

Herbal and Natural Product Interventions in Animal Models of Antibiotic-Associated Diarrhea and Their Effects on Gut Microbiota: a protocol for systematic review

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Objectives: Antibiotic-associated diarrhea (AAD) is a frequent complication of antibiotic use and is commonly used to investigate gut microbiota dysbiosis and potential therapeutic interventions in animals. Herbal medicines and natural product-derived compounds have shown promising effects in restoring microbial balance; however, no systematic review has yet synthesized the preclinical evidence. Therefore, this review aimed to systematically identify, evaluate, and synthesize animal studies examining herbal and natural product interventions for AAD, with a particular focus on gut microbiota restoration and related functional outcomes.

Methods: This protocol has been registered in PROSPERO (CRD420251136553). A systematic search was performed in PubMed, Embase, Web of Science, Scopus, CNKI, and other major Korean medical databases from inception to the search date. Controlled preclinical studies that evaluated herbal or natural product interventions for AAD in animal models and reported gut microbiota outcomes were also included. Two reviewers independently screened the studies, extracted the data, and assessed the risk of bias.

Results: This systematic review was conducted in accordance with the PRISMA guidelines. The findings were synthesized narratively and, where appropriate, organized by intervention type, animal model, and microbiome analytic method.

Conclusion: This review systematically evaluates the effects of herbal and natural products on the gut microbiota in animal models of antibiotic-associated diarrhea. These findings provide foundational preclinical evidence to support microbiome-directed development of herbal, polysaccharide-based, and synbiotic interventions for antibiotic-associated dysbiosis.

Keywords: antibiotic-associated diarrhea, gut microbiota, herbal medicine, natural products, microbiome, systematic review

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INTRODUCTION

Antibiotic therapy is central to modern infection management; however, the global rise in antibiotic use has raised concerns regarding antimicrobial resistance and disruption of host-microbiota homeostasis [1]. One clinically significant consequence is antibiotic-associated diarrhea (AAD), which is

driven by antibiotic-induced dysbiosis. This condition is characterized by a loss of microbial diversity, depletion of short-chain fatty acids (SCFAs), disruption of the epithelial barrier, and mucosal immune imbalance, ultimately facilitating opportunistic pathogen overgrowth and delaying intestinal recovery [2-4].

Commensal microorganisms play an essential role in main-

taining mucosal immunity, in part by regulating the secretion of antimicrobial peptides and the production of immunoglobulin A (IgA) in gut-associated lymphoid tissues [5]. Consequently, therapies that restore microbial ecological balance and mucosal defense have attracted increasing interest for the management of AAD.

Gut microbiotas influence a wide range of physiological and pathological processes beyond gastrointestinal diseases, including immune and metabolic regulation and neurodevelopment, highlighting their systemic relevance [6–10]. Notably, microbial function, particularly SCFA production, is as important as microbiota composition, given the role of SCFAs in pathogen control, epithelial barrier maintenance, inflammatory regulation, and metabolic homeostasis [11–13]. Since antibiotics disrupt both microbial communities and SCFA metabolism, restoring microbiota-derived metabolic and immunological functions has emerged as a key therapeutic target in AAD.

Herbal and natural products have traditionally been used to treat gastrointestinal disorders. Meanwhile, for this review, herbal and natural products are defined as an overarching category encompassing plant-derived substances, including standardized extracts, isolated natural compounds, and polysaccharide-rich preparations. Emerging preclinical evidence suggests that plant-derived compounds, especially polysaccharides, can beneficially modulate the gut microbiome, enhance SCFA production, strengthen epithelial junctions, and improve mucosal immunity [14–16]. In particular, *Zingiber officinale* and *As-tragalus* polysaccharides have shown protective effects in AAD models. More recently, synbiotic strategies that combine herbal polysaccharides with probiotics have demonstrated synergistic improvements in microbial composition and barrier integrity [17], supporting the translational development of microbiota-directed natural therapeutics.

Despite the growing body of evidence, studies have used diverse herbal compositions, extraction methods, and micro-

biome assessment platforms [18, 19]. Moreover, many studies have focused primarily on immune or barrier markers, providing only limited systematic microbiome profiling via molecular sequencing. Therefore, a structured synthesis of animal evidence is needed to evaluate the effects of natural products on the microbiota and host responses in AAD.

