

Phytochemical Constituents from the seeds of *Capsella bursa-pastoris* and their antioxidant activities

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Abstract

Phytochemical investigation of 70% EtOH extract of the seeds of *Capsella bursa-pastoris* led to the isolation of a new cyclobutane organic acid (**1**), and fourteen known compounds, including two organosulfur compounds (**2**, **3**), two quinonoids (**4**, **5**), five flavonoids (**6-10**), three steroidal (**11-13**) and two other types (**14**, **15**). The structures of the compounds were elucidated by extensive spectroscopic analyses as well as comparison of their spectroscopic data with those reported in the literature. The antioxidant capacities of all the compounds and the extractive fractions were evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging test and ferric reducing antioxidant power (FRAP) assay. Then the antioxidative substances were evaluated for their neuroprotective effects against H₂O₂-induced HT22 cell injury. The results indicated the extractive fractions and the compounds **1-3**, **8-10** and **13** had strong scavenging ability to free radical, and the reduction power of the extractive fractions and compounds **1-3**, **8** and **10** were close to or higher than that of the positive control trolox. The EtOAc fraction, n-BuOH fraction, and compounds **1**, **3** and **8** can protect HT-22 cells from oxidative damage.

Introduction

Capsella bursa-pastoris (L.) Medik commonly known as shepherd's purse is a cruciferous plant that is often used for both its edible and medicinal properties [1]. This family of plants has gained widespread attention due to their nutritional and medicinal value. Seeds from these plants, including rape seed [2], turnip seed [3], white mustard seed [4], semen lepidii [5], and orychofragmus violaceus seed [6], exhibit anti-cancer, anti-bacterial, anti-inflammation, anti-oxidation, anti-radiation and other beneficial biological activities. The "Compendium of Materia Medica" documents that shepherd's purse seed is gentle and non-toxic, and has the ability to improve eyesight, relieve eye pain, and supplement the deficiency of entrails such as the heart, liver, spleen, lung, and kidney. Despite numerous studies on shepherd's purse [7-9], the chemical constituents and biological activities of its seeds have not been systematically investigated.

In this study, fifteen components of shepherd's purse seed and their antioxidant activities were investigated. As a part of our ongoing research [10], a new cyclobutane organic acid (**1**) and fourteen known compounds, including two organosulfur compounds (**2**, **3**), two quinonoids (**4**, **5**), five flavonoids (**6-10**), three steroidal (**11-13**) and two other types (**14**, **15**) were isolated from the seed of *C. bursa-pastoris*. The antioxidant capacities of the extractive fractions and all the compounds were evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging test and ferric reducing antioxidant power (FRAP) assay. Then the neuroprotective effects of the antioxidative substances were tested against H₂O₂-induced HT22 cell injury.

Results And Discussion

Structure elucidation

Compound **1** was obtained as white amorphous powder. The HR-ESI-MS (negative-ion mode) m/z : 447.1290 $[M-H]^-$ (calcd. for $C_{22}H_{23}O_{10}^-$, 447.1291), 483.1059 $[M+Cl]^-$ (calcd. for $C_{22}H_{24}O_{10}Cl^-$, 483.1058) (Fig. S1 in the supplementary material), the molecular formula was deduced to be $C_{22}H_{24}O_{10}$, indicating eleven degrees of unsaturation. The UV spectrum (Fig. S2 and S3) exhibited the characteristic absorption peaks at 240 nm and 360 nm. The IR spectrum, as depicted in Fig. S4, confirmed the presence of benzene ring (1610, 1521, 1463, 839 and 803 cm^{-1}), C=O (1686 cm^{-1}), and OH (3422 cm^{-1}).

The 1H NMR (600 MHz, DMSO- d_6) spectrum (Fig. S5) of **1** showed two carboxyl proton signals at δ_H 12.02 (2H, s, 1-COOH and 2-COOH), two phenol hydroxyl proton signals at δ_H 8.20 (2H, s, 4'-OH and 4''-OH), four aromatic hydrogens proton signals at δ_H 6.58 (4H, s, H-2', 2'', 6', 6''), four methylene proton signals at δ_H 4.18 (1H, d, $J = 7.26$ Hz, H-3), 4.16 (1H, d, $J = 7.20$ Hz, H-4), 3.72 (1H, d, $J = 7.20$ Hz, H-1) and 3.70 (1H, d, $J = 7.38$ Hz, H-2), and four methoxys proton signals at δ_H 3.76 (12H, s, H-OCH₃).

