

ANTIMICROBIAL ACTIVITY OF FUNGI ISOLATED FROM TAN DAO MANGROVE FOREST IN KHANH HOA

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ABSTRACT

Mangrove-derived fungi are the potential source of novel metabolites, comprising unique molecular structures with diverse bioactivities. In this study, 32 fungal strains isolated from the Tan Dao mangrove forest in Khanh Hoa province were investigated for their ability to produce antimicrobial agents using the disc diffusion method. Seventy-eight percent of obtained fungi (25 out of 32 isolates) exhibited antimicrobial activity against at least two test pathogens. Of them, bacteria *Bacillus cereus* and *Streptococcus faecalis* were the most susceptible to the antimicrobial activity of all fungal isolates with inhibition zones ranging from 8 to 36 mm, respectively, followed by *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa*. There were only three fungal isolates that showed antimicrobial activity towards *Candida albicans* with the inhibition zone of 8–17 mm diameter. The strain LM.8.1 showing significant activity against all test microorganisms was identified as *Penicillium* sp. LM.8.1. The effect of culture conditions including pH, salinity, and incubation time on the production of antibacterial compounds of fungus *Penicillium* sp. LM.8.1 was also studied. The results indicated that fungi isolated from the Tan Dao mangrove forest possess potent antimicrobial properties and should be further investigated.

Keywords: Antimicrobial activity, culture conditions, mangrove-derived fungi, *Penicillium* sp.

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INTRODUCTION

The discovery of penicillin from *Penicillium notatum* by Sir Alexander Fleming in 1928 motivated the research groups to investigate fungi as a rich source of secondary metabolites with biological activity (Strobel et al., 2002). Fungi are the object of study worldwide since the discovery of their ecological role and capacity of synthesizing metabolites with different chemical structures. They can be found in several plants that live in regions of temperate, tropical climates, and inhospitable environments such as deserts, swamps, and estuaries (Gunatilaka et al., 2006). Novel secondary metabolites from fungi have also been reported and even exploited for pharmaceutical products, and drug development (Strobel et al., 2004). In recent years, most new natural products described in the literature have been isolated from fungi (Saleem et al., 2007). The variety of substances produced by fungi has not still estimated, but the expectation is high due to their versatility (Silva et al., 2011).

Since the need for new antimicrobial compounds continues to grow, mangrove fungi could represent diverse and abundant resources. The studies of mangroves since the 17th century have indicated that this ecosystem encompasses a vast number of fungal species with the potential to produce bioactive metabolites (Elsa et al., 2013). Mangroves are considered a dynamic ecotone between terrestrial and marine habitats (Cheng et al., 2009). Therefore, they play an important role in the ecological balance, being responsible for the supply of nutrients to the marine environment and forming rich biodiversity sites with a plethora of macro- and microorganisms, including mangrove fungi (Silva et al., 2011). Due to the adaptability to the conditions of an extreme environment, such as pH, salinity, oxygen, pressure, and nutrients, mangrove fungi can synthesize a number of substances with different chemical structures and great biotechnological potential (Bugni et al., 2004). Mangrove-associated fungi show interesting and attractive properties such as antibacterial, antifungal,

anti-inflammatory, anticancer, antiproliferative, antioxidant, insecticidal, and extracellular enzymatic activities (Chaeprasert et al., 2010; Bhimba et al., 2011; Salini et al., 2015; Abraham et al., 2015; Lumbreras-Martínez et al., 2018; Sibero et al., 2019; Rahaman et al., 2020). Hence, mangrove fungi are considered promising sources for screening natural products in exploring new drugs (Murugaiyan et al., 2014).

Among the South Central coastal provinces in Vietnam, Khanh Hoa has a 400-kilometer coastline with many large bays and approximately 104 hectares of mangroves scattered in the coastal region with about 34 mangrove species (Nguyen Xuan Hoa, 2009). Therefore, this area can host a wide variety of fungal strains with promising bioactivities. This study was conducted to screen antimicrobial activity and determine the effect of culture conditions on antimicrobial metabolite production of the fungi isolated from Tan Dao mangrove forest in Khanh Hoa province. The preliminary screening would serve as a basis for discovering new antibiotics from these mangrove fungi.

MATERIAL AND METHODS

Sample collection

Samples including leaves, trunks, foams, seagrasses, and sediments were collected from Tan Dao mangrove forest in Ninh Hoa district, Khanh Hoa province. All samples were kept in sterile plastic bags, stored in an icebox at 4–8 °C, and transported to the laboratory for the isolation of fungi.

