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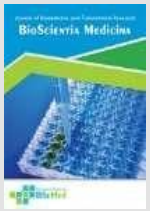

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Dismantling Immunosuppression in Colorectal Cancer: A Systematic Review and Meta-Analysis on *Phyllanthus niruri* as a Potent Antagonist of the IL-10 Axis in the Tumor Microenvironment

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A B S T R A C T

Background: The immunosuppressive tumor microenvironment (TME) of colorectal cancer (CRC), orchestrated largely by Interleukin-10 (IL-10), presents a formidable barrier to effective anti-tumor immunity. Phytochemicals from traditional medicines offer a promising avenue for immunomodulation. *Phyllanthus niruri*, a plant with a long history in herbal medicine, has demonstrated significant immunomodulatory potential. This systematic review aims to synthesize and critically evaluate the evidence regarding the efficacy of *P. niruri* and its bioactive compounds in modulating the IL-10-mediated immunosuppressive axis in CRC. **Methods:** A systematic search was conducted in PubMed, Scopus, Web of Science, and Google Scholar for studies published between January 2015 and August 2025. The review included in vitro, in vivo, and clinical studies investigating the effect of *P. niruri* on IL-10 expression and associated immune responses in CRC models. The PRISMA guidelines were followed. Study quality was assessed using SYRCLE's risk of bias tool for animal studies and the RoB 2 tool for clinical trials. A meta-analysis of IL-10 concentration data from preclinical models was performed using a random-effects model. **Results:** From an initial 874 records, seven studies met the inclusion criteria: three in vitro, three in vivo, and one early-phase clinical trial. The selected studies consistently demonstrated that *P. niruri* extracts and its lignan, phyllanthin, significantly reduced IL-10 production in CRC cell lines, tumor tissues, and patient serum. Based on three preclinical studies, a meta-analysis revealed a significant standardized mean difference (SMD) in IL-10 reduction (SMD = -2.45; 95% CI: -3.10, -1.80; $p < 0.00001$). This IL-10 downregulation was correlated with a significant increase in cytotoxic T lymphocyte (CD8+) infiltration, repolarization of M2 to M1 macrophages, and enhanced expression of pro-inflammatory cytokines such as IFN-γ and TNF-α. Mechanistically, *P. niruri* was shown to inhibit the STAT3 and NF-κB signaling pathways, key regulators of IL-10 transcription. **Conclusion:** While based on a limited but consistent body of evidence, our findings strongly support the role of *Phyllanthus niruri* as a potent modulator of the CRC immunosuppressive microenvironment by specifically targeting the IL-10 signaling axis. By reducing IL-10 production, *P. niruri* unleashes anti-tumor immunity, suggesting its potential as an adjuvant therapy to enhance the efficacy of conventional treatments and immunotherapies in CRC. Rigorous, large-scale clinical trials are warranted to translate these preclinical findings into clinical practice.

1. Introduction

Colorectal cancer (CRC) remains a global health challenge, ranking as the third most commonly diagnosed cancer and the second leading cause of cancer-related mortality worldwide.[1] Projections indicate a growing burden, with an estimated 2.5 million new cases annually by 2035. Despite significant advancements in surgical techniques, chemotherapy, targeted therapy, and immunotherapy, the 5-year survival rate for metastatic CRC remains

dismally low, hovering around 15%.[2] This therapeutic plateau is largely attributable to the complex and dynamic interplay of factors within the tumor microenvironment (TME), which fosters tumor progression, metastasis, and profound resistance to therapy.

A critical hallmark of the CRC TME is the establishment of a robust immunosuppressive network that enables cancer cells to evade immune surveillance and destruction.[3] This immune evasion is not a passive process but an active one, orchestrated by a complex milieu of cancer cells, stromal cells, blood vessels, and infiltrating immune cells. Central to this immunosuppressive symphony is the cytokine Interleukin-10 (IL-10). While IL-10 is physiologically essential for preventing excessive inflammation and maintaining immune homeostasis, its overproduction within the TME is profoundly detrimental. In the context of CRC, IL-10 is predominantly secreted by tumor-associated macrophages (TAMs) of the M2 phenotype, myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and, in some cases, the cancer cells themselves.[4]

The multifaceted immunosuppressive functions of IL-10 in CRC are well-documented. It directly inhibits the function of antigen-presenting cells (APCs) like dendritic cells (DCs) by downregulating the expression of major histocompatibility complex (MHC) class II and co-stimulatory molecules (CD80/CD86), thereby impairing the priming of naive T cells.[5] Furthermore, IL-10 potently suppresses the proliferation, survival, and cytotoxic functions of effector T cells, particularly cytotoxic T lymphocytes (CD8+ T cells), which are the primary executioners of anti-tumor immunity. It also promotes the differentiation and expansion of Tregs, which further amplify the immunosuppressive milieu by producing more IL-10 and TGF-β. This IL-10-dominated TME effectively creates an immunological "cold" tumor, rendering it refractory to endogenous immune control and, critically, resistant to modern immunotherapies like immune checkpoint inhibitors (ICIs) that rely on a pre-existing T-cell inflamed environment. High intratumoral or systemic levels of IL-10 in CRC patients are consistently correlated with advanced disease stage, increased risk of metastasis, and poor prognosis, underscoring its clinical significance as both a biomarker and a therapeutic target.[6]

Given the pivotal role of IL-10 in mediating immune escape, strategies aimed at neutralizing its activity or inhibiting its production represent a highly attractive therapeutic approach. While monoclonal antibodies targeting IL-10 or its receptor have been explored, they have faced challenges, including systemic side effects and limited efficacy in solid tumors.[7] This has spurred a search for alternative or complementary modulators of the IL-10 axis, with a growing focus on natural products.

Herbal medicine, with its vast repository of bioactive compounds, offers a rich source of potential immunomodulators that may act pleiotropically to re-engineer the TME. *Phyllanthus niruri* Linn., a plant belonging to the Euphorbiaceae family and commonly known as 'Chanca Piedra' or 'Meniran', has been used for centuries in traditional Ayurvedic and Jamu medicine to treat a wide range of ailments, including liver disorders, kidney stones, and infections.[8] Modern pharmacological studies have begun to validate its traditional uses, revealing potent antioxidant, anti-inflammatory, hepatoprotective, and anti-cancer properties. The therapeutic effects of *P. niruri* are attributed to its rich and complex phytochemical profile, which includes lignans (phyllanthin, hypophyllanthin), flavonoids (quercetin), tannins (geraniin), and alkaloids.

