Evaluation of Antibacterial and Toxicity Properties of Marine Endophytic Fungi from Red Algae *Gracilaria Salicornia* and *Chondrus* sp.

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Abstract. Marine fungi are widely recognized for producing bioactive compounds with diverse pharmaceutical applications. The study aimed to investigate the antibacterial and cytotoxic properties of methanolic extracts from endophytic fungi derived from *Gracilaria salicornia* and *Chondrus* sp., collected from Argani Beach, Bali, Indonesia. Fungal isolation yielded one and three fungal strains from each host, respectively. They were later identified as *Aspergillus unguis* A4-1-1, *Trichoderma yunnanense* A5-1-1, *Trichoderma asperellum* A5-1-2, and *Clonostachys pseudostriata* A5-2-1 through ITS or LSU region comparison. Each methanolic extract underwent testing for phytochemical content, antibacterial activities, and cytotoxic potentials. Phytochemical screening indicated the presence of alkaloids in all extracts. Notably, the extract of the non-salt-treated fungus *A. unguis* A4-1-1 demonstrated the most potent activity with MIC values of 15.6 µg/mL against *Staphylococcus aureus* and *Bacillus cereus* ATCC 11778, while other extracts showed no substantial activity. Additionally, the salt-treated *T. asperellum* A5-1-2 exhibited the most potent toxic activity compared to the others with LC_{50} of 104.9 µg/mL, according to the brine shrimp lethality test. Other extracts showed LC_{50} values starting from 107.0 to 792.9 µg/mL. Given their notable antibacterial and cytotoxic potentials, it is noteworthy to identify and characterize the bioactive compounds from these fungal strains in future study.

1 Introduction

The development of new drugs is currently a major global challenge, especially due to the increasing phenomenon of drug resistance. Secondary metabolites derived from natural

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products are still considered favourable sources of new drugs, including antimicrobial and anticancer agents [1]. The discovery of active compounds derived from marine natural materials is one approach to the discovery of new drugs.

Marine life is in principle tolerant of extreme situations caused by salinity, pollution, and climate change. Marine environmental conditions that are more diverse than terrestrial environments affect the bioactive properties produced [2]. For this reason, many researchers are more interested in studying marine organisms than terrestrial organisms in an effort to obtain new bioactive compounds. This unusual situation certainly leads to the evolution of organisms with new genes and unique properties [3].

Active compounds produced from marine natural materials can be produced by microorganisms associated with them, one of which is endophytic fungi [4]. Endophytic fungi are fungi that live and associate in plant tissues asymptomatically and do not cause any negative effects on their hosts [5]. The association that occurs is mutualism, in this case the endophytic fungi obtain nutrients from the host while the endophytic fungi produce metabolites that protect the host from pests and diseases [6]. Endophytic fungi activate silent genes as an adaptation to extreme environmental changes [7]. Different environments, locations, and stress will produce a diversity of metabolites [8].

One source of endophytic fungi in marine natural materials is algae. Endophytic fungi derived from algae are capable of producing promising new bioactive compounds for marine bioprospection [9]. This can be seen that algae hold the highest percentage contribution as a source of endophytic fungi, which is 17%, followed by sponges, corals, and mangroves with percentages of 12, 10, and 10%, respectively [10]. Therefore, in this study, antibacterial and toxicity tests of endophytic fungal extracts from red algae *Gracilaria salicornia* and *Chondrus* sp. collected from Argani Beach were carried out. Rhodophyta or red algae is a group of algae that has a dominant red colour caused by phycobilin pigments in the form of allo-phycocyanin, phycoerythrin, and phycocyanin [11]. Endophytic fungi from red algae showed the ability to produce diverse bioactive compounds including curvularin-type of antimicrobial macrolides [12] and cytotoxic polyketides [13].

Metabolites production from fungi can be influenced by the presence of salts. Incorporating salts, such as seawater salt, into the fermentation media can enhance the metabolites production of fungi. This phenomenon was observed by Overy et al. (2017) who reported that under elevated NaCl concentration, the production of secalonic acid D, aspergillusol, aculene C, and another aculene derivative were found to be increased from the fungus *Aspergillus aculeatus* [14]. Thus, in the present study, we established fungal fermentation conditioned with and without the presence of seawater salts.

