

REVIEW ARTICLE

Antioxidant and Anti-Inflammatory Effects of Blue Honeysuckle Anthocyanins: From Compositional Predominance to Functional Attribution

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ABSTRACT: Blue honeysuckle (*Lonicera caerulea* L.) is rich in anthocyanins, with cyanidin-3-O-glucoside (C3G) generally being the predominant constituent. Although blue honeysuckle extracts and anthocyanin-rich preparations exhibit antioxidant and anti-inflammatory activities in various in vitro and animal models, an important question remains: are the compounds most abundant in the starting material, or most effectively retained during processing, those primarily responsible for the observed protective effects? This review examines three findings that challenge a straightforward composition–activity relationship. First, the predominance of C3G does not consistently correspond to the strongest statistical association with chemical radical-scavenging measurements. Second, parent anthocyanins decline during simulated digestion, whereas the resulting mixtures retain certain in vitro activities. Third, microencapsulated anthocyanin-rich fractions have not demonstrated superior overall protection in a colitis model, while whole pomace has produced greater improvements than isolated polyphenol fractions in selected outcomes. By comparing chemical composition, gastrointestinal transformation, cellular signaling, and evidence from animal and human studies, we identify two unresolved links: that between compounds measured before consumption and those reaching relevant sites of action, and that between changes in signaling markers and mechanisms necessary for functional protection. Evidence of C3G–TAK1 interactions and intervention studies implicating the Nrf2–GPX7 axis in specific ferroptosis models has advanced mechanistic understanding, but these findings have not yet been integrated under comparable oral-exposure conditions. Component depletion and add-back experiments, quantitative analysis of parent compounds and metabolites, and targeted mechanistic interventions are needed to identify which compounds contribute to biological effects, at which sites, and through which mechanisms.

Keywords: Blue Honeysuckle; Anthocyanins; Cyanidin-3-O-Glucoside; Antioxidant Activity; Anti-Inflammatory Activity; Gastrointestinal Transformation; Functional Attribution

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1. INTRODUCTION

Blue honeysuckle (*Lonicera caerulea* L.) has attracted considerable interest because of its high anthocyanin content,

with cyanidin-3-O-glucoside (C3G) usually being the predominant anthocyanin. Extracts from different cultivars have demonstrated radical-scavenging activity and effects in cellular models of oxidative injury and inflammation [1–3]. Consequently, selecting anthocyanin-rich cultivars and improving extraction yield and stability have become common objectives in product development. These properties are useful measures of raw-material quality or processing performance, but leave a fundamental question

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unanswered: does a higher anthocyanin content translate into greater protection after consumption? Answering that question first requires identifying the molecules responsible for the observed effects.

Three recent findings make this question particularly relevant. Dharmawansa et al. compared free and microencapsulated anthocyanin-rich fractions in mice with dextran sulfate sodium (DSS)-induced colitis and found no superior overall protection with the encapsulated preparation [4]. Zhou et al. reported larger improvements in selected colitis endpoints with whole blue honeysuckle pomace than with an isolated polyphenol-rich fraction [5]. Simulated digestion studies, meanwhile, have shown that samples can retain certain cytoprotective or in vitro enzyme-inhibitory activities even after marked decreases in C3G and other parent anthocyanins [6, 7]. These studies used different materials and models and cannot establish the general

superiority of any processing strategy. Together, however, they demonstrate that a chemical advantage measured in the starting material need not be reflected directly in the final biological outcome.

Guo et al. [1] have reviewed the composition, extraction, functions, and applications of blue honeysuckle anthocyanins. Rather than cataloging additional bioactivities, the present review uses these counterintuitive observations to examine two related disconnects: the difference between the compounds measured before consumption and those actually present at sites of action, and the difference between changes in signaling markers and processes that are necessary for protection. We therefore examine functional attribution, processing and gastrointestinal transformation, antioxidant and anti-inflammatory mechanisms, and evidence from animal and human studies within a single analytical framework.

Core question: Are the major anthocyanins measured in raw materials the same molecules that actually drive biological effects?

