Common key ingredient concluded in Baitouweng decoction, Gegen Qinlian decoction and Shaoyao decoction prevents radiation enteritis by regulating gut microbial network

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Author contributions

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
RE, radiation enteritis; GM, gut microbiota; HPLC, high performance liquid chromatography; TCM, traditional Chinese medicine; CA, Centella asiatica; BTWD, Baitouweng decoction; GQD, Gegen Qinlian decoction; SYD, Shaoyao decoction; TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor-β; IL-1β, interleukin-1β; IL-10, interleukin-10; BP, biological process; CC, cellular component; MF, molecular function; PPI, protein-protein interactions.

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**Highlights**
The current study excavated the similarities and differences of the effective active ingredients, targets, and possible signaling pathways involved in regulating GM after radiation to expound “different treatments for the same disease” in RE.

**Medical history of objective**
The three Chinese herbs, Baitouweng decoction (BTWD), Gegen Qinlian decoction (GQD), and Shaoyao decoction (SYD), derived from * Treatise on Febrile Diseases of Zhang Zhongjing of the Eastern Han Dynasty or Inner Canon of Huangdi from Pre Qin to Han Dynasty, are recorded in the consensus of TCM experts suggesting “different treatments for the same disease” in RE. In ancient times, BTWD, GQD and SYD are frequently used in ancient writings to treat diarrhea, stomach discomfort, and other ailments.

**Background**
Radiation enteritis (RE) is one of the common intestinal problems produced by radiation for malignant malignancies in the abdominal cavity, pelvic cavity, or retroperitoneum [1]. Intestinal diseases are widespread, making treatment difficult and ineffective [1]. RE might present as a chronic or acute condition. The acute form appears within hours to days of radiation exposure and usually disappears within a few weeks. Chronic RE is distinguished by increasing oblitative endarteritis with increased submucosal fibrosis. It might appear as tightening, fistula development, local abscesses, perforation, and bleeding [2]. One of every 5 radiation treatment patients will develop clinical indicators of RE [3].

The gut microbiota (GM) plays an essential role in radiation-induced RE [1]. The GM is the largest and most complex micro-ecosystem in the human body, with many functions. Gut microbial dysbiosis may contribute to the development of RE. The GM might offer a set of biomarkers for prediction, disease activity evaluation, and treatment selection in RE [4]. Abdominal radiation also changes the microbial composition. Radiation also reduces microbial diversity, as evidenced by the decrease in *Lactobacillus* spp. and *Bifidobacterium* spp., and the increase in *Escherichia coli* and *Staphylococcus* spp. Gut microbial dysbiosis aggravates RE, weakens intestinal epithelial barrier function, and promotes inflammatory factor expression. GM could be a potential biomarker for the disease [5]. Moreover, localized irradiation dramatically altered the gut microbial composition, resulting in a decreased ratio of *Bacteroidetes* to *Firmicutes* [6].

Traditional Chinese medicine (TCM) application has achieved a certain effect on RE. Prospective study investigated the effect of TJ-14 (herbal medicine) for RE [7]. Microarray analysis explores the underlying mechanisms of Tong-Xie-Yao-Fang-mediated efficacy for RE [8]. *Centella asiatica*, an herbal medication, is a potent radio-mitigator against radiation-induced enteritis. It restores the epithelial integrity that leads to leaky gut, alleviates endothelial dysfunction, and reduces radiation-induced enteritis [9]. Resveratrol could protect radiation-induced injuries in the intestines [10]. The interaction between GM and TCM is crucial for host health [11]. TCM theory of “different treatments for the same disease”, a treatment in TCM, refers to treating the same disease with different TCM prescriptions according to the patient’s actual situation, time, and place [12]. Liu et al. investigated 87bukang granules, Xianling Gubao capsules, and Er-xian decoction are widely employed in the clinic for osteoporosis therapy, in accordance line with the compatibility principle of “different treatments for the same disease” in herbal medicine [13]. In this study, the three Chinese herbs, Baitouweng decoction (BTWD), Gegen Qinlian decoction (GQD), and Shaoyao decoction (SYD), are recorded in the consensus of TCM experts suggesting “different treatments for the same disease” in RE [14]. However, the specific pharmaceutical ingredients and molecular mechanisms are still unclear. Herein, we aimed to investigate the specific pharmaceutical ingredients and molecular mechanisms of the three formulas on RE from the perspective of GM through multi-bioinformatics analysis and mouse experiment. These findings show that quercetin controlled the GM for RE therapy, which is a pharmaceutical substance and mechanism of “different treatments for the same disease” on RE. This study also offers methodological recommendations to provide a basis for clinicians to identify the different characteristics, trends, and prognosis of the same symptom, and thus, to prescribe in a personalized and dynamic manner to improve clinical efficacy.

**Methods and materials**

**Target library of RE and gut microbial dysbiosis**
The OMIM, DisGeNET, TTD, and GeneCards databases were used to search with “radiation enteritis”, “radiation intestinal injury”, and “radiation-induced intestinal injury” as keywords to obtain the target library of RE. The OMIM, DisGeNET, TTD, and GeneCards databases were used for the target library of gut microbial dysbiosis with the keywords “gut microbial dysbiosis” and “dysbiosis of gut microbiota”. The obtained targets have been merged using Excel software and the search deadline is as of July 18, 2022.