This protocol describes a systematic review designed to evaluate the effects of herbal and natural products, including polysaccharide-based and synbiotic preparations, on gut microbiota composition and immune-metabolic pathways in animal models of AAD. Indeed, by prioritizing molecular microbiome outcomes (16S rRNA sequencing or metagenomics), this review aimed to describe the translational development of microbiota-modulating natural therapeutics for antibiotic-induced dysbiosis.

MATERIALS AND METHODS

1. Study design and registration

This protocol has been registered in PROSPERO (CRD 420251136553) and follows the PRISMA-P 2015 guidelines [20]. The completed systematic review is reported in accordance with the PRISMA 2020 guidelines. This review focuses on pre-clinical animal studies that evaluate herbal and natural product interventions for AAD.

2. Eligibility criteria

Eligible studies were controlled *in vivo* animal experiments that modeled AAD and evaluated herbal or natural product interventions using quantitative microbiota outcomes. Study eligibility was determined according to the pre-developed population, intervention, comparison, outcomes, and study (PICOS) design framework (Table 1).

Table 1. Eligibility criteria

Category	Inclusion	Exclusion
Study design	Controlled <i>in vivo</i> animal studies	Human studies, reviews, protocols, conference abstracts
Population	Antibiotic-induced diarrhea models	Non-AAD models (colitis, constipation, obesity, tumors)
Intervention	Single compounds; polysaccharide-rich preparations; multi-component herbal formulas; herbal–probiotic synbiotics	Probiotics alone; chemical drugs without a herbal basis; undefined crude mixtures
Comparator	Vehicle, no-treatment, antibiotic-only, positive controls	No comparator
Outcomes	Primary: gut microbiota (16S rRNA, metagenomics); Secondary: SCFAs, bile acids; TJ proteins, mucins, SIgA, cytokines	No microbiota data; qualitative microbiome reporting only
Language	No language restrictions	-

1) Inclusion criteria

(1) Population: Animal models of antibiotic-induced diarrhea, without restrictions on species, sex, or age.

(2) Intervention: Interventions were categorized for synthesis as single compounds, polysaccharide-rich preparations, multi-component herbal formulas, and herbal-probiotic synbiotics. While these categories are established to facilitate descriptive and interpretive clarity, all eligible studies were ultimately synthesized within a unified analytical framework rather than treated as strictly isolated sub-analyses.

(3) Comparator: Vehicle, no treatment, antibiotic only, or established positive controls (e.g., probiotics).

(4) Outcomes: Gut microbiota composition was assessed using 16S rRNA sequencing or metagenomics, and was treated as the primary outcome. Secondary outcomes included SCFAs, barrier proteins, cytokines, and markers of mucosal immunity. Functional and host-related outcomes were interpreted in relation to observed changes in the microbiota, rather than as inde-

pendent primary endpoints.

(5) Study design: Controlled *in vivo* studies published in peer-reviewed journals.

(6) Special note: Standardized multi-herbal formulas were analyzed separately owing to biological heterogeneity.

2) Exclusion criteria

(1) Human studies, *in vitro* studies, conference abstracts, reviews.

(2) Disease models other than AAD (e.g., colitis, IBS).

(3) Interventions without herbal or natural product components (e.g., probiotics alone, chemical drugs).

(4) Studies lacking extractable microbiome data.

3) Language

No language restrictions were applied, and non-English articles were included if extractable data were available.

