MINISTRY OF EDUCATION AND TRAINING
VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

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STUDY ON THE CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY OF THREE FUNGAL STRAINS: *Aspergillus* sp. DVXT-221, *Trichoderma* sp. GXT-22.1 AND *Aspergillus* sp. BCXT-22.1

SUMMARY OF DISSERTATION ON SCIENCES OF MATTER

Major: Organic Chemistry

Code: 9 44 01 14

Hanoi – 2026

The dissertation is completed at: Graduate University of Science and Technology, Vietnam Academy Science and Technology

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INTRODUCTION

In recent decades, the search for bioactive natural compounds from biological resources has become a key focus of life sciences research, particularly given the rise of drug resistance and the need to develop new natural-origin pharmaceutical agents. Among the organisms under investigation, microfungi are recognized as one of the richest and most diverse sources of secondary metabolites, offering a wealth of novel chemical structures and valuable biological activities.

The mangrove ecosystem is a unique environment subject to the simultaneous influence of salinity, waterlogging, tidal fluctuations, and high biological pressure. These harsh conditions have driven microorganisms—particularly endophytic fungi associated with mangrove plants—to develop specialized adaptive mechanisms, resulting in the capacity to biosynthesize a wide range of unique secondary metabolites. Numerous studies worldwide have demonstrated that fungi derived from mangrove plants are a promising source of compounds exhibiting antibacterial, antifungal, antiviral, antioxidant, and anticancer activities.

Xuan Thuy National Park is Vietnam's first Ramsar site, serving as a prime example of a coastal wetland ecosystem characterized by high biodiversity and rich mangrove flora. However, research in this area to date has primarily focused on the diversity of higher flora and fauna and on ecological conservation; meanwhile, microbial resources—particularly fungi associated with mangrove plants—have not yet been studied comprehensively or systematically, especially regarding the chemical composition and biological activity of the compounds they produce.

Stemming from that practice, the study of isolating microorganisms from mangrove plants in Xuan Thuy National Park, combined with chemical composition analysis and assessment of biological activity of the obtained compounds, not only contributes to supplementing the scientific database on the chemical diversity of fungi but also opens up application prospects in the fields of medicine, pharmacy and biotechnology. On that basis, topic **"Study on the chemical constituents and biological activity of three fungal strains: *Aspergillus* sp. DVXT-221, *Trichoderma* sp. GXT-22.1, and *Aspergillus* sp. BCXT-22.1"** is carried out to contribute to the rational exploitation of biological resources, linking basic research with application orientation and sustainable development. The thesis includes the following objectives and contents:

- Objectives of the thesis:

1. Isolation and structural elucidation of secondary metabolites from three fungal strains associated with mangrove plants collected at Xuan Thuy National Park.

2. Evaluated the cancer cell cytotoxicity against two cell lines liver cancer (Hep-G2) and breast cancer (MCF-7) and the inhibition of NO production in RAW264.7/BV2 cell lines of the isolated pure compounds.

- Contents of the thesis:

1. Research on the isolation of pure compounds from three fungal strains isolated from mangrove plants.

2. Determine the chemical structures of the isolated pure compounds.

3. The study evaluated the cancer cell cytotoxicity using liver cancer (Hep-G2) and breast cancer (MCF-7) cell lines and the inhibition of NO production in RAW264.7/BV2 cell lines of the isolated compounds.

CHAPTER 1. OVERVIEW

1.1. Secondary metabolites from microfungi associated with mangrove plants in Xuan Thuy National Park

Global research on mangrove-associated fungi reveals a diverse range of species inhabiting mangrove plants and highlights them as a rich source of novel, bioactive compounds. Notably, studies on the chemical composition and biological activity of fungi associated with dominant species in Xuan Thuy National Park such as *Sonneratia caseolaris*, *Rhizophora stylosa*, and *Excoecaria agallocha* have yielded intriguing results and demonstrated significant potential for pharmaceutical development [20, 45].

Studies on endophytic fungi associated with *S. caseolaris* have led to the isolation of several new, bioactive compounds. According to Ebrahim et al. (2012), the peptides pullularin A (**83**) and C (**84**), as well as verticilin (**85**) isolated from the fungus *Bionectria ochroleuca* exhibit potent cytotoxic activity against the L5178Y cell line (IC_{50} = 0.1-6.7 µg/mL) [17]. Research by Rönsberg et al (2013) isolated and identified a number of new α-pyrone compounds, pestalotiopyrone I–L (**83-86**) from the endophytic fungus *Pestalotiopsis virgatula* in *S. Caseolaris* [46]. However, none of the compounds exhibited cytotoxicity against L5178Y cells at a concentration of 10 µg/mL, nor

did they show antibacterial activity (MIC > 125 µg/mL). According to Liu et al. (2016), five new altenusin derivatives and several known compounds were isolated from the fungal strain *Alternaria* sp. SK6YW3L (derived from *S. caseolaris* fruit); among these, compounds **87**–**89** demonstrated inhibitory activity against the enzyme α-glucosidase, with IC_{50} values of 78.2, 78.1, and 64.7 µM, respectively [47]. Theo Zhu và cs (2016), 2 cặp đồng phân mới là campyridone A (**90**) và B (**91**) và campyridone C (**92**) và D (**93**) đã được phân lập từ chủng vi nấm *Campylocarpon* sp. HDN13-307, trong đó hợp chất **93** có hoạt tính gây độc đối với dòng tế bào HeLa (IC50 = 8.8 µM) [48].

