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Anti-Inflammatory and Antioxidant Potential of Corn Silk Extract in Carrageen an-Induced Inflammation in Albino Rats

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ABSTRACT
This study investigates the anti-inflammatory and antioxidant potential of corn silk (Stigma maydis) extract in the context of carrageenan-induced inflammation in albino rats. Renowned for its rich array of bioactive phytochemicals, including polyphenols, flavonoids, and carotenoids, corn silk has demonstrated significant therapeutic properties in traditional medicine. The extract's active components, such as tannins and phenols, contribute to its diverse pharmacological activities, including antioxidant, anti-inflammatory, and antidiabetic effects. Notably, corn silk can activate peroxisome proliferator-activated receptors, suggesting its role in diabetes management. With its high antioxidant capacity, corn silk extract may mitigate oxidative stress and offer therapeutic options for various diseases. Overall, the findings underscore the potential of corn silk extract as a valuable resource for anti-inflammatory and antioxidant applications in healthcare.

Keywords: Corn Silk, Anti-Inflammatory, Antioxidant, Carrageenan-Induced Inflammation, Albino Rats.

I. INTRODUCTION

Corn silk, the thread-like styles and stigmas of the maize plant (Zea mays L.), has garnered considerable attention for its medicinal properties, particularly its anti-inflammatory and antioxidant effects. Traditionally used in herbal medicine, corn silk is rich in bioactive compounds, including polyphenols, flavonoids, glycosides, and carotenoids, which contribute to its therapeutic potential. Inflammation is a complex biological response that can lead to chronic diseases when dysregulated, often exacerbated by oxidative stress. Carrageenan-induced inflammation is a widely accepted model for studying acute inflammatory responses, as carrageenan triggers the release of pro-inflammatory mediators and promotes edema. This model allows for the evaluation of potential anti-inflammatory agents and their mechanisms of action. Recent studies have indicated that corn silk extract exhibits significant antioxidant activity, which can help neutralize free radicals and reduce oxidative damage in tissues. By exploring the anti-inflammatory and antioxidant potential of corn silk extract in carrageenan-induced inflammation in albino rats, this research aims to provide insights into its therapeutic benefits and underlying mechanisms. The findings could pave the way for the development of corn silk as a natural remedy for managing inflammation and oxidative stress-related disorders, contributing to the growing body of evidence supporting the use of plant-based therapies in modern medicine.

II. METHODOLOGY

Corn silk (Zea mays L.) contains alkaloids, vitamins, saponins, proteins, carbohydrates, essential minerals (Na, K, Mg, Ca), fixed and volatile oils, steroids (e.g., sitosterol, stigmasterol), tannins, and flavonoids. Inflammation arises from infections (bacteria, viruses, fungi) or conditions like tissue injury, cell death, cancer, ischemia, and degeneration. Numerous studies highlight the biological activities of corn silk's constituents.

Chemical/Kit	Drugs	Instrument
Chloroform ethanol Petroleum ether	Indomethacin Carrageenangriess reagent (1% Sulphanilamide, 2% Phosphoric acid and 0.1% Naphtyl ethylenediamine dihydrochloride) aqueous plant extract	mercury displacement Plethysmograph

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Collection and Authentication: Corn silk was obtained from the local market in Bhopal.

Physico-Chemical Analysis
Determination of Ash
The ash content of medicinal plant material is measured using three methods: total ash, acid-insoluble ash, and water-soluble ash.

- **Total Ash:** Measures the total residue remaining after ignition.
- **Acid-Insoluble Ash:** The residue obtained by boiling total ash with dilute hydrochloric acid and igniting the insoluble matter, indicating the presence of silica, such as sand and siliceous earth.
- **Water-Soluble Ash:** The difference in weight between total ash and the residue after treating total ash with water.

Total Ash:
Weigh 2-4g of dried material in a pre-ignited crucible. Ignite at 500-600°C until the ash turns white. Cool in a desiccator and weigh. If carbon remains, add ~2 mL of water or saturated ammonium nitrate, dry, and re-ignite to constant weight. Cool for 30 minutes and weigh promptly. Calculate the total ash in mg per g of air-dried material.

