Original Article

Antimicrobial, Antioxidant and Cytotoxic Activities of Different Fractions Obtained from Endophytic *Fusarium solani* F01 Isolated in *Catharanthus roseus* Collected in Vietnam

Tu Nguyen[1,2,*], Vinh Doan[1,2], Anh Nguyen[1,2]

[1]School of Biotechnology, International University, Ho Chi Minh, VIETNAM
[2]Vietnam National University - Ho Chi Minh, VIETNAM.

ABSTRACT

Aim: Finding out the biological sources is essential for pharmaceutical field. **Materials and Methods:** Endophytic fungus was isolated from *Catharanthus roseus* originated in Vietnam and then identified by comparing, phylogeny analyzing its 18S sRNA sequence using Blast search and GenomeNet. The supernatant and sonicated mycelia were fractionated with methanol, ethyl acetate, hexane. All the fractions were used to test antimicrobial, antioxidant, cytotoxicity activities. Antimicrobial activities on *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Serratia marcescens* ATCC 14756, *Vibrio parahaemolyticus* ATCC 17802, and *Escherichia coli* ATCC 25922 were performed by well diffusion method. In terms of antioxidant activities, reducing power and DPPH radical scavenging assay were done. The purification from methanol extract was performed. The purified compounds were used to test antioxidant activity. For cytotoxicity activity, sulforhodamine B assay was used to test the extracts and purified compounds on the breast cancer cell line (MCF-7). **Results:** *Fusarium solani* F01 was identified. The treated methanol extract from cell-free supernatant showed the highest activities and wider spectrum than ethyl acetate and hexane extracts. Mycelial extracts in these solvents did not show activities except ethyl acetate extract. The methanol crude extract from cell-free supernatant gave the higher reducing power (1.416 ± 0.067) than ascorbic acid (0.577 ± 0.01) while others were very low at the concentration (100 μg/ml). Only methanol extract obtained from supernatant showed moderate scavenging activity when comparison of their IC_{50} (9.17 g/ml) with IC50 of ascorbic acid (1.28 g/ml). Four compounds were obtained that showed antioxidant activities, but there was one purified compound showed low effects on MCF-7 while methanol extract gave the highest inhibition (80.34 ± 1.28). **Conclusion:** The study suggested fungus isolated from *Catharanthus roseus* could show and give many biological compounds that will be a potential source for pharmaceutical field.

Key words: *Catharanthus roseus*, Identification, Molecular analysis, Biological activities, Extraction, Purification.

INTRODUCTION

Fusarium is one of the filamentous fungi which are often found in soil and plant tissues.[1] Some of *Fusarium* spp. are endophytic fungi,[2,3] a type of fungi that reside in plant tissues that have healthy relationship with the host plants rather than causing diseases.[4] The bioactive compounds produced by endophytic fungi can increase the adaptability of both themselves and their host plants to the environment stresses.[4] They encourage the plant growth, protect them from microbial attack, increase the accumulation of secondary metabolites in host plants and boost the plant defense against herbivores.[4,5] Endophytic *Fusarium* spp. are rich sources of secondary metabolites that have important biological activities. For example, crude

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Correspondence:
Ass. Prof. Tu Nguyen
School of Biotechnology,
International University,
Ho Chi Minh, VIETNAM.
E-mail: nhktu@hcmiu.edu.vn

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extracts of *Fusarium solani* isolated from *Taxus baccata*,[6] *Fusarium oxysporum* isolated from *Cananga odorata*,[7] *Fusarium oxysporum* isolated from *Rhizophora apiculata*,[8] have antimicrobial activitities. Antioxidant activities have been reported for methanol extract of *Fusarium lateritium* endophyte on *Rhizophora mucronata*,[9] ethyl acetate extract of *Fusarium oxysporum* isolated from the *Dendrobium lindleyi* flower,[10] and crude metabolites from endophytic *Fusarium tricinctum*.[11] Endophytic *Fusarium oxysporum* and *Fusarium solani* isolated from *Catharanthus roseus* have been reported to vincristine and vinblastine which have anticancer effects.[12,13]

Catharanthus roseus, an important medicinal plant belonged to *Apocynaceae* family, is a popular source of indole alkaloids.[14] At least 130 terpenoids indole alkaloids (TIA) are found in different organs of *Catharanthus roseus,* two of which are vinblastine and vincristine that have been used clinically in cancer treatment. Indole alkaloids also exhibit important pharmaceutical activities such as antioxidant, antimicrobial, anticancer and antidiabetic effects.[14] Endophytic fungi can also produce similar bioactive compounds to their host,[15] suggesting that endophytic fungi isolate from the medicinal plants can secret valuable medicinal compounds. Endophytic fungi isolated from *Catharanthus roseus* have been reported to produce bioactive compounds similar to the compounds in plant hosts, such as vincristine and vinblastine produced by *Fusarium oxysporum*,[12] *Fusarium solani*,[13] and *Talaromyces radicus*.[16] Furthermore, according to Radu (2002) and Wang (2007), the endophytic fungi isolated from medicinal plants including *Catharanthus roseus* could produce antifungal, antimicrobial and cytotoxic metabolites.[17,18]

