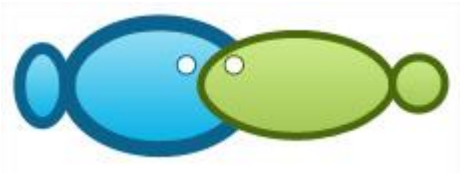

Unveiling the antibacterial potential and metabolite profile of fungal endophytes of *Caulerpa* spp. from Teluk Awur Beach, Jepara, Indonesia

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Abstract. The exploration of endophytic fungi and their associated hosts has provided valuable insights into the discovery and application of bioactive compounds derived from secondary metabolites, particularly as antibacterial agents. Recently, there has been growing interest in *Caulerpa* spp., a type of green macroalgae found in aquatic environments, due to its potential as a source of endophytic fungi with antibacterial properties. This study aims to obtain a profile of bioactive compounds of endophytic fungus *Caulerpa* spp. and molecular identification was carried out for the species with the highest antibacterial activity. Nine endophytic isolates of *Caulerpa* spp. were selected using the dual-culture antagonist test against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, followed by disc-filter diffusion for the supernatant antibacterial test. Three isolates from the screening process demonstrated positive results, indicating antibacterial activity. Among these isolates, isolate KEC 1 exhibited the highest activity. Liquid chromatography–mass spectrometry (LC-MS) analysis of KEC 1 extract exhibited several antibacterial compounds such as 1,2,4-Triazole, mycophenolic acid, cyclo (phenylalanyl-prolyl), and 2-Acrylamido-2-methyl-1-propanesulfonic acid. These isolates were identified as *Penicillium citrinum.* Overall, this research provides a foundation for the development of *Penicillium citrinum* utilization in the field of pharmacology. The discovery holds the potential in contributing to the development of new and more effective drugs for combating bacterial infections. Furthermore, it opens up new opportunities for research and utilization of endophytic microorganisms from the coastal waters.

Key Words: antibacterial compound, endophytic fungi, *Penicillium citrinum*.

Introduction. An antibacterial compound refers to a substance that can impede or inhibit the growth of bacteria. These compounds are commonly employed to control the proliferation of pathogenic bacteria that pose a threat to human health. Bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*, which are commonly found in various environments, including the surroundings of human populations, can be effectively targeted by antibacterial compounds (Pandey et al 2014; Heredia & García 2018). Natural antibacterial prospecting by researchers is an ongoing attempt due to bacterial resistance to antibiotics. Plants, animals, and microbes including fungi and bacteria itself are organisms that produce these antibacterial compounds (Moloney 2016).

One of the known antibacterial-producing agents, fungi, are multicellular microorganism that form mycelium, are microscopic, and reproduce by spores. Fungi inhabit a broad range of environments, even making symbiotic relations with the

organisms residing in soil, freshwaters, and saline waters. Aquatic organisms, like *Caulerpa* spp., can form symbiotic relationships with other organisms, such as fungi. *Caulerpa* spp. is a type of macroalgae characterized by its green thallus, which can take the shape of grapes, ferns, or horns. It typically grows up to 8.5 cm in length and is commonly found in tropical and subtropical regions (Klein & Verlaque 2008).

Caulerpa spp. holds significant potential in the health industry due to its bioactive compounds, which possess anticancer properties. These compounds have been studied for their ability to inhibit the growth and proliferation of cancer cells, highlighting their potential as therapeutic agents (Mehra et al 2019). The bioactive compounds derived from *Caulerpa* spp. offer promising prospects for the development of new treatments and interventions in the field of oncology. Several species from the group, like *Caulerpa racemosa* and *Caulerpa lentillifera*, were proven to have antibacterial and antioxidant activity in restricting neuropathogenic MRSA *E. coli* K1 (Yap et al 2019). These attractive features are expected to be retained in their endophytes.

Endophytic fungi are a group of fungi that live part or all their lives in living plant tissue and usually do not harm their host. Endophytic fungi have a role in the host defence mechanism against predators, and growth hormone production and increase their resistance towards abiotic and biotic stresses (Singh et al 2011; Kivlin et al 2013). Research by Flewelling et al (2013) reported that endophytic fungi derived from Atlantic macroalgae possessed antibacterial, antifungal, and even larvicidal activity.

