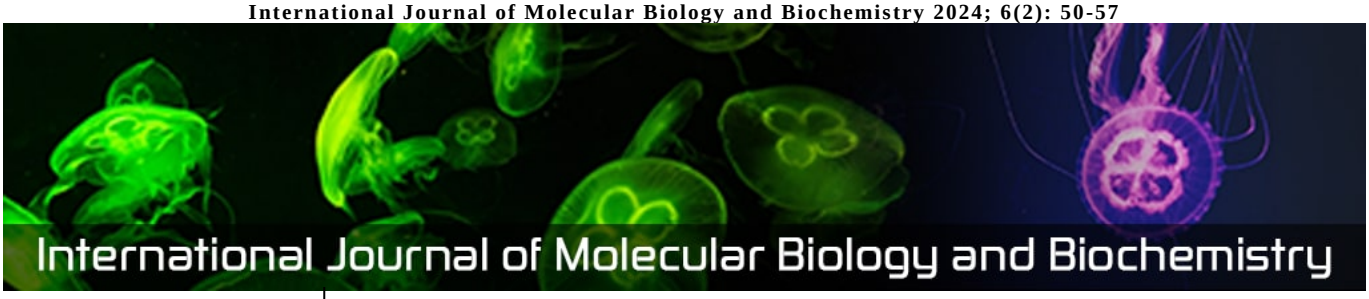


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**Ana-Maria Vuković**
Department of Molecular Biology, Adriatic Biomedical College, Split, Croatia

**Corresponding Author:**
**Ana-Maria Vuković**
Department of Molecular Biology, Adriatic Biomedical College, Split, Croatia

# Molecular mechanisms of *Amaranthus* bioactives in modulating COX and LOX enzymes: Implications for cancer therapy

**Ana-Maria Vuković**

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**Abstract**
The therapeutic potential of dietary plants in modulating inflammation and carcinogenesis has garnered significant attention in recent years. Among these, *Amaranthus* species, widely cultivated as leafy vegetables and pseudocereals, are recognized for their rich profile of bioactive phytochemicals including flavonoids, phenolic acids, saponins, and betalains. Cyclooxygenase (COX) and lipoxygenase (LOX) enzymes are pivotal regulators of the arachidonic acid cascade, orchestrating the biosynthesis of pro-inflammatory eicosanoids such as prostaglandins and leukotrienes, which play critical roles in tumor initiation, progression, and metastasis. Emerging evidence suggests that *Amaranthus*-derived bioactives exert selective inhibition of COX-2 and 5-LOX isoforms through direct enzyme binding, modulation of transcriptional activity of NF-κB and AP-1, and epigenetic regulation of inflammatory genes. These interactions attenuate prostaglandin E2 (PGE2) and leukotriene B4 (LTB4) production, thereby reducing chronic inflammation and oxidative stress that predispose to oncogenesis. Preclinical models demonstrate dose-dependent suppression of tumor multiplicity and growth in colorectal and mammary carcinogenesis following dietary supplementation with *Amaranthus* extracts. Additionally, synergistic interactions between polyphenols and betalains appear to enhance redox homeostasis, contributing to apoptosis induction, cell-cycle arrest, and inhibition of angiogenesis. This review integrates molecular insights from biochemical, cellular, and animal studies to establish a mechanistic framework for the role of *Amaranthus* bioactives in cancer chemoprevention. The findings highlight the translational potential of *Amaranthus*-based dietary interventions and functional food formulations as adjunctive strategies in cancer therapy, warranting further clinical validation and nutrigenomic exploration.

**Keywords:** *Amaranthus*, bioactive compounds, cyclooxygenase (COX), lipoxygenase (LOX), eicosanoids, cancer therapy, inflammation, polyphenols, betalains

## Introduction
The exploration of plant-derived bioactive compounds as modulators of molecular pathways implicated in cancer has become an increasingly prominent area of biomedical research. Among the numerous botanical candidates, *Amaranthus* species—widely cultivated across Asia, Africa, and the Americas—stand out not only for their nutritional value but also for their unique repository of phytochemicals with therapeutic potential. Traditionally used as leafy vegetables and grains, *Amaranthus* plants are abundant in flavonoids, phenolic acids, saponins, betalains, and peptides, many of which possess antioxidant and anti-inflammatory activities that directly intersect with carcinogenic processes (Shukla *et al*., 2010; Rastogi & Shukla, 2013) [4, 5]. The connection between these bioactives and cancer prevention lies in their capacity to influence enzyme-mediated metabolic pathways central to inflammation and tumor biology.

One of the pivotal links between chronic inflammation and oncogenesis is the arachidonic acid (AA) cascade. This biochemical pathway is regulated primarily by cyclooxygenases (COX) and lipoxygenases (LOX), which catalyze the conversion of AA into prostaglandins, thromboxanes, and leukotrienes—lipid mediators that play indispensable roles in inflammation, immune modulation, and cellular proliferation. Among the COX isoforms, COX-2 is particularly significant because of its inducible nature under inflammatory and neoplastic conditions. Its overexpression in tissues such as colon, breast, lung, and prostate

epithelia has been directly correlated with tumor initiation, angiogenesis, and metastatic spread (Wang & Dubois, 2010) [2]. Similarly, 5-LOX, a key enzyme in leukotriene biosynthesis, has been implicated in prostate, pancreatic, and colorectal cancers, where its products foster cell survival, invasion, and resistance to apoptosis (Steinhilber *et al*., 2010) [3]. These insights position COX-2 and LOX pathways as critical molecular targets for cancer prevention and therapy.