Studies on endophytic fungi associated with *R. stylosa* have also yielded intriguing results regarding chemical composition and biological activity. According to Peng et al. (2011), five new cerebrosides, chrysogesides A-E (**94**–**98**) and two new 2-pyridone alkaloids, chrysogedones A and B (**99** and **100**) were isolated from the *Penicillium chrysogenum* strain PXP-55 (derived from *R. stylosa* roots in China); notably, compound **95** exhibited antibacterial activity against Enterobacter aerogenes (MIC = 1.72 µM) [49]. According to Zang et al. (2012), nine new sesquiterpenoids diaporols A-I (**101**-**109**) were isolated from the fungal strain *Diaporthe* sp. found on *R. stylosa* leaves in China; however, these compounds did not exhibit cytotoxic activity at a concentration of 20 µM [50]. From this same fungal strain, ten new brasilane-type sesquiterpenoids diaporols J–S (**110**–**119**) were also isolated; however, these compounds showed no cytotoxicity at a

concentration of 30 µM, with the exception of compound **118**, which exhibited moderate activity against SW480 cells (IC_{50} = 8.72 ± 1.32 µM) [51]. Several polyketide compounds were isolated from the fungal strain *Cytospora heveae* NSHSJ-2 isolated from the stem of *S. caseolaris*) and exhibited DPPH radical-scavenging activity [52].

From the fungus *Aspergillus nidulans* MA-143 isolated from *R. stylosa* leaves, six new 4-phenyl-3,4-dihydroquinolone compounds aniduquinolones A-C (**120**-**122**), 6-deoxyaflaquinolone E (**123**), isoaflaquinolone E (**124**), and 14-hydroxyaflaquinolone F (**125**) were isolated; among these, compounds **121** and **122** exhibited toxicity against *Artemia salina* larvae, with IC_{50} values of 7.1 and 4.5 µM, respectively [53]. From the same fungal strain, An et al. (2013) isolated four quinazolinone alkaloid compounds aniquinazolines A-D (**126**-**129**) all of which demonstrated toxicity against *Artemia salina* (IC_{50} = 1.27–4.95 µM) [54]. From the fungus *Mucor irregularis* QEN-189 isolated from *R. stylosa* roots on Hainan Island, China, six new indole-diterpenoid compounds rhizovarins A-F were isolated; specifically, rhizovarins A (**130**), B (**131**), and E (**132**) exhibited cytotoxic activity against HL-60 and A-549 cell lines (IC_{50} = 6.3-11.5 µM) [55]. According to Zhang et al. (2015), two new alkaloid compounds penioxamide A (**133**) and 18-hydroxydecaturin B (**134**) were isolated from the *Penicillium oxalicum* EN-201 strain (derived from *R. stylosa* leaves), and both compounds showed toxicity against *Artemia salina* (LD_{50} = 5.6 and 2.3 µM) [56]. Several fungal strains isolated from *R. stylosa* exhibit antibacterial activity against

Pseudomonas adaceae Enterococcus faecalis, and methicillin-resistant *Staphylococcus aureus* (MRSA), as well as antifungal activity against the pathogen *Monilia albicans* and cytotoxic activity against certain cancer cell lines such as A549, HeLa, and HepG2 [57].

Studies on endophytic fungi associated with *E. agallocha* have yielded various new compounds with potential biological activity. According to Wang et al. (2012), four new polyphenolic compounds expansols C-F (**135**-**138**) along with the diphenyl ether 3-*O*-methyldiorcinol (**139**) and several known compounds, were isolated from the strain *Penicillium expansum* 091006; among these, expansols C (**135**) and E (**137**) exhibited cytotoxic activity against HL-60 cells, with IC_{50} values of 18.2 and 20.8 μM, respectively [58]. From the same fungal strain, two new polyphenolic compounds featuring a bisabolane sesquiterpene core expansols A (**140**) and B (**141**) were also isolated; compound 140 demonstrated cytotoxicity against HL-60 cells (IC_{50} = 15.7 μM), while compound **141** showed antiproliferative activity against A549 and HL-60 cell lines, with IC_{50} values of 1.9 and 5.4 μM, respectively [59]. Additionally, a study by Wang et al. (2018) reported the isolation of five new polyketide compounds (**142**–**146**) and several known compounds from the fungal strain *Cladosporium* sp. OUCMDZ-302: the compounds methyl (3*S*)-3-(2,3-dihydroxyphenyloxy)butanoate (**144**), 3-(2,3-dihydroxyphenoxy)butanoic acid, (2*S*,4*S*)-4-methoxy-2-methylchroman-5-ol, and (2*S*,4*S*)-2-methylchroman-4,5-diol exhibited DPPH radical scavenging activity (IC_{50} = 0.24–6.67 μM),

while the compound (3*E*,8*E*,6*S*)-undeca-3,8,10-trien-1,6-diol (**146**) showed cytotoxic activity against the H1975 cell line (IC_{50} = 10.0 µM) [60].

CHAPTER 2. RESERCH SUBJECTS AND METHODS

2.1. Research subjects

- ***The fungal strain XT37 (Aspergillus sp. DVXT-221)***

The fungal strain XT37 (*Aspergillus* sp. DVXT-221) was isolated from the leaves of *Rhizophora stylosa* collected in Xuan Thuy National Park. Its scientific identity was determined using molecular methods, specifically PCR and ITS rDNA sequencing. The ITS rDNA sequence of this strain has been registered in GenBank under accession number PQ119563. The strain is currently preserved at the Department of Molecular Biochemistry, Institute of Chemistry, Vietnam Academy of Science and Technology.