Further research of the isolates is expected to have other compounds with antivirus and anticancer properties (Flewelling et al 2015), thus making more experiments in macroalgae endophytic fungi worth conducting. The study of *Caulerpa* spp., its tropical habitat, and endophytic fungi associated with *Caulerpa* spp. is an area of research that is still not fully explored. Exploring the relationship between *Caulerpa* spp., its endophytic fungi, and the bioactive compounds they produce holds promise for discovering new therapeutic agents or applications in various fields such as medicine, agriculture, and environmental science. Further studies and research efforts are required to uncover the full potential of *Caulerpa* spp. and its associated endophytic fungi.

This study aimed to isolate the endophytic fungi from *Caulerpa* spp., test their antibacterial potential against *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. subtilis*, obtain the profile of bioactive compounds using liquid chromatography–mass spectrometry (LC-MS) analysis, and conduct molecular identification for the species with a highest antibacterial activity using internal transcribed spacer (ITS) marker.

Material and Method

Isolation and morphological characterization of endophytic fungi from Caulerpa spp. Research took place from May 2022 to January 2023. The collection of *Caulerpa* spp. samples was conducted along the coastline of Teluk Awur Beach in Jepara, located in Central Java, Indonesia. The sample was kept in an ice-cold container, had a weight of 1 gram, and soaked in alcohol (70%) for 1 minute. Sterilized thallus was rinsed using sterile distilled water, dried, and ground using mortar and pestle. The sample was diluted up to 10^{-5} using sterilized seawater, and 0.1 mL sample was grown in seawater potato dextrose agar (PDA) media containing 50 ppm chloramphenicol and incubated at 25-28°C for 3-7 days.

Purified colonies were observed at macroscopic and microscopic levels. Macroscopic observations included colony colour, the reverse of the colony, growing zone, radial furrow, colony surface texture, soluble pigment, and colony diameter. Microscopic structures observed included hyphae, conidial shape, presence or absence of septa, and several other structures. The characterization results were then matched with references (Klich 2002; Samson et al 2010; Watanabe 2010).

Screening of potential antibacterial fungi isolates. Fresh cultures of fungi (7 days old) and overnight-grown antagonistic bacteria (*E. coli*, *S. aureus*, *B. subtilis*, and *P. aeruginosa*) were prepared. The bacteria were adjusted to McFarland 0.5 using sodium chloride (NaCl) 0.9% and inoculated on Mueller Hinton agar (MHA) using the swab

method. The fungi were taken using a 6 mm cork borer and placed on the corresponding area of test agar. Clear zones formed after 24 hours at 25-28°C were then measured by Vernier calliper.

Secondary metabolite production and antibacterial test of potential fungal extract. One-week-old fungi cultures were taken using cork borer and inoculated in potato dextrose yeast-extract broth (PDYB) media. The cultures were grown for 14 days and were then harvested using filter papers. The filtered concentrate was centrifuged, and supernatant was used for further antibacterial test.

The density of the test bacterial suspension was adjusted based on the McFarland turbidity standard 0.5 using NaCl 0.9% and inoculated on MHA media. The supernatant containing fungal secondary metabolite extract (40 µL) were let infiltrated in test disc paper and placed on the MHA media. Positive control of 30 µg chloramphenicol was used, while PDYB media were used as a negative control. Clear zones formed after 24 hours at 37°C were then measured by Vernier calliper.

Liquid chromatography-mass spectrophotometry (LC-MS) analysis. Selected isolates were fermented by inoculating 3 pieces of 6 mm mycelium plug into 200 mL seawater PDB media at 25°C for 14 days. Mycelium biomasses were harvested using filter-paper Whatman No. 1, the filtrate was extracted using ethyl acetate (EtOAc) 1:1 (v/v) and left to sit until the filtrate formed 2 phases. The upper phase (ethyl acetate fraction) was taken and evaporated using rotary evaporator at 45°C until a concentrated extract formed (Ameen et al 2022).

As much as 1 mg of concentrated extract was dissolved in 10 mL of ethyl acetate solvent. Samples were tested in the Diponegoro University Integrated Laboratory for LC-MS analysis. The results were then analysed and matched with the database on the MassBank (2023) website and ChemSpider (2023) website.

