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Isolation of two flavonoids and mannitol from *Lagotis integrifolia* (Willd.) Schischk (Scrophulariaceae)

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Objectives: *Lagotis integrifolia* (Willd.) Schischk is an ingredient in many traditional Mongolian medicine prescriptions used for the treatment of hot-natured disorder, impure blood caused disorder, disorders of the vital organs, diphtheria, anthrax, and pneumonia. The objective of this study was to isolate and identify the compounds contained in *Lagotis integrifolia*. **Methods:** The compounds were isolated and purified using column chromatography, crystallization, and recrystallization methods. Chemical structures were elucidated using MS, ^{1}H, ^{13}C, HSQC, HMBC, and ^{1}H-^{1}H COSY NMR. **Results:** We identified 7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl) chromen-4-one (diosmetin), luteolin-7-O-glucopyranoside (cynaroside), and mannitol. **Conclusion:** Diosmetin and cynaroside were obtained from *Lagotis integrifolia* for the first time in Mongolia.

Keywords: Diosmetin, Cynaroside, Mannitol, *Lagotis integrifolia*

Introduction

In Mongolia, the genus Lagotis *(Scrophulariaceae)* is represented by one species which mostly grows in the northwestern part of the country, on mountains 3000 m above sea level and higher [1]. Several species such as *L.yunnanesis, L.glauca, L.integra,* and *L.brachystachya* have long been used in Tibetan traditional medicine for the treatment of hot-natured disorder, high blood pressure, and acute and chronic hepatitis [2, 3]. The chemical compositions of two species of Lagotis have been studied; flavonoids have been isolated from *L.brevituba* and phenylpropanoid glycosides and iridoid glycosides have been found in *L.stolonifera* [4-5].

In *Lagotis integrifolia*, iridoids and phenylethanoids,

such as mannitol, aucubin, and gardoside have been isolated [6]. *Lagotis integrifolia* has bitter taste and cool and coarse potency. In Mongolian traditional medicine, it is used for the treatment of hot-natured disorder, impure blood caused disorder, disorders of the vital organs, diphtheria, anthrax, and pneumonia. It is an ingredient in the following traditional prescriptions: *agar*-19, -35, *skyuru ra*-4, *dpa' po*-13, *ba li ka*-4, *spang rgyan*-10, -12, -15, -25, *spangrtsi do*-11, -12, *bashaka*-4, *wag lo*-25 [7]. The objective of this study was to isolate and identify the compounds contained in *Lagotis integrifolia*.

Materials and methods

General

The isolation of the compounds in *Lagotis integrifolia* was conducted at the Laboratory of Chemical and Technology, Institute of Traditional Medicine and Technology and School of Pharmacy and Biomedicine, Mongolian National University of Medical Sciences. The identification was performed at the Laboratory of Chemistry, Inner Mongolian University (IMU). Three compounds were isolated and purified using crystallization and recrystallization methods. Chemical structures were elucidated using mass spectrometry (MS), proton (^{1}H), carbon-13 (^{13}C), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC), and ^{1}H-^{1}H correlation spectroscopy (COSY) nuclear magnetic resonance (NMR) spectrometry (BrukerUltrashield 500 Plus NMR spectrometer, Beijing, China). Autospec-3000 ESI-mass spectrometry, China and EX 1600 HP / PUMP HPLC-DAD, China were used for this study.

Methanol and chloroform (extra pure grade) were purchased from Duksan Pure Chemicals (Sungkok-dong, Korea), and ethanol (pure grade) was purchased from Unionlab (China).

Plant material

L.integrifolia was collected in Arbulag, Khuvsgul province, in Mongolia in August 2015. The plant was identified by Prof. E.Ganbold, at Ulaanbaatar University, and a voucher specimen (No. 08) was deposited at the Institute of Traditional Medicine and Technology, Ulaanbaatar, Mongolia.

Extraction and isolation

The dried and powdered plant material (600 g) was extracted six times with 1 L 70% EtOH using the Soxhlet apparatus, and the combined extract was concentrated under reduced pressure. The residue was suspended in H_2O and then successively partitioned with hexane (4×0.5 L), chloroform (4×0.5 L), ethyl acetate (4×0.5 L), and butanol (6×0.5 L) [8]. The ethyl acetate extract (6.4 g) was subjected to chromatography over silica gel and eluted with chloroform/methanol (100:1→1:1). Fractions containing similar content was combined and concentrated. The E-1 compound was isolated with 10 mg yield.

