Chiang Mai J. Sci. 2015; 42(1) : 70-78
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Biological Activities of Integric Acid Isolated from the Wood-Decay Fungus *Xylaria feejeensis* 2FB-PPM08M

Totsaporn Srisapoomi [a], Tsuyoshi Ichiyanagi [c], Hiromitsu Nakajima [c], Tadanori Aimi [c] and Sophon Boonlue*[a, b]

[a] Department of Microbiology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.

[b] Natural Products Research Unit, Department of Chemistry, Faculty of Science, Khon Kaen University, 123 Moo 16 Mitrapap Road, Khon Kaen 40002, Thailand.

[c] Faculty of Agriculture, Tottori University, 4-101 Koyama-choMinami, Tottori, 680-8553 Japan.

*Author for correspondence; e-mail: bsopho@kku.ac.th

Received: 6 February 2013
Accepted: 22 April 2014

ABSTRACT

Integric acid was isolated from the wood-decay fungus *Xylaria feejeensis* strain 2FB-PPM08M grown on potato dextrose broth. The compound showed weak antimicrobial activity against Gram-positive bacteria. However, it was inactive against *M. tuberculosis* and had no antiviral effects on HSV-1 and H5N1 viruses. Notably, the metabolite exhibited inhibitory activity against the malarial parasite *Plasmodium falciparum* K1 strain, with IC_{50} values of 6.91 μM, and non-cytotoxicity against African green monkey kidney fibroblasts (Vero cells). This is the first report on its activity against *P. falciparum*.

Keywords: biological activities, integric acid, wood-decay fungus, *Xylaria feejeensis*

1. INTRODUCTION

Wood-decay fungi are important to ecological systems, as they are responsible for the recycling of plant materials and are key components of global carbon cycling [1]. Their capacity to degrade wood is crucial to the wood decomposition process. *Xylaria* is a genus of ascomycetes fungi commonly found on decaying wood. The species of the genus *Xylaria* primarily function as wood- and litter- decay fungi. In addition, *Xylaria* species are productive sources of secondary metabolites with diverse chemical structures and potent biological activities, including roles as antibiotics, toxins, anticancer compounds, hallucinogens [2], cytotoxic cytochalasins [3], antimalarial benzoquinones [4], and antifungal lactones [5].

Among the bioactive compounds produced by the wood-decay fungus *Xylaria feejeensis* 2FB-PPM08M, of particular interest is integric acid, a member of the eremophilane family of sesquiterpenoids, which was identified by comparison with data in the literature. Integric acid and its derivatives from *Xylaria* sp. have been previously reported to exhibit HIV-1 integrase

inhibitory activity [6, 7]. Therefore, the present study describes the bioactivities of integric acid - including antimicrobial, antimycobacterial, antiviral, antimalarial, and cytotoxicity activity - isolated from mycelial culture of *X. feejeensis* 2FB-PPM08M. Additionally, we report on the optimal result of integric acid: against the malarial parasite *Plasmodium falciparum* (K1, multidrug-resistant strain).

2. MATERIALS AND METHODS

2.1 Fungal Materials

Xylaria sp. 2FB-PPM08M was collected from the forest surrounding the Phu Pha Man National Park in Khon Kaen province, Thailand (latitude 16°38′39″N, longitude 101°54′16″E). The perithecial stromata from the fruiting body were surface-disinfected by 0.5% sodium hypochlorite for 4 min and washed three times in sterile distilled water [8]. Surface-disinfected stromata were subsequently placed on potato dextrose agar (PDA) containing 100 mg/L chlortetracycline and incubated at 30°C for 7 d. After incubation, the fungal mycelium was isolated as a pure culture and maintained at 30°C on PDA medium. A voucher specimen was deposited in the National Center for Genetic Engineering and Biotechnology (BIOTEC) Culture Collection in Thailand (voucher no. BCC63553).