**Target library of the three herbal formulas**
The TCMSp database was searched for BTWD, GQD, and SYD using the screening criteria of oral utilization > 30% and > 0.018 to obtain herbal ingredients and potential targets of the three preparations [15]. Using Cytoscape 3.8.2, the correspondence was portrayed in the network. Venn plots were created using venny 2.1.0 to obtain the intersecting genes [16].

**GO and KEGG pathway enrichment analysis**
The intersecting genes were uploaded to the Database for Annotation, Visualization, and Integrated Discovery [17]. The gene identifier was selected as official gene symbol, and the species was set as *Homo sapiens*. Biological process (BP), cellular component (CC), and molecular function (MF) were annotated. Finally, the top 20 entries of GO and KEGG were selected for the graph or transformed into bubble plots.

**Protein-protein interactions (PPI)**
The intersecting genes were uploaded to the STRING database for protein interaction network construction. The species was set to “Homo sapiens”, the minimum interaction score was set to 0.4, and the other parameters were kept as default. The results were stored in TSV format, imported into Cytoscape 3.8.2, visualized the network. The node size and color were used to reflect the size of degree; the larger the node, the larger the value of degree. The thickness of the edge was used to reflect the size of the combined score; the thicker the edge, the larger the combined score. The key targets were selected to create the protein interaction network graph.

**Molecular docking**
The target proteins and ligand molecules were downloaded by searching the PDB database and TCMSp. The binding energy of ligands and receptors was calculated using the software AutoDockTools 1.5.6. The binding energy between the ligand and the receptor was less than −4.25, −5.0, and −7.0 kcal/mol, representing existence, mildly, and strong docking activity, respectively. The molecular docking results were visualized using VMD software.

**Literature**
The China biomedical literature data system, CNKI, VIP, Chinese science and technology journal database, and Wanfang database were searched using the keywords “radiation enteritis”, “radiation intestinal injury”, “radiation-induced intestinal injury”, “gut microbial...
dysbiosis,” and “dysbiosis of gut microbiota”. The relevant literature data (animal and clinical experiments using the same methods) were included for sorting and summary to provide scientific evidence of gut microbial dysbiosis in RE. The deadline for searching the above literature was September 20, 2022.

Content of quercetin in the three formulas using high performance liquid chromatography (HPLC)

BTWD contains Coptis chinensis, Phellodendron amurense, Fraxini Cortex, Pulsatilliae Radix (6:12:12:15). GQD contains licorice, Scutellariae Radix, Capsidis Rhihoma, Radix Pueraria (6:9:9:24). SYD contains Angelicae Sinensis Radix, Glycyrrhiza Radix et Rhihoma, Aucklandiae Radix, Scutellariae Radix, Capsidis Rhihoma, Paeoniae Radix Alba, Radix Rhe Et Rhihoma, Cimnannomi Cortex, Arecae Semen (15:6:6:15:15:30:9:5:6). The three formulas were bought from the Wuxi Hospital Affiliated to Nanjing University of Chinese Medicine and made into decoctions by the department of Chinese medicine at the hospital. Quantitative research on quercetin in Chinese medicine solutions were carried out using an HPLC approach (Welsh Ultimate PLUS C18 250 × 4.6 mm, 5 μm, DAD detector, flow rate 1 mL/min, column temperature 35°C, injection volume 5 μL). To create a standard solution with a final concentration of 1 mg/mL, a certain amount of quercetin standard solution was weighed, dissolved in methanol, and then machine tests were run. The chromatogram was then recorded. The solutions were uniformly mixed through vortex, and an appropriate amount was taken and filtered with a 0.22 μm filter membrane. The solutions were then tested on the machine, and the chromatogram was recorded. The collection and integration of chromatograms were processed by Chemstation software. Finally, in order to calculate the amount of quercetin present in the three formulations, we constructed a curve with peak area (y) and content (x).

Animal experiments

Animal grouping. With 10 mice in each group, 40 male SPF C57/B16J (12–14 weeks old, weighing 25–30 g) mice were randomly assigned to the following groups: normal control group (Control group), radiation group (Model group), radiation plus quercetin low concentration (50 mg/kg, Quercetin1), and radiation plus quercetin high concentration (100 mg/kg, Quercetin1). For three days, the mice, which were given by Beijing Vital River Laboratory Animal Technology Co., Ltd., were fed a free and adapted diet. The qualification certificate number of the experimental animal was SCXX2021-006, and the ethics number of the animal experiment was JN.No20220515c0360630[207]. The animal experiment was performed in line with the Guidelines for the Management of Use of Laboratory Animals by the Chinese National Institutes of Health.

Radiation. The experimental group of mice received a single 6 Gy dose of radiation using a 60 Co-irradiation device (model: GM–11–03–A). The radiation exposure for the mice lasted for a total of 6 min at a dosage rate of 1 Gy/min, at a distance of less than 30 cm from the radiation source.

