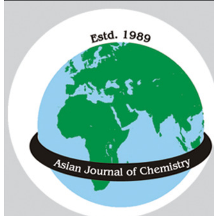
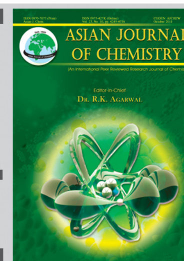


Asian Journal of Chemistry; *Vol. 24, No. 2 (2012), 885-889*

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Chemical Constituents from the Fruits of *Lycium chinense* and Antioxidant Activity

ILL-MIN CHUNG, NAGELLA PRAVEEN, SUN-JIN KIM and ATEEQUE AHMAD*

Department of Applied Life Science, Konkuk University, Seoul 143-701, South Korea

*Corresponding author: Fax: +82 2 4467856; Tel: +82 2 4503730; E-mail: ateeque97@gmail.com

(*Received*: 22 April 2011; *Accepted*: 21 October 2011) AJC-10552

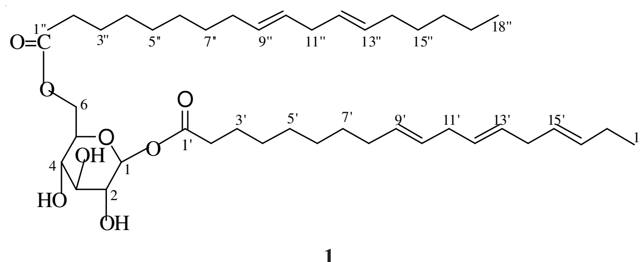
Three compounds glucopyranosyl-1-octadec-9',12',15'-trienoyl-6-octadec-9'',12''-dienoate (**1**), glyceryl-1-octadec-9',12',15'-trienoyl-2-octadec-9'',12''-dienoyl-3-hexadecanoate (**2**), glyceryl-1-octadec-9',12',15'-trienoyl-2-octadec-9''-enoyl-3-eicosanoate (**3**), have been isolated from the ethyl acetate extract of fruits of *Lycium chinense*. Their structures have been elucidated with the help of 600 MHz NMR using 1D spectral method *viz.*: ^{1}H and ^{13}C, aided by FAB MS and IR spectroscopy. The compounds **1-3** are reported for the first time in the fruits of *L. chinense* and data also compared with similar compounds. The compounds from the ethyl acetate extract of the *Lycium* fruits were investigated for scavenging of the diphenylpicrylhydrazyl (DPPH) radical activity and the reducing power and the results demonstrate that the compound **1** has potential as a natural antioxidant whereas the compounds **2** and **3** exhibited low antioxidant activity.

Key Words: *Lycium chinense*, Solanaceae, Fruits, Constituents.

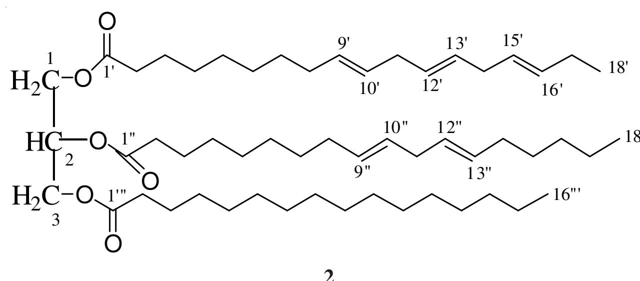
INTRODUCTION

The dried ripe fruits of *Lycium chinense* Miller (Solanaceae), distributed in northeast Asia, specially China, Japan, Korea and Taiwan, have been widely used as a tonic in traditional therapy. Oriental medicines reported to exhibit hypotensive, hypoglycemic and antipyretic activities[1,2]. Several compounds, steroids and alkaloids in this plant are known to various bioactivities[3-6]. Potentially hepatoprotective glycolipid constituents and determination of betain in *L. chinense* fruits have been reported[7,8]. Antimicrobial compounds are also reported from *L. chinense* roots[9]. Specific α-galactosidase inhibitors, N-methylcalystegines structure/activity relationship of calystegines from *L. chinense* have been reported[10]. The *L. chinense* plant is well known in northeast Asia and nowadays has been widely used as a popular functional food with a large variety of beneficial effects, such as antibacterial, antipyretic, cancer, haemostatic, hepatic, kidney, ophthalmic, tonic *etc*. Other useful references of *L. chinense* regarding compounds and activity also reported[11-16].