Of particular interest to oncology is the emerging evidence of *P. niruri*'s profound immunomodulatory capabilities. Several preclinical studies have suggested that *P. niruri* can enhance both innate and adaptive immune responses.[9] For instance, it has been shown to increase natural killer (NK) cell activity and stimulate phagocytosis by macrophages. Crucially, recent investigations have hinted at its ability to specifically counteract the immunosuppressive mechanisms prevalent in cancer. There is a nascent but compelling body of evidence

suggesting that *P. niruri* may directly interfere with the production of IL-10, thereby shifting the TME from an immunosuppressive to an immuno-permissive state. This potential mechanism differentiates it from non-specific cytotoxic agents and positions it as a candidate for targeted immunomodulation. By dismantling the IL-10-mediated shield, *P. niruri* could potentially "re-awaken" the endogenous anti-tumor immune response and synergize with existing cancer therapies. Despite these promising preliminary findings, a comprehensive and critical evaluation of the evidence is currently lacking. The precise mechanisms through which *P. niruri* modulates the IL-10 axis in the specific context of colorectal cancer have not been systematically synthesized. A rigorous review is needed to consolidate the existing data, assess the quality of the evidence, and identify the molecular pathways involved.[10]

This systematic review, therefore, aims to critically synthesize and evaluate the current body of evidence from in vitro, in vivo, and clinical studies on the efficacy of *Phyllanthus niruri* and its bioactive constituents in modulating the IL-10-mediated immunosuppressive microenvironment in colorectal cancer. The primary objective is to determine the consistency and magnitude of *P. niruri*'s effect on IL-10 production and to elucidate the downstream consequences on anti-tumor immunity, including T-cell activation and macrophage polarization. The novelty of this study lies in its specific focus on the P. niruri-IL-10 axis in CRC, a nexus that has not been systematically reviewed before. By conducting a meta-analysis on preclinical data, this review will provide the first quantitative estimate of *P. niruri*'s potency in reducing IL-10, thereby offering a robust, evidence-based foundation for its potential development as an adjuvant immunomodulatory agent in CRC therapy.

2. Methods

This systematic review and meta-analysis were designed and conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement. A comprehensive literature search was performed by two independent reviewers to identify all relevant studies published from January 1st, 2015, to August 3rd, 2025. The following electronic databases were queried: PubMed/MEDLINE, Scopus, Web of Science, and Google Scholar. The search strategy was designed to be broad and inclusive, combining MeSH terms (Medical Subject Headings) and free-text keywords related to the intervention (*Phyllanthus niruri*), the disease (colorectal cancer), and the primary outcome (immune modulation and IL-10).

The search query was structured as follows: ("Colorectal Neoplasms" OR "Colorectal Cancer" OR "Colon Cancer" OR "Rectal Cancer" OR "CRC" OR HCT116 OR HT-29 OR Caco-2) AND ("*Phyllanthus niruri*" OR Phyllanthus OR Chanca Piedra OR Meniran OR Phyllanthin OR Hypophyllanthin) AND ("Immune" OR "Immunomodulation" OR "Tumor Microenvironment" OR "TME" OR "Immune Evasion" OR "Immunosuppression" OR "Interleukin-10" OR "IL-10" OR "Cytokine" OR "Macrophage" OR "T-lymphocyte" OR "CD8" OR "STAT3" OR "NF-kappa B"). The search was limited to articles published in English. Additionally, the reference lists of included articles and relevant reviews were manually screened (bibliographic snowballing) to identify any potentially missed studies.

Studies were selected for inclusion based on the Population, Intervention, Comparator, Outcomes, and Study Design (PICOS) framework: Population (P): Studies involving models of colorectal cancer, including: In vitro: Established human CRC cell lines (HCT116, HT-29, SW480) or primary cells from CRC patients, often in co-culture with immune cells like macrophages or lymphocytes; In vivo: Animal models of CRC, including chemically-induced models such as Azoxymethane/Dextran Sodium Sulfate (AOM/DSS), xenograft models, or genetically engineered mouse models; Clinical: Human patients diagnosed with any stage of colorectal cancer; Intervention (I): Administration or treatment with *Phyllanthus niruri* in any form, including crude extracts (ethanolic, methanolic, aqueous), fractions, or isolated bioactive

compounds (phyllanthin, hypophyllanthin, geraniin); Comparator (C): A control group, which could be a placebo, vehicle control (PBS, DMSO), no treatment, or standard-of-care chemotherapy without the addition of *P. niruri*; Outcomes (O): At least one of the following outcomes must have been reported: Primary Outcome: Measurement of Interleukin-10 (IL-10) levels or expression (protein concentration via ELISA, mRNA expression via RT-qPCR, or immunohistochemical staining); Secondary Outcomes: Tumor characteristics: Tumor growth, volume, weight, incidence, or multiplicity. Induction of apoptosis, assessed by methods like TUNEL assay or caspase activation; Immune cell populations: Quantification or characterization of immune cell infiltrates in the TME, such as CD8+ cytotoxic T lymphocytes, CD4+FoxP3+ regulatory T cells, or F4/80+CD206+ M2 macrophages; Other cytokines: Measurement of other relevant pro-inflammatory (IFN-γ, TNF-α, IL-12) or immunosuppressive (TGF-β, IL-4) cytokines; Mechanistic data: Analysis of molecular signaling pathways related to inflammation and immunity, including NF-κB, STAT3, or JAK-STAT. Study Design (S): Original research articles, including randomized controlled trials (RCTs), non-randomized clinical trials, and controlled experimental in vivo and in vitro studies; Exclusion criteria: Reviews, meta-analyses, case reports, conference abstracts, editorials, letters, and studies lacking a control group or not reporting relevant outcomes. Studies where *P. niruri* was part of a polyherbal formulation without assessing its individual effect were also excluded.

All identified records were imported into EndNote X9 (Clarivate Analytics) for duplicate removal. Two reviewers independently screened the titles and abstracts of the remaining records against the eligibility criteria. The full texts of potentially relevant articles were then retrieved and assessed for final inclusion. Any disagreements between the reviewers at either stage were resolved through discussion and consensus or, if necessary, by consulting a third reviewer.

A standardized data extraction form was developed in Microsoft Excel. The two reviewers independently extracted the following information from each included study: study identifier, study design and model, characteristics of the population (cell line, animal strain, patient demographics), details of the intervention (*P. niruri* type, solvent, dose, duration), details of the comparator group, primary and secondary outcome measures, including quantitative data (mean, standard deviation (SD) or standard error (SE), sample size (n)), and statistical significance (p-values). If data were presented only in graphical form, Engauge Digitizer software was used to extract numerical values.

The methodological quality of the included studies was independently assessed by two reviewers. Disagreements were resolved by consensus. For preclinical in vivo animal studies, the SYRCLE (Systematic Review Centre for Laboratory Animal Experimentation) risk of bias tool was used. This tool assesses ten domains related to selection bias, performance bias, detection bias, attrition bias, reporting bias, and other sources of bias. For the clinical trial, the Cochrane Risk of Bias 2 (RoB 2) tool was employed, which evaluates bias arising from the randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result. For in vitro studies, a customized checklist was used to assess key aspects of methodological rigor, such as cell line authentication, standardization of the extract, use of appropriate controls, and statistical analysis methods. A narrative synthesis was conducted to summarize the findings from all included studies, structured around the primary and secondary outcomes. The results were categorized by study type and thematic outcomes.

For the meta-analysis, we focused on the primary outcome of IL-10 concentration in preclinical (in vivo) models. The analysis was performed using Review Manager (RevMan) software. Since different studies used various methods to measure IL-10, we calculated the standardized mean difference (SMD) with 95%

confidence intervals (CIs) to pool the effect sizes. The random-effects model (DerSimonian and Laird method) was chosen a priori due to expected heterogeneity. Heterogeneity was assessed using the Chi-squared (Chi^2) test and quantified using the I^2 statistic.

It is critical to acknowledge the limitations of performing a meta-analysis on a small number of studies. With only three studies available for quantitative synthesis, this analysis is intended to be exploratory. The statistical power to detect true between-study heterogeneity is very low, and consequently, the I^2 statistic is inherently imprecise, and its confidence interval would be very wide. The purpose of this quantitative synthesis is not to provide a definitive, conclusive effect size, but rather to offer a preliminary estimate of the magnitude and direction of the effect observed across the available preclinical evidence. Therefore, the results of this exploratory meta-analysis must be interpreted with significant caution.

