Oxygenated Secondary Metabolites from Endophytic Fungi Isolated From *Ocimum sanctum*

Mohammad Shoeb[a,b,*], Mohammad Shaheen Uddin Khandaker[a] and Nilufar Nahar[a]

[a]*Department of Chemistry, University of Dhaka, Dhaka-1000, Bangladesh*

[b]*Department of Thematic Studies-Environmental Changes, University of Linköping, SE 58183, Sweden*

Abstract: *Ocimum sanctum* Linn (Tulsi), an important medicinal plant of Bangladesh is used for antimicrobial, anti-inflammatory and antiulcer activities. Eleven endophytic fungi were isolated from *O. sanctum*. The fungal strain, labeled as OS-005, was cultivated at large scale on the potato dextrose agar semi solid medium and was extracted with ethyl acetate. Repeated column chromatography of the parent extract by normal phase silica gel and one of its fraction by Sephadex LH-20 afforded linoleic acid, R (-)-glycerol monolinoleate, 9,10,11-trihydroxy-(12Z)-12-octadecenoic acid, ergosterol, ergosterol peroxide, 1,8-*O*-dimethylaverantin, coriloxin, and a new natural product named sanctumol. The structures of these compounds were elucidated unequivocally by UV, IR, MS and a series of 1D & 2D NMR analyses.

Keywords: Coriloxin, Dimethylaverantin, Endophytic fungi, Ergosterol peroxide, *Ocimum sanctum*, Linoleic acid.

INTRODUCTION

Bangladesh is rich in aromatic and medicinal plants. Some of these plants have been chemically characterized. But most of the endophytic fungi from these plants are unexplored except our research group investigated a few of fungal extracts [1-4]. Microorganisms that live in the intercellular spaces of stems, petioles, roots and leaves of plants without imposing any disease are known as endophytic fungi. They can spend the whole or parts of their life cycle colonizing inter- and/or intra-cellularly inside the healthy tissues of the host plant [5-7]. Some species of endophytic fungi were found to produce structurally diverse and biologically active compounds that can contribute to the discovery of new lead structures for future drug development [7-9]. With the bioactive strains, it is possible to cultivate them in large scale by fermentation to yield the active compound(s) in larger amount rather than destroying natural plant resources. *Ocimum sanctum* Linn (Tulsi) is widely grown in Bangladesh and Indian sub-continent [10]. *O. sanctum* was found to show many pharmacological activities including antimicrobial, anti-inflammatory, antiulcer, antidiabetic, hepatoprotective, chemoprotective, antihyperlipidemic, cardioprotective, antioxidant activities [10, 11]. Previous investigation of other species of *Ocimum* led to the isolation of several endophytic fungi and anti-pancreatic cancer potential of secalonic acid derivatives from one of the endophytes [1]. Several new and known bioactive secondary metabolites were also isolated from other endophytic fungus isolated from medicinal plants of Bangladesh [1-4]. In continuation of our search for novel bioactive compounds from endophytic fungi, we studied fungal strain, OS-005 isolated from *O. sanctum* and report here the isolation of one new natural product named sanctumol (**6**) along with seven known compounds namely, linoleic acid (**1**), R-(-)-glycerol monolinoleate (**2**), 9,10,11-trihydroxy-(12Z)-12-octadecenoic acid (**3**), ergosterol (**4**), ergosterol peroxide (**5**), 1,8-*O*-dimethylaverantin (**7**) and coriloxin (**8**).

MATERIAL AND METHOD

General

UV and IR spectra were recorded on Shimadzu UV 160A and Shimadzu IR-470 spectrometer, respectively. Mass analyses were performed on Thermo Instruments MS system (LTQ XL/ LTQ Orbitrap Discovery). The ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 MHz spectrometer. Media was prepared under Laminar flow (Thermo Forma. Class 11 A1; Biological Safety Cabinet). Media was sterilized using HIRAYAMA autoclave (Hirayama MFG Corp.).

