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PHYTOCHEMICAL SCREENING AND EVALUATE THE ANTI-INFLAMMATORY ACTIVITY OF THE ETHANOLIC PEEL EXTRACT DERIVED FROM PUMPKINS

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ABSTRACT

This study evaluates the physicochemical and anti-inflammatory properties of pumpkin peel extract, highlighting its potential applications in pharmaceuticals and herbal formulations. The extract exhibited solid or semi-solid consistency, greenish color, and earthy aroma, with extractive values of approximately 12% w/w for ethanol and 15% w/w for aqueous solutions. Loss on drying was measured at 8.25% w/w, and the extract contained 1.25% w/w of foreign organic matter, indicating its purity and stability. Phytochemical screening revealed a diverse chemical composition. The extract demonstrated significant inhibition of albumin denaturation and heat-induced hemolysis in a concentration-dependent manner, achieving a maximum inhibition rate of 49.25% at 500 µg/ml. In a rat model, both low and high-dose formulations significantly reduced edema, while acute toxicity studies showed no mortality at administered doses of 150 mg/kg and 200 mg/kg. These findings support the extract's therapeutic potential and safety for further preclinical and clinical investigations.

Keywords: Pumpkin peel extract, anti-inflammatory activity, phytochemical screening, extractive values

INTRODUCTION

The use of natural products and plant-based remedies has a long-standing tradition in medicine, with plant-derived compounds offering significant therapeutic benefits. Among the various plant-based sources, pumpkins (*Cucurbita spp.*) have attracted attention due to their wide range of bioactive compounds, particularly in their peels, which are often discarded as waste.[1] The peel of the pumpkin, in particular, is recognized for its rich phytochemical profile, including flavonoids, tannins, alkaloids, saponins, and phenolic compounds, all of which are known to exhibit various pharmacological activities. In recent years, the investigation into the medicinal properties of pumpkin peel extracts has increased, with particular interest in their anti-inflammatory potential.[2]

Inflammation is a biological response to harmful stimuli, such as pathogens, damaged cells, or irritants, and is a protective mechanism designed to eliminate the initial cause of cell injury. However, chronic inflammation is linked to a wide range of diseases, including arthritis, diabetes, cardiovascular disorders, and cancer. As synthetic anti-inflammatory drugs are often associated with adverse effects, there is an increasing demand for safer, more effective alternatives derived from natural sources. Pumpkin peel, a waste product of pumpkin consumption, presents an appealing option due to its bioactive constituents with potential anti-inflammatory properties.[3]

Phytochemical Composition of Pumpkin Peels

Phytochemicals are naturally occurring compounds in plants that have been shown to possess therapeutic effects, contributing to their medicinal value. Pumpkin peels are particularly rich in bioactive compounds, which contribute to both their nutritional and therapeutic profiles. Research has shown that pumpkin peels contain significant amounts of flavonoids, phenolic acids, saponins, tannins, and alkaloids, which have been associated with antioxidant, antimicrobial, and anti-inflammatory properties.[4]

Flavonoids and phenolic compounds are well-known for their ability to scavenge free radicals and reduce oxidative stress, which is a key contributor to inflammation and the development of chronic diseases. For example, studies have indicated that the high phenolic content in pumpkin peel extract correlates with strong antioxidant activity, which can directly or indirectly modulate

inflammatory pathwaysponins and tannins present in pumpkin peels may inhibit the release of inflammatory mediators, such as prostaglandins and cytokines, reducing the overall inflammatory response.[4]

Anti- inflammatory mechanism of Pumpkin Peel Extract

The anti-inflammatory properties of plant extracts are largely attributed to their ability to inhibit key enzymes and mediators involved in the inflammatory process, such as cyclooxygenase (COX), lipoxygenase (LOX), nitric oxide (NO), and tumor necrosis factor-alpha (TNF-α). Ethanolic extracts are widely used for extracting these bioactive compounds due to their ability to solubilize both polar and non-polar compounds, providing a more comprehensive extract of the plant's phytochemicals.[5]

Pumpkin peel extract may inhibit the activity of enzymes like COX and LOX, which are involved in the synthesis of pro-inflammatory mediators such as prostaglandins and leukotrienes . Prostaglandins, in partiy a central role in the development of pain and swelling associated with inflammation. By inhibiting these enzymes, pumpkin peel extract could potentially reduce inflammation and its associated symptoms. Furthermore, flavonoids and other phenolic compounds present in pumpkin peel have been shown to modulate various signaling pathways involved in the inflammatory response, such as the nuclear factor-kappa B (NF-κB) pathway, which plays a crucial role in regulating the expression of inflammatory cytokines.[6]

