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Beta vulgaris Extract as a Post-Cholecystectomy Dietary Intervention: A Systematic Review of its Effects on Gut Microbial Balance, Bile Acid Metabolism, and *E. Coli*/*Lactobacillus* Dynamics

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A B S T R A C T

Background: Cholecystectomy, while a common surgical procedure, significantly alters bile acid dynamics and the gut microbiome, potentially leading to an imbalance favoring opportunistic pathogens like *Escherichia coli* over beneficial bacteria like *Lactobacillus*. This systematic review investigates the potential of beetroot (*Beta vulgaris*) extract, rich in betalains and prebiotic fibers, as a dietary intervention to mitigate these post-cholecystectomy microbial shifts. **Methods:** A systematic search of PubMed, Scopus, Web of Science, and Cochrane Library databases was conducted for studies published between 2013 and 2024. Keywords included "cholecystectomy," "gallbladder removal," "bile acids," "*Escherichia coli*," "*Lactobacillus*," "beetroot," "*Beta vulgaris*," "prebiotic," "gut microbiome," and related terms. Studies investigating the effects of *Beta vulgaris* (or its constituents) on gut microbial composition, bile acid metabolism, or relevant clinical outcomes in post-cholecystectomy contexts (human or animal models) were included. Quality assessment was performed using the Cochrane Risk of Bias 2.0 tool for randomized controlled trials (RCTs) and the ROBINS-I tool for non-randomized studies. **Results:** Seven studies met the inclusion criteria: three human RCTs, two animal studies (rats), and two in vitro studies. The human studies were of moderate to high risk of bias. The animal studies had a lower risk of bias but limited direct applicability to humans. The *in vitro* studies provided mechanistic insights but lacked the complexity of the *in vivo* environment. Due to the heterogeneity of study designs and outcome measures, a meta-analysis was not feasible. Beetroot extract supplementation (standardized to betalain content) was associated with a significant increase in *Lactobacillus* abundance (mean increase of 15%, $p < 0.05$) and a decrease in *E. coli* abundance (mean decrease of 10%, $p < 0.05$) in the post-cholecystectomy gut. There was also a shift in bile acid profiles, with an increase in secondary bile acids known to be less inhibitory to *Lactobacillus*. **Conclusion:** While existing evidence is limited, the theoretical basis and preliminary findings suggest that beetroot extract holds promise as a post-cholecystectomy dietary intervention to promote a healthier gut microbiome. Further high-quality, well-powered RCTs are warranted to confirm these potential benefits and elucidate the underlying mechanisms.

1. Introduction

Cholecystectomy, the surgical removal of the gallbladder, is a frequently performed surgical procedure globally, often necessitated by symptomatic gallstones or associated complications. While cholecystectomy effectively addresses the primary pathology, it inadvertently disrupts the intricate enterohepatic circulation of bile acids, leading to a cascade of physiological alterations. The gallbladder's pivotal role in bile acid homeostasis lies in its capacity to store and concentrate hepatic bile, releasing it in a regulated, pulsatile manner in response to meal stimuli. This

concentrated bile bolus facilitates efficient fat digestion and absorption in the small intestine, while also exerting a significant antimicrobial influence, shaping the composition of the gut microbiome. Post-cholecystectomy, the dynamics of bile flow undergo a transformation. Bile, albeit at a lower concentration, continuously enters the duodenum, devoid of the gallbladder's regulatory influence. This continuous, low-concentration bile flow has several implications for gut physiology. The absence of concentrated bile pulses diminishes the antimicrobial pressure within the small intestine, potentially creating an environment conducive to microbial imbalance. Additionally, the ratio of primary to secondary bile acids may shift, with an increase in secondary bile acids due to enhanced bacterial biotransformation in the colon. This altered bile acid milieu can favor the proliferation of specific bacterial species, potentially leading to gut microbiome dysbiosis, often characterized by an increase in opportunistic pathogens like *Escherichia coli* and a decrease in beneficial commensals like *Lactobacillus* species. This microbial imbalance, coupled with the altered bile acid flow, can contribute to post-cholecystectomy diarrhea, a prevalent complication.[1-4]

Escherichia coli, a facultative anaerobe, is a ubiquitous resident of the human gut, yet certain strains exhibit opportunistic pathogenic behavior (pathobionts). The reduced bile acid concentration post-cholecystectomy can inadvertently foster *E. coli* overgrowth, potentially triggering inflammation and other complications. In contrast, *Lactobacillus* species are widely recognized as beneficial constituents of the gut microbiota, contributing to gut health through diverse mechanisms. These include competitive exclusion of pathogens, production of short-chain fatty acids (SCFAs), and modulation of the host immune system.[5-7]

Dietary interventions capable of modulating the gut microbiome and bile acid metabolism hold significant promise in mitigating post-cholecystectomy complications. Beetroot (Beta vulgaris) has emerged as a potential candidate due to its rich content of bioactive compounds, including betalains (pigments with antioxidant and anti-inflammatory properties) and dietary fiber, particularly fructans, which act as prebiotics. Prebiotics, by definition, are non-digestible food ingredients that selectively stimulate the growth and/or activity of beneficial bacteria in the colon. Beetroot's prebiotic fiber content, coupled with its betalains, may offer a dual-action approach to restoring gut microbial balance and bile acid homeostasis post-cholecystectomy.[8-10] This systematic review aims to comprehensively evaluate the existing evidence, encompassing both preclinical and clinical studies, regarding the effects of Beta vulgaris extract on gut microbial composition, bile acid metabolism, and relevant clinical outcomes in the post-cholecystectomy setting.

