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Thesis for Master Degree

Isolation and structure determination of AMPK activators from *Saururus chinensis*

Chosun University Graduate School

College of Pharmacy

Kang Hu Won

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삼백초*(Saururus chinensis)*로부터 AMPK 활성화 화합물의 분리와 구조분석

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지도교수 오원근

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조선대학교 대학원

약학과

강후원

This thesis is examined and approved for

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List of Abbreviations

$[\alpha]^{T}_{D}$: specific rotation

AMPK: AMP- activated protein kinase

ACC: acetyl- CoA carboxylase

LKB1: serine/threonine kinase 11

CaMKKs: calmodulin-dependent protein kinase kinases

DMSO: dimethyl sulfoxide

EtOH: ethanol

MeOH: methanol

HPLC: high performance liquid chromatography

HREIMS: high resolution electro impact mass spectroscopy

EIMS: impact mass spectroscopy

MS: mass spectrum

m/z: mass to charge ratio

NMR: nuclear magnetic resonance

ppm: parts per million

RP: reverse phase

UV: ultraviolet absorption

THF: tetrahydrofuran

HFD: high-fat diets

ND: normal fed diets

GPT: glutamate pyruvate transaminase

BUN: blood urea nitrogen

GLUC: glucose

CHOL: cholesterol

HDLC: high-density lipoprotein cholesterol

LDLC: low-density lipoprotein cholesterol

TRIG: triglyceride.

(국문 초록)

삼백초*(Saururus chinensis)*로부터 AMPK 활성화 화합물의 분리와 구조분석

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최근 경제발전에 따른 생활수준의 향상으로 인하여 위생환경이 개선되고 식생활의 향상으로 섭취 열량 또한 급속한 증가가 이루어지고 있다. 그러나 과도한 음식으로 섭취 열량이 증가하는 반면 운동 부족 등으로 소비되는 열량이 적어 비만이 증가하는 경향을 보이고 있다.

AMP-activated protein kinase (AMPK) 효소는 세포 및 개체의 에너지 대사를 조절하는 효소로서 대사과정의 필수적인 조절자이다. 최근의 많은 연구는 운동을 모방한 효과를 보일 수 있는 AMPK 활성화 물질이 항비만, 항당뇨 및 대사증후군 질환의 강력한 약물 목표점으로 사용될 수 있음을 제시하고 있다. 본 연구자는 새로운 AMPK 활성화 물질을 천연물로부터 발굴하려는 탐색과정에서 삼백근 뿌리 추출물이 분화된 근육전구세포인 C2C12 세포주에서 AMPK 효소를 활성화 함을 발견하였다.

활성물질로서 리그난계 화합물 골격을 갖는 11종의 화합물을 크로마토그래피와 고압액체크로마토그래피 (HPLC)를 사용하여 분리하였다. 분광학적인 방법을 이용하여 결정한 화합물의 구조는 sauchinone (**1**), di-*O*-methyltetrahydrofuriguaicinB (**2**), rel-(7R,8R,7'-R,8'-R)-3',4'Methylenedioxy-3,4,5,5'-teramethoxy-7,7'-epoxylignan (**3**), saucerneol D (**4**), saucerneol E (**5**), machilin D (**6**), manassantin B (**7**), manassantin A (**8**), 4-*O*-demethylmanassantin B (**9**), 1,3-Benzodioxole-5-methanol,a-[1-[2,6-dimethoxy-4-(2-propenyl)phenoxy]ethyl]-7-methoxy-, (R*,R*)- (9CI) (**10**), 1,3-Benzodioxole-5-methanol,a-[1-[2,6-dimethoxy-4-(2-propenyl)phenoxy]ethyl]-7-methoxy-, acetate, (R*,R*)- (9CI) (**11**)로 확인하였고, 그 중 화합물 **10**과 **11**은 삼백초에서 처음 분리하였고, 화합물 **11**은 합성된 화합물로 보고되었으나 천연물에서는 처음으로 분리하였다. 화합물 **4, 7**과 **8**은 AMPK 효소를 활성화 하였다.

ABSTRACT

Isolation and structure determination of AMPK activators from *Saururus chinensis*

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The recent increase in obesity due to high calorie foods and sedentary lifestyles has led to an increase in the prevalence of type 2 diabetes and other metabolic disorders. AMP-activated protein kinase (AMPK), a heterotrimeric enzyme complex that works as a fuel gauge to regulate cellular and whole body energy homeostasis, may act as a key player in metabolic control. Many studies indicate that AMPK activators, which mimic or potentiate the exercise-related effects, are regarded as potential candidates for development of anti-obesity, anti-diabetic agents as well as drugs for the treatment of other metabolic diseases.

In our program to search new AMPK activators from plants, we found that a total extract of *S. chinensis* activated AMPK enzyme in differentiated C2C12 cells. As the active constituents, sauchinone (**1**), di-*O*-methyltetrahydrofuriguaicin B (**2**), rel-(7R,8R,7'-R,8'-R)-3',4'Methylenedioxy-3,4,5,5'-teramethoxy-7,7'-epoxylignan (**3**), saucerneol D (**4**), saucerneol E (**5**), machilin D (**6**), manassantin B (**7**), manassantin A (**8**), 4-*O*-demethylmanassantin B (**9**),

1,3-Benzodioxole-5-methanol,a-[1-[2,6-dimethoxy-4-(2-propenyl)phenoxy]ethyl]-7-methoxy-, (R*,R*)-(9CI) (**10**),1,3-Benzodioxole-5-methanol,a-[1-[2,6-dimethoxy-4-(2-propenyl)phenoxy]ethyl]-7-methoxy-, acetate, (R*,R*)- (9CI) (**11**) were isolated from this extract. Compounds **10** and **11** were isolated from this plant for the first time. This is the first report of compound **11** isolated from natural product. Among isolates, compounds **4**, **7,** and **8** showed a strong stimulation on AMPK enzyme.

1. Introduction

1.1. Metabolic syndrome

The original description of the metabolic syndrome includes obesity, insulin resistance, hypertension, impaired glucose tolerance or diabetes, hyperinsulinemia, and dyslipidemia[1]. A variety of names like the pluri- metabolic syndrome, the insulin resistance syndrome, syndrome X, the dysmetabolic syndrome, the metabolic syndrome have been associated with this condition. [1]

Consequences of the syndrome are diverse, including cardiovascular disease, hypertension, and chronic renal disease. Moreover, the metabolic disorders carry much higher risk for diabetes and obesity which are now becoming an epidemic in the developed and developing countries[1]. Today, more than 1.1 billion adults worldwide are overweight, and 312 million of them are obese. In the past 20 years, the rates of obesity have tripled in developing countries that have been adopting a Western life style involving decreased physical activity and over consumption of cheap, energy-dense food. In the developed world, 2 to 7% of total health care costs are attributable to obesity. In the United States alone, the combined direct and indirect costs of obesity were estimated to be $123 billion in 2001. The increase in the prevalence of type 2 diabetes is closely linked to the upsurge in obesity. About 90% of type 2 diabetes is attributable to excess weight. Furthermore, approximately 197 million people worldwide have impaired glucose tolerance, most commonly because of obesity and the associated metabolic syndrome. This number is expected to increase to 420 million by 2025[3].

The combination of the modern diet and sedentary lifestyle has resulted in an increase in obesity, type-2 diabetes, and other metabolic disorders. It is evident that if people would eat fewer calories and increase their activity level, the abnormalities of the metabolic

syndrome would be reserved. However, compliance with such a strategy is clearly difficult. If society is not willing to make major lifestyle to changes, we need other interventions available. Some promising approaches have recently been introduced by the pharmaceutical industry. Recent studies indicate that AMPK may play an important role in exercise-related effects. Therefore, compounds activating AMPK, which have been claimed as "exercise in a pill", are paid significant attention as potential drugs for the treatment of metabolic diseases.

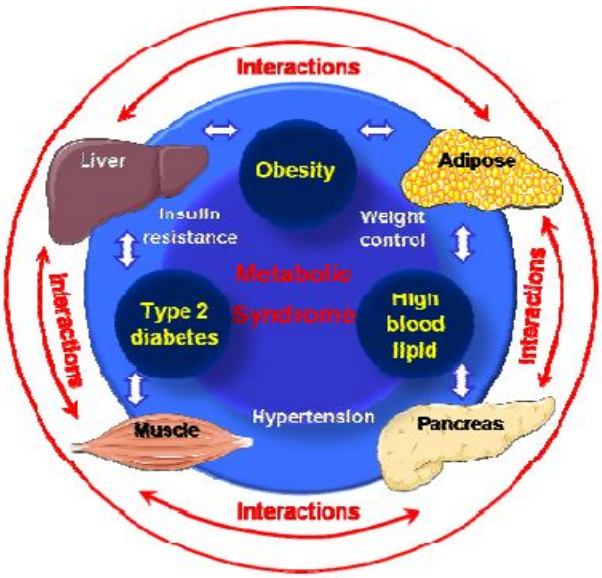


Fig. 1 Summary of the metabolic syndrome

1.2. AMP- activated protein kinase(AMPK): a target for total metabolic control

AMP- activated protein kinase (AMPK) was originally discovered as an enzyme by its ability to inactivate HMG- CoA and acetyl- CoA carboxylase. When it became clear that the kinase had multiple physiolosical substrates, it was renamed AMPK after its allosteric activator.[4] AMPK is now known to exist as heterotrimeric complexes comprising a catalytic α subunit and regulatory β and γ subunits. The catalytic subunit contains a conventional

serine/threonine protein kinase domain at the N- terminus and a C- terminal region that is required for the formation of the complex with the other two subunits. The β subunit contains a C-terminal domain that is required for complex formation and a central domain that is related to noncatalytic domains. The γ subunit contain variable N- terminal regions, followed by four tandem repeats of sequence known as a CBS motif, terms two Bateman domains, which bind ligans containing adenosine.[5]

AMPK is inactive unless phosphorylated by upstream kinases with the critical phosphorylation site being Thr^{172} within the activation loop of the kinase domain on the α subunit. As its name imply, AMP activates AMPK due to binding to the Bateman domains on the γ subunit via a complex mechanism involving three effects: (a) promotes phosphorylation by the upstream kinase; (b) causes allosteric activation of the phosphorylated kinase and (c) inhibits dephosphorylation of Thr^{172} by protein phosphatase. All three effects are also antagonized by binding of ATP, which binds to Bateman domains with a lower affinity than AMP and in a mutually exclusive manner. Because the adenylate kinase reaction is maintained close to the equilibrium in all eukaryotic cells, the cellular AMP: ATP ratio is a very sensitive indicator of compromised cellular energy status.[4]

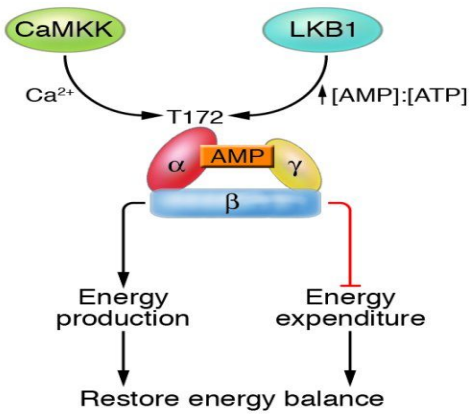


Fig. 2. Structure and regulation of AMPK[6]

The major upstream kinase of AMPK in most mammalian cells is a complex between the serine/threonine kinase 11 (LKB1) and two accessory subunits, STRAD and MO25. The LKB1 is a classical tumor suppressor and also acts upstream of at least 12 other AMPK-related kinase. The STRAD subunit is essential for the ability of the LKB1 complex to phosphorylate Thr^{172} on AMPK. The MO25 subunit contains a helical repeat that is distantly related to the armadillo proteins and appears to stabilize the LKB1-STRAD complex. Moreover, calmodulin-dependent protein kinase kinases (CaMKKs) which are upstream kinases for calmodulin-dephendent protein kinases I and IV would also phosphorylate and activate AMPK.[5]

Thus, phosphorylation of Thr^{172} in the activation loop of the catalytic α subunit is an absolute requirement for AMPK activity and is mediated by at least 2 upstream kinases: LKB1 and CaMKKs or by the increase of intracellular AMP: ATP ratio.[6] In general, activated AMPK switches off ATP-consuming anabolic pathways, switches on ATP-generating catabolic pathways, and reestablishes a proper energy balance in the cell.[5] Many downstream targets of AMPK have been identified to prove the role of AMPK in glucose and lipid metabolism, such as stimulating glucose uptake in muscle, suppressing hepatic glucose production, improving insulin sensitivity and reducing ectopic lipid accumulation.[7] Among them, AMPK regulation on lipid homeostasis through acetyl- coA carboxylases (ACCs) is paid much attention because dys-regulation of fatty acid metabolism is strongly associated with the development of insulin resistance and type-2 diabetes.[8] AMPK-mediated phosphorylation and inactivation of ACC1 and ACC2 lead to the acute inhibition of fatty acid synthesis and increases fatty acid oxidation, respectively, and as a result, reduce triglyceride storage, lower plasma fatty acid and triglyceride levels.[7] AMPK not only plays an important role in mediating whole-body glucose and lipid homeostasis but also functions to regulate food intake, energy expenditure and control weight.[9] For all these reasons, AMPK activators are regarded as promising candidates for the

discovery of anti-obesity, anti-diabetes agents as well as drugs for the treatment of other metabolic diseases.[5, 6, 7, 9]

1.3. *Saururus chinensis*

Saururus chinensis Baill (Saururaceae) is a perennial distributed in China, Japan, and Korea. The scented flowers are hermaphrodite, having both male and female organs, and flowers from July to August. The *Saururus chinensis* prefers light sandy, medium loamy, heavy clay soils and acid, neutral and basic(alkaline) soils. It can grow in semi-shade of no shade, requires wet soil and even grow in shallow water. This plant has been used in Korean folk medicine for the treatment of various diseases such as edema, jaundice, gonorrhea, anti-pyretic, diuretic, hepatoma, and anti-inflammatory agent.[31]

Previous chemical studied of the genus *Saururus* have shown the presence of more than 20 lignans as well as flavonoids ,anthraquinones ,alkaloids and furanoditerpens. The lignan derivatives isolated from this plant, which are tetrahydrofuran type, are known to have a

variety of biological activities, such as cell adhesion inhibitory anti-inflammatory, murine neuroleptic , hepatoprotective, and antifeedant activities.[17-20]

The lignans including six types: dibenzylbutane lignans, monoepoxylignans (substituted tetrahydrofurans), sauchinone-type lignans, alkyl aryl ether neolignans, sesquineolignans, and dineolignans; 2'-hydroxydihydroguaiaretic acid, meso-dihydroguaiaretic acid,austrobailignan-5, erythro-austrobailignan-6,(+)-saururinone, and saururin A, monoepoxylignans(substituted tetrahydrofurans): rel-(8R,8'R)-dimethyl-(7S,7'R)- bis(3,4-methmethylenedioxyphenyl),tetrahydrofuran, nectandrin B, (+)-saucernetin, galbacin, and di-*O*-methyltetrahydrofuruguaiacin B; alkyl aryl ether neolignans: machilin D; and virolin; sesquineolignans: saucerneols A-C , saucerneols D and E, (–)-saucerneol methyl ether , and (–)-saucerneol , dineolignans: 4-O-demethylmanassantin A, 4-O-demethylmanassantin B, manassantins A and B, saucernetin-7, saucernetin-8, threo, erythro-manassantin A, and erythro, erythro-manassantin A; sauchinone-type lignans: sauchinone, sauchinone A, 1'-epi-sauchinone, ent-sauchinone , and sauchinone B.[20-26,31]

In this study, the roots of *S. chinensis* were extracted with 70% ethanol and then directly subjected on a Diaion HP-20 column to give five fraction. Bioassay of five fractions on AMPK revealed that the 100% ethanol and the acetone-eluted fraction were active. 100% ethanol and the acetone-eluted fraction has led to the isolation of series of sauchinone-type lignan (**1**), monoepoxylignans(substituted tetrahydrofurans) lignan (**2-3**), alkyl aryl ether neolignans (**6, 10, and 11**), sesquineolignans (**4-5**), dineolignans (**7-9**). In this thesis, I would like to describe the isolation and structure elucidation of these compounds and the evaluation of their AMPK acticity.

