UHPLC–Q/Orbitrap/MS/MS fingerprinting of Bai-Hu-Jia-Ren-Shen-Tang Decoction and evaluation of its antioxidant activity in streptozotocin-induced diabetic rats

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Received: June 6, 2022 • Accepted: August 25, 2022

ABSTRACT

Bai-Hu-Jia-Ren-Shen-Tang Decoction (BHJSTD) is one of the oldest classic Chinese medicine prescriptions which used in the field of treatment of diabetes. However, to the best of our knowledge, the ingredients of this prescription have not been identified, and there are very few studies on the anti-diabetic mechanism of this prescription. Therefore, BHJSTD was detected and identified by ultra-high-performance liquid chromatography coupled with Quadrupole-Exactive Focus Orbitrap MS (UHPLC–Q/Orbitrap/MS/MS). We identified 74 compounds, including flavonoids, alkaloids, chalcones, xanthones, phenols, phenylpropanoids, terpenes, triterpenes, amino acid derivatives, etc. Then, Sprague Dawley rats were fed with a high-fat and high-sugar diet for two months and injected with streptozotocin (STZ) to induce type 2 diabetes (T2DM). The diabetic rats were randomized to given metformin (200 mg kg⁻¹·d⁻¹, n = 15), BHJSTD extracts (40 g kg⁻¹·d⁻¹) and BHJSTD extracts (10 g kg⁻¹·d⁻¹) by gavage for 8 weeks. The results confirmed that BHJSTD significantly decreased the level of MDA and increased levels of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), it shows that the prescription has significant antioxidant activity in the treatment of T2DM.

KEYWORDS
Bai-Hu-Jia-Ren-Shen-Tang Decoction, UHPLC–Q/Orbitrap/MS/MS, Type 2 diabetes, antioxidant activity

INTRODUCTION

In recent years, the advantages of Herbal and Traditional Chinese Medicine in the prevention and treatment of diabetes mellitus (DM) have been widely recognized around the world [1, 2]. Bai-Hu-Jia-Ren-Shen-Tang Decoction (BHJSTD), composed of Ginseng Radix, Rhizoma Anemarrhenae, Radix Glycyrrhizae Preparata, Gypsum Fibrosum and japonica rice is a traditional Chinese medicine prescriptions (TCMP) described in the Chinese medicine book “Treatise on Febrile and Miscellaneous Diseases”, which has been used in China for over 1,800 years [3]. The indications of BHJSTD include infectious diseases, such as influenza, lung infection, encephalitis, enteric typhoid and hospital infection; thermoplegia; acute cerebrovascular disease, severe hyperosmolarity, diabetes mellitus [4], intractable hypotension, hypernatremia, shock and other internal diseases. This TCMP has been widely used to treat acute and severe cases in the cardiovascular intensive care ward. Refractory
hypotension and hypovolemic shock that need large dose of supplemental fluid to maintain blood pressure and may also belong to the extension of the BHJRSTD formula syndrome.

Insulin resistance (IR) and pancreatic β-cell function defects were considered to be the main mechanisms of the pathogenesis of T2DM [5]. Oxidative stress can directly or indirectly aggravate IR and the damage of pancreatic β-cells, which is closely associated with the occurrence and development of T2DM [6, 7]. Studies have shown that compounds with antioxidant properties are more effective in treating T2DM [8, 9]. Nowadays, BHJRSTD is frequently used clinically in DM, especially as a complementary therapy [4]. The application of BHJRSTD proves to be more effective in the treatment of type 2 diabetes mellitus (T2DM) than using routine treatment only.

To our knowledge, there are no reports on chemical characterization and antioxidant activity studies of BHJRSTD, which is the usual way of consumption in traditional medicine in China. The complexity of chemicals in TCMP presents significant challenges to rapidly identify and characterize components. Over the past few decades, many natural product extracts, botanical ingredients and TCMP are analyzed using quadrupole Orbitrap spectrometry [10–12]. In this study, the main goals are develop an efficient method for rapid chemical characterization using UHPLC-Q-Exactive Orbitrap MS and measurement of the antioxidant activity of the BHJRSTD in diabetic rats, which are very beneficial for the further research and application of this TCMP including the material basis and pharmacological activity study.

