약학석사학위논문

개옻나무뿌리의 위암 세포독성 성분

Cytotoxic Constituents of *Rhus trichocarpa* Roots on Human Gastric Adenocarcinoma AGS Cells

2012 년 8 월

서울대학교 대학원
약학과 생약학전공
최 지 훈

Abstract

Gastric cancer is a major health issue and the second cause of cancer-related deaths worldwide. For its therapy, *Rhus trichocarpa* (Anacardiaceae) has been used in traditional East Asia medicine, although the mechanism for the biological activity remains to be clarified. In Korean folk medicine, it has been used for the treatment of anti-inflammatory and anti-cancer, and urushiols, flavonoids and polyphenolics have been reported from *Rhus* species. This study aims to investigate the cytotoxic constituents of *Rhus trichocarpa* roots on AGS human gastric cells. It was found that an 80% MeOH extract of roots of *Rhus trichocarpa* showed cytotoxic effects on AGS cells proliferation. In the present study, its bioassay-guided fractionation resulted in the isolation of thirteen compounds. The isolated compounds were identified as 3-methoxy semialactone (**1**), 4-(2,6-dihydroxy-4-methoxyphenyl)-4-oxobutanoic acid (**2**), gallic acid (**3**), 4-*O*-methylgallic acid (**4**), pentagalloyl glucose (**5**), (-)-fustin (**6**), (+)-taxifolin (**7**), 3,3',5,5',7-pentahydroxyflavanonol (**8**), fisetin (**9**), 3-methoxy-7,3',4'-trihydroxyflavone (**10**), sulfuretin (**11**), 5,7-dihydroxy-4H-chromen-4-one (**12**) and Isolariciresinol (**13**) respectively. Compounds **3** and **11** were newly reported in nature. Among the isolated compounds, compounds **4, 5** and **9** significantly showed the cytotoxic effect in on Human Gastric Adenocarcinoma AGS Cells

Student ID Number: 2010-23670

Contents

List of Schemes

List of Tables

List of Figures

List of Abbreviations

$[\alpha]_D$: specific rotation

MeCN: acetonitrile

n-BuOH: *n*-butanol

CC: column chromatography

$CHCl_3$: chloroform

COSY: correlation spectroscopy

d: doublet

dd: doublet of doublet

DMSO: dimethylsulfoxide

EI-MS: electron impact mass spectrometry

ESI-MS: electron spray impact mass spectroscopy

EtOAc: ethyl acetate

FAB-MS: fast atom bombardment mass spectrometry

HMBC: heteronuclear multi-bond correlation

HMQC: heteronuclear multiple quantum correlation

Hz: hertz

IC_{50}: the half maximal inhibitory concentration

m: multiplet

MeOH: methanol

NMR: nuclear magnetic resonance

NOESY: nuclear Overhauser enhancement spectroscopy

R_f: migration distance relative to solvent front in TLC

RP-HPLC: reverse phase high performance liquid chromatography

s: singlet

t: triplet

TLC: thin layer chromatography

UV: ultraviolet absorption spectroscopy

I. Introdution

As the leading cause of death, cancer, complex disease having various biological capabilities such as sustaining proliferative signaling, activating invasion and metastasis, and tumor-promoting inflammation etc., is a major public health problem in Korea and many other parts of the world (Statistics Korea, 2011). Also, as the current cancer statistics, more than 180,000 new cancer cases are originated every year in Korea, and one of four deaths is due to it (Jung, Park, Kong, et al., 2012). Especially, gastric cancer incidence has highly occurred among Asian populations, and this cancer is the second most frequent causes of death from cancer (Table 1) (Jung, Park, Won, et al., 2012). It is estimated 20.8% of all malignancies, and 24.5% in males and 15.8% in females are occurred in the Korean population (Shin, Jung, Won, & Park, 2004). The mechanism for gastric carcinogenesis is exactly not studied still, but it is reported that some elements such as *Helicobacter pylori* infection, genetic and environmental factors, and dietary habits are related with the cancer (Yuasa, 2003).

AGS cell line, a human gastric adenocarcinoma, was established as one of clones derived from a single biopsy specimen of an untreated human adenocarcinoma of the stomach. Because AGS cell has the gastric epithelial and unique characteristics, it is highly susceptible to antineoplastic substances and antitumor therapeutic recombinant viruses, and also, this cell line has been used in research in the fields of oncology, virology, bacteriology and immunology (Ooi et al., 2011).

Anacardiaceae, especially, *Rhus trichocarpa* and *Rhus verniciflua* Stokes, has

1

been used as the folk medicine. It usually contains urushiol, flavonoids and tannins, etc. Recently studies have shown that flavonoids, the major compounds from *R. verniciflua* Stokes have antioxidative, antiproliferative and apoptotic activities on various tumor cell lines including human lymphoma, breast cancer, osteosarcoma, and transformed hepatoma cells (Son et al., 2005). Because of the strong allergenic component, urushiol, which causes serious dermatitis, the clinical application of this Anacardiaceae family has been limited, but it has been traditionally used for the therapy of gastritis, stomach cancer and atherosclerosis in Eastern Asian including Korea, Japan and China (J. C. Lee, Lim, & Jang, 2002). Therefore, in this study, cytotoxic constituents of *R. trichocarpa* roots were identified, and the AGS human gastric cancer cell line was used for this investigation.

Table 1. Estimated new cancer cases & deaths during 2012 in Korea

Sites	Estimated new cases			Estimated deaths		
	Both genders	Male	Female	Both genders	Male	Female
All sites	234,727	118,304	116,423	73,313	45,356	27,957
Lip, oral cavity, and pharynx	2,801	2,102	699	1,024	754	270
Esophagus	2,262	2,075	187	1,223	1,139	84
Stomach	32,455	22,054	10,401	8,685	5,630	3,055
Colon and rectum	32,215	19,669	12,546	8,195	4,648	3,547
Liver	16,910	12,585	4,325	10,973	8,186	2,787
Gallbladder[a)]	5,481	2,697	2,784	3,884	1,879	2,005
Pancreas	5,396	2,911	2,485	4,663	2,445	2,218
Larynx	1,077	1,036	41	226	210	16
Lung	22,737	15,991	6,746	16,168	11,819	4,349
Breast	16,436	63	16,373	2,146	16	2,130
Cervix uteri	3,292	-	3,292	819	-	19
Corpus uteri	2,085	-	2,085	259	-	259
Ovary	2,124	-	2,124	987	-	987
Prostate	11,016	11,016	-	1,540	1,540	-
Testis	215	215	-	18	18	-
Kidney	4,310	2,949	1,361	846	558	288
Bladder	3,969	3,185	784	1,026	737	289
Brain and CNS	1,837	975	862	1,061	575	486
Thyroid	46,021	7,158	38,863	360	100	260
Hodgkin lymphoma	253	160	93	41	26	15
Non-Hodgkin lymphoma	4,506	2,502	2,004	1,493	865	628
Multiple myeloma	1,236	648	588	841	424	417
Leukemia	2,885	1,609	1,276	1,676	964	712
Other and ill defined	13,208	6,704	6,504	5,159	2,823	2,336

II. Materials and Methods

1. Materials

1.1. Plant material

R. trichocarpa roots were collected at Gimhae, Korea, in December 2010 and dried in the Medicinal herb garden, College of Pharmacy, Seoul National University.

1.2. Reagents

1.2.1. Reagents for column chromatography

Silica gel: Kiesgel 60 (40-63 μM, 230-400 mesh, Art. 9385, Merck, Germany)

Sephadex LH-20: bead size 25-100 μm, Pharmacia, Sweden

HP-20: bead size 250-600 μm, Mitsubishi Chemical Co., Japan

Analytical TLC: Kiesgel 60 F_{254}, Art. 5715, Merck, Germany

First grade solvent for extraction, fractional and isolation

: Dae Jung Pure chemical Eng. Co. Ltd., Korea

HPLC grade solvent: Fisher Scientific, Pittsburgh, PA, U.S.A.

1.2.2. Reagents for cell cultures

RPMI-1640 was purchased from Thermo Science (Logan, UT, U.S.A.). Penicillin-streptomycin, and 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Fetal bovine serum (FBS) was obtained from Hyclone (Logan, UT, U.S.A.). Multi well culture plate and cell culture dishes were purchased from Corning (New York, NY, U.S.A.)

1.3. Equipments

Analytical balance: Mettler AE 50, Switzerland

Autoclave: Sanyo MLS 3000, Japan

Centrifuge: Effendroff, Germany

Drying Oven: CO-2D-1S, Wooju Sci. Co., Korea

Elisa reader: Molecular Devices E_{max}, U.S.A.

Evaporator: EYELA NE, Japan

ESI-MS: Finnigan LCQ ion-trap mass, USA

FAB-MS: JEOL JMS AX 505 WA Spectrometer, Japan

Fluorometer: SPECTRAFlour, Tecam, Austria

Freeze-dryer: DURA-DRY, Fts system Inc., U.S.A.

HPLC: Gilson 321 pump, UV/Vis-151 detector, USA

NMR: JEOL LA 300 Spectrometer (300 MHz), Japan

Bruker GPX 400 Spectrometer (400 MHz), Germany

Bruker AMX 500 Spectrometer (500 MHz), Germany

Phase contrast inverted microscope: Olympus CK2-Japan

Polarimeter: JASCO, DIP-100, Japan

Rotary evaporator: Shimadzu 2101, Japan

Sonicator: Branson 5200, UK

UV spectrometer: Shimadzu UV-2101 Spectrophotometer, Japan

UV lamp: UVP UVGL-58, U.S.A.

Spectrofluorometer: JASCO, FT-6500, Japan

2. Methods

2.1. Extraction and fractionation of *R. trichocarpa*

Dried roots (6.8kg) of *R. trichocarpa* were extracted with 80 % MeOH in an ultrasonic apparatus. After removal of the solvent *in vacuo,* the total extract (819.2g) was suspended in H_2O and partitioned into *n-hexane* fraction (1.9g), EtOAc fraction (346.7g) and *n*-BuOH fraction (73.7g), respectively (Scheme 1).
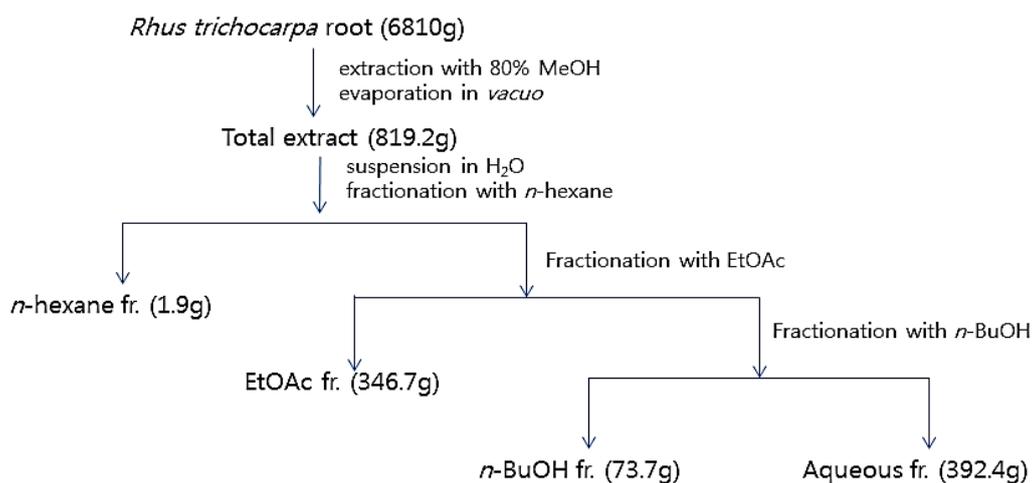
Scheme 1. Extraction and fractionation of *R. trichocarpa* root

2.2. Isolation of the compounds from the EtOAc fraction of *R. trichocarpa root*

Among these fractions, *n-hexane* and EtOAc fractions showed the cytotoxic effect on AGS cells. EtOAc fraction was subjected to silica gel column chromatography (CC) eluted with mixtures of chloroform-methanol (from 30:1 to 1:1), MeOH; v/v to yield sixteen fractions (E1-E16) (Scheme 2). Thirteen compounds including new compounds **1** and **2** were isolated from EtOAc fraction of *R. trichocarpa roots.*

EtOAc fr. (289.9g)

A (CM 30:1)

E5 — A (HE 7:1->3:1->CM 15:1) — Compound1 (11.4mg)

E6 — A (CM 100:1->1:1) — E6-5 — B (MeOH) — Compound12 (3.4mg)

E11&12 — C (MW 1:9->10:0)
- Compound6 (113.7mg) / Compound7 (18.1mg) / Compound9 (93.3mg)
- E11-12 — B (MeOH) — Compound8 (7.1mg) / Compound13 (13.4mg)
- E11-14 — B (MeOH) — E11-14-5 — D (60%MeOH) — Compound10 (13.0mg)
- E11-16 — B (MeOH) — Compound11 (5.9mg)

E16 — C (MW 2:8->10:0)
- Compound3 (0.3g) / Compound5 (2.5g)
- E16-4 — C (MW 2:8->10:0) — Compound2 (3.4mg)
- E16-9 — C (MW 2:8->10:0) — Compound4 (8.9mg)

A: Silica gel C.C
B: Sephadex LH-20
C: MPLC RP$_{18}$
D: HPLC RP$_{18}$

Scheme 2. Isolation of the compounds from the EtOAc fraction of *R. trichocarpa* root

2.2.1. Isolation of compound **1**

Compound **1** (11.4mg) was isolated from E5 using the silica gel CC eluted with mixtures of *n*-hexane-EtOAc (= 7:1, 3:1), CHCl$_3$-MeOH (= 15:1), MeOH. The E5 was further divided into twenty three sub-fractions (E5-1 to E5-23), and E5-7 was compound **1**.

