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ANTIOXIDANT, ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF HYDROALCOHOLIC EXTRACT OF CORN SILK IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objective: The hydroalcoholic extract of corn silk (Zea mays L.) was evaluated phytochemical constituents and its in vitro antioxidant, in vivo analgesic and anti-inflammatory activities.

Materials and Methods: Hydroalcoholic solvent was used to extract corn silk, and qualitative phytochemical screening was conducted to know the bioactive constituents present in corn silk. Several in vitro assays were performed to determine the antioxidant potential, namely DPPH free radical scavenging and metal chelating assays. In vivo, the analgesic activity was assessed in the hot plate test and anti-inflammatory activity was measured in carrageenan-induced paw edema model. The extract pharmacological effects were paralleled with the standard drugs, pentazocine (analgesic) and indomethacin (anti-inflammatory).

Results: Phytochemical analysis revealed the presence of flavonoids, tannins, terpenoids, steroids and carbohydrates in hydroalcoholic extract of corn silk. The antioxidant activity of the extract was very high in the DPPH assay and metal chelating assays. It also exhibited appreciable anti-inflammatory and antinociceptive properties in experimental animals, mostly at 200 mg/kg. The activities observed were similar to the standard drugs, pentazocine and indomethacin.

Conclusion: The hydroalcoholic extract of corn silk shows a high antioxidant, analgesic and anti-inflammatory activity, likely due to the high content of phytochemicals. The results indicate that corn silk may be a good source of therapeutic agents in the natural world. Another research is required to identify and purify the bioactive compounds and find their mechanisms of action.

KEYWORDS: Corn silk, Phytochemical analysis, Biological activities, Antioxidant, Traditional medicine

INTRODUCTION

Inflammation is a multifaceted biological reaction of the immune system to detrimental stimuli, including viruses, injuries, or irritants. Acute inflammation serves as an essential protective mechanism; however, persistent inflammation may result in numerous severe ailments, such as arthritis, cardiovascular diseases, and neurological disorders. Although several synthetic anti-inflammatory medications are accessible, many are linked to considerable adverse effects, including gastrointestinal discomfort and organ harm. This has heightened the pursuit of natural alternatives that have potent anti-inflammatory properties and minimal adverse effects. In this context, phytochemicals have garnered considerable attention as a source of safe and effective therapeutic compounds (Vikas et al.2025, Chen et al. 2017).

Paw edema model in rats induced by carrageenan is a proven experimental technique for assessing the anti-inflammatory properties of plant extracts. This is a model of acute inflammation, which causes localized edema, triggered by the production of inflammatory mediators, including histamine, prostaglandins, and cytokines. It gives a comprehensive platform to evaluate the effectiveness of anti-inflammatory drugs and demystify their action mechanisms (Xiang et al. 2023, Azarbaijani et al. 2021).

The utilization of medicinal plants as possible therapeutic agents to relieve pain and inflammation has attracted a lot of attention in recent years. corn silk is an annual herb and is a member of the Poaceae family. It is extracted from the stigmas and style of the female flower of maize plants. corn silk is a by-product of maize cultivation and can be easily obtained. It is known to be rich in phenolic compounds, particularly flavonoids (Maksimovic et al. 2005). Macronutrients (carbohydrates, fats, protein, fibres) compose corn silk in addition to vitamins, minerals and bioactive compounds (Liu et al. 2011, Tyskiewicz et al.2018). corn silk has a longstanding history of use in various systems of medicine and is

employed as a significant natural medicine. It has been used as a traditional medicine in various parts of the world, such as China, Turkey, the United States and France, often prepared in the form of infusions, decoctions or as a herbal preparation using a variety of parts of the plant, such as roots, leaves, stems, barks, fruit peels and immature fruits.
The aim behind this study is to examine the bioactive compounds and therapeutic effects of corn silk. The extensive research that employs numerous models to determine the effects of the extract on inflammatory biological indicators aims to confirm its traditional application and give it a scientific basis when developing it as a natural anti-inflammatory drug. This research enhances the domain of plant-based treatments and underscores the effectiveness of corn silk in the treatment of inflammatory disorders.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