The ^{13}C NMR (150 MHz, DMSO- d_6) spectrum (Fig. S6) showed 22 carbon signals, including two carboxyl groups at δ_C 173.3 (1-COOH, 2-COOH), twelve aromatic carbon signals at δ_C 129.7 (C-1', 1''), 105.3 (C-2', 6', 2'', 6''), 147.6 (C-3', 5', 3'', 5''), 134.4 (C-4', 4''), four methoxys carbon signals at δ_C 56.0 (3', 3'', 5', 5''-OCH₃), four methylene carbon signals at δ_C 46.9 (C-1, 2), 41.1 (C-3, 4). In the 1H - 1H COSY spectrum of **1**, homonuclear vicinal coupling correlations of H-1/H-4, H-2/H-3 (Fig. 2 and Fig. S10), combined with HMBC correlations of 4'-OH/C-3', 4', 5', 4''-OH/C-3'', 4'', 5'', H-1/C-1', C-4, 1-COOH, H-2/C-1'', C-3, 2-COOH, H-3/C-1'', 2'', 6'', 2, 2-COOH (Fig. 2 and Fig. S11), suggested that the whole planar structure of **1** was confirmed as shown in Fig. 1. In addition, the correlations between 4'-OH and H-1, 4''-OH and H-2, 5'-OCH₃ and H-6', 5''-OCH₃ and H-6'', 3'-OCH₃ and H-2', 3''-OCH₃ and H-2'', 4'-OH and 2-COOH, 4''-OH and 1-COOH in the NOESY spectrum (Fig. 3 and Fig. S12) indicated that 1-COOH is in the same axis with the 3-Ar, and 2-COOH is in the opposite axis with the 4-Ar. Thus, the structure of compound **1** was established and named capselllic acid A.

From the seeds of *C. bursa-pastoris*, additional fourteen known compounds were isolated for the first time. They were identified using ESI-MS and NMR data analyses (Fig. S13-S54), and comparison with those had been previously described in the literature to be pinoresinol-4-sulfate (**2**) [11], descurainoside (**3**) [12], munjistin (**4**), 2,6-dimethoxy-1,4-benzoquinone (**5**) [13], diosmin (**6**) [14], isorhamnetin-3,7-di- O - β -D-glucopyranoside (**7**) [15], quercetin (**8**) [16], quercetin-3- O - β -D-glucopyranosyl-7- O - α -L-rhamnopyranoside (**9**) [17], isorhamnetin (**10**) [18], β -sitosterol-3- O - β -D-glycoside (**11**) [19], β -sitosterol (**12**) [20], campesteryl ferulate (**13**) [21], allantoin (**14**) [22], ent-16 α ,17-dihydroxy-kauran-19-oic acid (**15**) [23].

Cellular oxidative stress and inflammation play a vital role in the pathological process of neural damage. Oxidative stress occurs in the vast majority of degenerative diseases of the nervous system and pathological process of neural damage [24, 25]. It is essential to develop diets and drugs that target inflammation and oxidative stress in the nervous system. It is well known that cruciferous plants have a wide range of antioxidant compounds [26]. Antioxidant capacity is one of the key indicators of the

nutritional and therapeutic potential of cruciferous plants. Based on the aforementioned objectives, DPPH radical-scavenging test and FRAP assay were used in this study to assess the antioxidant capacity of all compounds and extractive fractions. The neuroprotective properties of the antioxidative substances against H₂O₂-induced HT22 cell damage were then assessed.

DPPH radical-scavenging assay

The sample solution (1 mg/mL of the extractive fractions or 1 mmol/L of the compounds) and the DPPH working solution (0.1 mmol/L) were prepared. Vc was the positive control (1 mmol/L). To add respectively 100 µL preparing sample solution or Vc solution and 100 µL DPPH working solution into a 96-well plate, the absorbance was measured at 520 nm after the above reaction at room temperature of 25 °C for 30 min. The test sample's DPPH radical reducing activity was calculated as $I = [1 - (A_1 - A_2) / A_0] \times 100\%$ (A_0 : Absorption with 100 µL DPPH solution + 100 µL methanol; A_1 : Absorption with 100 µL DPPH solution + 100 µL sample solution; A_2 : Absorption with 100 µL sample solution + 100 µL methanol).