2 Materials and methods

2.1 Sample collection and isolation of endophytic fungi

The fresh red algae samples of *G. salicornia* and *Chondrus* sp. were collected from Argani Beach, Bali, Indonesia in November 2022. Samples were washed using running tap water for 5 minutes and then sterilized with 70% alcohol for 120 seconds. Samples were soaked in sterile aquadest. Each alga was cut with a size of 1 × 1 cm using a sterile scalpel. After that, four pieces of the samples were inoculated on isolation media. Each treatment on the sample was carried out aseptically in a laminar air flow (LAF). Furthermore, they were incubated at room temperature for several days until fungal growth was observed. Each fungal colony with a different morphological appearance was transferred to a new agar plate containing medium without chloramphenicol for the purpose of fungal purification. For long-term

fungal maintenance, a medium containing malt extract, Bacto agar, yeast extract, and glycerol in demineralized water was utilized [1].

2.2 Molecular Identification of endophytic fungi

Species determination of the isolated fungal strains was achieved through the analysis of the fungal ITS region. Firstly, the fungal DNA was extracted using Quick-DNA Fungal/Bacterial Miniprep Kit in accordance to the instruction from the manufacturer. The ITS region of the extracted DNA was amplified using the forward primer ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The isolate that could not be amplified with ITS primers, its large subunit (LSU) region was amplified with forward NL1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and reverse NL4 (5'-GGTCCGTGTTTCAAGACGG -3') primers. The PCR mixture and condition were prepared and performed according to the protocol previously described [1]. The PCR product was subsequently sent for sequencing analysis to the 1st BASE (Malaysia). To identify the closest species of the obtained DNA sequence, it was compared to the deposited DNA sequences in GenBank database employing BLAST for nucleotide program. Phylogenetic tree of the referred species was built with assistance of MEGA version 11.0.11 by the Neighbour-joining algorithm with 1000 replication bootstraps.

2.3 Fermentation and extraction

Each of the isolated fungus was cultivated both in non-salted and salted rice media. The non-salted rice media were made by putting 100 g of rice into a 1 L Erlenmeyer flask and then adding 110 mL of distilled water. Meanwhile, rice media with salt were prepared in the same way but with the addition of 3.5 g seawater salt. The flasks were closed with a cork lid and wrapped with aluminium foil. After that, it was sterilized using an autoclave for 30 minutes at 121°C, 1 atm pressure, and 15 minutes dry time. Endophytic fungal isolates in Petri dishes were cut into small pieces (1 × 1 cm), then 10 pieces were taken and put into rice media. Fermentation was carried out for 3-4 weeks until the entire surface of the rice media was covered with mycelia. Extraction was done by pouring 500 mL of ethyl acetate into the fungal culture. Extraction was carried out on an orbital shaker at 150 rpm for 8 hours. The liquid portion was then filtered and separated from the solvent with a rotary evaporator until a thick extract was obtained. The yielding extracts were then subjected to liquid-liquid extraction using *n*-hexane and 90% aqueous MeOH. The methanol and *n*-hexane phases were then evaporated into dryness *in vacuo* using a rotary evaporator. The obtained methanolic extracts were subjected for further research [1].

2.4 Phytochemical screening

2.4.1 Alkaloids

A total of 100 µL of the test extract solution was mixed with 100 µL of 2N HCl. The resulting solution was subjected into microplate wells with four replicates. The first well was designated as a blank. In the second well, 3 drops of Dragendorff reagent were added, followed by the addition of 3 drops of Wagner reagent to the third well, and 3 drops of Bouchardat reagent to the fourth well. The formation of an orange precipitate in the second well, a white to yellowish precipitate in the third well, and a brown to black precipitate in the fourth well indicated the presence of alkaloids [15].

2.4.2 Triterpenes

A volume of 200 µL of the extract was evaporated and added with 50 µL of dichloromethane. The solution was subsequently mixed with 50 µL of acetic anhydride. Following this, the mixture was spot-tested using 240 µL of concentrated sulfuric acid applied along the inner wall of the tube. The appearance of a brown or violet ring at the interface of the two solvents indicated the presence of triterpenoids [15, 16].

