Academia Journal of Medicinal Plants 6(11): 000-000, November 2018
DOI: 10.15413/ajmp.2018.0173
ISSN 2315-7720

Academia Publishing

CrossMark click for updates

Research Paper

Acetylcholinesterase inhibitory active metabolites from *Eurotium* sp. GLGS-4

Chengmang Liu[1], Qiaomei Zheng[1] and Keqin Zhang Guohong Li[2]*

[1]Key Laboratory for Conservation and Utilization of Bio-resource, and Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University, Kunming, 650091, Yunnan, China.
[2]Yunnan University, No.2 Cuihu North Road, Kunming, China.

*Corresponding author. E-mail: ligh@ynu.edu.cn. Tel: 86-871-65033805. Fax: 86-871-65034838.

Accepted 8th November, 2018

ABSTRACT

A fungus GLGS-4 was isolated from Gaoligongmountain and identified as *Eurotium* sp. based on the result of ITS sequence analysis. From the EtOAc extract of the culture broth of strain GLGS-4, eight compounds including flavoglaucin (**1**), 7-OH-emodin (**2**), 2,4-dihydroxy-6-methylacetophenone (**3**), orcinol (**4**), 3-acetyl-5-hydroxy-7-methoxy-2-methoxyl-1,4-naphthalenedione (**5**), physcion (**6**), 1-(2-hydroxy-4-methoxy-6-methylphenyl) ethanone (**7**) and 1,6-dihydroxy-3-(hydroxymethyl)-8-methoxy-9,10-anthracenedione (**8**) were obtained. Their structures were elucidated on the basis of their spectroscopic data. These compounds were evaluated for acetylcholinesterase inhibitory activities *in vitro*. Compounds **7** and **8** showed moderate acetylcholinesterase inhibitory activities with IC_{50} from 52.39 ± 1.4 to 121.61± 1.6 μM.

Key words: *Eurotium* sp. GLGS-4, metabolites, acetylcholinesterase inhibitory.

INTRODUCTION

Alzheimer's disease (AD) is the most predominant form of dementia among the elderly. The deficit of cholinergic functions leads to memory impairment of AD patients (Lahiri et al., 2010). Acetylcholinesterase (AChE) is an enzyme regulating the level of acetylcholine (ACh) in the brain, which plays an important role in the healthy brain (Cahlíková et al., 2010). Currently, acetylcholinesterase inhibitor (AChEI) is the mainstay for treating AD (Lleó et al., 2007). In the screening of natural inhibitors of acetylcholinesterase, various natural products isolated from microbial sources have demonstrated AChE inhibitory activity (Marston et al., 2006).

Microorganism is a good source of natural bioactive compounds (Schulz et al., 2002; Pratiwi et al., 2017). Fungi of the genus *Eurotium*, belonging to family Eurotiaceae, are osmophilic, which usually live in hyperosmotic environments, such as high sugar and salt, and are mainly distributed in Dead Sea, plateau and desert. Secondary metabolites from *Eurotium* are diverse and interesting, including: anthraquinone derivatives (Parameswaran et al., 2004), indolediketopiperazine alkaloids (Slack et al., 2009), benzaldehyde derivatives (Li et al., 2008; Slack et al., 2009) and other types of structures. Compounds obtained from *Eurotium* have showed antibacterial activity (Basu et al., 2005), antifungal activity (Podojil et al., 1978), DPPH radical scavenging activity (Li et al., 2009) and cytotoxicity (Umeda et al., 1974; Ali et al., 1989). As a part of our ongoing research on active compounds from fungi, a fungus (*Eurotium* sp. GLGS-4) was isolated from Gaoligong Mountain, located in Yunnan province of China. From the strain, we have purified eight compounds, and report the isolation, structural identification, and acetylcholinesterase inhibitory activities about these compounds.

