약학석사학위논문

Isolation and Structure Identification of Chemical Constituents of *Spatholobus suberectus* Dunn.

계혈등의 성분연구

2016년 2월

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Abstract

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For the investigation of bioactive natural products with sortase A (SrtA) inhibitory effect, various extracts of Korean herbal medicines were evaluated. Based upon the results of bioactivity screening, the dried vine stem of *Spatholobus suberectus* Dunn. which is an oriental folk medicine used mainly for the improvement of blood circulation and treatment for dysmenorrhea, anemia, paralysis, arthralgia and bacterial infections was selected for chemical investigation. The large-scale extraction followed by the bioactivity-guided partition and chromatographic separation yielded twenty compounds. Based upon the results of combined spectroscopic analyses, these compounds were structurally identified as eighteen flavonoids, one flavonoid dimer and a

phenolic compound. Among these three flavonoids were found from this plant for the first time.

Key Word : Spatholobus suberectus Dunn, flavonoids, flavonoid dimer, phenolic compound, sortase A inhibitory effect.

Student Number : 2014-21059

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Introduction

Spatholobus suberectus Dunn. (Leguminosae) which is an abundant plant in south of China is one of the most popular traditional herbal medicine. The vine stem of *S. spatholobus* has been used for improvement of blood circulation and treatment for dysmenorrhea, anemia, paralysis, arthralgia and bacterial infections.[1]

Gram-positive pathogenic bacteria display surface proteins that play important roles in their adhesion to specific organ tissues, invasion of host cells, or the evasion of host-immune responses.[2] These virulence-associated proteins are covalently anchored to bacterial cell wall peptidoglycans through a general sorting mechanism catalyzed by a super family of membrane-associated trans-peptidases termed sortases.[3] Two sortase isoforms, sortase A (SrtA) and sortase B (SrtB), have been identified in Staphylococcus aureus.[4] The SrtA isoform plays a criticalrole in the pathological effects of gram-positive bacteria by modulating the ability of the bacterium to adhere to host tissue via the covalent anchoring of adhesion molecules and other virulence-associated proteins to cell wall peptidoglycans. *S.aureus* mutants lacking sortase fail to display surface proteins and are defective in the establishment of infections but microbial viability is not affected.[5] There have only been a few reports in the literature describing inhibitors of sortase, due in part to the fact that the importance of sortase as a new target hasonly recently been acknowledged.[6] Therefore, inhibitors of SrtA might be promising candidates for the treatment and prevention of gram-positive bacterial infections.[7]

In the preliminary study on the SrtA inhibitors from Korean herb medicines, more than two-hundreds of plant extracts were tested. As

a result the crude extract of dry stem of *spatholobus suberectus* Dunn. was selected as one of the prime target with the inhibition value at 48.5% against SrtA at the concentration of 100 ㎍/mL.

The organic extracted from the stem of *spatholobus suberectus* Dunn. was separated by employing solvent-partitioning. Fractionation guided by SrtA inhibitory activity (50%, 40%, 30%, and 20% aqueous MeOH exhibited 11.97%, 13.97%, 32.64%, 14.58% inhibition, respectively, at the concentration of 100 ㎍/mL), followed by various chromatography methods yielded eighteen flavonoids, one phenolic compound, one flavonoid dimer, in total twenty compounds. The structure of the isolated compounds were identified on the basis of combined spectroscopic analyses. Among the isolated compounds, **3, 5, and 6** compounds were found in this plant first time.

Experimental Section

1. General Experimental Procedures

Optical rotations were measured on a JASCO P-1020 polarimeter and using a 1 cm cell. CD data were obtained on a JASCO J-715 spectrop olarimeter in MeOH solutions. IR spectra were obtained on a JASCO F T/IR-300E spectrophotometer. UV spectra were recorded on a Hitachi U-3010 spectrophotometer. NMR spectra were recorded in DMSO, and CD_3OD solutions, on a Bruker AMX-500 and 125 ㎒, respectively. Mas s spectra were provided by the Korea Basic Science Institute, Daegu Branch, Korea. All solvents used were spectral grade or were distilled from glass prior to use.

2. Plant Material

The vine stem of *Spatholobus suberectus* Dunn were purchased from the Kyungdong-Market, Seoul, Korea, in November, 2014. A voucher specimen is on deposit at the Natural Products Research Institute, College of Pharmacy, Seoul National University.

3. Extraction and Isolation

The vine stem of *Spatholobus suberectus* Dunn. was repeatedly extra cted with Methyl chloride (10 L x 3) and MeOH (10 L x 3). The com bined crude extract (210.0 g) were partitioned between water (156.1 g) and *n*-butanol (49.5 g). 49.5 g of *n*-butanol was repartitioned between *n*-hexane (3.95 g) and 15% aqueous MeOH (44.4 g). 20.8 g of 15% aq ueous MeOH layer from solvent partitioning was subjected to reversed

-phase vacuum flash chromatography using sequential mixtures of H_2O and MeOH (elution order: 50%, 40%, 30%, 20%, 10% aqueous MeOH, and 100% MeOH) and 100% acetone as eluents.

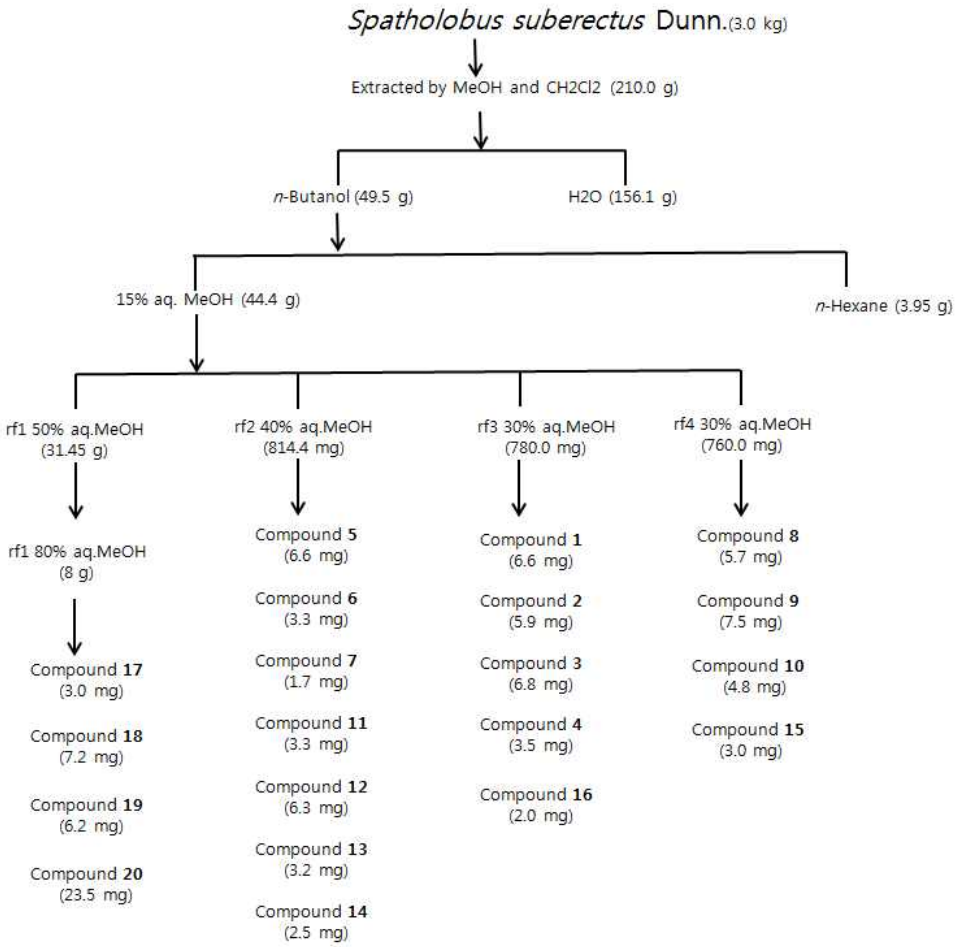
Base on the combined bioactivity test and TLC analysis, 31.5 g of the fraction which was eluted with 50% aqueous MeOH was subjected to reversed-phase vacuum flash chromatography again using sequential m ixtures of H_2O and MeOH (elution order: 80%, 70%, 60%, 50% aqueou s MeOH), then 8.0 g of the fraction which was eluted with 80% aqueo us MeOH was separated by semi-preparative reversed-phase HPLC (Y MC ODS-A column, 10 mm x 250 mm, 85% aqueous MeOH) to yield, in order of elution, compounds 17, 18, 19 and 20. Final purification of t he individual compound was then accomplished by HPLC (95% aqueou s ACN to afford 3.0, 7.2, 6.2 and 23.5 mg of compounds **17**, **18**, **19**, an d **20**, respectively).

A portion (814.4 mg) of the fraction eluted with 40% aqueous MeOH f rom vaccum flash chromatography was separated by reversed-phase H PLC (55% aqueous MeOH) to yield, in order of elution, compounds **5**, **6**, **7**, **11**, **12**, **13**, and **14**. Purification of each of these was then accom plished by reversed-phase HPLC (58% aqueous MeOH) to afford 6.6, a nd 3.3 mg of compounds 5, and 6 respectively, and reversed-phase HP LC (80% aqueous ACN) to afford 1.7, 3.3, 6.3, 3.2 and 2.5 mg of comp ounds **7**, **11**, **12**, **13** and **14** respectively.

A portion (780.0 mg) of the fraction eluted with 30% aqueous MeOH f rom flash chromatography was separated by reversed-phase HPLC (4 5% aqueous MeOH) to yield, in order of elution, compounds **1**, **2**, **3**, 4, and **16** as yellow color gums. Purification of each of these was then accomplished by reversed-phase HPLC (65% aqueous ACN) to afford 6.6, 5.9, 6.8, 3.5 and 2.0 mg of compounds **1**, **2**, **3**, **4** and **16** respectivel

y.

A portion (760.0 mg) of the fraction eluted with 20% aqueous MeOH f rom flash chromatography was separated by reversed-phase HPLC (3 5% aqueous MeOH) to yield, in order of elution, compounds **8**, **9**, **10**, a nd **16** as yellow color gums. Purification of each of these was then a ccomplished by reversed-phase HPLC (63% aqueous ACN) to afford 5. 7, 7.5, 4.8, and 3.0 mg of compounds **8**, **9**, **10**, and **15** respectively.



Scheme 1. Isolation of Compounds from *Spatholobus suberectus* Dunn

Results

1. Compound 1

The ^{1}H NMR spectrum indicated a chalcone, with aromatic signals at δ_H 7.59 (2H, d, J = 8.4 Hz), and δ_H 6.83 (2H, d, J = 8.4 Hz), δ_H 6.41 (1H, dd, J = 9.0, 2.4 Hz), δ_H 6.28 (1H, d, J = 2.4 Hz), δ_H 7.96 (1H, d, J = 9.0 Hz). a hydrogenated methine signal at δ_H 7.74 (1H, d, J = 14.2 Hz), an olefinic signal at δ_H 7.60 (1H, d, J = 14.2 Hz). ^{13}C NMR spectrum showed the presence of ketone signal at δ_C 191.4.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **1** was identified as isoliquiritigenin.[8]

2. Compound 2

The ^{1}H NMR spectrum indicated a flavanone, with aromatic signals at δ_H 7.63 (1H, d, J = 8.8 Hz), δ_H 7.52 (2H, d, J = 8.7 Hz), δ_H 7.42 (2H, dd, J = 8.7, 7.0 Hz), δ_H 7.37 (1H, m), δ_H 6.47 (1H, dd, J = 8.7, 2.2 Hz), δ_H 6.31 (1H, d, J = 2.2 Hz), an oxygenated methine signal at δ_H 5.57 (1H, dd, J = 12.6, 3.0 Hz) and two methylene signals at δ_H 3.08 (1H, dd, J = 16.7, 12.7 Hz), δ_H 2.70 (1H, dd, J = 16.7, 3.0 Hz). ^{13}C NMR showed the presence of ketone signal at δ_C 189.4. The appearance of meta-coupled doublet which is small (J = 2.2 HZ) at C-8 and C-6 in the ^{1}H NMR spectrum of **2** indicated meta position in phenyl ring.

The absolute configuration at C-2 is assigned by CD experiment from CD spectrum (positive cotton effect at 290 nm and negative cotton effect at 330 nm), the absolute configuration at C-2 was assigned to

be 2*R* configuration.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **2** was identified as (2*R*)-7-hydroxyflavanone.[9]

3. Compound 3

The ^{1}H NMR spectrum of compound **3** indicated isoflavan-4-ol, with aromatic signals at δ_H 7.25 (1H, d, *J* = 8.4 Hz), δ_H 6.79 (1H, s), δ_H 6.47 (1H, dd, *J* = 8.4, 2.4 Hz), δ_H 6.36 (1H, s), δ_H 6.29 (1H, d, *J* = 2.5 Hz), oxygenated methylene signals at δ_H 4.21 (1H, dd, *J* = 10.7, 4.8 Hz), δ_H 3.55 (1H, d, *J* = 10.7 Hz), two methine signals at δ_H 3.49 (1H, m), δ_H 5.44 (1H, d, *J* = 6.8 Hz), The appearance of para-singlet at C-3′, C-6′ in the ^{1}H NMR spectrum of **3**. The characteristic signal was methylene dioxy at δ_H 5.87 (1H, s), and δ_H 5.84 (1H, s).

The relative configuration defined from the *J* values of olefinic protons at C-3, C-4 (*J* = 6.8 Hz) is suggested the trans (*E*) geometry.

For absolute configuration at C-3, C-4 is assigned by CD experiment. From CD spectrum (positive cotton effect at 310 nm and negative cotton effect at 210, 240, 280 nm), the absolute configuration at C-3, C-4 was assigned to be 3*S*, 4*R* configuration.

Based upon the results of combined spectroscopic analysis and compari son of the spectral data with the previously reported data, the structur e of compound **3** was identified as (3*S*, 4*R*)-7,2'-dihydroxy-4,5'methyle ne dioxyisoflavan-4-ol.[10] This compound was isolated for the first time in *Spatholobus suberectus* Dunn.

4. Compound 4

The ^{1}H NMR spectrum of this compound were very similar to those of compound **3**. The most noticeable difference was the presence of ar omatic signals at δ_H 7.16 (1H, d, J = 8.4 Hz), δ_H 6.47 (1H, dd, J = 8.4, 2.4 Hz), δ_H 6.37 (1H, d, J = 2.4 Hz) and a methoxy signal at δ_H 3.53 (3H, s).

The relative configuration defined from the J values of olefinic protons at C-3, C-4 (J = 6.0 Hz) is suggested the trans (E) geometry.

For absolute configuration at C-3, C-4 is assigned by CD experiment. From CD spectrum (positive cotton effect at 290 nm and negative cott on effect at 210, 238 nm), the absolute configuration at C-3, C-4 was assigned to be 3*S*, 4*R* configuration.

Based upon the results of combined spectroscopic analysis and compari son of the spectral data with the previously reported data, the structur e of compound **4** was identified as (3*S*, 4*R*)-4,7,2'-dihydroxy-4'-methox y-isoflavanol.[11]

5. Compound 5

The ^{1}H NMR spectrum indicated an isoflavone structure, with an oygenated methine signal at δ_H 8.20 (1H, s), aromatic signals at δ_H 8.13 (1H, d, J = 8.8 Hz), δ_H 7.46 (2H, d, J = 8.8 Hz), δ_H 7.23 (1H, d, J = 2.2 Hz), δ_H 7.20 (1H, dd, J = 8.8, 2.2 Hz), δ_H 6.96 (2H, d, J = 8.8 Hz). one anomeric signal which was assigned at δ_H 5.09 (1H, d, J = 7.2 Hz) suggested the presence of glucose, a glucose signal at δ_H 3.89 (1H, dd, J = 1.6, 12.0 Hz), δ_H 3.71 (1H, dd, J = 12.0, 5.4 Hz), δ_H 3.57 (1H, m), δ_H 3.50 (2H, m), δ_H 3.42 (1H, m). On the basis of ^{1}H and ^{13}C

NMR spectrum, the sugar moiety was identified as a β-glucopyranosyl. In addition, the HMBC correlations between the anomeric proton and C-7 were confirmed. ^{13}C NMR data showed the presence of ketone signal at δ_C 178.0. and one methoxy signal at δ_C 55.7. The appearance of meta-coupled doublet which is (J = 2.2 Hz) at C-8 and C-6 in the ^1H NMR spectrum of **5** indicated meta position in phenyl ring.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **5** was identified as formononetin 7-O-β-D-glucoside.[12] This compound was isolated for the first time in *Spatholobus suberectus* Dunn.

6. Compound 6

The ^1H NMR spectrum of this compound were very similar to those of compound **5**. ^1H NMR data indicated an isoflavone structure, with an oxygenated methine proton at δ_H 8.28 (1H, s), aromatic proton signals. The most noticeable difference was the presence of two methoxy signals at δ_H 4.02 (3H, s), δ_H 3.82 (3H, s).

Based upon the results of spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **6** was identified as 8-O-methylretusin-7-O-β-D-glucopyranoside.[13] This compound was isolated for the first time in *Spatholobus suberectus* Dunn.

7. Compound 7

The ^1H NMR spectrum indicated a flavanone, with aromatic signals at

δ_H 7.54 (2H, d, J = 8.0 Hz), δ_H 7.41 (2H, m), δ_H 7.39 (1H, m), δ_H 7.28 (1H, s), δ_H 6.39 (1H, s), an oxygenated methine signal at δ_H 5.07 (1H, d, J = 11.8 Hz), an olefinic signal at δ_H 4.50 (1H, d, J = 11.8 Hz). ^{13}C NMR data showed the presence of ketone signal at δ_C 194.2. The appearance of para-singlets at C-8 and C-5 in the ^1H NMR spectrum of **7**.

The relative configuration defined from the J values of olefinic protons (11.8 Hz) at C-2, C-3 are suggested the cis (Z) geometry.

The absolute configurations at C-2, C-3 is assigned from CD spectrum (positive cotton effect at 220, 240, 350 nm and negative cotton effect at 310 nm), the absolute configuration at C-2, C-3 was assigned to be 2R, 3R configuration.

Based upon the results of combined spectroscopic analysis and compari son of the spectral data with the previously reported data, the structur e of compound **7** was identified as (2R, 3R)-3, 7-dihydroxy-6-methoxy flavanone.[14]

8. Compound 8

The ^1H NMR spectrum indicated an isoflavone structure, with an oxygenated methine signal at δ_H 8.15 (1H, s), aromatic signals at δ_H 7.56 (1H, s) δ_H 7.47 (1H, d, J = 8.6 Hz), δ_H 6.98 (2H, d, J = 8.6 Hz), δ_H 6.92 (1H, s). two methoxy group at δ_H 3.95 (3H, s), δ_H 3.81 (3H, s). ^{13}C NMR data showed the presence of ketone signal at δc 177.8. The appearance of para-singlets at C-8 and C-5 in the ^1H NMR spectrum of **8**.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **8** was identified as afromosin.[15]

9. Compound 9

The ^{1}H NMR spectrum of this compound were very similar to those of compound **8**. The ^{1}H NMR data indicated an isoflavone structure, with an olefinic signal at δ_H 8.28 (1H, s), aromatic proton signals. The most noticeable difference was the presence of one methoxy signal at δ_H 4.02 (3H, s).