The results indicated that the extractive fractions and the compounds **1-3**, **8-10** and **13** have strong scavenging ability to free radical. The DPPH radical scavenging rates of PE, DCM, EtOAc and n-BuOH fractions exceeded 84%, and that of compounds **1** and **8** were more than 91%, which were close to that of the positive control Vc (Fig. 4).

FRAP assay

the sample solution (5 µL), standard solution (0.15, 0.3, 0.6, 0.9, 1.2, 1.5 mM) and positive control solution (1 mmol/L) were added into each well of a 96-well plate after 180 µL of FRAP working liquid had been added. The control utilized was distilled water. Each well's solution was thoroughly mixed before the reaction was carried out for 5 minutes. Following this, the absorbance of each group of samples was then determined at 593 nm.

The results (Fig. 5) indicated that the reduction power of the extractive fractions and compounds **1-3**, **8** and **10** were close to or higher than that of the positive control trolox. FeSO₄ equivalent value range of total, DCM, EtOAc, n-BuOH and H₂O fractions was 1.23-2.53 (compared with control, $P < 0.01$), and that of compounds **1-3**, **8** and **10** respectively was 1.19-4.03 (compared with control, $P < 0.01$).

The neuroprotective effects were evaluated using H₂O₂-induced HT22 cell injury model

HT22 cells were cultured in Dulbecco's Modified Eagle's Medium (Macgene, Beijing, China) supplemented with 10% (v/v) Fetal Bovine Serum (Gibco, California, USA) 100 U/mL penicillin (Macgene, Beijing, China) and 100 µg/mL Streptomycin sulfate (Macgene, Beijing, China), at 37 °C under 5% CO₂. HT22 cells were seeded in 96-well plates at a density of 7000 cells per well and incubated overnight. Then, the cells were treated with extractive fractions at a concentration of 100 µg/mL or compounds at a concentration of 100 µM, respectively for 1 h, and then stimulated with H₂O₂ (600 µM). After incubating under the same

conditions for 24 h, 10 μ L CCK-8 solution was added to each well. After 2 h incubation, the absorbance of each well was measured at 450 nm using a microplated reader (Multiskan MK-3, Thermo, USA).

The results (Fig. 6) indicated that the EtOAc fraction, n-BuOH fraction, and compounds **1**, **3** and **8** can protect HT-22 cells from oxidative damage. Cell survival rates of that exceeded 81% (compared with model, $P < 0.05$ or $P < 0.01$).

Experimental

General experimental procedures

The HR-ESI-MS was recorded on an Agilent G6230A TOF LC/MS (Agilent Technologies Co., Ltd., CA, US). IR spectra were recorded on a Frontier FT-IR with KBr pellets. 1D and 2D NMR experiments were recorded on an AVANCE NEO 600 NMR and a Bruker ECA-400 NMR (Bruker BioSpin, MA, US). The chemical shifts were given in ppm (d), relative to TMS as an internal standard, and coupling constants are in Hz. A surveyor high performance liquid chromatography (Thermo, US) were used to record the UV spectra. Preparative HPLC separation was carried out on an LC-3000 liquid chromatography system equipped with a UV detector (Beijing Chuangxin Hengtong Technology Co., Ltd., Beijing, China). The column applied in this work was an Innoval C₁₈ column (21.2 $\times$ 250 nm, 5 μ m, Tianjin Bona Aijieer Technology Co., Ltd., Tianjin, China). Column chromatography (CC) was carried out using silica gel (200–300 mesh, Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia, Sweden), and macroporous resin AB-8 (Nan Kai College Chemical Inc., Tianjin, China), respectively. TLC was performed on GF254 plates pre-coated with silica gel 60 (5–20 μ m, Yantai Huayang New Material Technology Co., Ltd., China).

Plant material

The seeds of *Capsella bursa-pastoris* were purchased from Bozhou Chinese Medicine Exchange Center in Anhui province China in October 2021 and were identified by Prof. Bin Li, Beijing Institute of Radiation Medicine. A voucher specimen (No. 2021-1006) was deposited in the specimen cabinet of the Beijing Institute of Radiation Medicine.