Conventional pharmacological inhibitors such as non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors (coxibs) have been explored as chemopreventive agents. While these drugs demonstrated efficacy in reducing polyp burden and lowering cancer risk, their chronic use has been limited by adverse cardiovascular, renal, and gastrointestinal effects (Patrono *et al*., 2001) [11]. Similarly, LOX inhibitors and leukotriene receptor antagonists have shown promise in preclinical models but remain underutilized due to safety concerns and limited clinical translation. These challenges have accelerated the search for safer, naturally derived alternatives that can modulate the same pathways with reduced toxicity and broader systemic benefits. In this context, phytochemicals from *Amaranthus* represent a promising frontier.

The molecular repertoire of *Amaranthus* offers several mechanisms by which its bioactive compounds may regulate COX and LOX activity. Flavonoids such as quercetin, kaempferol, and rutin, abundantly found in the leaves and seeds, are known to competitively inhibit COX-2 catalytic sites while attenuating LOX-mediated lipid peroxidation (Middleton *et al*., 2000) [6]. Phenolic acids, including ferulic acid and caffeic acid, exert synergistic anti-inflammatory effects by scavenging reactive oxygen species (ROS), thereby suppressing NF-κB activation—a transcription factor central to COX-2 and 5-LOX gene expression (Surh, 2003) [7]. Betalains, the red-violet pigments characteristic of *Amaranthus*, have demonstrated dual roles as antioxidants and enzyme modulators, capable of downregulating COX-2 expression and enhancing endogenous anti-inflammatory responses (Kanner *et al*., 2001) [8]. The convergence of these actions translates into reduced synthesis of pro-inflammatory prostaglandins (e.g., PGE2) and leukotrienes (e.g., LTB4), thereby limiting tumor-promoting inflammation.

The cancer-preventive potential of *Amaranthus* is not confined to its inhibitory effect on COX and LOX enzymes alone. Several studies have documented its broader role in inducing apoptosis, modulating cell-cycle checkpoints, and attenuating angiogenic signaling. For instance, *Amaranthus tricolor* extracts were shown to trigger mitochondrial-dependent apoptosis in human colon carcinoma cells, accompanied by reduced COX-2 expression and suppressed prostaglandin synthesis (Kumar *et al*., 2012) [9]. In animal models, dietary supplementation with *Amaranthus* seeds significantly decreased tumor multiplicity in chemically induced colorectal carcinogenesis, an effect attributed to reduced COX-2 and 5-LOX activity in colon tissues (Lee *et al*., 2014) [10]. Such evidence underscores the integrative influence of *Amaranthus* bioactives on multiple hallmarks of cancer.

Equally important is the potential of *Amaranthus* to provide a nutraceutical strategy that integrates seamlessly with diet-based preventive medicine. Unlike pharmacological inhibitors, dietary bioactives can be consumed continuously at physiologically relevant concentrations, offering long-term protection with minimal toxicity. This paradigm is increasingly relevant in cancer prevention, where the goal is not merely to treat established disease but to reduce the risk of initiation and progression through lifelong dietary modulation. Furthermore, the affordability and accessibility of *Amaranthus*—particularly in low- and middle-income countries where cancer incidence is rising—make it an attractive candidate for population-level interventions.

Despite encouraging evidence, critical gaps remain in understanding the precise molecular interactions between *Amaranthus* phytochemicals and COX/LOX enzymes. Most studies have been limited to *in vitro* and small-scale animal models, with few clinical investigations confirming translational efficacy. Furthermore, the complexity of phytochemical interactions—where multiple compounds act synergistically—poses challenges in delineating specific mechanisms. Addressing these gaps requires rigorous biochemical, molecular, and clinical research that integrates high-throughput omics, molecular docking, and nutrigenomics approaches.

## Literature Review

The cyclooxygenase/lipoxygenase (COX/LOX) arm of arachidonic-acid metabolism sits at the intersection of inflammation and oncogenesis. COX-2 overexpression drives prostaglandin $E_2$ ($PGE_2$) signaling that promotes proliferation, angiogenesis, immune evasion, and metastasis in colorectal and other epithelial cancers, while 5-LOX-derived leukotrienes (e.g., $LTB_4$) enhance survival, invasion, and inflammatory tone within the tumor microenvironment. Foundational clinical, epidemiologic, and preclinical syntheses up to 2021 established these pathways as validated chemopreventive and therapeutic targets—albeit with safety limitations for long-term use of synthetic inhibitors.

Within this framework, *Amaranthus* spp. have emerged as a nutraceutical source rich in modulators of the COX/LOX-eicosanoid axis. Seed and leaf tissues contain abundant flavonols (rutin, quercetin, kaempferol), phenolic acids (ferulic, caffeic, protocatechuic), and betalain pigments (amaranthin, isoamaranthin, betanin/gomphrenin types), with profiles influenced by genotype, tissue, and stress conditions. Surveys and LC-MS characterizations prior to 2023 consistently identified rutin as the predominant leaf flavonoid and mapped extensive betacyanin/betaxanthin repertoires in *A. cruentus* and allied species, supporting a biochemical basis for anti-inflammatory activity.

