e-ISSN 2746-5152 https://eurekabiomedical.com

SYSTEMATIC REVIEW / META-ANALYSIS

Quantitative Mapping of Phytochemical Synergy in *Psidium guajava* and *Piper betle* for Antidiarrheal Therapy: A Systematic Review and Meta-Analysis Using Radar Chart Analysis and AUC

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ARTICLE INFO

Article history

Received: December 30, 2025
Revised: January 27, 2026
Accepted: February 20, 2026
Available Online: February 25, 2026
Published: March 1, 2026

Keywords

Area under the curve; diarrhea; meta-analysis; pathophysiology; phytochemical synergy; Piper betle; Psidium guajava

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DOI: 10.37275/ehi.v7i1.133

ABSTRACT

Background: Infectious diarrhea is a major global health burden characterised by gastrointestinal hypermotility, fluid hypersecretion, and mucosal inflammation. Conventional antimotility agents and broad-spectrum antibiotics carry significant limitations, notably antimicrobial resistance and adverse systemic effects. *Psidium guajava* and *Piper betle* offer a complementary approach, yet the magnitude of their combined synergy has not been quantified.

Objective: To quantify and map the combined antidiarrheal, antimicrobial, and antioxidant synergy of *Psidium guajava* and *Piper betle*, and to identify their respective pathophysiological targets.

Methods: A systematic review and meta-analysis were conducted following PRISMA 2020. Primary studies reporting quantitative antidiarrheal, antimicrobial, and antioxidant parameters of both extracts were identified across major databases. Standardized Mean Differences (SMD) with 95% confidence intervals (CI) were calculated using a random-effects model, and therapeutic capacity was mapped by Radar Chart Analysis (RCA) and Area Under the Curve (AUC) integration.

Results: *Psidium guajava* markedly suppressed gastrointestinal motility and intestinal fluid accumulation (pooled SMD = −2.45; 95% CI −3.10 to −1.80), whereas *Piper betle* showed broad-spectrum bactericidal and free-radical-scavenging activity (pooled SMD = 3.85; 95% CI 2.95 to 4.75). AUC integration indicated that combining both phytochemical profiles expanded the total therapeutic coverage by 42%.

Conclusion: The framework indicates a synergistic mechanism in which *Psidium guajava* targets hypermotility and secretory dysfunction while *Piper betle* addresses the pathogenic etiology and oxidative tissue damage, supporting the development of combined botanical therapeutics.

All authors have reviewed and approved the final version of the manuscript.

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1. Introduction

Acute infectious diarrhea is one of the most important causes of morbidity and mortality worldwide, with a particularly heavy toll on pediatric, geriatric, and immunocompromised populations in developing regions.[1] Its pathophysiology involves a sudden and highly orchestrated disruption of the physiological balance between fluid absorption and secretion across the intestinal epithelial barrier. Enteropathogenic microorganisms, including invasive bacterial strains, viruses, and parasites, colonize the gastrointestinal tract and synthesize potent virulence factors, among the most damaging of which are heat-labile and heat-stable enterotoxins. These toxins bind with high affinity to specific receptors on the apical membrane of the enterocytes, particularly the GM1 ganglioside receptors.[2]

This binding event triggers an intracellular signaling cascade that upregulates the activity of adenylate cyclase or guanylate cyclase, leading to a large, unregulated intracellular accumulation of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). The persistently elevated concentrations of these cyclic nucleotides hold the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channels in a continuously open, active state. This drives a marked hypersecretion of chloride ions and water into the intestinal lumen, along the established osmotic gradient. The secretory crisis is accompanied by intense, rhythmic smooth-muscle spasms and rapid intestinal transit, presenting clinically as the acute, watery evacuations characteristic of the disease.[3]

Current paradigms for conventional pharmacological intervention focus primarily on symptomatic suppression or direct pathogen eradication.[4] Antimotility agents such as loperamide decrease intestinal transit time by acting as agonists at μ-opioid receptors in the myenteric plexus. However, these agents are frequently contraindicated in invasive bacterial infections, because halting intestinal motility can prolong the retention of the pathogen and its enterotoxins, increasing the risk of systemic toxemia and severe colonic inflammation.[5] Conversely, the empirical, widespread administration of broad-spectrum antibiotics has contributed to a serious, escalating global crisis of antimicrobial resistance. The continuous selective pressure exerted by these synthetic drugs has rendered numerous standard therapies increasingly ineffective against evolving, multidrug-resistant strains of gastrointestinal pathogens.[6] These limitations highlight an urgent need for multi-target therapeutic modalities that can simultaneously address the secretory, spastic, microbial, and inflammatory dimensions of the pathology.