2.2 Internal Transcribed Spacer Sequencing of *Xylaria* sp. 2FB-PPM08M

Xylaria sp. 2FB-PPM08M was grown at 30°C on PDA. DNA was extracted using a slightly modified protocol and reagents based on Hoge *et al*. [9]. The internal transcribed spacer (ITS) rDNA was analyzed for identification of the fungal isolate. The primers used for ITS amplification were ITS1 (52-TCC GTA GGT GAA CCT GCG G-32) and ITS4 (52-TCC TCC GCT TAT TGA TAT GC-32), according to White *et al*. [10]. A Gene Q thermal cycler, model TC-24H/(b) (BIOER Technology, Tokyo, Japan) was employed in a thermal cycle with an initial denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 54°C for 1 min, 72°C for 2 min, and a final extension at 72°C for 5 min. DNA sequencing was performed using the custom-sequencing services of First BASE Laboratories SdnBhd (Selangor, Malaysia). The DNA sequence was submitted to GenBank for homology analysis using the BLASTN program. The partial sequence of the ITS1-ITS4 region of *Xylaria* sp. 2FB-PPM08M was deposited in the DNA Data Bank of Japan (DDBJ accession number AB809464).

2.3 Fermentation and Preparation of Mycelium

Xylaria sp. 2FB-PPM08M, cultured on PDA at 30°C for 7 d, was then cut into small pieces (1 cm^3) and inoculated into two 1,000 mL Erlenmeyer flasks, each containing 500 mL of potato dextrose broth (PDB; potato 200 g and dextrose 20 g per L, with 10 potato pieces for each flask). Cultures were incubated at 30°C for 21 d under static conditions. The mycelium cultures were separated from the fluid by filtration through filter paper using a Buchner funnel. Cultured mycelia were dried at 50°C for 5 d and ground to a powder.

2.4 Extraction and Isolation

Dried mycelial powder was extracted with ethyl acetate (EtOAc) with shaking for 24 h at 25°C. Mycelia were removed by filtration and the solvent was evaporated to produce a crude extract (0.5085 g). The extract was chromatographed over silica gel 60 (<0.063 mm mesh size; Merck) and eluted

successively with 250 mL of EtOAc: *n*-hexane (50:50, v/v) and 250 mL of methanol (MeOH) to yield four fractions (F1-F4). The fourth fraction (F4: 0.3830 g) was the most active against *P. falciparum*. F4 was dissolved in MeOH (1 mg/mL) and subjected to high-performance liquid chromatography (HPLC). A pure compound was identified from the HPLC chromatogram of the crude extract by evaluating the retention times (t_R) and UV spectra (acquired with a diode array detector). The HPLC system included a Waters separations module and UV/Vis detector, and the analytical column used was a Mightysil Si60 (250 × 3.0 mm; Kanto, Taiwan). The mobile phase consisted of 2-propanol:*n*-hexane (10:90, v/v) and 0.1% (v/v) acetic acid. The flow rate was set at 1 mL/min at 40°C, and the detector was set at 254 nm. Under these conditions, the compound of interest had a retention time of 6.3 min.

2.5 General Experimental Procedures

FTIR spectra were recorded for a thin film using a Bruker Tensor 27 spectrophotometer (Bruker, Billerica MA). ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 25°C on a Bruker Avance II 600 MHz NMR spectrometer and referenced to d_H 7.24 and d_C 77.0, respectively. Mass spectrometry (MS) was performed by positive- and negative-mode electrospray ionization on a Waters LCT Premier mass spectrometer (Waters, Milford MA). For high-precision measurements, the spectra were obtained by voltage scanning over a narrow mass range at 10,000 resolution. An HPLC system was employed, consisting of a Waters e2695 separations module and 2489 UV/Visible detector. Optical rotation was measured with a Horiba SEPA-500 polarimeter (Horiba, Kyoto, Japan).

2.6 Biological Activities

Antimicrobial activity was determined by the disk diffusion method [11]. The resazurin assay utilizing a microtiter plate as described by Sarker *et al*. [12] was slightly modified to allow determination of the minimum inhibitory concentration (MIC). The tested microorganisms, including 11 bacterial species (*Acinetobacter baumannii* ATCC 19606, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, Methicillin-Resistant *Staphylococcus aureus* (MRSA) DMST 20654, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* DMST 22842, *Shigella sonnei* ATCC 11060, *Staphylococcus aureus* ATCC 25923, *Staphylococcus saprophyticus* ATCC 15305, and *Vibrio cholerae* non O1/non O139 DMST 2873) and two yeast species (*Candida albicans* TISTR 5779 and *Cryptococcus neoformans* DMST 15319). The commercial antimicrobial agents vancomycin (Oxoid), oxytetracycline hydrochloride, and nystatin were employed as positive controls.

