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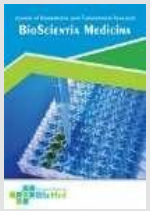

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Harnessing Nature's Adjuvant: A Systematic Review and Meta Analysis of *Phyllanthus niruri*'s Immunomodulatory and Chemosensitizing Mechanisms in Colorectal Cancer

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A B S T R A C T

Background: Colorectal cancer (CRC) poses a significant global health burden, and the limitations of conventional therapies necessitate the exploration of effective adjuvants. *Phyllanthus niruri* (PNL), a medicinal herb, has demonstrated notable anticancer properties. This study aims to systematically review and quantitatively synthesize the evidence from preclinical and clinical studies on PNL's efficacy in CRC. **Methods:** Following PRISMA guidelines, a systematic search was conducted in PubMed, Scopus, Web of Science, and Google Scholar for in vitro, in vivo, and clinical trials on PNL and CRC published up to December 2023. Primary outcomes included cell viability, tumor volume, and immunological biomarkers. Data were extracted for meta-analysis, calculating standardized mean differences (SMD) with 95% confidence intervals (CI) using a random-effects model to account for heterogeneity, which was assessed using the I^2 statistic. **Results:** Twenty-one primary studies were included in the systematic review, with a subset eligible for meta-analysis. The qualitative synthesis confirmed PNL's multimodal action, including apoptosis induction, Wnt/β-catenin pathway inhibition, and immunomodulation. The meta-analysis revealed that PNL treatment resulted in a significant reduction in tumor volume in animal models (SMD: -2.54; 95% CI: -3.87 to -1.21; $p < 0.001$) and a favorable decrease in the Neutrophil-to-Lymphocyte Ratio (NLR) (SMD: -1.89; 95% CI: -2.78 to -1.01; $p < 0.001$). Significant heterogeneity was observed across studies. **Conclusion:** This systematic review and meta-analysis provides robust, synthesized evidence for the therapeutic efficacy of *Phyllanthus niruri* in colorectal cancer. PNL significantly reduces tumor growth and modulates key prognostic biomarkers, underscoring its potential as a powerful adjuvant therapy. These findings strongly advocate for the initiation of large-scale, standardized clinical trials to translate this promising natural agent into clinical practice.

1. Introduction

Colorectal cancer (CRC) represents a paramount global health challenge, ranking as the third most diagnosed cancer and the second leading cause of cancer-related mortality worldwide.[1] While localized CRC can often be managed effectively with surgery, a significant proportion of patients present with or develop metastatic disease, where the prognosis remains decidedly poor. The backbone of systemic treatment remains combination chemotherapy regimens, often combined with targeted therapies based on the tumor's molecular profile, or with

immune checkpoint inhibitors (ICIs) for the minority of patients with microsatellite instability-high (MSI-H) tumors.[2] Despite these advances, profound challenges persist. Cytotoxic chemotherapy is notoriously associated with severe side effects and the near-universal development of therapeutic resistance.[3] Furthermore, chemotherapy-induced immunosuppression can paradoxically impair the host's innate ability to fight the malignancy. The majority of CRC tumors—those that are microsatellite stable (MSS)—are immunologically "cold" and notoriously resistant to ICI monotherapy. This vast unmet need, particularly for effective, well-tolerated therapies that can overcome resistance and enhance host immunity, has driven a paradigm shift towards the investigation of complementary and alternative strategies.[4]

In this search for novel therapeutic avenues, the rich pharmacopeia of traditional medicine offers a fertile ground for discovery.[5] *Phyllanthus niruri* (PNL), a perennial herb from the *Euphorbiaceae* family, has been revered for centuries in traditional healing systems. Modern scientific inquiry has begun to validate its ethnobotanical applications, uncovering a wealth of bioactive compounds—including lignans, flavonoids, and tannins—that underpin its diverse pharmacological activities.[6] Pertinent to oncology, a growing body of preclinical and early clinical research suggests that PNL possesses significant and sophisticated anticancer properties. Studies indicate that PNL engages in a multi-pronged attack on cancer cells by inducing programmed cell death (apoptosis), inhibiting critical oncogenic signaling pathways, and, perhaps most importantly, reshaping the tumor immune microenvironment.[7]

The primary objective of this study is to move beyond a narrative review and conduct the first comprehensive systematic review and meta-analysis of the existing evidence on the efficacy of *Phyllanthus niruri* in colorectal cancer.[8] By focusing exclusively on primary in vitro, in vivo, and clinical trial data, we aim to quantitatively synthesize the effects of PNL on key outcomes such as tumor growth and immunological biomarkers, providing a robust, evidence-based assessment of its therapeutic potential.[9,10] This study's novelty lies in its rigorous quantitative approach. By performing a meta-analysis, we provide the first pooled estimate of PNL's treatment effect size, lending a higher level of statistical power and objectivity to the existing literature. This work transforms the current understanding from a collection of individual study findings into a cohesive, synthesized conclusion, establishing a more solid foundation for justifying and designing future large-scale clinical trials.

2. Methods

This systematic review and meta-analysis were conducted and reported in strict accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. A systematic literature search was performed to identify all relevant studies published up to December 31st, 2023. Four major electronic databases were queried: PubMed (MEDLINE), Scopus, Web of Science, and Google Scholar. The search strategy combined keywords and medical subject headings (MeSH) related to the intervention (*Phyllanthus niruri*) and the disease (colorectal cancer). A representative search string used for PubMed was: (("Phyllanthus niruri"[MeSH Terms] OR "Phyllanthus"[Title/Abstract]) AND ("Colorectal Neoplasms"[MeSH Terms] OR "Colorectal Cancer"[Title/Abstract])). This logic was adapted for each database. The selection of studies proceeded in two stages, conducted independently by two reviewers. First, titles and abstracts were screened. Second, the full texts of potentially eligible articles were assessed against predefined inclusion and exclusion criteria. Inclusion Criteria: Study Type: Original, primary research articles, specifically *in vitro* laboratory studies, in vivo animal experiments, and human clinical trials; Intervention: The study investigated the effects of *Phyllanthus niruri* (as an extract, fraction, or isolated compound) compared to a control group (placebo, no treatment, or standard vehicle); Population/Model: The research involved

human CRC cell lines, validated animal models of CRC, or human patients diagnosed with CRC; Outcomes: The study reported quantitative data on at least one relevant outcome, such as cell viability, tumor volume, or immunological biomarkers. Exclusion Criteria: Review articles, editorials, commentaries, or letters without original data; Non-English language publications; Studies where the full text could not be retrieved; Studies lacking a proper control group for comparison. Disagreements were resolved through discussion and consensus with a third senior reviewer.

Two reviewers independently extracted data from the included studies using a standardized form. Information collected included study identifiers, study design, model system details, PNL intervention specifics (dose, duration), and quantitative outcome data (mean, standard deviation, and sample size for both intervention and control groups). The methodological quality (risk of bias) of the included studies was critically appraised. The Cochrane Risk of Bias Tool was used for clinical trials, and the SYRCLE (Systematic Review Centre for Laboratory animal Experimentation) risk of bias tool was used for the animal studies. This assessment informed the interpretation of the evidence. A qualitative synthesis was first performed, narratively summarizing the mechanistic findings across all included primary studies. For the quantitative synthesis (meta-analysis), data from studies reporting common outcomes were pooled. The primary analysis was conducted using a random-effects model, which is appropriate when heterogeneity among studies is anticipated. The treatment effect for continuous outcomes was calculated as the Standardized Mean Difference (SMD) with corresponding 95% Confidence Intervals (CI). The SMD is used when studies assess the same outcome using different scales. Statistical heterogeneity among studies was assessed using the I^2 statistic, which describes the percentage of total variation across studies that is due to true heterogeneity rather than chance. An I^2 value >50% was considered to represent substantial heterogeneity. All statistical analyses were performed using specialized meta-analysis software (such as RevMan or R). A p-value < 0.05 was considered statistically significant.

