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Article

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Antioxidant and myocardial preservation activities of natural phytochemicals from Mung Bean (*Vigna radiata* L.) seeds

Yan Bai*, Jiawei Chang*, Yan Xu*, Dan Cheng*, Hongxin Liu†, Yunli Zhao*, Zhiguo Yu*

*Department of Pharmaceutical Analysis, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China

†Dalian Weida Pharmacy Co., Limited, 12-7 Zhuhai Street, Xigang District, Dalian 116011, China

ABSTRACT

Mung bean (*Vigna radiata* L.) seeds (MBS) contain abundant nutrients with biological activities. This study was aimed to isolate key bioactive components from MBS with antioxidant and myocardial preservation activities. A new flavonoid *C*-glycoside, isovitexin-6"-*O*-*α*-*L*-glucoside, and fourteen known compounds were obtained. Their structures were identified by extensive 1D and 2D NMR and FT-ICR-MS spectroscopic analyses. The antioxidant activities of these compounds were evaluated. **1-5**, **7-10** displayed $ABTS^{\bullet+}$ scavenging activity, but only **5** and **7** exhibited $DPPH^{\bullet}$ scavenging activity. The myocardial preservation effect of **2**, **3** and MBS were investigated by measuring the serum levels of LDH, CK and AST as well as the tissue level of MDA and SOD. The results demonstrated that **2**, **3** and MBS had a significant protective effect against ISO-induced myocardial ischemia. MBS can be regarded as a potential new source of antioxidants and myocardial preservation agents.

KEYWORDS: mung bean seeds; vitexin; isovitexin; myocardial preservation; antioxidant; functional foods.

INTRODUCTION

Reactive oxygen species (ROS) are free radicals found naturally in the human body, which are well recognised for playing a dual role as both deleterious and beneficial species. Beneficial effects of ROS occur at low/moderate concentrations.[1] When environmental conditions or stress result in excession of ROS, damage to cellular systems and DNA can occur.[2] The damage caused by ROS is linked to several diseases, such as cancer, rheumatoid arthritis, diabetes, neurological disorders, aging, and cardiovascular diseases.[1] Among these, ROS-induced oxidative stress plays a role in various cardiovascular diseases, such as atherosclerosis, congestive heart failure, hypertension, cardiomyopathies, cardiac hypertrophy, and ischemic heart disease.[3] Ischemic heart disease secondary to acute myocardial infarction is a severe health problem in the world, and is a primary cause of morbidity and mortality.[4] The intake of foods rich in antioxidants can result in a lower risk of cardiovascular disease.[5] In recent years, there has been increasing interest in identifying new sources of natural antioxidants and other health-promoting compounds.[6] Many natural bioactive constituents, including flavones from vegetables, fruits, or grains, have been used experimentally as effective protection against ROS or myocardial ischemic injury.[7]

Mung bean, the seeds of *Vigna radiata* L. (MBS), are popular food legumes in China, India, Korea, Japan, and other parts of Southeast Asian countries. It is well known for its detoxification activities and is used to refresh mentality, alleviate heat stroke, and reduce swelling in the summer.[8] The seeds and sprouts of mung beans are widely used as a fresh salad vegetable or common food in India, Bangladesh, Southeast Asia, and western countries. In China, they not only have been widely consumed as a food, in the form of soup, cake, or ice cream, but also used as a cosmetic, such as mung bean mask. As a food, MBS contain abundant nutrients with

biological activities, including proteins, phenolic acids, fatty acid, and minerals.[9] In recent years, the sprouts of mung beans have more bioactive components, including flavonoids, phenolic acids, organic acids, amino acids, carbohydrates, and lipids.[10, 11] Interest in MBS as a functional food ingredient is increasing for its physiological functionalities, such as antiinflammatory,[12] antibacterial,[13] antitumor,[14] hypolipidemic,[15] antidiabetic,[16] detoxication,[17] hepatoprotective effect,[18] and antioxidant activity.[19, 20] However, until now, reports of antioxidant activity in MBS extract focus on two active components, vitexin and isovitexin.[20] Besides these findings, there is no literature published on a systematic phytochemical study of MBS. Accordingly, the active ingredients in the management of myocardial health from MBS are not yet clear.

This contribution investigates the phytochemical profiles of MBS and studies isolated key bioactive components from MBS with antioxidant and myocardial preservation activities firstly. These results suggest that bioactive components from MBS are new sources of natural active compounds that can be administered to prevent myocardial ischemic.

MATERIALS AND METHODS

Experimental animals. Healthy male Sprague-Dawley rats weighing 200-220 g, 7-8 weeks old, obtained from the Central Animal House of Shenyang Pharmaceutical University (Shenyang, China) were used in this study. They were housed (three rats/cage) in polypropylene cages (47 × 34 × 20 cm) lined with husk, renewed every 24 h under a 12:12 h light and dark cycle at around 22 °C. The rats had free access to standard laboratory water and food. Animal experiments were carried out according to the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University and approved by the Animal Ethics Committee of this institution.

Chemicals and Reagents. All of the solvents (analytical grade) for the extraction were purchased from Qingdao Haiyang Chemical Co., Ltd (Qingdao, China). Methanol and glacial acetic acid (chromatography grade) were purchased from Concord Chemical Reagents Co. (Tianjin, China). The water used during HPLC analysis and for sample preparation was obtained from Wahaha Group Co., Ltd. (Hangzhou, China). DPPH (2, 2-diphenyl-1-picrylhydrazyl, purity ≥ 98.0%), 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), lactate dehydrogenase (LDH), creatine kinase (CK), aspartate aminotransferase (AST), methane dicarboxylic aldehyde (MDA), and superoxide dismutase (SOD) test kits were obtained from the Jiancheng Institute of Biotechnology (Nanjing, China).

Instrumentation. ^{1}H NMR (400 MHz and 600 MHz), ^{13}C NMR (100 MHz and 150 MHz) and 2D NMR were acquired on Bruker-ARX-400 and Bruker-AV-600 NMR spectrometers with TMS as an internal standard. FT-ICR-MS spectra were obtained on a Bruker Solarix 7.0T FT-ICR-MS system. Semipreparative HPLC separations were carried out on a LC-10AT instrument with an SPD-10A VP UV/vis detector using YMC-Pack-ODS-A column (250 × 10 mm, 5 μm) at 210 nm. The UPLC-MS/MS analysis was carried out on an Acquity UPLC I-Class System (Waters Corp., Milford, MA, USA) combined with a Xevo TQ-S mass spectrometer (Waters Corp., Milford, MA, USA). The compositions of samples were analyzed by a Thermo Hypersil GOLD C18 column (2.1 × 50 mm, 1.9 μm). GC was performed in an Agilent 7890A gas chromatograph system (Agilent technologies, Inc., Santa Clara, CA, USA) coupled to a flame ionization detector (FID) by use of a HP-5 capillary column (30 m × 0.32 mm × 0.25 μM). Antioxidant and myocardial preservation activities were measured by a microplate reader (imark, BIO-RAD, USA). Column chromatography was performed using silica gel (100-200 and 200-300 mesh; Qingdao Ocean

Chemical Co. Ltd., Qingdao, China), C18 reversed-phase silica gel (50 μm; YMC Co. Ltd., Kyoto, Japan), and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden). TLC plates were precoated with silica gel GF254 (Qingdao Ocean Chemical Co. Ltd., Qingdao, China).

Plant Material. Mung bean (*Vigna radiata* L.) seeds in Jilin Province of China were purchased from Carrefour supermarket in Shenyang, Liaoning, China in March, 2014. A voucher specimen (No. 20140801) has been deposited in the Herbarium of Shenyang Pharmaceutical University, Liaoning, China.