White powder

C$_{31}$H$_{46}$O$_4$

$[\alpha]^{25}_D$ +62.7 (c 1.02, CHCl$_3$)

HR-FAB-MS: *m/z* 482.3396 [M]$^+$ (calcd. for. 482.3396)

^{1}H-NMR (500MHz, CDCl$_3$): δ 6.57 (1H, d, *J* = 5.9 Hz, H-24), 5.24 (1H, s, H-21b), 5.20 (1H, s, H-21a), 4.72 (1H, dd, *J* = 12.4, 3.3 Hz, H-22), 4.22 (1H, dd, *J* = 8.8, 2.4 Hz, H-19b), 3.69 (1H, d, *J* = 8.8, H-19a), 3.23 (3H, s, H-31), 2.93 (1H, dd, *J* = 18.9, 9.2, H-17), 2.50 (1H, m, H-23b), 2.30 (1H, m, H-23a), 2.10 (3H, m, H-2b, H-16b, H-13), 1.94 (1H, m, H-1b), 1.93 (3H, s, H-27), 1.67 (6H, m, H-1a, H-6b, H-11, H-12b, H-16a), 1.45 (4H, m, H-6a, 9, 15), 1.24 (2H, m, H-15), 1.21 (1H, m, H-12a), 1.13 (1H, m, H-5), 1.03 (1H, m, H-2a), 0.95 (6H, s, H-29, 28), 0.87 (3H, s, H-30), 0. 86 (3H, s, H-18) ppm

^{13}C-NMR (125MHz, CDCl$_3$): δ 166.0 (C-26), 149.3 (C-20), 139.1 (C-24), 128.4 (C-25), 113.4 (C-21), 100.6 (C-3), 80.8 (C-22), 67.9 (C-19), 50.3 (C-5), 49.4 (C-14), 49.3 (C-31), 45.3 (C-9), 45.0 (C-13), 41.0 (C-10), 40.1 (C-17), 39.8 (C-8), 35.4 (C-2), 35.0 (C-4), 33.2 (C-15), 33.1 (C-7), 30.1 (C-16), 29.1 (C-23), 26.7 (C-28), 25.4 (C-12), 23.1

(C-1), 22.5 (C-11), 19.8 (C-6), 18.6 (C-29), 17.0 (C-27), 16.6 (C-30), 15.4 (C-18) ppm

2.2.2. Isolation of compound **2**

E16 was subjected to MPLC RP_{18} gradient eluting with mixtures of MeOH-H_2O (= 2:8, 4:6, 6:4, 8:2 and 10:0, respectively), and the fraction was further divided into nine sub-fractions (E16-1 to E16-9). Compound **2** (3.4mg) from E16-4 was isolated using MPLC RP_{18} eluted with mixtures of MeOH-H_2O (= 2:8, 4:6 and 10:0, respectively).

Yellow amorphous powder

$C_{11}H_{12}O_6$

$[\alpha]^{25}_D$ -41.2 (c 0.1, MeOH)

HR-FAB-MS (positive mode): m/z 241.0717 $[M+H]^+$ (calcd. for. 241.0712)

UVλ_{max} : 256, 296 nm

^{1}H-NMR (500MHz, CD_3OD): δ 5.92 (2H, s, H-3', 5'), 3.76 (3H, s, H-7'), 3.39 (2H, t, H-3), 2.61 (2H, t, H-2) ppm

^{13}C-NMR (125MHz, CD_3OD): δ 205.8 (C-4), 178..0 (C-1), 168.3 (C-4'), 166.4 (C-2',6'), 106.8 (C-1'), 95.1 (C-3',5'), 56.6 (C-7), 40.7 (C-3), 30.0 (C-2) ppm

2.2.3. Isolation of compound **3**

Compound **3** (0.3g) from E16 was isolated using MPLC RP_{18} eluted with mixtures of $MeOH-H_2O$ (= 2:8, 4:6 and 10:0, respectively).

White amorphous powder

$C_7H_6O_5$

ESI-MS (negative mode): *m/z* 169 [M-H]⁻

$UV\lambda_{max}$: 272nm

¹H-NMR (400MHz, $(CD_3)_2SO$): See Table 2

¹³C-NMR (100MHz, $(CD_3)_2SO$): See Table 3

2.2.4. Isolation of compound **4**

Compound **4** (8.9g) from E16-9 was isolated using MPLC RP_{18} eluted with mixtures of $MeOH-H_2O$ (= 2:8, 4:6 and 10:0, respectively).

White amorphous powder

$C_8H_8O_5$

ESI-MS (negative mode): *m/z* 183 [M-H]⁻

$UV\lambda_{max}$: 272nm

¹H-NMR (300MHz, CD_3OD): See Table 2

¹³C-NMR (75MHz, CD_3OD): See Table 3

2.2.5. Isolation of compound **5**

Compound **5** (2.5g) from E16 was isolated using MPLC RP$_{18}$ eluted with mixtures of MeOH-H$_2$O (= 2:8, 4:6 and 10:0, respectively).

Yellow amorphous powder

C$_{41}$H$_{32}$O$_{26}$

ESI-MS (positive mode): *m/z* 941 [M+H]$^+$

UVλ$_{max}$: 214, 271 nm

^{1}H-NMR (500MHz, (CD$_3$)$_2$SO): δ 6.97 (2H, s, H-2'''', 6''''), 6.91 (2H, s, H-2''', 6'''), 6.85 (2H, s, H-2'', 6''), 6.82 (2H, s, H-2''''', 6'''''), 6.77 (2H, s, H-2',6'), 6.37 (1H, d, *J* = 8.3 Hz, H-1), 5.95 (1H, t, *J* = 9.7 Hz, H-3), 5.43 (2H, m, *J* = 21.0, 9.7 Hz, H-2,4), 4.58 (1H, d, *J* = 9.8 Hz, H-5), 4.31 (2H, s, H-6a, 6b) ppm

^{13}C-NMR (125MHz, (CD$_3$)$_2$SO): δ 166.34 (C-7''''') 165.43 (C-7'''), 164. 82 (C-7''''), 164.47 (C-7''), 163.94(C-7'), 145.69 (C-3'''', 5''''), 145.57 (C-3''', 5''') 145.51 (C-3'', 5''), 145.47 (C-3''''', 5'''''), 145.39 (C-3', 5'), 139.71 (C-4''''), 139.17 (C-4'''), 138.94 (C-4''), 138.80 (C-4'''''), 138.46 (C-4'), 119.30 (C-1''''), 118.89 (C-1'''), 118.07 (C-1''), 117.93 (C-1'''''), 117.33 (C-1'), 109.03 (C-2'''', 6''''), 108.90 (C-2''', 6'''), 108.80 (C-2'', 6''), 108.74 (C-2''''', 6'''''), 108.53 (C-2', 6'), 91.71 (C-1), 72.14 (C-5), 71.94 (C-3), 70.59 (C-2), 67.77 (C-4), 61.49 (C-6) ppm

2.2.6. Isolation of compound **6**

Compound **6** (113.7mg) from E11 was isolated using MPLC RP_{18} eluted with mixtures of $MeOH-H_2O$ (= 1:9, 3:7, 5:5, 7:3 and 10:0, respectively).

Yellow amorphous powder

$C_{15}H_{12}O_6$
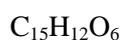
ESI-MS (negative mode): *m/z* 287 [M-H]⁻

$[\alpha]^{25}_D$ -1.5 (c 0.1, MeOH)

$UV\lambda_{max}$: 276nm

^{1}H-NMR (300MHz, CD_3OD): See Table 4

^{13}C-NMR (75MHz, CD_3OD): See Table 5

2.2.7. Isolation of compound **7**

Compound **7** (18.1mg) from E11 was isolated using MPLC RP_{18} eluted with mixtures of $MeOH-H_2O$ (= 1:9, 3:7, 5:5, 7:3 and 10:0, respectively).

Pale yellow needle

$C_{15}H_{12}O_7$
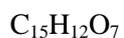
$[\alpha]^{25}_D$ +12.8 (c 0.1, MeOH)

ESI-MS: *m/z* 304 [M]⁺

$UV\lambda_{max}$: 291nm

^{1}H-NMR (300MHz, CD_3OD): See Table 4

^{13}C-NMR (75MHz, CD$_3$OD): See Table 5

2.2.8. Isolation of compound **8**

  E11 was subjected to MPLC RP$_{18}$ gradient eluting with mixtures of MeOH-H$_2$O

(= 1:9, 3:7, 5:5, 7:3 and 10:0, respectively), and the fraction was further separated into

sixteen sub-fractions (E11-1 to E11-16). Compound **8** (7.1mg) was obtained from E11-

12 using Sephadex LH-20 with 100% MeOH.

Yellow amorphous powder

C$_{15}$H$_{12}$O$_7$

ESI-MS (negative mode): *m/z* 303 [M-H]$^-$

[α]^{25}$_D$ +3.3 (c 0.1, MeOH)

UVλ$_{max}$: 289 nm

^{1}H-NMR (400MHz, (CD$_3$)$_2$SO): See Table 6

^{13}C-NMR (100MHz, (CD$_3$)$_2$SO): See Table 7

2.2.9. Isolation of compound 9

Compound **9** (93.3mg) from E11 was isolated using MPLC RP$_{18}$ eluted with mixtures of MeOH-H$_2$O (= 1:9, 3:7, 5:5, 7:3 and 10:0, respectively).

Yellow amorphous powder

C$_{15}$H$_{10}$O$_6$

ESI-MS (negative mode): *m/z* 287 [M-H]$^-$

UVλ_{max}: 256, 372nm

^{1}H-NMR (300MHz, CD$_3$OD): See Table 8

^{13}C-NMR (75MHz, CD$_3$OD): See Table 9

2.2.10. Isolation of compound 10

E11 was subjected to MPLC RP$_{18}$ gradient eluting with mixtures of MeOH-H$_2$O (= 1:9, 3:7, 5:5, 7:3 and 10:0, respectively), and the fraction was further separated into sixteen sub-fractions (E11-1 to E11-16). E11-14 was divided into five fractions (E11-14-1 to E11-14-5) using Sephadex LH-20 with 100% MeOH. Compound **10** (13.0mg) was purified from E11-14-5 by HPLC RP$_{18}$ using MeOH-H$_2$O (6:4, 2mL/min).

Yellow amorphous powder

C$_{16}$H$_{12}$O$_6$
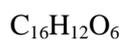
ESI-MS (negative mode): *m/z* 299 [M-H]$^-$

UVλ_{max}: 350nm

16

^{1}H-NMR (500MHz, CD$_3$OD): See Table 8

^{13}C-NMR (125MHz, CD$_3$OD): See Table 9

2.2.11. Isolation of compound 11

E11 was subjected to MPLC RP$_{18}$ gradient eluting with mixtures of MeOH-H$_2$O (= 1:9, 3:7, 5:5, 7:3 and 10:0, respectively), and the fraction was further separated into sixteen sub-fractions (E11-1 to E11-16). Compound **11** (5.9mg) was obtained from E11-16 using Sephadex LH-20 with 100% MeOH.

Yellow amorphous powder

C$_{15}$H$_{10}$O$_5$

ESI-MS (negative mode): *m/z* 269 [M-H]$^-$

UVλ$_{max}$: 256, 393nm

^{1}H-NMR (400MHz, CD$_3$OD): δ 7.58 (H, d, *J* = 9.0 Hz, H-5), 7.51 (H, d, *J* = 1.8 Hz, H-2'), 7.21 (H, dd, *J* = 8.2, 1.8 Hz, H-6'), 6.83 (H, d, *J* = 8.2 Hz, H-5'), 6.67 (2H, m, H-6 and H-8), 6.67 (H, s, H-2) ppm

^{13}C-NMR (100MHz, CD$_3$OD): δ 185.1 (C-4), 170.7 (C-7), 170.0 (C-9), 150.1 (C-4'), 148.6 (C-3), 147.5 (C-3'), 127.6 (C-5), 127.1 (C-6'), 126.4 (C-1'), 119.7 (C-2'), 117.5 (C-5'), 115.3 (C-2,6,10), 100.2 (C-8) ppm

2.2.12. Isolation of compound **12**

E6 was subjected to the silica gel CC eluted with mixtures of $CHCl_3$-MeOH (= 100:1), MeOH, and it was further divided into five sub-fractions (E6-1 to E6-5). Compound **12** (3.4mg) was obtained from E6-5 using Sephadex LH-20 with 100% MeOH.

Yellow amorphous powder

$C_9H_6O_4$

ESI-MS (negative mode): *m/z* 179 [M-H]⁻

$UV\lambda_{max}$: 256, 296 nm

^{1}H-NMR (400MHz, CD$_3$OD): δ 7.95 (1H, d, *J* = 6.0 Hz, H-2), 6.32 (1H, d, *J* = 2.1 Hz, H-8), 6.19 (1H, d, *J* = 2.1 Hz, H-6), 6.18 (1H, d , *J* = 5.9 Hz, H-3) ppm

^{13}C-NMR (100MHz, CD$_3$OD): δ 184.2 (C-4), 167.1 (C-7), 164.3 (C-5), 160.7 (C-9), 158.9 (C-2), 112.4 (C-3), 107.4 (C-10), 101.1 (C-6), 95.9 (C-8) ppm

2.2.13. Isolation of compound **13**

E11 was subjected to MPLC RP$_{18}$ gradient eluting with mixtures of MeOH-H$_2$O (= 1:9, 3:7, 5:5, 7:3 and 10:0, respectively), and the fraction was further separated into sixteen sub-fractions (E11-1 to E11-16). Compound **8** (13.4mg) was obtained from E11-12 using Sephadex LH-20 with 100% MeOH.

