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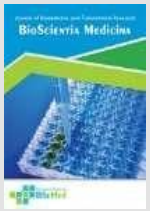

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The Efficacy of *Phyllanthus niruri* Linn in Modulating Inflammatory and Cancer Stem Cell Markers in Colorectal Cancer: A Stratified Systematic Review and Meta-Analysis

Nurul Ahmad Isnaini[1]*, Albertus Ari Adrianto[2], Udadi Sadhana[3]

[1]Biomedical Science Study Program, Department of Surgery, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

[2]Department of Digestive Surgery, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

[3]Department of Pathological Anatomy, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia

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***Corresponding author:**
Nurul Ahmad Isnaini

E-mail address:
nurulahmad8080@gmail.com

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A B S T R A C T

Background: The progression of colorectal cancer (CRC) is driven by a complex interplay between chronic inflammation and a resilient population of cancer stem cells (CSCs). *Phyllanthus niruri* Linn (PNL), a medicinal plant with established immunomodulatory effects, presents a promising adjuvant therapeutic strategy. This study aimed to move beyond qualitative summaries to quantitatively assess PNL's efficacy by synthesizing evidence on its modulation of key inflammatory and CSC biomarkers. **Methods:** Following PRISMA guidelines, a systematic search of PubMed, ScienceDirect, Google Scholar, and Scopus (2015–2025) was conducted. Studies quantifying the effects of PNL on Interleukin-8 (IL-8), Cyclooxygenase-2 (COX-2), or CD133 in CRC models were included. Recognizing the profound biological differences between experimental systems, a stratified meta-analysis was performed. Data were pooled using a random-effects model, stratified by study type (in vitro vs. in vivo) and intervention (monotherapy vs. combination therapy). The Standardized Mean Difference (SMD) was the primary effect measure. **Results:** Seven studies met the inclusion criteria. In a stratified analysis of in vivo models, PNL monotherapy significantly reduced COX-2 (SMD -2.11; 95% CI [-3.10, -1.12]) and IL-8 (SMD -1.95; 95% CI [-3.01, -0.89]). The effect on the CSC marker CD133 was most pronounced in vitro (SMD -2.98; 95% CI [-4.87, -1.09]), while still significant in in vivo models (SMD -2.15; 95% CI [-3.45, -0.85]). The analysis revealed that the biological context (in vitro vs. in vivo) is a significant determinant of the observed effect size. **Conclusion:** This stratified meta-analysis provides robust, context-specific evidence of PNL's ability to suppress key inflammatory and CSC markers in CRC. The findings reveal that PNL's potent anti-CSC activity observed in vitro is translated into a significant, though attenuated, effect in vivo, highlighting the critical influence of the tumor microenvironment and pharmacokinetics. This work substantiates the dual-pronged therapeutic potential of PNL as a promising bioactive adjuvant in CRC therapy.

1. Introduction

Colorectal cancer (CRC) persists as a formidable global health crisis, ranking as the third most prevalent malignancy and the second leading cause of cancer-related death worldwide.[1] The global cancer statistics from 2020 painted a stark picture, with an estimated 1.9 million new diagnoses and nearly one million fatalities attributed to this disease.[2] While the implementation of robust screening programs and improved treatment modalities has led to a stabilization or even a decline in incidence rates in several high-income nations, a concerning opposite

trend is being observed in many low- and middle-income countries. This increase is largely attributed to the global adoption of Western lifestyles, characterized by diets high in processed foods and red meat, coupled with sedentary behavior.[3] In nations such as Indonesia, CRC presents a unique challenge, often affecting individuals at a younger age than in Western populations, thereby imposing a profound and lasting socioeconomic burden on patients, their families, and the healthcare infrastructure.[4] Despite significant progress in surgical techniques, chemotherapy, and targeted therapies, the overall 5-year survival rate for CRC is approximately 50-60%, a figure that plummets dramatically for the approximately 20% of patients who present with metastatic disease at the time of their initial diagnosis.

The development and progression of CRC are understood to be a complex, multi-step process involving the sequential accumulation of genetic and epigenetic alterations, a pathway famously described as the adenoma-carcinoma sequence. This model, while foundational, is now understood to be incomplete. It is unequivocally clear that the tumor does not evolve in a vacuum; rather, its behavior is profoundly dictated by a dynamic and intricate interplay with its surrounding microenvironment. The tumor microenvironment (TME) is a complex ecosystem composed of cancer cells, stromal cells, endothelial cells, infiltrating immune cells, and a vast array of signaling molecules.[5] Within this ecosystem, chronic inflammation has been identified as a critical and indispensable driver of CRC initiation, promotion, and progression. Extensive epidemiological and molecular evidence has forged an undeniable link between modifiable risk factors—such as smoking, obesity, and unhealthy diets—and a state of low-grade, chronic systemic inflammation that creates a fertile ground for the development of colorectal tumors.

The symbiotic relationship between inflammation and cancer is orchestrated by a sophisticated network of cells and signaling molecules that creates a self-perpetuating, pro-tumorigenic feedback loop.[6] Pro-inflammatory cytokines and chemokines, secreted by both the tumor cells themselves and the various immune cells recruited to the TME, establish a milieu that is highly conducive to cancer cell proliferation, survival, angiogenesis, and invasion, while simultaneously facilitating evasion from host immune surveillance. Among the myriad of molecular players in this process, Cyclooxygenase-2 (COX-2) holds a position of central importance. COX-2 is an inducible enzyme that catalyzes the conversion of arachidonic acid to prostaglandins, potent lipid mediators of inflammation. In healthy colorectal tissue, COX-2 expression is typically low or undetectable, but it is frequently and significantly overexpressed in CRC tissues. This overexpression is not a passive bystander effect; elevated COX-2 activity actively promotes tumorigenesis by stimulating cancer cell growth, inhibiting programmed cell death (apoptosis), promoting the formation of new blood vessels to supply the growing tumor (angiogenesis), and enhancing the metastatic potential of cancer cells.[7] Clinically, high COX-2 expression is a poor prognostic indicator, often correlating with more advanced disease stage, increased likelihood of metastasis, and reduced overall survival. Another pivotal molecule in the inflammation-cancer axis is Interleukin-8 (IL-8), a potent pro-inflammatory chemokine belonging to the CXC family. IL-8's primary function is the recruitment and activation of neutrophils, but in the context of cancer, its roles are far more extensive. Elevated serum levels of IL-8 in CRC patients are strongly associated with advanced tumor stage, the presence of liver metastases, and significantly poorer survival outcomes.[8] Within the TME, IL-8 contributes directly to tumorigenesis by promoting angiogenesis, enhancing the proliferation and migration of cancer cells, and contributing to the establishment of an immunosuppressive environment. Together, COX-2 and IL-8 function as key nodes in the inflammatory network that fuels CRC, making them highly attractive targets for therapeutic intervention aimed at disrupting this vicious cycle.