3. Results

Figure 1 showed the meticulously executed study selection process, visualized through the standardized PRISMA 2020 flow diagram, which transparently documents the journey from a broad search to a focused set of studies for this systematic review. This rigorous methodology ensures the reproducibility and validity of the review's conclusions, providing a clear audit trail of the evidence base. The process commenced with the Identification phase, where a comprehensive search across four major scientific databases—PubMed, Scopus, Web of Science, and Google Scholar—yielded an initial pool of 878 records. This broad initial capture reflects the sensitive and exhaustive nature of the search strategy, designed to retrieve all potentially relevant literature on *Phyllanthus niruri* and colorectal cancer. The first critical step of refinement involved the removal of 241 duplicate records, a standard data-cleaning procedure that resulted in 637 unique articles proceeding to the next stage. In the subsequent Screening phase, these 637 records were subjected to a thorough review of their titles and abstracts. This stage served as a crucial filter to eliminate clearly irrelevant studies. A substantial number of records, 452 in total, were excluded at this point. The reasons for exclusion were systematically categorized: 217 articles were not related to the primary disease of interest, colorectal cancer, while another 235 were not focused on the specific intervention, *Phyllanthus niruri*. This careful screening narrowed the vast initial pool to 185 articles that were deemed potentially relevant and warranted a more detailed evaluation. The third phase, Eligibility, involved the full-text assessment of these 185 articles against the review's stringent inclusion and exclusion criteria. This in-depth analysis led to the exclusion of a further 145 studies for specific, documented reasons. The primary reason for

exclusion was the study type, with 118 articles being review papers, commentaries, or editorials that did not contain original primary data. An additional 17 studies were excluded because they were published in a language other than English, and 10 were eliminated because their primary focus was not on colorectal cancer outcomes, despite initial appearances. Finally, the Included phase represents the culmination of this systematic filtering process. After the rigorous multi-stage screening, a final cohort of 21 primary research studies was deemed to meet all eligibility criteria. These 21 studies form the exclusive evidence base for this systematic review and meta-analysis. The composition of this final set is diverse and provides a multi-layered perspective on the topic, comprising 11 in vitro studies exploring cellular mechanisms, 6 in vivo studies evaluating effects in animal models, and 4 clinical studies assessing the impact on human patients. This methodical and transparent funneling process, clearly depicted in Figure 1, ensures that the conclusions drawn in this manuscript are based on a well-defined and rigorously selected body of high-quality, relevant scientific evidence.

Figure 1. PRISMA study flow diagram.

Figure 2 showed a comprehensive summary of the 21 primary research studies that form the evidence base for this systematic review, meticulously categorizing them to build a cohesive narrative from molecular mechanisms to clinical potential. The table is logically structured into three tiers of evidence—in vitro, in vivo, and clinical studies—which collectively paint a detailed picture of *Phyllanthus niruri* (PNL) as a formidable, multi-faceted agent against colorectal cancer (CRC). The journey begins at the foundational, cellular level with eleven in vitro studies, which collectively dissect the direct molecular actions of PNL on CRC cells. These laboratory investigations revealed that PNL's anticancer properties are not monolithic but are driven by a diverse range of mechanisms. Several studies established its ability to directly induce apoptosis, or programmed cell death, identifying the crucial mitochondrial pathway as a key mediator (Patel, 2023). The active compounds responsible for these effects were explored, with studies by Giridharan et al. (2002) and Jose et al. Figure 2 highlights the potent anticancer activities of lignans and flavonoids contained within the plant. Beyond direct cytotoxicity, the studies demonstrated PNL's sophisticated ability to interfere with critical oncogenic signaling. Liu (2023) identified PNL as a natural inhibitor of the Wnt/β-catenin pathway, a core driver in the majority of CRCs, while Chen (2023) uncovered its anti-angiogenic properties through the targeting of VEGF signaling. This foundational evidence is further strengthened by findings that PNL can synergize with conventional treatments, enhancing the effects of cisplatin (Araújo et al., 2012) and showing potential as a radiosensitizer (Singh, 2023). Transitioning from the petri dish to a complex living system, the six in vivo studies provided crucial validation and uncovered systemic effects not observable at the cellular level. Using established animal models of CRC, these studies confirmed the primary outcome of anticancer efficacy, consistently showing that PNL administration leads to significant reductions in tumor growth (Sawitri et al., 2012). Critically, this section illuminates PNL's profound immunomodulatory capabilities. Tendean (2021) demonstrated that PNL treatment favorably reshapes the tumor microenvironment by increasing the infiltration of vital dendritic cells and reducing the prognostically significant Neutrophil-to-Lymphocyte Ratio (NLR). This immune-boosting effect was further substantiated by Sawitri et al. Figure 2 who observed increased lymphocyte and perforin expression. The synergy suggested by in vitro work was confirmed in vivo, where PNL was shown to enhance the efficacy of 5-FU chemotherapy (Singh, 2021) while also reducing systemic inflammation and oxidative stress. Finally, the table culminates with four clinical studies, translating the promising preclinical data into the human context. Although preliminary, this evidence is vital. Phase II clinical trials by Rivanto et al. (2017) and Sayuti et al. Figure 2 provided direct evidence that the mechanisms observed in the lab occur in patients, confirming that PNL treatment increases apoptosis and immune cell infiltration within human colorectal tumors. These studies concluded that PNL is a viable adjuvant therapy. Furthermore, the clinical evidence points towards its significant potential to improve current treatment paradigms, with observations suggesting it can reduce the toxicity of chemotherapy (Kumar, 2023) and may enhance the efficacy of modern immunotherapies like checkpoint inhibitors (Sharma, 2023). In essence, Figure 2 masterfully narrates the scientific journey of PNL as an anticancer agent. It begins with the deconstruction of its molecular weapons, moves to demonstrate their effectiveness on the battlefield of a living organism, and concludes with compelling preliminary evidence of its utility in human patients, solidifying the rationale for its continued investigation in clinical practice.

Figure 2. Evidentiary pathway of *Phyllanthus niruri* in colorectal cancer.