White powder

C$_{20}$H$_{24}$O$_6$

FAB-MS *m/z*: 360 [M]$^+$

$[\alpha]^{25}_D$ +7.1 (c 0.1, MeOH)

UVλ_{max}: 212, 284nm

^{1}H-NMR (400MHz, (CD$_3$)$_2$SO): δ 6.68 (1H, d, *J* = 7.6Hz, H-5'), 6.64 (1H, d, *J* = 1.1Hz, H-2'), 6.60 (1H, s, H-5), 6.49 (1H, dd, *J* = 8.0, 1.2 Hz, H-6'), 6.09 (1H, s, H-8), 3.73-3.82 (4H, m, H-1, 12 11a), 3.71 (6H, s, H-13, 14), 3.43 (1H, m, H-11b), 2.66(2H, d, *J* = 10.1Hz, H-4), 1.84 (1H, m, H-3), 1.62 (1H, m, H-2) ppm

^{13}C-NMR (100MHz, (CD$_3$)$_2$SO): δ 147.3 (C-6), 145.5 (C-3'), 144.6 (C-7), 144.1 (C-4'), 137.1 (C-9), 132.7 (C-10), 127.1 (C-1'), 121.4 (C-6'), 116.2 (C-8), 115.2 (C-5'), 113.2 (C-2'), 111.8 (C-5), 63.6 (C-12), 59.8 (C-11), 55.6, 55.5 (C-13, 14), 48.6 (C-2), 45.9 (C-1), 38.1 (C-3), 32.2 (C-4) ppm

Table 2. ^{1}H-NMR data of compounds **3** and **4**

position	δ (J = Hz)	
	3^a	**4^b**
2,6	6.89 (2H, s)	7.03 (2H, s)
OCH$_3$		3.80 (3H, s)

^{s}Recorded in 400MHz, (CD$_3$)$_2$SO. ^{b}Recorded in 300MHz, CD$_3$OD.

Table 3. ^{13}C-NMR data of compounds **3** and **4**

position	δ	
	3^a	**4^b**
1	122.0	122.2
2	108.7	110.8
3	145.5	147.2
4	137.7	140.5
5	145.5	147.2
6	108.7	110.8
7	168.5	169.8
OCH$_3$		53.1

^{s}Recorded in 400MHz, (CD$_3$)$_2$SO. ^{b}Recorded in 300MHz, CD$_3$OD.

Table 4. ^{1}H-NMR data of compounds **6** and **7**

position	δ (J = Hz)	
	6^a	**7^b**
2	4.92 (1H, d, 11.7)	4.93 (1H, d, 11.7)
3	4.47 (1H, d, 11.7)	4.49 (1H, d, 11.1)
5	7.71 (1H, d, 8.7)	
6	6.52 (1H, dd, 8.4, 2.1)	5.91 (1H, d, 2.1)
8	6.32 (1H, d, 2.4)	5.87 (1H, d, 2.1)
2'	6.97 (1H, d, 1.8)	6.97 (1H, d, 2.1)
5'	6.79 (1H, d, 8.1)	6.79 (1H, d, 8.1)
6'	6.85 (1H, dd, 8.4, 2.1)	6.84 (1H, dd, 8.1, 1.8)

^{s}Recorded in 300MHz, CD$_3$OD. ^{b}Recorded in 300MHz, CD$_3$OD.

Table 5. ^{13}C-NMR data of compounds **6** and **7**

position	δ	
	6[a]	**7**[b]
2	86.4	85.9
3	75.3	74.5
4	195.3	199.2
5	130.9	169.5
6	112.9	98.1
7	165.9	166.1
8	104.5	97.1
9	167.6	165.3
10	114.2	102.6
1'	130.9	130.9
2'	116.7	116.9
3'	147.1	147.9
4'	147.9	147.1
5'	116.8	116.7
6'	121.7	121.7

[s]Recorded in 300MHz, CD$_3$OD. [b]Recorded in 300MHz, CD$_3$OD.

Table 6. ^{1}H-NMR data of compound **8**

position	δ (J = Hz)
2	4.97 (1H, d, 11.1)
3	4.48 (1H, d, 11.1)
6	5.89 (1H, d, 1.8)
8	5.85 (1H, d, 1.6)
2'	6.74 (2H, s)
4'	6.89 (1H, s)
6'	6.74 (2H, s)

Recorded in 400MHz, $(CD_3)_2SO$

Table 7. ^{13}C-NMR data of compound **8**

position	δ
2	83.0
3	71.5
4	197.7
5	163.9
6	95.9
7	166.8
8	94.9
9	162.5
10	100.4
1'	128.0
2'	115.3
3'	145.7
4'	119.3
5'	144.9
6'	115.1

Recorded in 400MHz, $(CD_3)_2SO$

Table 8. ^{1}H-NMR data of compounds **9** and **10**

position	δ (J = Hz)	
	9^a	**10^b**
5	7.97 (1H, d, 9.3)	7.95 (1H, d, 8.8)
6	6.89 (3H, m)	6.86 (3H, m)
8	6.89 (3H, m)	6.86 (3H, m)
2'	7.76 (1H, d, 2.4)	7.63 (1H, d, 2.0)
5'	6.89 (3H, m)	6.86 (3H, m)
6'	7.66 (1H, dd, 8.4 2.1)	7.54 (1H, dd, 8.5, 2.1)
OCH$_3$		3.75 (3H, s)

^{s}Recorded in 300MHz, CD$_3$OD. ^{b}Recorded in 500MHz, CD$_3$OD.

Table 9. ^{13}C-NMR data of compounds **9** and **10**

position	δ	
	9^a	**10^b**
2	149.5	158.5
3	139.4	141.9
4	175.2	177.3
5	128.3	128.4
6	116.8	117.3
7	165.2	167.4
8	103.8	104.1
9	159.3	159.7
10	117.0	117.9
1'	125.2	124.1
2'	116.8	117.7
3'	147.0	147.3
4'	148.3	150.7
5'	116.2	117.2
6'	122.4	123.0
OCH$_3$		61.1

^{s}Recorded in 300MHz, CD$_3$OD. ^{b}Recorded in 500MHz, CD$_3$OD.

2.3. Evaluation of the cytotoxic effect *in vitro* model

2.3.1. Cell line culture

Human Gastric Adenocarcinoma AGS cell line was provided by Elcom Sci. Co.. AGS cells were maintained in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin at 37^0C in a humidified incubator containing 5% CO_2 gas.

2.3.2. Cell viability assay

3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium (MTT, 2mg/ml) assay was used to evaluate the effect of the test drugs on cell growth, as described previously (Alley et al., 1988). For this assay, the AGS cells were seeded in 96-well plates (2.5 x 10^5 cell/well) and incubated for 24hr. The growth medium was changed, and AGS cells were treated with vehicle or compounds at the concentration from 10 to 1000 uM for 24hr. After 24hr, MTT was added and incubated for 1hr. Reduction of MTT to formazan was assessed in an ELISA plate reader at OD 540nm. Data were analyzed at percent cell viability relative to control cultures as the mean of three independent experiments. Vinblastine was used as a positive control.

$$\text{Cell Viability } (\%) = \frac{\text{Abs.of sample treated}}{\text{Abs.of control}} \text{ x } 100$$

2.3.3. Statistical analysis

All data were expressed as means $\pm$ S.D. The evaluation of statistical significance was determined by an 'one-way ANOVA' test using computerized statistical package, with $p<0.05^{*}$, $p<0.01^{**}$ and $p<0.001^{***}$ considered to be statistically significant.

III. Results

1. Elucidation of chemical structure of compounds from *R. trichocarpa* roots

1.1. Compound **1**

Compound **1** was obtained as white amorphous powder, $[\alpha]^{25}_D$ +62.7 (c=1.02, CHCl$_3$). The molecular formula was C$_{31}$H$_{46}$O$_4$ by HRFABMS (*m/z* 482.3396 [M]$^+$, calcd. for 482.3396). The ^{1}H-NMR spectrum (CDCl$_3$) showed four methyl groups at δ 0.95 (6H, s, H-29, 28), 0.87 (3H, s, H-30), 0. 86 (3H, s, H-18), one vinyl methyl singlet at δ 1.93 (3H, s, H-27), one exo exo methylene group at δ 5.24 (1H, s, H-21b) and 5.20 (1H, s, H-21a), and one CH$_2$O group at δ 4.22 (1H, dd, *J* = 8.8, 2.4 Hz, H-19b) and 3.69 (1H, d, *J* = 8.8, H-19a). The protons of a methylene at δ 2.50 (1H, m, H-23b) and 2.30 (1H, m, H-23a) and two methines at δ 6.57 (1H, d, *J* = 5.9 Hz, H-24) and 4.72 (1H, dd, *J* = 12.4, 3.3 Hz, H-22) with a carbonyl group indicated the presence of an α,β-unsaturated-δ-lactone. In ^{13}C-NMR and DEPT spectra, an oxy-methine carbon at δ 80.8 (C-22) was found, and it was linked with a carboxyl (δ 166.0, C-26) as an ester linkage (Figure 1). The ^{1}H- and ^{13}C-NMR spectra showed that compound **1** had a triterpene skeleton of semialacton, and its spectra from *R. javanica* have been previously assigned, but revisions based upon 2D-NMR were found to be necessary to some of the ^{1}H- and ^{13}C-NMR assignments because there was a methoxy group (Lee et al., 2001). In the HMBC spectrum, H-19 was correlated with C-2 (δ 35.4), C-4 (δ 35.0)