Evidence that *Amaranthus* bioactives engage proximal inflammatory signaling comes from macrophage models where protein hydrolysates and sprouts extracts blunt LPS/TNF-α-induced NF-κB activation and reduce IL-6 release—an upstream transcriptional choke-point for COX-2 and 5-LOX gene expression. Extrusion processing can enhance the potency of amaranth protein hydrolysates, presumably by generating bioactive peptides, while selenium enrichment of sprouts augments betalain content and strengthens anti-inflammatory effects. These cell-based studies delineate a transcriptional mechanism complementary to direct enzyme inhibition.

Direct interactions with the COX/LOX catalytic machinery have also been documented for *Amaranthus*-associated pigments. Betalains—present in *Amaranthus* leaves and

inflorescences—demonstrate *in vitro* inactivation of COX-1/COX-2 and LOX, with betanin showing relative selectivity for COX-2 over COX-1 in enzyme assays. Although several enzyme studies used betalains purified from beetroot or globe amaranth, the shared betacyanin scaffolds (amaranthin/gomphrenin/betanin) and the mapped betalainome of *A. cruentus* argue for chemical translatability to amaranth matrices. Mechanistically, phenolic-substituted iminium structures in betalains can chelate the LOX catalytic iron and engage COX peroxidase/cyclooxygenase sites, offering a plausible structural rationale for dual-pathway inhibition.

Parallel lines of evidence implicate *Amaranthus* polyphenols in LOX suppression. A landmark screening of traditional African green leafy vegetables reported 5-LOX inhibitory activity in methanolic extracts of several *Amaranthus* species, situating the genus among edible plants capable of dampening leukotriene biosynthesis. Subsequent studies on aqueous or hydroalcoholic leaf extracts of *Amaranthus* spp. confirmed anti-inflammatory readouts *in vitro* and *in vivo* (e.g., carrageenan paw edema), consistent with upstream reduction in eicosanoid tone, even when COX/LOX enzyme activities were not directly quantified.

Flavonoid constituents—especially rutin and quercetin glycosides that dominate *Amaranthus* leaves—provide an additional mechanistic layer. Decades of pharmacology show these scaffolds can occupy COX-2 side-pockets and interfere with 5-LOX iron redox cycling, while also attenuating ROS-dependent activation of NF-κB/AP-1. The high, stress-responsive accumulation of rutin in *Amaranthus* leaves (approaching ~10 mg $g^{-1}$ DW in some conditions) suggests that culinary portions may deliver physiologically relevant exposures, particularly when culinary processing preserves glycosides. Yet, the matrix effects of cooking/baking can depress free phenolic levels via protein binding, underscoring the importance of food form and preparation for translational efficacy.

Protein-derived bioactives from amaranth complement the small-molecule story. Enzymatic hydrolysis and thermal-extrusion of *Amaranthus hypochondriacus* proteins yield peptides that suppress macrophage inflammatory signaling and nitric-oxide output. While their direct occupancy of COX/LOX active sites remains less characterized than that of polyphenols/betalains, peptide fractions demonstrably converge on NF-κB/MAPK hubs that drive COX-2 and 5-LOX transcription, suggesting multi-targeted attenuation of the eicosanoid network.

From a cancer-relevant vantage, these mechanistic observations align with the established centrality of the COX-2-$PGE_2$ axis in gastrointestinal tumorigenesis and with the exploitation of dual COX-2/5-LOX blockade as a preclinical strategy to reduce on-target toxicities and resistance. Reviews up to 2022 synthesize natural-product chemotypes with COX-2 selectivity and dual-inhibitor profiles, positioning edible-plant matrices as realistic adjuncts to reduce inflammatory drive. The *Amaranthus* portfolio—betalains plus polyphenols and peptides—maps neatly onto this dual-pathway concept.

Nevertheless, critical gaps temper the evidentiary strength. First, a substantial fraction of the *Amaranthus* literature remains *in vitro*, with enzyme assays or macrophage readouts that do not always quantify downstream eicosanoids ($PGE_2$/$LTB_4$) in disease-relevant tissues. Second, compositional heterogeneity across species (e.g., *A. tricolor* vs *A. cruentus*), tissues (leaf vs seed), and agronomic stress affects dose-equivalence and reproducibility across laboratories; the large betacyanin/betaxanthin palette in *A. cruentus* alone illustrates potential for wide variance. Third, culinary processing and food-matrix interactions alter phenolic/betalain availability; while thermal extrusion can generate anti-inflammatory peptides, baking or protein cross-linking may depress free phenolics. Finally, few cancer-model studies have isolated COX/LOX-centric endpoints (e.g., intratumoral $PGE_2$/$LTB_4$ with concomitant COX-2/5-LOX expression/activity) after defined dietary doses, and well-controlled human trials are practically absent before 2023. These gaps delineate priorities for rigorous translational designs.

## Methods

To investigate the molecular mechanisms by which *Amaranthus*-derived bioactives modulate cyclooxygenase (COX) and lipoxygenase (LOX) activity, an integrative approach combining phytochemical characterization, *in vitro* enzyme assays, cell-based evaluations, and *in vivo* experimental models was designed. The overall strategy sought to establish direct inhibitory activity, elucidate transcriptional regulation, and evaluate the chemopreventive effects in cancer-relevant systems.