Isolation of mangrove-derived fungi

The collected plant materials were washed thoroughly with sterile water to remove extraneous substances, surface-sterilized by sequential washes in 95% ethanol for 30 s, and finally rinsed with sterilized distilled water. The samples were cut into 0.5 cm × 0.5 cm pieces and placed in Petri dishes containing Tubaci agar medium (yeast extract, 0.5 g; glucose, 30 g; agar-agar, 18 g; K₂HPO₄, 1 g; MgSO₄·7H₂O, 0.5 g; FeSO₄·7H₂O, 0.001 g, dissolved in 1000 mL seawater) and

supplemented with streptomycin (0.03 mg/mL). After incubation at 28 °C for one week, pure colonies were isolated, by hyphal tip isolation, and transferred onto Tubaci agar plates without antibiotics. The purity of each fungal strain was examined by assessing the colony morphology and then transferred into 40% glycerol in seawater for preservation at -80 °C.

Preparation of crude extracts from fungal isolates

Each fungal isolate was grown on a Tubaci agar slant for two weeks at 28 °C, then the biomass and culture medium were extracted by maceration with ethyl acetate for 24 hours. The crude extracts were obtained by evaporation to dryness and used for screening antimicrobial activity.

Screening for antimicrobial activity of the crude extracts

Test microorganisms

Seven clinical pathogens including Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), Gram-positive bacteria (*Bacillus cereus* ATCC 11778, *Streptococcus faecalis* ATCC 19433, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19111), and yeast (*Candida albicans* ATCC 10231) were purchased from ATCC (Manassas, USA) and stored at Nha Trang Institute of Technology Research and Application and used as test microorganisms due to their clinical importance.

Paper disk diffusion assay

The test microorganism cell suspensions were prepared from 24 hours old cultures of test bacteria and yeast. The inoculum concentration was adjusted to 0.5 McFarland standard and swabbed on Mueller-Hinton Agar plates. The crude extracts were dissolved in ethyl acetate and loaded onto 6 mm diameter sterile Whatman No1 paper discs at a concentration of 100 µg/disc. Each paper disk was air-dried before placing onto the inoculated culture plates. Standard streptomycin discs (100 µg/disc) served as

positive control and discs containing only solvent (ethyl acetate) served as a negative control. The experiments were done in triplicate and antimicrobial activities were assessed by measuring the inhibition zone diameter after the plates were incubated at 37 °C for 24 hours (Bauer et al., 1966).

Fungal identification

The selected fungi were identified based on both morphological and ITS gene sequence analysis. Morphological traits identification was done based on selected culture, microscopic study, and consulting the relevant literature. This isolate was classified into a genus according to the classification keys of “A manual of Penicillia” by Kenneth B. Raper & Charles Thom (1949).

Genomic DNA was isolated following the protocol proposed by Fredricks et al. (2005). The nuclear ribosomal DNA of the fungal isolate was amplified using the forward primer, ITS1 (5'-CTTGGTCATTTAGAGGAAGTAA-3' and the reverse primer, ITS4 (5'-CTTGGTCATTTAGAGGAAGTAA-3') (White et al., 1990). Sequences of fungal ITS-rDNA regions were compared with those in the NCBI (National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov>). Multiple alignments of the ITS gene region were generated using MUSCLE (default conditions for gap opening and gap extension penalties) and implemented in MEGA 7.0 (Molecular Evolutionary Genetics Analysis). The phylogenetic tree was generated using neighbor-joining (NJ) with bootstrap analysis using 1,000 replicates.

Effect of culture conditions on antibiotics production of mangrove fungi

Fermentation was carried out in 500 mL Erlenmeyer flasks, containing 40 mL of natural seawater collected in Nha Trang Bay (pH 8.0, salinity of 30 g/L) supplemented with 20 g of rice, 20 mg of yeast extract, and 10 mg of KH₂PO₄ (Sobolevskaya et al., 2016). The parameters studied included different initial values of pH (4-9), salinity (10-45 g/L), and incubation periods (10-24 days)

using univariate analysis. All experiments were conducted at 28 °C. At the end of the cultivation, the mycelia and culture media were homogenized and extracted twice with equal volumes of ethyl acetate. The extract of the fungus was concentrated to dryness using rotary evaporators at 40 °C. The residues were obtained and used as a crude extract for the test of antimicrobial activity.