29

and C-5 (δ 50.3); H-22 was correlated with C-17 (δ 40.1), C-20 (δ 149.3), C-21 (δ 113.4) and C-23 (δ 29.1); H-23 was correlated with C-24 (δ 139.1) and C-25 (δ 128.4); H-27 was correlated with C-24, C-25, and C-26; and H-31 (δ 3.23, 3H, s) was correlated with C-3 (δ 100.6) (Figure 2). The cofiguration at C-17 was deduced as S, based on the H-22 being positioned as an axial by it coupling constants (dd, J = 12.4, 3.3 Hz) and observation of the unclear Overhauser effect spectroscopy (NOESY) between the following proton signals; H-18 and H-19a,b; H-17 and H-22; H-16a and H-23b (Figure 3). Therefore, the structure of compound **1** was determined to be 3-methoxy semialactone, and it was isolated for the first time from nature.
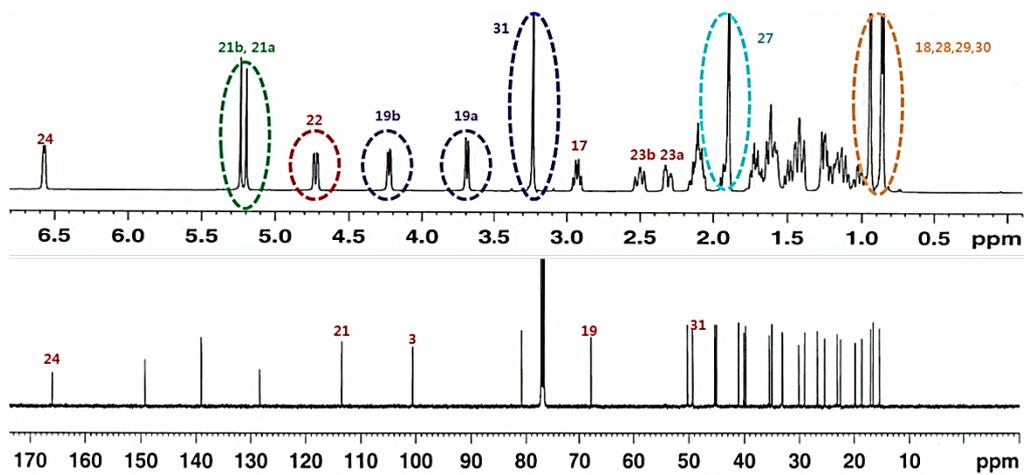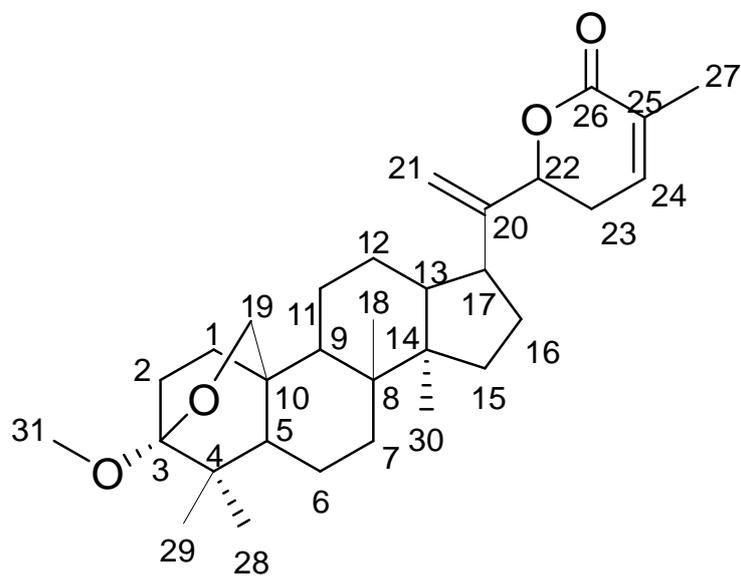
Figure 1. ^{1}H and ^{13}C NMR spectra of compound **1**
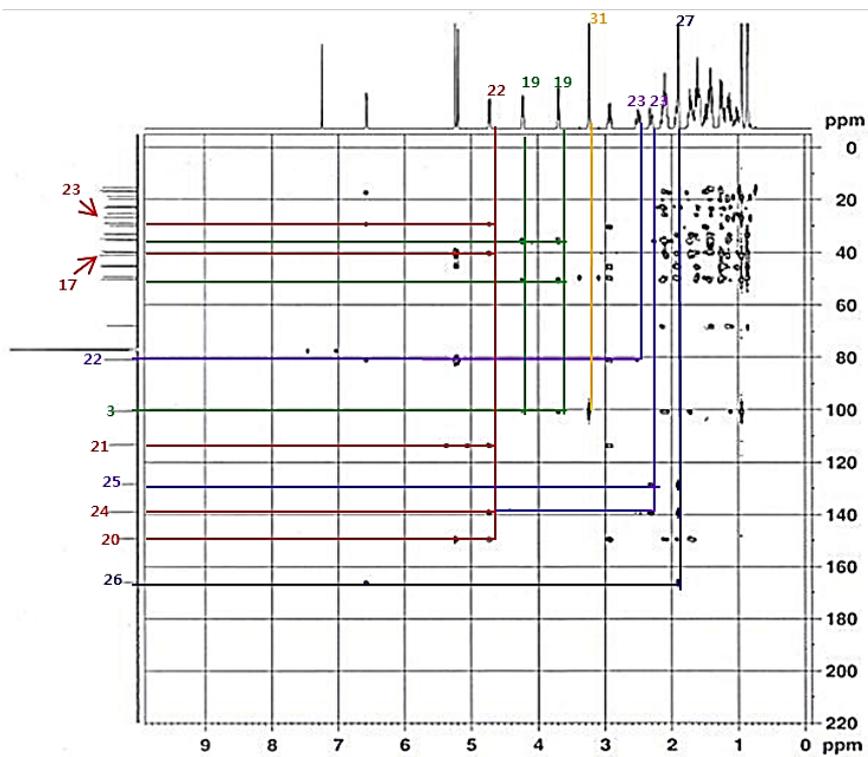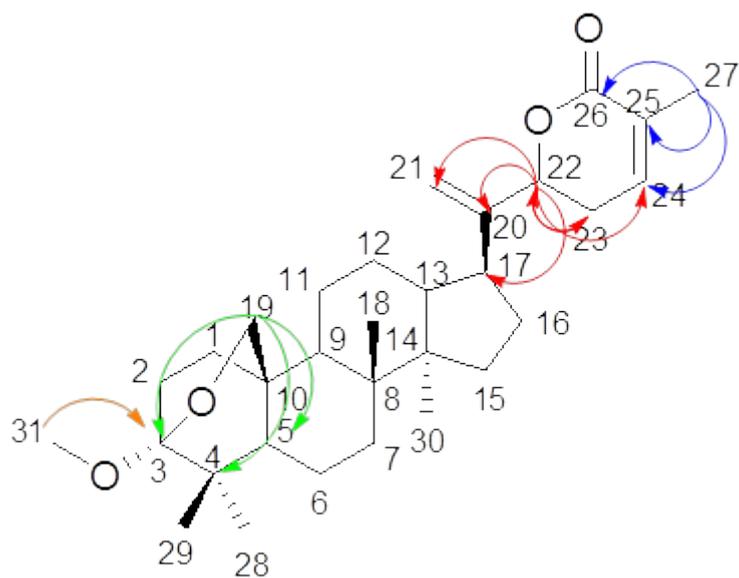
Figure 2. HMBC spectrum of compound **1**
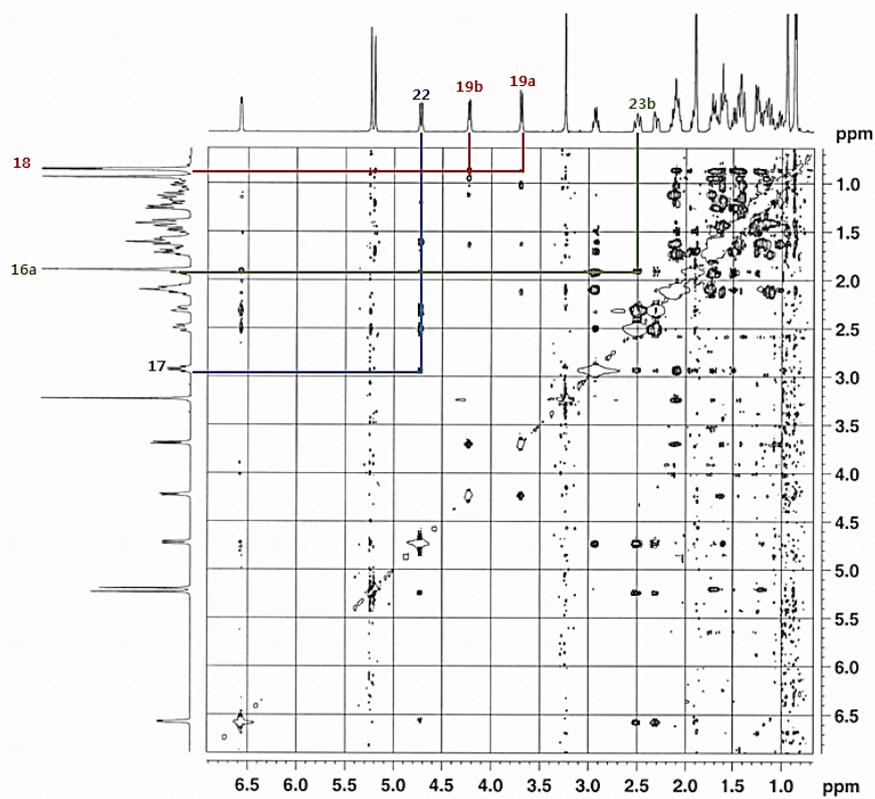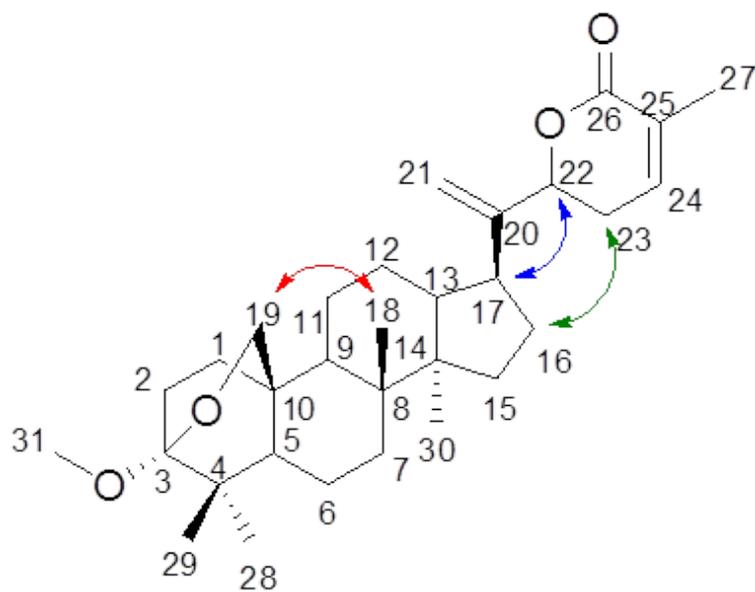
Figure 3. NOESY spectrum of compound **1**

1.2. Compound **2**

Compound **2** was characterized as yellow amorphous powder, $[\alpha]^{25}_{D}$ -41.2 (c=0.1, MeOH). The molecular formula was $C_{11}H_{12}O_6$ by HRFABMS (m/z 241.0717 [M+H]$^+$, calcd. for 241.0712). ^{1}H NMR spectrum showed that there were one aromatic moiety with two protons at δ 5.92 (2H, s, H-3', 5'), two methylene groups at δ 3.39 (2H, t, H-3) and 2.61 (2H, t, H-2), one methoxy group at δ 3.76 (3H, s, H-7'). The signals of the ^{13}C NMR spectrum at δ 205.8 (C-4) and 178.0 (C-1) revealed the presence of a carbonyl carbon and a carboxylic carbon, respectively (Figure 4). In the HMBC spectrum, H-2 was correlated with C-1, C-3 (δ 40.7) and C-4; H-3 was correlated with C-1, C-2 (δ 30.0) and C-4; H-3' and H-5' were correlated with C-1' (δ 106.8), C-2' & C-6' (δ 166.4), C-3' & 5' (δ 95.1) and C-4' (δ 168.3); H-7' was correalted with C-4' (Figure 5). Previous report has showed that there was a similir structure compared with compound **2**, but it did not have a hydoxy moiety at C-2 position (Cignarella et al., 1990). With above described data, compound **2** was identified as 4-(2',6'-dihydroxy-4'-methoxyphenyl)-4-oxobutanoic acid and was newly reported from nature.

)
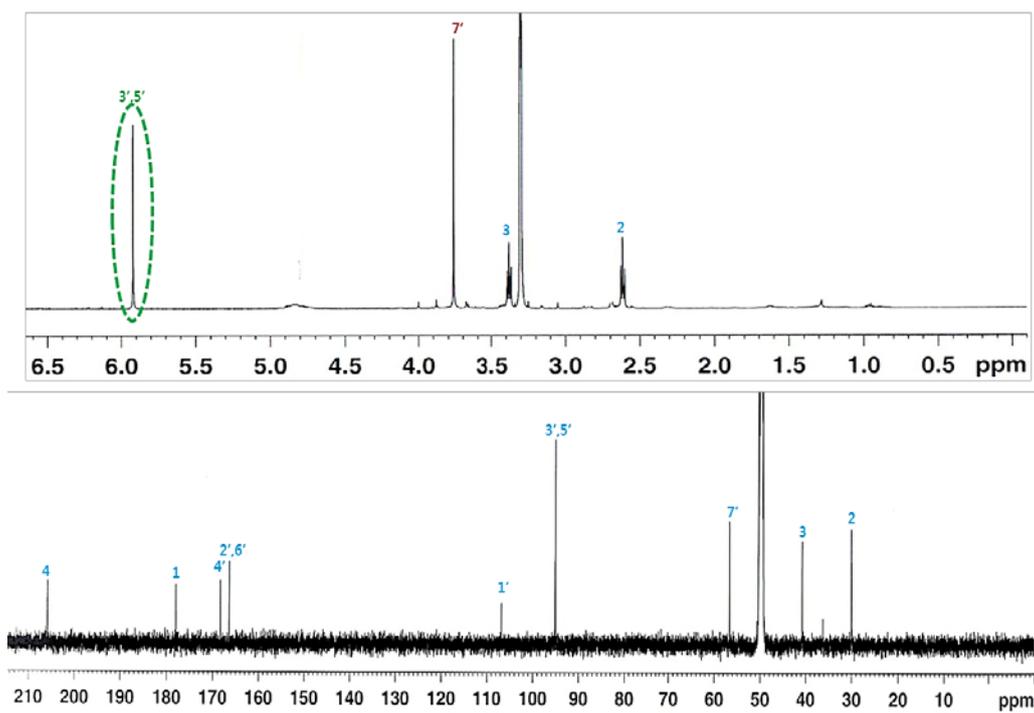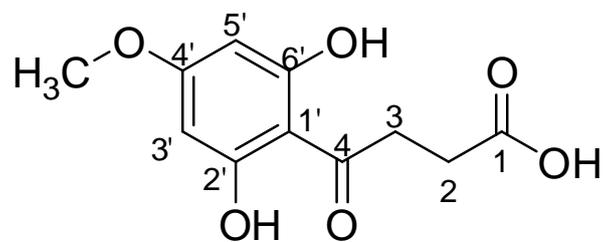
Figure 4. ^{1}H and ^{13}C NMR spectra of compound **2**
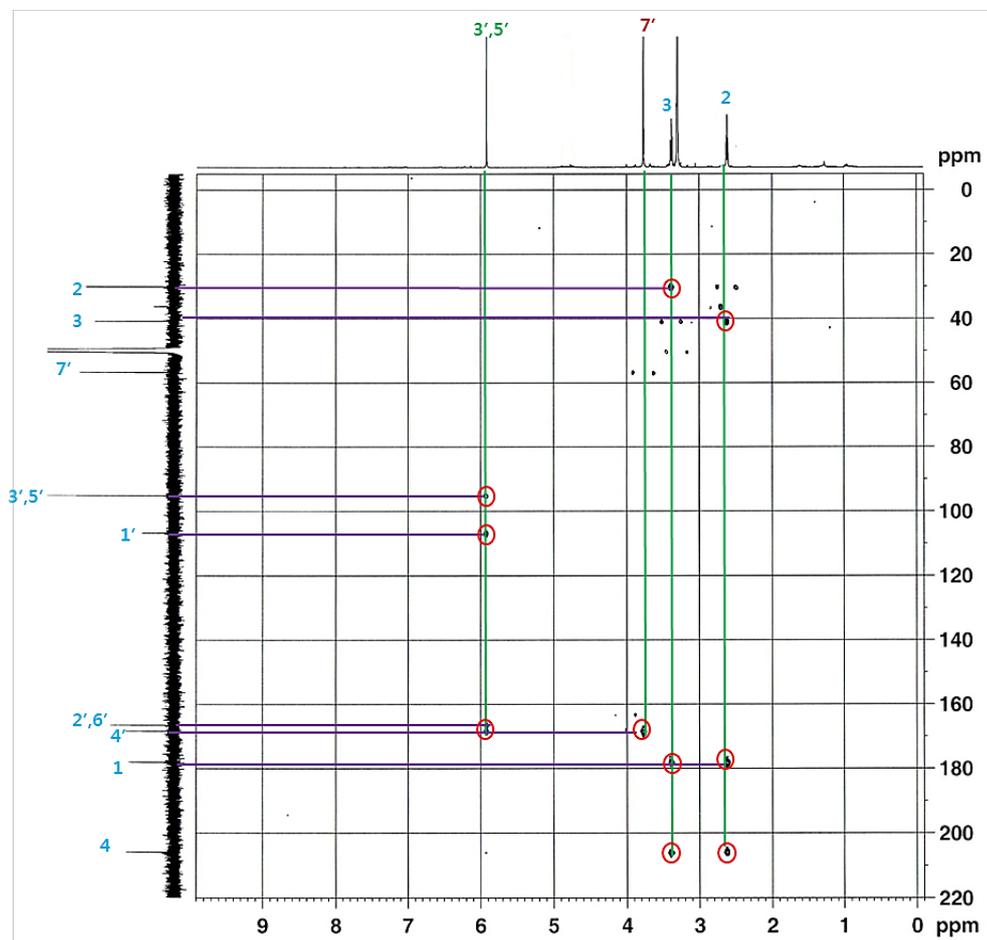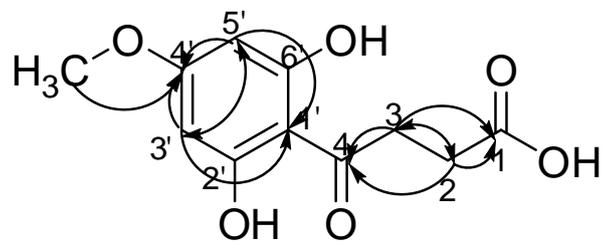
Figure 5. HMBC spectrum of compound **2**

1.3. Compounds **3** and **4**

Compound **3** was isolated as white amorphous powder, and the ESI-MS (negative mode) exhibited m/z 169 [M-H]⁻ indicating $C_7H_6O_5$ as its molecular formula. ¹H NMR spectrum showed two protons in an aromatic moiety at δ 6.89 (2H, s, H-2, H-6), and compound **3** was identified as gallic acid by comparison with previously reported literature (Jain et al., 2011).