2. Materials and Methods

2.1. Materials

2.1.1. Plant

The dried root of *Saururus chinensis* Baill. (Saururuaceae) were purchased at Kang Won medical herbs company in Kangwon city, Republic of Korea. The sample was identified by Professor YH Moon at Chosun University, and its specimen (No. 0010) has been deposited at the Department of Pharmacy, Chosun University, Republic of Korea.

2.1.2. Chemicals, reagents, and chromatography

Column chromatography was conducted on silica gel (Merck, 63 – 200 μm particle size) and reversed phase (ODS-A, Merck, 120 μm particle size) from Merck. TLC was carried out with silica gel 60 F254 plates from Merck. The solvent for NMR analysis was purchased from CIL (Cambridge Isotope Lab., USA). HPLC solvents were from Burdick & Jackson, USA.

AICAR was purchased from Sigma Chemical Company (St Louis, MO, USA), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and trypsin were purchased from GIBCO-BRL (Grand Island, NY, USA). Antibodies against phospho AMPKα Thr^{172}, phosphospecific ACC Ser^{79}, β-actin, anti-mouse, and anti-rabbit IgG antibodies were purchased from Cell Signaling Technology (Beverly, MA)

2.1.3. General experimental procedures

UV spectra were taken in MeOH using a Shimadzu spectrometer, and the optical rotations were obtained in MeOH using a Rudolph Autopol IV polarimeter. The nuclear magnetic resonance (NMR) spectra were obtained on Varian Unity Inova 500 MHz

spectrometer at Korea Basic Science Institute (KBSI, Gwangju Center, Korea). EIMS and HREIMS data were performed on a Micromass QTOF2 (Micromass, Wythenshawe, UK) mass spectrometer. HPLC was carried out using a Gilson System with UV detector and an RP-C18 column (10 × 250 mm, 5 μm particle size, RS Tech Optima Pak C18 column, Korea).

2.2. Methods

2.2.1. Cell culture

Mouse C2C12 skeletal myoblasts were maintained in DMEM supplemented with 10% fetal bovine serum in an atmosphere of 95% air and 5% CO_2 at 37 ^{o}C. To prepare for each assay, cells were seeded in 12-well plates with 10^5 cells/well in 2 mL growth medium. Differentiation of C2C12 myoblasts was induced by replacing growth medium with DMEM containing 5% horse serum when the cells were confluent. The medium were changed every 48 h until the formation of myotubes. Cells were used in experiments at 4-5 days after differentiation.

2.2.2. AMPK assay by Western blot analysis

C2C12 myotubes were incubated with appropriate concentration of compounds for 30 minutes and then lysed in EBC lysis buffer [50 mM Tris-HCl (pH 7.6), 120 mM NaCl, 1 mM EDTA (pH 8.0), 0.5% NP-40, and 50 mM sodium fluoride]. Cell debris was removed by centrifugation at 12,000 rpm for 15 min, at 4 ^{o}C. Protein concentrations in the cell lysates were determined using a Bio-rad protein assay kit. About 30 μg proteins of total cell extracts were subjected to western blot analysis using anti-phosphospecific AMPKα Thr^{172}, anti-phosphospecific ACC Ser^{79}. β-Actin protein levels were used as a control for equal protein loading. The immunoreactive antigen was then recognized by using a horseradish peroxidase-

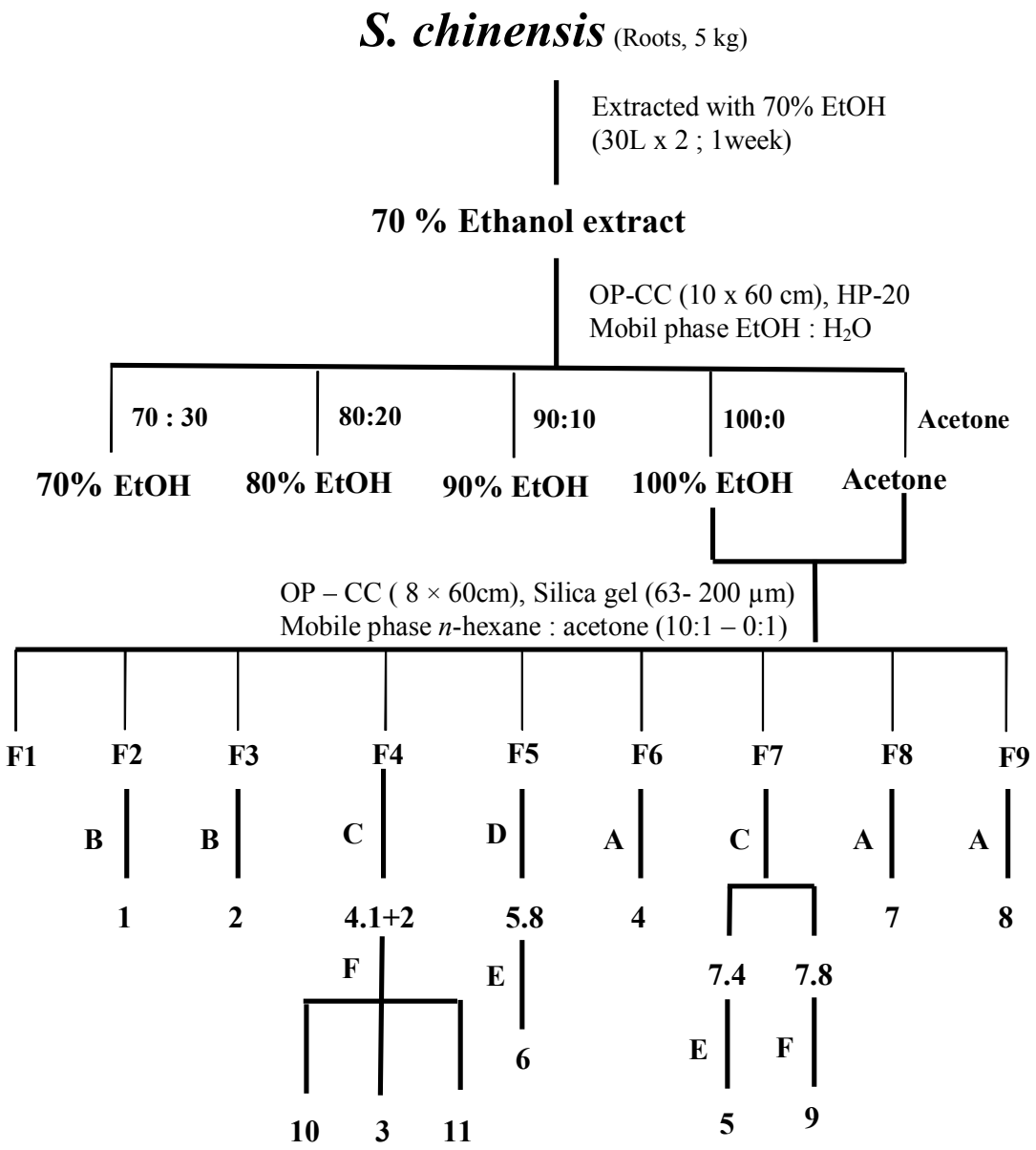
labeled anti-rabbit IgG and an enhanced chemiluminescence detection kit.[18]

2.2.3. Extraction and isolation of active compounds on AMPK from *Saururus chinensis* .

The roots of *S. Chinensis* (5 kg) were extracted with 70% EtOH at room temperature for 7days. The 70% ethanol-soluble extract was filtered, and then directly subjected on a Diaion HP-20 column (10 × 60 cm), eluted with H_2O/EtOH (30:70, 20:80, 10:90, 0:100, each 5L), and finally washed by acetone (5L) to give five fractions. Bioassay of five fractions on AMPK revealed that the 100% ethanol and the acetone-eluted fraction were active. This was further chromatography over silica gel (8 × 60 cm; 63–200 μm particle size) using a gradient of *n*-hexane/acetone (from 10:1 to 0:1), to yield nine fractions (F.1 – F.9) according to their TLC profiles. Compound **1** (973 mg) and Compound **2** (2 g) were purified from a part of fraction 2 and fraction 3 by crystallized with 100% MeOH, respectively. Fraction 4 was subjected to column chromatography over RP-C18 silica gel (4 × 50) eluted using MeOH / H_2O (1:1 – 6:1) as the eluting solvent to give six subfractions (F4.1 – F4.6). Purification of fraction F4.1-2 by preparative Gilson HPLC systems [using YMC JH ODS-H80 150 x 20 mm I.D, 4 *u*m particle size); mobile phase MeCN/H_2O containing 0.1% formic acid (65:35); flow rate 3mL/min; UV-detections at 205 and 254 nm] resulted in the isolation of compound **10** (21 mg, t_R 41.5 min), compound **3** (136 mg, t_R 48 min), and compounds **11** (18 mg, t_R 55 min), respectively. Fraction 5 was applied to column chromatography over a Sephadex LH-20 column(4 × 40cm), using MeOH as the eluting solvent to give ten subfractions (F5.1 – F5.10). Further separation F.5.8 by preparative Gilson HPLC [using RS Tech OptimaPak C18 column (10 × 250 mm, 10 μm particle size); mobile phase MeOH/H_2O(containing 0.1% formic acid)(77:23); flow rate 2 mL/min; UV-detections at 205 and 254 nm] resulted in the isolation of compound **6** (88 mg, t_R 13.4 min). Compound **4** (1.8 g) was purified from a part of fraction 6

by chromatography on a silica gel column (5.0 × 60 cm, 63–200 μm particle size) using a gradient of *n*-hexane/EtOAc (from 6:1 to 0:1). Fraction 7 was subjected to column chromatography over RP-C18 silica gel (5 × 60) eluted using MeOH / H_2O (1:2 – 5:1) as the eluting solvent to give ten subfractions (F7.1 – F7.10). Further separation of F.7.4 by preparative Gilson HPLC [using RS Tech OptimaPak C18 column (10 × 250 mm, 10 μm particle size); mobile phase MeOH/H_2O H_2O containing 0.1% formic acid (74:26); flow rate 2 mL/min; UV-detections at 205 and 254 nm] resulted in the isolation of compound **5** (7 mg, t_R 13.4 min) and separation of F.7.8 by preparative Gilson HPLC systems [using YMC JH ODS-H80 150 x 20 mm I.D, 4 *u*m particle size); mobile phase MeCN/H_2O containing 0.1% formic acid (72:38); flow rate 3mL/min; UV-detections at 205 and 254 nm] resulted in the isolation of compound **9** (22 mg, t_R 15 min), respectively. Compound **7** (13.2 g) was purified from a part of fraction 8 by chromatography on a silica gel column (5.0 × 60 cm, 63–200 μm particle size) using a gradient of *n*-hexane/EtOAc (from 5:1 to 0:1). Compound **8** (7.6 g) was purified from a part of fraction 9 by chromatography on a silica gel column (5.0 × 60 cm, 63–200 μm particle size) using a gradient of *n*-hexane/EtOAc (from 5:1 to 0:1).**(Scheme 1)**

Scheme 1. Isolation of compounds (**1** – **11**) from the *S. chinensis*



A; Silica gel CC, *n*-Hexane:EtOAC (gredient) B; Crystallization, MeOH 100%

C; RP-C18 CC, MeOH:H_2O (gredient) D; Sephadex LH-20, MeOH 100%

E; Gilson HPLC System, RP-C18 (10 × 250 mm, 10 μm, MeOH / H_2O + 0.1%Formic acid

F; Gilson HPLC System, RP-C18 (20 × 250 mm, 4 μm, MeCN / H_2O + 0.1%Formic acid

Sauchinone (1)

: Colorless powder : $C_{20}H_{20}O_6$; $[\alpha]^{26}_D$ -140° (c =1, $CHCl_3$); UV λ_{max}(MeOH) nm (log ε) 300 ,253.IR υ_{max} (KBr) cm^{-1}: 2916, 1676, 1664, 1418, 1433, 1321, 1240, 1184, 1155, 979, 926, 892, 756 ;EI-MS m/z (rel int) 356 $[M]^+$, 270 , 257, 205 , 175, 151, 138; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see **Table 1** and **2**.

Di-*O*-methyltetrahydrofuriguaicin B (2)

: Amorphous powder, $C_{22}H_{28}O_5$; $[\alpha]_D$ +43° (c=0.5, $CHCl_3$), UV λ_{max} (MeOH) nm (log ε): 280, 235. IR υ_{max} (KBr) cm^{-1}: 2959, 1591, 1515, 1417, 1255, 1235, 1159, 1136, 1028, 814, 761. EI-MS m/z (rel. int.): 372 $[M]^+$ 206 , 191, 175 , 165, 91, 77; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see **Table 1** and **2**.

rel-(7R,8R,7'-R,8'-R)-3',4'Methylenedioxy-3,4,5,5'-teramethoxy-7,7'-epoxylignan (3)

: Pale yellow oil, $C_{23}H_{28}O_7$; $[\alpha]^{21}_D$ -4.5° (c=0.01, MeOH;) UV λ_{max} (MeOH) nm (log ε): 254 ,273 , IR υ_{max} (KBr) cm^{-1}: 2957, 2924, 1634, 1506, 1457, 1435, 1091,1031. EI-MS m/z (rel. int.):416 $[M]^+$, 236, 224, 220, 208,205, 192, 175,165, 147, 135, 91, 77; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see **Table 1** and **2**.

Saucerneol D (4)

: Colorless powder; $C_{31}H_{36}O_8$; $[\alpha]^{25}_D$ -88.1° (c=1.2, $CHCl_3$), UV λ_{max} (MeOH) nm (log ε): 208.5, 235.6, 286.2. IR υ_{max} (KBr) cm^{-1}: 3490, 2960, 2920, 2900, 1605, 1595.; FABMS m/z 536 $[M]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see **Table 3** and **4**.

