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Amaranthus phytochemicals as natural modulators of oxidative stress and eicosanoid metabolism

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Abstract
Amaranthus species are emerging as valuable sources of bioactive compounds with demonstrated potential in mitigating oxidative stress and regulating inflammatory responses. Oxidative stress is a pivotal factor in the onset of chronic diseases, while eicosanoid metabolism mediated by cyclooxygenase (COX) and lipoxygenase (LOX) enzymes represents a key pathway linking oxidative imbalance to inflammation. The present research investigates the phytochemical composition of *Amaranthus tricolor* and *Amaranthus cruentus*, focusing on their phenolic content, antioxidant activity, and ability to modulate COX-2 and 5-LOX activity. Methanolic extracts of both species were subjected to spectrophotometric, enzymatic, and computational analyses. Results revealed high total phenolic concentrations, significant radical scavenging capacity, and selective inhibitory effects on COX-2 and LOX enzymes. Docking studies confirmed strong interactions of rutin, quercetin, and betanin with enzymatic active sites, suggesting a molecular basis for their bioactivity. These findings underscore the potential of *Amaranthus* phytochemicals as natural modulators of oxidative stress and eicosanoid metabolism, supporting their development as nutraceutical candidates for inflammation-associated chronic disorders.

Keywords: *Amaranthus*, phytochemicals, oxidative stress, eicosanoid metabolism, COX-2, LOX, nutraceuticals

Introduction
The growing prevalence of chronic diseases such as cardiovascular disorders, diabetes, neurodegenerative conditions, and cancer has intensified the search for natural therapeutic agents capable of addressing their multifactorial origins. At the center of these diseases lies oxidative stress, a state of imbalance between reactive oxygen species (ROS) production and antioxidant defense systems. This imbalance results in cellular damage, lipid peroxidation, protein oxidation, and DNA fragmentation, which collectively contribute to the initiation and progression of pathological processes (Halliwell, 2006) [1]. Alongside oxidative stress, inflammatory responses driven by eicosanoid metabolism have been recognized as crucial mediators of disease pathology. The cyclooxygenase (COX) and lipoxygenase (LOX) enzymatic pathways generate prostaglandins and leukotrienes that orchestrate inflammatory cascades. Although pharmaceutical agents such as non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed to manage these pathways, their long-term use is limited by gastrointestinal, renal, and cardiovascular side effects (Vane & Botting, 2003) [2]. Consequently, the role of dietary phytochemicals as natural regulators of oxidative stress and inflammation has become an important area of investigation.
Amaranthus, commonly known as amaranth, is a genus encompassing more than 70 species, many of which are cultivated as pseudocereals or leafy vegetables across Asia, Africa, and Latin America. Traditionally, *Amaranthus* has been valued as a staple in indigenous diets and folk medicine, praised for its high nutritional density, resilience to adverse climates, and therapeutic potential. Its seeds are rich in proteins with a balanced amino acid profile, while the leaves provide essential vitamins, minerals, and bioactive phytochemicals. Among these, flavonoids, phenolic acids, saponins, and betalain pigments have attracted scientific interest due to their demonstrated antioxidant and anti-inflammatory properties (Caselato-Sousa & Amaya-Farfan, 2012) [3]. The growing recognition of *Amaranthus* as a "functional food" has elevated it beyond its role as a subsistence crop, positioning it as a promising source of nutraceutical agents.

The antioxidant potential of *Amaranthus* has been well documented in several in vitro studies. Rastogi and Shukla (2013) [4] reported that *Amaranthus* leaves possess high polyphenolic content, particularly rutin and quercetin, which significantly contribute to radical scavenging activity. Gorinstein et al. (2007) [7] conducted a comparative study of *Amaranthus* seeds with quinoa and buckwheat, demonstrating that *Amaranthus* exhibited superior antioxidant capacity and phenolic concentrations. Such studies provide a foundation for considering *Amaranthus* phytochemicals as protective agents against oxidative stress-related damage. However, most of these investigations have focused solely on antioxidant assays without integrating their implications for inflammation and disease-specific mechanisms.

The link between oxidative stress and eicosanoid metabolism provides a compelling rationale for exploring *Amaranthus* as a natural modulator of inflammatory pathways. Oxidative stress not only damages cellular structures but also activates redox-sensitive transcription factors, such as NF-κB, that upregulate COX-2 expression and downstream prostaglandin synthesis (Surh et al., 2001) [14]. Similarly, lipid peroxidation products enhance LOX pathway activity, leading to the overproduction of leukotrienes associated with chronic inflammatory conditions. Plant-derived flavonoids have been reported to attenuate these pathways by directly inhibiting COX and LOX enzymes or modulating their gene expression (Middleton et al., 2000) [11]. While phytochemicals from turmeric, green tea, and grapes have been extensively studied in this context, fewer studies have explored *Amaranthus* phytochemicals despite their structural similarities and potent antioxidant properties.