Fig. 2.1. The fungal strain XT37 (*Aspergillus* sp. DVXT-221)

- ***The fungal strain XT47 (Trichoderma sp. GXT-22.1)***

The fungal strain XT47 (Trichoderma sp. GXT-22.1) was isolated from the roots of *Excoecaria agallocha* collected in Xuan Thuy National Park. The strain was identified using PCR and ITS rDNA gene sequencing (GenBank accession number: PQ559788). It

is currently preserved at the Department of Molecular Biochemistry, Institute of Chemistry, Vietnam Academy of Science and Technology..

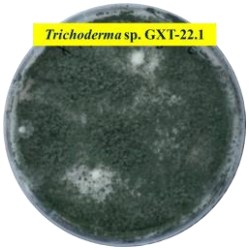

Fig. 2.2. The fungal strain XT47 (*Trichoderma* sp. GXT-22.1)

- ***The fungal strain XT49 (Aspergillus sp. BCXT-22.1)***

The fungal strain XT49 (Aspergillus sp. BCXT-22.1) was isolated from the roots of *Sonneratia caseolaris* collected in Xuan Thuy National Park. The strain was identified using PCR and ITS rDNA gene sequencing (GenBank accession number: PV425981). It is currently preserved at the Department of Molecular Biochemistry, Institute of Chemistry, Vietnam Academy of Science and Technology..

Fig. 2.3. The fungal strain XT49 (*Aspergillus* sp. BCXT-22.1)

CHAPTER 3. RESEARCH RESULTS

3.1. Results of biomass fermentation and production of crude extracts from fungal strains.

The fungal strains XT37, XT47, and XT49 were cultured on a large scale for biomass production, followed by extraction with ethyl acetate (EtOAc) to obtain crude extracts. Crude EtOAc extracts were successfully obtained from the three strains: XT37 (6.8 g), XT47 (20 g), and XT49 (7.0 g). These crude extracts were stored at 0–4°C pending further study.

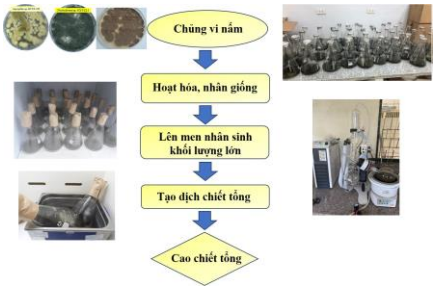

Fig. 0.1. Flowchart of large-scale biomass fermentation and total extract production for fungal strains XT37, XT47 and XT49