Based upon the results of spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **9** was identified as formononetin.[16]

10. Compound 10

The ^{1}H NMR spectrum indicated an isoflavan with aromatic proton signals at δ_H 7.02 (1H, d, J = 8.6 Hz), δ_H 6.53 (1H, d, J = 2.3 Hz), δ_H 6.45 (1H, dd, J = 8.6, 2.3 Hz), δ_H 6.30 (1H, dd, J = 8.2, 2.4 Hz), δ_H 6.21 (1H, d, J = 2.4 Hz), two oxygenated methylene signals at δ_H 4.17 (1H, m), δ_H 3.92 (1H, m), an methine signal at δ_H 3.44 (1H, m), a methylene signal at δ_H 2.90 (1H, dd, J = 17.0, 10.0 Hz), δ_H 2.76 (1H, dd, J = 17.0, 4.7 Hz), two methoxy group signals at δ_H 3.82, (3H, s), δ_H 3.76, (3H, s). The appearance of meta-coupled doublet which is small J value at C-3′ and C-5′ in the ^{1}H NMR spectrum of **10** indicated meta position in phenyl ring.

The absolute configuration was predictably assigned as 3*R* from the negative optical activity.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the

structure of compound **10** was identified as sativan.[17]

11. Compound 11

The ^{1}H NMR spectrum of this compound were very similar to those of compound **2**. The most difference was two aromatic signals at δ_H 7.31 (2H, d, J = 8.5 Hz), δ_H 6.80 (2H, d, J = 8.5 Hz).

The absolute configuration at C-2 is assigned by CD spectrum (positiv e cotton effect at 330 nm and negative cotton effect at 300 nm), the a bsolute configuration at C-2 was assigned as 2*S* configuration.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **11** was identified as (2*S*)-liquiritigenin.[18]

12. Compound 12

The ^{1}H NMR spectrum of this compound were very similar to those of compound **2**. the most difference was aromatic signal at δ_H 5.89 (2H, d, J = 2.2 Hz). The appearance of meta-coupled doublet which is small J value at C-8 and C-6 in the ^{1}H NMR spectrum of **12** indicated meta position in phenyl ring.

According to these combined spectral data, the structure of **12** was determined as (2*S*)-naringenin.[19]

13. Compound 13

The ^{1}H NMR spectrum indicated an isoflavone structure, with an olefinic signal at δ_H 8.00 (1H, s), aromatic signals at δ_H 7.36 (2H, d, J = 8.9 Hz), δ_H 6.84 (2H, d, J = 8.7 Hz), δ_H 6.31 (1H, d, J = 2.3 Hz), δ_H 6.20 (1H, d, J = 2.3, Hz). The appearance of meta-coupled doublet

which is small J value at C-8 and C-6 in the ^{1}H NMR spectrum of **13** indicated meta position in phenyl ring. Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **13** was identified as genistein.[20]

14. Compound 14

The ^{1}H NMR spectrum of this compound were very similar to those of compound **13**. the most difference was aromatic signals at δ_H 8.03 (1H, d, J = 8.8 Hz), δ_H 6.91 (1H, dd, J = 8.8, 2.2 Hz), δ_H 6.81 (1H, d, J = 2.2 Hz). The appearance of meta-coupled doublet which is small J value at C-8 and C-6 in the ^{1}H NMR spectrum of **14** indicated meta position in phenyl ring.

According to this spectral data, the structure of **14** was determined as daidzein.[21]

15. Compound 15

The ^{1}H NMR spectrum of this compound were very similar to those of compound **13**. the most difference characteristic was an methoxy signal at δ_H 3.82 (3H, s).

According to this spectral data, the structure of **15** was determined as 5, 7-dihydroxy-4′-methoxy-isoflavone.[22]

16. Compound 16

The ^{1}H NMR spectrum indicated an flavanone structure, with an oxygenated signal at δ_H 5.46 (1H, dd, J = 13.0, 3.0 Hz), methylene signals at δ_H 3.05 (1H, dd, J = 17.0, 13.0 Hz), δ_H 2.79 (1H, dd, J = 17.0, 3.0 Hz), aromatic signals at δ_H 7.52(2H, d, J = 7.2 Hz), δ_H 6.43

(2H, d, J = 7.2 Hz), δ_H 7.32 (1H, s), δ_H 6.43 (1H, s), methoxy group signal at δ_H 3.87 (1H, s), The appearance of para-singlets at C-8 and C-5 in the ^{1}H NMR spectrum of **16**.

Based upon the results of combined spectroscopic analysis and compari son of the spectral data with the previously reported data, the structur e of compound **16** was identified as (2*S*)-7-hydroxy-6-methoxy-flavan one.[23]

17. Compound 17

The ^{1}H NMR spectrum indicated a phenolic compound, with aromatic signals at δ_H 7.42 (1H, d, J = 2.1 Hz), δ_H 7.38 (1H, dd, J = 8.4, 2.1 Hz), δ_H 6.74 (1H, d, J = 8.4 Hz). ^{13}C NMR data showed the presence of aldehyde signal at δ_C 178.0.

According to these combined spectral data, the structure of **17** was determined as protocatechuic acid.[24]

18. Compound 18

The ^{1}H NMR spectrum indicated a flavan-3-ol structure, with aromatic proton signals at δ_H 6.83 (1H, d, J = 1.8 Hz), δ_H 6.75 (1H, d, J = 8.1 Hz), δ_H 6.71 (1H, d, J = 8.1, 1.8 Hz), δ_H 5.91 (1H, d, J = 2.1 Hz). δ_H 5.84 (1H, d, J = 2.1 Hz) with an oxygenated methine proton signal at δ_H 4.55 (1H, d, J = 7.5 Hz), a methine proton signal at δ_H 3.96 (1H, m), methylene proton signals at δ_H 2.84.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **18** was identified as (+)-catechin.[25]

19. Compound 19

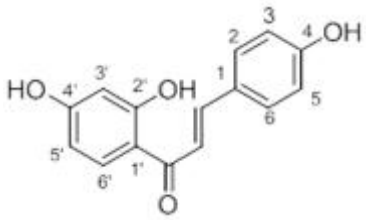
The ^{1}H NMR spectrum indicated a flavan-3-ol structure, with aromatic proton signals at δ_H 6.96 (1H, d, J = 1.9 Hz), δ_H 6.79 (1H, d, J = 8.1 Hz), δ_H 6.75 (1H, d, J = 8.1, 1.8 Hz), δ_H 5.93 (1H, d, J = 2.2 Hz). δ_H 5.91 (1H, d, J = 2.2 Hz) with an oxygenated methine proton signal at δ_H 4.81 (1H, d, J = 1.8 Hz), a methine proton signal at δ_H 4.17 (1H, m), methylene proton signals at δ_H 2.85 (1H, dd, J = 16.4, 7.7 Hz), δ_H 2.73 (1H, dd, J = 16.4, 2.1 Hz).

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **19** was identified as (-)-epicatechin.[26]

20. Compound 20

The ^{1}H NMR and ^{13}C NMR spectra of this compound provided a complete assignment of the A and B units. It was shown that this compound had an (-)-epicatechin unit as the A unit. because methylene signals δ_H 2.76 and δ_H 2.92 and the carbon signal δ_C 29.2 at C-4″, δ_C 82.3 at C-2″ and δ_C 155.2 at C-5″ of A unit were assigned. It was clear that the interflavanoid bond between the A and B units was 4→8″, because of the correlation between the proton signal δ_H 4.60 at C-4 of the B unit and the carbon signal δ_C 108.9 at C-8″ of the A unit, observed in the HMBC spectrum.

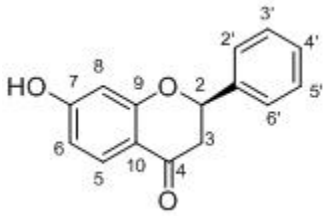
Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **20** was identified as procyanidin B2.



1

Table 1. ^{1}H and ^{13}C NMR Assignment for compound 1 in MeOD

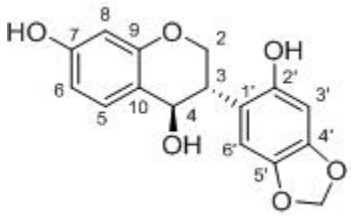
Position	^{1}H	^{13}C
1		129.8
2	7.59, d (8.4)	131.7
3	6.83, d (8.4)	115.9
4		160.3
5	6.83, d (8.4)	115.9
6	7.59, d (8.4)	131.7
1′		112.9
2′		165.8
3′	6.28, d (2.4)	102.6
4′		165.9
5′	6.41, dd (9.0, 2.4)	108.4
6′	7.96, d (9.0)	133.0
α	7.74, d (14.2)	144.1
β	7.58, d (14.2)	117.4
C=O		191.4



2

Table 2. ^{1}H and ^{13}C NMR Assignment for compound 2 in MeOD

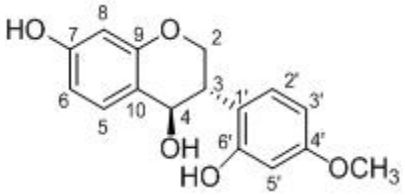
Position	^{1}H	^{13}C
2	5.57, dd (12.7, 3.0)	78.8
3	3.08, dd (16.7, 12.7); 2.70, dd (16.7, 3.0)	43.3
4		189.3
5	7.63, d (8.8)	128.3
6	6.47, dd (8.8, 2.2)	111.1
7		165.6
8	6.31, d (2.2)	102.6
9		163.0
10		112.8
1′		129.2
2′	7.52, d (7.5)	126.5
3′	7.42, d (7.5)	128.5
4′	7.37, m	128.3
5′	7.42, d (7.5)	128.5
6′	7.52, d (7.5)	126.5



3

Table 3. ^{1}H and ^{13}C NMR Assignment for compound 3 in MeOD

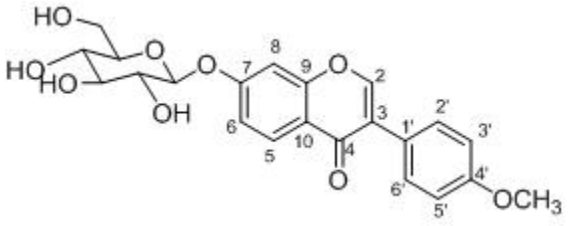
Position	^{1}H	^{13}C
2	4.21, dd (10.7, 4.8); 3.55, d (10.7)	67.4
3	3.49, m	41.6
4	5.44, d (6.8)	80.1
5	7.25, d (8.4)	133.1
6	6.47, dd (8.8, 2.2)	110.8
7		160.4
8	6.29, d (2.5)	104.1
9		158.0
10		112.7
1′		119.9
2′		155.6
3′	6.36, s	94.2
4′		149.5
5′		143.1
6′	6.79, s	105.0
$-OCH_2O-$	5.87, s ; 5.84, s	102.5



4

Table 4. ^{1}H and ^{13}C NMR Assignment for compound 4 in MeOD

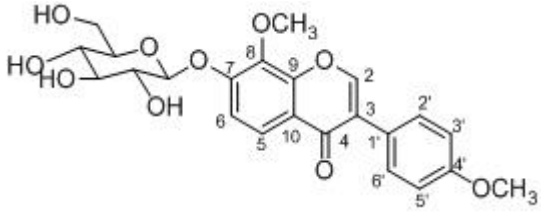
Position	^{1}H	^{13}C
2	4.20, m ; 3.53, m	67.6
3	3.49, m	40.9
4	5.45, d (6.0)	80.1
5	7.28, d (8.4)	133.2
6	6.48, dd (8.3, 2.4)	110.7
7		160.2
8	6.29, d (2.3)	104.1
9		158.1
10		112.9
1′		120.9
2′	7.16, d (8.4)	126.0
3′	6.47, dd (8.3, 2.4)	107.2
4′		162.6
5′	6.37, d (2.3)	97.6
6′		162.0
OCH_3	3.53, s	55.9



5

Table 5. ^{1}H and ^{13}C NMR Assignment for compound 5 in MeOD

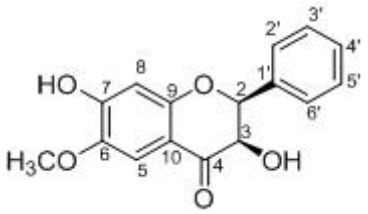
Position	^{1}H	^{13}C
2	8.20, s	155.2
3		126.0
4		178.0
5	8.13, d (8.8)	128.3
6	7.20, dd (8.8, 2.2)	117.1
7		163.5
8	7.23, d (2.2)	105.0
9		159.3
10		120.2
1′		125.8
2′	7.46, d (8.8)	131.4
3′	6.96, d (8.8)	114.9
4′		161.2
5′	6.96, d (8.8)	114.9
6′	7.46, d (8.8)	131.4
OCH_3	3.81, s	55.7
Glc 1	5.09, d(7.2)	101.8
2	3.50, m	74.8
3	3.48, m	77.9
4	3.39, m	71.8
5	3.53, m	78.4
6	3.91, dd (12.1, 2.2); 3.69, dd (12.1, 5.7)	62.5



6

Table 6. ^{1}H and ^{13}C NMR Assignment for compound 6 in MeOD

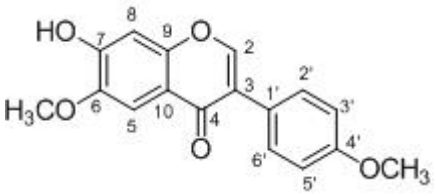
Position	^{1}H	^{13}C
2	8.28, s	155.1
3		125.8
4		178.0
5	7.92, d (9.0)	125.8
6	7.39, d (9.2)	115.7
7		163.8
8		139.0
9		155.9
10		121.2
1′		125.2
2′	7.48, d (9.0)	131.4
3′	6.99, d (9.2)	114.9
4′		161.2
5′	6.99, d (9.2)	114.9
6′	7.48, d (9.0)	131.4
OCH_3 X 2	4.02, s; 3.82, s	55.8, 55.6
Glc 1	5.46, d (7.6)	102.3
2	3.50, m	74.9
3	3.48, m	78.1
4	3.42, m	71.2
5	3.57, m	78.4
6	3.89, dd (12.0, 1.6); 3.71, dd (12.0, 5.4)	62.4



7

Table 7. ^{1}H and ^{13}C NMR Assignment for compound 7 in MeOD

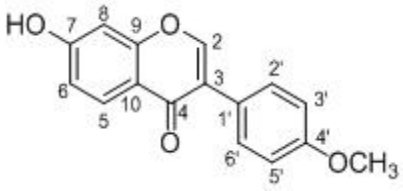
Position	^{1}H	^{13}C
2	5.07, d (11.8)	85.9
3	4.50, d (11.8)	74.7
4		194.2
5	7.28, s	108.1
6		145.8
7		157.7
8	6.39, s	104.6
9		159.9
10		111.7
1′		138.9
2′	7.54, d (8.0)	128.9
3′	7.41, m	129.4
4′	7.39, m	129.8
5′	7.41, m	129.4
6′	7.54, d (8.0)	128.9



8

Table 8. ^{1}H and ^{13}C NMR Assignment for compound 8 in MeOD

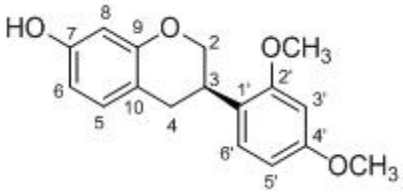
Position	^{1}H	^{13}C
2	8.15, s	154.6
3		125.2
4		177.8
5	7.56, s	105.5
6		148.7
7		155.4
8	6.92, s	104.0
9		154.3
10		117.9
1′		125.8
2′	7.47, d (8.6)	131.5
3′	6.98, d (8.6)	114.9
4′		161.2
5′	6.98, d (8.6)	114.9
6′	7.47, d (8.6)	131.5
OCH_3 X 2	3.95, s; 3.81, s	56.7, 55.7



9

Table 9. ^{1}H and ^{13}C NMR Assignment for compound 9 in MeOD

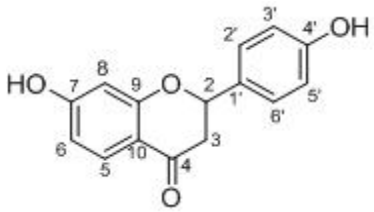
Position	^{1}H	^{13}C
2	8.09, s	153.1
3		126.6
4		174.6
5	8.01, d (9.3)	127.3
6	6.96, dd (9.3, 2.0)	115.5
7		163.3
8	6.90, d (2.0)	102.2
9		157.6
10		116.4
1′		124.3
2′	7.42, d (9.2)	130.1
3′	6.98, d (9.2)	113.7
4′		159.1
5′	6.98, d(9.2)	113.7
6′	7.42, d (9.2)	130.1
OCH_3	3.83, s	55.2



10

Table 10. ^{1}H and ^{13}C NMR Assignment for compound 10 in MeOD

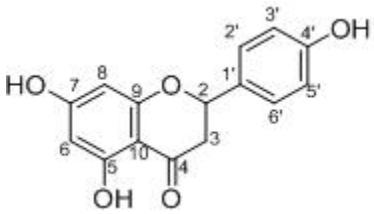
Position	^{1}H	^{13}C
2	4.17, m; 3.92, t (10.0)	31.5
3	3.44, m	33.0
4	2.90, dd (17.0, 10.0); 2.76, dd (17.0. 4.7)	71.1
5	6.85, d (8.6)	131.2
6	6.30, dd (8.2, 2.4)	109.1
7		157.6
8	6.21, d (2.4)	103.8
9		156.4
10		123.1
1′		114.7
2′		159.6
3′	6.53, d (2.3)	99.5
4′		161.3
5′	6.45, dd (8.5, 2.3)	105.7
6′	7.02, (8.6)	128.6
OCH_3 X 2	3.82, s; 3.76, s	55.9, 55.8



11

Table 11. ^{1}H and ^{13}C NMR Assignment for compound 11 in MeOD

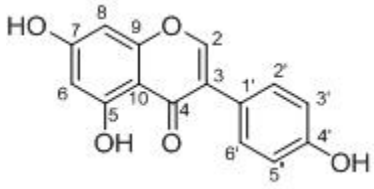
Position	^{1}H	^{13}C
2	5.37, dd (13.0, 2.8)	81.0
3	3.04, m; 2.68, dd (17.0, 3.0)	45.0
4		193.5
5	7.72, d (8.7)	129.8
6	6.48, dd (8.7, 5.3)	111.9
7		165.6
8	6.34, d (2.2)	103.9
9		167.2
10		114.6
1′		131.4
2′	7.31, d (8.5)	129.0
3′	6.80, d(8.5)	116.3
4′		159.0
5′	6.80, d (8.5)	116.3
6′	7.31. d (8.5)	129.0



12

Table 12. ^{1}H and ^{13}C NMR Assignment for compound 12 in MeOD

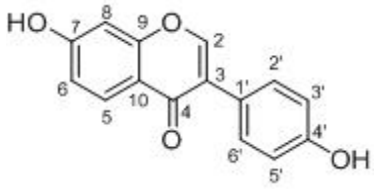
Position	^{1}H	^{13}C
2	5.33, dd (12.9, 2.9)	81.0
3	3.12, m; 2.68, dd (17.0, 3.0)	45.0
4		193.5
5		129.8
6	5.89, d (2.2)	111.9
7		165.6
8	5.89, d (2.2)	103.9
9		167.2
10		114.6
1′		131.4
2′	7.31, d (8.6)	129.0
3′	6.81, d(8.6)	116.3
4′		159.0
5′	6.81, d (8.6)	116.3
6′	7.31. d (8.6)	129.0