Saucerneol E (5)

: Colorless powder, $C_{30}H_{34}O_8$; [α] $^{25}_D$ -83.0° (c=0.3, $CHCl_3$), UV λ_{max} MeOH nm (log ε): 210.0, 232.5, 282.5 , IR └max (KBr) cm^{-1}: 3460, 2962, 2910, 2900, 1610, 1595; HRFABMS m/z: 540.2593, $C_{30}H_{34}O_8$, calcd 540.2597) ; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see **Table 3** and **4**.

Machilin D (6)

: Colorless oil, $C_{20}H_{24}O_5$; [α]$_D$ -160° (c=50.7, $CHCl_3$). UV λ_{max} (MeOH) nm (log ε): 260. IR (KBr) cm^{-1}: 3444 (OH), 2950, 2870, 1590, 1490; EI-MS m/z (rel. int.): 344 $[M]^+$, 191, 164 ,153 ,91, 77, 57; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see **Table 1** and **2**.

Manassantin B (7)

: White amorphous powder; $[\alpha]^{25}_D$ −51.7° (c=0.33, MeOH); UV λ_{max} (MeOH) nm (log ε): 208, 234, 282nm; IR (KBr) cm^{-1}: 3473, 2962, 2931, 1590, 1511, 1450, 1259, 1139, 1037, 935, 809, 736; HREIMS data(m/z [M]+, 716.3198, calcd 716.3197 for $C_{41}H_{48}O_{11}$); ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see **Table 3** and **4**.

Manassantin A (8)

: Brown powder**;** $[\alpha]^{25}_D$ −102.1° (c=0.5, $CHCl_3$); UV λ_{max} (MeOH) nm (log ε): 233, 280nm; IR (KBr) cm^{-1};3481, 3477, 2962, 2931, 1590, 1511, 1450, 1259, 1139, 1037, 935, 809, 736; HREIMS data (m/z [M]+ 732.3506, (calcd for $C_{42}H_{52}O_{11}$ 732.3510);^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see **Table 3** and **4**.

4-*O*-demethylmanassantin B (9)

: Clear powder; $[\alpha]^{25}_{D}$ −45° (c= 0.08, $CHCl_3$); UV λ_{max} (MeOH) nm (log ε); 208, 234, 282; IR (KBr) cm^{-1}: 3450, 2970, 2930, 1610, 1590, 1510, 1460, 1420, 1275, 1255, 1235, 1140, 1040, 935, 860, 820, 760; HREIMS data (m/z $[M-H]^-$, 701.2978, (calcd for $C_{40}H_{45}O_{11}$ 701.2962)); ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see **Table 3** and **4**.

1,3-Benzodioxole-5-methanol,a-[1-[2,6-dimethoxy-4-(2-propenyl)phenoxy]ethyl]-7-methoxy-, (R*,R*)-(9CI) (10)

: Green oil, IR (KBr) cm^{-1}: 3480, 1635, 1590, 1505, 1240, 1130, 1040; $C_{22}H_{26}0_7$; EIMS m/z (rel mt) 402, 221, 220, 209, 208, 205, 194, 193, 181, 179, 165, 163, 161, 151, 147, 137, 135, 133, 131, 123, 121; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see **Table 1** and **2**.

1,3-Benzodioxole-5-methanol,a-[1-[2,6-dimethoxy-4-(2-propenyl)phenoxy]ethyl]-7-methoxy-, acetate, (R*,R*)- (9CI) (11)

: Colorless powder, IR(KBr) cm^{-1} : 3480, 1635, 1590, 1505, 1240, 1130, 1040; $C_{24}H_{28}0_8$; EIMS m/z (rel mt) 402, 221, 220, 209, 208, 205, 194, 193, 181, 179, 165, 163, 161, 151, 147, 137, 135, 133, 131, 123, 121, 103, 102; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see **Table 1** and **2**.

3. Results and Discussions

3.1. Structure determination of lignans from *Saururus chinensis*

3.1.1. Structure determination of compound 1

Compound **1** was obtained as colorless powder with the molecular formula $C_{20}H_{20}O_6$. The NMR spectra showed the presence of two aromatic protons (δ 6.38 and 6.82) and two methylenedioxy groups, one (δ_H 5.90, 5.87; δ_C 101.24) attached to an aromatic ring and the other (δ_H 5.65, 5.60; δ_C 98.19) attached to aliphatic carbons.[13]C NMR signals at δ 143.26, 145.70, and 146.72 indicated that, in addition to aromatic carbon oxygen bonds for a methylenedioxy group, there is an additional oxygen attached to the aromatic ring. The [13]C NMR signal at δ 194.69 was attributed to the carbonyl group of an enone, while the [13]C NMR signal at δ 168.66 indicated the presence of a methylenedioxy group attached to the γ-carbon of an enone.[1]H NMR signals at δ 1.22 (d, 7.3 Hz) and 0.71 (d, 7.4 Hz) showed two methyl groups, each coupled to a vicinal proton. Comparison of [1]H and [13]C NMR spectral data suggested that compound are stereoisomers. Compound **1** was determined as sauchinone by [1]H and [13]C NMR spectroscopic data analyses, and comparison with those published in literatures.[14,30]

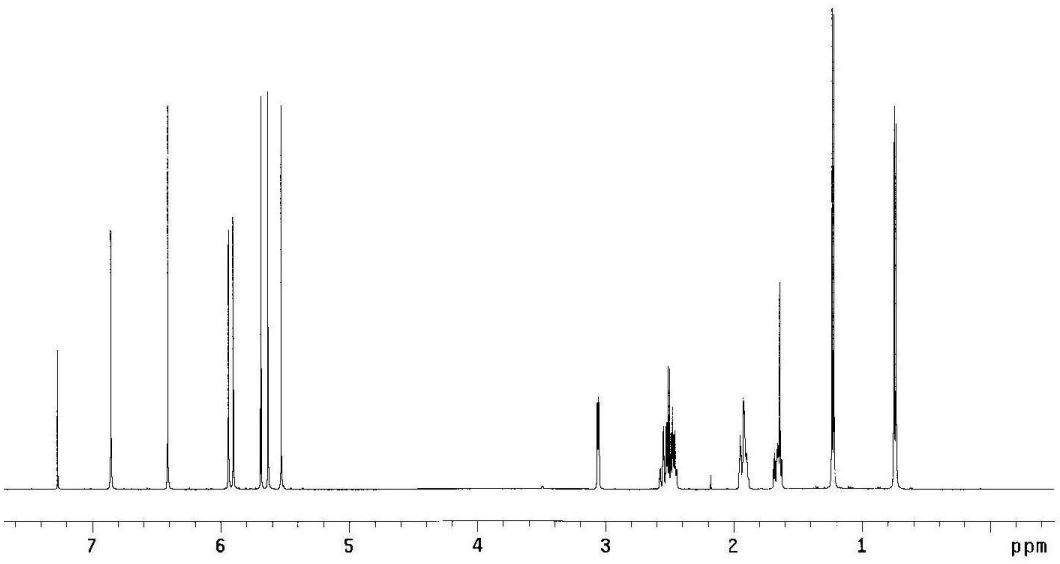


Fig. 3. ^{1}H-NMR spectrum of compound **1** (500 MHz, $CDCl_3$)

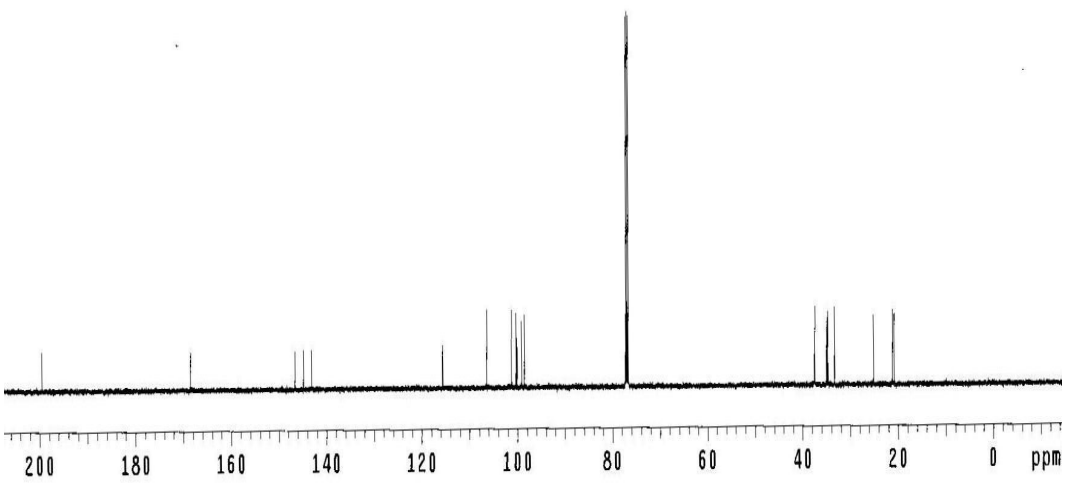


Fig. 4. ^{13}C-NMR spectrum of compound **1** (125 MHz, $CDCl_3$)

3.1.2. Structure determination of compound 2

Compound **2** was purified as amorphous powder, with the molecular formula $C_{22}H_{28}O_5$. as determined by the high resolution mass spectrum [HREIMS *m/z* 372.1645 (calcd for $C_{20}H_{28}O_5$, 372.1619)]. The ^1H and ^{13}C NMR spectra of compound **2** showed the symmetric nature of the molecular structure and corresponding to two identical sets of protons and carbon atoms, respectively. Furthermore, the ^1H NMR spectral data of compound **2** showed characteristic signals arising from the tetrasubstituted tetrahydrofuran ring system comprising of two secondary methyl groups at δ_H 0.69 (2H, d, J=6.6 Hz), a two proton multiplet at δ_H 2.24 (2H, m) due to the H-3, H-4 methine protons and two protons doublet at δ_H 5.44 (H, d, J=6.4 Hz) due to the two oxymethine protons, H-2 and H-5.[20] The coupling constant 6.6 Hz of the doublet at δ_H 0.69 for 3-methyl and 4-methyl suggested that these two methyl groups are in a *cis*-configuration with the adjacent protons H-2 and H-5, respectively[21]. The remaining six aromatic protons indicated the presence of two sets of 3-methoxy-4-hydroxyphenyl systems. ^{13}C NMR spectrum corroborated the assignments made for the structural determination of both aromatic rings. Comparison of spectroscopic data and physiochemical values between compound **2** and published data[22] led the structure of compound **2** to be di-*O*-methyltetrahydrofuriguaiacin B.[26,35]

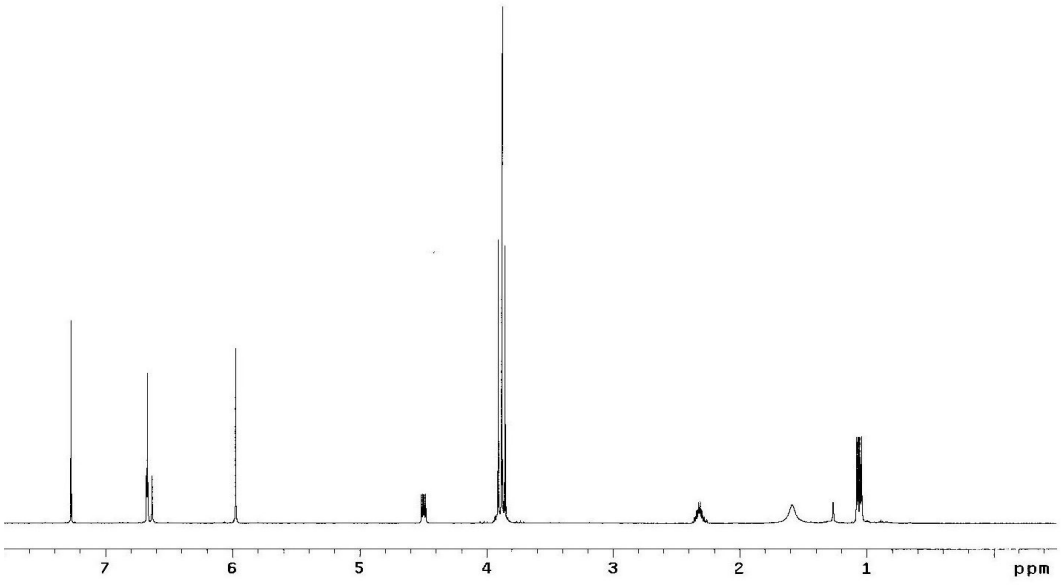


Fig. 5. ^{1}H-NMR spectrum of compound **2** (500 MHz, $CDCl_3$)

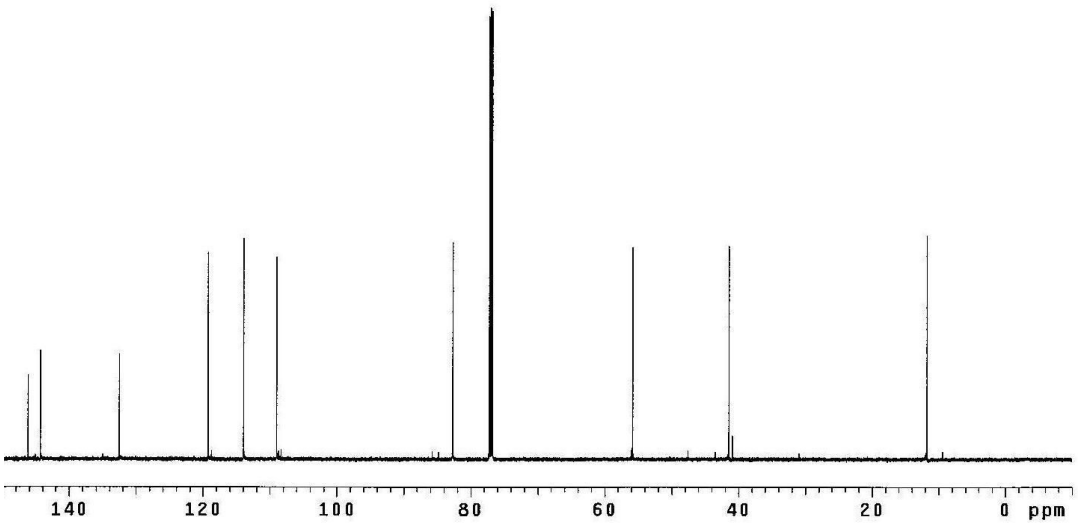


Fig. 6. ^{13}C-NMR spectrum of compound **2** (125 MHz, $CDCl_3$)