3.2. Results of compound isolation

3.2.1. Result of compound isolation from the fungal XT37

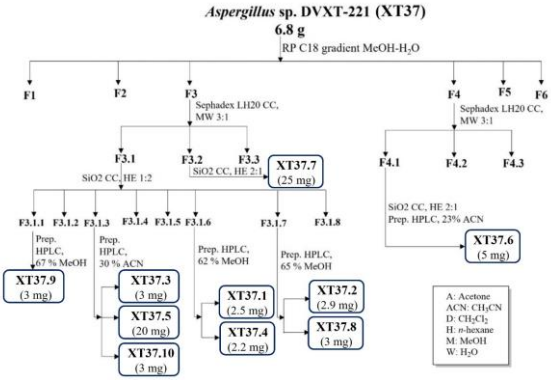

Fig. 3.2. Flowchart for the isolation of compounds from the fungal strain XT37

3.2.2. Result of compound isolation from the fungal XT47

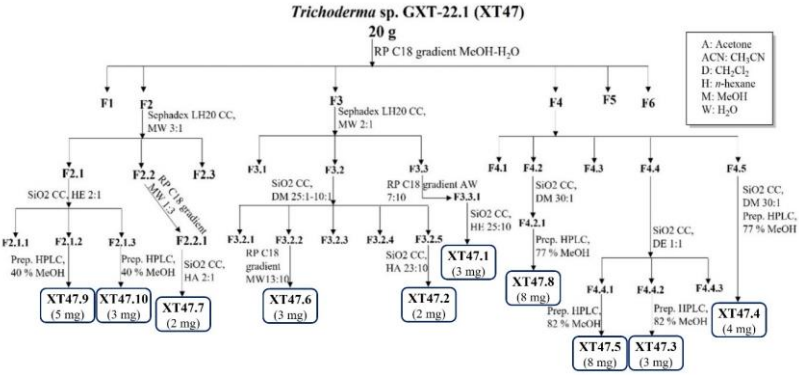

Fig. 3.3. Flowchart for the isolation of compounds from the fungal strain XT47

3.2.3. Result of compound isolation from the fungal XT49

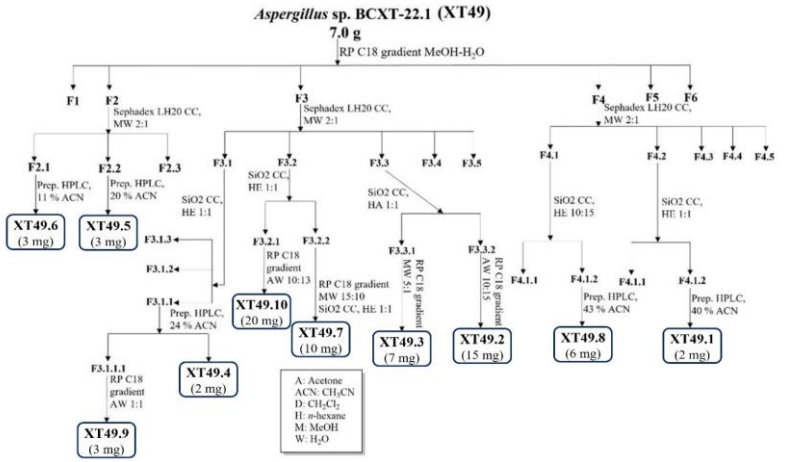

Fig. 3.4. Flowchart for the isolation of compounds from the fungal strain XT49\

CHAPTER 4. DISCUSS RESULTS

3.1. Determine the structure of the isolated compounds

3.1.1. Determination of the structures of compounds isolated from fungal strain Aspergillus sp. DVXT-221 (XT37)

3.1.1.1. Compound XT37.1: Fumigaclavine K (new)

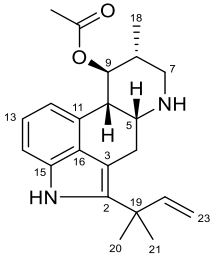

Fig. 0.1. Structure of **XT37.1**

Compound **XT37.1** was obtained as a white powder. Its molecular formula was determined to be $C_{22}H_{28}N_2O_5$ based on the protonated molecular ion signal $[M+H]^+$ at *m/z* 353.2226 (calculated value for $C_{22}H_{29}N_2O_2$: 353.2229) in the HRESIMS spectrum (Fig. 4.5). The ^{1}H NMR spectrum displayed characteristic aromatic proton signals forming an ABC spin system at δ_H 6.71 (dd, J = 7.2 Hz, H-12), 7.03 (t, J = 7.8 Hz, H-13), and 7.18 (d, J = 7.8 Hz, H-14); signals corresponding to a double bond at δ_H 6.18 (dd, J = 10.8, 17.4 Hz, H-22), 5.12 (dd, J = 2.4, 17.4 Hz, Ha-23), and 5.10 (dd, J = 1.8, 10.8 Hz, Hb-23); and four methyl groups at δ_H 1.39 (d, J = 7.8 Hz, H-18), 1.56 (s, H-20), 1.55 (s, H-21), and 1.89 (s, 9-OAc) (Table 4.1). The ^{13}C NMR spectrum displayed 22 carbon signals, including a carbonyl group at δ_C 172.0 (9-OAc), 10 sp^2 carbons, and 11 sp^3 carbons. Among the sp^2 carbons in addition to signals from double bonds and three methine carbons five non-protonated carbons were observed,

including two nitrogen-bearing carbons at δ_C 139.5 (C-2) and 134.8 (C-15); this suggests that compound **XT37.1** contains an indole ring (Table 4.1) [14, 15]. HSQC spectral analysis revealed the presence of sp^3 carbons, comprising two methylene carbons [including a nitrogen-bearing carbon at δ_C 46.4 (C-7)], four methine carbons [including a nitrogen-bearing carbon at δ_C 55.1 (C-5) and an oxygen-bearing carbon at δ_C 70.5 (C-9)], four methyl groups, and one non-protonated carbon at δ_C 40.3 (C-19). Detailed analysis of COSY and HSQC spectra allowed for the identification of connectivity fragments spanning positions C-4/C-5 and from C-7 to C-10. Based on these analyses, combined with HMBC correlations observed from H-18 to C-7, C-8, and C-9, the methyl group was localized at C-8. An HMBC correlation from the methyl group at δ_H 1.89 (s) to the carbonyl at δ_C 172.0 indicated the presence of an acetyl group. This acetyl group was assigned to position C-9, supported by the significant downfield shift observed at C-9 (δ_C 70.5 / δH 5.77). Furthermore, HMBC correlations from H-23 to C-19 and C-22, from H-22 to C-19 and C-2, and from H-20 to C-2, C-19, and C-21 indicate the presence of a 2-methylbut-3-en-2-yl substituent attached at position C-2 (Figure 4.3). Based on these spectral analysis results, a comparison of the NMR data for compound **XT37.1** with the known ergot alkaloid fumigaclavine D revealed a high degree of similarity, with the exception of significant differences in the chemical shifts of the 3-methyldecahydroquinolin-4-yl acetate moiety; this suggests that **XT37.1** is a diastereoisomer of fumigaclavine D [15]. This finding is consistent with the NOESY spectral analysis (Figure 4.3). Specifically, H-9 exhibits an NOE correlation with H-18 but not with H-5, indicating that H-18 and H-9 are oriented towards the α-face, whereas H-5 and H-8 are oriented

towards the β-face. This analysis indicates that XT37.1 and fumigaclavine D share similar relative configurations at positions C-5, C-8, and C-9 [15]. However, H-8 shows an NOE correlation with H-10, indicating that these two protons share the same spatial orientation. Based on the NOESY spectral analysis results, combined with differences in chemical shifts for the methyldecahydroquinolin-4-yl acetate moiety, compound **XT37.1** was identified as the 10-epimer of fumigaclavine D. Using this relative configuration, a theoretical ECD spectrum was calculated via the TD-DFT method using Gaussian 16 W software. The results showed that the calculated ECD spectrum for the 5R8R9S10S configuration matched the experimental ECD spectrum perfectly, thereby establishing the absolute configuration of compound **XT37.1** (Figure 4.4). Based on these spectral analysis results, the chemical structure of compound **XT37.1** was determined and named fumigaclavine K (Figure 4.1).