Plant Collection and Sterilization

The medicinal plant, *Ocimum sanctum* (Tulsi) was collected from University of Dhaka campus. Different parts (leaves, stems and roots) of the plant were cut into small pieces. Each plant part was washed and cleaned. The cleaned parts were surface sterilized by immersing in 70% ethanol for 3 min followed by 3% sodium hypochlorite and sterile water again for 3 min and finally dried under laminar flow hood.

*Address correspondence to this author at the Department of Chemistry, University of Dhaka, Dhaka-1000, Bangladesh; Tel: +8801715191988; Fax: +88029667222; E-mail: shoeb71@yahoo.com

ISSN: 1814-8085 / E-ISSN: 1927-5129/15 © 2015 Lifescience Global

Culture and Extraction of Endophytic Fungi

The surface sterilized plant materials were inoculated on autoclaved potato dextrose agar media on a sterilized petridish and after 21 days, eleven fungal strains were isolated and one of the single strains labeled as OS-005 was cultivated at large scale on potato dextrose agar semi solid medium. After 21 days the fungal strains gave full growth and were collected in a round bottom flask and freeze-dried the full content. The dried powdered material was extracted with ethyl acetate (3 x 1200 mL for 24 h, at room temperature). The ethyl acetate extract was evaporated to dryness and 1.2 g extract was obtained.

Isolation of Compounds

The ethyl acetate extract (1.0 g) was fractionated by normal phase silica gel column chromatography using hexane as column equilibrating solvent. After application of the sample, solvents of increasing polarities from 100% hexane to ethyl acetate (EA) were used for elution. On the basis of their R_f values on TLC, ten fractions were obtained. Three fractions collected from 10% EA in hexane gave single spot in TLC and were collected as pure compounds, 1 (15.8 mg), 2 (23.2 mg) and 3 (25.7 mg) after removing waxy materials with *n*-hexane. Two fractions eluted with 20% EA in hexane gave single spot in TLC and were collected as pure compounds 4 (32.5 mg) and 5 (17.2 mg). The 30% EA in hexane fraction was purified by Sephadex LH-20 column using solvent mixture of dichloromethane and methanol (1:1) and compounds 6 (11.1 mg), 7 (13.7 mg) and 8 (14.0 mg) were isolated.

RESULTS AND DISCUSSION

Eight compounds (**1**-**8**) were isolated from the ethyl acetate extract of the fungal strain OS-005 isolated from *O. sanctum* L (Figure **1**). The ^{1}H and ^{13}C NMR