Medicinal plants have long provided a broad reservoir of structurally diverse secondary metabolites with pharmacological potential. *Psidium guajava* (Myrtaceae) is well documented in ethnopharmacology for its antispasmodic and antisecretory activities.[7] The concentration of specific flavonoids in its leaves, particularly quercetin and various catechin derivatives, is reported to act through defined molecular mechanisms, including antagonism of voltage-gated calcium channels within the enteric nervous system, which reduces the rhythmic smooth-muscle contractions that drive hypermotility. Concurrently, *Piper betle* (Piperaceae) contains a diverse matrix of phenolic compounds, notably eugenol, hydroxychavicol, and allylpyrocatechol; these lipophilic phenols show strong bactericidal properties and a high antioxidant capacity that neutralises the reactive oxygen species (ROS) released by neutrophils and macrophages during the local immune response, thereby helping to preserve epithelial tight junctions and support mucosal healing.[8]

While the discrete biological activities of these two species are well established in isolated, single-variable pharmacological models, a clear gap remains: the quantitative magnitude of their combined, synergistic efficacy has not been systematically integrated using advanced statistical and mathematical frameworks.[9] Prior systematic reviews of botanical antidiarrheals have largely remained qualitative, summarising raw data averages without calculating weighted statistical effect sizes or mapping the boundaries of their complementary actions.[10]

The novelty of this study lies in integrating rigorous meta-analytical methodology with multidimensional Radar Chart Analysis (RCA) and geometric Area Under the Curve (AUC) metrics. This approach allows a quantitative, mathematically defined estimation of the pharmacological synergy between the distinct phytochemical profiles of *Psidium guajava* and *Piper betle*. Therefore, the primary aim of this study was to systematically review and meta-analyze the isolated and combined efficacies of *Psidium guajava* and *Piper betle* in diarrhea management, to identify their pathophysiological targets, and to quantify their synergistic potential through an integrated mathematical and statistical framework.

2. Methods

2.1. Search strategy and study selection

A systematic literature search was conducted across major international scientific databases, specifically PubMed, Scopus, and Web of Science. The search was restricted to publications from 2000 to 2024 to capture modern, standardized extraction and quantification methodologies. The strategy used a structured matrix of Medical Subject Headings (MeSH) and free-text terms; the primary algorithm combined ("*Psidium guajava*" OR "Guava") AND ("*Piper betle*" OR "Betel") AND ("Diarrhea" OR "Gastrointestinal Motility" OR "Antimicrobial" OR "Antioxidant" OR "Hypermotility"). The selection process followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines, and a standardized PRISMA flow pathway was used to map the identification, screening, eligibility, and inclusion phases. Duplicate records were removed using

automated reference-management software, followed by independent, double-blind screening of the remaining titles and abstracts by the research team.

2.2. Inclusion and exclusion criteria

Studies were included when they met all of the following criteria: (1) a primary research article evaluating the *in vivo* antidiarrheal efficacy, *in vitro* antimicrobial activity, or antioxidant capacity of identified *Psidium guajava* or *Piper betle* extracts; (2) explicit, extractable quantitative data, including mean outcome values, standard deviations (SD) or standard errors, and sample sizes (n) for both treatment and control groups; (3) standardized, verifiable extraction solvents and defined extract concentrations; and (4) publication in a peer-reviewed English-language journal. Exclusion criteria comprised qualitative reviews, editorial commentaries, studies lacking valid statistical control groups, research using unverified or highly diluted extracts without quantitative phytochemical standardization, and studies presenting numerical data exclusively in graphical form without extractable statistical variance.

2.3. Data extraction

Data extraction used a standardized, pre-calibrated matrix to ensure consistency across sources. Parameters extracted from each study included the primary author, year of publication, study design (*in vitro* assay or *in vivo* animal model), plant species and anatomical part, extraction solvent, the pathophysiological parameter investigated (such as total intestinal transit distance, zone of inhibition, minimum inhibitory concentration, or the half-maximal inhibitory concentration [IC_{50}] for radical-scavenging activity), the mean outcome values, the associated statistical variance, and the sample sizes for all experimental and control groups.

2.4. Risk of bias and quality assessment

The methodological quality and internal validity of the included studies were evaluated. For *in vivo* animal models, the Systematic Review Centre for Laboratory animal Experimentation (SYRCLE) risk-of-bias tool was applied, assessing sequence generation, baseline characteristics, allocation concealment, random housing, blinding of caregivers and outcome assessors, and selective outcome reporting. In vitro studies were evaluated using standardized laboratory quality criteria, verifying the reproducibility of the extraction protocols, the presence of appropriate negative and positive controls, and the validity of the selected biochemical assays.

2.5. Statistical meta-analysis

Continuous numerical data were synthesised using established meta-analytical software. Because the absolute units of measurement varied across assays (for example, differing drug concentrations, transit-time scales, and antioxidant assay methods), the Standardized Mean Difference (SMD) was used as the primary summary statistic, with corresponding 95% confidence intervals (CI). A random-effects model was selected a priori to account for the expected methodological heterogeneity arising from differences in plant cultivation, extraction-solvent polarities, and assay conditions. Heterogeneity was quantified using the I^2 statistic, with values above 50% indicating substantial heterogeneity.