Extraction and Isolation. MBS (50 kg) were extracted three times with 95% EtOH (3 × 8 L) under reflux (2 h each). After concentrating the extract solutions under reduced pressure at 45 °C, the crude residue was suspended in water (1.5 L), which was successively partitioned with petroleum ether (3 × 1.5 L), dichloromethane (3 × 1.5 L), and *n*-butanol (3 × 1.5 L) to obtain petroleum ether, dichloromethane, and *n*-butanol fractions. The *n*-butanol fraction (63.2 g) was chromatographed over a silica gel column (80 × 650 mm, 386 g) by use of a gradient system of increasing polarity with CH_2Cl_2-MeOH (from 100:0 to 1:1, *v*/*v*) to afford five fractions (A-E), and compounds **2** (8.2 g), **6** (108 mg), **9** (1.6 g), **13** (28 mg), and **15** (159 mg). The fraction A (3.1 g) was subjected to a Sephadex LH-20 (25 × 1300 mm) eluting with MeOH to afford two fractions: frs. A_1 to A_2. Fraction A_1 was purified by preparative TLC, eluting with PE-Acetone-HAc (400:50:1, *v*/*v*/*v*) to provide compounds **10** (37 mg) and **11** (14 mg). Fr. A_2 was passed over an ODS column (50 × 500 mm, 60 g) eluting with MeOH-H_2O (from 1:4 to 1:1, *v*/*v*) to afford three fractions: frs. A_{2a} to A_{2c}. Fractions A_{2a} to A_{2c} were purified by preparative TLC, eluting with CH_2Cl_2-MeOH (30:1, *v*/*v*) to obtain **7** (19 mg), **12** (6 mg), and **14** (29 mg), respectively. The fraction D (7.5 g) was subjected to an ODS column (50 × 500 mm, 60 g) eluting with MeOH-

H_2O (from 1:9 to 1:1, *v*/*v*) to obtain fraction D_1 and to yield **3** (4.2 g). Fraction D_1 was purified by preparative TLC, eluting with CH_2Cl_2-MeOH-HAc (250:50:1, *v*/*v*/*v*) to give **5** (7 mg). The fraction E (6.8 g) was subjected to a Sephadex LH-20 (25 × 1300 mm) with MeOH to afford two fractions: frs. E_1 to E_2. Fraction E_1 was purified by preparative TLC, eluting with CH_2Cl_2-MeOH-HAc (150:50:1, *v*/*v*/*v*) to give **1** (253 mg), and **4** (14 mg). Fraction E_2 was separated by preparative HPLC (MeOH/H_2O, 45:55) to afford **8** (85 mg, t_R 19.2 min).

Isovitexin-6"-*O*-*α*-*D*-glucoside (**1**): yellow oil; UV (MeOH) λ_{max} (log ε) 334 (0.59) and 271 (0.51); IR (KBr) ν_{max} 3409, 2924, 1653, 1510, 1355, 1020, 835, and 780 cm^{-1}. FT-ICR-MS *m*/*z*: 593.151194 $[M-H]^+$ ($C_{27}H_{29}O_{15}$, calculated for 593.150365). For ^1H and ^{13}C NMR, see Table 1.

Acid Hydrolysis of Compound 1. Compound **1** (2 mg) was hydrolyzed with 2 M HCl (2 mL) at 90°C for 3 h. After cooling, the mixture was extracted with EtOAc (3 × 3.0 mL). The EtOAc layer and the aqueous layer were then evaporated under vacuum to give residues. The solutions were examined by TLC and compared with authentic samples. Flavone was detected in the EtOAc layer solution and glucose was detected in the aqueous layer solution. The remaining sugar residue was dissolved in anhydrous pyridine (2 mL) and mixed with *L*-cysteine methyl ester hydrochloride (2 mg). The mixture was kept at 60 °C for 3 h, and 0.5 mL of trimethylsilyimidazole (TMSI) was added. The reactant was then cooled to ambient temperature and partitioned between *n*-hexane and H_2O (2 mL each). The *n*-hexane fraction was analysed by GC under the following conditions: column (HP-5, 30 m × 0.32 mm × 0.25 μm, Agilent); detection (FID); detector temperature (280 °C); injection temperature (270 °C); injection volume (1 mL); column temperature (120 to 280 °C at the rate of 8 °C/min); carrier gas (N_2, 1.4 mL/min). The peaks of authentic samples of

160 *D*-glucose and *L*-glucose after treatment in the same manner were detected at 20.25
161 min and 21.48 min, respectively.

162 **UPLC-MS/MS Analysis.** The mass spectrometry was operated in positive scan mode.
163 The mobile phase was composed of methanol-0.1% formic acid in water (28:72, *v*/ *v*).
164 The total running time was 5 min with a flow rate of 0.3 mL/min. The main working
165 parameters were set as follows: cone voltage, 30 V; the desolvation temperature, 350
166 °C; ion source temperature, 150 °C; gas flow (N_2), 700 L/h.

167 **Assay for ABTS Radical Scavenging Activity.** The ABTS 2, 2′-azinobis (3-
168 ethylbenzothiazoline-6-sulphonic acid) diammonium salt assay was carried out
169 according to the method of Cai et al.[21] with slight modifications. The ABTS radical
170 cation solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium
171 persulphate. The mixture was incubated in darkness at room temperature for 12 h
172 before use, and the radical was stable in this form for more than 2 days when stored in
173 the dark at room temperature. The ABTS cation radical solution was then diluted with
174 water to obtain an absorbance of 0.70 ± 0.02 at 734 nm. One hundred microlitres of
175 test sample with various concentrations were added to 3.0 mL of diluted
176 $ABTS^{\bullet+}$ solution and mixed vigorously, incubated in darkness for 6 min at room
177 temperature and the absorbance was measured at 734 nm. *L*-Ascorbic acid was used
178 as the positive control. The negative control used 100 μL ethanol instead of the
179 sample. The test was carried out in triplicate and the results were mean values. The
180 $ABTS^{\bullet+}$ scavenging effect was calculated as follows: $ABTS^{\bullet+}$ scavenging effect (%) =
181 $[1 - (S-S_b)/(C-C_b)] \times 100\%$, where S, S_b, C, and C_b are the absorbances of the sample,
182 blank sample, negative control, and blank control, respectively.

183 **Assay for DPPH Radical Scavenging Activity.** The DPPH free radicals scavenging
184 activity of fifteen compounds was determined by the method mentioned by Hatano[22]

with slight modifications. One hundred microlitres of different concentrations of the ethanol solution of the sample (the negative control used 100 μL ethanol instead of the sample) was added to 96-wells, followed by 100 μL of 0.15 mM DPPH. The mixtures were incubated for 30 min in darkness at room temperature. The reduction of the DPPH radical was determined by measuring the absorbance at 517 nm with a microplate reader (imark, BIO-RAD, USA). *L*-Ascorbic acid was used as the positive control. The test was carried out in triplicate and the results were mean values. The percentage of DPPH free radical scavenging activity was calculated using the same formula as for the ABTS assay.