Intervention. Mice in the intervention group received varying dosages of quercetin via gavage 30 min before radiation. Low concentrations of quercetin (50 mg/kg) or high concentrations of quercetin (100 mg/kg) were administered as interventions depending on the dose for human administration, whereas physiological saline intervention was supplied to the control group in the same quantities. Isoflurane anesthesia was utilized to prevent discomfort and collect materials after 7 days of operation.

Hematoxylin-eosin (HE) staining. A mouse from each group was randomly selected, and intestinal tissues were collected, routinely harvested, dehydrated, embedded, prepared, and stained with HE, observed and described under an optical microscope, and the lesion sites corresponding to different types in the main description were photographed.

Inflammatory cytokines. The mice were anesthetized with isoflurane using a small animal ventilator to avoid suffering. Blood was drawn from the eyeball and set in an aseptic blood collection vessel. It was centrifuged for 5 min at 3,000 rpm after 2–4 h. The supernatant was collected in a sterilized 1.5 mL EP tube for detection according to the operating instructions for the tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), interleukin-1β (IL-1β), interleukin-10 (IL-10) ELISA kits which were provided by Meimian Co., Ltd. (Yancheng, China).

16S rRNA analysis. Following the manufacturer’s instructions, microbial DNA was extracted from colon tissues using the E.Z.N.A.- Soil DNA kit (Omega Bio-tek Co., Ltd., Norcross, GA, USA). By using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific Co., Ltd., Wilmington, DE, USA), the final DNA concentration and purity were measured, and DNA quality was examined using 1% agarose gel electrophoresis. With the use of the thermocycler PCR equipment (GeneAmp 9700, ABI, Carlsbad, CA, USA) and the primers 338F (5′-ACTCTACGGGAGGCAGCAG-3′) and 806R (5′-GACTACHVGGGTWTCTAAT-3′), the V3-V4 hypervariable portions of the bacteria’s 16S rRNA gene were amplified. The PCR reactions were carried out using the AxyPrep DNA Gel Extraction kit (Axygen Biosciences Co., Ltd., Union City, CA, USA), further purified using it, and quantified using QuantifluorTM-ST (Promega, Madison, Wisconsin, USA) in accordance with the manufacturer’s instructions. According to the established methods, Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) pooled purified amplicons in an equimolar fashion and sequenced them (2 × 300) using an Illumina MiSeq platform (Illumina, San Diego, CA, USA). Operational taxonomic units (OTUs) were grouped with a 97% similarity criterion using the unique “greedy” technique of UPARSE 7.1, which concurrently conducts chimera screening and OTU clustering. Using a confidence level of 70%, the RDP Classifier algorithm examined the taxonomy of each 16S rRNA gene sequence against the Silva (SSU123) 16S rRNA database.

Real-time Quantitative PCR (qPCR) detection of key genes.

Following the manufacturer’s directions, total RNA isolation was obtained using Trizol reagent (Thermo Fisher Scientific, Waltham, MA, USA). Thermo Fisher Scientific's RNase-free DNase I was used to digest samples made up of 2 μL of total RNA from each group. Takara, Japan’s Transcriptor reverse transcriptase was then used to reverse-transcribe the samples into cDNA. A 20 μL final volume was used for the triplicate real-time PCR analysis (a CFX96 real-time PCR instrument, Bio-Rad). 10 μL of 2× SYBR Green qPCR SuperMix (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), 5 μL of cDNA, 4 μL of dhH2O, and 1 μL of primer mix were used in each experiment. The housekeeping control also made use of the Gapdh gene. Table 1 contains a list of the PCR primers.

Statistical analysis. A practical t-test and one-way ANOVA method for comparison between two groups or comparison between multiple groups were conducted, and statistical charts were created using GraphPad Prism software. All data were presented as means with standard error of the mean (SEM). The difference was deemed statistically significant if the P values were less than 0.05.

Results

Identify gut microbial dysbiosis and RE targets library

A total of 5,915 targets related to RE were identified in OMIM, DisGeNET, TTD, and GeneCards databases. Meanwhile, the OMIM, DisGeNET, TTD, and GeneCards databases contained 5,784, 5,915, 5,915, and 5,915 targets, respectively (Supplementary Figure S1). Moreover, 470 targets were obtained related to gut microbial dysbiosis. The OMIM, DisGeNET, TTD, and GeneCards contained 532, 4, 8, and 2 targets, respectively. Finally, we discovered 99 gut microbial dysbiosis targets related to RE (Supplementary Figure S1, Supplementary Table S1). This target library construction indicated the important role of CM in RE.