EXPERIMENTAL

Materials

Streptozotocin (STZ) was MP Biomedicals, Trisodium citrate and citric acid were purchased from Sigma Aldrich Inc. (St Louis, MO, USA). Enzyme-linked immune adsorbent assay (ELISA) kits, of Malondialdehyde (MDA), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) were obtained from R&D Systems Inc. (Minneapolis, MN, USA). Ginseng Radix, Rhizoma Anemarrhenae, Radix Glycyrrhizae Preparata, Gypsum Fibrosum and japonica rice were purchased from Xi’an Grass Plant Technology Co. Ltd. (Xi’an, Shaanxi, China). Methanol and acetonitrile of LC-MS grade were purchased from Fisher (Fisher Corp., Fair lawn, NJ, USA). All other chemicals were of analytical grade available commercially. ALL Chinese herbal medicine were obtained from Department of Chinese Materia Medica and Natural Medicines, School of Pharmacy, Fourth Military Medical University, and botanically identified by Professor Xiao-mei Song a traditional Chinese Medicine chemist at College of pharmacy, Shaanxi University of Chinese Medicine.

Preparation of BHJRSTD extracts

Ginseng Radix (10 g), Rhizoma Anemarrhenae (18 g), Radix Glycyrrhizae Preparata (6 g), Gypsum Fibrosum (30 g) and japonica rice (9 g) were mixed in a classical dosage ratio used in the Han Dynasty. The mixture was soaked in distilled water (750 mL) for 30 min in a beaker (2,000 mL), then refluxed at 100 °C for 1 h twice under nitrogen protection in a round-bottomed flask (3,000 mL). The extraction solutions were combined and to be filtered, partial moisture was removed by rotary evaporator under reduced pressure. Then, the residuary solution was condensed to crude drug (2 g mL⁻¹), the extracts were stored at 4 °C and were brought to room temperature before use [8].

Sample preparation

1 mL of BHJRSTD extracts was vortexed for 30 s at 4 °C and centrifuged at 12,000 rpm for 15 min. Then take 200 μL of supernatant into an EP tube, added 1 mL of extract (methanol: water = 4:1); Vortexed for 30 s and sonicated in ice-water bath for 5 min; After standing at −40 °C for 1 h, the samples were centrifuged at 4 °C and 12,000 rpm for 15 min; The supernatant was carefully filtered through a 0.22 μm microporous filter and stored at −20 °C until assayed.

Instruments and conditions

LC-MS/MS analysis was performed on an Agilent ultra-high performance liquid chromatography 1290 UPLC system with a Waters UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm). The flow rate was set at 0.4 mL min⁻¹ and the sample injection volume was set at 5 μL. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The multi-step linear elution gradient program was as follows: 0–3.5 min, 95–85% A; 3.5–6 min, 85–70% A; 6–6.5 min, 70–70% A; 6.5–12 min, 70–30% A; 12–12.5 min, 30–30% A; 12.5–18 min, 30–0% A; 18–25 min, 0–0% A; 25–26 min, 0–95% A; 26–30 min, 95–95% A.

An Q Exactive Focus mass spectrometer coupled with an Xcalibur software was employed to obtain the MS and MS/MS data based on the IDA acquisition mode. During each acquisition cycle, the mass range was from 100 to 1,500, and the top three of every cycle were screened and the corresponding MS/MS data were further acquired. Sheath gas flow rate: 45 Arb, Aux gas flow rate: 15 Arb, Capillary temperature: 400 °C, Full ms resolution: 70,000, MS/MS resolution: 17,500, Collision energy: 15/30/45 in NCE mode, Spray Voltage: 4.0 kV (positive) or −3.6 kV (negative).

Animals and treatment

All the studies on animals were conducted in strict compliance in accordance with the Guide for the Care and Use of Laboratory Animals. The experimental protocol (2021-9-11) involving animals was reviewed and approved by the Institutional Animal Care and Use Committee of the Fourth Military Medical University. One-month-old, healthy, specific pathogen-free, Sprague Dawley rats weighing 120 ± 10 g were supplied by the Experimental Animal Center of Air Force Military Medical University. All rats were housed in a tidy animal room with constant temperature (23 °C ± 2 °C) and humidity (55% ± 10%), and a
12-h light/dark cycle. The rats had unrestricted access to water and food. After a week of feeding, all rats were randomly divided into two groups: (1) control group (n = 15); fed with normal diet and water; (2) model group (n = 60); fed with high-fat and high-sugar feed for two month. After these, model group rats were starved overnight and injected 40 mg kg\(^{-1}\) streptozotocin (STZ) to induce diabetes, and the control group was injected with an equal volume of citric acid-sodium citrate buffer solution (pH = 4.2). Seventy-two hours after injection, fasting for 12 h, blood glucose was measured, after one week, a re-examination was performed 1 week later. The fasting blood glucose (FBG) was greater than 11.1 mmol L\(^{-1}\) twice, and then the T2DM model was considered successfully established. T2DM rats were randomly divided into 4 groups: (1) T2DM (DM) model group (n = 15) (2) metformin (Met) group (200 mg kg\(^{-1}\)d\(^{-1}\), n = 15); (3) high-dose (H) BHJRSTD group (40 g kg\(^{-1}\)d\(^{-1}\), n = 15); (4) low-dose (L) BHJRSTD group (10 g kg\(^{-1}\)d\(^{-1}\), n = 15). The model groups were given high-fat and high-sugar diet every day, and the normal control (NC) group continued to be given the normal diet and water. Each group was administration through gastric gavage for one month to verify the hypoglycemic effect. Body weight, food consumption, and FBG levels were monitored and recorded during the administration period.