The n-BuOH extract (70 g) was subjected to chromatography over silica gel and eluted with $CHCl_3$/MeOH/H_2O (10:1:0.1→ 0:1:0) to give BA-1 and BB-1. BA-1, which was isolated from 151-170 fractions, yielded 129 mg, and BB-1, which was isolated from 207-339 fractions, yielded 15 mg.

The E-1 compound was dissolved in methanol, BA-1 and BB-1 were dissolved in DMSO for MS.

Results

1. E-1: Diosmetin

The E-1 substance was a white, amorphous powder and had a molecular formula of $C_{16}H_{12}O_6$ (300.26 g/mol), which was determined using MS. The ^{1}H NMR spectrum exhibited the presence of six aromatic protons at 6.20 (1H, d, J1.5, H-6), 6.44 (1H, d, J1.5, H-8), 6.57 (1H, s, H-3), 7.06 (1H, d, J8.5, H-5`), 7.06 (1H, dd, J8.5 and 1.5, H-6`), 7.38 (1H, d, J1.5, H-2`) and three methoxy protons at 3.93 (3H, s, OCH_3) (Table 1). The ^{13}C NMR spectrum detected 16 carbons at 56-185 ppm, aromatic carbons at 95-185 ppm, and a methoxy at 56,55 ppm (Table 2).

In the HMBC spectrum, the 3 methoxy protons at 3.93 (3H, s, OCH_3) correlated with a carbon at 152.70 ppm. Furthermore, a proton at H-6` correlated with carbons at 112.76, 114.0, 152.70, and 166.02 ppm, and a proton at H-2` correlated with carbons at 120.80, 148.31, 152.70, and 166.02 ppm. The data suggested that the methoxy group was located in the C-4` (Figure 1).

^{1}H-^{1}H COSY was used to find that H-8, H-6 protons and H-6', H-5', H-2' protons correlated with each other (Figure 2). In the HSQC spectrum, we observed the following carbon-proton correlations: H-6 to carbon at 100.22 ppm, H-8 to carbon at 95.08 ppm, H-3 to carbon at 105.43 ppm, H-5`to carbon at

Table1. ^{1}H NMR (δ, ppm) spectrum of E-1 and BA-1 (ppm from TMS, in DMSO-d6).

Position	E-1 (J in Hz)	BA-1 (J in Hz)
H-3	6.57 (1H, s)	6.75 (1H, s)
H-6	6.20 (1H, d, 1.5)	6.45 (1H, d, 2.0)
H-8	6.44 (1H, d, 1.5)	6.8 (1H, d, 2.0)
H-2`	7.38 (1H, d, 1.5)	7.43 (1H, d, 2.0)
H-5`	7.06 (1H, d, 8.5)	6.92 (1H, d, 8.5)
H-6`	7.49 (1H, dd, 1.5, 8.5)	7.46 (1H, dd, 8.5, 2.0)
OCH_3	3.93 (3H, кв)	-
H-1``	-	5.09 (1H, d,7.3)
H-2``	-	3.2 (1H, t, 9.3)
H-3``	-	3.2-3.3 (1H, m)
H-4``	-	3.2 (1H, m)
H-5``	-	3.2-3.3 (1H, m)
H-6``a	-	3.7 (1H, overlapped)
H-6``b	-	3.4 (1H, overlapped)

Table 2. ^{13}C NMR (δ, ppm) spectrum of E-1 and BA-1 (ppm from TMS, in DMSO-d6).

Position	E-1	BA-1
C-2	166.02	164.84
C-3	105.43	103.71
C-4	183.92	182.28
C-5	163.30	161.30
C-6	100.22	99.93
C-7	166.12	163.36
C-8	95.08	95.23
C-9	159.41	157.42
C-10	105.43	105.77
C-1`	125.10	121.89
C-2`	114.0	113.95
C-3`	148.31	146.05
C-4`	152.70	150.15
C-5`	112.76	116.36
C-6`	120.08	119.63
OCH_3	56.55	-
C-1``	-	100.31
C-2``	-	73.45
C-3``	-	76.67
C-4``	-	69.87
C-5``	-	77.57
C-6``	-	60.95

112.76 ppm, H-2`to carbon at 114.0 ppm, and H-6`to carbon at 120.08 ppm (Figure 3).

