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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF DICHLOROMETHANE AND ETHYL ACETATE EXTRACTS OF BINTARO LEAF (CERBERA MANGHAS, LINN) AGAINST STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI

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ABSTRACT

Sea mango leaf and fruit (*Cerbera manghas, Linn*) are very toxic, therefore some people in Southeast Asia and South Asia use them as fish poisons and rat poisons, besides that some people have also used them to treat several diseases, such as, diabetes, digestion, fever, malaria and infectious diseases. This study aims to determine the potential of dichloromethane and ethyl acetate extracts of sea mango (Cerbera manghas, linn) leaf against *Staphylococcus aureus and Escherichia coli*. The leaves of Cerbera manghas, Linn was obtained from Research Institute for Spices and Medicinal Plants (BALITRO) Bogor, Indonesia. Sea mango leaves were extracted by multistage extraction using dichloromethane and ethyl acetate solvents. The obtained extracts were prepared with concentrations of 10 mg/ml, 20 mg/ml, and 30 mg/ml. Test preparation activity were carried out against *Escherichia coli and Staphylococcus aureus* by using paper disc and dilution methods, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined and ampicillin antibiotic was used as a comparison. In this cases was obtained the MIC values of ethyl acetate extract against *E coli and S aureus* was 5 mg/ml and 2,5 mg/ml respectively and for dichloromethane extract was 2,5 mg/ml and 1,5 mg/ml respectively, while the MBC values of ethyl acetate extract against *E coli and S aureus* was 10 mg/ml and 5 mg/ml respectively and for dichloromethane extract was 5 mg/ml and 2,5 mg/ml respectively. Equality value for 10 - 30 mg/ml extract of ethyl acetate was same with 26,12 - 36,20 µg/ml ampicillin in against *E. coli* and 10,37 - 15,55µg/ml in against S. aureus respectively. Equality value for 10 - 30 mg/ml extract of dichloromethane was same with 20,55 - 34,98 µg/ml l ampicillin in against *E. coli* and 5,17 - 13,4 µg/ml in against *S. aureus* respectively. Based on the results of this study was obtained, that extracts of ethyl acetate and dichloromethane sea mango (*Cerbera manghas, Linn*) leaves have antibacterial activity against *S aureus and E coli,* where, extracts dichloromethane leaf of sea mango has antibacterial activity a little stronger than the extract ethyl acetate sea mango leaf.

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INTRODUCTION

Antimicrobial resistance (AMR) has threatened the prevention and effective treatment of a variety of ever-increasing infections caused by bacteria, parasites, viruses and fungi. In the coming decades, more common infections and minor injuries can kill, because there are no more effective antibiotics to fight infectious diseases, because antibiotics are generally resistant. Therefore, efforts to prevent the causes of infectious diseases become resistant to antibiotics and the discovery of new drugs to fight infectious diseases must be increasingly activated (WHO, 2011, Palhares, 2015).

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According to the World Health Organization (WHO), about 65% - 80% of the populations of developing countries currently use medicinal plants as remedies. It is estimated that of the 300,000 plant species that exist in the world, only about 15% have been evaluated to determine their pharmacological potential. About 25% of modern drugs and approximately 60% of antitumor drugs are derived from natural products (WHO, 2014). Since ancient times, today and also for the future, the main source of medicinal ingredients is from natural products. One of the natural ingredients that has become a major concern of researchers as an infectious drug is sea mango. Due to sea mango is a plant that is all toxic, but from some preliminary studies it is good enough to fight infectious diseases In traditional medicine, some people have used sea mango to treat diseases, such as, analgesic, anticonvulsant,

cardiotonic, hypotensive activity (Chang *et al*., 2000), anticancer, antioxidant, antifungal, antilarva, and antibacterial. Rahman *et al*. (2011). Sea mango contains several chemical compounds that was produced from secondary metabolites, such as saponins, polyphenols, terpenoids, and alkaloids. The seeds of the Sea mango contain cerberin compounds that have the potential to anti-Carcinogenic (Amarpreet, 2014; Liu *et al*., 2008). Cerberin is a type of cardiac glycoside, a steroidal class found in the seeds of sea mango. This class includes digitalis-like agents, channel-blockers that work as cardiac treatments, but which at higher doses are extremely toxic and can cause it to occur nausea, vomiting, and abdominal pain, often leading to death. The natural product has been structurally characterized, its toxicity is clear, it is often used as an intentional human poison in third-world countries, and accidental poisonings with fatalities have resulted from individuals even indirectly consuming the agent. but the pharmacological effects of cerberin compounds have not been much explained (Bari *et al*., 2017).