2.4.3 Polyphenols

A volume of 200 µL of the test extract solution was reacted with a 10% FeCl3 solution. The occurrence of a dark blue, dark bluish-black, or greenish-black colour indicated the presence of polyphenolic compounds [15].

2.4.4 Flavonoids

A volume of 200 µL of the test extract solution was evaporated, and the residue was added with 200 µL of acetone P. A small amount of boric acid and oxalic acid powder were added, followed by heating. The remaining residue was mixed with 200 µL of ether. Observation was conducted under UV light at 366 nm; the presence of an intense yellow fluorescence indicated the presence of flavonoids [17].

2.5 Antibacterial assay

The antibacterial assay was conducted against *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Bacillus cereus* ATCC 11778, and *Escherichia coli* ATCC 8739 using the microdilution method in accordance with the instruction of Clinical and Laboratory Standards Institute (CLSI). Prior to the test, the methanolic extract from each fungal isolate was dissolved in DMSO. Following this, the fungal extracts were subjected to a two-fold serial dilution in 96-microwell plates, resulting in a concentration range from 1,000 to 1.95 µg/mL. This experimental procedure was replicated three times. The test results were incubated at a temperature of 35±2°C for a duration of 18-24 hours. Subsequently, a visual examination was performed across all wells to ascertain the presence of a clear zone, indicating the absence of bacterial growth. The minimal concentration demonstrating the absence of bacterial growth was defined as the Minimum Inhibitory Concentration (MIC) [18]. Chloramphenicol was used as the positive control, while a medium containing 1% DMSO was included as the negative control.

2.6 Toxicity

The toxicity assay method used was the brine shrimp lethality test (BSLT) by using a 24-well microplate. Before the process of hatching shrimp eggs, artificial seawater salt was prepared by dissolving 9.5 g artificial seawater in 300 mL of distilled water. A total of 20 mg of *Artemia salina* shrimp eggs were incubated in a brine incubator containing 300 mL artificial seawater salt [1]. The eggs were illuminated and aerated for 36-48 hours until they hatched to be nauplii. For the toxicity assay, ten mature nauplii were added into each plate containing the test extract with a concentration series of 1000; 500; 250; 125, 62.5; and 31.3 µg/mL as well as a negative control containing only artificial seawater salt and DMSO. Each treatment was carried out in triplicate, and all treatments were illuminated. After 24 hours, the number of dead larvae was counted and compared with the control [19, 20]. With the help of SPSS,

a probit analysis of concentration *vs.* mortality was used to calculate the LC_{50} value for each tested methanolic extract.

3 Results and discussion

3.1 Isolation of endophytic fungi

Fungal isolation yielded a fungal strain from red alga *G. salicornia,* recorded as A4-1-1, together with three fungal isolates, coded as A5-1-1, A5-1-2, and A5-2-1, derived from *Chondrus* sp. Each isolate showed a different morphology. Macroscopically, the fungal isolates are depicted in Figure 1.

More endophytic fungal isolates afforded from *Chondrus* sp. than *G. salicornia* may be influenced by the host specificity of endophytic fungi. Some hosts may provide more suitable condition for the colonization and growth of fungi, resulting in the higher diversity and more endophytic fungal species that can be isolated from a particular host [21, 22].

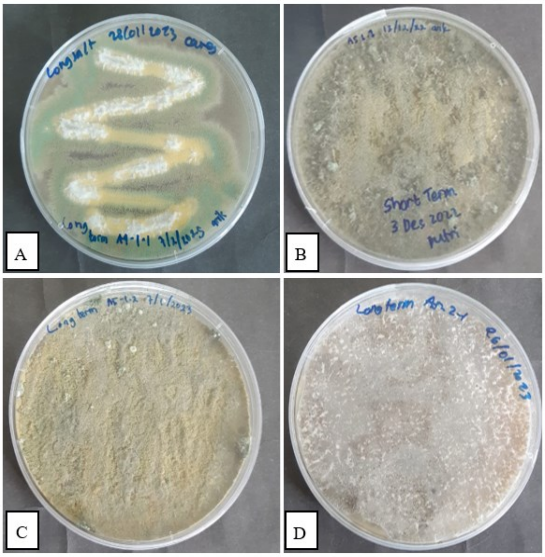

Fig 1. The colony appearance of endophytic fungi of *Gracilaria salicornia* and *Chondrus* sp. cultured on media consisting of malt extract, yeast extract, glycerol, and Bacto agar in demineralized water. (A) A4-1-1. (B) A5-1-1. (C) A5-1-2. (D) A5-2-1.