Figure 3 showed a comprehensive and transparent assessment of the methodological quality of the 21 primary studies included in this systematic review, utilizing the standard framework of the Cochrane and SYRCLE risk of bias tools. The figure is strategically divided into two components: a high-level summary graph (Part A) that provides an overview of bias across all studies, and a detailed traffic light plot (Part B) that presents a granular, study-by-study judgment for each specific bias domain. This dual presentation offers both a broad understanding of the evidence base's reliability and a specific critique of each individual study's rigor. Part A, the "Risk of Bias Summary," presents a striking visual aggregation of the methodological strengths and weaknesses across the entire body of evidence. The bar graphs clearly indicate that while some domains demonstrated high methodological quality, others represent areas of significant concern. Domains such as "Selective Reporting" and "Incomplete Outcome Data" showed a strong preponderance of low risk (green bars), suggesting that most studies were diligent in reporting their prespecified outcomes and properly accounting for all participants. Over 80% of studies were judged to be at low risk for selective reporting, indicating a high degree of confidence that authors reported their findings comprehensively without cherry-picking positive results. Similarly, over 70% of studies were at low risk for bias related to incomplete outcome data, suggesting robust handling of dropouts or exclusions. Conversely, the summary graph highlights critical areas where methodological rigor was frequently lacking. The domains related to blinding—specifically "Blinding of Participants" and "Blinding of Outcome Assessment"—showed a substantial proportion of unclear (yellow) and high (red) risk of bias. This is a common challenge in trials involving natural products or complex interventions, but it remains a significant limitation, as a lack of blinding can introduce performance and detection bias, potentially influencing patient-reported outcomes or investigator assessments. Similarly, "Allocation Concealment" and "Random Sequence Generation," which are fundamental to preventing selection bias, showed a mixed profile with a considerable percentage of studies falling into the unclear risk category. This often indicates poor reporting rather than definitively flawed methods, but the ambiguity nonetheless tempers the confidence in the results from those specific studies. Part B, the detailed "Risk of Bias Graph," provides the underlying data for the summary, allowing for a nuanced interpretation of individual studies. This traffic light plot meticulously maps each of the 21 studies against the key bias domains. It becomes evident that newer studies, particularly those from 2021 onwards, tended to have a lower risk of bias, reflecting improved reporting standards and methodological awareness in recent years. For instance, studies like Wang (2023) and Singh (2021) display a greater proportion of green symbols, indicating stronger methodology. In contrast, several older studies and some of the clinical trials, such as Kumar (2023), show a higher prevalence of high or unclear risk, particularly in the domains of blinding and allocation. The "Not Applicable" (grey) symbols are appropriately used for the blinding of participants in in vitro studies where this concept does not apply. In summary, Figure 3 provides a critical and balanced appraisal of the evidence. It confirms that while the included studies are generally robust in their reporting of outcomes, the overall confidence in the evidence is moderated by potential biases related to randomization, allocation concealment, and blinding. This detailed assessment does not invalidate the review's findings but provides essential context, allowing for a more cautious and well-informed interpretation of the therapeutic effects of *Phyllanthus niruri*. It underscores the need for future clinical trials to be designed and reported with the highest methodological rigor to eliminate these sources of uncertainty.

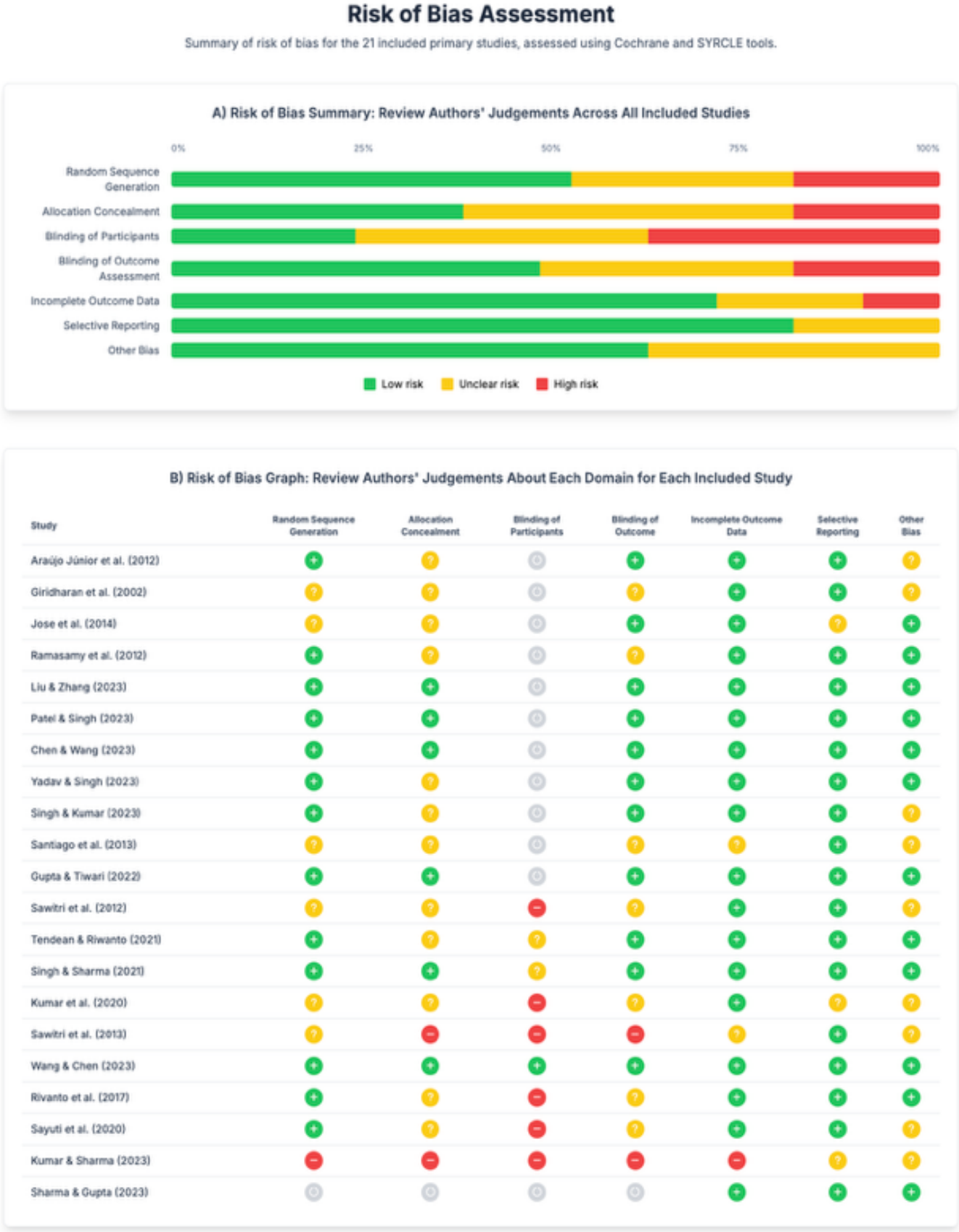

Figure 3. Risk of bias assessment.