Extraction and isolation

The dried seeds of *C. bursa-pastoris* (80 kg) were extracted with 70% EtOH. The pooled extracts were concentrated to yield a residue (6.69 kg) and were suspended in water. Total extract was then extracted with petroleum ether (PE), dichloromethane (DCM), EtOAc and n-BuOH successively, yielding DCM extract (220 g) and n-BuOH extract (1000 g). The DCM extract was subjected to silica gel column chromatography (CC) eluted with dichloromethane-methanol (from 100:0 to 1:1) to afford 17 fractions (A–Q) and a precipitation. Fraction G (20 g) was further separated by column chromatography over silica gel, Sephadex LH-20 gel, and preparative HPLC to afford compound **1** (13.3 mg) and compound **11** (7.6 mg). Some white sediment precipitated from fraction D (13 g), and the precipitation was further purified by Sephadex LH-20 gel to afford compound **12** (11.6 mg). Fraction B (15 g) was subjected to silica gel

column chromatography eluted with Petroleum ether-Ethyl acetate gradient to yield 6 fractions (B1-B6). Preparative thin-layer chromatography was used to separate fraction B4 and obtain compound **13** (20.8 mg).

A viscous substance precipitated from the n-BuOH extract, and the precipitation was further separated by silica gel column chromatography and recrystallization to afford compound **6** (12.7 mg) and compound **14** (21.0 mg). The n-BuOH extract was fractionated by macroreticular resin attraction separation (AB-8) into four fractions (25% ethanol, 50% ethanol, 70% ethanol and 95% ethanol). The 25% ethanol fraction of the n-BuOH extract (160 g) was further separated by CC over silica gel to afford fractions A-F. After removing the precipitate, fraction B (15 g) was further separated by Sephadex LH-20 gel to afford 9 fractions (B1-B9), and by preparative thin-layer chromatography and preparative HPLC for the separation of fraction B2 to afford compound **2** (20.2 mg), Fraction B3 was separated by recrystallization to afford compound **15** (5.2 mg). Yellow powder was precipitated from fractions B7 and B9 to obtain compound **9** (5.6 mg) and compound **4** (14.4 mg). Fraction C (30 g) was further separated by column chromatography (CC) over silica gel to afford fractions C1-C16. Fractions C1-C9 were further separated by Sephadex LH-20 gel to afford compound **10** (5.2 mg). Fractions C10-C16 were further separated by Sephadex LH-20 gel and preparative HPLC to afford compound **3** (785.8 mg) and compound **8** (11.3 mg). Fraction A (10 g) was further separated by CC over silica gel and preparative HPLC to afford compound **5** (5.0 mg). Some substances precipitated from fraction E were further separated by Sephadex LH-20 gel to afford compound **7** (7.6 mg).

Statistical analysis

Data were represented as mean $\pm$ SD of three independent experiments. The statistical analyses were performed using the one-way analysis of ANOVA in GraphPad Prism 8. Differences of 5% were considered significant (* P 0.05, ** P 0.01).

Conclusion

In this study, the chemical constituents of shepherd's purse seeds were systematically separated to afford one new compound and fourteen known compounds, and their antioxidant activities were evaluated *in vitro*. The antioxidant capacities of all compounds and the extractive fractions were evaluated by the DPPH radical-scavenging test and FRAP assay. The data indicated that the reduction power of the extractive fractions and compounds **1–3**, **8** and **10** were close to or higher than that of the positive control trolox. The extractive fractions and compounds **1–3**, **8–10** and **13** have strong scavenging ability to free radical. Subsequently, the neuroprotective effects of all the isolates against H₂O₂-induced injury in HT22 cells were evaluated *in vitro* by CCK8 assay. The EtOAc fraction, n-BuOH fraction, and compounds **1**, **3**, and **8** can protect HT-22 cells from oxidative damage at 100 μ M.

Declarations

Ethical Approval and Consent to Participate

Not applicable

Consent for publication

Written informed consent for publication was obtained from all authors.

Conflict of Interest

No potential conflict of interest was reported by the authors.

Authors' contributions

Ti-Qiang Zhou and Zhen-Zhen Wei wrote the main manuscript text and prepared figures 1-6; Jin-Rui Zhang, Jia-Hui Dong, Chun-Ying Liu and Cong-Zhi Jiang taken the formal analysis of the manuscript; Zi-Ming Xia, Si-Fan Liu, Min Li and Guang-jie Zhang curated the related data; and Li Chen, Ying Tian, Bin Li, and Shu-Chen Liu reviewed and edited the manuscript. All authors reviewed the manuscript.