RESULTS AND DISCUSSION

Isolation of mangrove-derived fungi

The present work is the first report of fungi isolated from the Tan Dao mangrove forest in Khanh Hoa province. A total of 32 fungal isolates was obtained from various mangrove samples including leaves, trunks, seagrasses, foams, and sediments (Table 1). In this study, due to a limited number of collected samples, we have not evaluated the distribution of fungal strains in different sources of this mangrove. However, the previous report about mangrove fungi from Sundarbans, Bangladesh indicated that the isolation rates of fungi from leaves were higher than that of bark, root, and fruits. The isolation rates for root, bark, fruits and leaves were 46, 43, 33, and 70%, respectively (Nurunnabi et al., 2020). Moron et al. (2018) also reported that stems from Philippines mangroves had a higher number of fungi compared to roots. Besides, those studies

demonstrated that the abundance and diversity of mangrove fungi were dependent on mangrove plant species and their tissue types, i.e., stem, leaf, or root (Chi et al., 2019a). Likewise, fungal diversity and abundance in xylem parts are lower than in the bark, shoot, and foliar parts (Stone et al., 2000). These differences in fungal species composition and frequency of occurrence in fungal communities might explain that the fungi that colonize different tissues have diverse substrates utilization abilities (Carroll & Petrini, 1983). Besides, regarding the symbiotic interaction between the fungi and the host mangrove plant, fungi can colonize inside tissues and organs without causing any symptoms or apparent injury to the host (Petrini, 1991). The host plant protects and gives nutrients to the fungi while fungi produce several secondary metabolites, increasing the growth and competitiveness of the host plant in the presence of herbivores and other pathogens (Dreyfuss & Chapela, 1994). However, some genera such as *Cladosporium*, *Colletotrichum*, *Fusarium*, *Phyllosticta*, and *Botryosphaeria* were reported to potentially cause diseases of mangrove plants (Chi et al., 2019a; Osorio et al., 2017). The association of fungi isolated with the mangrove plant is not known, but future studies should focus on the ecological roles of these fungi.

Table 1. List of fungal strains isolated from mangrove sources

Order	Samples	Number of samples	Number of isolated fungal strains
1	Foam	1	2
2	Sediment	1	3
3	Sea grass	1	3
4	Leave	6	12
5	Trunk	8	12

Antimicrobial activity of isolated mangrove fungi

The extracts of mangrove fungi originating from Khanh Hoa province were investigated

for antimicrobial activity against seven different pathogens by the disc diffusion method. The antimicrobial activity of crude extracts with inhibition zones ranging from 8 mm to 50 mm in diameter is shown in Table 2.

Table 2. Antimicrobial activity of isolated mangrove fungi

Strains	Zone of inhibition in mm (D, mm)						
	Gram (+)				Gram (-)		Yeast
	<i>B. cereus</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>	<i>S. faecalis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
FM.1.1	17	-	13	17	12	12	-
FM.1.3	10	15	8	9	-	-	-
SM.1.1	14	-	-	14	9	13	-
SM.1.2	-	11	10	-	-	-	-
SM.1.3	14	13	16	-	-	-	-
SGM.1.1	19	-	-	10	-	-	-
SGM.1.2	16	-	16	19	10	15	-
SGM.1.3	14	9	15	13	-	-	-
LM.1.1	10	11	13	8	-	-	8
LM.3.1	8	15	10	11	-	-	-
LM.4.1	-	-	15	7	-	-	-
LM.4.4	7	-	-	8	-	-	-
LM.4.8	-	-	-	8	-	-	-
LM.5.2	-	-	10	-	-	-	-
LM.5.3	9	-	-	12	-	-	-
LM.5.4	7	10	17	8	-	-	-
LM.5.5	15	-	-	15	-	-	-
LM.6.2	18	30	26	18	22	-	-
LM.8.1	36	46	50	35	40	16	17
LM.8.2	20	18	16	20	-	-	-
TM.2.1	18	23	26	16	20	17	-
TM.3.2	12	-	-	-	-	-	-
TM.4.3	16	16	17	13	-	-	-
TM.4.6	-	-	10	-	-	-	-
TM.7.2	8	-	-	-	-	-	15
TM.8.1	-	-	-	-	-	-	-
TM.8.2	13	-	9	8	-	-	-
TM.9.1	-	-	12	-	-	-	-
TM.10.1	11	13	12	11	-	-	-
TM.10.2	21	21	17	18	-	-	-
TM.12.1	-	-	16	-	-	-	-
TM.12.2	-	10	-	10	-	-	-

Note: "-": no antimicrobial activity.