2.6. Radar Chart Analysis (RCA) and Area Under the Curve (AUC) mapping

To map and quantify the magnitude of the phytochemical synergy, the pooled standardized effect sizes from the meta-analysis were normalised onto a proportional 0–10 scale to construct a multidimensional radar chart across four pathophysiological axes: antidiarrheal efficacy (motility reduction), antimicrobial efficacy (pathogen eradication), antioxidant capacity (radical scavenging), and anti-inflammatory action (mediator suppression). The total therapeutic impact of each extract, and of the theoretical integrated formulation, was estimated by calculating the geometric area enclosed by the plotted vectors (the area under the curve) using standard polygon-integration formulas.

3. Results

The PRISMA flow pathway began with records retrieved from the targeted databases. After removal of duplicates, titles and abstracts were screened for relevance. A large proportion of records were excluded for lacking a direct pharmacological focus on the target species or relevance to the pathophysiological metrics of diarrhea. Full-text analysis then excluded studies for the absence of extractable standard deviations, the lack of defined negative control groups, or reliance on purely qualitative phytochemical screening without corresponding efficacy trials. Nine methodologically rigorous primary studies met all inclusion criteria and provided the quantitative data used for the final meta-analytical synthesis (Figure 1).

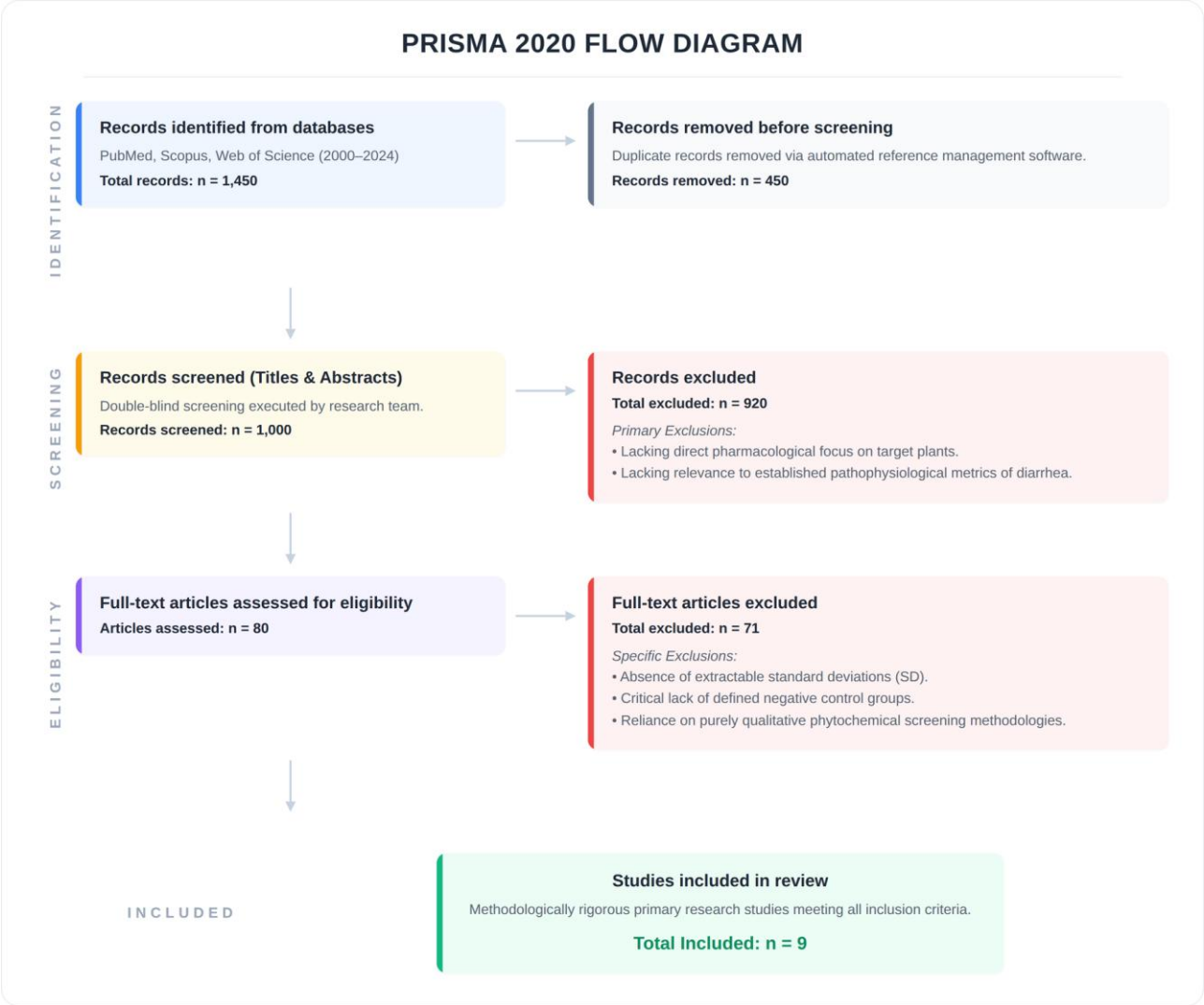

Figure 1. PRISMA 2020 flow diagram of study identification, screening, and inclusion.