3.2 Molecular identification of endophytic fungi

Amplification of ITS region from isolate A4-1-1, A5-1-1, and A5-2-1 resulting in a single DNA band with a size of 539, 577, and 535 base pair (bp), respectively. Despite being the gold standard used in fungal identification [23], the variability of this region is not consistent among all fungal species [24]. Thus, we attempted to identify the A5-1-2 isolate through the amplification of its LSU region, as it is also a valuable tool for DNA-based fungal species determination [25]. This attempt yielded a 533 bp DNA band on gel electrophoresis (Figure 2).

As shown in Table 1, comparison analysis of the obtained sequences suggested that A4-1-1 had closest similarity with *Aspergillus unguis* (JF731256.1), A5-1-1 with *Trichoderma yunnanense* (OP800836.1), A5-1-2 with *Trichoderma asperellum* (MK411201.1), and A5-2-

1 with *Clonostachys pseudostriata* (LC499621.1). These findings are also supported by each of the isolate phylogenetic reconstruction with bootstrap support of more than 90% (Figure 3). Therefore, in the remaining section of this article, we refer fungal strain A4-1-1 as *A. unguis* A4-1-1, A5-1-1 as *T. yunnanense* A5-1-1, A5-1-2 as *T. asperellum*, and A5-2-1 as *C. pseudostriata* A5-2-1.

Table 1. The result of comparison sequences of endophytic fungi derived from *Gracilaria salicornia* and *Chondrus* sp. utilizing GenBank's Basic Local Alignment Search Tool (BLAST) for nucleotides.

Fungal Code	Taxon with closest similarity	Percent identity	Query coverage	GenBank Accession Number*
A4-1-1	*Aspergillus unguis* (JF731256.1)	99.4%	98%	PP218350
A5-1-1	*Trichoderma yunnanense* (OP800836.1)	100%	99%	OR462072
A5-1-2	*Trichoderma asperellum* (MK411201.1)	98.9%	90%	PP291901
A5-2-1	*Clonostachys pseudostriata* (LC499621.1)	100%	90%	PP230471