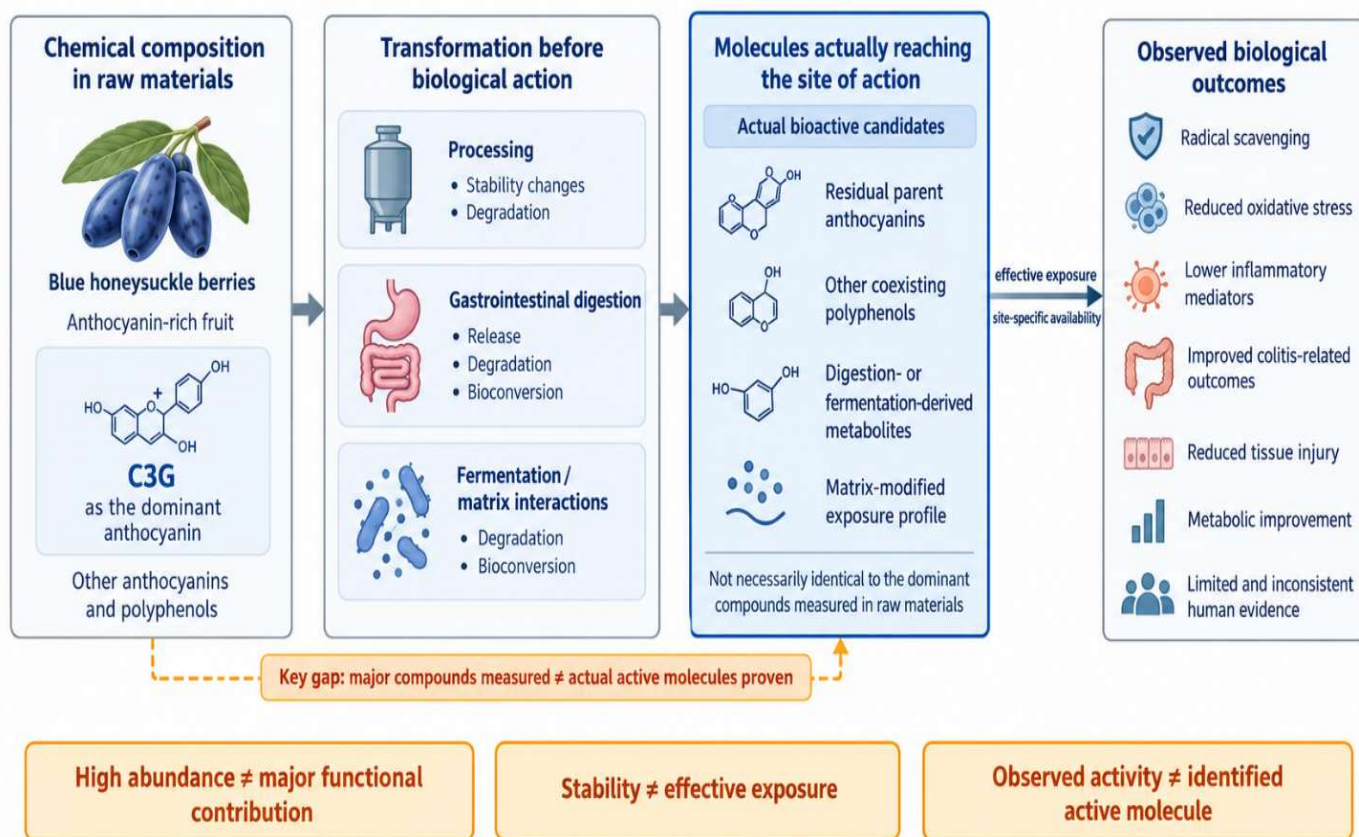


Fig. 1. Conceptual framework linking anthocyanin composition to biological effects in blue honeysuckle. C3G is usually the predominant anthocyanin in raw berries or extracts, but processing, gastrointestinal digestion, fermentation, and food-matrix interactions may change the compounds available at relevant sites of action. Antioxidant and anti-inflammatory effects have been reported for whole extracts, anthocyanin-rich fractions, and certain isolated compounds. Nevertheless, the relationship between the constituents most abundant in the starting material and the molecules responsible for biological effects remains unresolved. The review evaluates this relationship in terms of functional contribution, relevant exposure, and mechanistic evidence.

2. COMPOSITIONAL PREDOMINANCE VERSUS FUNCTIONAL CONTRIBUTION

2.1. What Cultivar Differences Reveal about Composition–Activity Relationships

Although C3G predominates in blue honeysuckle, it is not invariably the anthocyanin most strongly associated with every activity endpoint. Wang et al. compared four cultivars and reported differences in anthocyanin and other polyphenol profiles [2]. Fan et al. characterized 61 genotypes from northeastern China, identified nine major anthocyanins, and reported total anthocyanin concentrations ranging from 158.44 to 1751.44 mg/100 g fresh weight, an approximately 11-fold difference [3]. Notably, gray relational analysis identified cyanidin-3,5-O-diglucoside, rather than the more abundant C3G, as having the strongest association with the measured reactive oxygen species (ROS)-scavenging endpoints. This is a statistical association between composition and chemical assay results, not an independent

ranking of the compounds' biological potency. It raises an experimentally testable question: is the most abundant constituent also the largest contributor to activity in the mixture?

Simply expanding the number of cultivars will not resolve this question. In a comparison of 20 blue honeysuckle cultivars, Zhang et al. found differing relationships of anthocyanins, phenolic acids, and other flavonoids with DPPH, ABTS, FRAP, and α -amylase inhibition assays [8]. Ponder et al. likewise documented concurrent effects of cultivar, cultivation system, and year on polyphenol composition and antioxidant measurements [9]. An increase in a particular constituent alongside higher activity may reflect its own contribution, but it may also arise because several phenolic compounds change together under the same genetic or environmental conditions. Cultivar comparisons can identify candidate contributors; their independent contributions require controlled comparisons of fractions within a common chemical background.