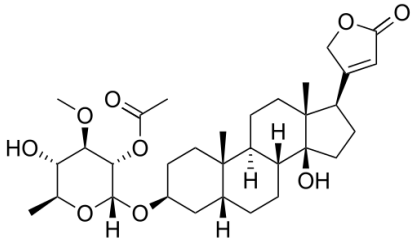

Figure 1. Cerberin

MATERIALS AND METHODS

Sea mango (*Cerbera manghas, Linn* or *Cerbera odollam*) leaves was obtained from the Spice and Medicine Research Center (BALITRO), Bogor, Indonesia and to prove this plant was sea mango leaf, determination was made at the Biology Research Center, Indonesian Institute of Sciences, Bogor, Indonesia. sea mango (*Cerbera manghas, Linn*) in Indonesian is called Bintaro. The dried powder of sea mango leaves was macerated with 100% methanol and dried by using a rotary evaporator. The extract obtained was dissolved with dichloromethane until it was clear, then dried with a rotary evaporator and used as a test preparation (dichloromethane extract). While the residue from the extract was added with 80% methanol and n-hexane with the same volume, thus forming 2 phases. Namely the n-hexane fraction and the methanol fraction. In the methanol fraction was added ethyl acetate with a ratio of 1: 1.

The ethyl acetate fraction that was obtained, then was dried by using a rotary evaporator and used as a test preparation (ethyl acetate extract). Phytochemical screening was carried out by the Harborne methods (1998) on the class of chemical compounds, i.e, alkaloids, flavonoids, saponins, tannins, steroids and Triterpenoids. (Harborne, 1998). Bacteria used for experiments was carried out the process of bacterial regeneration by cultured on Trypticasein Soy Agar (TSA) media and incubated at 37 ° C for 24 hours, then was inoculated as much as 3 ose into 30 ml of Triptocasein Soy Broth (TSB) media then incubated on temperature of 37 ° C for 24 hours. This culture was used for making mid log culture. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the test bacteria were carried out by dilution method by using agar plate and droptest.

In 12 test tubes with variation of concentration of test solution was made; 10, 50, 100, 500, 1000, 1500, 2500, 5000, 10000, 20000, 50000, 100000 ppm, each were diluted with ethyl acetate and dichloromethane until 1 ml of the test solution. Then each tube were added with 3.9 ml of bacterial growth media (TSB), and 0.1 ml of inoculum. Then incubated for 24 hours at 37 ° C. Observe the turbidity of the tube to determine the MIC. Whereas to determine MBC, each test tube was taken 10 µl and dropped on TSA agar media. Incubated for 24 hours at 37 °C. MBC values were known by the absence of bacterial colony growth on TSA media. Ampicillin was used as a comparison with a concentration of 30µg/ml, 40 µg/ml, and 53.33 µg/ml.

RESULTS AND DISCUSSION

The results of plant determination at the Center for Biological Research, Indonesian Institute of Sciences, Cibinong, Bogor, Indonesia, showed that the plants used for the study of *Cerbera manghas*, linn from the family of Apocynaceae. Phytochemical screening was performed on the dichloromethane and ethyl acetate extract of sea mango leaves by the Harborne method (1998), which was found in the groups of chemical compounds, as shown in Table 1. The result of phytochemical screening of ethanol extract *Cerbera manghas* leaf that was done by Monjour *et al* (2014), They was also found biologically active chemical groups i.e, alkaloids, glycoside, steroids, flavonoids and tannins. Based on these two studies, there was a difference in the content of *Cerbera manghas* leaves between dichloromethane and ethyl acetate extracts with ethanol extract, namely, dichloromethane and ethyl acetate extracts did not have alkaloids, whereas in ethanol extracts there were alkaloids.