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not applicable

Data Availability

All relevant data are within the manuscript and its supplementary material, and are also available from the corresponding author on reasonable request.

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Figures

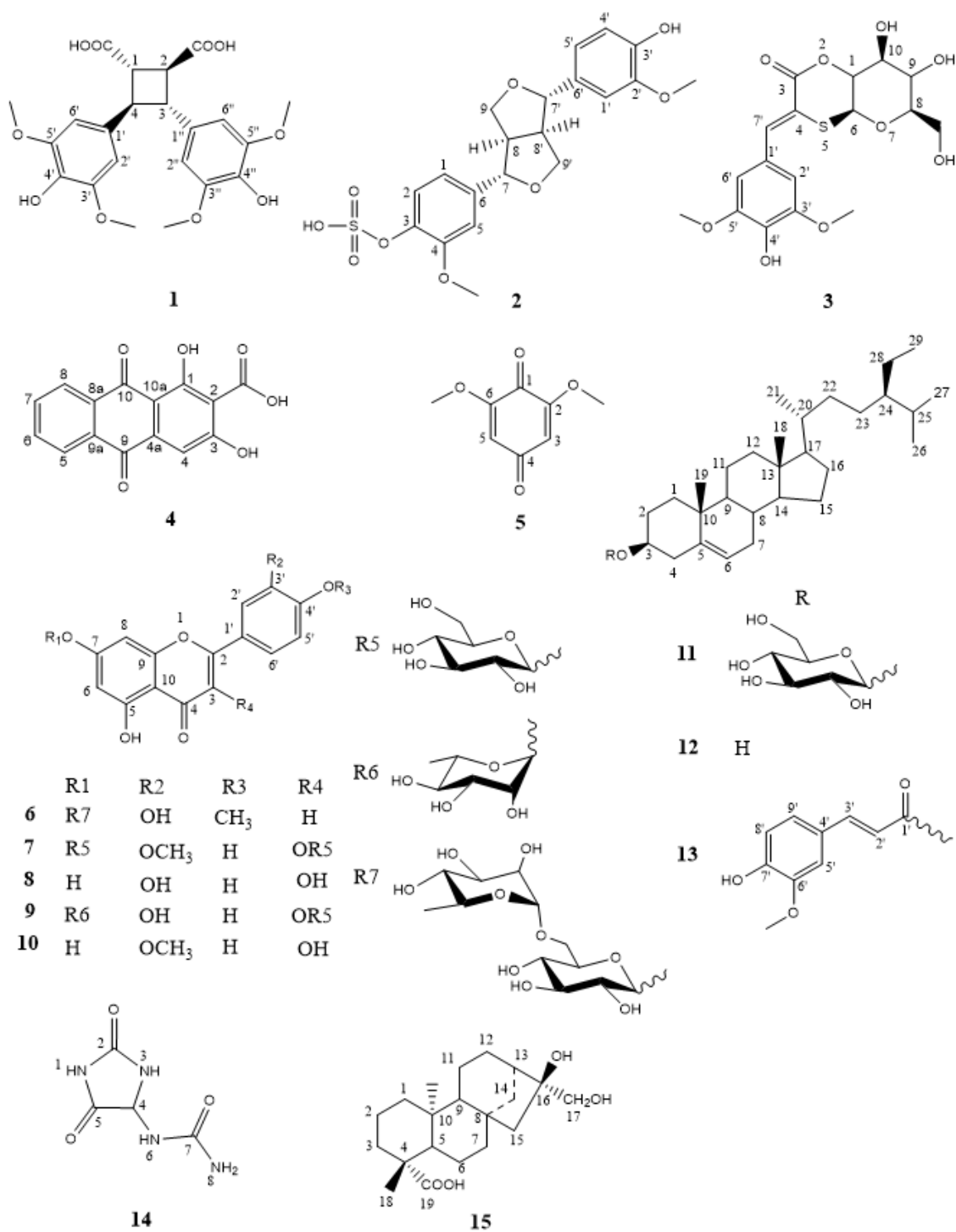


Figure 1

Chemical structures of compounds 1-15

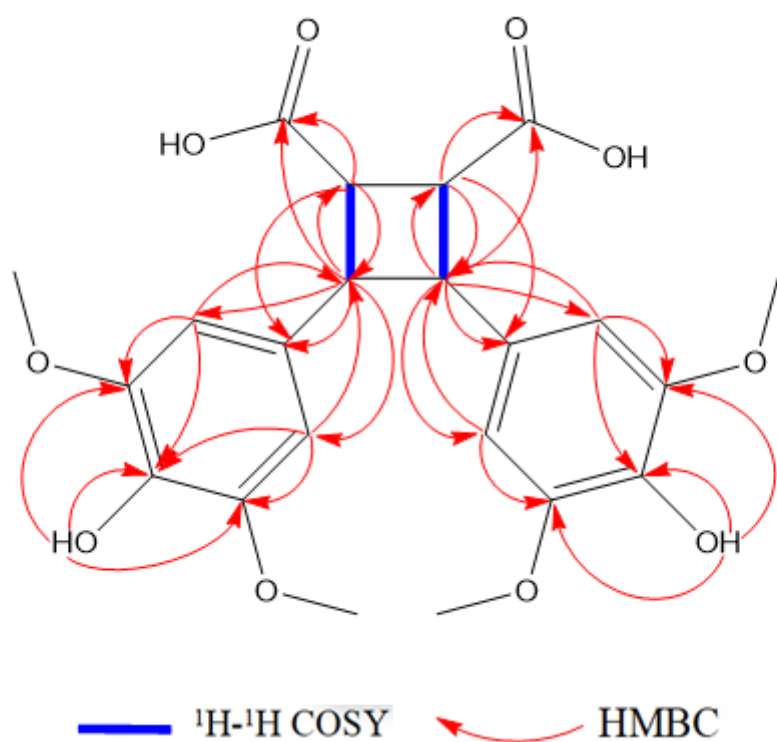


Figure 2

Key ¹H-¹H COSY and HMBC correlations of compound 1.

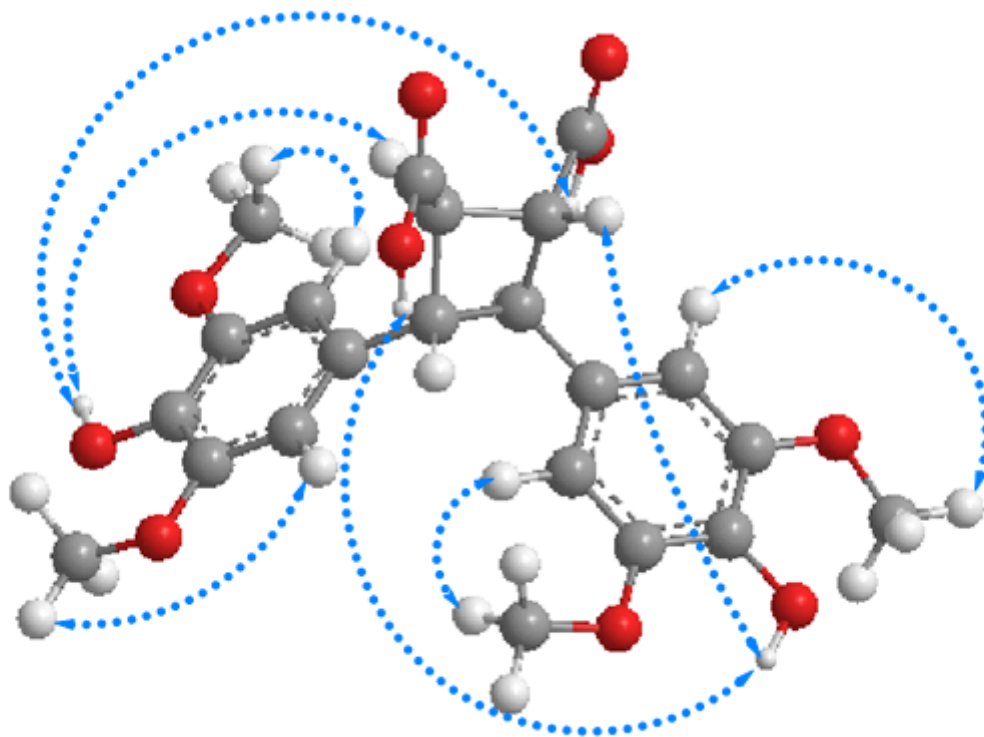


Figure 3

NOESY correlation of compound 1 (gray: carbon atom; red: oxygen atom; white: hydrogen atom).