Compound **4**, white amorphous powder, was fulfilled ESI-MS (negative mode), ¹H and ¹³C NMR expriments to assign its molecular formular with $C_8H_8O_5$. ¹H and ¹³C NMR spectra were very similar to those of compound **3**, except for a methoxy group at δ 3.80 (3H, s). These data indicated that compound **4** was 4-*O*-methylgallic acid by comparison with previously reported literature (Wang et al., 2011).

1.4. Compound **5**

Compound **5** was obtained yellow amorphous powder, and its molecular formula was identified as $C_{41}H_{32}O_{26}$ by the positive mode ESI-MS (m/z 941 [M+H]$^+$) and the ^{13}C NMR spectum. ^1H NMR spectrum showed the presence of five aromatic moieties between δ 6.77 and 6.97 (H-2 and H-6 in each aromatic ring) and one glucose. The signal of ^{13}C NMR experiment between δ 108.5 and 166.3 revealed the presence of galloy moiety (Figure 6). In 2D expreiments, H-1 (δ 6.37, 1H, d, J = 8.3 Hz), H-2,4 (δ 5.43, m, J = 21.0, 9.7 Hz), H-3 (δ 5.95, t, J = 9.7 Hz), and H-6 (δ 4.31, 2H, s) of compound **5** were correlated with C-7' (δ 163.9), C-7'' (δ 164.5), C-7'''' (δ 164.8), C-7''' (δ 165.4) and C-7'''''(δ 166.3), respectively (Figure 7). These data indicated that compound **5** was identified as pentagalloy glucose (Beretta et al., 2011; Cho et al, 2010)
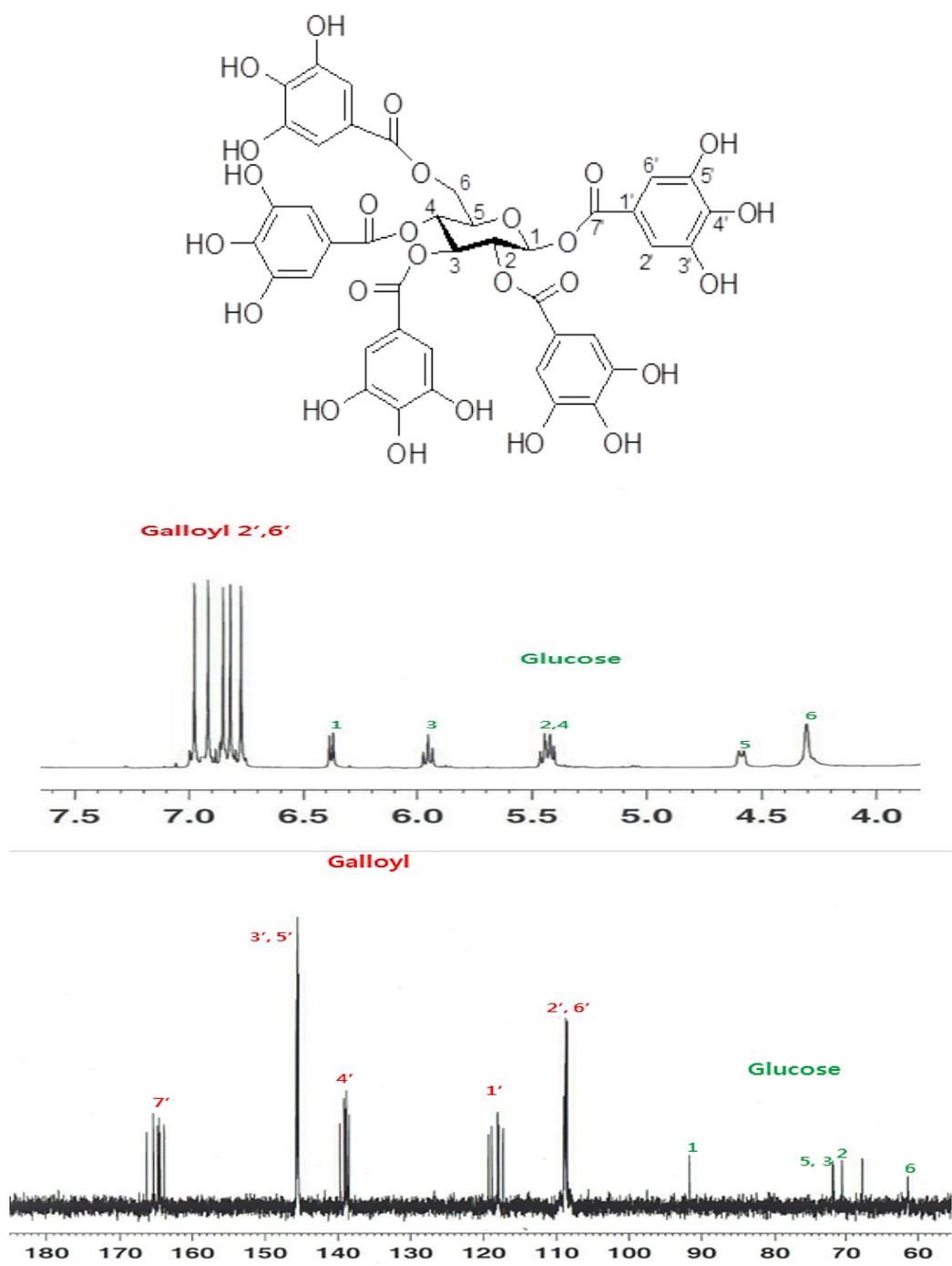
Figure 6. ^{1}H and ^{13}C NMR spectra of compound **5**
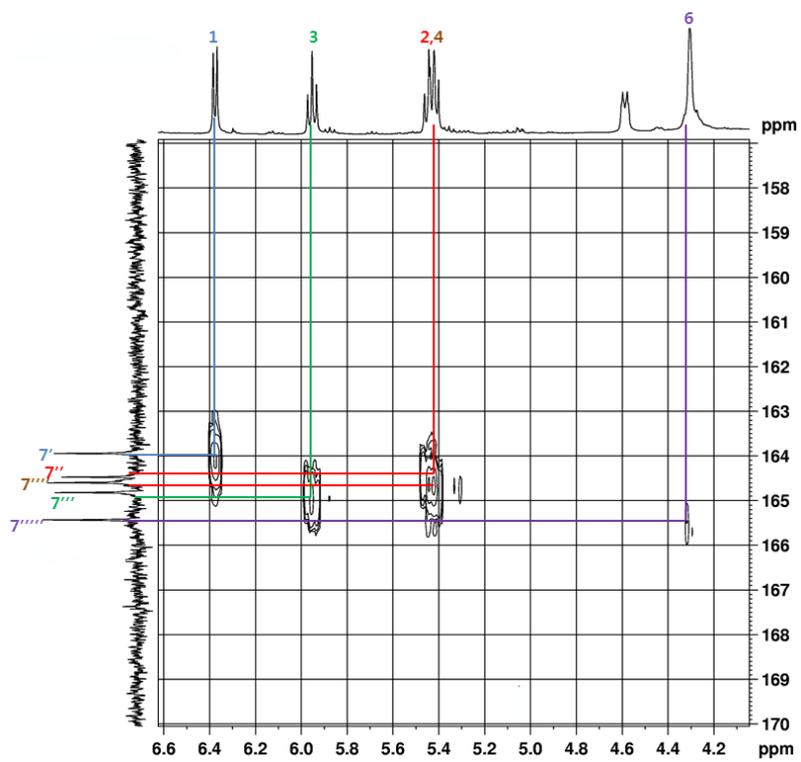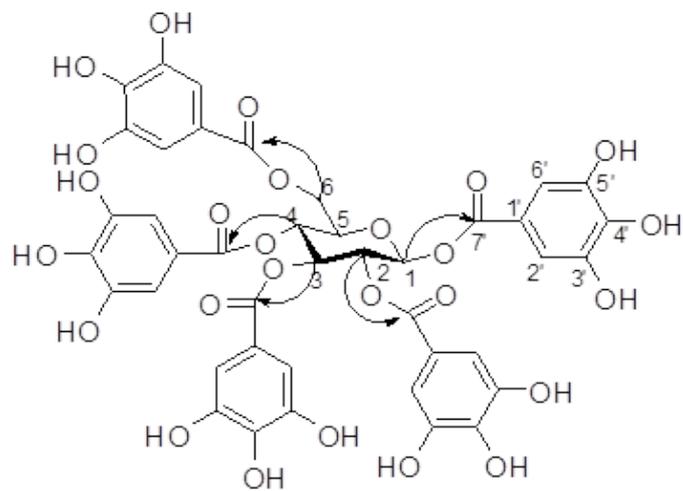
Figure 7. HMBC spectrum of compound **5**

40

1.5. Compounds **6** and **7**

Compound **6** was obtained as yellow amorphous powder, and its molecular formula was identified as $C_{15}H_{12}O_6$ by the negative mode ESI-MS (*m/z* 287 [M-H]⁻). The ^{1}H NMR spectrum displayed three *ortho-, meta*-coupling signals at δ 6.97 (1H, d, *J* = 1.8 Hz, H-2'), 6.85 (1H, dd, *J* = 8.4, 2.1 Hz, H-6') and 6.79 (1H, d, *J* = 8.1 Hz, H-5') and another *ortho-, meta*-coupling signals at δ 7.71 (1H, d, *J* = 8.7 Hz, H-5), 6.52 (1H, dd, *J* = 8.4, 2.1 Hz, H-6) and 6.32 (1H, d, *J* = 2.4 Hz, H-8) ascribable to two benzene rings. Also, the signals at δ 4.92 (1H, d, *J* = 11.7 Hz, H-2) and 4.47 (1H, d, *J* = 11.7 Hz, H-3) showed two oxygenated methine protons. The ^{13}C NMR spectrum was displayed the peaks of two oxygenated carbons at δ 86.4 (C-2) and 75.3 (C-3), and an oxo group at δ 195.3 (C-4). With these data, compound **6** was defined as (-)-fustin (Nishino et al., 1987).

Compound **7** was isolated as pale yellow needle and its molecular formula was $C_{15}H_{12}O_7$ decided by the ion peak at *m/z* 304 [M]⁺ of ESI-MS. The ^{1}H and ^{13}C NMR spectral data of compound **7** showed similar patterns with those of compound **6** except for the downfield exhibited by C-5 (δ 169.5). It suggested tha tthe proton at C-5 of compound **6** was replaced by a hydroxy grooup. Thus, compound **7** was identified as (+)-taxifolin by comparison with previously reported literature (Han et al., 2007).

1.6. Compound **8**

Compound **8** was obtained as a yellow amorphous powder, and the negative ESI-MS showed a peak at *m/z* 303 [M-H]⁻. The molecular formula was $C_{15}H_{12}O_7$. The ^1H NMR spectrum showed three *ortho-, para*-coupling signals at at δ 6.89 (1H, s, H-4') and 6.74 (2H, s, H-2', 6'), and two meta-coupling signals at δ 5.89 (1H, d, J = 1.8 Hz, H-6) and 5.85 (1H, d, J = 1.6 Hz, H-8) meant two benzene rings. Two oxygenated methine protons were showed at δ 4.97 (1H, d, J = 11.1 Hz, H-2) and 4.48 (1H, J = 11.1 Hz, H-3). One oxo group was observed at δ 197.7 (C-4), and on this basis of above results, compound **8** was conclued as 3,3',5,5',7-pentahydroxyflavanonol by comparison with previously reported literature (Zhang et al., 2007).

1.7. Compound **9** and **10**

The negative mode ESI-MS of compound **9**, yellow amorphous powder, displayed at m/z 287 [M-H]$^-$ corresponding to $C_{15}H_{10}O_6$ as its molecular formula. The ^{1}H spectrum showed that the coupling constants at δ 7.76 (1H, d, J = 2.4 Hz, H-2'), 7.66 (1H, dd, J = 8.4, 2.1 Hz, H-6') and 6.89 (3H, m) in B ring, and at δ 7.97 (1H, d, J = 9.3 Hz, H-5) and 6.89 (3H, m) in A ring indicated that compound **9** was a flavonol substituted as hydroxy groups at position 3, 7, 3' and 4'. Thus, with above observed data, compound **9** was identified as fisetin (Hasan et al., 2010).