Table 1. Results of Phytochemical Screening of Dichloromethane and Ethyl Acetate Extract of sea mango leaves

No	Chemical groups	Dichlorometan extract	Ethyl Acetate extract
1.	Alkaloid	+++	+++
2.	Flavonoids	++++	++++
3.	Saponin	+++	+++
4.	Tanin	+++	+++
5	Steroids	-	-
6	Triterpenoids	+++	+++
7	Phenolic	+	+
8	Glycoside	++++	++++

Note :
-: Negative
+: Positive weak
++: Positive
+++: Strong positive
++++: Very strong positive

Based on the table 2 above, the results of the dichloromethane extract of sea mango leaves with a concentration of 30 mg/ml showed the highest antibacterial potency against *Escherichia coli.* This was indicated by the amount of inhibition zone obtained was greater than the concentration of 10 mg/ml and 20 mg/ml. Based on this table was obtained a straight-line equation to know the equivalence of the concentration of dichloromethane extract of sea mango leaves with ampicillin, i.e. Y = 0.077x + 0.761. Based on the curves and tables 3 above it can be seen that 10 mg/ml of dichloromethane extract was equivalent to 20.55 µg/ml of ampicillin, 20 mg/ml of dichloromethane extract equivalent to 25.27 µg/ml, 30 mg/ml of dichloromethane extract equivalent to 34 , 98 µg/ml of ampicillin. Antibacterial activity test results of sea mango leaf dichloromethane extract against *Staphy*

Table 2. Inhibitio zone diameter of dchlorometan extract against Escherichia coli (Gram negative) bacteria on several concentrations

Petri dish	Ampicillin (mm)			Diklorometane Extract (mm)		
	S1	S2	S3	U1	U2	U3
	30 µg/ml	40 µg/ml	53.3 µg/ml	10 mg/ml	20 mg/ml	30 mg/ml
I	10	11	12	7	8	10
II	8.5	11.5	12	7.5	8.5	10
III	9	11	13	7	8.5	10.5
Mean	9.166	11.166	12.333	7.166	8.333	10.166

Table 3. Equivalence between dichloromethane extracts and ampicillin against E. coli

Extract consentration (mg/ml)	10	20	30
Inhibition Zone (mm)	7,17	8,33	10,17
Equivalence of anpicillin (µg/ml)	20,55	25,27	34,98

Table 4. Inhibition zone diameter of dchlorometane extract against *S. aureus* on several concentrations

Petri dish	Ampicillin (mm)			Diklorometan Extract (mm)		
	S1	S2	S3	U1	U2	U3
	30 µg/ml	40 µg/ml	53.3 µg/ml	10 mg/ml	20 mg/ml	30 mg/ml
I	21	22,5	25	10	13	15,5
II	21	23	24	9,5	14	15
III	21	22,5	25	10	13	17
Means	21,00	22,67	24,67	9,83	13,33	15,83

Table 5. Equivalence of Dichloromethane Extracts with ampicillin against *S.aureus.*

Extract concentration (mg/ml)	10	20	30
Inhibition zone (mm)	9,83	13,33	15,83
Equivalence with ampisilin. (µg/ml)	5,17	8,87	13,04

Table 6. Inhibition zone diameter of ethyl acetate extract against *E.coli* on several concentrations

Petri dish	Ampicillin (mm)			Etil acetate extract (mm)		
	S1	S2	S3	U1	U2	U3
	30 µg/ml	40 µg/ml	53.3 µg/ml	10 mg/ml	20 mg/ml	30 mg/ml
I	9	12	13,5	8	9,5	10
II	9	12,5	14	8	9	10,5
III	10	11,5	14	9,5	9	10,5
Mean	9,33	12,00	13,83	8,50	9,17	10,33