In recent years, people are interested in the use of fungi or plant that can extract new and efficient components which may exhibit biological activities such as antioxidant and antimicrobial and cytotoxicity activities. Nowadays, antibiotic resistance in both pathogenic and non-pathogenic bacteria is increasing, which become one of the most difficult global problems to be solved.[19] Increasing level of antibiotic resistance has encouraged the search for new natural antibiotic compounds to control diseases. Along with antibiotic resistance, oxidation stress is a problem to concern. Oxidative stress is caused by the loss in balance between the accumulation and production of oxygen reactive species (ROS) and the ability of neutralizing ROS of a biological system.[20] Oxidative stress can result in severe damages in the body, causing health problems such as cancer, cardiovascular disease, neurological disease, respiratory disease and others.[20] Every biological system must evolve defense mechanisms to prevent and repair the damage caused by oxidative stress and antioxidants are the first line defense.[21] Antioxidants are compounds that can neutralize free radicals at appropriate concentration and prevent the oxidation. The antioxidants can be endogenous or exogenous (obtained from diets or dietary supplements). Although not all dietary compounds neutralize free radicals, they are also called antioxidants as they enhance endogenous antioxidant activity.[21] Plants are primary source for those antimicrobial and antioxidant compounds, but their growth is much slower compared to fungi. On the other hands, in ideal set up, fungi grow at a higher rate, and they can produce the biological compounds with higher yield and consistency than the original plant sources.[22] Combined with the ability to produce similar biological active compounds to host plants, endophytic fungi are a promising source for natural antimicrobial and antioxidant compounds.

As the above state, *Fusarium solani* F01 isolated from *Catharanthus roseus* were carried out. The supernatant and sonicated mycelia of fungus were fractionated with methanol, ethyl acetate, hexane. All the fractions were used to test for antimicrobial, antioxidant, cytotoxicity activities. Antimicrobial activity test was performed on the tested pathogens (*Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Serratia marcescens* ATCC 14756, *Escherichia coli* ATCC 25922, *Vibrio parahaemolyticus* ATCC 17802). For cytotoxicity activity, all the extracts were tested on the proliferation of MCF-7. For antioxidant activities, reducing power and DPPH radical scavenging assay were done. In term of antioxidant activities and cytotoxicity, purified crystals were obtained from fractionating the extract which had the highest activities through column chromatography, and they were used to screen for antioxidant activities. The study contributed fungus isolated from *Catharanthus roseus* could show and give many biological compounds that will be a potential source for pharmaceutical field.

MATERIALS AND METHODS

Cultivation of *Fusarium solani* isolated from *Catharanthus roseus*

Catharanthus roseus collected in Binh Thuan province (Vietnam). The outside of the trunk was cleaned. The inside of the trunk was taken out and cultured in potato dextrose broth (PDB) in 3 - 5 days and then spread on PDA. The single colony was picked for DNA extraction using Qiamp DNA minikit (Quigen). The extracted DNA was checked the purity and determined concentration, and then used for sequencing 18S rRNA of the extracted DNA. The 18S rRNA sequence was

aligned and compared to the sequences of well-known strains published in gene bank using BLAST tool in the web together with application of Environment for Tree Exploration (ETE3) v3.1.1,[23] and PhyML v20160115,[24] fungus was identified. The 18S rRNA sequence was registered in DDBJ (DNA data bank of Japan).

Crude extract preparation from cell-free supernatant

The 14-day-old culture of *Fusarium solani* was centrifuged to separate mycelia and cell-free supernatant in the suitable condition with the speed of 10,000 rpm within 10 min at room temperature. Mycelia and cell-free supernatant were used for further tests.

All collected samples were evaporated at 50°C to obtain condensed sample (CS) before extraction with methanol, ethyl acetate, n-hexane.

CS was extracted with methanol, ethyl acetate and n-hexane with the ratio (1:1) repeated three times to get extracts labeled as (M) for methanol extract, (E) for ethyl acetate extract and (H) for n-hexane extract. All solvents in M, E, H samples were removed by evaporator, weighed and mixed with sterile distilled water before storing at -20°C for further use.