Among 32 mangrove fungi, 31 isolates showed activity against bacteria or yeast. More than 20 isolates were active against *B. cereus*, *S. faecalis*, and *S. aureus*, while 15 isolates inhibited the growth of *L. monocytogenes*, and fewer isolates inhibited *E. coli* and *P. aeruginosa*. The yeast *C.*

albicans could not grow in the presence of the extracts from three isolates LM.1.1, LM.8.1, and TM.7.2. It is worth noting that the isolates FM.1.1, SGM.1.2, LM.6.2, LM.8.1, and TM.2.1 exhibited significant activity against almost all the test microorganisms (Fig. 1 & Table 2). Our

findings also indicated that the fungal strains isolated from mangroves showed a different spectrum of antimicrobial activity. The highest number of isolates (96.8%) were active against Gram-positive bacteria, meanwhile, 18.7% of the fungi were active against Gram-negative bacteria, and only 9.3% active against the yeast pathogen. Differences in the cell wall structure of the tested microorganisms are supposed to relate to the difference in susceptibility (Moron et al., 2018). Besides, it is possible that the

fungal extracts contained compounds more toxic to Gram-positive bacteria than to the Gram-negative cells and the yeast (Ahmad et al., 2018). Furthermore, the nature of the compounds produced by the fungi and the mechanism of action is not known yet, so further research is needed. Similar results are found in other reports which indicated that fungi from mangrove plants show more potential antibacterial activity towards Gram-positive than Gram-negative cells (Chareprasert et al., 2010; Chi et al., 2019b).

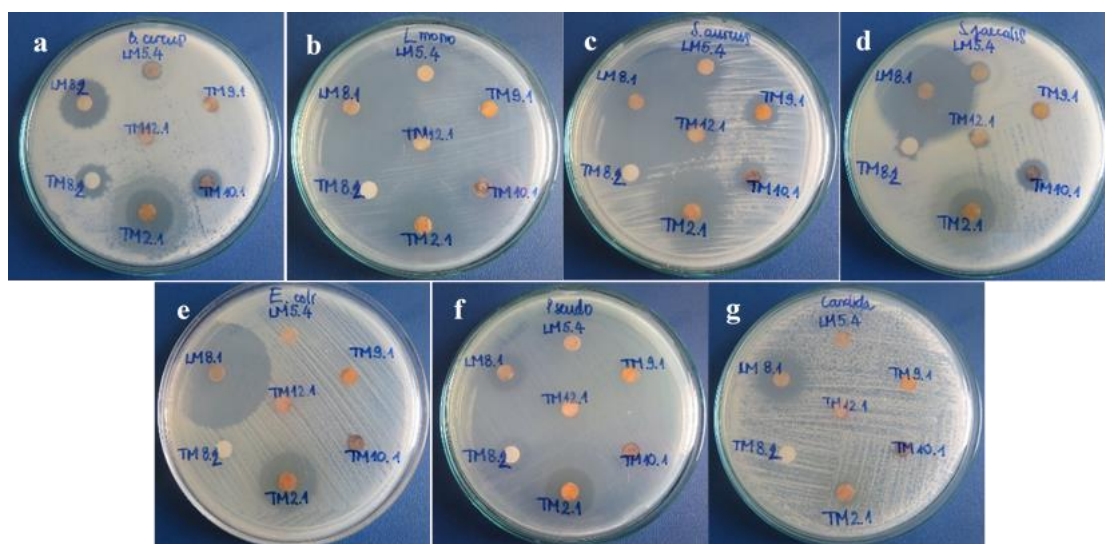


Figure 1. Antimicrobial activity of isolated strains against seven different pathogens. (a) *Bacillus cereus*; (b) *Listeria monocytogenes*; (c) *Staphylococcus aureus*; (d) *Streptococcus faecalis*; (e) *Escherichia coli*; (f) *Pseudomonas aeruginosa*; (g) *Candida albicans*

The present study has revealed that the Tan Dao mangrove forest harbors fascinating fungi that produce potential antimicrobial substances. This report suggests that fungi from harsh and competitive environments, such as the mangrove ecosystem, might be an attractive source for new antimicrobial compounds. The presence of fungal strains with high antimicrobial activity could protect the mangrove plants from microbial diseases.

Identification of the active mangrove fungi

According to antimicrobial activity, isolate LM.8.1 was found to be the most effective of all the fungal isolates and seems

to have a broad spectrum of activity as it inhibited the growth of all test pathogens. Therefore, the fungus LM.8.1 was selected as a good candidate for secondary metabolite production, and its identification was carried out.