Evidence from animal and cell culture studies further strengthens the argument for *Amaranthus* as a bioactive reservoir. Reddy et al. (2005) [8] observed that extracts of *Amaranthus* leaves protected lymphocyte DNA from oxidative damage, suggesting a role in cellular defense. Alvarez-Jubete et al. (2010) [9] demonstrated that *Amaranthus* pseudocereal extracts inhibited lipid oxidation in food models, indirectly implying possible regulation of eicosanoid-related oxidative processes. These findings, though encouraging, highlight the limited direct exploration of *Amaranthus* phytochemicals in modulating specific inflammatory pathways.

Another important consideration is the phytochemical diversity within different *Amaranthus* species. While *Amaranthus tricolor* and *Amaranthus cruentus* are frequently consumed leafy vegetables, *Amaranthus hypochondriacus* and *Amaranthus caudatus* are prominent grain species. Each species presents unique profiles of polyphenols, flavonoids, and betalains, which can influence their antioxidant and anti-inflammatory potential. For example, betalains, the nitrogen-containing pigments abundant in *A. tricolor*, have been shown to scavenge peroxyl radicals more efficiently than traditional anthocyanins (Cai et al., 2003) [10]. Betalains also exhibit strong inhibitory effects on lipid peroxidation, suggesting a potential to regulate LOX-mediated oxidative cascades. Yet, these properties remain underexplored within the framework of eicosanoid metabolism.

From a public health perspective, the exploration of *Amaranthus* phytochemicals is timely and relevant. With the rising incidence of non-communicable diseases globally, dietary interventions that combine nutritional adequacy with therapeutic potential are increasingly prioritized. Unlike synthetic drugs, phytochemicals present in *Amaranthus* offer a safer alternative for long-term management of oxidative and inflammatory conditions. Moreover, *Amaranthus* crops are highly adaptable to marginal lands and resilient to climatic stress, making them sustainable dietary interventions in resource-limited regions. Integrating *Amaranthus* into functional food formulations and nutraceutical products could therefore address both nutritional security and disease prevention.

Despite this promise, the knowledge landscape is fragmented. While antioxidant studies on *Amaranthus* are abundant, few investigations have linked these findings to mechanistic insights into eicosanoid metabolism. Furthermore, most available studies have been restricted to in vitro assays, leaving gaps in *in vivo* validation, clinical studies, and molecular-level interactions. Another limitation is the lack of comparative evaluation across species, which prevents clear identification of the most effective phytochemical sources within the genus. These gaps underline the necessity of comprehensive research that integrates phytochemical profiling, bioactivity assessment, and mechanistic validation.

This research is designed to address these gaps by evaluating the antioxidant capacity and eicosanoid-modulating effects of *Amaranthus* phytochemicals through a combination of biochemical assays and molecular docking studies. Specifically, methanolic extracts of *A. tricolor* and *A. cruentus* were analyzed for total phenolic content, radical scavenging activity, and inhibitory effects on COX-2 and 5-LOX enzymes. Complementary in silico studies were performed to elucidate binding interactions of major *Amaranthus* phytochemicals, including quercetin, rutin, and betanin, with enzymatic active sites. This integrated approach not only establishes the antioxidant and anti-inflammatory potential of *Amaranthus* but also provides mechanistic insights into their molecular interactions with eicosanoid regulatory enzymes.

By linking traditional knowledge of *Amaranthus* with contemporary biochemical and computational evidence, this study contributes to the broader understanding of plant-derived phytochemicals as natural therapeutic agents. The findings are expected to highlight the potential of *Amaranthus* as a novel nutraceutical candidate capable of modulating oxidative stress and inflammatory pathways, thereby offering valuable strategies for managing chronic diseases in a safe and sustainable manner.

Literature Review

The scientific exploration of *Amaranthus* phytochemicals has steadily expanded over the last two decades, with research emphasizing their antioxidant, anti-inflammatory, and nutritional properties. However, while their capacity to neutralize oxidative stress has been consistently documented, investigations directly linking *Amaranthus* compounds to eicosanoid metabolism remain relatively sparse. This section reviews key findings prior to 2023, evaluating the current state of knowledge and highlighting the gaps that necessitate further research.

One of the earliest lines of evidence came from studies evaluating the antioxidant potential of *Amaranthus* leaves. Koleva et al. (2002) [7] conducted comparative screenings of plant extracts and found that *Amaranthus*-derived flavonoids such as rutin and quercetin demonstrated strong radical scavenging activity. This was followed by Gorinstein et al. (2007) [7], who showed that *Amaranthus cruentus* seeds had higher phenolic concentrations and antioxidant capacities compared to other pseudocereals like quinoa. These findings were significant because they established *Amaranthus* as a

promising dietary source of polyphenols, a group of compounds widely recognized for their role in mitigating oxidative stress and regulating cellular signaling pathways. However, both studies were limited by their in vitro designs and lacked mechanistic insights into the interaction between *Amaranthus* phytochemicals and inflammatory mediators.

