Jurnal Ilmu Kelautan **P-ISSN: 2460-0156**
SPERMONDE (2021) 7(2): 38-43 **E-ISSN: 2614-5049**

ANTIBACTERIAL ACTIVITY OF *CAULERPA RACEMOSA* ENDOPHYTIC FUNGI FROM LEMUKUTAN ISLAND WATERS

Syarif Irwan Nurdiansyah[1], Mega Sj Sofiana[1], Melia Trianasta[1], Warsidah[1]*, Maulana Hidayat[1]

Submitted: September 4, 2021 Accapted: December 23, 2021 Online Published: February 14, 2022

[1]Department of Marine Science, FMIPA, University Tanjungpura
Corresponding Author:
*Warsidah
E-mail:warsidah@fmipa.untan.ac.id

ABSTRACT

Green macroalgae *Caulerpa racemosa* from the waters of Lemukutan Island was isolated to obtain endophytic fungi. Endophytic fungi were explored to determine the antibacterial activity. Isolation of endophyte fungi was carried out by dilution method using PDA (Potato Dextrose Agar) seawater media. Endophyte fungi with different morphology were obtained 11 isolates, namely CRF01, CRF02, CRF03, CRF04, CRF05, CRF06, CRF07, CRF08, CRF09, CRF10 and CRF11. Antibacterial activity testing was carried out by agar diffusion method. Endophyte fungi isolate CRF09 showed the highest activity against both Escherichia coli and Staphylococcus aureus test bacteria with inhibition zone diameters of 15.96 mm and 16.47 mm respectively. Endophyte fungi isolates identified from the green macroalgae *Caulerpa recemosa* were of the genus *Trichocladium* sp., *Aspergilus* sp., *Chaetomium* sp., *Coprinus* sp., *Hymenochaete* sp., *Rhizopus* sp., *Trenella* sp. and *Zygorhynchus* sp.

Keywords: antibacterial. endophyte fungi, *Caulerpa racemosa*, Lemukutan Island, activity

INTRODUCTION

Macroalgae (seaweed) is a plant-like organism that lives in coastal waters and grows attached to substrates (Meriam et al., 2016; Kepel and Mantiri, 2019). Based on macroalgae morphology including Thallophyta plants where roots, stems and leaves have not been clearly identified (not yet true) (Meriam et al., 2016). According to Rahmat et al. (2020), macroalgae generally consists of three classes namely Chlorophyta class (green macroalgae), Phaeophyta (brown macroalgae) and Rhodophyta (red macroalgae). Green macroalgae have branched filament-shaped thallus. It has chlorophyll pigments a, b, carotene, lutein and zeaxanthin (Meriam et al., 2016).

One of the green macroalgae species in coastal waters is Caulerpa racemosa. It has green thallus composed of ramuli, stolon and holdfast. Macroalgae produces secondary metabolites as a form of self-defense (Minarti et al., 2019). According to Ridhowati and Asnani (2016), there are about 500 chemical compounds derived from macroalgae have been identified and most of them are bioactive compounds derived from secondary metabolites. C. racemosa has secondary metabolite compounds such as alkaloids, phenols, flavonoids, tannins, saponins, terpenoids and steroids. They showed antibacterial activities (Wulandari, 2017; Marfuah et al., 2018; Indayani et al., 2019).

Secondary metabolites produced from marine organisms also produced by associated microorganisms such as fungal endophyte. Endophytic fungi are fungi that live intracellularly in plant tissues by forming colonies in tissues without causing harmful effects on their hosts (Murdiyah, 2017). They protect their hosts and produce secondary metabolite compounds that can kill pathogens (Andriani, 2015). Endophytic fungi can produce bioactive compounds that have biological activities such as antioxidants, anticancer, antibacterial, antifungi, antimalaria and antivirus (Rollando et al., 2017).

Antibacterial is a compound that can be used to control the growth of pathogenic bacteria (Marfuah et al., 2018). Based on research conducted by Andriani (2015) that C. racemosa from Takalar Regency, South Sulawesi obtained 1 isolate of endophytic fungi that have antibacterial activity against Escherichia coli bacteria and Staphylococcus aureus with diameter of inhibition zone of 18.5 mm and 18.35 mm, respectively. The ability of endophytic fungi to produce secondary metabolite compounds similar to their host plants is a solution to find antibacterial sources without damaging existing ecosystems. The aim of this research is exploring of antibacterial fungi endophytes from the macroalgae C. racemosa in the waters of Lemukutan Island, Bengkayang Regency, against E. coli and S. aureus.