Myocardial Preservation Experimental Design. The animals were grouped into five groups of six rats each, including a normal control, a isoproterenol (ISO) model control, a vitexin group (VG), an isovitexin group (IVG), and a mung bean seeds extract group (MBSG). Rats in the VG and the IVG were given an oral dose of vitexin and isovitexin saline solution at dosages of 6 mg/kg body weight. Rats in the MBSG were given an oral dose of 750 mg/kg (6.75 mg for **2** and 11.25 mg for **3**) saline solution. Rats in the normal control and ISO model control groups were given equal volumes saline (5 mL/kg). Then, all of the rats, except for the normal control group were injected subcutaneously with ISO (6 mg/kg body weight) at an interval of 24 h for 2 days to induce myocardial infarction (MI) (on 6th and 7th day).[23] All treatments were performed once daily for 7 consecutive days. Two hours after the second dose of ISO injection (on 7th day), all of the rats were anesthetized with diethyl ether. Blood was collected in dry tubes without anticoagulant. The blood samples were centrifuged at 10000 × g for 10 min at 4 °C to obtain the serum. The serum samples were stored at -80 °C until required for the assay of LDH, CK, and AST. All of the rats were sacrificed by cervical decapitation. Heart tissues were excised immediately and rinsed

in ice-chilled saline. The homogenate was centrifuged at 10000 × g for 10 min and the supernatant was collected for determination of MDA content and SOD activity. A portion of the heart was retained for histopathologic observation.

Biochemical Analyses. LDH, CK, AST, MDA, and SOD in heart tissues were measured by the detection kits according to the manufacturer's instructions (Nanjing Jiancheng Institute of Biotechnology, Nanjing, China).

Histopathological Examination. Portions of the heart tissues were fixed in 10% formalin. After the proper dehydration, the tissues were embedded in paraffin wax. Sections (5 μm) were prepared and stained with hematoxylin and eosin.

Statistical Analysis. The compound content of MBS and antioxidant activities were repeated in triplicate and data were analyzed by an ANOVA test with SPSS 17.0 (Statistical Program for Social Sciences, SPSS Inc., Chicago, IL, USA). All statistical comparisons were made by means of a one-way ANOVA test followed by Dunett's t-test. All of the results were expressed as mean ± standard deviation (SD). P values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow oil; FT-ICR-MS m/z: 593.151194 $[M-H]^+$ ($C_{27}H_{29}O_{15}$, calculated for 593.150365). Its UV spectrum showed absorbances at λ_{max} 271 and 334 nm, and the IR spectrum showed absorption bands for hydroxyl (3409 cm^{-1}) and carbonyl (1653 cm^{-1}) groups. Its ^{1}H-NMR (600 MHz, DMSO-d_6) spectrum showed signals for two phenolic hydroxyl δ_H 13.52 and 10.35, two aromatic protons signals at δ_H 6.75 (1H, s) and 6.48 (1H, s), and an AA′BB′ system at δ_H 7.90 (2H, d, J = 8.6 Hz) and 6.92 (2H, d, J = 8.6 Hz) of ring B, which indicated it was a flavone, with apigenin as the aglycone. The proton signals at δ_H 4.57 (1H, d, J = 9.8 Hz) and 4.72 (1H, d, J = 3.5 Hz) were assigned to a β-glucopyranosyl and α-glucopyranosyl,

respectively. The ^{13}C NMR spectrum of **1** (Table 1) also suggested the presence of *β*-glucopyranosyl and *α*-glucopyranosyl residues. The chemical shift value of C-1″ (δ_c 73.3 ppm) suggested that **1** was a *C*-glycoside. In addition, the (1→6) glycosidic bond of the outside glucose to the inner glucose was characterized by the HMBC correlations of δ 3.60 and 3.70 (H-6″) to δ 98.6 (C-1‴). The linkage position of the glucose chain to flavone nucleus was determined at C-6 from the HMBC correlations of δ 4.57 (H-1″) to δ 160.6 (C-5), 108.8 (C-6) and 163.4 (C-7) (Fig. 2). After acid hydrolysis, isovitexin was detected in the EtOAc solution (Isovitexin was a flavonoid *C*-glycoside, which was hard to be hydrolyzed under acid condition). The *L*-configuration of the glucopyranosyl moiety was determined by acid hydrolysis followed by derivatization and GC analysis. The structure of compound **1** was therefore characterized as a new flavone *C*-glycoside and named isovitexin-6"-*O*-*α*-*L*-glucoside (Fig. 1).

Fourteen known compounds (Fig. 1) were identified as vitexin (**2**),[24] isovitexin (**3**),[25] dulcinoside (**4**),[26] (2*R*, 3*R*)-taxifolin (**5**),[27] scopolin (**6**),[28] *p*-coumaric acid (**7**),[29] *L*-tryptophan (**8**),[30] *D*-3-*O*-methyl inositol (**9**),[31] muconic acid (**10**),[32] benzoic acid (**11**),[33] 2H-1, 3-thiazine-2, 4 (3H)-dione (**12**),[34] nicotinic acid (**13**),[35] nicotinamide (**14**),[36] and methyl-*α*-*D*-glucoside (**15**).[37]

Quantitation is the key issue for quality control of functional foods. In this study, content analysis was conducted to determine the content of **1-4, 8,** and **13** in 95% EtOH extract by UPLC-MS/MS analysis. Product ion scan spectra and representative multiple-reaction monitoring (MRM) chromatograms of the six analytes are shown in Fig. 3. The contents of the six analytes exhibited remarkable differences. Among the analytes, the content of **3** with 5.32 mg/g was the highest. The second one is **2** with 2.97 mg/g. In the remaining analytes, **8** showed a content of 2.29 mg/g, followed by **1**

with 1.70 mg/g, **13** with 0.38 mg/g, while **4** showed the lowest content with 0.13 mg/g (Table 2). Given these results, **2** and **3** were the major secondary metabolite components in MBS.

In the present study, the $ABTS^{\bullet+}$ scavenging assay and $DPPH^{\bullet}$ scavenging assay were performed to study the antioxidant properties of MBS extract and isolated compounds. As shown in Table 3, **1**-**5**, **7**-**10** demonstrated $ABTS^{\bullet+}$ scavenging activity with EC_{50} values ranging from 2.21 to 71.12 μM. Significantly, **3**-**5**, **7**, **8**, and **10** exhibited significant $ABTS^{\bullet+}$ scavenging activity with EC_{50} values less than 10 μM, which were more potent than the positive control, *L*-ascorbic acid (EC_{50} = 11.06 μM). However, most of isolates (EC_{50} > 100 μM) were found to be less active than the positive control *L*-ascorbic acid (EC_{50} = 21.55 μM) in the $DPPH^{\bullet}$ scavenging assay except for **5** (EC_{50} = 17.32 μM) and **7** (EC_{50} = 19.33 μM). This finding is in accordance with the knowledge that the compounds with more phenolic hydroxyl groups show stronger radical scavenging activity.[38] Significant differences were observed between $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging assays. The antioxidant capacity detected by $ABTS^{\bullet+}$ scavenging assay was markedly higher than by $DPPH^{\bullet}$ scavenging assay for fruits, vegetables, and beverages,[29] which is in agreement with our results.

Vitexin (**2**) has a reported myocardial preservation effect.[7] Thus, the question arises, whether isovitexin (**3**) and extract from MBS could have a similar effect. LDH, CK, and AST are major metabolic enzymes in the heart and can be released into the serum during cardiac damage. As shown in Fig. 4, serum LDH, CK, and AST levels increased quickly in the model control group (P < 0.01), indicating that the ISO-induced myocardial ischemia model in rats was successfully established. Vitexin, isovitexin, or MBS extract preconditioning showed a significant protective effect on

the ISO-induced myocardial ischemia by markedly preventing any elevation in the serum levels of LDH, CK, and AST ($P < 0.01$). However, both VG and IVG showed a better protective effect than MBSG ($P < 0.05$). The MDA levels and SOD activities in heart tissues are shown in Fig. 4. Compared with the levels in the normal control group, there was a significant increase in the MDA level and a marked reduction in SOD activity in the ISO-induced myocardial ischemia model group ($P < 0.01$). The administration of vitexin, isovitexin or MBS extract resulted in a significant decrease in the level of MDA and a marked increase in the level of SOD, compared with the ISO-induced myocardial ischemia model group ($P < 0.01$). The changed trend of MDA and SOD is in accordance with the serum LDH, CK, and AST levels.