13

Table 13. ^{1}H and ^{13}C NMR Assignment for compound 13 in MeOD

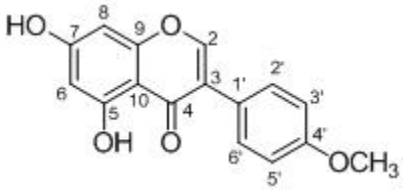
Position	^{1}H	^{13}C
2	8.01, s	154.8
3		123.3
4		182.3
5		159.8
6	6.20, d (2.3)	100.2
7		163.9
8	6.31, d (2.3)	94.8
9		158.8
10		106.3
1′		123.3
2′	7.36, d (8.9)	131.4
3′	6.84, d (8.7)	116.3
4′		159.6
5′	6.84, d (8.7)	116.3
6′	7.36, d (8.9)	131.4



14

Table 14. ^{1}H and ^{13}C NMR Assignment for compound 14 in MeOD

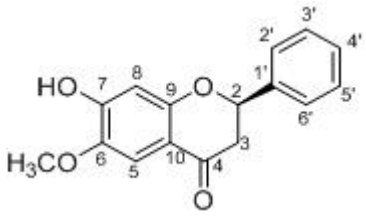
Position	^{1}H	^{13}C
2	8.11, s	152.4
3		122.8
4		178.7
5	8.03, d (8.8)	127.3
6	6.91, dd (8.8, 2.2)	115.1
7		162.3
8	6.81, d (2.2)	102.2
9		157.8
10		116.9
1′		124.0
2′	7.36, d (8.7)	130.0
3′	6.84, d (8.7)	115.2
4′		157.5
5′	6.84, d (8.7)	115.2
6′	7.36, d (8.7)	130.0



15

Table 15. ^{1}H and ^{13}C NMR Assignment for compound 15 in MeOD

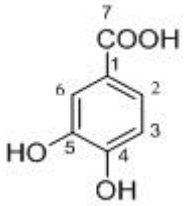
Position	^{1}H	^{13}C
2	8.07, s	155.0
3		124.5
4		182.2
5		163.9
6	6.22, d (2.2)	100.2
7		166.2
8	6.34, d (2.2)	94.9
9		161.2
10		106.3
1′		124.6
2′	7.46, d (8.8)	131.4
3′	6.97, d (8.8)	114.9
4′		159.7
5′	6.97, d (8.8)	114.9
6′	7.46, d (8.8)	131.4
OCH_3	3.82, s	55.8



16

Table 16. ^{1}H and ^{13}C NMR Assignment for compound 16 in MeOD

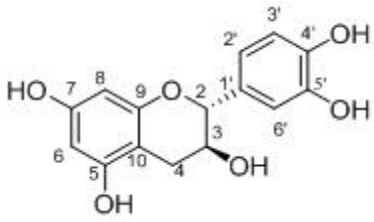
Position	^{1}H	^{13}C
2	5.46, dd (13.0, 3.0)	81.6
3	3.05, dd (17.0, 13.0); 2.79, dd (17.0, 3.0)	45.6
4		193.5
5	7.32, s	108.5
6		146.1
7		161.2
8	6.43, s	105.3
9		158.1
10		114.0
1′		141.4
2′	7.52, d (7.2)	128.3
3′	7.43, m	130.2
4′	7.38, m	130.0
5′	7.43, m	130.2
6′	7.52, d (7.2)	128.3
OCH_3	3.87, s	57.1



17

Table 17. ^{1}H and ^{13}C NMR Assignment for compound 17 in MeOD

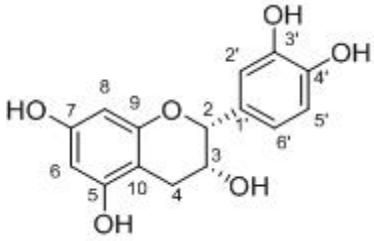
Position	^{1}H	^{13}C
1		127.5
2	7.42, d (2.1)	117.8
3		145.6
4		150.0
5	6.74, d (8.4)	115.4
6	7.38, dd (8.4, 2.1)	123.4
7		173.4



18

Table 18. ^{1}H and ^{13}C NMR Assignment for compound 18 in MeOD

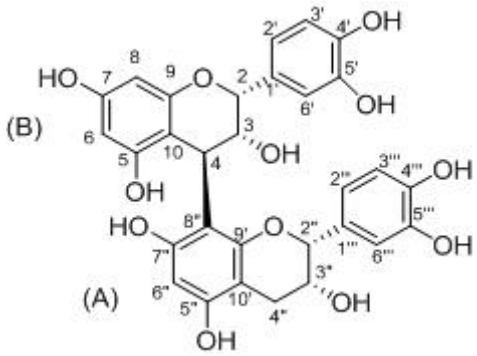
Position	^{1}H	^{13}C
2	4.55, d (7.5)	82.9
3	3.96, m	68.8
4	2.84, dd (16.1, 5.2); 2.49, dd (16.1, 8.1)	28.5
5		157.9
6	5.84, d (2.1)	96.3
7		157.6
8	5.91, d (2.1)	95.5
9		156.9
10		100.8
1′		115.3
2′	6.71, dd (8.1, 1.8)	132.2
3′	6.75, d (8.1)	116.1
4′		146.3
5′		146.2
6′	6.83, d (1.8)	120.0



19

Table 19. ^{1}H and ^{13}C NMR Assignment for compound 19 in MeOD

Position	^{1}H	^{13}C
2	4.81, d (1.8)	78.6
3	4.17, m	65.4
4	2.85, dd (16.4, 7.7); 2.73, dd (16.4, 2.1)	28.8
5		95.6
6	5.91, d (2.2)	94.6
7		157.1
8	5.93, d (2.2)	156.8
9		156.3
10		99.0
1′		131.1
2′	6.96, d (1.9)	118.5
3′		115.4
4′		145.0
5′	6.79, d (8.1)	144.9
6′	6.75, d (8.1, 1.9)	115.3



20

Table 20. ^{1}H and ^{13}C NMR Assignment for B unit of compound 20 in DMSO

Position	^{1}H	^{13}C
2	5.04, br s	78..8
3	3.77, br s	71.9
4	4.60, br s	37.9
5		156.1
6	5.93, d (2.0)	94.8
7		157.9
8	5.92. d (2.0)	97.5
9		100.1
10		154.8
1′		131.6
2′	6.67, dd (8.2, 2.0)	114.3
3′	6.62, d (8.2)	144.6
4′		144.3
5′		115.6
6′	6.81, d (2.0)	119.3

Table 20. ^{1}H and ^{13}C NMR Assignment for A unit of compound 20 in DMSO

Position	^{1}H	^{13}C
2″	4.93, br s	82.3
3″	4.22, m	65.7
4″	2.76, d (17.0), 2.92, d (17.4)	29.2
5″		155.2
6″	5.86, s	96.3
7″		156.6
8″		108.9
9′		96.5
10′		154.8
1‴		131.3
2‴	6.83, dd (8.0, 2.0)	115.3
3‴	6.70, d (8.0)	144.3
4‴		144.5
5‴		115.8
6‴	7.10, d (2.0)	119.5

Discussion

The phytochemical investigation of *Spatholobus suberectus* Dunn, afford a very diverse series of flavonoids, sterols and triterpenes.

Even though several flavonoids such as formononetin, daidzein and genistein have been reported from *Spatholobus suberectus* Dunn, the flavonoid dimer and flavonoid glycosides from this plants are relatively rare.

Fractionation guided by inhibitory activity of sortase A, an enzyme that plays a key role in cell wall protein anchoring and virulence in *Staphylococcus aureus*, the flavonoid-containing fraction of 80% aqueous MeOH, 40% aqueous MeOH exhibited the most potent inhibitory activity. Therefore, isolation of flavonoids from *S. spatholobus* can be good starting candidates for biomedical purposes.

In this study, the isolated compounds were structurally identified to be eighteen of flavonoids and one of phenolic and one of flavonoid dimer. Among the isolated compounds, three flavonoids were isolated for the first time in this plant.

The result of detailed biological activities of these compounds, nine flavonoids showed strong inhibitory activity of sortase A.

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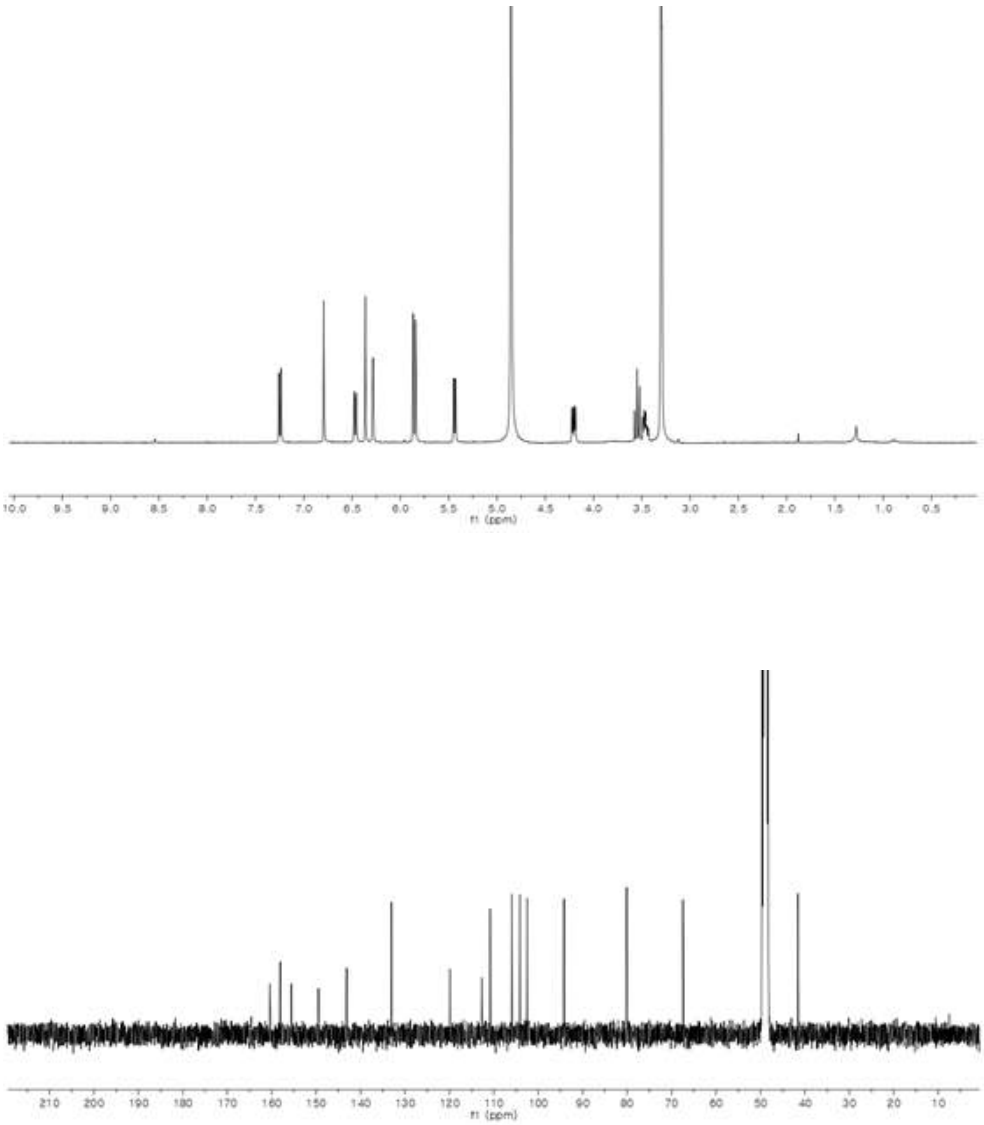