Table 0.1. ^{1}H, ^{13}C NMR data of **XT37.1**

Position	δ_C[a,b]	δ_H[a,c] (***J*** **= Hz)**
2	139.5	-
3	103.6	-
4	29.1	3.51 (m) 3.03 (m)
5	55.1	3.67 (m)
7	46.4	3.52 (m) 3.27 (br d, 7.2)
8	32.8	2.41 (m)
9	70.5	5.77 (br s)
10	38.8	3.52 (m)
11	126.6	-
12	113.3	6.71 (d, 7.2)
13	122.8	7.03 (t, 7.8)
14	110.1	7.18 (d, 7.8)

15	134.8	-
16	128.7	-
18	15.3	1.39 (d, 7.8)
19	40.3	-
20	28.1	1.56 (s)
21	28.0	1.55 (s)
22	147.2	6.18 (dd, 10.8, 17.4)
23	112.0	5.12 (dd, 2.4, 17.4) 5.10 (dd, 1.8, 10.8)
9-OAc	172.0	
	20.7	1.89 (s)

[a] *Recorded in CD_3OD;* [b] *150 MHz,* [c] *600 MHz*

3.1.2. Compounds isolated from Aspergillus sp. DVXT-221 (XT37)

Based on a detailed analysis of NMR spectroscopic data (^{1}H, ^{13}C, HSQC, HMBC, COSY, NOESY), HRESIMS, ECD spectra, and optical rotation, combined with comparisons to published data, the chemical structures of the compounds isolated from the *Aspergillus* sp. strain DVXT-221 (**XT37**) were elucidated, including three new compounds. fumigaclavine K (**XT37.1**), fumigaclavine L (**XT37.2**), and fumigaclavine M (**XT37.3**) and the 7 known compounds are: fumigaclavine I (**XT37.4**) [14], fumigaclavine C (**XT37.5**) [15], và fumigaclavine E (**XT37.6**) [15], monomethylsulochrin (**XT37.7**) [16], 8′-*O*-methylasterric acid (**XT37.8**) [17], 2,3′-dimethylosoate (**XT37.9**) [18] and chaetominine (**XT37.10**) [17] (Fig. 4.2).

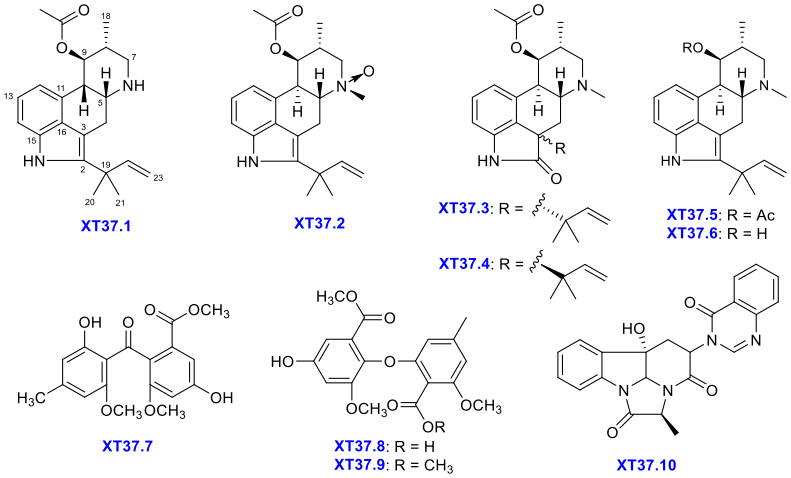

Fig. 0.2. Structure compounds from *Aspergillus* sp. DVXT-221 (**XT37**)

4.1.2. Determination of the structures of compounds isolated from fungal strain Trichoderma sp. GXT-22.1

The chemical structures of compounds **XT47.1**–**XT47.10** were determined using combined spectroscopic methods including NMR and HRESIMS—and by comparison with literature data. Two new compounds were identified: 5′-(4-methoxyphenyl)-1′,3′-oxazole (**XT47.1**) and (*E*)-6,10-dimethyl-5-undecene-2,9,10-triol (**XT47.2**) and eight known compounds: tricholumin A (**XT47.3**) [19], harzianol J (**XT47.4**) [20], cyclonerodiol (**XT47.5**) [21], 10,11-dihydro-11-hydroxycyclonerodiol (**XT47.6**) [22], cyclonerodiol B (**XT47.7**) [23], epicyclonerodiol oxide (**XT47.8**) [24], cyclo(Val-Pro) (**XT47.9**) [25] and cyclo-(4-hydroxyprolinyl-leucine) (**XT47.10**) [26] (Fig. 4.53)

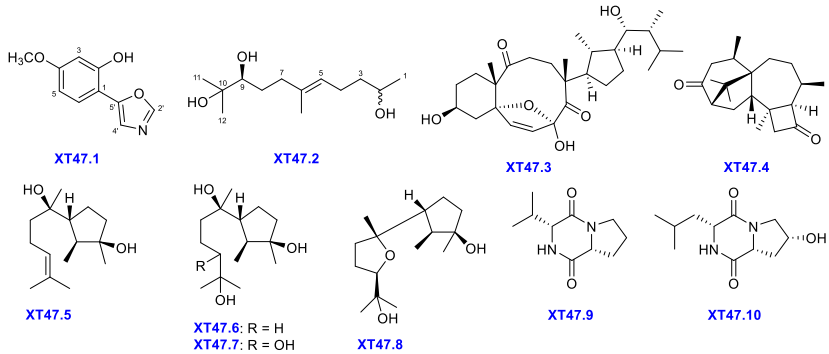

Fig. 0.2. Structure compounds *Trichoderma* sp. GXT-22.1 (XT47)

4.1.3. Determination of the structures of compounds isolated from fungal strain Aspergillus sp. BCXT-22.1 (XT49.1)

Based on NMR and HRESIMS spectroscopic analysis, combined with comparisons to known compounds, the chemical structures of 10 pure compounds were determined, including two new compounds: polyporusterone H (**XT49.1**) và stellatinol A (**XT49.4**) và 8 hợp chất đã biết: panuosterone (**XT49.2**) [27], makisterone A (**XT49.3**) [28], stellatin (**XT49.5**) [29], embeurekol C (**XT49.6**) [30], oxepinamide D (**XT49.7**) [31], oxepinamide F (**XT49.8**) [31], oxepinamide J (**XT49.9**) [32] and protubonine B (**XT49.10**) [33] (Hình 4.79)