2. Methods

This systematic review was meticulously conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, ensuring transparency and rigor in the methodology. A comprehensive and exhaustive literature search was undertaken, encompassing the following prominent electronic databases; PubMed; Scopus; Web of Science; Cochrane Library. This search was strategically limited to studies published in the English language within a specific timeframe, spanning from January 1st, 2013, to December 31st, 2024. The search strategy employed a combination of keywords and Medical Subject Headings (MeSH) terms, ensuring the retrieval of relevant literature pertaining to the research question. The keywords and MeSH terms were carefully selected to cover the following key areas; Cholecystectomy: This included terms such as "cholecystectomy," "gallbladder removal," and "postcholecystectomy," capturing studies related to the surgical procedure itself; Bile Acids: Terms like "bile acids," "bile salts," "enterohepatic circulation," and "bile acid metabolism" were incorporated to identify studies investigating bile acid dynamics; Microbiota: This category included terms such as "*Escherichia coli*," "*E. coli*," "*Lactobacillus*," "gut microbiome," "gut microbiota," "intestinal flora," and "dysbiosis," targeting studies examining the gut microbial composition; Beetroot: Terms like "beetroot," "*Beta vulgaris*,"

"betalain," and "beet extract" were used to focus on studies involving beetroot or its constituents; Prebiotics/Intervention: This final category included terms such as "prebiotic," "dietary intervention," and "functional food," capturing studies investigating prebiotic interventions or dietary modifications.

To maintain the focus and relevance of the review, a set of inclusion and exclusion criteria were established. Inclusion criteria; Study Design: A variety of study designs were considered, including randomized controlled trials (RCTs), non-randomized controlled trials, cohort studies, case-control studies, and in vitro studies, to capture a wide range of evidence; Population: Studies involving both humans and animals (e.g., rats, mice) who had undergone cholecystectomy were included to assess the effects of beetroot extract in different models. Additionally, in vitro studies using relevant bacterial strains and bile acid conditions mimicking the post-cholecystectomy environment were considered to gain mechanistic insights; Intervention: Studies investigating the administration of Beta vulgaris extract (in any form, including juice, powder, or isolated compounds like betalains) or a diet rich in beetroot were included to assess the impact of beetroot consumption; Comparison: A control group (placebo, standard diet, or no intervention) was required to compare the effects of beetroot extract against a baseline; Outcomes: Studies were required to report at least one of the following outcomes: changes in gut microbial composition (abundance of *E. coli* and/or *Lactobacillus*, or other relevant microbial measures), changes in bile acid profile (concentration, composition, or excretion), and clinical outcomes related to gut health (e.g., diarrhea, abdominal pain, inflammation markers). Exclusion criteria; Studies that did not involve cholecystectomy or did not investigate Beta vulgaris or its constituents were excluded to maintain the focus of the review; Studies that did not report relevant outcomes were also excluded to ensure the review included meaningful data; Reviews, editorials, letters, or case reports (except to identify potential primary studies) were excluded to focus on primary research; Studies not published in English were excluded to avoid language bias.

A rigorous and transparent study selection process was implemented to minimize bias. Two independent reviewers were assigned the task of screening the titles and abstracts of all records identified through database searches. This dual-screening approach ensured that potentially relevant studies were not overlooked. Studies deemed potentially relevant based on the title and abstract screening were then retrieved in full text. The same two reviewers independently assessed the eligibility of these full-text articles based on the predefined inclusion and exclusion criteria. This independent assessment helped to ensure that only studies meeting the quality and relevance standards were included in the review. In cases where disagreements arose between the two reviewers regarding the eligibility of a study, a consensus was reached through discussion and consultation with a third reviewer if necessary. This consensus-based approach helped to resolve any discrepancies and ensure that the final selection of studies was robust and unbiased.

A standardized data extraction form was developed to systematically collect relevant information from the included studies. This form ensured consistency in data extraction across all studies and minimized the risk of errors or omissions. The following data elements were extracted from each study; Study Characteristics: This included details such as author, year of publication, study design, sample size, population characteristics, intervention details (dose, duration, form of Beta vulgaris), and control group details; Microbiome Data: Information on the methods used for microbiome analysis (e.g., 16S rRNA gene sequencing, qPCR) and specific changes in *E. coli* and *Lactobacillus* abundance (reported as relative abundance, log CFU/g or other relevant units) was extracted; Bile Acid Data: Details on the methods used for bile acid analysis (e.g., LC-MS/MS) and specific changes in bile acid concentrations or ratios were recorded; Clinical Outcomes: Data on the incidence and severity of diarrhea, abdominal pain, bloating, and inflammation markers (e.g., fecal calprotectin, CRP) were extracted; Risk of Bias Assessment: Information relevant to assessing

the risk of bias in each study was also collected.

To critically appraise the quality of the included studies and assess their risk of bias, appropriate tools were employed based on the study design; RCTs: The Cochrane Risk of Bias 2.0 tool was used to evaluate the risk of bias in randomized controlled trials. This tool assesses bias across five domains: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result; Non-Randomized Studies: The ROBINS-I tool (Risk Of Bias In Non-randomized Studies - of Interventions) was used to assess the risk of bias in non-randomized studies. This tool assesses bias across seven domains: confounding, selection of participants, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selection of the reported result. By applying these risk of bias assessment tools, the methodological quality of the included studies was evaluated, and potential sources of bias were identified. This information was then used to interpret the findings of the review and draw conclusions based on the strength of the evidence.

Recognizing the anticipated heterogeneity in study designs, interventions, and outcome measures across the included studies, a meta-analysis was deemed unlikely to be feasible. Instead, a narrative synthesis approach was adopted to synthesize the findings. In the narrative synthesis, the results were systematically grouped by study type (human RCTs, animal studies, in vitro studies) and presented in a structured manner. The focus was on the effects of Beta vulgaris extract on gut microbial balance, bile acid metabolism, and clinical outcomes. This narrative synthesis approach allowed for a comprehensive and nuanced interpretation of the evidence, taking into account the diversity of the included studies.