Figure 1. ^{1}H and ^{13}C NMR spectra of Compound 3

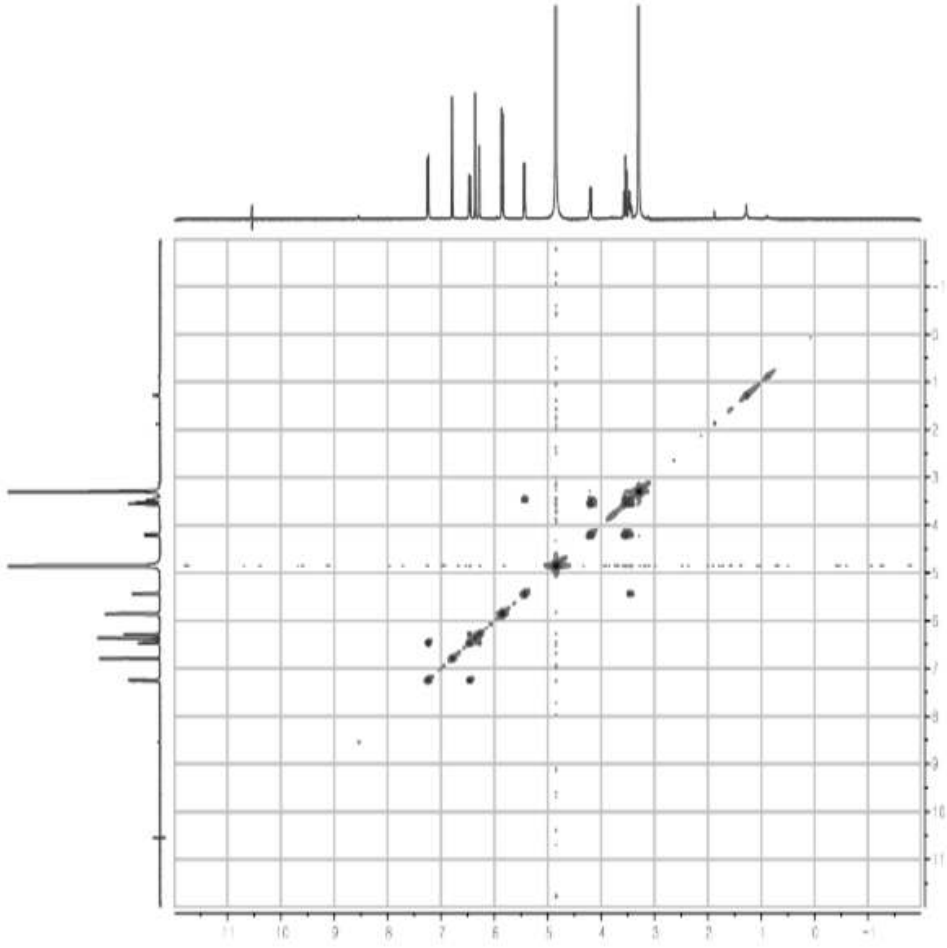

Figure 2. COSY spectrum of Compound 3

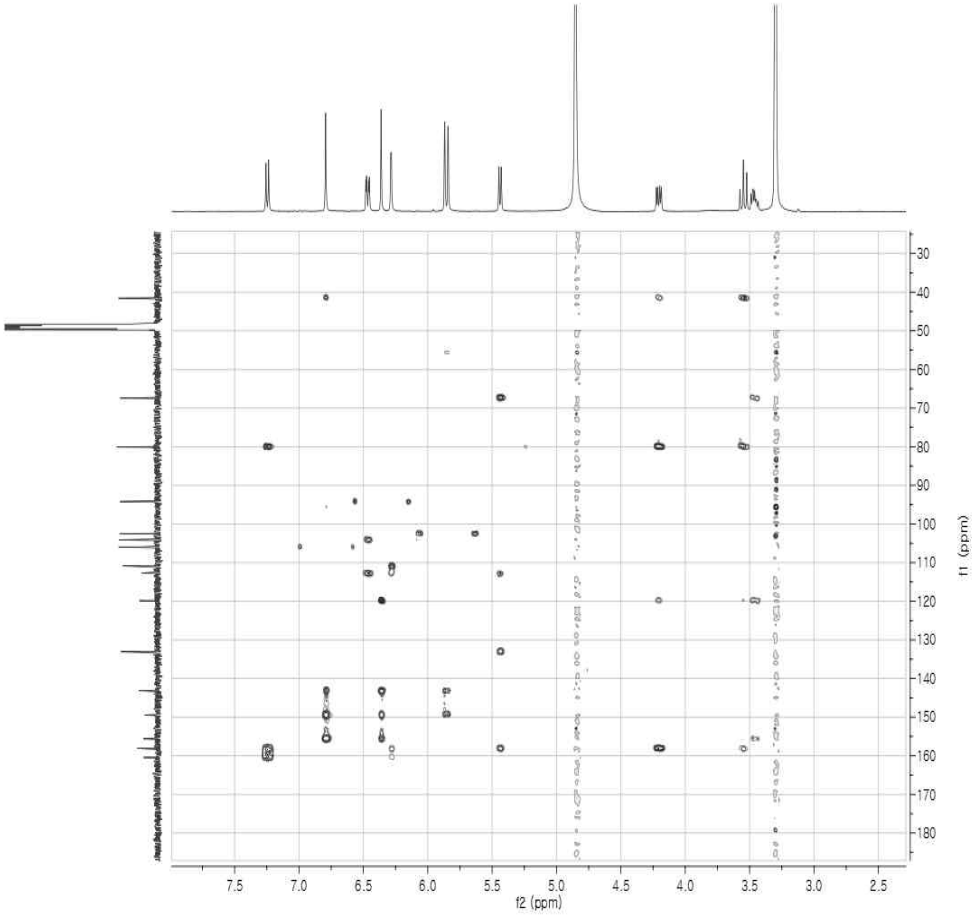

Figure 3. *g*HMBC spectrum of Compound 3

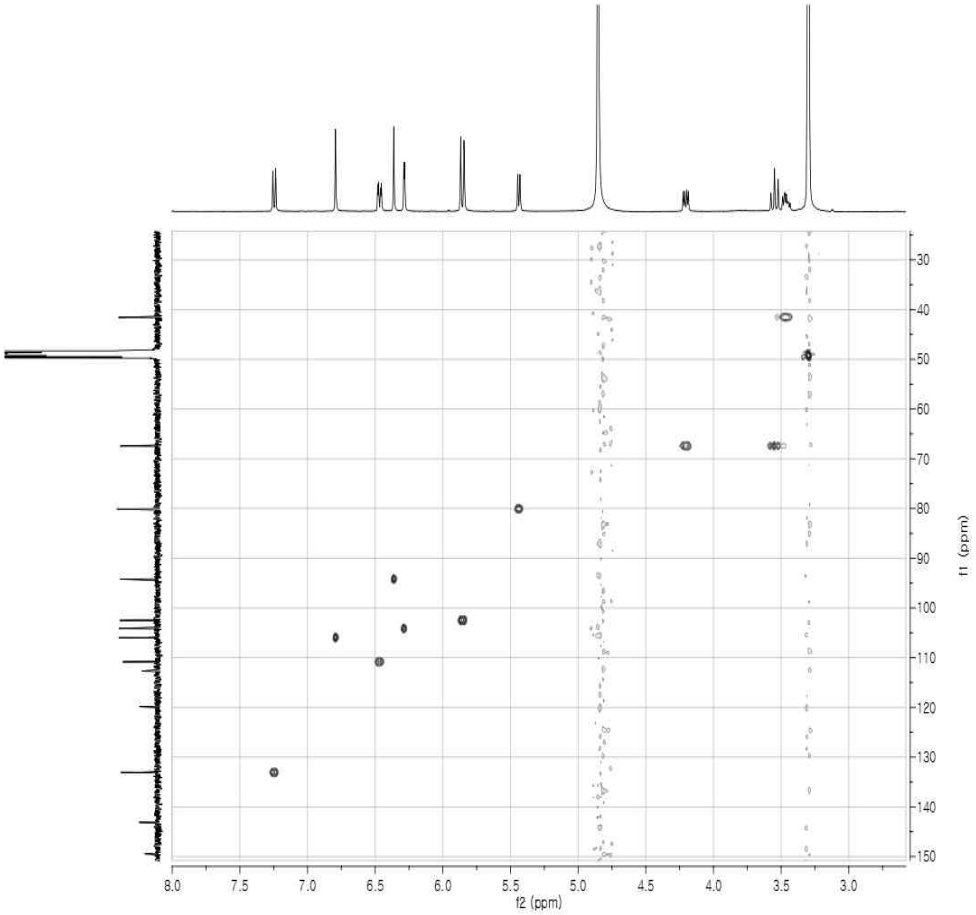

Figure 4. HSQC spectrum of Compound 3

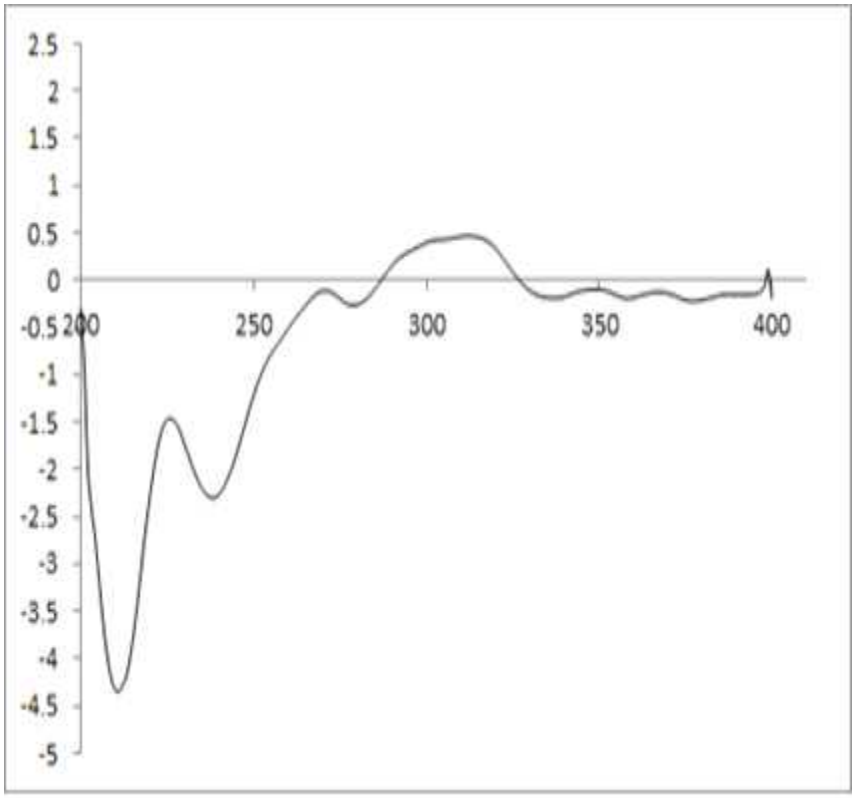

Figure 5. CD spectrum of Compound 3

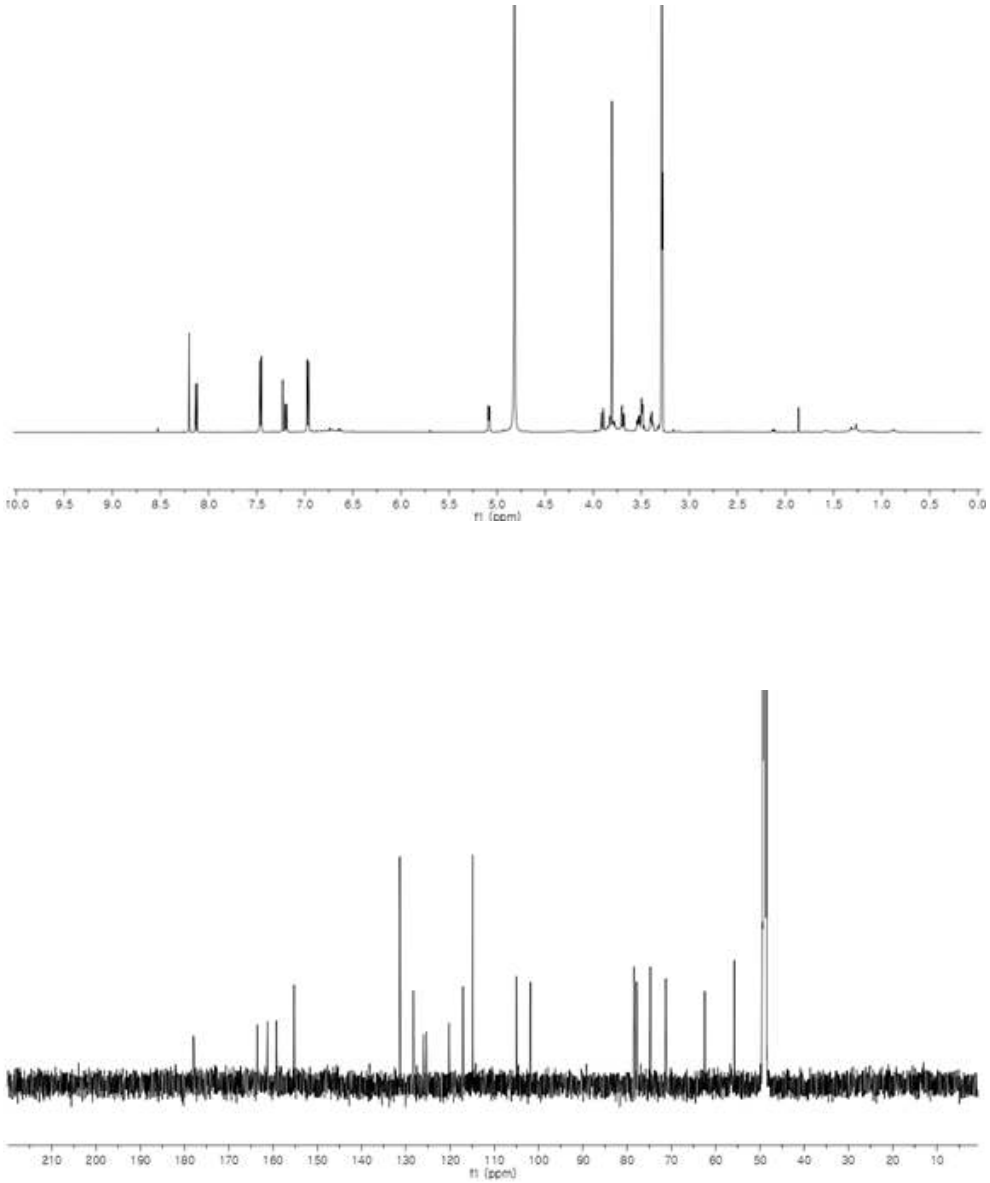

Figure 6. ^{1}H and ^{13}C NMR spectra of Compound 5

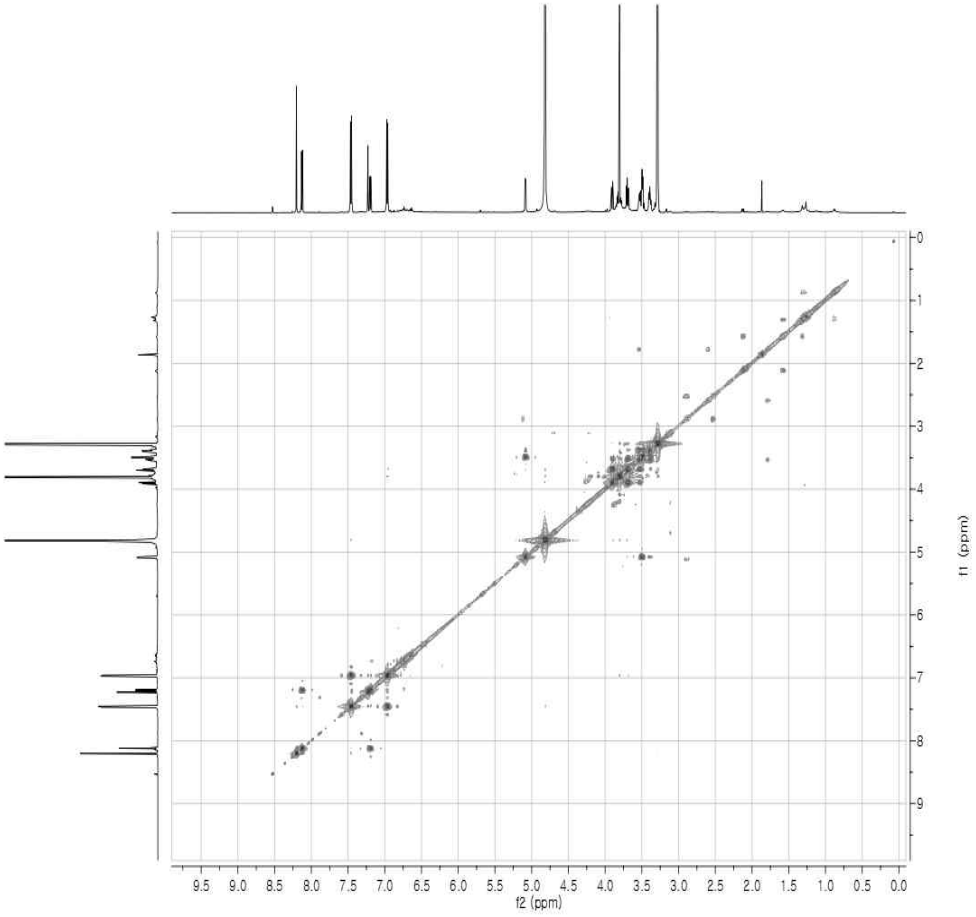

Figure 7. COSY spectrum of Compound 5

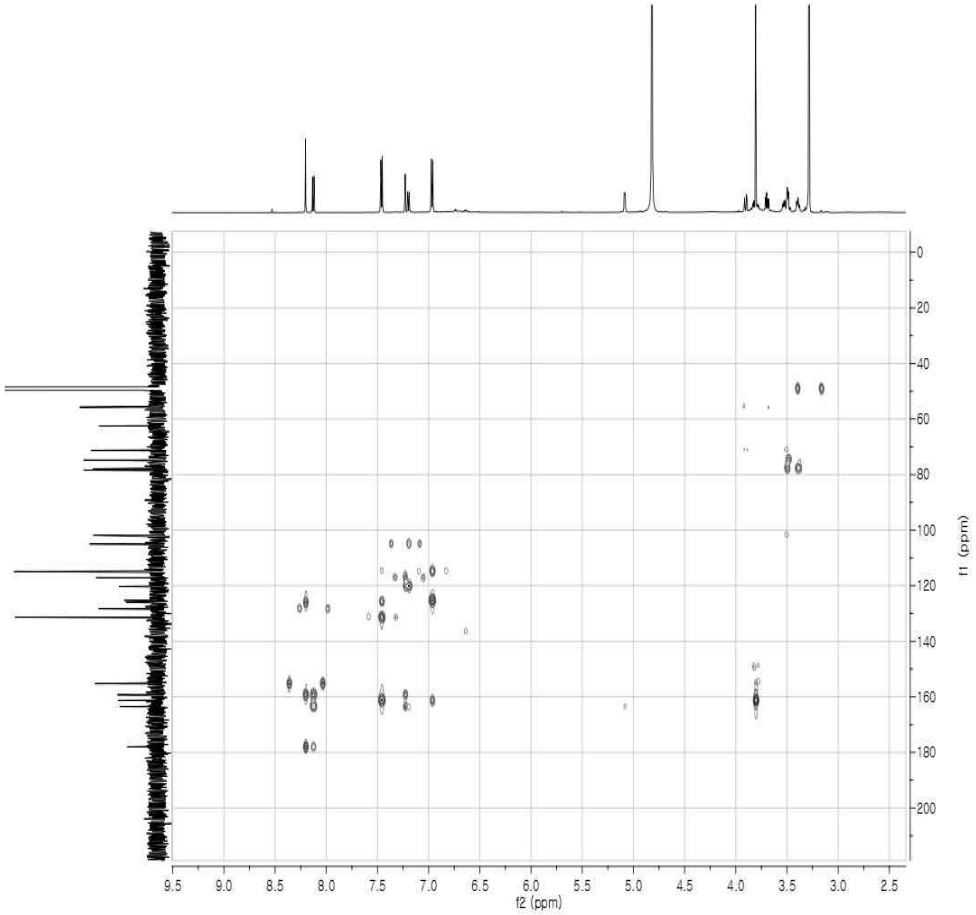

Figure 8. *g*HMBC spectrum of Compound 5

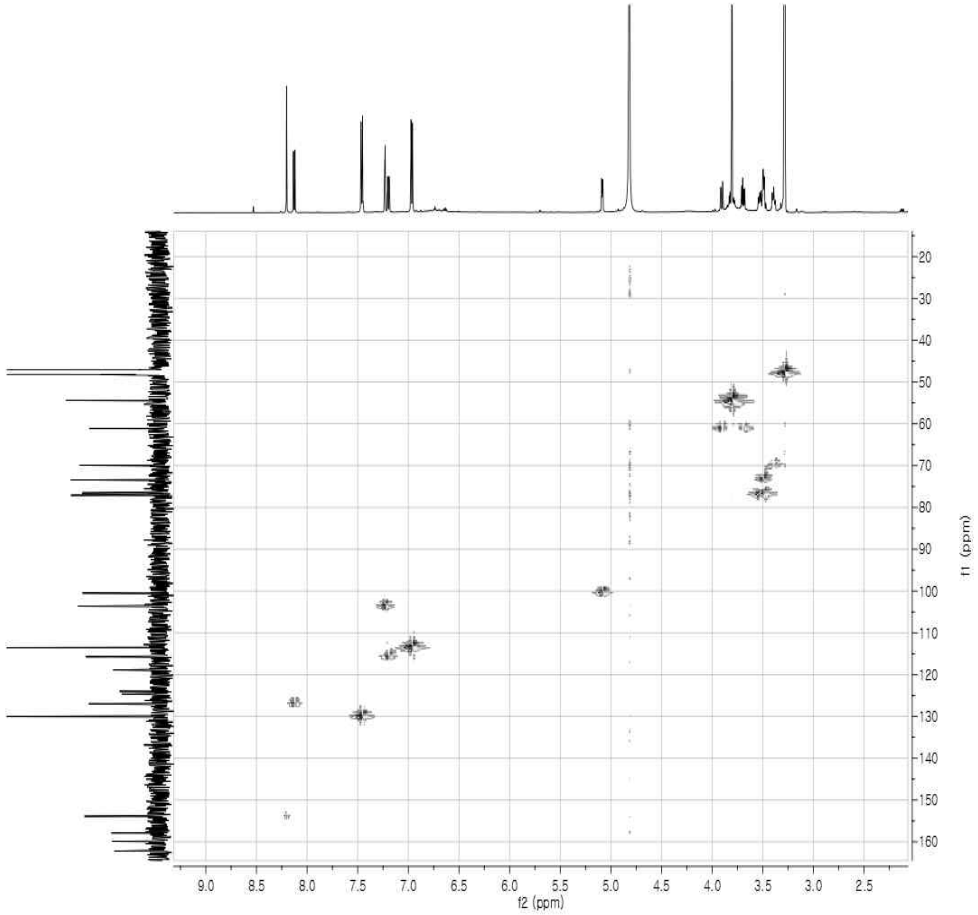

Figure 9. HSQC spectrum of Compound 5

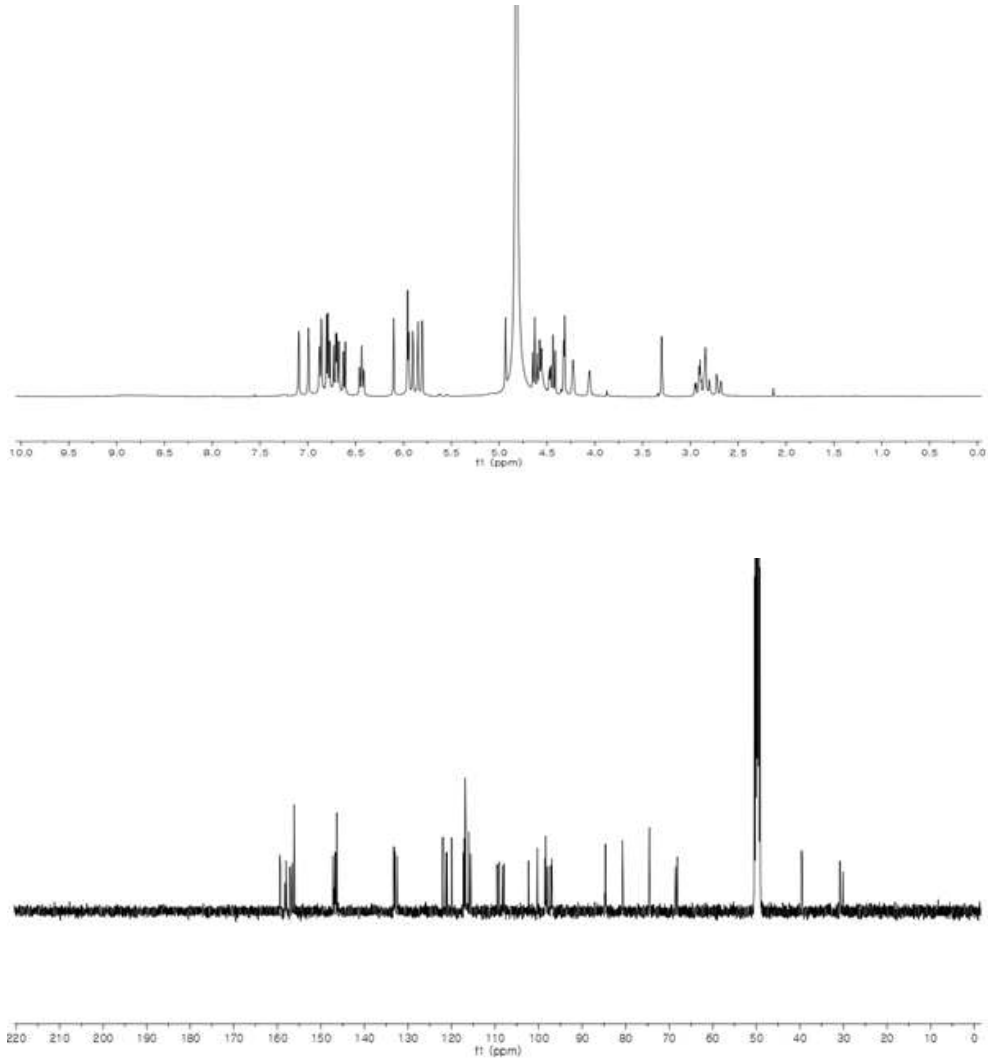

Figure 10. ^{1}H and ^{13}C NMR spectra of Compound 20

국문초록

계혈등의 성분연구

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효소 sortase A에 대해 저해 생리활성을 가지는 천연 물질을 찾고자 다양한 생약 추출물을 평가하였다. 생리활성 검색 결과에 따라 계혈등을 연구대상으로 선택하였는데 이는 혈액순환 개선과 생리통, 빈혈, 마비, 관절통 및 세균 감염에 사용되는 전통 생약이다. Sortase A에 다하여 높은 저해활성을 보이는 계혈등 추출물의 분획에 대하여 다양한 크로마토그래피 분리 기법으로 총 20개의 물질을 분리하였다. 복합적 분광학적 분석의 결과를 토대로 분리된 물질들이 18종의 flavonoids, 1종의 phenolic, 1종의 flavonoid dimer 임을 동정하였다. 이들 중 3종의 flavonoid 계열 물질이 계혈등에서는 처음 발견된 물질임을 확인하였다.

주요어 : 계혈등, flavonoids, sortase A 활성저해.
학번 : 2014-21059

약학석사학위논문

Isolation and Structure Identification of Chemical Constituents of *Spatholobus suberectus* Dunn.