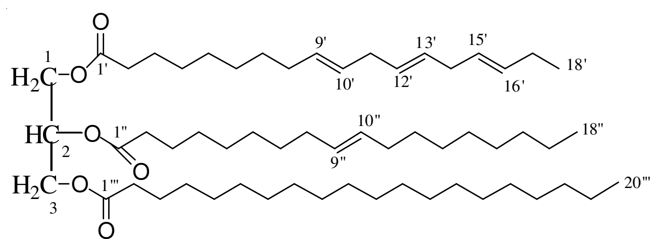
This paper deals with the isolation and structure elucidation of three compounds, glucopyranosyl-1-octadec-9',12',15'-trienoyl-6-octadec-9'',12''-dienoate (**1**), glyceryl-1-octadec-9', 12',15'-trienoyl-2-octadec-9'',12''-dienoyl-3-hexadecanoate (**2**), glyceryl-1-octadec-9',12',15'-trienoyl-2-octadec-9''-enoyl-3-eicosanoate (**3**), on the basis of ^{1}H and ^{13}C NMR, spectroscopic studies, including FAB MS and IR for the first time from the fruits of *L. chinense*. This is the first report of the isolated compounds (**1-3**) along with other known compound β-sitosterol from the fruits of *L. chinense*. Due to significance of fruits of this plant as a medicinal, the work in this area has already been done. Isolated compounds data are compared with previously reported similar compounds like glycolipid[7], glycerogalactolipids[17], glycosidic[18] compounds. The aim of the present investigation is to report compounds (**1-3**) for the first time in the form of natural products from the fruits of *L. chinense* and its antioxidant activity.



1



2



3

Chemical structures of compounds **1-3**

EXPERIMENTAL

All chemicals used were of analytical grade. Hexane, ethyl acetate, methanol, ethanol, water, sulphuric acid and vanillin were purchased from Daejung Chemicals and Metals Co. Ltd., Korea. Pre-coated TLC plates (layer thickness 0.25 mm), silica gel for column chromatography (70-230 mesh ASTM) and LiChroprep RP-18 (40-63 μm) were from Merck (Darmstadt, Germany). Previously isolated authentic standard of β-sitosterol, is available. Both ^{1}H and ^{13}C NMR spectra were obtained on a Bruker Avance 600 high resolution spectrometer operating at 600 and 150 MHz, respectively. This NMR machine was available at Seoul National University (SNU), Seoul, South Korea and all NMR spectra were recorded at SNU. NMR spectra were obtained in deuterated chloroform using tetramethylsilane (TMS) as an internal standard, with chemical shifts expressed in ppm (δ) and coupling constants (*J*) in Hz. FAB MS data were recorded on a JMS-700 (Jeol, Japan) spectrometer instrument which was available at Korea Basic Science Institute (KBSI), Daegu, South Korea. IR spectra were recorded on an Infinity Gold FT-IR (Thermo Mattson, USA) spectrophotometer, which was available at Korea Institute of Science and Technology, Seoul, South Korea.

Extraction of fruits: The fruits of *L. chinense* (3.1 kg) were immersed in methanol (8 L) for 3 days at room temperature and then the supernatant was concentrated under vacuum to yield 230 g of the extract, which was suspended in water and extracted with hexane, ethyl acetate and *n*-butanol successively to produce 20.0 g, 10.1 g and 40 g extract, respectively.

Isolation of the compounds from ethyl acetate extract: The entire ethyl acetate extract was subjected to normal phase column chromatography over silica gel (600 g) to yield 24 fractions (each of 500 mL) with the following eluants: fractions 1-2 with hexane, fractions 3-4 with hexane:EtOAc (9:1), fractions 5-6 with hexane:EtOAc (8:2), fractions 7-8 with hexane:EtOAc (7:3), fractions 9-10 with hexane:EtOAc (6:4), fractions 11-12 with hexane:EtOAc (1:1), fractions 13-14 with hexane:EtOAc (4:6), fractions 15-16 with hexane:EtOAc (3:7), fractions 17-18 with hexane:EtOAc (2:8), fractions 19-20 with hexane:EtOAc (1:9) and fractions 21-24 with EtOAc. All fractions were examined by TLC. Fractions 1-4 were not further separated due to the low amount of the substance. Fractions 5-6 (0.9 g) were crystallized after the purification by column chromatography, yielding β-sitosterol (37 mg) whose identity was confirmed through the comparison of TLC and spectroscopic data with those of an authentic sample. Fractions 17-20 (4.4 g) was re-chromatographed over LiChroprep RP-18 (ODS silica gel; 40-63 μm: 200 g; each fraction 100 mL). The elution was sequentially performed with methanol and water to yield 20 fractions. Fractions 1-4 with water:methanol (8:2), fractions 5-8 with water:methanol (6:4), fractions 9-12 with water:methanol (4:6), fractions 13-16 with water: methanol (2:8), 17-20 with methanol. Fractions 13-16 after rechromatography over Lichroprep RP18 ODS (80 g, each fraction of 50 mL). The elution was sequentially performed with methanol containing 80, 60, 40, 20, 10 and 0 % of water to yield three compounds **1**, **2** and **3**.