The fungal isolate LM.8.1 grows on Czapek agar comparatively rapidly, attaining a diameter of 28–30 mm after 7th days at room temperature. The colony consists of a tough basal felt and loose aerial hyphae, producing dark green to dark bluish-green conidia. The reverse is creamish dull yellow; the marginal areas are whitish. Conidiophores have smooth

wall and branches of $15\ \mu\text{m} \times 3\ \mu\text{m}$, metulae of $10\ \mu\text{m} \times 3\ \mu\text{m}$, in groups of 2 to 4; sterigmata in groups of 5 to 10. The conidia at first elliptical of about $4\ \mu\text{m} \times 3\ \mu\text{m}$, with individual smooth spores up to $5.5\ \mu\text{m}$ in length. Identification of the isolate LM.8.1 was carried out by comparison of its morpho-cultural characteristics with published literature, specifically the classification keys of “A manual of *Penicillia*” by Kenneth B. Raper and Charles Thom (1949). Based on the results of characterization, strain LM.8.1 was

determined to belong to the genus *Penicillium*. Macroscopic and microscopic characteristics of the strain LM.8.1 are presented in Figure 2. Moreover, the BLAST search result indicated that the ITS gene sequence of the fungus LM.8.1 was 98–99% similar to the sequences of *Penicillium solitum* (JX290030) and other fungi of genus *Penicillium* from GenBank (Fig. 3). Thus, this fungal strain was assigned the name *Penicillium* sp. LM.8.1 (GenBank accession number MZ411574).

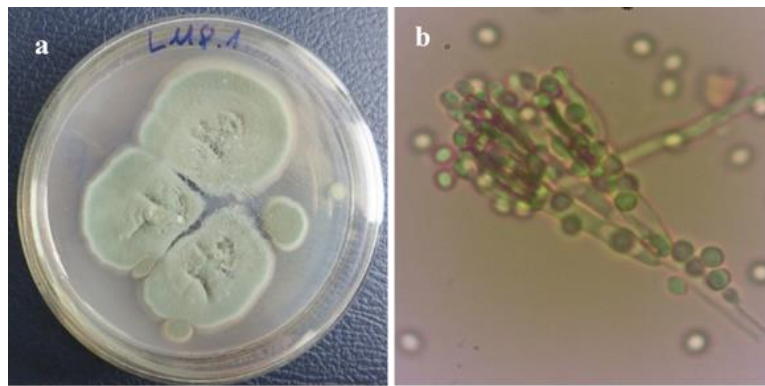


Figure 2. *Penicillium* sp. LM.8.1: (a) Colony appearance; (b) Micromorphology

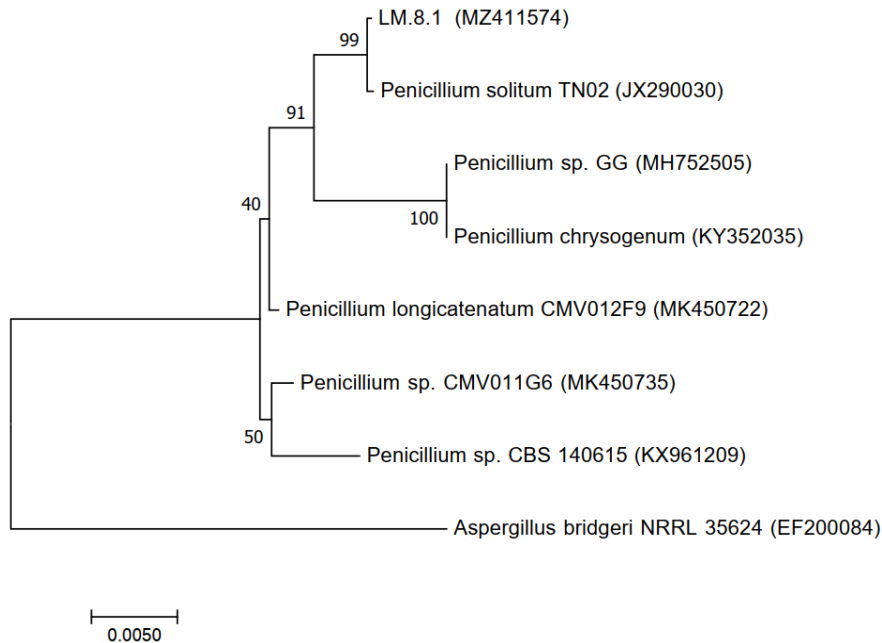


Figure 3. Phylogenetic tree based on rDNA-ITS sequences of the fungus *Penicillium* sp. LM.8.1 and related members of the genus *Penicillium* from GenBank

Effects of culture conditions on antimicrobial activity of *Penicillium* sp. LM.8.1

The effect of initial medium pH

The initial pH of the culture medium is an important parameter affecting the biosynthesis of secondary metabolites since it can indirectly act on the fungal growth by affecting the availability of medium nutrients. The hydrogen or hydroxyl ion concentration of the medium directly affects the permeability characteristics of the cell wall and membrane. Thus, the change of pH is related to the enzyme activity and the intermediate products by varying the degree of dissociation and solubility of substances in the medium (Gogoi et al., 2008). Both the metabolite production and antibacterial activity of the fungus *Penicillium* sp. LM.8.1 were gradually enhanced with the increase of pH from 4 to 6. Further increase of pH, particularly under strongly alkaline conditions, resulted in a significant decrease in the production and antibacterial activity. Maximum solid crude extract weight (349 mg) and antibacterial activity (the zone of inhibition of 31 mm against *E. coli* and 33 mm against *S. aureus*) were obtained at pH 6 (Fig. 4).