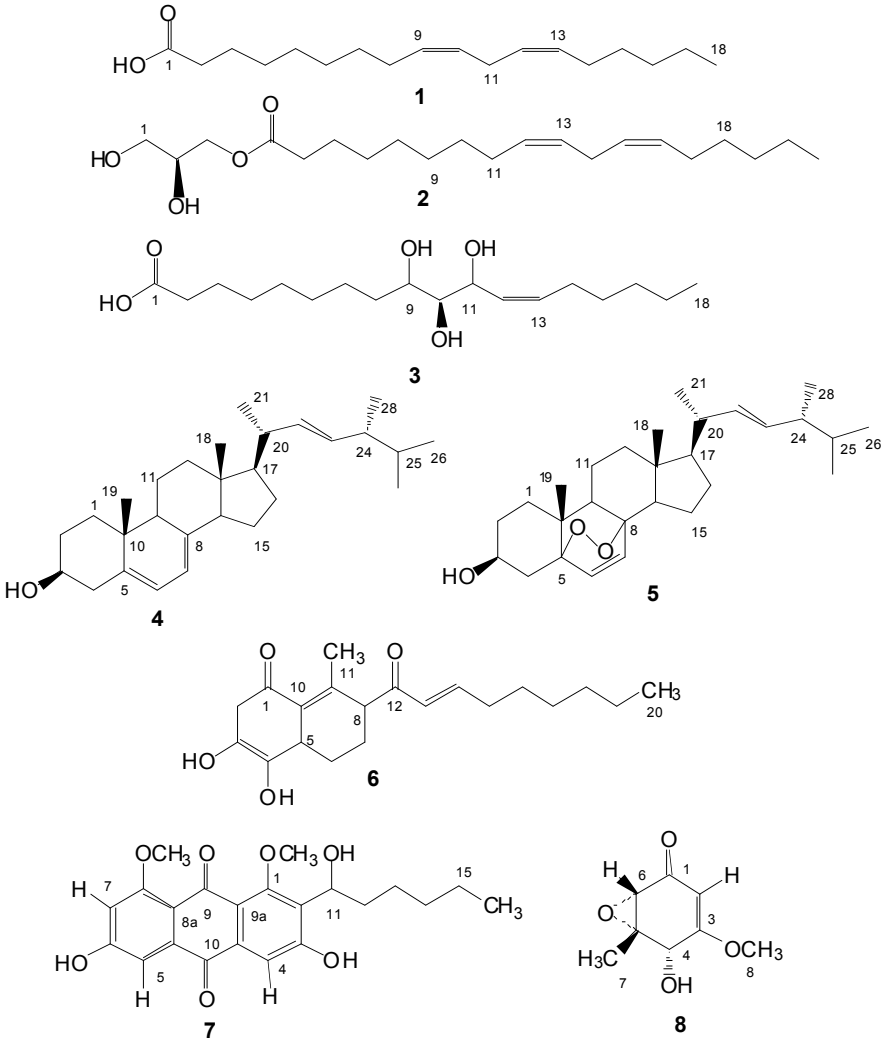

Figure 1: Structure of secondary metabolites isolated from fungal strain OS005.

spectra data of compounds **1, 2 and 3** indicated that they are similar type of compounds and might be fatty acid metabolites. The ^{1}H NMR of **1** gave signals at $\delta_{H=}$ 5.31 (m) and 5.27 (m) for olefinic protons and at $\delta_{H=}$ 0.88 (d, *J*=7.2 Hz) for methyl group. Signals between 2.27-1.25 ppm were for methylene groups. The ^{13}C NMR spectrum gave signals at 174.0, 130.4 & 128.1, 34.3-22.0 and 14.3 ppm for oxygenated carbonyl carbons, olefinic carbons, methylene and methyl group, respectively. The NMR data confirmed that **1** is linolenic acid [12]. The ^{1}H and ^{13}C NMR data of **2** and **3** gave additional signals at $\delta_{H=}$ 4.18, 4.15, 3.90, 3.70, 3.60 and δ_c=70.4, 65.3, 63.5 ppm, and $\delta_{H=}$ 4.32 (2H, dd, H-11), 4.23 (1H, dd, H-10), 3.73 and δ_c=94.1 (10), 70.9 (9), 61.0 (11), respetively for the presence of oxygenated carbons. The spectral data of **2** and **3** were compared with reported values and found identical with glycerol monlinoleate [12] and 9,10,11-trihydroxy-(12Z)-12-octadecenoic acid [13], respectively.

The ^{1}H and ^{13}C NMR spectral data of compounds **4** and **5** also indicated that they were similar types of compounds. The spectral data of **4** were compared with metabolite isolated previously from the fungal strain derived from *O. basilicum* and found identical with ergosterol [1]. Ergosterol was previously isolated from many endophytic fungi and other fungus and showed cytotoxic activities [1, 2, 4, 14]. Comparing spectrsocopic data with reference data [14, 15], compound **5** was identified as ergosterol peroxide.