MATERIALS AND METHODS

General experimental procedures

NMR spectra were obtained with a Bruker Avance III-600 NMR spectrometer with TMS as an internal standard. ESI-MS were recorded on a mass spectrometer (Finnigan LCQ-Advantage). Column chromatography (CC) was performed on silica gel G (200-300 mesh; Qingdao Marine Chemical Factory, China) and Sephadex LH-20 (Amersham Pharmacia, Sweden). Precoated silica gel GF254 plates

(Qingdao Marine Chemical Factory) were used for thin-layer chromatography.

Microorganisms, media and culture conditions

Eurotium sp. GLGS-4 was isolated from Gaoligong Mountain, Yunnan province of China. It was deposited in the Key Laboratory for Conservation and Utilization of Bio-resources of Yunnan Province, China. In our previous experiment, the strain was cultured on PDB medium and SDB medium respectively. The extract was compared by TLC (Thin Layer Chromatography), and the result showed that the compounds in the extract of SDB medium was more than those in the extract of PDB medium, so SDB medium was selected to culture strain GLGS-4. Then the fungus was grown in shake culture (250 ml per 500 ml triangular flask) on a SDB medium consisting of glucose (40 g/l), peptone (10 g/l), and fermented for 20 days at 180 rpm at 28°C. The culture was harvested for further study.

Identification of strain GLGS-4

Fungal identification was carried out on the basis of the ITS sequence of rDNA. Using ITS4 primer (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS5 primer(5'-GGA AGT AAA AGT CGT AAC AAG G-3'), the nucleotide sequences of the ITS5-5.8S rDNA-ITS4 region was amplified by PCR and subsequently sequenced for comparison with GenBank.

Isolation and purification of compounds

The EtOAc extract (12 g) of the culture broth (30 L) was subjected to silica gel G CC (petroleum ether/acetone 50:1–1:1, v/v) to yield fractions A1-A24 according to TLC analysis. Fraction A5 (35 mg) was first subjected to Sephadex LH-20 CC ($CHCl_3$–MeOH; 1:1), and was further purified by semi-preparative HPLC (LC3000 Semi-preparation Gradient HPLC System, Beijing, China) to yield compound **1** (2.5 mg) (Sample was performed in a RPC_{18} column (250 mm × 10 mm) at ambient temperature with a detection wavelength at 254 nm and a mobile phase of methanol/water at a flow rate of 3 ml/min). Fraction A8 (65 mg) was first purified by Sephadex LH-20 CC ($CHCl_3$–MeOH; 1:1), and then was further purified by silica gel G CC (200-300 mesh; petroleum ether/acetone 90:1–40:1, v/v) and Sephadex LH-20 CC (acetone) to yield compound **2** (3 mg). Fraction A9 (32 mg), was first purified by Sephadex LH-20 CC ($CHCl_3$–MeOH; 1:1), and then was further purified by silica gel G CC (200-300 mesh; petroleum ether/acetone 50:1–30:1, v/v) and Sephadex LH-20 CC (methanol) to yield compound **3** (3 mg). Fraction A11 (23 mg) was purified by Sephadex LH-20 CC and eluted with methanol to yield compound **4** (2 mg). Fraction A13 (63 mg) was first subjected to Sephadex LH-20 CC ($CHCl_3$–MeOH; 1:1), and then was further purified by silica gel G CC (200-300 mesh; petroleum ether/acetone 30:1–10:1, v/v) and Sephadex LH-20 CC (methanol) to yield compound **5** (3.6 mg). Fraction A15 (30 mg) was subjected to Sephadex LH-20 CC ($CHCl_3$–MeOH; 1:1) and Sephadex LH-20 CC (methanol) to yield compound **6** (4.8 mg). Fraction A18 (25 mg) was first purified by Sephadex LH-20 CC and eluted with methanol, and then was further purified by silica gel G CC (200-300 mesh; petroleum ether/acetone 20:1–8:1, v/v) and Sephadex LH-20 CC (methanol) to yield compound **7** (3 mg). Fraction A21 (123 mg) was first subjected to Sephadex LH-20 CC and eluted with methanol, and then was further purified by silica gel G CC (200-300mesh; petroleum ether/acetone 20:1–8:1, v/v) and Sephadex LH-20 CC (methanol) to yield compound **8** (3 mg).