MATERIALS AND METHODS

This research was conducted in December 2020-March 2021. Macroalgae sampling of C. racemosa in the waters of Lemukutan Island, Bengkayang Regency, West Kalimantan (Figure 1). Isolation and testing of antibacterial activity was conducted in the Laboratory of Microbiology, Technical

Implementation Unit of The Implementation of Quality of Fishery Products, West Kalimantan Province.

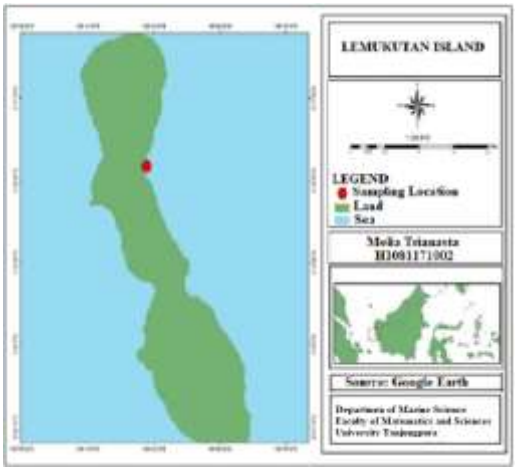

Figure 1. The research site

Sampling of Macroalgae *C. Racemosa*

Sampling of *C. racemosa* was taken using basic diving equipment. Samples are taken at a depth of 1-2 m, then washed with sea water. The sample is put in a sterile plastic sample containing sea water, then stored in a cool box containing ice cubes with the aim of maintaining the freshness of the sample and subsequently in the analysis in the laboratory.

Isolation of Endophyte Fungi

Macroalgae samples of *C. racemosa* as the source of isolates to be isolated weighed as much as 10 g (Handayani et al., 2019). Furthermore, macroalgae samples are washed with sterilized water and soaking the sample into a solution of sodium hypochlorite 1% for 5 minutes, ethanol 70% 1 minute then cleaned with sterile aquades (Nurzakiyah, 2016). The sample was smoothed using mortal after fine samples were inserted into the erlenmeyer and added sterile sea water until 100 mL. Samples suspended in stock solution taken 1 mL then put in 9 mL sterile sea water to produce dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. Then, 1 mL samples suspended by dilution 10^{-3}, 10^{-4} and 10^{-5} were inoculated on Potato Dextrose Agar (PDA) media dissolved in seawater by pouring method. Chloramphenicol is added as much as 50 mg/L as an antibiotic (Rizky et al., 2019) to the PDA media with the aim of inhibiting bacterial growth. Then, samples incubated for 5-7 days at 25-27 °C.

Antibacterial activity test

Antibacterial activity test conducted using diffusion method agar. The isolate colony of endophytic fungi *C. racemosa* was grown for 7 days then the colony was cut round with a diameter of 6 mm and placed in the media NA (Nutrient Agar) which had previously been scratched with test bacteria and incubated at a temperature of 37 °C for 2 days. Isolates grown in petri dish are analyzed with diameter inhibition zone measurement (Rizky et al., 2019).

Identification of endophyte fungi

The identification of endophyte fungal isolates were carried out based on macroscopic and microscopic observations. Macroscopic observations based on colony color and colony shape (Yunaedi et al., 2016). Microscopic observations of endophytic fungi isolates are seen directly under binocular microscopes with magnification of 100x. The identification of isolation-based endophytic fungi based on the pictoral atlas of soil and seed fungi morphologies of Cultured Fungi and Key to Species, Watanabe (2010), referring to the journal Nie et al. (2017) and Zhao et al. (2019).

RESULTS AND DISCUSSION

Sampling of green macroalgae *C. racemosa*

Sampling of green macroalgae *C. racemosa* was taken in the waters of Lemukutan island, Bengkayang Regency of West Kalimantan (Figure 2). The sampling location was carried out at N 00'46'48.46" E 108°42'23.981". The *C. racemosa* sample was taken at a depth of 0.9-1.3 m with murky water conditions. The conditions coincide with high tides and large ocean waves.

Figure 2. *C. racemosa* in the waters of Lemukutan Island

Isolation of endophytic fungi in *C. racemosa*

The endophyte fungal of *C. racemosa* were isolated using a multilevel dilution method. A series dilution aims to minimize or decrease the number of microbes suspended in the sample. Isolation of endophytic fungi using PDA media (Potato Dextrose Agar) dissolved using seawater. PDA media is a common medium used for the isolation and cultivation of fungi (Rohmi et al., 2019). The isolation of endophytic fungi from *C. racemosa* was obtained 11 isolates, namely CRF01, CRF02, CRF03, CRF04, CRF05, CRF06, CRF07, CRF08, CRF09, CRF10, and CRF11 (Figure 3).