Molecular identification of potential isolate. DNA isolation of potential isolate was carried out using the cetyltrimethylammonium bromide (CTAB) method (Bartlett & Stirling 2003). About 200 mg of one-week-old fungi hyphae were put into 1.5 mL microtube and 600 µL CTAB buffer solution was added. The mixture was ground on mortar using a pestle and was put back into a microtube prior to incubation at 65°C for 1 hour. Chloroform: isoamyl alcohol (CIA) 24:1 as much as 600 µL was added and centrifuged at 12,000 rpm for 20 minutes. Supernatant was transferred into new tube and 300 µL of isopropanol were added, inverted several times to homogenization and was incubated at -20°C for 1 hour. The supernatant was dissolved, the precipitate containing DNA was washed using alcohol 70% and centrifuged at 12,000 rpm for 20 minutes at 4°C. TE buffer (50 µL) was used to dilute the DNA. The concentration and purity were measured using NanoDrop spectrophotometer (Thermo Scientific NanoDrop 2000).

DNA amplification process was carried out using ITS gene with primer sequences: ITS 5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and ITS 4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and reaction: pre-denaturation (96°C, 2 min), followed by 35 cycles of denaturation (96°C, 1 min), annealing (54°C, 1 min), and extension (72°C, 1 min). Confirmed PCR product with bands were sequenced at 1st Base Sequencing Service and the results were processed with Mega XI software for phylogenetic analysis using neighbour-joining method and 1000 replication bootstraps.

Data analysis. Data were analysed to determine the antibacterial activity of endophytic fungi. Data were presented as mean ± standard deviation of triplicates (n = 3). Data was analysed using one-way analysis of variance (ANOVA). P value < 0.05 was considered as significant using SPSS statistical software package version 26.0.

Results

Morphological observation of Caulerpa spp. endophytic fungi. The result of endophytic fungi isolation showed 9 distinct colonies grown on seawater PDA media at 25°C. Macroscopic and microscopic features of fungal isolates can be observed in Figure 1 and Figure 2. These features were compared according to Samson et al (2010).

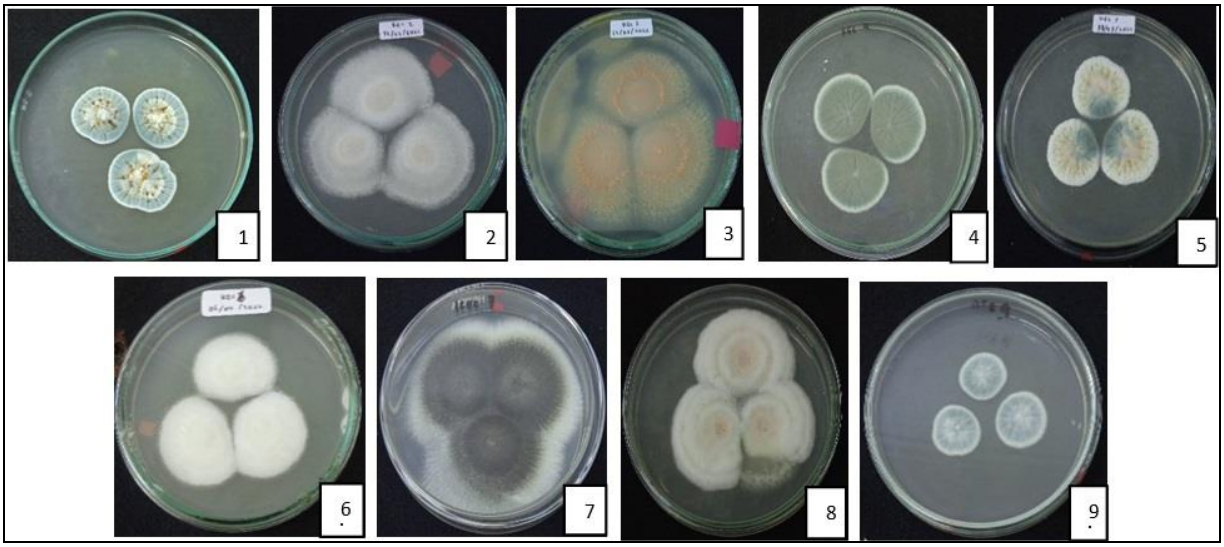

Figure 1. Macroscopic colony of *Caulerpa* spp. endophytic fungus isolates. Isolate KEC 1, KEC 2, KEC 3, KEC 4, KEC 5, KEC 6, KEC 7, KEC 8 and KEC 9.