Collection and processing of test samples

After 8 weeks of administration, then all rats were killed, the excised pancreas tissues from the normal and experimental rats were immediately rinsed with normal saline and fixed in 4% paraformaldehyde. The tissues were cut into 5μm thick sections after embedding in paraffin and then stained with hematoxylin/eosin staining (HE) for histopathological examination.

Antioxidant activity

The CAT, SOD, GSH-Px are important ones of antioxidant defense systems. MDA is formed as an end product of lipid peroxidation. Briefly, portions of pancreatic tissue were homogenized in cool phosphate-buffered saline (pancreatic tissue to normal saline, 1:4). The levels of CAT, SOD, GSH-Px and MDA in pancreatic tissue was measured using a commercially available ELISA kit, according to the manufacturer’s instructions.

Statistical analysis

The results of the antioxidant assays were statistically processed using GraphPad Prism 6.0 (P < 0.05; Dunnett’s test) to determine significant differences between multiple groups.

RESULTS AND DISCUSSION

Identification of BHJRSTD extracts

To comprehensively characterize the BHJRSTD extract, an analytical method based on UHPLC Q-Exactive Focus Orbitrap MS was established in this research. Firstly, the prepared sample was injected into the UHPLC Q-Exactive Focus Orbitrap MS to obtain full-scan high-resolution MS data. Then, these data were processed using the Q Exactive Focus mass spectrometer’s control software (Xcalibur, Thermo Fisher Scientific) to acquire, detect and predict various types of molecules. Third, the MS\(^{+}\) of the predicted molecule was obtained in parallel reaction monitoring mode via UHPLC Q-Exactive Focus Orbitrap MS. Finally, compounds were identified by full scan MS data, MS\(^{+}\) data, retention times and references. Details of the 74 major compounds are shown in Table 1 and Fig. 1.

Effects of BHJRSTD on the histomorphology of pancreatic islets in T2DM rats

As shown in Fig. 2, the pancreatic islets in the NC group were oval in shape, with regular structure, different size of cell clusters, regular shape, complete structure, clear edge, and scattered among the pancreatic vesicles. The β-cells in pancreatic islets were abundant, plentiful in shape, uniform in size, and tightly arranged, no pathological changes were found. In contrast, the shape of pancreatic islets in the DM group was blurred, irregular, and structurally disordered. The β-cells in the pancreatic islets were deformed and swollen, and the cytoplasm was lightly stained. Compared with the pancreatic islets of the DM group, the number of islet β-cells in the MET group, H group and L group was significantly increased, meanwhile, the structure of β-cells was regular and no obvious necrosis was seen. The islet borders were clear and the morphological structure was improved.