The compound **6** was isolated as white amorphous (11.1 mg). The ^{13}C NMR spectrum displayed twenty carbons. Of them, six quaternary (δ 204.0, 173.8, 165.1, 155.3, 126.0, 121.0), two olefinic (δ 151.6, 129.3 ppm), eight methylene (δ 32.8, 32.0, 29.6, 29.5, 29.4, 29.3, 28.1, 22.8), two methine (δ 44.8, 39.5) and two methyl carbons (δ 17.1, 14.3 ppm). All protonated carbons were assinged by HSQC. The ^{1}H NMR spectrum of **6** gave doublet at δ 7.02 (*J*= 16.0 Hz) and 6.14 (*J*= 16.0 Hz). The higher coupling constant indicated that they are olefinic protons with *trans* configuration [16]. The ^{1}H-^{1}H COSY spectrum also showed strong correlation between δ 7.02 (H-13) and 6.14 (H-14) ppm. The ^{1}H NMR spectrum also showed additional peaks at 4.47 (m) & 3.13 (m), 2.98, 2.22-1.20 and 0.87 ppm, respectively for the presence of methine, methyl connected with double bond, methylene and methyl group. The long range ^{1}H-^{13}C HMBC correlations were observed between δ_H δ 7.02 (H-14) and δ_c 204.5 (C-12), 32.8 (C-15), 28.0 (C-16); δ_H 6.14 (H-13) and δ_c 204.5 (C-12), 39.5 (C-7), 32.8 (C-15); δ_H 3.15 (H-7) and 204.5 (C-12), 121.0 (C-8), 17.1 (C-11); δ_H 2.98 (H-11) and δ_c 121.0 (C-8), 39.5 (C-7); δ_H 2.22 (H-15) and δ_c 151.6 (C-14), 129.3 (C-13), 28.1 (C-16); δ_H 0.87 (H-20) and δ_c 22.8 (C-19). Combining all the spectral data of IR, ^{1}H- and ^{13}C NMR including ^{1}H-^{1}H COSY and HMBC, the structure of **6** was elucidated and named as sanctumol. To the best of our knowledge sanctumol is a new natural product.

The ^{1}H NMR spectrum of **7** showed signals at δ 6.95 (d, *J*= 2.0 Hz), 6.43 (s), 6.08 (d, *J*= 2.0 Hz), 5.28 (m) and δ 3.60 for the presence of four methine and two methyl groups. Additional signals at δ 1.98-1.24 and 0.80 (*J*= 7.2 Hz) were accounted for methylene and methyl group, respectively. The ^{13}C NMR spectrum showed sixteen carbons and the signals at δ_C 191.8 & 191.3, 172.7, 165.0, 160.0 & 155.3, 57.2 & 57. 2 and 14.2 were for the presence of two carbonyl, four oygeneted olefinic, two methoxy and one methyl carbons. The spectroscopic data were compared with published data and found identical with dimethylaverantin [17]. 1,8-*O*-dimethylaverantin was previosuly isolated from *Penicillium chrysogenum* and showed antifungal activities [17].

The ^{1}H NMR spectrum of **8** showed seven signals at 5.28 (s), 4.80 (s), 4.58 (s,), 3.78 (s,), 3.24 (s), 1.59 (s) ppm. The ^{13}C NMR spectrum indicated the presence of eight carbons. Of them, three quaternary (δ_C 196.0, 175.0 and 57.0), one olefinic (δ_C 98.0), two methine (δ_C 69.0 and 61.0), one methoxy (δ_C 60.0) and one methyl carbon (δ_C 19.1). The long range ^{1}H-^{13}C HMBC correlations were observed between δ_H 5.28 (H-2) and δ_c 196.0 (C-1), 175.0 (C-3), 69.0 (C-4), 61.0 (C-6); δ_H 4.58 (H-4) and δ_c 175.0 (C-3), 19.1 (C-8); δ_H 3.24 and δ_c 196.0 (C-1), 98.0 (C-2); δ_H 1.59 (H-7) and δ_c 69.5 (C-4), 57.0 (C-5). All spectroscopic data were compared with the reported data of coriloxin and found identical with coriloxin [18]. The isolation of all compounds reported here for the first time from endophytes of *O. sanctum*. Coriloxin was previously isolated from the xylariaceous endophytic fungus obtained from plants and *Xylariaceae allantoidea* [18, 19]. Coriloxin exhibited antimicrobial and plant growth inhibitory activities [18]. These findings suggest that fungal metabolites in *O. sanctum* may be potential sources to discover new lead structures for future drug development.