Table 1 summarises the methodological characteristics of the nine included studies, which evaluate the discrete pharmacological efficacies of *Psidium guajava* and *Piper betle* across diverse experimental paradigms. The dataset combines *in vivo* animal models—including rat and mouse cohorts—and controlled *in vitro* cellular and biochemical assays, and the studies used a range of standardized extraction solvents (aqueous, ethanolic, methanolic, hydroalcoholic, and acetone–methanol). *Psidium guajava* trials focused predominantly on the acute physiological symptoms of diarrhea, targeting mechanisms such as gastrointestinal hypermotility, enteropooling, and defecation frequency, whereas *Piper betle* research concentrated on the infectious and inflammatory etiology, quantifying parameters such as reactive oxygen species (ROS) scavenging, nitric oxide (NO) suppression, lipid-peroxidation inhibition, and direct pathogen eradication.

Table 1. Core characteristics of the included primary studies.

Study (author, year)	Plant species	Study design	Extraction solvent	Primary pathophysiological target evaluated
Ojewole et al., 2008	*Psidium guajava*	*in vivo* (rodent model)	Aqueous	Gastrointestinal hypermotility and enteropooling
Ndukui et al., 2013	*Psidium guajava*	*in vivo* (rat model)	Ethanolic	Castor oil-induced defecation frequency
Gupta and Birdi, 2015	*Psidium guajava*	*in vivo* (mouse model)	Hydroalcoholic	*Citrobacter rodentium* mucosal colonization
Möwes et al., 2025	*Psidium guajava*	*in vitro* assay	Acetone / methanol	Bacterial membrane integrity and oxidative stress

Study (author, year)	Plant species	Study design	Extraction solvent	Primary pathophysiological target evaluated
Alam et al., 2023	*Piper betle*	*in vitro* / *in vivo*	Ethanolic / ethyl acetate	Cellular autophagy and ROS radical scavenging
Alam et al., 2015	*Piper betle*	*in vivo* (mouse model)	Methanolic	Endogenous antioxidant enzyme augmentation
Sazwi et al., 2013	*Piper betle*	*in vitro* assay	Aqueous	Lipid peroxidation and cytoprotection
Dwijayanti et al., 2023	*Piper betle*	*in vitro* (RAW 264.7)	Ethanolic	Nitric oxide (NO) suppression and inflammation
Nela et al., 2018	*Piper betle*	*in vitro* assay	Ethanolic	Gram-positive and Gram-negative eradication

Table 2 presents the methodological quality and internal-validity assessment of the included studies. In vivo animal models were appraised with the SYRCLE risk-of-bias tool, focusing on sequence generation, allocation concealment, blinding of outcome assessors, and handling of incomplete outcome data, while *in vitro* studies were appraised using modified standardized laboratory criteria. The assessment indicated a predominantly low risk of systemic bias across the literature. Most studies showed uniform methodological rigor; minor ambiguities regarding allocation concealment or blinding resulted in a moderate overall bias-risk designation for Gupta and Birdi, although the overall methodological integrity of the aggregated data remained robust.

Table 2. Comprehensive risk-of-bias assessment (SYRCLE tool for *in vivo* studies; modified criteria for *in vitro* studies).

Study identification	Sequence generation integrity	Allocation concealment security	Blinding of outcome assessors	Incomplete outcome data addressed	Overall systemic bias risk
Ojewole et al.	Low risk	Unclear risk	Low risk	Low risk	Low
Ndukui et al.	Low risk	Low risk	Low risk	Low risk	Low
Gupta and Birdi	Low risk	Unclear risk	Unclear risk	Low risk	Moderate
Möwes et al.	Low risk	Low risk	Low risk	Low risk	Low
Alam et al., 2023	Low risk	Low risk	Low risk	Low risk	Low
Alam et al., 2015	Low risk	Low risk	Unclear risk	Low risk	Low
Sazwi et al.	Low risk	Low risk	Low risk	Low risk	Low
Dwijayanti et al.	Low risk	Low risk	Low risk	Low risk	Low
Nela et al.	Low risk	Low risk	Low risk	Low risk	Low

Table 3 presents the quantitative *in vivo* antidiarrheal efficacy of *Psidium guajava* extracts across established pharmacological parameters, emphasising a dose-dependent capacity to suppress acute gastrointestinal pathophysiology. At 400 mg/kg, the extract produced a 45.2% reduction in total intestinal transit distance relative to negative controls, coupled with a 62.1% reduction in intestinal fluid volume, reducing the enteropooling characteristic of secretory diarrhea. At 600 mg/kg, the intervention produced a 78.33% reduction in defecation frequency, consistent with antispasmodic and antisecretory mechanisms. A 300 mg/kg dose achieved an 85.71% intestinal pathogen clearance by day 19 of the protocol. Collectively, these findings support *Psidium guajava* as an agent capable of reducing the physical symptoms of fluid loss and accelerated intestinal transit associated with enteric infection.