Figure 2. Microscopic colony of *Caulerpa* spp. endophytic fungus isolates. Isolate KEC 1, KEC 2, KEC 3, KEC 4, KEC 5, KEC 6, KEC 7, KEC 8 and KEC 9.

KEC 1, KEC 4, KEC 5, and KEC 9 were grouped into *Penicillium* with characteristics such as green-coloured colony and yellow reverse morphology with velvety texture and radial furrow. The conidia were shaped like globose and had a smooth surface. The hyphae have a flat wall with no septate and biverticillate type of branching. Both conidia and hyphae have no colour (hyaline). Isolate KEC 2 and KEC 6 were grouped into *Geotrichum* that has white colonies with yellow reverse and smooth texture. Microscopic structures observed has cylindrical arthrospores with flat wall and no colour. The hyphae were also flat-walled, no colour, and no septate. Isolate KEC 3 and KEC 8 were grouped into *Fusarium.* The characteristics of KEC 3 was an orange-coloured colony and reverse, with floccose surface, while KEC 8 has white colony and orange reverse, with surface that resembled cotton. Both isolates had oval-shaped macroconidia with 4-5 septate. On the other hand, the microconidia had a slight difference in which KEC 3 has transparent elliptical microconidia, while KEC 8 has globose microconidia. Isolate KEC 7 was grouped into *Aspergillus* according to its black-and-white colony with yellow reverse. The surface texture was granular and had radial furrow. The microscopic structure showed

transparent globose-shaped conidia and flat wall. The hyphae had no colour, was flat-walled, and with no septate. The vesicle was globose shaped, and uniseriate.

Screening of antibacterial activity of Caulerpa spp. endophytic fungi. Screening of antibacterial activity of the endophytes was carried out against *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. subtilis* using dual culture method. The inhibition activity was determined based on the diameter of clear zone (Figure 1, Table 1). Based on the activity measured, KEC 1 and KEC 2 were the only isolates that showed inhibition against all bacteria tested. Isolate KEC 4 and KEC 5 were able to inhibit all of them except for *E. coli*, while KEC 6 and KEC 9 also can inhibit 3 bacteria, except for *S. aureus*. Isolate KEC3, KEC 7, and KEC 8 showed no inhibition activity.

Table 1

Measurement of inhibition activity of *Caulerpa* spp. endophytic fungi

Isolate	*Diameter of inhibition (mm)*			
	E. coli	*S. aureus*	*B. subtilis*	*P. aeruginosa*
KEC 1	11.7 ± 1.75	11.47 ± 1.25	5.03 ± 1.56	1.87 ± 0.58
KEC 2	9.4 ± 1.05	8.37 ± 2.86	4.53 ± 2.15	6.07 ± 1.05
KEC 3	-	-	-	7.03 ± 0.76
KEC 4	-	9.4 ± 0.56	2.57 ± 0.8	3.5 ± 0.44
KEC 5	-	7.07 ± 1.88	3.67 ± 0.96	8.5 ± 0.17
KEC 6	4.96 ± 1.46	-	2.47 ± 1.31	3.9 ± 1.91
KEC 7	-	-	-	-
KEC 8	7.4 ± 1.59	-	-	-
KEC 9	-	-	5.63 ± 2.44	9.2 ± 2.23

Antibacterial activity of fungal supernatant extract. The supernatant of endophytic fungi was extracted and tested on *E. coli*, *S. aureus*, *P. aeruginosa*, and *B. subtilis* using disc diffusion Kirby-Bauer method. Disc paper containing the supernatant was placed on the agar media with inoculated bacteria. The antibacterial compounds would diffuse into the agar and formed a clear zone indicating inhibition activity (Table 2).

Table 2

Measurement of inhibition activity from supernatant of *Caulerpa* spp. endophytic fungi

Isolate	*Diameter of inhibition (mm)*			
	E. coli	*S. aureus*	*B. subtilis*	*P. aeruginosa*
KEC 1	5.47 ± 0.15	10.93 ± 0.59	16.80 ± 1.31	8.27 ± 1.15
KEC 2	-	-	-	-
KEC 3	-	-	-	-
KEC 4	8.72 ± 0.20	-	13.03 ± 0.80	13.25 ± 1.13
KEC 5	-	-	-	-
KEC 6	-	-	-	-
KEC 9	5.59 ± 0.18	-	6.57 ± 0.21	9.97 ± 0.60
Positive control	11.23 ± 0.64	-	13.10 ± 0.26	12.20 ± 0.45
Negative control	-	21.03 ± 0.50	-	-