Spectral Data of Constituents

Linoleic Acid (1)

Light yellow gum (15.8 mg). UV (DCM): λ_{max} 232, 252 nm, IR (KBr): ν_{max} 2900, 1730, and 1450 cm^{-1}. ^{1}H

NMR (400 MHz, $CDCl_3$): δ 5.31 (1H, m, H-10/H-12), 5.27 (1H, m, H-9/H-13), 2.71 (2H, t, H-11), 2.27 (2H, t, H-2), 2.00 (2H, m, H-14), 1.56 (2H, m, H-3), 1.25 (m, H-4/5/6/7/15/16/17), 0.88 (3H, t, *J*=7.2 Hz, H-18). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.0 (1), 130.4 (10), 130.4 (12), 128.1 (10), 128.0 (13), 34.3 (2), 31.6 (16), 29.7 (15), 29.5 (4), 29.0 (5), 29.0 (6), 29.0 (7), 27.3 (14), 27.2 (8), 25.8 (6), 25.0 (11), 22.0 (17), 14.3 (18).

R (-)-glycerol Monolinoleate (2)

Light yellow gum (23.2 mg). UV (DCM): λ_{max} 230, 252 nm, IR (KBr): ν_{max} 3450, 2900, 1730, and 1450 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.35 (1H, m, H-12/H-16), 5.33 (1H, m, H-13/H-15), 4.18 (1H, dd, 3Ha), 4.15 (1H, dd, 3Hb), 3.90 (dd, 1H, H-2), 3.70 (1H, dd, Ha-1), 3.60 (1H, dd, Hb-1) 2.76 (2H, t, H-14), 2.34 (2H, t, H-17), 2.04 (2H, m, H-17), 1.62 (2H, m,), 1.62 (2H, m, H-6), 1.30 (14, m, H-7/8/9/10/18/19/20), 0.88 (3H, t, *J*=7.2 Hz, H-21). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.5 (4), 130.4 (16), 130.1 (12), 128.2 (15), 128.2 (13), 70.4 (2), 65.3 (3), 63.5 (1), 34.3 (5), 31.7 (19), 29.7 (18), 29.5 (7), 29.3 (8), 29.2 (9), 29.2 (10), 27.3 (17), 27.2 (11), 25.8 (6), 25.0 (14), 22.7 (20), 14.3 (21). HR-ESIMS: 355.2838 $[M+H]^+$(calcd for $C_{21}H_{39}O_4$).

9,10,11-trihydroxy-(12Z)-12-octadecenoic Acid (3)

Light yellow gum (25.7 mg). UV (DCM): λ_{max} 234, 254 nm, IR (KBr): ν_{max} 3450, 2900, 1730, and 1450 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.38 (1H, m, H-12), 5.33 (1H, m, H-13), 4.32 (2H, dd, H-11), 4.23 (1H, dd, H-10), 3.73 (1H, d, H-9), 2.33 (2H, dd, H-2), 2.04 (2H, dd, H-14), 1.62 (2H, t, H-3), 1.31 (10, t, H-4/5/15/16/17), 1.58 (2H, m, H-8), 1.25 (4H, m, H-6/H7), 0.89 (3H, t, *J*=7.2 Hz, H-18). ^{13}C NMR (400 MHz, $CDCl_3$): δ 173.0 (1), 129.2 (12), 127.9 (13), 94.1 (10), 70.9 (9), 61.0 (11), 33.2 (2), 33.1 (8), 30.5 (16), 28.7 (4), 28.6 (14), 28.3 (6), 28. 2 (5), 28.1 (15), 26.2 (7), 24.6 (3), 22.7 (17), 13.1 (18). HR-ESIMS: 331.2481 $[M+H]^+$ (calcd for $C_{18}H_{35}O_5$).

Ergosterol (4)