Table 7. Equivalence of ethyl acetate extract and ampicillin against *E. coli*

Extract concentration (mg/ml)	10	20	30
Inhibition zone (mm)	8,50	9,17	10,33
Equivalence of ampicillin (µg/ml)	26,12	28,37	32,80

Table 8. Inhibition zone diameter diameter of ethyl acetate extract against Gram positive bacteria *Staphylococcus aureus* at several concentrations

Petri dish	Ampicillin (mm)			Ethyl acetate extract (mm)		
	S1	S2	S3	U1	U2	U3
	30 µg/ml	40 µg/ml	53.3 µg/ml	10 mg/ml	20 mg/ml	30 mg/ml
I	19	21,5	25	11	12	14,5
II	20	22	24	10	11,5	13
III	19,5	22,5	25	9	10,5	13,5
Mean	19,33	22,00	24,67	10,00	11,33	13,67

Table 9. Equivalence of ethyl acetate extract with ampicillin against *S aureus*

Extract concentration (mg/ml)	10	20	30
Inhibition zona (mm)	10	11,33	13,67
Ampicilln equivalence concentration (µg/ml)	10,37	12,02	15,55

Table 10. The MIC values and MBC of dichloromethane and ethyl acetate extracts of sea mango leaves against *S.aureus* and *E.coli.*

No	Concentration (ppm)	Dichorometane extract		Ethyl Acetate Extract	
		E. coli	*S. aureus*	*E. coli*	*S. aureus*
1	10	+	+	+	+
2	50	+	+	+	+
3	100	+	+	+	+
4	500	+	+	+	+
5	1000	+	+	+	+
6	1500	+	+	+	+
7	2500	+	0	+	+
8	5000	0	0	+	0
9	10000	0	0	0	0
10	20000	0	0	0	0
11	50000	0	0	0	0
12	100000	0	0	0	0