계혈등의 성분연구

2016년 2월

서울대학교 대학원
약학대학 약학과
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Abstract

Isolation and Structure Identification of Chemical Constituents of *Spatholobus suberectus* Dunn.

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For the investigation of bioactive natural products with sortase A (SrtA) inhibitory effect, various extracts of Korean herbal medicines were evaluated. Based upon the results of bioactivity screening, the dried vine stem of *Spatholobus suberectus* Dunn. which is an oriental folk medicine used mainly for the improvement of blood circulation and treatment for dysmenorrhea, anemia, paralysis, arthralgia and bacterial infections was selected for chemical investigation. The large-scale extraction followed by the bioactivity-guided partition and chromatographic separation yielded twenty compounds. Based upon the results of combined spectroscopic analyses, these compounds were structurally identified as eighteen flavonoids, one flavonoid dimer and a

phenolic compound. Among these three flavonoids were found from this plant for the first time.

Key Word : Spatholobus suberectus Dunn, flavonoids, flavonoid dimer, phenolic compound, sortase A inhibitory effect.

Student Number : 2014-21059

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Introduction

Spatholobus suberectus Dunn. (Leguminosae) which is an abundant plant in south of China is one of the most popular traditional herbal medicine. The vine stem of *S. spatholobus* has been used for improvement of blood circulation and treatment for dysmenorrhea, anemia, paralysis, arthralgia and bacterial infections.[1]

Gram-positive pathogenic bacteria display surface proteins that play important roles in their adhesion to specific organ tissues, invasion of host cells, or the evasion of host-immune responses.[2] These virulence-associated proteins are covalently anchored to bacterial cell wall peptidoglycans through a general sorting mechanism catalyzed by a super family of membrane-associated trans-peptidases termed sortases.[3] Two sortase isoforms, sortase A (SrtA) and sortase B (SrtB), have been identified in Staphylococcus aureus.[4] The SrtA isoform plays a criticalrole in the pathological effects of gram-positive bacteria by modulating the ability of the bacterium to adhere to host tissue via the covalent anchoring of adhesion molecules and other virulence-associated proteins to cell wall peptidoglycans. *S.aureus* mutants lacking sortase fail to display surface proteins and are defective in the establishment of infections but microbial viability is not affected.[5] There have only been a few reports in the literature describing inhibitors of sortase, due in part to the fact that the importance of sortase as a new target hasonly recently been acknowledged.[6] Therefore, inhibitors of SrtA might be promising candidates for the treatment and prevention of gram-positive bacterial infections.[7]

In the preliminary study on the SrtA inhibitors from Korean herb medicines, more than two-hundreds of plant extracts were tested. As

a result the crude extract of dry stem of *spatholobus suberectus* Dunn. was selected as one of the prime target with the inhibition value at 48.5% against SrtA at the concentration of 100 ㎍/mL.

The organic extracted from the stem of *spatholobus suberectus* Dunn. was separated by employing solvent-partitioning. Fractionation guided by SrtA inhibitory activity (50%, 40%, 30%, and 20% aqueous MeOH exhibited 11.97%, 13.97%, 32.64%, 14.58% inhibition, respectively, at the concentration of 100 ㎍/mL), followed by various chromatography methods yielded eighteen flavonoids, one phenolic compound, one flavonoid dimer, in total twenty compounds. The structure of the isolated compounds were identified on the basis of combined spectroscopic analyses. Among the isolated compounds, **3, 5, and 6** compounds were found in this plant first time.

Experimental Section

1. General Experimental Procedures

Optical rotations were measured on a JASCO P-1020 polarimeter and using a 1 cm cell. CD data were obtained on a JASCO J-715 spectrop olarimeter in MeOH solutions. IR spectra were obtained on a JASCO F T/IR-300E spectrophotometer. UV spectra were recorded on a Hitachi U-3010 spectrophotometer. NMR spectra were recorded in DMSO, and CD_3OD solutions, on a Bruker AMX-500 and 125 ㎒, respectively. Mas s spectra were provided by the Korea Basic Science Institute, Daegu Branch, Korea. All solvents used were spectral grade or were distilled from glass prior to use.

2. Plant Material

The vine stem of *Spatholobus suberectus* Dunn were purchased from the Kyungdong-Market, Seoul, Korea, in November, 2014. A voucher specimen is on deposit at the Natural Products Research Institute, College of Pharmacy, Seoul National University.

3. Extraction and Isolation

The vine stem of *Spatholobus suberectus* Dunn. was repeatedly extra cted with Methyl chloride (10 L x 3) and MeOH (10 L x 3). The com bined crude extract (210.0 g) were partitioned between water (156.1 g) and *n*-butanol (49.5 g). 49.5 g of *n*-butanol was repartitioned between *n*-hexane (3.95 g) and 15% aqueous MeOH (44.4 g). 20.8 g of 15% aq ueous MeOH layer from solvent partitioning was subjected to reversed

-phase vacuum flash chromatography using sequential mixtures of H_2O and MeOH (elution order: 50%, 40%, 30%, 20%, 10% aqueous MeOH, and 100% MeOH) and 100% acetone as eluents.

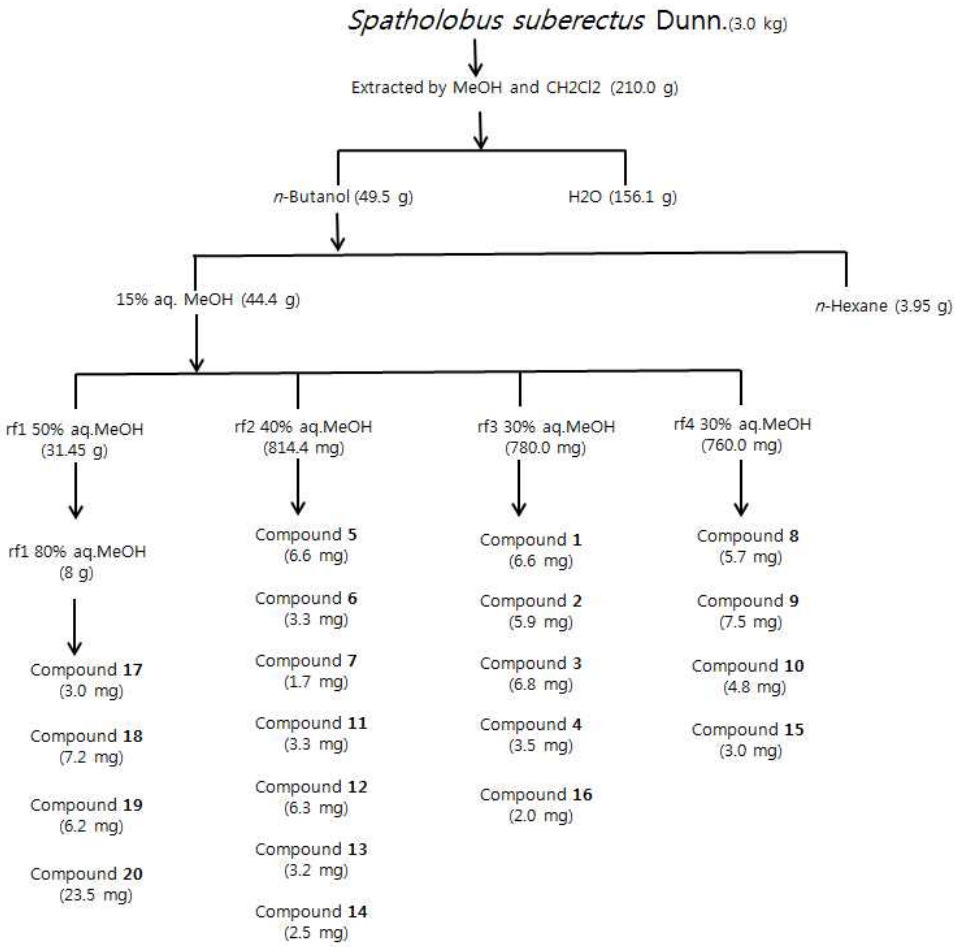
Base on the combined bioactivity test and TLC analysis, 31.5 g of the fraction which was eluted with 50% aqueous MeOH was subjected to reversed-phase vacuum flash chromatography again using sequential m ixtures of H_2O and MeOH (elution order: 80%, 70%, 60%, 50% aqueou s MeOH), then 8.0 g of the fraction which was eluted with 80% aqueo us MeOH was separated by semi-preparative reversed-phase HPLC (Y MC ODS-A column, 10 mm x 250 mm, 85% aqueous MeOH) to yield, in order of elution, compounds 17, 18, 19 and 20. Final purification of t he individual compound was then accomplished by HPLC (95% aqueou s ACN to afford 3.0, 7.2, 6.2 and 23.5 mg of compounds **17**, **18**, **19**, an d **20**, respectively).

A portion (814.4 mg) of the fraction eluted with 40% aqueous MeOH f rom vaccum flash chromatography was separated by reversed-phase H PLC (55% aqueous MeOH) to yield, in order of elution, compounds **5**, **6**, **7**, **11**, **12**, **13**, and **14**. Purification of each of these was then accom plished by reversed-phase HPLC (58% aqueous MeOH) to afford 6.6, a nd 3.3 mg of compounds 5, and 6 respectively, and reversed-phase HP LC (80% aqueous ACN) to afford 1.7, 3.3, 6.3, 3.2 and 2.5 mg of comp ounds **7**, **11**, **12**, **13** and **14** respectively.

A portion (780.0 mg) of the fraction eluted with 30% aqueous MeOH f rom flash chromatography was separated by reversed-phase HPLC (4 5% aqueous MeOH) to yield, in order of elution, compounds **1**, **2**, **3**, 4, and **16** as yellow color gums. Purification of each of these was then accomplished by reversed-phase HPLC (65% aqueous ACN) to afford 6.6, 5.9, 6.8, 3.5 and 2.0 mg of compounds **1**, **2**, **3**, **4** and **16** respectivel

y.

A portion (760.0 mg) of the fraction eluted with 20% aqueous MeOH f rom flash chromatography was separated by reversed-phase HPLC (3 5% aqueous MeOH) to yield, in order of elution, compounds **8**, **9**, **10**, a nd **16** as yellow color gums. Purification of each of these was then a ccomplished by reversed-phase HPLC (63% aqueous ACN) to afford 5. 7, 7.5, 4.8, and 3.0 mg of compounds **8**, **9**, **10**, and **15** respectively.



Scheme 1. Isolation of Compounds from *Spatholobus suberectus* Dunn

Results

1. Compound 1

The ^{1}H NMR spectrum indicated a chalcone, with aromatic signals at δ_H 7.59 (2H, d, J = 8.4 Hz), and δ_H 6.83 (2H, d, J = 8.4 Hz), δ_H 6.41 (1H, dd, J = 9.0, 2.4 Hz), δ_H 6.28 (1H, d, J = 2.4 Hz), δ_H 7.96 (1H, d, J = 9.0 Hz). a hydrogenated methine signal at δ_H 7.74 (1H, d, J = 14.2 Hz), an olefinic signal at δ_H 7.60 (1H, d, J = 14.2 Hz). ^{13}C NMR spectrum showed the presence of ketone signal at δ_C 191.4.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **1** was identified as isoliquiritigenin.[8]

2. Compound 2

The ^{1}H NMR spectrum indicated a flavanone, with aromatic signals at δ_H 7.63 (1H, d, J = 8.8 Hz), δ_H 7.52 (2H, d, J = 8.7 Hz), δ_H 7.42 (2H, dd, J = 8.7, 7.0 Hz), δ_H 7.37 (1H, m), δ_H 6.47 (1H, dd, J = 8.7, 2.2 Hz), δ_H 6.31 (1H, d, J = 2.2 Hz), an oxygenated methine signal at δ_H 5.57 (1H, dd, J = 12.6, 3.0 Hz) and two methylene signals at δ_H 3.08 (1H, dd, J = 16.7, 12.7 Hz), δ_H 2.70 (1H, dd, J = 16.7, 3.0 Hz). ^{13}C NMR showed the presence of ketone signal at δ_C 189.4. The appearance of meta-coupled doublet which is small (J = 2.2 HZ) at C-8 and C-6 in the ^{1}H NMR spectrum of **2** indicated meta position in phenyl ring.

The absolute configuration at C-2 is assigned by CD experiment from CD spectrum (positive cotton effect at 290 nm and negative cotton effect at 330 nm), the absolute configuration at C-2 was assigned to

be 2*R* configuration.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **2** was identified as (2*R*)-7-hydroxyflavanone.[9]

3. Compound 3

The ^{1}H NMR spectrum of compound **3** indicated isoflavan-4-ol, with aromatic signals at δ_H 7.25 (1H, d, *J* = 8.4 Hz), δ_H 6.79 (1H, s), δ_H 6.47 (1H, dd, *J* = 8.4, 2.4 Hz), δ_H 6.36 (1H, s), δ_H 6.29 (1H, d, *J* = 2.5 Hz), oxygenated methylene signals at δ_H 4.21 (1H, dd, *J* = 10.7, 4.8 Hz), δ_H 3.55 (1H, d, *J* = 10.7 Hz), two methine signals at δ_H 3.49 (1H, m), δ_H 5.44 (1H, d, *J* = 6.8 Hz), The appearance of para-singlet at C-3′, C-6′ in the ^{1}H NMR spectrum of **3**. The characteristic signal was methylene dioxy at δ_H 5.87 (1H, s), and δ_H 5.84 (1H, s).

The relative configuration defined from the *J* values of olefinic protons at C-3, C-4 (*J* = 6.8 Hz) is suggested the trans (*E*) geometry.

For absolute configuration at C-3, C-4 is assigned by CD experiment. From CD spectrum (positive cotton effect at 310 nm and negative cotton effect at 210, 240, 280 nm), the absolute configuration at C-3, C-4 was assigned to be 3*S*, 4*R* configuration.

Based upon the results of combined spectroscopic analysis and compari son of the spectral data with the previously reported data, the structur e of compound **3** was identified as (3*S*, 4*R*)-7,2'-dihydroxy-4,5'methyle ne dioxyisoflavan-4-ol.[10] This compound was isolated for the first time in *Spatholobus suberectus* Dunn.

4. Compound 4

The ^{1}H NMR spectrum of this compound were very similar to those of compound **3**. The most noticeable difference was the presence of ar omatic signals at δ_H 7.16 (1H, d, J = 8.4 Hz), δ_H 6.47 (1H, dd, J = 8.4, 2.4 Hz), δ_H 6.37 (1H, d, J = 2.4 Hz) and a methoxy signal at δ_H 3.53 (3H, s).

The relative configuration defined from the J values of olefinic protons at C-3, C-4 (J = 6.0 Hz) is suggested the trans (E) geometry.

For absolute configuration at C-3, C-4 is assigned by CD experiment. From CD spectrum (positive cotton effect at 290 nm and negative cott on effect at 210, 238 nm), the absolute configuration at C-3, C-4 was assigned to be 3*S*, 4*R* configuration.

Based upon the results of combined spectroscopic analysis and compari son of the spectral data with the previously reported data, the structur e of compound **4** was identified as (3*S*, 4*R*)-4,7,2'-dihydroxy-4'-methox y-isoflavanol.[11]

5. Compound 5

The ^{1}H NMR spectrum indicated an isoflavone structure, with an oygenated methine signal at δ_H 8.20 (1H, s), aromatic signals at δ_H 8.13 (1H, d, J = 8.8 Hz), δ_H 7.46 (2H, d, J = 8.8 Hz), δ_H 7.23 (1H, d, J = 2.2 Hz), δ_H 7.20 (1H, dd, J = 8.8, 2.2 Hz), δ_H 6.96 (2H, d, J = 8.8 Hz). one anomeric signal which was assigned at δ_H 5.09 (1H, d, J = 7.2 Hz) suggested the presence of glucose, a glucose signal at δ_H 3.89 (1H, dd, J = 1.6, 12.0 Hz), δ_H 3.71 (1H, dd, J = 12.0, 5.4 Hz), δ_H 3.57 (1H, m), δ_H 3.50 (2H, m), δ_H 3.42 (1H, m). On the basis of ^{1}H and ^{13}C

NMR spectrum, the sugar moiety was identified as a β-glucopyranosyl. In addition, the HMBC correlations between the anomeric proton and C-7 were confirmed. ^{13}C NMR data showed the presence of ketone signal at δ_C 178.0. and one methoxy signal at δ_C 55.7. The appearance of meta-coupled doublet which is (J = 2.2 Hz) at C-8 and C-6 in the ^1H NMR spectrum of **5** indicated meta position in phenyl ring.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **5** was identified as formononetin 7-O-β-D-glucoside.[12] This compound was isolated for the first time in *Spatholobus suberectus* Dunn.

6. Compound 6

The ^1H NMR spectrum of this compound were very similar to those of compound **5**. ^1H NMR data indicated an isoflavone structure, with an oxygenated methine proton at δ_H 8.28 (1H, s), aromatic proton signals. The most noticeable difference was the presence of two methoxy signals at δ_H 4.02 (3H, s), δ_H 3.82 (3H, s).

Based upon the results of spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **6** was identified as 8-O-methylretusin-7-O-β-D-glucopyranoside.[13] This compound was isolated for the first time in *Spatholobus suberectus* Dunn.

7. Compound 7

The ^1H NMR spectrum indicated a flavanone, with aromatic signals at

δ_H 7.54 (2H, d, J = 8.0 Hz), δ_H 7.41 (2H, m), δ_H 7.39 (1H, m), δ_H 7.28 (1H, s), δ_H 6.39 (1H, s), an oxygenated methine signal at δ_H 5.07 (1H, d, J = 11.8 Hz), an olefinic signal at δ_H 4.50 (1H, d, J = 11.8 Hz). ^{13}C NMR data showed the presence of ketone signal at δ_C 194.2. The appearance of para-singlets at C-8 and C-5 in the ^1H NMR spectrum of **7**.

The relative configuration defined from the J values of olefinic protons (11.8 Hz) at C-2, C-3 are suggested the cis (Z) geometry.

The absolute configurations at C-2, C-3 is assigned from CD spectrum (positive cotton effect at 220, 240, 350 nm and negative cotton effect at 310 nm), the absolute configuration at C-2, C-3 was assigned to be 2R, 3R configuration.

Based upon the results of combined spectroscopic analysis and compari son of the spectral data with the previously reported data, the structur e of compound **7** was identified as (2R, 3R)-3, 7-dihydroxy-6-methoxy flavanone.[14]

8. Compound 8

The ^1H NMR spectrum indicated an isoflavone structure, with an oxygenated methine signal at δ_H 8.15 (1H, s), aromatic signals at δ_H 7.56 (1H, s) δ_H 7.47 (1H, d, J = 8.6 Hz), δ_H 6.98 (2H, d, J = 8.6 Hz), δ_H 6.92 (1H, s). two methoxy group at δ_H 3.95 (3H, s), δ_H 3.81 (3H, s). ^{13}C NMR data showed the presence of ketone signal at δc 177.8. The appearance of para-singlets at C-8 and C-5 in the ^1H NMR spectrum of **8**.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **8** was identified as afromosin.[15]

9. Compound 9

The ^{1}H NMR spectrum of this compound were very similar to those of compound **8**. The ^{1}H NMR data indicated an isoflavone structure, with an olefinic signal at δ_H 8.28 (1H, s), aromatic proton signals. The most noticeable difference was the presence of one methoxy signal at δ_H 4.02 (3H, s).

