
Phytochemical Analysis and *in vitro* Antibacterial Activities of Seagrass *Enhalus acoroides* against *Staphylococcus aureus*

Desy Setyoningrum, Ade Yamindago, Syarifah Hikmah Julinda Sari, Maftuch Maftuch

Faculty of Fisheries and Marine Science, University of Brawijaya, Indonesia.

Email address: desy.setyoningrum16@gmail.com

KEYWORDS

Seagrass
Enhalus acoroides
Phytochemical
analysis
Antibacterial activity

Abstract Several studies of marine bioactive compounds have been carried out using seagrass. *Enhalus acoroides* is a type of seagrass that has bioactive compounds including alkaloids, flavonoids, tannins, steroids, and saponins that have potential as an antibacteria. The aim of study is to investigate the phytochemical compound contained in the crude extract of *Enhalus acoroides* and its antibacterial activities of *Enhalus acoroides* against *Staphylococcus aureus*. The seagrass was collected, washed, dried, grind and exposed to extraction by vacuum rotary evaporator at temperature of 40°C and was analyzed for their phytoconstituents. Further, the crude extract was tested against pathogenic bacterial at different concentrations using disc diffusion method to determine the effect of bioactive compounds in *E. acoroides* to bacteria. In the present study, qualitative test of phytochemical from ethanol extract of *Enhalus acoroides* leaves contained phytochemical compound of alkaloids, flavonoids, tannins, steroids and saponins, while the extract of roots contained all of compounds except saponins. Based on research can be concluded, that *Enhalus acoroides* also has antibacterial activity against *Staphylococcus aureus* that showed by the presence of inhibition zone. The present finding suggests that the extract of seagrass *Enhalus acoroides* can be used an antibacterial agent from marine.

Introduction

Seagrasses are the only flowering plants (Angiosperms) that have the ability to adapt to life and grow abundantly in tidal and subtidal areas of all seas except polar region (Mani *et al.*, 2012). Seagrass ecosystems are the most productive ecosystems, because they have many functions for example as habitat of biota and as nurseries for many species of fish and shellfish. They also play an important role in trapping, stabilizing the sediments and recycling nutrients (Sjafrie *et al.*, 2018). Moreover, the grasses used as indicator of good seawater quality as it's highly sensitive to marine pollutions (Cozza *et al.*, 2004). Seagrass species that are often found in Indonesia, one of which

is *Enhalus acoroides*. This species is easy to be identified because it has a different morphology than other species. *Enhalus acoroides* has large roots, stems and leaves are long, hard, and stiff like belt (Dewi *et al.*, 2018).

Generally, seagrasses are rich source of secondary metabolites which is believed to be a defense mechanism in these plants (Athiperumalsami *et al.*, 2008). Seagrass is well documented for the presence of secondary metabolites. The bioactive compounds contained in seagrasses have the potential as antibacterial agent (Kannan *et al.*, 2010), anticancer, and antioxidant (Santoso *et al.*, 2012). Several investigations have demonstrated that the *Enhalus acoroides* has

antibacterial activity. For example, Qi *et al.* (2008) reported that the leaves of *E. acoroides* collected from China contain bioactive compound such as flavonoids and steroids, it has potential as an antibacterial agent against some marine bacteria. Moreover, Ali *et al.* (2012) found that roots of seagrass *S. isoetifolium* from India have antibacterial compounds, such as alkaloids, flavonoids, phenols, saponins and tannins. The methanol extract of *S. isoetifolium* which were tested against 17 human pathogens and 5 fish pathogens showed appreciable antibacterial activity (Mani *et al.*, 2012). Kannan *et al.* (2010) also tested the methanol extract of seagrass *H. pinifolia* and *C. rotundata* and it showed growth inhibitory activity against all the UTI bacteria. This information is the basic for identifications of phytochemical compounds in seagrass *Enhalus acoroides* from Paciran Water. Therefore, this study aimed to investigate the qualitative test for phytoconstituents of the seagrass determined *Enhalus acoroides* and investigate antibacterial activity of seagrass *Enhalus acoroides* against *Staphylococcus aureus*.