All isolates with positive results from previous tests were tested and the result confirmed that only 3 out of 7 isolates produced antibacterial compounds. The antibacterial activity produced by the isolates were classified as moderate to strong (Table 2). Compared to all endophytic fungi isolates, KEC 1 had the highest antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* with inhibition zones of 10.93 mm and 16.80 mm, while KEC 4 had the highest antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa* with inhibition zones of 8.72 mm and 13.25 mm. The statistical results of the ANOVA test showed that there was a significant difference ($p<0.05$)

between each supernatant of the isolates against bacteria tested. Therefore, the positive isolates of the *Caulerpa* spp. endophytic fungi (KEC 1, KEC 4, and KEC 9) possessed compounds with significant effect of antibacterial activity.

LC-MS analysis of secondary metabolites found in potential isolate KEC 1. The extraction of fungal supernatant using ethyl acetate obtained as much as 1.7-gram brownish yellow extract. The extract was then analysed using LC-MS to determine the compounds contained. The LC-MS results can be seen in the chromatogram and the compounds were listed in Table 3.

Table 3

KEC 1 metabolite compound profile

Number	*RT (min)*	*Molecular weight (m/z)*	*Compound*	*Molecular formula*	*Reference*
1	2.69	82.7	Unidentified	-	-
2	6.36	68.9	1,2,4- Triazole	$C_2H_3N_3$	MassBank 2023
3	13.36	92.6	Unidentified	-	-
4	2.68	320.1	Mycophenolic acid	$C_{17}H_{20}O_6$	Oh et al 2015
5	15.12	244.1	Cyclo (phenylalanyl-prolyl)	$C_{14}H_{16}N_2O_2$	Kannabiran 2016
6	3.62	205	5-Methoxy-3-indoleacetic acid	$C_{11}H_{11}NO_3$	MassBank 2023
7	4.31	202	2-Naphthoxyacetic acid	$C_{12}H_{10}O_3$	MassBank 2023
8	13.41	207	2-Acrylamido-2-methyl-1-propanesulfonic acid	$C_7H_{13}NO_4S$	MassBank 2023

The LC-MS results of the KEC 1 supernatant extract showed 8 peaks of identified compounds with different molecular weight value and retention time (Table 3). The LC-MS successfully identified 6 compounds produced by KEC 1. The other two compounds were labelled as unidentified though a molecular peak was present, as it was possible that the compounds are new metabolites produced by KEC 1 isolates.

Molecular identification of potential isolate KEC 1. Isolate KEC 1 was identified as the isolate with the highest potential as an antibacterial agent. Molecular identification of KEC 1 was successfully carried out and visualized using marker gene ITS (Figure 3). The BLAST result of sequences identified by NCBI (2023) showed that KEC 1 has the closest relation to *Penicillium citrinum* (GenBank Accession Number: LT558897.1) with a similarity percentage of 99%. The high percentage (>97%) indicates that both species are identical (Setyati et al 2019). This finding was supported by the construction of a phylogenetic tree (Figure 3). The tree appeared to line up KEC 1 and *Penicillium citrinum* in the same clade (bootstrap value of 86), meaning they have the closest relation among other species with high percentage of similarity.

Figure 3. Reconstruction of the phylogenetic tree of KEC 1 endophytic fungi isolates (NCBI 2023).

Discussion. Four distinct groups of endophytic fungi isolated from *Caulerpa* spp. in this study belong to *Aspergillus*, *Penicillium*, *Fusarium*, and *Geotrichum*. Macroalgae-associated fungi were previously reported from various groups such as *Caulerpa* spp., *Gracilaria* spp., *Gelidium* spp., and *Sargassum* spp. Those endophytes were identified as *P. citrinum*, *A. sydowii*, *A. niveus*, *A. westerdijkiae*, *A. terreus*, and *A. versicolor* (Kawaroe et al 2015). *Fusarium* was also one of the endophytes found on algae like *Fucus gardneri* (Granchinho et al 2002). While several strains of *Geotrichum* were reported to have pathogenicity towards plants, in this study they thrive as endophytes on *Caulerpa* spp. and even had antibacterial activity against *E. coli*, *B. subtilis*, and *P. aeruginosa*. These properties were also found in *G. candidum* isolated from red macroalgae *Padina pavonica* (Hawas & Al-Farawati 2017).