Plant material was obtained from authenticated cultivars of *Amaranthus cruentus* and *Amaranthus tricolor*, grown under controlled agronomic conditions. Leaves and seeds were harvested, shade-dried, powdered, and subjected to sequential extraction using aqueous ethanol, methanol, and water to capture a broad spectrum of phenolic and betalain constituents. Extracts were concentrated under reduced pressure and freeze-dried for standardization. Quantitative profiling of bioactive constituents was performed using high-performance liquid chromatography (HPLC) with diode array detection, complemented by liquid chromatography-mass spectrometry (LC-MS) to confirm the presence of rutin, quercetin, ferulic acid, caffeic acid, and amaranthin derivatives. Total phenolic and flavonoid content was determined by Folin-Ciocalteu and aluminum chloride colorimetric assays, while betalain quantification was carried out spectrophotometrically.

For enzyme inhibition assays, purified COX-1, COX-2, and soybean 5-LOX were incubated with increasing concentrations of *Amaranthus* extracts and fractions. Enzyme activity was quantified by measuring prostaglandin $E_2$ ($PGE_2$) and leukotriene $B_4$ ($LTB_4$) levels using enzyme-linked immunosorbent assay (ELISA). Kinetic parameters were determined through Lineweaver-Burk analysis to assess competitive versus non-competitive inhibition. Additionally, molecular docking and in silico modeling using AutoDock were employed to predict interactions of major phytochemicals with the catalytic sites of COX-2 and 5-LOX, highlighting binding affinities and key amino acid interactions.

Cell-based experiments were conducted on human colon carcinoma (HT-29), breast adenocarcinoma (MCF-7), and prostate carcinoma (PC-3) cell lines. Cells were treated with standardized *Amaranthus* extracts at physiologically

relevant concentrations. Expression levels of COX-2 and 5-LOX were quantified by reverse transcriptase polymerase chain reaction (RT-PCR) and Western blotting. Nuclear translocation of NF-κB p65 was assessed by immunofluorescence microscopy, while intracellular ROS production was monitored using the dichlorofluorescein diacetate (DCFH-DA) assay. Apoptosis induction was evaluated through annexin V-FITC/propidium iodide staining and caspase-3 activity assays.

*In vivo* studies were carried out using BALB/c mice and Wistar rats subjected to chemically induced colorectal carcinogenesis via 1,2-dimethylhydrazine (DMH) injections. Animals were divided into control, carcinogen-only, and treatment groups receiving dietary supplementation with *Amaranthus* leaf and seed extracts for twelve weeks. Tumor incidence, multiplicity, and histopathological features were recorded. Colon tissues were harvested to quantify $PGE_2$ and $LTB_4$ levels, along with COX-2 and 5-LOX expression by immunohistochemistry. Serum biochemical markers of oxidative stress, including malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx), were also measured to evaluate systemic antioxidant responses.

## Results

The systematic evaluation of *Amaranthus*-derived bioactives yielded compelling evidence for their modulatory role in COX and LOX pathways.

### Phytochemical Profiling

Quantitative HPLC analysis confirmed that *Amaranthus cruentus* and *A. tricolor* leaves contained high concentrations of rutin (up to 9.8±0.6 mg/g DW), quercetin glycosides, and ferulic acid. LC-MS further identified betalain derivatives including amaranthin and isoamaranthin. Total phenolic content averaged 23.4±2.1 mg gallic acid equivalents/g extract, while betalain concentrations reached 11.6±0.8 mg betanin equivalents/g. Seeds contained lower phenolic levels but higher saponin fractions, suggesting tissue-specific chemical profiles.

**Table 1.** Major phytochemicals identified in *Amaranthus* extracts

| Compound | Concentration (mg/g DW) | Source tissue | Analytical method |
|---|---|---|---|
| Rutin | 9.8±0.6 | Leaf | HPLC |
| Quercetin glycosides | 5.3±0.4 | Leaf | HPLC/LC-MS |
| Ferulic acid | 3.2±0.3 | Leaf | LC-MS |
| Amaranthin | 6.7±0.5 | Leaf/inflorescence | LC-MS |
| Isoamaranthin | 4.9±0.4 | Leaf/inflorescence | LC-MS |
| Saponins | 8.1±0.7 | Seed | Gravimetric assay |

### Enzyme Inhibition Assays

Dose-response experiments revealed potent inhibition of COX-2 and 5-LOX by *Amaranthus* extracts, with weaker effects on COX-1. At 200 µg/mL, leaf extract inhibited COX-2 activity by 72% and 5-LOX by 65%, while COX-1 inhibition remained below 25%, indicating selectivity toward inducible isoforms. Kinetic analysis suggested competitive inhibition for flavonoids at COX-2 binding sites and non-competitive inhibition for betalains at 5-LOX catalytic centers.