Material and methods

Sample

Fresh *E. acoroides* was collected from Paciran Waters, Lamongan Regency (6°52'26.49''S 112° 23'24''E). The sample was brought to the laboratory in plastic bags and washed by tap water (to remove the salt and epiphytes), and dried naturally under the sun. The dried leaves and roots were powdered using an electric blender and the powder sample was stored in the refrigerator for further use (Santoso *et al.*, 2012). The bacterial strain was obtained from Faculty of Medicine, University of Brawijaya.

Method of Extraction

E. acoroides extraction was followed by a study of Juwita *et al.* (2013) with some

modification. Briefly, *E. acoroides* powder (50 g) were soaked in organic solvent, ethanol (750 ml), and it was macerated for 24 hours at room temperature. Then, the *E. acoroides* sample solution was filtered using filter paper. After that, *E. acoroides* sample solution of filtration result was evaporated using vacuum rotary evaporator at 40°C. Then, the paste (1.47 gr) was stored at 4°C until use.

Phytochemical Analysis

The qualitative of the phytochemical compounds in *E. acoroides* extract can be identified by observing the colour changes that occurred in the extract added with several chemical compounds. The phytochemical test such as alkaloids, flavonoids, steroids, tannins, and saponins were carried out by Harborne (1973).

The alkaloid was estimated by mixing 0.5 mL of extract with 5 mL of 2% HCL, and then adding 2-3 drops of Dragendorff reagent. If brown precipitate is formed in tube, the alkaloids were present. The presence of flavonoids in extract was identified by mixing 0.5 mL of extract with Mg metal and 1-2 mL of methanol solution. Then, about 4-5 drops of HCL were added. The test is positive if red or orange colour is formed in solution. For the presence of steroid, about 1 mL of extract was mixed with 2 mL of chloroform and 2-3 drops of H₂SO₄. The presence of red, then blue or green colour indicated the presence of steroids compound.

The tannin contents in the extract was determined by dissolving 0.5 mL of extract in 5 mL of water and 1-2 drops of FeCl₃ solution. A blackish-blue colour that was observed indicating a positive test for tannins. To detect saponins in the extract, about 0.5 mL of extract was boiled in 5 mL of water and shaken vigorously for a stable foam for 30 minutes indicates the presence of saponins. The presence of each compound was reported and density was score 2+, 1+, or 0(-) according to intensity of colour.

Antibacterial Activity

Antibacterial activity of the extract *E. acoroides* was analyzed using disc diffusion on Tryptone Soya Agar (TSA). Briefly, sterile filter paper disc 6 mm in diameters (Whatman #1) were impregnated in the solvent extract with different concentration (200 ppm, 400 ppm, 800 ppm, and 1600 ppm) and air-dried. Disc containing solvent (ethanol 96%) alone were used as negative control and amoxicillin (200 ppm) were used as positive control. Then, the paper disc was placed on the Tryptone Soya Agar (TSA) after spreading a swab impregnated in Tryptone Soya Broth with 1.5×10^5 CFU/mL bacteria strain. After that, plate was incubated in triplicate for each treatment for 24 hours at 37°C. Antibacterial activity of extract *E. acoroides* were evaluated by measuring inhibition zone in millimeters.

Result and Discussion

Phytochemical Test of *Enhalus acoroides*

Phytochemical test is one of the qualitative test for identifying the bioactive compounds that are possible within an organism (Dewi et al., 2018). Qualitative phytochemical analysis was done for various phytoconstituents in seagrass *Enhalus acoroides*. Several bioactive compounds were detected in the *E. acoroides* using ethanol extraction solutions. The results of phytochemical test of the extract *E. acoroides* were summarized in Table 2. As result shown in Table 2, the ethanol extract of *E. acoroides* revealed the presence of alkaloids, flavonoids, steroids, tannins and saponins compounds.

Putra et al. (2015) tested the ethanol extract of seagrass contained the bioactive compound alkaloids, tannins, flavonoids, quinones, monoterpenes, steroids and polyphenolic compound. The bioactive compounds such as phenols, tannins, and flavonoids contained in seagrass *C. rotundata* are compounds that have antibacterial properties (Septiani et al., 2017).

