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The phenylpropanoid network in pearl millet: Antioxidant functions and biological roles of polyphenols

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Abstract

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a resilient, nutrient-dense cereal increasingly recognized for its rich polyphenolic composition and potent antioxidant potential, yet current knowledge remains scattered across biochemical, nutritional, and functional studies. This review consolidates advances published between 2010 and 2025, identified through structured searches of Web of Science, Scopus, PubMed, and Google Scholar using targeted keywords related to millet polyphenols, antioxidant activity, and functional foods. It integrates present understanding of composition, biosynthesis, analytical characterization, and biological relevance of pearl millet polyphenols, highlighting their role in oxidative stress modulation and health promotion. The dominant compounds include phenolic acids (ferulic, caffeic, pcoumaric) and flavonoids (tricin, luteolin, quercetin) derived from the phenylpropanoid pathway regulated by PAL, C4H, and CHS enzymes. Ethanol based extraction combined with UPLC QTOF MS profiling enhances recovery and structural identification of these metabolites. Functionally, pearl millet polyphenols operate through hydrogen atom transfer, single electron transfer, and metal chelating mechanisms, activate Nrf2 ARE signalling, and inhibit pro oxidant enzymes, producing strong radical scavenging, DNA protective, and membrane stabilizing effects linked to antidiabetic, cardioprotective, anti-inflammatory, and gut modulatory benefits. By unifying molecular and functional perspectives, this review establishes a framework for standardized research and supports the development of pearl millet as a sustainable nutraceutical and phytomedicine resource.

1. Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a nutritionally rich and agronomically strategic cereal predominantly cultivated in semi-arid and arid regions. It accounts for a major share of global millet production, with India serving as the principal cultivation hub (Kaila and Muthukumar, 2025; Singh and Nara, 2023). Owing to its exceptional tolerance to heat, drought, and nutrient-poor soils, pearl millet has become a cornerstone of climate-resilient agriculture. Beyond ecological adaptability, it is recognized for its dense nutritional profile, offering significant quantities of macronutrients, essential minerals such as iron and zinc, and a broad array of bioactive phytochemicals that underpin its reputation as a functional grain (Tomar *et al*., 2021). Compared with major cereals such as wheat, rice, and maize, pearl millet has been shown to contain higher concentrations of bound phenolic compounds, contributing to its potent antioxidant potential (Tomar *et al*., 2021). In addition, phenolic acids and flavonoids in millets occur in both free and bound forms, with the bound phenolics predominantly in the outer layers of the grain, which are retained when consumed as whole grain (Chandrasekara and Shahidi, 2010), enhancing antioxidant availability relative to refined cereals.

Recent advances in genomics and metabolomics have deepened our understanding of the crop's nutritional and biochemical diversity. Multivariate analyses of indigenous germplasm have revealed substantial variation in nutrient and phytochemical profiles, supporting the identification of nutrition-oriented ideotypes (Tomar *et al*., 2021). Metabolite-genome-wide association studies (metabolite-GWAS) have mapped several loci linked to health-benefiting metabolites, notably genes encoding UDP-glycosyl-transferases (UGTs) and L-ascorbate oxidase, which are implicated in flavonoid glycosylation and antioxidant metabolism (Yadav *et al*., 2021). These insights highlight opportunities to integrate nutritional quality with stress-resilience traits in next-generation pearl millet breeding programs.

From a biochemical perspective, pearl millet grains, particularly their bran and bound phenolic fractions are credible sources of natural antioxidants and polyphenolic compounds. These molecules display a wide range of biological functions, including free-radical scavenging, digestive-enzyme inhibition, DNA protection, and anti-inflammatory or immunomodulatory activities (Singh *et al*., 2025; Krishnan *et al*., 2022; Salar and Purewal, 2017; Nani *et al*., 2015). Quantitative profiling across diverse genotypes has identified more than forty

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individual phenolics, with total phenolic contents ranging from 286 to 338 mg gallic acid equivalents (GAE)/100 g, demonstrating enhanced bioaccessibility during simulated digestion (Singh *et al*., 2025). Antioxidant potency, strongly influenced by solvent polarity and extraction methodology, has been verified through DPPH, ABTS, FRAP, and ORAC assays (Marmouzi *et al*., 2018; Salar and Purewal, 2017).

Beyond antioxidant capacity, mechanistic studies indicate that pearl millet polyphenols contribute to glycaemic regulation and immune modulation. Polyphenol-enriched extracts inhibit α-amylase and α-glucosidase while enhancing hepatocyte glucose uptake, with molecular docking implicating flavonoids such as acacetin and 3,4-dimethoxy-luteolin derivatives as key ligands (Krishnan *et al*., 2022). Similarly, phenolic and lipid fractions modulate T-cell signalling by attenuating Ca^{2+} flux and ERK1/2 phosphorylation (Nani *et al*., 2015). Processing interventions, including germination and fermentation, further amplify antioxidant and anti-inflammatory properties by improving phenolic bioavailability and reducing antinutritional factors (Gabriele *et al*., 2024; Bheemaiah *et al*., 2024).

Despite the growing body of evidence supporting the nutritional and functional potential of pearl millet, critical research gaps persist. The dynamic behaviour of free and bound phenolics during digestion, the impact of antinutrients such as phytic acid on mineral bioaccessibility, and the absence of standardized extraction and analytical protocols hinder cross-study comparability (Singh *et al*., 2025; Kaila and Muthukumar, 2025). Moreover, while *in vitro* and cell-based assays consistently demonstrate strong antioxidant and bioactive responses, human pharmacokinetic and clinical efficacy data for defined pearl millet phenolic fractions are lacking (Bheemaiah *et al*., 2024). Future research integrating genomic, biochemical, and nutritional frameworks is essential to elucidate structure-function relationships, refine processing strategies, and translate antioxidant mechanisms into tangible dietary and therapeutic outcomes.

This review synthesizes current knowledge on the polyphenolic diversity and antioxidant mechanisms of pearl millet. It integrates compositional, mechanistic, and processing perspectives, while identifying research priorities and technological opportunities to advance the utilization of pearl millet as a functional grain for nutrition security and health-oriented cereal innovation.

2. Polyphenolic composition and determinants of diversity in pearl millet

2.1 Classification of polyphenols

Polyphenols in pearl millet comprise three major groups: phenolic acids, flavonoids, and polymeric tannins, also known as proanthocyanidins. Together, these compounds define the cereal's antioxidant and bioactive potential (Nambiar *et al*., 2012; Shahidi and Chandrasekara, 2013; Singh *et al*., 2025). Phenolic acids include both hydroxybenzoic and hydroxycinnamic derivatives, encompassing compounds such as *p*-hydroxybenzoic, vanillic, syringic, protocatechuic, *p*-coumaric, caffeic, ferulic, sinapic, and chlorogenic acids. A considerable proportion of these acids occurs in a bound form, linked to the cell-wall matrix of the grain, which influences their extractability and physiological availability (Shahidi and Chandrasekara, 2013; Nambiar *et al*., 2012). Among the flavonoids, the predominant subclasses are flavones and flavonols, represented by compounds such as tricin, acacetin, luteolin derivatives, vitexin, isovitexin, quercetin, kaempferol, and myricetin, most of which are present as glycosides or methylated forms (Nambiar *et al*., 2012; Shahidi and Chandrasekara, 2013). Condensed tannins or proanthocyanidins form another significant group of polyphenols, synthesized through the polymerization of flavan-3-ols and typically assessed using the vanillin-HCl spectrophotometric method (Salar and Purewal, 2017). Advanced UPLC-QTOF analyses of traditional pearl millet foods, including *chapatis* prepared from multiple genotypes, have revealed large reserves of alkali-liberated bound phenolics that contribute substantially to the overall antioxidant capacity of the grain (Singh *et al*., 2025).