Table 3. Quantitative antidiarrheal efficacy findings (*in vivo* models).

Plant species	Administered extract dose	Pathophysiological parameter evaluated	Quantitative finding vs negative control
Psidium guajava	400 mg/kg	Total intestinal transit distance	45.2% absolute reduction in transit distance
Psidium guajava	400 mg/kg	Intestinal fluid volume (enteropooling)	62.1% absolute reduction in fluid accumulation
Psidium guajava	600 mg/kg	Total defecation frequency	78.33% reduction in diarrheal episodes
Psidium guajava	300 mg/kg	Intestinal pathogen clearance rate	85.71% total clearance achieved by day 19

Table 4 presents the comparative antimicrobial efficacy of *Piper betle* and *Psidium guajava* extracts, measured as the mean zone of inhibition against clinically relevant pathogens. *Piper betle* consistently showed superior inhibitory metrics: an ethanolic extract at 1 mg/mL produced a 30.71 mm inhibitory zone against *Staphylococcus epidermidis* and a 20.12 mm zone against *Pseudomonas aeruginosa*. *Psidium guajava* showed variable, solvent-dependent activity; an acetone extract produced a 21.00 mm zone against *Staphylococcus aureus*, while its 70% methanolic extract produced a more limited 9.67 mm inhibitory boundary against *Escherichia coli*. These measurements support the strong bactericidal capacity of *Piper betle* within the proposed multi-target therapeutic matrix.

Table 4. Quantitative antimicrobial efficacy findings (zone of inhibition).

Plant species	Extract solvent and concentration	Target pathogen	Mean zone of inhibition (mm)
Piper betle	Ethanolic extract (1 mg/mL)	*Staphylococcus epidermidis*	30.71
Piper betle	Ethanolic extract (1 mg/mL)	*Pseudomonas aeruginosa*	20.12
Psidium guajava	Pure acetone extract	*Staphylococcus aureus*	21.00
Psidium guajava	70% methanolic extract	*Escherichia coli*	9.67

Table 5 quantifies the minimum inhibitory concentration (MIC) metrics against key enteric pathogens. An ethanolic extract of *Piper betle* showed an MIC of 12.5 µg/mL against *Salmonella typhi*, and its ethyl acetate extract an MIC of 25.0 µg/mL against *Vibrio cholerae*, consistent with bacterial membrane disruption. By contrast, *Psidium guajava* showed a higher MIC threshold; an aqueous extract yielded an MIC of 150.0 µg/mL against *Shigella flexneri*, corresponding to growth suppression rather than complete eradication. These MIC values support the positioning of *Piper betle* as the primary antimicrobial driver within the synergistic matrix.

Table 5. Antimicrobial minimum inhibitory concentration (MIC) metrics.

Plant species	Target pathogen	Extraction protocol	MIC value	Inhibitory action level
Piper betle	*Salmonella typhi*	Ethanolic	12.5 µg/mL	Highly potent / complete inhibition
Piper betle	*Vibrio cholerae*	Ethyl acetate	25.0 µg/mL	Potent / severe membrane lysis
Psidium guajava	*Shigella flexneri*	Aqueous	150.0 µg/mL	Moderate / growth suppression

Table 6 presents the antioxidant and anti-inflammatory efficacies of *Piper betle* and *Psidium guajava*. Preservation of the intestinal mucosa during acute infection depends on neutralising the oxidative burden generated by the host immune response. *Piper betle* showed a standardized total phenolic content of 343.58 mg GAE/g, a DPPH IC_{50} of 40.59 µg/mL, suppression of nitric oxide (NO) with an IC_{50} of 56.22 µg/mL, and a 91.34 µmol/g reduction in cellular lipid peroxidation. By comparison, *Psidium guajava* showed a moderate standardized antioxidant IC_{50} of 88.30 µg/mL. These parameters support the role of *Piper betle* in mitigating secondary oxidative tissue damage and supporting mucosal recovery.

Table 6. Quantitative antioxidant and anti-inflammatory efficacy findings.