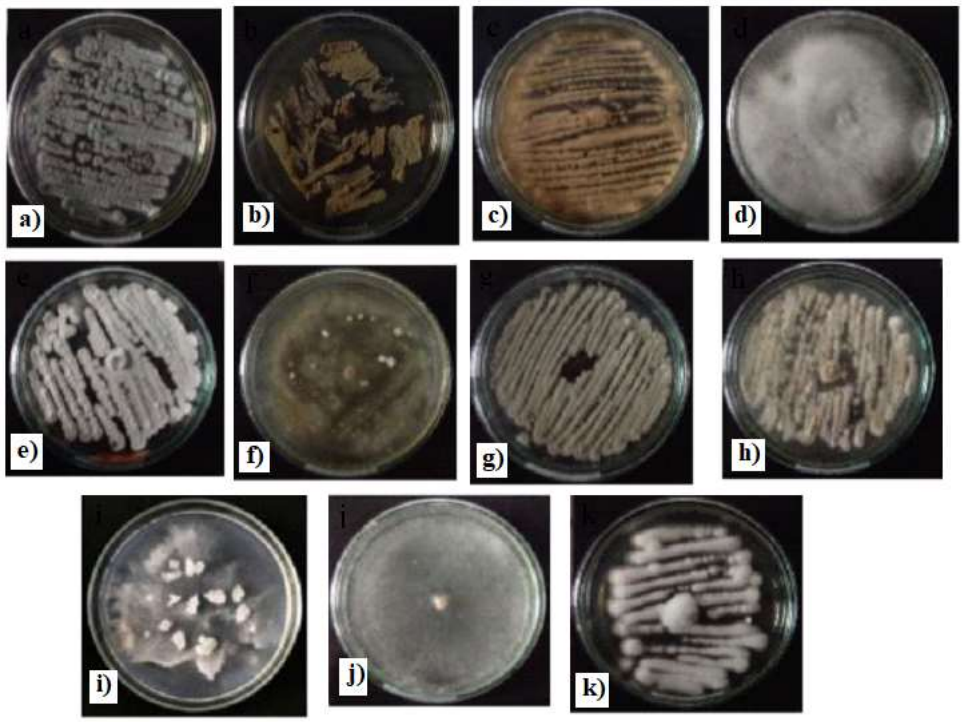

Figure 3. Endophytic fungi of *C. racemosa* a) CRF01; b) CRF02; c) CRF03; d) CRF04; e) CRF05; f) CRF06; g) CRF07; h) CRF08; i)CRF09; j)CRF10 and k) CRF11

Antibacterial Activity Test

Antibacterial activity tests of endophytic fungi isolate *C. racemosa* showed that 11 endophytic fungi isolates had antibacterial activity against *S. aureus* bacteria. Six of 11 isolates were active against *E. coli* bacteria. Antibacterial activity was characterized by the presence of clear zones and fog zones. The clear zone indicates the presence of antibacterial activity, while the fog zone indicates weak antibacterial activity.

CRF03, CRF06 and CRF09 isolates were active against *E. coli* with clear zones diameter of 9.62 mm, 7.45 mm, 15.96 mm, respectively. CRF01, CRF10 and CRF11 isolates forms fog zone. While CRF02, CRF04, CRF05, CRF07 and CRF08 isolates do not form clear zones indicating that there is no antibacterial activity. CRF09 isolates were inhibited *S. aureus* growth with clear zone diameter of 16.47 mm (Table 1).

Test antibacterial activity using diffusion agar method. The antibacterial compounds diffuse into the medium and inhibit the growth of test bacteria. The category of antibacterial activity determined by measuring the clear zone. According to Djakatara et al. (2019), the clear zone >21 mm (antibacterial activity is very strong), 11-20 mm (strong antibacterial activity), 6-10 mm (moderate antibacterial activity) and the <5 mm (weak antibacterial activity). Based on the results of endophytic fungi isolate *C. racemosa* have antibacterial activity against *E. coli* and *S. aureus* test bacteria with categories of antibacterial activity ranging from moderate to strong.