Compound **1:** pale-yellow solid; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 2.23 (3H, s, 6-CH_3), 6.67 (1H, m, H-9/H-10/H-11/H-12), 1.57 (2H, m, H-8), 1.70 (3H, s, H-17), 1.76 (3H, s, H-18), 2.89 (2H, t, *J* = 7.9 Hz, H-7), 3.30 (2H, d, *J* = 7.3 Hz, H-14), 5.28 (1H, t, *J* = 7.3 Hz, H-5), 6.88 (1H, s, H-4), 10.2 (1H, s, H-19); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 117.3 (s, C-1), 155.8 (t, C-2), 128.6 (s, C-3), 125.6 (d, C-4), 144.9 (s, C-5), 128.5 (s, C-6), 23.9 (t, C-7), 29.1 (t, C-8), 29.6 (t, C-9), 32.0 (t, C-10), 31.8 (t, C-11), 22.6 (t, C-12), 14.1 (q, C-13), 27.0 (t, C-14), 121.1 (t, C-15), 133.7 (q, C-16), 17.8 (q, C-17), 25.8 (q, C-18), 195.5 (d, C-19); ESI-MS: 303 $[M - H]^-$.

Compound **2:** yellow solid; ^{1}H-NMR (600 MHz, CD_3COCD_3) δ: 2.04 (s), 6.66 (d, *J* = 2.5 Hz, H-2), 7.21 (d, *J* =2.5 Hz, H-4), 7.31 (s, H-5), 2.83 (s); ^{13}C-NMR (150 MHz, CD_3COCD_3) δ: 16.2, 109.1, 109.2, 130, 136.4, 141.3, 158, 158.4, 166.3, 187.4, 189.7, 200.8, 218. ESI-MS: 285 $[M - H]^-$.

Compound **3:** yellow solid; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 2.38 (3H, s, H-9), 2.55 (3H, s, H-8), 6.12 (1H, d, *J* = 2.2 Hz, H-3), 6.18 (1H, d, *J* = 2.2 Hz, H-5); ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 23.3 (q, C-9), 32.9 (q, C-8), 101.7 (d, C-3), 112.0 (d, C-5), 118.2 (s, C-1), 142.6 (s, C-6), 163.1 (s, C-2), 164.3 (s, C-4), 206.3 (s, C-7); ESI-MS: 165 $[M - H]^-$.

Compound **4:** red solid; ^{1}H-NMR (600 MHz, CD_3OD) δ: 6.10 (2H, d, *J* = 1.7 Hz, H-2/H-6), 6.05 (1H, d, *J* = 1.7 Hz, H-4), 2.15 (3H, CH_3); ^{13}C-NMR (CD_3OD, 150 MHz) δ: 159.3 (C-3, C-5), 141.1 (C-1), 108.5 (C-2, C-6), 100.7 (C-4), 21.6 (C-7); ESI-MS:123 $[M -H]^-$.

Compound **5:** greyish-green solid; ^{1}H-NMR (600 MHz, CD_3OD) δ: 1.94 (3H, s, 2-CH_3), 2.40 (3H, s, 3-$COCH_3$), 3.88 (3H, s, 7-OCH_3), 6.89 (1H, s, H-6), 7.07 (1H, s, H-8); ^{13}C-NMR (150 MHz, CD_3OD) δ: 12.5 (q, 3-CH_3), 31.7 (q, 3-$COCH_3$), 56.7 (q, 7-OCH_3), 105.2 (d, C-6), 108.3 (d, C-8), 113.0 (s, C-4a), 136.9 (s, C-8a), 140.3 (d, C-3), 148.8 (s, C-2), 164.1 (s, C-5), 166.2 (s, C-7), 182.0 (s, C-1), 186.4 (s, C-4), 204.3 (3-$COCH_3$); ESI-MS: 259 $[M - H]^-$.