From Anthocyanin Abundance to Functional Contribution in Blue Honeysuckle

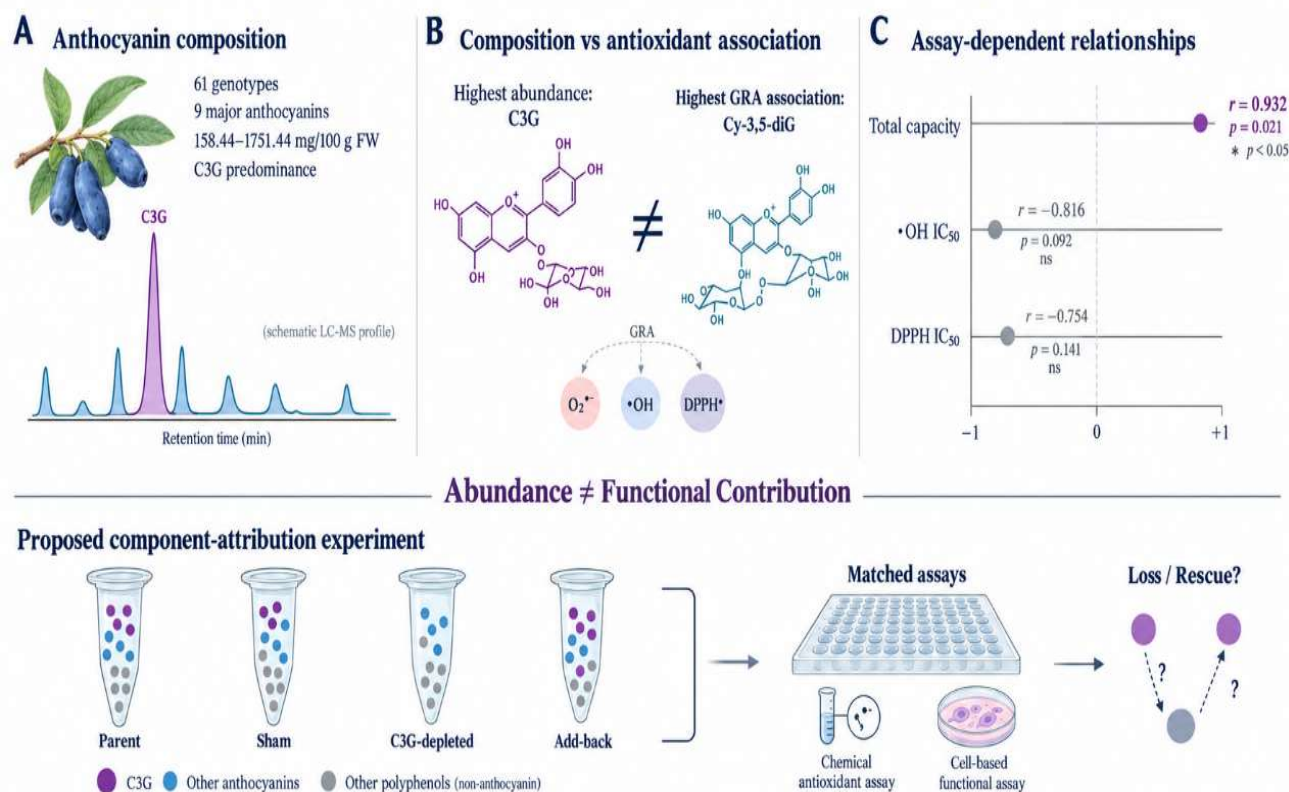


Fig. 2. Distinguishing anthocyanin abundance, chemical antioxidant associations, and functional contribution in blue honeysuckle. Anthocyanin content varies substantially across genotypes, although C3G generally predominates. Grey relational analysis nevertheless identified cyanidin-3,5-O-diglucoside as the constituent most strongly associated with selected chemical ROS-scavenging endpoints. Correlations between anthocyanin purity and chemical antioxidant assays also differed in magnitude and statistical significance. These observations distinguish compositional abundance and assay-dependent associations from independently established functional contributions. The lower panel illustrates a proposed strategy combining selective C3G depletion, add-back, and matched functional testing. Molecular and chromatographic symbols are schematic and not to scale; numerical annotations are taken from the cited studies.

2.2. Why Purified Fractions Produce Different Activity Rankings?

Li et al. compared anthocyanin-rich fractions and found a significant positive correlation between anthocyanin purity and total antioxidant capacity ($r = 0.932$, $P = 0.021$) [10]. By contrast, correlations with the IC_{50} values for hydroxyl radical scavenging ($r = -0.816$, $P = 0.092$) and DPPH scavenging ($r = -0.754$, $P = 0.141$) did not reach statistical significance. This distinction matters: even for fractions obtained from the same starting material, conclusions depend on how 'antioxidant activity' is defined. Total antioxidant capacity and individual radical-scavenging assays rely on different reaction principles. Collapsing their outcomes into a single-activity ranking obscures, rather than resolves, differences in constituent contribution.

Dose definition is another source of ambiguity. An AB-8 resin-enriched blue honeysuckle preparation produced by Zhang et al. contained measured total anthocyanins of 24.046% and total phenolics of 38.075% [11]. Because the two values were obtained by different assays, subtracting the percentages would not yield an exact proportion of non-anthocyanin phenolics. Similarly, '200 $\mu\text{g/mL}$ extract', '6.2 mg C3G equivalents per mouse per day', and 'a given $\mu\text{mol/L}$ concentration of purified C3G' describe the mass of a mixture, an equivalent-based dose, and the molar concentration of a defined compound, respectively. They are not interchangeable exposure measures. Without this distinction, studies described as investigating 'blue honeysuckle anthocyanins' may actually be testing chemically different materials.

C3G should therefore be treated as a priority candidate, not an established dominant effector. A more informative approach would begin with a single chemically standardized extract, selectively remove C3G, restore it at a matched amount, and compare the resulting preparations with mixtures reconstructed from other anthocyanins and non-anthocyanin polyphenols. Under a largely unchanged chemical background, a decline in activity after depletion followed by recovery after add-back would provide evidence of functional contribution beyond compositional predominance.

The central conclusion of this section is thus not that C3G has been established as the principal bioactive constituent. Rather, its abundance makes it an obvious first candidate, while evidence from cultivar comparisons, composition–activity associations, and extract assays remains insufficient for compound-specific attribution. Figure 2 distinguishes compositional predominance, statistical association, and experimentally demonstrated contribution, and outlines the controls needed to assess C3G's role.

3. FROM INGESTED CONSTITUENTS TO MOLECULES POTENTIALLY RESPONSIBLE FOR BIOLOGICAL ACTIVITY

3.1. Processing and Digestion Reshape Chemical Composition

Identifying the active constituents in an extract would still not establish which compounds exert effects after oral consumption. Zhang et al. reported pronounced pH dependence of the apparent half-life of an anthocyanin-rich blue honeysuckle preparation heated at 90 °C: approximately 7.37 h at pH 2.0 versus 0.70 h at pH 7.0 [11]. These values, derived from total anthocyanin measurements under specified heating conditions, demonstrate the influence of formulation conditions on apparent stability. They neither represent independent degradation rates for each anthocyanin nor describe actual gastrointestinal kinetics. Changes in color may also reflect pH-dependent structural equilibria or complex formation rather than exclusively irreversible molecular degradation.