Antimycobacterial activity was assessed against *M. tuberculosis* H37Ra using the Green fluorescent protein microplate assay (GFPMA) [13]. The standard drugs employed were rifampicin, streptomycin, isoniazid, ofloxacin, andethambutol.

Antiviral activity was assessed against *Herpes simplex* virus type-1 (HSV-1) using the green fluorescent protein (GFP)-based assay [14]. Acyclovir and 0.5% DMSO were used as positive and negative controls, respectively. In addition, fluorometric determination (MUNANA-based enzyme inhibition assay) [15] using for influenza A virus subtype H5N1. Oseltamivir carboxylate and 0.5% DMSO were used as positive and negative controls, respectively.

Antimalarial activity was evaluated against the parasite *P. falciparum* (K1, multidrug-resistant strain) according to the method of

Trager and Jensen [16]. Quantitative assessment of malarial activity *in vitro* was determined by means of microculture radioisotope technique, based on the method described by Desjardins *et al*. [17], where IC_{50} represents the concentration that causes a 50% reduction in parasite growth as indicated by the *in vitro* uptake of [^{3}H]-hypoxanthine by *P. falciparum*. The standard compound were dihydroartemisinin and mefloquine.

Cytotoxicity activity was assessed by using Vero cells, derived from the kidney of the African green monkey, adhering to 96-well plates, were used to evaluate the toxicity of the compound on the basis of Green Fluorescent Protein (GFP)-based assay according to a modified protocol of Halter *et al*. [14]. Ellipticine and 0.5% DMSO were used as positive and negative controls, respectively. The cells were incubated at 37°C for 72 h in 5% CO_2. The cytotoxicity was expressed as 50% inhibitory concentration (IC_{50}), the concentration of compound that inhibited cell growth by 50%, compared with untreated cells [4].

3. Results and Discussion

3.1 Identification of *Xylaria* sp. 2FB PPM08M

Slender stromata were characteristic of *Xylaria* sp. 2FB-PPM08M. The stromata were attached to a substrate, ≤5 mm in diameter and 10-40 mm high, and had a fine, blackish surface and a white interior (Figure 1A). The colony of *Xylaria* sp. 2FB-PPM08M was initially white with a regular margin; it formed stromata around the center of the PDA plate (Figure 1B). The reverse of the PDA plate colony was reddish-orange (Figure 1C). In PDB, the colonies also developed black stromata with white heads (Figure 1D). These features correlated well with descriptions of *Xylaria* species [18]. The *Xylaria* isolate was identified as *X. feejeensis* based on its nuclear ribosomal RNA sequences (ITS). Sequence analysis of strain 2FB-PPM08M revealed a 99% sequence similarity to *X. feejeensis* (GU322454.1) from GenBank (data not shown), and therefore was identified as *X. feejeensis.*

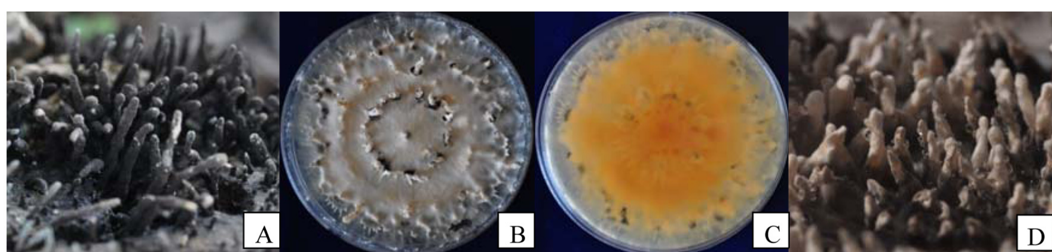

Figure 1. Colony and stromata of *X. feejeensis* 2FB-PPM08M.
A, stromata in nature; B, stromata around the center and margins of the PDA plate; C, the reverse of the PDA plate; D, stromata in PDB medium.

