

Phytochemical Profiling, Green Extraction, Separation and Characterization of Bioactive Compounds from Corn Silk (*Zea mays* L.): A Review

Dr. Aangothu Sreenivasa Rao

Department of Chemistry, Government College (Autonomous), Rajahmundry, Andhra Pradesh, India-53310

Abstract—Corn silk, the stigmatic and stilar tissue of maize (*Zea mays* L.), is an abundant but underutilized agricultural by-product with a chemically diverse profile. Modern phytochemical investigations have identified flavonoids, phenolic acids, terpenoids, sterols, fatty acids, polysaccharides and nitrogen-containing metabolites in corn-silk preparations. This review consolidates current knowledge on the chemical composition of corn silk and emphasizes the relationship between extraction strategy, fractionation, chromatographic separation and structural characterization. Particular attention is given to environmentally preferable extraction using water and aqueous ethanol, ultrasound-assisted extraction, solvent–solvent partitioning, thin-layer chromatography, column chromatography, HPLC-DAD, LC-MS/MS, GC-MS, FTIR and NMR. High-resolution LC-MS studies have substantially expanded the known metabolite space; one detailed UHPLC/HRMS/MS investigation proposed 104 molecules, including 67 phenolic, 28 lipidic and 7

nitrogen-containing compounds. The review also summarizes reported antioxidant, anti-inflammatory, metabolic and other biological activities while distinguishing chemical evidence from pharmacological evidence. Finally, the paper identifies major research gaps, including standardization of plant material, harmonization of extraction procedures, confirmation of tentative LC-MS annotations with authentic standards, assessment of matrix and seasonal effects, and translation from in-vitro or preclinical findings to validated applications. The integrated evidence supports corn silk as a promising feedstock for sustainable phytochemical recovery and agricultural by-product valorization.

Index Terms—Corn silk; *Zea mays* L.; Phytochemical Profiling; Green xtraction; Ultrasound-assisted extraction; Flavonoids; Phenolic acids; HPLC; LC-MS/MS; GC-MS; Chromatography; Agricultural by-products.

Graphical Abstract:

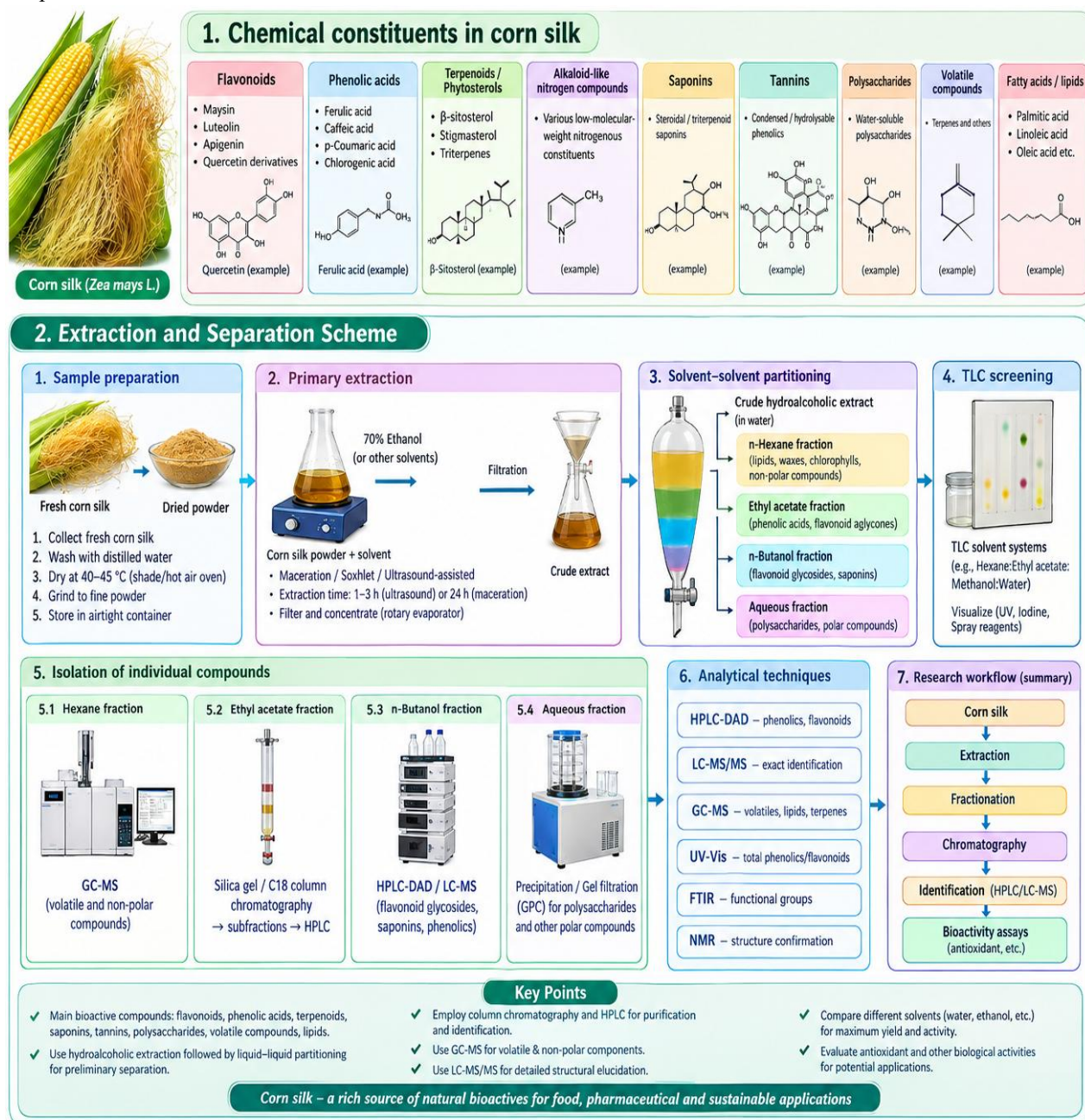


Figure 1. Integrated workflow for phytochemical profiling, green extraction, fractionation, chromatographic separation and characterization of corn-silk constituents.

I. INTRODUCTION

Corn silk (*Stigma maydis*) comprises the long, thread-like stigmas and styles associated with the female inflorescence of maize. Although routinely discarded during maize processing, it has a long history of traditional use and has become a subject of

modern natural-product research. Earlier reviews emphasized flavonoids, terpenoids and related constituents, while more recent reviews have broadened the chemical picture to include phenolic acids, polysaccharides, sterols, fatty acids and other metabolites.

Interest in corn silk is increasingly aligned with the principles of agricultural by-product valorization and green chemistry. Rather than treating corn silk solely as waste, extraction can be designed to recover selected phytochemicals using lower-toxicity solvents, reduced energy demand and scalable separation strategies. However, reported composition varies with maize genotype, harvest maturity, geographical origin, drying conditions and extraction protocol. A 2014 study, for example, found significant variation in total phenolics, flavonoids and antioxidant activity among varieties and maturity stages, demonstrating why sample standardization is essential.

The analytical landscape has also changed substantially. Conventional colorimetric assays and HPLC methods remain useful for targeted analysis, but UHPLC-HRMS/MS and molecular-networking approaches now permit broader metabolite mapping. A 2023 study combining UHPLC/HRMS and HRMS/MS proposed 104 molecules in a corn-silk extract, illustrating the complexity of the matrix and the value of complementary analytical workflows.

This review therefore focuses on the complete research chain: raw-material selection, green extraction, fractionation, separation, chemical profiling, structural characterization and biological evaluation. The objective is not to assume that every compound reported in the literature occurs in every maize sample, but to provide a practical framework for experimentally verifying composition in a defined material.

3.1 Major phytochemical classes:

II. REVIEW SCOPE AND LITERATURE-SELECTION APPROACH

This narrative review was organized around peer-reviewed literature on corn silk (*Zea mays* L.) published through 2026. Priority was given to papers describing phytochemical composition, extraction optimization, chromatographic or mass-spectrometric characterization, polysaccharide isolation, antioxidant/biological evaluation and recent reviews. Particular emphasis was placed on analytical studies that provide identifiable chemical evidence and on reviews that synthesize broader pharmacological findings. The literature was conceptually grouped into: (i) phytochemical composition; (ii) extraction and green-extraction technologies; (iii) fractionation and purification; (iv) instrumental characterization; (v) biological activities; and (vi) research gaps and future prospects..

