



Invitro Antioxidant and Anti-Inflammatory Activities of Corn Silk

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Abstracts

A new, improved, more potent antioxidant and anti-inflammatory agent is required since very little or no effective variants of those elements available to be used during treatment of responses and diseases. This study evaluated the phytochemical composition, antioxidant activity, and anti-inflammatory potential of ethanol and n-hexane extracts of corn silk, comparing their activities with standard reference compounds. Phytochemical screening revealed the presence of saponins, alkaloids, quinones, terpenoids, and cardiac glycosides. Antioxidant activity was assessed using the DPPH free radical scavenging and ferric reducing antioxidant power (FRAP) assays, with ascorbic acid as the standard. The ethanol extract exhibited stronger DPPH radical scavenging ($IC_{50} = 277.33 \mu\text{g/mL}$) than the n-hexane extract ($IC_{50} = 343.51 \mu\text{g/mL}$), although both were less potent than ascorbic acid ($IC_{50} = 57.38 \mu\text{g/mL}$), a trend consistent in FRAP results. Anti-inflammatory activity, evaluated through anti-lipoxygenase and membrane stabilization assays, revealed that the n-hexane extract ($IC_{50} = 239.31 \mu\text{g/mL}$) matched Indomethacin ($IC_{50} = 239.52 \mu\text{g/mL}$) in lipoxygenase inhibition and was more active than the ethanol extract ($IC_{50} = 327.85 \mu\text{g/mL}$). In membrane stabilization, the n-hexane extract ($IC_{50} = 321.78 \mu\text{g/mL}$) outperformed the ethanol extract ($IC_{50} = 457.01 \mu\text{g/mL}$) but was less potent than Aspirin ($IC_{50} = 305.13 \mu\text{g/mL}$). These results suggest that corn silk possesses moderate antioxidant capacity and notable anti-inflammatory properties, particularly in its non-polar fraction, with activity profiles approaching those of standard drugs. The findings support its potential as a natural, low-cost adjunctive therapeutic for oxidative stress and inflammation, and highlight the need for further bioactive compound isolation, mechanistic studies, and in vivo validation.

Keywords: Maize silk extract, Antioxidant activity, Anti-inflammatory activity, Ethanol extract, Phytochemical analysis

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Introduction

Inflammation is a complex and highly regulated biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or chemical irritants (Calhelha et al., 2023). It is characterized by cardinal signs including fever, discomfort, edema, redness, and loss of function, and involves the migration of leukocytes from the circulatory system to the site of injury, followed by the secretion of pro-inflammatory cytokines. These mediators promote vasodilation and increase blood flow, resulting in localized warmth and erythema, while enhanced vascular permeability facilitates fluid leakage into tissues, causing swelling

(Akinwumi & Oyedapo, 2015; Stone et al., 2024). In more severe or persistent cases, this process may contribute to tissue injury, nerve irritation, and functional impairment. A well-established link exists between oxidative stress and inflammation, as activated neutrophils and macrophages generate reactive oxygen species (ROS) that perpetuate inflammation by stimulating cytokine production and further free radical release (Sobhon et al., 2023). Persistent oxidative stress increases vascular permeability, promotes protein denaturation, and alters membrane integrity, contributing to the pathophysiology of numerous inflammatory diseases. In neurodegenerative conditions, oxidative damage is evidenced by oxidized

proteins, glycated products, and lipid peroxidation (Olufunmilayo et al., 2023). Inflammation may arise from mechanical, chemical, microbial, or systemic insults and is mediated by a complex interplay between cellular and humoral immune components (Damyanova & Paunova-Krasteva, 2025). Mast cells, located near autonomic nerves, play a central role by releasing histamine, serotonin, and bradykinin during degranulation, which orchestrates vascular and immune responses (Dileepan et al., 2023).

Antioxidants are chemical compounds capable of delaying or preventing oxidative damage even at low concentrations (Martemucci et al., 2022). They form the body's primary defense against ROS, neutralizing free radicals before they can damage biomolecules. Both enzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and non-enzymatic antioxidants, including vitamins C and E, carotenoids, flavonoids, and phenolic acids, are essential in maintaining redox balance (Anwar et al., 2024; El-Lateef et al., 2023). While endogenous antioxidants provide baseline protection, dietary sources supply additional defense, particularly under conditions of increased oxidative stress caused by pollution, smoking, illness, or physical exertion (Chandimali et al., 2025). Natural antioxidants are widely valued in the pharmaceutical, food, and cosmetic industries for their ability to prevent oxidative degradation and support human health (Zulhendri et al., 2021).