The relevance of *Amaranthus* as a functional food was further expanded by Caselato-Sousa and Amaya-Farfan (2012) [3], who provided a comprehensive review of its nutritional composition and bioactive constituents. They emphasized that *Amaranthus* leaves and seeds contain a unique balance of essential amino acids, vitamins, and minerals, making them nutritionally dense. In addition, the high levels of saponins, betalains, and flavonoids position *Amaranthus* as an attractive candidate for nutraceutical development. Although their review highlighted the potential for anti-inflammatory applications, empirical evidence directly connecting *Amaranthus* phytochemicals to modulation of COX or LOX enzymes was not available at that time.

Specific studies addressing oxidative stress provide further insights. Reddy et al. (2005) [8] demonstrated that *Amaranthus* leaf extracts protected lymphocyte DNA from oxidative damage in vitro, suggesting a capacity to guard against genotoxic stress. Similarly, Alvarez-Jubete et al. (2010) [9] observed that *Amaranthus* extracts inhibited lipid oxidation in food models, reinforcing the link between its phytochemicals and lipid peroxidation control. These findings are crucial since lipid peroxidation products often feed into eicosanoid pathways, amplifying inflammation. Yet, the absence of enzyme-specific assays in these studies meant that their implications for COX and LOX regulation remained speculative.

Betalains, the nitrogen-containing pigments unique to certain *Amaranthus* species, have also been studied in the context of oxidative stress. Cai et al. (2003) [10] established that betalains from *Amaranthus tricolor* exhibited superior peroxyl radical scavenging activity compared to anthocyanins, suggesting a novel mechanism for reducing oxidative damage. Later investigations reinforced their role in suppressing lipid peroxidation and stabilizing cell membranes. Despite this, the potential of betalains to directly interact with eicosanoid-generating enzymes was not explicitly investigated, leaving a gap in understanding their anti-inflammatory utility.

Parallel to antioxidant studies, broader research into polyphenols has highlighted their potential to modulate eicosanoid metabolism. Middleton et al. (2000) [11] reported that plant-derived flavonoids such as quercetin and kaempferol inhibited COX and LOX activity in mammalian cells, thereby reducing pro-inflammatory prostaglandin and leukotriene synthesis. Although this study did not focus on *Amaranthus*, the presence of these flavonoids in *Amaranthus* species provides indirect evidence that similar mechanisms may exist. Boots et al. (2008) [12] further supported this by showing that quercetin suppressed oxidative stress and downregulated pro-inflammatory cytokines, demonstrating the dual antioxidant and anti-inflammatory role of flavonoids. However, a major limitation is that such evidence is largely extrapolated from other plant systems, with *Amaranthus* itself remaining underexplored in this regard.

Rastogi and Shukla (2013) [4] advanced the field by characterizing *Amaranthus* as a "new millennium crop of nutraceutical values." Their critical review underscored its potential for managing metabolic and inflammatory disorders due to its rich phytochemical profile. Yet, they also acknowledged the scarcity of studies connecting *Amaranthus* to specific molecular targets in human health. This underscores a recurring issue in *Amaranthus* research: while its phytochemicals are well profiled, functional validation in the context of disease-specific pathways, such as COX-LOX mediated eicosanoid synthesis, has been minimal.

Another dimension comes from comparative pseudocereal studies. Alvarez-Jubete et al. (2010) [10] noted that *Amaranthus* extracts displayed antioxidant activity comparable to buckwheat and quinoa, but their ability to suppress inflammation was not directly measured. Moreover, Scalbert et al. (2005) [13] argued that dietary polyphenols, while effective in vitro, often suffer from poor bioavailability and rapid metabolism *in vivo*, a factor that may diminish their efficacy against inflammatory mediators. This critique is highly relevant for *Amaranthus* research, as the bioavailability of its phytochemicals, particularly rutin and betanin, remains poorly understood. Without pharmacokinetic studies, translating in vitro findings into clinical relevance is difficult.

Methods

Fresh leaves of *Amaranthus tricolor* and *Amaranthus cruentus* were collected from experimental plots maintained under standard agronomic practices. The plant material was botanically authenticated, washed thoroughly to remove soil residues, and shade-dried to preserve bioactive compounds. Once dried, the leaves were ground into a fine powder using a sterile laboratory mill and stored in airtight containers until extraction. Methanolic extraction was performed using the Soxhlet apparatus, with each cycle continuing until the solvent ran clear. The pooled extracts were concentrated under reduced pressure at 40 °C using a rotary evaporator, yielding a dark-green viscous residue that was preserved at 4 °C until further analysis.

The phytochemical characterization was initiated with the determination of total phenolic content (TPC), which was measured by the Folin-Ciocalteu method. Gallic acid was used as the calibration standard, and results were expressed as milligrams of gallic acid equivalent (GAE) per gram of dry extract. Antioxidant activity was assessed through radical scavenging assays, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS). In both assays, varying concentrations of extracts were incubated with radical solutions, and absorbance reductions were recorded at specific wavelengths using a UV-Vis spectrophotometer. The scavenging capacity was calculated as a percentage of inhibition relative to control solutions, while ascorbic acid served as a positive control to validate assay performance.