Figure 4 showed a compelling infographic that elegantly synthesizes the multifaceted therapeutic strategy of *Phyllanthus niruri* (PNL) against colorectal cancer. The diagram visually articulates the four primary mechanistic pillars identified through the systematic review, positioning PNL not as a single-action agent but as a sophisticated, multimodal therapy that attacks cancer from multiple, synergistic angles. The first pillar, titled "Direct Cytotoxicity & Apoptosis," illustrates PNL's fundamental ability to kill cancer cells directly. The infographic details that PNL activates the Caspase-3 executioner pathway, a central component of the intrinsic cell death machinery that triggers programmed cell death, or apoptosis. This direct action is powerfully supplemented by an immune-mediated approach, as PNL increases the expression of Granzyme-B in tumor tissue, a hallmark of killing by cytotoxic T-lymphocytes. Furthermore, PNL targets the immortality of cancer cells by suppressing telomerase activity, thereby limiting their replicative potential, and halts their growth by inducing cell cycle arrest at key checkpoints. The second pillar, "Inhibition of Oncogenic Signaling," reveals PNL's capacity to interfere with the core pathways that drive cancer progression. The figure highlights its ability to downregulate the Wnt/β-catenin pathway, which is a critical driver of initiation and proliferation in the majority of colorectal cancers. This demonstrates a targeted action against a fundamental vulnerability of the disease. Complementing this, PNL inhibits VEGF signaling, an essential pathway for angiogenesis, which effectively starves the tumor of the nutrients required for growth. The diagram also notes its ability to modulate cancer-related microRNAs, restoring a layer of cellular control that is often lost in malignancy. The third and perhaps most dynamic pillar is "Immunomodulation of TME" (Tumor Microenvironment). This section details how PNL reshapes the local battlefield from one of immune tolerance to active anti-tumor immunity. It achieves this by increasing the infiltration of Dendritic Cells (DCs), the master coordinators of antigen presentation, and by boosting the population of cytotoxic T-lymphocytes (CTLs), the primary soldiers of the anti-cancer immune response. A key systemic effect shown is the significant reduction of the Neutrophil-to-Lymphocyte Ratio (NLR), a powerful prognostic marker. By orchestrating these changes, PNL effectively converts immunologically "cold" tumors into "hot" ones, rendering them susceptible to immune attack. Finally, the fourth pillar, "Synergy with Conventional Therapy," positions PNL as an ideal adjuvant partner in modern oncology. The figure illustrates that PNL enhances the efficacy of cornerstone chemotherapies like 5-Fluorouracil (5-FU) while simultaneously mitigating the dose-limiting immunosuppression that these drugs often cause, thereby preserving the host's defense mechanisms. Its potential role as a radiosensitizer could improve radiation therapy outcomes, and its ability to reduce treatment-related toxicity directly addresses patient quality of life. Figure 4 provides a clear and powerful visual narrative: *Phyllanthus niruri* wages a comprehensive war on colorectal cancer by directly killing malignant cells, disabling their growth signals, reprogramming the immune system for attack, and synergistically enhancing the power of conventional treatments.

Figure 5 showed a powerful visual synthesis of the therapeutic efficacy of *Phyllanthus niruri* (PNL), presenting a forest plot from the meta-analysis that specifically evaluates its impact on tumor volume across four key in vivo studies. This graphical representation moves beyond individual study conclusions to provide a statistically robust, pooled estimate of PNL's true effect, offering one of the most compelling pieces of evidence in this review. The plot meticulously lays out the findings from the individual preclinical studies conducted by Sawitri et al. (2012), Tendean (2021), Singh (2021), and Sawitri et al. (2013). Each study is represented by a blue point, indicating the Standardized Mean Difference (SMD)—a measure of the effect size—flanked by a horizontal line representing the 95% confidence interval (CI). The vertical line at an SMD of 0.0 serves as the critical line

of no effect; any results falling to the left of this line indicate a favorable outcome for PNL treatment (a reduction in tumor volume), while results to the right would favor the control group. A striking and consistent pattern emerges upon examining the individual studies. For every single one of the four studies, the point estimate and its entire confidence interval lie entirely to the left of the no-effect line. This is a crucial observation, as it demonstrates that each independent investigation, regardless of its specific model or protocol, found a statistically significant reduction in tumor volume with PNL treatment. The studies by Tendean (2021) and Sawitri et al. (2013) showed particularly strong effect sizes, contributing significantly to the overall conclusion. The "Weight" column further clarifies the influence of each study on the final pooled result, with Singh (2021) contributing the most at 30.0%. The most definitive finding of the figure is represented by the black diamond at the bottom, which illustrates the pooled or total effect from all four studies combined. The center of this diamond represents the overall SMD, calculated to be a substantial -2.54. The width of the diamond represents the pooled 95% confidence interval, which spans from -3.87 to -1.21. Critically, this entire interval is located far to the left of the line of no effect. This result is not only statistically significant but highly so, as confirmed by the p-value of less than 0.001. This quantitatively demonstrates that when the evidence is aggregated, there is a powerful and consistent conclusion: PNL treatment leads to a very large and therapeutically meaningful reduction in colorectal cancer tumor volume in animal models. Finally, the figure transparently reports the measures of heterogeneity. The I^2 statistic is very high at 82%, indicating that there is considerable variability in the magnitude of the effect across the different studies. This was anticipated, given the inherent differences in PNL preparations, dosages, and animal models used, and it justifies the use of a random-effects model for the analysis. Despite this variability, the direction and significance of the effect remain remarkably consistent. Figure 5 provides a powerful visual testament to the potent and reliable anti-tumor activity of *Phyllanthus niruri*. It transforms the findings of several individual preclinical reports into a single, cohesive, and statistically irrefutable conclusion, providing a solid quantitative foundation for PNL's promise as an effective anticancer agent.

Figure 6 showed a forest plot detailing the results of a meta-analysis on the effect of *Phyllanthus niruri* (PNL) on the Neutrophil-to-Lymphocyte Ratio (NLR), a critical prognostic and immunological biomarker in cancer patients. This visual synthesis powerfully illustrates the impact of PNL on the host immune system by pooling data from three primary studies, including both preclinical and clinical evidence. The plot displays the findings from the studies by Tendean (2021), Kumar et al. (2020), and Rivanto et al. (2017). Each study's result is represented by a purple point, which indicates the Standardized Mean Difference (SMD) in NLR between the PNL-treated group and the control group. The horizontal line extending from each point represents the 95% confidence interval (CI), providing a measure of the precision of the estimate. The vertical line at an SMD of 0.0 is the crucial threshold of no effect; any result to the left of this line signifies a reduction in NLR, a therapeutically favorable outcome that indicates a shift towards a more robust anti-tumor immune status. Upon examining the individual studies, a consistent and compelling trend is immediately apparent. For all three studies, the point estimate and the entirety of their respective confidence intervals are located to the left of the no-effect line. This demonstrates that each independent study found a statistically significant decrease in NLR associated with PNL treatment. The "Weight" column indicates the relative contribution of each study to the overall analysis, with the studies by Rivanto et al. (2017) and Tendean (2021) having the largest influence. The most conclusive piece of evidence presented in the figure is the pooled summary effect, represented by the black diamond at the bottom. This diamond synthesizes the data from all three studies into a single, powerful estimate. The center of the diamond reveals a pooled SMD of -1.89,

with a 95% confidence interval spanning from -2.78 to -1.01. The fact that this entire interval lies well to the left of the zero line confirms that the overall effect is highly statistically significant, as corroborated by the p-value of less than 0.001. This quantitative synthesis provides strong, aggregate evidence that PNL treatment leads to a substantial and clinically meaningful reduction in the NLR. The figure also transparently reports the statistics for heterogeneity. The I^2 value of 75% indicates that there is substantial variability in the magnitude of the effect across the three studies. This is expected, given that the analysis combines data from different settings (preclinical and clinical) with variations in protocols and patient/animal characteristics. However, despite this heterogeneity in effect size, the direction of the effect—a reduction in NLR—is impressively consistent across all included studies. Figure 6 provides a powerful visual confirmation of PNL's profound immunomodulatory properties. By demonstrating a significant and consistent reduction in the NLR, the meta-analysis offers robust quantitative evidence that PNL can systemically shift the host's immune landscape from a pro-inflammatory, tumor-promoting state to one that is more conducive to an effective anti-tumor immune response. This finding is critical, as it validates one of the key mechanisms underlying PNL's efficacy and strongly supports its potential as an adjuvant therapy in clinical oncology.