Based upon the results of spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **9** was identified as formononetin.[16]

10. Compound 10

The ^{1}H NMR spectrum indicated an isoflavan with aromatic proton signals at δ_H 7.02 (1H, d, J = 8.6 Hz), δ_H 6.53 (1H, d, J = 2.3 Hz), δ_H 6.45 (1H, dd, J = 8.6, 2.3 Hz), δ_H 6.30 (1H, dd, J = 8.2, 2.4 Hz), δ_H 6.21 (1H, d, J = 2.4 Hz), two oxygenated methylene signals at δ_H 4.17 (1H, m), δ_H 3.92 (1H, m), an methine signal at δ_H 3.44 (1H, m), a methylene signal at δ_H 2.90 (1H, dd, J = 17.0, 10.0 Hz), δ_H 2.76 (1H, dd, J = 17.0, 4.7 Hz), two methoxy group signals at δ_H 3.82, (3H, s), δ_H 3.76, (3H, s). The appearance of meta-coupled doublet which is small J value at C-3′ and C-5′ in the ^{1}H NMR spectrum of **10** indicated meta position in phenyl ring.

The absolute configuration was predictably assigned as 3*R* from the negative optical activity.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the

structure of compound **10** was identified as sativan.[17]

11. Compound 11

The ^{1}H NMR spectrum of this compound were very similar to those of compound **2**. The most difference was two aromatic signals at δ_H 7.31 (2H, d, J = 8.5 Hz), δ_H 6.80 (2H, d, J = 8.5 Hz).

The absolute configuration at C-2 is assigned by CD spectrum (positiv e cotton effect at 330 nm and negative cotton effect at 300 nm), the a bsolute configuration at C-2 was assigned as 2*S* configuration.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **11** was identified as (2*S*)-liquiritigenin.[18]

12. Compound 12

The ^{1}H NMR spectrum of this compound were very similar to those of compound **2**. the most difference was aromatic signal at δ_H 5.89 (2H, d, J = 2.2 Hz). The appearance of meta-coupled doublet which is small J value at C-8 and C-6 in the ^{1}H NMR spectrum of **12** indicated meta position in phenyl ring.

According to these combined spectral data, the structure of **12** was determined as (2*S*)-naringenin.[19]

13. Compound 13

The ^{1}H NMR spectrum indicated an isoflavone structure, with an olefinic signal at δ_H 8.00 (1H, s), aromatic signals at δ_H 7.36 (2H, d, J = 8.9 Hz), δ_H 6.84 (2H, d, J = 8.7 Hz), δ_H 6.31 (1H, d, J = 2.3 Hz), δ_H 6.20 (1H, d, J = 2.3, Hz). The appearance of meta-coupled doublet

which is small J value at C-8 and C-6 in the ^{1}H NMR spectrum of **13** indicated meta position in phenyl ring. Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **13** was identified as genistein.[20]

14. Compound 14

The ^{1}H NMR spectrum of this compound were very similar to those of compound **13**. the most difference was aromatic signals at δ_H 8.03 (1H, d, J = 8.8 Hz), δ_H 6.91 (1H, dd, J = 8.8, 2.2 Hz), δ_H 6.81 (1H, d, J = 2.2 Hz). The appearance of meta-coupled doublet which is small J value at C-8 and C-6 in the ^{1}H NMR spectrum of **14** indicated meta position in phenyl ring.

According to this spectral data, the structure of **14** was determined as daidzein.[21]

15. Compound 15

The ^{1}H NMR spectrum of this compound were very similar to those of compound **13**. the most difference characteristic was an methoxy signal at δ_H 3.82 (3H, s).

According to this spectral data, the structure of **15** was determined as 5, 7-dihydroxy-4′-methoxy-isoflavone.[22]

16. Compound 16

The ^{1}H NMR spectrum indicated an flavanone structure, with an oxygenated signal at δ_H 5.46 (1H, dd, J = 13.0, 3.0 Hz), methylene signals at δ_H 3.05 (1H, dd, J = 17.0, 13.0 Hz), δ_H 2.79 (1H, dd, J = 17.0, 3.0 Hz), aromatic signals at δ_H 7.52(2H, d, J = 7.2 Hz), δ_H 6.43

(2H, d, J = 7.2 Hz), δ_H 7.32 (1H, s), δ_H 6.43 (1H, s), methoxy group signal at δ_H 3.87 (1H, s), The appearance of para-singlets at C-8 and C-5 in the ^{1}H NMR spectrum of **16**.

Based upon the results of combined spectroscopic analysis and compari son of the spectral data with the previously reported data, the structur e of compound **16** was identified as (2*S*)-7-hydroxy-6-methoxy-flavan one.[23]

17. Compound 17

The ^{1}H NMR spectrum indicated a phenolic compound, with aromatic signals at δ_H 7.42 (1H, d, J = 2.1 Hz), δ_H 7.38 (1H, dd, J = 8.4, 2.1 Hz), δ_H 6.74 (1H, d, J = 8.4 Hz). ^{13}C NMR data showed the presence of aldehyde signal at δ_C 178.0.

According to these combined spectral data, the structure of **17** was determined as protocatechuic acid.[24]

18. Compound 18

The ^{1}H NMR spectrum indicated a flavan-3-ol structure, with aromatic proton signals at δ_H 6.83 (1H, d, J = 1.8 Hz), δ_H 6.75 (1H, d, J = 8.1 Hz), δ_H 6.71 (1H, d, J = 8.1, 1.8 Hz), δ_H 5.91 (1H, d, J = 2.1 Hz). δ_H 5.84 (1H, d, J = 2.1 Hz) with an oxygenated methine proton signal at δ_H 4.55 (1H, d, J = 7.5 Hz), a methine proton signal at δ_H 3.96 (1H, m), methylene proton signals at δ_H 2.84.

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **18** was identified as (+)-catechin.[25]

19. Compound 19

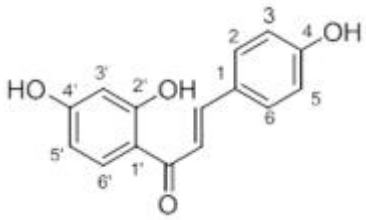
The ^{1}H NMR spectrum indicated a flavan-3-ol structure, with aromatic proton signals at δ_H 6.96 (1H, d, J = 1.9 Hz), δ_H 6.79 (1H, d, J = 8.1 Hz), δ_H 6.75 (1H, d, J = 8.1, 1.8 Hz), δ_H 5.93 (1H, d, J = 2.2 Hz). δ_H 5.91 (1H, d, J = 2.2 Hz) with an oxygenated methine proton signal at δ_H 4.81 (1H, d, J = 1.8 Hz), a methine proton signal at δ_H 4.17 (1H, m), methylene proton signals at δ_H 2.85 (1H, dd, J = 16.4, 7.7 Hz), δ_H 2.73 (1H, dd, J = 16.4, 2.1 Hz).

Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **19** was identified as (-)-epicatechin.[26]

20. Compound 20

The ^{1}H NMR and ^{13}C NMR spectra of this compound provided a complete assignment of the A and B units. It was shown that this compound had an (-)-epicatechin unit as the A unit. because methylene signals δ_H 2.76 and δ_H 2.92 and the carbon signal δ_C 29.2 at C-4″, δ_C 82.3 at C-2″ and δ_C 155.2 at C-5″ of A unit were assigned. It was clear that the interflavanoid bond between the A and B units was 4→8″, because of the correlation between the proton signal δ_H 4.60 at C-4 of the B unit and the carbon signal δ_C 108.9 at C-8″ of the A unit, observed in the HMBC spectrum.

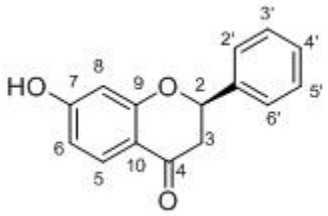
Based upon the results of combined spectroscopic analysis and comparison of the spectral data with the previously reported data, the structure of compound **20** was identified as procyanidin B2.



1

Table 1. ^{1}H and ^{13}C NMR Assignment for compound 1 in MeOD

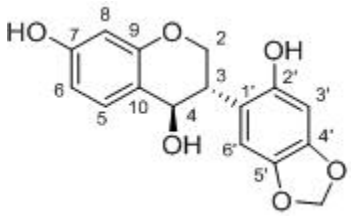
Position	^{1}H	^{13}C
1		129.8
2	7.59, d (8.4)	131.7
3	6.83, d (8.4)	115.9
4		160.3
5	6.83, d (8.4)	115.9
6	7.59, d (8.4)	131.7
1′		112.9
2′		165.8
3′	6.28, d (2.4)	102.6
4′		165.9
5′	6.41, dd (9.0, 2.4)	108.4
6′	7.96, d (9.0)	133.0
α	7.74, d (14.2)	144.1
β	7.58, d (14.2)	117.4
C=O		191.4



2

Table 2. ^{1}H and ^{13}C NMR Assignment for compound 2 in MeOD

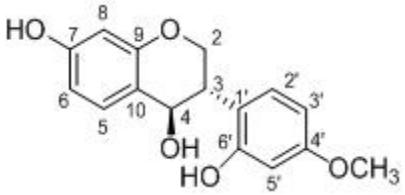
Position	^{1}H	^{13}C
2	5.57, dd (12.7, 3.0)	78.8
3	3.08, dd (16.7, 12.7); 2.70, dd (16.7, 3.0)	43.3
4		189.3
5	7.63, d (8.8)	128.3
6	6.47, dd (8.8, 2.2)	111.1
7		165.6
8	6.31, d (2.2)	102.6
9		163.0
10		112.8
1′		129.2
2′	7.52, d (7.5)	126.5
3′	7.42, d (7.5)	128.5
4′	7.37, m	128.3
5′	7.42, d (7.5)	128.5
6′	7.52, d (7.5)	126.5



3

Table 3. ^{1}H and ^{13}C NMR Assignment for compound 3 in MeOD

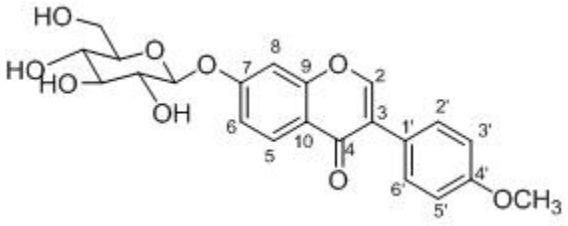
Position	^{1}H	^{13}C
2	4.21, dd (10.7, 4.8); 3.55, d (10.7)	67.4
3	3.49, m	41.6
4	5.44, d (6.8)	80.1
5	7.25, d (8.4)	133.1
6	6.47, dd (8.8, 2.2)	110.8
7		160.4
8	6.29, d (2.5)	104.1
9		158.0
10		112.7
1′		119.9
2′		155.6
3′	6.36, s	94.2
4′		149.5
5′		143.1
6′	6.79, s	105.0
$-OCH_2O-$	5.87, s ; 5.84, s	102.5



4

Table 4. ^{1}H and ^{13}C NMR Assignment for compound 4 in MeOD

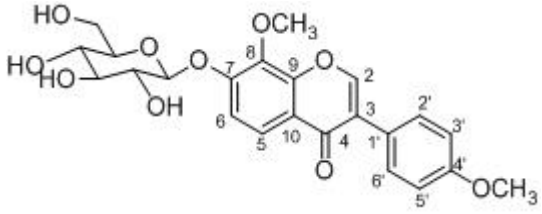
Position	^{1}H	^{13}C
2	4.20, m ; 3.53, m	67.6
3	3.49, m	40.9
4	5.45, d (6.0)	80.1
5	7.28, d (8.4)	133.2
6	6.48, dd (8.3, 2.4)	110.7
7		160.2
8	6.29, d (2.3)	104.1
9		158.1
10		112.9
1′		120.9
2′	7.16, d (8.4)	126.0
3′	6.47, dd (8.3, 2.4)	107.2
4′		162.6
5′	6.37, d (2.3)	97.6
6′		162.0
OCH_3	3.53, s	55.9



5

Table 5. ^{1}H and ^{13}C NMR Assignment for compound 5 in MeOD

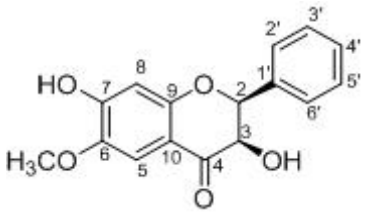
Position	^{1}H	^{13}C
2	8.20, s	155.2
3		126.0
4		178.0
5	8.13, d (8.8)	128.3
6	7.20, dd (8.8, 2.2)	117.1
7		163.5
8	7.23, d (2.2)	105.0
9		159.3
10		120.2
1′		125.8
2′	7.46, d (8.8)	131.4
3′	6.96, d (8.8)	114.9
4′		161.2
5′	6.96, d (8.8)	114.9
6′	7.46, d (8.8)	131.4
OCH_3	3.81, s	55.7
Glc 1	5.09, d(7.2)	101.8
2	3.50, m	74.8
3	3.48, m	77.9
4	3.39, m	71.8
5	3.53, m	78.4
6	3.91, dd (12.1, 2.2); 3.69, dd (12.1, 5.7)	62.5



6

Table 6. ^{1}H and ^{13}C NMR Assignment for compound 6 in MeOD

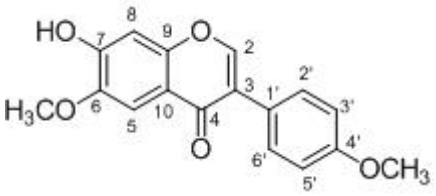
Position	^{1}H	^{13}C
2	8.28, s	155.1
3		125.8
4		178.0
5	7.92, d (9.0)	125.8
6	7.39, d (9.2)	115.7
7		163.8
8		139.0
9		155.9
10		121.2
1′		125.2
2′	7.48, d (9.0)	131.4
3′	6.99, d (9.2)	114.9
4′		161.2
5′	6.99, d (9.2)	114.9
6′	7.48, d (9.0)	131.4
OCH_3 X 2	4.02, s; 3.82, s	55.8, 55.6
Glc 1	5.46, d (7.6)	102.3
2	3.50, m	74.9
3	3.48, m	78.1
4	3.42, m	71.2
5	3.57, m	78.4
6	3.89, dd (12.0, 1.6); 3.71, dd (12.0, 5.4)	62.4



7

Table 7. ^{1}H and ^{13}C NMR Assignment for compound 7 in MeOD

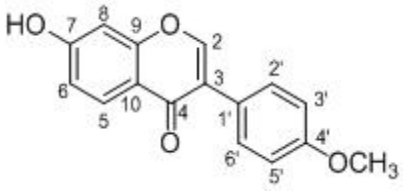
Position	^{1}H	^{13}C
2	5.07, d (11.8)	85.9
3	4.50, d (11.8)	74.7
4		194.2
5	7.28, s	108.1
6		145.8
7		157.7
8	6.39, s	104.6
9		159.9
10		111.7
1′		138.9
2′	7.54, d (8.0)	128.9
3′	7.41, m	129.4
4′	7.39, m	129.8
5′	7.41, m	129.4
6′	7.54, d (8.0)	128.9



8

Table 8. ^{1}H and ^{13}C NMR Assignment for compound 8 in MeOD

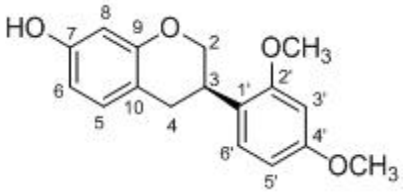
Position	^{1}H	^{13}C
2	8.15, s	154.6
3		125.2
4		177.8
5	7.56, s	105.5
6		148.7
7		155.4
8	6.92, s	104.0
9		154.3
10		117.9
1′		125.8
2′	7.47, d (8.6)	131.5
3′	6.98, d (8.6)	114.9
4′		161.2
5′	6.98, d (8.6)	114.9
6′	7.47, d (8.6)	131.5
OCH_3 X 2	3.95, s; 3.81, s	56.7, 55.7



9

Table 9. ^{1}H and ^{13}C NMR Assignment for compound 9 in MeOD

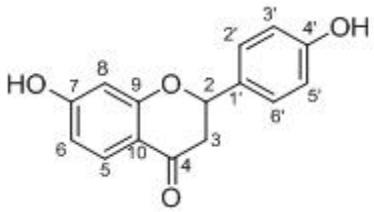
Position	^{1}H	^{13}C
2	8.09, s	153.1
3		126.6
4		174.6
5	8.01, d (9.3)	127.3
6	6.96, dd (9.3, 2.0)	115.5
7		163.3
8	6.90, d (2.0)	102.2
9		157.6
10		116.4
1′		124.3
2′	7.42, d (9.2)	130.1
3′	6.98, d (9.2)	113.7
4′		159.1
5′	6.98, d(9.2)	113.7
6′	7.42, d (9.2)	130.1
OCH_3	3.83, s	55.2



10

Table 10. ^{1}H and ^{13}C NMR Assignment for compound 10 in MeOD

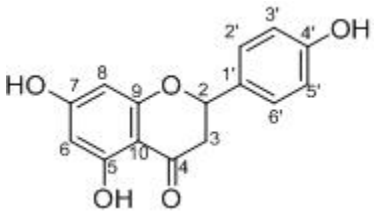
Position	^{1}H	^{13}C
2	4.17, m; 3.92, t (10.0)	31.5
3	3.44, m	33.0
4	2.90, dd (17.0, 10.0); 2.76, dd (17.0. 4.7)	71.1
5	6.85, d (8.6)	131.2
6	6.30, dd (8.2, 2.4)	109.1
7		157.6
8	6.21, d (2.4)	103.8
9		156.4
10		123.1
1′		114.7
2′		159.6
3′	6.53, d (2.3)	99.5
4′		161.3
5′	6.45, dd (8.5, 2.3)	105.7
6′	7.02, (8.6)	128.6
OCH_3 X 2	3.82, s; 3.76, s	55.9, 55.8



11

Table 11. ^{1}H and ^{13}C NMR Assignment for compound 11 in MeOD

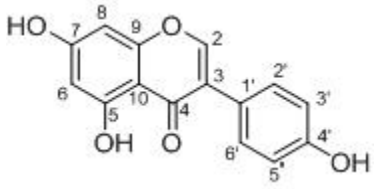
Position	^{1}H	^{13}C
2	5.37, dd (13.0, 2.8)	81.0
3	3.04, m; 2.68, dd (17.0, 3.0)	45.0
4		193.5
5	7.72, d (8.7)	129.8
6	6.48, dd (8.7, 5.3)	111.9
7		165.6
8	6.34, d (2.2)	103.9
9		167.2
10		114.6
1′		131.4
2′	7.31, d (8.5)	129.0
3′	6.80, d(8.5)	116.3
4′		159.0
5′	6.80, d (8.5)	116.3
6′	7.31. d (8.5)	129.0



12

Table 12. ^{1}H and ^{13}C NMR Assignment for compound 12 in MeOD

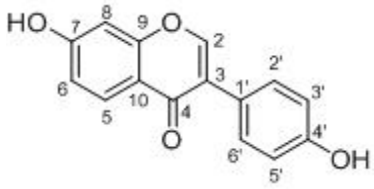
Position	^{1}H	^{13}C
2	5.33, dd (12.9, 2.9)	81.0
3	3.12, m; 2.68, dd (17.0, 3.0)	45.0
4		193.5
5		129.8
6	5.89, d (2.2)	111.9
7		165.6
8	5.89, d (2.2)	103.9
9		167.2
10		114.6
1′		131.4
2′	7.31, d (8.6)	129.0
3′	6.81, d(8.6)	116.3
4′		159.0
5′	6.81, d (8.6)	116.3
6′	7.31. d (8.6)	129.0