Obvious morphological changes were observed in the heart cells of the myocardial ischemia rats. As shown in Fig. 5, in the hearts of the normal rats, cells had clear borders with intact cytoplasm and prominent nuclei. In addition, no inflammatory cell infiltrates were observed (Fig. 5A). In contrast, changes in the features of the myocardium appeared after injection of ISO. Cardiac interstitial edema and infiltration of inflammatory cells were observed in the ISO-induced myocardial ischemia model group (Fig. 5B). After administration of vitexin or isovitexin, cardiac interstitial edema diminished and less infiltration of inflammatory cells was observed (Fig. 5C and D). Rats fed with MBS extract exhibited still less cardiac interstitial edema and infiltration of inflammatory cells, but the histopathologic changes were markedly improved compared with ISO-induced myocardial ischemia model group (Fig. 5E). These data suggest a myocardial preservation effect of MBS and vitexin as well as isovitexin appear to be the major compounds responsible for the effect. Administertion of either vitexin or isovitexin alone showed much better effects on myocardial preservation than treatment with MBS extract. Isovitexin-6"-*O*-*α*-*L*-

glucoside (**1**) would be hydrolyzed *in vivo* to produce the isovitexin (**3**). Thus, **1** may has effect on myocardial preservation as well, which should be further studied.

The correlation between antioxidant and cardioproctetive activities of flavone *C*-glycosides has been reported. Antioxidant activity of orientin and isoorientin (two flavone *C*-glycosides) appears to reduce the *L*-NAME induced damage in rats to make *Lagenaria siceraria* fruit possess antihypertensive and cardioprotective activity.[39] Therefore, the antioxidant property of vitexin and isovitexin may contribute to the myocardial preservation effect. Further studies should be carried out to assess the link between antioxidant activity and myocardial preservation action of vitexin and isovitexin.

In conclusion, we report here the structures of 15 compounds isolated from MBS, as well as their *in vitro* antioxidant activities for the first time. **1**-**5**, **7**-**10** displayed $ABTS^{\bullet+}$ scavenging activity, and only **5** and **7** exhibited $DPPH^{\bullet}$ scavenging activity. By UPLC-MS/MS analysis, **2** and **3** were the major secondary metabolite components in the MBS extract. In the investigation of the myocardial preservation effect, **2**, **3**, and MBS extract showed significantly decreased the activities of LDH, CK, and AST of the myocardial ischemic, MDA contents decreased and the activity of SOD increased in the hearts of ISO-induced myocardial ischemic rats. In addition, histopathologic changes induced by ISO were markedly improved by **2**, **3**, and MBS extract. **2** and **3** are the major constituents responsible for the myocardial preservation effect in MBS. This finding is in accordance with the knowledge that many natural bioactive constituents have protection effect against myocardial ischemic injury. The results obtained in our study provide a potential justification for the use of the seeds from *Vigna radiata* L. industrial byproducts as a valuable source of raw material for new antioxidant and myocardial preservation agents.

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website at DOI:

UV, IR, NMR, and FT-ICR-MS data of compound **1**. (PDF)

AUTHOR INFORMATION

Corresponding Author

*(Z.-G.Y.) Mail: School of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang Liaoning 110016, China. Phone: +86-24-23986295.

E-mail: zhiguo-yu@163.com.

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Notes

The authors declare that there are no conflicts of interest.

ABBREVIATIONS USED

NMR, nuclear magnetic resonance spectrometry; HPLC, highperformance liquid chromatography; HSQC, heteronuclear single-quantum coherence; HMBC, heteronuclear multiplebond correlation; FT-ICR-MS, fourier transform ion cyclotron resonance mass spectrometry; UV, ultraviolet spectrometry; IR, infared absorption spectrum; DPPH, 2, 2-Diphenyl-1-picrylhydrazyl; $ABTS^{+}$, 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical cation.

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Figure captions

Figure 1. Chemical structures of the isolated compounds **1-15** from mung bean seeds.

Figure 2. Key HMBC correlations of compound **1**.

Figure 3. Product ion scan spectra and representative multiple-reaction monitoring (MRM) chromatograms of six analytes.

Figure 4. Effects of vitexin, isovitexin and extract from MBS on serum and tissue biochemical paramters in rats with ISO-induced myocardial ischemia. Normal: normal control group; Model: ISO model control group; VG: vitexin group; IVG: isovitexin group; MBSG: mung bean seeds extract group. Values were presented as mean ± standard deviation (n = 6). ** $P < 0.01$ vs. normal control group; # $P < 0.05$ vs. model control group; ## $P < 0.01$ vs. model control group by one-way ANOVA.

Figure 5. HE-stained sections of heart tissues from the different experimental groups. (A) normal control group, (B) ISO model control group, (C) VG (6 mg/kg), (D) IVG (6 mg/kg), (E) MBSG (750 mg/kg).

Table 1 ^{1}H and ^{13}C NMR data of compound **1** (in DMSO-d_6)

position	δ_C	δ_H	position	δ_C	δ_H
2	163.4		2″	70.1	4.02 m
3	102.7	6.75 s	3″	79.0	3.20 m
4	181.7		4″	70.0	3.06 m
5	160.6		5″	79.6	3.32 m
6	108.8		6″	66.8	3.60 m, 3.70 m
7	163.4		1‴	98.6	4.72 d (3.5)
8	93.8	6.48 s	2‴	72.0	3.15 m
9	156.3		3‴	73.1	3.39 m
10	103.1		4‴	70.2	3.30 m
1′	121.1		5‴	72.5	3.37 m
2′, 6′	128.4	7.90 d (8.6)	6‴	60.6	3.39 m, 3.51 m
3′, 5′	116.0	6.92 d (8.6)	5-OH		13.52 s
4′	161.2		7-OH		10.35 br s
1″	73.3	4.57 d (9.8)			

NMR spectroscopic data were recorded at 600 MHz (^{1}H NMR) and 150 MHz (^{13}C NMR)

Table 2 Calibration and quantitation of six analytes (n = 3)[a]

No.	Analytes	Regression equation	Correlation coefficient (r^2)	X ranges (μg/mL)	Contents (mg/g)[b]
1	isovitexin-6"-*O*-α-*L*-glucoside	Y = 68294X-2422	0.9998	0.1397-5.5897	1.70 ± 0.22
2	vitexin	Y = 1E+06X+2E+06	0.9991	0.1884-7.5370	2.97 ± 2.00
3	isovitexin	Y = 2E+06X+8E+06	0.9985	0.4373-17.4904	5.32 ± 0.27
4	dulcinoside	Y = 30760X+29378	0.9993	0.0103-0.4130	0.13 ± 0.08
8	*L*-tryptophan	Y = 3E+06X+4E+07	0.9985	0.2447-9.7893	2.29 ± 0.25
13	nicotinic acid	Y = 136549X+434939	0.9997	0.0031-1.2333	0.38 ± 0.09

[a] n = number of samples; data represent the mean ± SD of triplicate tests for each sample.

[b] Milligram per 1 g of 95% EtOH extract.