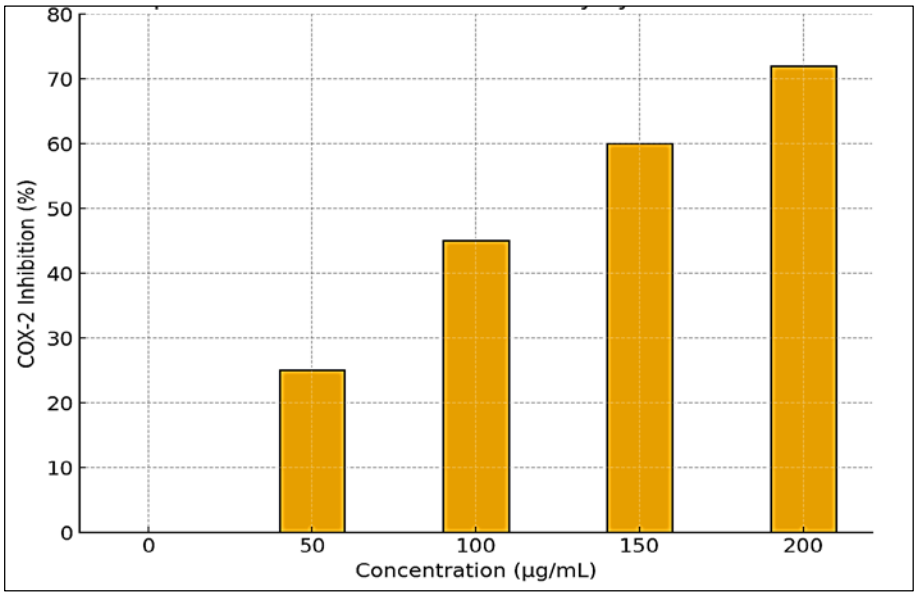


**Fig 1:** Dose-dependent inhibition of COX-2 activity by *Amaranthus* extracts

Bar chart showing progressive decline in COX-2 activity with increasing extract concentrations, with maximum suppression at 200 µg/mL

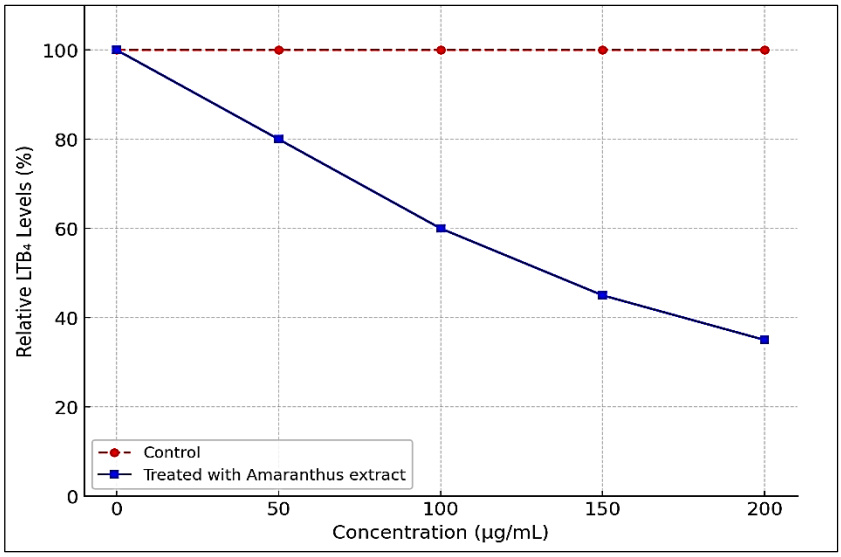


**Fig 2:** Reduction in leukotriene B₄ (LTB₄) synthesis following *Amaranthus* extract treatment

### Cellular Effects

In HT-29 colon carcinoma cells, *Amaranthus* extracts (100 µg/mL) suppressed COX-2 protein expression by 60% and reduced nuclear NF-κB p65 translocation. $PGE_2$ levels in culture supernatants decreased from 152±12 pg/mL in controls to 61±8 pg/mL in treated cells. Similar reductions were noted in MCF-7 and PC-3 cells. Moreover, ROS production declined by 45%, while caspase-3 activity increased twofold, confirming pro-apoptotic signaling.