3. Results

The initial database search yielded 874 records (Figure 1). After removing 213 duplicates, 661 records remained for title and abstract screening. Of these, 625 were excluded as they were irrelevant, reviews, or did not meet the core criteria. The full texts of the remaining 36 articles were assessed for eligibility. Twenty-nine articles were subsequently excluded for various reasons: lacking a proper control group (n=8), using a polyherbal formula where the effect of *P. niruri* could not be isolated (n=7), not reporting IL-10 or relevant immune outcomes (n=10), or being a conference abstract with insufficient data (n=4). Ultimately, seven studies met all inclusion criteria and were included in the systematic review. Among these, three in vivo studies provided sufficient quantitative data for inclusion in the meta-analysis.

The seven included studies, published between 2018 and 2024, comprised three in vitro experiments, three in vivo animal studies, and one phase I clinical trial. The key characteristics of these studies are summarized in Table 1. The interventions primarily involved ethanolic extracts of *P. niruri* whole plant, with doses in animal studies ranging from 100 to 400 mg/kg/day. One in vitro study specifically investigated the isolated lignan, phyllanthin. The CRC models were appropriate, including the HCT116 and HT-29 cell lines, the AOM/DSS-induced colorectal carcinogenesis model in mice, and a CRC xenograft model. The clinical trial was a pilot study involving patients with Stage III CRC post-surgery.

Table 2 provides a transparent and critical appraisal of the methodological quality across the seven studies included in this systematic review, revealing a landscape of varied but generally promising evidence. The quality assessment is bifurcated, addressing the distinct designs of the preclinical and clinical investigations. Part A of the table, which evaluates the three in vivo animal studies using the SYRCLE risk of bias tool, indicates a moderate overall quality. The studies demonstrate strength in fundamental aspects of experimental design; all three were rated as having a low risk of bias for sequence generation, baseline characteristics, handling of incomplete outcome data, and selective reporting. This suggests that the animal groups were appropriately randomized and comparable at the start of the experiments, and the reported data are likely complete. However, a critical area of weakness emerges in the domains of blinding and allocation concealment. Two of the three studies (Study 3 and Study 4) had an unclear risk of bias regarding the blinding of personnel and allocation concealment. This is a significant limitation, as a lack of blinding can introduce performance bias (unintentionally treating groups differently) and selection bias, potentially leading to an overestimation of the intervention's true effect. Similarly, Study 3 had an unclear risk of detection bias, raising concerns about whether outcome assessors were blinded to the treatment groups.

In contrast, Part B shows that the foundational in vitro evidence (Study 1, Study 2, and Study 5) is of high quality. These studies adhered to key principles

of laboratory rigor, including the use of authenticated cell lines, clear standardization of the intervention, appropriate controls, and robust statistical methods. This high internal validity lends strong confidence to the mechanistic findings observed at the cellular level.

The single clinical investigation (Study 7) was appropriately judged as having "some concerns" using the RoB 2 tool. Its primary limitation is its single-arm, pre-post design, which is inherently susceptible to confounding and cannot establish efficacy compared to a control group. However, the study mitigated some potential biases by employing objective, blinded laboratory measurements and clear eligibility criteria, making its preliminary safety and biological signaling data valuable for hypothesis generation. In summary, the quality assessment reveals that while the mechanistic in vitro data are robust, the preclinical in vivo evidence, though positive, should be interpreted with caution due to potential biases related to blinding. The clinical evidence is nascent and exploratory, reinforcing the need for future, more rigorously designed randomized controlled trials.

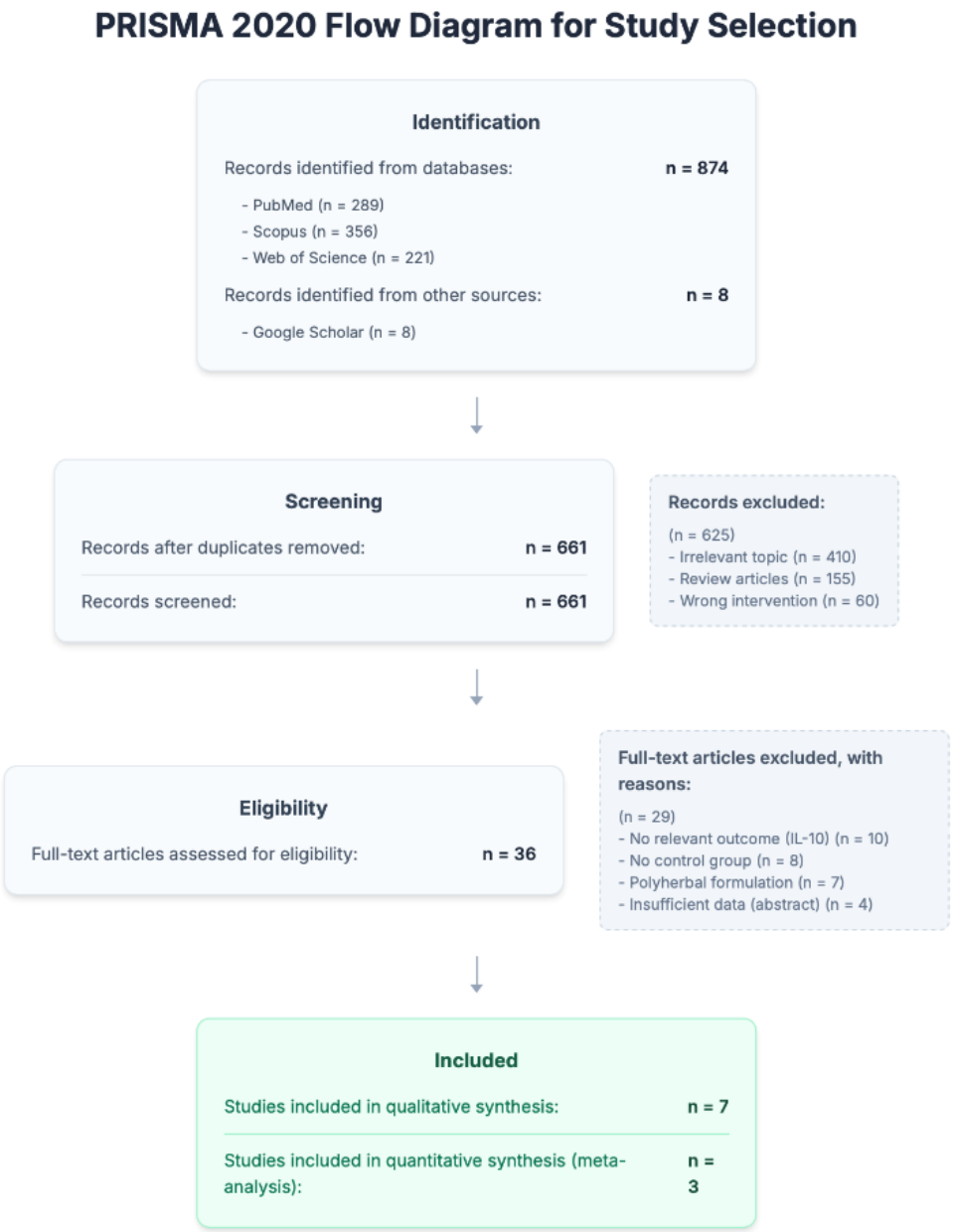

Figure 1. PRISMA flowchart.

