Sains Malaysiana 41(3)(2012): 333–337

Chemical Components of Marine Sponge Derived Fungus *Fusarium proliferatum* Collected from Pulau Tinggi, Malaysia

(Komponen Kimia Kulat *Fusarium proliferatum* diperolehi daripada Span Marin di Perairan Pulau Tinggi, Malaysia)

Z. MOSADEGHZAD*, ZURIATI ZAKARIA, A. ASMAT, U. GIRES , R. WICKNESWARI, P. PITTAYAKHAJONWUT & G.H.N. FARAHANI

ABSTRACT

Marine fungus Fusarium proliferatum *derived from marine sponge collected along Pulau Tinggi, Malaysia was cultivated on Potato Dextrose Broth and incubated for 7 days at 30°C. The liquid cultures were then extracted using ethyl acetate. The crude extract was investigated for its anti-microbial activity and was passed through Sephadex column and the fractions were collected. Reverse phase HPLC was used to monitor the component of crude extract. HPLC guided purification of crude extract resulted in the isolation of linoleic acid, 4-hydroxy phenethyl alcohol, 2,5-furandimethanol and adenosine. Their structures were elucidated by spectroscopic methods.*

Keywords: Antimicrobial activity; Fusarium proliferatum*; marine fungi*

ABSTRAK

Kulat marin Fusarium proliferatum *yang diperolehi daripada span marin dikutip di perairan Pulau Tinggi, Malaysia telah dieramkan pada Agar Potato Dextrose (PDA) pada 30°C selama 7 hari. Kultur cecair diekstrak dengan pelarut etil asetat. Ekstrak mentah dikaji untuk aktiviti antimikrob dan dimasukkan ke dalam turus Sephadex dan fraksi dikumpulkan. HPLC fasa terbalik digunakan untuk memantau komponen ekstrak mentah. Penulenan ekstrak mentah berpandukan HPLC telah menghasilkan pemencilan asid linoleik, alcohol 4-hidroksifenetil, 2,5-furan dimetanol dan adenosine. Struktur telah ditentukan dengan kaedah spektroskopi.*

Kata kunci: Aktiviti antimikrob; Fusarium proliferatum*; kulat marin*

INTRODUCTION

The marine environment is a rich source of both biological and chemical diversity, where it has been reported that the oceans contain nearly 300,000 described species (Malakoff 1997; Pomponi 1999). The ocean comprises more than 70% of the earth surface, and each drop of water taken from the ocean will contain microbial species unknown to humans (Colwell 2002). Almost all forms of life in the marine environment e.g. algae, sponges, corals, ascidians have been investigated for their biological active content (Faulkner 2000a; Faulkner, 2000b). Most efforts have been directed towards chemical studies of marine microorganisms. Although marine microorganisms are not well defined taxonomically, preliminary studies indicated that the wealth of microbial diversity in the world oceans, make this a promising frontier for the discovery of new medicines (Rovirosa et al. 2006).

As interests have turned to marine microorganisms, the fungus is being recognized, as likely source of potentially useful natural products. Most of these microorganisms grow in a unique and extreme habitat and therefore, they have the capability to produce unique and unusual active metabolite. It is believed that these metabolites possibly act as a chemical defense as an adaption of fungi competing for substrates (Fenical & Jensen 1993; Gallo et al. 2004).

Nowadays, there is a need for the discovery and development of new classes of antimicrobial fractions, due to recent trends in antibiotic resistance among different strains of bacteria (methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*), which are causing serious problems in the contaminant of infectious diseases (Dominguez 2004; Nagaraju et al. 2004; Neu 1992).

In this study, the chemical components of Malaysian marine *Fusarium proliferatum* were investigated. The chemical structure of pure compounds was identified and then the bioactivity of isolated metabolites was evaluated against pathogenic bacteria and fungi.

MATERIALS AND METHODS

CULTURING

Sponge derived fungal isolate which was identified as *Fusarium proliferatum* were cultivated on PDA (Potato Dextrose Agar) containing 30% NaCl and was incubated

at 30°C for 3-4 days. Cultures were then scrubbed and transferred to 10L PDB (Potato Dextrose Broth) containing 30% NaCl. Liquid cultures were shaken on a rotary shaker at 220 rpm for 7 days at 30°C.