Effects of BHJRSTD on the level of oxidative stress in T2DM rats

The human body is constantly exposed to different types of agents, resulting in the production of reactive species called free radicals [13], which lead to the oxidation of cellular machinery through the transfer of their free unpaired electrons [14]. In response to the harmful effects of these species, the body has acquired an endogenous antioxidant system or obtained exogenous antioxidants from the diet to
| Peak no. | Components name | Formula | InChIKey | RT (min) | Measured Mass ([m/z]) | Quasi-Molecular Ion ([m/z]) | Class |
|---------|-----------------|---------|----------|----------|-----------------------|----------------------------|-------|
| 1       | Isoleucine      | C6H13NO2 | AGPKZVTJTNPAG-UHFFFAOYSA-N | 0.75     | 132.1020527           | 132.102[M+H]+               | Amino acid |
| 2       | L-Proline       | C3H6NO2  | ONIBWKKTOPVIA-BYPZUCNCSA-N | 0.75     | 116.0706856           | 116.07[M+H]+               | Amino acid |
| 3       | Nicotinic acid  | C6H5NO2  | PYNIMMLHAYWP-UHFFFAOYSA-N | 0.87     | 124.0394663           | 124.039[M+H]+              | Vitamin  |
| 4       | L-Isoleucine    | C6H13NO2 | AGPKZVTJTNPAG-UHFFFAOYSA-N | 1.03     | 132.1020398           | 132.102[M+H]+              | Amino acid |
| 5       | L-Tryptophan    | C11H12N2O2 | QIVBCDIJAPG-VIPYBQESA-N | 2.34     | 205.0974881           | 205.097[M+H]+              | Organoheterocyclic compounds |
| 6       | Neomangiferin   | C25H28O16 | VUWOVGXVRYBSGI-UHFFFAOYSA-N | 2.62     | 585.1453811           | 585.145[M+H]+              | Xanthones |
| 7       | Mangiferin      | C19H18O11 | AEDDIBAIWPIIBD-ZJKJAXBQSA-N | 3.07     | 423.092836            | 423.092[M+H]+              | Xanthones |
| 8       | Isomangiferin   | C19H18O11 | CDYBOKJASDEORM-HBVDMOISA-N | 4.76     | 423.0929111           | 423.093[M+H]+              | Xanthones |
| 9       | Liquiritin      | C21H22O9  | DEMKZLAVQYISIA-UZQFATADSA-N | 5.30     | 441.114928            | 441.115[M+Na]+             | Flavonoids |
| 10      | Liquiritigenin  | C15H12O4  | FURUXTVZLHCCNA-AWEZNOCLCSA-N | 5.96     | 257.0808213           | 257.081[M+H]+              | Flavonoids |
| 11      | Licochalcone B  | C16H14O5  | DRDRYGIIYOPBBZ-XBXARRHUSA-N | 6.21     | 287.0911089           | 287.091[M+H]+              | Chalcones |
| 12      | Pseudoginsenoside F11 | C42H72O14 | JBGYSAVRIDZNKA-UHFFFAOYSA-N | 7.01     | 801.5010963           | 801.501[M+H]+              | Terpenoids |
| 13      | Isoliquiritigenin | C15H12O4 | DXDRHHKMWQZJHT-FPYGCLRLSA-N | 7.64     | 257.0808108           | 257.081[M+H]+              | Chalcones |
| 14      | Ginsenoside Rg1 | C15H12O4  | YURJSTAIMNSZAE-ILDRPQKSSA-N | 8.40     | 823.4817834           | 823.482[M+H]+              | Terpenoids |
| 15      | Kaempferide     | C16H12O6  | SQFSKOYWJBQGKQ-UHFFFAOYSA-N | 8.48     | 301.0710347           | 301.071[M+Na]+             | Flavonoids |
| 16      | Ginsenoside Rg3 | C16H12O13 | RXWIFXNRLCMLQCD-XXKJBRESA-N | 8.75     | 807.4895478           | 807.486[M+Na]+             | Terpenoids |
| 17      | Ginsenoside Rg5 | C14H10O2  | NJUXRKMKOFPXR-UHFFFAOYSA-N | 8.85     | 767.4950304           | 767.495[M+H]+              | Terpenoids |
| 18      | Glabrone        | C20H16O5  | COMLVFWKZOOP-UHFFFAOYSA-N | 9.11     | 337.1070099           | 337.107[M+H]+              | Terpenoids |
| 19      | Ginsenoside Re  | C18H12O18 | WPACOOSDJMUFQOKB-DAQSNDRCSA-N | 9.22     | 969.5380583           | 969.538[M+Na]+             | Terpenoids |
| 20      | Licoricesaponin G2 | C14H12O17 | WBPVQHPYRYEUEBQ-OJYVLISWSA-N | 9.29     | 839.4050082           | 839.405[M+H]+              | Terpenoids |
| 21      | Ginsenoside Rf  | C16H12O14 | UZIOUZHBHULYLDWE-KIOKIGKZSA-N | 9.59     | 801.4973674           | 801.497[M+H]+              | Terpenoids |
| 22      | Licollavone A   | C20H18O4  | HJURDBGPPIKERR-UHFFFAOYSA-N | 10.35    | 323.1278005           | 323.128[M+H]+              | Terpenoids |
| 23      | Chikusetsu saponin IVa | C16H10O14 | YOSRMIUOCHBEA-SNHRWUJAS-A-UHFFFAOYSA-N | 10.57     | 817.4358779           | 817.436[M+Na]+             | Terpenoids |
| 24      | Licochalcone A  | C21H22O4  | UZIOUZHBHULYLDWE-KIOKIGKZSA-N | 10.76    | 339.1586959           | 339.159[M+H]+              | Flavonoids |
| 25      | Sarsasapogenin  | C27H44O3  | GMBSQZIUCVWOCDD-WWASVFQGSA-N | 11.49    | 417.33681             | 417.337[M+H]+              | Terpenoids |
| 26      | Timosaponin A-III | C34H41O13 | MMTXWQXLMQLPACP-HRISIQGESA-N | 11.49    | 741.4428077           | 741.443[M+H]+              | Terpenoids |
| 27      | Ginsenoside Rk1 | C24H20O12 | KWDWBAISZWOAHD-MHOSXIPRSA-N | 12.35    | 789.4744795           | 789.474[M+Na]+             | Terpenoids |
| 28      | 20(S)-Ginsenoside Ck | C26H20O8  | FVIZARNDLVOMSU-IRFNNABS-N | 12.60    | 645.4331453           | 645.433[M+Na]+             | Terpenoids |