We discovered that BTWD consists of 31 active ingredients and 197 potential targets, after removing the duplicate items (Supplementary Table S2). The intersection with the targets of RE yielded 172 targets of BTWD involved in RE treatment (Figure 1A). Four targets, IL10, STAT1, IL1B, and IL1A, were involved in RE treatment through the
improvement of gut microbial dysbiosis (Figure 1A). We obtained that GQD consists of 122 active ingredients and 242 potential targets after removing the duplicate items (Supplementary Table S2). The intersection with the targets of RE yielded 208 targets of GQD involved in RE treatment (Figure 1B). Five targets, IL10, STAT1, IL1B, IL1A, and APOB, impacted dysbiosis (Figure 1B). We achieved that SYD consists of 184 active ingredients and 305 potential targets after removing the duplicate items (Supplementary Table S2), and 245 targets involved in RE treatment (Figure 1C). Five targets, IL10, STAT1, IL1B, IL1A, and APOB, were involved in RE treatment by influencing gut microbial dysbiosis (Figure 2). Three formulas have 169 common targets for RE treatment, and the network pharmacology diagram of the three formulas presents the correspondence (Figure 2). GM regulated four key targets, including IL10, STAT1, IL1B, and IL1A (Figure 2).

Table 1 Primers

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<tr>
<td>M-GAPDH-F</td>
<td>CCTCGTCCCGTACGACAAAAATG</td>
</tr>
<tr>
<td>M-GAPDH-R</td>
<td>TGAGGCTCAATGAAGGGGTCTG</td>
</tr>
<tr>
<td>M-IL1A-F</td>
<td>GTCGGGAGGAGAGCCTCTAA</td>
</tr>
<tr>
<td>M-IL1A-R</td>
<td>GTTTCTGCAACTCTTCAGC</td>
</tr>
<tr>
<td>M-STAT1-F</td>
<td>GATCGCTTGCCCAACTCTTG</td>
</tr>
<tr>
<td>M-STAT1-R</td>
<td>GCAGAGCTGAAACGACCTAGA</td>
</tr>
<tr>
<td>M-IL-1β-F</td>
<td>GCCACCTTTTGAGGATGAGG</td>
</tr>
<tr>
<td>M-IL-1β-R</td>
<td>GACAGCCAGGTCAAAGTT</td>
</tr>
<tr>
<td>M-IL-10-F</td>
<td>GTAGAAGTGTGCCCCAGG</td>
</tr>
<tr>
<td>M-IL-10-R</td>
<td>CACCTGGTCTTTGGAGGTTT</td>
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Figure 1 “Formula-herbs-compounds-targets” network pharmacology of the three formulas. (A) BTWD, Baitouweng decoction. (B) GQD, Gegen Qinlian decoction. (C) SYD, Shaoyao decoction.
Figure 2 Network pharmacology of the three formulas for RE. (A) Targets of three formulas for the treatment of RE. (B) "Formula-herbs-compounds-targets-disease" network pharmacology of the three formulas. (C) Common gut microbial targets of three formulas for the treatment of RE. (D) The Sankey diagram of the detailed herb-compounds-targets information. BTWD, Baitouweng decoction; GQD, Gegen Qinlian decoction; SYD, Shaoyao decoction.

Function enrichment analysis for 99 gut microbial dysbiosis targets related to RE
A total of 159 GO items were screened, of which 118 were related to BP, and 30 were selected using the standard of $P < 0.05$. It mainly involves positive gene expression regulation, negative cell proliferation regulation, immune responses, cell surface receptor signaling pathway, apoptosis, inflammatory response, and immune response regulation. There were 22 CC-related genes, including the lateral side of the plasma membrane, plasma membrane, neuronal cell body, extracellular body, nuclear plasma, and 19 of them were related to MF, including IgG binding, protein binding, interleukin-1 receptor binding, cytokine activity, low-affinity IgG receptor activity, cell adhesion molecule binding, and integrin binding (Supplementary Figure S2A).

A total of 30 KEGG pathways were enriched, including leishmaniasis, tuberculosis, osteoclast differentiation, inflammatory bowel disease, Staphylococcus aureus infection, hematopoietic cell lineage, N-Glycan biosynthesis, Fc gamma R-mediated phagocytosis, non-alcoholic fatty liver disease, cytokine-cytokine receptor interaction, JAK-STAT signaling pathway, C-type lectin receptor signaling pathway, systemic lupus erythematosus, measles, signaling pathways regulating pluripotency of stem cells, cell adhesion molecules, phagosome, various types of N-glycan biosynthesis, hepatitis B, protein processing in endoplasmic reticulum, Th17 cell differentiation, valine, leucine and isoleucine degradation, intestinal immune network for IgA production, neutrophil extracellular trap formation, endocrine and other factor-regulated calcium reabsorption, transcriptional misregulation in cancer, fatty acid metabolism, Alzheimer disease, chemical carcinogenesis-receptor activation, and Yersinia infection (Supplementary Figure S2B).

Function enrichment analysis of 169 common targets of three formulas for the RE treatment
A total of 7,002 GO entries were screened, 5,730 were related to BP, and 3,734 were screened for significantly enriched biological function major entries using $P < 0.05$ as the criterion. Supplementary Figure S3A presents the top 20 entries primarily involving the single-organism process, cellular process, biological regulation, stimulus-response, biological process regulation, metabolic process, positive biological process regulation, multicellular organismal process, signaling, developmental process, localization, and negative biological process regulation. Two hundred and eleven entries related to CC were screened for significantly enriched biological functions using $P < 0.05$ as the criterion, mainly with the presence of cell, cell part, organelle, organelle part, membrane, and other components.
Moreover, 801 were related to MFs, and 440 were screened for significantly enriched.