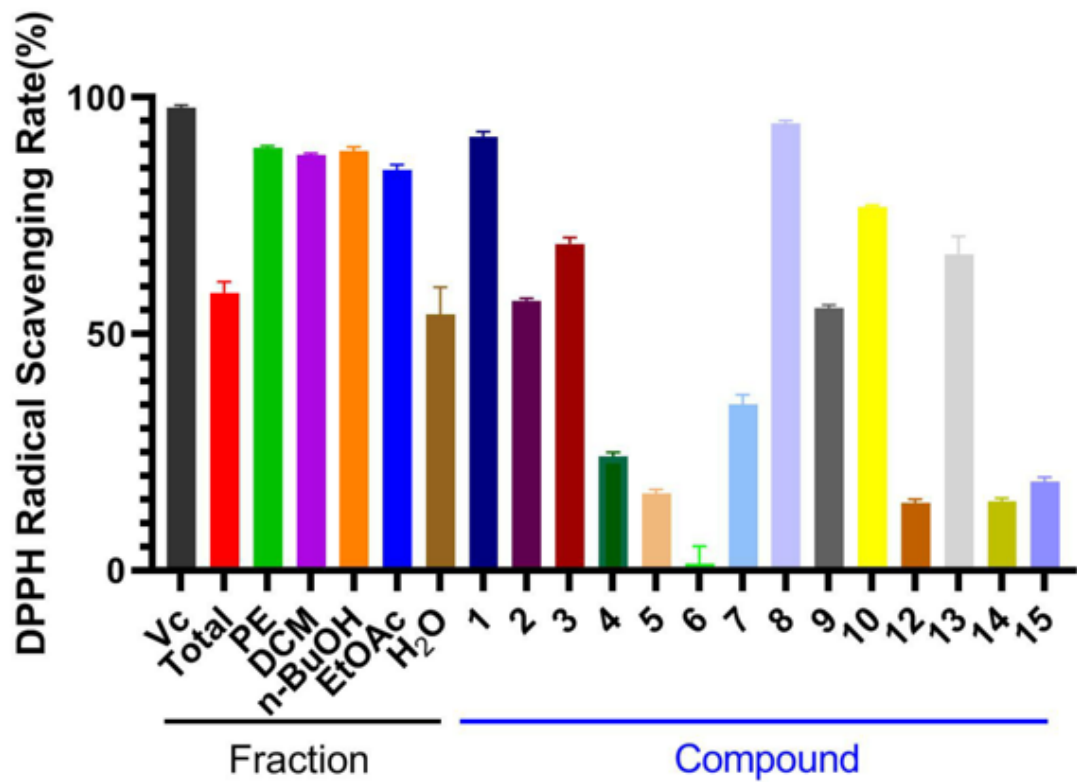


Figure 4

DPPH radical scavenging rate of the extractive fractions and compounds ($\pm$ s, n=4)