The molecular formula of compound **10** was $C_{16}H_{12}O_6$ by ^{1}H, ^{13}C NMR spectra and the ESI-MS (m/z 299 [M-H]$^-$). In the comparison with ^{1}H, ^{13}C NMR spectral data of compound 9, those of compound **10** were only different from the existence of a methoxy moiety at δ 3.75 (3H,s), and it was correlated with C-3 (δ 141.9). On the basis of these data, compound **10** was determined as 3-methoxy-7,3',4'-trihydroxyflavone (Wu et al., 2008).

1.8. Compound **11**

Compound **11** was obtained yellow amorphous powder, and its molecular formula was identified as $C_{15}H_{10}O_5$ by the negative mode ESI-MS (*m/z* 269 [M-H]⁻) and the ^{13}C NMR spectum. In the 1H NMR spectrum, a singlet at δ 6.67 could be assigned to H-2, and the spectrum showed that three *ortho-, meta*-coupling signals at δ 7.51 (1H, d, *J* = 1.8 Hz, H-2'), 7.21 (1H, dd, *J* = 8.2, 1.8 Hz, H-6') and 6.83 (1H, d, *J* = 8.2 Hz, H-5'), and another *ortho-, meta*-coupling signals at δ 7.58 (1H, d, *J* = 9.0 Hz, H-5) and 6.67 (2H, m, H-6, 8) ascribable to two benzene rings (Figure 8). Analysis of the HMBC spectrum showed that there was a correlation between H-2 and C-4/C-2'/C-6', and this could occur with an aurone structure as proposed. Thus, with these data, compound **11** was defined as sulfuretin in comparison with previously reported literature (Junior et al., 2008)
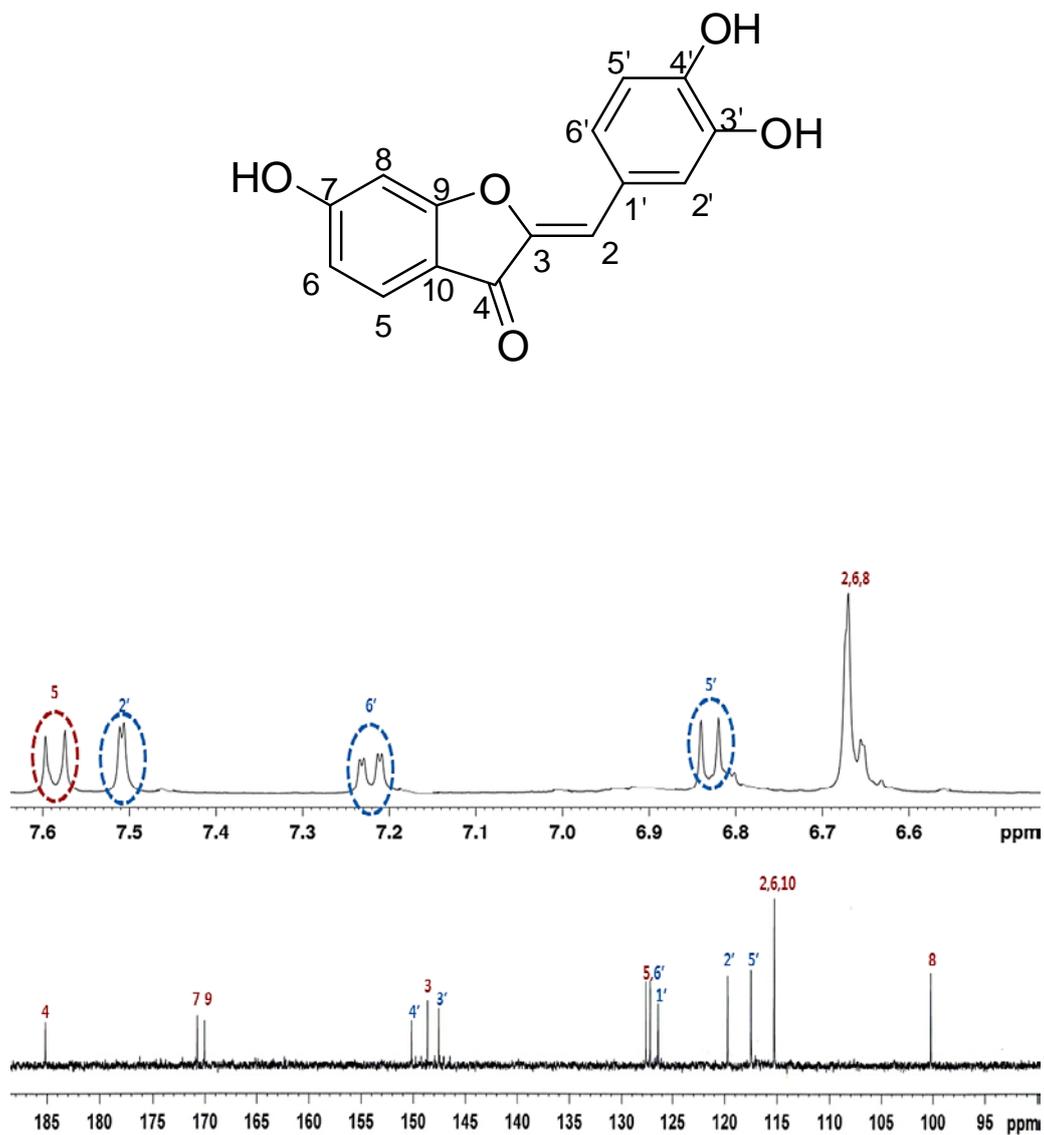
Figure 8. ^{1}H and ^{13}C NMR spectra of compound **11**

1.9. Compound **12**

Compound **12** was isolated as yellow amorphous powder. Its molecular formula was determined as $C_9H_6O_4$ by the negative mode ESI-MS (m/z 179 [M-H]$^-$) and ^{13}C NMR spectrum. The ^1H and ^{13}C NMR spetra showed *ortho*-coupling signals at δ 6.32 (1H, d, J = 2.1 Hz, H-8) and δ 6.19 (1H, d, J = 2.1 Hz, H-6) and an *ortho- and meta*-coupling signals at at δ 7.95 (1H, d, J = 6.0 Hz, H-2) and δ 6.18 (1H, d, J = 5.9 Hz, H-3), and one oxo group at δ 184.2 (C-4) (Figure 9). With these data, compound **12** was identified as 5,7-dihydroxy-4H-chromen-4-one in comparison with previously reported literature (Simon et al., 1994).
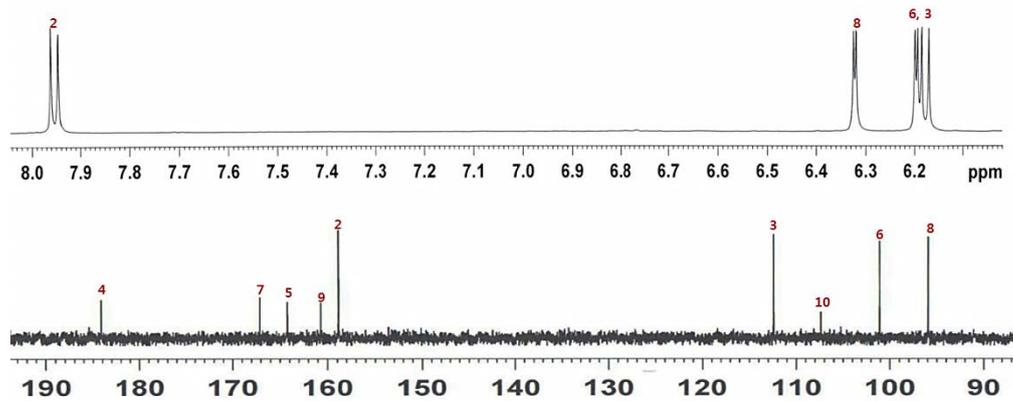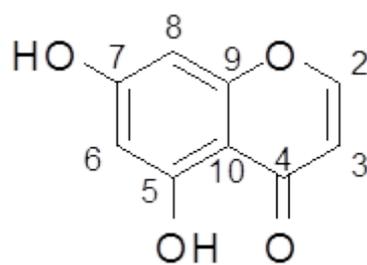
Figure 9. ^{1}H and ^{13}C NMR spectra of compound **12**

1.10. Compound **13**

Compound **13** was characterized as white powder, and the FABMS exhibited m/z 360 [M]$^+$ indicating $C_{20}H_{24}O_6$ as its molecular formula. The ^{1}H NMR spectrum showed two methoxy groups at δ 3.71, and in HMBC spectrum, these groups were corelated with C-6 (δ 147.3) and C-3' (δ 145.5) (Figure 10). Therefore, compound **13** was determined as (+)-isolariciresinol by comparison with previously reported literature (Hungerford et al, 1998).
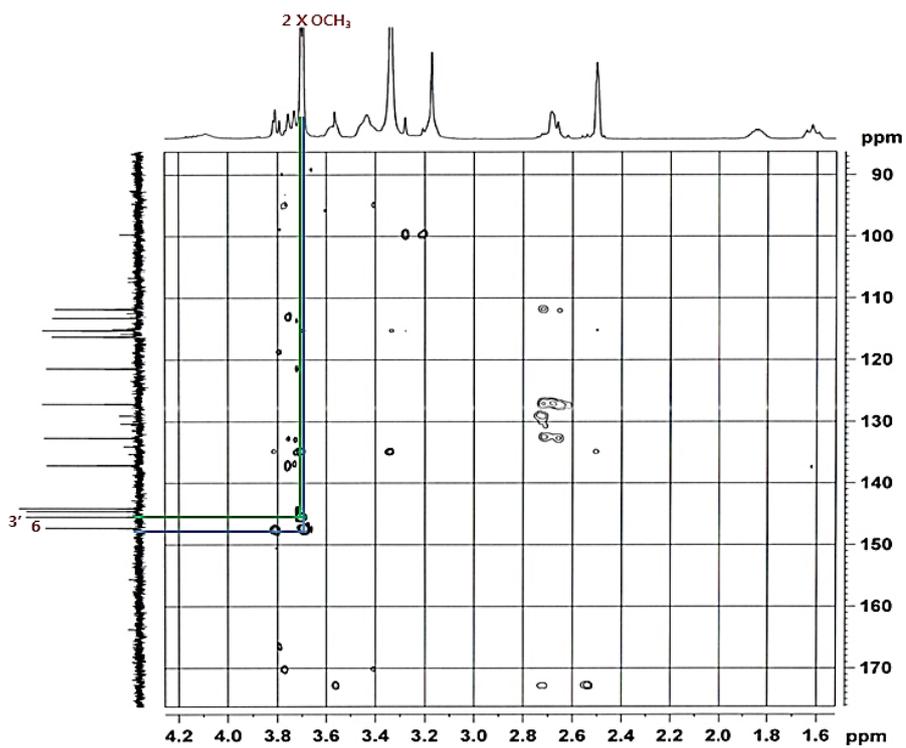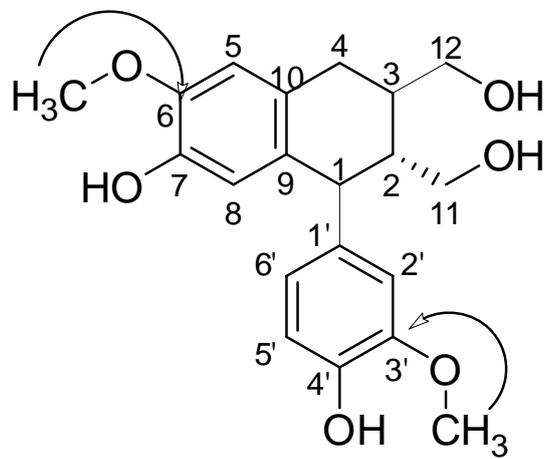
Figure 10. HMBC spectrum of compound **13**
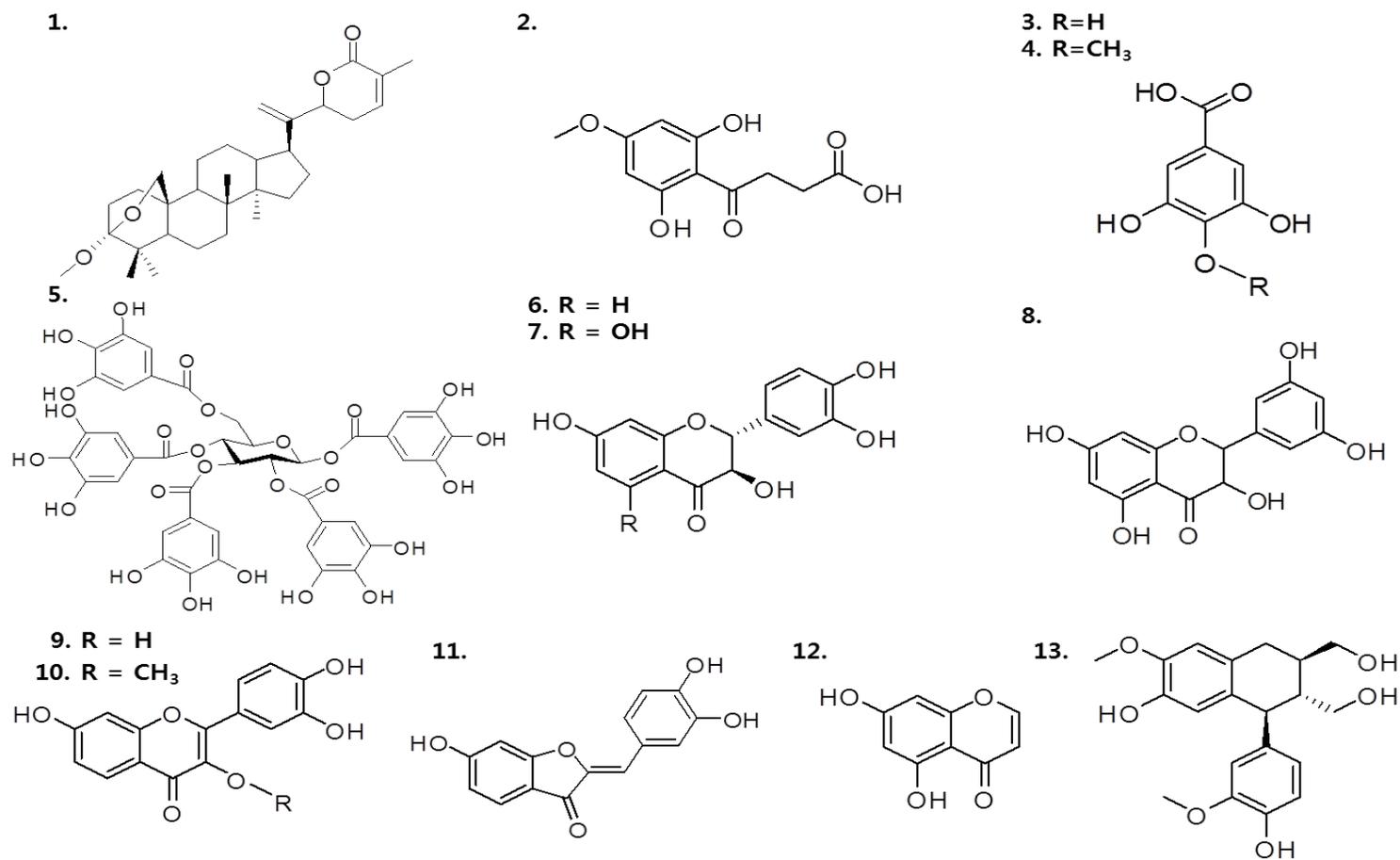
Figure 11. The structures of compounds isolated from *R. trichocarpa* roots