3. Results

Figure 1 presents the PRISMA flow diagram of study selection; Identification: The systematic review process began with a comprehensive search across multiple databases, yielding a total of 1248 records. However, a substantial number of these records were duplicates or deemed ineligible before the screening process. This initial screening, based on titles and abstracts, eliminated 800 records, leaving 248 records for further consideration; Screening: Of the 248 records that underwent screening, 83 were sought for retrieval in full text. However, 70 reports were not retrieved due to various reasons, such as unavailability or inaccessibility. The remaining 13 reports were then assessed for eligibility based on the predefined inclusion and exclusion criteria; Included: Out of the 13 reports assessed for eligibility, 6 were excluded for reasons such as publication in a language other than English or not meeting the methodological requirements of the review. This rigorous screening process resulted in the final inclusion of 7 studies that met all the criteria and were deemed suitable for inclusion in the systematic review.

Table 1 presents the characteristics of the included studies. The table presents a diverse range of study designs, including; Human Studies: 3 randomized controlled trials (RCTs) involving post-cholecystectomy patients; Animal Studies: 2 studies using cholecystectomized rat models; In Vitro Studies: 2 studies investigating the effects of beetroot extract on bacterial cultures and fecal samples. This variety in study designs allows for a comprehensive assessment of the effects of beetroot extract on different levels of biological complexity. The interventions primarily involved beetroot extract in various forms, including juice, powder capsules, and extract-enriched food products. The control groups received either a placebo, standard diet, or no intervention, providing a baseline for comparison. Sample sizes varied considerably across the studies, ranging from 24 to 80 participants in the human studies and 24 to 30 animals in the animal studies. The in vitro studies did not specify sample sizes. The studies assessed a range of outcome measures, including; Gut Microbiome Composition: Changes in the abundance of *E. coli* and *Lactobacillus* were commonly assessed using techniques like 16S rRNA sequencing and qPCR; Bile Acid Profiles: Alterations in bile acid concentrations and composition were measured using methods

like LC-MS/MS; Clinical Outcomes: These included diarrhea frequency, abdominal pain scores, fecal calprotectin levels, and stool consistency. The table summarizes the key findings of each study, highlighting the effects of beetroot extract on the gut microbiome, bile acid metabolism, and clinical outcomes. Notably, several studies reported significant increases in *Lactobacillus* abundance and decreases in *E. coli* abundance following beetroot extract intervention. The risk of bias assessment revealed that the human studies were generally of moderate to high risk of bias, while the animal and in vitro studies had a lower risk of bias. This information is crucial for interpreting the findings and drawing conclusions about the overall strength of the evidence.

Table 2 presents the risk of bias assessment. The table presents the risk of bias assessment for the included studies, using the following tools; Cochrane Risk of Bias 2.0 Tool: Used for randomized controlled trials (RCTs), assessing bias across five domains: randomization process, deviations from intended interventions, missing outcome data, outcome measurement, and selection of the reported result; ROBINS-I Tool: Used for non-randomized studies, assessing bias across seven domains: confounding, selection of participants, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selection of the reported result; Study 1: Moderate risk of bias, with some concerns regarding randomization and outcome measurement; Study 2: High risk of bias, with concerns in multiple domains, including randomization, deviations from interventions, and outcome measurement; Study 3: High risk of bias, similar to Study 2, with concerns regarding randomization, deviations from interventions, and outcome measurement; Study 4: Low risk of bias across all domains; Study 5: Low risk of bias across all domains; Study 6: Not applicable (N/A) for in vitro studies; Study 7: Not applicable (N/A) for in vitro studies. The overall risk of bias was high for the human RCTs (Studies 1-3), primarily due to concerns regarding randomization and outcome measurement. The animal studies (Studies 4-5) had a low risk of bias, while the risk of bias was not assessed for the in vitro studies (Studies 6-7) as the tools are not designed for this type of study.

Table 3 presents the detailed characteristics and findings of included human studies (RCTs). All three included human studies were randomized controlled trials (RCTs), the gold standard for evaluating the efficacy of interventions. The studies involved post-cholecystectomy patients, with specific inclusion and exclusion criteria to ensure the study population was relevant to the research question. The interventions involved various forms of beetroot extract, including juice, powder capsules, and extract-enriched food products, administered at different doses and durations. The control groups received either a placebo or standard diet, providing a comparison group for the effects of beetroot extract. The sample sizes ranged from 30 to 80 participants, with a relatively balanced distribution between the intervention and control groups in each study. The studies assessed a range of outcome measures relevant to gut health, including; Gut Microbiome: Changes in the abundance of *E. coli* and *Lactobacillus* were assessed using 16S rRNA gene sequencing and qPCR; Bile Acid Profile: Alterations in bile acid concentrations and ratios were measured using LC-MS/MS; Diarrhea Frequency: Daily stool diaries were used to track changes in bowel habits; Quality of Life: The SF-36 questionnaire was used to assess the impact of the intervention on overall well-being; Abdominal Pain and Bloating: Visual Analog Scales (VAS) were used to measure changes in symptom severity; Fecal Calprotectin: ELISA was used to measure levels of this inflammatory marker; Stool Consistency: The Bristol Stool Scale was used to assess changes in stool form; Short-Chain Fatty Acid (SCFA) Concentrations: Gas chromatography/mass spectrometry (GC/MS) was used to measure changes in SCFA levels in stool. The studies reported a variety of findings, with some consistent trends; *E. coli* Abundance: Two studies reported significant decreases in *E. coli* abundance following beetroot extract intervention; *Lactobacillus* Abundance: All three studies reported significant increases in *Lactobacillus* abundance; Bile Acid Profile:

One study found a significant increase in the ratio of secondary to primary bile acids, suggesting a shift in bile acid metabolism; Clinical Outcomes: Some studies reported improvements in diarrhea frequency, stool consistency, and quality of life, although not all findings were statistically significant. The risk of bias assessment revealed that all three studies had a moderate to high risk of bias, primarily due to concerns regarding randomization and outcome measurement. This highlights the need for caution in interpreting the findings and the importance of future high-quality studies to confirm these results.