KEC 1, KEC 4, and KEC 9 isolates from Jepara Coastal Waters were identified as *Penicillium*, a cosmopolite fungus found in broad range of habitats from aquatic and terrestrial. The coastal waters are no exception, and macroalgae are one of the most suitable habitats for them to form symbiosis to their substrate. It was reported that *Penicillium* was also found on 6 groups of algae (*Fucus vesiculosus*, *F. spiralis*, *F. serratus*, *Ulva lactuca*, *U. intestinalis*, and *Plocamium cartilagineum*) (Flewelling et al 2013). Observation on endophytes found in this study resulted in potential species, *Penicillium citrinum* KEC 1. This isolate successfully inhibits the growth of 4 potential pathogen bacteria. *P. citrinum* itself was known to have antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Xanthomonas campestris*, *Salmonella enterica*, *Klebsiella pneumonia*, and *Salmonella typhi* (Nischitha & Shivanna 2021; Kandou et al 2021). These species could be found in water, soil, or even extreme habitats (Yadav et al 2018). As endophytic fungi on plants, *P. citrinum* has been reported to colonize several plants such as *Ceratonia siliqua*, *Ocimum tenuiflorum*, and *Azadirachta indica* (El-Neketi et al 2013; Lai et al 2013; Kumari et al 2021). According to Bao et al (2013) *Penicillium* spp. was known to be able to produce various important bioactive compounds such as antibacterial and antifouling polyketides. This group had antibacterial activity against the bacteria *Staphylococcus aureus*, *Kocuria rhizophila*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Gonçalves et al 2021).

LC-MS analysis of the compounds produced by *P. citrinum* KEC 1 isolate was also known to have several bioactive potentials. Matin et al (2022) reported 1,2,4-Triazole as a bioactive compound with antimicrobial, antiviral, antitubercular, anticancer, anticonvulsant, analgesic, antioxidant, anti-inflammatory, and antidepressant activity. Mycophenolic acid is a known secondary metabolite produced by several species of *Penicillium.* It was first isolated in 1983 as an antibiotic against the bacterium *Bacillus anthracis* (Patel et al 2016). Cyclo (phenylalanyl-prolyl) is another known metabolite compound from the group that has antibacterial function (Kannabiran 2016). Previous research by Nischitha and Shivanna (2021) found cyclo (phenylalanyl-prolyl) with

antibacterial potential produced by *Penicillium citrinum*. Several compounds from phytohormone group were also found such as 5-Methoxy-3-indoleacetic acid, a compound from the indole-3-acetic acid (IAA) class wherein the hydrogen at position 5 of indole-3-acetic acid is replaced by a methoxy group and 2-Naphthoxyacetic acid which is known as a plant growth regulator. Both compounds possessed the ability as an antibacterial (PubChem NCBI 2023). The last compound is 2-Acrylamido-2-methyl-1-propanesulfonic acid which also has antibacterial and antifungal properties (Farag et al 2020).

Conclusions. Nine isolates of endophytic fungi from *Caulerpa* spp. were successfully isolated. Isolate KEC 1, KEC 4, and KEC 9 were proven to inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. Among the 3 isolates, KEC 1 isolate had the highest antibacterial activity. Based on LC-MS analysis, KEC 1 contains antibacterial compounds such as 1,2,4-Triazole, mycophenolic acid, cyclo (phenylalanyl-prolyl), and 2-Acrylamido-2-methyl-1-propanesulfonic acid. The result of molecular identification showed that KEC 1 isolate was *Penicillium citrinum*. Purification of the antibacterial compound produced by *P. citrinum* KEC1 for pharmacological preparation is needed for further research.

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Conflict of interest. The authors declare that there is no conflict of interest.

References

Ameen F., AlNAdhari S., Al-Homaidan A. A., 2022 Marine fungi showing multifunctional activity against human pathogenic microbes and cancer. PLoS ONE 17(11):e0276926. https://doi.org/10.1371/journal.pone.0276926.