Crude extract preparation from mycelia

Fungal mycelia were divided into 3 separated parts in order to be sonicated with methanol, ethyl acetate and n-hexane with the ratio (1:1). All sonicated samples were centrifuged at 10,000 rpm, 15 min to collect mycelial-free supernatant. All obtained supernatants were finally evaporated, weighed and mixed with sterile distilled water before storing at -20°C for further tests. All samples were labeled as Mp, Ep, Hp.

Antimicrobial activity

The strains including *Pseudomonas aeruginosa* ATCC 27853, *Vibrio parahaemolyticus* ATCC 17802, *Staphylococcus aureus* ATCC 25923, and *Escherichia coli* ATCC 25922, *Serratia marcescens* ATCC 14756 were used in the study. Each pathogen was grown in Luria broth (LB) until OD_{600} of 0.5. The antimicrobial testing was done by well diffusion method.[25] Sterile distilled water was used as the negative control and specific antibiotics were used for specific testing pathogens as the positive control for the test. The LB nutrient agar was prepared, and the wells (6 mm in diameter) were made on these LB agar dishes. Then, 100 µl of pathogen culture was added onto LB agar petri dishes and then spread by using sterilized cotton swab. The samples, positive and negative controls were loaded with approximately 45 µl into each well and then incubated at room temperatures. The aseptic technique was applied in whole process to avoid contaminating. The data recorded by measuring the inhibition zones around the wells in millimeters (including the diameter of wells) which appeared after ten to twelve hours of incubation. Each experiment was triplicated.

Reducing power assay

Antioxidant activity was quantified by reducing power assay. This assay was used to measure the reductive ability by transformation of Fe^{3+} to Fe^{2+} in the presence our extract and fractioned samples by according to the previous method with slight modification.[26] First of all, the standard solution was prepared by dissolving 100 mg of ascorbic acid in 100 ml of distilled water then diluted this solution with distilled water to get the concentration from 10 to 100 g/ml. In the next steps, the phosphate buffer (pH 6.6), potassium ferricyanide (1%), ferric chloride (0.1%) and trichloroacetic acid (10%) were prepared. Moving onto the preparation of test samples, the samples were diluted to the concentration of 10, 25, 50, 75 and 100 g/ml. According to this method, 150 l of various concentration of the standard solution and sample extracts and fractions were mixed with 100 l of prepared phosphate buffer (pH 6.6) and 100 l of potassium ferricyanide (1%) before incubating at 50°C in water bath for 20 min. After that, the mixture was supplied with 100 l of trichloroacetic acid (10 %) and then centrifuged at 3000 rpm for 10 min. Nextly, 80 l of the upper layer of this mixture was collected then mixed with 104 l of distilled water and 16 l of ferric chloride solution (0.1 %). The antioxidant activities were calculated with the absorbance at 700 nm wavelength. This method was done by on 96 well plates. All the replicated experiments were carried out to ensure the results.

DPPH radical scavenging activity

The samples' antioxidant potency could be determined based on its scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical molecules with radical progresses which is the results of hydrogen donation, the absorbance of DPPH radical decreased showing the antioxidant activity.[27] The DPPH solution was prepared in dark container containing 4.3 mg of DPPH (1,1-diphenyl-2-picrylhydrazyl) dissolved in 3.3 ml of methanol. Then 9l of this DPPH solution was added to 180 l methanol then measured at 517 nm by using ultraviolet spectrophotometer. Methanol was used as blank. Ascorbic acid was used as the standard solution for making the standard curve. The ascorbic acid solutions of different concentrations were prepared before taking 180 l to mix with 9l of this DPPH solution. Besides that, 180 l of each sample was mixed with 9 l then incubated 30

min at 37°C incubator before measuring the absorbance at 700 nm wavelength. This method was done by using 96 well plates. All the experiments were performed in triplicate. The determination of scavenging percentage was obtained based on the absorbance of the control (A_c) and the absorbance of the extracts or crystals (A_s) via the formular $(A_c - A_s)/A_c \times 100$.

Purification

In the study, the extract showing the highest activities were used to fractionate for purification. These extracts were loaded onto silica loaded column chromatography. After elution with solvents and detection of activities, the suitable fraction was selected for further steps. The fraction was kept on fractionating with the mixture of solvents to fractionate compounds in chromatography. The eluted fractions were collected and detected on thin layer chromatography. All suspected samples were used to extract with chloroform, detected on thin layer chromatography to check the purity. The purified samples were obtained after crystallization at cold condition. The crystals were collected, washed and recrystallized. In order to determine the purity, thin layer chromatography and LC-MS were used to analyze.