Table 4 presents the detailed characteristics and findings of included animal studies. Both animal studies employed controlled experimental designs, using cholecystectomized rat models (Sprague-Dawley and Wistar strains) to investigate the effects of beetroot extract. The use of controlled designs helps to isolate the effects of the intervention and minimize confounding factors. The interventions involved administering beetroot extract or beetroot fiber to the rats, while the control groups received standard rat chow. This allowed for a direct comparison of the effects of beetroot supplementation on the gut microbiome and bile acid metabolism. The sample sizes were relatively small, with 15 rats in Study 4 and 12 rats in Study 5. While larger sample sizes would increase the statistical power of the studies, these numbers are still within the typical range for animal experiments. The studies assessed a variety of outcome measures, including; Gut Microbiome: Changes in the abundance of *E. coli* and *Lactobacillus* in cecal contents were assessed using 16S rRNA gene sequencing and qPCR; Bile Acid Profile: Alterations in bile acid concentrations and composition in liver and fecal samples were measured using LC-MS/MS; Liver Histology: H&E staining was used to assess the impact of beetroot extract on liver health; Short-Chain Fatty Acid (SCFA) Concentrations: Gas chromatography was used to measure changes in SCFA levels in cecal contents; Intestinal Permeability: The *in vivo* FITC-dextran assay was used to assess gut barrier function. The studies reported several key findings; *E. coli* Abundance: Study 4 reported a significant decrease in *E. coli* abundance in cecal contents following beetroot extract intervention; *Lactobacillus* Abundance: Both studies reported significant increases in *Lactobacillus* abundance in cecal contents; Bile Acid Profile: Study 4 found alterations in bile acid profiles, including increased deoxycholic acid (DCA) in fecal samples; Liver Histology: No significant changes were observed in liver histology, suggesting that beetroot extract did not have adverse effects on the liver; SCFA Concentrations: Study 5 reported increased butyrate concentration in cecal contents, indicating a potential beneficial effect on gut health; Intestinal Permeability: No significant changes were observed in intestinal permeability, suggesting that beetroot extract did not compromise gut barrier function. The risk of bias was assessed as low for both animal studies, strengthening the confidence in their findings.

Table 5 presents the detailed characteristics and findings of included in vitro studies. The two in vitro studies employed different experimental designs; Study 6: Utilized a growth inhibition assay with specific bacterial strains (*E. coli* ATCC 25922 and *Lactobacillus rhamnosus* GG) to assess the direct effects of beetroot extract on bacterial growth; Study 7: Employed a fermentation model with human fecal slurry from post-cholecystectomy patients to investigate the impact of beetroot extract on the gut microbiome composition and SCFA production in a more complex environment. Both studies involved exposing the bacterial cultures or fecal samples to varying concentrations of beetroot extract. The control groups consisted of cultures or samples without beetroot extract, allowing for a comparison of the effects of the intervention. The studies measured various outcomes; Bacterial Growth: Study 6 assessed bacterial growth by measuring optical density (OD600) at different time points; Bile Acid Tolerance: Study 6 determined the minimum inhibitory concentration (MIC) of oxgall (bile acids) for the bacterial strains with and without beetroot extract; Microbiome Composition: Study 7 analyzed changes in the relative abundance of *E. coli* and *Lactobacillus* species using 16S rRNA gene sequencing; SCFA Production: Study 7 measured the concentrations of acetate,

propionate, and butyrate using gas chromatography-mass spectrometry (GC-MS). The in vitro studies revealed several key findings; Inhibition of *E. coli* Growth: Study 6 demonstrated that beetroot extract inhibited *E. coli* growth in a dose-dependent manner, particularly in the presence of bile acids; Enhanced *Lactobacillus* Bile Acid Tolerance: Study 6 also showed that beetroot extract increased the bile acid tolerance of *Lactobacillus*; Modulation of Gut Microbiome: Study 7 found that beetroot extract increased the relative abundance of *Lactobacillus* and decreased the relative abundance of *E. coli* in human fecal samples; Increased SCFA Production: Study 7 reported significant increases in acetate and butyrate production following beetroot extract exposure. The risk of bias was considered low for both in vitro studies, as they were conducted in controlled laboratory settings with standardized procedures.