2.2 Major polyphenolic constituents

Compositional analyses across diverse germplasm and processed products reveal a wide array of individual phenolic acids and flavonoids. Among the polyphenolic groups, bound hydroxycinnamic acids, particularly ferulic and caffeic acid derivatives, and flavones such as tricin and luteolin constitute the principal contributors to antioxidant capacity in pearl millet, accounting for much of the radical scavenging and reducing activity observed in whole grain extracts (Nambiar *et al*., 2012; Singh *et al*., 2025). Typical phenolic acids include vanillic, syringic, salicylic, p-hydroxybenzoic, o/p-coumaric, cis/trans-ferulic, caffeic, chlorogenic, quinic, and salvianolic acids, detected in both raw grains and processed foods (Nambiar *et al*., 2012; Triki *et al*., 2022).

In Tunisian germplasm, LC-MS profiling identified eight phenolic acids, most abundantly quinic, p-coumaric,caffeic acids and six flavonoids including quercetin, kaempferol, silymarin, apigenin, cirsiliol, and hyperoside, with free phenolic concentrations ranging from 506 to 1288 µg/g ferulic acid equivalents (Triki *et al*., 2022). Similarly, Moroccan seed extracts rich in vanillic and caffeic acids displayed strong DPPH, FRAP, and TEAC activities, illustrating solvent polarity as a critical determinant of phenolic yield and antioxidant potency (Marmouzi *et al*., 2018). In Indian chapatis, Singh *et al*. (2025) reported total phenolic contents between 286 and 338 mg GAE/100 g, identifying forty-three phenolic compounds. Phenolic bioaccessibility progressively increased from the oral to intestinal phases of simulated digestion, confirming the release of bound forms during digestion.

Flavonoid profiles mirror this diversity: tricin, acacetin, 3,4-dimethoxy-luteolin, 4-O-methyl-tricin, quercetin, and kaempferol are consistently reported across raw grains and processed foods (Nambiar *et al*., 2012; Triki *et al*., 2022). Among tannin-type phenolics, condensed tannins can reach up to ~173 mg/100 g in the Pakistani cultivar S. Bajra-2011, although anthocyanins are typically absent from tested varieties (Ahmad *et al*., 2018). Enzyme pretreatment of bran has been shown to enhance tannin and phenolic recovery by disrupting cell-wall linkages. Regional and varietal variations are also pronounced. North African accessions from inland Medenine contain higher flavonoid levels than coastal Djerba lines, while Indian genotypes differ in their acacetin and 3,4-dimethoxy-luteolin derivatives, that correspond with variation in enzyme-inhibition activity (Triki *et al*., 2022). The breadth of phenolic acids, flavonoids, tannins, and processing-derived phenolics reported across pearl millet germplasm and food products is systematically consolidated in Table 1, highlighting both compound diversity and analytical resolution.

Table 1: Representative polyphenolic compounds identified in pearl millet

Class	Compound examples	Analytical detection (method)	Reported range/notes	References
Hydroxybenzoic acids	*p*-Hydroxybenzoic, vanillic, syringic, protocatechuic	LC-MS,UPLC-QTOF	Detected in free and bound forms	Nambiar *et al*., 2012; Triki *et al*., 2022
Hydroxycinnamic acids	*p*-Coumaric, caffeic, ferulic, sinapic, chlorogenic, quinic	LC-MS, HPLC	286-338 mg GAE/100 g (total phenolics)	Marmouzi *et al*., 2018; Singh *et al*., 2025
Flavones/ flavonols	Tricin, acacetin, luteolin, quercetin, kaempferol, myricetin	LC-MS, UV-Vis	Major contributors to antioxidant activity	Krishnan *et al*., 2022; Nambiar *et al*., 2012
Tannins (proanthocyanidins)	Catechin polymers, condensed tannins	Vanillin-HCl assay	Up to ~173 mg/100 g in *S. Bajra*-2011	Ahmad *et al*., 2018; Salar and Purewal, 2017
Processing-derived phenolics	Gallic acid, rutin (post-fermentation)	UPLC-QTOF	Appear after fermentation due to microbial hydrolysis	Gabriele *et al*., 2024

2.3 Factors influencing polyphenolic diversity

The diversity and abundance of polyphenols in pearl millet are governed by genetic, environmental, analytical, and processing factors. At the genetic level, metabolite-GWAS of 197 inbred lines linked 897 SNPs with 396 metabolite features, identifying 738 candidate genes related to phenylpropanoid and antioxidant pathways. These include UGTs, L-ascorbate oxidase, and isoflavone 22 -monooxygenase, suggesting a strong heritable basis for phenolic composition (Yadav *et al*., 2021). Multi-location trials across West Africa further demonstrated significant genotype × environment interactions for 54 metabolites, confirming that environmental modulation influences metabolite accumulation and stability (Yadav *et al*., 2022).

Extraction methodology also exerts a marked influence on apparent phenolic composition and bioactivity. Ethanolic solvents typically yield higher recoveries of catechin, rutin, kaempferol, vanillic, and caffeic acids and produce stronger antioxidant and anti-inflammatory effects than methanol or acetonitrile (Kaila and Muthukumar, 2025). Processing techniques, such as fermentation and thermal treatment, further modulate polyphenolic profiles. Sourdough fermentation increased total polyphenols from 1.83 to 3.25 mg GAE/g and total flavonoids from 1.29 to 2.84 mg CE/g, with the release of gallic acid, quercetin, and rutin previously undetected in unfermented samples (Gabriele *et al*., 2024; Wang *et al*., 2022). Likewise, roasting, boiling, and pressure cooking enhanced DPPH scavenging and reducing power, though FRAP activity remained relatively stable, reflecting assay-specific responses (Pushparaj and Urooj, 2014). Bran-enriched fractions consistently showed higher reducing capacity, consistent with the concentration of bound phenolics.Food-matrix interactions also determine phenolic bioaccessibility. *In vitro* digestion models show that phenolic release increases from ~12-24% during the oral phase to 67-90%, with phytic acid influencing both mineral and phenolic availability (Singh *et al*., 2025).

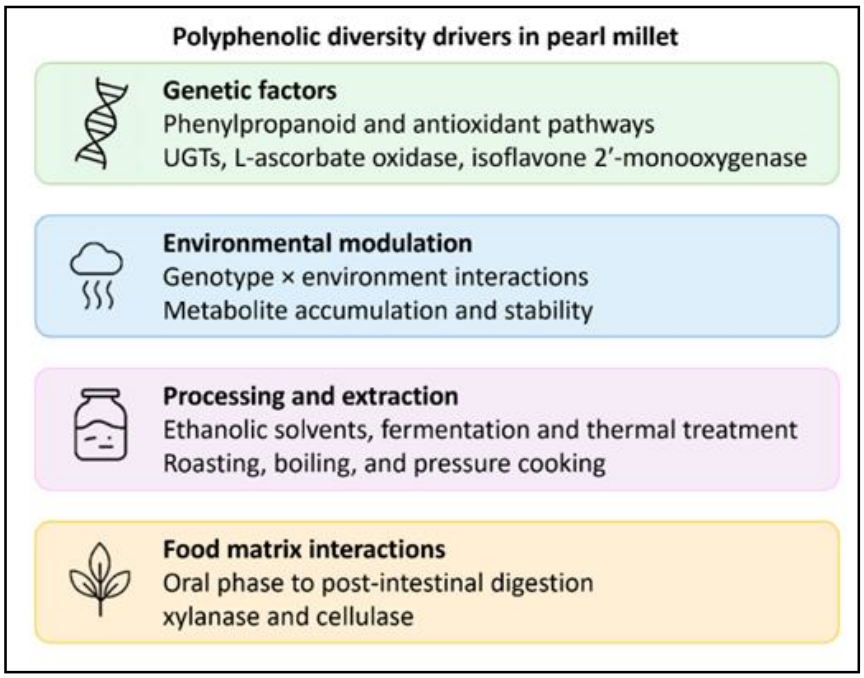

Figure 1: Conceptual overview of polyphenolic diversity drivers in pearl millet.