Table 3 Antioxidant activities of MBS extract and compounds 1-15

compound	ABTS (EC_{50}, [a]μM)	DPPH (EC_{50}, [a]μM)
1	12.20 ± 2.66	>100
2	38.29 ± 3.86	>100
3	4.09 ± 0.37	>100
4	3.70 ± 0.12	>100
5	2.21 ± 0.15	17.32± 2.01
6	>100	>100
7	3.02 ± 0.18	19.33 ± 1.99
8	4.17 ± 0.62	>100
9	71.12 ± 4.28	>100
10	9.08 ± 0.07	>100
11	>100	>100
12	>100	>100
13	>100	>100
14	>100	>100
15	>100	>100
MBS extract	0.13 ± 0.26 mg/mL	3.00 ± 0.23 mg/mL
L-Ascorbic acid	11.06 ± 0.62	21.55 ± 1.61

[a] EC_{50} values correspond to the sample concentration achieving 50% of activity

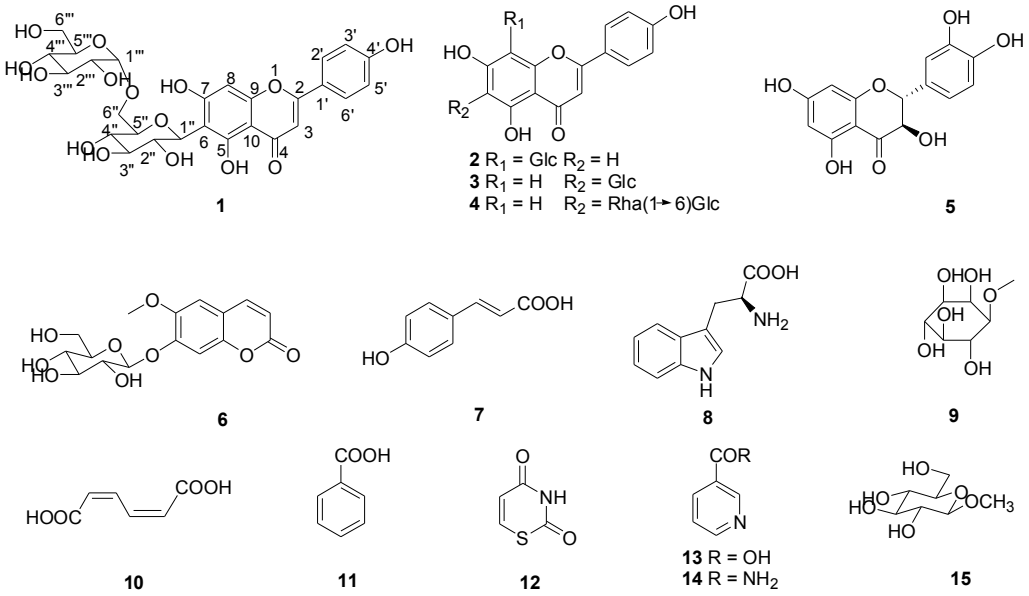


Figure 1

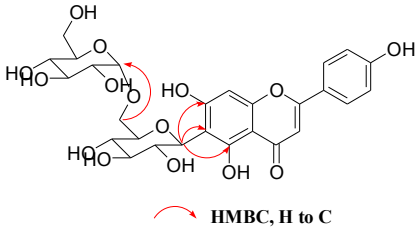
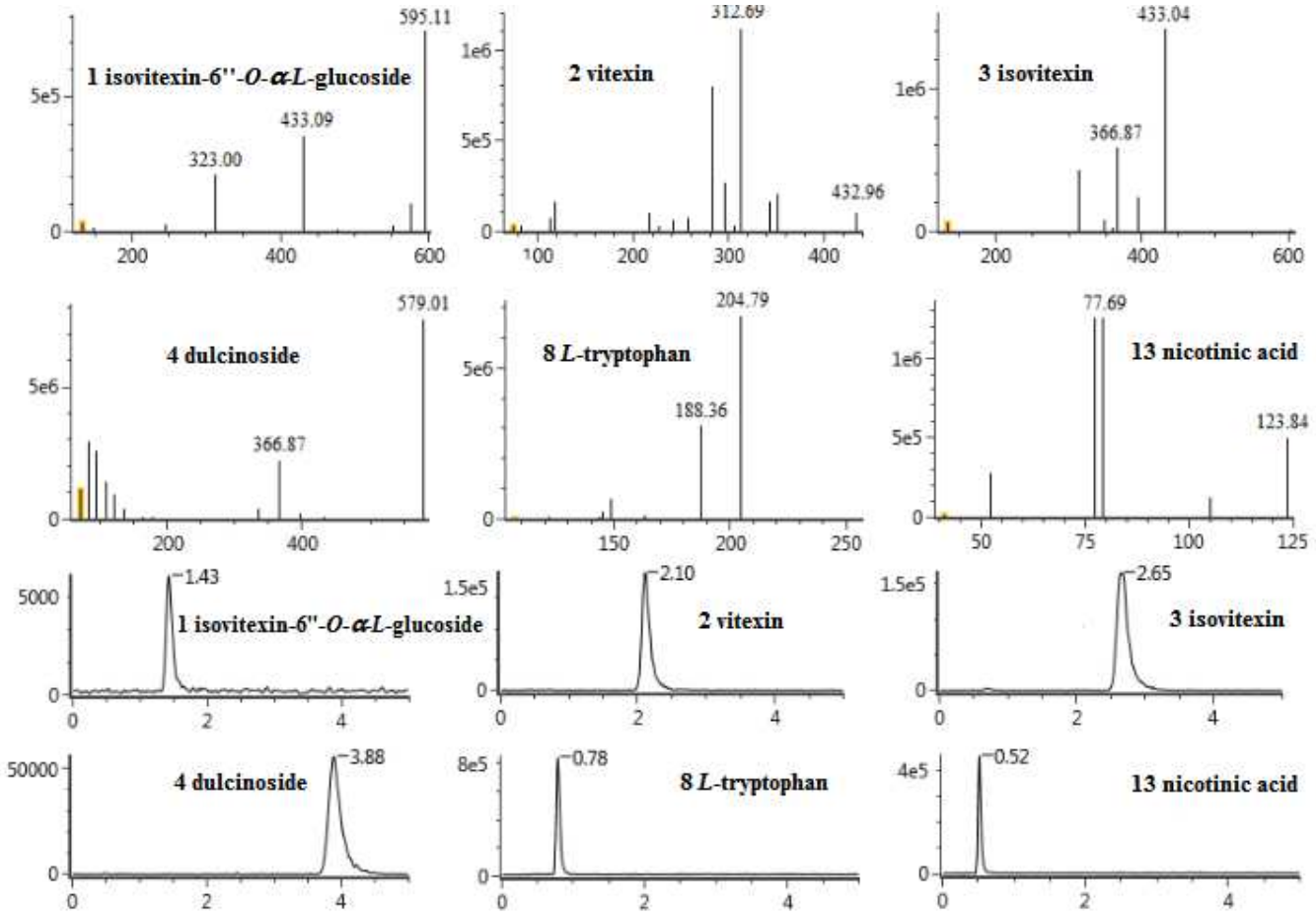


Figure 2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433.09
323.00
2 vitexin
312.69
432.96
3 isovitexin
433.04
366.87
4 dulcinoside
579.01
366.87
8 L-tryptophan
204.79
188.36
13 nicotinic acid
77.69
123.84
1 isovitexin-6''-O-α-L-glucoside
1.43
2 vitexin
2.10
3 isovitexin
2.65
4 dulcinoside
3.88
8 L-tryptophan
0.78
13 nicotinic acid
0.52