3.1.3. Structure determination of compound 3

Compound **3** was isolated as pale yellow oil, with the molecular formula $C_{23}H_{28}O_{7}$. The ^{1}H NMR spectrum of Compound **3** was very similar to that of grandisin. The set of signals at δ_H 0.96 (2 CH_3, d, 4.5Hz), δ_H 1.67 (2CH, m), and δ_H 4.52 (2CH, d, 7.2 Hz), whose connectivities were established by HMBC spectrum, confirmed the symmetric tetrahydrofuran ring moiety. The remaining signals at δ_H 5.87 (s, 2H), δ_H 3.84 (3H), δ_H 3.80 (6H) and δ_H 3.76 (3H) were assigned to a methylenedioxyphenyl and four aromatic methoxyl groups. This information, associated to aromatic hydrogens signals at δ_H 6.54 (s, 2H) and δ_H 6.52 (s, 2H), determined the two aromatic rings as 3,4,5-trimethoxyphenyl and 5′-methoxy-3′, 4′-methylenedioxyphenyl for this lignan. ^{1}H and ^{13}C NMR spectra of Compound **3** lacked the signals of two methoxy groups and showed the signal of one additional methylenedioxy group (δ_H ; 5.87, 2H , 3H, δ_c ; 101.3). The relative stereo-chemistry in the tetrahydrofuran ring was defined as all trans based on the coupling constants values observed in ^{1}H NMR spectrum. Compound **3** was determined as rel-(7R,8R,7'-R,8'-R)-3',4'Methylenedioxy-3,4,5,5'-teramethoxy-7,7'-epoxylignan by ^{1}H and ^{13}C NMR spectroscopic data analyses, and comparison with those published in literatures.[15]

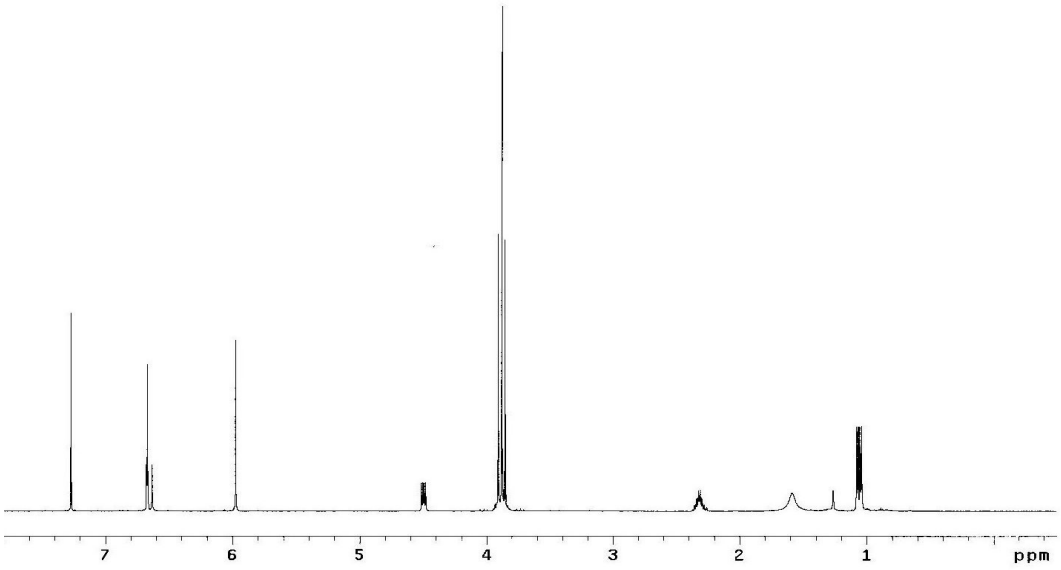


Fig. 7. ^{1}H-NMR spectrum of compound **3** (500 MHz, $CDCl_3$)

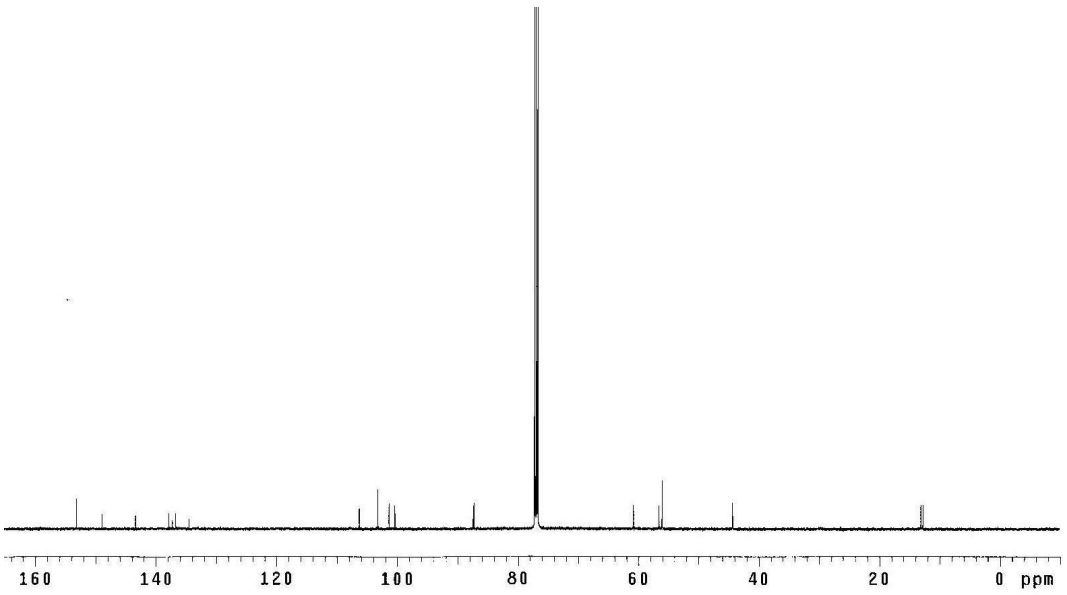


Fig. 8. ^{13}C-NMR spectrum of compound **3** (125 MHz, $CDCl_3$)

3.1.4. Structure determination of compound 4

Compound **4** was isolated as colorless powder with the molecular formula $C_{31}H_{36}O_{8.}$ by the high resolution mass spectrum [HREIMS *m/z* 544.2751 (calcd for $C_{30}H_{34}O_8$ 554.2754)]. The ^1H and ^{13}C NMR spectra of compound **4** showed two methine groups at δ_H 2.26 (H-8) and δ_H 2.28 (H-8') coupled with oxymethine groups at δ_H 5.42 (H-7) and 5.43 (H-7') as well as with methyl groups at δ_H 0.71 (H-9) and 0.70 (H-9'), respectively. These signals were assigned to those protons in the asymmetric 8,8'-dimethyl-7,7'-disubstitutedtetrahydrofuran moiety of compound 4. Additionally, the proton of an oxygenatedmethine group at δ_H 4.13 (H-8'') was coupled with that of another oxygenated methine group at δ_H 4.65 (H-7") and a methyl group at δ_H 1.17 (H-9"), indicating the presence of third phenyl propanoid moiety. The remaining nine aromatic protons exhibited characteristic signals belonging to three sets of 1,3,4-trisubstitutedbenzene rings. Two doublets at δ_H 6.81 (*J*=1.7 Hz, H-2) and δ_H 6.79 (*J*=8.0 Hz, H-5), one broad doublet at δ_H 6.76 (*J*=8.0 Hz, H-6), and two proton singlets at δ 5.95 suggested that one substituent was a 3,4-methylenedioxyphenyl group. In addition, three methoxyl groups at δ_H 3.93 (3'-OCH_3), 3.89 (3''-OCH_3),and 3.88 (4''-OCH_3) and another two sets of 1,3,4-tri-substituted benzene protons at δ_H 6.90 (d, *J*=1.6 Hz, H-2'), 6.99 (d, *J*=8.0 Hz, H-5'), 6.82 (dd, *J*=8.0, 1.6Hz, H-6'), 6.95 (d, *J*=1.7 Hz, H-2''), 6.84 (d, *J*=8.2Hz, H-5''), and 6.93 (dd, *J*=8.2, 1.7 Hz, H-6'') indicated that the presence of an 8''-*O*-4'-neolignan containing a 3'-methoxyphenyl group and a 3'',4''-dimethoxylphenyl group. This known compound was characterized as saucerneol D by comparing their physicochemical values, ^1H and ^{13}C NMR spectra data with those published in literature. [15, 16, 25]

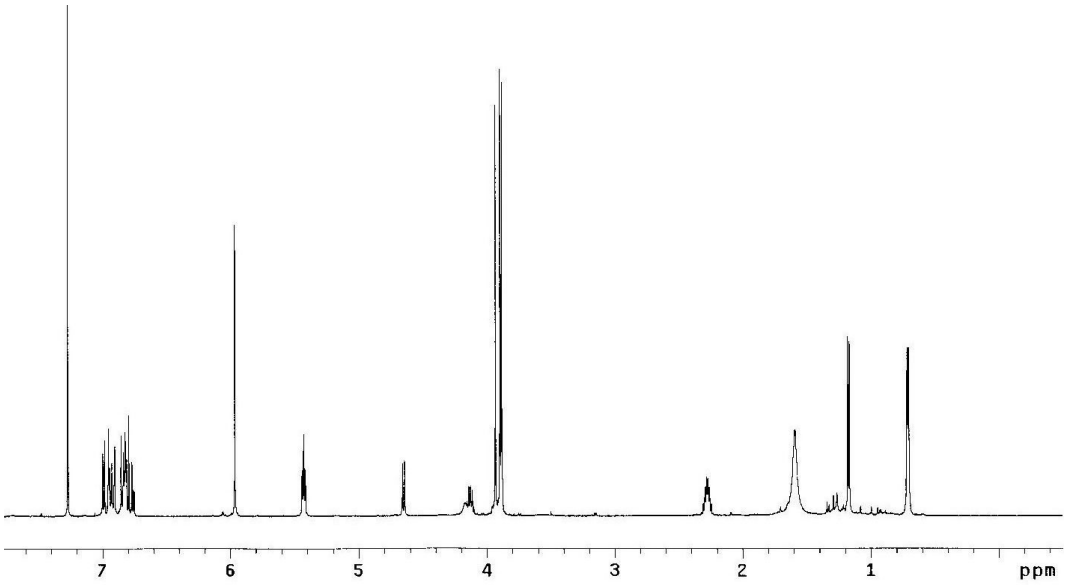


Fig. 9. ^{1}H-NMR spectrum of compound **4** (500 MHz, $CDCl_3$)

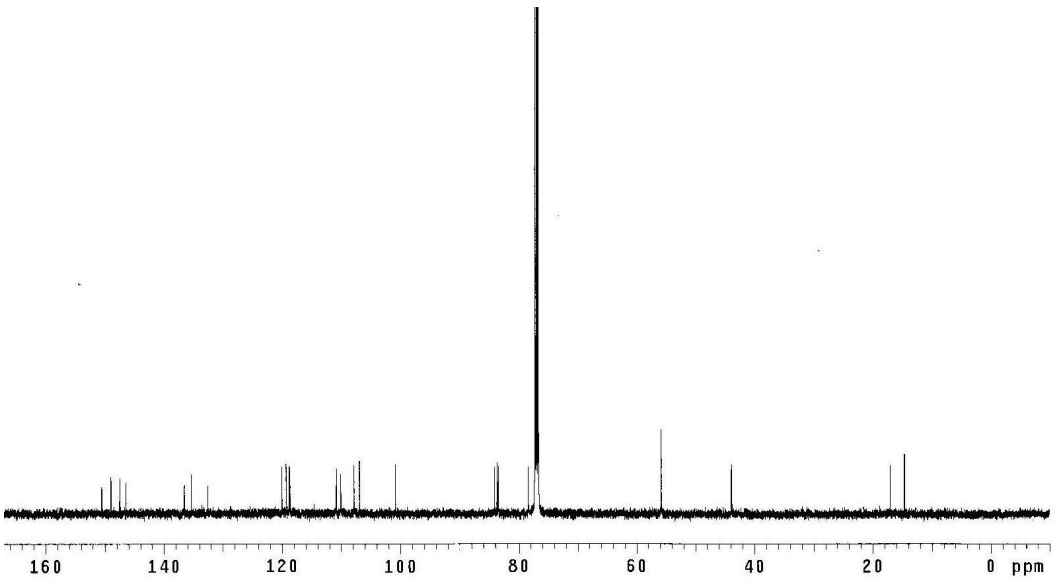


Fig. 10. ^{13}C-NMR spectrum of compound **4** (125 MHz, $CDCl_3$)

3.1.5. Structure determination of compound 5

Compound **5** was isolated as colorless powder with the molecular formula $C_{30}H_{34}O_8$ by the high resolution mass spectrum [HREIMS *m*/*z* 540.2593 (calcd for $C_{30}H_{34}O_8$ 540.2597)]. Comparison of the ^1H and ^{13}C NMR spectra of compound **5** were very similar to those of compound **4**, except for the absence of a singlet signal of a phenyl methoxyl group, suggesting that compound **5** was the demethylated form of compound **4**. In the ^{13}C spectrum of compound **4**, the methoxylated aromatic carbons at C-3″ and C-4″ resonate at δ_C 149.0 and 148.8, respectively. However, in the structure of compound **5** these signals were shifted upfield to δ_C 145.5 and 146.6, respectively. Further, the aromatic C-5″ resonance at δ_C 110.9 in compound **4** was shifte downfield to δ_C 114.1 in compound **5**. This known compound was characterized as saucerneol E by comparing their physicochemical values, ^1H and ^{13}C NMR spectra, and MS data with those published in literature.[15,16, 25]

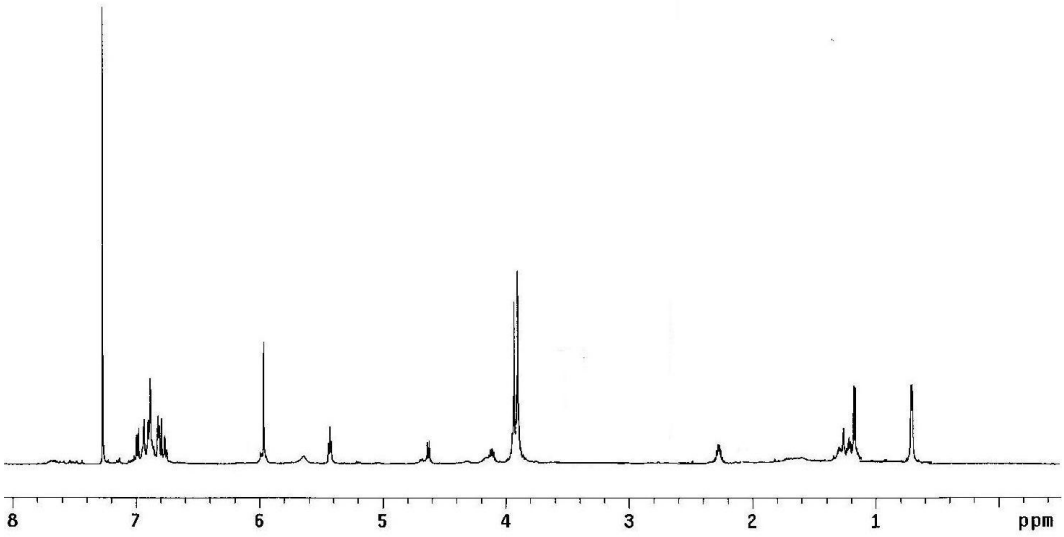


Fig. 11. ^{1}H-NMR spectrum of compound **5** (500 MHz, $CDCl_3$)