Corn silk (*Zea mays* L., stigma maydis), the dried stigmas of maize flowers, is a readily available agricultural byproduct belonging to the Poaceae family. Historically, it has been used in traditional medicine in countries such as China, Turkey, and the United States for its diuretic, anti-inflammatory, and wound-healing properties (Li et al., 2020). Phytochemical studies have shown that corn silk contains bioactive compounds such as flavonoids, phenolic acids, vitamins, and minerals, which contribute to its antioxidant and anti-inflammatory effects (Li et al., 2021; Yang et al., 2024). Its high yield, low cost, and diverse pharmacological activities make it a promising natural resource for therapeutic applications aimed at combating oxidative stress and inflammation.

The rising global prevalence of inflammation-related disorders and oxidative stress-mediated diseases highlights the urgent need for safe, effective, and affordable therapeutic agents (Sobhon et al., 2023). Conventional anti-inflammatory and antioxidant drugs,

though effective, can have undesirable side effects and are often costly to produce, limiting their accessibility in resource-limited settings (Olufunmilayo et al., 2023). Corn silk, as a low-cost and widely available agricultural byproduct, offers a sustainable source of bioactive compounds with reported antioxidant and anti-inflammatory properties. However, despite its extensive use in traditional medicine, there is limited systematic *in vitro* evidence to validate and quantify these biological activities under controlled conditions. Therefore, this study aims to evaluate the *in vitro* antioxidant and anti-inflammatory activities of corn silk in order to provide a scientific basis for its pharmacological potential, elucidate its mechanisms of action, and support its possible application in functional foods, nutraceuticals, and complementary therapeutic strategies.

Materials and Methods

Materials

Corn silk, glassware, electric blender, distilled water, ethanol, airtight bag, rotary evaporator, measuring cylinder, beaker, and conical flask.

Chemicals and Equipment

In this experiment, analytical grade of chemicals and reagents were applied. Some of the materials and equipment were availed by Department of Biochemistry, Faculty of Pure and Applied Sciences, The Federal Polytechnic Ilaro, Ogun State, Nigeria

Sample Collection and Preparation

Corn was gotten from Sayedero market, Ilaro, Ogun state. The corn silk shaft was removed from the whole maize plant and air-dried. The dried corn silk was grind into a fine powder using an electric blender.

Extract Preparation

The material of the powder plant was immersed in 80 percent ethanol water during 72 hours of time. The mixture was thereafter filtered and the filtrate re-concentrated using reduced pressure at 45°C using a rotary evaporator to produce an extract.

Qualitative Analysis of Phytochemicals

The major groups of secondary metabolites including glycosides, phenols, anthraquinone, tannins, alkaloids, saponins, flavonoids, terpenoids and steroids were

tested with the standard procedures (Dubale et al., 2023; Singh et al., 2023).

Quantitative Analysis of Phytochemicals

The ethanol extract of corn silk was used to determine the amount of some phytochemicals including but not limited to, total phenol content and total flavonoid content (Farhana et al., 2023).

Antioxidant Assays

Ferric ion reducing power (FRAP) Assay

We tested the antioxidant activity of the extract in order to evaluate the Lapčík et al. (2023) technique (the ferric (Fe³⁺) reducing power assay (FRAP), and we created the following extract concentrations: 1 to 100 µg/mL. After the addition of 1% potassium ferricyanide and 2.5 mL of phosphate buffer (200 mM, pH 6.6) to each concentration of the extract. After 20 minutes of rapid cooling in 50 Celsius in a water bath, the mixture was combined with 2.5 milliliters of 10 per cent trichloroacetic acid after which the mixture was centrifuged in 10 minutes at a rate of 3, 000 g. 2.5 milliliters of a sterilized distilled water and 0.5 milliliters, 0.1 percent of ferric chloride (FeCl₃) were added into the supernatant.

