Chemical Constituents and Biological Activities from a Fungus *Chaetomium longirostre*

องค์ประกอบทางเคมีและสารออกฤทธิ์ทางชีวภาพจากรา *Chaetomium longirostre*

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ABSTRACT

The chromatographic separation of the crude EtOAc extract from a fungus *C. longirostre* provided seven compounds. They were four new azaphilones, longirostrerones A-D (**4-7**), together with three known steroids, ergosterol (**1**), ergosterylpalmitate (**2**), and 24(*R*)-5α,8α-epidioxyergosta-6-22-diene-3β-ol (**3**). The complete interpretations of these structures were established on the conclusive result of IR, 1D and 2D NMR, CD, and MS. The Circular Dichroism (CD) experiment was used to determine the stereochemistry at C-7 of azaphilones, **4-7**. The bioactivity assay of the isolated compounds indicated that compounds **4-7** showed strong cytotoxicity against KB cancer cell line with IC_{50} 1.04, 1.52, 0.23, and 6.38 μM, respectively. Among these, compound **4** exhibited potent cytotoxicity against MCF7 and NCI-H187 cell lines with IC_{50} 0.24 and 1.04 μM, whereas compound **7** exhibited moderated cytotoxicity against these two cancer cell lines with IC_{50} 38.22 and 18.39 μM. In addition, compounds **4-6** displayed antimalarial activity against *Plasmodium falciparum* with IC_{50} 0.63, 3.73, and 0.62 μM, respectively.

บทคัดย่อ

จากการแยกส่วนสกัดหยาบเอทิลอะซิเตตของเชื้อรา *C. longirostre* ด้วยวิธีทางโครมาโทกราฟี ได้สารทั้งหมด 7 สาร เป็นสารใหม่ในกลุ่ม azaphilones 4 สารคือ longirostrerones A-D (**4-7**) และสารที่เคยรายงานโครงสร้างแล้ว ซึ่งเป็นสเตียรอยด์ 3 สารคือ ergosterol (**1**) ergosterylpalmitate (**2**) และ 24(*R*)-5α,8α-epidioxyergosta-6-22-diene-3β-ol (**3**) การวิเคราะห์โครงสร้างที่สมบูรณ์ของสารเหล่านี้ อาศัยข้อมูลทางเปกโทร สโกปี ได้แก่ IR 1D และ 2D NMR CD และ MS โดย Circular Dichroism (CD) เป็นเทคนิคที่ใช้ในการระบุ absolute stereochemistry ที่คาร์บอน 7 ของสาร **4-7** จากการทดสอบฤทธิ์ทางชีวภาพของสารที่แยกได้ พบว่าสาร **4-7** มีความเป็นพิษสูงต่อเซลล์มะเร็งชนิด KB ด้วยค่า IC_{50} เท่ากับ 1.04 1.52 0.23 และ 6.38 μM ตามลำดับ ในจำนวนนี้สาร **4** ยังมีความเป็นพิษสูงต่อเซลล์มะเร็งชนิด MCF-7 และ NCI-H187 ที่ค่า IC_{50} 0.24 และ 1.04 μM นอกจากนี้ สาร **4-6** ยังแสดงฤทธิ์ยับยั้งเชื้อ *Plasmodium falciparum* สาเหตุของไข้มาลาเรียที่ค่า IC_{50} เท่ากับ 0.63 3.73 และ 0.62 μM ตามลำดับ

KEYWORDS: azaphilone, *chaetomium longirostre*, cytotoxic activities

คำสำคัญ: azaphilone *chaetomium longirostre* สารต้านมะเร็ง

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Introduction

Natural products have been used in treating and preventing human diseases for more than thousand years. It was expected that about 40% of all medicines were natural products or their derivatives. This data may not be surprising, as herbal medicines have became a traditional health care since ancient time. They have played a key role in pharma research. Until now, natural extract screening has been one of the roots of pharma research. Thus, natural products play an important role in the drug discovery and development process (Newman, et al, 2007; Menezes, et al, 2009). *Chaetomium* belongs to the family Chaetomiaceae, order Chaetomiales, series Pyrenomycetes, subclass Hymenoascomycetidae II (Xylaria type of centrum). It is a dematiaceous filamentous fungus found in soil, air, and plant debris. This family includes more than 300 species worldwide, and 24 species have been found in Thailand (*Chaetomium*, 2008; *Chaetomium*, 2009; Soytong, et al, 2008). Secondary metabolites from *Chaetomium* species resulted in numerous types of isolated compounds such benzoquinone derivatives