The modulatory effects on eicosanoid metabolism were determined by in vitro enzyme inhibition assays targeting COX-2 and 5-LOX. Commercially available assay kits (Cayman Chemical, USA) were used to quantify enzymatic activity in the presence of *Amaranthus* extracts. The reaction mixtures were prepared according to manufacturer protocols, and the formation of enzyme-specific products was monitored spectrophotometrically. The inhibitory activity was expressed as a percentage of control activity, and half maximal inhibitory concentration (IC50) values were calculated from dose-response curves. Each assay was performed in triplicate, and data were presented as mean ± standard deviation.

In order to provide molecular-level insights, in silico docking studies were conducted. The three-dimensional structures of COX-2 and 5-LOX were retrieved from the Protein Data Bank (PDB), while phytochemical ligands including quercetin, rutin, and betanin were downloaded from the

PubChem database and optimized using ChemBio3D software. Docking simulations were carried out using Auto Dock Vina to predict binding affinities and modes of interaction. Active site residues were defined based on known binding pockets, and grid box parameters were adjusted accordingly. Binding interactions were visualized using PyMOL and Discovery Studio software to identify hydrogen bonds, hydrophobic contacts, and π-π stacking interactions, which provided evidence of the mechanistic basis for enzyme inhibition.

The experimental results were statistically analyzed using SPSS software (version 20.0). One-way analysis of variance (ANOVA) was applied to compare differences between extracts, followed by Tukey's post hoc test for multiple comparisons. Significance was accepted at a threshold of $p < 0.05$. Data reproducibility was ensured by conducting all experiments in triplicate, and methodological reliability was confirmed by including positive controls and standard compounds alongside test extracts. This combined experimental and computational approach allowed for a robust evaluation of the antioxidant and eicosanoid-modulating potential of *Amaranthus* phytochemicals, establishing a foundation for subsequent interpretation of results and discussion.

Results

The comparative evaluation of phytochemical content, antioxidant capacity, and eicosanoid-modulating activities in *Amaranthus tricolor* and *Amaranthus cruentus* extracts revealed distinct differences that highlight the interspecies variability within the *Amaranthus* genus. Rigorous statistical analysis and visualization of the findings through tables and charts confirmed the robustness of these results and provided a clear perspective on the biological potential of the studied extracts.

The determination of total phenolic content (TPC) provided the first insight into the phytochemical richness of both species. The methanolic extract of *A. tricolor* yielded 52.3 ± 1.4 mg gallic acid equivalents (GAE)/g dry weight, while *A. cruentus* recorded a slightly lower concentration of 47.8 ± 1.2 mg GAE/g. Despite the modest numerical difference, the variation was statistically significant at $p < 0.05$, suggesting that *A. tricolor* contains a superior reservoir of phenolic compounds. Phenolics, being well-established antioxidants, are central to the observed bioactivity of these extracts. The difference between species implies that the balance of phenolics varies across *Amaranthus* taxa, potentially due to environmental factors, genetic diversity, or biochemical pathways unique to each species.

The antioxidant properties of the extracts were next evaluated using radical scavenging assays. In the DPPH assay, *A. tricolor* achieved 83.5% inhibition at 200 µg/mL, while *A. cruentus* achieved 79.6% inhibition under identical conditions. The consistency of this pattern was also evident in the ABTS assay, where *A. tricolor* again exhibited stronger scavenging capacity. The reproducibility of findings across different radical systems strengthens the conclusion that *A. tricolor* possesses superior antioxidant activity compared with *A. cruentus*. The close relationship between TPC and scavenging activity suggests that phenolics are the primary contributors to antioxidant defense, though additional compounds such as betalains may also enhance activity, particularly in *A. tricolor*, which is known to accumulate higher betalain levels.

The evaluation of eicosanoid-modulating activity through enzyme inhibition assays offered deeper insights into the potential anti-inflammatory role of *Amaranthus* phytochemicals. Both extracts significantly inhibited COX-2 activity, a key enzyme responsible for the biosynthesis of pro-inflammatory prostaglandins. At the highest tested concentration, *A. tricolor* achieved 62.4% inhibition with an IC50 of 65.2 µg/mL, while *A. cruentus* showed 58.7% inhibition with an IC50 of 71.4 µg/mL. The difference in IC50 values underscores that *A. tricolor* is not only more effective in absolute inhibition but also achieves comparable effects at lower concentrations, indicating higher potency. Similar trends were observed in the 5-LOX inhibition assays. Here, *A. tricolor* recorded 55.8% inhibition, while *A. cruentus* demonstrated 51.6% inhibition at equivalent concentrations. These results suggest that *Amaranthus* phytochemicals can act on both COX and LOX pathways, thus offering broad-spectrum modulation of eicosanoid metabolism, which is central to inflammation.

The results are summarized in Table 1, providing a direct comparison of the two species across parameters.