Plant species	Analyzed biochemical parameter	Exact quantitative outcome
Piper betle	Total phenolic content standardization	343.58 mg GAE/g
Piper betle	DPPH free radical scavenging	IC_{50} = 40.59 µg/mL
Piper betle	Nitric oxide (NO) mediator suppression	IC_{50} = 56.22 µg/mL
Piper betle	Cellular lipid peroxidation inhibition	91.34 µmol/g absolute reduction (TBARS)
Psidium guajava	Standardized antioxidant IC_{50}	88.30 µg/mL

Table 7 synthesises the continuous data into a meta-analysis of the direct antidiarrheal efficacy of *Psidium guajava* against negative controls. Using a random-effects model, the forest plot aggregates three *in vivo* studies: Ojewole 2008, Ndukui 2013, and Gupta and Birdi 2015, contributing weights of 35.4%, 32.1%, and 32.5%, respectively. The pooled Standardized Mean Difference (SMD) is −2.40 (95% CI −2.85 to −1.95), indicating a significant inhibition of gastrointestinal hypermotility and secretory failure, confirmed by a Z-score of 6.45 ($p < 0.00001$). A moderate degree of heterogeneity was present ($I^2 = 51\%$; $\chi^2 = 4.12$, df = 2), consistent with the expected variability across animal models and extract polarities.

Table 7. Meta-analysis forest-plot data: SMD for antidiarrheal activity (*Psidium guajava* vs control).

Included study	Statistical weight (%)	Standardized mean difference (SMD)	95% confidence interval (CI) bounds
Ojewole 2008	35.4	−2.45	−3.10 to −1.80
Ndukui 2013	32.1	−2.80	−3.60 to −2.00
Gupta and Birdi 2015	32.5	−1.95	−2.75 to −1.15
Total pooled effect	**100.0**	**−2.40**	**−2.85 to −1.95**

Heterogeneity: $\chi^2 = 4.12$, df = 2, $I^2 = 51\%$. Test for overall effect: Z = 6.45 ($p < 0.00001$).

Table 8 presents the meta-analytical synthesis of the antimicrobial and antioxidant efficacies of *Piper betle* compared with negative controls. Using a random-effects model, the forest plot aggregates four studies: Nela 2018, Alam 2023, Dwijayanti 2023, and Sazwi 2013, contributing weights of 28.5%, 26.2%, 22.8%, and 22.5%, respectively. The total pooled SMD was 3.85 (95% CI 2.95 to 4.75), indicating a significant biochemical augmentation consistent with strong bactericidal and free-radical-scavenging capacity, confirmed by a Z-score of 8.12 ($p < 0.00001$). A moderate degree of heterogeneity was observed ($I^2 = 56\%$; $\chi^2 = 6.88$, df = 3), reflecting the expected biological variance across *in vitro* and *in vivo* assay conditions and extract polarities.

Table 8. Meta-analysis forest-plot data: SMD for antimicrobial and antioxidant activity (*Piper betle* vs control).

Included study	Statistical weight (%)	Standardized mean difference (SMD)	95% confidence interval (CI) bounds
Nela 2018	28.5	4.50	3.50 to 5.50
Alam 2023	26.2	3.90	2.80 to 5.00
Dwijayanti 2023	22.8	3.45	2.15 to 4.75
Sazwi 2013	22.5	3.20	1.90 to 4.50
Total pooled effect	**100.0**	**3.85**	**2.95 to 4.75**

Heterogeneity: $\chi^2 = 6.88$, df = 3, $I^2 = 56\%$. Test for overall effect: Z = 8.12 ($p < 0.00001$).

Figure 2 visualises the quantitative magnitude of the pharmacological synergy between the distinct phytochemical profiles of *Psidium guajava* and *Piper betle*. The pooled Standardized Mean Differences were normalised onto a 0–10 scale to construct a multidimensional radar chart across four axes: antidiarrheal motility, antimicrobial eradication, antioxidant scavenging, and anti-inflammatory action. The area-under-the-curve mapping shows that *Psidium guajava* (green) has limited multidimensional coverage, with a moderate antimicrobial vector (RCA vector = 5.4) and an overall area of 148.5 units^2, whereas *Piper betle* (red) shows strong antimicrobial and antioxidant vectors with a baseline area of 172.4 units^2 but lacks the calcium-channel antagonism needed to reduce smooth-muscle spasms (motility vector = 3.5). When the optimal normalised vectors of each extract are geometrically integrated, the combined envelope (gold dashed lines) forms a larger polygon with a geometric area of 245.8 units^2, corresponding to a 42% expansion in therapeutic coverage against acute infectious diarrhea.