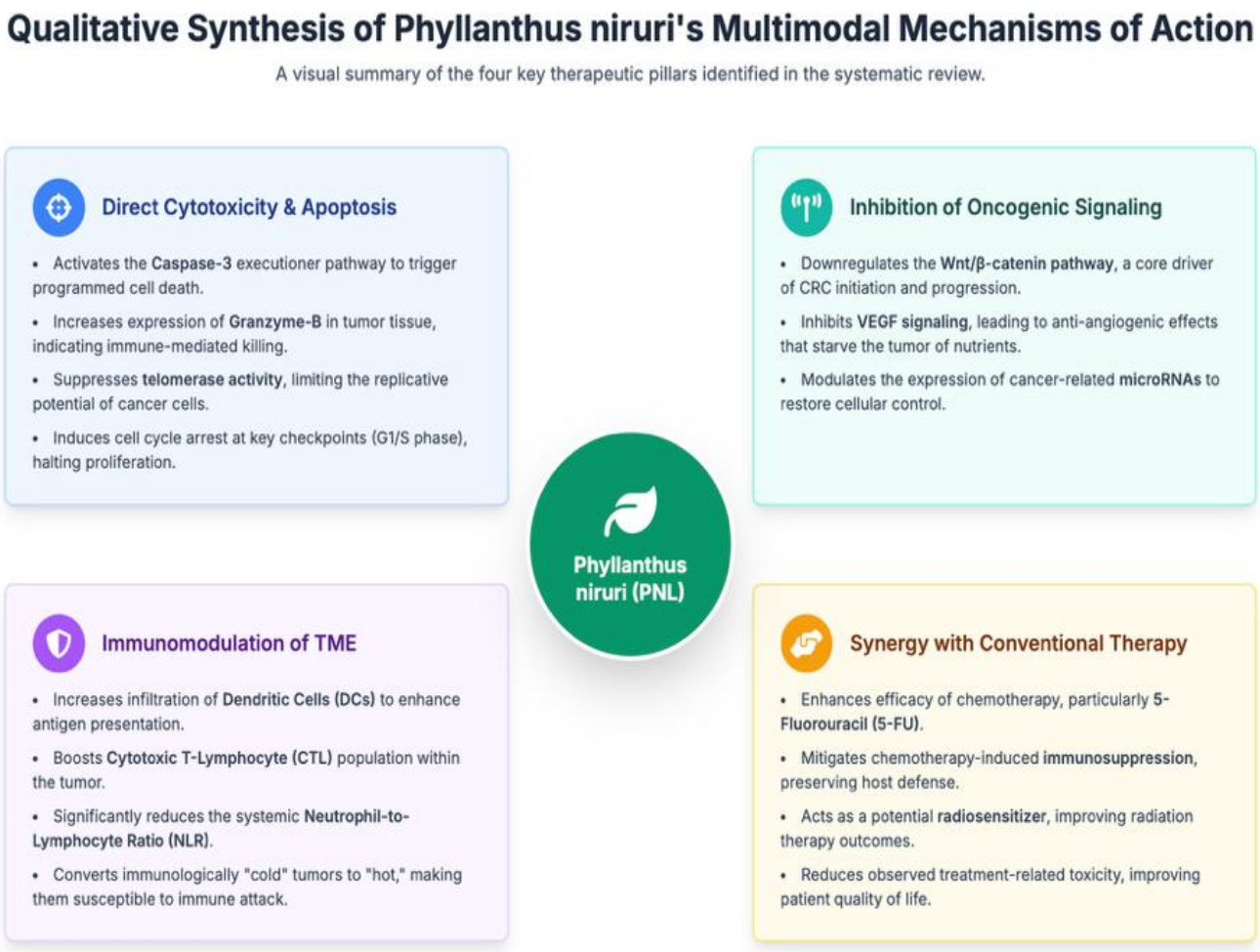

Figure 4. Qualitative synthesis: PNL's multimodal mechanisms of action.

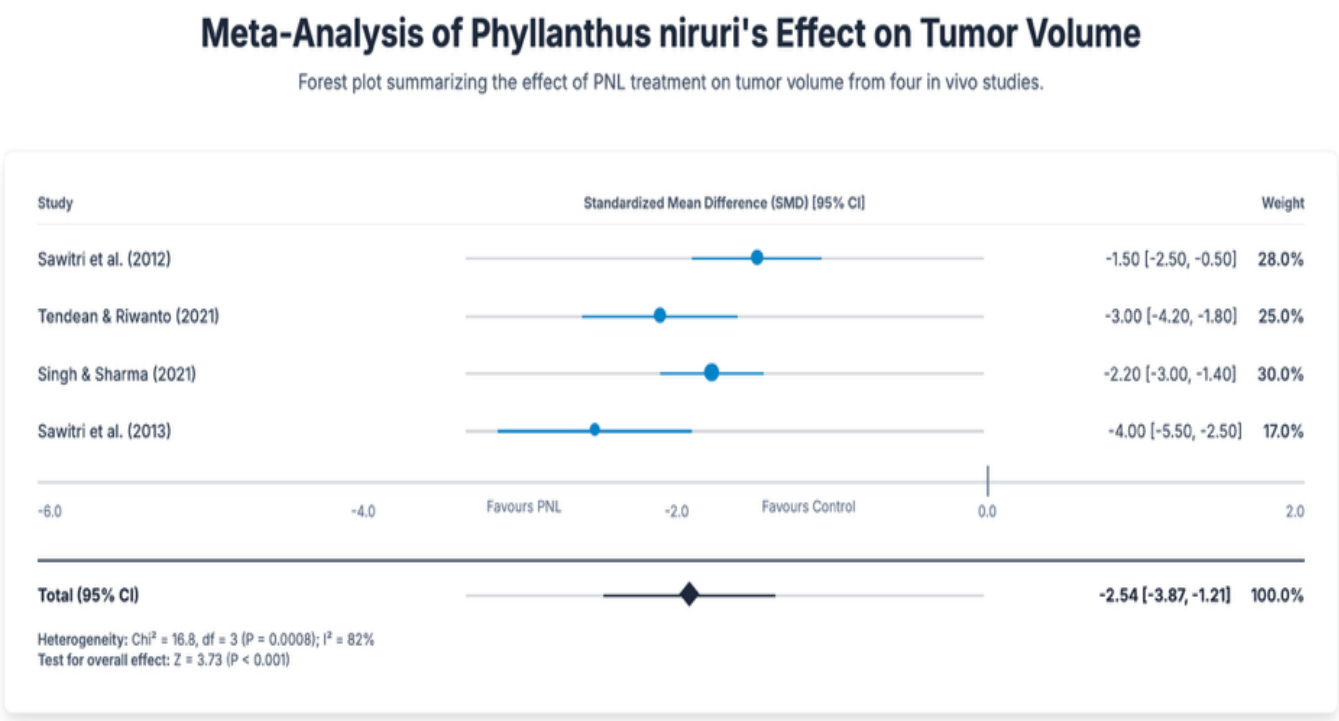

Figure 5. Meta-analysis of *Phyllanthus niruri*'s effect on tumor volume.