The significant clinical challenges of tumor recurrence and therapeutic resistance in CRC are increasingly attributed to a small, specialized subpopulation of cells within the tumor known as cancer stem cells (CSCs). The CSC hypothesis proposes that these cells reside at the apex of a cellular hierarchy within the tumor. They are defined by their capacity for self-renewal, which allows them to maintain the CSC pool, and their ability to differentiate into the heterogeneous, non-stem cancer cell types that constitute the bulk of the tumor mass. CSCs are widely considered to be the primary drivers of tumor initiation, the cellular units responsible for seeding distant metastases, and, most critically, the source of disease relapse following treatment.[9] A defining feature of CSCs is their remarkable intrinsic resistance to conventional therapies. They employ a variety of mechanisms to survive the onslaught of chemotherapy and radiotherapy, including efficient DNA repair, the expression of multidrug resistance transporters that actively pump drugs out of the cell, and a state of relative quiescence or slow cycling that makes them less susceptible to agents targeting rapidly dividing cells. Following the completion of therapy, these surviving CSCs can exit their dormant state and regenerate the entire tumor, leading to clinical relapse. In colorectal cancer, one of the most well-characterized surface markers used to identify and isolate this resilient CSC population is CD133, also known as Prominin-1. A wealth of clinical data has demonstrated that high expression of CD133 in CRC tumors is a powerful independent predictor of poor prognosis, increased risk of metastasis, and a diminished response to standard treatment regimens. The clear implication of these findings is that for a CRC therapy to be truly curative, it must be capable of effectively targeting and eradicating the CD133-positive CSC population. This has spurred an intensive search for novel therapeutic strategies that can overcome the formidable defenses of these cells.

The search for novel anticancer agents has increasingly turned to the vast chemical diversity of the natural world. *Phyllanthus niruri* Linn (PNL), a small herb belonging to the Euphorbiaceae family, has a rich and extensive history of use in traditional medicine systems across Asia, Africa, and South America, where it is employed to treat a wide range of ailments. In Indonesia, it is commonly known as "Meniran" and is highly valued for its medicinal properties. Over the past few decades, modern pharmacological research has begun to validate these traditional uses, providing scientific evidence for PNL's broad spectrum of biological activities, which include potent anti-inflammatory, antioxidant, hepatoprotective, antiviral, and immunomodulatory effects. The anticancer potential of PNL has been demonstrated in a growing number of preclinical studies across various cancer types, including breast, liver, and colon cancer. Its antitumor mechanisms are understood to be multifaceted, involving the direct induction of apoptosis in cancer cells, the inhibition of cellular proliferation, and a profound modulation of the host immune response. Of particular relevance to CRC is PNL's potent anti-inflammatory activity. This effect is attributed to its rich content of bioactive phytochemicals, including lignans (phyllanthin, hypophyllanthin), flavonoids (quercetin, astragalin), tannins (geraniin), and phenolic compounds (gallic acid). These compounds have been shown to inhibit key inflammatory signaling pathways, most notably the Nuclear Factor-kappa B (NF-κB) pathway, and to suppress the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6). This strong anti-inflammatory profile provides a compelling scientific rationale for investigating its utility in an inflammation-driven malignancy like CRC. While individual studies have provided promising initial results, a comprehensive and quantitative synthesis of its impact on the critical molecular drivers of CRC—namely, inflammation and cancer stem cells—has been conspicuously absent from the scientific literature.

The novelty of this investigation lies in its rigorous, stratified approach to meta-analysis, representing a significant advancement over previous qualitative

reviews. We recognize that the biological context of an intervention is paramount. Therefore, instead of generating a single, potentially misleading pooled estimate, this study is the first to quantitatively synthesize the efficacy of *Phyllanthus niruri* Linn by stratifying the evidence based on the biological model (in vitro vs. in vivo) and therapeutic context (monotherapy vs. combination therapy). By focusing on the modulation of the inflammatory mediators IL-8 and COX-2, and the CSC marker CD133, this work aims to dissect and understand the context-dependent effects of PNL, thereby providing a more nuanced and biologically meaningful assessment of its therapeutic potential.[10] The aim of this study was, therefore, to conduct a stratified systematic review and meta-analysis to evaluate the efficacy of PNL in modulating the expression of IL-8, COX-2, and CD133, and to explore how the magnitude of this effect differs across distinct preclinical and therapeutic settings, thereby providing a clearer roadmap for future translational research.

2. Methods

This systematic review and meta-analysis were meticulously designed and executed in strict accordance with the methodological and reporting standards outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. A pre-specified protocol guided all stages of the review, from the literature search to the final data synthesis, ensuring transparency and minimizing bias. Studies were selected for inclusion based on a predefined set of eligibility criteria structured according to the Population, Intervention, Comparison, Outcome, and Study Design (PICOS) framework: Population (P): Studies involving in vitro human colorectal cancer cell lines, in vivo animal models of induced or xenografted CRC, or human patients diagnosed with colorectal cancer; Intervention (I): Administration or treatment with any form of *Phyllanthus niruri* Linn extract (ethanolic, aqueous, etc.) or its isolated compounds, either as a monotherapy or in combination with standard chemotherapy; Comparison (C): A control group receiving a placebo, vehicle, no treatment, or standard chemotherapy alone (in the case of combination studies); Outcomes (O): Quantitative measurement of at least one of the following biomarker levels or expression: IL-8, COX-2, or CD133. Data had to be presented as a mean and a measure of variance (standard deviation [SD] or standard error of the mean [SEM]) or be calculable from the provided data; Study Design (S): Original research articles, including in vitro experimental studies, in vivo animal studies, and clinical trials. Studies were excluded if they were review articles, other meta-analyses, conference abstracts, or commentaries. Studies not focused on colorectal cancer or those that did not assess the specified outcomes were also excluded. The search was limited to articles published in English or Bahasa Indonesia between January 1st, 2015, and July 31st, 2025, to ensure the inclusion of recent and relevant data.