A pathway enrichment analysis was performed, and 245 KEGG pathways were enriched. A total of 169 pathways were screened for significant enrichment using $P < 0.05$, and the top 20 were visualized. Among them, AGE-RAGE signaling pathway in diabetic complications, fluid shear stress and atherosclerosis, bladder cancer, hepatitis B, prostate cancer, Kaposi sarcoma-associated herpesvirus infection, human cytomegalovirus infection, IL-17 signaling pathway, pancreatic cancer, TNF signaling pathway, hepatitis C, non-small cell lung cancer, platinum drug resistance, endocrine resistance, hepatocellular carcinoma, proteoglycans in cancer, cellular senescence, HIF-1 signaling pathway, and colorectal cancer (Supplementary Figure S3B).

**Function enrichment analysis for four GM-regulated targets of the three formulas for RE treatment**
A total of 1,647 GO entries were screened, of which 1,469 were related to BP, and 1,010 were screened for significantly enriched major entries using $P < 0.05$ as the criterion, mainly involving blood vessel morphogenesis, positive hemopoiesis regulation, blood vessel development, vasculature development, angiogenesis regulation, and response to lipopolysaccharide (Supplementary Figure S4A). A total of 100 CC-related entries were screened, and four were significantly enriched, mainly with mature chylomicron, chylomicron remnant, extracellular space, and intermediate-density lipoprotein particle (Supplementary Figure S4A). Fifteen entries, including mature chylomicron, chylomicron remnant, extracellular space, and intermediate-density lipoprotein particle, were screened for significantly enriched (Supplementary Figure S4A). Pathway enrichment analysis was performed, 39 pathways were screened for significant enrichment using $P < 0.05$, and the top 15 were visualized. They were mainly involved in inflammatory bowel disease, leishmaniasis, tuberculosis, pertussis, AGE-RAGE signaling pathway in diabetic complications, C-type lectin receptor signaling pathway, and osteoclast differentiation (Supplementary Figure S4B).

**PPI and key target analysis**
The interaction network of 99 gut microbial dysbiosis targets related to RE was analyzed, and then the dominant targets were screened by degree. The network result revealed 78 nodes and 186 edges and the top six targets, IL1B, IL10, CD34, STAT1, FGFR3A, and MTOR (Figure 3A, Supplementary Table S3). The PPI network of the three formula targets had 168 nodes and 2,993 edges, with the top 25 targets filtered based on degree value being AKT1, TP53, TNF, IL6, VEGFA, JUN, CASP3, IL1B, EGFR, MYC, ESR1, HIF1A, PTGS2, EGF, MMP9, PPAR, FOS, PTEN, CCND1, CXCL8, CCL2, ERBB2, IL10, MMP2, and HMOX1 (Figure 3B, Supplementary Table S3). ILB1, ILA1, IL10, and STAT1 were common targets of GM regulation (Figure 3C, Supplementary Table S3). Moreover, PPI based on 172 targets of BTWD involved in RE treatment was performed. There were 172 nodes and 3,022 edges, and the top 25 targets were filtered based on degree value as AKT1, TP53, TNF, IL6, VEGFA, JUN, CASP3, IL1B, EGFR, MYC, ESR1, PTGS2, HIF1A, EGF, MMP9, PPAR, FOS, PTEN, CCND1, CXCL8, CCL2, ERBB2, IL10, MMP2, and HMOX1 (Supplementary Figure S5A, Supplementary Table S3). The network of GQD targets involved in RE treatment had 208 nodes and 4,198 edges, with the top 25 targets filtered based on degree value: AKT1, TP53, TNF, IL6, JUN, VEGFA, CASP3, IL1B, MAPK3, MYC, EGFR, ESR1, HIF1A, PTGS2, MMP9, STAT3, EGF, PPAR, FN1, FOS, PTEN, CCND1, CXCL8, CAT, and CCL2 (Supplementary Figure S5B, Supplementary Table S3). There were 244 nodes and 5,056 edges in SYD treating RE target of the network, with the top 25 being AKT1, IL6, TNF, TP53, JUN, IL1B, CASP3, VEGFA, MAPK3, EGFR, STAT3, MYC, PTGS2, PPAR, ESR1, HIF1A, MMP9, EGF, CXCL8, FN1, FOS, CCL2, CAT, PTEN, and IL10 (Supplementary Figure S5C, Supplementary Table S3).

**Molecular docking**
The selected compounds queretin were further assessed for their stability and coherence with STAT1, IL1B, IL1A, and IL10. The binding energy was $-4.44, -6.22, -7.37$, and $-5.14$ kcal mol$^{-1}$, respectively. Other molecular docking results revealed that the binding energy of kaempferol coherence with STAT1, aloe-emodin, and aloe-emodin jointing IL1B were $-5.01, -6.06$, and $-6.88$ kcal mol$^{-1}$, respectively. The binding forces are hydrogen bonding and van der Waals forces (Figure 4, Table 2). The stability of the combination increases with the absolute value of the compound and the target's lowest binding energy. The molecular docking results revealed that small molecule ligands have the lowest binding energies, indicating a better binding environment and strong binding and high affinity between the corresponding ligands and receptors.