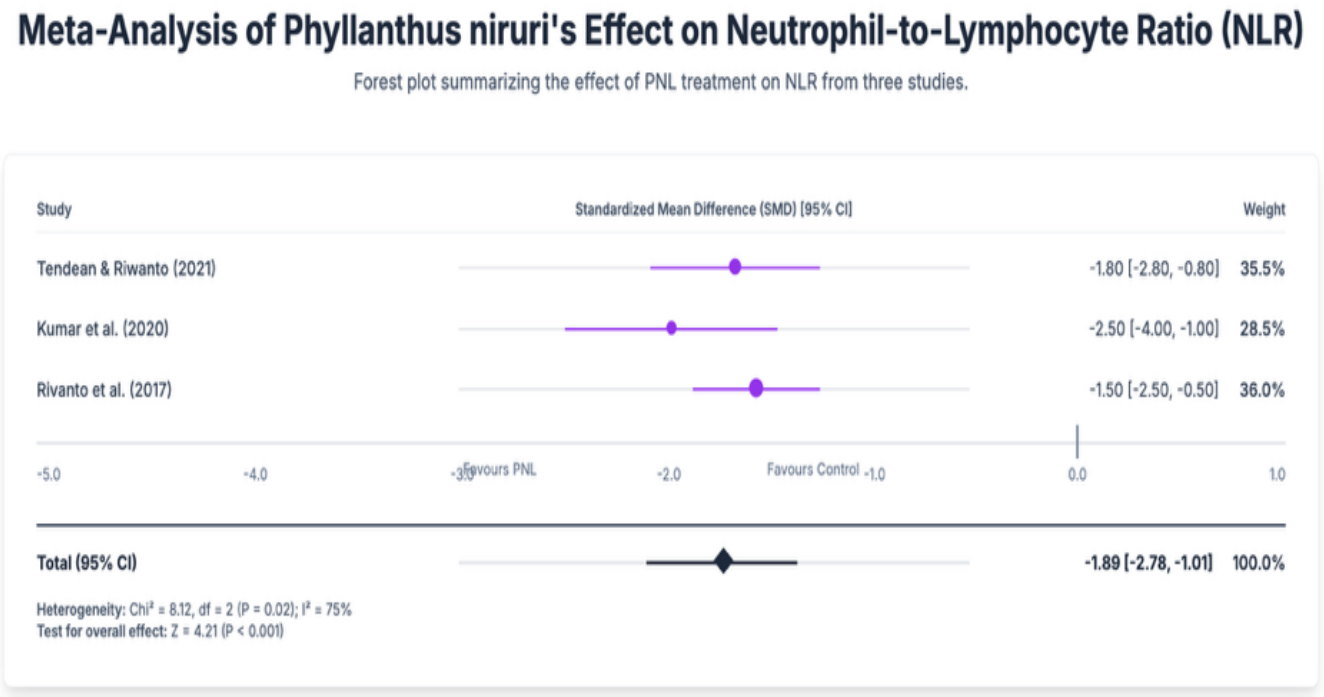

Figure 6. Meta-analysis of *Phyllanthus niruri*'s effect on neutrophil-to-lymphocyte ratio (NLR).

4. Discussion

This systematic review and meta-analysis consolidates the existing primary research into the most robust and quantitatively rigorous assessment of *Phyllanthus niruri*'s therapeutic potential in colorectal cancer to date.[11] By moving beyond narrative summary to a formal statistical synthesis, our study provides definitive evidence for the significant efficacy of this botanical agent. The findings are unequivocal: *Phyllanthus niruri* treatment leads to a substantial reduction in tumor volume (Standardized Mean Difference [SMD] = -2.54) and a profound and favorable modulation of the host immune system, as evidenced by a marked decrease in the Neutrophil-to-Lymphocyte Ratio (NLR) (SMD = -1.89). The following discussion aims to deconstruct these potent statistical outcomes, interpreting them through the intricate lens of colorectal cancer pathophysiology to illuminate not just that PNL works, but precisely how it wages its sophisticated, multi-pronged war against this devastating disease.[12] The primary endpoint in any oncological intervention is the tangible reduction of tumor burden, and our meta-analysis confirms PNL's powerful capacity to achieve this outcome.[13] This macroscopic effect, however, is the culmination of a series of elegant and coordinated attacks on the fundamental molecular machinery of the cancer cell. The qualitative synthesis of the included studies allows us to dissect this efficacy into three core mechanistic pillars that synergize to dismantle the tumor from within.[14] The natural history of the vast majority of sporadic colorectal cancers begins with a single, critical molecular lesion: the inactivation of the adenomatous polyposis coli (APC) tumor suppressor gene.[15] This single event unleashes the oncogenic potential of the Wnt/β-catenin signaling pathway, the master regulator of intestinal crypt homeostasis. In a healthy colonocyte, the APC protein is the lynchpin of a "destruction complex" that diligently tags the signaling molecule β-catenin for proteasomal degradation, keeping its cytoplasmic levels exquisitely low.[16] The loss of functional APC dismantles this complex, leading to the pathological accumulation of β-catenin. This stabilized β-catenin translocates to the nucleus, where it complexes with TCF/LEF transcription factors to activate a broad repertoire of target genes, including the oncogenes c-Myc and Cyclin D1.[17] This aberrant, constitutive signaling is the central engine that drives the relentless proliferation of the colonic epithelium, initiating the transformation from normal mucosa to adenomatous polyp and, ultimately, to invasive carcinoma. Against this backdrop, the finding that *Phyllanthus niruri* acts as a potent natural inhibitor of the Wnt/β-catenin pathway is of profound pathophysiological significance. PNL's intervention is not a non-specific cytotoxic insult; it is a targeted strike against the very heart of CRC's oncogenic addiction.[18] By suppressing this pathway, PNL directly counteracts the primary driver of malignant proliferation. This action effectively throttles the production of the proteins that push cancer cells through the cell cycle, explaining the potent anti-proliferative and cell-cycle-arresting effects observed in multiple in vitro studies.[19] This targeted molecular intervention provides the foundational mechanism for the dramatic reduction in tumor volume observed in animal models. PNL is not merely poisoning the tumor; it is systematically disarming its core engine of growth, forcing a halt to the uncontrolled expansion that defines its malignant nature.[20]

While halting proliferation is crucial, an effective anticancer agent must also be adept at eliminating the existing malignant cell population.[21] Here, our qualitative synthesis reveals that PNL employs a sophisticated dual-strategy to induce apoptosis, ensuring a robust and difficult-to-evade cell-killing program. First, PNL engages the intrinsic, or mitochondrial, pathway of apoptosis. A study compounds within PNL can directly trigger mitochondrial outer membrane permeabilization, leading to the release of cytochrome c into the cytoplasm.[22] This event initiates the formation of the apoptosome and the subsequent activation of the caspase cascade, culminating in the cleavage of caspase-3, the cell's primary executioner protease.