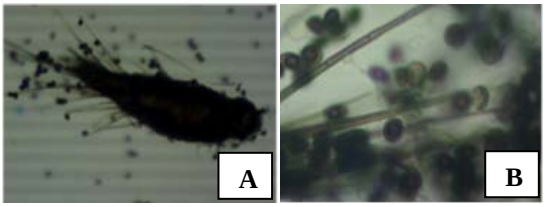

Figure 1 The characteristic of *C. longirostre*;
A) ascomata (fruiting structure) with terminal hairs
B) terminal hairs with septum and ascospores.

(Brewer, et al, 1968), azaphilones (Takahashi, et al, 1990; Kanokmedhakul, et al., 2006; Phonkerd, et al, 2008), and chaetogobosin analogues (Sekita, et al, 1976), etc. As part of our search for bioactive compounds from fungi isolated from Thai soil, an EtOAc extract of *C. longirostre* (Figure 1) showed cytotoxicity against KB, MCF7, and NCIH-187 cell lines with 60.2%, 31.9%, and 22.5% inhibition, respectively, at a concentration of 50 μg/mL.

Extraction and isolation

Dried fungal biomass of *C. longirostre* (273 g) was ground into powder and then extracted successively with EtOAc (3 x 400 mL) and MeOH (3 x 400 mL). Removal of solvents under reduce pressure gave crude EtOAc (11.49 g, 4.2%) and MeOH (20.0 g, 7.3%) extracts, respectively. The EtOAc extract 11.49 g was applied on silica gel FCC, gradient eluting with EtOAc-CH_2Cl_2 and MeOH-EtOAc to give 33 fractions (100 mL per each fraction). According to TLC patterns, these fractions were combined to provide 11 fractions, L_1-L_{11}. Fraction L_1 was dissolved with hexane to give compound **2** (1.5360 g). Fraction L_2 was further dissolved with hexane to yield compound **1** (53.4 mg). Fraction L_4 was purified by silica gel FCC, eluted with an isocratic of 60% EtOAc-hexane to give 5 subfractions, designated as $L_{4.1}$-$L_{4.4}$. Subfraction $F_{4.3}$ was purified by preparative TLC and developed with 60% EtOAc-hexane (x3) to yield compound **5** (10 mg). Subfraction $L_{4.4}$ was purified by preparative TLC using 60% EtOAc-hexane as eluent (x4) to give compound **4** (23.2 mg). Fraction L_5 was separated by silicagel FCC and eluted with isocratic system of 1% MeOH-CH_2Cl_2 to give compound **3** (47.8 mg). Fraction L_8 was purified by silica gel FCC, eluted with gradient system of MeOH-CH_2Cl_2 to obtained 3 fractions ($L_{8.1}$-$L_{8.3}$). Subfraction $L_{8.1}$ was purified by

preparative TLC and developed three times with 1% MeOH-CH_2Cl_2 as eluent to give compound **6** (6.1 mg). Fraction L_{10} was applied on silica gel FCC, eluted with isocratic system of 2% MeOH-CH_2Cl_2 to give 5 subfractions, designated as $L_{10.1}$-$L_{10.4}$. Subfraction $L_{10.1}$ was separated by preparative TLC and developed with 3% MeOH-CH_2Cl_2 to yield an additional amount of compound **7** (5 mg). Subfraction $F_{10.2}$ was purified by FCC, eluted with isocratic system of 1% MeOH-CH_2Cl_2 to give an additional amount of compounds **4** (7 mg) and **7** (82.7 mg). Fraction $L_{10.3}$ was separated by using FCC, isocratic eluting with 1% MeOH-CH_2Cl_2 gave an additional amount of compounds **4** (111.0 mg) and **7** (53.6 mg).