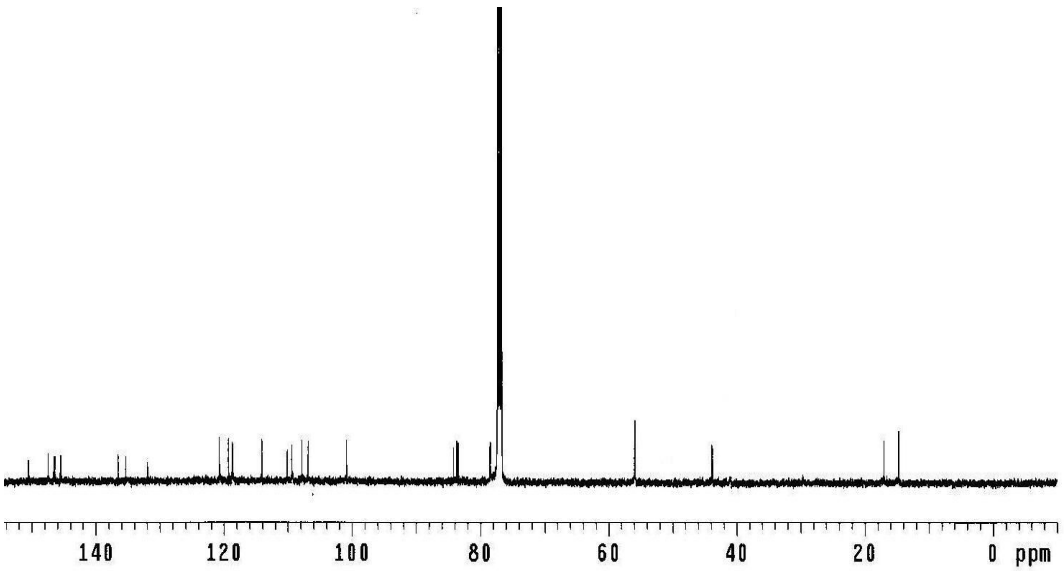


Fig. 12. ^{13}C-NMR spectrum of compound **5** (125 MHz, $CDCl_3$)

3.1.6. Structure determination of compound 6

Compound **6** was isolated as colorless oil with the molecular formula $C_{20}H_{24}O_5$ by the high resolution mass spectrum [HREIMS data (m/z [M]+, 344.1628, calcd 344.1624 for $C_{20}H_{24}O_5$)]. The ^{1}H NMR spectrum of compound **6** revealed the presence of methyl protons at δ_H 1.09 (s, 3H, H-9), allylicmethyl protons at δ_H 1.80 (s, 3H, H-9′), two methoxy protons at δ_H 3.81 (s, 3H), and 3.84 (s, 3H), the methine proton at δ_H 4.06 (m, 1H, H-8), a benzylic methine proton at δ_H 4.54 (d, 1H, H-7), and two vinyl protons at δ_H 6.09 (m, 1H, H-7′) and 6.28 (d, 1H, H-8′), respectively. This known compound was characterized as machilin D by comparing their physicochemical values, ^{1}H and ^{13}C NMR spectra date with those published in literature.[17,]

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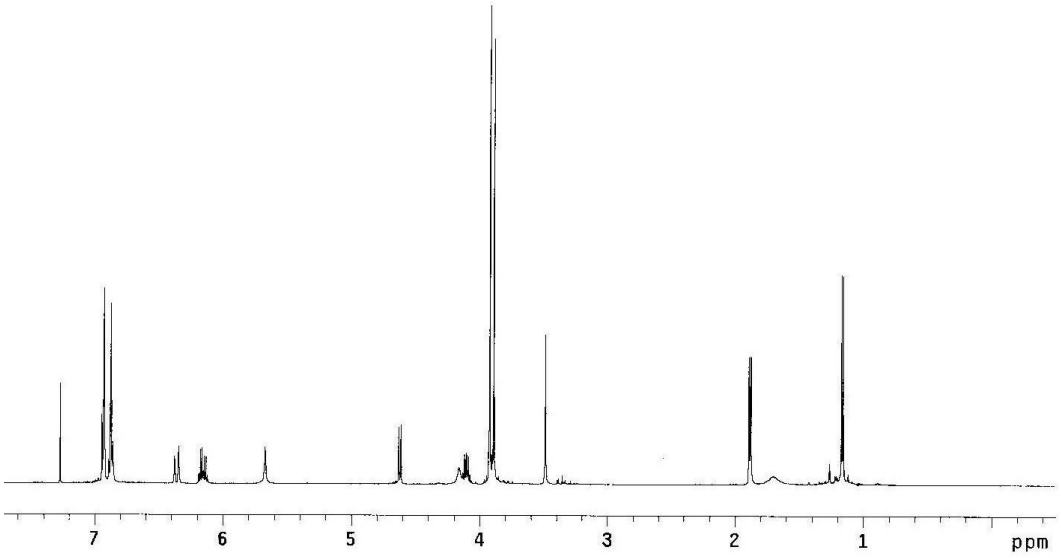

Fig. 13. ^{1}H-NMR spectrum of compound **6** (500 MHz, $CDCl_3$)

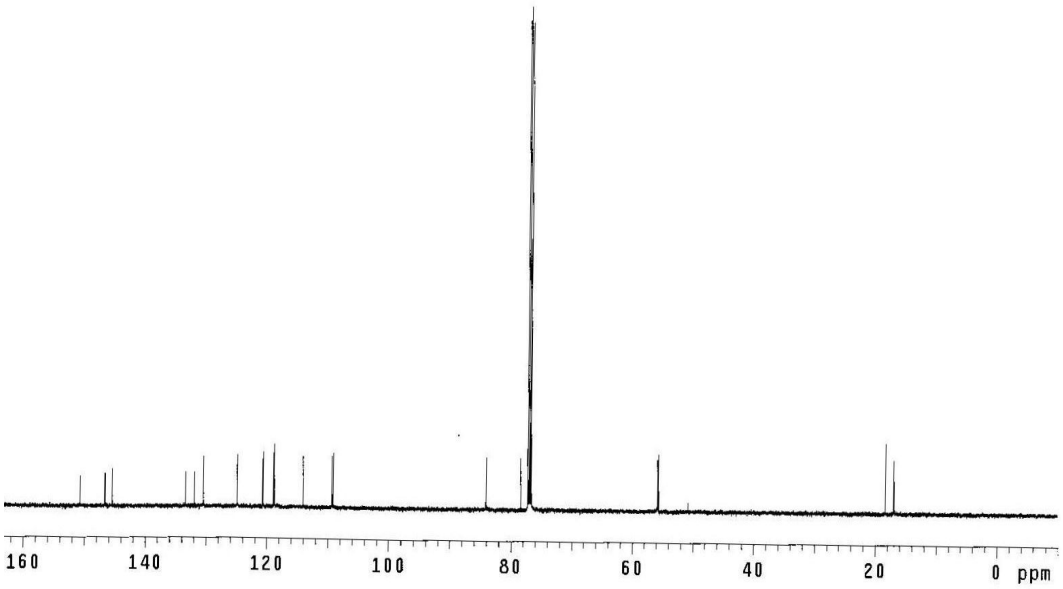

Fig. 14. ^{13}C-NMR spectrum of compound **6** (125 MHz, $CDCl_3$)

3.1.7. Structure determination of compound 7

Compound **7** was isolated as colorless powder with the molecular formula $C_{41}H_{48}O_{11}$ by the high resolution mass spectrum [HREIMS data(m/z [M]+, 716.3198, calcd 716.3197 for $C_{41}H_{48}O_{11}$). Comparison of the ^{1}H and ^{13}C NMR spectra of Compound **7** and Compound **8** were very similar. However, ^{1}H and ^{13}C NMR spectra of Compound **7** lacked the signals of two methoxy groups and showed the signal of one additional methylenedioxy group (δ_H; 5.92, 2H , 3H, δ_c; 101.0). The ^{1}H NMR spectrum of Compound **7** showed singlets at δ_H 3.85, 3.87, 3.89, 3.90 and 5.92corresponding to 14H that were attributed to four methoxy and methylenedioxy groups. Three aromatic signals were displayed between δ_H 6.73 and 6.99 (12H, m). Additionally, doublet at δ_H 0.71 (6H, d, *J*=6.0Hz, H-9,9′) of the methyl groups at C-8 and C-8′ with four methine groups at δ_H 2.27 (2H, m, H-8,8′) and 5.45 (2H, d, *J*=5.8 Hz, H-7,7′) pointed to tetrahydrofuran moiety. Also, the relative stereochemistry of the tetrahydrofuran ring. This known compound was characterized as manassantin B by comparing their physicochemical values, ^{1}H and ^{13}C NMR spectra, and MS data with those published in literature.[18,,21, 22-24]

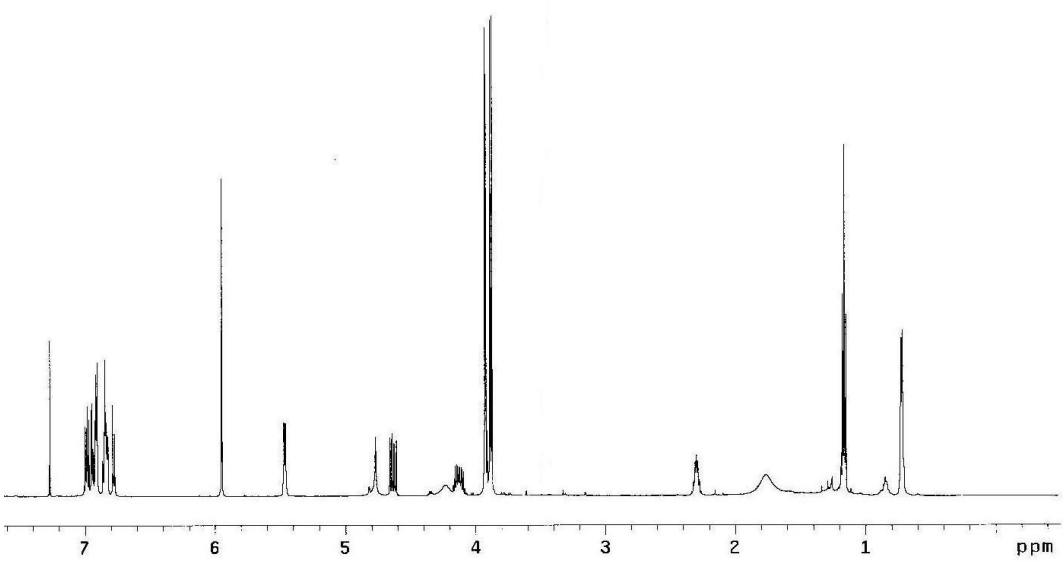

Fig. 15. ^{1}H-NMR spectrum of compound **7** (500 MHz, $CDCl_3$)

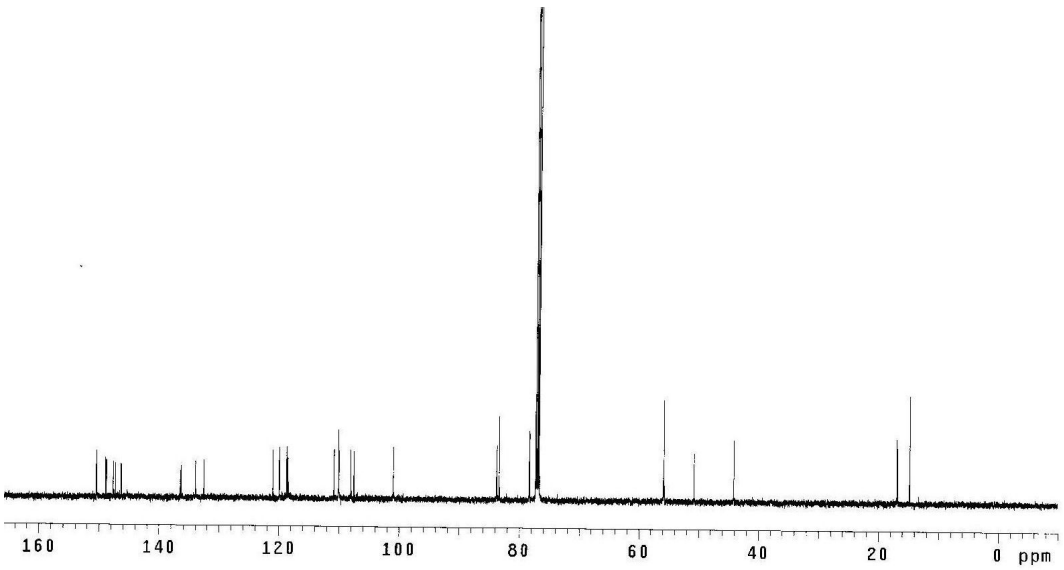

Fig. 16. ^{13}C-NMR spectrum of compound **7** (125 MHz, $CDCl_3$)

3.1.8. Structure determination of compound 8

Compound **8** was isolated as colorless powder with the molecular formula $C_{42}H_{52}O_{11}$ by the high resolution mass spectrum [HREIMS data (m/z [M]+ 732.3506, calcd 732.3510 for $C_{42}H_{52}O_{11}$)]. Comparison of the ^{1}H and ^{13}C NMR spectra of compound **7** and compound **8** were very similar. However, ^{1}H and ^{13}C NMR spectra of Compound **7** lacked the signals of two methoxy groups and compound **8** showed two methoxy groups singlets at δ_H 3.90(6H,s, $-OCH_3 \times 2$) and 3.85(6H, s , $-OCH_3 \times 2$) corresponding to 18H that were attributed to six methoxy groups. Three aromatic signals were displayed between δ_H 6.79 and 6.98 (12H, m). Additionally, doublet at δ_H 0.71(6H, d, *J*=6.4Hz, H-9, 9′) of the methyl groups at C-8 and C-8′ with four methine groups at δ_H 2.27 (2H, m, H-8,8′) and 5.45 (2H, d, *J*=5.8 Hz, H-7,7′) pointed to tetrahydrofuran moirty. Also the relative stereochemistry of the tetrahydrofuran ring. This known compound was characterized as manassantin A by comparing their physicochemical values, ^{1}H and ^{13}C NMR spectra data with those published in literature. [18,,21, 22-24]