Figure 3

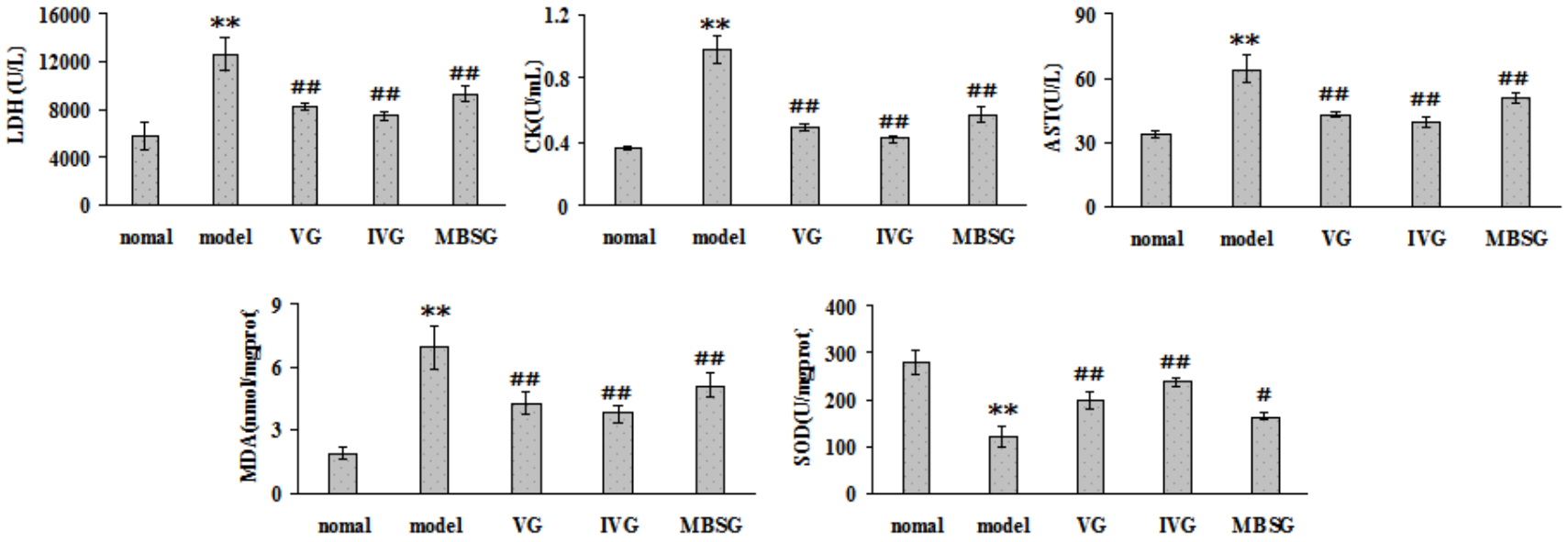

LDH (U/L)
16000
12000
8000
4000
0
CK(U/mL)
1.2
0.8
0.4
0
AST(U/L)
90
60
30
0
MDA(nmol/mgprot
9
6
3
0
SOD(U/mgprot
400
300
200
100
0
**
##
#
nomal
model
VG
IVG
MBSG

Figure 4

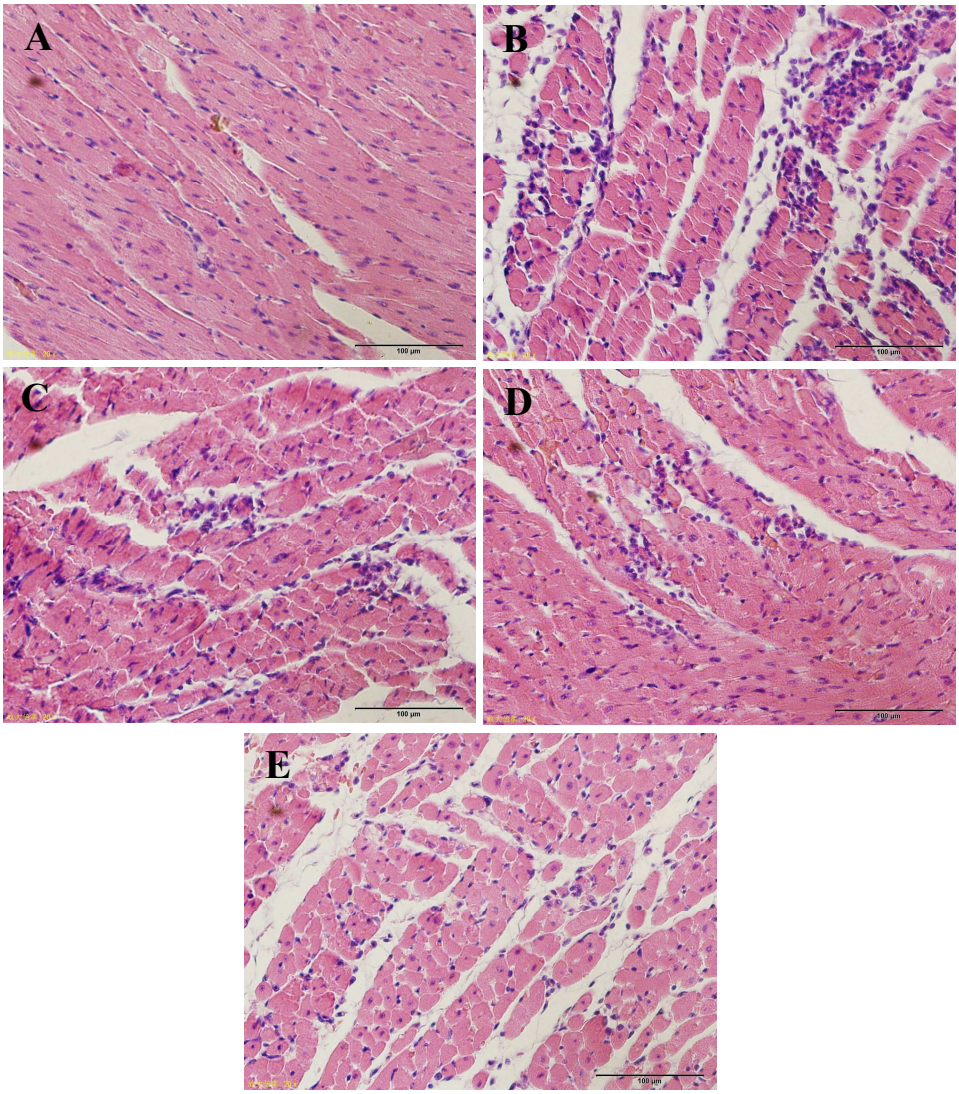
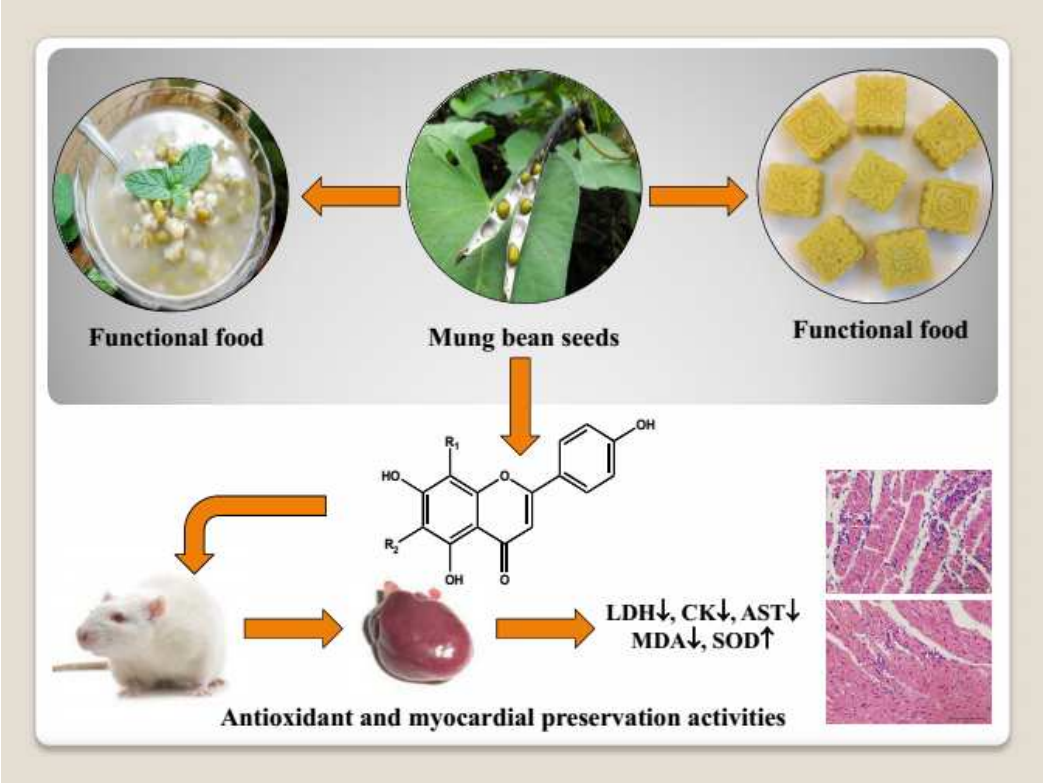