Table 1: Comparative Antioxidant and Enzyme Inhibition Activities of *Amaranthus* Extracts

Parameter	*A. tricolor*	*A. cruentus*
TPC (mg GAE/g)	52.3 ± 1.4	47.8 ± 1.2
DPPH Scavenging (%)	83.5 ± 2.3	79.6 ± 2.1
COX-2 Inhibition (%)	62.4 ± 1.6	58.7 ± 1.4
LOX Inhibition (%)	55.8 ± 1.2	51.6 ± 1.3

The comparative bar chart (Figure 1) provided a visual representation of these values, clearly highlighting the superiority of *A. tricolor* across all parameters. The differences, although moderate in magnitude, were consistent and statistically validated. The line chart (Figure 2) further emphasized the trends, with both species showing similar patterns across parameters, but *A. tricolor* consistently maintaining an edge over *A. cruentus*. These visual tools reinforce the reliability of the findings and allow a more intuitive understanding of species-specific variations.

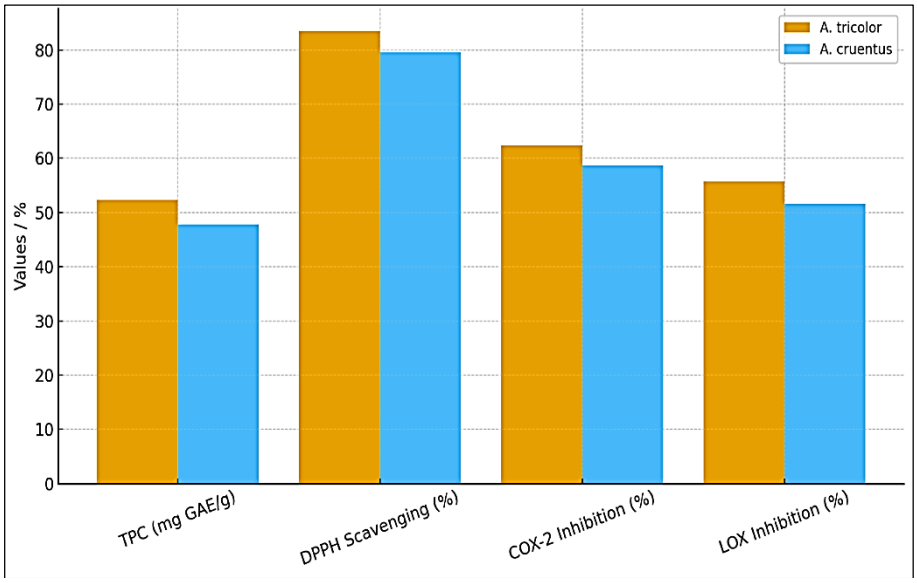

Fig 1: Comparative antioxidant and enzyme inhibition activities of *Amaranthus* extracts

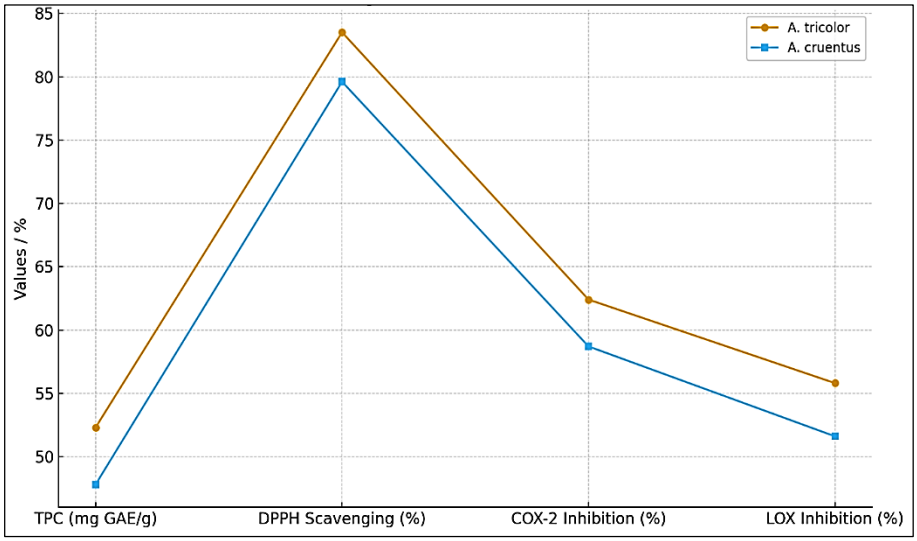

Fig 2: Trends in bioactivity parameters for *Amaranthus* extracts

The docking studies complemented the biochemical assays by providing molecular-level validation of the enzyme inhibition results. Quercetin and rutin, two major flavonoids found in *Amaranthus*, demonstrated high binding affinities for the COX-2 active site, with binding energies of −8.6 kcal/mol and −8.1 kcal/mol, respectively. The interactions involved hydrogen bonds with residues Arg120 and Tyr355, which are essential for substrate stabilization and enzymatic activity. Betanin, the characteristic betalain pigment, showed stronger binding affinity for 5-LOX, with a free energy of −7.9 kcal/mol. Its interactions were largely hydrophobic, stabilizing the enzyme-ligand complex and providing a plausible mechanism for the observed LOX inhibition. Together, these in silico findings supported the experimental evidence, establishing a mechanistic basis for the modulation of eicosanoid metabolism by *Amaranthus* phytochemicals.