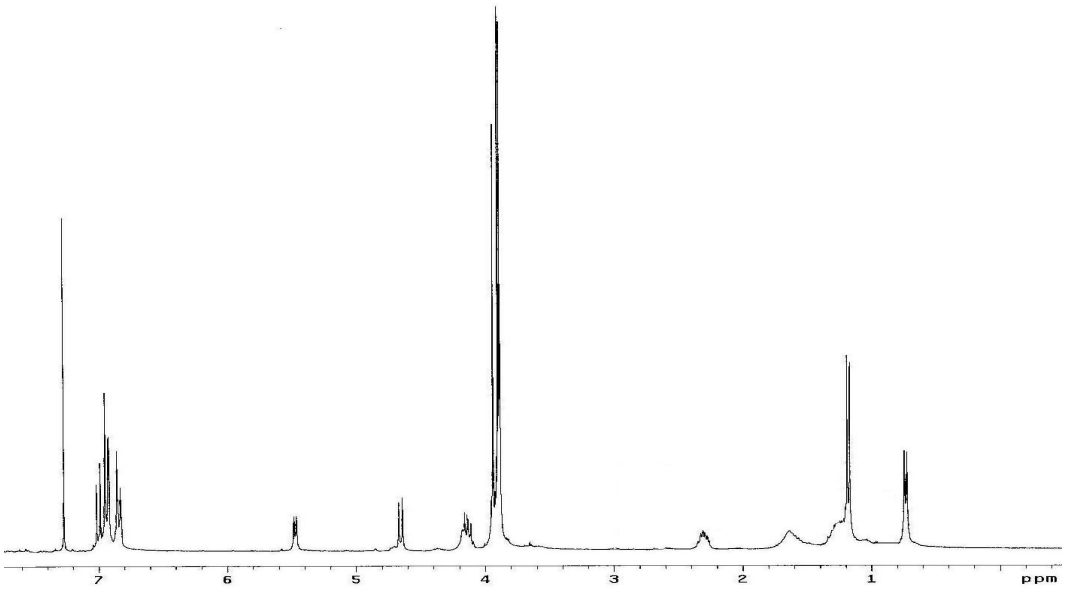

Fig. 17. ^{1}H-NMR spectrum of compound **8** (500 MHz, $CDCl_3$)

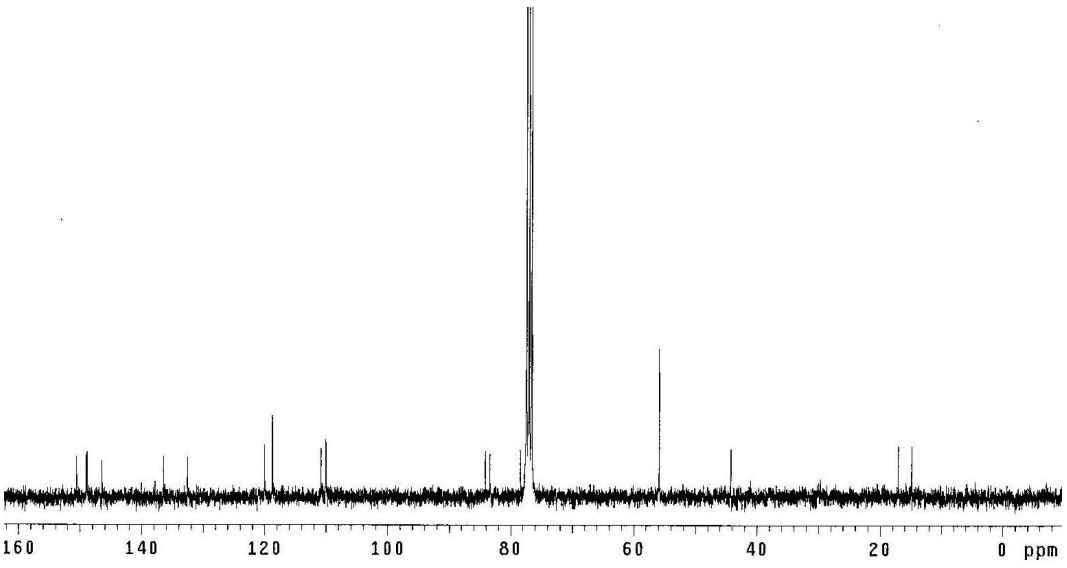

Fig. 18. ^{13}C-NMR spectrum of compound **8** (125 MHz, $CDCl_3$)

3.1.9. Structure determination of compound 9

Compound **9** as isolated as clear powder with the molecular formula $C_{40}H_{45}O_{11}$ by the high resolution mass spectrum [HREIMS data (m/z $[M-H]^-$, 701.2978 (calcd for $C_{40}H_{45}O_{11}$ 701.2962)] Comparison of the ^1H and ^{13}C NMR spectra of compound **9** were very similar to those of compound **7**, except for the absence of a singlet signal of a phenyl methoxyl group, suggesting that compound **9** was the demethylated form of compound **7**. In the ^{13}C spectrum of compound **7**, the methoxylated aromatic carbons at C-3″ and C-4″ resonate at δ_C 147.3 and 148.9, respectively. However, these signals in the structure of compound **9** were shifted upfield to δ_C 147 and 145.9, respectively. Further, the aromatic C-5″ resonance at δ_C 118.6 in compound **7** was shifted as downfield to δ_C 114.5 in compound **9**. This known compound was characterized as 4-*O*-Demethyl manassantin B by comparing their physicochemical values, ^1H and ^{13}C NMR spectra, and MS data with those published in literature. [18,,21, 32]

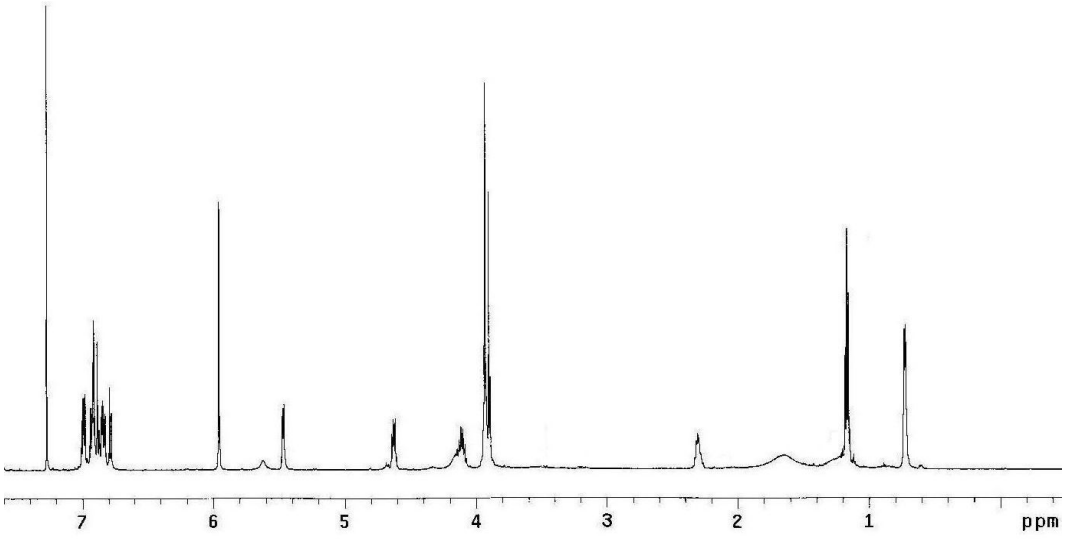

Fig. 19. ^{1}H-NMR spectrum of compound **9** (500 MHz, $CDCl_3$)

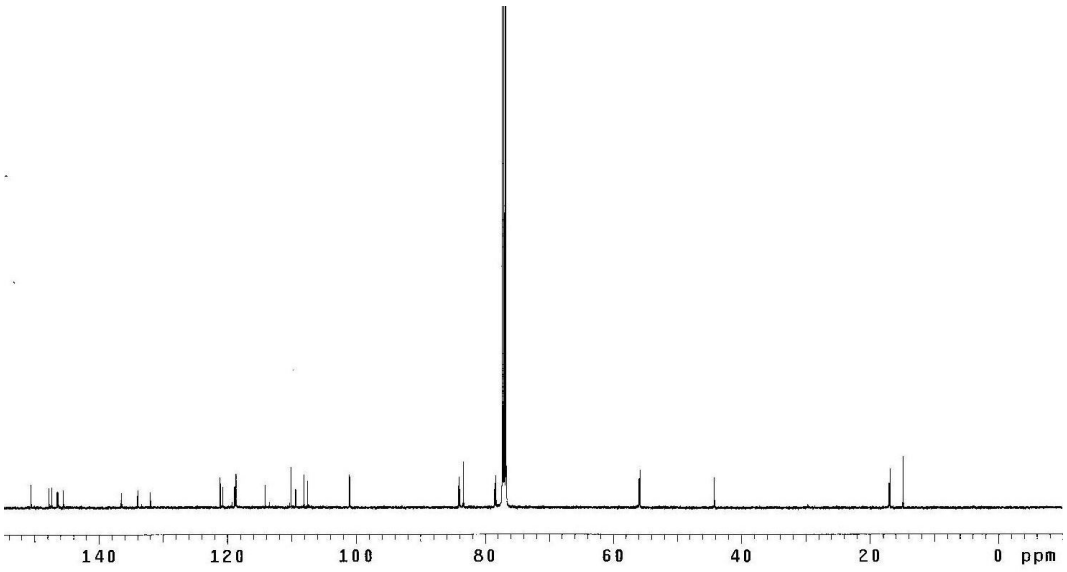

Fig. 20. ^{13}C-NMR spectrum of compound **9** (125 MHz, $CDCl_3$)

3.1.10. Structure determination of compound 10

Compound **10** as isolated as green oil with the molecular formula $C_{22}H_{26}O_7$ by the impact mass spectrum EIMS m/z (rel mt) 402, 221, 220, 209, 208, 205, 194, 193, 181, 179, 165, 163, 161, 151, 147, 137, 135, 133, 131, 123, 121. The ^{1}H and ^{13}C NMR spectra revealed 1,3,4-trisubstttuted δ_H 6.67 (br s, H-2, 5, 6), δ_C 130.00 (C-l), δ_C 107.91 (CH-2), δ_C 147.32 (C-3, C-4), δ_C 109 94 (CH-5) δ_C 122.31 (CH-6) and 1,3,4,5-tetrasubstrtuted [δ_H 6.38 (s, H-2', 6'); 6, δ_C 135 35 (C-l'), δ_C 105.80 (CH-2', CH-6') δ_C 153.67 (C-3', C-5', δ_C 132 00 (C-4')] aromatic rings, one methylenedioxy [δ_H 5.88 (s), δ_C 100.58 and two methoxy δ_H 3 70 (s), $\delta_{C\,c}$ 56.02 groups. The signals at δ_H3.20 (d, *J*=7 Hz, CH_2-7'), δ_H 6.2-5.80 (m. H-8') and δ_H 5.20-4.80 (m, CH,-9') and at δ_C 40.51 (CH,-7'), δ_C 137.32 (CH-8') and 115 83 (CH_2-9') were consistent with the presence of an allyl group The chemical shafts of methylene [δ_H 3.05-2.50 (m), δ_C 42 96], methine OCH-8 [S, 4.20-3.90 (m), δ_C 79.721 and secondary methyl [δ_H 1.10(d, *J*=7 Hz), δ_C 19.51) groups and the observed multiplicity showed the attachment of the 3,4-OCH_2O C_6H_3-CH_2-CH-Me moiety to the oxygen atom of C-4'. This known compound was characterized as 1,3-benzodioxole-5-methanol,a-[1-[2,6-dimethoxy-4-(2-propenyl)phenoxy]ethyl]-7-methoxy-,(R*,R*)-(9CI) by comparing their physicochemical values, ^{1}H and ^{13}C NMR spectra, and MS data with those published in literature.[32-34]

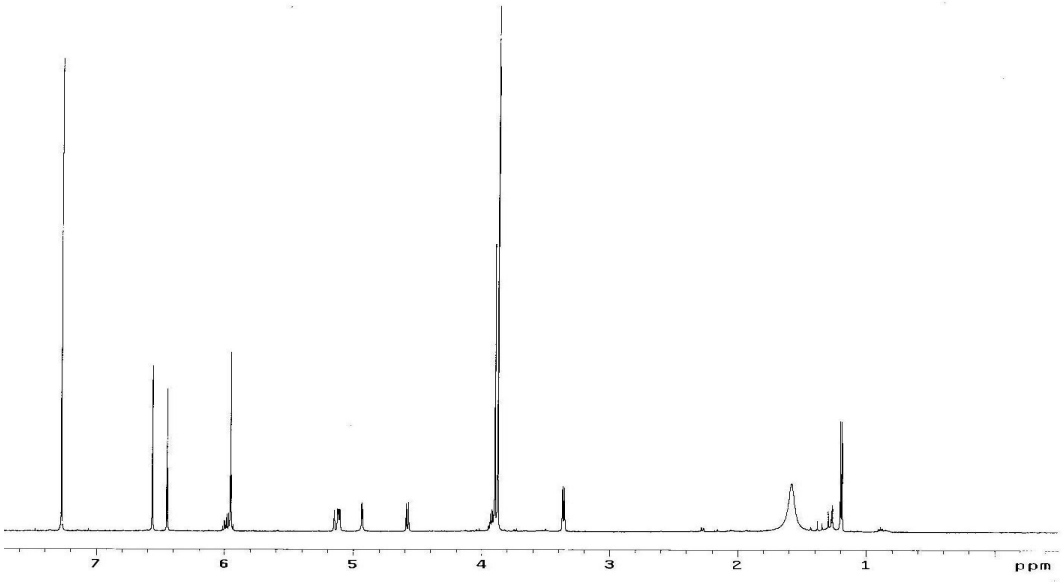

Fig. 21. ^{1}H-NMR spectrum of compound **10** (500 MHz, $CDCl_3$)

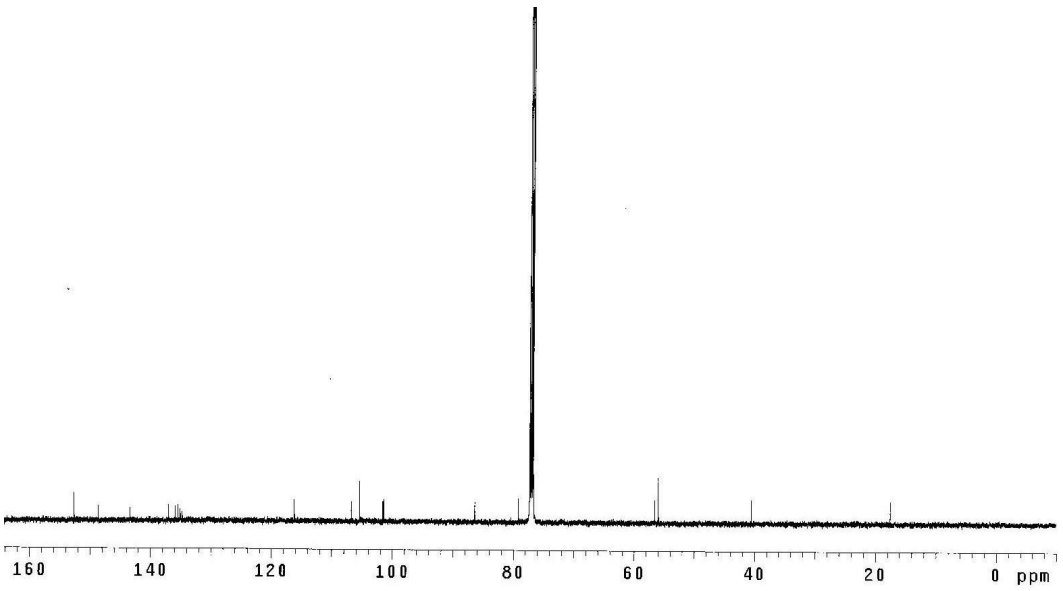

Fig. 22. ^{13}C-NMR spectrum of compound **10** (125 MHz, $CDCl_3$)