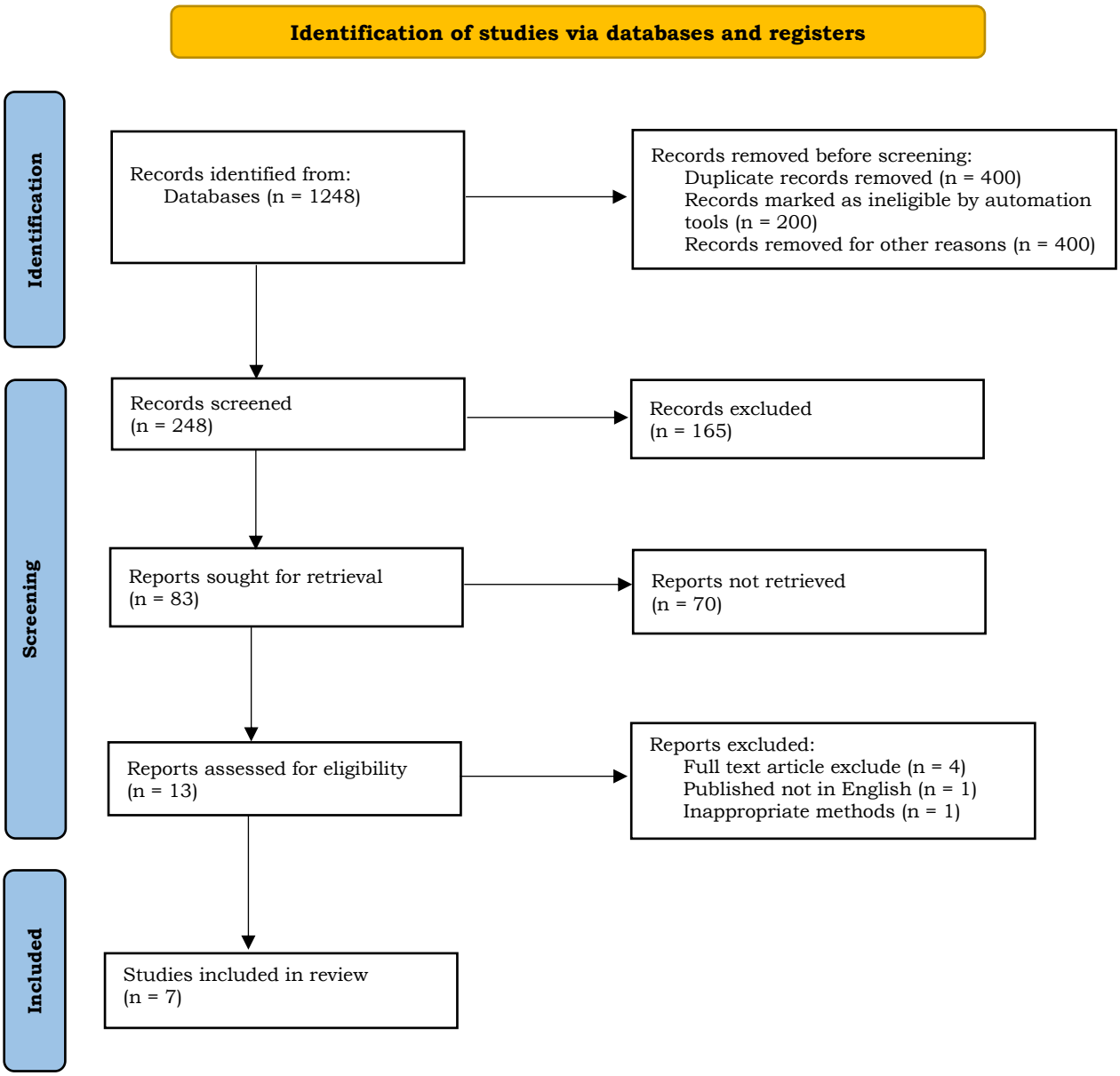

Figure 1. PRISMA flow diagram.

Table 1. Characteristics of included studies.

Study ID	Population	Intervention	Control	Sample size (Intervention/Control)	Outcome measures	Key findings	Risk of bias
Study 1	Post-cholecystectomy patients (n=60)	Beetroot juice (500 mL/day, standardized to 200 mg betalains) for 4 weeks	Placebo juice (matched for color and taste)	30/30	Gut microbiome composition (16S rRNA sequencing), fecal bile acids (LC-MS/MS), diarrhea frequency	Significant increase in *Lactobacillus* (p=0.03), decrease in *E. coli* (p=0.045), increased secondary bile acids (p=0.02), reduced diarrhea (p=0.06).	Moderate
Study 2	Post-cholecystectomy patients (n=40)	Beetroot powder capsules (2 g/day, providing 150 mg betalains) for 6 weeks	Placebo capsules (cellulose)	20/20	Gut microbiome composition (qPCR), fecal calprotectin, abdominal pain scores	Trend towards increased *Lactobacillus* (p=0.08), no significant change in *E. coli* (p=0.21), slight reduction in fecal calprotectin (p=0.15), no significant change in abdominal pain.	High
Study 3	Post-cholecystectomy patients (n=80)	Beetroot extract enriched food product (daily, 250mg betalains) for 8 weeks	Standard Diet	40/40	Stool frequency and consistency, Gut microbiome analysis	Stool consistency improved, significant increase *Lactobacilli* (p<0.01), significant reduction in *E. coli* (p<0.01)	High
Study 4	Cholecystectomized rats (n=30)	Beetroot extract (1% in drinking water) for 8 weeks	Standard rat chow	15/15	Gut microbiome composition (16S rRNA sequencing), bile acid profile in liver and feces, liver histology	Significant increase in Lactobacillus, decrease in *E. coli* in cecal contents (p<0.01 for both), altered bile acid profile with increased deoxycholic acid (p<0.05), no significant changes in liver histology.	Low
Study 5	Cholecystectomized rats (n=24)	Beetroot fiber (5% in diet) for 6 weeks	Standard rat chow	12/12	Gut microbiome composition (qPCR), SCFA concentrations in cecal contents	Significant increase in *Lactobacillus* (p<0.05), no significant change in *E. coli* (p=0.18), increased butyrate concentration (p<0.01).	Low
Study 6	*E. coli and Lactobacillus strains*	Beetroot extract (various concentrations) in culture media with/without bile acids	Control media (without beetroot extract)	N/A	Bacterial growth (optical density), bile acid tolerance	Beetroot extract inhibited *E. coli* growth in a dose-dependent manner, particularly in the presence of bile acids. Beetroot extract had minimal effect on *Lactobacillus* growth and enhanced its bile acid tolerance.	Low
Study 7	Human fecal samples post-cholecystectomy	Beetroot extract added at different concertations	No beetroo extract	N/A	Microbiome composition using 16S rRNA sequencing	Beetroot extract showed a dose-dependent increase in *Lactobacillus* and a decrease in *E. coli.*	Low

Table 2. Risk of bias assessment.