EXTRACTION AND ISOLATION OF METABOLITES

The culture broth was filtered to separate mycelium and supernatant. The mycelium was extracted with ethyl acetate (2×200 mL) and once with acetone. The supernatant was extracted with ethyl acetate (3×300 mL for every 500 mL of media). The organic fractions combined and the solvent removed at reduced pressure at 30°C in order to obtain the crude extract. Crude extracts have been partitioning between MeOH and CH_2Cl_2. The CH_2Cl_2 part was in oil form and was not biologically active. The crude extract (690 mg) having an antimicrobial activity was subjected to various separation steps using Sephadex LH20 and silica gel column chromatography in order to isolate metabolites (Figure 1).

THIN LAYER CHROMATOGRAPHY (TLC)

Twenty microlitre of the fungal extract and fractions resulted from the column were applied to a silica gel 60 F_{254} TLC plate (Merck) and developed with different solvent systems. Metabolites were detected under UV-light (254 and 366 nm) followed by treatment with anisaldehyde/ sulphuric acid and heated at 100°C to visualize most of the organic compounds.

UV-VISIBLE SPECTROPHOTOMETER

The crude extract was scanned from 200-400 nm using Varian-Cary 50 spectrophotometer to obtain a range of wavelengths on maximum absorptions.

NMR ANALYSES

The structure of chemical compounds was identified through the Nuclear Magnetic Resonance; ^{1}H NMR, ^{13}C NMR by Bruker DRX 400 and Bruker AV 500 D instrument at a probe temperature of 30°C. Chemical shifts are reported relative to internal TMS.

ESI-MS ANALYSIS

The mass spectra were determined using Bruker Micro TOF instrument through ESI-MS technique.

ANTIMICROBIAL ASSAY

Antimicrobial activity for crude extracts and pure compounds was determined against cultures of *Escherichia coli*, *MRSA, Aeromonas hydrophila, Candida albicans*