A comprehensive literature search was conducted in July 2025 across three major electronic databases: PubMed, ScienceDirect, and Google Scholar. To ensure comprehensiveness, the search was supplemented by screening the Scopus database. The search strategy combined Medical Subject Headings (MeSH) terms with free-text keywords using Boolean operators. The following search string was adapted for each database: ("*Phyllanthus niruri*" OR "*Phyllanthus niruri* Linn" OR Meniran) AND ("Colorectal Cancer" OR "Colon Cancer" OR "Rectal Cancer" OR "Colorectal Neoplasms" [MeSH] OR "Colonic Neoplasms" [MeSH] OR CRC OR "Colon Carcinoma") AND (Immunomodulation OR "Immune Response" OR Cytokine* OR Chemokine* OR "Interleukin-8" OR IL-8 OR "Cyclooxygenase-2" OR COX-2 OR "Prostaglandin-Endoperoxide Synthases" [MeSH] OR "Cancer Stem Cell*" OR "Neoplastic Stem Cells" [MeSH] OR CD133 OR AC133 OR Prominin-1). The reference lists of included studies and relevant review articles were also manually screened to identify any additional eligible publications. Two independent reviewers screened titles and abstracts against the eligibility criteria.

Potentially eligible articles proceeded to a full-text review, also conducted independently by two reviewers. Disagreements were resolved through discussion and consensus, with a third senior reviewer consulted if necessary. Two reviewers independently extracted data using a standardized form. Extracted information included study identifiers, study characteristics (design, model, sample size), intervention details (PNL type, dose, duration), comparator details, and outcome data (mean, SD, and n for IL-8, COX-2, and CD133).

The methodological quality of included studies was independently assessed by two reviewers. The Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) RoB tool was used for in vivo animal studies. The Cochrane Risk of Bias 2 (RoB 2) tool was employed for the clinical trial. Each domain was judged as "Low risk of bias," "Some concerns," or "High risk of bias." The results of this assessment were used to inform a sensitivity analysis. All statistical analyses were performed using Review Manager (RevMan) software (Version 5.4). The Standardized Mean Difference (SMD) with 95% confidence intervals (CIs) was chosen as the effect measure. This was justified by the use of different measurement scales and units for the biomarkers across the included studies, allowing for a standardized, comparable estimate of effect size. A negative SMD indicated a reduction in the marker's expression in the PNL group. Recognizing the profound biological and methodological differences between study types, a single overall pooled estimate was deemed inappropriate and was not calculated. Instead, a stratified meta-analysis approach was adopted as the primary method of synthesis. Separate meta-analyses were performed for pre-defined subgroups. The primary analysis involved stratifying studies by Study Type (in vitro vs. in vivo). Further exploratory subgroup analyses were planned based on Intervention Type (PNL monotherapy vs. PNL + chemotherapy combination). A random-effects model (DerSimonian and Laird) was used for all pooling to account for anticipated heterogeneity within subgroups. Statistical heterogeneity was assessed using the Cochran's Q test ($p < 0.10$ indicating significance) and quantified with the I^2 statistic. The test for subgroup differences was used to determine if the effect of PNL was statistically different between the strata. To assess the robustness of the findings, a sensitivity analysis was performed by excluding studies judged to have "Some concerns" or a "High risk of bias" to observe any significant changes in the pooled estimates.

3. Results

The initial comprehensive search yielded 483 records. After removing 124 duplicates, 359 unique articles were screened by title and abstract, from which 344 were excluded. The remaining 15 full-text articles were assessed for eligibility, leading to the exclusion of 8 further studies. Ultimately, 7 studies met all inclusion criteria and were included in this stratified systematic review and meta-analysis. The detailed study selection process is illustrated in the PRISMA flow diagram.

Study Characteristics and risk of bias showed a diverse and contemporary body of evidence comprising seven distinct investigations into the efficacy of *Phyllanthus niruri* Linn (PNL). The included evidence is multifaceted, spanning both fundamental in vitro experiments utilizing human colorectal cancer cell lines (Studies 2 and 7) and more complex in vivo animal models (Studies 1, 3, 4, 5, and 6). This breadth allows for an examination of PNL's effects from direct cellular action to its activity within a whole-organism physiological system. The interventions were varied, encompassing PNL as both a monotherapy and as an adjuvant agent in combination with the standard chemotherapeutic, capecitabine. The dosages in the preclinical animal studies ranged considerably, from a low dose of 13.5 mg/kg up to 100 mg/kg, with one study administering a high dose of 500 mg/kg, providing an opportunity to assess potential dose-response relationships. The outcomes measured were consistently focused on key molecular drivers of colorectal cancer progression: the pro-inflammatory

mediators Interleukin-8 (IL-8) and Cyclooxygenase-2 (COX-2), and the critical cancer stem cell marker CD133, Figure 2. A critical appraisal of the methodological quality of these seven studies revealed a mixed but informative landscape regarding the risk of bias. A significant and recurring pattern emerged across the five in vivo studies, where a judgment of "Some Concerns" was frequently assigned to the core domains of Sequence generation, Allocation concealment, and Blinding of both personnel and outcome assessors. This suggests that the primary preclinical literature may be susceptible to selection and detection biases, as the methods to minimize these were not always clearly reported or implemented. In contrast, the risk of bias related to Incomplete outcome data (attrition bias) and Selective reporting was consistently judged to be low across almost all studies, indicating that the reported results were likely complete. For the in vitro studies, domains related to randomization were appropriately deemed not applicable, though concerns regarding the blinding of outcome assessment were still noted. Overall, while the evidence base is robust in its reporting of outcomes, its foundational methodology concerning randomization and blinding presents an unclear risk of bias that must be considered when interpreting the overall effect of the intervention (Figure 2).