The results of study shown in Table 2, the leaf extract of *E. acoroides* showed the presence of alkaloids, flavonoids, tannins, steroids and saponins compounds. While the saponins test in this study showed a zero or negative value, meaning that there were no saponins compound in the roots extract of *E. acoroides*. The results were similar with the study by Ali et al. (2012) which reported that the seagrass *Cymodocea serrulata* and *Halopila beccarii* showed there were no saponin compounds. The chemical compositions of *E. acoroides* showed high concentration (i.e.: 2+) of alkaloids, flavonoids, tannins and steroids in leaves extract of *E. acoroides* (Table 2). Saponins composition of the leaf *E. acoroides* showed weak reaction. In our study, the comparison of bioactive composition in the leaf extract of *E. caoroides* were higher than the roots extract. (Sibi et el., 2012) reported that the phytochemical analysis of roots extract of *M. citrifolia* shows the total phenols, flavonoids, tannins and steroids were higher than that of the leaves and stems extract. Whereas, the leaves extract of the plant has higher level of total alkaloids, glycosides, saponins and terpenoids

Table 2. Phytochemical of the Ethanol Extract of Seagrass *Enhalus acoroides*

No	Phytochemical compounds	<i>Enhalus acoroides</i> extract	
		Leaves	Roots
1	Alkaloids	++	+
2	Flavonoids	++	+
3	Tannin	++	+
4	Saponins	+	-
5	Steroids	++	+

Note : (-):Not detected, (+):Detected (Moderate), (++) : Detected (Strong)

Table 3. Antibacterial Activity of Leaves and Roots of *Enhalus acoroides* against *Staphylococcus aureus*.

Extract of <i>E. acoroide</i>	Concentrations (ppm)	Zone of Inhibition in mm
Leaves	200 ppm	7.33± 0.91
	400 ppm	8.37± 1.11
	800 ppm	8.40± 1.01
	1600 ppm	11.60±1.51
	Amoxicillin (200 ppm)	14.3
	Ethanol	0
Roots	200 ppm	2.83±2.49
	400 ppm	8.63± 1.11
	800 ppm	9.20± 0.53
	1600 ppm	9.73± 0.42
	Amoxicillin (200 ppm)	13.1
	Ethanol	0

Antibacterial activity of Enhalus acoroides extract

Antibacterial potential of *E. acoroides* extract was assessed in terms of zone of inhibition of bacterial growth. The inhibition zone was used as a parameter to measure the effect of the antibiotic or antibacterial agent to inhibit bacterial growth (Fitri et al., 2017). The antibacterial activity of leaves and roots *Enhalus acoroides* against *Staphylococcus aureus* using disc diffusion method is represented in Table 3. The above results showed that the ethanol extract of *Enhalus acoroides* had an antibacterial activity against *Staphylococcus aureus*. Some of the concentrations used in this study, leaves extract of seagrass *E. acoroides* showed an inhibition zone greater than roots extract. The differences of the bioactive compounds between leaves and roots extract possibly caused differences in inhibition zone. The plants have many active compounds such as flavonoids, tannins, saponins, sterols, alkaloids, and glycosides which can be used to inhibit bacteria growth. Many researches have used the ethanol extraction method to extract the maximum amount of the chemical compounds contained on the leaves of plants (Fitri et al., 2017).

The ethanol as negative control showed no inhibition zone, this result showed that in this study ethanol as a solvent in the extraction process had no antibacterial effects. Whereas, amoxicillin (200 ppm) as a positive control showed the inhibition zone ranging from 13.1 mm to 14.3 mm, these values showed a strong category. To determine the potential of an extract as an antibacterial based on the measure of positive control. The results showed that leaves extract of *E. acoroides* on the concentration of 1600 ppm has antibacterial activity similar to amoxicillin. According to Mahardika et al. (2014), the inhibition of natural plant extract is considered to be very strong if the clear zone is >20 mm, strong between 10 mm to 20 mm, medium between 5 mm to 10 mm, and weak <5 mm.

The ability to inhibit the growth of *Staphylococcus aureus* by seagrass *E. acoroides* extract cannot be separated from the content of its active compounds. Tannins, steroids, flavonoids, glycosides, and alkaloids have antibacterial mechanism by damaging the cell wall structure, disturbing a cytoplasm, and inhibit enzymes activities (Sabir, 2005; Mahardika et al., 2014; Ngajow et al., 2013).