Glucopyranosyl-1-octadec-9',12',15'-trienoyl-6-octadec-9'',12''-dienoate (1): Light yellow viscous mass; R_f 0.45; hexane:EtOAc; (1:9); IR (KBr, ν_{max}, cm^{-1}): 3406, 3350, 2924, 2853, 1739, 1722, 1645, 1464, 1335, 1258, 1172, 1025; FAB MS (positive mode) m/z (rel. int.): 703 $[M + H]^+$ ($C_{42}H_{71}O_8$) (3.5), 441 (7.8), 439 (6.3), 422 (8.1), 280 (12.9), 263 (20.6), 261 (34.8); ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR (Table-1).

TABLE-1
^{1}H (600 MHz) AND ^{13}C NMR (150 MHz) NMR DATA FOR COMPOUND **1** IN $CDCl_3$ (*J* IN Hz IN PARENTHESIS)

Position	^{1}H NMR	^{13}C NMR
1	5.26 d (7.8)	103.59
2	3.60 dd (7.8, 6.0)	72.40
3	3.67 m	71.49
4	3.94 m	68.32
5	4.37 m	73.10
6	4.28 d (6.6), 4.26 d (6.3)	62.36
1’	–	173.39
2’	2.78 d (7.2), 2.75 d (7.1)	34.25
3’	1.34 br s	27.17
4’	1.30 br s	29.66
5’	1.25 br s	29.46
6’	1.25 br s	29.32
7’	1.19 br s	29.11
8’	2.05 m	31.89
9’	5.38 m	127.86
10’	5.37 m	128.27
11’	2.32 m	34.25
12’	5.41 m	129.96
13’	5.40 m	131.92
14’	2.29 m	34.10
15’	5.34 m	130.19
16’	5.32 m	127.08
17’	1.61 m	31.49
18’	0.89 t (6.0)	14.25
1’’	–	173.91
2’’	2.80 d (7.5), 2.77d (7.3)	34.10
3’’	1.67 m	27.17
4’’	1.25 br s	29.66
5’’	1.25 br s	29.46
6’’	1.25 br s	29.32
7’’	1.19 br s	29.11
8’’	2.22 m	25.59
9’’	5.35 m	127.73
10’’	5.33 m	128.19
11’’	2.41 m	34.21
12’’	5.37 m	128.05
13’’	5.33 m	127.09
14’’	2.18 m	24.85
15’’	1.19 br s	22.66
16’’	1.25 br s	22.54
17’’	1.30 br s	20.53
18’’	0.97 t (7.2)	14.09

Coupling constant in hertz are provided in parenthesis.