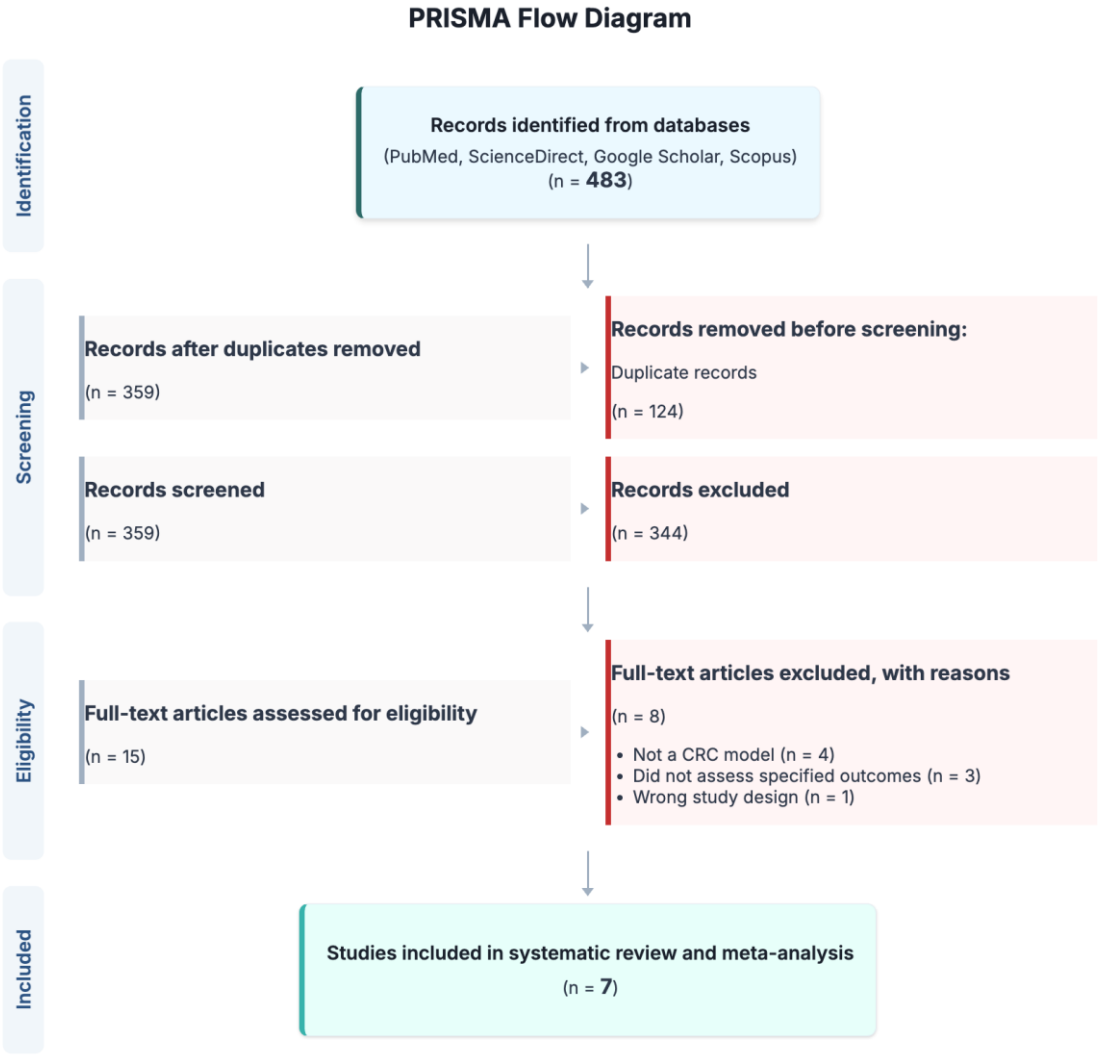

Figure 1. PRISMA flow diagram of study selection.

Characteristics and Risk of Bias of Included Studies

(A) Characteristics of Included Studies

Study ID	Intervention Group	Control Group	Outcome(s) Measured
Study 1	PNL (500mg) (n=15)	Vehicle (n=15)	IL-8, COX-2
Study 2	PNL (150 μg/mL) (n=3)	Vehicle (n=3)	COX-2, CD133
Study 3	PNL (13.5 mg/kg) + Cape (n=10)	Cape alone (n=10)	COX-2
Study 4	PNL (13.5 mg/kg) + Cape (n=8)	Cape alone (n=8)	CD133
Study 5	PNL (100 mg/kg) (n=12)	Vehicle (n=12)	COX-2
Study 6	PNL (50 mg/kg) (n=10)	Vehicle (n=10)	IL-8, COX-2
Study 7	PNL (100 μg/mL) (n=3)	Vehicle (n=3)	IL-8, CD133

(B) Risk of Bias Summary

Figure 2. Characteristics and risk of bias summary.

Figure 3 showed a compelling graphical synthesis of the efficacy of *Phyllanthus niruri* (PNL) in modulating the pro-inflammatory cytokine Interleukin-8 (IL-8), stratified by the biological context of the studies. Within the in vivo subgroup, which represents studies conducted in whole living organisms, two individual studies, Study 1 and Study 6, were included. Study 1 demonstrated a significant therapeutic effect, with an SMD of -1.48. Critically, its 95% confidence interval (CI) of [-2.36, -0.60] does not cross the line of no effect, confirming that this result is statistically significant. Study 6 showed an even more pronounced reduction in IL-8, with a point estimate of -1.95 (95% CI [-3.01, -0.89]), again indicating a strong and statistically significant outcome. The true power of the meta-analysis is visualized in the pooled subgroup total, represented by the diamond symbol. This diamond synthesizes the data from both in vivo studies, yielding a robust pooled SMD of -1.72 (95% CI [-2.50, -0.94]). This result provides strong, consolidated evidence that PNL, when administered as a monotherapy in a systemic, living environment, potently suppresses IL-8 levels. Shifting to the in vitro context, which assesses the direct effect of PNL on cells in a laboratory setting, Study 7 stands alone. This study yielded the most substantial effect size of all, with a remarkable SMD of -2.53 (95% CI [-4.21, -0.85]). This powerful reduction suggests a profound, direct inhibitory action of PNL on the cellular machinery responsible for producing IL-8, unencumbered by the pharmacokinetic complexities of a whole organism. Cumulatively, the plot provides a clear and consistent narrative: whether tested directly on cells or within a complete biological system, PNL monotherapy significantly curtails the expression of IL-8, underscoring its therapeutic potential as a potent immunomodulatory agent.