III. BOTANICAL SOURCE AND CHEMICAL DIVERSITY

Maize is a major cereal crop and corn silk is generated at the ear as the receptive female floral tissue. The material is chemically dynamic because the concentration of secondary metabolites changes with genotype and developmental stage. Studies comparing varieties and maturity stages have reported statistically significant differences in total phenolics, total flavonoids, anthocyanins and antioxidant activity.

Table: 1. Major phytochemical classes

Chemical class	Representative constituents reported	Likely polarity	Principal analytical approaches
Flavonoids	Maysin and related C-/O-glycosides; luteolin-, apigenin-, quercetin- and kaempferol-type compounds	Polar to moderately polar	HPLC-DAD; LC-MS/MS; NMR
Phenolic acids	Caffeic, ferulic, p-coumaric, chlorogenic and related acids	Polar	HPLC-DAD; LC-MS/MS
Lipids/fatty acids	Palmitic, oleic, linoleic and related derivatives	Non-polar	GC-MS; LC-MS
Sterols/terpenoids	β -Sitosterol, stigmasterol and related compounds	Non-polar	GC-MS; LC-MS
Polysaccharides	Water-soluble heterogeneous polysaccharide fractions	Highly polar	Colorimetry; SEC/GPC; FTIR; NMR
Saponins	Steroidal/triterpenoid-type saponin	Polar	TLC; HPLC; LC-MS

	fractions reported in literature		
Nitrogen-containing compounds	Benzoxazinoid/benzoxamine-related and other nitrogenous metabolites	Variable	LC-MS/MS

High-resolution mass spectrometry has shown that the chemical space is broader than the small set of traditional marker compounds. In the 2023 UHPLC/HRMS/MS study by Fougère and co-workers, 104 molecules were proposed, comprising 67 phenolic compounds, 28 lipidic compounds and 7 nitrogen-containing compounds; the study also highlighted 55 flavonoids and 13 organic acids in the detected profile.

IV. EXTRACTION OF CORN-SILK PHYTOCHEMICALS

Extraction is the first major determinant of the final chemical profile. Solvent polarity, solid-to-liquid ratio, temperature, time, particle size and extraction energy can alter both extraction yield and the relative abundance of individual compounds. Consequently, 'total extract yield' alone is not an adequate optimization criterion; a suitable process should also consider chemical selectivity, phenolic/flavonoid recovery, solvent safety, energy use and downstream purification.

4.1 Conventional extraction

Maceration and reflux/Soxhlet-type extraction have historically been used for plant materials. They are straightforward but can require longer extraction times and relatively large volumes of solvent. Prolonged heating may also alter thermolabile constituents. Hydroalcoholic systems are frequently used because water improves extraction of polar components while ethanol increases recovery of moderately polar constituents.

4.2 Ultrasound-assisted extraction

Ultrasound-assisted extraction (UAE) uses acoustic cavitation to enhance solvent penetration and mass transfer. Corn-silk studies have investigated ultrasound extraction by varying ethanol concentration, temperature, time and solid-to-liquid ratio. UAE is attractive for green extraction because it can shorten extraction time and potentially reduce solvent consumption, although the optimum conditions must be established for the target compound class.

4.3 Green extraction principles

For corn silk, an experimentally realistic green strategy is to begin with water and aqueous ethanol, compare extraction efficiency under mild conditions, and recover solvent where feasible. The process should minimize unnecessary chlorinated solvents and avoid excessive energy-intensive steps. Green metrics can be incorporated at process-development stage, including solvent volume, solvent hazard, energy requirement, extraction time, mass yield and waste generation.

V. FRACTIONATION AND SEPARATION STRATEGY

Crude extracts are chemically complex, so polarity-based fractionation can simplify downstream chromatographic analysis. A common conceptual sequence is aqueous suspension of the hydroalcoholic extract followed by partitioning into n-hexane, ethyl acetate, n-butanol and an aqueous residue. The exact order and solvent system should be adapted to the target compounds and validated for recovery.