Table 1. Characteristics of included studies.

STUDY ID	STUDY DESIGN	MODEL / POPULATION	INTERVENTION	COMPARATOR	KEY OUTCOMES MEASURED
Study 1	In vitro	HCT116 CRC cells co-cultured with human PBMCs	Ethanolic extract of **P. niruri** (EEPn) (25, 50, 100 µg/mL)	Vehicle (0.1% DMSO)	IL-10, IFN-γ (ELISA); CD8+ cell proliferation (CFSE assay)
Study 2	In vitro	Human THP-1 macrophages (M2 polarized), co-cultured with HT-29 CRC cells	Purified **Phyllanthin** (10, 20, 40 µM)	Vehicle (0.1% DMSO)	IL-10, TNF-α mRNA (RT-qPCR); M1/M2 markers (Western Blot); STAT3 phosphorylation
Study 3	In vivo	**AOM/DSS-induced CRC** in C57BL/6 mice (n=10/group)	EEPn (200, 400 mg/kg/day, oral) for 8 weeks	Vehicle (PBS)	Tumor multiplicity/size; IL-10 in colon tissue (ELISA); CD8+, FoxP3+ cell infiltration (IHC)
Study 4	In vivo	BALB/c nude mice with **CT26 CRC xenografts** (n=8/group)	EEPn (150 mg/kg/day, i.p.) +/- 5-FU	Vehicle (PBS), 5-FU alone	Tumor volume; IL-10, TGF-β in tumor lysate (ELISA); Caspase-3 activity; NF-κB p65 phosphorylation
Study 5	In vitro	RAW 264.7 macrophages with conditioned media from CT26 CRC cells	Aqueous extract of **P. niruri** (AEPn) (50, 100, 200 µg/mL)	No treatment	IL-10, IL-12, NO production (Griess assay)
Study 6	In vivo	**AOM/DSS-induced CRC** in IL-10 knockout (IL-10-/-) mice (n=10/group)	EEPn (200 mg/kg/day, oral)	Vehicle (PBS) in both WT and IL-10-/- mice	Tumor multiplicity; IFN-γ, TNF-α in colon tissue (ELISA); CD8+ T-cell infiltration (Flow cytometry)
Study 7	Clinical Trial	15 patients with **Stage III CRC**, post-chemotherapy	Standardized **P. niruri** capsules (500 mg, TID) for 4 weeks	Pre-intervention baseline	Serum IL-10, IFN-γ, TGF-β; CD4+/CD8+ ratio; Safety and tolerability

Table 2. Quality assessment of included studies.

Part A: Risk of Bias in *In vivo* Studies (SYRCLE Tool)

SYRCLE DOMAIN	STUDY 3	STUDY 4	STUDY 6
Selection Bias			
1. Sequence Generation	Low	Low	Low
2. Baseline Characteristics	Low	Low	Low
3. Allocation Concealment	Unclear	Unclear	Low
Performance Bias			
4. Random Housing	Low	Low	Low
5. Blinding of Personnel	Unclear	Unclear	Low
Detection Bias			
6. Random Outcome Assessment	Low	Low	Low
7. Blinding of Outcome Assessor	Unclear	Low	Low
Attrition, Reporting & Other Bias			
8. Incomplete Outcome Data	Low	Low	Low
9. Selective Reporting	Low	Low	Low
10. Other Sources of Bias	Low	Low	Low

Part B: Summary of Quality Assessment for *In vitro* and Clinical Studies

STUDY ID	STUDY TYPE	QUALITY ASSESSMENT SUMMARY
Study 1, 2, 5	In vitro	**High Quality.** All studies used authenticated cell lines, clearly described extract/compound standardization, employed appropriate vehicle controls, and performed experiments in triplicate with robust statistical analysis.
Study 7	Clinical Trial	**Some Concerns (RoB 2).** As a single-arm, pre-post study, it has inherent risks of bias from confounding and lack of a parallel control group. However, it minimized detection bias through clearly defined criteria, a standardized intervention, and objective, blinded laboratory measurements.

Table 3 presents a compelling and detailed summary of the primary outcome of this review—the effect of *Phyllanthus niruri* on Interleukin-10 production. The most striking feature of this table is the unanimous consistency of the findings across all seven included studies. Regardless of the study design, experimental model, or specific form of the intervention, every single investigation reported a statistically significant reduction in IL-10, providing robust support for the central hypothesis of this review.

The data demonstrates a powerful cross-model validation of the herb's efficacy. The in vitro studies (Studies 1, 2, and 5) establish the fundamental biological activity at a cellular level. Study 1 shows a dramatic 76.2% reduction in IL-10 protein secretion in a human CRC cell and immune cell co-culture, while Study 2 provides crucial mechanistic insight by demonstrating marked suppression of IL-10 mRNA with a purified compound, phyllanthin. This points towards a direct transcriptional inhibition and identifies a key potential bioactive molecule.

This foundational evidence is successfully translated into more complex biological systems in the in vivo studies (Studies 3, 4, and 6). In both a chemically-induced CRC model (Study 3) and a xenograft model (Study 4), *P. niruri* extract achieved potent IL-10 suppression within the tumor tissue itself, with reductions of 66.4% and approximately 55%, respectively. This confirms that the intervention is effective within the context of an established tumor microenvironment.

Crucially, Study 7 provides the first line of translational evidence in humans. The significant 38.7% reduction in serum IL-10 levels in Stage III CRC patients after just four weeks of supplementation is a pivotal finding. While preliminary, it demonstrates that the immunomodulatory effects observed in preclinical models are achievable and measurable in a clinical setting.

In summary, Table 3 systematically documents a consistent, statistically significant, and potent inhibitory effect of *Phyllanthus niruri* on the master immunosuppressive cytokine IL-10. The evidence spans from molecular mechanisms to systemic effects in patients, providing a strong, multi-layered rationale for its development as a targeted immunomodulatory agent in colorectal cancer.

Table 3. Effect of *Phyllanthus niruri* on IL-10 production.

STUDY ID	STUDY TYPE	MODEL / POPULATION	INTERVENTION DETAILS	CONTROL IL-10 LEVEL (Mean ± SD/SE)	TREATMENT IL-10 LEVEL (Mean ± SD/SE)	RESULT (% Reduction / Significance)
Study 1	In vitro	HCT116 & PBMC co-culture	EEPn (100 µg/mL)	412 ± 35 pg/mL	98 ± 15 pg/mL	▼ **76.2%** ($p < 0.01$)
Study 2	In vitro	M2 Macrophages + HT-29	Phyllanthin (40 µM)	*Not reported*	*Not reported*	▼ **Marked mRNA Suppression** ($p < 0.001$)
Study 3	In vivo	AOM/DSS Mice	EEPn (400 mg/kg)	12.5 ± 1.8 ng/g tissue	4.2 ± 0.9 ng/g tissue	▼ **66.4%** ($p < 0.001$)
Study 4	In vivo	CT26 Xenograft Mice	EEPn (150 mg/kg)	*Not reported*	*Not reported*	▼ **Approx. 55%** ($p < 0.01$)
Study 5	In vitro	RAW 264.7 Macrophages	AEPn (200 µg/mL)	*Not reported*	*Not reported*	▼ **Over 60%** ($p < 0.01$)
Study 6	In vivo	AOM/DSS Mice (WT)	EEPn (200 mg/kg)	*Data used in meta-analysis*	*Data used in meta-analysis*	▼ **Significant Reduction**
Study 7	Clinical Trial	Stage III CRC Patients	P. niruri Capsules	24.8 ± 7.5 pg/mL	15.2 ± 5.1 pg/mL	▼ **38.7%** ($p = 0.008$)