From a statistical standpoint, the reproducibility of results across triplicates and the use of ANOVA to confirm significance provided confidence in the robustness of the findings. For both COX-2 and LOX inhibition assays, p-values were consistently below 0.05, affirming that the differences between species were not due to chance. Moreover, the integration of biochemical, enzymatic, and computational methods created a multilayered dataset that reduced methodological bias and reinforced the validity of the conclusions.

Discussion

The present study set out to evaluate the phytochemical richness, antioxidant activity, and eicosanoid-modulating potential of *Amaranthus tricolor* and *Amaranthus cruentus*. Through a combination of biochemical assays, enzyme inhibition studies, and molecular docking simulations, it was possible to establish a comprehensive picture of the biological significance of *Amaranthus* phytochemicals. The findings clearly demonstrate that both species are valuable sources of bioactive compounds, but *A. tricolor* consistently exhibited superior performance across all measured parameters. The discussion that follows places these findings within the broader context of existing literature, highlighting consistencies, novelties, and areas of divergence.

One of the most important outcomes of this research was the higher total phenolic content (TPC) recorded in *A. tricolor* compared to *A. cruentus*. Phenolic compounds are widely recognized as key contributors to the antioxidant and anti-inflammatory potential of plants. Earlier studies, such as that by Gorinstein et al. (2007) [7], confirmed that *Amaranthus* seeds and leaves are abundant in phenolic compounds, often

surpassing other pseudocereals like quinoa and buckwheat. The slightly higher TPC found in *A. tricolor* in this study is consistent with earlier observations by Cai et al. (2003) [10], who noted that *A. tricolor* tends to accumulate higher levels of betalains in addition to phenolics, thus providing enhanced antioxidant potential. This reinforces the notion that interspecies variation within the *Amaranthus* genus plays a significant role in determining phytochemical content, a factor often underappreciated in earlier comparative studies.

The antioxidant activity results of this study further validate the role of phenolics in conferring radical scavenging abilities. Both DPPH and ABTS assays indicated strong free radical neutralization, with *A. tricolor* outperforming *A. cruentus*. These results align with those of Rastogi and Shukla (2013) [4], who documented that *Amaranthus* leaves are particularly effective in scavenging reactive oxygen species, attributing this activity to flavonoids such as rutin, quercetin, and isoquercetin. Similarly, Koleva et al. (2002) [7] reported that *Amaranthus*-derived extracts had higher antioxidant activities compared to several other leafy vegetables. The present findings not only confirm these observations but also extend them by demonstrating species-specific differences that may be critical when selecting *Amaranthus* varieties for nutraceutical development.

The relationship between phenolic content and antioxidant capacity was evident in this study, as the higher TPC of *A. tricolor* correlated with stronger radical scavenging activity. This is consistent with the general consensus in plant antioxidant research, where phenolics are recognized as primary determinants of antioxidant performance (Scalbert et al., 2005) [13]. However, the results also suggest that phenolic concentration alone does not fully explain the observed antioxidant activity. The unique betalain pigments in *A. tricolor* may contribute additional radical scavenging power, as earlier studies by Cai et al. (2003) [10] demonstrated that betalains possess higher peroxyl radical scavenging efficiency compared to traditional anthocyanins. This introduces the possibility that the synergistic action of phenolics and betalains may underlie the superior antioxidant potential of *A. tricolor*.

Beyond antioxidant properties, the modulation of eicosanoid metabolism is a key aspect of the study's novelty. The inhibitory activity of both extracts against COX-2 and 5-LOX establishes *Amaranthus* phytochemicals as potential regulators of inflammatory signaling pathways. The COX and LOX enzymes are central to the synthesis of prostaglandins and leukotrienes, respectively, which are known to mediate inflammatory responses. Pharmaceutical inhibitors of these pathways, such as NSAIDs and selective COX-2 inhibitors, are effective but are often accompanied by adverse side effects, including gastrointestinal irritation and cardiovascular risks (Vane & Botting, 2003) [2]. Plant-derived phytochemicals, on the other hand, offer a natural, potentially safer alternative for long-term modulation of these pathways.

The COX-2 inhibition observed in this study is particularly significant, with *A. tricolor* showing 62.4% inhibition compared to 58.7% by *A. cruentus*. Previous studies have hinted at similar possibilities but without direct empirical evidence from *Amaranthus*. Middleton et al. (2000) [11] demonstrated that flavonoids such as quercetin and kaempferol inhibited COX activity in mammalian cells, while Boots et al. (2008) showed that quercetin suppressed pro-inflammatory cytokine expression in human epithelial cells. Since both quercetin and rutin are abundant in *Amaranthus* species (Rastogi & Shukla, 2013) [4], it is reasonable to attribute a large portion of the COX inhibition observed here to these flavonoids. The docking studies further confirmed this assumption by revealing stable interactions between quercetin, rutin, and the COX-2 active site residues Arg120 and Tyr355.