3.2 Structure Determination of the Pure Compound

A pure compound was obtained as a colorless powder and determined to have the molecular formula $C_{25}H_{34}O_6$ by high-resolution electrospray ionization mass spectrometry analysis (HRMS *m/z* = 429.2272 [M-H]$^-$, calcd 429.2277). This formula suggested 9 units of unsaturation in the molecule, which was supported by ^{1}H, ^{13}C and DEPT NMR spectra. The IR spectrum revealed a broad absorption band, suggesting an OH group (3040 cm^{-1}) and four carbonyl functional groups (1720, 1719, 1688 and 1645

cm^{-1}). Optical rotation of the compound yielded $[a]_D^{24}$ +38.0 (*c* 0.40, MeOH). Analysis of the ^{1}H NMR spectral data indicated signals for one aldehyde (δ_H 9.53), four olefinic protons (δ_H 6.54, 6.35, 6.24 and 6.09), and four methyl groups (δ_H 1.83, 1.51, 0.99 and 0.86). In the ^{13}C NMR and DEPT spectra, two carbonyl groups (δ_C 196.3), two oxygen-connected carbonyl groups (δ_C 166.8 and 178.1), four methyl groups (δ_C 19.9, 19.5, 14.0 and 12.6), four quaternary groups (δ_C159.0, 147.6, 125.8 and 38.2), one oxygen-connected methine group (δ_C 72.6), three methyl groups (δ_C 53.3, 43.0 and 33.3), and six methylene carbons (δ_C 43.1, 36.5, 29.7, 29.6, 22.7 and 20.0) were identified. Analysis of heteronuclear single-quantum coherence (HSQC) data allowed the assignment of all proton and carbon signals and the assignment of a terminal olefin functional group. The presence of a spin system involving had shown correlation in two-dimensional H-H correlation spectroscopy (2D H-H COSY). In the heteronuclear multiple-bond correlation (HMBC) spectrum, which indicated a methacrolein structure, a bicyclo[4.4.0]-9-decene-8-one structure was indicated by five sets of correlations and an acyclic unsaturated carboxylate structure was indicated by two sets of correlations. Key HMBC correlations disclosed the planar structure of the compound. Comparisons of the spectral data with values from the literature led to the identification of the pure compound as integric acid, as shown in Figure 2 [6]. The yield of integric acid obtained was 0.3830 g. The synthesis of integric acid and its C4′ diastereoisomers revealed conclusive evidence of (*S*)-stereochemistry at the C4′ position [19].

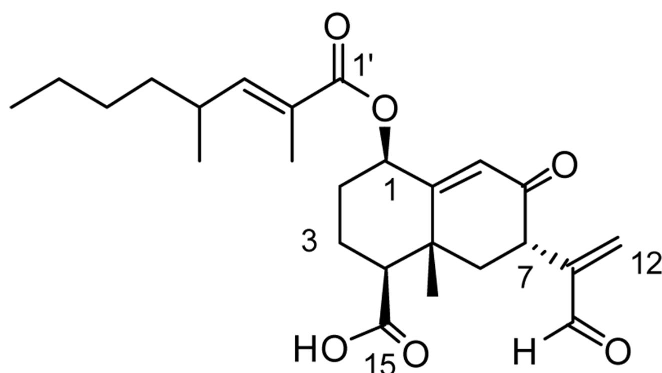

Figure 2. Structure of integric acid isolated from *X. feejeensis* 2FB-PPM08M.

3.3 Biological Activities of Integric Acid from *X. feejeensis* 2FB-PPM08M

In order to investigate the biological activity of integric acid from *X. feejeensis* 2FB-PPM08M, tests were carried out to ascertain its antimicrobial, antimycobacterial, antiviral, antimalarial, and cytotoxic properties. The compound exhibited marginal inhibitory activities against *B. subtilis* ATCC 6633, *S. saprophyticus* ATCC 15305, *S. aureus* ATCC 25923, MRSA DMST 20654, and *E. faecalis* ATCC 29212 at concentrations of 0.312 mg/ml, 0.833 mg/ml, 1.250 mg/ml, 1.250 mg/ml, and 2.500 mg/ml, repectively. Compared with standard drugs, the low potency of this compound may limit its potential as an antibacterial agent.

In vitro evaluation of antimycobacterial activity revealed that the compound was inactive against *M. tuberculosis* H37Ra. Also,

the compound showed no antiviral activity against HSV-1 and H5N1 viruses, in comparison with standard antiviral drugs (data not shown).