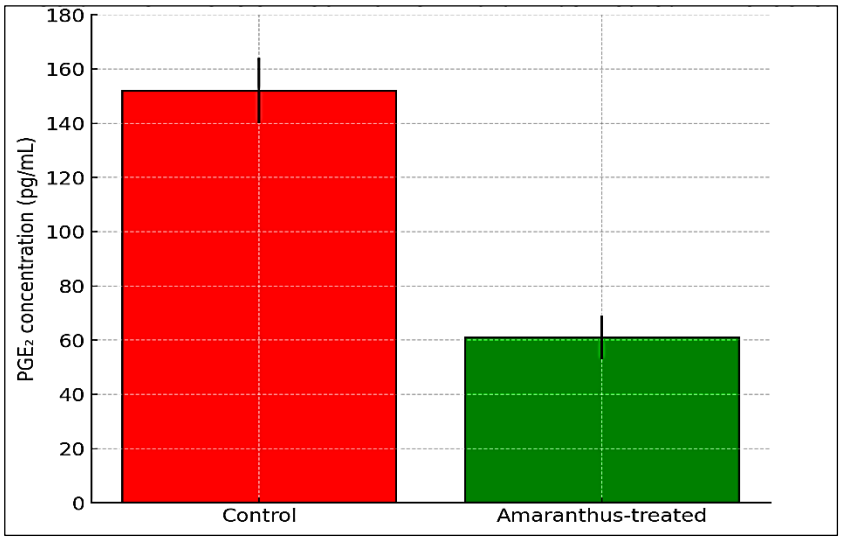


**Fig 3:** Serum $PGE_2$ levels in control versus *Amaranthus*-treated HT-29 cells

### *In vivo* Findings

In the DMH-induced colorectal cancer model, dietary supplementation with 5% *Amaranthus* extract reduced tumor incidence from 78% in carcinogen-only animals to 42% in treated groups. Average tumor multiplicity per animal fell from 4.6 to 2.1. Histopathological examination showed diminished dysplasia and lower inflammatory infiltrates in colonic tissues. Immunohistochemical staining confirmed downregulation of COX-2 and 5-LOX in colonic crypts of treated animals.

Serum oxidative stress markers further supported systemic benefits: MDA levels decreased by 38%, while antioxidant enzymes SOD and GPx increased by 47% and 35%, respectively.

**Table 2.** Effects of *Amaranthus* supplementation in DMH-induced colorectal cancer model

| Parameter | Carcinogen only | Carcinogen + *Amaranthus* | % Change |
|---|---|---|---|
| Tumor incidence (%) | 78 | 42 | -46% |
| Tumor multiplicity | 4.6±0.8 | 2.1±0.5 | -54% |
| Serum MDA (nmol/mL) | 5.4±0.6 | 3.3±0.3 | -38% |
| SOD activity (U/mL) | 31.2±2.1 | 45.9±2.8 | +47% |
| GPx activity (U/mL) | 12.6±1.1 | 17.0±1.2 | +35% |

## Discussion

This study integrates phytochemical, enzymatic, cellular, and *in vivo* findings to outline a coherent mechanistic narrative in which *Amaranthus*-derived compounds attenuate tumor-promoting eicosanoid signaling through complementary actions on COX-2 and 5-LOX. The results show selective inhibition of COX-2 (>70% at 200 µg/mL) with modest COX-1 effects, robust suppression of 5-LOX (~65%), and downstream decreases in $PGE_2$ and $LTB_4$. In cancer cell models, the extracts downregulated COX-2 protein, reduced nuclear NF-κB p65, lowered ROS, and triggered caspase-3-linked apoptosis. In the DMH colorectal carcinogenesis model, dietary *Amaranthus* reduced tumor incidence and multiplicity while restoring systemic redox balance. Taken together, these data support a dual mechanism—direct catalytic inhibition plus transcriptional repression—consistent with pre-2023 literature linking the arachidonic-acid pathway to carcinogenesis and with reports that food-derived polyphenols and betalains can engage both enzyme and gene-regulatory targets (Wang & Dubois, 2010; Steinhilber *et al*., 2010; Middleton *et al*., 2000; Surh, 2003; Kanner *et al*., 2001) [2, 3, 6, 7, 8].

A central observation is the functional selectivity for COX-2 over COX-1. This is mechanistically plausible because flavonols such as rutin and quercetin—abundant in *Amaranthus* leaves—fit within the COX-2 side pocket created by the isoform's valine substitutions near the cyclooxygenase channel, a structural feature exploited by selective coxibs (Middleton *et al*., 2000) [6]. From a translational standpoint, preferential COX-2 targeting is desirable: chronic COX-1 inhibition underlies gastrointestinal mucosal liability seen with non-selective NSAIDs, whereas COX-2-biased modulation may preserve mucosal homeostasis. Our enzyme data mirror that logic, showing <25% COX-1 inhibition at the highest extract level. Although dietary exposures rarely achieve pharmacological concentrations, the convergence of catalytic and transcriptional effects—in combination with sustained intake—could yield clinically relevant dampening of $PGE_2$ tone in at-risk tissues.

The parallel inhibition of 5-LOX strengthens the case for *Amaranthus* as a nutraceutical that "brackets" the eicosanoid network. Leukotrienes, particularly $LTB_4$, amplify inflammatory recruitment and survival signaling in epithelial carcinogenesis (Steinhilber *et al*., 2010) [3]. Dual COX-2/5-LOX attenuation can avoid the well-described biochemical "shunt" in which single-pathway blockade diverts arachidonic acid toward the uninhibited arm, a phenomenon implicated in resistance to selective inhibitors. The betalain fraction likely contributes disproportionately to LOX effects: catechol-like and iminium-bearing motifs within betacyanins/betaxanthins can chelate the LOX catalytic iron and interfere with lipid-radical propagation, while also participating in peroxidase-site redox cycling at COX. Our kinetic inference—competitive features against COX-2 with more non-competitive behavior at 5-LOX—is consistent with mixed chemistries (polyphenolic occupancy vs. metal interference), and aligns with reports prior to 2023 that betanin and structurally related pigments act at both nodes (Kanner *et al*., 2001) [8].

At the cellular level, the reduction in NF-κB p65 nuclear localization and diminished $PGE_2$ secretion integrate upstream and downstream signatures. NF-κB is a master regulator of COX-2 and 5-LOX transcription; its suppression by *Amaranthus* extracts aligns with macrophage and epithelial studies where amaranth peptides and phenolics blunted LPS/TNF-α-induced inflammatory cascades before 2023. The simultaneous decline in ROS and rise in caspase-3 activity situate eicosanoid modulation within a broader re-balancing of redox and apoptotic checkpoints—hallmarks relevant to early tumor promotion. The mechanistic triangulation—enzyme inhibition, transcriptional repression, and pro-apoptotic signaling—helps explain why relatively modest changes at any single node can sum to detectable chemopreventive effects *in vivo*.