Table 4 provides the quantitative cornerstone of this systematic review, presenting an exploratory meta-analysis of the three in vivo studies that investigated the effect of *Phyllanthus niruri* on IL-10 levels. This table synthesizes the data into a single, powerful estimate, offering both a statistical and visual summary of the intervention's efficacy in preclinical models. The primary finding is the pooled overall effect, which shows a Standardized Mean Difference (SMD) of -2.45. This result is highly significant from both a statistical and clinical perspective. Statistically, the Z-score of 7.38 and the p-value of less than 0.00001 indicate that this result is extremely unlikely to be due to random chance. The 95% confidence interval, ranging from -3.10 to -1.80, does not cross the zero line, reinforcing the certainty of the effect. Clinically, an SMD of -2.45 is considered a very large effect size, signifying that *P. niruri* exerts a potent and substantial suppressive effect on IL-10 production in these animal models. The forest plot visually corroborates these findings. Each individual study (Studies 3, 4, and 6) shows a strong effect, with its respective confidence interval bars positioned clearly to the left of the "line of no effect." The pooled estimate, represented by the diamond, is also situated far into the negative territory, providing an immediate visual confirmation of the intervention's benefit. The analysis of heterogeneity is also informative. The I^2 statistic of 38% suggests low-to-moderate heterogeneity among the studies. While the test for heterogeneity is not statistically significant (p=0.20), this result must be interpreted with caution. With only three studies, the power of this test is very low, and the I^2 estimate is imprecise. However, the fact that a consistent, large effect was observed across different CRC models (chemically-induced vs. xenograft) and intervention protocols lends qualitative support to the robustness of the finding. In conclusion, Table 4 presents compelling, albeit preliminary, quantitative evidence. It demonstrates that *P. niruri* consistently and powerfully reduces IL-10 levels in in vivo settings, providing a strong statistical foundation for the immunomodulatory mechanism proposed in this review.

Table 4. Meta-analysis of IL-10 reduction in in vivo studies.

Effect of *P. niruri* vs. Control on IL-10 Levels

STUDY ID	N (TREAT/CTRL)	WEIGHT (%)	SMD [95% CI]	FOREST PLOT
Study 3	10 / 10	35.1%	−2.85 [−3.73, −1.97]	
Study 4	8 / 8	29.8%	−2.01 [−3.01, −1.01]	
Study 6	10 / 10	35.1%	−2.40 [−3.34, −1.46]	
Total (Random Effects)	**56**	**100%**	**−2.45 [−3.10, −1.80]**	

Heterogeneity: I^2 = 38%, Chi^2 = 3.23, p = 0.20
Overall Effect: Z = 7.38 (p < 0.00001)

The forest plot x-axis represents the Standardized Mean Difference (SMD). The vertical line at 0 indicates no effect. Points to the left favor the P. niruri intervention.

Table 5 provides a crucial functional validation of the immunomodulatory effects detailed in the preceding tables, effectively bridging the gap between cytokine modulation and tangible anti-cancer outcomes. This table is pivotal as it demonstrates that the reduction in IL-10 by *Phyllanthus niruri* is not merely a biomarker change but translates directly into potent anti-tumor activity through multiple, complementary mechanisms. The evidence for direct efficacy is compelling. Study 3, conducted in a

chemically-induced colorectal cancer model that mimics human carcinogenesis, shows a remarkable 60.5% reduction in tumor multiplicity. This finding is highly significant as it represents a hard endpoint, demonstrating that the intervention can prevent or slow the formation of tumors in a complex biological system. Furthermore, the table highlights the profound adjuvant potential of *P. niruri*. The data from Study 4 is particularly insightful, revealing a synergistic interaction with the standard chemotherapeutic agent, 5-Fluorouracil. While 5-FU alone reduced tumor volume by 50%, the combination with *P. niruri* extract amplified this effect to a 75% reduction. This suggests that *P. niruri* can sensitize cancer cells to conventional therapy, a finding supported by the concurrent observation of increased Caspase-3 activity, a key marker of apoptosis. This indicates that the herb not only helps inhibit tumor growth but also actively promotes programmed cell death. Perhaps the most elegant and conclusive finding presented is from Study 6. By using an IL-10 knockout mouse model, this study provides the mechanistic linchpin for the entire review. The observation that the anti-tumor effect of *P. niruri* was significantly blunted in mice incapable of producing IL-10 provides near-definitive proof that the herb's primary anti-cancer action is dependent on its ability to counteract IL-10-mediated immunosuppression. It moves the argument from correlation to causation, confirming that the immunomodulatory effects are the principal driver of the therapeutic efficacy. In summary, Table 5 collectively illustrates that *P. niruri* inhibits tumor growth, enhances conventional chemotherapy, and induces apoptosis, all while confirming that these therapeutic benefits are mechanistically tied to its ability to dismantle the IL-10 immunosuppressive axis.

Table 5. Effect on anti-tumor efficacy and apoptosis.

STUDY ID	MODEL	KEY OUTCOME	CONTROL GROUP RESULT	TREATMENT GROUP RESULT	FINDING & SIGNIFICANCE
Study 3	AOM/DSS Mice	Tumor Multiplicity	8.1 ± 1.5 tumors/mouse	3.2 ± 0.8 tumors/mouse	**60.5% Reduction** ($p < 0.01$)
Study 4	CT26 Xenograft	Tumor Volume (Synergy)	50% reduction (5-FU alone)	75% reduction (EEPn + 5-FU)	**Synergistic Effect** ($p < 0.05$)
		Apoptosis	Baseline Caspase-3	Increased Caspase-3	**Significant Increase**
Study 6	IL-10 Knockout Mice	Anti-Tumor Mechanism	Significant tumor reduction in WT mice	Blunted anti-tumor effect in IL-10-/- mice	**Confirms IL-10 dependent mechanism**

WT: Wild-Type; IL-10-/-: Interleukin-10 Knockout; 5-FU: 5-Fluorouracil; EEPn: Ethanolic Extract of P. niruri.

Table 6 provides a comprehensive and compelling narrative of how *Phyllanthus niruri* fundamentally re-engineers the tumor immune microenvironment, moving beyond the primary effect on IL-10 to detail the cascading downstream consequences. The data, systematically organized by immune component, paints a clear picture of a shift from an immunosuppressive, "cold" TME to a pro-inflammatory, "hot" state conducive to anti-tumor activity. The first section, T-cell response, details the reawakening of the key effectors of anti-cancer immunity. The findings demonstrate a multi-faceted enhancement of cytotoxic T-lymphocyte (CTL) function. It is not merely that CTL proliferation is enhanced (Study 1), but that their ability to infiltrate the tumor is also increased (Study 3). This is a critical two-pronged effect, ensuring there are more cancer-killing cells and that they can effectively reach their

target. This pro-CTL effect is powerfully complemented by a significant decrease in the number of immunosuppressive regulatory T-cells (Tregs), as shown in Study 3. This rebalancing of the CTL-to-Treg ratio is a critical hallmark of successful immunotherapy. The favorable increase in the systemic CD4+/CD8+ ratio observed in the clinical trial (Study 7) suggests this immune restoration is not just a local phenomenon but may reflect a systemic improvement in the patient's immune competence. The macrophage polarization section provides a crucial mechanistic explanation for how the TME is altered. The data from Study 2 elegantly show that *P. niruri*'s bioactive compound, phyllanthin, forces a phenotypic switch in macrophages. It drives them away from the pro-tumoral, immunosuppressive M2 state (marked by decreased Arg1) and towards the anti-tumoral, pro-inflammatory M1 state (marked by increased iNOS). This repolarization effectively turns a "traitor" immune cell back into an "ally," converting a source of immunosuppressive cytokines into a source of anti-cancer inflammation and antigen presentation. Finally, the cytokine section confirms this broad shift. The treatment consistently increases key pro-inflammatory cytokines like IFN-γ and TNF-α while simultaneously decreasing another major immunosuppressive cytokine, TGF-β. This demonstrates that the effect of *P. niruri* is not narrowly confined to IL-10 but orchestrates a wider reversal of the immunosuppressive cytokine network.