Tatal ash % = $\frac{(W_2 - W_1)}{W} \times 100$

Where W = Weight of sample, W_1 = Weight of empty crucible W_2 = Final weightof crucible.

Acid-Insoluble Ash Determination:
Add 25 mL of dilute HCl to the crucible with total ash, cover, and gently boil for 5 minutes. Rinse the watch glass with hot water and add the rinse to the crucible. Collect the insoluble matter on ashless filter paper and wash until the filtrate is neutral. Transfer the filter paper with residue back to the crucible, dry on a hot plate, and ignite to a constant weight. Cool in a desiccator for 30 minutes and weigh promptly. Calculate the acid-insoluble ash in mg per g of dried material.

Acid Insoluble ash % = $\frac{W_2 - W_1}{W} \times 100$
Where, W = Weight of sample, W_1 = Weight of empty crucible W_2 = Final weightof crucible.

Water-Soluble Ash Determination:
Add 25 mL of dilute HCl to the crucible with total ash and boil for 5 minutes. Collect the insoluble matter in a sintered glass crucible or on ashless filter paper, then wash with hot water. Ignite at ≤450°C for 5 minutes. Subtract the residue weight (mg) from the total ash weight and calculate the water-soluble ash in mg per g of air-dried material.

Moisture Content Determination:
Sample Preparation: Break seeds into smaller pieces without high-speed mills.
Procedure: Weigh 10 g of the drug in an evaporating dish and dry in a hot air oven at 105°C for 5 hours. Weigh and continue drying, checking at 1-hour intervals until the weight difference is ≤0.25%. Constant weight is reached when two weighings after 30 minutes in a desiccator differ by no more than 0.01 g. Calculate the percentage loss on drying based on the air-dried drug.

Table 1: Evaluation of crude drug

S.No	Parameter of crude drug	Result% (w/w)
1	TotalAsh	5.87
2	Acidinsolubleash	2.5
3	Watersolubleash	1.7
4	Moisturecontent	7.98

Extraction Method: Corn silk was dried at room temperature (24.2 ± 1.0°C). Aqueous extraction involved adding 100 mL of boiling water to 10 g of corn silk for 20 minutes, followed by centrifugation at 12,000 × g for 30 minutes at 4°C. The supernatant was lyophilized to form a powder.
Aqueous Extract: The marc from the aqueous extraction was dried in a hot air oven at below 50°C, then packed into a percolator. Cold maceration in distilled water for three days yielded an aqueous extract, which was collected and concentrated by evaporation in a water bath. The extract was distilled under reduced pressure to remove the solvent, dried, and stored in a desiccator until experimentation. The yield was

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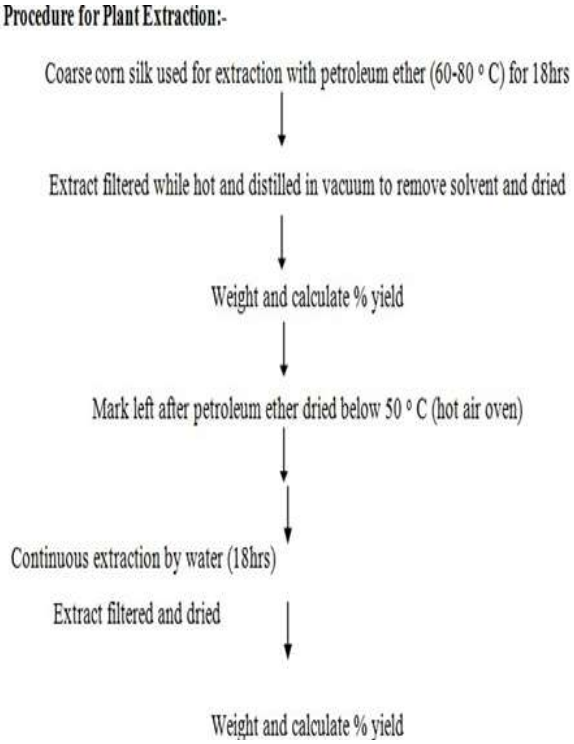
weighed, and the percentage yield was calculated based on the air-dried powdered crude material.