3.1.11. Structure determination of compound 11

Compound **11** as isolated as colorless powder with the molecular formula $C_{22}H_{26}O_7$ by the impact mass spectrum EIMS m/z (rel mt) 402, 221, 220, 209, 208, 205, 194, 193, 181, 179, 165, 163, 161, 151, 147, 137, 135, 133, 131, 123, 121, 103, 102. Comparison with compound **10** of the ^1H and ^{13}C NMR spectra were very similar. However, the signal of acetate showed (δ_H ; 1.90, s, δ_c ; 21.0 and 169.9). This known compound was characterized as 1,3-benzodioxole-5-methanol,a-[1-[2,6-dimethoxy-4-(2-propenyl)phenoxy]ethyl]-7-methoxy-, acetate, (R*,R*)- (9CI) by comparing their physicochemical values, ^1H and ^{13}C NMR spectra, and MS data with those published in literature.[32-34]

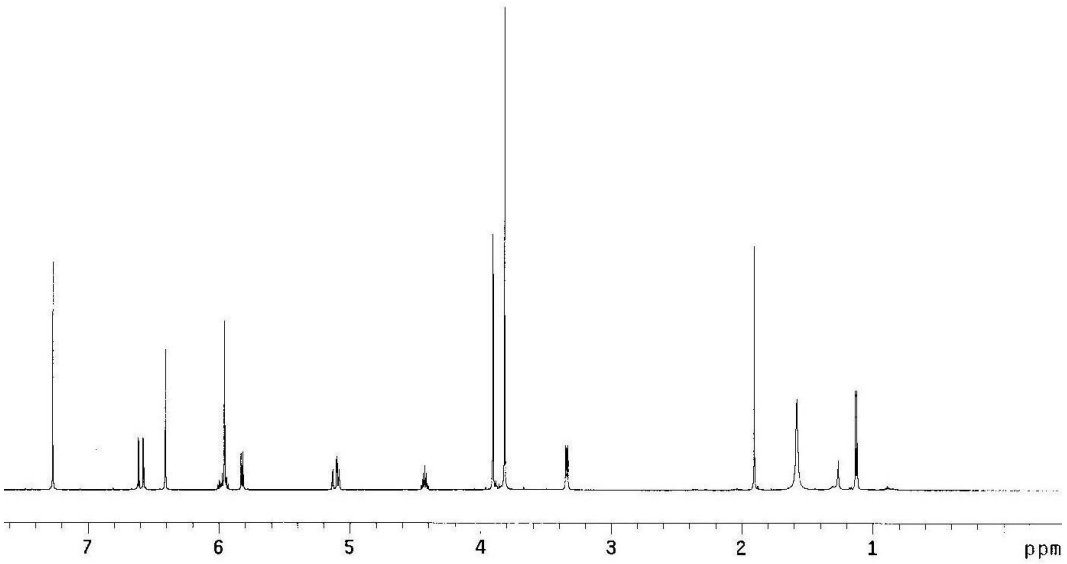

Fig. 23. ^{1}H-NMR spectrum of compound **11** (500 MHz, $CDCl_3$)

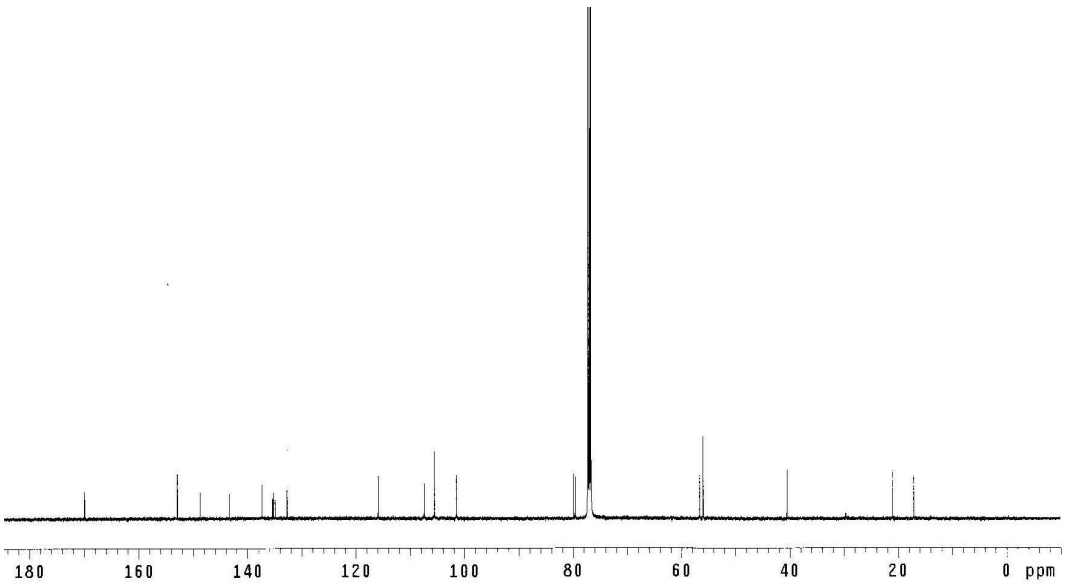

Fig. 24. ^{13}C-NMR spectrum of compound **11** (125 MHz, $CDCl_3$)

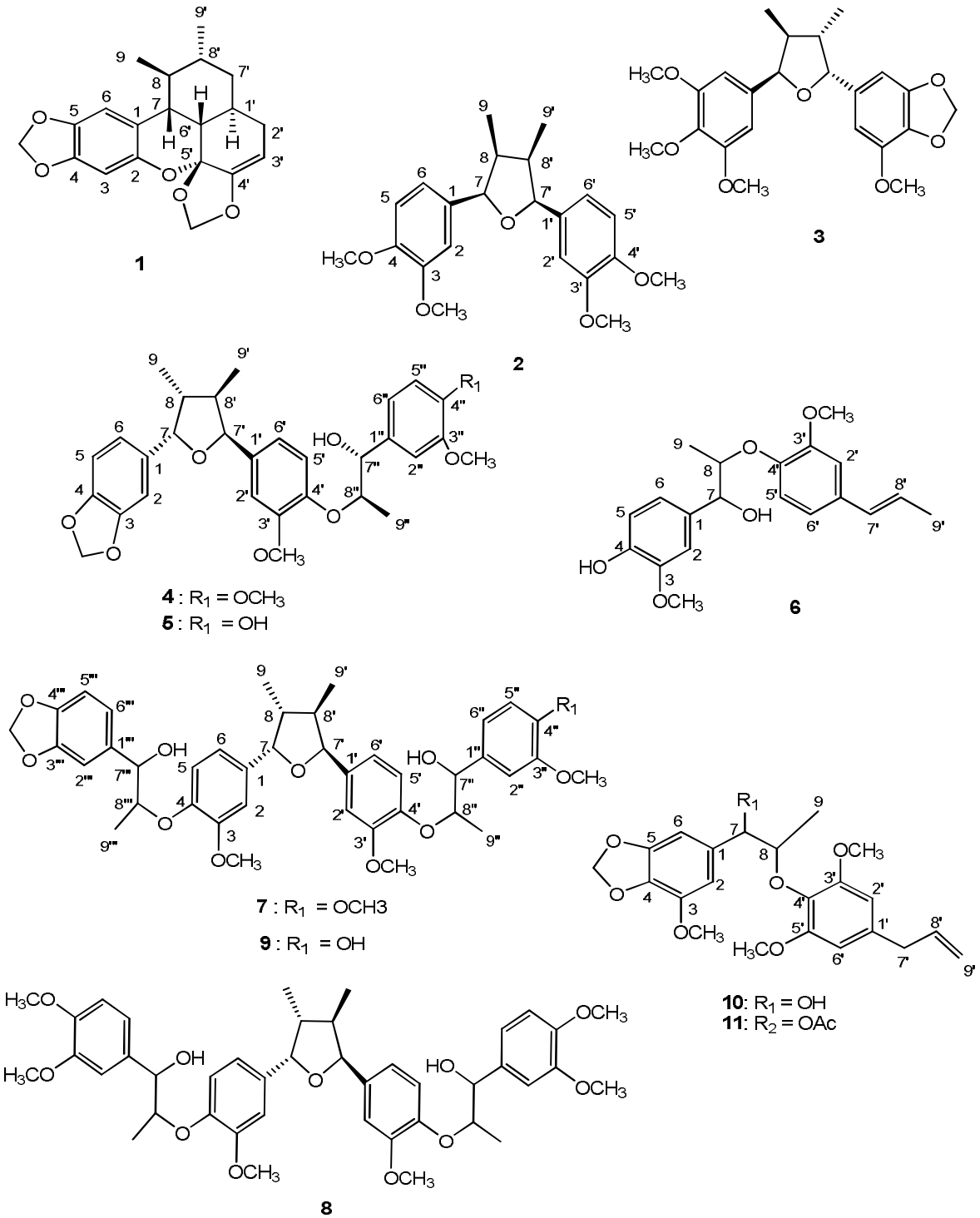

Fig.24. Chemical structures of compounds **1–11** isolated from *Saururus chinensis*

Table 1. ^{1}H (500 MHz) data of isolated compounds **1, 2, 3, 6, 10, 11** from *S. chinensis*.in $CDCl_3$

position	**1**	**2**	**3**	**6**	**10**	**11**
	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)
1						
2			6.54, s		6.40, s	6..61, s
3	6.38, s					
4						
5						
6	6.82, s		6.54, s		6.50,, s	6.57, s
7	3.03, d, 5.4	5.44, d, 6.4	4.52, d, 7.2	4.62, d, 8.3	4.56, d, 8	5.83, d
8	2.44, m	2.24, m	1.67, m	4.09, m	3.91 , m	4.43, m
9	1.22, d, 7.3	0.69, d, 6.6	0.96, d, 4.5	1.15, d, 6.1	1.20, d, 7	1.11, d, 7
1′	2.52, td,11.9					
2′			6.52, s		6.40, s	6.40, s
3′	5.57, s					
4′						
5′						
6′	2.48, d, 5.4		6.52, s		6.40, s	6.40, s
7′	1.74,m (eq) 1.92,m (ax)	5.44, d, 6.4	4.52, d, 7.2	6.37, d, 1.5	3.35, d, 7	3.33, d, 7
8′	1.88, m	2.24, m	1.67, m	6.12, m	6.00-5.80, m	6.00-5.80, m
9′	0.71, d, 7.4	0.69, d, 6.6	0.96, d, 4.5	1.87, dd, 1.5, 6.7	5.12, m	5.09, m
3-OMe		3.88	3.80	3.84	3.80	3.80
4-OMe		3.90	3.76	3.88		
5-OMe			3.80	3.91		
3′-OMe		3.88				
4′-OMe		3.90				
5′-OMe			3.84			
OCH_2O-	5.90, 5.87, s (Al) 5.65, 5.60, s (Ar)		5.87, s		5.88, s	5.88, s
3′, 5′- OMe					3.80, s	3.80, s
C=O						
CH_3						1.90, s

Table 2. ^{13}C (125 MHz) date of isolated compounds **1, 2, 3, 6, 10, 11** from *S. chinensis*.in $CDCl_3$

position	**1**	**2**	**3**	**6**	**10**	**11**
	δ_C	δ_C	δ_C	δ_C	δ_C	δ_C
1	115.7	134.0	137.8	131.9	135.0	132.7
2	145.7	109.6	103.1	109.1	106.8	107.3
3	99.5	148.6	153.2	146.5	143.4	143.3
4	143.2	147.9	137.4	145.5	134.7	134.9
5	146.7	110.7	153.2	114.1	148.6	148.6
6	105.5	118.4	103.1	120.7	101.5	101.4
7	35.0	83.5	88.3	78.4	79.1	79.5
8	34.8	44.0	50.8	84.2	86.3	79.9
9	21.2	14.7	13.8	17..0	17.1	17.1
1′	37.5	134.0	134.5	133.4	135.4	135.2
2′	194.6	109.6	105.7	109.3	152.6	152.9
3′	101.3	148.6	143.4	150.7	105.4	105.5
4′	168.6	147.9	137.1	146.7	135.9	135.3
5′	100.4	110.7	148.8	118.7	105.4	105.5
6′	37.6	118.4	100.2	118.9	152.6	152.9
7′		83.5	88.4	130.4	40.2	40.2
8′	33.4	44.0	51.1	124.9	137.0	137.3
9′	20.9	14.7	13.9	18.4	116.1	115.8
3-OMe		55.9	56.1	55.7	56.5	
4-OMe		55.9	60.7	55.9		
5-OMe			56.1	55.7		
3′-OMe		55.9		131.9		
4′-OMe		55.9		109.1		
5′-OMe			56.6	146.5		
OCH_2O-	98.1(Al.) 101.2(Ar)		101.3		101.3	101.4
3′, 5′- OMe					55.9	55.9
C=O						169.9
CH_3						21.0