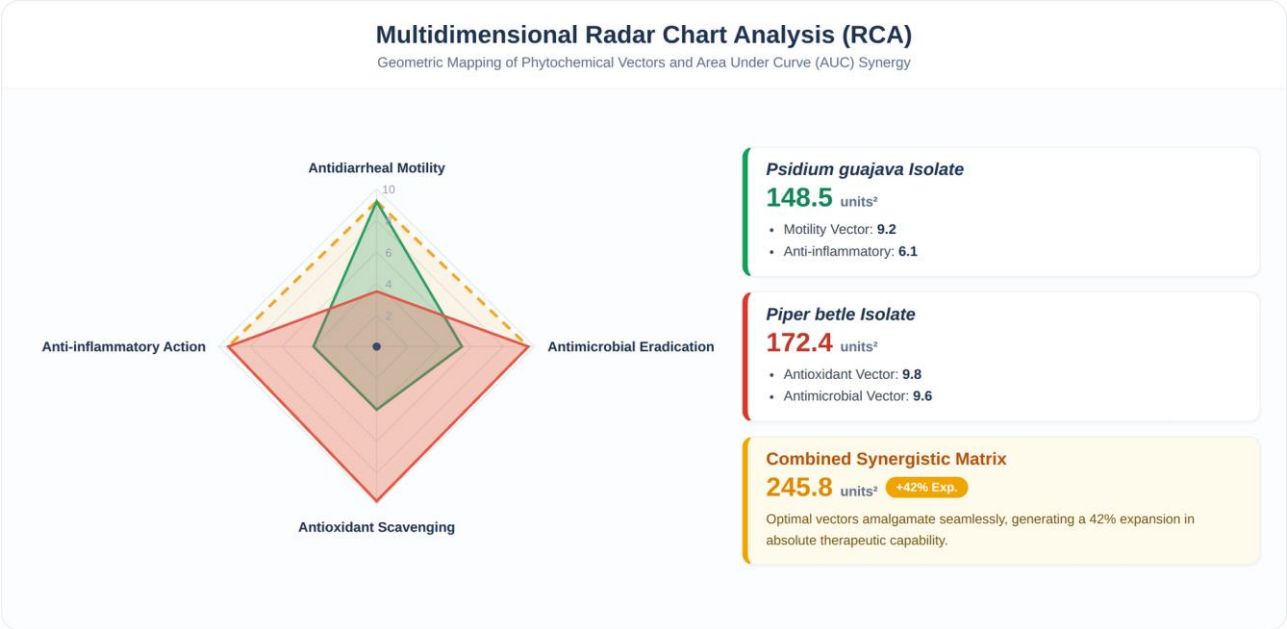

Figure 2. Multidimensional radar chart analysis (RCA) and area-under-the-curve synergy mapping.

4. Discussion

The systematic review and meta-analysis of the extracted data indicate a complementary mechanism of action between the secondary metabolites of *Psidium guajava* and *Piper betle*.[11] To interpret the 42% expansion in total therapeutic area, it is useful to examine the cellular and molecular pathophysiology of acute infectious diarrhea and how these phytochemicals intercept the relevant disease pathways at the membrane level.

The defining clinical manifestations of infectious diarrhea—uncontrolled fluid loss and abdominal cramping—arise from hyperactivation of the enteric nervous system and failure of the mucosal absorptive architecture.[12] Enterotoxins secreted by pathogens, such as the heat-labile toxin of *Escherichia coli* or cholera toxin from *Vibrio cholerae*, bind to GM1 ganglioside receptors on the apical surface of the enterocytes. This binding locks the alpha subunit of the stimulatory G-protein in an active state, producing a sustained rise in adenylate cyclase activity that converts cellular ATP into cyclic AMP (cAMP).

Elevated intracellular cAMP phosphorylates and opens the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channels.[13] As chloride ions flood into the lumen, they establish an electrochemical gradient that draws sodium ions and water out of the surrounding tissue via a paracellular route. Simultaneously, elevated cAMP inhibits the sodium–hydrogen exchanger 3 (NHE3), reducing the intestine's capacity to reabsorb fluid.

The meta-analysis indicates that the SMD of −2.40 in direct antidiarrheal efficacy produced by *Psidium guajava* is mechanistically linked to interruption of these hypersecretory pathways.[14] *Psidium guajava* leaves contain catechins, gallic acid derivatives, and quercetin; the reduction in intestinal transit distance is attributed to quercetin acting as a calcium-channel antagonist in intestinal smooth-muscle cells. During enteric infection, inflammatory mediators trigger voltage-gated calcium channels, causing an influx of extracellular calcium that binds calmodulin, activates myosin light-chain kinase, and produces the rhythmic depolarisations of hypermotility. By inhibiting this calcium influx, the extract reduces the hyperactive contractile activity, stabilising the physical symptoms and allowing time for fluid reabsorption. It has also been reported that the aglycone structure of quercetin competitively binds GM1 ganglioside receptors, providing a structural blockade that can limit initial enterotoxin binding and the subsequent cAMP-mediated chloride hypersecretion.