Procedure for Plant Extraction



III. RESULT & DISCUSSION

Phytochemical Analysis

Tests for Alkaloids: To test for alkaloids, dissolve 0.5 g of leaf extract in 10 mL of dilute hydrochloric acid (0.1 N) and filter the solution. The filtrate will be used for the following tests:

- **Mayer's Test:** Add Mayer's reagent. A yellow cream-colored precipitate indicates alkaloids. **Mayer's Reagent:** Mix 1.36 g of mercuric chloride in 60 mL of distilled water with 5 mg of potassium iodide in 20 mL of distilled water, then adjust the volume to 100 mL.
- **Dragendorff's Test:** Add Dragendorff's reagent. A reddish-brown precipitate indicates alkaloids.
  Dragendorff's Reagent: Dissolve 8 g of bismuth nitrate in 20 mL of nitric acid and 27.2 g of potassium iodide in 50 mL of distilled water, then combine and adjust the volume to 100 mL.
- **Wagner's Test:** Add Wagner's reagent. A reddish-brown precipitate indicates alkaloids. **Wagner's Reagent:** Dissolve 1.27 g of iodine and 2 g of potassium iodide in 5 mL of water, then dilute to 100 mL.
- **Hager's Test:** Add Hager's reagent. A yellow precipitate indicates alkaloids.
- **Hager's Reagent:** Use a saturated solution of picric acid in distilled water.

Tests for carbohydrates

Molisch's Test: Add a few drops of Molisch's reagent to the filtrate, then carefully add concentrated H_2SO_4. After standing for two minutes, dilute with 5 mL of distilled water. A red or dull violet color at the interface indicates a positive result.
Molisch's Reagent: 10 g of α-naphthol in 100 mL of 95% alcohol.
Barfoed's Test: Dissolve 1 mL of the extract in distilled water and filter. Mix 1 mL of the filtrate with 1 mL of Barfoed's reagent and heat for 2 minutes. A reddish precipitate of cuprous oxide indicates a positive result.
Barfoed's Reagent: 13.3 g of crystalline neutral copper acetate in 200 mL of 1% acetic acid.
Fehling's Test: Heat 1 mL of the filtrate with 5 mL of Fehling's solution A and B. A red precipitate of cuprous oxide indicates reducing sugars.

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Benedict's Test: Add a few drops of Benedict's reagent to the filtrate and boil. A reddish-brown precipitate indicates reducing sugars.

Tests for Glycosides:
Hydrolyze 0.5 g of leaf extract with 20 mL of dilute HCl (0.1 N) and filter.

- **Modified Borntrager's Test:** Add ferric chloride to the filtrate, heat, cool, and shake with benzene. A rose pink or cherry red color in the ammonical layer indicates anthraquinones.
- **Keller-Killiani Test:** Shake the filtrate with glacial acetic acid and ferric chloride, then transfer to concentrated sulfuric acid. A reddish-brown layer with bluish-green indicates digitoxose.
- **Legal Test:** Add sodium nitroprusside to the corn seed extract in pyridine. A pink or red color indicates cardiac glycosides.
- **Baljet Test:** Treat 1 mL of the filtrate with sodium picrate reagent. A yellow to orange color indicates cardiac glycosides.

Tests for Protein and Amino acids:
Millon's Test: Combine 2 mL of filtrate with 2 mL of Millon's reagent and heat in a water bath for 5 minutes. Cool and add a few drops of $NaNO_2$. A white precipitate that turns red upon heating indicates proteins and amino acids.
Millon's Reagent: Mercury in nitric acid (mercuric and mercurous nitrates).
Ninhydrin Test: Add 0.25% Ninhydrin reagent to 2 mL of filtrate and boil for 2 minutes. A blue color indicates the presence of amino acids.
Ninhydrin Reagent: 0.25% ninhydrin in n-butanol.