Table: 2. Fractionation and Separation Strategy

Fraction	Compounds expected to be enriched	Advantages	Recommended next analysis
n-Hexane	Lipids, waxes, sterols, non-polar constituents	Removes hydrophobic matrix components	GC-MS; TLC
Ethyl acetate	Phenolic acids; relatively less-polar flavonoids	Useful enrichment of many phenolics	TLC; HPLC-DAD; LC-MS/MS
n-Butanol	Polar flavonoid glycosides and some saponins	Enriches moderately/highly polar secondary	HPLC; LC-MS/MS

		metabolites	
Aqueous	Polysaccharides and highly polar compounds	Retains highly water-soluble constituents	SEC/GPC; FTIR; NMR; carbohydrate assays

VI. CHROMATOGRAPHIC TECHNIQUES FOR PROFILING AND ISOLATION

6.1 Thin-layer chromatography:

TLC is a low-cost screening tool for comparing crude extracts and fractions, monitoring column fractions and selecting samples for instrumental analysis. Visualization under UV light and appropriate derivatization can provide rapid fingerprints for phenolic, flavonoid, terpenoid and other compound classes.

6.2 Column chromatography:

Silica-gel chromatography is particularly useful for non-polar to moderately polar constituents, whereas reversed-phase C18 materials are well suited to phenolic acids and flavonoid glycosides. Fraction collection should be guided by TLC or online chromatographic monitoring rather than arbitrary pooling.

6.3 HPLC-DAD:

HPLC with diode-array detection is appropriate for targeted profiling and quantification of UV-absorbing phenolics and flavonoids. Authentic standards are preferable for definitive identification and quantification. Retention time plus UV spectrum provides stronger evidence than retention time alone.

Technique	Primary information	Role in corn-silk research
HPLC-DAD	Retention time and UV/Vis spectral profile	Targeted phenolic/flavonoid profiling and quantification
LC-MS/MS	Accurate mass and fragmentation	Broad metabolite annotation and glycoside characterization
GC-MS	Mass spectra of volatile/derivatized compounds	Fatty acids, sterols, volatile and non-polar constituents
FTIR	Functional groups	Rapid chemical-class confirmation
NMR	Molecular framework and connectivity	Structure confirmation of purified compounds

VIII. BIOLOGICAL ACTIVITIES AND PHYTOCHEMICAL RELEVANCE

Published studies describe antioxidant, anti-inflammatory, hypoglycemic, hypolipidemic, antihypertensive and other biological effects of corn-silk preparations. Recent reviews summarize a broad preclinical literature, but the strength of evidence

6.4 LC-MS/MS:

LC-MS/MS provides accurate-mass and fragmentation information for compounds that are difficult to identify by UV detection alone. It is particularly useful for glycosylated flavonoids, phenolic acids, organic acids and other semi-polar metabolites. However, database/library matches should be treated as tentative unless supported by authentic standards, diagnostic MS/MS spectra or orthogonal structural evidence.

6.5 GC-MS:

GC-MS complements LC-based methods for volatile compounds, fatty acids and other thermally stable/derivatizable constituents. Non-polar fractions may be especially suitable for this approach.

VII. STRUCTURAL CHARACTERIZATION

Reliable compound characterization is best achieved through orthogonal analytical evidence. FTIR can support functional-group assignment, but it rarely provides complete structural identification for complex plant metabolites. NMR spectroscopy is particularly valuable for purified compounds because it provides direct information about the molecular framework and substitution pattern.

varies considerably by endpoint and experimental model.

Antioxidant activity is among the most consistently investigated properties. DPPH, ABTS and FRAP assays have been widely used, and higher phenolic/flavonoid content is often associated with greater antioxidant capacity. Nevertheless, such correlations do not establish that a single phenolic

compound is responsible, because extracts contain mixtures of compounds that may act additively or synergistically.

Metabolic effects, including reported glucose- and lipid-related activities, are supported mainly by preclinical evidence. A recent systematic review and meta-analysis of preclinical studies specifically examined the anti-diabetic potential of corn silk and reported pooled effects for selected metabolic and oxidative-stress parameters. Translation to human therapeutic use requires appropriately designed clinical studies.