Bao J., Sun Y. L., Zhang X. Y., Han Z., Gao H. C., He F., Qian P. Y., Qi S. H., 2013 Antifouling and antibacterial polyketides from marine gorgonian coral-associated fungus *Penicillium* sp. SCSGAF 0023. J. Antibiot. (Tokyo) 66(4):219-223. doi: 10.1038/ja.2012.110.

Bartlett J. M. S., Stirling D., 2003 PCR protocols: second edition. Methods in Molecular Biology Vol. 226. 556 pp.

El-Neketi M., Ebrahim W., Lin W., Gedara S., Badria F., Saad H. E. A., Lai D., Proksch P., 2013 Alkaloids and polyketides from *Penicillium citrinum*, an endophyte isolated from the Moroccan plant *Ceratonia siliqua*. Journal of Natural Products 76(6):1099-1104.

Farag R. K., Atta A. M., Labena A., AlHawari S. H., Safwat G., Diab A., 2020 Antibacterial and anti-fungal biological activities for acrylonitrile, acrylamide and 2-Acrylamido-2-Methylpropane sulphonic acid crosslinked terpolymers. Materials (Basel) 13(21):4891. doi: 10.3390/ma13214891.

Flewelling A. J., Currie J., Gray C. A., Johnson J. A., 2015 Endophytes from marine macroalgae: promising sources of novel natural products. Current Science 109:88-111.

Flewelling A. J., Johnson J. A., Gray C. A., 2013 Isolation and bioassay screening of fungal endophytes from North Atlantic marine macroalgae. Botanica Marina 56(3):287-297.

Gonçalves M. F. M., Paço A., Escada L. F., Albuquerque M. S. F., Pinto C. A., Saraiva J. A., Duarte A. S., Rocha-Santos T. A. P., Esteves A. C., Alves A., 2021 Unveiling biological activities of marine fungi: the effect of sea salt. MDPI Applied Science 11(13):6008. https://doi.org/10.3390/app11136008.

Granchinho S. C., Franz C. M., Polishchuk E., Cullen W. R., Reimer K. J., 2002 Transformation of arsenic (V) by the fungus *Fusarium oxysporum melonis* isolated from the alga *Fucus gardneri*. Applied Organometallic Chemistry 16(12):721-726.

