function of cell membrane was by forming complex compound with the dissolved extracellular protein, therefore it could damage cell membrane of bacteria and followed by the exit of intracellular compound (Rijayanti et al., 2014). Another research revealed that flavonoid mechanism inhibited the function of cell membrane by hampering the permeability of cell membrane and enzyme bond such as ATPase and phospholipase. Tannin had antibacterial activity that related to its ability to deactivate adhesin of microbial cell, deactivate enzyme, and hamper protein transport in the inner layer of cell (Li et al., 2003). Tannin also had the target on cell wall polypeptide, so the formation of cell wall became less perfect. This caused bacteria cell to subject to lysis due to either osmotic or physical pressure so that bacteria cell will be mortal (Sari & Muktiana, 2011)bakteri, maupun virus menyebabkan kebutuhan antibiotik semakin meningkat. Berbagai macam tanaman herbal berusaha untuk dimanfaatkan secara maksimal sebagai bahan baku antibiotik. Salah satunya adalah antibiotik dari Jatropha Multifida. Proses untuk produksi antibiotik adalah ekstraksi untuk mengambil zat aktif yaitu tanin dan alkaloid yang terdapat dalam Jatropha Multifida. Namun, proses ekstraksi secara maserasi tanpa memperhatikan kondisi operasi menyebabkan kualitas dan kuantitas produk tidak sesuai dengan yang diharapkan. Pada penelitian ini dilakukan pengujian zat aktif tanin yang diperoleh dari ekstraksi Jatropha Multifida

menggunakan metode ekstraksi berpengaduk dengan solvent metanol.Tujuan dari penelitian ini adalah untuk mengetahui kondisi optimum ekstraksi tanaman jatropha multifida, serta mengetahui efektivitas hasil ekstraksi terhadap berbagai jenis mikroorganisme pathogen penyebab berbagai macam penyakit. Alat yang digunakan adalah alat ekstraksi. Variabel berubah yang digunakan antara lain rasio (massa sampel/volume solven.

CONCLUSION

According to this study, *ethyl acetate* fraction extract demonstrated a more activity than the methanol and n-heksan fraction extract in antibacterial activity. Phytochemical screening of the ethyl acetate fraction extract showed the presence of alkaloids, anthraquinone, flavonoids and tannin. The phytochemicals are responsible for the antibacterial activity of the extracts.

ACKNOWLEDGEMENT

This research was funded by a grant from PDP DIKTI (117/SP2H/LT/DRPM/2019). The author would like to thank head of Laboratory of Faculty of Pharmacy UM Banjarmasin for providing the equipment and materials required in this research.

REFERENCES

Darsana, I.G.O., Besung, I.N.K., Mahatmi, H. (2012). Potentials of binahong (*Anredera cordifolia* (Tenore) Steenis) in inhibiting in vitro bacterial growth of *Escheria coli*. *Indonesia Medicus Vaterinus*, 1(3), 337-351

Cushnie, T. P. T., & Lamb, A. J. (2005). Antibacterial activity of flavonoids. *International Journal of Antibacterial Agents*, *26*(5), 343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002

Endra Tirtana1*, Nora Idiawati1, Warsidah1, A. J. (2013). Analysis of Proximate, Phytochemical Test, and Antioxidant Activity. *Jurnal Kajian Komunikasi*, *2*(1), 42–45.

Fadzelly, M., Bakar, A., Ahmad, N. E., Karim, F. A., & Saib, S. (2014). Phytochemicals and Antioxidative Properties of Borneo Indigenous Liposu (*Baccaurea lanceolata*) and Tampoi (*Baccaurea macrocarpa*) Fruits, 516–525. https://doi.org/10.3390/antiox3030516

Fair, R. J., & Tor, Y. (2014). Perspectives in Medicinal Chemistry Antibiotics and Bacterial Resistance in the 21st Century. *Perspectives in Medicinal Chemistry*, 25–64. https://doi.org/10.4137/PMC.S14459.Received

Li, H. Wang, Z. Liu, Y (2003). Review in the studies on tannins activity of cancer prevention and anticancer. Zhong-Yao-Cai. 26(6): 444-448.