The data suggested that nine protons had straight correlations to the carbons, and the data of ^{13}C and ^{1}H NMR spectrum confirmed that the three remaining protons are belonged to a hydroxyl group. Thus, the E-1 compound was identified as the flavone, diosmetin (Figure 8).

2. BA-1: Cynaroside

The BA-1 compound was a yellow, amorphous powder and had the molecular formula of $C_{21}H_{20}O_{11}$ (448.37 g/mol), which was determined using MS. In the ^{1}H NMR spectrum, six aromatic proton and six glucose protons were recorded at 3.24-4.2 in (Table 1). In the ^{13}C spectrum, 21 carbons were detected at 60-183 ppm and 5 carbons of monoglycoside at 60-80 ppm (Table 2). In the ^{1}H-^{1}H COSY, we observed correlations of H-8 to H-6; H-6′ to H-5′, H-2′ protons; and an anomeric proton at 5.09 ppm to a glucose proton (Figure 4).

In the HSQC spectrum, we observed the following carbon-proton correlations: H-6 proton to carbon at 99.93 ppm, H-8 proton to carbon at 95.23 ppm, H-3 proton to carbon at 103.7 ppm, H-5` proton to carbon at 116.36ppm, H-2` proton to carbon at 113.95 ppm, H-6` proton 119.63 ppm, and anomeric proton to carbon at 100.31 ppm (Figure 6). In the HMBC spectrum, an anomeric proton at 5,09 ppm only correlated with a carbon at 163,36 ppm (Figure 5). Thus, the BA-1 compound was concluded as luteolin-7-O-glucopyranoside (cynaroside) (Figure 7).

3. BB-1: Mannitol

The BB-1 compound was a white, amorphous powder, isolated from the butanol layer. In ^{1}H NMR spectrum, seven proton signals were recorded at 4.4 (2H, d, J 5.5 Hz, 2OH), 4.33 (2H, t, J 5.5Hz, 2OH), 4.13 (2H, d, J 7Hz, 2OH), 3.6 (2H, m, H-1a, H-6a), 3.54 (2H, m, H-3, H-4), 3.46 (2H, m, H-2, H-5), and 3.38 (2H, m, H-1b, H-6b). The ^{1}H NMR signals were observed between δ 3.3 to 4.4, and only three carbons at δ 71.24, 69.63, and 63.75 were evident in the ^{13}C NMR spectrum. The ^{1}H and ^{13}C NMR spectrums of the BB-1 compound were in good agreement with those of mannitol (Figure 9) [15].