The following chemicals were utilized: 2, 2-Diphenyl-1-1 Picryl hydrazyl, Glacial Acetic Acid, Sulphuric Acid, and Lead Acetate Solution, all obtained from Sigma, Mumbai. Additional reagents included Iodine Solution, Nitric acid, and Potassium Dichromate (Sigma, Mumbai), as well as Pyridine, Ammonium Molybdate, Ascorbic acid, and Sodium Phosphate (Sarabhai, Baroda). Ferrous Sulphate was sourced from SD Fine Chemical, Mumbai, while Ethylene Diamine Tetraacetic Acid and Ferrozine were acquired from Sisco, Mumbai. Sodium Nitroprusside (Sisco, Mumbai) and Fetal Bovine Serum (SRH Bioscience, Inc., USA) were also employed.

2.2 Plant Material

Corn silk material was collected from Aeswarg Creations, an Amazon seller based in Bareilly, Uttar Pradesh, with the order number 405-4102769-0801966. The plant sample was authenticated by Dr. Praveen Kumar Joshi, Plant Taxonomist, Department of Botany, Shri Narayan Prasad Awasthi Government Ayurved College, Raipur, Chhattisgarh, India. After collecting the authentication certificate, the extraction procedure was performed.

2.3 Preparation of Extract

The plant material was air-dried and extracted using a hydroalcoholic solvent (ethanol: water, 50:50 v/v) in a Soxhlet apparatus for 8 hours at 55–60°C. The resulting extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at 44 ± 1°C using a rotary evaporator (IKA® RB 10, India). The concentrated extract was then lyophilized using a freeze dryer (Thermo Fisher, Germany; Thermo Heto LL 3000). The lyophilized plant powder was stored at 4°C until further use (Hamoudi et al. 2021).

2.4 Experimental animals

Wistar rats (125–150 g) were used for in vivo studies. The animals were obtained from Ghosh Enterprises, Kolkata, and Registration No 1433/TO/D/11/CPCSEA. To start the experiment, the animal was kept for one week in the pharmacology research laboratory. Under the environmental condition (12:12 h day/light cycle at 23±2ºC), animals were kept in groups of eight per cage and administered an accepted pellet diet and water given ad libitum, followed by 12 hour before the experiment, the food was withdrawn. All preliminary protocols were reviewed and approved by the Animal Ethics Committee of Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg (C.G.) (Approval No.: SRIP/IAEC/2020-21/06).

2.5 Phytochemical Studies

A qualitative phytochemical analysis of plant extracts was performed to ascertain the principal classes of compounds (cardiac glycosides, alkaloids, tannins, phenols) contained in the extracts using standardized methods (Dethe et al. 2014, Sileshi et al. 2023).

2.6 Determination of In-Vitro Antioxidant Activity

2.6.1 Determination of DPPH Radical Scavenging Activity

The antioxidant activity of the corn silk hydroethanolic extract (CSHE) was evaluated based on its hydrogen-donating and free radical scavenging ability using the stable DPPH assay (Singh et al. 2017). As standard references, gallic acid and ascorbic acid were used. A 0.1 mM DPPH aqueous solution (3 mL) was added to various concentrations of the extract (20, 40, 60, 80, 100 and 120 ug/mL) to prepare the solution. The mixture was shaken thoroughly and then kept in a dark room for 90 minutes. The absorbance was measured at 517 nm, and all experiments were performed in triplicate. The percentage of inhibition was determined using the subsequent formula:

$$\text{Inhibition (\%)} = (A0 - A1 / A0) \times 100$$

A0 represents the absorbance of the control, while A1 denotes the absorbance in the presence of the CSHE. This activity was quantified as the IC50, which denotes the concentration of the sample necessary to block 50% of the action caused by DPPH.

2.6.2 Determination of Metal Chelating Activity

The metal chelating activity of ferrous ion ($Fe2^{+}$) was assessed using the ferrozine standard method and compared to that of EDTA (Singh et al. 2023). All tests were conducted in duplicate. EDTA served as the reference chemical. The inhibition % was determined using the subsequent formula:

$$\text{Inhibition (\%)} = (A0 - A1 / A0) \times 100$$

Where A0 is the absorbance of the control
A1: the absorbance in the presence of CSHE.