Note + = there was bacterial growth 0 = no bacterial growth

lococcus aureus, as shown in Table 3. Based on the results of this test was obtained a straight line equation Y = 0.067x + 0.055 and can be used to know concentration of dichloromethane extract of sea mango leaves which is equivalent to ampicillin. Based on the curve and table 5 above it can be seen that 10 mg/ml of dichloromethane extract was equivalent to 5.17 µg/ml of ampicillin, 20 mg/ml of dichloromethane extract equal to 08.87 µg/ml, 30 mg/ml of dichloromethane extract equivalent to 13.04 µg/ml of ampicillin. The results of antibacterial activity test of sea mango leaf ethyl acetate extract against *E.coli* are as shown in Table 5. Based on the above data can be made the equation of the straight line Y = 0.054x + 0.958, to determine the concentration of sea mango leaf ethyl acetate extract which is equivalent to Ampicillin. Based on the curves and table 7 above it can be seen that 10 mg/ml of ethyl acetate extract was equivalent to 26.12 µg/ml of ampicillin, 20 mg/ml of ethyl acetate extract equivalent to 28.37 µg/ml, 30 mg/ml of ethyl extract acetate is equivalent to 32.8 µg/ml of ampicillin antibiotics. Antibacterial activity test of sea mango leaves ethyl acetate extract against *S. aureus* as shown in table 8. From table 7 it is obtained the straight line equation Y = 0.048x + 0.536, to determine the equality of the concentration of ethyl acetate extract of sea mango leaves with ampicillin Based on the curves and Table 8 above, it can be seen that 10 mg/ml of ethyl acetate extract was equivalent to 10.37 µg/ml of ampicillin and 20 mg/ml of ethyl acetate extract equivalent to 12.02 µg/ml and 30 mg/ml of ethyl acetate extract equivalent to 1,192 µg/ml ampicillin. The MIC was determined based on the lowest concentration of test solution which could inhibit bacterial growth, while the determination of MBC was based on the lowest concentration where at this concentration there was no more bacterial growth. Observations were based on the presence or absence of bacterial growth in the media. The result of MIC and MBC were as shown in table 9. The MIC value for dichloromethane extract of sea mango leaf against *E.coli* was 2500 ppm or 2.5 mg/ml against *S.aureus* was 1500 ppm or 1.5 mg/ml. The MBC value for the dichloromethane extract of sea mango leaves against *E.coli* was 5000 ppm/5 mg/ml against *S.aureus* was 2500 ppm or 2.5 mg/ml. The MIC value for sea mango leaf ethyl acetate extract against *E. coli* was 5000 ppm or 5 mg/ml against *S. aureus* was 2500 ppm or 2.5 mg/ml. MBC value for sea mango leaf ethyl acetate extract against *E.coli* was 10000 ppm or 10 mg/ml while for *S.aureus* was 5000 ppm or 5 mg/ml. The antibacterial activity of these two extracts were not so much different this might be in both extracts containing substances that work as antibacterials. On the other hand, Arockiya *et al*. (2014) also conducted a study, the antibacterial effect of sea mango leaves made into nano particles, the results of their study also found that the water extract of sea mango leaves was effective against *E. coli, S. aureus, S. pyogenes, V. cholera and S. Typhi.* Then the results of the study by Lestari *et al*. (2016) found that sea mango flower extract was also effective against *S. aureus* bacteria (Lestari *et al*., 2016), while the research from Sahoo *et al*. (2018) showed that methanol extract of sea mango had various antibacterial activities against *Bacillus subtilis, Staphylococcus aureus, Salmonella typhiand Escherichia coli* with inhibitory zone ranging from 2 mm to 3 mm, whereas the aqueous extract showed no activity. High antifungal activity was found against *Saccharomyces cerevisiae* and *Candida albicans* for methanol extract (Sahoo *et al*., 2018). Antibacterial Effects of Sea mango, besides the leaves, the fruit also has an antibacterial effect, as research conducted by Ahmad *et al.* (on several bacteria found that extracts of fruit Sea mango quite effective against gram-positive bacteria such as, *Staphylococcus aureus, Streptococcus saprophyticus, Streptococcus pyogenes* and was not effective in against *Staphylococcus epidermis,* while against the gram-negative bacteria was obtained quite effective against *Salmonella typhi, Shigella flexneri, Shigella dysenteriae* and was not effective in against *Shigella boydii, Shigella sonnie.* On the other hand, the results of research from Dalia Sukmawati (2016) had also found that *Sea mango* leaf water extract has the effect of killing fungi that contaminate animal feed, namely *Aspergillus sp* and *Penicillium sp.* (Dalia Sukmawati, 2016).

Conclusion

Dichloromethane and ethyl acetate extracts of sea mango leaves have antibacterial activity both against Gram positive (*S.aureus*) and Gram negative (*E.coli*) bacteria. Equivalence of dichloromethane extract with ampicillin against *E.coli* at a concentration of 10 mg/ml, 20 mg/ml, and 30 mg/ml was 20.55 µg/ml, 25.27 µg/ml, 34.98 µg/ml respectively, and for *S .aureus* 5.17 µg/ml, 8.87 µg/ ml and 13.04 µg/ml respectively. Whereas the Equivalence of ethyl acetate extract with ampicillin agaist *E.coli* at a concentration of 10 mg / ml, 20 mg / ml, and 30 mg / ml was 26.12 µg/ml, 28.37 µg/ml, 32.80 µg/ml and for S. aureus 10.37 µg/ml, 12.02 µg/ml and 15.55 µg/ml respectively. The MIC value of the dichloromethane extract of sea mango leaves against *E.coli* was 2.5 mg/ml and the MBC value was 5 mg/ml. The MIC value against *S. aureus* was 1.5 mg/ml while the MBC value was 2.5 mg/ml. The MIC value of the sea mango leaf ethyl acetate extract against *E.coli* was 5 mg/ml and the MBC value was 10 mg/ml. The MIC value of *S. aureus* is 2.5 mg/ml while the KBM value was 5 mg/ml.

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Declaration of conflicts of interest: The authors declare no have conflicts of interest

REFERENCES

Ahmed, F., Amin, R., Shahid, IZ. and Sobhani, MME. 2008. Antibacterial, cytotoxic and neuropharmacological activities of *Cerbera odollam* seeds, *J. Oriental Pharmacy and Experimental Medicine,* (4), 323-328, DOI 10.3742/ OPEM.2008.8.4.323.