Glyceryl-1-octadec-9',12',15'-trienoyl-2-octadec-9'',12''-dienoyl-3-hexadecanoate (2): Dark yellow semi-solid; R_f: 0.43; hexane:EtOAc; (1:9); IR (KBr, ν_{max}, cm^{-1}): 2925, 2854, 1725, 1640, 1463, 1350, 1281, 1135, 943, 723; ^1H NMR ($CDCl_3$): δ 5.38 (1H, m, H-12'), 5.36 (1H, m, H-13'), 5.35 (1H, m, H-10'), 5.34 (1H, m, H-12'' and H-16'), 5.32 ((1H, m, H-10''), 5.30 (1H, m, H-9'), 5.28 (1H, m, H-15'), 5.27 (1H, m, H-9''), 5.26 (1H, m, H-13''), 4.13 (1H, m, H-3), 3.94 (1H, d, *J* = 4.2 Hz, H_2-1a), 3.92 (1H, d, *J* = 5.4 Hz, H_2-1b), 3.70 (1H, d, *J* = 3.6 Hz, H_2-3a), 3.68 (1H, d, *J* = 3.6 Hz, H_2-3b), 2.79 (2H, d, *J* = 6.0 Hz, H_2-2'), 2.76 (2H, d, *J* = 7.2 Hz, H_2-2''), 2.74 (2H, d, *J* = 6.6 Hz, H_2-2'''), 2.32 (2H, m, H_2-2'''), 2.30 (2H, m, H_2-14'), 2.07 (4H, m, H_2-11', H_2-11''), 2.04 (4H, m, H_2-8', H_2-8''), 2.01 (2H, m, H2-17'), 1.98 (2H, m, H_2-14''), 1.61 (6H, m, 3x CH_2), 1.38 (2H, m, CH_2), 1.35 (2H, m, CH_2), 1.33 (2H, m, CH_2), 1.30 (2H, m, CH_2), 1.29 (6H, br s, 3 x CH_2), 1.28 (4H, br s, 2 x CH_2), 1.24 (10H, br s, 5 x CH_2), 0.95 (3H, t, *J* = 6.8 Hz, Me-18'), 0.88 (3H, t, *J* = 6.6 Hz, Me-18''), 0.86 (3H, t, *J* = 7.2 Hz, Me-18'''); ^{13}C NMR ($CDCl_3$): 179.75 (C-1'), 174.12 (C-1''), 172.45 (C-1'''), 131.74 (C-15'), 129.99 (C-12'), 129.81 (C-12''), 129.55 (C-10''), 128.11 (C-16'), 128.08 (C-10'), 127.93 (C-9'), 127.77 (C-9''), 127.63 (C-13''), 126.99 (C-13'), 64.81 (C-1), 70.12 (C-2), 63.13 (C-3), 44.12 (C-2'), 42.78 (C-2''), 35.60 (C-2'''), 33.98 (C-11'), 31.82 (C-14'), 31.41 (C-11''), 29.59 (CH_2), 29.56 (CH_2), 29.47 (CH_2), 29.35 (CH_2), 29.26 (CH_2), 29.23 (CH_2), 29.16 (CH_2), 29.04 (20 x CH_2), 28.97 (CH_2), 29.94 (CH_2), 27.07 (CH_2), 25.50 (CH_2), 25.40 (CH_2), 24.73 (CH_2), 24.57 (CH_2), 22.58 (CH_2), 22.46 (CH_2), 20.42 (CH_2), 14.12 (Me-18'), 13.97 (Me-18''), 13.92 (Me-16'''); FAB MS (positive mode) m/z 853 $[M + H]^+$ ($C_{55}H_{97}O_6$) (1.8), 597 (10.8), 577 (6.3), 279 (93.6), 277 (34.0), 263 (58.2), 261(17.5), 255 (10.8), 239 (53.1).

Glyceryl-1-octadec-9',12',15'-trienoyl-2-octadec-9''-enoyl-3-eicosanoate (3): Light yellow semi-solid R_f: 0.39; hexane:EtOAc; 1:9); IR (KBr, ν_{max}, cm^{-1}): 2928, 2654, 1741, 1645, 1463, 1378, 1260, 1171, 724; ^1H NMR ($CDCl_3$): δ 5.39 (1H, m, H-12'), 5.37 (1H, m, H-13'), 5.35 (2H, m, H-9', H-15'), 5.33 (1H, m, H-9''), 5.31 ((1H, m, H-16'), 5.07 (1H, m, H-10''), 4.18 (1H, m, H-2), 4.14 (2H, m, H_2-1), 4.07 (2H, m, H_2-3), 2.86 (2H, m, H_2-2'), 2.79 (2H, m, H_2-2''), 2.76 (2H, m, H_2-2'''), 2.33 (2H, m, H_2-11'), 2.30 (2H, m, H_2-14'), 2.08 (2H, m, H_2-8'), 2.05 (2H, m, H_2-16'), 2.03 (2H, m, H_2-8?'), 1.99 (2H, m, H_2-11''), 1.61 (6H, m, 3 x CH_2), 1.36 (4H, m, 2 x CH_2), 1.35 (4H, m, 2 x CH_2), 1.32 (6H, m, 3 x CH_2), 1.30 (26H, br s, 13 x CH_2), 1.25 (20H, br s, 10 x CH_2), 0.95 (3H, t, *J* = 6.8 Hz, Me-18'), 0.89 (3H, t, *J* = 6.5 Hz, Me-18''), 0.87 (3H, t, *J* = 6.6 Hz, Me-20'''); ^{13}C NMR ($CDCl_3$): δ 173.81 (C-1'), 173.67 (C-1''), 173.34 (C-1'''), 131.87 (C-12'), 130.13 (C-13'), 129.92 (C-10'), 128.21 (C-15'), 128.01 (C-9'), 127.83 (C-9''), 127.69 (C-16'), 127.05 (C-10''), 64.95 (C-1), 68.22 (C-2), 61.37 (C-3), 34.20 (C-2'), 34.02 (C-2'', C - 2'''), 31.86 (C-11'), 31.46 (C-13', C-8', C-8'', C-16', C-11''), 29.63 (CH_2), 29.59 (CH_2), 29.53 (CH_2), 29.40 (CH_2), 29.28 (CH_2), 29.09 (7 x CH_2), 29.03 (8 x CH_2), 27.13 (6 x CH_2), 25.56 (CH_2), 25.46 (CH_2), 24.80 (CH_2), 22.62 (CH_2), 22.51 (2 x CH_2), 20.48 (CH_2), 14.20 (Me-18'), 14.05 (Me-18''), 14.01 (Me-20'''); FAB MS (positive mode) m/z 911 $[M + H]^+$ ($C_{59}H_{107}O_6$) (2.1), 647 (2.8), 645 (3.9), 599 (45.8), 311 (19.2), 295 (8.0), 281 (21.6), 279 (8.5), 265 (7.3), 263 (42.5).