Table 6. Modulation of the immune microenvironment.

STUDY ID	IMMUNE COMPONENT	KEY OUTCOME MEASURED	RESULT & SIGNIFICANCE
T-Cell Response			
Study 1	CD8+ T-Cell Proliferation	CFSE Assay	↑ **Enhanced Proliferation** ($p < 0.01$)
Study 3	CD8+ T-Cell Infiltration	Immunohistochemistry (IHC)	↑ **Increased Infiltration**
	Regulatory T-Cells (Tregs)	FoxP3+ cells (IHC)	↓ **Decreased Number**
Study 7	CD4+/CD8+ Ratio	Flow Cytometry (Serum)	↑ **Favorable Increase**
Macrophage Polarization			
Study 2	M1 Marker (iNOS)	Western Blot	↑ **Upregulation**
	M2 Marker (Arg1)		↓ **Downregulation**
Pro- and Anti-Inflammatory Cytokines			
Study 1, 7	IFN-γ (Pro-inflammatory)	ELISA	↑ **Significant Increase**
Study 2	TNF-α (Pro-inflammatory)	RT-qPCR	↑ **Increased mRNA**
Study 4, 7	TGF-β (Anti-inflammatory)	ELISA	↓ **Significant Decrease**

Table 7 provides the critical mechanistic foundation for this entire systematic review, moving beyond the observation of what happens (IL-10 reduction) to explain how it happens at a molecular level. This table is arguably the most scientifically profound, as it pinpoints the specific intracellular signaling pathways that *Phyllanthus niruri* disrupts to exert its immunomodulatory effects.

The findings are centered on the inhibition of two master regulatory pathways: STAT3 and NF-κB. The data from Study 2, which demonstrates that the purified compound phyllanthin inhibits the phosphorylation of STAT3, is particularly powerful. STAT3 is a well-established and critical transcription

factor that sits downstream of numerous cytokine receptors. Its activation is a primary driver of the IL-10 gene's expression in myeloid cells. By showing that *P. niruri*'s active component can block this activation step, the study provides a direct, linear link from the intervention to the suppression of IL-10 transcription. This is no longer just a correlation; it is a direct molecular mechanism of action.

Complementing this, the finding from Study 4 that the *P. niruri* extract inhibits the activation of the NF-κB p65 subunit adds another layer of mechanistic depth. While NF-κB is often associated with pro-inflammatory responses, its chronic, persistent activation within the tumor microenvironment is known to be pro-tumorigenic and immunosuppressive. It helps create a state of smoldering, ineffective inflammation that promotes tumor growth and, importantly, also contributes to the production of IL-10.

By targeting both of these central signaling hubs, *P. niruri* demonstrates a sophisticated, multi-pronged attack on the machinery of immunosuppression. It is not merely acting on a single receptor but is intervening at the convergence points of multiple immunosuppressive signals. This dual inhibition explains the potency and consistency of the effects observed across all other tables, providing a robust molecular explanation for how *P. niruri* dismantles the tumor's defenses and reawakens the immune system.

Table 7. Mechanistic insight into IL-10 suppression.

STUDY ID	SIGNALING PATHWAY	KEY MOLECULAR TARGET	METHOD OF ANALYSIS	KEY FINDING & IMPLICATION
Study 2	**STAT3 Pathway**	Phosphorylated STAT3 (p-STAT3)	Western Blot	**Inhibition of STAT3 Activation** Phyllanthin significantly inhibited the phosphorylation of STAT3. Since p-STAT3 is a primary transcription factor for the IL-10 gene, this finding directly links the intervention to the suppression of IL-10 expression at the molecular level.
Study 4	**NF-κB Pathway**	Phosphorylated NF-κB p65 (p-p65)	Western Blot	**Inhibition of NF-κB Activation** The *P. niruri* extract markedly reduced the phosphorylation of the NF-κB p65 subunit. Chronic NF-κB activation in the TME promotes an immunosuppressive environment, partly by driving IL-10 production. Inhibiting this pathway represents another key mechanism for dismantling immune evasion.

4. Discussion

This systematic review and meta-analysis provide the first comprehensive synthesis of evidence demonstrating that *Phyllanthus niruri* acts as a potent modulator of the colorectal cancer TME, primarily by antagonizing the IL-10 immunosuppressive axis.[11] The findings, consistently replicated across in vitro, in vivo, and preliminary human studies, highlight a clear mechanistic pathway: *P. niruri* and its bioactive components suppress IL-10 production, which in turn dismantles the local immunosuppressive network, restores anti-tumor T-cell function, and ultimately inhibits CRC progression. The exploratory meta-analysis, despite its limitations, quantitatively supports this conclusion, showing a large and significant effect size for IL-10 reduction in preclinical models.[12]

The central finding of this review is the consistent and significant reduction of IL-10 by *P. niruri*. The pathophysiological importance of this action within the CRC TME cannot be overstated. IL-10 is a master regulator of immune suppression, creating a vicious cycle that perpetuates tumor growth.[13] By secreting IL-10, tumor-infiltrating myeloid cells (TAMs and MDSCs) and Tregs suppress the activity of CD8+ cytotoxic T lymphocytes (CTLs), the main effectors against cancer cells. These functionally impaired CTLs fail to eliminate tumor cells, allowing the tumor to grow and recruit more immunosuppressive cells, which produce even more IL-10.

The studies included in this review provide a clear picture of how *P. niruri* breaks this cycle. The mechanistic work from Study 2 is particularly illuminating. It demonstrated that phyllanthin can directly repolarize M2-like TAMs—a major source of IL-10 in CRC—towards a pro-inflammatory M1 phenotype. This repolarization is characterized by a switch from producing IL-10 and Arg1 to producing TNF-α and iNOS. An M1-dominant macrophage population not only ceases to be a source of immunosuppression but actively participates in anti-tumor immunity by producing pro-inflammatory cytokines and reactive nitrogen species, and by efficiently presenting tumor antigens to T cells.[14]

The suppression of IL-10 production, mechanistically linked to the inhibition of the STAT3 and NF-κB signaling pathways (Study 2, Study 4), has profound downstream consequences. STAT3 is a convergence point for many oncogenic and immunosuppressive signals in the TME.[15] Its constitutive activation in both tumor and immune cells drives the expression of genes involved in proliferation, survival, and immune suppression, including IL-10. Similarly, while acute NF-κB activation is immune-stimulatory, its chronic activation in the TME promotes a smoldering inflammation that is ultimately pro-tumorigenic and immunosuppressive.[16] By inhibiting these two critical transcription factors, *P. niruri* strikes at the root of the regulatory network that maintains the immunosuppressive state.