Similar results have been reported by Bhavani & Muvva (2020), the initial pH of 6.0 of the medium was the most suitable for *Cladosporium cladosporioides* isolated from mangrove plant *Lumnitzera racemosato* to have the highest bioactivity. According to our previous investigation, the pH of 6.0 was also determined as the best pH for sponge-associated fungus *Aspergillus flocculosus* 01NT.1.1.5 produce antibiotics (Trinh et al., 2017). Nevertheless, the pH of 7.0 was detected to be optimum for maximum production of antimicrobial metabolites by two marine fungi *Penicillium chrysogenum* 045-357-2 and *Penicillium* sp. 1901NT-1.11.1 (Trinh et al., 2016; Trinh et al., 2020). The fungus *Arthrinium c.f. saccharicola* isolated from seawater in a mangrove habitat in Yung Shue O, Hong Kong exhibited higher antibacterial activity at pH 7.5 (Miao et al., 2006). The reports indicated that pH affects not only growth but also antimicrobial agent production and most microorganisms synthesize antimicrobial compounds at pH ranging from 5.5 to 8.5 (Thongwai & Kunopakarn, 2007).

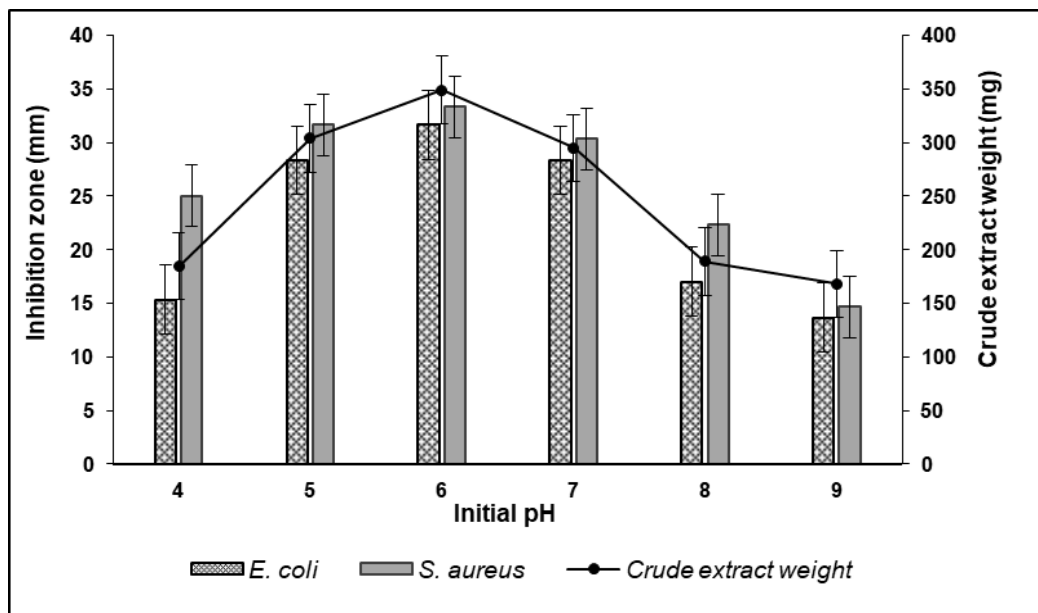


Figure 4. Effect of initial pH on the production of antibacterial metabolites

The effect of salt concentration

Salt concentration has a profound effect on the growth and metabolite production of antibiotics from mangrove fungi due to its influence on the osmotic pressure of the medium (Pelczar et al., 1993). In the present study, the production of antimicrobial metabolites by *Penicillium* sp. LM.8.1 was evaluated in culture media with salinity ranging from 10 g/L to 45 g/L. The results showed that the amount of crude ethyl acetate extract from this fungus and its antimicrobial activity gradually increased with the increase of NaCl concentration. The salinity of 35 g/L was recorded as optimal, giving the highest metabolite production (the crude extract of 352 mg) and improved bioactivity (the zone of inhibition of 31 mm against *E. coli* and 34 mm against *S. aureus*). The lowest crude extract weight was observed in the medium with a NaCl concentration of 45 g/L (Fig. 5). The marine fungus *Penicillium chrysogenum*, obtained from Ca Na Bay, Ninh Thuan province, also exhibited excellent growth and bioactive metabolite production at the salinity of 35 g/L (Trinh et al., 2016).