**)* Accession number obtained upon the submission of the sequence to the NCBI's GenBank

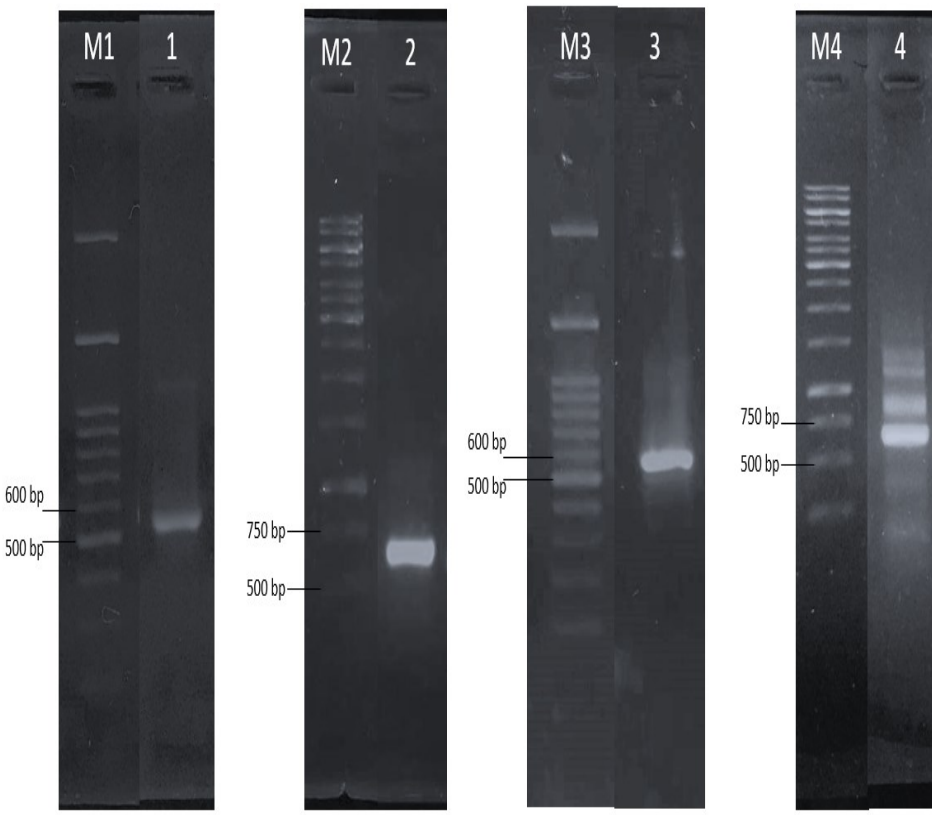

Fig 2. Result of amplification of ITS region from (1) A4-1-1, (2) A5-1-1, and (4) A5-2-1 isolate, and LSU region from (3) A5-1-2, displayed on electrophoresis gel. M1,3: 1000 bp DNA ladder, M2,4: 100 bp DNA ladder.