Polyphenolic diversity in pearl millet is shaped by multiple interacting biological and technological drivers. Genetic architecture defines the inherent biosynthetic capacity of the phenylpropanoid and antioxidant pathways, including genes encoding UDP glycosyl transferases, L ascorbate oxidase, and related monooxygenases. Environmental modulation further influences metabolite accumulation through genotype × environment interactions that affect the stability and expression of phenolic compounds. Processing and extraction strategies such as ethanol based solvents, fermentation, and thermal treatments regulate the liberation and measurable recovery of polyphenols. In addition, food matrix interactions during digestion, supported by enzymatic breakdown of cell wall components, determine phenolic bioaccessibility from oral to post intestinal phases. Together, these layered influences illustrate that antioxidant potential arises from dynamic biological and technological interactions rather than genotype alone (Figure 1).

Enzymatic pretreatments with xylanase and cellulase further enhance phenolic extraction from bran by disrupting cell-wall polymers (Ahmad *et al*., 2018). Functional studies connect compositional diversity to bioactivity magnitude. Extracts rich in acacetin and 3,4-dimethoxy-luteolin inhibited α-amylase and α-glucosidase ($IC_{50} \approx 73$ µg/ml) and increased hepatocyte glucose uptake by up to 241%, with structure activity relationships implicating Ar-OH, OCH_3, and COOH groups as key pharmacophores (Krishnan *et al*., 2022). Collectively, these findings position pearl millet as a polyphenol-rich cereal whose compositional diversity stems from genetic background, processing, and analytical context each shaping its measured antioxidant and functional potential. Empirical evidence supporting these interacting determinants, along with their documented biochemical and phenotypic outcomes, is summarized in Table 2.

2.4 Genotypic variation in phenylpropanoid composition

Pearl millet exhibits substantial genotypic variation in phenylpropanoid composition, reflecting both genetic architecture and adaptive selection. Comparative metabolomic studies across diverse germplasm panels demonstrate wide ranges in total phenolic content, flavonoid subclasses, and antioxidant capacity. Variations in acacetin, tricin, luteolin derivatives, and bound ferulic acid fractions are particularly genotype-dependent and correspond with differences in enzyme-inhibition and radical-scavenging potential. Metabolite-GWAS analyses further confirm that phenolic diversity is heritable and linked to specific SNP clusters associated with UDP-glycosyl transferase and oxidative stress response genes. These findings position pearl millet as a genetically tractable system for breeding antioxidant-enriched cultivars. The extent of genotypic variation in phenylpropanoid composition and antioxidant capacity across representative germplasm panels is summarized in Table 3.

Table 2: Key determinants of polyphenolic diversity in pearl millet

Determinant	Examples/mechanisms	Observed outcome	References
Genetic variation	SNP metabolite associations; UGT and L-ascorbate oxidase loci	Defines heritable phenolic profiles	Yadav *et al*., 2021
Environment (GEI)	Location and season effects on metabolite accumulation	Alters stability of key metabolites	Yadav *et al*., 2022
Extraction solvent	Ethanol vs. methanol/acetonitrile	Ethanol yields higher phenolic recovery and bioactivity	Kaila and Muthukumar, 2025; Marmouzi *et al*., 2018
Processing methods	Fermentation, roasting, boiling, enzymatic pretreatment	Enhances release of bound phenolics	Gabriele *et al*., 2024; Pushparaj and Urooj, 2014
Matrix interactions	Cell-wall linkages, phytic acid interference	Modulate bioaccessibility and mineral availability	Singh *et al*., 2025; Ahmad *et al*., 2018

Table 3: Representative genotypic variation in polyphenolic composition and antioxidant activity in pearl millet

Germplasm set	Genotypes evaluated	Major phenylpropanoid classes detected	Key individual compounds	Range/genotypic extremes	References
Tunisian germplasm	10 landraces (Medenine + Djerba)	Phenolic acids + flavonoids	Quinic acid, caffeic acid, p-coumaric acid, ferulic acid; quercetin, kaempferol, silymarin, cirsiliol	Total phenolics: ~519-1135 µg/g DM; highest in Med. AG3.1; lowest in Jer.AG5.2	Triki *et al*., 2022
Indian breeding + landrace panel	13 diverse genotypes	Free + bound phenylpropanoids (broad phenolic spectrum)	43 compounds identified including cinnamic acid derivatives, apigenin glycosides, quercetin derivatives, epicatechin	Total phenolics: 286-338 mg GAE/ 100 g; Dhanshakti highest bound fraction; PUSA 1201 highest total	Singh *et al*., 2025
Pakistani millet panel	2 millet varieties	Condensed tannins (proanthocyanidins); negligible anthocyanins	Catechin-derived condensed tannins	Tannins up to ~173 mg/100 g in	S. Bajra-2011 Ahmad *et al*., 2018

3. Biosynthesis and regulation of polyphenols in pearl millet

The biosynthesis of polyphenols in pearl millet is governed by the phenylpropanoid pathway, a central metabolic route linking primary metabolism to the production of phenolic acids, flavonoids, lignins, and tannins. This pathway begins with the deamination of phenylalanine by phenylalanine ammonia-lyase (PAL), yielding trans-cinnamic acid. Sequential hydroxylation and methylation reactions mediated by cinnamate-4-hydroxylase (C4H) and 4-coumarate-CoA ligase (4CL) generate key intermediates such as p-coumaroyl-CoA, which serve as precursors for downstream branches of phenolic and flavonoid synthesis (Cheynier *et al*., 2013; Vogt, 2010).

3.1 Core pathway and key enzymes

Within this framework, chalcone synthase (CHS) catalyses the condensation of p-coumaroyl-CoA with malonyl-CoA to form chalcones, which act as the structural backbone for flavonoid biosynthesis. These chalcones are subsequently isomerized by chalcone isomerase (CHI) into naringenin, a central intermediate diverted toward multiple flavonoid subclasses through the action of flavanone 3-hydroxylase (F3H), flavonol synthase (FLS), flavone synthase (FNS), and anthocyanidin synthase (ANS).The phenylpropanoid pathway and its major enzymatic branch points governing phenolic acid and flavonoid biosynthesis in pearl millet are schematically illustrated in Figure 2.