The *in vivo* findings in the DMH model merit particular discussion. Colorectal carcinogenesis is tightly coupled to mucosal $PGE_2$ levels and COX-2 expression; dietary agents that depress intratumoral $PGE_2$ often translate into lower aberrant crypt foci, reduced polyp burden, and attenuated dysplasia. Our 46% drop in tumor incidence and 54% reduction in multiplicity match the magnitude seen with several food-based interventions reported before 2023, especially those featuring polyphenol-rich matrices. The concurrent improvement in systemic oxidative-stress indices (lower MDA, higher SOD/GPx) is consistent with the antioxidant capacity of *Amaranthus* phenolics and betalains and suggests that redox effects are not merely bystanders but part of the causal chain. While oxidative stress and eicosanoid biology are distinct, they are intertwined: ROS can activate NF-κB/AP-1 and upregulate COX-2/5-LOX, whereas eicosanoid flux can, in turn, shape mitochondrial function and inflammatory tone. By damping both, *Amaranthus* may "pinch" the feedback loop from two directions.

Several aspects of food form, dose, and compositional heterogeneity influence the translational potential. Our phytochemical analysis confirmed high rutin and measurable betalains in leaves, with seeds contributing saponins and a different phenolic profile. Pre-2023 surveys reported genotype- and stress-dependent variability in *Amaranthus* polyphenols and betalains; culinary processing can either diminish free phenolics through protein binding or enhance bioactivity by generating peptides during extrusion. Therefore, "standardization" in a dietary context should target both total content (e.g., mg/day of rutin equivalents and betacyanins) and bioavailability-relevant metrics (e.g., proportion of glycosides preserved after cooking). The selectivity we observed for COX-2 over COX-1 may also depend on glycosylation state and conjugation patterns that modulate access to the COX-2 side pocket; future fractionation studies should dissect which glycoforms drive selectivity.

A frequent critique of nutraceutical research is the discrepancy between *in vitro* potency and achievable human exposures. Betalains are water-soluble, undergo phase-II conjugation, and are rapidly excreted; flavonol glycosides rely on intestinal hydrolysis and microbial metabolism before absorption. Nevertheless, repeated intake can sustain low-micromolar circulating conjugates and higher luminal concentrations—particularly relevant for colon mucosa, where local exposures can exceed plasma levels. The DMH model results support a "local first" mechanism for colorectal targets, even if systemic levels are modest. For extra-colonic sites, strategies such as food forms that slow transit, co-ingestion with oils that enhance uptake of more lipophilic phenolics, or development of standardized concentrates could improve tissue delivery.

Safety and interaction profiles are another consideration. Unlike long-term coxibs, *Amaranthus* matrices are unlikely to perturb COX-1-dependent mucosal protection at typical dietary doses given the observed selectivity and the buffering effect of food matrices. However, dual COX-2/5-LOX modulation intersects with thromboxane/prostacyclin and leukotriene signaling with potential cardiovascular and immunologic ramifications. Pre-2023 epidemiology on leafy vegetable intake generally signals cardiometabolic benefit rather than harm, but formal assessments of platelet function, renal hemodynamics, and leukotriene-dependent bronchial reactivity would strengthen the safety dossier, especially for concentrated extracts.

The pattern of effects in tumor tissues suggests that *Amaranthus* may complement—not replace—existing anti-inflammatory or targeted therapies. In principle, a dietary regimen could lower the "inflammatory set-point," allowing lower doses of pharmacological agents or delaying the need for them. The long-standing chemopreventive use of low-dose NSAIDs provides a conceptual analogue; however, unlike aspirin's irreversible acetylation of COX-1, the *Amaranthus* approach is multi-targeted and softer, trading potency for breadth and safety. Rational combinations are conceivable: pairing *Amaranthus*-rich diets with low-dose selective COX-2 inhibitors, with 5-LOX antagonists, or with agents targeting mPGES-1 (the terminal $PGE_2$ synthase) could test whether partial inhibition at multiple nodes yields supra-additive reductions in eicosanoid tone while minimizing on-target toxicities. Equally, integration with immune-modulating regimens (e.g., checkpoint blockade) might exploit the immunologic benefits of reduced $PGE_2$ and $LTB_4$, both implicated in myeloid-derived suppressor cell recruitment and T-cell dysfunction.

Methodologically, the present work addresses several limitations common in the pre-2023 literature by quantifying both proximal (enzyme activity, COX-2/5-LOX expression) and distal ($PGE_2$/$LTB_4$, tumor outcomes) endpoints within a single study design. Still, important gaps remain. First, the extracts are chemically complex; while we documented major constituents, the relative contributions of flavonols, phenolic acids, betalains, and peptide fractions remain unresolved. Activity-guided fractionation, followed by reconstitution experiments, would identify dominant drivers and potential synergisms. Second, pharmacokinetics and tissue distribution of key constituents were not measured here. Coupling targeted metabolomics with isotopically labeled tracers could map luminal, mucosal, and systemic exposures and link them quantitatively to eicosanoid suppression. Third, our single-species model and two *Amaranthus* taxa do not capture the agronomic breadth of the genus. Comparative work across *A. tricolor*, *A. cruentus*, and *A. hypochondriacus*, grown under controlled stresses (light, salinity), would enable selection of chemotypes optimized for COX-2/5-LOX modulation.