**Figure 3** Protein-protein interactions (PPI). (A) The PPI of the 99 gut microbial dysbiosis targets related to RE. (B) The PPI of the 169 common targets of three formulas for treating RE. (C) The PPI of common 4 GM regulated targets of the three formulas for treating RE.
Figure 4 Molecular docking. Active pockets and 3D structure of docking results of kaempferol, quercetin, aloe-emodin, and alpha-humulene with the common 4 GM regulated targets of the three formulas for the treatment of RE.

Table 2 Molecular docking result with binding energy

<table>
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<th>MOL ID</th>
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Verification of gut microbial dysbiosis related to RE
We firstly verified the above findings through five studies, including two animal experiments and three clinical studies (one for each abdominal tumor, cervical cancer, and gynecologic cancer to demonstrate the important role of GM in RE [4, 18–21]). By retrieving recently published experimental data relating to changes in the GM associated with RE, we found that animal and clinical experiments validating that radiation-induced RE results in altered GM abundance, with Bacteroidetes predominating (Table 3). These data indicate that GM plays an essential role in RE.

Further experimental verification
Content of quercetin in the three formulas using HPLC. To reveal the content of quercetin in the three formulas, we performed the HPLC on the BTWD, QGD, SYD with the quercetin standard. As shown in Figure 5, with the chromatogram of quercetin standard, the concentration of quercetin in the three formulations was quantified. We found the concentration of quercetin in GQD > SYD > BTWD (P < 0.001). The result indicates that quercetin does exist in the three formulas and is one of their common ingredients.

Active ingredient quercetin of the three formulas protects inflammatory response in radiation induced mice. To further reveal the research results of the previous work, quercetin, the common active ingredient of the three prescriptions, was selected for follow-up animal experiment. As a validation experiment, we used quercetin with a concentration gradient to prevent radiation induced mouse models, as our experimental design generally (Figure 6A). As shown in Figure 6B–6E, the serum inflammatory factor levels of TNF-α, TGF-β, and IL-1β in model group significantly increased compared with control group, while the level of IL-10 in model group significantly decreased. Compared with the model group, the levels of TNF-α, TGF-β, IL-1β of each administration groups decreased, and the IL-10 level increased, with a statistically significant difference. Thus,

Table 3 Changes of gut microbial community abundance in RE

<table>
<thead>
<tr>
<th>Samples</th>
<th>Intervention</th>
<th>Alterations in the GM composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feces of C57BL/6J mice [18]</td>
<td>A single dose of 6.5 Gy gamma ray at a rate of 1.0</td>
<td>↓ Bacteroides</td>
</tr>
<tr>
<td></td>
<td>Gy/min</td>
<td>↑ Proteobacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Bacteroidete</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Firmicutes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Actinobacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Verrucomicrobia</td>
</tr>
<tr>
<td>Feces of C57BL/6J mice [19]</td>
<td>High-dose abdominal precision radiation with a single</td>
<td>↑ Bacilli</td>
</tr>
<tr>
<td></td>
<td>dose of 10 Gy</td>
<td></td>
</tr>
<tr>
<td>Feces of patients [20]</td>
<td>Abdominal radiotherapy</td>
<td>↑ Actinobacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Clostridia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Proteobacteria</td>
</tr>
<tr>
<td>Feces of patients [4]</td>
<td>Pelvic radiotherapy</td>
<td>↑ Gammaprote-obacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ Coprococcus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Bacteroides</td>
</tr>
<tr>
<td>Feces of patients [21]</td>
<td>Pelvic radiotherapy, five times a week during a 5</td>
<td>↑ Gram-negative bacilli</td>
</tr>
<tr>
<td></td>
<td>weeks period</td>
<td>↑ Eubacteriaceae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Prevotellaceae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Oscillospiraceae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↓ Fusobacteriaceae</td>
</tr>
</tbody>
</table>

![Figure 5 Content of quercetin in the three formulas using HPLC. (A–D) Chromatogram of quercetin standard, BTWD, QGD, SYD. (E) Content of quercetin in the three formulas. **P < 0.001. BTWD, Baitouweng decoction; QGD, Gegen Qinlian decoction; SYD, Shaoyao decoction.](image-url)

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Figure 6 The common active ingredient quercetin of the three formulas protects inflammatory response in radiation induced mice. (A) Design diagram for experimental verification. (B–E) Changes in inflammatory factors. (F) Changes in HE pathology (50 μm). ***P < 0.001 vs control group, ###P < 0.001 vs model group, & P < 0.001.

The anti-inflammatory effect of low-dose quercetin group is better than that of high-dose quercetin group (Figure 6B–6E). Then, HE staining was used to reveal colon injuries intuitively. The colonic villi in the control group were neatly aligned, the mucosal surface was flat, and the crypts (black arrows, C) were parallel (Figure 6F). The mucosal layer and mucosal muscle layer were stripped in the model group, the colonic villi were disordered, the crypt structure was altered (black arrow, C), a large region of inflammatory cell infiltration (yellow arrow), and intracellular mucus production was substantially decreased (Figure 6F).