Table 1. Antibacterial activity of endophytic fungi *C. racemosa* against *E.coli* and *S. aureus*

Isolate	Diameter zone of inhibition (mm)	
	E. coli	*S. aureus*
CRF01	±	±
CRF02	-	±
CRF03	9,62	±
CRF04	-	±
CRF05	-	±
CRF06	7,46	±
CRF07	-	±
CRF08	-	±
CRF09	15,96	16,47
CRF10	±	±
CRF11	±	±

The highest antibacterial activity possessed by CRF09 isolates against posisive gram bacteria, namely *S. aureus* (Figure 4). Gram-positive bacteria have a cell wall with a peptidoglycan layer located on the outer membrane. Testing the antibacterial activity of endophytic fungi isolates begins with preparing a 7 day old fungi isolate, then it tested for antibacterial with an incubation time of 24 hours. The purpose of its were get secondary metabolite compounds in the stationary phase (Roosheroe et al., 2016).

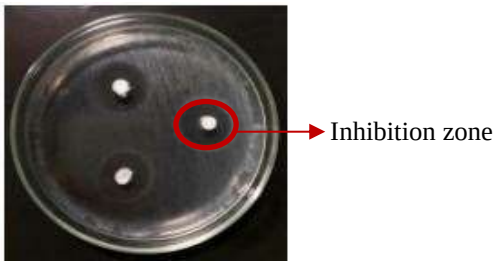

Figure 4. Antibacterial activity test of CRF09 isolate against *S. aureus* bacteria

Identify Endophytic Fungi Isolate C. racemosa

The CRF01 isolated morphological micrographs have oval-shaped spores and directly in laterally arranged hyphae. Its belongs to the genus *Trichocladium*. The morphological micrographs of CRF02 and CRF05 isolates have spores shaped like flowers and form round vesicles with 4 apical fragments, while CRF11 isolates have flower-shaped spores with circles located on hyphae. Morphology in all three isolates belongs to the genus *Aspergillus*. The CRF03 isolates have round-shaped spores with spore surfaces covered by hyaline hair, these features are owned by the genus *Chaetomium*.

The CRF04 isolated morphological micrographs have radially aligned hyaline and subhyaline hyphae. Hyphae does not clump, narrow and does not debauch, these characteristics belong to the genus *Coprinus*. The morphological micrographs of CRF06 and CRF08 isolates have dense hyphae and arranged in a branching frame with cylindrical form. Spores are short and curved cylindrical. The characteristics exhibited by both isolates belong to the genus *Hymenochaete*. CRF09 isolated morphological micrographs have a single, branched and grouped hyaline hyphae. Spores are round in shape, have cells 2-4 and a smooth spore surface. These features belong to the genus *Tremella*.

The morphological micrograph of CRF07 isolates has hiphae that is upright branched, rooted and has spores. Spores are located at the top of hyphae with a triangular shape with sharp ends such as thorns. These features belong to the genus *Rhizopus*. The morphological micrograph of CRF10 isolate has upright, branched hyaline hyphae. Spores are located in hyphae with a rough surface and have spiral hair. These traits belong to the genus *Zygorhynchus*. Isolation and identification of fungi in ocean waters has been widely reported. The genus *Aspergillus* is a fungi found in many marine waters. The fungi genus *Aspergillus* is found in the macroalgae *C. racemosa* in the Indian Sea (Suryanarayanan et al., 2010; Venkatachalam et al., 2015). Morphology of endophytic fungal colonies can be seen in Figure 5.