Tests for Phytosterols and Triterpenoids
Liebermann's Test: Add 2 mL of filtrate to a test tube and mix in 2-3 drops of acetic anhydride. Gently heat the mixture, then cool and add a few drops of concentrated sulfuric acid from the side of the test tube. A blue color indicates the presence of sterols.
Libermann-Burchard Test: Dissolve 2 mL of filtrate in chloroform, add a few drops of acetic anhydride, and then introduce concentrated sulfuric acid from the side. A brown ring at the junction of the layers, along with a green upper layer, indicates sterols, while a deep color indicates triterpenoids.
Salkowski Test: Mix 2 mL of filtrate with 2 mL of chloroform and add 2 mL of concentrated sulfuric acid from the side of the test tube. Shake for a few minutes. A red color in the chloroform layer indicates sterols, while a yellow lower layer indicates triterpenoids.

Tests for Phenolic Compounds and Tannins
Ferric Chloride Test: Add a few drops of 1% ferric chloride solution to 2 mL of filtrate. A blue-black, green, or blue-green precipitate indicates the presence of tannins.
Lead Acetate Test: Combine 2 mL of filtrate with a few drops of lead acetate solution in a test tube. Formation of a yellow precipitate indicates the presence of tannins.
Tests for Flavonoids
Shinoda's Test: Dissolve 0.5 g of the sample in ethanol, warm, and filter. Add a few magnesium chips and a few drops of concentrated HCl. A pink, orange, or red to purple coloration indicates the presence of flavonoids.
Alkaline Reagent Test: Add a few drops of sodium hydroxide to the test solution. Formation of a yellow color that turns colorless upon the addition of dilute hydrochloric acid indicates the presence of flavonoids.

Test for Saponins
Foam Test: Dilute 1 mL of leaf extract with distilled water and shake vigorously for about 5 minutes. Persistent frothing upon warming indicates the presence of saponins.

Table 2: Phytochemical tests of aqueous extract of Corn Silk

S.No.	ChemicalTest	Inference
1.	**TestsforAlkaloids**	
	Mayer'sreagent:	Positive
	Dragendroff'sreagent:	Positive
	Wagner'sreagent	Positive
	Hager'sreagent	Positive
2.	**Testsforcarbohydrates**	
	Molisch'stest	Positive

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	Barfoed'stest	Positive
	Fehling'stest	Positive
	Benedict'stest	Positive
3.	**TestsforGlycosides**:	
	ModifiedBorntrager'stest	Positive
	Kellerkilliani'stest	Positive
	Legaltest	Positive
	Baljettest	Positive
4.	**TestsforProtein**	
	Millon'stest	Positive
5.	**Aminoacids:**	
	Ninhydrintest	Positive
6.	**TestsforphytosterolsandTriterpenoids**	
	Liebermann'sTest	Positive
	Libermann-Burchardtest	Positive
	SalkowaskiTest	Positive
7.	**TestforPhenolicandTannins**	
	FerricChlorideTest	Positive
	LeadacetateTest	Positive
8.	**Testforflavonoids**	
	Shinoda'stest	Positive
	Alkalinereagenttest	Positive
9.	**TestsforSaponins**	
	FoamTest	Positive

Pharmacological Activity

Dose Selection:

Doses were based on OECD guidelines 423, with significant effects at 1000 and 2000 mg/kg. The 1/10 doses calculated were 100 and 200 mg/kg.

Group Descriptions:

- **Group 1:** Positive control (Vehicle treated)
- **Group 2:** Negative control (Disease induced)
- **Group 3:** Standard (Indomethacin 10 mg/kg)
- **Group 4:** Test Group I (Aqueous extract 100 mg/kg)
- **Group 5:** Test Group II (Aqueous extract 200 mg/kg)

Study Model: Carrageenan-Induced Paw Edema

Carrageen an, introduced by Winter et al. in 1962, induces edema by releasing histamine, serotonin, and prostaglandins. To induce paw edema, 0.1 mL of 1% w/v carrageenan was injected into the left hind paw of each rat, divided into four groups of six.The effects of 200 and 400 mg/kg of Ficus virens extract, 40 mg/kg ibuprofen, or saline (10 mL/kg) on edema were evaluated. Swelling was measured at 0, 1, 2, and 3 hours using a plethysmometer. Animals were treated with the extract one hour before carrageenan injection, and percent inhibition was calculated against the vehicle control (100%).