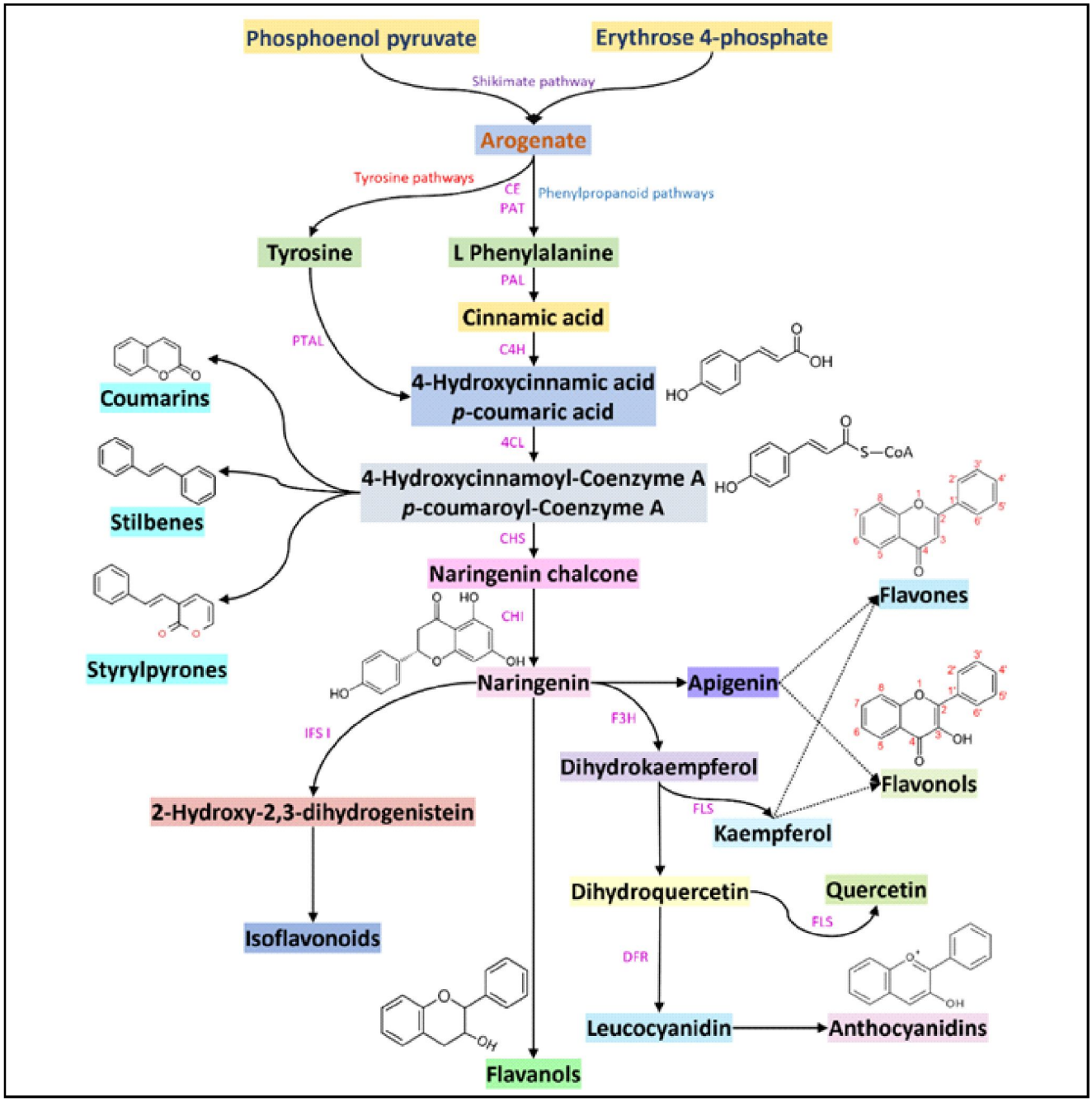

Figure 2: Phenylpropanoid pathway leading to polyphenolic compound biosynthesis in pearl millet (Yadav *et al*., 2020).

In pearl millet, these enzymatic steps culminate predominantly in the accumulation of flavones and flavonols such as tricin, acacetin, luteolin, and quercetin rather than anthocyanins, which are largely absent (Nambiar *et al*., 2012; Ahmad *et al*., 2018). The relative abundance of each subclass reflects the transcriptional balance between key enzymes such as FNS and F32 H, which modulate the metabolic flux through distinct branch points of the phenylpropanoid network (Vogt, 2010; Li *et al*., 2020).

3.2 Genetic regulation and metabolic control

Metabolomic and genomic studies in pearl millet reveal a strong genetic basis for polyphenolic diversity. A metabolitegenome-wide association study (mGWAS) involving 197 inbred lines identified 897 significant SNPs linked to 396 bioactive metabolites, pinpointing 738 candidate genes related to antioxidant and flavonoid pathways (Yadav *et al*., 2021). Notably, these include UDP-glycosyltransferases (UGTs) that stabilize flavonoid aglycones through glycosylation, L-ascorbate oxidase involved in redox regulation, and isoflavone 22 - monooxygenase, which catalyses hydroxylation steps linked to bioactivity modulation.

The expression of structural genes such as PAL, C4H, CHS, and FLS is transcriptionally regulated by MYB, bHLH, and WD40 transcription factors, a conserved complex also known as the MBW regulatory module (Xu *et al*., 2015). Variations in promoter activity or gene copy number within this regulatory framework contribute to genotype-specific differences in phenolic accumulation and antioxidant capacity.

Epigenetic and post-transcriptional factors also appear to influence metabolic plasticity. Differential expression of small RNAs and methylation changes near phenylpropanoid gene clusters have been implicated in stress-responsive activation of the pathway, though detailed molecular evidence in pearl millet remains limited (Kaila and Muthukumar, 2025).

3.3 Transcriptomic and proteomic perspectives

Emerging transcriptomic and proteomic approaches offer valuable opportunities to refine understanding of polyphenol biosynthesis in pearl millet beyond pathway-level descriptions. RNA sequencing enables genome-wide profiling of phenylpropanoid gene expression and can link variation in PAL, C4H, CHS, and downstream enzymes with genotype-specific phenolic accumulation (Cheynier *et al*., 2013; Vogt, 2010). Integration of transcriptomic data with metabolite association studies in pearl millet has already highlighted candidate UDP-glycosyltransferases and redox-related genes associated with antioxidant metabolites (Yadav *et al*., 2021). Proteomic analyses complement these findings by revealing enzyme abundance, post-translational regulation, and metabolic channeling that are not always predicted from mRNA levels. Together, multi-omics frameworks provide a systems-level strategy to connect gene regulation with phenolic diversity and support breeding of antioxidant-enriched millet cultivars (Xu *et al*., 2015; Kaila and Muthukumar, 2025).

4. Analytical and extraction approaches in polyphenol characterization of pearl millet

Accurate characterization of polyphenolic compounds in pearl millet depends on the effectiveness of extraction techniques and the precision of analytical platforms used to detect and quantify them. Because phenolics occur in free, soluble-conjugated, and insoluble-bound forms, comprehensive profiling requires sequential extraction and advanced spectroscopic or chromatographic validation. Over the past decade, methodological convergence across studies has led to more reproducible phenolic recovery and quantification protocols, integrating solvent-assisted extraction, enzymatic pretreatment, and high-resolution mass spectrometry (Singh *et al*., 2025; Triki *et al*., 2022; Gabriele *et al*., 2024).

4.1 Extraction strategies and solvent optimization

Extraction efficiency depends largely on solvent polarity, matrix disruption, and pretreatment methods. Among commonly employed solvents, methanol, ethanol, acetone, and acetonitrile, ethanol consistently offers the best balance between polarity, recovery efficiency, and food-grade compatibility. Comparative studies demonstrate that 70-80% ethanol maximizes recovery of phenolic acids (vanillic, caffeic, ferulic) and flavonoids (catechin, rutin, kaempferol), while also yielding superior DPPH and FRAP antioxidant activity relative to other solvents (Kaila and Muthukumar, 2025; Marmouzi *et al*., 2018).

To access bound phenolics, alkaline or acid hydrolysis is typically performed following free-phenolic extraction. Singh *et al*. (2025) used a sequential extraction solvent-based recovery followed by alkaline hydrolysis to isolate both free and bound pools prior to UPLC-QTOF analysis, revealing that bound phenolics represent a dominant antioxidant fraction in whole grains.

Processing-enhanced extractions, such as enzyme-assisted techniques, improve phenolic yield by dismantling cell-wall linkages. Enzymes like xylanase and cellulase increase the recovery of ferulate-derived phenolics and condensed tannins by degrading hemicellulose and lignin barriers (Ahmad *et al*., 2018). Similarly, fermentation and mild heat treatment act as biological or thermal catalysts, liberating conjugated phenolics that are otherwise inaccessible in untreated grains (Gabriele *et al*., 2024; Pushparaj and Urooj, 2014).

4.2 Chromatographic and spectrometric identification

Advances in chromatography-mass spectrometry have revolutionized the profiling of polyphenols in pearl millet, shifting the focus from bulk quantification to compound-level resolution. Ultra-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOF-MS) enables highly sensitive detection and annotation of more than forty distinct phenolic compounds, including tricin, acacetin, luteolin, and caffeic acid derivatives (Singh *et al*., 2025). Complementary analyses using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in Tunisian germplasm have identified both free and conjugated phenolic acids such as quinic, caffeic, *p*-coumaric, and ferulic acids, along with flavonoids including quercetin, kaempferol, apigenin, and cirsiliol, providing molecular fingerprints useful for varietal differentiation (Triki *et al*., 2022). Post-fermentation metabolomic profiling further revealed the emergence of novel phenolic compounds, including gallic acid, rutin, and quercetin, which were absent in unfermented controls, illustrating the dynamic biotransformation of phenolic matrices during microbial processing (Gabriele *et al*., 2024). These advanced chromatographic platforms are routinely complemented by HPLC-UV-Vis for quantitative analysis, while gas chromatography-mass spectrometry (GC-MS) is occasionally applied to characterize volatile phenolic derivatives generated during thermal processing.