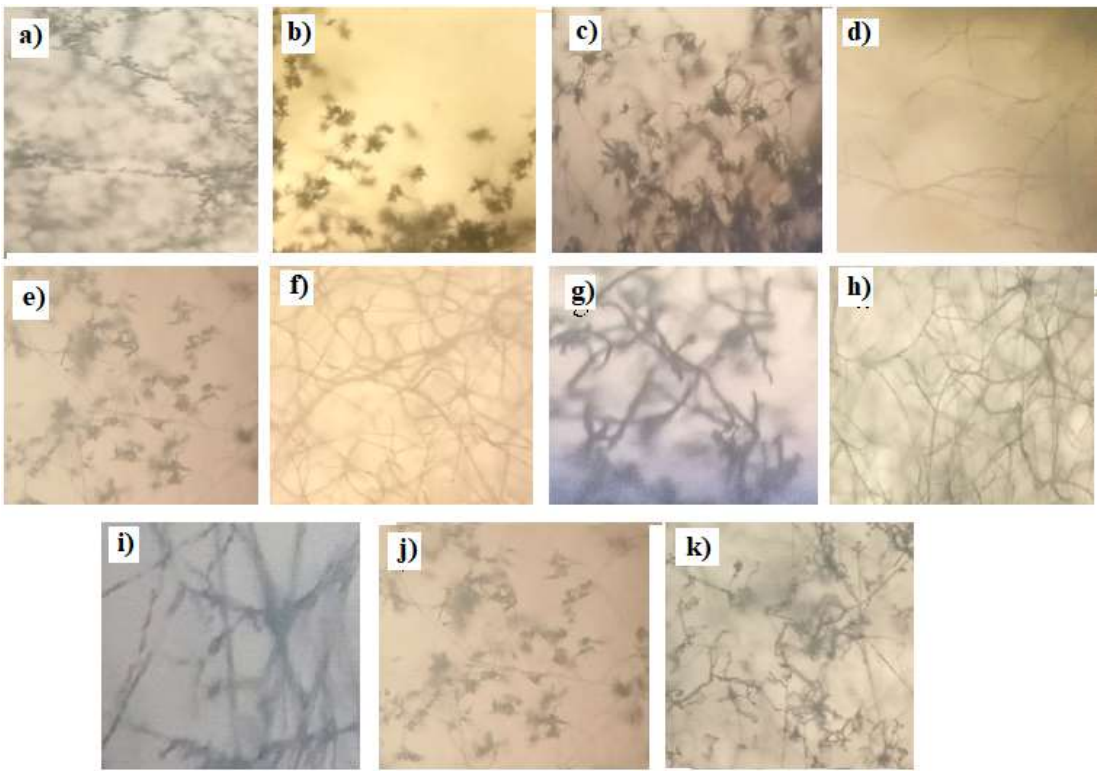

Figure 16. Morphological micrographs isolate endophytic fungi with light microscop binocular of 100x CRF01 (a), CRF02 (b), CRF03 (c), CRF04 (d), CRF05 (e), CRF06 (f), CRF07 (g), CRF08 (h), CRF09 (i), CRF10 (j) and CRF11 (k)

CONCLUSION

Isolation of fungal endophyte *C. racemosa* from the waters of Lemukutan Island, Bengkayang Regency West Kalimantan obtained as many as 11 isolates. The best antibacterial activity of the green macroalgae endophytic fungi *C. racemosa* against *E. coli* and *S. aureus* bacteria is the CRF09 Isolate with a clear zone diameter of 15.96 mm and 16.47 mm, respectively. Identification of the green macroalgae endophyte fungi isolate *C. racemosa* from the waters of Lemukutan Island is known to be the genus *Trichocladium*, *Aspergillus*, *Chaetomium*, *Coprinus*, *Hymenochaete*, *Rhizopus*, *Tremella* and *Zygorhynchus*.

REFERENCES

Andriani. 2015. Aktivitas Antibakterial Fungi Endofit *Caulerpa racemosa* Terhadap Bakteri *Escherichia coli* dan *Staphylococcus aureus*. Prosiding Seminar Nasional Mikrobiologi Kesehatan dan Lingkungan. Makassar: 29 Januari 2015 Jurusan Biologi, Fakultas Sains dan Teknologi, UIN Alauddin, Makassar.

Djakatara, R.S., D.S. Wewengkang, H. Rotinsulu. 2019. Uji Aktivitas Antimikroba dari Jamur Laut yang Berasosiasi dengan Alga *Halimeda opuntia*. Pharmacon J. Ilmiah Farmasi, 8(1) : 41-50.

Handayani, D., N. Ananda, M.A. Artasasta, R. Ruslan, O. Fadriyanti, and T.E. Tallei. 2019. Antimicrobial Activity Screening of Endophytic Fungi Extracts Isolated from Brown Algae *Padina* sp. *J. of Applied Pharmaceutical Science*, 9(3): 9-13.

Indayani, M.K., Asnani, Suwarjoyowirayatno. 2019, Pengaruh Metode Pengeringan yang Berbeda Terhadap Komposisi Kimia, Vitamin C, dan Aktivitas Antioksidan Anggur Laut *Caulerpa racemosa*. J. Fish Protech, 2(1) : 100-108.

Kepel, R.C. dan Mantiri, D.M.H. 2019. Biodiversitas Makroalga di Perairan Pesisir Kora-Kora, Kecamatan Lembean Timur, Kabupaten Minahasa. J. Ilmiah Platax, 7(2) : 383-393.