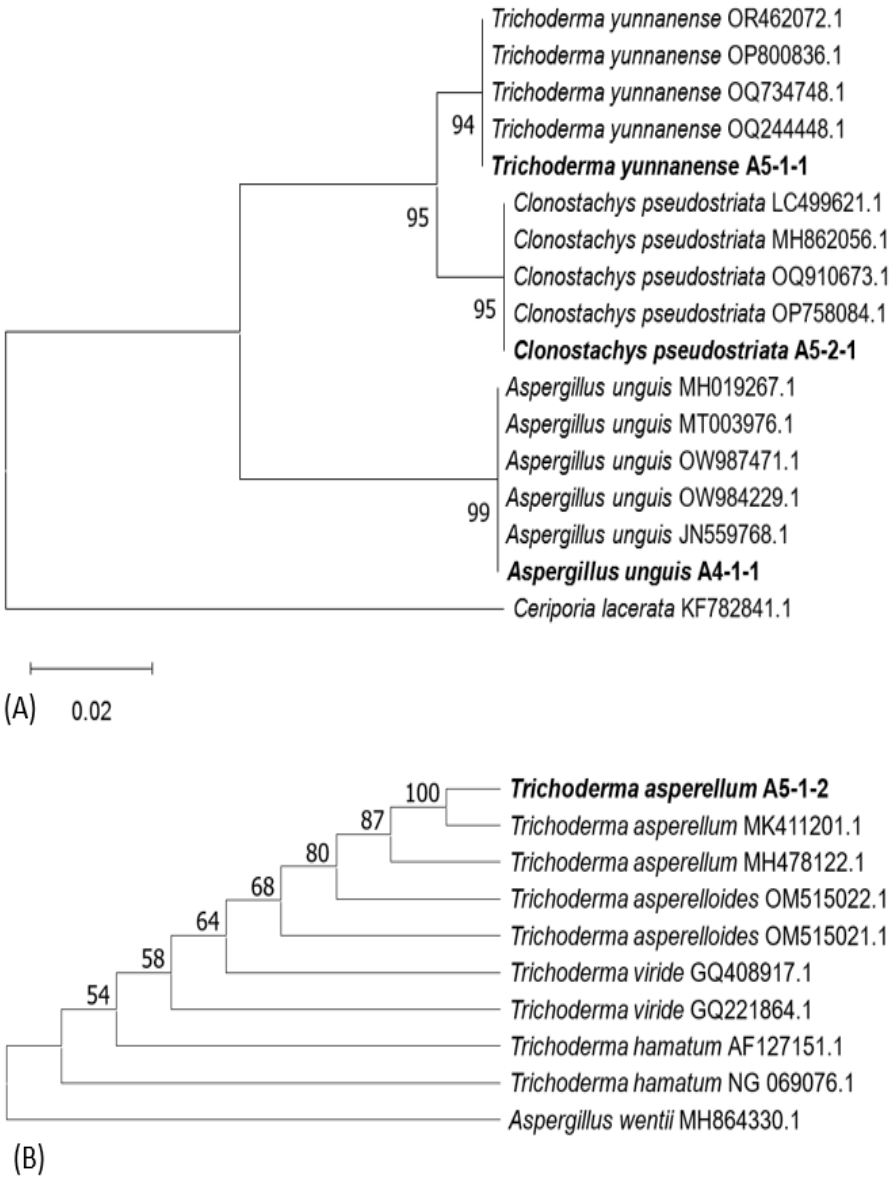

Fig 3. Phylogenetic tree of the ITS region of isolate (A) A4-1-1, A5-1-1, A5-2-1, and (B) the LSU region of A5-1-2 employing Neighbour-joining method with 1,000 bootstrap supports.

3.3 Phytochemical screening

Phytochemical screening of extracts from eight extracts are shown in Table 2. All of these methanolic extracts contain secondary metabolites including alkaloids, triterpenes, polyphenols, and flavonoids. In this study, all methanolic extracts were positive of alkaloids.

Table 2. Phytochemical screening of methanolic extracts from endophytic fungi of *Gracilaria salicornia* and *Chondrus* sp.

Methanolic extracts of endophytic fungi	Phytochemical screening			
	Alkaloids	Triterpenes	Polyphenols	Flavonoids
A. unguis A4-1-1 salt	(+)	(-)	(-)	(+)
A. unguis A4-1-1 non-salt	(+)	(-)	(+)	(+)
T. yunnanense A5-1-1 salt	(+)	(+)	(-)	(-)
T. yunnanense A5-1-1 non-salt	(+)	(-)	(-)	(-)
T. asperellum A5-1-2 salt	(+)	(+)	(+)	(+)
T. asperellum A5-1-2 non-salt	(+)	(+)	(+)	(-)
C. pseudostriata A5-2-1 salt	(+)	(-)	(+)	(+)
C. pseudostriata A5-2-1 non-salt	(+)	(+)	(-)	(-)

Secondary metabolites produced by endophytic fungi act as host plant defence mechanisms against pathogens. Based on previous research, red alga *Chondrus crispus* has been reported to contain chemical constituents in the form of flavonoids, tannins, and phenolics [26]. Meanwhile, *G. salicornia* alga contains chemical content in the form of tannins, alkaloids, saponins, and flavonoids[27]. Factors that play a role in the amount of secondary metabolite content produced from endophytic fungi include temperature, salinity, alkalinity, UV radiation, nutrient deficiency, and pathogen infection [28]. The diverse content of secondary metabolites in endophytic fungi can also be influenced by several genetic mechanisms such as gene clustering, transcription factors, changes in the genetic makeup of the host plant, and horizontal gene transfer that play a role in the biosynthesis of secondary metabolites from endophytic fungi [29].

The presence of secondary metabolites in the methanolic extracts of salt-treated and non-salt-treated fungus is likely influenced by the fungi's osmoregulatory mechanisms. In order for fungi to thrive in the marine environment, they must possess osmoregulatory mechanisms that signal the production of polyols and amino compounds while concurrently elevating the concentration of cytoplasmic ions. Given the energetically demanding nature of biosynthesizing these solutes for osmoregulation, fungi may exhibit reduced secondary metabolite production or slower rates of metabolite production in the presence of high salt concentrations [30]. Salt stress also stimulates gene expression to increase the activity of the phenylpropanoid biosynthetic pathway to produce various phenolic compounds that have strong antioxidant activity [31].