(continued)
| Peak no. | Components name | Formula | InChIKey                      | RT (min) | Measured Mass (m/z) | Quasi-Molecular Ion (m/z) | Class               |
|---------|-----------------|---------|-------------------------------|----------|---------------------|---------------------------|----------------------|
| 29      | L-Valine        | C_{5}H_{11}NO_{2} | KZSJWJFQEVHDFM-BYPYUZCNNSA-N | 26.14    | 118.0862706         | 118.086                   | [M+H]^+              | Amino acid          |
| 30      | Cinnamic acid   | C_{9}H_{8}O_{2} | WBYWAXHAXSNVQTXGWSA-N         | 2.58     | 147.045177          | 147.045                   | [M-H]^−              | Phenylpropanoids    |
| 31      | Phenylacetic acid | C_{8}H_{8}O_{2} | WLIYXDMOQOGPHL-UHFFAOYSA-N    | 2.63     | 135.045226          | 135.045                   | [M-H]^−              | Aromaticity          |
| 32      | Gallic acid     | C_{7}H_{6}O_{3} | LNTHTIQWEHAMDL-UHFFAOYSA-N    | 2.77     | 169.014344          | 169.014                   | [M-H]^−              | Phenols             |
| 33      | Cryptochlorogenic acid | C_{16}H_{18}O_{9} | GYFFKZTYYAPFCTR-UXIPKLUSA-N  | 3.16     | 353.088036          | 353.088                   | [M-H]^−              | Phenylpropanoids    |
| 34      | 2-Hydroxycinnamic acid, predominantly trans Daidzin | C_{21}H_{20}O_{9} | KYQZWONCHDNQDP-QNDHHXLSA-N    | 4.45     | 461.1109435         | 461.110                   | [M-H]^−              | Flavonoids           |
| 35      | Homoorientin    | C_{21}H_{20}O_{11} | ODBRNRZJSYPI-DVXVPBNA-N      | 4.58     | 447.094523          | 447.095                   | [M-H]^−              | Flavonoids           |
| 36      | Gentiopicroside | C_{16}H_{20}O_{9} | DUAGQYUORDTXOR-UHFFAOYNA-N    | 4.65     | 355.1038508         | 355.104                   | [M-H]^−              | Iridoids            |
| 37      | Gossypetin-8-C-glucoside Orientin | C_{16}H_{20}O_{13} | SXRVLZXMDCNG-UHFFAOYSA-N    | 4.75     | 479.0832296         | 479.083                   | [M-H]^−              | Flavonoids           |
| 38      | Pseudolaric acid B | C_{23}H_{28}O_{8} | VDGOFNYMYZUDT-VAFVZMPKSA-N   | 4.77     | 431.1691084         | 431.169                   | [M-H]^−              | Terpenoids           |
| 39      | Eleutheroside E | C_{34}H_{46}O_{18} | FFDULTAFAQRCT-UHFFAOYSA-N    | 5.00     | 787.2695132         | 787.270                   | [M-H]^−              | Phenylpropanoids    |
| 40      | Timosaponin B II | C_{45}H_{70}O_{29} | URYNWXJFQOCHMPCK-LXGDFEPSPA-N | 6.21     | 1227.566017         | 1227.566                  | [M-H]^−              | Terpenoids           |
| 41      | Atractyloside A | C_{21}H_{36}O_{10} | SQBLVYVWDWDM-BLJXOBSA-N      | 6.44     | 445.1151963         | 445.115                   | [M-H]^−              | Flavonoids           |
| 42      | Hesperetin      | C_{16}H_{12}O_{5} | FTVWIRXFLQPL-HOFFAOYNA-N     | 6.45     | 343.098513          | 343.099                   | [M-H]^−              | Flavonoids           |
| 43      | Ginsenoside F3  | C_{41}H_{70}O_{13} | QNVLVYVWDWDM-BLJXOBSA-N      | 6.47     | 431.1737251         | 431.174                   | [M-H]^−              | Flavonoids           |
| 44      | Terrestrosin K  | C_{31}H_{32}O_{14} | TVRRDUFXKROMDX-CXJPAHDSA-N   | 6.55     | 417.1155898         | 417.120                   | [M-H]^−              | Chalcones           |
| 45      | Naringenin      | C_{15}H_{12}O_{3} | FTVWIRXFLQPL-HOFFAOYNA-N     | 6.77     | 919.4922594         | 919.492                   | [M-H]^−              | Terpenoids           |