Hawas U. W., Al-Farawati R., 2017 Chemical constituents and antiviral activity from marine endophytic fungi from Red Sea alga *Padina pavonica*. J. Chem. Soc. Pak. 39(03):478-483.
Heredia N., García S., 2018 Animals as sources of food-borne pathogens: a review. Animal Nutrition, 4(3):250-255.
Kandou F. E. F., Mangindaan R. E. P., Rompas R. M., Simbala H. I., 2021 Molecular identification and antibacterial activity of marine-endophytic fungi isolated from sea fan Annella sp. from Bunaken waters, Manado, North Sulawesi, Indonesia. AACL Bioflux 14(1):317-327.
Kannabiran K., 2016 Bioactivity-guided extraction and identification of antibacterial compound from marine *Actinomycetes* strains isolated from costal soil samples of Rameswaram and Dhanushkodi, Tamil Nadu. India Asian J. Pharm. 10(04). https://doi.org/10.22377/ajp.v10i04.885.
Kawaroe M., Sunuddin, A., Hwangbo, B., Shaumi A., 2015 Characteristics and selulotic activities of endophytic fungi in macroalgae (*Sargassum* sp., *Gracilaria* sp., *Gelidium* sp., and *Caulerpa* sp.) from seagrass habitat in Pari Island, Thousand Islands, Jakarta. International Journal of Sciences: Basic and Applied Research 22(2):149-160.
Kivlin S. N., Emery S. M., Rudgers J. A., 2013 Fungal symbionts alter plant responses to global change. American Journal of Botany 100(7):1445-1457.
Klein J., Verlaque M., 2008 The *Caulerpa racemosa* invasion: a critical review. Marine Pollution Bulletin 56(2):205-225.
Klich M. A., 2002 Identification of common *Aspergillus* species. Centraalbureau voor Schimmelcultures, Utrecht. 116 pp.
Kumari P., Singh A., Singh D. K., Sharma V. K., Kumar J., Gupta V. K., Bhattacharya S., Kharwar R. N., 2021 Isolation and purification of bioactive metabolites from an endophytic fungus *Penicillium citrinum* of *Azadirachta indica*. South African Journal of Botany 139:449-457.
Lai D., Brötz-Oesterhelt H., Müller W. E., Wray V., Proksch P., 2013 Bioactive polyketides and alkaloids from *Penicillium citrinum*, a fungal endophyte isolated from *Ocimum tenuiflorum*. Fitoterapia 91:100-106.
Matin M. M., Matin P., Rahman M. R., Ben Hadda T., Almalki F. A., Mahmud S., Ghoneim M. M., Alruwaily M., Alshehri S., 2022 Triazoles and their derivatives: chemistry, synthesis, and therapeutic applications. Front, Mol, Biosci. doi: 10.3389/fmolb.2022.864286.
Mehra R., Bhushan S., Bast F., Singh S., 2019 Marine macroalga *Caulerpa*: role of its metabolites in modulating cancer signaling. Molecular Biology Reports 46(3):3545-3555.
Moloney M. G., 2016 Natural products as a source for novel antibiotics. Trends in Pharmacological Sciences, 37(8):689-701.
Nischitha R., Shivanna M. B. 2021 Antimicrobial activity and metabolite profiling of endophytic fungi in *Digitaria bicornis* (Lam) Roem. and Schult. and *Paspalidium favidum* (Retz.) A. Camus. 3 Biotech 11(2):53. doi: 10.1007/s13205-020-02590-x.
Oh S. Y., Mead P. J., Sharma B. S., Quinton V. M., Boermans H. J., Smith T. K., Swamy H. V. L. N., Karrow N. A., 2015 Effect of *Penicillium* mycotoxins on the cytokine gene expression, reactive oxygen species production, and phagocytosis of bovine macrophage (BoMacs) function. Toxicol in Vitro 30(1):446–453.
Pandey P. K., Kass P. H., Soupir M. L., Biswas S., Singh V. P., 2014 Contamination of water resources by pathogenic bacteria. AMB Express 4(51):1-16. https://doi.org/10.1186/s13568-014-0051-x.
Patel G., Patil M. D., Soni S., Khobragade T. P., Chisti Y., Banerjee U. C., 2016 Production of mycophenolic acid by *Penicillium brevicompactum* - a comparison of two methods of optimization. Biotechnol. Rep. (Amst). 11:77–85.
Samson R. A., Houbraken J., Thrane U., Frisvad J. C., Anderson B., 2010 Food and indoor fungi. 2nd ed. Fungal Biodiversity Centre Utrecht, Neterlands. 481 pp.
Setyati W. A, Martani E., Triyanto, Zainuddin M., Puspita M., Suryono C. A., Subagyo, Pringgenies D., 2019 Genetic heterogeneity of proteolytic bacteria isolated from

sediments mangrove areas based on repetitive sequence-based polymerase chain reaction and 16S-rRNA gene sequences. Biodiversitas 20(11):3256-3261.
Singh L. P., Gill S. S., Tuteja N., 2011 Unraveling the role of fungal symbionts in plant abiotic stress tolerance. Plant Signaling & Behavior 6(2):175-191.
Watanabe T., 2010 Pictorial atlas of soil and seed fungi. CRC Press, Boca Raton. 426 pp.
Yadav A. N., Verma P., Kumar V., Sangwan P., Mishra S., Panjiar N., Gupta V. K., Saxena A. K., 2018 Biodiversity of the genus *Penicillium* in different habitats. In: New and Future Developments in Microbial Biotechnology and Bioengineering. Elsevier. 3-18 p.
Yap W. F., Tay V., Tan S. H., Yow Y. Y., Chew J., 2019 Decoding antioxidant and antibacterial potentials of Malaysian green seaweeds: *Caulerpa racemosa* and *Caulerpa lentillifera*. Antibiotics 8(3):152. doi: 10.3390/antibiotics8030152.
*** ChemSpider, 2023 www.chemspider.com [Last accessed on 20 January 2023].
*** MassBank, 2023 www.massbank.eu [Last accessed on 20 January 2023].
*** National Center for Biotechnology Information (NCBI), 2023 BLAST software databases. www.ncbi.nlm.nih.gov [Last accessed on 20 January 2023].
*** PubChem National Center for Biotechnology Information (PubChem NCBI), 2023 PubChem compound summary for CID 18986,5-Methoxyindole-3-aceticacid. https://pubchem.ncbi.nlm.nih.gov/compound/5-Methoxyindole-3-acetic-acid [Last accessed on 20 January 2023].

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