Figure 5

Graphic Abstract



Supporting Information

Antioxidant and myocardial preservation activities of natural phytochemicals from Mung Bean (*Vigna radiata* L.) seeds

Yan Bai*, Jiawei Chang*, Yan Xu*, Dan Cheng*, Hongxin Liu†, Yunli Zhao*, Zhiguo Yu*

*Department of Pharmaceutical Analysis, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, China

†Dalian Weida Pharmacy Co., Limited, 12-7 Zhuhai Street, Xigang District, Dalian 116011, China

Contents

Figure S1. The UV spectrum of compound **1**

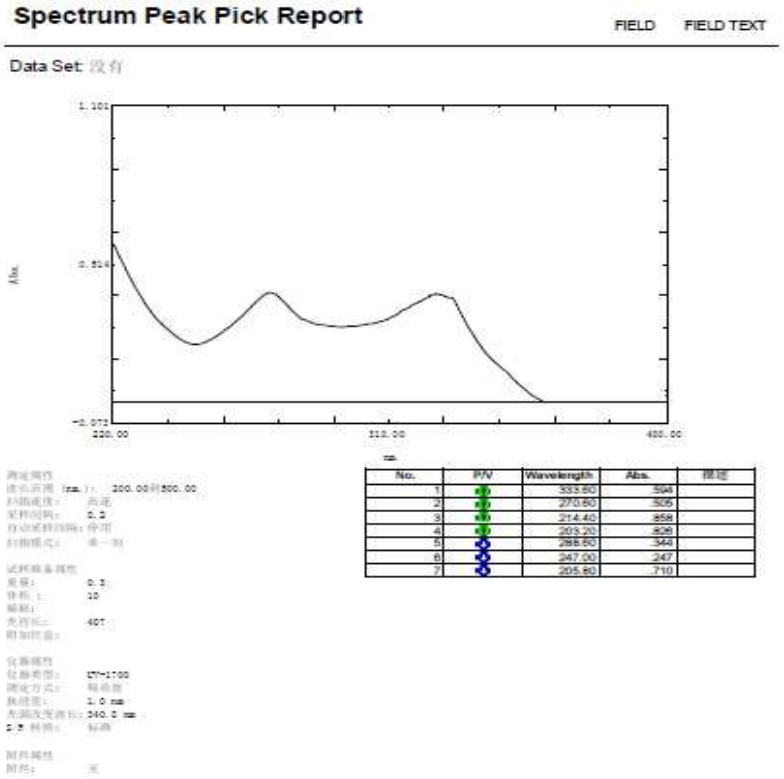