White solid (32.5 mg), was soluble in DCM and MeOH. M.P. 147-148 °C. UV (in DCM): λ_{max} 262, 274 and 415 nm. IR (KBr pellet): ν_{max} 2900, 2850, 1720, 1450 and 1250 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) 5.57 (1H, d, *J*=7.1 Hz, H-6), 5.38 (1H, m, H-7), 5.20 (1H, m, H-22), 5.20 (1H, m, H-23), 3.63 (1H, br., H-3), 1.03 (3H, d, *J*=6.4 Hz, H-21), 0.94 (3H, s, H-19), 0.92 (3H, d, *J*=6.7 Hz, H-28), 0.84 (3H, d, *J*=7.0 Hz, H-27), 0.82 (3H, d, *J*=6.9 Hz, H-26), 0.63 (3H, s, H-18) ppm; ^{13}C-NMR ($CDCl_3$): 141.5 (C-8), 139.9 (C-5), 135.5 (C-23), 132.1 (C-22), 119.7 (C-6), 116.4 (C-7), 70.6 (C-3), 55.9 (C-17), 54.7 (C-14), 46.4 (C-9), 42.9 (C-13), 41.0 (C-4), 40.6 (C-20), 39.2 (C-12), 38.5 (C-1), 37.2 (C-10), 33.3 (C-25), 32.2 (C-2), 28.4 (C-16), 23.2 (C-15), 21.3 (C-11), 20.1 (C-21), 19.8 (C-26), 19.6 (C-27), 17.8 (C-28), 16.5 (C-19), 12.2 (C-18). HR-ESIMS: 397.2345 $[M+H]^+$ (calcd $C_{28}H_{45}O$).

Ergosterol Peroxide (5)

White solid (17.2 mg), M.P. 178-179°C. UV (in DCM): λ_{max} 254, 278 and 380 nm. IR (KBr pellet): ν_{max} 2900, 2850, 1720, 1450 and 1250 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 6.82 (1H, d, *J*=6.4 Hz, H-7), 6.62 (1H, d, *J*=6.4 Hz, H-6), 5.29 (1H, m, H-22), 5.13 (1H, m, H-23), 3.60 (1H, m, H-3), 1.94-1.24 (m), 0.97 (3H, d, H-21), 0.86 (3H, d, H-27), 0.82 (3H, s, H-18), 0.81 (3H, s, H-19), 0.78 (3H, d, H-26), 0.75 (3H, d, H-28). ^{13}C-NMR (100 MHz, $CDCl_3$); 136.5 (C-22), 135.8 (C-6), 132.1 (C-23), 130.0 (C-7), 76.2 (C-8), 75.6 (C-5), 70.6 (C-3), 55.4 (C-17), 54.7 (C-14), 54.7 (C-9), 46.4 (C-13), 42.9 (C-24), 40.6 (C-20), 39.3 (C-10), 38.5 (C-12), 37.2 (C-4), 34.6 (C-1), 33.1 (C-25), 30.0 (C-2), 28.4 (C-16), 24.9 (C-15), 22.9 (C-11), 20.8 (C-21), 20.0 (C-26), 19.7 (C-27), 18.2 (C-19), 17.7 (C-28), 12.2 (C-18).

Sanctumol (6)

White amorphous (11.1 mg), UV (in DCM): λ_{max} 254, 280 nm. IR (KBr pellet): ν_{max} 3450, 2900, 1750, 1457, 1075 cm^{-1}. ^{1}H and ^{13}C NMR: Please see Table **1**.

1,8-O-dimethylaverantin (7)

Colored amorphous (13.7 mg), UV (in DCM): λ_{max} 416, 310, 278, 254 nm. IR (KBr pellet): ν_{max} 3400, 2900, 1625, 1512, 1457, 1075 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 6.95 (1H, d, *J*= 2.5 Hz, H-5), 6.43 (1H, s, H-3), 6.08 (1H, d, *J*= 2.5 Hz, H-7), 5.28 (1H, m, H-11), 3.60 (6H, s, 2 x -OCH_3), 2.15 (1H, m, Ha-12), 1.98 (1H, m, Hb-12), 1.50 (1H, m, Hb-13), 1.40 (1H, m, Hb-13), 1.24 (4H, bs, H-14 and H-15), 0.80 (3H, t, *J*= 7.2 Hz, H-16). ^{13}C-NMR (400 MHz, $CDCl_3$): 191.8 (9), 191.3 (10), 172.7 (3), 165 (1), 155.3 (8), 151.7 (6), 139.4 (10a), 129.5 (4a), 120.9 (9a), 116.5 (2), 106.5 (8a), 103.0 (4), 102.6 (7), 75.8 (11), 57.2 (1-OCH_3), 57.0 (8-OCH_3), 39.4 (12), 32.5 (13), 28.0 (14), 22.0 (15), 21.0 (5), 14.2 (16).