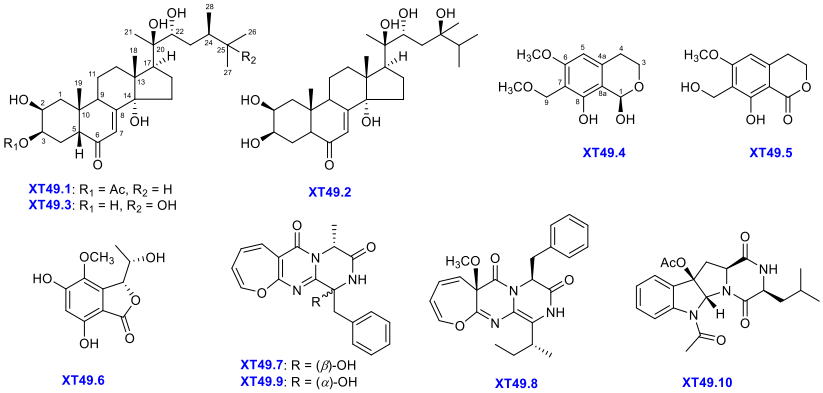

Fig. 0.3. Structure compounds from *Aspergillus* sp. BCXT-22.1 (**XT49.1**)

4.2. Biological activity of the compounds

4.2.1. Inhibition of NO production and cytotoxicity against cancer cells of compounds from Aspergillus sp. DVXT-221 (XT37)

Evaluation of the NO production inhibitory and cancer cell cytotoxic activities of ten pure compounds isolated from the *Aspergillus* sp. strain DVXT-221 revealed that two compounds, **XT37.1** and **XT37.9**, strongly inhibited NO production in LPS-stimulated RAW264.7 macrophages (Table 3.1). A comparison of the structures and biological activities of several ergot alkaloid isomers (**XT37.1**-**XT37.5**) indicated that factors such as the H-10(β) configuration and the α-orientation of the prenyl group at the C-3 position positively influenced NO inhibitory capacity. Additionally, the presence of a methyl ester group contributed to superior NO inhibitory activity compared to a carboxyl group in compounds **XT37.8** and **XT37.9**.

Based on the results regarding their ability to inhibit NO production, compounds **XT37.1** and **XT37.9** were investigated for their binding affinity to the active site of the iNOS enzyme the enzyme that catalyzes NO synthesis in macrophages. Specifically, molecular docking simulations of compounds **XT37.1** and **XT37.9** with the crystal structure of iNOS (PDB ID: 3E6T) were performed using AutoDock 4.2 (Figure 4.112). The results indicate that **XT37.1** binds effectively to the enzyme's active site (binding energy of -12.71 kcal/mol), evidenced by the formation of a hydrogen bond with the Val346 residue, alongside multiple hydrophobic interactions with key amino acid residues such as Ala345, Phe363, Gly365, Trp366, Glu371, Pro344, Tyr367, Asp376, Tyr341, Gln257, and Asn364. The simultaneous presence of these hydrogen bonds and hydrophobic interactions suggests that **XT37.1** can effectively occupy the active site, thereby inhibiting the catalytic activity of iNOS. Additionally, compound **XT37.9** demonstrated interation with the iNOS active site with a binding energy of -6.62 kcal/mol involving hydrogen bonds with Phe363, Pro344, and Gln257, as well as hydrophobic interactions with residues such as Tyr341, Tyr367, Val346, Ala345, and Asn364. Molecular docking results revealed the simultaneous presence of hydrogen bonding and hydrophobic interactions, indicating that compounds **XT37.1** and **XT37.9** can effectively occupy the active site, thereby potentially inhibiting the catalytic activity of the iNOS enzyme in NO production. This supports the hypothesis that both compounds inhibit excessive NO production by modulating iNOS activity in LPS-stimulated RAW264.7 cells. These findings highlight the potential for further research into the anti-inflammatory activity of compounds **XT37.1** and **XT37.9**.

Based on previously published findings regarding the cytotoxic activity of compounds **XT37.5**, **XT37.7**, **XT37.8**, and **XT37.10** [17, 34, 35], the cytotoxicity of compounds **XT37.1**–**XT37.4**, **XT37.6**, and **XT37.9** against the HepG2 and MCF7 carcinoma cell lines was evaluated using the SRB assay [36, 37]. The results indicated that compounds **XT37.1** and **XT37.3** exhibited weak cytotoxicity against both HepG2 and MCF7 cell lines, with IC_{50} values ranging from 61.1 ± 3.1 to 77.2 ± 1.5 µM (Table 3.1), whereas compounds **XT37.2**, **XT37.4**, **XT37.6**, and **XT37.9** showed no cytotoxic activity against either cell line (IC50 > 100 µM).