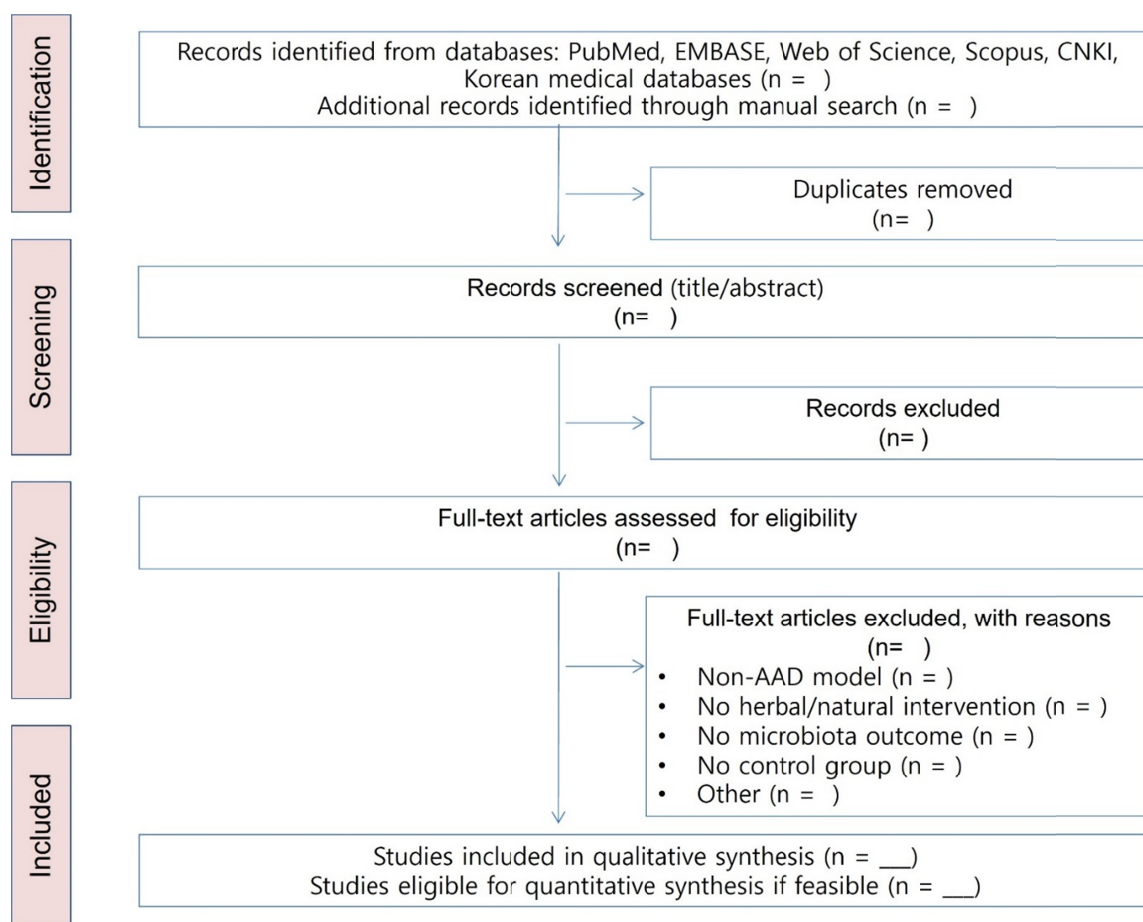


Figure 1. PRISMA flow diagram.

3. Information sources

The databases searched included PubMed/MEDLINE, EMBASE, Web of Science, Scopus, CNKI, and major Korean medical databases (OASIS, KISS, and KMBase) from inception through September 2025. A manual search of reference lists was also performed to complement the database search.

4. Search strategy

A comprehensive search strategy was developed using both MeSH and free-text terms. The full search strategies for all databases, including Embase, Scopus, and Web of Science, are provided as supplementary material upon completion of the review to ensure reproducibility.

PubMed example: (“antibiotic-associated diarrhea” OR “antibiotic-induced diarrhea” OR AAD OR dysbiosis) AND (“herbal medicine” OR herb* OR phytotherapy OR “natural product*” OR “plant extract*” OR polysaccharide OR ginseng OR “Zingiber officinale” OR “Astragalus”) AND (“gut microbiota” OR microbiome OR “16S rRNA” OR metagenom*) AND (animals[mh] NOT humans[mh]).

CNKI example: 主题=(抗生素相关性腹泻 OR 抗生素诱导性腹泻) AND 主题=(中药 OR 植物提取物 OR 多糖) AND 主题=(肠道微生物 OR 肠道菌群) AND 主题=(动物模型 OR 小鼠 OR 大鼠).

5. Study selection

Two reviewers (JHH and YKC) independently screened all retrieved records. Duplicate citations were removed, titles and abstracts were screened, and full texts were assessed against predefined eligibility criteria. Discrepancies were resolved through discussion and consensus. The study selection process is illustrated in the PRISMA flow diagram (Fig. 1).

6. Data extraction

Data were independently extracted by two reviewers using a standardized extraction form. Extracted information included study characteristics (author, year, funding), animal details (species, sex, age/weight), antibiotic regimens (agent, dose, route, duration), intervention characteristics (herbal component, extraction type, dose, route, duration), and microbiome assessment methods (16S rRNA sequencing or metagenomics).