IX. CORN-SILK POLYSACCHARIDES AS A SEPARATE RESEARCH STREAM

Polysaccharides deserve separate consideration because their extraction and purification differ fundamentally from low-molecular-weight phenolics. Water extraction, concentration, ethanol precipitation, deproteinization, dialysis and chromatographic purification are commonly used conceptual steps. A 2024 review summarized extraction, purification, structural characterization, biological activities and toxicity considerations of corn-silk polysaccharides and emphasized their reported antioxidant, hypoglycemic, hypolipidemic, hepatorenal-protective, immunomodulatory and other activities.

For a complete phytochemical project, it is therefore useful to decide early whether polysaccharides are a primary target. Combining a phenolic/flavonoid workflow with a separate polysaccharide workflow can prevent the extraction conditions optimized for one chemical class from being incorrectly applied to another.

X. INTEGRATED GREEN EXTRACTION AND CHARACTERIZATION WORKFLOW

A practical review-derived workflow for future experimental studies is summarized below:

1. Authentication and documentation of maize variety, location, harvest stage and sample voucher.
2. Low-temperature drying and standardized milling.
3. Screening of water and aqueous ethanol as primary extraction solvents.

4. Ultrasound-assisted extraction under controlled mild conditions.
5. Filtration and reduced-pressure concentration.
6. Calculation of extraction yield together with total phenolic and flavonoid contents.
7. Polarity-based fractionation into hexane, ethyl acetate, n-butanol and aqueous fractions.
8. TLC fingerprinting and pooling of chemically similar fractions.
9. HPLC-DAD profiling and targeted quantification is using authentic standards.
10. LC-MS/MS-based untargeted or semi-targeted metabolite characterization.
11. GC-MS examination of suitable non-polar/volatile fractions.
12. FTIR and NMR confirmation for purified or enriched compounds.
13. Bioactivity testing and correlation with chemical profiles.
14. Assessment of solvent use, energy demand, waste generation and recovery potential for green-process evaluation.

XI. CHALLENGES AND RESEARCH GAPS:

11.1 Botanical and harvest variability:

Genotype, maturity stage, environmental conditions and post-harvest handling can alter phytochemical levels. Future studies should report these variables explicitly.

11.2 Lack of harmonized extraction protocols:

Differences in solvent composition, solid-to-liquid ratio, temperature and time make direct comparison between studies difficult. Standardized reporting is required.

11.3 Tentative metabolite annotations;

Accurate mass and library matching can generate strong hypotheses but do not always prove structure. Authentic standards and NMR should be used for high-confidence confirmation where feasible.

11.4 Matrix complexity:

Co-extracted sugars, pigments, lipids and polymers can suppress ionization or interfere with chromatographic separation. Fractionation and matrix-aware method validation are important.

11.5 Biological evidence versus clinical evidence:

Most reported biological effects arise from in-vitro or preclinical studies. Such evidence should not be presented as proof of clinical efficacy.

11.6 Green-chemistry assessment:

Calling a process 'green' solely because ethanol is used is insufficient. Solvent quantity, energy, waste, process yield, solvent recovery and scale-up should all be considered.

XII. FUTURE PERSPECTIVES

Future corn-silk research is likely to benefit from integrated metabolomics, chemometrics and sustainable-process engineering. UHPLC-HRMS/MS can generate broad chemical fingerprints, while multivariate statistics can relate chemical patterns to variety, maturity, extraction condition and bioactivity. Machine-learning-assisted annotation and molecular networking may accelerate discovery, but experimental confirmation remains essential.

From a green-chemistry perspective, aqueous ethanol, water, ultrasound, microwave-assisted extraction, pressurized liquid extraction and other intensified techniques deserve systematic comparison using common performance metrics. At process scale, solvent recovery, energy demand and fractionation efficiency should be evaluated alongside phytochemical yield.

Corn silk also fits a circular-bioeconomy concept in which an agricultural by-product is converted into standardized extracts, purified phytochemicals, functional ingredients or other value-added products. Future work should combine chemical standardization with safety assessment, reproducibility, stability studies and appropriate toxicological evaluation.