4.2.2. Inhibition of NO production and cytotoxicity against cancer cells of compounds from Trichoderma sp. BCXT-22.1 (XT47)

Compounds XT47.1–XT47.8 and XT47.10, isolated from the *Trichoderma* sp. strain BCXT-22.1 (XT47), were evaluated for their inhibitory activity against NO production in LPS-stimulated RAW264.7 cells using the Griess assay. The results demonstrated that compound **XT47.1** exhibited significant biological activity, inhibiting NO production with an IC_{50} value of 37.5 ± 2.6 µM; in contrast, the remaining compounds (excluding **XT47.6** and **XT47.7**, which showed no activity) displayed relatively low inhibitory effects, with IC_{50} values ranging from 60.2 to 86.5 µM (Table 3.2). Molecular docking simulations revealed that compound **XT47.1** effectively interacts with the active site of the iNOS enzyme, exhibiting a binding energy of -6.34 kcal/mol (Figure 4.113). Specifically, compound **XT47.1** forms a hydrogen bond with the Glu371 residue and engages in hydrophobic interactions with several other key amino acid residues, such as Tyr341, Gln257, Tyr367, Ala345, Pro344, Val346, Gly365, Phe363, and Asn364. These interactions suggest that the NO-inhibitory activity

of compound **XT47.1** may be closely linked to its ability to bind to iNOS and modulate the enzyme's activity.

Compounds **XT47.1**-**XT47.8** were evaluated for cytotoxic activity against two cancer cell lines: MCF-7 and HepG2. Compared to the positive control ellipticine (which achieved cell death rates of 80.7% for MCF-7 and 84.6% for HepG2 at a concentration of 2 µg/mL), all tested compounds exhibited lower cell death rates at a concentration of 100 µM (Table 3.2). For the MCF-7 cell line, compound **XT47.4** demonstrated the highest activity, with a cell death rate of 39.3 ± 2.3%, followed by XT47.1 (32.9 ± 1.9%) and **XT47.3** (25.4 ± 1.9%), while compounds **XT47.7**, **XT47.5**, **XT47.6**, and **XT47.8** induced cell death rates ranging from 15% to 22%. In the HepG2 cell line, compound **XT47.1** showed the highest activity, with a cell death rate of 41.5 ± 3.0%, whereas compounds **XT47.3**, **XT47.4**, and **XT47.7** induced lower levels of cell death, ranging from 21% to 23%. The results indicate that only compounds **XT47.1** and **XT47.4** exhibited weak cytotoxic activity; the remaining compounds did not significantly affect cell viability and were considered non-toxic at the tested concentration.

4.2.3. Inhibition of NO production and cytotoxicity against cancer cells of compounds from Aspergillus sp. GXT-22.1 (XT49)

Based on screening results indicating that the *Aspergillus* sp. strain GXT-22.1 (**XT49**) exhibited potent cytotoxic activity against tested cancer cell lines, compounds **XT49.1**-**XT49.10** isolated from this strain were evaluated for cytotoxicity against two human cancer cell lines MCF-7 and HepG2 using the SRB assay. The results showed that among the tested compounds, **XT49.1** demonstrated cytotoxic activity against both MCF-7 and HepG2 cell lines, with IC_{50} values of

75.41 ± 5.76 μM and 87.65 ± 3.49 μM, respectively (Table 3.3). Compounds **XT49.2**-**XT49.7**, **XT49.9**, and **XT49.10** exhibited only weak cytotoxic activity against the MCF-7 cell line, with IC_{50} values ranging from 78.05 ± 2.24 to 94.20 ± 3.41 μM. However, with the exception of **XT49.1**, none of the compounds showed activity against the HepG2 cell line (IC_{50} > 100 μM).

Additionally, all the investigated compounds were evaluated for anti-inflammatory activity based on their ability to inhibit NO production in LPS-stimulated BV2 microglial cells. At a non-cytotoxic concentration (40 μM), most compounds displayed only weak inhibitory effects, with NO inhibition rates ranging from 10.4 ± 0.9% to 36.6 ± 1.3% (Table 3.3). Among them, compounds **XT49.4** and **XT49.8** exhibited markedly higher inhibition levels than the other compounds, reaching 36.6 ± 1.3% and 31.7 ± 2.9%, respectively. Based on these screening results, **XT49.4** and **XT49.8** were selected for molecular docking simulations to investigate their binding affinity to the active site of the iNOS enzyme (PDB ID: 3E6T). Docking results revealed that both compounds could interact with the iNOS active site via hydrogen bonding and hydrophobic interactions with specific amino acid residues; their binding energies were -7.02 and -10.31 kcal/mol, respectively, indicating affinity for the enzyme's active site (Figure 4.114). Consistent with international studies, compound **XT49.4** formed a hydrogen bond with Glu371, an amino acid considered crucial to the iNOS catalytic mechanism, and engaged in hydrophobic interactions with Gln257, Tyr367, Pro344, and Val346. Meanwhile, compound **XT49.8** formed hydrogen bonds with Tyr367 and the Hem901 group, while also exhibiting hydrophobic interactions with key amino acids such as Gln257, Arg260, Glu371,

Tyr341, and Pro344. These findings suggest that the NO-inhibitory activity of compounds **XT49.4** and **XT49.8** may be linked to their ability to modulate iNOS activity through interactions with the enzyme's active site.

CONCLUSIONS

- Thirty pure compounds were isolated and their structures elucidated from three fungal strains *Aspergillus* sp. DVXT-221, *Trichoderma* sp. BCXT-22.1, and *Aspergillus* sp. GXT-22.1. These included six new compounds: fumigaclavine K–M (**XT37.1**–**XT37.3**), 5'-(4-methoxyphenyl)-1',3'-oxazole (**XT47.1**), polyporusterone H (XT49.1), and stellatinol A (**XT49.4**); as well as one compound isolated from a natural source for the first time: (*E*)-6,10-dimethyl-5-undecene-2,9,10-triol (**XT47.2**).