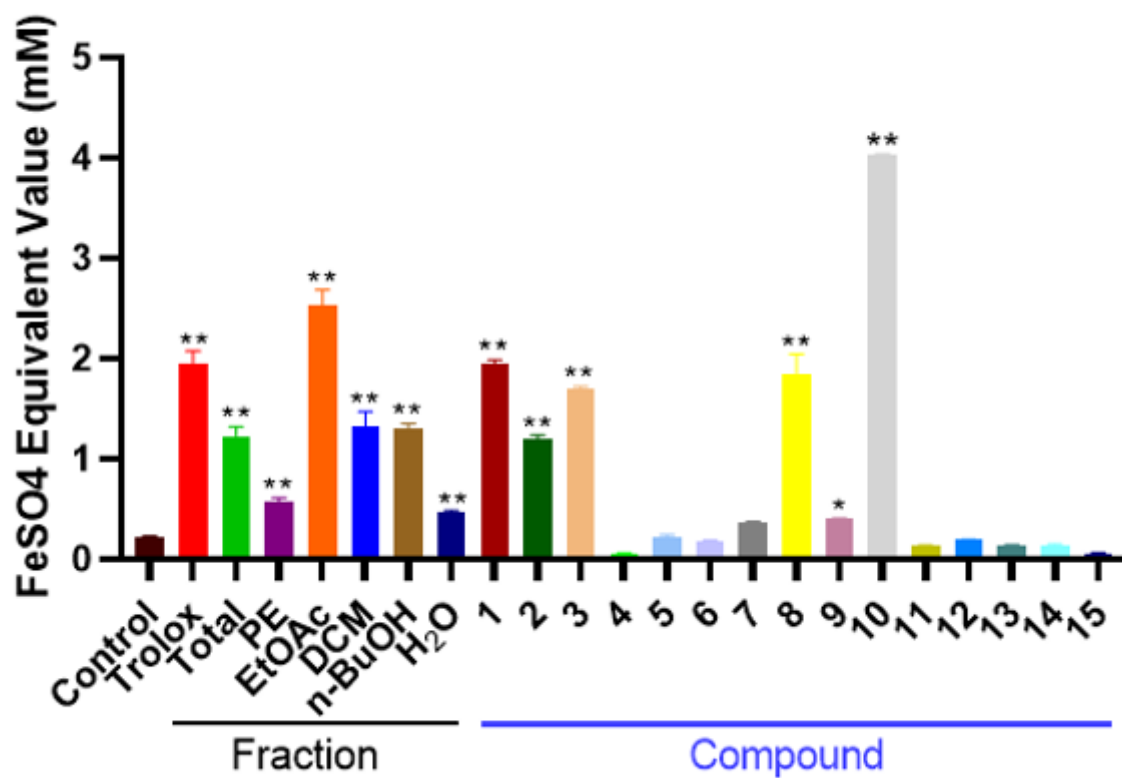


Figure 5

Ferric reducing antioxidant power of extractive fractions and compounds ($\pm s$, $n=3$)

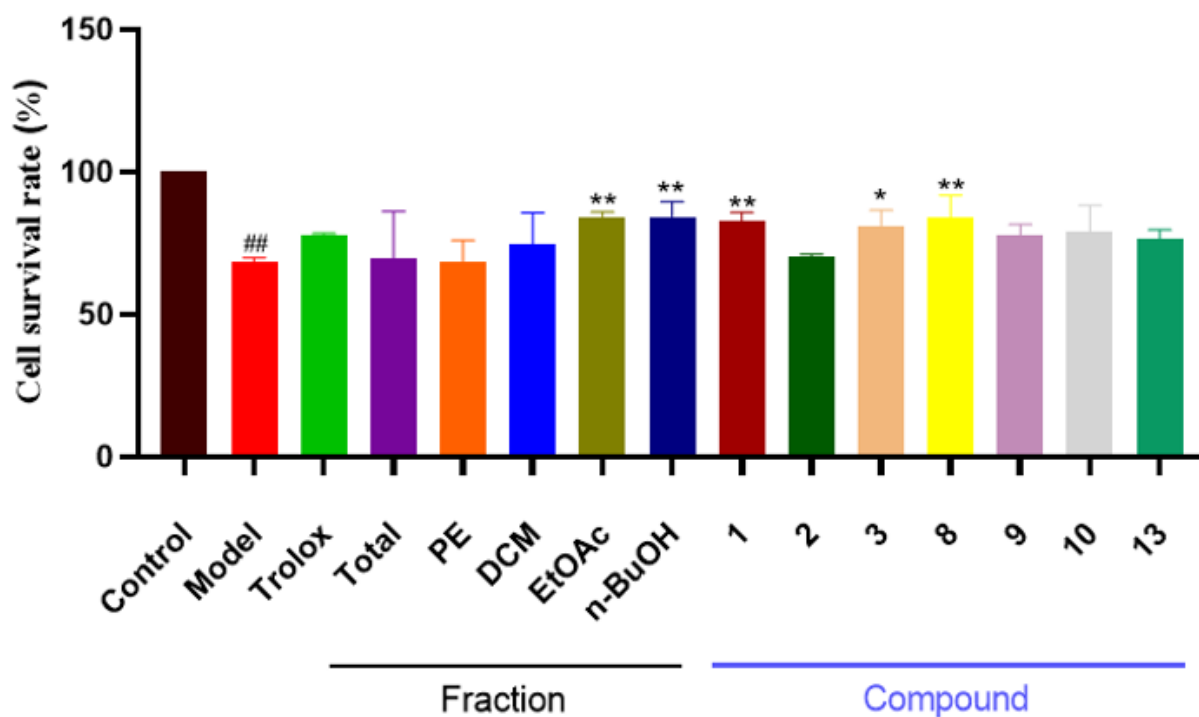


Figure 6

The protective effects on H₂O₂-induced HT22 cell injury model ($\pm$ s, n=3)

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