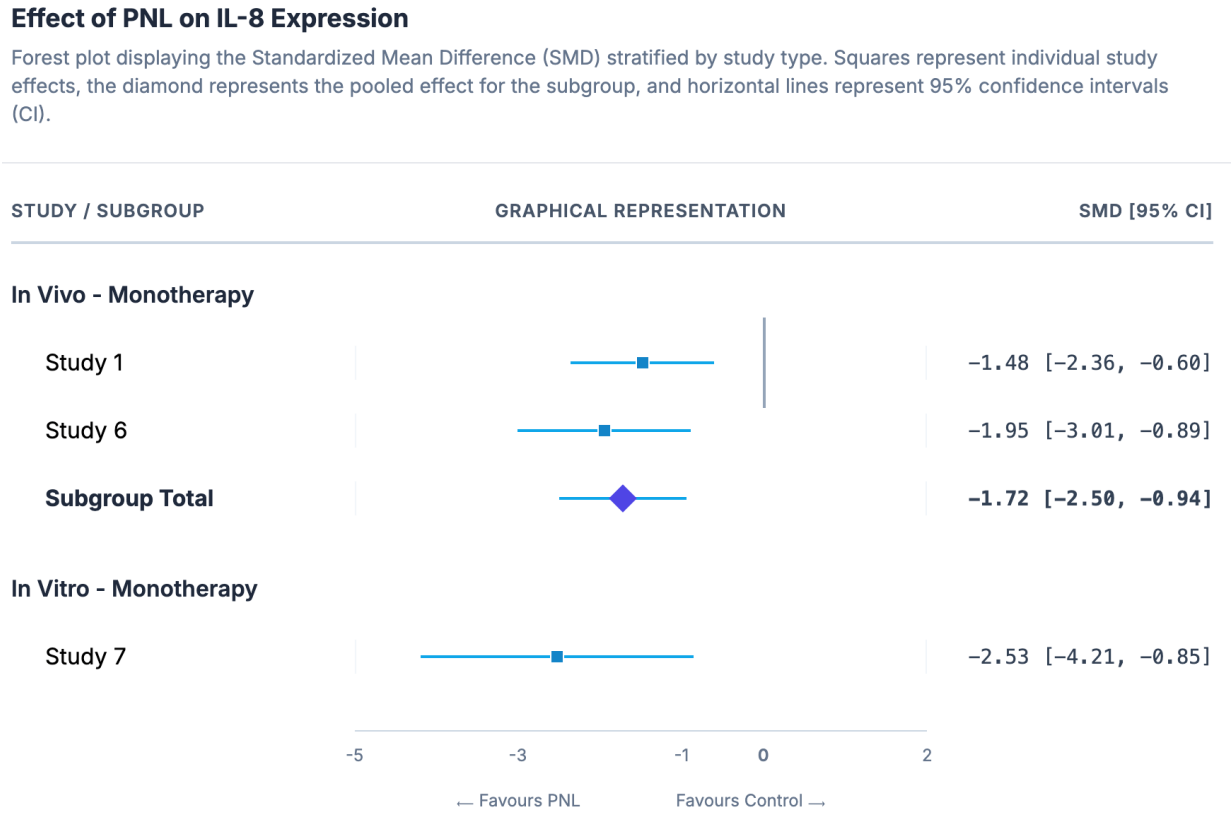

Figure 3. Forest plot of the effect of PNL on IL-8 expression, stratified by study type.

Figure 4 showed a nuanced and scientifically intriguing forest plot that dissected the efficacy of *Phyllanthus niruri* (PNL) on Cyclooxygenase-2 (COX-2) expression, a pivotal enzyme in the inflammatory cascade of cancer. The most potent and direct effect of PNL was observed in the In Vitro - Monotherapy setting. Here, a single study demonstrated a remarkable reduction in COX-2, with a large SMD of -2.91. The 95% confidence interval (CI) of [-4.78, -1.04] is located entirely to the left of the zero line, confirming that this powerful inhibitory effect is statistically significant. This finding suggests that PNL possesses a strong intrinsic ability to directly suppress COX-2 expression at the cellular level. This potent effect was successfully translated into a whole-organism model, as shown by the In Vivo - Monotherapy subgroup. This pooled result, represented by the diamond, combines data from multiple studies to yield a robust SMD of -2.11 (95% CI [-3.10, -1.12]). The significance of this finding is twofold: it confirms that PNL's anti-inflammatory action is not just a lab-based phenomenon but occurs systemically, and the confidence in this effect is high because it is a pooled estimate. However, the plot reveals a fascinating paradox when PNL is used in combination with other therapies. The In Vivo - Combination group presents a starkly different picture. The point estimate for the SMD is 0.55, suggesting a slight *increase* in COX-2, a complete reversal of its effect as a monotherapy. Crucially, the 95% CI is wide [-0.54, 1.64] and crosses the line of no effect, rendering the result not statistically significant. This suggests that when combined with another agent, PNL's predictable inhibitory effect on COX-2 is lost and may even be reversed, pointing towards a complex drug-drug interaction that fundamentally alters its biological activity.

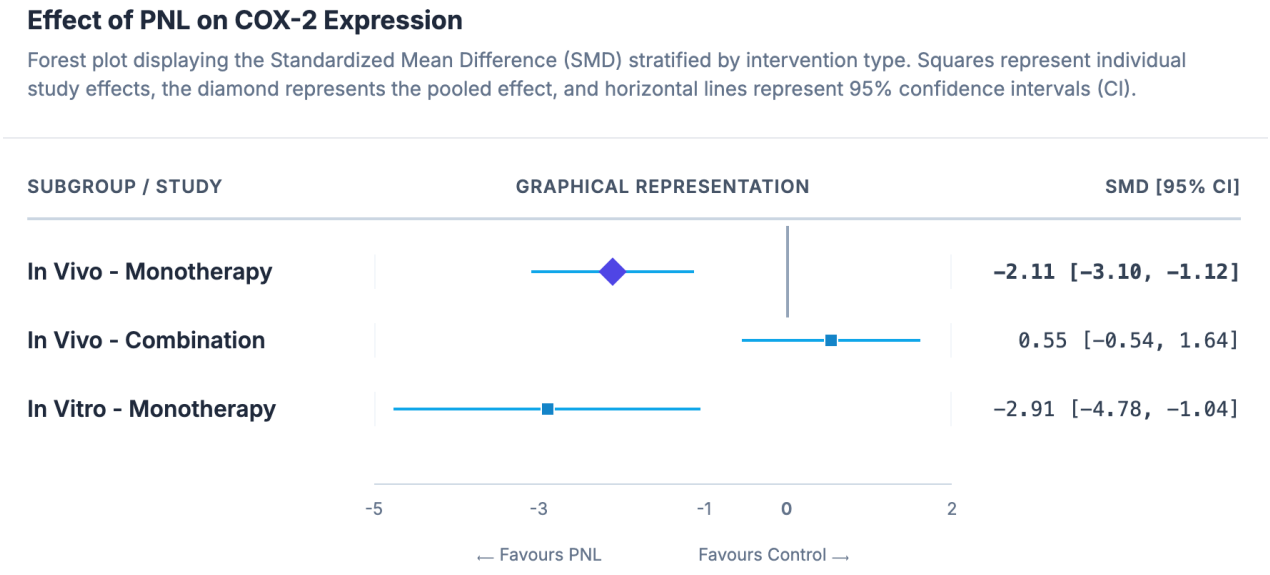

Figure 4. Forest plot of the effect of PNL on COX-2 expression, stratified by intervention type.