The colon morphology was restored to normal in the low-dose quercetin group, with the colonic villi placed reasonably orderly and flat, the crypts parallel (black arrow, C), and just a few inflammatory cells and edema apparent. The morphology of the colon recovered in the high-dose quercetin group, and the mucosal layer rupture was relieved, but the arrangement of colonic villi remained uneven and atrophic, and the crypt structure was disordered (black arrow, C) (Figure 6F). These results indicated that quercetin has significant preventive and protective effects on radiation-induced intestinal...
injury in mice. Changes in gut microbial composition of quercetin in radiation induced mice. To reveal the changing characteristics of GM, intestinal mucosal tissues were selected to continue 16S rDNA sequencing analysis. We found that evident changes in the number of OTUs in mice before and after quercetin intervention (Figure 7A). The result of PCoA on OTU level revealed there were significant differences among the microbial communities of control, model, quercetin L, and quercetin H group (Figure 7B). The box plot in Figure 7C represented the distribution dispersion of different groups on the PC1 axis, and the results showed significant differences in microbial communities among different groups. The columns of different colors represented the top phyla and genera, and the length of the columns represented the proportion of the phyla and genus (Figure 7D, 7E). The dominant phyla were Firmicutes, Proteobacteria, Bacteroidota in the four groups (Figure 7D). The dominant genera were Lachnospiraceae_NK4A136_group, Lactobacillus, Parabacteroides, unclassified_f_Lachnospiraceae, Turicibacter, Burkholderia-Caballeronia-Paraburkholderia, Ralstonia (Figure 7E). These results illustrated significant differences in microbial communities among different groups. Additionally, the detailed differential genera were found as Lachnospiraceae_NK4A136_group, unclassified_f_Lachnospiraceae, Clostridium_sensu_stricto_1, Bacteroides, norank_f_Ruminococcaceae, Lachnocostridiurn, Lachnospiraceae_UCG-006, NK4A214_group and GCA-900066575 in control, model, quercetin L and quercetin H groups (Figure 7F). All these findings explained the changes in gut microbial composition of quercetin in radiation induced mice. Correlations between the differential genera and inflammatory factors, signal transduction in KEGG pathways. The heat map showed the Spearman correlation coefficient between mucosal differential bacterial abundance and selected inflammatory factors levels, as well as enriched signaling pathways. The relationship between differential bacterial abundance and inflammatory factors after quercetin intervention was investigated by correlation thermogram analysis. Interestingly, the bacteria Lachnospiraceae_NK4A136_group enriched by quercetin after intragastric administration were positively correlated with the levels of TNF-α, TGF-β, and IL-1β, while negatively correlated with the level of IL-10 (Figure 8A). The bacteria Bacteroides enriched by quercetin after intragastric administration were negatively correlated with the levels of TNF-α, TGF-β, and IL-1β, while positively correlated with the level of IL-10 (Figure 8A). The relationship between 9 differential bacterial abundance and the enriched pathways after quercetin intervention was investigated by correlation thermogram analysis. The results showed the enriched pathways such as two-component system, HIF-1 signaling pathway, AMPK signaling pathway, phosphatidylinositol signaling system, PI3K-Akt signaling pathway, MAPK signaling pathway-plant, FoxO signaling pathway, MAPK signaling pathway-fly, phospholipase D signaling pathway, MAPK signaling pathway-yeast were related to many differential genera. Importantly, MAPK signaling pathway-plant and FoxO signaling pathway were found related to more than 6 or 7 of 9 differential genera (Figure 8B).
Changes in mRNA relative expression of the common four GM regulated targets of the three formulas for the treatment of RE. In order to further verify the genes closely related to quercetin found in previous bioinformatics analysis, we used qPCR to analyze the key genes in mouse colon tissues. As shown in Figure 9A, 9B, the mRNA relative expression of IL-10 and STATS in model group significantly decreased compared with control group, while the mRNA relative expression of IL-1A, IL-1B in model group significantly increased compared with control group. Compared with the model group, the mRNA relative expression of IL-10 and STATS of each administration group increased and the mRNA relative expression of IL-1A, IL-1B of each administration group decreased, with a statistically significant difference. All these findings verified the changes in mRNA relative expression of the common four GM regulated targets of the three formulas for the treatment of RE.

Discussion

Radiotherapy is a key treatment for malignancies that results in RE, a serious side effect [22]. Studies have demonstrated that GM affects cancer immunotherapy responses, maintaining a healthy microbiota to fight cancer and diseases and promoting gut healing [23–25].

TCM is closely related to gut microbial dysbiosis, especially its repair and regulation [26]. Gut microbial dysbiosis weakens intestinal permeability, attenuates intestinal barrier function, and loss of barrier integrity, and promotes inflammatory factor expression, inflammatory infiltration, and metabolic disorders [26]. Gut microbial dysbiosis can also aggravate radiological damage [5]. Chinese herbs have been used to cure various disorders for over a thousand years. Numerous studies have demonstrated that the external application of TCM for RE is a secure and affordable therapy [27–29]. The TCM theory of “different treatment for the same disease” is usually used for RE treatment in China. Based on the previous theory, multiple formulas exist for the same symptom. Therefore, it is vital to investigate new techniques for studying herbal formulae’s pharmacological foundation and molecular mechanism to provide a scientific justification. In view of the role of GM in intestinal radiation injury, we believe that by applying the theory of “different treatment for the same disease” to compare the similarities and differences of different herbal formulas, the more precise the correspondence between the evidence and herbal formulas can be, and the maximum advantage of different herbal formulas can be used to correct the microbial environment to treat and prevent RE.