In another study, a marine-derived fungus *Arthrinium. c.f. saccharicola* grew faster in freshwater than in seawater, however, the salinity condition of 34 g NaCl/L improved the antibacterial activity (Miao et al., 2006). The influence of NaCl on the biomass and bioactive compound production by the fungus *Cladosporium cladosporioides*, isolated from leaves of mangrove plant *Lumnitzera racemosa* in Gilakaladindi, India was investigated (Bhavani & Muvva, 2020). It was found that the highest growth was observed at 4% NaCl, and antimicrobial compound production was high at 5% NaCl. In contrast, Mathan et al. (2013) detected NaCl concentration of 5 g/L as optimum for the production of antibiotics by *Aspergillus terreus* KC 582297 isolated from seaweed *Codium decortiatum*. These results indicated that marine-derived fungi are significantly affected by salt concentration in the culturing media. Most studied fungi synthesize secondary metabolites with high antimicrobial activity at the salinity of 35–50 g/L.

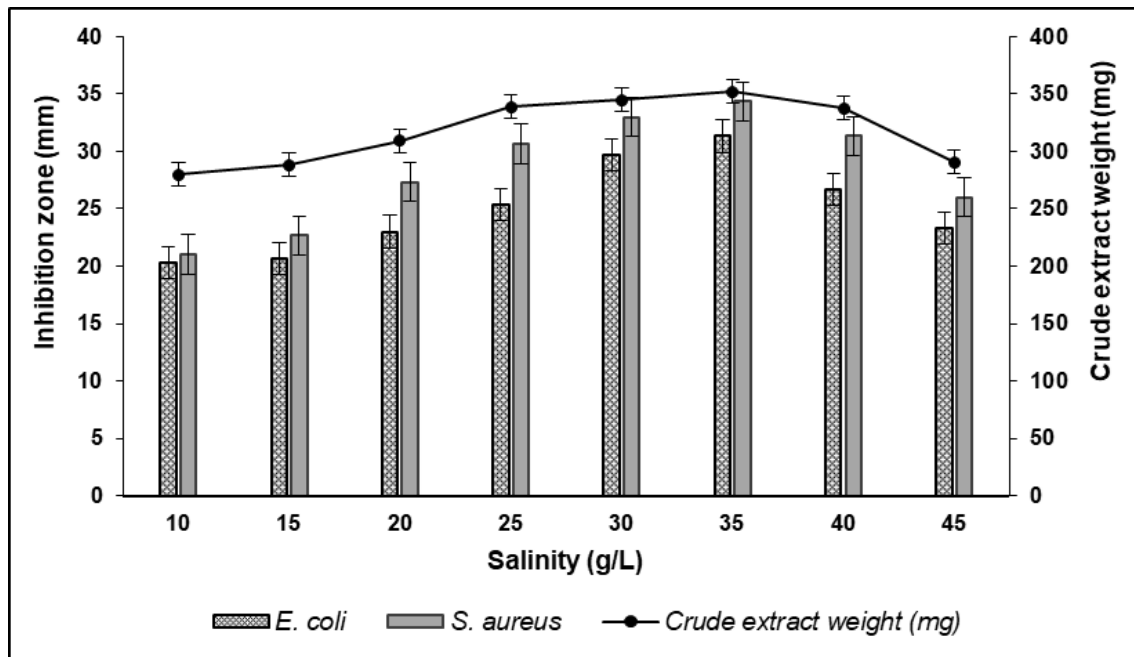


Figure 5. Effect of salinity on the production of antibacterial metabolites

The effect of cultivation time

The fermentation time is a significant factor that needs to be determined for having the maximum harvest of the desired metabolite(s). The duration of cultivation is impactful for the production of biomass and bioactive metabolite, but it happens very often that increasing cultivation time does not mean to enhance the harvest. Prolong cultivation may make more toxins which inhibit the production of antimicrobial metabolites (Song et al., 2012). Antibacterial metabolites production by the strain *Penicillium* sp. LM.8.1 was assayed after 10th days of incubation and till the 24th day. The antimicrobial activity and crude extract weight were highest on the

16th day of incubation, then gradually declined (Fig. 6). Here, the decrease in metabolites secretion observed from the 20th day of cultivation may be due to the exhaustion of some medium constituents or the production of inhibitory compounds. Thus, the most suitable cultivation time for the fungal isolate *Penicillium* sp. LM.8.1 was of 16 days when the fungus produce the highest amount of crude extract (374 mg) as well as antimicrobial activity (zone of inhibition of 33 mm against *E. coli*; and 36 mm against *S. aureus*) (Fig. 6). The effect of the incubation period on the production of the bioactive compound by the sponge-associated fungus *Penicillium* sp. 1901NT-1.11.1, obtained in Nha Trang Bay, was reported (Trinh et al., 2020).