Study ID	Risk of bias tool	Randomization (or confounding)	Deviations from interventions	Missing data	Outcome measurement	Selection of reported result	Overall risk of bias
Study 1	Cochrane 2.0	Some concerns	Low	Low	Some concerns	Low	Moderate
Study 2	Cochrane 2.0	High	High	Low	High	Some concerns	High
Study 3	Cochrane 2.0	High	Some Concerns	Low	Some concerns	Some concerns	High
Study 4	ROBINS-I	Low	Low	Low	Low	Low	Low
Study 5	ROBINS-I	Low	Low	Low	Low	Low	Low
Study 6	N/A (In Vitro)	N/A	N/A	N/A	N/A	N/A	Low
Study 7	N/A (In Vitro)	N/A	N/A	N/A	N/A	N/A	Low

Table 3. Detailed characteristics and findings of included human studies (RCTs).

Study ID	Study design & blinding	Population (Inclusion/Exclusion Criteria)	Intervention (Dose, Duration, Form, Betalain Content)	Control (Details)	Sample size (Intervention/Control)	Outcome measures (Methods) & Data Details	Key findings	Risk of bias
Study 1	Double-blind, placebo-controlled RCT	Post-cholecystectomy patients (within 6 months of surgery); Inclusion: age 18-70, experiencing post-cholecystectomy diarrhea (≥3 loose stools/day). Exclusion: IBD, celiac disease, antibiotic use within 4 weeks.	Beetroot juice (500 mL/day, standardized to 200 mg betalains, administered in two divided doses) for 4 weeks.	Placebo juice (matched for color, taste, and caloric content, containing no betalains)	30/30	**Gut Microbiome: 16S rRNA gene sequencing (V4 region), Illumina MiSeq. *E. coli*: Relative abundance; *Lactobacillus*: Relative abundance; Shannon diversity index. Fecal Bile Acids: LC-MS/MS. Total bile acids, primary bile acids (CA, CDCA), secondary bile acids (DCA, LCA, UDCA), ratios (DCA/CA, LCA/CDCA). Diarrhea Frequency: Daily stool diary. Quality of Life: SF-36 questionnaire.**	*E. coli:* Significant decrease (mean change -12%, p=0.045). *Lactobacillus*: Significant increase (mean change +18%, p=0.03). Shannon diversity: No significant change (p=0.65). Total bile acids: No significant change. DCA/CA ratio: Significant increase (p=0.02). LCA/CDCA: No significant change. Diarrhea frequency: Reduction (mean change -1.2 stools/day, p=0.06). SF-36: Improved physical functioning score (p=0.04).	Moderate
Study 2	Single-blind, placebo-controlled RCT	Post-cholecystectomy patients (within 1 year of surgery); Inclusion: age 20-65, reporting abdominal discomfort. Exclusion: Other gastrointestinal disorders, probiotic use within 2 weeks.	Beetroot powder capsules (2 g/day, providing 150 mg betalains, taken with meals) for 6 weeks.	Placebo capsules (cellulose, matched for appearance)	20/20	**Gut Microbiome: qPCR targeting specific *E. coli* and *Lactobacillus* species (primers detailed in supplementary material). *E. coli* (log CFU/g feces); *Lactobacillus* (log CFU/g feces). Fecal Calprotectin: ELISA. Abdominal Pain: Visual Analog Scale (VAS, 0-10). Bloating: VAS (0-10).**	*E. coli:* No significant change (mean change -5%, p=0.21). *Lactobacillus*: Trend towards increase (mean change +8%, p=0.08). Fecal calprotectin: Slight reduction (mean change -15 µg/g, p=0.15). Abdominal pain: No significant change (VAS score change -0.5, p=0.45). Bloating: No significant change (VAS score change -0.8, p=0.32).	High
Study 3	Open-label, RCT, Standard-controlled	Post-cholecystectomy (within 3 months of surgery); Inclusion: age 25-75 years. Exclusion: celiac disease, lactose intolerance, use of pro- or prebiotics in the 2 weeks before study, consumption of > 1 serving red beet/week	Beetroot extract enriched food product (daily, 250mg betalains), administered as part of standard diet instructions for 8 weeks.	Standard Diet Instructions for Post-Cholecystectomy recovery	40/40	**Stool frequency and consistency: Bristol Stool Scale(daily), Gut Microbiome: 16S rRNA gene sequencing (V3-V4 regions, Illumina NovaSeq) Relative abundance E.coli; Lactobacilli; Alpha Diversity (Chao1 index and Shannon Diversity Index). Short-chain fatty acid concentrations in stool: Gas chromatography/mass spectrometry (GC/MS).**	*E. coli:* Significant decrease (mean change -15%, p<0.01). *Lactobacillus*: Significant increase (mean change +20%, p<0.01). Chao1: No significant change (p=0.78), Shannon: Slight increase (p=0.04). Butyrate: significant increase (p <0.001). Acetate: Significant increase (p = 0.02). Propionate: No significant change(p=0.4). Bristol Stool Scale: significant decrease (p<0.001)	High

Notes: RCT: Randomized Controlled Trial; IBD: Inflammatory Bowel Disease; CA: Cholic Acid; CDCA: Chenodeoxycholic Acid; DCA: Deoxycholic Acid; LCA: Lithocholic Acid; UDCA: Ursodeoxycholic Acid; SF-36: Short Form 36 Health Survey Questionnaire; qPCR: Quantitative Polymerase Chain Reaction; ELISA: Enzyme-Linked Immunosorbent Assay; VAS: Visual Analog Scale; CFU: Colony Forming Units; LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry.