Our study demonstrated that quercetin, common active ingredient in the three formulas (BTWD, GQD, and SYD) for RE through gut microbial targets, acts on the targets IL10, ILA1, ILB1, and STAT1, which are involved in AGE-RAGE signaling pathway in diabetic complications and C-type lectin receptor signaling pathway. However, the molecular docking results indicated that the maximum binding energy was greater than –4.25, suggesting that a potential therapeutic mechanism existed. Quercetin is a unique flavonoid with numerous biological effects, including anticancer, anti-inflammatory, and antiviral activities [30]. Extensive evidence confirmed that quercetin reduces intestinal oxidative stress, pro-inflammatory cytokines, and chemokines and improves the ability of the GM to rebuild. The results of the present investigation are consistent with the previous study. The active components of alpha-humulene and...

Aloe-emodin are unique to SYD target IL1B. SYD and GQD share the active ingredient licorice, whose STAT1 target is kaempferol. Bian et al. discovered that kaempferol supplementation improved intestinal barrier integrity by reducing the TLR4/NF-κB pathway activation and inhibiting intestinal inflammation [31]. Therefore, kaempferol supplementation can alleviate the GM and metabolic disorders caused by radiation injury [31]. Rhubarb can reduce acute lung injury by regulating the dysbiosis of GM in mice, but few studies have investigated which active ingredient in rhubarb is the active ingredient [32]. Few studies have reported the effect of cinnamon on GM regulation. However, many studies have confirmed the therapeutic effects, including antibacterial, antiviral, antioxidant, antitumor, anti-hypertensive, anti-hippoxic, anti-diabetic, gastroprotective, and immunomodulatory effects of cinnamon [33, 34]. Further analysis revealed that the active ingredient for its action is alpha-humulene, and the target of action is ILB1, which is involved in various inflammatory response signaling pathways [35].

Literature research, including animal and clinical experiments, validated the presence of gut microbial dysbiosis during the RE. Previous studies have demonstrated that Bacteroidetes are downregulated in GM after radiation intervention [36]. Moreover, Bacteroidetes is related to adaptive radiation and the intestinal tract superiorty bacteria [37]. The data suggested that GM plays an essential role in RE. GM is an inevitable link for Chinese herbal metabolism. Studies have shown that the three formulas (BTWD, GQD, and SYD) recommended by the public regulate GM composition [38–41]. The results are consistent with the previous studies. The three formulas improved the gut microbial dysbiosis by targeting IL1B, IL10, STAT1, and IL1A, which are related to the inflammatory bowel disease, leishmaniasis, tuberculosis, pertussis, AGE-RAGE signaling pathway in diabetic complications, C-type lectin receptor signaling pathway, and osteoclast differentiation. These findings suggest that the GM-host-related chain of evidence may be the scientific foundation of the theory of “different treatments for the same disease” on RE. Finally, we validated the results of our previous bioinformatics analysis using mouse experiments. Quercetin was preferably used for validation experiments. Interestingly, our mouse experiment results are consistent with previous bioinformatics analysis results.

There are still many shortcomings: (1) Lack of validation of clinical samples. The current study aimed to reveal the GM-host-related chain of evidence of the theory of “different treatments for the same disease” on RE through multi-bioinformatics analysis, literature research, and mouse experiment. Therefore, no clinical research has been designed due to the theoretical research of TCM involved. (2) Only three herbal formulas were selected as research objects to study the theory of “different treatments for the same disease” according to the guidelines. Additionally, analyzing the similarities and differences between the three formulas provides a basis for clinicians to prescribe individualized medical advice.

The current study excavated the similarities and differences of the effective active ingredients, targets, and possible signaling pathways involved in regulating GM after radiation to expand “different treatments for the same disease” in RE. Multi-bioinformatics analysis, literature research, and experiment revealed the GM-host-related chain of evidence for “different treatments for the same disease” on RE (Figure 10). The experiment of quercetin intervention in mice revealed that quercetin could regulate the composition and structure of GM and the expression of key genes to prevent RE in mice. Additionally, it provides a basis for clinicians to prescribe individualized medical advice and offer methodological guidelines and approaches for the contemporary use of and research into TCM therapeutic principles.

Figure 9 Changes in mRNA relative expression of the common four GM regulated targets of the three formulas for RE. *P < 0.05, **P < 0.01, ***P < 0.001. ns, no significance.
Figure 10 Gut microbiota-host-related insight of the three formulas on RE. BTWD, Baitouweng decoction; QGD, Gegen Qinlian decoction; SYD, Shaoyao decoction; IL-1β, interleukin-1β; IL-10, interleukin-10.

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