Strength of the iron (II)-ferricyanide complex was determined by determining the absorbance of the Perl Prussian, blue color formation at the wavelength of 700 nm after a ten minutes reaction. Blank solution containing the solvent and reagent solutions required to liquefy the material was prepared and run on identical procedures. The greater the absorbance the mixture of the reaction, the more the reducing power. The same procedure was performed using geraniin and 1 100 µg/ml reference chemical, i.e. 2-tocopherol. It was done three times in order to ensure that the procedure was correct.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability assay

The free radical scavenge ability of extract was determined by the method given by Lapčík et al. (2023). 10 ml of extract quantity was to be weighed with 10 ml of sterile distilled water to provide 1 mg/ml of a stock solution. Two milliliters of the stock was then filled in a sterile test tube which was then diluted to two milliliters of distilled water. The serial dilution of the solution was completed twice till obtaining the

concentration of 0.019510 gg/ml. They added one milliliter of each concentration into three milliliter of freshly prepared DPPH solution (50 0 M) in methanol. In a UV spectrophotometer, absorbance at 517 nm was recorded after 30-min dark incubation at 25° C as opposed to the blank. The material dissolved in the solvent and reagent solution (50 299; DPPH) was used to prepare and treat blank solution. The experiment was done three times. The same thing was done to both geraniin and 2 (reference chemical) tocopherol. Ability to scavenge the DPPH radical can be determined with the following formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance}_{\text{DPPH}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{DPPH}}} \times 100 \quad (1)$$

Invitro Anti-inflammatory Assays

Anti-Lipoxygenase Inhibition Assay

Inhibition of lipoxygenase was carried out according to the guidelines of Belgharbi et al., (2025) where 10 mL of the extract, 10 uL lipoxygenase (final concentration 8000 U/mL) and 1 mL sodium borate buffer (0.1 M, pH 8.8) were added to a cuvette and incubated at room temperature (30 +/- 2 C) for 5 minutes. The reaction is initiated by adding ten microliter measure of linoleic acid substrate (10 mmol). Using a UV/VIS spectrometer the absorbance of the reaction solution was measured at 234 nm. With the phosphate buffer solution as the control, the inhibition of lipoxygenase was computed as of percentage through the following formula:

$$\% \text{ Inhibition} = 100 \times \frac{\text{absorbance of the control} - \text{absorbance of the sample}}{\text{absorbance of control}} \quad (2)$$

Human red blood cell (HRBC) Membrane stabilization assay

As Yesmin et al. (2020) note, the anti-inflammatory effect of the corn silk extract on the membrane stability was studied. The standard NSAIDs were to be assessed on the basis of inhibitory lysis percentages. Suspension absorbance was determined to determine the level of hemoglobin by making use of a spectrophotometer whose range was 560 nm. The predominant factor that was taken into consideration regarding the exclusion of healthy human volunteers willing to supply blood donating was the consumption of an NSAID two weeks prior to the experiment. An anticoagulant Na-oxalate was utilized. Each blood sample was stored at a

temperature of 4 °C, and before it was employed, we allowed it to stay at the constant temperature of 4°C in a full day. The supernatant was extracted in to by centrifugation a duration of five minutes at 2500 rpm. After washing that will be done using sterile saline solution (0.9% w/v NaCl) the centrifugation will be done in a five-minute duration of 2500 rpm. After three times washing of the supernatant, it was determined on the packed cell volume. To restore the cellular components, it was reconstituted with phosphate-buffered saline solution (10 mM and pH 7.4) containing 1 L of distilled water, 0.26 g of NaH₂PO₄.2H₂O, 1.15 g of Na₂HPO₄ and 9 g of sodium chloride by preparation of a 40% (v/v) suspension in a mixture of the component.

Statistical Analysis

Results were reported to be in the mean +/- SD. GraphPad Prism 6 was used to analyzed the data using one-way analysis of variance also known as ANOVA. The mean difference was compared in Turkey using a comparison test. Statistical significance is presented by p-value less than 0.05.

Results and Discussion

Results

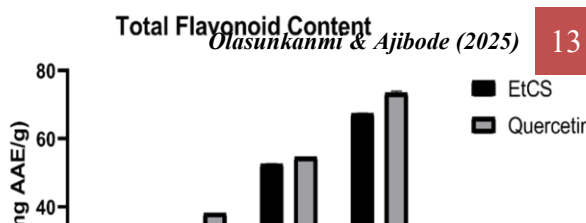
The qualitative phytochemical of Ethanol extract of Corn silk is shown in Table 1. The result shows that Saponin, Alkaloids, quinones, terpenoids are positive while Carbohydrate, Tannin, Flavonoid, Glycosides, Phenols, Steroids and Antraquinones are negative.