Discussion

The aqueous extract of corn silk exhibited significant anti-inflammatory effects on carrageenan-induced hind paw edema. At doses of 100 and 200 mg/kg, the extract significantly reduced inflammation, with results comparable to the control group. The maximum effect was observed at 400 mg/kg, yielding a 66.46% inhibition of edema. The 200 mg/kg dose also showed substantial inhibition, starting at 48% after 4 hours and increasing to 52%. While the extract demonstrated significant anti-inflammatory activity ($p<0.001$) compared to the control, it was less effective than indomethacin ($p<0.001$). Overall, the findings suggest that corn silk extract effectively reduces paw edema, particularly at the higher dose.

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Table 3: Carrageenan-Induced Paw Edema in rats

GROUP	Pawthicknessinmm				
	0 hr	1 hr	2 hr	3 hr	4 hr
Group-I Carrageen an (control)	1.8±0.03	3.7±0.06	4.6±0.05	6.5±0.06	4.6±0.03
Group-II Indometh acine (10mg/kg)	1.7±0.04	2.4±0.04**	2.8±0.05**	3.3±0.02**	2.3±0.03**
Group- III(100mg /kg)	1.2±0.02	3.3±0.03	4.3±0.05	4.5±0.02*	3.4±0.05**
Group- IV (200mg /kg)	1.3±0.01	2.6±0.05**	3.7±0.06*	3.4±0.05**	2.5±0.05**

Values were mean ± SEM, (n=6), *P<0.05, **P<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

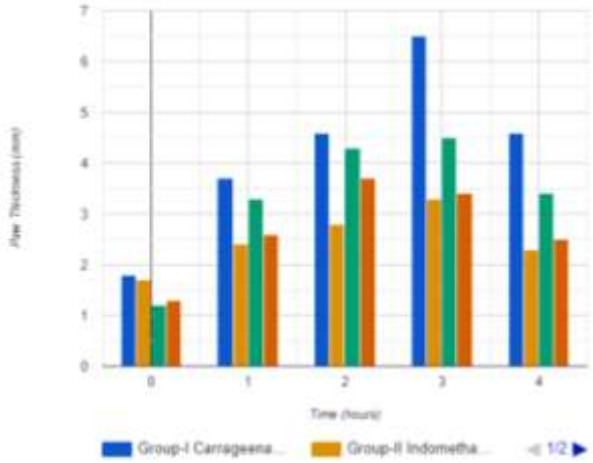

Fig. 1 Carrageenan-Induced Paw Edema in Rats

Nitric Oxide Scavenging Activity

The aqueous extract of corn silk was evaluated for free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is a violet free radical that turns yellow when scavenged by antioxidants in the sample. Stock solutions (1.0 mg/ml) were diluted to 50, 100, 200, 600, 800, and 1000 µg/ml. One milliliter of 0.3 mM DPPH solution was added to 2.5 ml of each sample concentration and reacted at room temperature for 30 minutes. Absorbance was measured at 518 nm using a UV-Vis spectrophotometer.

Discussion

Nitric oxide scavenging activity was assessed by mixing sodium nitroprusside (5 mM) in phosphate buffer with varying concentrations of the extract (25–800 µg/ml) and incubating at 25°C for 30 minutes, with a control group present. The aqueous extract of corn silk exhibited significant free radical scavenging effects against nitric oxide in a concentration-dependent manner. The reduction in DPPH absorbance confirmed its radical scavenging ability, with an IC_{50} value of 60 mg/ml.