3.4 Antibacterial

The MIC values of each fungal methanolic extract against *S. aureus* are shown in Table 3. All of the tested extracts showed strong to weak activity against *S. aureus*, *S. epidermidis*, and *B. cereus* with MIC values ranging from 15.62 to 500 µg/mL. With the exception of salt-treated *A. unguis* A4-1-1 and *T. yunnanense* A5-1-1, as well as non-salt-treated *T. yunnanense* A5-1-1 and *C. pseudostriata* A5-2-1 extracts, which showed no activity up to the tested concentration of 1,000 µg/mL. These extracts also did not show any activity against *E. coli*.

In this study, both the salt-treated and non-salt-treated fungal strains demonstrated antimicrobial activity, with the exception of the *T. yunnanense* A5-1-1 strain. The extract of *A. unguis* A4-1-1 strain without salt treatment exhibited strong antibacterial activity

compared to the salt-treated fungal strain. Another strain, *T. asperellum* A5-1-2, also displayed improved activity when not treated with salt. In contrast, the extract of *C. pseudostriata* A5-2-1 exhibited strong activity when treated with salt, compared to the non-salt treatment. The algae *Chondrus ocellatus* Holmes collected from the coast of Shonai, Japan, exhibited inhibition against *S. aureus* NBRC 13276 with a MIC value of 3.2 µg/mL [32]. These differences of antibacterial results may be influenced by differences of species and the endophytic fungal habitat. This is due to the fact that environmental conditions significantly affect the secondary metabolites produced by the host plant, consequently affecting the outcomes of the antibacterial assays [33].

Table 3. MIC values (µg/mL) of methanolic extracts from endophytic fungi of *Gracilaria salicornia* and *Chondrus* sp. against several bacterial strains

Methanolic extracts of endophytic fungi	MIC (µg/mL) against bacterial strains			
	S. aureus ATCC 25923	*S. epidermidis* ATCC 12228	*B. cereus* ATCC 11778	*E. coli* ATCC 8739
A. unguis A4-1-1 salt	1000	500	250	>1000
A. unguis A4-1-1 non-salt	15.6	>1000	15.6	>1000
T. yunnanense A5-1-1 salt	>1000	>1000	>1000	>1000
T. yunnanense A5-1-1 non-salt	>1000	>1000	>1000	>1000
T. asperellum A5-1-2 salt	500	>1000	1000	>1000
T. asperellum A5-1-2 non-salt	125	>1000	1000	>1000
C. pseudostriata A5-2-1 salt	125	>1000	>1000	>1000
C. pseudostriata A5-2-1 non-salt	>1000	>1000	>1000	>1000

Previous research by Masuma *et al*. (2001) observed the effect of salt concentration on fungal growth and associated antimicrobial activities. Fungal strains of the *Aspergillus* genus (FT-0104, FT-0449, and FT-0317) obtained from marine sponges collected from the coast of Pohnpei and seaweed collected from Palau's coastline demonstrated elevated antibacterial activity with the addition of salt concentrations of both 50% and 100%. This suggests that these strains possess a tendency to adapt more effectively to the marine environment [34]. Therefore, it is possible that the *C. pseudostriata* A5-2-1 strain also possesses a similar adaptation mechanism.

The addition of salt to fungal growth media can influence its antimicrobial activity. Prior studies have indicated that numerous marine fungi isolated tend to demonstrate enhanced growth with increasing seawater concentrations. This increased growth is frequently accompanied by a boost in the production of antimicrobial substances. Based on this study, the production of antibacterial metabolites in *C. pseudostriata* A5-2-1 might have been enhanced as a response to the potentially stressful high salinity conditions. This strain exhibits the traits of halotolerant fungi, which have developed a unique metabolism to adapt to changes in salinity [30, 35, 36].

3.5 Toxicity

The brine shrimp lethality test (BSLT) method is a simple preliminary biological screening test in determining toxicity and an initial testing method for the anticancer activity of a compound. According to Meyer et al. (1982) the toxicity level of an extract can be determined through its LC_{50} value [19]. The toxicity category of an extract based on the LC_{50} value includes very strong (<10 ppm), strong (10-100 ppm), moderate (100-500 ppm) and weak

(500-1000 ppm) [37]. The level of toxicity will give meaning to its potential activity as an anticancer. The smaller the LC_{50} value, the more toxic the compound.