Cytotoxicity test

The cytotoxicity was tested by sulforhodamine B assay,[27-29] which is a simple colorimetric method for determining sensitivity and cytotoxicity of a substance. Fibroblasts and the cancer cell line MCF-7 were used. These cells were mixed with EMEM in 10% FBS medium for cell line cultivation. Camptothecin (0.1 g/ml) was used as positive control. EMEM/10% FBS was used as negative control. The extracts were prepared at different concentration and then added in cancer cell line, fibroblasts, EMEM/10% FBS. After incubation, sulforhodamine B was added to the positive control, negative control and samples. The cell growth was detected using ultraviolet spectrophotometer at 560 nm. The ratios of cell growth were detected using the formular expressed as $(1 - A_s/A_c) \times 100\%$ whereas and A_c were the absorbance of sample and control, respectively.

FT-IR

FT-IR based on the previous method was used to detect functional group or chemical bonds presented in the purified samples.[30] FTIR spectrophotometer used in the study was named Nicolet-5700 (Thermo Electron Corporation, Madison, WI, USA). The frequency range was 400 - 4000 cm^{-1}.

Data analysis

The obtained results in this study were reported as mean standard deviation (SD). Statistical package for the social science (SPSS) software version 20.0 was applied for statistical analysis.

RESULTS AND DISCUSSION

Identification of *Fusarium solani* F01 isolated from *Catharanthus roseus*

After sequencing, the 18S rRNA sequence was analyzed using BLAST search, showing homology (98%) to 18S rRNA sequence of *Fusarium solani* AIBF 005-1 (Figure 1). By taxonomy analysis, 18S rRNA sequence of the isolated strain had the higher number of hit to *Fusarium solani* with 75 hits than other species. Phylogeny analysis based on neighbor joining with maximal sequence difference at 0.2 that showed the isolated strain to be closed *Fusarium solani* strains. Moreover, phylogeny analysis by using (ETE3) v3.1.1 and PhyML v20160115 also showed the related *Fusarium solani* strains (Figure 2). Therefore, the strain used in the study was confirmed as *Fusarium solani* F01. The 18S rRNA sequence of *Fusarium solani* F01 was deposited with accession number (LC642563) in DNA data bank of Japan (DDBJ).

Antimicrobial activity

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F01         AGGCGTCCGGACTGGCCCAGAGAGGTGGGCAACTACCACTCAGGGCCGGAAAGCTCTCCA
GQ229075.1  AGGCGTCCGGACTGGCCCAGGGAGGTGGGCAACTACCACCCAGGGCCGGAAAGCTCTCCA
            ********************* ********************* ********************

F01         AACTCGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGA
GQ229075.1  AACTCGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGA
            ************************************************************

F01         GGGATCATTACCGAGTTATACAACTCATCAACCCTGTGAACATACCTAAAACGTTGCTTC
GQ229075.1  GGGATCATTACCGAGTTATTCAACTCATCAACCCTGTGAACATACCTAAA-CGTTGCTTC
            ******************* ****************************** *********

F01         GGCGGGAACAGACGGCCCCGTAACACGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGT
GQ229075.1  GGCGGGAATAGACGGCCCCGTAAGACGGGCCGCCCCCGCCAGAGGACCCCT-TAACTCTGT
            ******** ************** ************************  *********

F01         TTCTATTATGTTTCTTCTGAGTAAAACAAGCAAATAAATTAAAACTTTCAACAACGGATC
GQ229075.1  TTCTATAATGTTTCTTCTGAGTAAAACAAGCAAATAAATTAAAACTTTCAACAACGGATC
            ****** ************************************************************

F01         TCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA
GQ229075.1  TCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGA
            ************************************************************

F01         ATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCAT
GQ229075.1  ATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCAT
            ************************************************************

F01         GCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCTGGCGTTGGGGATCGGCGAG
GQ229075.1  GCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCTGGCGTTGGGGATCGGCGGA
            ************************************************************

F01         GCGCCCCCTGCGGGCACACGCCGTCCCCCAAATACAGTGGCGGTCCCGCCGCAGCTTCCA
GQ229075.1  G--CCCCCCGTGGGCACACGCCGTCCCCCAAATACAGTGGCGGTCCCGCCGCAGCTTCCA
            *  ***** * ************************************************

F01         TTGCGTAGTAGCTAACACCTCGCAACTGGAGAGCGGCGCGGCCACGCCGTAAAACACCCA
GQ229075.1  TCGCGTAGTAGCTAACACCTCGCGACTGGAGAGCGGCGCGGCCACGCCGTAAAACACCCA
            * ********************* ************************************

F01         ACTTCTGAATGTTGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATA
GQ229075.1  ACTCTTCTGAAGTGACCTCGAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATA
            *** *      ************************************************

F01         AGCGGAGGAAAAGAAACCAACAGGGATTGCCCCAGTAACGGCGAGTGAAGCGGCAACAGC
GQ229075.1  AGCGGAGGAAAAGAAACCAACAGGGATTGCCCCAGTAACGGCGAGTGAAGCGGCAACAGC
            ************************************************************
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Figure 1: Alignment of 18S RNA sequence of isolate and *Fusarium solani* AIBF 005-1.