4.3 Spectrophotometric quantification and functional assays

While chromatographic methods provide high specificity and structural resolution, spectrophotometric assays remain indispensable for the comparative evaluation of total phenolic, flavonoid, and tannin contents, as well as their associated antioxidant capacities. The total phenolic content (TPC) is typically determined using the Folin-Ciocalteu assay and expressed as milligrams of gallic acid equivalents (GAE), whereas the total flavonoid content (TFC) is quantified through aluminum chloride colorimetry and reported as catechin equivalents (CE). Condensed tannins are commonly measured using the vanillin-HCl or butanol-HCl assays, with results also expressed as catechin equivalents (Ahmad *et al*., 2018; Salar and Purewal, 2017). To assess antioxidant potential, a suite of complementary assays including DPPH, ABTS, FRAP, ORAC, and TAC is employed to capture distinct aspects of redox behaviour. DPPH and ABTS assays evaluate radical scavenging through hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms, FRAP determines ferric-reducing power, ORAC measures peroxyl-radical scavenging capacity, and hydroxyl radical scavenging activity (HFRSA) quantifies protection against hydroxyl-induced oxidative damage. Because these assays differ in reaction kinetics and mechanistic bases, their parallel application is recommended to ensure accurate interstudy comparison and comprehensive characterization of antioxidant capacity (Pushparaj and Urooj, 2014; Gabriele *et al*., 2024). The principles, analytical outputs, and antioxidant dimensions captured by commonly employed spectrophotometric and chromatographic assays in pearl millet research are critically summarized in Table 4.

Table 4: Analytical and spectrophotometric assays used for polyphenol characterization and antioxidant evaluation in pearl millet

Assay/method	Principle of measurement	Expression of results (units)	Assessed property	Advantages/ remarks	References
Folin-Ciocalteu (TPC)	Reduction of the Folin-Ciocalteu reagent by phenolic hydroxyl groups forming a blue complex measured at 765 nm	mg gallic acid equivalents (GAE) per 100 g	Total phenolic content	Simple, reproducible; measures total reducing capacity but not specific to phenolics	Ahmad *et al*., 2018; Salar and Purewal, 2017
$AlCl_3$ colorimetry (TFC)	Complex formation between flavonoids and AP^+ ions producing a yellow chromophore detected at 430-510 nm	mg catechin equivalents (CE) per 100 g	Total flavonoid content	Sensitive to flavones and flavonols; may underestimate other flavonoid subclasses	Ahmad *et al*., 2018
Vanillin-HCl/ Butanol-HCl	Condensation of vanillin or butanol with flavan-3-ols forming red complexes measured at 500-550 nm	mg catechin equivalents (CE) per 100 g	Condensed tannins (proanthocyanidins)	Distinguishes polymeric phenolics; vanillin assay less accurate for high-molecular-weight tannins	Shahidi and Chandrasekara, 2013
DPPH radical scavenging	Reduction of the DPPH radical (λ = 517 nm) by antioxidants via HAT/SET reactions	% inhibition or IC_{50} (µg/ml)	Free radical scavenging capacity	Rapid and widely used; limited to lipophilic radicals	Pushparaj and Urooj, 2014
ABTS radical cation	Decolorization of $ABTS^{+}$• by antioxidants via electron or hydrogen donation	µmol Trolox equivalents (TE) per g or % inhibition	Radical scavenging activity	Suitable for both hydrophilic and lipophilic systems	Gabriele *et al*., 2024
FRAP (Ferric Reducing Antioxidant Power)	Reduction of Fe^{3+}-TPTZ complex to Fe^{3+}-TPTZ (λ = 593 nm)	µmol Fe^{3+} equivalents per g	Reducing potential	Reflects electron-donating capacity; not sensitive to thiols	Pushparaj and Urooj, 2014
ORAC (Oxygen Radical Absorbance Capacity)	Inhibition of fluorescein oxidation by peroxyl radicals generated from AAPH	µmol Trolox equivalents (TE) per g	Peroxyl-radical scavenging	Reflects chain-breaking antioxidant action; kinetically based	Gabriele *et al*., 2024
TAC (Total Antioxidant Capacity)	Cumulative electron transfer to molybdate or phosphomolybdenum reagents	µmol Trolox equivalents (TE) per g	Overall redox potential	Provides integrative antioxidant index	Salar and Purewal, 2017
HFRSA (Hydroxyl Radical Scavenging Activity)	Measurement of antioxidant-mediated inhibition of hydroxyl radical-induced damage	% inhibition or IC_{50} (µg/ml)	Hydroxyl radical quenching	Sensitive to hydroxyl-based oxidative stress	Pushparaj and Urooj, 2014

4.4 Structural and microstructural analyses

Structural characterization techniques provide valuable insight into the interactions between phenolic compounds and the grain matrix that govern extractability and recovery efficiency. Fourier transform infrared spectroscopy (FTIR) identifies characteristic absorption peaks associated with phenolic hydroxyl and carbonyl functional groups, thereby revealing covalent linkages between phenolics and cell-wall polysaccharides (Khalil *et al*., 2025). Scanning electron microscopy (SEM) complements this by visualizing the grain and flour microstructure, demonstrating that matrix porosity, granule integrity, and surface morphology strongly influence solvent penetration and phenolic release. The integration of FTIR and SEM analyses with extraction data has clarified the mechanisms underlying enhanced phenolic recovery in enzyme-assisted and fermented samples. These treatments promote enzymatic hydrolysis, which modifies the surface architecture and disrupts fibre-phenolic entrapment, resulting in greater liberation of bound phenolic compounds (Khalil *et al*., 2025; Ahmad *et al*., 2018).

4.5 Bioaccessibility and digestion models

To link analytical data with nutritional relevance, *in vitro* digestion models (INFOGEST protocol) simulate gastrointestinal release of phenolics. Singh *et al*. (2025) reported phenolic bioaccessibility

increasing from 12-24% in the oral phase to 67-90% after intestinal digestion, correlating with matrix disintegration and enzyme mediated hydrolysis. Such approaches bridge analytical chemistry with nutritional functionality, offering physiologically meaningful indicators of antioxidant availability.

Despite substantial methodological progress, several critical gaps continue to hinder the comparability and reproducibility of polyphenol analyses in pearl millet. Current studies often lack inter-laboratory validation of extraction reproducibility and compound quantification, leading to variable recovery rates across protocols. Additionally, inconsistent reporting of key analytical parameters such as the limit of detection (LOD), limit of quantification (LOQ), and recovery efficiency undermines cross-study reliability. Significant variation in solvent composition, extraction duration, temperature, and hydrolysis conditions further contributes to discrepancies in total phenolic yield and compound profiles.

To improve analytical coherence, standardized methodologies are needed that integrate ethanol-based sequential extraction for free and bound phenolics, harmonized reporting units (*e.g.*, mg gallic acid or catechin equivalents per 100 g), and a unified set of benchmark antioxidant assays (DPPH, FRAP, ABTS, ORAC). Establishing such consensus protocols will enhance inter-study comparability, support meta-analyses, and strengthen the reproducibility of polyphenol research in pearl millet and other cereals (Kaila and Muthukumar, 2025; Singh *et al*., 2025).

5. Antioxidant mechanisms of pearl millet polyphenols

Polyphenolic compounds in pearl millet play multifaceted antioxidant and protective roles, functioning through both direct chemical mechanisms and indirect biological modulation of oxidative processes. Their activity stems from diverse phenolic acids and flavonoids bearing hydroxyl and methoxy groups that enable radical scavenging, metal chelation, and redox regulation, ultimately contributing to metabolic health and food stability (Shahidi and Ambigaipalan, 2015).