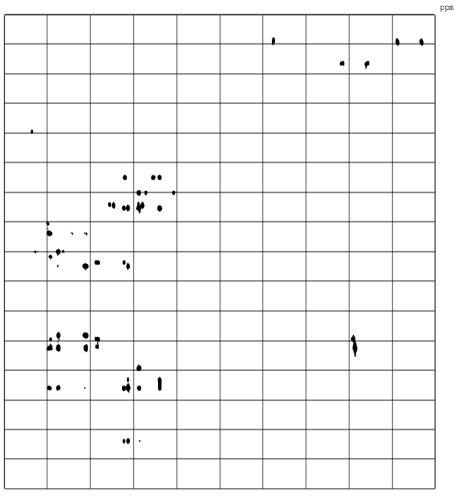

Figure 1. HMBC spectrum of E-1

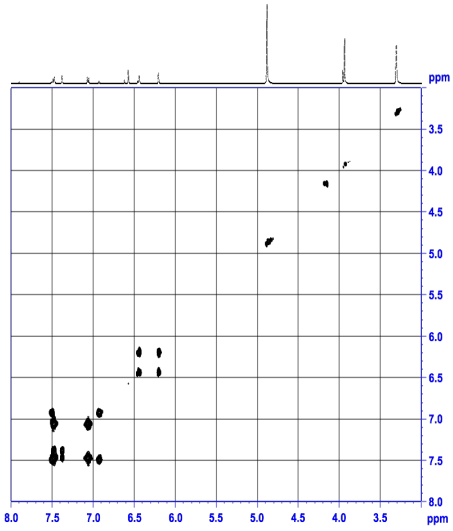

Figure 2. COSY spectrum of E-1

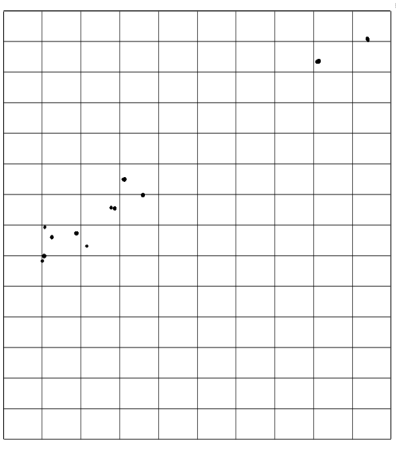

Figure 3. HSQC spectrum of E-1

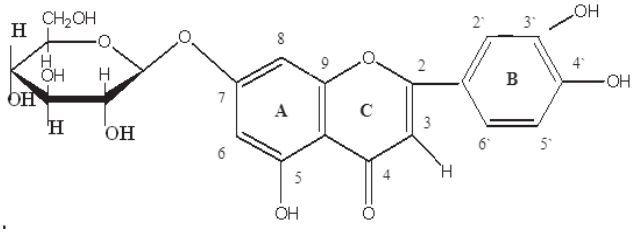

Figure 7. Luteolin-7-O-glucopyranoside (BA-1),

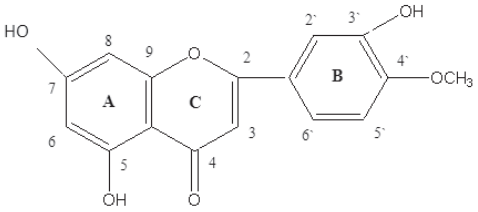

Figure 8. Diosmetin (E-1),

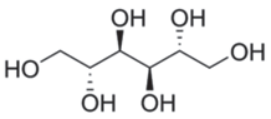

Figure 9. Mannitol (BB-1)

Discussion

For E-1, the ^{1}H NMR spectrum did not distinguish between flavone or a flavonol, but the ^{13}C NMR spectrum clearly distinguished between the two types of flavonoids. In the ^{13}C NMR spectrum, we detected 16 carbons at 56-185 ppm, aromatic carbons at 95-185 ppm and a methoxy at 56,55 ppm (Table 2). The ^{13}C NMR spectrum did not show signals at around δ136 to 139.0 ppm, which would correspond to C-3 in flavonols, nor did it show signals at δ172 to 177 ppm, which could correspond to C-4 in flavonols. However, it did show two characteristic signals of flavones: C-3 at δ103.0 to 111.8 and C-4 at δ177.3 to 184.0 [11]. Our literature search showed that the ^{13}C NMR spectrum of E-1 was the same as that of diosmetin. The methoxy group located at C-4` from HMBC spectrum, and data of ^{13}C NMR, COSY and HSQC spectrum suggested that E-1 was diosmetin.

For BA-1, the ^{1}H NMR spectrum detected the anomeric proton of glycoside at 5.09 ppm. The signal of the anomeric proton at 5.09 (1H, d, 7.3) with coupling constant (J = 8 Hz) suggested a β-configuration of glycoside [12, 13]. From

Table 3. ^{1}H and ^{13}C NMR (δ, ppm) spectrum of BB-1

Position	BB-1 (J in Hz)	BB-1
H-1a	3.6 (1H, m)	
H-1b	3.38 (1H, m)	63.75
H-2	3.46 (1H, m)	71.24
H-3	3.54 (1H, m)	69.63
H-4	3.54 (1H, m)	69.63
H-5	3.46 (1H, m)	71.24
H-6a	3.6 (1H, m)	63.75
H-6b	3.38 (1H, m)	
OH	4.4 (2H, d, 5.5, 2OH)	
OH	4.33 (2H, t, 5.5, 2OH)	
OH	4.13 (2H, d, 7, 2OH)	

the ^{1}H NMR, the BA-1 compound was determined to be 5,7,3`,4`-tetrasubstituted flavone-monoglycoside. Based he ^{1}H–^{1}H COSY data and other published date on carbon-proton chemical shifts, the sugar moiety was determined to be a glucopyranose [12-14]. The data from the ^{13}C NMR and HSQC spectrum suggested that BA-1 was luteolin-7-O-glucopyranoside (cynaroside).