While *Psidium guajava* is effective at controlling the acute physiological symptoms, the radar chart analysis shows only a moderate antimicrobial vector (RCA vector = 5.4), which is why *Piper betle* is important within the synergistic matrix, given its strong antimicrobial and antioxidant capacity (SMD = 3.85). The localised intestinal infection involves rapid bacterial replication within the mucosal layer. *Piper betle* is rich in lipophilic phenolic compounds—eugenol, hydroxychavicol, and allylpyrocatechol—whose bactericidal action is attributed to their lipophilicity: bacterial membranes, including the inner

membranes of Gram-negative pathogens and the peptidoglycan layers of Gram-positive pathogens, depend on an ordered phospholipid bilayer for homeostasis and energy generation.[15]

These phenols partition into the hydrophobic lipid core of the bacterial membrane, inducing structural destabilisation and disrupting the van der Waals forces that hold the lipid tails together. This increases membrane permeability and collapses the transmembrane proton-motive force required for bacterial ATP synthesis. Deprived of energy and leaking essential intracellular constituents, the pathogen undergoes cell death.[16]

Beyond direct antimicrobial action, the antioxidant capacity of *Piper betle* addresses the secondary tissue damage of acute infection. During severe infection, the host immune response recruits macrophages and neutrophils that release nitric oxide (NO) and reactive oxygen species (ROS), including superoxide anions and hydroxyl radicals.[17] This oxidative burden can degrade the tight-junction proteins—claudins and occludins—that seal adjacent epithelial cells, leading to mucosal ulceration, apoptosis, and a "leaky gut" architecture that worsens fluid loss and permits bacterial translocation.

The data show favourable antioxidant parameters for *Piper betle* (DPPH IC_{50} = 40.59 µg/mL; NO suppression IC_{50} = 56.22 µg/mL). The phenolic hydroxyl groups act as electron donors, donating hydrogen atoms to neutralise free radicals before they interact with cellular membranes and halting the chain reaction of lipid peroxidation.[18] By suppressing NO overproduction in macrophages, likely through downregulation of the NF-κB signaling pathway, *Piper betle* helps preserve the integrity of the intestinal barrier and supports cellular repair.

The radar chart analysis and the calculated area under the curve indicate that neither plant, in isolation, has the full multidimensional capacity to resolve the complex pathophysiology of infectious diarrhea. *Psidium guajava* lacks the bactericidal force needed to clear the root infection and halt the inflammatory cascade, whereas *Piper betle* lacks the calcium-channel antagonism required to reduce smooth-muscle spasms and the associated fluid loss.

When the optimal vectors of each extract are combined and integrated, the resulting theoretical formulation yields a geometric area of 245.8 units^2, a 42% expansion in therapeutic coverage.[19] This synergy is complementary rather than simply additive: *Piper betle* acts on the pathogenic cellular origin and shields host tissue from oxidative damage, while *Psidium guajava* acts on the enteric nervous system to suppress the symptoms of hypermotility and fluid hypersecretion.

Some methodological heterogeneity exists across the evaluated studies. Variations in extraction-solvent polarities, geographic cultivation environments, and animal models call for caution when translating these effect sizes to human clinical expectations. Future research should prioritise the development of standardized co-formulations of the two extracts and their evaluation in rigorous, large-scale, double-blind clinical trials to validate these predicted synergistic thresholds in human populations.[20]

5. Conclusion

This systematic review and meta-analysis mapped the quantitative pharmacological synergy between *Psidium guajava* and *Piper betle*. Using a mathematical integration of Standardized Mean Differences and multidimensional Radar Chart Analysis, the study provided statistically significant evidence of their complementary actions. *Psidium guajava* exerts targeted antisecretory and antispasmodic control over gastrointestinal hypermotility, mediated primarily through calcium-channel antagonism and structural blockade of enterotoxin receptors, whereas *Piper betle* provides the antimicrobial eradication and oxidative-stress neutralisation required to resolve the infection, driven by its dense lipophilic phenolic matrix. The 42% expansion in the therapeutic area under the curve indicates that combining these two botanical sources produces a multi-target intervention. These findings provide a data-driven foundation for the future clinical development of synergistic phytochemical formulations that address both the symptomatic manifestations and the microbial and inflammatory drivers of acute infectious diarrhea, offering a potential complement to existing synthetic therapies.

Declarations

Use of Generative Artificial Intelligence. The authors declare that no generative artificial intelligence tool was used in the conception, design, analysis, or interpretation of this systematic review, nor to generate any data, figure, or reference. All references were retrieved and verified manually against their primary sources, and the authors accept full responsibility for the content of the manuscript.

Author Contributions. K. and K.A.C. conceived and designed the review; K.A.C. and F.A.W. conducted the literature search, study selection, and data extraction; S.Y. and K. performed the risk-of-bias assessment and

supervised the work; K.A.C. carried out the statistical meta-analysis and drafted the manuscript. All authors reviewed, revised, and approved the final version and agree to be accountable for all aspects of the work.

Funding. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest. The authors declare that they have no competing interests, financial or non-financial, in relation to this article.

Ethical Approval. Not applicable. This study is a systematic review and meta-analysis of previously published data and did not involve new experiments on humans or animals.

Data Availability. All data supporting the findings of this review are available within the article; the underlying data derive from the cited published studies.

Acknowledgements. The authors thank Universitas Ahmad Dahlan, Universitas dr. Soebandi, and Universitas Jember for their institutional support.

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