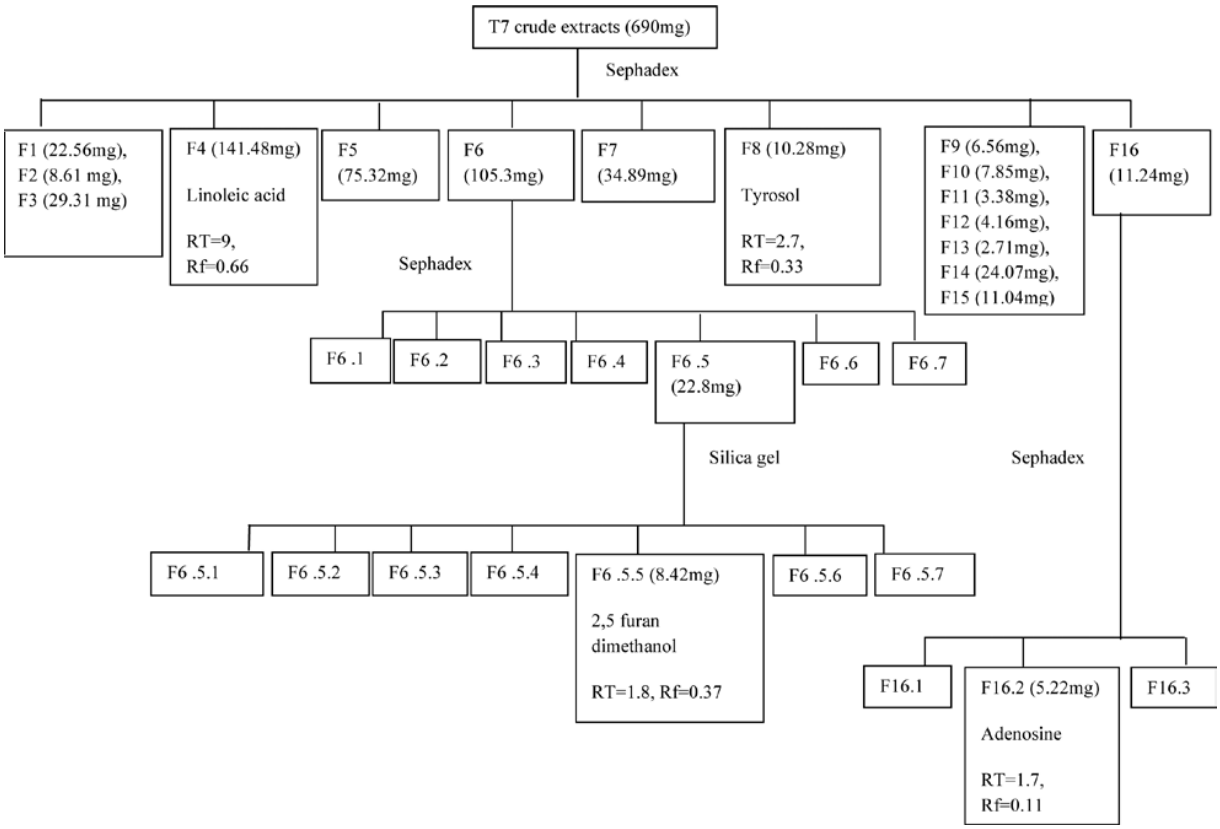


FIGURE1. Analytical scheme for purification of metabolites from *Fusarium proliferatum* crude extract

and *Aspergillus niger* using disk diffusion method. All pathogens were taken from culture stock of School of Bioscience and Biotechnology at Universiti Kebangsaan Malaysia. The crude extracts dissolved in MeOH (0.1 mL) were introduced onto the sterile disk (0.5 cm) and then allowed to dry. Thus the disk was completely saturated with the extract, then the disk was introduced onto the medium (Muller Hinton Agar) with the pathogen. The plates were incubated at 37°C for 24 h. MeOH were used as the control. Microbial growth was determined by measuring the diameter of the zone of inhibition.

HPLC ANALYSIS

In order to trace the chemical component of crude extract and all the fractions resulted from each column chromatography, they all were screened by High Performance Liquid Chromatography using Dionex Ultimate 3000, Chromeleon, C18 Merck column (55×2 cm, 3 µm) gradient mobile phase ACN 0-100% / H_2O with flow rate 0.5 mL/min.

RESULTS AND DISCUSSION

The UV absorption was screened on the crude extract from 200-600 nm. Figure 2 indicates that the crude has maximum UV absorbance at 277 and 286 nm. The HPLC chromatogram of crude extracts illustrates four major components (Figure 3).

The ESI-MS spectrum of F4, Rt=9, Rf=0.66 (10%MeOH in CH_2Cl_2) showed a peak at m/z 303 [M+ Na] that corresponds to the empirical formula $C_{18}H_{32}O_2$. The chemical structure of F4 was identified on the basis of a comparison with the data of literature as linoleic acid (Pouchert & Behnke 1993) which is one of the essential fatty acids that humans require (Figure 4).

F8, Rt=2.7, Rf=0.33 (10%MeOH in CH_2Cl_2) was identified as 4-hydroxy phenethyl alcohol (Figure 4). Its ESI-Ms spectrum showed a peak at m/z 161 [M+Na] that correspomds to the molecular formula of $C_8H_{10}O_2$. The ^{13}CNMR spectra of F8 indicated the presence of six carbones in the molecule whose mutiplicities determined by DEPT spectral data: two methylenes, two unsaturated