Table 1. Qualitative Phytochemical of Corn silk extract

SN	CONSTITUENT	TEST	RESULT
1	Carbohydrate	Molisch test	-ve
2	Tannin	Ferric chloride test	-ve
3	Saponin	Distilled water	+ve
4	Alkaloids	Wagners test	+ve
5	Flavonoid	Sodium hydroxide test	-ve
6	Glycosides	Ammonia test	-ve
7	Quinone	Sulphuric acid test	+ve
8	Phenols	Ferric chloride test	-ve
9	Terpenoids	Chloroform + Conc. Sulphuric acid test	+ve
10	Steroids	Conc. Sulphuric acid test	-ve
11	Antraquinones	Ammonia test	-ve
12	Coumarins	Sodium hydroxide test	-ve
13	Cardiac Glycosides	Glacia acetic acid + Ferric chloride+ Conc. Sulphronic acid test	+ve

+ve = positive, -ve = negative

Total Phenol and Total Flavonoid Content



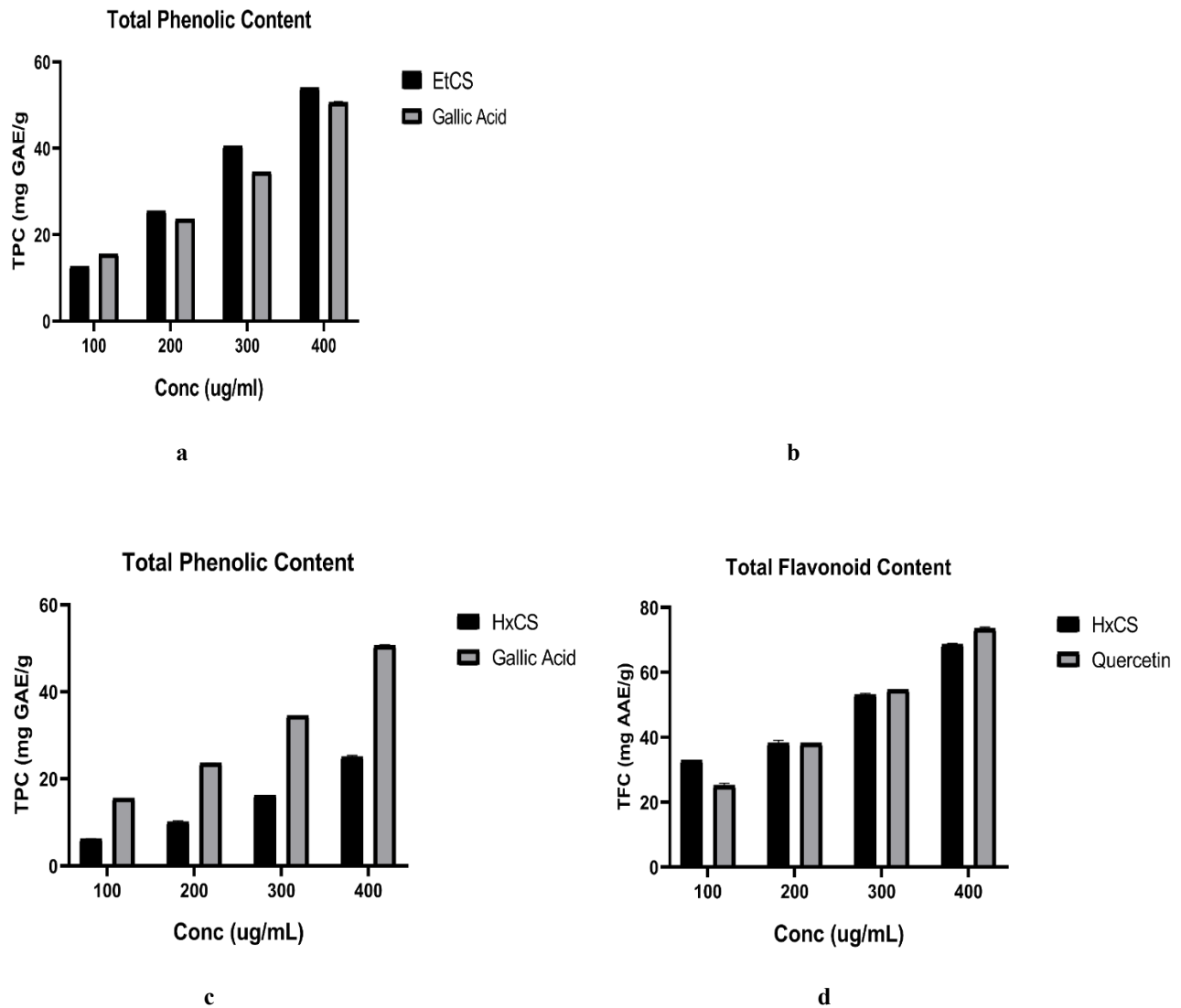


Figure 1: a) Total phenolic content of ethanol extract of *Corn silk*, b) Total flavonoid content of ethanol extract of *Corn silk*, c) Total phenolic content of n-hexane extract of *Corn Silk*, d) Total flavonoid content of n-hexane extract of *Corn silk*.

Antioxidant Activity

Table 2: Percentage inhibition and IC₅₀ value of invitro antioxidant activity of *Corn silk* ethanol and n-hexane extract using DPPH free radical scavenging assay.

Concentration (µg/ml)	Ethanol Extract	N-hexane Extract	Ascorbic acid
100	32.66±0.084	26.48±0.011	53.26±0.015

200	44.85±0.012	34.76±0.030	59.54±0.012
300	51.23±0.031	42.95±0.020	93.25±0.017
400	58.89±0.017	54.30±0.010	97.77±0.000
500	73.21±0.021	68.88±0.015	95.83±0.000
IC₅₀	277.33	343.51	57.38

Table 3: Percentage inhibition and IC₅₀ value of invitro antioxidant activity of *Corn silk* ethanol and n-hexane extract using FRAP

Concentration (µg/ml)	Ethanol Extract	N-hexane Extract	Ascorbic Acid
100	21.61±0.012	17.41±0.015	47.51±0.005