(continued)
neutralize these species and maintain the body’s homeostasis. Any imbalance between reactive species and antioxidants might cause a condition called “oxidative stress”, which can lead to the development of pathological conditions, one of which is diabetes [15]. The damage of these free radicals to cells can be quantified by measuring levels of MDA, which is diabetes [15]. The damage of these free radicals lead to the development of pathological conditions, one of which is diabetes [15].

In this study, the effect of BHJRSTD on the level of anti-oxidative stress in T2DM rats was evaluated via observing the contents of CAT, SOD, and GSH-Px in rat pancreatic tissue. Figure 3 illustrates that, compared with the NC group, the levels of CAT, SOD, and GSH-Px in the DM group were significantly decreased (P < 0.05), and the level of MDA was significantly increased (P < 0.05). Compared with the DM group, the levels of CAT, SOD and GSH-Px were significantly decreased (P < 0.05), and the level of MDA was significantly increased (P < 0.05). After administration of BHJRSTD extracts to rats for 8 weeks, the levels of SOD and GSH-Px in H and L groups were significantly increased (P < 0.05), and the level of MDA was significantly decreased (P < 0.05). The results indicated that BHJRSTD extracts had a certain anti-oxidative stress effect on pancreatic tissue of T2DM rats.

### Table 1. Continued

| Peak no. | Components name | Formula | InChIKey | RT (min) | Measured Mass (m/z) | Quasi-Molecular Ion (m/z) | Class |
|----------|-----------------|---------|----------|----------|---------------------|--------------------------|-------|
| 56       | Ginsenoside Rh1  | C_{62}H_{89}O_{9} | RAQNTCRNSXLYAH-UHFFFAOYSA-N | 7.99 | 683.4375706 | [M+HCOO]^- | Terpenoids |
| 57       | Kaempferol      | C_{28}H_{30}O_{6} | IYRMWWMYZSQQJKC-UHFFFAOYSA-N | 8.10 | 285.0404805 | [M-H]^+ | Flavonoids |
| 58       | Ginsenoside Rb1  | C_{52}H_{62}O_{23} | GZYPWOGIYAIIPV-UHFFFAOYNA-N | 8.54 | 1153.599607 | [M+HCOO]^- | Terpenoids |
| 59       | Ginsenoside Rc   | C_{52}H_{66}O_{22} | JDCPEQKWEDWQLI-UHFFFAOYSA-N | 8.67 | 1077.587843 | [M-H]^+ | Terpenoids |
| 60       | Ginsenoside Rg2  | C_{42}H_{63}O_{13} | AGBZIJAHARWNL-AUHFFFAOYNA-N | 8.75 | 783.4898787 | [M-H]^+ | Terpenoids |
| 61       | Ginsenoside Rg3  | C_{42}H_{62}O_{19} | NFZYDZXIKFHPGAT-QODHHTSITA-N | 8.80 | 955.4926865 | [M-H]^+ | Terpenoids |
| 62       | Ginsenoside Rb2  | C_{52}H_{66}O_{22} | NODILNFTIFURN-UHFFFAOYNA-N | 8.85 | 1123.595118 | [M+HCOO]^- | Terpenoids |
| 63       | Ginsenoside F1   | C_{52}H_{63}O_{9} | XNGWXFSJMQMC-UHFFFAOYNA-N | 8.96 | 683.4385504 | [M+HCOO]^- | Terpenoids |
| 64       | Araloside A      | C_{42}H_{29}O_{18} | KQSFNXMDCOFGW-UGBTYEQWQA-N | 9.03 | 925.4819032 | [M+HCOO]^- | Terpenoids |