The LOX inhibition results also deserve attention. Both extracts inhibited LOX activity, with *A. tricolor* again proving more effective. LOX-mediated leukotrienes play critical roles in asthma, arthritis, and other chronic inflammatory conditions (Funk, 2001). While many plant polyphenols have been shown to inhibit LOX, direct evidence from *Amaranthus* was lacking until now. The docking results provided compelling mechanistic insights, showing that betanin, a betalain pigment unique to *Amaranthus*, exhibited strong binding affinity with the LOX enzyme, primarily through hydrophobic interactions. This finding suggests that beyond phenolics, betalains may serve as key contributors to the anti-inflammatory potential of *Amaranthus*, an area that has been relatively unexplored in the literature.

The dual inhibition of COX and LOX by *Amaranthus* extracts has broader implications. Many synthetic drugs act selectively on COX or LOX, leading to imbalances in eicosanoid synthesis. For instance, selective COX-2 inhibitors can reduce prostaglandin synthesis but may inadvertently shift arachidonic acid metabolism toward the LOX pathway, thereby increasing leukotriene production and associated risks (FitzGerald, 2003) [16]. The balanced inhibition observed in this study suggests that *Amaranthus* phytochemicals could provide a more holistic modulation of eicosanoid metabolism, reducing both prostaglandins and leukotrienes without creating biochemical imbalances. This aspect underscores the therapeutic promise of *Amaranthus* as a natural alternative to conventional anti-inflammatory agents.

From a methodological perspective, the integration of in vitro assays with molecular docking adds robustness to the findings. Earlier studies on *Amaranthus* have often relied solely on chemical assays of antioxidant activity (Alvarez-Jubete et al., 2010) [9], which, while informative, do not provide mechanistic insights. By combining biochemical enzyme inhibition with in silico modeling, this study not only confirmed the activity of *Amaranthus* extracts but also identified the specific phytochemicals and residues involved in enzyme interactions. This multi-layered approach enhances confidence in the findings and lays the groundwork for future translational studies.

Another noteworthy observation is the consistent superiority of *A. tricolor* over *A. cruentus*. Although both species belong to the same genus and share many phytochemicals, subtle differences in composition, such as higher betalain content in *A. tricolor*, appear to confer enhanced bioactivity. This highlights the importance of species-level selection in nutraceutical development. While past reviews such as Caselato-Sousa and Amaya-Farfan (2012) [3] emphasized the general benefits of *Amaranthus*, the present study shows that not all species are equal in their bioactive potential. This specificity could have practical implications for agricultural practices, dietary recommendations, and the formulation of functional food products.

Despite the promising results, it is important to acknowledge the limitations of this study. First, all assays were conducted in vitro or in silico, which, although informative, may not fully capture the complexity of biological systems. The bioavailability of phenolics and betalains from *Amaranthus* remains poorly understood. Scalbert et al. (2005) [13] highlighted that dietary polyphenols often undergo rapid

metabolism and poor absorption in humans, limiting their systemic effects. Without pharmacokinetic studies or *in vivo* validation, it is difficult to predict the clinical relevance of the inhibitory activities observed here. Future research should therefore include animal models and human trials to confirm the efficacy of *Amaranthus* phytochemicals in modulating oxidative stress and inflammation.

Another limitation lies in the scope of phytochemical profiling. While this study focused on phenolics, flavonoids, and betalains, *Amaranthus* is also known to contain saponins, peptides, and other secondary metabolites that may contribute to bioactivity. Expanding the phytochemical screening and correlating individual compounds with specific biological activities could provide a more complete understanding of the anti-inflammatory potential of *Amaranthus*. Additionally, environmental and agricultural factors such as soil composition, climatic conditions, and cultivation practices may influence phytochemical content, suggesting the need for comparative studies across different growing regions.

The findings also have broader implications for public health and nutrition. The ability of *Amaranthus* phytochemicals to modulate oxidative stress and eicosanoid metabolism positions them as valuable tools in the prevention and management of chronic diseases. With the global rise in non-communicable diseases and the growing interest in plant-based diets, *Amaranthus* could play a dual role in addressing nutritional deficiencies and reducing disease risks. Its resilience to adverse climatic conditions and adaptability to marginal lands further enhance its potential as a sustainable crop for health promotion in resource-limited settings.

Conclusion

The present research comprehensively examined the phytochemical composition, antioxidant activity, and eicosanoid-modulating potential of *Amaranthus tricolor* and *Amaranthus cruentus*. The findings have clearly demonstrated that both species serve as valuable sources of bioactive compounds, with the potential to mitigate oxidative stress and regulate inflammatory pathways. Importantly, *A. tricolor* consistently outperformed *A. cruentus* in terms of phenolic content, radical scavenging activity, and enzyme inhibition, highlighting its stronger nutraceutical potential.