Bao et al. combined simulated gastrointestinal digestion with colonic fermentation and observed declines in parent anthocyanins, including C3G, alongside changes in several low-molecular-weight phenolic acids [6]. Nevertheless, the processed samples retained some protective activity in a Caco-2 cell model of oxidative injury. The key question is not whether degradation is intrinsically beneficial, but which compounds were present in the material to which the cells were exposed: residual anthocyanins, other phenolics, and transformation products were all present. Because different precursors can generate the same small phenolic molecules, these findings do not assign the protective effect to any individual metabolite.

Heng et al. similarly reported a substantial decrease in total anthocyanin content following simulated digestion of a whole blue honeysuckle extract [7]. At higher test concentrations (≥ 10 mg/mL), some DPPH and ABTS radical-scavenging rates and xanthine oxidase (XOD) inhibition rates of the digested preparation still exceeded 70%. These percentages describe assay responses under the stated conditions; they are neither anthocyanin retention rates nor percentages of the original activity preserved after digestion. They establish that the digested mixture retains measurable *in vitro* activity, but reveal nothing directly about the amount of anthocyanin absorbed in humans.

3.2. Fermentation Generates New Combinations of Candidate Bioactives

Liu et al. examined blue honeysuckle juice fermented with *Lactiplantibacillus plantarum* and reported increases in six phenolic constituents—C3G, cyanidin-3,5-O-diglucoside, peonidin-3-O-glucoside, 3-caffeoylquinic acid, quercetin, and catechin—accompanied by stronger *in vitro* XOD inhibition [12]. The IC_{50} values for XOD inhibition by C3G, peonidin-3-O-glucoside, and quercetin were 115.28, 105.34, and 88.75 $\mu\text{mol/L}$, respectively. Because both abundance and compound-specific activity differed, fermentation should not be assessed solely by the amount of C3G lost or retained: it can reshape the combination of potentially active

constituents. XOD inhibition remains an in vitro enzyme assay and does not, by itself, establish a urate-lowering or anti-inflammatory effect in humans.

These findings call for at least two chemical profiles when evaluating processing: one describing parent constituents before consumption and another characterizing the compounds that remain potentially available after digestion and transformation. Studies of local intestinal effects should measure parent compounds and metabolites in intestinal contents and near the mucosal surface. Claims concerning distant organs, such as the liver or kidney, require measurements of circulating and target-tissue exposure. Retention of C3G in the starting material alone cannot capture these spatial and temporal changes.

3.3. Animal Evidence from Free, Encapsulated, and Whole-Matrix Preparations

Dharmawansa et al. prepared microcapsules of an anthocyanin-rich fraction (AHF) using maltodextrin and inulin (3:1), achieving encapsulation efficiencies of approximately 54–60% [4]. They subsequently compared free and encapsulated AHF in DSS-induced colitis in mice (n = 5 per group), using a nominal daily dose of 6.2 mg C3G equivalents per mouse. Free AHF and some treatments combined with probiotics improved disease activity scores and selected serum inflammatory outcomes, whereas encapsulated AHF did not provide superior overall protection. Encapsulation efficiency measures how much material is entrapped; it does not establish where the formulation releases its contents in the gut or what local concentrations are achieved. The value of this study lies in separating a favorable formulation property from a demonstrated animal

outcome. Zhou et al. compared whole blue honeysuckle pomace (LCP), a polyphenol-rich extract (PEE), and a fiber-rich residue in a DSS-induced colitis model [5]. Relative to the DSS model group, reported reductions in histopathology scores were 87.80% (P < 0.01), 21.95% (P < 0.01), and 17.07% (P > 0.05), respectively. The numerical improvement was substantially larger in the whole-pomace group, although these percentages are comparisons with the model group and do not substitute for direct tests of significance between every pair of treatments. Zhang et al. also reported changes in mucosal and microbiota-related outcomes following pomace supplementation [13]. Because the research teams overlap, the independence of these datasets should be established before treating them as completely separate replications.

Although these animal studies did not compare identical preparations, they sharpen the same question: both encapsulation and extraction alter the physical and chemical environment of potentially active constituents, and final efficacy cannot be predicted from any single processing metric. The stronger responses to whole pomace in selected outcomes warrant attention to food-matrix effects, but a claim of 'matrix synergy' requires component depletion and add-back at matched effective doses, together with an appropriate analysis of combined effects. For food development, the priority is not simply to maximize enrichment, but to establish which exposures a processed product produces and how they relate to functional outcomes. Thus, changes in abundance, processing retention, and formulation properties acquire biological meaning only when linked to relevant effects. Table 1 compares representative primary studies in terms of preparation, model, direct result, and supported inference.

Table 1. Differences between measured properties and functional outcomes in representative studies of blue honeysuckle preparations.

Material and model	Key findings of the original study	Supported interpretation (limits of inference)	Study
61 genotypes; anthocyanin profiling and chemical ROS-scavenging assays	C3G generally predominated; cyanidin-3,5-O-diglucoside showed stronger statistical associations with selected ROS-scavenging endpoints.	Compositional predominance differs from association with a chemical endpoint; association does not rank the independent biological contributions of individual compounds.	Fan et al., 2023 [3]
Digested/fermented blue honeysuckle samples; Caco-2 oxidative injury model	Some parent anthocyanins declined, while the processed mixture retained cytoprotective activity.	The processed mixture can remain active; the contributions of individual metabolites are unidentified.	Bao et al., 2022 [6]