| 65       | 3-[(Carboxycarbonyl) amino]-1-alanine | C_{52}H_{63}O_{5} | NEEQFPMRODIQKX-REOHCLBHSA-N | 9.43 | 174.9563911 | [M-H]^+ | Alkaloids |
| 66       | Isoanhydroicarin  | C_{52}H_{29}O_{6} | RJNATGWWPNKAL-UHFFFAOYSA-N | 9.79 | 367.1197283 | [M-H]^+ | Flavonoids |
| 67       | Icaritin         | C_{52}H_{29}O_{6} | TUXBSASAOJECY-UHFFFAOYSA-N | 10.24 | 367.117729 | [M-H]^+ | Flavonoids |
| 68       | Neobavaisoflavone | C_{32}H_{18}O_{4} | OGBPEBHYGIFUFB-UHFFFAOYSA-N | 10.36 | 321.14183 | [M-H]^+ | Flavonoids |
| 69       | xanthohumol      | C_{32}H_{22}O_{5} | ORXQGJKIUDPHEAYRNVLUSQSA-N | 10.57 | 353.1398336 | [M-H]^+ | Flavonoids |
| 70       | Inermin          | C_{52}H_{12}O_{3} | HUKSITUSUGIDC-ZBEGNZNMSA-N | 10.64 | 283.0616985 | [M-H]^+ | Flavonoids |
| 71       | Ginsenoside Rg3(S-FORM) | C_{42}H_{29}O_{13} | RWXIFXNRCLMOQCD-UHFFFAOYNA-N | 10.96 | 783.4903466 | [M-H]^+ | Terpenoids |
| 72       | Momordin Ic      | C_{42}H_{29}O_{13} | HWYBGIDROCPYOE-WEAQAMGWSA-N | 11.14 | 763.4297182 | [M-H]^+ | Terpenoids |
| 73       | Galbradin        | C_{30}H_{13}O_{4} | LBJQVLKGVZRIW-ZDUSSCGKSA-N | 11.32 | 323.1290457 | [M-H]^+ | Flavonoids |
| 74       | Mulberrin        | C_{30}H_{13}O_{6} | UWQYBLOHTQWQD-UHFFFAOYSA-N | 13.01 | 421.1659774 | [M-H]^+ | Flavonoids |
Fig. 1. UHPLC–Q/Orbitrap/MS/MS chromatograms of BHJRSTD extracts (total ion current; a: positive ion mode; b: negative ion mode)

Fig. 2. Hematoxylin and eosin staining of T2DM rats pancreatic tissue
CONCLUSION

In the present investigation, the potential chemical composition and antioxidant effect of BHJRSTD extracts were evaluated via UHPLC-Q/Orbitrap/MS/MS and ELISA kit. We identified 74 compounds, including flavonoids, alkaloids, chalcones, xanthones, phenols, phenylpropanoids, terpenes, triterpenes, terpenes, amino acid derivatives, etc. It obviously and increased the content of CAT, SOD, GSH-Px and reduced level of MDA in T2DM rats after 8 weeks of administration. This study provides some reference information for the clinical use of the BHJRSTD, and also provides some basis for the development of Chinese herbal medicine

ACKNOWLEDGEMENT

This research was supported by the Scientific Research Program Funded by Education Department of Shaanxi Provincial Government, Shaanxi Province, China (Grant No: 21JK0578) and Basic Research plan of Natural Science of Shaanxi Province, China (Grant No: 2021JQ-887, 2021JQ-888).

REFERENCES

1. Xu, Y. X. Z.; Xi, S.; Qian, X. Evaluating traditional Chinese medicine and herbal products for the treatment of gestational diabetes mellitus. *J. Diabetes Res.* 2019, 2019, 9182595.

2. Wang, J.; Ma, Q.; Li, Y.; Wang, M.; Wang, T.; Zhao, B. Research progress on Traditional Chinese Medicine syndromes of diabetes mellitus. *Biomed. Pharmacother.* 2020, 121, 109565.