Table 3. ^{1}H (500 MHz) data of isolated compounds **4, 5, 7, 8, 9** from *S. chinensis*.in $CDCl_3$

position	**4**	**5**	7	**8**	**9**
	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)	δ_H (*J* in Hz)
1					
2	6.81, d, 1.7	6.82, d, 1.5	6.86, d, 1.8	6.92, d, 1.8	6.88, d, 1.8
3					
4					
5	6.79, d, 8.0	6.80, d, 8.0	6.84, d, 8.2	6.84, d, 8.2	6.84, d, 8.2
6	6.76, br d, 8.0	6.76, br d, 8.0	6.88, dd, 8.0, 1.6	6.83, dd, 8.3, 1.8	6.88, dd, 8.0, 1.6
7	5.42, d, 6.5	5.42, d, 6.4	5.46, d, 6	5.45, d, 6	5.46, d, 5.7
8	2.26, ddq,12.8, 6.5, 6.5	2.26, ddq,12.8, 6.4, 6.4	2.29,m	2.29,m	2.29,m
9	0.71, d, 6.5	0.71, d, 6.4	0.72, d, 6.4	0.71, d, 6.4	0.72, d, 6.4
1′					
2′	6.90, d, 1.6	6.91, d, 1.6	6.92, d, 1.8	6.94, d, 1.8	6.88, d, 1.8
3′					
4′					
5′	6.99, d, 8.0	6.99, d, 7.9	6.86 d, 7.9	6.84 d, 8.2	6.86 d, 7.9
6′	6.82, dd, 8.0, 1.6	6.82, dd, 7.9, 1.6	6.82, dd, 8.0, 1.6	6.83, dd, 8.3, 1.8	6.98, d, 8.2
7′	5.43, d, 6.5	5.43, d, 6.4	5.46, d, 6	5.45, d, 6	5.46, d, 5.7
8′	2.28, ddq,12.8 6.5, 6.5	2.29, ddq,12.8 6.4, 6.4	2.29,m	2.29,m	2.29,m
9′	0.70, d, 6.5	0.72, d, 6.4	0.72, d, 6.4	0.71, d, 6.4	0.72, d, 6.4
1″					
2″	6.95, d, 1.7	6.94, d, 1.5	6.84, d, 1.8	6.94, d, 1.8	6.88, d, 1.8
3″					
4″					
5″	6.84, d, 8.2	6.88, d, 8.6	6.99, d, 8.2	6.99, d, 8.2	6.99, d, 8.2
6″	6.93, dd, 8.2, 1.7	6.89, dd, 8.6, 1.5	6.94, dd, 1.8, 8.2	6.94, dd, 1.8, 8.2	6.99, d, 8
7″	4.65, d, 8.3	4.63, d, 8.3	4.64, d, 7.2	4.63, d, 7.2	4.61, d, 8.2
8″	4.13, dq, 8.3, 6.2	4.13, dq, 8.3, 6.2	4.10, m	4.11, m	4.12, m
9″	1.17, d, 6.2	1.17, d, 6.2	1.17, d, 6.4	1.16, d, 6.4	1.15, d, 6.4
1‴					
2‴			6.94, d, 1.8	6.94, d, 1.8	6.91, s
3‴					
4‴					
5‴			6.98, d, 7.2	6.99, d, 7.2	6.98, d, 7.2
6‴			6.92, dd, 1.8, 8.2	6.94, dd, 1.8, 8.2	6.92, dd, 1.8, 8.2
7‴			4.62, d, 7.2	4.63, d, 7.2	4.62, d, 7.2
8‴			4.10, m	4.11, m	4.10, m
9‴			1.15, d, 6.4	1.16, d, 6.4	1.17, d, 4.6
-OCH_2O-	5.95, s	5.96, s	5.94, s		5.95, s
3-OMe	3.93, s	3.93, s	3.92, s	3.92, s	3.92, s
3′-OMe	3.89, s		3.93, s	3.93, s	3.93, s
4′-OMe	3.88, s	3.90, s			
3″, 4″-OMe			3.88, 3.88, s	3.88, 3.88 s	3.90, 3.90, s
3‴ , 4‴-OMe				3.88, 3.88,s	

Table 4. ^{13}C (125 MHz) data of isolated compounds **4, 5, 7, 8, 9** from *S. chinensis*.in $CDCl_3$

position	**4**	**5**	7	**8**	**9**
	δ_C	δ_C	δ_C	δ_C	δ_C
1	135.3	135.4	136.5	136.5	137.0
2	106.9	106.9	110.2	110.1	110.2
3	147.5	147.5	146.4	146.4	146.9
4	146.4	146.4	150.6	149.0	151.0
5	107.8	107.8	108.1	110.1	119.1
6	119.3	119.3	118.8	118.8	119.1
7	83.6	83.7	83.4	83.4	83.4
8	43.9	44.0	44.3	44.2	44.3
9	14.7	14.7	14.9	14.9	14.9
1′	136.5	136.6	136.6	136.6	136.9
2′	110.2	110.2	110.2	110.1	110.0
3′	150.5	150.6	146.5	146.5	146.7
4′	146.4	146.4	150.6	149.0	151.0
5′	118.7	118.7	107.6	110.1	119.3
6′	118.7	118.8	118.8	118.8	119.1
7′	83.4	83.5	83.4	83.4	83.4
8′	43.9	43.9	44.3	44.2	44.3
9′	14.7	14.7	14.9	14.9	14.9
1″	132.6	132.0	132.6	132.6	132.4
2″	110.1	109.4	110.2	110.9	110.0
3″	149.0	145.5	147.4	148.9	146.7
4″	148.8	146.6	148.9	148.9	151.0
5″	110.9	114.1	118.7	118.7	119.3
6″	120.0	120.7	120.0	120.0	121.5
7″	78.3	78.5	78.4	78.4	78.4
8″	84.0	84.1	84.0	84.1	84.0
9″	17.0	17.0	16.9	17.1	16.9
1‴			134.0	132.6	134.4
2‴			110.9	110.9	109.8
3‴			147.8	148.9	147.0
4‴			149.0	149.0	145.9
5‴			119.0	118.7	114.5
6‴			121.1	120.0	121.1
7‴			78.4	78.4	78.4
8‴			84.1	84.1	84.1
9‴			17.1	17.1	17.1
-OCH_2O-	100.8	100.9	101.0		101.0
3′-OMe	55.8	55.8	55.8	55.8	55.8
3″-OMe	55.9				
4″-OMe	55.9	55.9	55.8	55.8	55.8
3″, 4″-OMe			55.9	55.9, 55.9	55.9
3‴ , 4‴-OMe			55.9, 55.9	55.9, 55.9	55.9, 55.9

3.2. Effect of tetrahydrofuran type-lignans from *Saururus chinensis* on AMPK activation

Fraction 1-9 isolated from 100% ethanol-eluted and the acetone-eluted fraction were tested using an *in vitro* assay to investigate their stimulation effects on AMPK. Among them, fraction**1, 2, 7, 8,** and **9** showed the activation, exhibiting strong stimulation at the concentration of 10 μg/mL on this enzyme assay.(**Fig. 25**)

Compound **1, 4, 5, 7, 8**, and **9** compound isolated from each fraction **1, 2, 7**, **8** and **9** effects on AMPK**.** Compound **1, 4, 5, 7, 8**, and **9** were tested using an in vitro assay to investigate its stimulation effect on AMPK. 5-Aminoimidazole-4-carboxamide ribonucleoside was used as a positive control at the concentration of 100 mM.(**Fig.26**)

As shown in Fig. 26, copmpound **1**, **4, 7,** and **8** exhibited strong stimulation effect at the concentration of 10 mg/mL on this assay. Compound **1** reported a novel AMPK-activating to previously.[34] The effect of copmpound **4, 7,** and **8** on AMPKa and its downstreamtarget ACC2 inculture skeletal cells were examined. Western blot analysis results indicated that the phosphorylation level of not only AMPKa Thr172 but also its intracellular substrate ACC2 Ser 79, was increased after treatment of this compound. Even though detailed investigation of the chemical and biological properties of copmpound **4, 7,** and **8** are not studied enough, our results indicated that tetrahydrofuran lignans may be responsible for its high effects on stimulating AMPK activity and also in downstream target ACC2 of AMPK.[36-37]

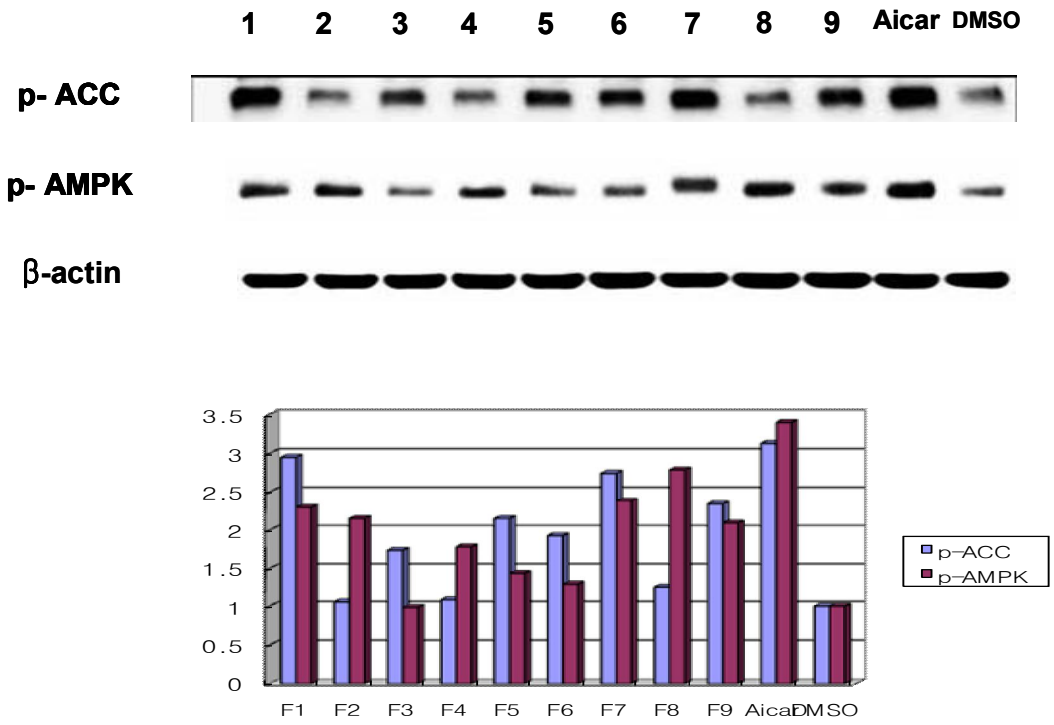

Fig. 25. Stimulatory effects of isolated fraction. **1**−**9** on AMPK activity. activity at the concentration of 10 μg/mL.

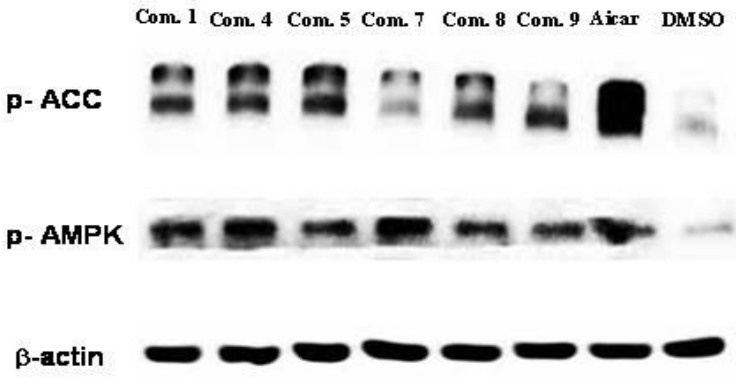

Fig. 26. Stimulatory effects of isolated compounds **1, 4, 5, 7, 8,** and **9** on AMPK activity activity at the concentration of 10 μg/mL.

3.5. Discussions

The species *S. chinensis* has been used for the treatment of edema, jaundice, and gonorrhea, and inflammatory diseases in the Oriental folk medicine. Because AMPK has recently drawn attention as a next generation target for total metabolic control[9], it has been suggested that AMPK activators can be used for treating not only type-2 diabetes but also obesity. In the course of our screening efforts on new AMPK activators from natural foods by a cell-based assay on AMPK phosphorylation, eleven tetrahydrofuran lignans were isolated from *S.chinensis* The effect of individual tetrahydrofuran-type lignans on AMPK and its downstream target ACCs in culture skeletal cells were examined. Western blot analysis results indicated that the phosphorylation level of not only AMPKα Thr^{172} but also its intracellular substrate, ACC2 Ser^{79}, was increased after treatment of these compounds. Although partial correlation between chemical structure and biological activity of individual compounds were discussed, the use of lignan-rich extract may provide better benefits for food industry to develop nutraceuticals for preventing metabolic syndrome. From the above data obtained, tetrahydrofuran lignans could be promised to be a new class of AMPK activators. Therefore, it is strongly suggested that tetrahydrofuran-type lignans and its enriched extract from *S.chinensis* can be used not only for development of agents for the treatment of type-2 diabetes and possibly obesity, but also be beneficially used for metabolic disorders as functional food. [36]

4. Conclusions

AMP-activated protein kinase (AMPK) has been considered as a therapeutic target for the treatment of metabolic syndrome including obesity and type-2 diabetes. In our program to search new AMPK activators from plants, we found that a total extract of *S.chinensis* activated AMPK enzyme in differentiated C2C12 cells. As the active constituents, tetrahydrofuran lignans sauchinone (**1**), di-*O*-methyltetrahydrofuriguaicin B (**2**), rel-(7R,8R,7'-R,8'-R)-3',4'Methylenedioxy-3,4,5,5'-teramethoxy-7,7'-epoxylignan (**3**), saucerneol D (**4**), saucerneol E (**5**), machilin D (**6**), manassantin B (**7**), manassantin A (**8**), 4-*O*-demethylmanassantin B (**9**), 1,3-benzodioxole-5-methanol,a-[1-[2,6-dimethoxy-4-(2-propenyl)phenoxy]ethyl]-7-methoxy-, (R*,R*)-(9CI) (**10**),1,3-benzodioxole-5-methanol,a-[1-[2,6-dimethoxy-4-(2-propenyl)phenoxy]ethyl]-7-methoxy-, acetate, (R*,R*)- (9CI) (**11**) were isolated from this extract. Compounds **10** and **11** were isolated from this plant for the first time. And this is the first report of compound **11** isolated from natural product. Among isolates, compounds **4, 7** and **8** showed a strong stimulation on AMPK enzyme. These lignans significantly prevented the increasing body weight and can be used for the treatment of metabolic disorders as functional foods and medicine.[31,37]

5. References

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6. Acknowledgments

지난 2년 동안 부족한 면이 많이 있었지만 본 논문이 완성되기까지 정성을 다해 지도해주시면서 때로는 정말 엄하신 교수님이셨지만 뒤에서는 아껴주시면서 무한한 정을 주시며 옆에서 많은 것을 보고 느끼고, 학문의 길이라는 것이 어떤 것인지 접하게 해주시고, 내적으로 배울 점이 너무 많은 오원근 교수님께 깊은 감사를 드립니다. 아울러 성심으로 심사하여 주신 우은란 교수님, 강건욱 교수님께도 진심으로 감사드립니다.

또한 본 논문을 위해 많은 조언을 해준 박사과정인 응구엔피흥과 다오트롱투완 형, 동기인 부이탄 텅, 지금은 졸업했지만 실험실 생활을 도와준 레티반트, 처음 저의 짧은 영어실력에 고생하고 논문실험을 도와준 응구엔 티녹안, 후배인 당타이 쭝, 트란티엔 람, 도 두안 퐁, 유일한 한국인 후배로서 저의 오지람을 잘 받아 준 김자연 실험실 모든 식구들에게 감사드립니다.

2년이란 시간이 정신없이 지나간 것 같습니다. 많은 일이 있었지만 힘들 때 마다 저녁에 물주가 되어주시고 좋은 조언을 해주신 조승식박사님과 힘들 때 마다 옆에서 힘내라고 해준 친구들에게 감사드립니다.

마지막으로 실험실 생활이 바쁘다는 핑계로 집안 일도 잘 보살피지 못했지만, 항상 옆에서 걱정해주신 영원한 멘토이신 부모님과 미국에 있으면서 힘내라고 해준 누나에게 사랑한다는 말을 전하고 싶습니다.

저작물 이용 허락서

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논문제목	한글: 삼백초*(Saururus chinensis)*로부터 AMPK 활성화 화합물의 분리와 구조분석. 영문: Isolation and structure determination of AMPK activators from *Saururus chinensis.*				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건 아래 -조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. (다만, 저작물의 내용 변경은 금지함.)
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의 여부: 동의 ( ) 반대 (o)

2011 년 2월

저작자: 강후원 (인)

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