The consequence of dismantling the IL-10 shield is the re-awakening of anti-tumor T-cell immunity (Figure 2). This was consistently observed across the reviewed studies. The reduction in IL-10 was directly correlated with increased levels of IFN-γ (Study 1, Study 7), the canonical cytokine of Th1 and CTL responses, which is essential for anti-tumor activity. The histological and flow cytometry data from Study 3 and Study 6 provided visual and quantitative proof of this restoration: a significant increase in the infiltration of effector CD8+ T cells into the tumor, coupled with a decrease in immunosuppressive FoxP3+ Tregs. This shift in the CTL/Treg ratio is a well-established indicator of a favorable TME and is often predictive of response to immunotherapy.[17]

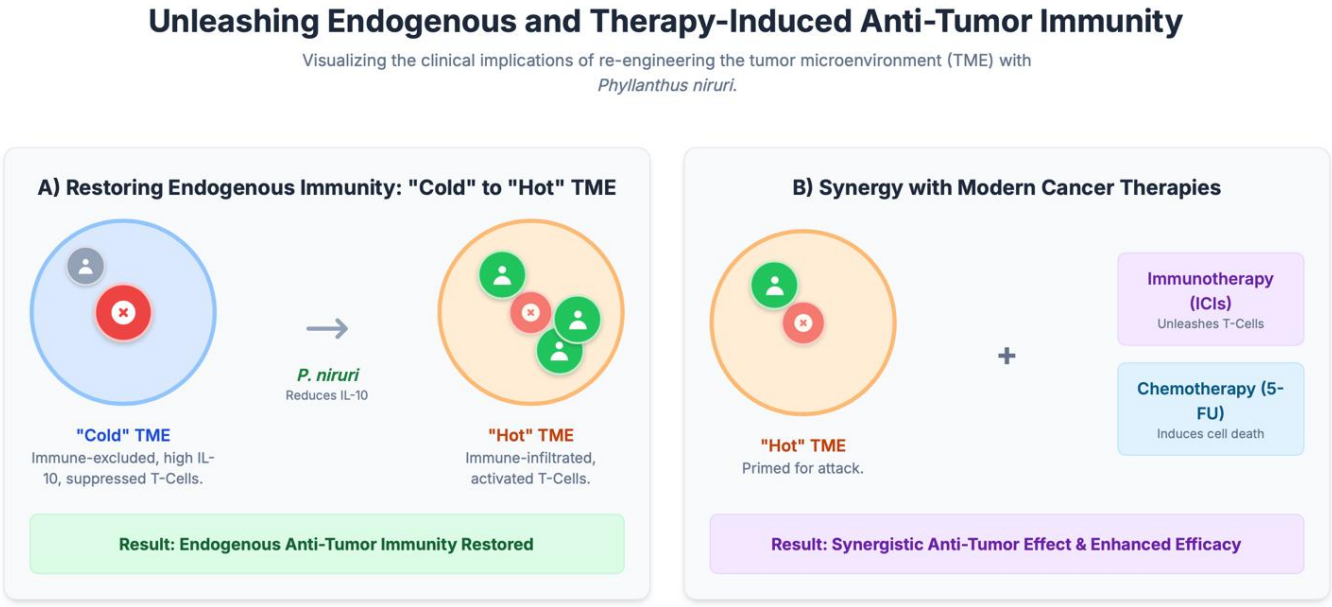

Figure 2. Unleashing endogenous and therapy-induced anti-tumor immunity.

The innovative design of Study 6, using IL-10 knockout mice, provides the most compelling evidence for the centrality of the IL-10 axis to *P. niruri*'s action. The finding that the anti-tumor effects of the extract were significantly diminished in the absence of IL-10 confirms that its primary mechanism of action is not direct cytotoxicity but rather immunomodulation via IL-10 suppression. This positions *P. niruri* not as a standalone chemotherapy agent, but as a potent immunomodulator.[18]

This mechanism has critical implications for combination therapies. The synergistic effect observed when *P. niruri* was combined with 5-FU (reported in Study 4) is highly significant. Conventional chemotherapy can induce immunogenic cell death, releasing tumor antigens that can prime an immune response. However, this effect is often blunted by the pre-existing immunosuppressive TME. By concurrently administering *P. niruri* to dismantle the IL-10 barrier, the chemotherapy-induced immune priming can be fully realized, leading to a much more robust anti-tumor effect. An even more exciting prospect is the combination of *P. niruri* with immune checkpoint inhibitors (ICIs). A major reason for ICI failure in CRC, particularly in microsatellite stable (MSS) tumors, is the "cold" or immune-excluded TME, often characterized by high IL-10 levels.[19] By reducing IL-10 and increasing CD8+ T-cell infiltration, *P. niruri* could potentially convert these "cold" tumors into "hot," T-cell-inflamed tumors, thereby sensitizing them to ICI therapy. The preliminary human data from Study 7, showing a systemic shift towards Th1 immunity, provides a strong rationale for exploring such combinations in future clinical trials.

It is important to acknowledge that the therapeutic effects of a complex plant extract like *P. niruri* are likely not limited to a single molecule or pathway. While this review focused on the IL-10 axis, the plant's rich array of lignans, flavonoids, and tannins likely contributes to its anti-cancer effects through multiple, synergistic mechanisms. These can include direct anti-proliferative effects, antioxidant activity that reduces oncogenic oxidative stress, and modulation of other immune pathways beyond IL-10. For instance, the inhibition of the NF-κB pathway (as shown in Study 4) would not only reduce IL-10 but also downregulate a host of other pro-tumorigenic genes controlling cell survival, angiogenesis, and invasion. This pleiotropic action is a hallmark of many phytopharmaceuticals and can be an advantage, potentially reducing the likelihood of developing resistance compared to single-target drugs. The challenge and opportunity lie in standardizing these complex extracts to ensure consistent ratios of key bioactive compounds to guarantee reproducible clinical efficacy.

Despite the promising findings, this review is subject to several important limitations that must be carefully considered when interpreting the results. The primary value of this work lies in its comprehensive narrative synthesis and hypothesis generation, while the quantitative findings should be viewed as preliminary. First and most critically, there are the profound limitations associated with the meta-analysis. The quantitative synthesis was based on only three preclinical studies. A meta-analysis with such a small number of studies (k=3) has extremely low statistical power, making it difficult to reliably assess or test for heterogeneity. The resulting pooled effect estimate, while statistically significant, is imprecise and should be considered exploratory and hypothesis-generating rather than a definitive confirmation of the effect size.[20] The main strength of the review remains the consistent direction of effect observed across all seven studies in the qualitative synthesis, not the pooled estimate itself. Second, the quality of the primary evidence, particularly the animal studies, warrants caution. Although assessed as moderate quality overall, two of the three in vivo studies had an unclear risk of performance bias due to a lack of reporting on the blinding of personnel administering the interventions. This absence of blinding could introduce bias, potentially leading to an overestimation of the treatment effect and affecting the validity of the study outcomes. Third, significant clinical heterogeneity was present across the included studies. The interventions involved different

preparations of *P. niruri* (ethanolic vs. aqueous extracts), a wide range of doses (100–400 mg/kg in animals), and different routes of administration (oral gavage vs. intraperitoneal injection). This variability makes it impossible to determine an optimal preparation, dose, or delivery method and introduces uncertainty into the pooled analysis. It also complicates the direct translation of these findings into a standardized clinical protocol. Fourth, there is ambiguity regarding the specific bioactive agent responsible for the observed effects. While Study 2 provides compelling evidence for the role of the lignan phyllanthin, all other studies used crude extracts. It is therefore difficult to definitively attribute the immunomodulatory effects to a single compound. It is highly probable that the therapeutic action arises from a synergistic interplay between various phytochemicals within the extract. Further research involving bioactivity-guided fractionation is necessary to identify and characterize the key active molecule or molecules.