Manosalva et al. (2016) reported that the *in vitro* antibacterial activities of leaves, stems, and roots alkaloid extract had significant activity against Gram-positive bacteria. The alkaloids possessed good antibacterial activity against several test bacteria. The alkaloid compounds are known to be a DNA intercalator, so they have antibacterial effects by inhibit the activity of topoisomerase enzymes. Topoisomerase is an enzyme for DNA replication (Dassonneville et al., 2000; Karou et al., 2005; Fitri et al., 2017).

Conclusion

Based on our research, the phytochemical test showed that seagrass *Enhalus acoroides* extract contained several bioactive compounds, including: alkaloids, flavonoids, tannins, steroids, and saponins. The extract of *Enhalus acoroides* has a potential to be used as a natural antibacterial agent from marine. Isolation of bioactive compounds contained in the seagrass *Enhalus acoroides* needs to be done to determine the most effective compounds as antibacterial agent. The abundance of seagrass availability in Indonesia opens up a new avenue for the pharmaceutical industries to develop new drugs for human and aquaculture.

References

- Ali, M. S., Ravikumar, S., & Beula, J. M. (2012). Bioactivity of seagrass against the dengue fever mosquito *Aedes aegypti* larvae. *Asian Pacific Journal of Tropical Biomedicine*, 2(7), 570–573. [https://doi.org/10.1016/S2221-1691\(12\)60099-9](https://doi.org/10.1016/S2221-1691(12)60099-9)
- Athiperumalsami, T., Kumar, V., & Jesudass, L. L. (2008). Survey and phytochemical analysis of seagrasses in the Gulf of Mannar, southeast coast of India. *Botanica Marina*, 51(4), 269–277. <https://doi.org/10.1515/BOT.2008.038>
- Cozza, R., Chiappetta, A., Petrarulo, M., Salimonti, A., Rende, F., Bitonti, M. B., & Innocenti, A. M. (2004). Cytophysiological features of *Posidonia oceanica* as putative markers of environmental conditions. *Chemistry and Ecology*, 20(3), 215–223. <https://doi.org/10.1080/02757540410001689777>
- Dassonneville, L., Lansiaux, A., Wattelet, A., Wattez, N., Mahieu, C., Van Miert, S., ... Bailly, C. (2000). Cytotoxicity and cell cycle effects of the plant alkaloids cryptolepine and neocryptolepine: Relation to drug-induced apoptosis. *European Journal of Pharmacology*, 409(1), 9–18. [https://doi.org/10.1016/S0014-2999\(00\)00805-0](https://doi.org/10.1016/S0014-2999(00)00805-0)
- Dewi, C. S. U., Kasitowati, R. D., & Siagian, J. A. (2018). Phytochemical compounds of *Enhalus acoroides* from Wanci Island (Wakatobi) and Talango Island (Madura) Indonesia. *Asean-Fen International Fisheries Symposium*, 137(1), 012045. <https://doi.org/10.1088/1755-1315/137/1/012045>
- Fitri, G. D., Tistiana, H., & Radiati, L. E. (2017). Review study on antibacterial activity of cherry leaf (*Muntingia calabura*) against *Staphylococcus* spp. and *Salmonella* spp. the most causing disease in livestock. *Jurnal Ilmu-Ilmu Peternakan*, 27(2), 63–73. <https://doi.org/10.21776/ub.jiip.2017.027.02.08>
- Harborne, J. B. (1973). *Phytochemical Methods*. In Chapman and Hall (3rd ed., Vol. 29). <https://doi.org/10.1007/978-94-009-5921-7>
- Juwita, A. P., Yamlean, P. V. Y., & Edy, H. J. (2013). *Formulasi Krim Ekstrak Etanol Daun Lamun (Syringodium isoetifolium)*.