2. Cytotoxic effect of total extract, fractions and the compounds from *R. trichocarpa* roots on AGS cells

2.1. Cytotoxic effect of total extract and fractions on AGS cells.

Since AGS cell, as a human gastric adenocarcinoma, has the gastric epithelial and unique characteristics, it has been used in research in the various fields including oncology, virology and immunology. Therefore, because of this reason, AGS cell line was used for the screening tool in the process of searchiong the antineoplastic substances, and in this study, the cytotoxic effect of total extract and each fraction was examined by it. The total extract, *n*-hexane and EtOAc fractions showed significant inhibitory effect on the growth of AGS cells by MTT assay (57.3, 33.2 and 57.4% at 100*u*g/ml, respectively). EtOAc fraction was subjected to repeated column chromatography and HPLC, and compound **1-13** were isolated.

* P<0.05, ** P<0.01, *** P<0.001 versus control group
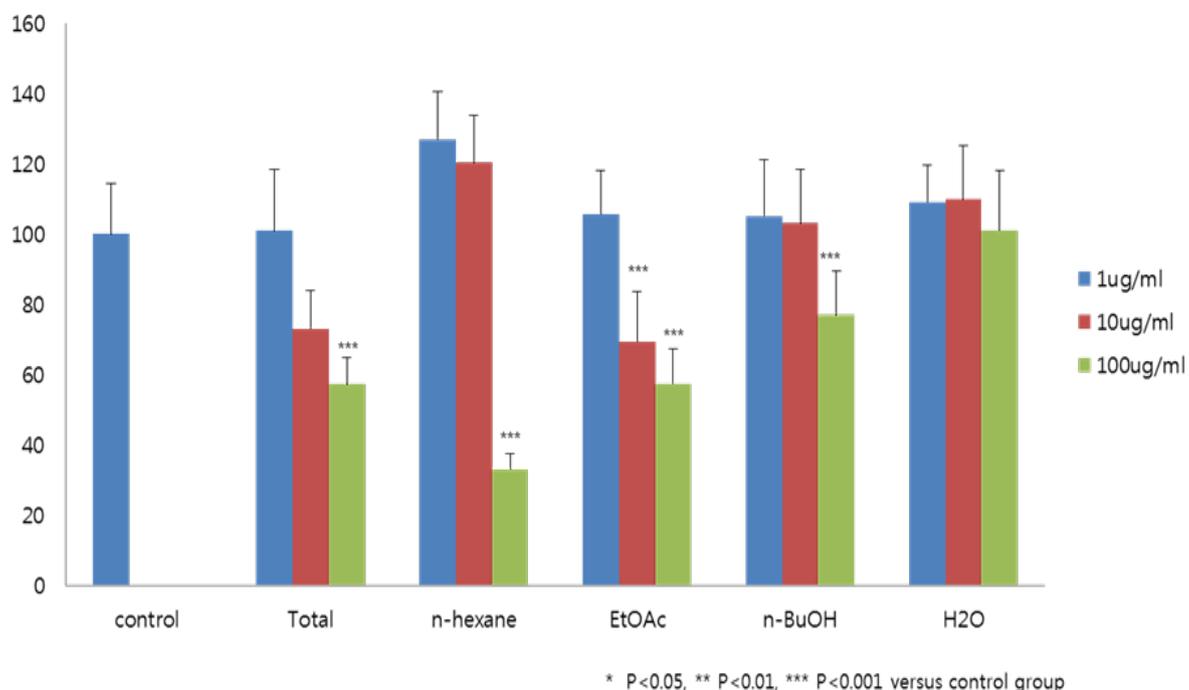
Figure 12. Cytotoxic effect of total extract and fractions from *R. trichocarpa* roots on AGS cells

Cell viability was measured by MTT assay. AGS cells were treated with total extract and fractions at concentration ranging from 1 to 100 ug/ml for 24 hr. Control was incubated with a vehicle alone. Results are expressed as the mean ± S.D. of three independent experiments, each performed using triplicate wells. *p<0.05, **p<0.01 and ***p<0.001 compared with control.

2.2. Cytotoxic effect of compounds **1-13** on AGS cells.

The cytotoxic effect of isolated compounds **1-13** from *R. Trichocaroa* root on AGS cells was evaluated at concentration ranging from 10 to 1000 uM for 24 hr (Figure 13). The AGS cell viability treated with vinblastine as a positive control was 73.8% at 10uM, 50.5% at 100uM, 21.2% at 500uM and 23.6% at 1000uM, respectively. It was found that compounds **1** to **12** except compound **13** showed the cytotoxic effect in AGS cells. Also, compound **4** having a methoxy moiety showed more potent cytotoxic effect than compound **3**.
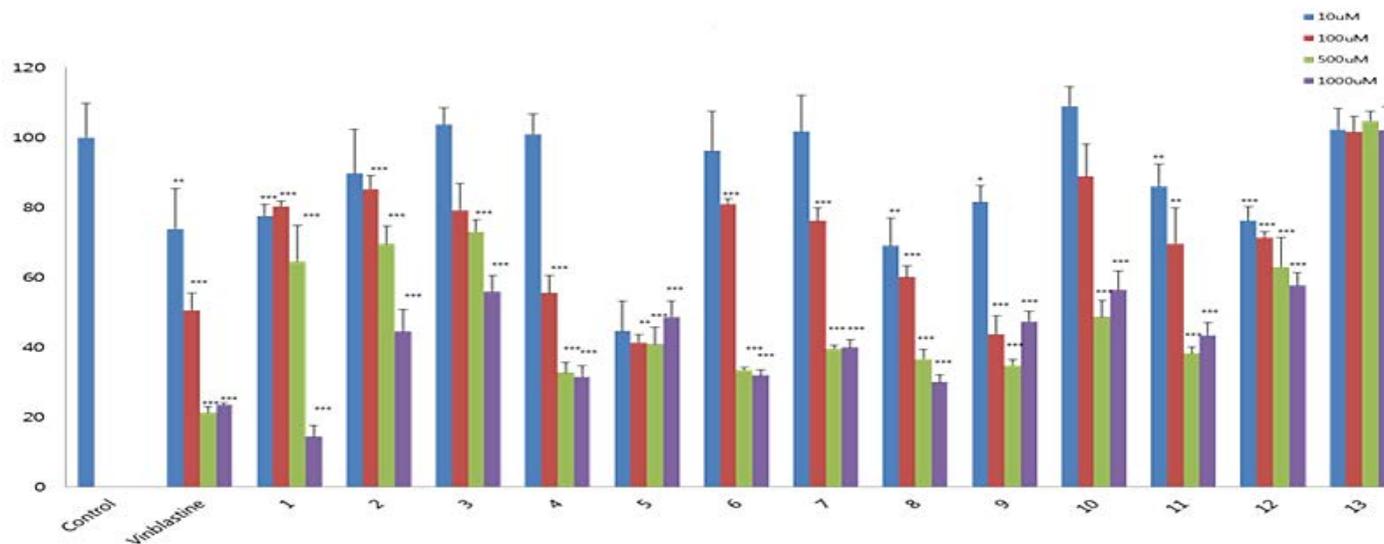
Figure 13. Cytotoxic effect of compounds **1-13** from *R. trichocarpa* roots on AGS cells

Cell viability was measured by MTT assay. AGS cells were treated with compound **1-13** at concentration ranging from 10 to 1000 uM for 24 hr. AGS cells were incubated with a vehicle (control). The cytotoxic effect of vinblastine as a positive control was 73.8% at 10uM, 50.5% at 100uM, 21.2% at 500uM and 23.6% at 1000uM, respectively. Results are expressed as the mean ± S.D. of three independent experiments, each performed using triplicate wells. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ compared with control.

2.3. Cytotoxic effect of compounds **3,4** and **5** on AGS cells for 24 and 48 hr.

The most types of polyphenol in the human diet are the phenolic acids and flavonoids, and polypehnols have been studied to possesss antioxidative and anti-tumoe promoting effects (Lin et al., 2007; Lin et al., 2005). Recent study demonstrated the cytotoxic effect of gallic acid (compound **3**) on AGS cells for 24 and 48hr (Ho et al., 2010). Thus, in this study, to investigate phenolic compounds about the time-dependent inhibitory effect on growth of AGS cells, similar compounds **3,4** and **5** were examined for 24 and 48 hr. (Figure 14). Compounds **3** and **4** showed time-depended cytotoxic effect on AGS cells, and compound **4** having a methoxy moiety had higher cytotoxic effect than compound **4.**
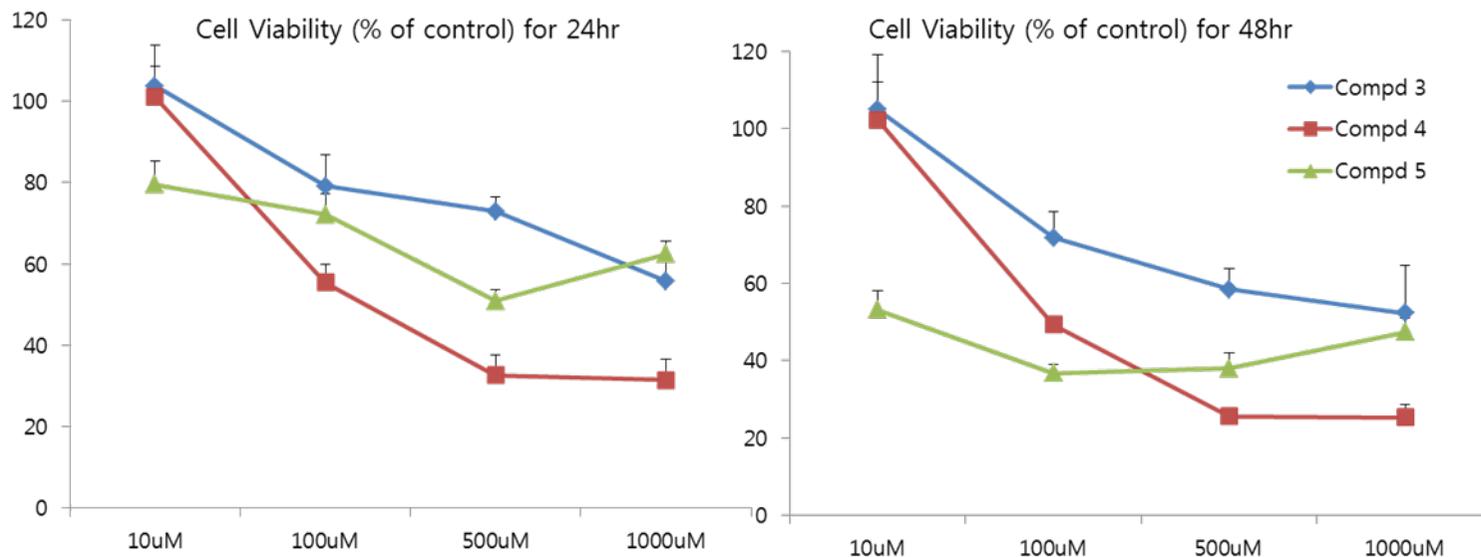
Figure 14. Cytotoxic effect of compounds **3,4** and **5** from *R. trichocarpa* roots on AGS cells for 24 and 48hr.

Cell viability was measured by MTT assay. AGS cells were treated with compound **3,4** and **5** at concentration ranging from 10 to 1000 *u*M for 24 and 48hr. AGS cells were incubated with a vehicle (control). Results are expressed as the mean ± S.D. of three independent experiments, each performed using triplicate wel

IV. Conclusion

1. Total extract, *n*-hexane and EtOAc fractions of *R. trichocarpa* roots exhibited cytotoxic effects on AGS cells.

2. Thirteen compounds were isolated from EtOAC fr. of *R. trichocarpa* roots.

3. A triterpene (**1**), four phenolic compounds (**2-5**), six flavonoids (**6-11**), a chromene (**12**) and a lignan (**13**) were isolated by the activity-guided isolated over EtOAc.