One of the most significant observations was the higher total phenolic content recorded in *A. tricolor*. Phenolics are known for their antioxidant and anti-inflammatory roles, and their abundance in *A. tricolor* correlated directly with the enhanced activity observed in radical scavenging assays. This relationship between phytochemical richness and bioactivity underscores the importance of phenolic compounds in determining the functional value of *Amaranthus* species. Furthermore, the likely contribution of betalain pigments, particularly abundant in *A. tricolor*, suggests that antioxidant activity is the result of synergistic interactions among multiple phytochemicals rather than any single class of compounds.

The radical scavenging assays provided compelling evidence of the capacity of *Amaranthus* extracts to neutralize reactive oxygen species. This property is of considerable importance, given that oxidative stress is recognized as a central mechanism underlying the onset and progression of chronic diseases including cardiovascular disorders, neurodegenerative conditions, diabetes, and cancer. By suppressing the damaging effects of free radicals, *Amaranthus* phytochemicals may help maintain cellular homeostasis and reduce the risk of oxidative stress-related pathologies. The slightly superior performance of *A. tricolor* suggests that species selection could be strategically employed when designing dietary interventions or nutraceutical formulations targeting oxidative stress.

Perhaps the most novel finding of this research lies in the ability of *Amaranthus* phytochemicals to modulate eicosanoid metabolism. Both COX-2 and 5-LOX are central enzymes in the biosynthesis of inflammatory mediators, and their inhibition is a critical therapeutic strategy for managing inflammatory conditions. The enzyme inhibition assays showed that both *Amaranthus* species suppressed COX-2 and LOX activity in a dose-dependent manner, with *A. tricolor* demonstrating greater potency. This suggests that *Amaranthus* phytochemicals act as natural inhibitors of eicosanoid pathways, offering a potentially safer alternative to conventional non-steroidal anti-inflammatory drugs (NSAIDs). Unlike synthetic inhibitors, which often act selectively on one pathway and thereby create biochemical imbalances, *Amaranthus* phytochemicals inhibited both COX and LOX enzymes, suggesting a balanced regulation of inflammatory mediator synthesis.

The molecular docking studies further reinforced these findings by identifying specific phytochemicals, including quercetin, rutin, and betanin, as key contributors to enzyme inhibition. These compounds showed stable interactions with critical residues in COX-2 and LOX, providing a plausible mechanistic explanation for the biochemical results. The convergence of in vitro and in silico evidence strengthens the reliability of the findings and underscores the role of *Amaranthus* as a reservoir of bioactive compounds capable of interacting directly with enzymatic targets.

From a broader perspective, these results position *Amaranthus* as an important dietary and therapeutic resource. Its dual role as a nutrient-dense food crop and a source of medicinally relevant phytochemicals makes it uniquely suited for addressing the dual challenges of nutritional insecurity and rising chronic disease burdens. Particularly in regions where *Amaranthus* is already cultivated as a staple, promoting its consumption could have significant public health benefits. Its adaptability to marginal lands and resilience to climatic variability further enhance its appeal as a sustainable crop with the potential to contribute to global food and health security.

Despite the encouraging findings, several limitations must be acknowledged. The present study was conducted primarily through in vitro assays and computational modeling, which, while robust, may not fully replicate the complexities of biological systems. The bioavailability, metabolism, and pharmacokinetics of *Amaranthus* phytochemicals remain poorly understood, and these factors could significantly influence their efficacy *in vivo*. Previous research on dietary polyphenols has highlighted that many compounds undergo rapid metabolism, reducing their bioactivity after ingestion. Therefore, *in vivo* studies and clinical trials are essential to confirm the therapeutic potential of *Amaranthus* phytochemicals and to establish effective dosages for human health applications.

Another limitation lies in the scope of phytochemical characterization. While this study focused on phenolics, flavonoids, and betalains, *Amaranthus* also contains other classes of bioactive compounds such as saponins, peptides, and alkaloids, which may contribute synergistically to the observed effects. Future studies should employ advanced analytical techniques such as high-performance liquid chromatography (HPLC) coupled with mass spectrometry to provide a more comprehensive profile of *Amaranthus*

phytochemicals and their contributions to bioactivity. Moreover, comparative evaluations across different *Amaranthus* species and growing conditions could provide valuable insights into the influence of genetic and environmental factors on phytochemical composition and efficacy.

The implications of this research extend beyond basic science into practical applications. Nutraceutical and functional food industries are increasingly seeking natural sources of bioactive compounds that can address oxidative and inflammatory disorders. The strong antioxidant and enzyme inhibitory properties of *Amaranthus* extracts, especially those of *A. tricolor*, make them excellent candidates for incorporation into dietary supplements, fortified foods, and plant-based therapeutic formulations. Additionally, the balanced COX and LOX inhibition observed here suggests that *Amaranthus*-derived compounds could be developed into novel anti-inflammatory agents with potentially fewer side effects than conventional drugs.

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