3. Liu, H.-K.; Hung, T.-M.; Huang, H.-C.; Huang, H. C.; Lee, I.; Chang, C. C.; Cheng, J. J.; Huang, C. Bai-Hu-Jia-Ren-Shen-Tang decoction reduces fatty liver by activating AMP-activated protein kinase in vitro and in vivo. *Evidence-Based Complement. Altern. Med.* 2015, 2015, 651734.

4. Tian, Y.; Zhong, W.; Zhang, Y.; Zhou, L.; Fu, X.; Wang, L.; Xie, C. Baihu Jia Renshen Decoction for type 2 diabetic mellitus: a protocol for systematic review and meta-analysis. *Medicine (Baltimore)* 2020, 99(19) e20210–e20210.

5. Abdul-Ghani, M. A.; Tripathy, D.; DeFronzo, R. A. Contributions of β-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. *Diabetes Care* 2006, 29(5), 1130–9.

6. Caimi, G.; Carollo, C.; Presti, R. L. Diabetes mellitus: oxidative stress and wine. *Curr. Med. Res. Opin.* 2003, 19(7), 581–6.

7. Patil, V. S.; Patil, V. P.; Gokhale, N.; Acharya, A.; Kangokar, P. Chronic periodontitis in type 2 diabetes mellitus: oxidative stress as a common factor in periodontal tissue injury. *J. Clin. Diagn. Res.* 2016, 10(4), BC12–6.

8. Unoufin, J. O.; Lebelo, S. L. Antioxidant effects and mechanisms of medicinal plants and their bioactive compounds for the prevention and treatment of type 2 diabetes: an updated review. *Oxidative Med. Cell Longevity* 2020, 2020, 1356893.

9. Dembinska-Kiec, A.; Mykkänen, O.; Kiec-Wilk, B.; Mykkänen, H. Antioxidant phytochemicals against type 2 diabetes. *Br. J. Nutr.* 2008, 99(E-S1), ES109–17.

10. Qiao, X.; Li, R.; Song, W.; Miao, W. J.; Liu, J.; Chen, H. B.; Ye, M. A targeted strategy to analyze untargeted mass spectral data: rapid chemical profiling of Scutellaria baicalensis using ultra-high
performance liquid chromatography coupled with hybrid quadrupole orbitrap mass spectrometry and key ion filtering. J. Chromatogr. A 2016, 1441, 83–95.

11. Nardin, T.; Piasentier, E.; Barnaba, C.; Larcher, R. Targeted and untargeted profiling of alkaloids in herbal extracts using online solid-phase extraction and high-resolution mass spectrometry (Q-Orbitrap). J. Mass Spectrom. 2016, 51(9), 729–41.

12. Gómez, J.; Simirgiotis, M. J.; Lima, B.; Paredes, J. D.; Villegas Gabutti, C. M.; Gamarra-Luques, C.; Tapia, A. Antioxidant, gastroprotective, cytotoxic activities and UHPLC PDA-Q orbitrap mass spectrometry identification of metabolites in baccharis grisebachii decoction. Molecules 2019, 24(6), 1085.

13. Feinendegen, L. E. Reactive oxygen species in cell responses to toxic agents. Hum. Exp. Toxicol. 2002, 21(2), 85–90.

14. Asmat, U.; Abad, K.; Ismail, K. Diabetes mellitus and oxidative stress—a concise review. Saudi Pharm. J. 2016, 24(5), 547–53.

15. Wei, W.; Liu, Q.; Tan, Y.; Liu, L.; Li, X.; Cai, L. Oxidative stress, diabetes, and diabetic complications. Hemoglobin 2009, 33(5), 370–7.

16. Tiwari, B. K.; Pandey, K. B.; Abidi, A. B.; Rizvi, S. I. Markers of oxidative stress during diabetes mellitus. J. Biomarkers 2013, 2013, 378790.

17. Yaribeygi, H.; Sathyapalan, T.; Atkin, S. L.; Sahebkar, A. Molecular mechanisms linking oxidative stress and diabetes mellitus. Oxidative Med. Cell Longevity 2020, 2020, 8609213.

18. Djordjevic, A.; Spasic, S.; Jovanovic-Galovic, A.; Djordjevic, R.; Grubor-Lajsic, G. Oxidative stress in diabetic pregnancy: SOD, CAT and GSH-Px activity and lipid peroxidation products. J. Maternal-Fetal Neonatal Med. 2004, 16(6), 367–72.