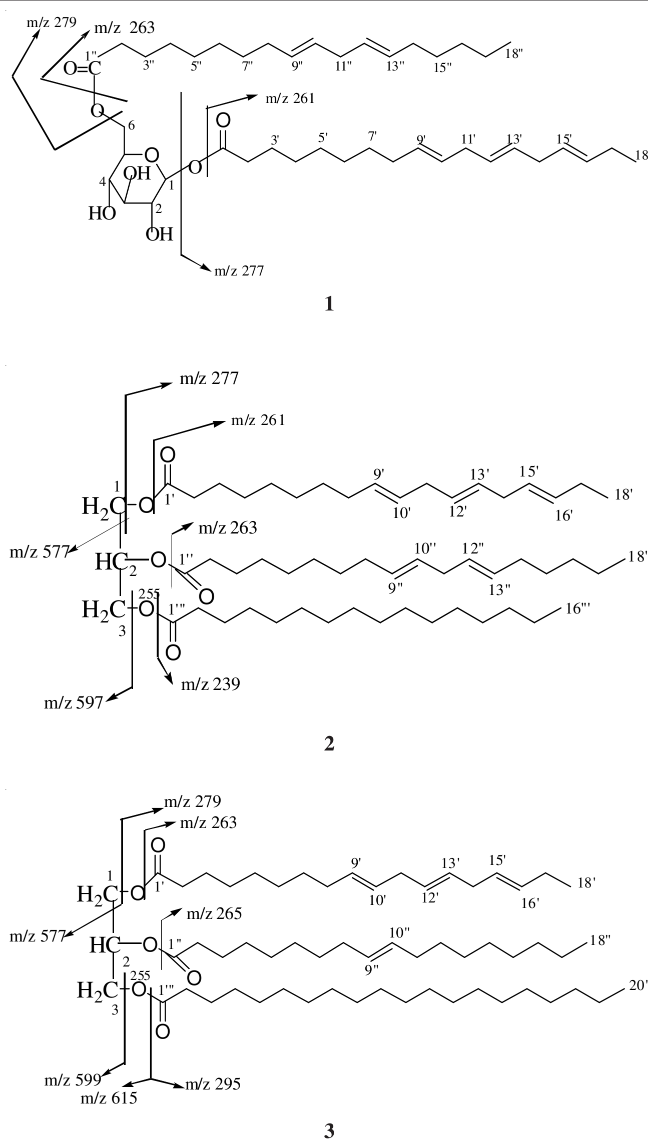


Fig. 1. Mass fragmentation pattern of compounds **1-3**

Free radical scavenging activity: The antioxidant activity of the different compounds (**1**, **2** and **3**), based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl ($DPPH^-$) free radical, was determined by the method described by Katerere and Eloff[19]. The different concentrations (100, 200, 300, 400, 500 and 1000 μg) of the tested samples (0.2 mL; compounds and tocopherol) were taken in different test tubes with 4 mL of a 0.006 % MeOH solution of $DPPH^-$. Water (0.2 mL) in place of the compound was used as control. Absorbance at 517 nm was determined after 0.5 h. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula, Radical scavenging activity (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the compound/standard.