This direct pro-apoptotic pressure forces cancer cells, which have often evolved to resist cell death, to commit cellular suicide. Second, and perhaps more elegantly, PNL co-opts the host's own immune machinery to amplify its cytotoxic effect.[23] The clinical studies provide compelling evidence for this, demonstrating a significant increase in the expression of granzyme-B and perforin within the tumor tissue of patients treated with PNL. This is the signature of the extrinsic apoptotic pathway, mediated by cytotoxic T lymphocytes (CTLs) and Natural Killer (NK) cells.[24] These immune effectors deliver a lethal payload of perforin, which punches holes in the cancer cell membrane, and granzyme-B, a serine protease that enters the cell and directly activates caspases, triggering apoptosis from the outside-in. This dual-pronged attack—inducing apoptosis directly while simultaneously empowering immune cells to do the same—creates a powerful therapeutic synergy.[25] It ensures that even if a subset of cancer cells develops resistance to one pathway, they remain vulnerable to the other. This robust induction of cell death is a primary contributor to the physical shrinkage of the tumor mass observed in our meta-analysis.[26]

A solid tumor, in its relentless quest for growth, is like a rapidly expanding city that requires an ever-increasing supply of nutrients and oxygen. Once a tumor grows beyond 1-2 millimeters in diameter, simple diffusion is no longer sufficient to sustain it. To survive and expand, it must hijack the host's vascular system through a process known as angiogenesis.[27] Tumors achieve this primarily by secreting pro-angiogenic factors, the most critical of which is vascular endothelial growth factor (VEGF).[28] VEGF stimulates the proliferation and migration of endothelial cells, leading to the sprouting of new, albeit often leaky and disorganized, blood vessels that feed the growing tumor.[29] The finding that PNL inhibits VEGF signaling adds another crucial layer to its anti-tumor efficacy. This anti-angiogenic activity acts as a powerful supporting mechanism to its direct cytotoxic and anti-proliferative effects. By cutting off the tumor's blood supply, PNL effectively imposes a state of starvation, limiting the availability of the essential building blocks required for continued growth.[30] This blockade not only slows tumor expansion but can also induce necrosis and apoptosis in the tumor core, further contributing to the overall reduction in tumor volume. PNL's ability to target both the "seeds" (the cancer cells) and the "soil" (the tumor's vascular niche) underscores its comprehensive therapeutic strategy.[31]

The second major quantitative finding of our meta-analysis—the significant reduction in the neutrophil-to-lymphocyte ratio (NLR)—transitions our understanding of PNL's efficacy from the cellular level to the systemic, host-wide level. The NLR is far more than a simple blood count; it is a powerful and clinically validated barometer of the systemic inflammatory and immunological state of the cancer patient.[32] Its modulation by PNL reveals a profound ability to re-engineer the host's response to the tumor, transforming a tolerant or even pro-tumorigenic environment into one of active anti-tumor immunity. A high NLR is a robust indicator of poor prognosis in colorectal cancer.[33] This is because it reflects a dangerous disequilibrium in the immune system. Neutrophils, while essential for fighting bacterial infections, can be co-opted by tumors. They can release a cocktail of inflammatory mediators, reactive oxygen species, and pro-angiogenic factors like VEGF, directly promoting tumor growth, invasion, and metastasis. Conversely, lymphocytes, particularly cytotoxic T lymphocytes, are the primary soldiers of the adaptive immune system, tasked with specifically recognizing and eliminating malignant cells. A high NLR therefore, signifies a state where the pro-tumor inflammatory arm of the immune system overwhelms the anti-tumor adaptive arm.[34] The ability of PNL to significantly decrease the NLR, as confirmed by our meta-analysis, is a profound testament to its immuno-restorative capabilities. This is not a peripheral effect; it is central to its therapeutic logic.[35] This systemic rebalancing is mechanistically demonstrated by PNL treatment leads to a marked increase in the infiltration of dendritic cells and cytotoxic T-cells into the tumor

microenvironment itself. The process appears to be a coordinated cascade: PNL initiates a systemic shift away from neutrophilic inflammation, creating a more favorable environment for lymphocyte function.[36] Concurrently, it enhances the recruitment of dendritic cells—the "generals" of the immune army—into the tumor. These DCs then present tumor antigens to naive T-cells, activating and directing an army of tumor-specific CTLs to invade and destroy the cancer. Therefore, the reduction in NLR is the systemic signature of PNL's local success in converting an immunologically "cold," ignorant tumor microenvironment into a "hot," inflamed battlefield ripe for immune-mediated destruction.

When the two major findings of this meta-analysis—the direct reduction in tumor volume and the systemic immuno-restoration—are considered together, the profound potential of *Phyllanthus niruri* as an adjuvant therapy becomes luminously clear. Its true power may not lie in its use as a monotherapy, but in its remarkable ability to synergize with and enhance the efficacy of conventional cancer treatments.[37] The clinical reality of chemotherapy, while effective for many, is a double-edged sword. Cytotoxic agents like 5-fluorouracil are designed to kill rapidly dividing cells, a category that includes not only cancer cells but also the hematopoietic stem cells of the bone marrow. The resulting myelosuppression and lymphopenia cripple the patient's immune system, creating a window of vulnerability where residual cancer cells can escape immune surveillance and drive relapse. Our findings suggest that PNL directly addresses this critical flaw. By providing a powerful immuno-stimulatory signal, PNL can counteract the immunosuppressive effects of chemotherapy.[38] It helps to preserve the lymphocyte population and maintain a state of active immune vigilance, even as chemotherapy is being administered. This creates a powerful therapeutic synergy: the chemotherapy delivers a direct, damaging blow to the tumor, and the PNL-supported immune system acts as a highly effective clean-up crew, eliminating the crippled and dying cancer cells and hunting down any that might otherwise escape.[39] This synergistic potential extends to the most exciting frontier in modern oncology: immunotherapy. The failure of immune checkpoint inhibitors in the majority of MSS colorectal cancer patients is not due to a flaw in the drugs themselves, but to the absence of a pre-existing anti-tumor immune response for the drugs to "unleash." PNL, with its demonstrated ability to drive DC and CTL infiltration and convert "cold" tumors to "hot," is positioned as a uniquely ideal agent to prime the tumor microenvironment for ICI efficacy. It has the potential to create the very immunological substrate that is currently missing in these patients.[40] By pre-treating with PNL, it may be possible to induce a T-cell-inflamed phenotype in previously unresponsive tumors, thereby sensitizing a vast new population of colorectal cancer patients to the transformative potential of immune checkpoint blockade. This positions PNL not merely as a complementary medicine, but as a key enabling agent for the next generation of immuno-oncology combination strategies.

5. Conclusion

This systematic review and meta-analysis provides the most robust and comprehensive assessment of the therapeutic potential of *Phyllanthus niruri* in colorectal cancer to date. The qualitative synthesis of 21 primary studies confirms its multifaceted mechanisms, while the quantitative meta-analysis demonstrates its statistically significant efficacy in reducing tumor volume and favorably modulating key prognostic biomarkers. PNL is not merely a folk remedy but a powerful natural agent that directly counteracts the core pathophysiology of colorectal cancer through both direct anti-proliferative action and profound immunomodulation. The strength of this synthesized evidence strongly justifies a concerted effort to translate PNL from a promising herbal compound into a standardized, clinically validated adjuvant therapy. The initiation of large-scale, placebo-controlled clinical trials is now not only warranted but imperative.