BB-1 was believed to be a monosaccharide, based on the ^{1}H NMR. The ^{1}H NMR signals were observed between δ 3.6 to 3.9, and only three carbons at δ 71.3, 69.7, and 63.9 were evident on the ^{13}C NMR spectrum. Based on an extensive literature search and the observations from the ^{1}H and ^{13}C NMR spectrums, this compound was identified as mannitol [15].

In conclusion, two flavonoids (diosmetin and cynaroside) and mannitol were isolated from *Lagotis integrifolia*. To the best of our knowledge, our study was the first to isolate diosmetin and cynaroside from *Lagotis integrifolia*. We recommend further studies on the pharmacological effects of *Lagotis integrifolia*.

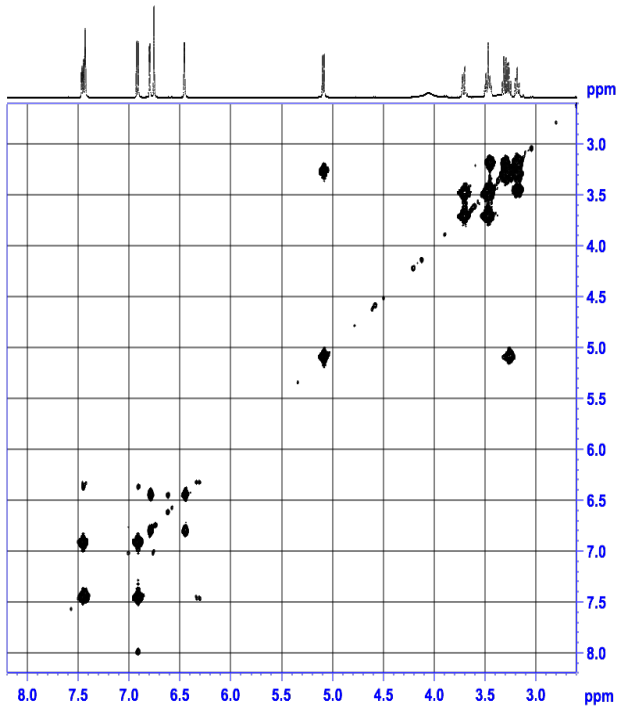

Figure 4. COSY spectrum of BA-1

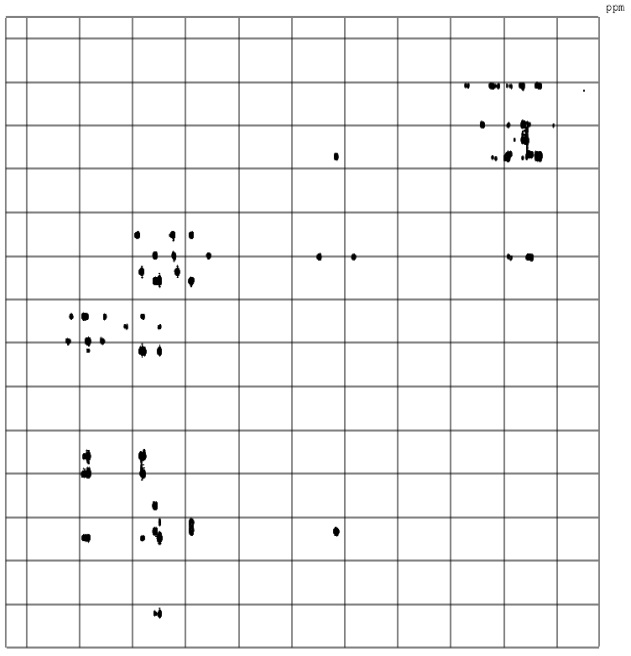

Figure 5. HMBC spectrum of BA-1\

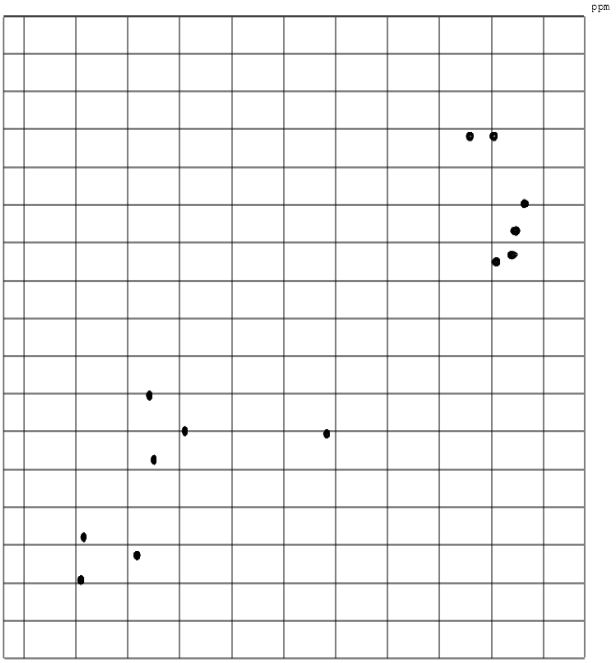

Figure 6. HSQC spectrum of BA-1

Conclusion

Two flavonoids and mannitol have been isolated from *Lagotis integrifolia*, and diosmetin and luteolin-7-O-glucopyranoside have been isolated from this plant for the first time.

Conflict of Interest

The authors have declared no conflict of interest.

Acknowledgements

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References

1. Ligaa U, Davaasuren B, Ninjil N. Mongolian Medicinal Plants Using in Western and Eastern Medicine. Ulaanbaatar, Mongolia: JKC Printing; 2005. p 355.
2. Gansu Health Bureau. Handbook of Traditional Chinese Medicines in Gansu. Lanzhou, China: Gansu People Press; 1974. p 121 – 129.
3. Yunnan Medicinal Material Company. Index Chinese Medicines Resources Yunnanensis. Beijing, China: Beijing Science Press; 1993. p 486 – 490.