- The inhibitory activity of the pure compounds against excessive NO production was evaluated. Compounds **XT37.1** and **XT37.9** exhibited the strongest activity (IC_{50} = 5.3 ± 0.2 and 8.7 ± 0.4 µM), while 16 other compounds (**XT37.1**–**XT37.4**, **XT37.8**–**XT37.10**, **XT47.1**–**XT47.5**, **XT47.8**, **XT47.10**, **XT49.4**, and **XT49.8**) showed weaker activity. Molecular docking studies revealed that compounds **XT37.1**, **XT37.9**, **XT47.1**, **XT49.4**, and **XT49.8** all demonstrated binding affinity for the active site of the iNOS enzyme, with binding energies ranging from -12.71 to -6.34 kcal/mol.

- The cytotoxic activity of the pure compounds was evaluated against two cancer cell lines: HepG2 and MCF7. Among them, compounds **XT37.1**, **XT37.3**, and **XT49.1** exhibited activity against both HepG2 and MCF7 cell lines (IC_{50} = 61.1 ± 3.1 – 87.65 ± 3.49 µM). Compounds **XT49.2**–**XT49.7**, **XT49.9**, and **XT49.10** showed

cytotoxicity against the MCF7 cell line (IC_{50} = 76.56 ± 4.70 – 94.20 ± 3.41 µM).

NEW CONTRIBUTIONS OF THE THESIS

1. Six new compounds were isolated and structurally elucidated—namely fumigaclavine K (**XT37.1**), fumigaclavine L (**XT37.2**), fumigaclavine M (XT37.4), 5'-(4-methoxyphenyl)-1',3'-oxazole (**XT47.1**), polyporusterone H (**XT49.1**), and stellatinol A (**XT49.4**) along with one compound isolated from a natural source for the first time: (*E*)-6,10-dimethyl-5-undecene-2,9,10-triol (**XT47.2**). These were obtained from large-scale fermentation extracts of three mangrove-associated fungal strains: *Aspergillus* sp. DVXT-221, *Trichoderma* sp. BCXT-22.1, and *Aspergillus* sp. GXT-22.1.

2. The cytotoxic activity of 19 compounds—including **XT37.1**, **XT37.3**, **XT47.1**–**XT47.8**, **XT49.1**–**XT49.7**, **XT49.9**, and **XT49.10** was evaluated. Among them, two ergot alkaloids (**XT37.1** and **XT37.3**) exhibited activity against both HepG2 and MCF7 cell lines (IC_{50} = 61.1 ± 3.1 – 87.65 ± 3.49 µM), while the new ecdysteroid compound **XT49.1** showed activity against all three cell lines: HepG2, MCF7, and SK-Mel-2 (IC_{50} = 75.41 ± 5.76 – 87.65 ± 3.49 µM). Compounds **XT49.2**–**XT49.7**, **XT49.9**, and **XT49.10** exhibited cytotoxic activity against both MCF7 and SK-Mel-2 cell lines (IC_{50} = 76.30 ± 1.96 – 94.20 ± 3.41 µM).

3. The inhibitory activity against excessive NO production was evaluated for 24 compounds, including **XT37.1**–**XT37.4**, **XT37.8**–**XT37.10**, **XT47.1**–**XT47.5**, **XT47.8**, **XT47.10**, and **XT49.1**–**XT49.10**. Among them, compounds **XT37.1** and **XT37.9** displayed the strongest activity (IC_{50} = 5.3 ± 0.2 and 8.7 ± 0.4 µM),

while 16 compounds **XT37.1**–**XT37.4**, **XT37.8**–**XT37.10**, **XT47.1**–**XT47.5**, **XT47.8**, **XT47.10**, **XT49.4**, and **XT49.8** exhibited weaker activity. Molecular docking modeling revealed that compounds **XT37.1**, **XT37.9**, **XT47.1**, **XT49.4**, and **XT49.8** all demonstrated binding affinity for the active site of the iNOS enzyme, with binding energies ranging from −12.71 to −6.34 kcal/mol.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Dang Viet Anh**, Tran Hong Quang, Ninh Thi Ngoc, Tran Thi Hong Hanh, Nguyen Xuan Cuong, Nguyen Thi Thanh Ngan, Nguyen Ngoc Tung, Nguyen Hoai Nam, Chau Van Minh. Fumigaclavines K-M, undescribed ergot alkaloids from the mangrove-derived fungus *Aspergillus* sp. DVXT-221 with cytotoxic and NO inhibitory activities. ***Tetrahedron***, 2025; 171: 134414. DOI: 10.1016/j.tet.2024.134414.

2. **Dang Viet Anh**, Do Hoang Anh, Le Thi Vien, Pham Thi Mai Huong, Nguyen Xuan Cuong, Nguyen Thi Thanh Ngan, Nguyen Ngoc Tung, Tran Hong Quang. An Oxazole Alkaloid, Terpenoids, and Cyclodipeptides with Cytotoxic and NO Inhibitory Effects from a Mangrove-Derived Fungus *Trichoderma* sp. GXT-22.1. ***Chemistry & Biodiversity***, 2025; 22(5): e202402986. DOI: 10.1002/cbdv.202402986.

3. **Dang Viet Anh**, Le Thi Vien, Pham Thi Cham, Nguyen Hoang Nam, Nguyen Quoc Vuong, Nguyen Thi Thanh Ngan, Do Thi Thao, Dong-Sung Lee, Tran Hong Quang. Ecdysteroids, Phenolics, and Alkaloids from a Mangrove-Derived Fungus *Aspergillus* sp. BCXT-22.1 with Cytotoxic and NO Inhibitory Activities. ***Chemistry & Biodiversity***, 2025; 22(11): e01213. DOI: 10.1002/cbdv.202501213.