<i>Lactiplantibacillus plantarum</i>-fermented juice; <i>in vitro</i> XOD inhibition	Several anthocyanins and other phenolics changed in abundance, accompanied by stronger XOD inhibition.	Fermentation reshapes the candidate bioactive mixture; it does not establish urate-lowering or anti-inflammatory efficacy in humans.	Liu et al., 2025 [12]
Free versus encapsulated AHF; DSS colitis in mice (n = 5 per group)	Encapsulation efficiency was approximately 54–60%, but overall protection was not superior to that of free AHF; several local protein endpoints were not significantly restored.	Formulation metrics alone cannot predict disease outcomes; the findings do not establish that microencapsulation is generally ineffective.	Dharmawansa et al., 2024 [4]
Whole pomace (LCP), polyphenol-rich extract (PEE), and fiber-rich residue; DSS colitis in mice	Histopathology scores were reduced by 87.80%, 21.95%, and 17.07%, respectively, relative to the DSS model group; the residue group was not statistically significant.	Whole-food matrix effects warrant investigation; the comparisons do not directly establish fiber–anthocyanin synergy.	Zhou et al., 2024 [5]
Blue honeysuckle polyphenols; LPS-stimulated cells and mouse inflammation model	Inflammatory endpoints and TAK1-associated signaling changed; C3G and (–)-epicatechin showed binding to TAK1.	TAK1 is a candidate mechanistic node, but the contribution of individual compounds to the whole-extract effect remains unresolved.	Wu et al., 2017 [16]
C3G/anthocyanin-rich preparation; neuronal ferroptosis models and MPTP-treated mice	Inhibition of Nrf2 or GPX7 weakened C3G protection in specific cell models; the animal study used intraperitoneal administration.	Intervention supports pathway involvement in specific models, not efficacy following routine oral intake.	Li et al., 2025 [18]
10 healthy volunteers; 165 g fresh fruit/day for 7 days	Selected phenolic acid metabolites, GSH-Px, and CAT increased, but TBARS also increased.	Human redox-related readouts changed in different directions; a consistent overall antioxidant benefit was not demonstrated.	Heinrich et al., 2013 [26]

4. FROM ANTIOXIDANT AND ANTI-INFLAMMATORY PHENOTYPES TO MECHANISMS OF ACTION

4.1. Chemical Antioxidant Activity Is Not Equivalent to Cellular Protection

At 300 µg/mL, Lee et al. measured DPPH scavenging rates of 86.1% and 92.96% for blue honeysuckle extracts from two geographical origins, but their protective effects against

oxidative injury in HepG2 cells did not follow the same ranking [14]. In H₂O₂-challenged RAW264.7 cells, Li et al. reported viabilities of 64.45% in the model group, 78.29% following treatment with 200 µg/mL aqueous extract, and 70.07% with the corresponding ethanolic extract; the vitamin C positive control reached 95.03% [15]. The improvement with the aqueous extract was statistically significant relative to the model group, whereas the ethanolic preparation did not achieve the same statistical result. Together, these observations show that radical-scavenging performance in a chemical assay cannot substitute for measuring cellular

survival and defense under oxidative stress.

Lee et al. also observed increased activity of an antioxidant response element (ARE) reporter and higher expression of genes including HO-1 and NQO1; at 300 $\mu\text{g/mL}$, the Korean extract increased ARE reporter activity to approximately 3.9 times the control level [14]. These observations support the ability of the extract to affect endogenous cellular antioxidant responses, but do not establish that the transcriptional changes are necessary for cytoprotection. Demonstrating a requirement for Nrf2-related signaling would entail perturbing a key pathway component and assessing whether protection is altered at the same functional endpoint.

4.2. From Pathway Associations to Mechanistic Necessity

In LPS-stimulated macrophage and mouse models, Wu et al. observed changes in TAK1-associated MAPK and NF- κB signaling, together with early Nrf2 and MnSOD responses, following treatment with blue honeysuckle polyphenols [16]. They also detected interactions between TAK1 and both C3G and epicatechin. Linking defined molecules to a candidate signaling node represents stronger mechanistic evidence than cytokine measurements alone. Nevertheless, multiple components of the complete extract may contribute to protection in animals, and a binding interaction does not establish the contribution of each constituent to the overall effect.

Using a standardized blue honeysuckle extract, Zima et al. reported reduced NF- κB p65 nuclear translocation in A549 cells and lower p65 phosphorylation and selected inflammatory cytokines in human peripheral blood mononuclear cells [17]. Changes in p65 localization and phosphorylation provide a more direct readout of pathway activation than changes in its mRNA abundance, but the preparation remained multicomponent. The study strengthens the evidence for NF- κB -associated effects without establishing this pathway as the mechanism by which purified C3G suppresses inflammation in humans.

Li et al. used purified C3G and an anthocyanin-rich fraction in neuronal injury models involving MPP⁺ and erastin, observing reductions in lipid peroxidation and ferroptosis-related phenotypes [18]. Inhibiting Nrf2 or GPX7 attenuated the protective effects of C3G, providing intervention-based evidence that the Nrf2–GPX7 axis participates in cytoprotection in the models examined. The authors also reported neuroprotection in MPTP-treated mice, but administered C3G intraperitoneally rather than orally. These findings therefore establish pathway involvement under specific injury and administration conditions; they do not demonstrate that dietary blue honeysuckle produces the same effect in the gut or other tissues.

4.3. Why Inflammatory Outcomes and Tissue-Level Markers Do Not Always Agree?

The DSS colitis study by Dharmawansa et al. illustrates the distinction clearly [4]. Free AHF and some combination treatments improved the disease activity index, and several intervention groups exhibited lower serum TNF. Significant improvements in serum IL-6 were mainly observed in the probiotic-only group and the group receiving free AHF plus probiotics, whereas serum IL-1 β did not change significantly. At the same time, measurements of colonic TNF, IL-6, BAX, BCL-2, and tight junction proteins showed no consistent significant restoration. The study therefore supports improvements in selected disease manifestations and circulating inflammatory endpoints, not a demonstrated recovery of local colonic barrier proteins. Divergence between local and systemic endpoints should remain part of the mechanistic interpretation rather than be averaged away.