Reducing power: The reducing power of the Lycium fruit compounds was determined according to the method of Oyaizu[20]. Different extracts of concentration (100, 200, 300, 400, 500 and 1000 μg) in 1 ml of distilled water and was mixed with phosphate buffer (2.5 mL, 0.2 M/L, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1 %). The mixture was

incubated at 50 ºC for 20 min. A portion (2.5 mL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 1000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1 %) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. All analysis were run in triplicate and averaged.

RESULTS AND DISCUSSION

The ethyl acetate extract of *L. chinense* fruits was chromatographed on a SiO_2 gel using hexane-ethyl acetate and then were further subjected to Lichroprep RP-18 (ODS silica gel) with water and methanol to yield three compounds (**1-3**).

Compound **1**, was obtained as a light yellow viscous mass from hexane-ethyl acetate (1:9) eluants. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3406, 3350, 3395 cm^{-1}), ester function (1739 cm^{-1}) and unsaturation (1645, 14641, 335, 1258 cm^{-1}). The FAB mass and ^{13}C NMR spectral data led to established molecular formula ion peak at m/z 702 consistent with the molecular formula $C_{42}H_{70}O_8$ of a monoglucoside esterified with two fatty acids. The ion peaks arising at m/z 441, 439, 422, 280, 261, indicated that the fatty acids attached to the glucose moiety were linoleic and linolenic acids.

The ^1H NMR spectrum of **1** showed a one proton doublet at δ 5.26 assigned to anomeric H-1 proton. One proton doublet at δ 3.60 (dd, 7.8, 6.0) was assigned for H-2. Two one-proton doublets δ 4.28 (J = 6.6 Hz) and 4.26 (J = 6.3 Hz) were attributed to oxygenated methylene protons H-6. The other protons of glucose moiety appeared as multiplets as δ 3.67, 3.94, 4.37 all integrated for one protons. Six protons signals at δ 2.78 (d, J = 7.2 Hz) and 2.75 (d, J = 7.1 Hz), 1.34, 1.30, 1.25, 1.19 were assigned for H-2',3',4',5',6',7'. Several protons appeared as multiplets as δ 2.05, 5.38, 5.37, 2.32, 5.41, 5.40, 2.29, 5.34, 5.32 and 1.61 for protons H-8' to H-17'. The ^{13}C NMR spectrum of **1** exhibited two deshielded carbon δ 173.39, 173.91 assigned to ester carbonyl carbon C-1' and C-1''. The carbon signals for the sugar moiety appeared between δ 103.59 - 68.23. The deshielding of the carbon signal of the oxygenated of the methylene group from δ 62.36 supported the presence of one of the fatty acid moiety at this group. The deshielded carbon signals at δ 127.09-130.19 were associated with the vinylic carbons of the fatty acids. More details of proton and carbon assignments are showed in Table-1. On the basis of spectral data analysis, the structure of **1** has been established as glucopyranosyl-1-octadec-9', 12', 15'-trienoyl-6-octadec-9'',12''-dienoate.

Compound **2**, was obtained as a dark yellow semi-solid from hexane-ethyl acetate (1:9) eluants. Its IR spectrum showed characteristic absorption bands for 2925, 2845 cm^{-1}, ester function (1725 cm^{-1}) and double bonds (1640, 1463 cm^{-1}). The FAB mass and ^{13}C NMR spectral data led to established molecular formula ion peak at m/z 852 consistent with the molecular formula $C_{55}H_{96}O_6$ of a glycerol esterified with two unsaturated and one saturated fatty acids. The ion peaks arising at m/z 597, 577, 279, 277, 263, 261, 255, 239 indicated that the fatty acids was linked to a glycerol unit.

The ^1H NMR spectrum of **2** showed a multiplets between δ 4.13-5.38 assigned to H-12', H-13', H-10', H-12'', H-16', H-10'', H-9', H-15', H-9'', H-13'' and H-3. Two protons double doublet at δ 3.94 and 3.92 (dd, J = 4.2, 5.4 Hz) was assigned for H_2-1a and H2-1b. The ^{13}C NMR spectrum of **2** exhibited three deshielded carbon at δ 179.75, (C-1'), 174.12 (C-1'') and 174.45 (C-1''') were assigned to ester carbonyl. More details of proton and carbon assignments are given in experimental. On the basis of spectral data analysis, the structure of **2** has been established as glyceryl-1-octadec-9',12',15'-trienoyl-2-octadec-9'',12''-dienoyl-3-hexadecanoate.