Table 4: Anti-oxidant activity using Nitric Oxide scavenging activity

S. No	Concentration(mg/ml)	% inhibition
1	20	32
2	40	46
3	60	50
4	80	54
5	100	60

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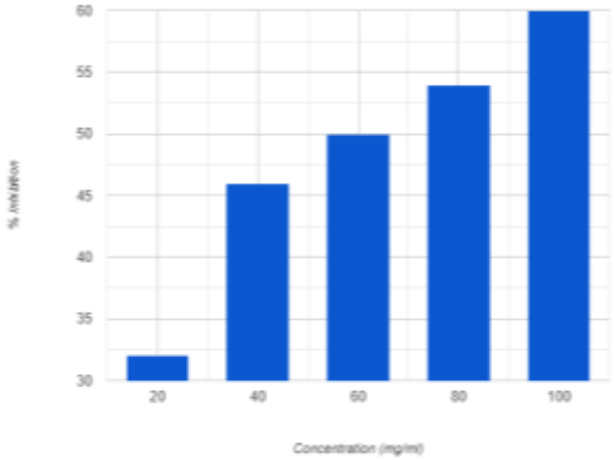

Fig. 2 Anti-oxidant Activity using Nitric Oxide Scavenging Activity

ABTS Assay

The ABTS+ radical (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)) was prepared by mixing 5 ml of 4.9 mM ammonium persulfate with 5 ml of 14 mM ABTS solution and incubating in the dark for 16 hours. This solution was diluted with 99.5% ethanol to achieve an absorbance of 0.70 ± 0.02 at 734 nm. For the assay, 950 µl of ABTS radical solution was mixed with 50 µl of extract solutions (25-500 µg/ml) and vortexed for 10 seconds. After 6 minutes, absorbance was measured at 734 nm against the control.

Discussion

The aqueous extract of corn silk demonstrated strong antioxidant properties by effectively scavenging ABTS radicals, with an IC_{50} value of 60 µg/ml.

Table 5: Anti-oxidant activity using ABTS assay

S. No	Concentration(mg/ml)	% inhibition
1	20	18
2	40	33
3	60	50
4	80	53
5	100	62

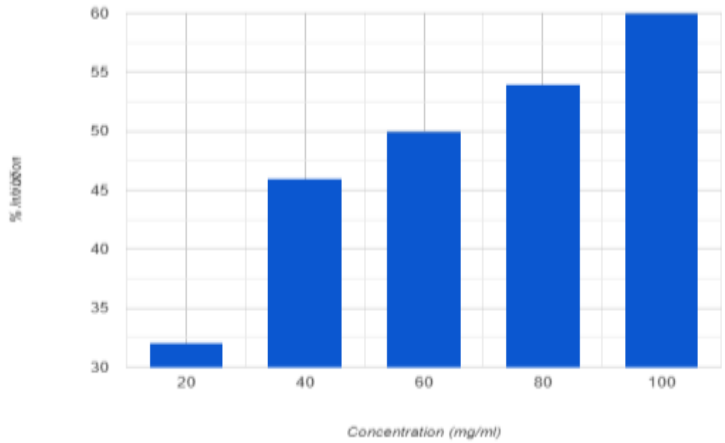

Fig. 3 Anti-oxidant Activity using Nitric Oxide Scavenging Activity

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Statistical Analysis

Statistical analysis will be conducted using standard methods. Results will be expressed as mean ± SEM. Data groups will be compared using analysis of variance (ANOVA), followed by Dunnett's t-test to determine statistical significance.

IV. CONCLUSION

In conclusion, corn silk (Stigma maydis) is a highly valued medicinal plant, renowned for its rich array of bioactive phytochemicals, including polyphenols, flavonoids, and carotenoids. These compounds confer significant antioxidant and anti-inflammatory properties, making corn silk a staple in traditional medicine for treating various ailments. The presence of active ingredients such as tannins, phenols, and flavonoids underscores its diverse pharmacological activities, including antioxidant, anti-inflammatory, antidiabetic, and antibacterial effects. Notably, research indicates that corn silk can activate receptors associated with human peroxisome proliferator-activated receptors, highlighting its potential in managing diabetes. Given its high antioxidant capacity, corn silk shows promise in mitigating oxidative stress and providing therapeutic options for a range of diseases. Overall, the findings support the utilization of corn silk extract as a valuable resource in both anti-inflammatory and antioxidant applications.

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