Compound **6:** yellow solid; ^{1}H-NMR (600 MHz, CD_3OD) δ: 2.41 (3H, s, 6-CH_3), 3.95 (3H, s, 3-OCH_3), 6.79 (1H, d, *J* = 1.9 Hz, H-2), 7.06 (1H, s, H-7), 7.25 (1H, d, *J* = 1.9 Hz, H-4), 7.50 (1H, s, H-5); ^{13}C-NMR (150 MHz, CD_3OD) δ: 21.9 (q, 6-CH_3), 56.8 (q, 3-OCH_3), 105.8 (d, C-2), 108.9 (d, C-4), 110.9 (s, C-

13), 116.0 (s, C-12), 120.5 (d, C-5), 125.3 (d, C-7), 133.9 (s, C-11), 138.8 (s, C-14), 163.7 (s, C-1), 165.3 (s, C-3), 184.2 (s, C-10), 188.5 (s, C-9). ESI-MS: 285 $[M + H]^+$.

Compound **7:** yellow solid; ^{1}H-NMR (600 MHz, CD_3OD) δ: 2.15 (3H, s, H-9), 2.41 (3H, s, H-8), 3.78 (3H, 7-OCH_3), 6.20 (1H, d, *J* = 2.2 Hz, H-3), 6.25 (1H, d, *J* = 2.2 Hz, H-5); ^{13}C-NMR (150 MHz, CD_3OD) δ: 20.4 (q, C-9), 32.6 (q, C-8), 55.9 (q, 7-OCH_3), 98.2 (d, C-3), 112.0 (d, C-5), 121.6 (s, C-1), 139.7 (s, C-6), 161.1 (s, C-2), 168 (s, C-4), 206.9 (s, C-7). ESI-MS: 180 $[M + H]^+$.

Compound **8:** yellow oil; ^{1}H-NMR (600 MHz, CD_3OD) δ: 4.58 (2H, s, 6-CH_2OH), 3.30 (3H, s, 3-OCH_3), 6.84 (1H, d, *J* = 1.9 Hz, H-2), 7.25 (1H, s, H-7), 7.30 (1H, d, *J* = 1.9 Hz, H-4), 7.69 (1H, s, H-5); ^{13}C-NMR (150 MHz, CD_3OD) δ: 64.1 (t, 6-CH_2OH), 56.8 (q, 3-OCH_3), 105.8 (d, C-2), 108.9 (d, C-4), 110.2 (s, C-4a), 116.9 (s, C-12), 117.4 (d, C-5), 122.4 (d, C-7), 134.2 (s, C-11), 138.8 (s, C-14), 151.8 (s, C-6), 165.3 (s, C-1), 166.7 (s, C-3), 184.0 (s, C-10), 188.7 (s, C-9); ESI-MS: 299 $[M - H]^-$.

Acetylcholinesterase inhibitory activities of compounds

The AChE inhibitory activities of the compounds were determined using the modified Ellman spectrophotometric method with DTNB (Dithiobisnitrobenzoic acid) color developing reagent (Ellman et al., 1961). Compounds were dissolved with 2% DMSO (Dimethylsulfoxide). The sample solution (10 µL) was combined with 110 µL0.1 M PBS, 40 µL 0.1 U/mLAChE, and added to 96 well plates. The mixture was incubated at 37°C for 20 min, and the optical density at 405 nm was read 2 times as background value. Thereafter, 40 µL equal volume mixture of 6.25 mM 5,5′-dithiobis-(2-nitrobenzoic acid) solution and 6.25 mM substrate acetylthiocholine iodide solution was added to start the reaction. The progress of the reaction was monitored using a microplate reader with detection at 405 nm every 30 s. Blank control of 0.1 M PBS, 2% DMSO, and negative control of 0.1 M PBS, 2% DMSO, 40 µL 0.1 U/mLAChE and positive control Tacrine were monitored at the same time. The above experiment was repeated three times. The AChE inhibiting activity (I) was expressed in percentage:

$I\ (\%) = 100\times(\Delta Abs_{NC} - \Delta Abs_{sample})/\Delta Abs_{NC}$

Where, $\Delta Abs_{sample} = Abs_{sample} - Abs_{blank\ sample}$

RESULTS

Identification of fungus

The nucleotide sequences of the ITS5-5.8S rDNA-ITS4 region of the fungus GLGS-4 was compared with the GenBank database,GLGS-4 (accession number MH499457) was identified as being closely related to *Eurotium amstelodami* (HQ728257.1), displaying 99% similarity. Thus, strain GLGS-4was identified as *Eurotium* sp.

Structure determination of compounds 1-8

The spectral data revealed that the compounds **1-8** were flavoglaucin (**1**) (Allen et al., 1978), 7-OH-emodin (**2**) (Morooka et al., 1990), 2,4-dihydroxy-6-methylacetophenone (**3**) (Königs et al., 2010), orcinol (**4**) (Lopes et al., 2008), 3-acetyl-5-hydroxy-7-methoxy-2-methoxyl-1,4-naphthalenedione (**5**) (Nishina et al., 1993), physcion (**6**) (Danielsen et al., 2011), 1-(2-hydroxy-4-methoxy-6-methylphenyl), ethanone (**7**) (Sarkar et al., 2011) and 1,6-dihydroxy-3-(hydroxymethyl)-8-methoxy-9,10-anthracenedione (**8**) (Fujimoto et al., 2004), respectively (Figure 1). Compounds **3**, **5** and **7** were obtained from the genus *Eurotium* for the first time.

AChE inhibitory activities

Compounds **1-8** were assayed for acetylcholinesterase (AChE) inhibitory activities. Compounds **7** and **8** showed AChE inhibitory activities. The IC_{50} value of 1-(2-hydroxy-4-methoxy-6-methyl-phenyl) ethanone (**7**) was 121.61± 1.6 µM (31.7 % of inhibition at 100 µg/mL); IC_{50} value of 1,6-dihydroxy-3-(hydroxymethyl)-8-methoxy-9,10-anthracenedione (**8**) was 52.39± 1.4 µM (46.6% of inhibition at 100 µg/mL). Other compounds did not show AChE inhibitory activities (<20% of inhibition at 100 µg/mL). Tacrine was the standard drug used for positive control (IC_{50} value was 0.234 ± 0.005 µM).

DISCUSSION

Flavoglaucin (**1**) was isolated from *E. amstelodami* (Slack et al., 2009) and *Aspergillus chevalieri* (Nazar et al., 1984) and showed a variety of activities, such as cytotoxic activity on HeLa cells (Umeda et al., 1974) and antioxidant activity (Ishikawa et al., 1984), which also caused hepatic damage in rabbits (Nazar et al., 1984). Orcinol (**4**) was isolated from *Parmotrema tinctorum* (Lopes et al., 2008) and *Ascomycota* sp. Ind19F07 (Tian et al.,2015), and showed antioxidant activity. Physcion (**6**) was isolated from *E. ruber* (Engstrom et al., 1980), and showed iron chelating capability and moderate cytotoxicity towards HeLa cells (Podojil et al., 1978).