Compound **3**, was obtained as a light yellow semi-solid from hexane-ethyl acetate (1:9) eluants. Its IR spectrum showed characteristic absorption bands for 2928, 2654 cm^{-1}), ester function (1739 cm^{-1}) and double bonds (1645, 1463 cm^{-1}). The FAB mass and ^{13}C NMR spectral data led to established molecular formula ion peak at m/z 910 consistent with the molecular formula $C_{59}H_{106}O_6$ of a glycerol esterified with two unsaturated and one saturated fatty acids. The ion peaks arising at m/z 647, 645, 615, 599, 311, 295, 281, 279, 265, 263 indicated that the fatty acid was linked to a glycerol unit. The ^1H and ^{13}C NMR of compound **3** discussion is as same as compound **2**. On the basis of spectral data analysis, the structure of **3** has been established as glyceryl-1-octadec-9',12',15'-trienoyl-2-octadec-9''-enoyl-3-eicosanoate.

Antioxidant activity

Free radical scavenging activity: The free radical-scavenging activity of the polysaccharides was tested through DPPH- method[19] and the results were compared with tocopherol. DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. The method is based on the reduction of methanolic $DPPH^-$ solution in the presence of a hydrogen donating antioxidant, due to the formation of the non-radical form DPPH-H by the reaction. The extract was able to reduce the stable radical DPPH- to the yellow-coloured diphenylpicrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (*e.g.*, hydroquinone, pyrogallol, gallic acid) and aromatic amines (*e.g.*, *p*-phenylene diamine, *p*-aminophenol), reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability[21]. Of the different compounds from the ethyl acetate extract from the lycium fruits, compound **1** exhibited the highest activity of 66 % at 1000 μg concentration when compared with other compounds (Table-2). The compounds **2** and **3** were very weak to reduce the stable radical $DPPH^-$ to the yellow coloured diphenylpicrylhydrazine. The DPPH activity of tocopherol showed higher degree of free radical-scavenging activity than that of the compounds at very low concentration points. Similar to our results reported[22] that the polysaccharide fraction from the fruits of *Lycium barbarum* exhibited a weak DPPH activity. This is similar to other studies wherein they have reported that only 0.3 mg/mL tocopherol, 0.23 mg/mL BHT and 0.1 mg BHA exhibited a free radical scavenging activity equivalent to 3.9 mg/mL of red bean and 10 mg/mL of sesame coat extract[23,24].

Reducing power: Antioxidant effect exponentially increases as a function of the development of the reducing power, indicating

TABLE-2
RADICAL SCAVENGING ACTIVITY OF THREE COMPOUNDS BY DPPH METHOD

Compounds	Inhibition (%)					
	100 µg	200 µg	300 µg	400 µg	500 µg	1000 µg
1	15.8	29.4	39.5	46.5	51.5	66.2
2	8.1	8.9	12.0	14.3	16.3	18.6
3	1.9	3.1	8.5	9.7	10.5	18.2

that the antioxidant properties are concomitant with the development of reducing power[25].Tanaka *et al*.[26] reported the reducing power of tannins from medicinal plants prevents liver injury by inhibiting formation of lipid peroxides. Reductones are believed not only to react directly with peroxides but also prevent peroxide formation by reacting with certain precursors. As seen in Fig. 2 reducing power of the different compounds from the ethyl acetate extract of Lycium fruit increased with increasing concentration from 100-1000 µg. Reducing power of the compounds from the ethyl acetate extract of lycium fruits followed the order: **1** < **3** < **2**. The activity of tocopherol was pronouncedly higher than the test samples. This is in line with the observations of several other workers wherein the reducing power of BHT and tocopherol[23] and BHA[27] was higher than the extracts. In the present study, though the compounds from the ethyl acetate extract of lycium fruits exhibited a moderate reducing power they did have an activity that reveals that the compounds from the ethyl acetate extract of lycium fruit are electron donors and can react with free radicals and convert them to stable products thus terminating the free radical chain reactions.

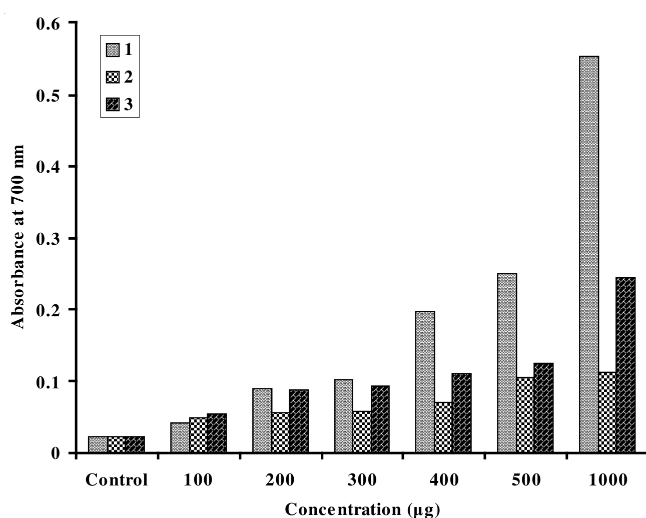


Fig. 2. Reducing power of compounds **1-3**

ACKNOWLEDGEMENTS

This work was carried out with the support of Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ 906938), Rural Development Administration, Republic of Korea.