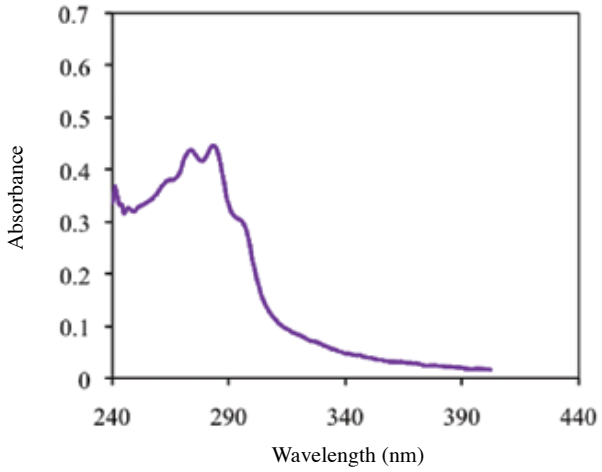


FIGURE 2. UV spectrum of *Fusarium proliferatum* extract

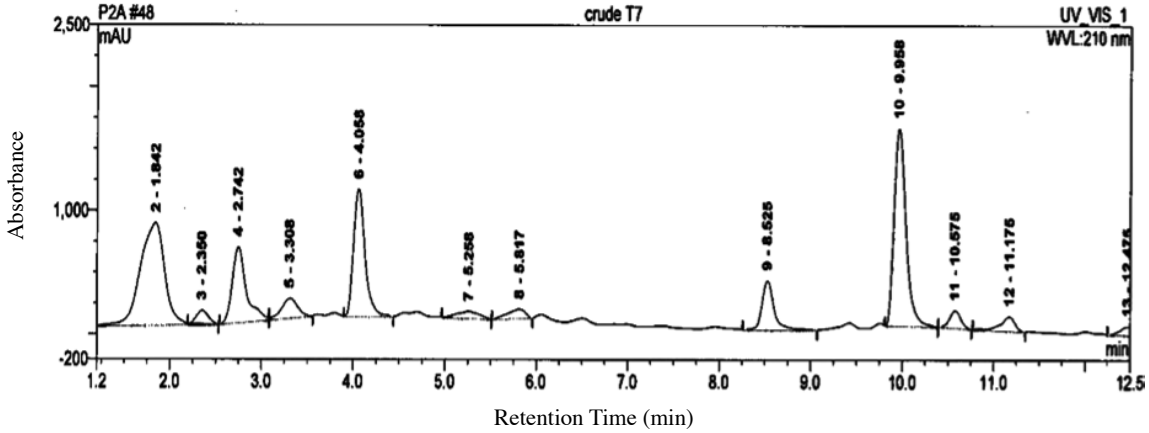


FIGURE 3. HPLC chromatogram on *Fusarium proliferatum* crude extract

methines and two tetrasustituted carbones. The aromatic nature of this compound was verified by means of ^{1}H NMR spectrum that showed two doublets at δ7.10 (2H, d, J=8.4 HZ) and δ6.78 (2H, d, J=8.4 HZ), complete this spectrum two methylenes at δ3.83 (1H, d, J=6.5 HZ) and δ2.80 (1H, d, J=6.5 HZ). F8 also known as tyrosol, was reported to be antibiotically weakly active against *Saccharomyces cerevisiae*, *Nematospora corlyi* and is moderately phytotoxic and antifungal (Laatsch 2003). The compound is widespread in fungi like *Candida*. 4-hydroxy phenethyl alcohol (tyrosol) was identified as quorom sensing molecule in the yeast *Candida albicans* (Chen et al. 2004). Quorom sensing molecules are released by cells to monitor their population density and stimulate coordinated behaviour when a threshold concentration of the molecule is reached. In the case of *Candida albicans*, the acumulation of tyrosol in the growth medium increases with increasing cell density when added to dilue cultures of *C. albicans*, tyrosol reduces the lack phase of growth (Serdyuk et al. 1995).

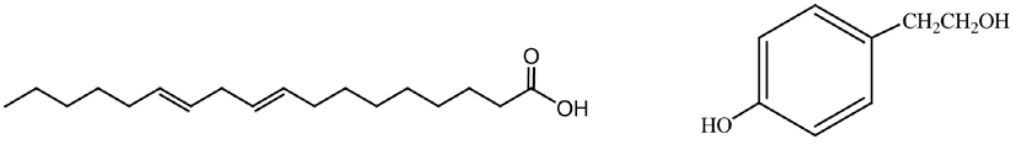
F6.5.5 (Figure 5) was afforded a colourless semi-solid which was identified as 2, 5 furan dimethanol. It was weakly UV absorbing substance which turned to brown by anisaldehyde/suphoric acid, Rf=0.37 (10% MeOH in $CHCl_3$) and Rt= 1.8. ^{1}H NMR, δ = 6.17 (s, 2 H, 3, 4-H), 5.09 (t, ^{3}J = 6.1 Hz, OH), 4.35 (d, ^{3}J = 6.1 Hz, 2, 5-CH_2). ^{13}C NMR (DMSO-d_6): δ 154.0 (Cq-2, 5), 108.4 (CH-3, 4), 56.8 (CH2-2, 5). Its ESI-MS spectrum showed a peak at m/z 151 [M+ Na] which confirms the molecular formula of $C_6H_8O_3$ with mw 128 g/mol for F6.5.5.