XIII. CONCLUSION

Corn silk is a chemically diverse maize by-product containing flavonoids, phenolic acids, lipids, sterols, terpenoids, polysaccharides and other metabolites. The evidence reviewed here supports an integrated analytical strategy rather than reliance on a single extraction or detection method. Aqueous ethanol and ultrasound-assisted extraction provide practical starting points for green process development, while

polarity-based fractionation can simplify the complex matrix. HPLC-DAD and LC-MS/MS are central to phenolic and flavonoid profiling, GC-MS complements the analysis of non-polar and volatile constituents, and FTIR/NMR provide orthogonal structural evidence.

The major opportunity is to move from descriptive phytochemical screening toward standardized, reproducible and sustainable processes that connect chemical composition with validated biological activity. Such an approach could contribute to the scientific valorization of corn silk and support its development as a source of bioactive and value-added products.

REFERENCES

- [1] Hasanudin K, Hashim P, Mustafa S. Corn silk (*Stigma maydis*) in healthcare: a phytochemical and pharmacological review. *Molecules*. 2012;17(8):9697–9715. doi:10.3390/molecules17089697.
- [2] Maksimović Z, Malenčić Đ, Kovačević N. Polyphenol contents and antioxidant activity of *Maydis stigma* extracts. *Bioresource Technology*. 2005;96:873–877. doi:10.1016/j.biortech.2004.09.006.
- [3] Liu J, Wang C, Wang Z, Zhang C, Lu S, Liu J. The antioxidant and free-radical scavenging activities of extract and fractions from corn silk (*Zea mays* L.) and related flavone glycosides. *Food Chemistry*. 2011;126:261–269.
- [4] Udomkun P, Innawong B, Khanongnuch C, et al. Influence of variety and harvest maturity on phytochemical content in corn silk. *Food Chemistry*. 2014.
- [5] Fougère L, Zubrzycki S, Elfakir C, Destandau E. Characterization of Corn Silk Extract Using HPLC/HRMS/MS Analyses and Bioinformatic Data Processing. *Plants*. 2023;12(4):721. doi:10.3390/plants12040721.
- [6] Li P, et al. Extraction Optimization, Preliminary Identification, and Bioactivities in Corn Silk. Evidence-Based Complementary and Alternative Medicine. 2023;2023:5685174. doi:10.1155/2023/5685174.
- [7] Wang Y, Mao J, Zhang M, et al. An Umbrella Insight into the Phytochemistry Features and Biological Activities of Corn Silk: A Narrative

- Review. *Molecules*. 2024;29(4):891. doi:10.3390/molecules29040891.
- [8] Extraction, purification, structural characteristics, and pharmacological activities of the polysaccharides from corn silk: A review. *International Journal of Biological Macromolecules*. 2024. doi:10.1016/j.ijbiomac.2024.133433.
- [9] Rapid analysis and characterization of multiple constituents of corn silk aqueous extract using ultra-high-performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry. 2019.
- [10] Antioxidant, Anti-Tyrosinase, and Anti-Skin Pathogenic Bacterial Activities and Phytochemical Compositions of Corn Silk Extracts, and Stability of Corn Silk Facial Cream Product. *Antibiotics*. 2023;12(9):1443. doi:10.3390/antibiotics12091443.
- [11] Ding L, Wang X, Liu T, et al. Corn Silk in Pharmaceutical Biotechnology: Bioactive Compounds, Pharmacological Activities, and Mechanistic Insights. *Current Pharmaceutical Biotechnology*. 2026. doi:10.2174/0113892010442889260302083802.
- [12] By-Products of *Zea mays* L.: A Promising Source of Medicinal Properties with Phytochemistry and Pharmacological Activities: A Comprehensive Review. 2023.
- [13] Unlocking Corn Silk's Potential: Bioactive Compounds Targeting Age-Related Diseases. *Molecular Nutrition & Food Research*. 2025.
- [14] Anti-Diabetic Potential of Corn (*Zea mays* L.) Silk: A Systematic Review and Meta-Analysis of Preclinical Studies. 2026.

Author Note for Manuscript Preparation

This document is a literature-based review draft. Reported compound names, biological activities and analytical observations should be checked against the cited primary literature before journal submission. Any experimental values, chromatograms, spectra, antioxidant IC₅₀ values, extraction yields or compound concentrations generated in future laboratory work should replace placeholders rather than being inferred from literature.