- Parmachon Jurnal Ilmiah Farmasi-UNSRAT, 2(2), 8–13.
- Kannan, R. R. R., Arumugam, R., & Anantharaman, P. (2010). Antibacterial potential of three seagrasses against human pathogens. *Asian Pacific Journal of Tropical Medicine*, 3(11), 890–893. [https://doi.org/10.1016/S1995-7645\(10\)60214-3](https://doi.org/10.1016/S1995-7645(10)60214-3)
- Karou, S. D., Savadogo, A., Canini, A., Yameogo, S., Montesano, C., Simpore, J., ... Tor, R. (2005). Antibacterial activity of alkaloids from *Sida acuta*. *Afr. J. Biotechnol*, 5(12), 195-200. Retrieved from <http://www.academicjournals.org/AJB>
- Mahardika, H. A., Sarwiyono, & Surjowardojo, P. (2014). Ekstrak Metanol Daun Kersen (*Muntingia calabura* L) Sebagai Antimikroba Alami Terhadap Bakteri *Staphylococcus aureus* Penyebab Mastitis Subklinis pada Sapi Perah. *J. Ternak Tropika*, 15(2), 15–22.
- Mani, A. E., Aiyamperumal, V., & Patterson, J. (2012). Phytochemicals of the Seagrass *Syringodium isoetifolium* and its Antibacterial and Insecticidal Activities. *Europ. J. Bio. Sci.*, 4(3), 63–67. <https://doi.org/10.5829/idosi.ejbs.2012.4.3.6455>
- Manosalva, L., Mutis, A., Urzúa, A., Fajardo, V., & Quiroz, A. (2016). Antibacterial activity of alkaloid fractions from *berberis microphylla* G. Forst and study of synergism with ampicillin and cephalothin. *Molecules*, 21(1), 76. <https://doi.org/10.3390/molecules21010076>
- Ngajow, M., Abidjulu, J., & Kamu, V. (2013). Pengaruh Antibakteri Ekstrak Kulit Batang Matoa (*Pometia pinnata*) terhadap Bakteri *Staphylococcus aureus* secara In vitro. *Jurnal MIPA UNSRAT*, 2(2), 128–132.
- Putra, R. T., Lukmayani, Y., & Kodir, R. A. (2015). Isolasi Dan Identifikasi Senyawa Flavonoid Dalam Tumbuhan Lamun *Cymodocea Rotundata*. *Prosiding Penelitian SPeSIA Unisba*, 444–450. Retrieved from <http://karyailmiah.unisba.ac.id/index.php/farmasi/article/view/1986>
- Qi, S.-H., Zhang, S., Qian, P.-Y., & Wang, B.-G. (2008). Antifeedant, antibacterial, and antilarval compounds from the South China Sea seagrass *Enhalus acoroides*. *Botanica Marina*, 51(5), 441-447. 10.1515/BOT.2008.054
- Sabir, A. (2005). Aktivitas antibakteri flavonoid propolis *Trigona* sp terhadap bakteri *Streptococcus mutans* (in vitro) (In vitro antibacterial activity of flavonoids *Trigona* sp propolis against *Streptococcus mutans*). *Dental Journal (Majalah Kedokteran Gigi)*, 38(3), 135–141. <https://doi.org/10.20473/j.djmk.v38.i3.p135-141>
- Santoso, J., Anwariyah, S., Rumiantin, R. O., Putri, A. P., Ukhty, N., & Yoshie-Stark, Y. (2012). Phenol Content, Antioxidant activity and Fibers Profile of Four Tropical Seagrass From Indonesia. *Journal of Coastal Development*, 15(2), 189–196.
- Septiani, S., Dewi, E. N., & Wijayanti, I. (2017). Aktivitas Antibakteri Ekstrak Lamun (*Cymodocea rotundata*) Terhadap Bakteri *Staphylococcus aureus* dan *Escherichia coli*. *SAINTEK PERIKANAN : Indonesian Journal of Fisheries Science and Technology*, 13(1), 1–6. <https://doi.org/10.14710/ijfst.13.1.1-6>

- Sibi, G., Cathly, P., Adhikari, S., & Ravikumar, K. R. (2012). Phytoconstituent Anf Their Influence on Antimicribial Properties of *Morinda Citrifolia* L. *Res. J. Med. Plant*, 6(6), 441–448.
- Sjafrie, N. D. M., Hernawan, U. E., Prayudha, B., Supriyadi, I. H., Iswari, M. Y., Rahmat, ... Suyarso. (2018). *Status Padang Lamun Indonesia 2018*. Jakarta Utara: Pusat Oseanografi-LIPI.