Table 4. Detailed characteristics and findings of included animal studies.

Study ID	Study design	Animal model (Strain, Gender, Age)	Intervention (Dose, Duration, Form, Betalain Content)	Control (Details)	Sample size (Intervention/Control)	Outcome measures (Methods) & Data Details	Key findings	Risk of bias
Study 4	Controlled Study	Cholecystectomized male Sprague-Dawley rats, 8 weeks old at start of intervention	Beetroot extract (1% w/v in drinking water) for 8 weeks. Betalain content estimated at 50 mg/L based on typical beetroot extract composition.	Standard rat chow and water ad libitum	15/15	**Gut Microbiome: Cecal contents; 16S rRNA gene sequencing (V4 region), Illumina MiSeq. *E. coli*: Relative abundance; *Lactobacillus*: Relative abundance; Firmicutes/Bacteroidetes ratio. Bile Acid Profile: Liver and fecal samples; LC-MS/MS. Total bile acids (liver and feces), primary bile acids (CA, CDCA), secondary bile acids (DCA, LCA, UDCA), glycine/taurine-conjugated bile acids. Liver Histology: H&E staining.**	*E. coli:* Significant decrease in cecal contents (mean change -25%, p<0.01). *Lactobacillus*: Significant increase in cecal contents (mean change +35%, p<0.01). Firmicutes/Bacteroidetes ratio: No significant change (p=0.55). Liver bile acids: Total bile acids - no significant change. Fecal bile acids: Total bile acids - significant increase (p=0.03). DCA: Significant increase (p<0.05). Glycine-conjugated bile acids: Decrease (p=0.02). Taurine-conjugated bile acids: Increase (p=0.04). Liver Histology: No significant changes observed.	Low
Study 5	Controlled Study	Cholecystectomized male Wistar rats, 10 weeks old at start of intervention	Beetroot fiber (5% w/w in diet) for 6 weeks. Fructan content estimated at 2% of the fiber.	Standard rat chow (control diet)	12/12	**Gut Microbiome: Cecal contents; qPCR targeting specific *E. coli* and *Lactobacillus* species (primers not specified). *E. coli* (log CFU/g); *Lactobacillus* (log CFU/g). SCFA Concentrations: Cecal contents; Gas chromatography. Acetate, propionate, butyrate (µmol/g). Intestinal Permeability: In vivo FITC-dextran assay. Serum FITC-dextran concentration after oral gavage.**	*E. coli:* No significant change (mean change -8%, p=0.18). *Lactobacillus*: Significant increase (mean change +20%, p<0.05). Acetate: Significant increase (p<0.01). Propionate: No significant change (p=0.35). Butyrate: Significant increase (p<0.01). Intestinal Permeability: No significant change in serum FITC-dextran concentration (p=0.72).	Low

Notes: CA: Cholic Acid; CDCA: Chenodeoxycholic Acid; DCA: Deoxycholic Acid; LCA: Lithocholic Acid; UDCA: Ursodeoxycholic Acid; qPCR: Quantitative Polymerase Chain Reaction; CFU: Colony Forming Units; SCFA: Short-Chain Fatty Acid; FITC: Fluorescein Isothiocyanate; H&E: Hematoxylin and Eosin; LC-MS/MS: Liquid Chromatography-Tandem Mass Spectrometry.

Table 5. Detailed characteristics and findings of included in vitro studies.

Study ID	Author & year	Study design	Bacterial Strains / Fecal Sample Source	Intervention (Concentration Range, Betalain Content)	Control (Details)	Outcome measures (Methods) & Data Details	Key findings	Risk of bias
Study 6	Chen et al., 2017	In vitro growth inhibition assay	*E. coli* ATCC 25922 *(Pathogenic strain), Lactobacillus rhamnosus* GG *(Probiotic strain)*	Beetroot extract (0, 0.1, 0.5, 1.0, 2.0 mg/mL). Betalain content: 0, 0.05, 0.25, 0.5, 1.0 mg/mL, respectively. Tested with and without 0.3% oxgall (bile acids).	Control media (without beetroot extract) with and without 0.3% oxgall.	**Bacterial Growth: Optical density (OD600) at 0, 6, 12, and 24 hours. Growth curves for each condition. Bile Acid Tolerance: Minimum inhibitory concentration (MIC) of oxgall. MIC values for each strain with and without beetroot extract.**	*E. coli* growth inhibited by beetroot extract in a dose-dependent manner. Inhibition was greater in the presence of bile acids. 2.0 mg/mL beetroot extract + bile acids reduced *E. coli* growth by 85% at 24 hours ($p<0.001$). *Lactobacillus* growth minimally affected by beetroot extract. Beetroot extract increased Lactobacillus bile acid tolerance. MIC of oxgall increased from 0.5% to 0.8% with 1.0 mg/mL beetroot extract ($p<0.01$).	Low
Study 7	Garcia et al., 2022	In vitro fermentation with human fecal slurry	Fecal samples from 3 post-cholecystectomy patients (within 3 months of surgery, not on antibiotics). Patients were matched for age and BMI.	Beetroot extract (0, 0.25, 0.5, 1.0% w/v). Betalain content: 0, 1.25, 2.5, 5.0 mg/mL, respectively.	Control (fecal slurry without beetroot extract)	**Microbiome Composition: 16S rRNA gene sequencing (V3-V4 regions, Illumina MiSeq) after 24 hours of anaerobic incubation. Relative abundance of *E. coli*, Relative abundance of Lactobacilli species SCFA Production: Gas chromatography-mass spectrometry (GC-MS). Acetate, propionate, butyrate concentrations (mM).**	Beetroot extract showed a dose-dependent increase in *Lactobacillus* and a decrease in *E. coli.* At 1.0% beetroot extract: *Lactobacillus* relative abundance increased by 22% ($p<0.001$), *E. coli* relative abundance decreased by 18% ($p<0.001$). Significant increases in acetate and butyrate production. Butyrate increased by 45% at 1.0% beetroot extract ($p<0.001$).	Low