200	33.02±0.015	27.84±0.011	65.71±0.000
300	41.22±0.020	40.77±0.025	83.52±0.017
400	57.13±0.025	61.03±0.035	87.61±0.015
500	63.42±0.010	72.56±0.011	96.46±0.000
IC₅₀	362.47	342.34	81.62

Anti-inflammatory Activity

Table 4: Percentage inhibition and IC₅₀ value of invitro antiinflammation activity of *Corn silk* ethanol and n-hexane extract using anti-lipoxygenase activity.

Concentration (µg/ml)	Ethanol Extract	N-Hexane Extract	Indomethacin
100	14.37±0.012	22.29±0.017	11.62±0.015
200	30.63±0.021	40.52±0.015	15.03±0.036
300	52.32±0.025	57.84±0.017	39.85±0.012
400	61.29±0.017	65.30±0.030	60.13±0.025
500	71.22±0.021	74.90±0.011	72.28±0.021
IC₅₀	327.85	239.31	239.52

Table 5: Percentage inhibition and IC₅₀ value of invitro anti-inflammation activity of *Corn silk* ethanol and n-hexane extract using membrane stabilizing activity.

Concentration (µg/ml)	Ethanol Extract	N-Hexane Extract	Aspirin
100	10.89±0.065	9.64±0.040	21.02±0.046

200	20.24±0.030	13.96±0.015	35.77±0.022
300	36.19±0.554	40.52±0.025	46.02±0.021
400	44.13±0.028	52.80±0.011	68.40±0.020
500	53.22±0.026	58.66±0.025	75.22±0.012
IC50	457.01	321.78	305.13

Discussion

Plants serve as a rich source of bioactive compounds with notable antioxidant capacity, enabling the neutralization of reactive oxygen species and the mitigation of oxidative stress (Yang et al. (2024)). Given the established link between oxidative stress and inflammation, these plant-derived antioxidants frequently exhibit complementary anti-inflammatory effects by modulating inflammatory pathways and preserving cellular integrity, thereby representing promising candidates for the development of safe, naturally derived therapeutic agents (El-Lateef et al., 2023).