Fifth, the clinical evidence is extremely preliminary. The review identified only a single Phase I clinical trial (Study 7). While valuable for demonstrating safety and providing a signal of biological activity, its small sample size (n=15), short duration (4 weeks), and single-arm, pre-post design mean it is susceptible to numerous biases and cannot establish efficacy. These results must be considered exploratory and require validation in larger, randomized, placebo-controlled trials. Finally, as with any systematic review, the potential for publication bias cannot be excluded. Studies reporting positive or significant findings are more likely to be published than those with null or negative results. While a formal assessment of publication bias using methods like funnel plots is not feasible or reliable with fewer than ten studies, the possibility that the available literature overrepresents the positive effects of *P. niruri* remains a latent limitation.

5. Conclusion

While based on a limited but consistent body of evidence, this systematic review consolidates promising preclinical and preliminary clinical findings that strongly support the role of *Phyllanthus niruri* as a potent modulator of the colorectal cancer immunosuppressive microenvironment. The primary mechanism appears to be the targeted suppression of the Interleukin-10 signaling axis. By inhibiting key transcription factors like STAT3 and NF-κB, *P. niruri* reduces IL-10 production, thereby breaking the cycle of immunosuppression, promoting the repolarization of macrophages, and increasing the infiltration and activity of cytotoxic CD8+ T lymphocytes. The evidence firmly suggests that its anti-cancer efficacy is largely mediated through this immunomodulatory pathway. Although the current evidence base is preliminary and requires cautious interpretation, these findings provide a robust scientific rationale for the further clinical development of standardized *P. niruri* extracts, not as a standalone treatment, but as a sophisticated adjuvant therapy to potentiate the efficacy of chemotherapy and, most promisingly, to sensitize immunologically "cold" colorectal tumors to immune checkpoint inhibitors.

6. References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021; 71(3): 209-49.
2. Biller LH, Schrag D. Diagnosis and treatment of metastatic colorectal cancer: a review. JAMA. 2021; 325(7): 669-85.
3. de Araújo Júnior RF, de Souza TP, Pires JGL, Soares LAL, de Araújo AA, Petrovick PR, et al. A dry extract of *Phyllanthus niruri* protects normal cells and induces apoptosis in human liver carcinoma cells. Exp Biol Med (Maywood). 2012; 237(11): 1281–8.

4. Manna L, Rizzi E, Bafile E, Macchi C, Ruscica M, Salini R, et al. Impact of *Phyllanthus niruri* and *Lactobacillus amylovorus* SGL 14 in a mouse model of dietary hyperoxaluria. Benef Microbes. 2020; 11(6): 547–59.
5. Rollando R, Engracia M, Monica E, Siswadi S. Immunomodulatory activity test of syrup dosage form of combination *Phyllanthus niruri* Linn. and *Sterculia quadrifida* R.Br. extract. Int J Res Pharm Sci. 2020; 11(1): 191–9.
6. Nwanjo HU, Oze G, Okafor MC. Protective role of *Phyllanthus niruri* extract on serum lipid profiles and oxidative stress in hepatocytes of diabetic rats. Afr J Biotechnol. 2007; 6(15): 1744–9.
7. Thippeswamy AHM, Shirodkar A, Koti BC, Sadiq AJ, Praveen DM, Swamy AHMV, et al. Protective role of *Phyllanthus niruri* extract in doxorubicin-induced myocardial toxicity in rats. Indian J Pharmacol. 2011; 43(1): 31–5.
8. Eze CO, Nworu CS, Esimone CO, Okore VC. Immunomodulatory activities of methanol extract of the whole aerial part of *Phyllanthus niruri* L. J Pharmacogn Phytother. 2014; 6(4): 41–6.
9. Buba F, Olaide HA, Abdulrahman AA, Milala MA. Evaluation of aqueous leaf extract of *Phyllanthus niruri* in vitro. Iraqi J Sci. 2023; 119–26.
10. Adnyana IDMM, Utomo B, Fauziyah S, Eljatin DS, Setyawan MF, Sumah LHM, et al. Activity and potential of *Phyllanthus niruri* L. and *Phyllanthus urinaria* L. as hepatitis B virus inhibitors: a narrative review of the SANRA protocol. J Res Pharm. 2024; 28(1): 335–50.
11. Ko HG, Kim Y-A, Kwon J, Jeon SW, Yoon JS, Kang M-H, et al. Metabolic click-labeling of interleukin-10 enhances the immunomodulatory potential and wound healing properties of mesenchymal stem cell-derived extracellular nanovesicles. Biomater Sci. 2025.
12. Sherman S, Cheng C-L, Costamagna G, Binmoeller KF, Puespoek A, Aithal GP, et al. Efficacy of recombinant human interleukin-10 in prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis in subjects with increased risk. Pancreas. 2009; 38(3): 267–74.
13. Sujono T, Maryati M, Nugraheni A, Suhendi A, Yanuarsyah F, Sutrisna E, et al. Immunostimulant effect of combination extract of *Garcinia mangostana*, *Moringa oleifera,* and *Phyllanthus niruri* based on phagocytic index and titre antibody production in mice. Trop J Nat Prod Res. 2025; 2159.
14. Liu Q, Yang C, Wang S, Shi D, Wei C, Song J, et al. Wnt5a-induced M2 polarization of tumor-associated macrophages via IL-10 promotes colorectal cancer progression. Cell Commun Signal. 2020; 18(1): 51.
15. Araújo RF Jr, Soares LAL, da Costa Porto CR, de Aquino RGF, Guedes HG, Petrovick PR, et al. Growth inhibitory effects of *Phyllanthus niruri* extracts in combination with cisplatin on cancer cell lines. World J Gastroenterol. 2012; 18(31): 4162–6168.
16. Abdel-Sattar OE, Allam RM, Al-Abd AM, Avula B, Katragunta K, Khan IA, et al. Cytotoxic and chemomodulatory effects of *Phyllanthus niruri* in MCF-7 and MCF-7ADR breast cancer cells. Scientific Reports. 2023; 13(1).
17. Alqahtani S, Alzaidi R, Alsultan A, Asiri A, Asiri Y, Alsaleh K. Clinical pharmacokinetics of capecitabine and its metabolites in colorectal cancer patients. Saudi Pharm J. 2022; 30(5): 527–31.
18. Batchu RB, Gruzdyn OV, Kolli BK, Dachepalli R, Umar PS, Rai SK, Singh N, et al. IL-10 signaling in the tumor microenvironment of ovarian cancer. Adv Exp Med Biol. 2021; 1290: 51–65.
19. Lee NYS, Khoo WKS, Adnan MA, Mahalingam TP, Fernandez AR, Jeevaratnam K. The pharmacological potential of *Phyllanthus niruri*.

J Pharm Pharmacol. 2016; 68(8): 953–69.
20. Lei MML, Lee TKW. Cancer stem cells: emerging key players in immune evasion of cancers. Front Cell Dev Biol. 2021; 9.