The ^{1}H NMR spectrum of F16.2 in DMSO-d_6 was that of adenosine with signals at δ 8.33 (s, 1H), 8.13 (s, 1H), 7.3 (s, 2H) for NH_2, 5.87 (d, 1H), 5.87 (d, 1H), 5.41 (d), 5.14 (d), 4.61 (m), 4.17 (m), 3.96 (m) and 3.61 (m) for the only CH_2 in adenosine structure. The ^{13}C NMR spectrum showed 10 types of carbon at δ 62.1 which was identified as the only methylene in adenosine structure by DEPT spectral data, δ 71.12, 73.90, 86.35, 88.41, 119.85, 140.36, 149.57, 152.84, 156.64. The ESI-MS spectrum of F16.2 showed a peak at m/z 268 [M+ H] which confirms the molecular formula of $C_{10}H_{13}N_5O_4$ with mw 267 g/mol for F16.2 (Figure 5).

Table 1 illustrates the antimicrobial activity of crude extract and pure fractions. As shown in Table 1, crude extract showed activity against Methicillin-resistant *Staphylococcus aureus* (MRSA), *Aeromonas hydrophila* and *candida albicans*. No activity was detected for *Escherichia coli*. F8 and F6.5.5 showed activity against MRSA. F16.2 showed resistant against *Aeromonas hydrophila*. F4 did not show any activity.

Conclusion

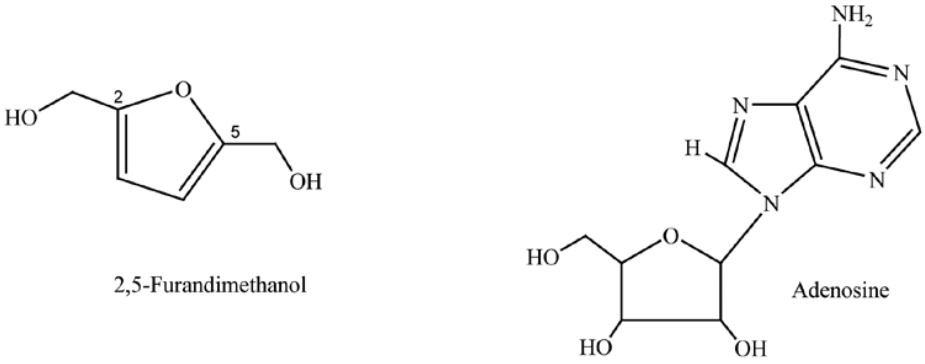
Four major metabolites, Linoleic acid, Tyrosol, 2, 5 Furandimethanol and Adenosine were isolated from marine fungus *Fusarium proliferatum*. The bio active UV absorption range was found from 270-290 nm. This marine fungus produces large amount of Linoleic acid. Although this compound showed no activity against any of tested pathogens, it is an essential fatty acid for



Linoleic acid — F4

4-Hydroxy phenethyl alcohol(Tyrosol) — F8

FIGURE 4. Chemical structures of F4 and F8 isolated from *Fusarium proliferatum* crude extract



2,5-Furandimethanol — F6.6.6

Adenosine — F16.2

FIGURE 5. Chemical structures of F6.5.5 and F16.2 isolated from *Fusarium proliferatum* crude extract

TABLE 1. Antimicrobial activity of metabolites isolated from Fusarium proliferatum