Results and Discussion

Isolation of a fungus, *Cheatomium longilostre* mainly by chromatographic method provided seven compounds (**1-7**). The structures of these compounds were determined on the basis of ^{1}H and ^{13}C NMR, including 2D NMR techniques, (COSY, HSQC, HMBC and NOESY), IR, HRMS, and CD spectral data. The results indicated that compound **1** was ergosterol, compound **2** was ergosteryl palmitate, compound **3** was 24(*R*)- 5α,8α-epidioxyergosta-6-22-diene-3β-ol, compound **4** was longirostrerones A, compound **5** was longirostrerones B, compound **6** was longirostrerones C, and compound **7** was longirostrerones D. These structures are shown in Figure 2.

The isolated compounds were sent to evaluate for their bioactivities at BIOTECH, Pathumthani, Thailand. The results of the biological activities of the new isolated azaphilones, longirostrenes **4-7**, indicated that all compounds exhibited strong cytotoxicity against KB cell lines with IC_{50} values of 1.04, 1.52, 0.23, and 6.38 μM, respectively. While only **4** showed potent cytotoxicity against MCF7 and NCI-H187 cell lines. In addition, compounds **4-6** showed antimalarail activity against *P. falciparum* with IC_{50} values of 0.63, 3.73, and 0.62 μM, respectively (Table 1).

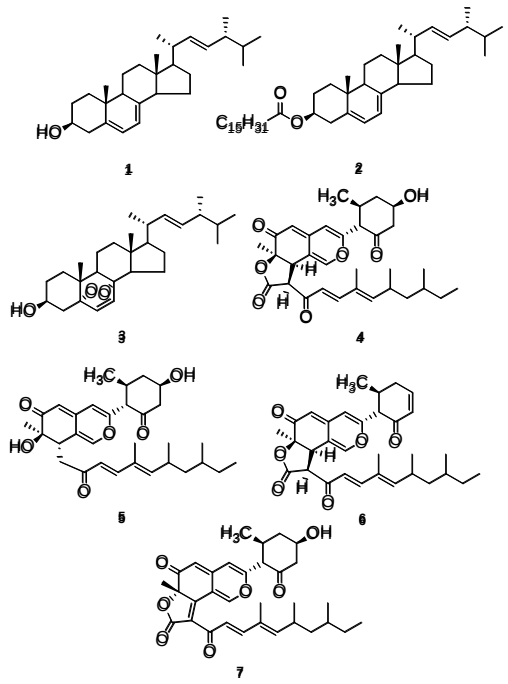

Figure 2 Structures of the isolated compounds from *C. longirostre*

Table 1 Biological activities of compounds **4-7**

compound	cytotoxicity (IC_{50} μM)			antimalarial
	KB[a]	MCF-7[b]	NCI-H187[c]	(IC_{50}, μM)
4	1.04	0.24	3.08	0.63
5	1.52	inactive	inactive	3.73
6	0.23	inactive	inactive	0.62
7	6.38	38.22	18.38	inactive
Dihydro-artemisinine	-	-	-	0.004
Doxoru-bicine	0.33	2.29	0.11	-
ellipticine	1.25	-	1.82	-

[a]Human epidermoid carcinoma of the mouth.

[b]Human breast adenocarcinoma (MCF-7).

[c]Human small cell lung cancer.

Conclusions

Chromatographic separation of EtOAc extract of *C. longirostre* gave seven compounds (**1-7**). Their structures were analyzed by spectroscopic data, IR, 1D and 2D NMR, CD, and MS. They were ergosterol (**1**), ergosteryl palmitate (**2**), 24(*R*)-5α,8α-epidioxyergosta-6-22-diene-3β-ol (**3**), longirostrerone A (**4**), longirostrerone B (**5**), longirostrerone C (**6**), and longirostrerone D (**7**). Among seven isolated compounds, four compounds were reported as new azaphilones, longirostrerones A-D (**4-7**). The result of bioactivity assays of these isolated compounds revealed that compounds **4-7** exhibited strong cytotoxicity against KB cancer cell lines, whereas compounds **4** and **7** were potent cytotoxic against all cell lines tests. Among these, **4** is the most active compound for antimalarial (IC_{50} 0.63 μM), and cytotoxicity against KB (IC_{50} 1.04 μM), MCF7 (IC_{50} 0.24 μM), and NCI-H187 (IC_{50} 3.08 μM). Compounds **4-6** also displayed antimalarial activity against *Plasmodium falciparum*.

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