Other animal models broaden the range of outcomes associated with blue honeysuckle preparations. Zhao et al. reported effects of an anthocyanin extract on MDA, SOD, and GSH-Px in irradiated mice [19]. Li et al. associated blue honeysuckle polyphenol supplementation with changes in intestinal oxidative stress, barrier-related proteins, and liver injury markers in high-fat-diet-fed rats [20]. Heng et al. observed improvements in urate metabolism, inflammatory markers, and liver–kidney–gut outcomes after treatment with a whole extract in hyperuricemic mice [7]. These findings support biological activity in diverse settings, but differences in material and pathological context prevent them from serving as direct independent confirmations of a single, cross-tissue mechanism mediated by purified C3G.

The distinction between materials becomes especially important in immune studies. LPS-based cell studies by Rupasinghe et al. and Li et al. examined reductions in inflammatory mediators after stimulation [15, 21], whereas Zhang et al. investigated restoration of immune-related outcomes by polyphenols in cyclophosphamide-immunosuppressed mice [22]. Huang et al. studied THP-2, a blue honeysuckle polysaccharide that increased costimulatory molecules and selected inflammation-associated readouts in certain immune cell models [23]. These experiments involve different chemical classes and immune states; together they do not establish 'bidirectional immunomodulation by anthocyanins'. Meaningful comparisons must first identify the tested substance, the cellular state, and the function being measured.

Mechanistic evidence in this field now spans three levels: cell or animal phenotypes establish that an effect occurs; signaling and protein measurements implicate particular processes; and perturbation of a pathway component can test whether that process is necessary for protection. TAK1 binding, p65 nuclear translocation, and Nrf2–GPX7 intervention each advance this progression, but were investigated using different preparations and models. The priority is to connect these levels through the same chemically defined active material under biologically plausible exposure conditions, not simply to add more pathway names.

The differences among current mechanistic studies reflect not only which signaling proteins were measured, but

also material identity and the extent of causal testing. To avoid combining single-compound mechanisms, cellular responses to mixed extracts, and animal disease phenotypes into an unverified pathway, Figure 3 presents these findings as three distinct evidence tiers.

5. WHAT DISCORDANT RESULTS REVEAL ABOUT THE LIMITS OF CURRENT EVIDENCE?

5.1. Model Dependence of Gut Microbiota Responses

Gut microbiota responses are particularly susceptible to overgeneralization. Wu et al. reported reduced relative

abundance of certain *Lactobacillus*-related taxa in high-fat-diet-fed mice treated with blue honeysuckle polyphenols [24], whereas Wang et al. observed increases in small-intestinal samples from high-fat-diet-fed rats [25]. In cyclophosphamide-immunosuppressed mice, Zhang et al. reported that the *Firmicutes/Bacteroidetes* relative-abundance ratio fell from approximately 3.40 in controls to 0.92 in the model group and rose to 1.41 after polyphenol supplementation; the relative abundance of *Akkermansia* increased from approximately 0.04% in controls to 14.81% in the model group, then shifted toward the control level with treatment [22]. By contrast, Heng et al. reported increased relative abundances of *Akkermansia* and *Lactobacillus* after whole-extract intervention in hyperuricemic mice [7].