Most notably, this compound displayed antimalarial activity against *P. falciparum*, with an IC_{50} value of 6.91 μM, and was not cytotoxic to Vero cells. The antiparasitic and cytotoxic activities of the compound are presented in Table 1. Previous studies on integric acid and its derivatives have only found inhibitory activity against human immunodeficiency virus-1 (HIV-1) integrase [6, 7]. The compound inhibits 3′-end processing, strand transfer, and disintegration reactions catalyzed by HIV-1 integrase [19]. The antimalarial activity of integric acid has not been previously reported. This is the first report on the effectiveness of integric acid isolated from *X. feejeensis* 2FB-PPM08M against *P. falciparum* K1 strain. We obtained integric acid with potent antimalarial activity, which justifies the use of the compound in the further development of antimalarial drugs. The ongoing development of new antimalarial agents is important in order to overcome the limitations related to the high toxicity of drugs currently available for treatment of this and other diseases caused by tropical parasites.

Table 1. Antiplasmodial and cytotoxic activities of integric acid.

Compound	Activity IC_{50} (μM)	
	P. falciparum [a]	Cytotoxicity [b]
Integric acid	6.91	-
Dihydroartemisinine	0.00253	ND
Mefloquine	0.0297	ND

[a] includes inhibitory concentration; mean value of three independent experiments;
[b] Experiments performed with Vero cells; ND = not determined; (-) = Non-cytotoxicity

There are several other compounds from xylariaceous fungi that have a structure similar to integric acid – such as Sch 420789, 07H239-A, and 1-(xylarenone A) xylariate A compounds-and that have shown great promise as biologically active agents. The Sch 420789 compound, which was obtained from an unidentified fungus, displayed phospholipase D activity [20]. Compound 07H239-A, from a marine-derived xylariaceous fungus, was shown to be cytotoxic toward a variety of cancer cell lines and also exhibited activation activity on α-glucosidase [21, 22]; its derivatives also reportedly showed cytotoxicity against cancer cell lines [23]. The compound 1-(xylarenone A) xylariate A, isolated from *Xylaria* sp. NCY2, demonstrated moderate antitumor and antibacterial activities *in vitro* [24]. Recently, a new eremophilanolide closely related to integric acid, named eremoxylarin C, was isolated from *X. allantoidea*. It was shown to exhibit antimalarial activity (*P. falciparum* K1) while it was non-cytotoxic to Vero cells [25].

The diverse and widespread fungal genus *Xylaria* is known to be a rich source of bioactive secondary metabolites. Some examples that have been reported include: xylarenals A and B, isolated from *X. persicaria*, which are selective ligands for the neuropeptide Y Y5 receptor [26]; five unique xyloketals, A-E, from the mangrove fungus *Xylaria* sp. 2508 (xyloketal A exhibited inhibitory activity against acetylcholinesterase) [27]; and multiplolides A and B from *X. multiplex*, active against *C. albicans* [5]. However, few reports have explored

xylariaceous fungi as a source of antimalarial agents. Only benzoquinone [4], phaseolinone and phomenone [28, 29] have been revealed to be active compounds against *P. falciparum*, K1 strain. Therefore, as the results of the present findings, integric acid produced by *X. feejeensis* might be a promising antimalarial compound.

4. Conclusion

Integric acid isolated from *X. feejeensis* 2FB-PPM08M showed weak antimicrobial activity against Gram-positive bacteria. In addition, the compound was inactive against *M. tuberculosis* and had no antiviral activity against HSV-1 and H5N1 viruses. To the best of our knowledge, this is the first report on the *in vitro* activity of an integric acid against a human parasite (*P. falciparum*). The present findings indicate that, in light of its outstanding antimalarial activity, structural modification via genetic manipulation of the biosynthetic pathways of this compound requires further investigation, with an eye toward the development of new antimalarial drugs. However, this study only indicates the concentration of the compound that was able to cause growth inhibition in *P. falciparum*.

Acknowledgments

We would like to thank the Global COE Program (Tottori University, Japan) on Advanced Utilization of Fungus/Mushroom Resources for their valuable support and analytical processes. We are also grateful to Associate Professor Dr. Somdej Kanokmedhakul, Head of the Natural Products Research Unit, Department of Chemistry, and the Center of Excellence for Innovation in Chemistry, Faculty of Science, Khon Kaen University, Thailand, for providing the laboratory facilities in the preliminary part of the experiment. We thank the Microbial Resources and Applications group for partial support.

References

[1] Odor P., Heilmann-Clausem J., Christensen M., Aude E., van Dort K.W., Piltaver A., Siller I., Veerkamp M.T., Walleyn R., Standovar T., van Hees A.F.M., Kosec J., Matocec N., Kraogher H. and Grebenc T., Diversity of dead wood inhabiting fungi and bryophytes in semi-natural beech forests in Europe, *Biol. Conserv.*, 2006; **131(1)**: 58-71. DOI 10.1016/j.biocon.2006.02.004.