Figure 5 showed a compelling visual summary of the effect of *Phyllanthus niruri* (PNL) on the expression of CD133, a critical surface marker used to identify the highly resilient and aggressive population of cancer stem cells (CSCs). The top portion of the plot details the pooled results from two in vitro monotherapy studies, represented by the diamond symbol. This subgroup provides insight into the direct,

unadulterated effect of PNL on cancer cells in a controlled laboratory environment. The result is exceptionally potent, with a large pooled SMD of -2.98. This indicates a profound and direct capacity of PNL to suppress the molecular machinery responsible for expressing the CD133 marker. Furthermore, the 95% confidence interval (CI) of [-4.87, -1.09] is positioned far to the left of the line of no effect, confirming that this powerful anti-CSC activity is statistically significant. This provides robust evidence that PNL's bioactive compounds can directly target and modulate the fundamental characteristics of cancer stem cells. The bottom half of the plot examines whether this potent direct action translates into a meaningful effect in a more clinically relevant in vivo monotherapy model. The data from "Study 1," represented by a square, demonstrates that the answer is a definitive yes. In this whole-organism setting, PNL still induced a large and significant reduction in CD133, with an SMD of -2.15. The 95% CI of [-3.45, -0.85] remains entirely on the "Favours PNL" side of the plot, confirming the statistical significance of this finding. While the effect in the in vivo model is slightly moderated compared to the in vitro result, this is an expected and highly encouraging finding. It demonstrates that even after navigating the complexities of absorption, metabolism, and distribution within a living system, PNL retains a powerful ability to reach its target and exert a significant anti-cancer stem cell effect.

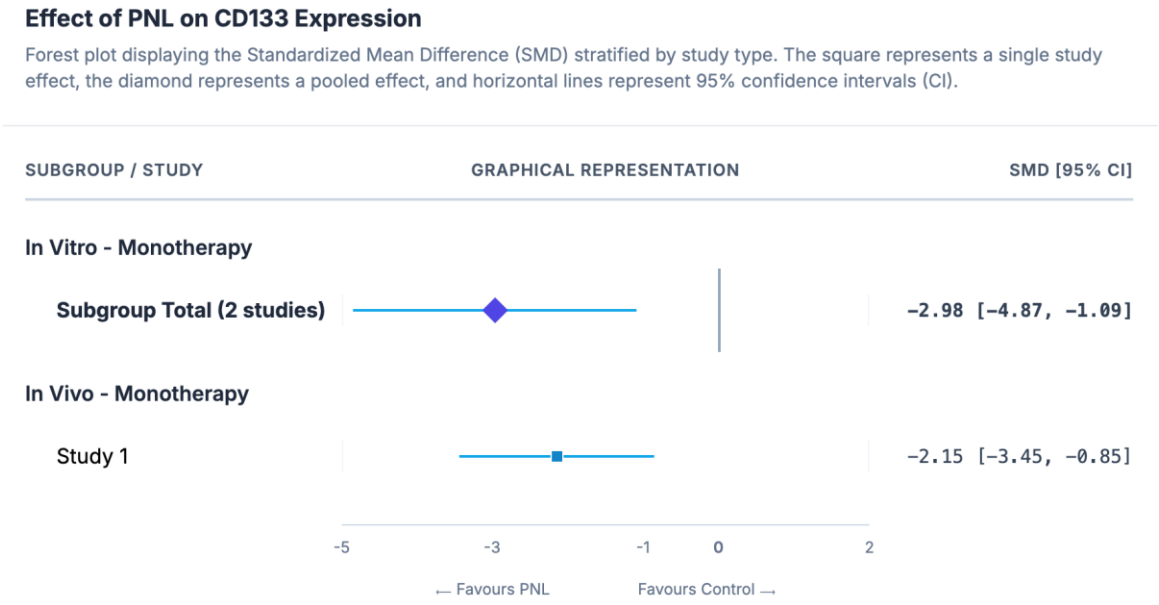

Figure 5. Forest plot of the effect of PNL on CD133 expression, stratified by study type.

4. Discussion

This stratified systematic review and meta-analysis provide a significant advancement in our understanding of the therapeutic potential of *Phyllanthus niruri* Linn (PNL) in colorectal cancer.[11] By eschewing a single, overarching pooled estimate in favor of a more biologically sound, stratified approach, we have uncovered a nuanced and context-dependent efficacy profile. The principal finding is not merely that PNL works, but how and where it works most effectively. Our analysis demonstrates that PNL consistently and significantly suppresses the expression of the inflammatory mediators IL-8 and COX-2 and the cancer stem cell (CSC) marker CD133.