The phytochemical screening of the ethanol extract of corn silk revealed the presence of saponins, alkaloids, quinones, terpenoids, and cardiac glycosides, while carbohydrates, tannins, flavonoids, glycosides, phenols, steroids, and anthraquinones were absent. This profile is consistent with previous reports by Li et al. (2021) and Yang et al. (2024), who also identified terpenoids and saponins as major classes of compounds in *Zea mays* stigma with documented antioxidant and anti-inflammatory activity. The absence of flavonoids in the ethanol extract, however, contrasts with the work of Li et al. (2020), where flavonoids were reported as abundant; this difference may be attributed to variations in plant source, harvest time, or extraction conditions.

In the DPPH radical scavenging assay, the ethanol extract demonstrated a concentration-dependent increase in antioxidant activity, achieving 73.21% inhibition at 500 µg/mL with an IC₅₀ of 277.33 µg/mL. While this is lower in potency compared to ascorbic acid (IC₅₀ = 57.38 µg/mL), it indicates moderate free radical scavenging potential. These findings are in line with reports by Martemucci et al. (2022) and Zuhendri et al. (2021), who emphasized the role of plant-derived antioxidants in neutralizing free radicals, but our results suggest that corn silk may be less potent than pure

antioxidant standards. The n-hexane extract, with an IC₅₀ of 343.51 µg/mL, exhibited lower activity, consistent with Anwar et al. (2024), who noted that polar fractions tend to yield higher antioxidant capacity due to the solubility of phenolic compounds in polar solvents.

The FRAP assay results further corroborated these trends, with both ethanol and n-hexane extracts displaying appreciable ferric-reducing power, although still below the activity of ascorbic acid. This aligns with the observations of El-Lateef et al. (2023), who reported that plant extracts can exhibit significant but sub-maximal ferric-reducing power when compared to pure antioxidants.

In anti-inflammatory assay using anti-lipoxygenase activity, the n-hexane extract (IC₅₀ = 239.31 µg/mL) was comparable in potency to indomethacin (IC₅₀ = 239.52 µg/mL) and more active than the ethanol extract (IC₅₀ = 327.85 µg/mL). This mirrors the findings of Dileepan et al. (2023), who described the role of lipoxygenase pathway inhibition in reducing inflammation, and suggests that the non-polar bioactives in corn silk likely terpenoids and quinones contribute significantly to this mechanism.

In the membrane stabilizing assay, the n-hexane extract again outperformed the ethanol extract, with an IC₅₀ of 321.78 µg/mL versus 457.01 µg/mL, though both were less potent than Aspirin (IC₅₀ = 305.13 µg/mL). The proximity of the n-hexane extract's activity to that of Aspirin is notable, as Aspirin is a well-established cyclooxygenase inhibitor with recognized membrane-stabilizing effects (Akinwumi & Oyedapo, 2015). While our results confirm the anti-inflammatory potential of corn silk, they contrast with some earlier literature such as Olufunmilayo et al. (2023) that reported higher potency in aqueous extracts. This difference likely arises from solvent-specific extraction of active compounds.

Overall, our findings support the conclusions of Calhelha et al. (2023) and Yang et al. (2024) that corn

silk possesses pharmacologically relevant antioxidant and anti-inflammatory activities. However, when benchmarked against standard agents such as ascorbic acid and Aspirin, corn silk extracts show reduced potency. Despite this, the combination of multiple bioactive phytochemicals and moderate efficacy across both oxidative and inflammatory pathways supports their potential as adjunctive therapeutics. This dual action is consistent with the multi-mechanistic approach to managing oxidative stress and inflammation described by Sobhon et al. (2023) and Damyanova & Paunova-Krasteva (2025).

Conclusion

This study establishes that corn silk (*Zea mays* L., stigma maydis) contains bioactive constituents such as saponins, alkaloids, quinones, terpenoids, and cardiac glycosides that contribute to measurable antioxidant and anti-inflammatory activities. The ethanol extract demonstrated greater antioxidant potential, while the n-hexane extract exhibited superior anti-inflammatory effects, with activity in membrane stabilization approaching that of Aspirin and matching Indomethacin in lipoxygenase inhibition. Although less potent than standard drugs, the multi-target action of corn silk supports its potential as a complementary therapeutic for oxidative stress and inflammation.

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