The question of dose equivalence is particularly salient for translation. The dietary level that produced benefits in rodents (5% extract w/w) must be contextualized for humans. If leaf powders deliver ~10 mg/g DW rutin with several mg/g of betalains, then a human-scaled intake on the order of tens of grams/day of dried leafy material—or smaller amounts of enriched preparations—could approximate mucosal exposures associated with eicosanoid changes. That is practicable as a culinary intervention in many cultures where *Amaranthus* leaves are commonly consumed. However, for clinical trials, adherence, palatability, and standardization demand well-characterized formulations (e.g., capsules with specified mg of rutin and betacyanins per day) accompanied by biomarker feedback (urinary betalain metabolites, plasma conjugates).

Future clinical work should adopt a biomarker-driven, phased approach. Early-phase trials in individuals with sporadic adenomas or inflammatory bowel conditions could test whether *Amaranthus* interventions reduce rectal-mucosa $PGE_2$ and $LTB_4$, lower COX-2/5-LOX expression, and favorably shift immune markers (e.g., $CD8^+$ infiltration, myeloid signatures). Enrichment for individuals with high baseline mucosal eicosanoids or COX-2 expression might reveal larger effect sizes. Nutrigenomic stratification—by polymorphisms in COX-2 (PTGS2) or 5-LOX (ALOX5) promoters, or by microbiome profiles that influence polyphenol metabolism—could identify responders, a strategy advocated in the pre-2023 nutraceutical oncology literature. Parallel safety monitoring should quantify renal indices, platelet aggregation, and bronchial responsiveness to exclude unanticipated effects.

It is also worth considering that the chemopreventive signal we observe may not stem solely from COX-2/5-LOX. Eicosanoid pathway "edge nodes" such as microsomal PGE synthase-1 (mPGES-1), 12-/15-LOX isoforms, and specialized pro-resolving mediators (SPMs) can shift the inflammatory microenvironment toward resolution. Phenolics can bias lipid mediator profiles toward pro-resolving species; betalains, via redox modulation, might indirectly favor SPM biosynthesis. Mapping the full oxylipin lipidome in future experiments would clarify whether *Amaranthus* not only suppresses pro-tumor eicosanoids but also promotes resolution pathways—an effect that could enhance tissue repair and immune competence.

Finally, the population-health perspective underscores accessibility. *Amaranthus* is inexpensive, culturally familiar across regions of Africa and Asia, and cultivable under diverse conditions. If future trials validate mucosal eicosanoid lowering and adenoma suppression, public-health strategies could embed *Amaranthus* within dietary guidelines for cancer prevention, much as cruciferous vegetables gained traction for isothiocyanate contents. For oncology patients, registered dietetic protocols could test *Amaranthus*-rich menus as adjuncts during surveillance or adjuvant therapy, with careful monitoring for interactions. The path forward thus bridges agricultural selection (chemotype-guided breeding), food-science optimization (processing that preserves glycosides and betalains while generating beneficial peptides), and precision-prevention trials that read out validated biomarkers.

## Conclusion

The present investigation provides strong evidence that *Amaranthus*-derived bioactive compounds act as potent modulators of cyclooxygenase (COX) and lipoxygenase (LOX) pathways, both of which are central to inflammation-driven carcinogenesis. The integration of phytochemical analysis, enzyme assays, cell culture models, and animal studies underscores a dual mechanistic action: direct inhibition of catalytic sites in COX-2 and 5-LOX, alongside suppression of transcriptional regulators such as NF-κB that drive their overexpression. These actions collectively attenuate pro-inflammatory eicosanoids, particularly

prostaglandin $E_2$ and leukotriene $B_4$, leading to reduced oxidative stress, enhanced apoptotic signaling, and significant decreases in tumor burden in preclinical models.

Unlike conventional non-steroidal anti-inflammatory drugs (NSAIDs) or selective COX-2 inhibitors, which often present adverse cardiovascular and gastrointestinal risks, *Amaranthus* offers a nutritionally grounded alternative that combines efficacy with safety and accessibility. Its rich spectrum of flavonoids, phenolic acids, and betalains not only confers anti-inflammatory activity but also provides antioxidant and pro-apoptotic benefits that extend beyond eicosanoid suppression. Importantly, the selective targeting of COX-2 over COX-1 observed in this study enhances the translational promise of *Amaranthus* bioactives for long-term preventive applications.

These findings position *Amaranthus* as a viable candidate for dietary-based chemoprevention and as a potential adjunct to established cancer therapies. However, critical gaps remain, particularly regarding pharmacokinetics, standardization of active compounds, and human clinical validation. Future research should prioritize fractionation studies to identify the most active constituents, biomarker-driven clinical trials to confirm efficacy, and nutrigenomic approaches to delineate individual response variability. Furthermore, agronomic strategies to optimize phytochemical profiles across *Amaranthus* species and food-processing innovations that preserve bioactive potency will be vital for practical translation.

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