	Inhibition zone (mm)			
	Methicillin-resistant *Staphylococcus aureus* (MRSA)	*Aeromonas hydrophila*	*Escherichia coli*	*Candida albicans*
Crude extract	10	6	-	6
F4	-	-	-	-
F8	7	-	-	-
F6.5.5	8	-	-	-
F16.2	-	9	-	-

human body. So it seems this marine microorganism could be an abundant source for Linoleic acid. Tyrosol inhibited only against *MRSA*. No activity detected against *Candida albicans* while the compound was already reported in the literatures as quorum sensing molecule in *Candida albicans*. 2.5 Furandimethanol also resisted only against *MRSA*. Adenosine inhibited against *Aeromonas hydrophila*. Few animals contain significant amounts of free nucleotides. A notable exception is sponges. This compound is mostly famous for its cardio depressor and anti-inflammatory activity.

REFERENCES

Chen, H., Fujita, M., Feng, Q., Clardy, J. & Fink, G.R. 2004. *Proc. Natl. Acad. Sci USA* 101: 5048-5052.

Colwell, R.R. 2002. Fufilling the promise of biotechnology. *Biotechnol. Adv.* 20: 215-228.

Dominguez, T.J. 2004. It's not a spider bite, its community-acquired methicililn-resistant *Staphylococcus aureus*. *J. Am. Board. Fam. Pract*. 17: 220-226.

Faulkner, D.J. 2000a. Highlights of marine natural products chemistry (1972-1999). *Nat. Prod. Rep*. 17: 1-6.

Faulkner, D.J. 2000b. Marine pharmacology. *Antonie van Leeuwenhoek* 77: 135-145.

Fenical, W. & Jensen, P.R. 1993. Marine microorganisms: a new biomedical resource. In *Marine Biotechnology*, Vol 1, edited by Attaway, D.H., Zaborsky, O.R. New York: Plenum Press.

Gallo, M.L., Seldes, A.M. & Cabrera, G.M. 2004. Antibiotic long-chain and α, β-Unsaturated aldehydes from the culture of the marine fungus *Cladosporium* sp. *Biochem. Syst. Ecol*. 32: 545-551.

Laatsch, H. 2003. A Data Base for Rapid Structural Determination of Microbial Natural Products, 2003 and annual updates, Chemical Concepts, Weinheim, Germany; http://www.user.gwdg. de/~ucoc/laatsch/AntiBase. htm.

Malakoff, D. 1997. Extinction on the high seas. *Science* 277: 486-488.

Nagaraju, U., Bhat, G., Kuruvila, M., Pai, G.S., Jayalakshmi & Babu R.P. 2004. Methicillin-resistant *Staphylococcus aureus* in community-acquared pyoderma. *Int. J. Dermatol*. 43: 412-414.

Neu, H.C. 1992. The crisis in antibiotic resistance. *Science* 257: 1064-1073.

Pomponi, S.A. 1999. The bioprocesstechnological potential of the sea. *J. Biotech*. 70: 5-13.

Pouchert, C.J. & Behnke, J. 1993. *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*. 1st ed Vol. 1. P785/B.

Rovirosa, J., Marrero, D.A., Darias, J., Painemal, K. & Martin, S.A. 2006. Secondary metabolites from marine *Penicilium brevicompactum*. *Journal of Chilean Chemical Society* 51: 1-6.

Serdyuk, O.P., Smolygina, L.D, Muzafarov, E.N., Adanin, V.M. & Arinbasarov, M.U. 1995. *FEBS Lett*. 10: 365.

Z. Mosadeghzad & G. H. N. Farahani
Department of Chemistry, Faculty of Science
Islamic Azad University-Sharekord Branch
166 Shahrekord-Iran

Zuriati Zakaria
School of Chemical Sciences and Food Technology
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600 Bangi, Selangor D.E.
Malaysia

A. Asmat, U. Gires & R. Wickneswari
School of Bio Sciences and Biotechnology
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600 Bangi, Selangor D.E.
Malaysia

P. Pittayakhajonwut
National Centre for Genetic Engineering and Biotechnology (BIOTEC)
National Science and Technology Development Agency (NSTDA)
113, Paholyothin Road, Klong 1, Klong Luang
Pathum Thani 12120
Thailand

*Corresponding author; email: zmzad79@gmail.com

Received: 26 April 2011
Accepted: 13 September 2011