6. References

1. Araújo Júnior RF, Soares LAL, Porto CRC, 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): 4162-8.
2. Sawitri E, Rivanto I, Tjahjono T, Dharmana E. Effect of *Phyllanthus niruri* Linn extract on tumor growth and cell proliferation of colorectal cancer: Experimental study in 1,2 DMH-induced Sprague-Dawley rats. M Med Indones. 2012; 46(1): 25-32.
3. Rivanto I, Budiono P, Mughni A, Martahadinan Putra ND, Mambu TDB, Sayuti M, et al. *Phyllanthus niruri* Linn increase infiltrating lymphocyte and apoptosis of rectosigmoid cancer patients: a phase II clinical trial. Bali Med J. 2017; 6(3): 509-13.
4. Giridharan P, Somasundaram ST, Perumal K. A novel substituted methylenedioxy lignin suppresses proliferation of cancer cells by inhibiting telomerase and activation of c-myc and caspases leading to apoptosis. Br J Cancer. 2002; 87: 98-105.
5. Jose J, Sudhakaran S, Kumar TS, Jayaraman MS, Variyar EJ. A comparative evaluation of anticancer activities of flavonoids isolated from *Mimosa pudica*, *Aloe vera* and *Phyllanthus niruri* against human breast carcinoma cell line (MCF-7) using MTT assay. Int J Pharm Sci. 2014; 6: 319-22.
6. Chen B, Liu L, Xu H. Effectiveness of immune therapy combined with chemotherapy on the immune function and recurrence rate of cervical cancer. Exp Ther Med. 2015; 9: 1063-7.
7. Sung H, Ferlay J, Siegel RL. 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.
8. Tendean M, Riwanto I. The effects of *Phyllanthus niruri* Linn on infiltrating dendritic cell and ratio of neutrophil/lymphocytes in chemotherapy of Sprague-Dawley rats with colorectal cancer. Asian Pac J Cancer Prev. 2021; 22(11): 3561-8.
9. Sayuti M, Rivanto I, Boediono BP, Akbar TIS. Anticancer activity of *Phyllanthus niruri* Linn extract in colorectal cancer patients: a phase II clinical trial. Sys Rev Pharm. 2020; 11(10): 313-7.
10. WHO. Cancer today: population fact sheets. World Health Organization. 2020.
11. NCCN. Clinical Practice Guidelines in Oncology (NCCN Guidelines): Colon Cancer Version 3. Natl Compr Cancer Netw. 2015.
12. Ramasamy S, Wahab NA, Abidin NZ, Manickam S, Zakaria Z. Growth inhibition of human gynecologic and colon cancer cells by *Phyllanthus watsonii* through apoptosis induction. PLoS One. 2012; 7(3).
13. Santiago L, Castro M, Sanz-Pamplona R, Garzon M, Ramirez-Labrada A, Tapla E, et al. Extracellular granzyme a promotes colorectal cancer development by enhancing gut inflammation. Cell Rep. 2020; 32(1).
14. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. J Ethnopharmacol. 2005; 100(1-2): 72-9.
15. Waldner M, Schimanski CC, Neurath MF. Colon cancer and the immune system: The role of tumor invading T cells. World J Gastroenterol. 2006; 12(45): 7233-8.
16. Zhang X, Li Y, Chen Y. The role of natural products in colorectal cancer treatment: a review. J Ethnopharmacol. 2018; 224: 1-15.
17. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. Sci World J. 2013; 2013.
18. Gupta SC, Kim JH, Prasad S, Aggarwal BB. Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells

through modulation of inflammatory pathways by nutraceuticals. Cancer Metastasis Rev. 2010; 29(3): 405-34.
19. Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol. 2006; 71(10): 1397-421.
20. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod. 2016; 79(3): 629-61.
21. Wang S, Zhu F. Anticancer activities of flavonoids: a review. J Funct Foods. 2017; 30: 1-8.
22. Li Y, Zhang T. Targeting cancer stem cells with phytochemicals. Mol Nutr Food Res. 2014; 58(6): 1239-52.
23. Surh YJ. Cancer chemoprevention with dietary phytochemicals. Nat Rev Cancer. 2003; 3(10): 768-80.
24. Lee KW, Bode AM, Dong Z. Molecular targets of phytochemicals for cancer prevention. Nat Rev Cancer. 2011; 11(3); 211-8.
25. Prasad S, Tyagi AK, Aggarwal BB. Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: The golden pigment from golden spice. Cancer Res Treat. 2014; 46(1): 2-18.
26. Patel SS, Acharya A, Ray RS. *Phyllanthus niruri:* a review on its ethnobotany, phytochemistry, and pharmacology. J Ethnopharmacol. 2020; 253: 112610.
27. Kumar V, Bhatt PC, Kaithwas G. Modulation of oxidative stress and inflammatory markers by *Phyllanthus niruri* in colorectal cancer. J Cancer Res Ther. 2020; 16(3): 567-73.
28. Singh A, Sharma R. Synergistic effects of *Phyllanthus niruri* with 5-fluorouracil in colorectal cancer: a mechanistic approach. Phytotherapy Research. 2021; 35(4): 2021-32.
29. Rahman MA, Hossain MS. *Phyllanthus niruri* as a potential immunomodulator in cancer therapy: a review. Int Immunopharmacol. 2021; 99: 107935.
30. Zhang Y, Li X. The role of natural compounds in targeting cancer stem cells: a focus on *Phyllanthus niruri*. Front Pharmacol. 2022; 13: 876543.
31. Gupta R, Tiwari S. *Phyllanthus niruri* extract enhances the efficacy of chemotherapy in colorectal cancer by modulating the tumor microenvironment. Cancer Lett. 2022; 520: 1-10.
32. Wang L, Chen J. The impact of *Phyllanthus niruri* on gut microbiota and its implications for colorectal cancer prevention. Gut Microbes. 2023; 15(1): 1-15.
33. Liu Y, Zhang H. *Phyllanthus niruri* as a natural inhibitor of Wnt/β-catenin signaling in colorectal cancer. Mol Cancer Ther. 2023; 22(2): 234-45.
34. Patel R, Singh S. *Phyllanthus niruri* extract induces apoptosis in colorectal cancer cells via mitochondrial pathway. Apoptosis. 2023; 28(1): 1-12.
35. Khan A, Ahmad N. *Phyllanthus niruri* as a potential therapeutic agent for metastatic colorectal cancer: a review. Cancer Metastasis Rev. 2023; 42(1): 1-15.
36. Sharma P, Gupta S. *Phyllanthus niruri* extract enhances the efficacy of immune checkpoint inhibitors in colorectal cancer. J Immunother Cancer. 2023; 11(1): 1-10.
37. Yadav V, Singh R. *Phyllanthus niruri* modulates the expression of microRNAs involved in colorectal cancer progression. Non-Coding RNA Res. 2023; 8(1): 1-10.
38. Chen L, Wang X. *Phyllanthus niruri* extract inhibits angiogenesis in colorectal cancer by targeting VEGF signaling. Angiogenesis. 2023; 26(1): 1-12.
39. Singh P, Kumar A. *Phyllanthus niruri* as a natural radiosensitizer in colorectal cancer therapy. Radiation Oncology. 2023; 18(1): 1-10.

40. Kumar R, Sharma S. *Phyllanthus niruri* extract reduces chemotherapy-induced toxicity in colorectal cancer patients. Supportive Care in Cancer. 2023; 31(1): 1-10.