AD is a neurodegenerative disease of the central nervous system (Wang et al., 2015), which has become the third major incurable disease after cardiovascular disease and cancer in many developed countries (Jiang et al., 2003). In the search for active compounds from fungi in the present study, eight compounds were purified from the fungus *Eurotium* sp. GLGS-4, and three structures are reported for the first time in the fungal genus *Eurotium*. We studied the

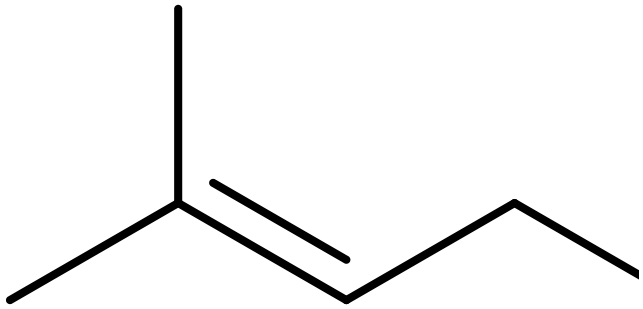

Figure 1: Structures of compounds 1-8.

AChE inhibitory activities of compounds, two of them show moderate activities. This is the first report on the AChE inhibitory activities of 1-(2-hydroxy-4-methoxy-6-methylphenyl)ethanone (**7**) and 1,6-dihydroxy-3-(hydroxymethyl)-8-methoxy-9,10-anthracenedione (**8**).

ACKNOWLEDGEMENTS

This work was supported by grants from the NSFC (31560016, 31760018).

REFERENCES

Ali M, Mohammed N, Alnaqeeb M, Hassan R, Ahmad H (1989). Toxicity of echinulin from *Aspergilluschevalieri* in rabbits.Toxicol.Lett. 48:235-241.

Allen JK, Barrow KD, Jones AJ, Hanisch P(1978). Biosynthesis of flavoglaucin.Stereochemistry of aromatic isoprenylation.J. Chem. Soc. *Perkin. T.* 2:152-154.

Basu S,GhoshAB (2005). Evaluation of the antibacterial activity of *ventilagomadraspatanagaertn.rubiacordifolialinn*. and*lantana camaralinn*.: isolation of emodin and physcion as active antibacterial agents.Phytother. Res.19:888-894.

Cahlíková L, Macáková K, Kuneš J, Kurfürst M, Opletal L,CvačKa J,Chlebeka J, Blundene G(2010). Acetylcholinesterase and butyrylcholinesterase inhibitory compounds from*Eschscholziacalifornica*(*papaveraceae*).Nat. Prod.Commun.5:1035-1038.

Danielsen K, Aksnes DW, Francis GW (2011). NMR study of some anthraquinones from rhubarb.Magn. Reson. Chem.30:359-360.

EllmanGL, Courtney KD, Andres V, Featherstone RM**(**1961).A new and rapid colorimetric determination of acetylcholinesterase activity.Biochem.Pharmacol. **7**: 88-95.

Engstrom GW, Mcdorman DJ, Maroney MJ (1980). Iron chelating capability of physcion, a yellow pigment from *Aspergillusruber*. J. Agr. *Food* Chem. 28:1139-1141.

Fujimoto H, Nakamura E, Okuyama E,Ishibashi M(2004). Six immunosuppressive features from an *ascomycete*, *Zopfiellalongicaudata*, found in a screening study monitored by immunomodulatory activity.Cheminform.52:1005-1008.

Ishikawa Y, Morimoto K, Hamasaki T (1984). Flavoglaucin, a metabolite of *Eurotiumchevalieri*, its antioxidation and synergism with tocopherol.J. Am. *Oil.* Chem. Soc. 61:1864-1868.

Jiang H, Luo X, Bai D(2003). Progress in clinical, pharmacological, chemical and structural biological studies of huperzine A: a drug of traditional Chinese medicine origin for the treatment of Alzheimer's disease.Curr. Med. Chem. 10:2231-2252.

König P, Rinker B, Maus L, NiegerM, Rheinheimer J,Waldvogel SR(2010). Structural revision and synthesis of altechromone A.J.Nat. Prod. 73:2064-2066.

Lahiri DK, Farlow MR, Greig NH,Sambamurti K(2010). Current drug targets for alzheimer's disease treatment.Drug Develop. Res.56:267-281.