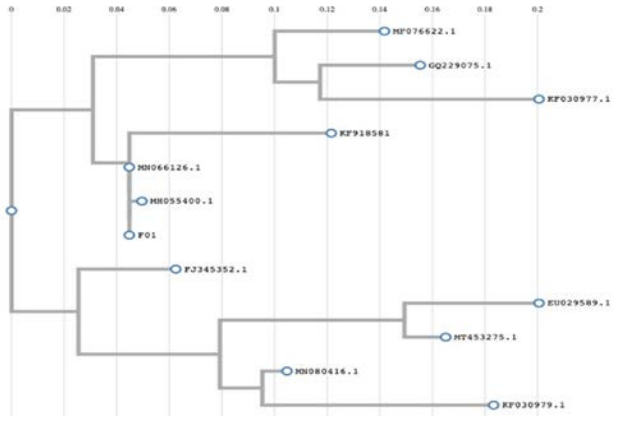

Figure 2: Phylogeny analysis. MF076622.1: *Fusarium solani* isolate S248; GQ229075.1: *Fusarium solani* strain AIBF 005-1; KF030977.1: *Fusarium solani strain* LMMM135; KF918581.1: *Fusarium solani* strain DI16; MN066126.1: *Fusarium solani* isolate NB366_07; MH055400.1: *Fusarium solani* isolate DSM 106836; FJ345352.1: *Fusarium solani* strain ATCC 56480; EU029589.1: *Fusarium solani* isolate S-0900; MT453275.1: *Fusarium solani* isolate DSM100290_DF32_RLCS13; MN080416.1: *Fusarium solani* strain CLR36; KF030979.1: *Fusarium solani* strain ATCC62877; F01: isolate.

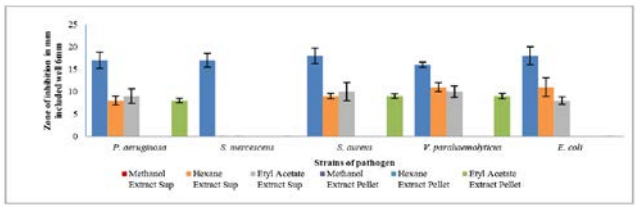

Figure 3: Diameter of inhibition zones of different extracts on pathogens.

All the samples (M, E, H) showed the effects on the most tested pathogens, except for *S. mercescens* which was only against by M extract. All samples (Mp, Hp) did not show activities, except for Ep that showed effect on *P. aeruginosa*, *S. aureus*, *V. parahemolyticus*, *E. coli*.

From the Figure 3, M extract showed the strongest antimicrobial activity against *P. aeruginosa*, which has the inhibition zone was the biggest and measured about 16.5 ± 1.80 mm. The weakest antimicrobial activity was expressed by H extract (8.00 ± 1.00 mm). For sonicated mycelia fungal, Ep showed the antimicrobial activity with the zone of inhibition measured about 7.50 ± 0.50 mm. For *S. mercesens*, M extract caused the inhibition zone measured around 16.5 ± 1.80 mm, showing the antimicrobial activity against this strain.

For *S. aureus*, M showed the strongest antimicrobial activity which had the inhibition zone was the biggest and measured about 18.00 ± 1.73 mm. The weakest antimicrobial activity expressed by H extract was 8.67 ± 0.58 mm. However, only Ep obtained from sonicated mycelia showed the antimicrobial activity with the inhibition zone of 8.50 ± 0.50 mm.

For *V. parahemolyticus*, M extract showed the strongest antimicrobial activity which had the inhibition zone was the biggest and measured about 16.33 ± 0.58 mm. H extract was higher than that of E extract. The weakest antimicrobial activity of E extract was based on the measured inhibition zone (10.17 ± 1.26 mm). In case of sonicated mycelia, only Ep showed the antimicrobial activity with the zone of inhibition measured about 8.67 ± 0.58 mm.

In *E. coli*, M extract showed the strongest antimicrobial activity which has the inhibition zone was the biggest and measured about 17.83 ± 2.02 mm. In this test pathogen, H extract was higher than that of E extract. The weakest antimicrobial activity was found in E extract based on the inhibition zone (8.00 ± 1.26 mm). For sonicated mycelia, all samples (Mp, Ep, Hp) did not show the antimicrobial activity.

Reducing power assay

Reducing power of ascorbic acid (standard), crude extracts (M, E, H) and mycelial extracts (Mp, Ep, Hp) was showed in Table 1 and 2. When the concentration of fungal samples and standard solution (ascorbic acid) increased, their reducing power increased.