Figure S2. The IR spectrum of compound **1**

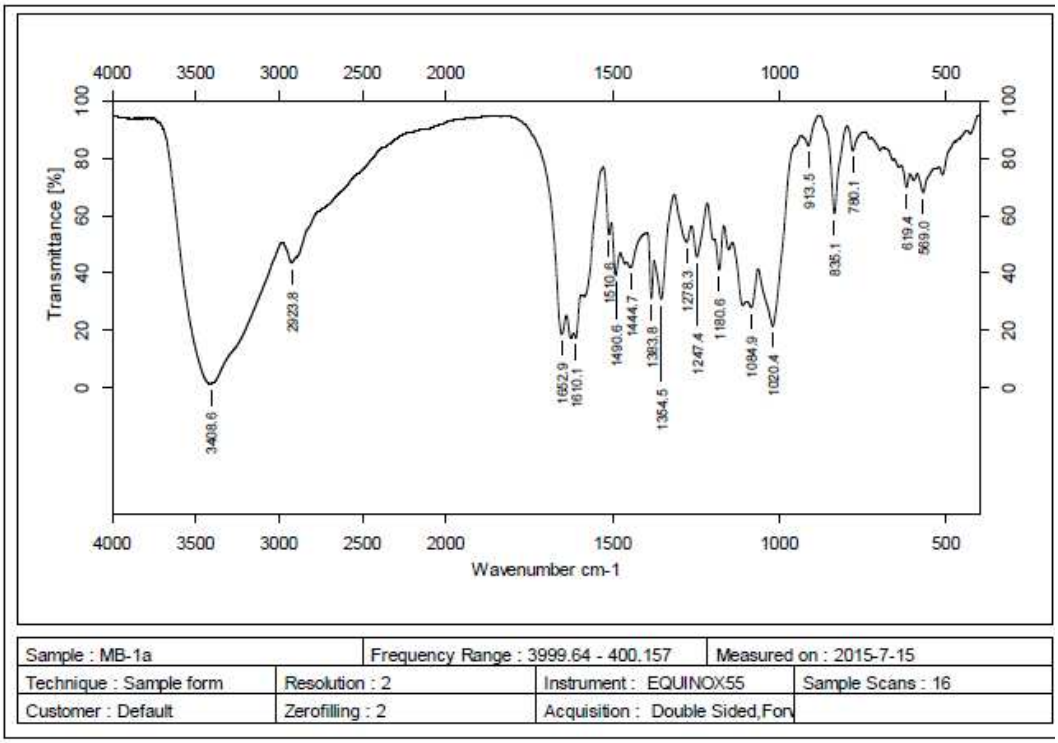


Figure S3. The FT-ICR-MS spectrum of compound **1**

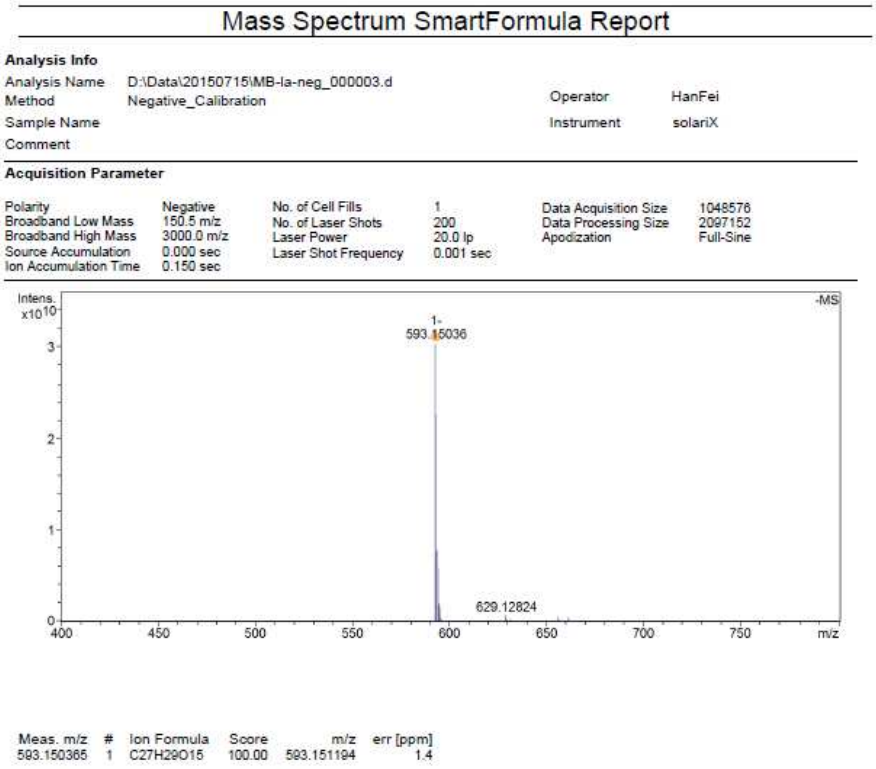


Figure S4. The ^{1}H-NMR spectrum of compound **1**

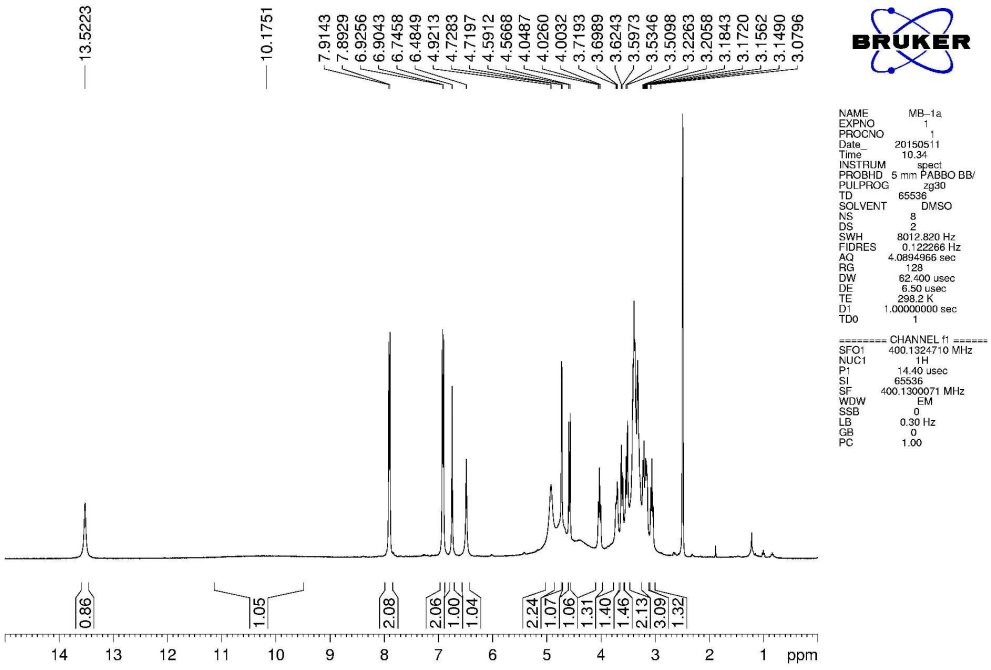


Figure S5. The ^{13}C-NMR spectrum of compound **1**

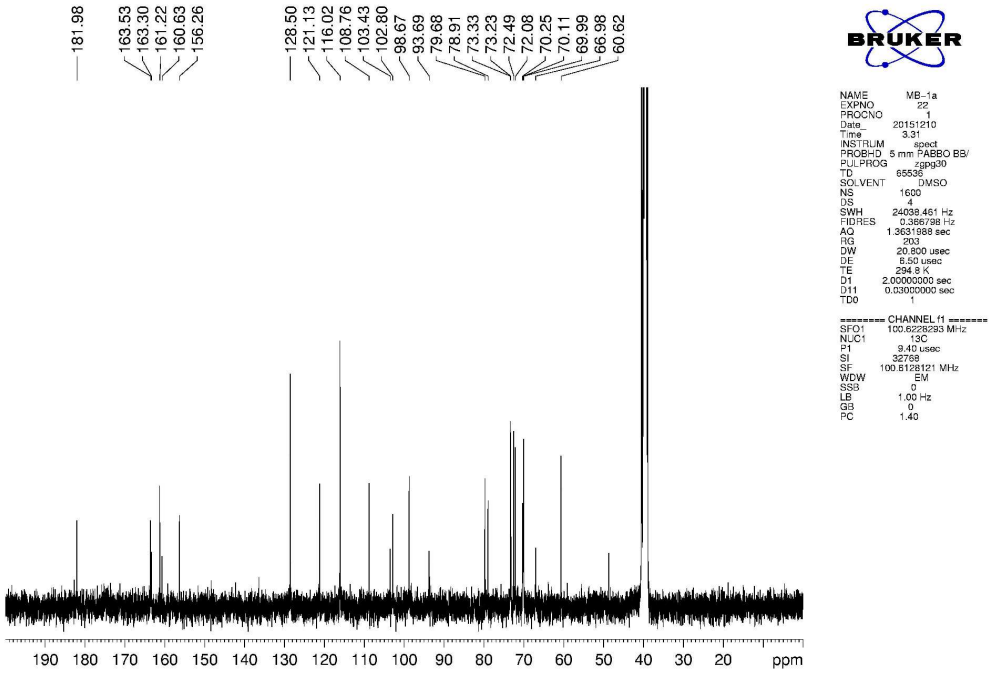


Figure S6. The HSQC spectrum of compound **1**

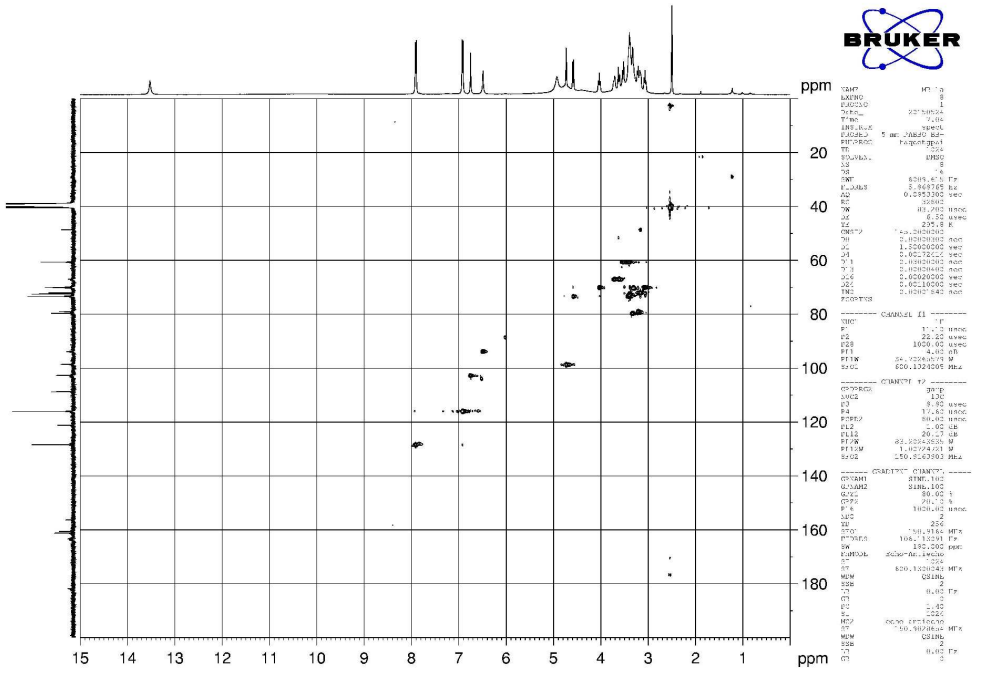


Figure S7. The HMBC spectrum of compound **1**