4. Compounds **1** (3-methoxy semialactone) and **2** (4-(2',6'-dihydroxy-4'-methoxyphenyl)-4-oxobutanoic acid) were newly reported from nature.

5. Compounds **1** to **12** except compound **13** showed the cytotoxic effect on AGS cells.

6. Compounds **3** (gallic acid) and **4** (4-*O*-methylgallic acid) showed time-dependent inhibitory effects on the growth of AGS cells, and compound **4** had higher cytotoxic effect than compound **3**

V. References

Alley, M. C., Scudiero, D. A., Monks, A., Hursey, M. L., Czerwinski, M. J., Fine, D. L., . . . Boyd, M. R. (1988). Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. [Research Support, U.S. Gov't, P.H.S.]. *Cancer Res, 48*(3), 589-601.

Beretta, G., Artali, R., Caneva, E., & Facino, R. M. (2011). Conformation of the tridimensional structure of 1,2,3,4,6-pentagalloyl-beta-D-glucopyranose (PGG) by H-1 NMR, NOESY and theoretical study and membrane interaction in a simulated phospholipid bilayer: a first insight. *Magnetic Resonance in Chemistry, 49*(3), 132-136.

Cho, J. Y., Sohn, M. J., Lee, J., & Kim, W. G. (2010). Isolation and identification of pentagalloylglucose with broad-spectrum antibacterial activity from Rhus trichocarpa Miquel. *Food Chemistry, 123*(2), 501-506. doi: DOI 10.1016/j.foodchem.2010.04.072

Cignarella, G., Barlocco, D., Curzu, MM., Pinna, GA., Cazzulani, P., Cassin, M., & Laumachi, B. (1990). Synthesis and pharmacological evaluation of 4,4a-dihydro-SH-[l]benzopyrano[4,3-c]pyridazin-3(2H)-ones bioisosters of antihypertensive and antithrombotic benzo[h]cinnolinones. *Eur J Med Chem*, 25, 749-756.

Han, X. H., Hong, S. S., Hwang, J. S., Lee, M. K., Hwang, B. Y., & Ro, J. S. (2007). Monoamine oxidase inhibitory components from Cayratia japonica. *Archives*

of Pharmacal Research, 30(1), 13-17.

Hasan, A., Sadiq, A., Abbas, A., Mughal, E., Khan, K. M., & Ali, M. (2010). Isolation and synthesis of flavonols and comparison of their antioxidant activity. *Natural Product Research, 24*(11), 995-1003.

Ho, H. H., Chang, C. S., Ho, W. C., Liao, S. Y., Wu, C. H., & Wang, C. J. (2010). Anti-metastasis effects of gallic acid on gastric cancer cells involves inhibition of NF-kappa B activity and downregulation of PI3K/AKT/small GTPase signals. *Food and Chemical Toxicology, 48*(8-9), 2508-2516.

Hungerford, N. L., Sands, D. P. A., & Kitching, W. (1998). Isolation and structure of some constituents of the Australian medicinal plant Tinospora smilacina ('snakevine'). *Australian Journal of Chemistry, 51*(12), 1103-1111.

Jain, R., Pancholi, B., & Jain, S. C. (2011). Radical Scavenging and Antimicrobial Activities of Convolvulus microphyllus. *Asian Journal of Chemistry, 23*(10), 4591-4594.

Jung, K. W., Park, S., Kong, H. J., Won, Y. J., Lee, J. Y., Seo, H. G., & Lee, J. S. (2012). Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2009. *Cancer Res Treat, 44*(1), 11-24. doi: 10.4143/crt.2012.44.1.11

Jung, K. W., Park, S., Won, Y. J., Kong, H. J., Lee, J. Y., Seo, H. G., & Lee, J. S. (2012). Prediction of cancer incidence and mortality in Korea, 2012. *Cancer Res Treat, 44*(1), 25-31.

Junior, G. M. V., Sotssa, C. M. D., Cavalheiro, A. L., Lago, J. H. G., & Chaves, M. H. (2008). Phenolic Derivatives from Fruits of Dipteryx lacunifera DUCKE and

Evaluation of Their Antiradical Activities. *Helvetica Chimica Acta, 91*(11), 2159-2167.

Lee, I. S., Oh, S. R., Ahn, K. S., & Lee, H. K. (2001). Semialactone, isofouquierone peroxide and fouquierone, three new dammarane triterpenes from Rhus javanica. *Chemical & Pharmaceutical Bulletin, 49*(8), 1024-1026.

Lee, J. C., Lim, K. T., & Jang, Y. S. (2002). Identification of Rhus verniciflua Stokes compounds that exhibit free radical scavenging and anti-apoptotic properties. [Research Support, Non-U.S. Gov't]. *Biochim Biophys Acta, 1570*(3), 181-191.

Lin, H. H., Chen, J. H., Kuo, W. H., & Wang, C. J. (2007). Chemopreventive properties of Hibiscus sabdariffa L. on human gastric carcinoma cells through apoptosis induction and JNK/p38 MAPK signaling activation. [Research Support, Non-U.S. Gov't]. *Chem Biol Interact, 165*(1), 59-75

Lin, H. H., Huang, H. P., Huang, C. C., Chen, J. H., & Wang, C. J. (2005). Hibiscus polyphenol-rich extract induces apoptosis in human gastric carcinoma cells via p53 phosphorylation and p38 MAPK/FasL cascade pathway. [Research Support, Non-U.S. Gov't]. *Mol Carcinog, 43*(2), 86-99.

Nishino, C., Enoki, N., Tawata, S., Mori, A., Kobayashi, K., & Fukushima, M. (1987). Antibacterial Activity of Flavonoids against Staphylococcus-Epidermidis, a Skin Bacterium. *Agricultural and Biological Chemistry, 51*(1), 139-143.

Ooi, Y., Daikoku, E., Wu, H., Aoki, H., Morita, C., Nakano, T., . . . Sano, K. (2011). Morphology and infectivity of virus that persistently caused infection in an AGS cell line. *Medical Molecular Morphology, 44*(4), 213-220.

Shin, H. R., Jung, K. W., Won, Y. J., & Park, J. G. (2004). 2002 annual report of the Korea Central Cancer Registry: based on registered data from 139 hospitals. *Cancer Res Treat, 36*(2), 103-114.

Simon, A., Chulia, A. J., Kaouadji, M., & Delage, C. (1994). Phytochemistry of the Ericaceae .7. Quercetin 3-[Triacetylarabinosyl(1-]6)Galactoside] and Chromones from Calluna-Vulgaris. *Phytochemistry, 36*(4), 1043-1045.

Son, Y. O., Lee, K. Y., Lee, J. C., Jang, H. S., Kim, J. G., Jeon, Y. M., & Jang, Y. S. (2005). Selective antiproliferative and apoptotic effects of flavonoids purified from Rhus verniciflua Stokes on normal versus transformed hepatic cell lines. [Research Support, Non-U.S. Gov't]. *Toxicol Lett, 155*(1), 115-125.

Wang, L. Q., Huang, R., Zhang, F. C., Fu, Q. X., & Chen, Y. G. (2011). CHEMICAL CONSTITUENTS OF Combretum punctatum spp. squamosum. *Chemistry of Natural Compounds, 47*(3), 470-471.

Wu, J. H., Tung, Y. T., Chien, S. C., Wang, S. Y., Kuo, Y. H., Shyur, L. F., & Chang, S. T. (2008). Effect of phytocompounds from the heartwood of Acacia confusa on inflammatory mediator production. *Journal of Agricultural and Food Chemistry, 56*(5), 1567-1573.

Yuasa, Y. (2003). Control of gut differentiation and intestinal-type gastric carcinogenesis. [Research Support, Non-U.S. Gov't [Review]. *Nat Rev Cancer, 3*(8), 592-600.

Zhang, Y. S., Que, S., Yang, X. W., Wang, B., Qiao, L. A., & Zhao, Y. Y. (2007). Isolation and identification of metabolites from dihydromyricetin. *Magnetic*

Resonance in Chemistry, 45(11), 909-916.

국문초록

암은 세포 자체의 조절 기능에 문제가 생기면서 정상적으로는 사멸되어야 할 비정상 세포들이 과다 증식하게 되며, 조직 및 장기에 침입하여 종괴를 형성하고 기존의 구조를 파괴하거나 변형시키는 질병이다. 특히 위암은 암 관련 사망 중 두 번째로 높은 비율을 차지하고 있으며, 그 원인은 *Helicobacter pylori* 감염이나 식습관, 환경적·유전적인 요인이라고 보고 되고 있다. 옻나무과 (Anacardiaceae)에 속하는 개옻나무 (*Rhus trichocarpa*)는 urushiols, flavonoid, polyphenolics 계열을 주성분으로 하며, 전통적으로 민간에서 위장 질환, 동맥경화등의 치료를 위해 사용 되어 왔으며, anti-apoptotic, anti-rhematoid, anti-mutagenic 등의 효과가 있다고 보고 되고 있다.

이에 본 연구에서는 위암 세포인 AGS cell을 이용하여 세포독성을 갖는 성분을 개옻나무의 뿌리로부터 활성 지향적 분리 기법을 이용하여 분리하고 그 구조를 규명하고자 하였다.

개옻나무 뿌리를 80% 메탄올로 추출한 다음, 추출물을 다시 *n*-hexane, EtOAc 및 *n*-BuOH 분획으로 나누었고, 이중 EtOAc 분획이 유의성 있는 세포독성을 확인 하였다. 각종 column chromatography 및 RP-HPLC를 이용하여 EtOAc 분획으로부터 총 13종의 화합물을 분리하였다. 분리한 화합물들은 각종 이화학적 성상 및 분광학적 데이터를 종합하여 그 구조를 각각

3-methoxy semialactone (**1**), 4-(2,6-dihydroxy-4-methoxyphenyl)-4-oxobutanoic acid (**2**), gallic acid (**3**), 4-*O*-methylgallic acid (**4**), pentagalloyl glucose (**5**), (-)-fustin (**6**), (+)-taxifolin (**7**), 3,3',5,5',7-pentahydroxyflavanonol (**8**), fisetin (**9**), 3-methoxy-7,3',4'-trihydroxyflavone (**10**), sulfuretin (**11**), 5,7-dihydroxy-4H-chromen-4-one (**12**) and Isolariciresinol (**13**) 으로 동정하였으며, 이들 화합물 중 **1** 과 **2** 은 천연에서 처음으로 분리, 보고 되는 물질이다. 분리한 총 13 종의 화합물에 대하여 AGS cell에서 세포독성을 확인한 결과, 화합물 **1-12** 가 유의성 있는 세포독성을 나타내었다.

감사의 글

2년 전, 아무것도 모른 체 옻나무 샘플을 채취하다 옻이 오른 일이 엊그제 같은데 어느덧 이렇게 대학원 석사 2년이라는 시간이 흘러 제가 감사의 글을 쓰게 되었습니다. 먼저, 부족한 저를 제자로 받아 주신 세분의 교수님께 감사 드립니다. 짧은 시간이었지만, 어머니 같이 따뜻하게 챙겨주시며 학자의 길로 인도해주신 김영중 교수님, 늘 인자한 모습으로 즐거운 분위기를 만들어 주시며 학문의 식견을 넓혀주신 김진웅 교수님, 그리고 많은 대화로 언제나 제자들에게 용기와 힘이 되어주시며 학문에 대한 열의를 가르쳐 주신 성상현 교수님께 감사의 말씀을 전하고 싶습니다.

2년의 시간 동안 너무나도 많은 추억과 좋은 인연을 만들어 주신 우리 생약실 식구들에게도 감사합니다. 실험부터 논문까지 친누나 같이 하나하나 꼼꼼하게 챙겨주신 이기용 박사님, 언제나 친절하게 가르쳐주며 학문적으로나 모든 것이 배울 점이 많은 양희정 박사님, 학문에 대한 열의와 언제나 남들보다 노력하는 이민아 박사님, 겉으로는 표현을 잘 하진 못 했지만 따뜻한 마음을 가지고 신앙심이 깊은 사수 남기 선배, 처음 실험실을 왔을 때부터 따뜻하게 하나하나 가르쳐 주며 챙겨준 태범 선배, 실험실을 위해 너무나도 수고가 많은 방장 동영 선배, 모르는 것이 있으면 그때마다 친절하게 가르쳐준 정무선배, 정말 똑똑하며 따뜻한 교빈 선배, 언제나 노력하며 최선을 다하는 정화, 웨이브 춤을 잘 추며 늘 해맑은

65

웃음과 긍정적인 생각을 가진 혜성이, 동생이지만 배울 점이 많은 귀재, 그리고 실험외적으로도 많은 도움을 준 현우, 생약실 궂은일을 하면서도 항상 밝은 미나와 은혜에게도 감사의 마음을 전합니다. 또한 한 분씩 감사의 표현을 하지 못해 죄송하지만 옆방의 종혜누나, 장규 선배, 이레 선배, 민지 선배, 정률 선배, 준철, 종민에게도 감사합니다.

제 인생에서 언제나 힘이 되어주시며 항상 저의 인생 길잡이가 되어주시는 사랑하는, 존경하는 아버지, 어머니께 감사 드리며, 저 멀리서 늘 걱정하며 자식처럼 아껴주는 사랑하는 누나, 매형 감사합니다. 또한, 항상 많은 힘이 되어주고 있는 소중한 친구들에게도 감사합니다. 마지막으로, 긴 시간 동안 이렇게 기다려준 사랑하는 나의 가장 오래된 친구 지언이에게도 고맙습니다.

저를 아껴주시며 도와주신 가족, 교수님, 선후배님들, 그리고 친구들의 건강과 행복을 바라며, 항상 감사하는 마음을 가지며 긍정적으로 살아가며 바른 사람이 되도록 노력하겠습니다. 다시 한번 감사 드립니다.

2012 년 8 월

최지훈 올림