From the Table 1 and 2, M extract giving the highest activities and then was used to fractionate to obtain purified compounds (C1, C2, C3, C4) after checking by TLC. These compounds were white powder. C1, C2, C3 and C4 were obtained among fractions of absolute methanol, methyl: ethyl acetate (8:2), methyl: ethyl acetate (50:50), methyl: ethyl acetate (4:2), respectively. The results were presented in Table 3. When comparing to standard and M extract, the reducing power of C1, C2, C3 and C4 was very low (Table 3), suggesting there was other compounds having high power in M extract.

Table 1: Absorbance of solvent crude extract of *Fusarium solani* at various concentrations (µg/ml) in ferric reducing power assay.

Concentration	Absorbance (ascorbic acid)	Absorbance (M extract)	Absorbance (E extract)	Absorbance (H extract)
10	0.112 ± 0.010	0.345 ± 0.051	0.047 ± 0.005	0.019 ± 0.015
25	0.239 ± 0.012	0.490 ± 0.040	0.062 ± 0.003	0.037 ± 0.013
50	0.303 ± 0.005	0.957 ± 0.043	0.134 ± 0.037	0.048 ± 0.003
75	0.459 ± 0.009	1.312 ± 0.039	0.295 ± 0.016	0.060 ± 0.001
100	0.577 ± 0.011	1.416 ± 0.067	0.361 ± 0.040	0.077 ± 0.015

Table 2: Absorbance of solvent mycelia fungal extract of *Fusarium solani* at various concentrations (µg/ml) in ferric reducing power assay.

Concentration	Absorbance (Mp extract)	Absorbance (Ep extract)	Absorbance (Ep extract)
10	0.095 ± 0.009	0.008 ± 0.002	0.023 ± 0.006
25	0.129 ± 0.013	0.014 ± 0.001	0.022 ± 0.003
50	0.162 ± 0.019	0.024 ± 0.005	0.029 ± 0.006
75	0.228 ± 0.040	0.035 ± 0.003	0.030 ± 0.004
100	0.317 ± 0.012	0.045 ± 0.005	0.035 ± 0.003

Table 3: Absorbance of four compounds at various concentrations (µg/ml) in ferric reducing power assay.

Concentration	Absorbance (CI)	Absorbance (CII)	Absorbance (CIII)	Absorbance (CIV)
10	0.021 ± 0.002	0.015 ± 0.005	0.013 ± 0.001	0.017 ± 0.008
25	0.031 ± 0.003	0.026 ± 0.004	0.013 ± 0.003	0.018 ± 0.006
50	0.038 ± 0.002	0.033 ± 0.004	0.016 ± 0.007	0.017 ± 0.017
75	0.047 ± 0.004	0.043 ± 0.004	0.020 ± 0.005	0.016 ± 0.006
100	0.064 ± 0.004	0.054 ± 0.002	0.017 ± 0.002	0.024 ± 0.010

DPPH radical scavenging assay

The free radical – scavenging activity of *Fusarium solani* along with standard ascorbic acid was determined and measured by the DPPH assay. Different kinds of samples exhibited a comparable antioxidant activity with the standard solution at varying concentration tested ranging from 1 g/ml to 20 g/ml. The samples (M, E, H, Mp, Ep, Hp, CI, CII, CIII, CIV) showed antioxidant activity depending on concentration by measuring the DPPH radical inhibition (Figure 4, 5 and 6). In order to evaluate the strongest or weakness of activities of each sample, IC_{50} should be determined.

The IC_{50} value of M extract which was 9.17 g/ml equivalent with 1.28 g/ml ascorbic acid based on the standard curve (y = 8.0795x + 39.646 with r^2 = 0.9899). Meanwhile, the IC_{50} values of E extract and H extract were 10.86 g/ml and 17.96 g/ml, respectively.

Mp extract had the highest IC_{50} value which was 10.81 g/ml, in comparison with 15.02 g/ml for Ep and 19.42 g/ml for Hp extract.

Moreover, the IC_{50} value of CI, CII, CIII, CIV ranged from 19.89 g/ml, 14.73 g/ml, 19.25 g/ml and 19.71 g/ml, respectively.

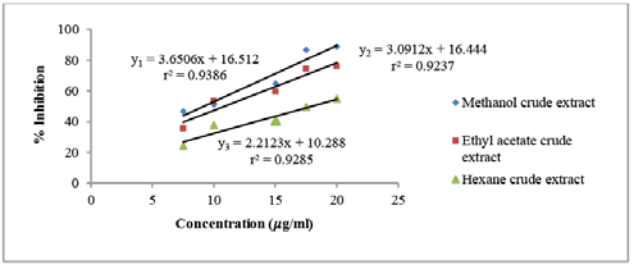

Figure 4: Percentage inhibition of crude extracts obtained from extraction of supernatant with different solvent.