2.7 In-vivo Activity

2.7.1 Determination of Analgesic Activity by Hot Plate Method

The mentioned method was utilized to evaluate the central analgesic efficiency of corn silk extract, employing Wistar rats situated in an open-ended cylindrical apparatus having a metallic floor heated by a thermode (Hijazi et al. 2017). A plate was kept at a stable temperature of 55°C±1°C, provoking behavioral reactions measured by reaction times, such as paw licking, paw withdrawal, and jumping. All reactions were considered to be integrated at the supraspinal level. Wistar rats were placed separately on a hot plate with a cut-off time of 15 seconds to avert harm to their paws. The duration required to lick the paw or jump off the burning surface was recorded as the reaction time. Reaction times were measured at 30, 60, 90, and 120 minutes post-administration of the vehicle (distilled water 10 mL/kg), standard drug (Pentazocine 3 mg/kg), and extract dosages of 100 mg/kg and 200 mg/kg. The percentage increase in reaction time or pain threshold inhibition was calculated as follows:

$$\% \text{ elongation} = \text{latency(test)} - \text{latency(control)} / \text{latency(test)} \times 100$$

2.7.2 Determination of Anti-inflammatory activity by Carrageenan-Induced Paw Edema

The methodology was modified to evaluate the in vivo anti-inflammatory activity of corn silk extract. This method depends on the inhibition of carrageenan-induced swelling in the hind paw of mice, utilizing a plethysmometer (Basile, Italy). All animals were categorized into four groups, each comprising six rats.
Group I: Negative Control (Inflammation Control)
Group II: Positive control (administered orally with Indomethacin at a dosage of 10 mg/Kg)
Group III -IV: Samples were administered orally with corn silk dosages of 100 mg/kg and 200 mg/kg, respectively
Treatment was provided one hour before edema induction, involving the injection of 100 µl of 2.0% w/v carrageenan into the left paw of each subject. The paw volume of each rat was precisely measured using a water paleothermometer at 0, 1, 2, 3, and 4 hours following injection. The dimensions of edema were documented using A and B, with A indicating measurements obtained before the injection of the inducing medication, and B representing measures acquired after induction (Razafindrakoto et al. 2021).

Statistical analysis

All results were expressed as means ± standard deviations of triplicate measurements. For statistical analyses, the one-way ANOVA (Analysis of Variance) and Duncan's test were applied using SPSS version 17 software. The significance level was set at *P<0.05, **P<0.01 compared to control.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Studies

The preliminary evaluation of chemical components derived from corn silk hydroalcoholic extracts was conducted using a standardized approach (Singh et al. 2017). Phytochemical analyses of plant species are a crucial factor in the standardization of crude pharmaceuticals. The existence of pharmacologically active secondary metabolites suggests the potential therapeutic capabilities of the plant. The majority of these compounds are recognized for their medicinal and restorative activities in the plant. The phytochemical investigation of the hydroalcoholic extract of corn silk shows that it has carbohydrates, glycosides, tannins, flavonoids, steroids and amino acids,as shown in Table 1.

Table 1: Qualitative examination of the phytochemicals present in corn silk.

S. No.	Phytochemical components	Hydroalcoholic Extract (50:50)
1	Carbohydrate	+
2	Proteins	-
3	Alkaloids	-
4	Glycosides	+
5	Tannins & Phenolic Compound	+
6	Flavonoids	+
7	Steroids	+
8	Vitamins	-
9	Amino Acids	+

3.2 In-vitro Activity:

3.2.1 DPPH Radical Scavenging Activity

Antioxidants' capacity to donate hydrogen is what makes them effective against DPPH. Using DPPH, a stable nitrogen-centered free radical, the ability of natural antioxidants to neutralize radicals has been assessed in a much shorter amount of time than with other techniques (Prathapan et al. 2011). As illustrated in Figure 1, the scavenging activity rose as the concentration of corn silk increased up to 120 µg before nearly leveling out as the concentration grew further. The estimated range of concentrations for the DPPH radical scavenging activity was 20–120 µg/ml. The IC50 value for corn silk extract was established at 56.81µg/ml. The concentrations of the standard substances ascorbic acid and gallic acid are

2.01±1.4µg/ml and 1.55±0.8µg/ml, respectively. The antioxidant potential exhibited an inverse correlation with the observed outcomes.