Li D, Li X, Li T, Dang H, ProkschP, Wang B(2008). Benzaldehyde derivatives from *Eurotiumrubrum*, an endophytic fungus derived from the mangrove plant *Hibiscus tiliaceus*. Chem. Pharm. Bull. 56:1282-1285.

Li DL, Li XM,Wang BG(2009). Natural anthraquinone derivatives from a marine mangrove plant-derived endophytic fungus *eurotiumrubrum*: structural elucidation and DPPH radical scavenging activity.J.Microbiol.Biotechnol.19:675-680.

Lleó A (2007). Current therapeutic options for alzheimer's disease.Curr.Genomics.8:550-558.

Lopes TI, Coelho RG, Yoshida NC, Honda NK(2008) Radical-scavenging activity of orsellinates.Chem. Pharm. Bull.56:1551-1554.

MarstonA,Urbain A,Borloz A, HostettmannK(2006). Natural product inhibitors of acetylcholinesterase.Curr. Org. Chem.10:825-847.

Morooka N, Nakano S, Itoi N, Ueno Y (1990). The chemical structure and the mutagenicity of emodinmetabolites. J. Agric. Chem. Soc.

Japan.54:1247-1252.
Nazar M, Ali M, Fatima T,Gubler CJ(1984). Toxicity of flavoglaucin from *aspergilluschevalieri* in rabbits.Toxicol.Lett. 23:233-237.
Nishina A, Suzuki H (1993). Naphthoquinone derivative of*Rumexjaponicus* and *Rheum* as microbicide for foods.Jpn. KokaiTokkyoKoho.4:16-17.
Parameswaran P, Gawas D, Tilvi S,Naik C (2004). Biological activeanthraquinone analogs from the fungus *Eurotium* sp. Nat. Inst.Ocean Technol. 1:3-9.
Podojil M, Sedmera P, Vokoun J, Betina V, Baráthová H,Duracková Z, HoráковáK,Nemec P (1978). *Eurotium* (*aspergillus*) *repens* metabolites and their biological activity. Folia Microbiol. 23:438-443.
Pratiwi RH, HidayatI, Hanafi M,Mangunwardoyo W(2017). Antibacterial compound produced by *Pseudomonas aeruginosa*strain UICC B-40, an endophytic bacterium isolated from *Neesiaaltissima*. J.Microbiol. 55:289-295.
Sarkar D,Venkateswaran RV (2011). Biomimetic type approach to the tricyclic core of xyloketals.application to a short, stereocontrolled synthesis of alboatrin and first synthesis of xyloketal G. Tetrahedron 67:4559-4568.
Schulz B, Boyle C, Draeger S, Römmer AK,Krohn K (2002). Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol. Res. 106:996-1004.
Slack GJ, Puniani E, Frisvad JC, Samson RA, Miller JD (2009). Secondary metabolites from *Eurotium*species, *aspergilluscalidoustus* and *A. insuetus* common in canadian homes with a review of their chemistry and biological activities. Mycol. Res. 113:480-490.
Tian YQ, Lin XP, Liu J, Kaliyaperumal K, Ai W,Ju ZR, Yang B, Wang J, Yang XW, Liu Y(2015). Ascomycotin A, a new citromycetin analogue produced by *ascomycota* sp. ind19F07 isolated from deep sea sediment. Nat. Prod. Res. 29:820-826.
Umeda M, Yamashita T, Saito M, Sekita S,Takahashi C (1974). Chemical and cytotoxicity survey on the metabolites of toxic fungi. Japanese J. Experimental Medicine 44: 83-96.
Wang Z, MaZ, Wang L, Tang C, Hu Z, Chou G, Li W (2015). Active anti-acetylcholinesterase component of secondary metabolites produced by the endophytic fungi of *huperziaserrata*.Electro. J. Biotechnol. 18: 399-405.