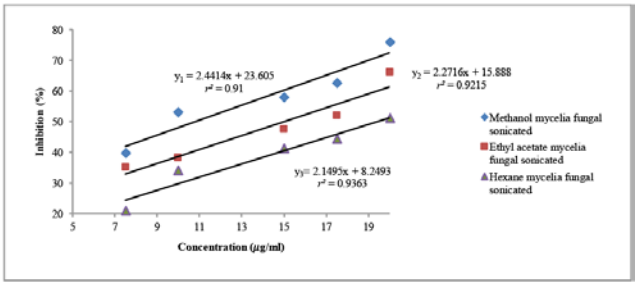

Figure 5: Percentage inhibition of solvent mycelia fungal sonicated at various concentrations.

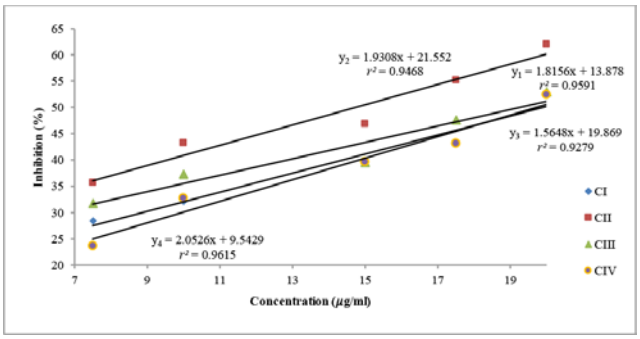

Figure 6: Percentage inhibition of purified compounds at various concentrations

Cytotoxicity

All the extracts were used to test cytotoxicity against MCF-7 cell line. From the Table 4, M extract showed the highest inhibition percentage (80.34 ± 1.28). The cytotoxicity percentage of Mp was approximately 28.07 ± 1.33 while CIII showed the lower effect determined based on the inhibition percentage (7.10 ± 1.59). Other extracts and CI, CII and CIV did not show the effects due to low concentrations.

FT-IR was performed for determination the groups presenting in CIII. By FT-IR analysis, there were OH- and COO- groups at 3739 cm^{-1} and 2349 cm^{-1}, respectively (Figure 7).

Table 4: Inhibitory effects of compound III, mycelia fungal sonicated in methanol and. methanol extract from supernatant on the growth of MCF-7 human cancer cell lines.

Cell line	CIII	Mycelia sonicated in methanol	Methanol extract
MCF – 7	7.10 ± 1.59	28.07 ± 1.33	80.34 ± 1.28

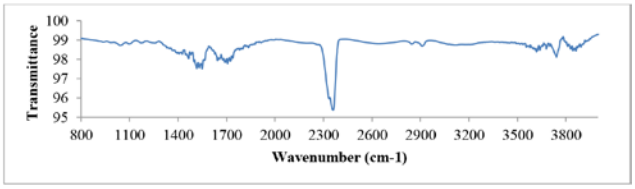

Figure 7: FT-IR spectrum of compound III.

DISCUSSION

Fusarium solani F01 was identified based on not only the alignment of the partial sequence of 18S rRNA of *Fusarium solani* F01 and others in Blast search, ETE and PhyML analysis in GenomeNet. BLAST search is used for homology and taxonomy based on DNA sequence information. In the study, *Fusarium solani* F01 had partial sequence of 18S rRNA with 98% identity to 18S rRNA *Fusarium solani* AIBF-005-1. ETE and PhyML contribute to phylogenetics by reconstructing, analyzing the phylogenetic trees. Phylogeny study clarifies the evolutionary origin of a taxonomic group of *Fusarium solani*. With the identity recognition and visualization of phylogeny tree, *Fusarium solani* AIBF-005-1 was identified.

When comparing the antimicrobial activities of extracts from supernatant and sonicated mycelia, fungus produced antimicrobial agents extracellularly. In fungus, there were the synthetic pathways for antimicrobial agents. Up to now, the antimicrobial synthesis pathway of *Fusarium solani* as well as *Fusarium* spp. was not studied well even searching data in KEGG website. The antimicrobial activity of M extract was the strongest and wide spectrum than E and H extracts. Obviously, there were the polar antimicrobial agents produced in the fungal culture. Based on the screening antimicrobial activity, M extract will be used to exploit polar antimicrobial agents. The higher polarity of antimicrobial agent will be convenient in formulation and pharmacokinetic improvement such as absorption and distribution. The results suggested that the importance of endophytic fungi with the mysterious genetic properties should be interesting not in science but in pharmaceutical industry.