4. Yuan X, Wen H, Cui Y, Fan M, Liu Z, Mei L, et al. Phenolics from Lagotis brevituba Maxim. Natural Product Research 2017; 31: 362-366.
5. Calis I, Tasdemir D, Wright AD, Sticher O. Lagotoside. Anew phenylpropanoid glycoside from Lagotis stolonifera. HelvChimActa 1991; 74: 1273 – 1277.
6. Jensen SR, Gotfredsen CH, Zidorn C. Iridoids and phenylethanoids in *Lagotis integrifolia* and Wulfeniopsis amherstiana (Plantaginaceae). BiochemSystEcol 2009; 37: 421-425.
7. WHO. Medicinal plants in Mongolia [accessed on 15 May 2013]. Available at: http://www.wpro.who.int/publications/Medicinal_Plants_in_Mongolia_VF.pdf?ua=1.
8. Yanga XD, Yang LJ, Yanga S, Zhao JF, Zhang HB, Li L. Two New Iridoid Glycosides from Lagotis yunnanensis. ZNaturforsch 2007; 62b: 749 – 752.
9. Markham KR. 13C NMR of flavonoids–II:Flavonoids other then flavone and flavonol aglycones. Tetrahedron 1976; 32: 2607 - 2612.
10. XieYY, Yuan D, Yang JY, Wang LH, Wu CF. Cytotoxic activity of flavonoids from the flowers of Chrysanthemum morifolium on human colon cancer Colon205 cells. J Asian Nat Prod Res 2009; 11: 771–778.
11. Bohm BA. Introduction to Flavonoids. Amsterdam, The Netherlands: Harwood Academic Publishers; 1998. p 206.
12. Markham KR. Carbon-13 NMR studies of flavonoids-III: Naturally occurring flavonoid glycosides and their acylated derivatives. Tetrahidron 1978: 34; 1389-1397.
13. Mabry TJ, Markham KR, Thomas MB. The Systematic Identification of Flavonoids. New York, USA: Springer-Verlag; 1970. p 285-287.
14. Antri AE, Messouri I, Tlemcani RC, Bouktaib M, Alami RE, Bali BE, et al. Flavone Glycosides from Calycoto mevillosa ssp. Intermed Mol 2004: 9; 568– 573.
15. Kim YS, Lee IK, Seok SJ, Yun BS. Chemical Constituents of the Fruiting Bodies of Clitocybenebularis and Their Antifungal Activity. Mycobiology 2008; 36: 110-113.