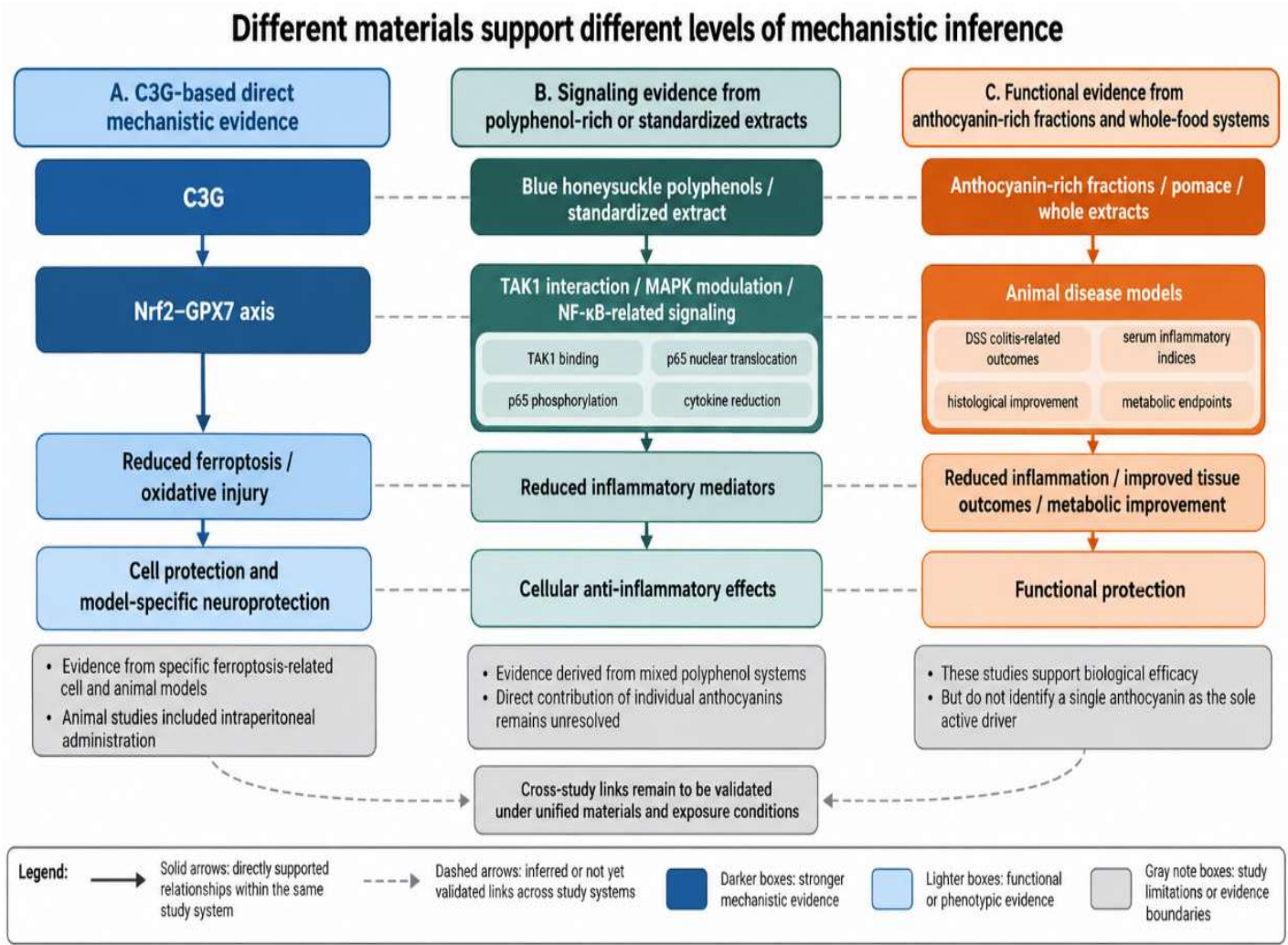


Fig. 3. Evidence hierarchy for antioxidant and anti-inflammatory mechanisms associated with blue honeysuckle anthocyanins and related preparations. Evidence derives from different materials and experimental systems. Lane A summarizes C3G-based mechanistic findings in specific ferroptosis-related cell and animal models. Lane B presents signaling observations from polyphenol-rich and standardized extracts, including TAK1 interactions and MAPK- and NF-κB-associated changes in cell models. Lane C summarizes selected functional outcomes reported for anthocyanin-rich fractions, pomace, and whole extracts in animal disease models. Solid connectors denote relationships investigated within the respective study systems, not necessarily established direct causation; dashed connectors denote proposed cross-study links requiring validation under standardized material and exposure conditions.

These differing directions first point to differences in models and materials, not necessarily contradictory findings. Host species, intestinal sampling site, disease state, and intervention formulation can all shape community responses. Moreover, 16S rRNA sequencing generally yields relative abundances, which do not directly quantify absolute bacterial counts or metabolic capacity. Rather than assuming that an increase in a named genus is invariably beneficial, studies should ask which microbial functions or metabolites are associated with relevant host inflammatory outcomes. Zhou et al. reported associations between bacterial taxa and serum metabolites [5], but these associations do not establish causality.

5.2. The Evidence Gap between

Intestinal Barrier Markers and Protection of Distant Organs
Intestinal barrier function may connect local Intestinal barrier function may connect local inflammation with distant organ responses, but the evidence from blue honeysuckle studies is not uniform. Whole pomace supplementation has coincided with improvements in mucosal and barrier-related outcomes in certain DSS colitis studies [5, 13], whereas AHF did not significantly restore the measured colonic tight junction proteins in a separate DSS model [4]. In hyperuricemic mice, Heng et al. observed increased intestinal expression of ZO-1, occludin, and ABCG2 after intervention with a whole extract, together with changes in renal urate transporter proteins [7]. These tissue-level expression data are informative, but protein abundance is not itself a direct measure of epithelial permeability or urate transport flux.

The strongest conclusion currently supported is that blue honeysuckle preparations alter selected intestinal, inflammatory, and distant-organ endpoints in different models. Whether intestinal changes mediate hepatic or renal protection remains unresolved because targeted measurements and interventions addressing barrier function, transporter function, and their causal contributions are lacking. Presenting Nrf2, NF- κ B, the gut microbiota, and distant organs as a single validated pathway would obscure the fact that the individual links were observed in different studies.

5.3. Human Evidence and Food Applications

Human data ultimately determine whether the proposed interpretation can be translated into health relevance. Heinrich et al. studied 10 healthy volunteers who consumed 165 g of fresh blue honeysuckle fruit per day for 7 days, providing approximately 208 mg of anthocyanins daily [26]. Urinary excretion of selected phenolic acid metabolites increased, as did erythrocyte glutathione peroxidase (GSH-Px) and catalase (CAT) activities. However, thiobarbituric acid-reactive substances (TBARS) also increased in plasma and erythrocytes, and overall plasma antioxidant status did

not change significantly. The intervention therefore elicited metabolic and redox-related responses without demonstrating a consistent reduction in oxidative damage, much less a defined anti-inflammatory effect attributable to anthocyanins in humans.

Bell and Williams reported changes in selected cognitive and blood-pressure outcomes in 20 older adults [27], while Howatson et al. observed improvements in certain exercise-performance outcomes among 30 male recreational runners [28]. These studies provide functional observations under actual ingestion conditions, but their endpoints were not inflammatory biomarkers and the compounds responsible were not established. Human trials explicitly designed to test anti-inflammatory effects should therefore include appropriate inflammatory and functional endpoints alongside measurements of parent anthocyanins and major metabolites.

Food applications likewise require distinguishing constituent abundance from actual functionality. Meng et al. reported an in vitro estimated glycemic index (eGI) of 54.58 for noodles supplemented with 12% blue honeysuckle polyphenols; this is not a glycemic index measured in humans [29]. Qi et al. reviewed the effects of cultivar and processing on acidity, bitterness, astringency, and aroma [30], while Ponder et al. reported cultivar-dependent differences in measured proteins associated with potential allergenicity [9]. These findings do not directly demonstrate anti-inflammatory mechanisms of anthocyanins, but indicate that formulation quality must also account for composition, acceptability, and safety-related assessment.