All the extracts showed the reducing power. The reducing power occurred because there was the presence of reducers in the assay. When the concentration of fungal samples and standard solution (ascorbic acid) increased, their activities increased. Endophytic *Fusarium solani* F01 could produce many antioxidant agents in the extracts, especially in M extract. The activity of M extract was higher than ascorbic acid. Moreover, the sonicated mycelia activities were as low as four purified compounds. In scavenging assay, IC_{50} was used to evaluate the strength and weakness. IC_{50} values of the extracts and the purified compounds were higher than ascorbic acid. Therefore, their activities were low. However, reducing power associated to human health.

By FT-IR, C3 had OH^- and COO^- groups. Those functional groups involve the biological activities will be studied in future.

For cytotoxicity, the screening was tried different concentration (from 0.1 to 1 mg/ml) of the extracts and four compounds prepared in the study. At the concentration (1 mg/ml), the activity of M extract showed the highest. There were the polar compounds with many biological activities produced by fungus. The concentration increased, the cytotoxicity on MCF-7 increased. The study gave a promising source for pharmaceutical field. However, CIII and sonicated mycelia in methanol (Mp) was low while the others did not give cytotoxicity. To confirm CIII and Mp whether vincristine/vinblastine could show the cytotoxicity, LC-MS was used to detect these compounds. However, vincristine/vinblastine were not detected. As a result, the cytotoxicity in Mp might be due to any novel compounds that should be determined in future.

CONCLUSION

In the study, antimicrobial, antioxidant, cytotoxic activities from solvent extracts (methanol, ethyl acetate, hexane) were obtained from cell-free supernatant and mycelium of *Fusarium solani* F01 isolated from *Catharanthus roseus* originated in Vietnam. Moreover, four purified crystals obtained from methanol extract also showed DPPH antioxidant activity. One crystal showed low effect on MCF-7 due to low concentration. Mycelia extracted with methanol and partial purification showed effect on MCF-7. The study suggest fungus isolated from herbal medicine like *Catharanthus roseus* in Vietnam can show biological activities. The study also points out that endophytic *Fusarium solani* can produce many kinds of valuable activities if it is symbiotic with the valuable medicinal herbs that let us look again the importance of fungi besides, we only

conclude that fungi is harmful for human health and the growth of medicinal herbs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ATCC: American Type Culture Collection; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **ETE:** Environment for Tree Exploration; **IC_{50}:** Half-maximal inhibitory concentration; **LC-MS:** Liquid chromatography - mass spectrometry; **OD:** Optical density; **mm:** Milimeter; **ml:** Mililiter; **nm:** Nanometer; **°C:** degree Celcius; **ROS:** Oxygen reactive species; **TIA:** Terpenoids indole alkaloid; **µg:** Microgram.

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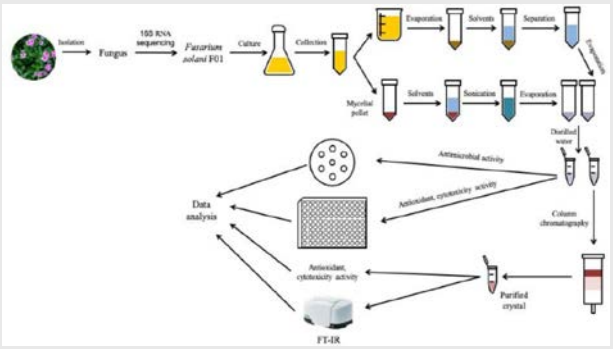
PICTORIAL ABSTRACT



SUMMARY

Endophytic *Fusarium solani* F01 was identified from *Catharanthus roseus* gave many important activities such as antimicrobial, antioxidant and cytotoxicity activities after extracting mycelia and fungal cultures with methanol, ethylacetate and hexan together with purification of methanol extract to get four compounds. The study demonstrated properties which provided valuable data on the activities found due to those enhancing its symbiotic activities with significant medicinal herbs that are not usually found in fungi. Furthermore, there were interesting and diverse results not covered completely during the present study, therefore, further research would be needed to deepen on the understanding of the potential found during our research that could be of great importance in different fields that have not studied about these properties at this moment.

About Authors

Associate Professor. Nguyen Hoang Khue Tu got PhD degree of Biomedical Science certified by Hiroshima University (Japan). She is now working at School of Biotechnology, International University, Vietnam National University, Ho Chi Minh city (Vietnam). She had many publications on drug deliverry, natural products, gene expression, microbial identification, and microbial resistance. She is intersting in pharmaceutical research and development.

Msc. Doan Thi Thanh Vinh worked at School of Biotechnology, International University, Vietnam national university, Ho Chi Minh city. At present, she is studying PhD program of Biomedical Science in Alabama University, USA.

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