[2] Stadler M. and Hellwig V., Chemotaxonomy of the *Xylariaceae* and remarkable bioactive compounds from *Xylariales* and their associated asexual stages, *Rec. Res. Devel. Phytochem.*, 2005; **9**: 41-93.

[3] Pongcharoen W., Rukachaisirikul V., Isaka M. and Sriklung K., Cytotoxic metabolites from the wood-decayed fungus *Xylaria* sp. BCC 9653, *Chem. Pharm. Bull.*, 2007; **55(11)**: 1647-1648. DOI 10.1248/cpb.55.1647.

[4] Tansuwan S., Pornpakakul S., Roengsumran S., Petsom A., Muangsin N., Sihanonta P. and Chaichit N., Antimalarial benzoquinones from an endophytic fungus, *Xylaria* sp., *J. Nat. Prod.*, 2007; **70(10)**: 1620-1623. DOI 10.1021/np0701069.

[5] Boonphong S., Kittakoop P., Isaka M., Pittayakhajonwut D., Tanticharoen M. and Thebtaranonth Y., Multiplolides A and B, new antifungal 10-membered lactones from *Xylaria multiplex*, *J. Nat. Prod.*, 2001; **64(7)**: 965-967. DOI 10.1021/np000291p.

[6] Singh S.B., Zink D., Polishook J., Valentino D., Shafiee A., Silverman K., Felock P., Teran A., Vilella D.,

Hazuda D.J. and Lingham R.B., Structure and absolute stereochemistry of HIV- 1 integrase inhibitor integric acid. A novel eremophilane sesquiterpenoid produced by a *Xylaria* sp, *Tetrahedron Lett*., 1999; **40(50)**: 8775-8779. DOI 10.1016/S0040-4039(99)01878-X.

[7] Singh S.B., Felock P. and Hazuda D.J., Chemical and enzymatic modifications of integric acid and HIV-1 integrase inhibitory activity, *Bioorg. Med. Chem. Lett*., 2000; **10(3)**: 235-238. DOI 10.1016/S0960-894X(99)00666-6.

[8] Jordan E.G., Manandhar J.B., Thapliyal P.N. and Sinclair J.B., Factors affecting soybean seed quality in Illinois, *Plant Dis*., 1986; **70(3)**: 246-248. DOI 10.1094/PD-70-246.

[9] Hoge J.H.C., Springer J., Zantige B. and Wessels J.H.C., Absence of differences in polysomal RNA from vegetative monokaryotic and dikaryotic cells of the fungus *Schizophyllum commune*, *Exp. Mycol*., 1982; **6(3)**: 225-232. DOI 10.1016/0147-5975(82)90114-1.

[10] White T.J., Bruns T., Lee S. and Taylor T., Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics; in Innis M.A., Gelfand D.H., Sninsky J.J. and White T.J., eds., *PCR Protocols: A Guide to Methods and Applications*, Academic, San Diego, 1990: 315-322.

[11] Omar S., Lemonnier B., Jones N., Ficker C., Smith M.L., Neema C., Towers G.H.N., Goel K and Arnason J.T., Antimicrobial activity of extracts of eastern north American hardwood trees and relation to traditional medicine, *J. Ethnopharmacol*., 2000; **73(1-2)**: 161-170. DOI 10.1016/S0378-8741(00)00294-4.

[12] Sarker S.D., Nahar L. and Kumarasamy Y., Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals, *Methods*, 2007; **42(4)**: 321-324. DOI 10.1016/j.ymeth.2007.01.006.

[13] Changsen C., *A Green Fluorescent Protein Microplate Assay (GFPMA) for Screening of Antimycobacterial Compounds*, PhD Thesis, Mahidol University, Thailand, 2004.

[14] Halter M., Almeida J.L., Tona A., Cole K.D., Plant A.L. and Elliott J.T., A mechanistically relevant cytotoxicity assay based on the detection of cellular GFP, *Assay Drug Dev. Technol*., 2009; **7(4)**: 356-365. DOI 10.1089/adt.2009.192.

[15] McKimm-Breschkin J.L., Selleck P.W., Usman T.B. and Johnson M.A., Reduced sensitivity of influenza A (H5N1) to oseltamivir, *Emerg. Infect. Dis*., 2007; **13(9)**: 1354-1357. DOI 10.3201/eid1309.07-0164.