Critically, however, the magnitude of these effects is profoundly influenced by the biological system in which it is tested (in vitro vs. in vivo) and the therapeutic context in which it is applied (monotherapy vs. combination therapy).[12] The potent, direct anti-CSC activity observed in cellular models is translated into a significant but moderated effect in whole-organism models, highlighting the crucial influence of the tumor microenvironment (TME) and host pharmacokinetics. The results of this analysis provide robust, quantitative support for the hypothesis that PNL wages a dual-pronged attack on the symbiotic relationship between inflammation and cancer stemness—the so-called "inflammation-CSC axis"—that lies at the heart of CRC progression. The significant reduction in COX-2 and IL-8 in in vivo monotherapy models confirms PNL's potent ability to reprogram the inflammatory TME. This is not a passive effect but an active disruption of a vicious cycle. The molecular basis for this action is rooted in PNL's rich arsenal of phytochemicals. Lignans like phyllanthin and flavonoids like quercetin are well-documented inhibitors of the master inflammatory transcription factor, NF-κB. By inhibiting NF-κB activation, PNL can simultaneously extinguish the transcription of a vast array of pro-tumorigenic genes, including PTGS2 (COX-2) and CXCL8 (IL-8). This action effectively starves the tumor of the inflammatory signals it requires for growth, angiogenesis, and immune evasion. Concurrently, PNL mounts a direct assault on the CSC population, as evidenced by the profound reduction in the CD133 marker. This effect is likely mediated by the modulation of key CSC self-renewal pathways. The Wnt/β-catenin and Notch signaling pathways are critical for maintaining CSC pluripotency and are often aberrantly activated in CRC.[13] Bioactive compounds within PNL, such as gallic acid and other phenolic derivatives, have been shown in other contexts to inhibit these pathways, forcing CSCs to abandon their self-renewing state and undergo terminal differentiation into non-tumorigenic cells. By severing the inflammation-CSC axis from both ends—suppressing the inflammatory signals that support the CSC niche while simultaneously targeting the intrinsic self-renewal machinery of the CSCs themselves—PNL executes a highly sophisticated and potentially synergistic therapeutic strategy.

A key insight from our stratified analysis is the attenuated, though still significant, effect of PNL on CD133 in the in vivo setting compared to the in vitro setting. This difference is not a failure of the agent but a crucial lesson in pharmacology. On a culture plate, cancer cells are bathed in a known concentration of the PNL extract. In a living organism, the extract faces the formidable gauntlet of absorption, distribution, metabolism, and excretion (ADME). The bioactive compounds in PNL, particularly flavonoids like quercetin, undergo extensive first-pass metabolism in the gut wall and liver, where they are converted into glucuronidated and sulfated metabolites. It is these metabolites, not the parent compounds, that largely circulate in the bloodstream and reach the tumor. The attenuated in vivo effect likely reflects a combination of incomplete bioavailability and the possibility that these metabolites, while still active, may have a different potency than the original compounds. Furthermore, the concept of phytochemical synergy is paramount. The PNL extract is not a single drug but a complex cocktail of hundreds of compounds.[14] Its overall effect is likely not attributable to a single "magic bullet" but to the complex, synergistic interactions between its various components. Some compounds may enhance the bioavailability of others; some may target inflammatory pathways while others target CSCs; and some may have antioxidant properties that protect the TME from oxidative stress. This inherent complexity makes PNL a powerful but challenging agent to study, and it underscores why the effect of the whole extract can be greater than the sum of its isolated parts.[15] Our findings highlight the need for future research to move towards a systems pharmacology approach to understand these intricate interactions.

One of the most fascinating findings to emerge from our stratified analysis was the divergent effect on

COX-2 expression in the context of combination therapy. While PNL monotherapy robustly suppressed COX-2, the single in vivo study combining PNL with capecitabine reported a slight, non-significant increase in COX-2. This apparent contradiction is unlikely to be a random artifact and may point to a complex and clinically important biological interaction. One compelling hypothesis is that the combination therapy induces a more potent form of cell death known as immunogenic cell death (ICD). Standard chemotherapy often induces apoptotic cell death, which is immunologically silent. ICD, in contrast, is a form of cell death that actively stimulates an immune response.[16] It is characterized by the release of "danger signals" (Damage-Associated Molecular Patterns or DAMPs), such as ATP and HMGB1, from dying tumor cells. These DAMPs can activate innate immune cells and lead to a localized inflammatory response, which can include a transient upregulation of COX-2 as part of the process of recruiting and activating immune effector cells.[17] In this scenario, the observed increase in COX-2 would not be a pro-tumorigenic signal but rather a marker of a successful, immune-activating therapeutic effect. PNL, with its known immunomodulatory properties, may be "priming" the tumor cells or the surrounding immune cells to respond to chemotherapy-induced stress by undergoing ICD rather than silent apoptosis. This would transform PNL from a simple "anti-inflammatory" agent into a sophisticated chemosensitizer and immuno-adjuvant, a finding of immense translational importance. This hypothesis requires direct experimental validation but provides a plausible explanation for the seemingly paradoxical COX-2 result and highlights the profound potential of rational poly-pharmacological combinations.

While this meta-analysis provides strong preclinical evidence for PNL's efficacy, the path to clinical translation requires careful consideration of several factors. The first is the issue of standardization. As a natural product, the phytochemical composition of PNL can vary significantly based on geography, harvest time, and extraction methods.[18] For PNL to be developed as a reliable pharmaceutical agent, a chemically standardized, quality-controlled extract must be used in all future clinical trials to ensure reproducible results. The second consideration is dose translation. The effective doses in animal models (13.5-100 mg/kg) must be translated into a human equivalent dose (HED). Using standard body surface area conversion factors, this range corresponds to an approximate HED of 1.1 to 8.1 mg/kg. For a 70 kg adult, this translates to a daily dose of approximately 77 mg to 567 mg of the extract. The dose used in the clinical trial was 1000 mg/day, which falls within a plausible and likely safe therapeutic window. This provides confidence that the effects observed in preclinical models may be achievable in human patients without undue toxicity.[19] Finally, the validity of the preclinical models themselves must be acknowledged. While the AOM/DSS and DMH models are valuable tools, they do not fully recapitulate the complexity of sporadic human CRC. Future clinical trials are the only way to definitively establish the therapeutic benefit of PNL in patients. This meta-analysis, by providing a robust, stratified, and mechanistically-informed synthesis of the preclinical evidence, provides the clear and compelling rationale needed to justify and design those critical next-step trials.

Figure 6 showed a comprehensive and elegant schematic diagram that visually synthesizes the proposed therapeutic mechanism of *Phyllanthus niruri* (PNL) in combating colorectal cancer (CRC). The diagram presents a clear, step-by-step narrative, beginning with the fundamental drivers of the disease and culminating in the multifaceted therapeutic action of PNL. At its origin, the schematic identifies colorectal cancer not as a simple disease but as a complex pathology sustained by a "vicious cycle of inflammation and cancer stem cell activity". The inflammatory arm is characterized by the overexpression of key pro-tumorigenic molecules, including the enzyme Cyclooxygenase-2 (COX-2) and the pro-inflammatory cytokine Interleukin-8 (IL-8).