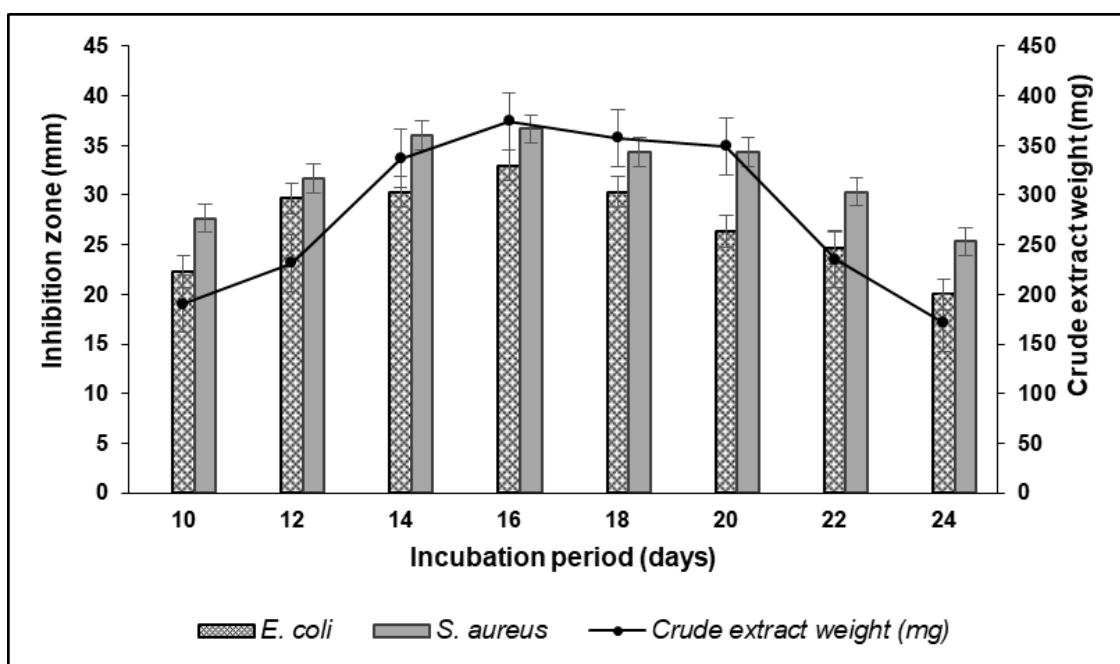


Figure 6. Effect of incubation period on the production of antibacterial metabolites

They observed that the production of metabolites was commenced after 20 days. Similarly, Mabrouk et al. (2011) reported that the marine fungus *Penicillium brevicompactum*, associated algae *Pterocladia* sp., produced maximum bioactivity at 12 days of incubation. However, the marine fungus *Aspergillus terreus* var. *africanus* showed

optimal growth time with high antimicrobial activity on the 6th day of cultivation (Barakat & Gohar, 2012). Generally, fungal strains have different optimal culturing times for the growth and synthesis of bioactive compounds. Besides, types of fermentation processes that are carried out such as solid-state, submerged, and liquid fermentation, along with growth

characteristics of each fungal strain also affect the suitable cultivation time.

These results revealed that fermentation conditions remarkably affect antibiotic biosynthesis of *Penicillium* sp. LM.8.1. The maximum growth and antimicrobial metabolites production by this isolate was achieved in the rice medium with initial pH of 6.0, the salinity of 35 g/L, and after 16 days of incubation at 28 °C. The studies indicated that determining suitable fermentation conditions is one of the necessary steps before scaling up the production process to obtain bioactive compounds from potential strains.

CONCLUSION

The present study discovered a high diversity of fungi with potential antimicrobial activity from the Tan Dao mangrove forest in Khanh Hoa province. Out of 32 fungal isolates, 31 (96.8%) exhibited antimicrobial activity against at least one of the test pathogens. Noticeably, the fungus *Penicillium* sp. LM.8.1 displayed significant activity towards seven tested microorganisms. Besides, this report also indicated suitable cultural conditions for producing a crude extract from the fungal culture with high antimicrobial activity. These results revealed the potential of locally isolated fungi as promising sources of bioactive secondary metabolites that need further exploitation for new antibiotic discovery.

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