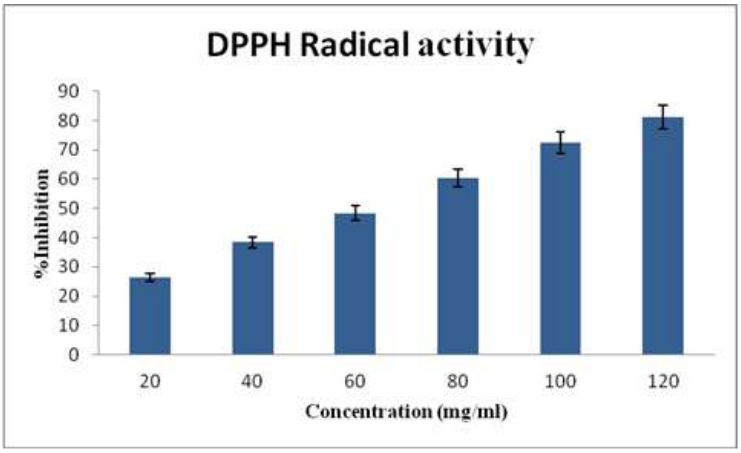

Figure 1: DPPH radical scavenging activity of CSHE.

3.2.2 Metal Chelating Assay

Ferrozine was used to measure the procedure, and the results were compared with those of EDTA. Ferrozine and Fe2+ combine to produce a complex in this process, which is quantitative in nature. The combination that formed between Fe2+ and ferrozine produced a violet color. In a dose-dependent manner, the complex's absorbance was linearly suppressed (100-600µg/ml) in Figure 2.

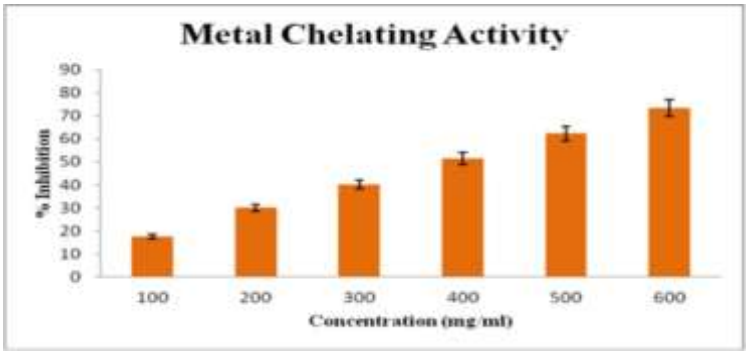

Figure 2: Metal chelating activity of the of CSHE.

At 600µg/ml, corn silk exhibited a metal chelating action of 76.26% in comparison to the reference compound of EDTA, which is 15.61±0.65µg/ml.

3.3 *In vivo* studies

3.3.1 Determination of Analgesic activity of cornsilk extract

3.3.1.1 Eddie's Hot Plate Method

Eddie's hot plate method was used to evaluate the analgesic effectiveness of a hydroalcoholic extract of corn silk in Wistar rats. At concentrations of 100 and 200 mg/kg, the hydroalcoholic extract of corn silk demonstrated notable analgesic benefits. The analgesic effect resembled that of pentazocine, a prevalent drug. At a reaction time of 120 minutes, the 200 mg/kg dosage demonstrated the greatest analgesic efficacy of the two doses (12.4±0.61), which is somewhat inferior to that of the standard medicine pentazocine (15.9±0.93) as presented in Table 2.

Table 2: Analgesic efficacy of corn silk in the hot plate assay using Wistar rats.