5.1 Mechanistic basis of antioxidant action

The antioxidant behaviour of millet polyphenols primarily involves hydrogen atom transfer (HAT) and single electron transfer (SET) reactions. In hydrogen atom transfer (HAT), phenolic hydroxyl groups quench reactive radicals such as DPPH• and ROO• by donating hydrogen atoms, whereas single electron transfer (SET) involves electron donation to oxidants including ABTS•z and Fe^{3+}, resulting in radical neutralization and restoration of redox balance (Apak *et al*., 2016). The efficiency of these reactions depends on the molecular structure of individual compounds. Flavonoids such as quercetin, luteolin, and tricin exhibit strong dual-mode activity due to ortho-dihydroxyl groups, C2=C3 double bond conjugation, and 4-oxo groups that stabilize phenoxyl radicals. Methoxy substitutions enhance lipophilicity and radical interception in lipid environments, whereas glycosylation improves solubility and transport despite slightly reducing direct radical-quenching capacity (Krishnan *et al*., 2022).

Beyond scavenging, millet phenolics chelate transition metals such as Fe^{2+} and Cu^{2+}, suppressing Fenton-type reactions that produce hydroxyl radicals. Phenolic acids including caffeic, ferulic, and chlorogenic acids are particularly effective due to their catechol and methoxy-hydroxy configurations (Marmouzi *et al*., 2018). These combined effects prevent oxidative degradation of biomolecules and reinforce the grain's natural defence systems.

5.2 Biological and functional implications

In biological systems, pearl millet phenolics also function as secondary antioxidants by regulating intracellular redox signalling and enzyme activity. They activate the Nrf2-ARE pathway, leading to upregulation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Alzahrani *et al*., 2022). Simultaneously, they inhibit pro-oxidant enzymes like xanthine oxidase and NADPH oxidase, reducing ROS generation at the source. These molecular responses translate into tangible health benefits including protection against oxidative stressrelated inflammation, lipid peroxidation, and metabolic disorders. Extracts enriched in flavones and phenolic acids have been shown to enhance glucose uptake, attenuate oxidative stress in hepatocytes, and exhibit anti-inflammatory and cardioprotective potential (Krishnan *et al*., 2022; Shahidi and Chandrasekara, 2013).

In food systems, polyphenols extend their antioxidant roles by stabilizing lipids, delaying rancidity, and enhancing storage quality. During processing and digestion, bound phenolics are released through fermentation or enzymatic hydrolysis, generating bioaccessible antioxidant metabolites that continue to act in the gastrointestinal tract (Singh *et al*., 2025). Together, these chemical and biological mechanisms underpin the functional versatility of pearl millet polyphenols, positioning them as key contributors to both nutritional quality and chronic disease mitigation.

6. *In vitro* and *ex vivo* antioxidant performance

Pearl millet polyphenols demonstrate potent *in vitro* antioxidant activity, as evidenced by strong radical-scavenging, reducing, and DNA protective effects across multiple assays. In twelve north indian cultivars, total phenolic content correlated positively with DPPH, ABTS, FRAP, TAC, and hydroxyl radical scavenging capacity, confirming the consistency of redox activity across assay types (Salar and Purewal, 2017). Ethanolic extracts effectively protected pBR322 plasmid DNA from oxidative cleavage, providing direct evidence that solution-phase radical quenching translates into biological protection. Similarly, Singh *et al*. (2025) reported DPPH inhibition ranging from 36-48% in chapatis prepared from thirteen genotypes, where total phenolics reached 286-338 mg GAE/100 g. Simulated digestion studies revealed a progressive increase in phenolic bioaccessibility from 12-24% during oral and gastric phases to 67-90% post-intestinal digestion demonstrating that matrix disintegration enhances physiological relevance of millet phenolics.

Processing methods further amplify antioxidant performance. In Burkina Faso varieties, sourdough fermentation nearly doubled total phenolics (1.83 → 3.25 mg GAE/g) and flavonoids (1.29 → 2.84 mg CE/g), while lowering DPPH EC_{50} and elevating FRAP and ORAC values (Gabriele *et al*., 2024). These concurrent gains suggest that microbial hydrolysis releases bound phenolics and converts precursors into more redox-active forms. Comparable improvements in antioxidant activity were achieved with ethanolic extraction, which yielded higher concentrations of catechin, rutin, kaempferol, vanillic, and caffeic acids than methanol or acetonitrile systems (Kaila and Muthukumar, 2025). Thermal processing, roasting, boiling, and pressure cooking similarly enhanced DPPH and reducing power but

produced variable FRAP responses, indicating assay-specific sensitivities and the possible influence of Maillard derived reductants (Pushparaj and Urooj, 2014).

The biological translation of these *in vitro* effects is supported by *ex vivo* and *in vivo* studies. Fermented pearl millet extracts improved erythrocyte stability under oxidative stress, suggesting membrane-level protection linked to elevated ORAC and FRAP values (Gabriele *et al*., 2024). In a highfat diet rat model, whole grain and ethanolic millet extracts lowered fasting glucose, improved insulin sensitivity, reduced hepatic steatosis, and mitigated inflammation (Alzahrani *et al*., 2022). Although, direct biomarkers of systemic antioxidant status were not quantified, these physiological benefits are consistent with phenolic-mediated modulation of redoxsensitive metabolic pathways. Additionally, the DNA-protective effects observed by Salar and Purewal (2017) reinforce the mechanistic continuum between chemical antioxidant activity and genoprotective potential. A mechanistic integration of dominant pearl millet polyphenols, their primary antioxidant modes of action, and associated functional outcomes is provided in Table 5.

Overall, the findings support the view that HAT and SET based redox mechanisms underlie the *in vitro* and *ex vivo* antioxidant performance of pearl millet polyphenols. These effects are magnified by processing and solvent optimization, yet constrained by limited data on metal chelation, kinetic stability, and physiological biomarkers. Future research integrating electrochemical assays, redox potential mapping, and controlled human studies will be crucial to confirm whether these chemical and cellular antioxidant activities yield clinically relevant oxidative-stress mitigation.

Table 5: Major polyphenolic compounds in pearl millet and their associated antioxidant mechanisms and functional roles

Compound /class	Representative structures/examples	Primary antioxidant mechanisms	Documented or proposed biological functions	References
Phenolic acids	Ferulic, *p*-coumaric, caffeic, sinapic, chlorogenic acids	Hydrogen atom transfer (HAT); single electron transfer (SET); metal chelation	Radical scavenging; inhibition of lipid peroxidation; protection of DNA and membrane lipids	Nambiar *et al*., 2012; Shahidi and Chandrasekara, 2013
Hydroxybenzoic acids	Vanillic, syringic, *p*-hydroxybenzoic, protocatechuic acids	Electron donation and Fe^{3+} /Cu^{2+} chelation	Anti-inflammatory activity; maintenance of redox balance; antimicrobial defence	Shahidi and Chandrasekara, 2013
Flavones	Tricin, apigenin, luteolin, acacetin	HAT/SET; modulation of Nrf2-ARE signalling; inhibition of xanthine oxidase	Anti-inflammatory and antidiabetic effects; vascular protection; modulation of oxidative enzyme activity	Singh *et al*., 2025; Pushparaj and Urooj, 2014
Flavonols	Quercetin, kaempferol, myricetin, isorhamnetin	ROS scavenging; metal ion chelation; enzyme inhibition (LOX, COX, NADPH oxidase)	Antioxidant, cardioprotective, and antiageing effects; maintenance of endothelial function	Nambiar *et al*., 2012; Shahidi and Chandrasekara, 2013
C-glycosyl flavones	Vitexin, isovitexin, orientin	Chain-breaking antioxidant action; stabilization of cellular redox systems	Cytoprotective and anti-inflammatory roles; enhanced stability under digestion	Ahmad *et al*., 2018
Polymeric tannins (proanthocyanidins)	Catechin and epicatechin polymers	Radical scavenging; metal chelation; protein complexation	Antioxidant and antimicrobial effects; modulation of gut microbiota and digestive enzyme activity	Shahidi and Chandrasekara, 2013
Phenolic mixtures (fermented/ enzymatically treated)	Ferulic acid, gallic acid, rutin, quercetin (formed post-fermentation)	Enhanced redox potential; release of bound phenolics; microbial biotransformation	Improved bioaccessibility and bioavailability; protection against oxidative stress in biological systems	Gabriele *et al*., 2024; Singh *et al*., 2025

7. Physiological and nutraceutical relevance of pearl millet polyphenols

The antioxidant mechanisms and bioactivities of pearl millet polyphenols translate into measurable physiological and nutraceutical benefits, underpinning their value as functional food constituents. By modulating redox balance, enzyme activity, and inflammatory signalling, these bioactives contribute to the prevention and management of oxidative stress-related metabolic disorders.