Marfuah, I., E.N. Dewi, L. Rianingsih. 2018. Kajian Potensi Ekstrak Anggur Laut (*Caulerpa racemosa*) Sebagai Antibakteri Terhadap Bakteri *Escherichia coli* dan *Staphylococcus aureus*. J. Peng. & Biotek. Hasil Pi., 7(1) : 7-14.

Meriam, W.P.M., R.C. Kepel, L.J.L. Lumingas. 2016. Inventarisasi Makroalga Di Perairan Pesisir Pulau Mantehage Kecamatan Wori, Kabupaten Minahasa Utara, Provinsi Sulawesi Utara. J. Ilmiah Platax, 4(2) : 84-108.

Murdiyah, S.. 2017. Fungi Endofit pada Berbagai Tanaman Berkhasiat Obat di Kawasan Hutan Evergreen Taman Nasional Baluran dan Potensi Pengembangan Sebagai Petunjuk Praktikum Mata Kuliah Mikologi. J. Pendidikan Biologi Indonesia, 3(1) : 64-71.

Nie, T., Y. Tian, S.L. Liu, J. Yang, S.H. He, S.H. 2017. Species of Hymenochaete (Hymenochaetales, Basidiomycota) on Bamboos from East Asia, with Descriptions of Two New Species. J. Myco Keys, 20: 51-65.

Nurzakiyah. 2016. Isolasi dan Identifikasi Molekuler Bakteri Endofit *Caulerpa racemosa* serta Aktivitas Antibakterinya Terhadap *Staphylococcous aureus* dan *Methicillin Resistant Staphylococcous aureus* (MRSA), Skripsi Fakultas Sains dan Teknologi, Universitas Islam Negeri Alauddin, Makassar.

Ridhowati, S. dan Asnani. 2016. Potensi Anggur Laut Kelompok *Caulerpa racemosa* Sebagai Kandidat Sumber Pangan Fungsional Indonesia. J. Oseana, 41(4) : 50–62.

Amelia, R. D., Warsidah dan M.S.J. Sofiana. 2019. Isolasi dan Identifikasi Fungi Berasosiasi Lamun *Thalassia hemprichii* dari Perairan Pulau Kabung, J. Laut Khatulistiwa, 2(3) : 102-106.

Rollando, M., D. Aditya, D. Notario, E. Monica, dan R. Sitepu, R., 2017, Kajian Aktivitas Antibakteri, Antioksidan dan Sitotoksik Fungi Endofit Genus *Fusarium* sp. Isolat Daun Meniran (*Phyllantus niruri* Linn.). J. Pharmaciana, 7(1) : 95-104.

Roosheroe, I.G., W. Sjamsuridzal dan A. Oetari. 2006. Mikologi Dasar dan Terapan. Yayasan Pustaka Obor Indonesia. Jakarta.

Suryanarayanan, T.S., A. Venkatachalam, N. Thirunavukkarasu, J.P. Ravishankar, M. Doble, and V. Geetha. 2010. Internal Mycobiota of Marine Macroalgae from the Tamilnadu Coast: Distribution, Diversity and Biotechnological Potential. J. Botanica Marina, 53: 457-468.

Venkatachalam, A., R.M.B. Govinda, N. Thirunavukkarasu and T.S. Suryanarayanan. 2015. Endophytic Fungi of Marine Algae and Seagrasses: a Novel Source of Chitin Modifying Enzymes. J. Mycosphere, 6(3) : 345-355.

Watanabe, T. 2010. Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species, CRC Press LLC, USA.

Wulandari, Z. 2017. Uji Aktivitas Antibakteri Ekstrak Kasar Caulerpa racemosa dengan Pelarut dan Bagian Alga yang Berbeda Terhadap Pertumbuhan *Escherichia coli* dan *Staphylococcus aureus*, Skripsi FPIK, Universitas Brawijaya, Malang.

Yunaedi, Y.F. Victoria, L. Meylina, dan R. Rusli. 2016. Isolasi dan Karakterisasi Jamur Endofit Akar Merung (*Captosapelta tomentosa*). Prosiding Seminar Nasional Kefarmasian Ke-4, Samarinda: 20-21 Oktober 2016, Laboratorium Penelitian dan Pengembangan FARMAKA TROPIS Fakultas Farmasi Universitas Mulawarman Samarinda, Kalimantan Timur.

Zhao, Y., X.Z. Liu and F.Y. Bai. 2019. Four New Species of Tremella (*Tremellales*, *Basidiomycota*) Based on Morphology and DNA Sequence Data. J. Myco Keys, 47: 75-95.