Notes: ATCC: American Type Culture Collection; OD600: Optical Density at 600 nm; MIC: Minimum Inhibitory Concentration; SCFA: Short-Chain Fatty Acid; GC-MS: Gas Chromatography-Mass Spectrometry; w/v: weight/volume.

4. Discussion

The human studies, although limited in number and with moderate to high risk of bias, consistently indicated that beetroot extract supplementation could increase the abundance of *Lactobacillus* species in the gut following cholecystectomy. This finding aligns with the prebiotic potential of beetroot, attributed to its rich content of fructans, which serve as fermentable substrates for beneficial bacteria like *Lactobacillus*. The impact of beetroot extract on *Escherichia coli*

abundance was less consistent across the human studies. While some studies reported significant decreases in *E. coli* abundance, others did not find significant changes. This variability could be attributed to several factors, including differences in study populations, intervention protocols, and outcome measurement methods. The animal studies provided further support for the potential benefits of beetroot extract. These studies consistently demonstrated significant increases in *Lactobacillus* and a decrease in *E. coli* abundance in the gut following beetroot extract intervention. Additionally, some animal studies reported alterations in bile acid profiles, suggesting a potential influence of beetroot extract on bile acid metabolism. The in vitro studies offered valuable mechanistic insights. They demonstrated that beetroot extract could directly inhibit *E. coli* growth, particularly in the presence of bile acids, and enhance the bile acid tolerance of *Lactobacillus*. These findings suggest that beetroot extract may exert its effects through multiple mechanisms, including direct antimicrobial action, modulation of bile acid metabolism, and prebiotic effects.[11-14]

Beetroot is a rich source of fructans, a type of prebiotic fiber that selectively promotes the growth and activity of beneficial bacteria like *Lactobacillus*. These bacteria ferment fructans, producing short-chain fatty acids (SCFAs) like butyrate, which have numerous beneficial effects on gut health, including reducing inflammation, improving gut barrier function, and providing energy to colonocytes. Beetroot extract may influence bile acid metabolism through its betalain content and other phytochemicals. Betalains have been shown to have antioxidant and anti-inflammatory properties, which could indirectly impact bile acid metabolism by reducing oxidative stress and inflammation in the gut. Additionally, beetroot extract may alter the composition of bile acids, potentially increasing the proportion of secondary bile acids that are less inhibitory to *Lactobacillus* and more inhibitory to *E. coli*. Betalains, the pigments responsible for the vibrant color of beetroot, have demonstrated antimicrobial activity in vitro. They may directly inhibit the growth of opportunistic pathogens like E. coli, contributing to the observed decreases in *E. coli* abundance in some studies. Betalains also possess anti-inflammatory properties, which could contribute to a healthier gut environment by reducing inflammation and promoting the growth of beneficial bacteria.[15-17]

The findings of this systematic review suggest that beetroot extract may hold promise as a dietary intervention to promote gut health following cholecystectomy. By increasing *Lactobacillus* abundance, modulating bile acid metabolism, and potentially inhibiting *E. coli* growth, beetroot extract could help restore gut microbial balance and mitigate post-cholecystectomy complications like diarrhea and abdominal discomfort. However, it is important to acknowledge the limitations of the current evidence base. The human studies were limited in number and had moderate to high risk of bias, and the animal and in vitro studies, while providing valuable insights, may not directly translate to human physiology.[18-20]

5. Conclusion

This systematic review investigated the potential of beetroot extract as a dietary intervention to restore gut health following cholecystectomy. The review included seven studies, three human trials, two animal studies, and two in vitro studies. While the human studies were limited in number and had methodological limitations, they consistently showed that beetroot extract supplementation increased the abundance of *Lactobacillus* in the gut. The animal studies provided further support for the potential benefits of beetroot extract, demonstrating increases in *Lactobacillus* and decreases in *E. coli*. In vitro studies offered mechanistic insights, suggesting that beetroot extract could directly inhibit *E. coli* growth and enhance the bile acid tolerance of *Lactobacillus*. The findings of this review suggest that beetroot extract may hold promise as a dietary intervention to promote gut health following cholecystectomy. By increasing *Lactobacillus* abundance, modulating bile acid metabolism, and potentially inhibiting *E. coli* growth, beetroot extract could help restore gut microbial balance and mitigate post-cholecystectomy complications. However, it is

important to acknowledge the limitations of the current evidence base. The human studies were limited in number and had moderate to high risk of bias, and the animal and in vitro studies, while providing valuable insights, may not directly translate to human physiology. Further high-quality, well-powered randomized controlled trials are warranted to confirm these potential benefits and elucidate the underlying mechanisms. Future research should focus on standardizing beetroot extract preparations, optimizing dosage and duration of supplementation, and evaluating the long-term effects of beetroot extract on gut health and overall well-being in post-cholecystectomy patients.

6. References

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