[16] Trager W. and Jensen J.B., Human malaria parasites in continuous culture, *Science*, 1976; **193(4254)**: 673-675. DOI 10.1126/science.781840.

[17] Desjardins R.E., Canfield C.J., Haynes J.D. and Chulay J.D., Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique, *Antimicrob. Agents Chemother*., 1979; **16(6)**: 710-718. DOI 10.1128/AAC.16.6.710.

[18] Rogers J.D., Anamorphs of *Xylaria*: Taxonomic considerations, *Sydowia*, 1985; **38:** 255-262.

[19] Waalboer D.C.J., Kalkeren H.A., Schaapman M.C., Delft F.L. and Rutjes F.P.J.T., Total synthesis and absolute stereochemistry of integric acid, *J. Org. Chem*., 2009; **74(22)**: 8878-8881. DOI 10.1021/jo901845r.

[20] Puar M.S., Barrabee E., Hallade M. and Patel M., Sch 420789: A novel fungal metabolite with phospholipase D inhibitory activity, *J. Antibiot.*, 2000; **53(8)**: 837-838. DOI 10.7164/antibiotics. 53.837.

[21] McDonald L.A., Barbieri L.R., Bernan V.S., Janso J., Lassota P. and Carter G.T.,07H239-A, a new cytotoxic eremophilane sesquiterpene from the marine-derived Xylariaceous fungus LL-07H239, *J. Nat. Prod.*, 2004; **67(9)**: 1565-1567. DOI 10.1021/np049924g.

[22] Song Y.X., Wang J.J., Huang H., Ma L., Wang J., Gu Y.C., Liu L. and Lin Y.C., Four eremophilane sesquiterpenes from the mangrove endophytic fungus *Xylaria* sp. BL321, *Mar. Drugs*, 2012; **10(2)**: 340-348. DOI 10.3390/md10020340.

[23] Song Y.X., Cheng B., Zhu X., Qiao L.T., Wang J.J., Gu Y.C., Li M.F., Liu L. and Lin Y.C., Synthesis and cytotoxic evaluation of eremophilane sesquiterpene 07H239-A derivatives, *Chem. Pharm. Bull.*, 2011; **59(9)**: 1186-1189. DOI 10.1248/cpb.59.1186.

[24] Hu Z.Y., Li Y.Y., Lu C.H., Lin T., Hu P. and Shen Y.M., Seven novel linear polyketides from *Xylaria* sp. NCY2, *Helvetica Chimica Acta*, 2010; **93(5)**: 925-932. DOI 10.1002/hlca.200900323.

[25] Isaka M., Yangchum A., Supothina S., Chanthaket R. and Srikitikulchai P., Isopimaranes and eremophilanes from the wood-decay fungus *Xylaria allantoidea* BCC 23163, *Phytochem. Lett.*, 2014; **8**: 59-64. DOI 10.1016/j.phytol.2014.01.011.

[26] Smith C.J., Morin N.R., Bills G.F., Dombrowski A.N., Salituro G.M., Smith S.K., Zhao A. and MacNeil D.J., Novel sesquiterpenoids from the fermentation of *Xylaria persicaria* are selective ligands for the NPY Y5 receptor, *J. Org. Chem.*, 2002; **67(14)**: 5001-5004. DOI 10.1002/chin.200246181.

[27] Lin Y., Wu X., Feng S., Jiang G., Luo J., Zhou S.L., Vrijmoed L.P., Jones E.B.G., Krohn K., Steingrover K. and Zsila F., Five unique compounds: xyloketals from mangrove fungus *Xylaria* sp. from the south China sea coast, *J. Org. Chem.*, 2001; **66(19)**: 6252-6256. DOI 10.1021/jo015522r.

[28] Isaka M., Jaturapat A., Kladwang W., Punya J., Lertwerawat Y., Tanticharoen M. and Thebtaranonth Y., Antiplasmodial compounds from the wood-decayed fungus *Xylaria* sp. BCC 1067, *Planta Med.*, 2000; **66(5)**: 473-475. DOI 10.1055/s-2000-8588.

[29] Kaur K., Jain M., Kaur T. and Jain R., Antimalarials from nature, *Bioorg. Med. Chem.*, 2009; **17(9)**: 3229-3256. DOI 10.1016/j.bmc.2009.02.050.