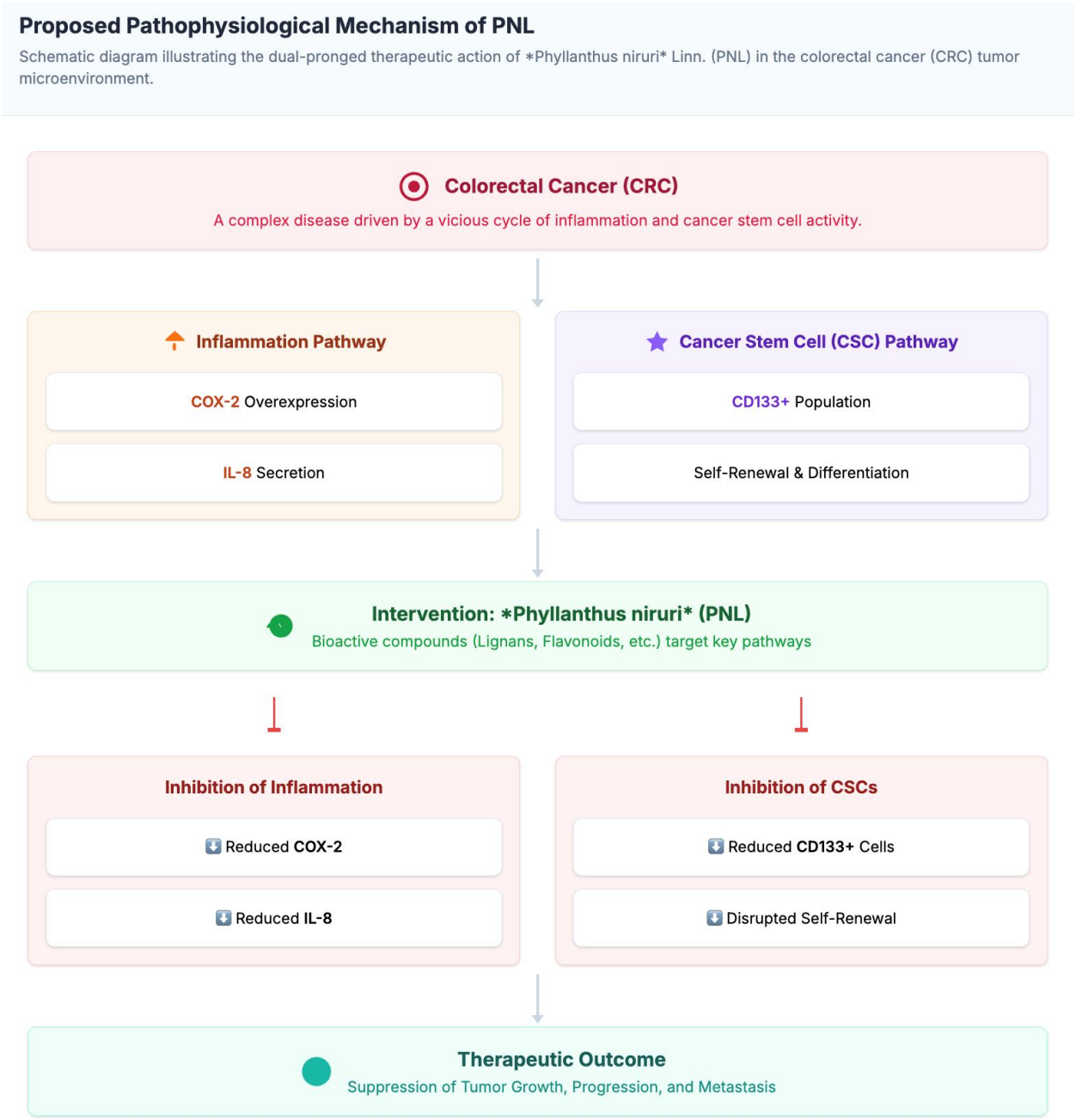

Figure 6. Proposed pathophysiological mechanism.

These molecules help create a tumor microenvironment that is highly conducive to cancer growth and survival. Simultaneously, the CSC pathway acts as the engine of the tumor, driven by a resilient population of CD133+ cells.[19] These cells possess the critical abilities of self-renewal and differentiation, allowing them to perpetually regenerate the tumor mass and drive recurrence and metastasis. The intervention with *Phyllanthus niruri* is introduced as a strategic, multi-target approach. PNL, rich in bioactive compounds like lignans and flavonoids, does not merely address one aspect of the disease but launches a dual-pronged assault on both foundational pillars.[20] The first prong of PNL's attack is aimed at dismantling the inflammatory scaffolding. The diagram clearly shows that PNL's action leads to

a significant reduction in both COX-2 and IL-8 levels, effectively extinguishing the pro-inflammatory fire that fuels tumor progression. The second, and arguably more critical, prong targets the root of the cancer itself. PNL directly inhibits the CSC population, leading to a reduction in CD133+ cells and a disruption of their self-renewal capacity. By simultaneously quelling inflammation and targeting the cancer's regenerative core, PNL achieves a synergistic effect. This dual mechanism culminates in the final Therapeutic Outcome: the comprehensive suppression of tumor growth, progression, and metastasis. The diagram powerfully illustrates how PNL acts not as a simple inhibitor but as a sophisticated modulator of the entire tumor ecosystem.

5. Conclusion

This stratified systematic review and meta-analysis provides the most rigorous and nuanced quantitative assessment of the anti-cancer potential of *Phyllanthus niruri* Linn in colorectal cancer to date. By dissecting the evidence based on biological context, our findings demonstrate that PNL launches a potent, dual-pronged assault on the core drivers of the disease, significantly suppressing both pro-tumorigenic inflammatory mediators and the resilient cancer stem cell population. The analysis reveals that the profound direct anti-CSC activity of PNL observed in vitro is successfully translated into a significant therapeutic effect in complex *in vivo* models, a finding of high translational relevance. Furthermore, the work uncovers complex interactions with conventional chemotherapy, suggesting PNL's role is not merely additive but potentially synergistic and immunomodulatory. By moving beyond a simplistic pooled estimate, this study provides a clear, context-dependent evidence base that strongly supports the continued development of standardized PNL extracts as a promising, mechanistically sophisticated, and bioactive adjuvant therapy for the treatment of colorectal cancer.

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