REFERENCES

1. S. Funayama, K. Yoshida, C. Konno and H. Hikino, *Tetrahedron Lett*., **21**, 1355 (1980).
2. J. Yamahara, M. Kim, T. Sawada and H. Fujimua, *Shoyakugaku Zasshi*, **18**, 33 (1964).
3. A. Sannai, T. Fujimori and K. Kato, *Agric. Biol. Chem*., **47**, 2397 (1983).
4. S.Y. Kim, Y. Choi, H. Huh, J. Kim, Y.C. Kim and H.S. Lee, *J. Nat. Prod*., **60**, 274 (1997).
5. M. Noguchi, K. Mochida, M. Shingu, K. Kozuka and K. Fujitani, *Chem. Pharm. Bull*., **32**, 3584 (1984).
6. L.L. Yanfg, K.Y. Yen, Y. Kiso and H. Kikino, *J. Ethnopharmacol*., **19**, 103 (1987).
7. K. Jung, Y.W. Chin, Y.C. Kim and J. Kim, *Arch. Pharm. Res*., **28**, 1381 (2005).
8. Y.G. Shin, K.H. Cho, J.M. Kim, M.K. Park and J.H. Park, *J. Chromatogr. A*, **857**, 331 (1999).
9. D.G. Lee, H. J. Jung and E.R. Woo, *Arch. Pharm. Res*., **28**, 1031 (2005).
10. T. Itoh, T. Tamura and T. Matsumoto, *Phytochemistry*, **17**, 971 (1978).
11. Y.W. Chin, S.W. Lim, S.H. Kim, D.Y. Sin, Y.G. Sur, Y. Kim, Y.C. Kim and J. Kim, *Bioorg. Biomed. Chem. Lett*., **13**, 79 (2003).
12. A. Sannai, T. Fujimori and K. Kato, *Phytochemisty*, **21**, 2986 (1982).
13. K.H. Miean and S. Mohamed, *J. Agric. Food Chem*., **49**, 3106 (2001).
14. S.J. Han, H.H. Lee, I.S. Lee, Y.S. Moon and E.R. Woo, *Arch. Pharm. Res*., **25**, 433 (2002).
15. P. Weller and D.E. Breithaupt, *J. Agric. Food Chem*., **51**, 7044 (2003).
16. J.Z. Dong, D.Y. Lu and Y. Wang, *Plant Foods Hum. Nutr*., 64, 199 (2009).
17. Z. Gao, Z. Ali and I.A. Khan, *Phytochemistry*, **69**, 2856 (2008).
18. I.M. Chung, H.Y. Park, S.C. Chun, J.J. Kim and A. Ahmad, *Chem. Nat. Compd*., **43**, 417 (2007).
19. D.R. Katerere and J.N. Eloff, *Phytother Res*., **19**, 779 (2005).
20. M. Oyaizu, *Japan. J. Nutr*., **44**, 307 (1986).
21. M.S. Blois, *Nature*, **181**, 1199 (1958).
22. X.M. Li, X.L. Li and A.G. Zhou, *Eur. Polym. J*., **43**, 488 (2007).
23. L.W. Chang, W.J. Yen, S.C. Huang and P.D. Duh, *Food Chem*., **78**, 347 (2002).
24. Y.C. Chung, C.T. Chang, W.W. Chao, C.F. Lin and S.T. Chu, *J Agric. Food Chem*., **50**, 2454 (2002).
25. T. Okuda, Y. Kimura, T. Yoshida, T. Hatano, H. Okuda and S. Arichi, *Chem. Pharm. Bull*., **31**, 1625 (1983).
26. M. Tanaka, C.W. Kuie, Y. Nagashima and T. Taguchi, *Nippon Suisan Gakkaishi*, **54**, 1409 (1988).
27. M. Oktay, I. Culcin and O.I. Kufrevioglu, *Lebensm. Wissens. Technol*., **36**, 263 (2003).