7.1 Metabolic and cardioprotective benefits

Polyphenolrich millet consumption has been associated with improved glycaemic control, lipid regulation, and vascular protection. The synergistic action of phenolic acids (ferulic, caffeic) and flavonoids (quercetin, tricin, luteolin) supports the inhibition of α-amylase and α-glucosidase, thereby delaying carbohydrate digestion and glucose absorption (Krishnan *et al*., 2022). Concurrently, antioxidant modulation of the Nrf2-ARE and NF-κB pathways improves insulin signalling and reduces systemic inflammation (Matzinger *et al*., 2018). In animal models, pearl millet supplementation lowered plasma glucose and triglycerides, enhanced HDL levels, and attenuated hepatic lipid accumulation (Alzahrani *et al*., 2022). These effects align with reduced oxidative stress markers and improved antioxidant enzyme activities, highlighting a clear redox-metabolic link. The chelation of Fe^{2+} and Cu^{2+} ions by phenolic acids further contributes to the prevention of LDL oxidation and

endothelial dysfunction two major risk factors for cardiovascular disease (Shahidi and Chandrasekara, 2013).

7.2 Gut health and microbiome interaction

Pearl millet phenolics also exhibit prebiotic potential by influencing gut microbial composition and metabolism. Bound phenolics that resist digestion in the upper gastrointestinal tract reach the colon, where they undergo microbial biotransformation into smaller, more bioavailable metabolites such as phenylpropionic and benzoic acids (Singh *et al*., 2025). These metabolites enhance short-chain fatty acid (SCFA) production, modulate intestinal redox state, and suppress gut inflammation. Fermented millet foods have shown increased abundance of *Lactobacillus* and *Bifidobacterium* species, suggesting that phenolic-microbiota crosstalk may contribute to both digestive and systemic antioxidant effects (Gabriele *et al*., 2024). This interplay reinforces the role of pearl millet as a symbiotic substrate, integrating fibre-bound phenolics and fermentable carbohydrates that support microbial health and host immunity.

7.3 Functional food and nutraceutical potential

Given their biochemical stability, extractability, and bioactivity, pearl millet phenolics present a valuable opportunity for functional food formulation and nutraceutical development. The high concentration of flavones and phenolic acids in whole-grain and fermented millet products offers a natural source of antioxidants comparable to those from more established functional cereals like oats and barley. Incorporating millet polyphenols into bakery, beverage, and snack matrices could enhance shelf-life through lipid stabilization while providing intrinsic antioxidant and metabolic benefits. Furthermore, encapsulation and nanoformulation approaches can be employed to improve phenolic bioavailability and stability during digestion, enhancing their nutraceutical efficacy. To realize these applications, consistent standardization of extraction, quantification, and bioefficacy testing is essential to translate *in vitro* potency into reliable functional claims. Such integration of millet phenolics into mainstream food systems supports global efforts toward sustainable nutrition, oxidative stress management, and non-communicable disease prevention.

7.4 Translational perspective

Despite promising results, translating the antioxidant potential of millet polyphenols into clinical relevance remains a major research frontier. Current data primarily derive from *in vitro* and animal studies, with limited human intervention trials. Future work should focus on establishing bioavailability, metabolic fate, and doseresponse relationships using controlled feeding or supplementation studies. Elucidating the kinetics of phenolic absorption, plasma transport, and tissue distribution will clarify their physiological thresholds for antioxidant and anti-inflammatory actions. Integrating omics-based profiling (metabolomics, transcriptomics) with redox biomarkers could further validate their nutraceutical potential and guide targeted food design. Ultimately, understanding the systemic and molecular interactions of pearl millet polyphenols will enable their effective utilization in personalized nutrition and therapeutic strategies.

7.5 Pharmaceutical and therapeutic applications

Beyond their dietary role, pearl millet polyphenols show emerging relevance in phytomedicine due to their capacity to modulate oxidative stress, inflammatory pathways, and carbohydrate metabolism. Flavones and phenolic acids isolated from millet exhibit enzyme inhibitory, anti-inflammatory, and cytoprotective properties that position them as potential adjuncts in the management of metabolic syndrome, diabetes, and cardiovascular disorders. Their ability to activate Nrf2-mediated antioxidant defences and suppress NF-êB signalling suggests therapeutic utility in oxidative stress driven diseases (Matzinger *et al*., 2018). From a pharmaceutical perspective, standardized millet-derived polyphenol extracts could be developed as nutraceutical formulations or complementary therapies. Encapsulation technologies, nano-delivery systems, and controlled-release matrices offer opportunities to enhance bioavailability and stability of these compounds. However, clinical validation, toxicological safety assessment, and pharmacokinetic profiling remain essential steps before therapeutic translation. Establishing evidence-based dosage frameworks will be critical for integrating pearl millet bioactives into modern phytomedicine.

8. Conclusion and future perspectives

Pearl millet emerges from this review as a climate-resilient cereal with exceptional biochemical value, driven largely by its phenyl propanoid-derived polyphenols. These compounds act through complementary antioxidant mechanisms, including hydrogen atom transfer, electron donation, metal chelation, and redox-sensitive signalling, translating into antidiabetic, cardioprotective, anti-inflammatory, and gut-modulatory benefits. Advances in metabolomic profiling and high-resolution analytical platforms have improved our understanding of phenolic diversity, while fermentation and enzyme-assisted processing have demonstrated practical routes to enhance phenolic bioaccessibility. However, inconsistencies in extraction protocols and antioxidant evaluation methods continue to limit cross-study comparability. Establishing standardized analytical frameworks and reference methodologies is essential to strengthen reproducibility and to validate functional claims surrounding millet polyphenols.

Future research must move beyond *in vitro* antioxidant characterization toward human-relevant validation. Controlled clinical and pharmacokinetic studies are needed to clarify absorption, metabolic fate, doseresponse relationships, and long-term safety of millet-derived phenolics. Integrating metabolomics, transcriptomics, and microbiome profiling will provide systems-level insight into how these compounds influence host physiology and redox homeostasis. At the crop level, genomic and metabolite-associated markers linked to phenylpropanoid pathways present opportunities to breed pearl millet varieties enriched for nutraceutical traits without compromising agronomic performance. Such omics-guided breeding strategies could position pearl millet as a flagship model for combining climate resilience with functional metabolite enhancement.

From a translational perspective, pearl millet polyphenols hold significant promise for functional foods, nutraceutical formulations, and sustainable food systems. Bran-rich fractions and processing by-products represent underexploited reservoirs of natural antioxidants suitable for clean-label preservation and value-added ingredient development. Advances in encapsulation and delivery technologies may further improve phenolic stability and bioavailability. At a global scale, expanding millet utilization supports dietary diversification, non-communicable disease prevention, and climate-adaptive agriculture. Bridging plant biochemistry, food science, and clinical nutrition will be crucial to reposition pearl millet

as a strategic functional grain capable of contributing simultaneously to sustainable agriculture and preventive healthcare.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Ahmad, F.; Pasha, I.; Saeed, M. and Asgher, M. (2018). Biochemical profiling of Pakistani sorghum and millet varieties with special reference to anthocyanins and condensed tannins. International Journal of Food Properties, **21**(1):1586-1597.