Mikail, M. A., Ahmed, I. A., Ibrahim, M., Hazali, N., Abdul Rasad, M. S. B., Abdul Ghani, R., … Adros Yahya, M. N. (2016). Baccaurea angulata fruit inhibits lipid peroxidation and induces the increase in antioxidant enzyme activities. *European Journal of Nutrition*, *55*(4), 1435–1444. https://doi.org/10.1007/s00394-015-0961-7

Momand, L. (2014). Antibacterial Effect of Baccaurea angulata Fruit Extracts against Human Pathogenic Microorganisms. *Medicinal & Aromatic Plants*, *03*(04), 3–7. https://doi.org/10.4172/2167-0412.1000172

Paterson, I. K., Hoyle, A., Ochoa, G., Baker-Austin, C., & Taylor, N. G. H. (2016). Optimising antibiotic usage to treat bacterial infections. *Scientific Reports*, *6*(November), 1–10. https://doi.org/10.1038/srep37853

p-ISSN: 2477-3328
e-ISSN: 2615-1588

Permatasari GAAA, Besung INK & Mahatmi H. (2013). Inhibition power of soursop leaf on the growth of *Escherichia coli* bacterium. *Indonesia Medicus Veterinus* 2(2):162-169.

Pratiwi, R. H. (2017). Mechanism of Pathogen Bacteria on Antibiotics. *Jurnal Pro-Life*, *4*(3), 425.

Raini, M. (2017). Fluoroquinolon Antibiotic: Advantages and Disadvantages. *Media Penelitian Dan Pengembangan Kesehatan*, *26*(3), 163–174. https://doi.org/10.22435/mpk.v26i3.4449.163-174

Rijayanti, R. P., Luliana, S., & Trianto, H. fajar. (2014). In vitro Antibacterial Activity test Of Ethanol Extracts Bacang mango (*Mangifera foetida* L.) Leaves Against Staphylococcus aureus. *Naskah Publikasi Universitas Tanjungpura*, *1*(1), 10–12.

Sari, F. P., & Muktiana, S. S. (2011). Extraction of Active Antibacterial Substance of Iodine Plant (*Jatropha Multifida* Linn) as Raw Materials of Natural Antibiotic. 1–7.

Septiadi, T., Pringgenies, D., & Radjasa, O. K. (2013). Phytochemical Test and Antifungal Activity of Sea Cucumber (*Holoturia atra*) Extract from Bandengan Beach on *Candida albicans* fungi. *Journal of Marine Research*, *2*(2), 76–84

Sheppard, D. and Lampiris, H. W., 2015. Antifungi Agents. In : *Basic and Clinical Pharmacology.* Ed 13th. Editors : Katzung, B.G. and Trevor, A.J. New York :McGraw-Hill Companies, Inc, Chapter 48, p. 825-834.

Timbrell, J. (2001). Natural Products. *Introduction to Toxicology, Third Edition*, *5*(2), 143–150. https://doi.org/10.1201/b13946-11

Umar A, Krihariyani D & Mutiarawati DT. (2012). Effect of binahong (*Anredera cordifolia*) extract on the cure of infection wound of Staphylococcus aureus on mouse. *Analis Kesehatan Sains* 01(02):68-75.

Volk WA. (1992). *Basic Microbiology*. New York: Harper Collins Publisher

Yunus, R., Alimuddin, H. A., & Ardiningsih, P. (2014). Antibacterial activity assay on the extract of Tampoi skin (*Baccaurea macrocarpa*) on *Escherichia coli* and *Staphylococcus aureus* bacteria. Renos Yunus 1*, Andi Hairil Alimuddin 1, Puji Ardiningsih 1, Chemistry Department, Faculty of Mathematics and Science, Universitas Tanjungpu. *Jurnal Kajian Komunikasi*, *3*(3), 19–24.