Taken together, these apparently divergent findings repeatedly expose the same limitation: starting-material composition, formulation performance, or an individual biomarker cannot stand in for a final functional outcome. Table 2 aligns these observations with the interpretations they support and the tests needed to distinguish among explanations.

5.4. Future Directions: Establishing Active Constituents, Relevant Exposure, and Mechanisms

The first priority is functional attribution. Using a single standardized extract, researchers should quantify individual constituents and compare the parent preparation with selectively C3G-depleted and concentration-matched add-back preparations. If the removal of C3G weakens a predefined functional outcome and its restoration reverses that effect, the comparison would support a specific contribution. Other anthocyanins and coexisting polyphenols can be examined using analogous reconstruction experiments. Synergy should be evaluated against an explicitly selected additivity model, rather than inferred from statistical differences between combined and individual treatment groups [31].

Table 2. Common expectations, contrasting observations, supported interpretations, and validation priorities in blue honeysuckle anthocyanin research

Common expectation	What the original studies observed	Interpretation supported by the evidence	Priority for validation
As the most abundant anthocyanin, C3G should contribute the most.	Other anthocyanins showed stronger statistical associations with selected chemical ROS-scavenging endpoints [3].	Chemical abundance is distinct from independent functional contribution; association does not establish attribution.	Equimolar single-compound testing; depletion and add-back; mixture reconstitution.
Loss of parent anthocyanins should imply proportional loss of activity.	Parent compounds decreased after digestion, but the processed mixtures retained certain <i>in vitro</i> activities [6, 7].	The chemical mixture changed, but the compounds responsible for the residual activity remain unidentified.	Quantification after digestion; metabolite fractionation; paired exposure and activity measurements.
Higher encapsulation efficiency should improve protection <i>in vivo</i>.	AHF microcapsules with approximately 54–60% encapsulation efficiency did not provide better overall colitis protection than free AHF [4].	Encapsulation efficiency does not establish release, relevant exposure, or animal efficacy.	Matched C3G-equivalent doses; blank-wall controls; intestinal release and local exposure measurements.
Purifying polyphenols should increase anti-inflammatory efficacy.	Whole pomace showed larger improvements in selected histopathology endpoints than an isolated polyphenol-rich fraction [5].	The food matrix may affect functional outcomes, but the difference does not establish synergy.	Matched-dose depletion, add-back, and an appropriate combined-effect model.
Reduced serum inflammatory markers indicate repair of the intestinal barrier.	Selected serum endpoints improved with free AHF, but measured colonic tight junction proteins were not significantly restored [4].	Circulating and local tissue readouts need not change in parallel and cannot substitute for each other.	Direct permeability testing, local protein measurements, and histopathology.
Higher antioxidant enzyme activity means lower overall oxidative damage.	GSH-Px/CAT and TBARS both increased after short-term human consumption [26].	Redox-related markers do not all indicate benefit in the same direction.	Human trials with predefined, complementary functional endpoints and exposure measurements.

The second priority is relevant exposure. The standardized INFOGEST static digestion protocol offers a basis for comparable initial digestion experiments [32]. Coupled with targeted chemical analysis, it can help trace parent anthocyanins and transformation products; measurements of their concentration–time profiles should then distinguish intestinal contents, mucosa, blood, and target tissues. Comparisons of free fractions, encapsulated preparations, and whole foods should match nominal doses of the relevant active constituents and include wall-material and food-matrix controls. Such designs can establish whether processing changes chemical retention, the compounds reaching sites of action, or both.

The final priority is mechanistic testing. At concentrations consistent with measured exposure, genetic perturbation and functional rescue of candidate nodes such as TAK1 and Nrf2 should be accompanied by tissue- or disease-relevant outcomes. Claims of microbial mediation likewise require causal experiments in addition to microbiota profiling and metabolite associations. A consensus on microbiome research emphasizes choosing causal models to fit the question and accounting for the effects of interventions such as transplantation and antibiotics themselves [33]. Combining component attribution, exposure measurements, and mechanistic testing would make it possible to distinguish an active extract from a defined compound acting under biologically relevant conditions.

6. CONCLUSIONS

C3G is the predominant anthocyanin in blue honeysuckle, and anthocyanin-rich fractions and other polyphenol preparations have shown activity in chemical antioxidant assays, cellular oxidative injury models, and selected animal models of inflammation. Studies of TAK1/MAPK–NF- κ B and Nrf2 signaling, as well as Nrf2–GPX7 in specific ferroptosis models, provide evidence ranging from pathway responses to targeted molecular interventions. These findings establish a meaningful basis for further bioactivity research, but protection conferred by complex extracts cannot be assigned directly to C3G.

The most informative observation across the literature is that chemical advantages do not invariably translate into greater biological effects. Parent anthocyanins decrease during simulated digestion while some processed mixtures retain in vitro activity; microencapsulation has not yielded superior overall protection in a colitis model; and whole pomace has outperformed an isolated polyphenol fraction for selected endpoints. Thus, constituent abundance, purification, and formulation stability alone cannot predict biological outcomes. Relating chemical composition to actual exposure and functional endpoints offers a more useful basis for explaining these discrepancies.

Future work should establish the contributions of individual compounds using standardized materials, quantify gastrointestinal transformation and tissue exposure, and test candidate mechanisms through targeted interventions.

Human studies should integrate exposure measurements with predefined functional outcomes. This approach can move the field from recognizing that blue honeysuckle is rich in anthocyanins to establishing which compounds exert specific effects, under what conditions, and through which mechanisms.

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

Availability of data and material

All of the data obtained or analyzed during this study is included in the report that was submitted.

Conflicts of Interest

The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Authors' contributions

All authors contributed equally to this work.

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