Alzahrani, N. S.; Alshammari, G. M.; El-Ansary, A.; Yagoub, A. E. A.; Amina, M.; Saleh, A. and Yahya, M. A. (2022). Anti-hyperlipidemia, hypoglycemic, and hepatoprotective impacts of pearl millet (*Pennisetum glaucum* L.) grains and their ethanol extract on rats fed a high-fat diet. Nutrients, **14**(9):1791.

Apak, R.; Ozyurek, M.; Guclu, K. and Capanoglu, E. (2016). Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. Journal of Agricultural and Food Chemistry, **64**(5):997-1027.

Bheemaiah B, S.; Gowda, N. N.; Subbiah, J.; Chakraborty, S.; Prasad, P. V. and Siliveru, K. (2024). Physiochemical, bio, thermal, and non-thermal processing of major and minor millets: A comprehensive review on antinutritional and antioxidant properties. Foods, **13**(22):3684.

Chandrasekara, A. and Shahidi, F. (2010). Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. Journal of Agricultural and Food Chemistry, **58**(11):6706-6714.

Cheynier, V.; Comte, G.; Davies, K. M.; Lattanzio, V. and Martens, S. (2013). Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. Plant Physiology and Biochemistry, **72**:1-20.

Gabriele, M.; Cavallero, A.; Tomassi, E.; Arouna, N.; Arvay, J.; Longo, V. and Pucci, L. (2024). Assessment of sourdough fermentation impact on the antioxidant and anti-inflammatory potential of pearl millet from Burkina Faso. Foods, **13**(5):704.

Kaila, N. H. and Muthukumar, S. P. (2025). Antioxidant, anti-inflammatory and enzyme inhibition potential of pearl millet (*Pennisetum glaucum*). Journal of Food Measurement and Characterization, **19**(6):4034-4047.

Khalil, I.; Bashir, S.; Saeed, K.; Alsulami, T.; Rafique, H. and Mukonzo, E. K. L. (2025). Phytochemical and structural portrayal of barley and pearl millet through FTIR and SEM. Food Science and Nutrition, **13**(5):70120.

Krishnan, V.; Verma, P.; Saha, S.; Singh, B.; Vinutha, T.; Kumar, R. R.; Kulshreshta, A.; Singh, S.P.; Sathyavathi, T.; Sachdev, A. and Praveen, S. (2022). Polyphenol-enriched extract from pearl millet (*Pennisetum glaucum*) inhibits key enzymes involved in post prandial hyper glycemia (α-amylase, α-glucosidase) and regulates hepatic glucose uptake. Biocatalysis and Agricultural Biotechnology, **43**(2022):102411.

Li, Zhonghua.; Qian Su.; Mingqi Xu.; Jiaqi You.; Anam Qadir Khan.; Junyi Li.; Xianlong Zhang.; Lili Tu. and Chunyuan You. (2020). Phenylpropanoid metabolism and pigmentation show divergent patterns between brown colour and green colour cottons as revealed by metabolic and gene expression analyses. Journal of Cotton Research, **3**(1):27.

Marmouzi., Ilias.; Kaddafi Ali.; Hicham Harhar.; Said Gharby.; Karima Sayah.; Nadia El Madani.; Yahia Cherrah. and My El Abbes Faouzi. (2018). Functional composition, antibacterial and antioxidative properties of oil and phenolics from Moroccan *Pennisetum glaucum* seeds. Journal of the Saudi Society of Agricultural Sciences, **17**(3):229-234.

Matzinger, M.; Fischhuber, K. and Heiss, E. H. (2018). Activation of Nrf2 signaling by natural products-can it alleviate diabetes?. Biotechnology Advances, **36**(6):1738-1767.

Nambiar, V. S.; Sareen, N.; Daniel, M. and Gallego, E. B. (2012). Flavonoids and phenolic acids from pearl millet (*Pennisetum glaucum*) based foods and their functional implications. Functional Foods in Health and Disease, **2**(7):251-264.

Nani, Abdelhafid, Meriem Belarbi, WidedKsouri-Megdiche, Souleymane Abdoul-Azize, Chahid Benammar, François Ghiringhelli, Aziz Hichami, and Naim Akhtar Khan. (2015). Effects of polyphenols and lipids from *Pennisetum glaucum* grains on T-cell activation: Modulation of Ca^{2+} and ERK1/ERK2 signaling. BMC Complementary and Alternative Medicine, **15**(1):426.

Pushparaj, F. and Urooj, A. (2014). Antioxidant activity in two pearl millet (*Pennisetum typhoideum*) cultivars as influenced by processing. Antioxidants, **3**(1):55-66.

Salar, R. K. and Purewal, S. S. (2017). Phenolic content, antioxidant potential and DNA damage protection of pearl millet (*Pennisetum glaucum*) cultivars of North Indian region. Journal of Food Measurement and Characterization, **11**(1):126-133.

Shahidi, F. and Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects-A review. Journal of Functional Foods, **18**:820-897.

Shahidi, F. and Chandrasekara, A. (2013). Millet grain phenolics and their role in disease risk reduction and health promotion: A review. Journal of Functional Foods, **5**(2):570-581.

Singh, M. and Nara, U. (2023). Genetic insights in pearl millet breeding in the genomic era: Challenges and prospects. Plant Biotechnology Reports, **17**(1):15-37.

Singh, T.; Goswami, S.; Ali, A.; Munibyrappa, S.; Dutta, M.; Thimmegowda, V.; Kumar, R.R.; Bansal, N.; Kundu, A.; Meena, M.C. and Mishra, G.P. (2025). Characterization of phenolics and influence of phytic acid content on iron and zinc bioaccessibility in chapati prepared from diverse pearl millet genotypes. Molecular Nutrition and Food Research, **13**:e70130

Tomar, M.; Bhardwaj, R.; Kumar, M.; Singh, S.P.; Krishnan, V.; Kansal, R.; Verma, R.; Yadav, V.K.; Ahlawat, S.P.; Rana, J.C. and Bollinedi, H. (2021). Nutritional composition patterns and application of multivariate analysis to evaluate indigenous Pearl millet (*Pennisetum glaucum* (L.) R. Br.) germplasm. Journal of Food Composition and Analysis, **103**:104086.

Triki, T.; Tlahig, S.; Benabderrahim, M.A.; Elfalleh, W.; Mabrouk, M.; Bagues, M.; Yahia, H.; Belhouchette, K.; Guasmi, F. and Loumerem, M. (2022). Variation in phenolic, mineral, dietary fiber, and antioxidant activity across

southern tunisian pearl millet germplasm. Journal of Food Quality, **2022**(1):1437306.

Vogt, T. (2010). Phenylpropanoid biosynthesis. Molecular Plant, **3**(1):2-20.

Wang, H.; Fu, Y.; Zhao, Q.; Hou, D.; Yang, X.; Bai, S.; Diao, X.; Xue, Y. and Shen, Q. (2022). Effect of different processing methods on the millet polyphenols and their antidiabetic potential. Frontiers in Nutrition, **9**:780499.

Xu, W.; Dubos, C. and Lepiniec, L. (2015). Transcriptional control of flavonoid biosynthesis by MYB-bHLH-WDR complexes. Trends in Plant Science, **20**(3):176-185.

Yadav, C. B.; Gangashetty, P. I.; Beckmann, M.; Mur, L. A. and Yadav, R. S. (2022). Genotype-by-environment interaction analysis of metabolites in pearl millet genotypes with high concentrations of slowly digestible and resistant starch in their grains. Cells, **11**(19):3109.

Yadav, C. B.; Srivastava, R. K.; Gangashetty, P. I.; Yadav, R.; Mur, L. A. and Yadav, R. S. (2021). Metabolite diversity and metabolic genome-wide marker association studies (MGWAS) for health benefiting nutritional traits in pearl millet grains. Cells, **10**(11):3076.

Yadav, V.; Wang, Z.; Wei, C.; Amo, A.; Ahmed, B.; Yang, X. and Zhang, X. (2020). Phenylpropanoid pathway engineering: An emerging approach towards plant defense. Pathogens, **9**(4), 312.

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