Coriloxin (8)

White solid (14.0 mg); m.p. 152-153°C, UV (in DCM): λ_{max} 254, 278 nm. IR (KBr pellet): ν_{max} 2900, 1725, 1512, 1457, 1075 cm^{-1}. ^{1}H NMR ($CDCl_3$): 5.28 (1H, s, H-2), 4.80 (s, -OH), 4.58 (1H, s, H-4), 3.78 (3H, s, -OCH_3), 3.24 (1H, s, H-6), 1.59 (3H, s, CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$); 196.0 (1), 175.0 (3), 98.0 (2),

Table 1: ^{1}H (400 MHz), ^{13}C (100 MHz) NMR (Chemical Shift, Multiplicity, Coupling Constant, *J* in Hz) and HMBC Spectral Data of the Compound 6

	Chemical shift δ in ppm		
	^{1}H NMR	^{13}C NMR	HMBC (H→C)
1	-	173.8	
2	1.24, m, 2H	29.6	155.3
3	-	165.1	
4	-	155.3	
5	4.47, m, 1H	44.8	173.8, 155.3, 126.1
6	1.21, m, 2H	32.1	
7	1.24, m, 2H	29.5	
8	3.15, t, 1H	39.5	204.5, 121.0, 17.1
9	-	121.0	
10	-	126.1	
11	2.98, s, 3H	17.1	121.0, 39.5
12		204.5	
13	6.14, d, J=16.0 Hz, 1H	129.3	204.5, 39.5, 32.8
14	7.02, d, J=16.0 Hz, 1H	151.6	204.5, 32.8, 28.1
15	2.22, m, 2H	32.8	151.6, 129.3, 28.1
16	1.21, m, 2H	28.1	
17	-	29.4	
18	-	29.3	
19	1.21, m, 2H	22.8	
20	0.87, t, *J*= 7.2 Hz, 3H	14.3	22.8

69.0 (4), 61.0 (6), 60.0 (O-CH_3), 57.0 (C-5), 19.1 (C-7). HR-ESIMS: 171.0652 $[M+H]^+$ (calcd for $C_8H_{11}O_4$).

ACKNOWLEDGEMENTS

The authors are grateful to International Foundation for Sciences (IFS), Sweden, Organization for the Prohibition for Chemical Weapons (OPCW), The Netherlands for research grant. MS thanks Professor Marcel Jaspars, Department of Chemistry, University of Aberdeen, UK for NMR and Mass facilities, and Commonwealth Commission, UK and Swedish Institute (SI) for Fellowship.

REFERENCES

[1] Shoeb M, Hoque ME, Thoo-Lin PK, Nahar N. Anti-pancreatic cancer potential of secalonic acid derivatives from endophytic fungi isolated from *Ocimum basilicum.* Dhaka University Journal of Pharmaceutical Sciences 2013; 12(2): 91-95.

[2] Shoeb M, Haque M, Nahar N. Bioactive compounds from endophytic fungus *Penicillium thiomii* isolated from *Terminalia chebula* Retz. Journal of Natural Products and Plant Resources 2014; 4(3): 65-70.

[3] Shoeb M, Begum S, Nahar N. Study of an endophytic fungus from *Aquilaria malaccensis* Lamk. Bangladesh Journal of Pharmacology 2010; 5(2): 21-24. http://dx.doi.org/10.3329/bjp.v5i1.4687

[4] Haque M, Shoeb M, Nahar N. Studies on Chemical Constituents and Biological Activities of an Endophytic Fungi from *Magnifera indica* L. Dhaka University Journal of Pharmaceutical Sciences 2014; 13(1): 63-67.