Harbone, A.J. 1998. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis*, Publisher Springer Netherlands*,

Amarpreet Kour, 2014. Plants Exhibiting Potential for Cancer Treatment, *Int. J. Pharm. Sci. Rev. Res*., 27(2), July – August, Article No. 06, Pages: 23-53.

Arockiya Aarthi Rajathi, F. and Subramanyam Nambaru, V.R.M. 2014. Phytofabrication of nano-crystalline platinum particles by leaves of Cerbera manghas and its antibacterial efficacy. *Int J Pharm Bio Sci.,* 5(1): (P) 619 – 628.

Bari, IN. and Kato-Noguchi, H. 2017. Phytotoxic efects of Cerbera manghas L. leaf extracts on seedling elongation of four monocot and four dicot test species. *Acta Agrobot*., 70(3):1720. https://doi.org/10.5586/aa.1720.

Chang, L.C., Gills, J.J., Bhat, K.P.L., Luyengi,L., Farnsworth, N.R., Pezzuto, J.M. and Kinghorn, A.D. 2000. Activity-

Guided Isolation of Constituents of Cerbera manghaswith Antiproliferative and Antiestrogenic Activities, Bioorganic and Medicinal Chemistry Letters, 10, 2431-2434.

Cheenpracha, S., Karalai, C., Rat-A-Pa, Y., ponglimanont, C. and Chantrapomma, K. 2004. new cytotoxic cardenoline glycoside from the seeds of *Cerbera manghas*. *Chem. Pham.Bull.,* 52: 1023-5.

Dalia Sukmawati, 2016. Antagonism Mechanism of Fungal Contamination Animal Feed using Phylloplane Yeasts Isolated from the Bintaro Plant (Cerbera manghas) Bekasi in Java, Indonesia. Int.J.Curr.Microbiol.App.Sci. 5(5): 63-74. doi:http://dx.doi.org/10.20546/ijcmas.2016. 505.007.

Lestari, DRS., Lisa, S. and Liliek, SH. 2016. Antibacterial and antibiofilm potential of ethanolic extract from bintaro flower (Cerbera odollam) against Staphylococcus aureus ATCC 6538, *Proceeding ICMHS*., 17-19.

Liu, P.C., Liu, M.H., Chen, S.Y., Cherng, W.J. and Wang, C.H. 2008. Sea mango cardiac intoxication. *Acta Cardiol Sin.,* 24: 9-56.

Monjur-Al-Hossain, A.S.M., Sumon, S., Sanjib, S., Lokman, H Md. and Mahadhi, H Md. 2013. Biological assessment on Cerbera manghas (linn.), J. *Pharmacology Online*, vol.1, pp,155 – 160.

Palhares, RM. and Gonçalves Drummond, M, dos Santos Alves Figueiredo Brasil B, Pereira Cosenza G, das Graças Lins Brandão M, Oliveira G. 2015. Medicinal Plants Recommended by the World Health Organization: DNA Barcode Identification Associated with Chemical Analyses Guarantees Their Quality. PLoS ONE 10(5): e0127866. doi:10.1371/journal.pone.0127866.

Rahman, M. D.A., Paul, P. and Rahman, A.A. 2011. Antinociceptive, Antibacterial & Diuretic Activities of Cerbera odollam Gaertn Roots, Research Journal of Pharmaceutical, Biological and Chemical ciences, 2(3), 16-23.

Sahoo A, Marar T, 2018. Phytochemical Analysis, Antioxidant Assay and Antimicrobial Activity in Leaves Extracts of Cerbera odollam Gaertn. *Pharmacog J.*, 10(2):285-92.

WHO (World Health Organization), 2011, The World Traditional Medicines Situation, in Traditional medicines: Global Situation, Issues and Challenges. Geneva 3:1–14.

WHO, 2014, Antimicrobial resistance: global report on surveillance, p.12-30

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