13

Table 13. ^{1}H and ^{13}C NMR Assignment for compound 13 in MeOD

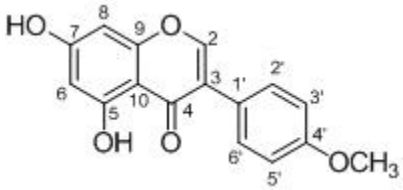
Position	^{1}H	^{13}C
2	8.01, s	154.8
3		123.3
4		182.3
5		159.8
6	6.20, d (2.3)	100.2
7		163.9
8	6.31, d (2.3)	94.8
9		158.8
10		106.3
1′		123.3
2′	7.36, d (8.9)	131.4
3′	6.84, d (8.7)	116.3
4′		159.6
5′	6.84, d (8.7)	116.3
6′	7.36, d (8.9)	131.4



14

Table 14. ^{1}H and ^{13}C NMR Assignment for compound 14 in MeOD

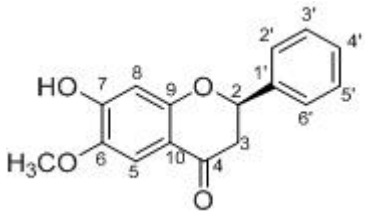
Position	^{1}H	^{13}C
2	8.11, s	152.4
3		122.8
4		178.7
5	8.03, d (8.8)	127.3
6	6.91, dd (8.8, 2.2)	115.1
7		162.3
8	6.81, d (2.2)	102.2
9		157.8
10		116.9
1′		124.0
2′	7.36, d (8.7)	130.0
3′	6.84, d (8.7)	115.2
4′		157.5
5′	6.84, d (8.7)	115.2
6′	7.36, d (8.7)	130.0



15

Table 15. ^{1}H and ^{13}C NMR Assignment for compound 15 in MeOD

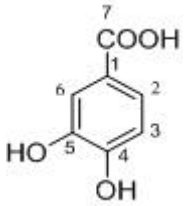
Position	^{1}H	^{13}C
2	8.07, s	155.0
3		124.5
4		182.2
5		163.9
6	6.22, d (2.2)	100.2
7		166.2
8	6.34, d (2.2)	94.9
9		161.2
10		106.3
1′		124.6
2′	7.46, d (8.8)	131.4
3′	6.97, d (8.8)	114.9
4′		159.7
5′	6.97, d (8.8)	114.9
6′	7.46, d (8.8)	131.4
OCH_3	3.82, s	55.8



16

Table 16. ^{1}H and ^{13}C NMR Assignment for compound 16 in MeOD

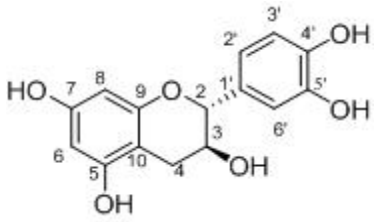
Position	^{1}H	^{13}C
2	5.46, dd (13.0, 3.0)	81.6
3	3.05, dd (17.0, 13.0); 2.79, dd (17.0, 3.0)	45.6
4		193.5
5	7.32, s	108.5
6		146.1
7		161.2
8	6.43, s	105.3
9		158.1
10		114.0
1′		141.4
2′	7.52, d (7.2)	128.3
3′	7.43, m	130.2
4′	7.38, m	130.0
5′	7.43, m	130.2
6′	7.52, d (7.2)	128.3
OCH_3	3.87, s	57.1



17

Table 17. ^{1}H and ^{13}C NMR Assignment for compound 17 in MeOD

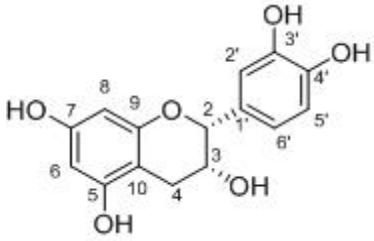
Position	^{1}H	^{13}C
1		127.5
2	7.42, d (2.1)	117.8
3		145.6
4		150.0
5	6.74, d (8.4)	115.4
6	7.38, dd (8.4, 2.1)	123.4
7		173.4



18

Table 18. ^{1}H and ^{13}C NMR Assignment for compound 18 in MeOD

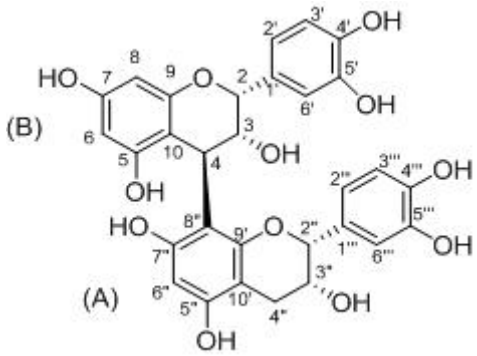
Position	^{1}H	^{13}C
2	4.55, d (7.5)	82.9
3	3.96, m	68.8
4	2.84, dd (16.1, 5.2); 2.49, dd (16.1, 8.1)	28.5
5		157.9
6	5.84, d (2.1)	96.3
7		157.6
8	5.91, d (2.1)	95.5
9		156.9
10		100.8
1′		115.3
2′	6.71, dd (8.1, 1.8)	132.2
3′	6.75, d (8.1)	116.1
4′		146.3
5′		146.2
6′	6.83, d (1.8)	120.0



19

Table 19. ^{1}H and ^{13}C NMR Assignment for compound 19 in MeOD

Position	^{1}H	^{13}C
2	4.81, d (1.8)	78.6
3	4.17, m	65.4
4	2.85, dd (16.4, 7.7); 2.73, dd (16.4, 2.1)	28.8
5		95.6
6	5.91, d (2.2)	94.6
7		157.1
8	5.93, d (2.2)	156.8
9		156.3
10		99.0
1′		131.1
2′	6.96, d (1.9)	118.5
3′		115.4
4′		145.0
5′	6.79, d (8.1)	144.9
6′	6.75, d (8.1, 1.9)	115.3



20

Table 20. ^{1}H and ^{13}C NMR Assignment for B unit of compound 20 in DMSO

Position	^{1}H	^{13}C
2	5.04, br s	78..8
3	3.77, br s	71.9
4	4.60, br s	37.9
5		156.1
6	5.93, d (2.0)	94.8
7		157.9
8	5.92. d (2.0)	97.5
9		100.1
10		154.8
1′		131.6
2′	6.67, dd (8.2, 2.0)	114.3
3′	6.62, d (8.2)	144.6
4′		144.3
5′		115.6
6′	6.81, d (2.0)	119.3

Table 20. ^{1}H and ^{13}C NMR Assignment for A unit of compound 20 in DMSO

Position	^{1}H	^{13}C
2″	4.93, br s	82.3
3″	4.22, m	65.7
4″	2.76, d (17.0), 2.92, d (17.4)	29.2
5″		155.2
6″	5.86, s	96.3
7″		156.6
8″		108.9
9′		96.5
10′		154.8
1‴		131.3
2‴	6.83, dd (8.0, 2.0)	115.3
3‴	6.70, d (8.0)	144.3
4‴		144.5
5‴		115.8
6‴	7.10, d (2.0)	119.5

Discussion

The phytochemical investigation of *Spatholobus suberectus* Dunn, afford a very diverse series of flavonoids, sterols and triterpenes.

Even though several flavonoids such as formononetin, daidzein and genistein have been reported from *Spatholobus suberectus* Dunn, the flavonoid dimer and flavonoid glycosides from this plants are relatively rare.

Fractionation guided by inhibitory activity of sortase A, an enzyme that plays a key role in cell wall protein anchoring and virulence in *Staphylococcus aureus*, the flavonoid-containing fraction of 80% aqueous MeOH, 40% aqueous MeOH exhibited the most potent inhibitory activity. Therefore, isolation of flavonoids from *S. spatholobus* can be good starting candidates for biomedical purposes.

In this study, the isolated compounds were structurally identified to be eighteen of flavonoids and one of phenolic and one of flavonoid dimer. Among the isolated compounds, three flavonoids were isolated for the first time in this plant.

The result of detailed biological activities of these compounds, nine flavonoids showed strong inhibitory activity of sortase A.

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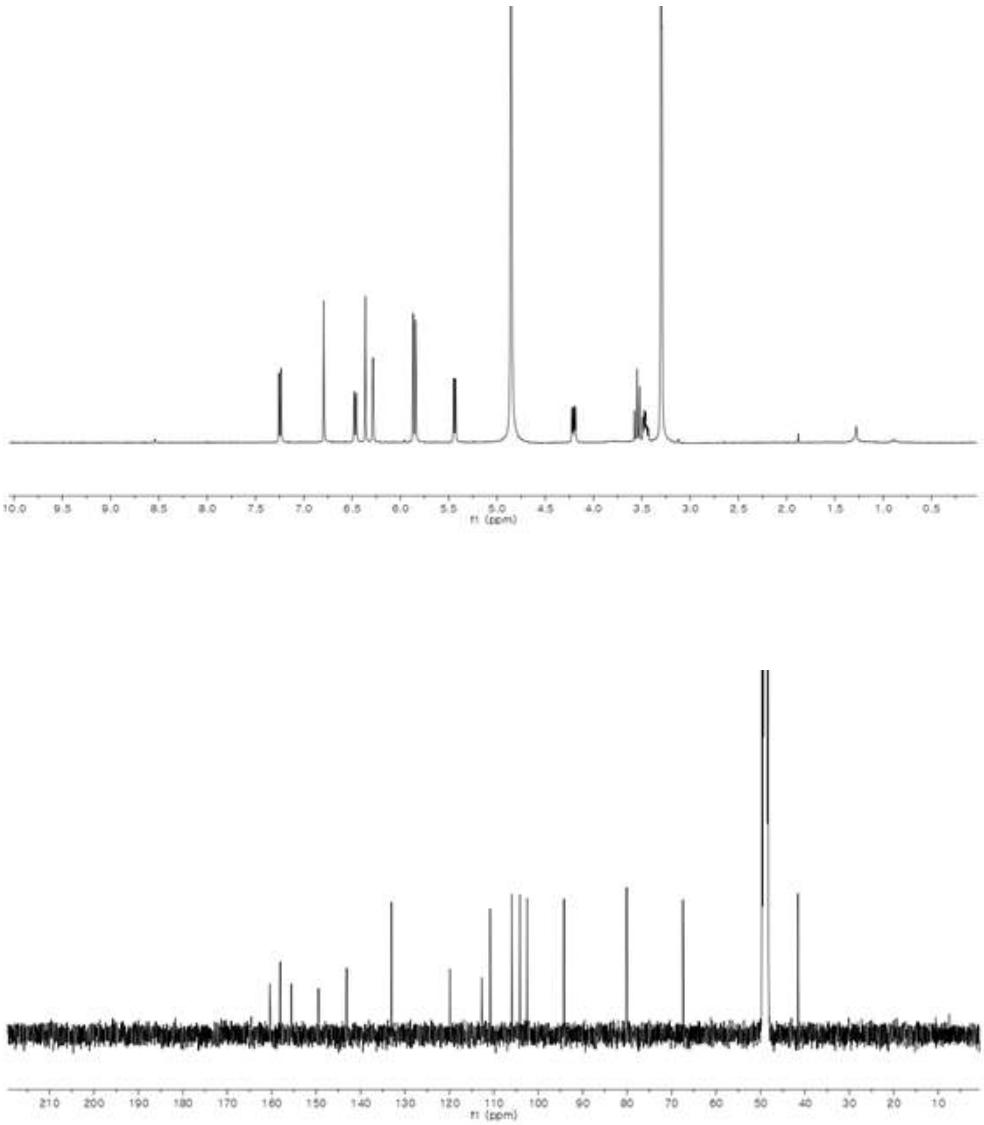