The Lethal Concentration 50 (LC_{50}) values of each fungal methanolic extract from *G. salicornia* and *Chondrus* sp. are shown in Table 3. All of the tested extracts showed LC_{50} values < 1000 ppm. Based on the toxicity category by Mclaughlin and Rogers (1998), the toxicity obtained in this study is classified into the moderate category with LC_{50} values ranging from 104.9 to 792.9 µg/mL [37].

Table 4. LC_{50} values (µg/mL) of methanolic extracts from endophytic fungi of *Gracilaria salicornia* and *Chondrus* sp.

Methanolic extracts of endophytic fungi	LC_{50} (µg/mL)
A. unguis A4-1-1 salt	110.0
A. unguis A4-1-1 non-salt	617.2
T. yunnanense A5-1-1 salt	254.8
T. yunnanense A5-1-1 non-salt	406.3
T. asperellum A5-1-2 salt	104.9
T. asperellum A5-1-2 non-salt	107.1
C. pseudostriata A5-2-1 salt	792.9
C. pseudostriata A5-2-1 non-salt	223.9

According to previous study by Li et al. (2014), the endophytic fungus *Penicillium echinulatum* isolated from *C. ocellatus* algae collected from Pingtan Island, China, is able to produce bioactive compounds in the form of arisugacins G, J, and K, and territrem C. The compound arisugacin K is reported to show toxic effects on *A. salina* shrimp larvae with an LC_{50} value of 48.6 µg/mL [38]. Meanwhile, no studies have reported the toxic effects of endophytic fungi isolated from the algae *G. salicornia.* The differences in the results shown can be influenced by the adaptation mechanism of each endophytic fungus to different microecological habitats.

Both the salt-treated and non-salt-treated fungal strains in this study displayed moderate toxic activity. The extract of *T. asperellum* A5-1-2 strain with salt treatment exhibited the most potent toxic activity compared to the others. Another extract, from the strain *A. unguis* A4-1-1 and *T. yunnanense* A5-1-1 with salt addition, also exhibited stronger activity compared to the strain without salt addition. In contrast, *C. pseudostriata* A5-2-1 extract showed stronger toxic activity when treated without salt, compared to the fungal strain with salt treatment.

With the addition of salt to fungal growth media may affect the toxic activity produced. Endophytic fungi activate silent genes as an adaptation to extreme environmental changes [7]. According to Cui et al. (2019), changes in carbon and nitrogen metabolism under salt stress not only affect plant growth but also promote the biosynthesis of secondary metabolism and the accumulation of secondary metabolites [39]. With the occurrence of increased production of secondary metabolites, it could have an impact on the results of greater activity. Because, the more metabolite compounds a sample has, the higher the cytotoxic effect will be [33].

Rodriguez et al. (2008) referred to this adaptation as 'habitat-adapted symbiosis' and showed that endophytes isolated from salinity-adapted plants exhibited salt tolerance [40]. Although the mechanism by which endophytes can confer salt tolerance on their host plants is unclear, it has been suggested that this may involve the synthesis of host stress-responsive hormones, upregulation of host stress-responsive genes and also actively maintaining a low Na^+: K^+ ratio [41]. In addition, increasing K^+ content is related to mechanisms that enhance

salt tolerance, which may decrease toxic ion levels under NaCl stress. Endophytic fungi negate salt stress in plants by activating antioxidant systems, increasing osmoprotectant levels, modulating phytohormone profiles, and reducing salt-induced root respiration [42].

4 Conclusion

Fungal isolation yielded three and one fungal strains from *G. salicornia* and *Chondrus* sp. Based on phytochemical screening, indicated the presence of alkaloids in all extracts. In antibacterial assay, the extract of the non-salt-treated fungus *A. unguis* A4-1-1 demonstrated the most potent activity with MIC values of 15.6 µg/mL against *Staphylococcus aureus*. For the toxicity, the salt-treated fungus *T. asperellum* A5-1-2 exhibited the most potent toxic activity compared to the others with LC_{50} of 104.9 µg/mL, according to the brine shrimp lethality test. With the addition of salt to the treatment, it could have an effect on increasing the activity produced, however, further research is needed to confirm this.

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