GROUP	Paw licking or jumping in minutes			
	30	**60**	**90**	**120**
Test-I Control	4.4±0.34	6.8±0.66	9.8±0.86	8.8±0.77
Test-II Pentazocine(3mg/kg)	5.7±0.64	10.8±0.44**	14.9±0.66**	15.9±0.93**
Test-III (100mg/kg)	3.9±0.64	6.9±0.22**	8.8±0.78**	9.6±0.81**
Test-IV (200mg/kg)	4.7±0.44	7.8±0.33**	11.2±0.62**	12.4±0.61**

Pentazocine validated centrally acting medications by significantly prolonging the reaction time of rats, accompanied by an extended duration of stimulation in this analgesic testing type. All extracts in the present study demonstrated significant analgesic effects ($p<0.05$ and $p<0.01$); however, at a reaction time of 120 minutes, the 200 mg/kg dosage exhibited the highest analgesic activity among the two dosages.

3.3.2 Determination of the inflammation of cornsilk extract

3.3.2.1 Carrageenan-Induced Paw Edema in Rats

The experimental animals were employed to evaluate the anti-inflammatory properties of corn silk hydroalcoholic extract on carrageenan-induced hind paw edema. The hydroalcoholic extract of corn silk significantly reduced carrageenan-induced inflammation at doses of 100 and 200 mg/kg, as indicated in Table 3.

Table 3: Anti-inflammatory effects of corn silk assessed by the Carrageenan-induced paw edema model in rats.

GROUP	Paw thickness in mm					%
	0 h	1h	2h	3h	4h	Inhibition at 3h
Test-I Carrageenan (control)	1.6±0.05	3.7±0.08	4.9±0.03	6.8±0.02	4.7±0.05	-
Test-II Indomethacin (10mg/kg)	1.5±0.05	2.6±0.04**	2.8±0.06**	3.5±0.03**	2.4±0.03**	57
Test-III (100mg/kg)	1.4±0.01	3.2±0.05	4.5±0.03	4.9±0.02*	3.8±0.03**	27
Test-IV (200mg/kg)	1.2±0.02	2.5±0.03**	3.8±0.03*	3.7±0.07**	3.7±0.02**	49

After three hours, the 200 mg/kg dosage demonstrated a notable inhibition of 49%, which increased to 57%, as seen in the Table. The hydroalcoholic extract of corn silk exhibited notable anti-inflammatory properties similar to indomethacin (10 mg/kg).

4. CONCLUSION

The study paper thoroughly explores the phytochemical profile, antioxidant, analgesic and anti-inflammatory properties of the hydroalcoholic extract of corn silk (Zea mays L.). The phytochemical analysis indicated the presence of flavonoids, tannins, terpenoids, steroids, and carbohydrates, which means that the extract could become a good source of bioactive compounds. The extract showed a strong in vitro antioxidant activity, as it was shown to scavenge the DPPH radical and metal chelate with DPPH and metal chelation IC50 of 56.81 µg/ml and 56.81 µg/ml, respectively. The in vivo experiment proved that it exhibits a strong analgesic activity in the hot plate test, with the highest effect observed in the highest dose (200 mg/kg) at 120 minutes and gave the highest effect of 12.4±0.61 seconds, which is similar to pentazocin. The extract was also shown to have a significant anti-inflammatory effect in the carrageenan-induced paw edema model, where 200 mg/kg gave 48-57 percent inhibition after 3 and 4 hours, respectively, similar to indomethacin. These findings indicate that the corn silk hydroalcoholic extract is a promising natural medicinal extract with antioxidant, analgesic, and anti-inflammatory effects. The presence of flavonoids and other phenolic compounds probably facilitates the pharmacological activities. The corn silk extract could be a useful alternative or supplement to the traditional methods of pain and inflammatory disease treatment, due to its historical use and the safety of its active components. It is justified to conduct further studies to isolate and identify certain bioactive chemicals, understand their action mechanisms, and investigate their clinical uses. In addition, toxicological tests and standardization need to be carried out to determine the safety and efficiency of the extract. corn silk shows great promise as a natural source of medicine compounds to treat oxidative stress, pain, and inflammation.

Conflict of Interest

The authors declare no conflict of interest.

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