Figure 1. ^{1}H and ^{13}C NMR spectra of Compound 3

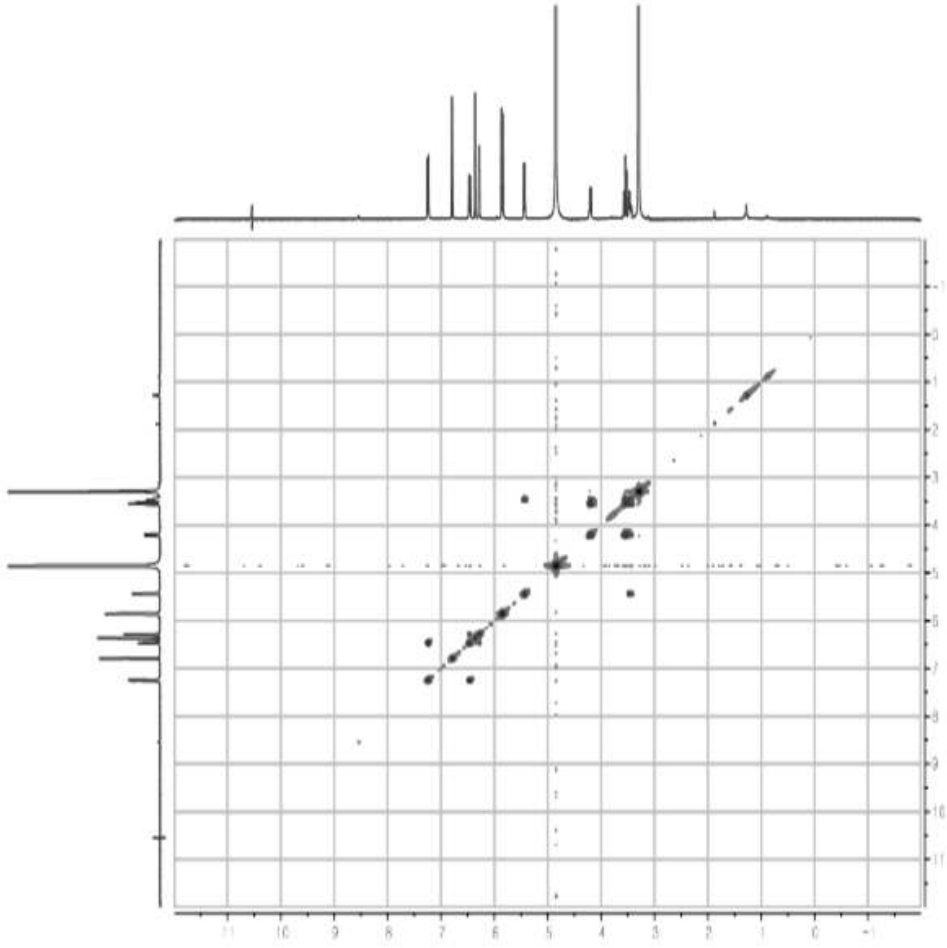

Figure 2. COSY spectrum of Compound 3

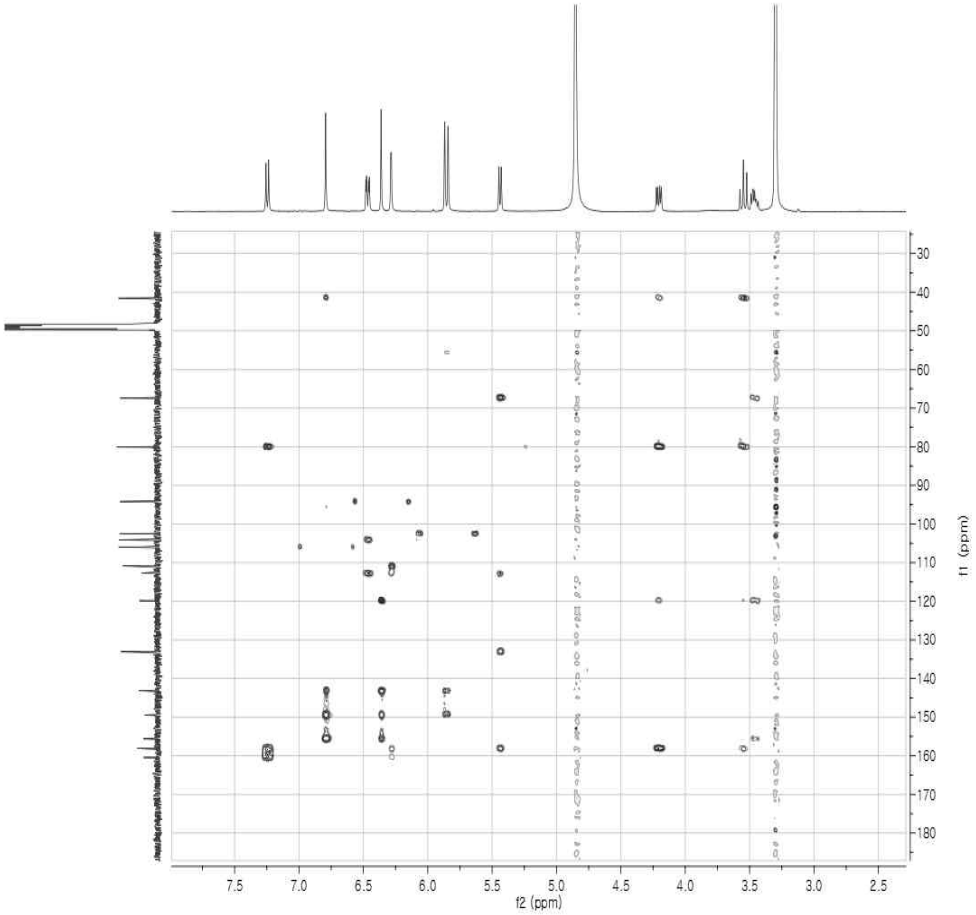

Figure 3. *g*HMBC spectrum of Compound 3

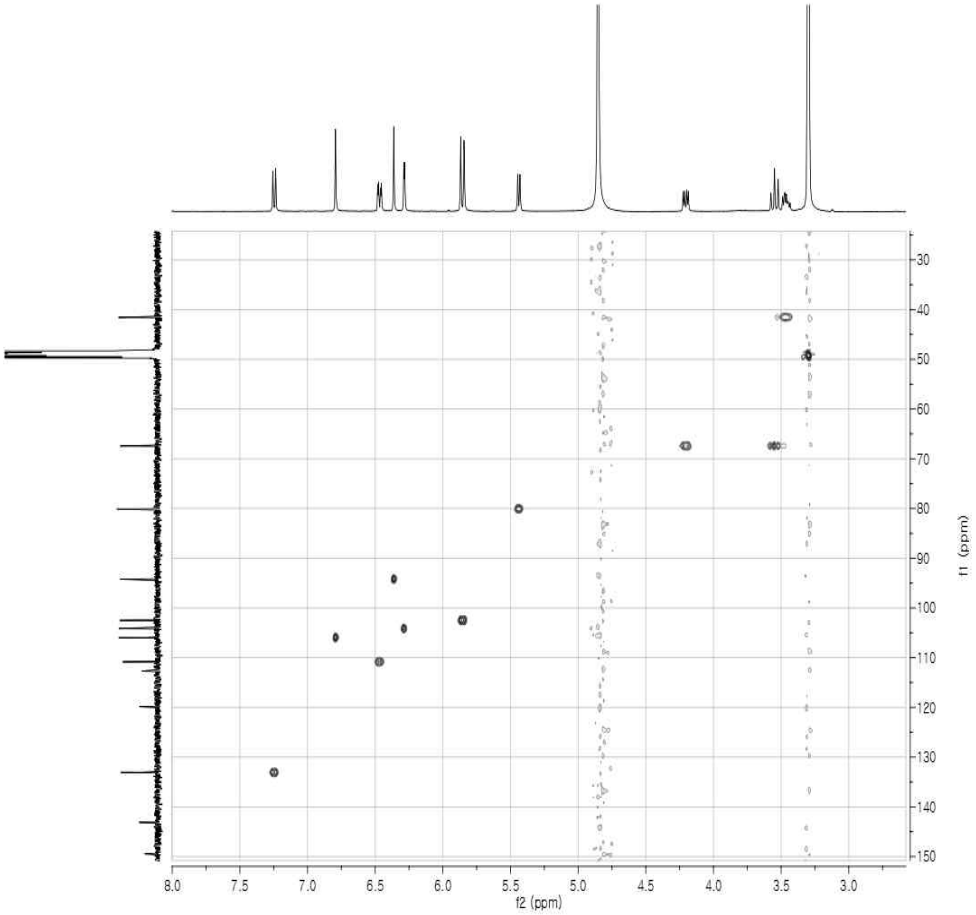

Figure 4. HSQC spectrum of Compound 3

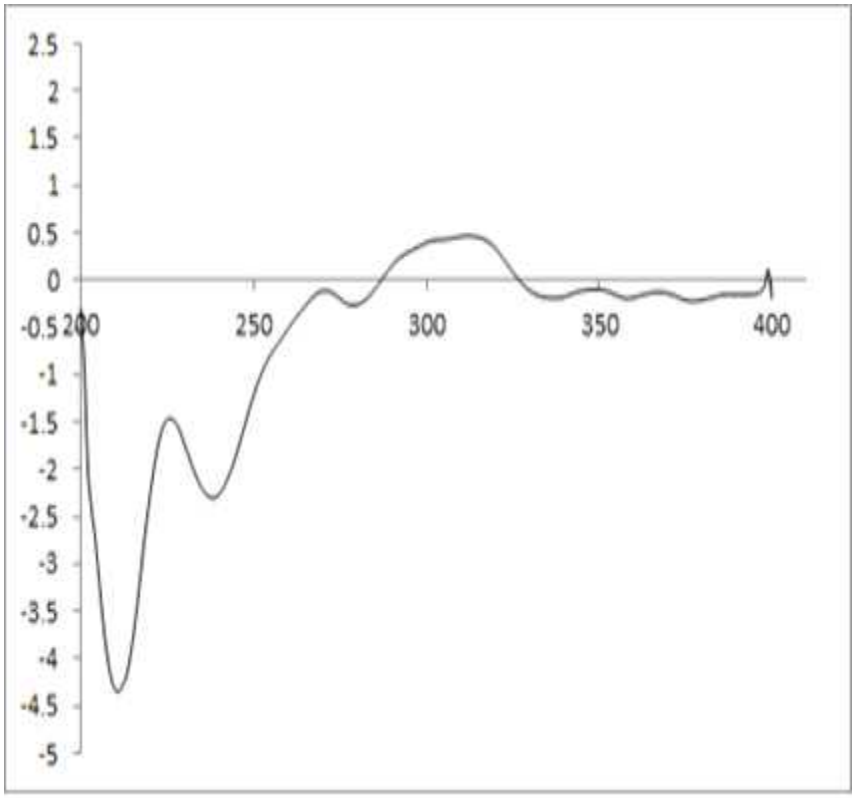

Figure 5. CD spectrum of Compound 3

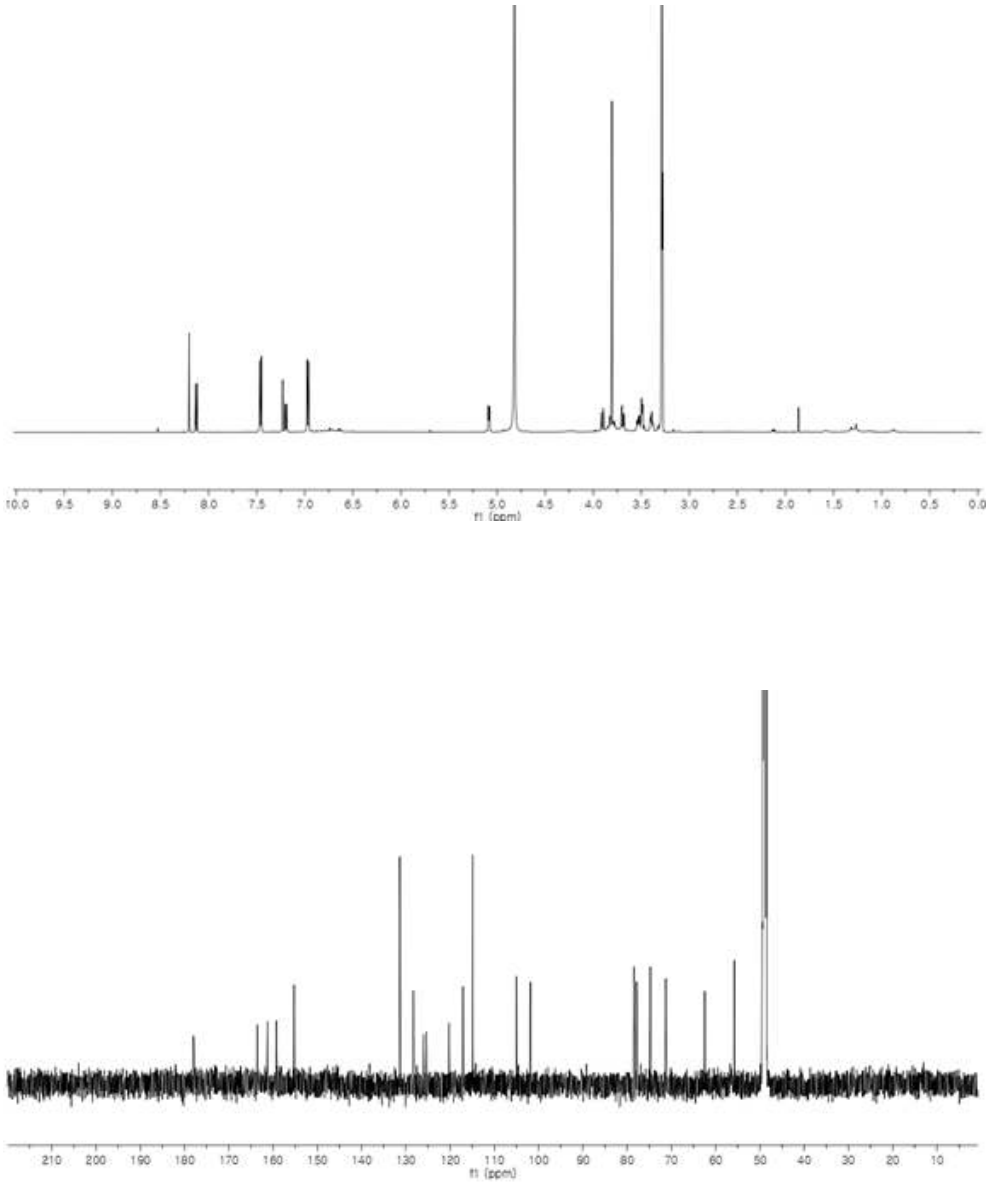

Figure 6. ^{1}H and ^{13}C NMR spectra of Compound 5

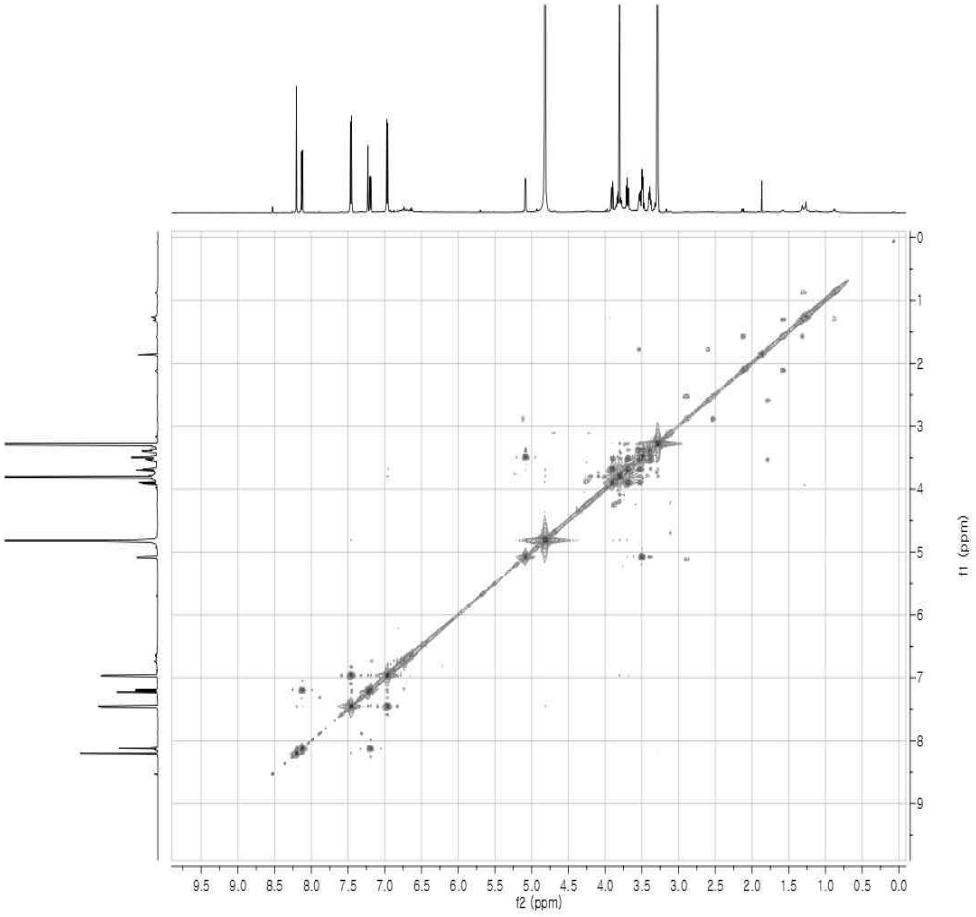

Figure 7. COSY spectrum of Compound 5

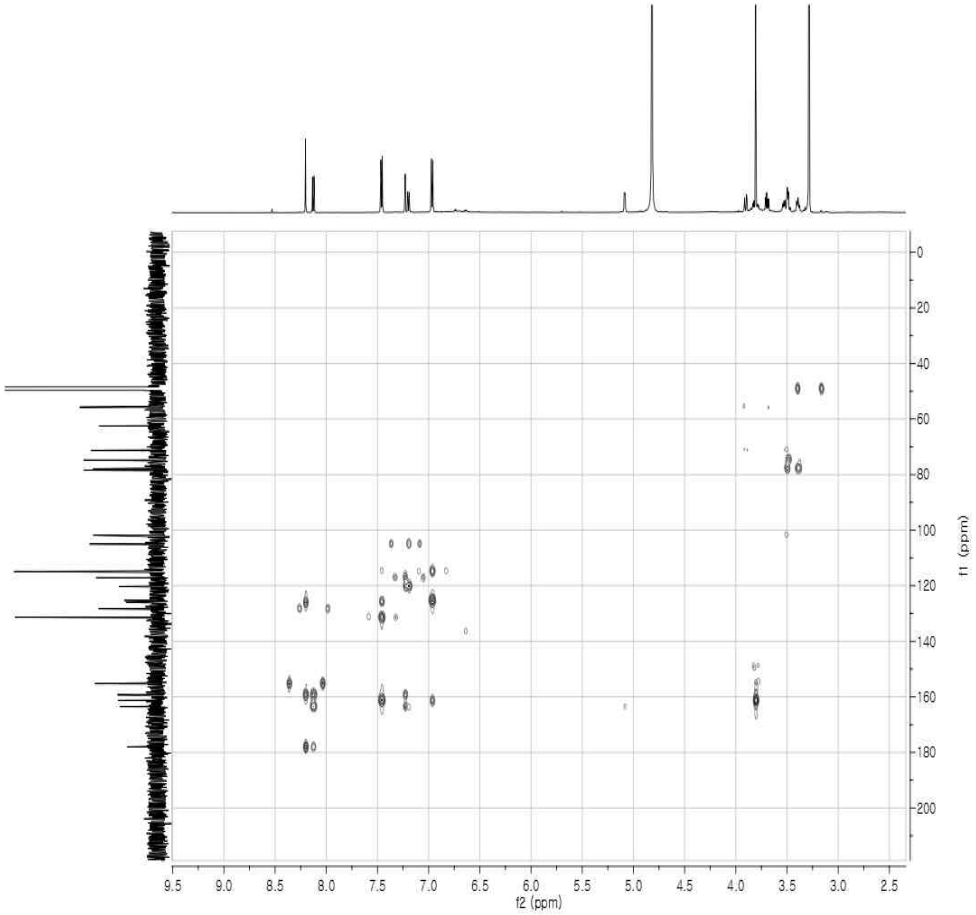

Figure 8. *g*HMBC spectrum of Compound 5

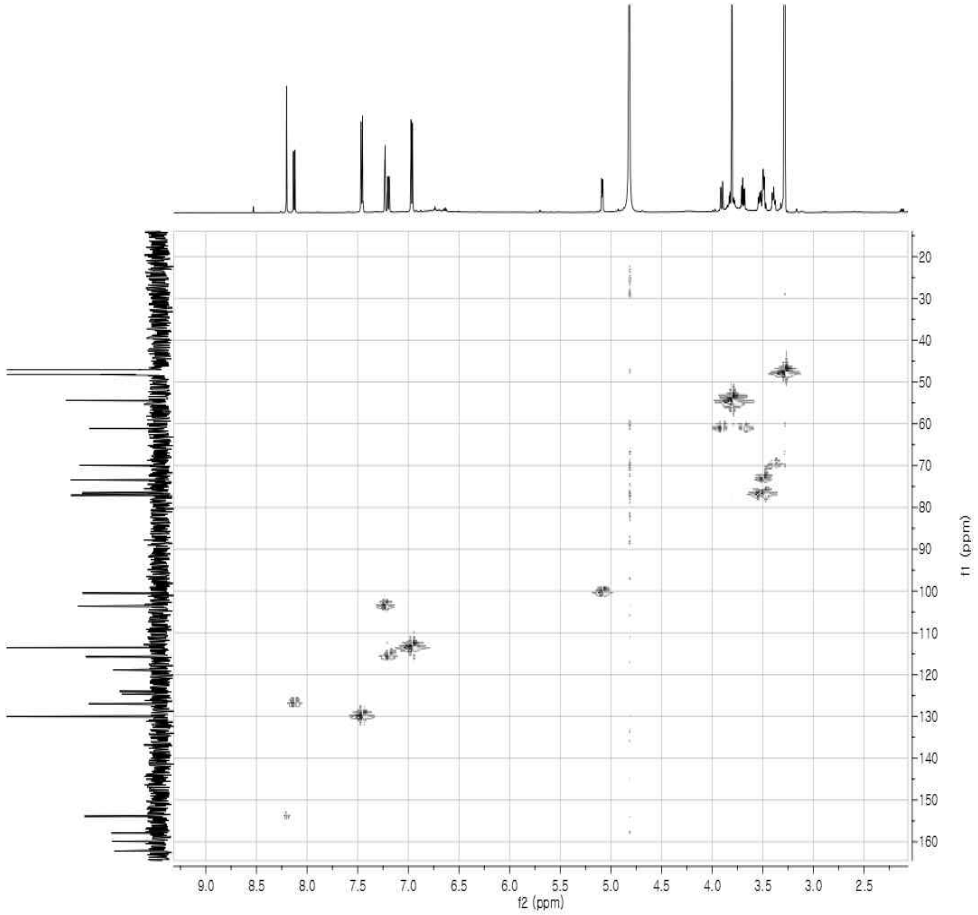

Figure 9. HSQC spectrum of Compound 5

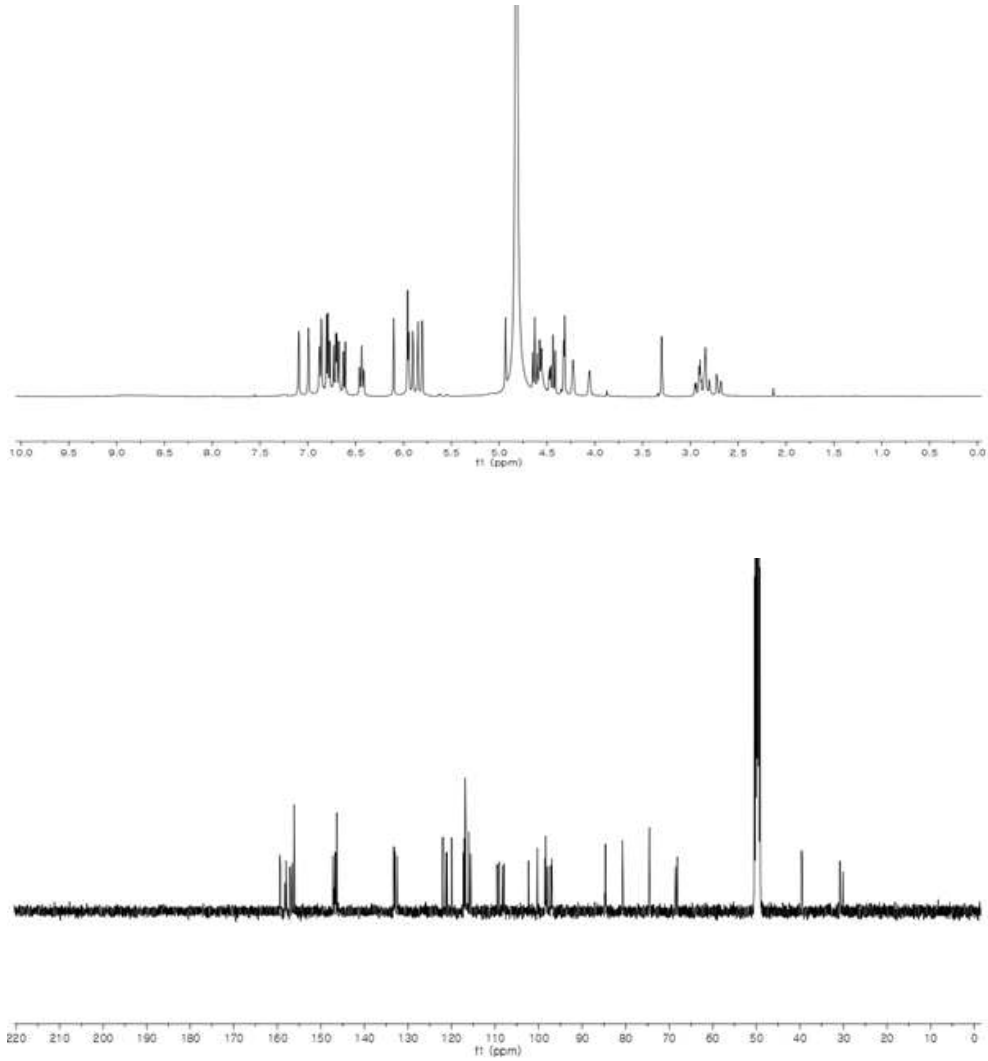

Figure 10. ^{1}H and ^{13}C NMR spectra of Compound 20

국문초록

계혈등의 성분연구

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조 현 주

효소 sortase A에 대해 저해 생리활성을 가지는 천연 물질을 찾고자 다양한 생약 추출물을 평가하였다. 생리활성 검색 결과에 따라 계혈등을 연구대상으로 선택하였는데 이는 혈액순환 개선과 생리통, 빈혈, 마비, 관절통 및 세균 감염에 사용되는 전통 생약이다. Sortase A에 다하여 높은 저해활성을 보이는 계혈등 추출물의 분획에 대하여 다양한 크로마토그래피 분리 기법으로 총 20개의 물질을 분리하였다. 복합적 분광학적 분석의 결과를 토대로 분리된 물질들이 18종의 flavonoids, 1종의 phenolic, 1종의 flavonoid dimer 임을 동정하였다. 이들 중 3종의 flavonoid 계열 물질이 계혈등에서는 처음 발견된 물질임을 확인하였다.

주요어 : 계혈등, flavonoids, sortase A 활성저해.
학번 : 2014-21059