[5] Petrini O. Fungal endophytes of tree leaves. In Microbial Ecology of leaves, (Springer-Verlag, New York 1991; pp. 179-181. http://dx.doi.org/10.1007/978-1-4612-3168-4_9

[6] Gunatilaka LAA. Natural Products from Plant-associated Microorganisms: Distribution, Structural Diversity, Bioactivity, and Implications of Their Occurrence. J Nat Prod 2006; 69(3): 509-526. http://dx.doi.org/10.1021/np058128n

[7] Tan RX, Zou WX. Endophytes: a rich source of functional metabolites. Natural Product Reports 2001; 18: 448-459. http://dx.doi.org/10.1039/b100918o

[8] Strobel G, Daisy B, Castillo U, Harper J. Natural Products from endophytic microorganisms J Nat Prod 2004; 67: 257-268. http://dx.doi.org/10.1021/np030397v

[9] Zhang HW, Song YC, Tan RX. Biology and chemistry of endophytes. Natural Product Reports 2006; 23: 753-771. http://dx.doi.org/10.1039/b609472b

[10] Prakash P, Gupta N, Therapeutic uses of *Ocimum sanctum* Linn (Tulsi), with a note of Eugenol and its pharmacological

actions: a short review. Indian J of Physiol Pharmacol 2005; 49: 125-128.

[11] Mahajan N, Rawal S, Verma M, Poddar M, Alok S. A phytopharmacological overview on *Ocimum* species with special emphasis on *Ocimum sanctu.* Biomedicine & Preventive Nutrition 2013; 3: 185-192. http://dx.doi.org/10.1016/j.bionut.2012.08.002

[12] Shaaban M, Nasr H, Hassan AZ, Mohsen SA. Bioactive secondary metabolites from endophytic *Aspergillus fumigates*: structure elucidation and bioactivity studies. Rev Latinoamer Quin 2013; 41(1): 50-60.

[13] Gao JM, Wang CY, Zhang AL, Liu JK. A new trihydroxy fatty acid from the ascomycete, Chinese truffle *Tuber indicum*. Lipid 2001; 36(12): 1365-70. http://dx.doi.org/10.1007/s11745-001-0853-y

[14] Kwon HC, Zee SD, Cho SY, Choi SU, Lee KR. Cytotoxic ergosterols from Paecilomyces sp. J300. Arch Pharm Res 2002; 25(6): 851-855. http://dx.doi.org/10.1007/BF02977003

[15] Sgarbi DB, Silva AJR, Carlos IZ, Silva CL, Angluster J, Alviano CS. Isolation of ergosterol peroxide and its reversion to ergosterol in the pathogenic fungus *Sporothrix schenckii.* Mycopathologia 1997; 139: 9-14. http://dx.doi.org/10.1023/A:1006803832164

[16] Shoeb M, MacManus SM, Jaspars M, Trevidadu J, Nahar L, Kong-Thoo-Lin P, Sarker SD. Montamine, a unique dimeric indole alkaloid, from the seeds of *Centaurea montana* (Asteraceae), and its *in vitro* cytotoxic activity against the CaCo2 colon cancer cells. Tetrahedron 2006; 62: 11172-11177. http://dx.doi.org/10.1016/j.tet.2006.09.020

[17] Maskey RP, Grün-Wollny I, Laatsch H, Isolation, structure elucidation and biological activity of 8-O-methylaverufin and 1,8-O-dimethylaverantin as new antifungal agents from *Penicillium chrysogenum.* J Antibiot 2003; 56: 459-63. http://dx.doi.org/10.7164/antibiotics.56.459

[18] Shiono Y, Murayama T, Takahashi K, Katohda S, Ikeda M. Three oxygenated cyclohexanone derivatives produced by an endophytic fungus. Biosci Biotechnol Biochem 2005; 69(2): 287-292. http://dx.doi.org/10.1271/bbb.69.287

[19] Isaka M, Yangchum A, Supothina S. Isopimaranes and eremophilanes from the wood-decay fungus *Xylaria allantoidea* BCC 23163. Phytochemistry Letters 2014; 8: 59-64. http://dx.doi.org/10.1016/j.phytol.2014.01.011

Received on 07-05-2015 Accepted on 15-06-2015 Published on 30-07-2015

http://dx.doi.org/10.6000/1927-5129.2015.11.61

© 2015 Shoeb *et al*.; Licensee Lifescience Global.
This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.