Primary microbiome outcomes (alpha and beta diversity, and taxonomic composition) and secondary outcomes (short-chain fatty acids, bile acids, tight-junction proteins (ZO-1, occludin, claudin-1, and MUC2), cytokines (TNF- α , IL-6, IL-10), and SIgA) were also systematically collected (Table 2).

Data reported numerically were extracted, and graphical data were used only when values were clearly identifiable.

7. Risk of bias assessment

Risk of bias was evaluated using the SYRCLE tool, in accordance with our registered PROSPERO protocol (CRD 420251136553). Two reviewers independently assessed each domain and resolved discrepancies by consensus. These risk-of-bias assessments were used to contextualize and narratively weight study findings during data synthesis. Studies were not excluded solely based on risk-of-bias judgments; however, methodological limitations were explicitly considered when interpreting the strength and consistency of the evidence.

8. Data synthesis

Given the expected heterogeneity in animal models, antibiotic regimens, herbal preparations, and microbiome platforms, the findings were synthesized qualitatively, with subgroup analyses conducted where feasible. A meta-analysis was considered if sufficient methodologically comparable data were available. Where data were available, exploratory subgroup analyses were conducted by animal species (mouse vs. rat), antibiotic class, exposure duration, and microbiome analytical platform (16S rRNA sequencing vs. metagenomics).

Table 2. Planned data extraction structure

Category	Items
Study information	Author, year, funding source
Animal model	Species, strain, sex, age/weight
AAD induction	Antibiotic type, dose, route, duration
Intervention	Herbal or natural agent, preparation, dose, duration, route
Comparator	Vehicle, antibiotic-only, or positive control
Microbiota outcomes	α -diversity, β -diversity, taxa shifts (16S/metagenomics)
Secondary outcomes	SCFAs, barrier proteins, cytokines, SIgA
Key findings	Summary of main effects
Notes	Limitations and risk-of-bias elements

RESULTS

This systematic review was conducted in accordance with the PRISMA guidelines. The findings were synthesized qualitatively and summarized by intervention type, animal model, and microbiome analyses. Where feasible, subgroup analyses will be performed.

DISCUSSION

This systematic review synthesized preclinical evidence on herbal and natural products for antibiotic-associated diarrhea, with an emphasis on microbiota-centered outcomes. Although individual studies suggest that natural compounds can restore microbial diversity, enhance SCFA production, reinforce tight-junction integrity, and modulate mucosal immune signaling [3, 4, 14-17], the current evidence remains fragmented and methodologically variable. Therefore, a structured synthesis that clarifies therapeutic mechanisms, prioritizes promising candidates, and identifies key gaps for future research is warranted.

Thus, by integrating studies that employ molecular microbiome analyses [11, 21], this review provides a comprehensive overview of how natural products influence microbial ecology in AAD. In particular, polysaccharide-rich preparations and ginger-based interventions were examined as candidate intervention categories of interest, based on prior reports describing associations with mucosal immunity and microbial-metabolic pathways [14-17]. Meanwhile, evidence regarding synbiotic combinations was also explored, as existing studies report preliminary associations between combined herbal-probiotic interventions and microbiota- or host-related outcomes [17, 22, 23]. Nonetheless, we anticipate substantial heterogeneity among the included studies arising from differences in animal species, antibiotic regimens, herbal preparation methods, and microbiome sequencing platforms [3, 4, 17, 21, 24]. Therefore, the results are synthesized narratively, and subgroup analyses are considered where feasible to address this limitation. Additionally, application of the risk-of-bias tool by SYRCLE can help contextualize the findings and highlight methodological limitations within the current preclinical literature [25].

The findings of this review can support the rational development of future interventions that integrate traditional herbal medicines, modern polysaccharide research, and emerging synbiotic strategies. This study can also provide methodological guidance for designing animal studies and clinical translation

pipelines focusing on microbiome restoration in AAD and related dysbiosis-associated disorders.

Furthermore, this review can aid in identifying methodological gaps in preclinical microbiome research, including the need for standardization of antibiotic induction regimens, sequencing platforms, and reporting practices [21, 24], thereby promoting higher-quality experimental designs in future studies.

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AUTHORS' CONTRIBUTIONS

CYK and JHH conceived the study, developed the criteria, searched the literature, and analyzed the data; JHH wrote the protocol, and JHH and CYK revised the manuscript. All authors have read and approved the final manuscript.

ETHICAL APPROVAL

This study is a protocol for a systematic review of previously published animal studies. As it does not involve any new animal or human experimentation, ethical approval was not required.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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