Importance of Exploring Natural Anti-Inflammatory Agents

The search for natural anti-inflammatory agents has gained momentum due to the limitations of current synthetic drugs, which are often associated with significant side effects, such as gastrointestinal disturbances, cardiovascular risks, and immunosuppression . Nonsteroidal anti-inflammatory drugs (NSAIDs), commonly useding inflammation, are notorious for their adverse effects, particularly with long-term use. Thus, the exploration of plant-based anti-inflammatory agents is an attractive alternative for safer, long-term management of inflammatory conditions.[7]

Pumpkin peel, as an underutilized byproduct, holds immense potential not only for reducing food waste but also for providing a sustainable source of bioactive compounds with therapeutic

benefits. By harnessing the anti-inflammatory properties of pumpkin peel extract, this study aims to contribute to the growing body of evidence supporting the use of natural products in inflammation management.[8]

The primary objectives of this study are to conduct a comprehensive phytochemical screening of the ethanolic peel extract of pumpkin to identify key bioactive compounds. Evaluate the anti-inflammatory activity of the ethanolic pumpkin peel extract using established in vitro and in vivo models.

Material and Methodology

The materials used in this study included Cucurbita pepo (pumpkin), which was sourced from a botanical garden. Ethanol, methanol, sodium bicarbonate, and acetic acid were procured from Sigma Aldrich, India. Sodium chloride and catalase were obtained from the college laboratory. All other chemicals used in the study were of analytical grade, ensuring the precision and accuracy of the experimental procedures.

The instruments used in this study included a hot plate, digital caliper, micrometer, muffler furnace, vacuum evaporator, digital weighing balance, pH meter, vortex shaker, water bath, volumetric pipette, test tubes, and beaker, all sourced from the college laboratory. Additionally, an electric homogenizer (Ever Shine, Model no: 607) and a cold centrifuge machine (Remi, Model no: C-24 BL) were utilized, along with a micro-centrifuge (Remi, Model no: C-24 BL) for various experimental procedures.

Collection, Identification and Authentication of Plant Specimens

Plants are increasingly studied for their therapeutic potential due to their potent pharmacological effects, low toxicity, and cost-effectiveness. In this context, the current study focuses on evaluating the anti-inflammatory action of pumpkin, drawing on knowledge from regional vaidhyas and traditional medicine practitioners who have long used pumpkin for its medicinal properties.

Physicochemical Evaluation

Loss on drying

Mass loss during drying was measured using Mass % (m/m). The drug powder (5.6–7 g) in a Petri dish was weighed, then heated in a hot air oven at 105°C for 4-5 hours. After cooling in a desiccator, the weight loss was noted, and the process was repeated until a stable weight was reached.

Loss on drying (%) = loss in weight X 100/ W

W= weight of the drugs in grams.

Determination of Ash Value

Total ash value

A silica plate was used to weigh 2-3 grams of powdered extract. The powder was evenly spread, and heat was gradually increased to burn the carbon without exceeding dull red heat. After cooling, it was weighed. If carbon-free ash wasn't obtained, hot water was used to wash the residue, which was collected, filtered, and reheated at low temperatures. An empty silica crucible was pre-heated at 600°C for 30 minutes, weighed, and 2 grams of drug powder was added. The sample was baked in a muffle furnace at 500-600°C for 2-3 hours until white ash formed. The final weight was measured to calculate the total ash percentage in the air-dried sample:

Total ash value = (z-x/y) × 100

Where,

X = "weight of the silica crucible"

Y = "weight of the drug powder (g)"

Z = "weight of the silica cruicible with powder ash"

Acid-insoluble ash

The ash was boiled in about 30 mL of diluted hydrochloric acid for 10-15 minutes, then the insoluble material was collected, washed with hot water, and ignited. The acid-insoluble ash percentage was calculated based on the air-dried sample. After producing ash, it was mixed with 25 mL of diluted HCl, boiled for 5 minutes, and the residue was filtered. This residue was then burned in a muffle furnace at 560°C for an hour to determine the percentage of acid-insoluble ash using the air-dried sample as a reference.

Acid insoluble ash value % = (a /y) × 100

where,

A = "weight of the remaining residue"

Y = "weight of crude powder taken (g)"

Water-soluble ash

Boil the total ash in 25 mL of water for five minutes. The insoluble material is then filtered using ash-free filter paper and ignited at a low temperature to retain its weight. The weight of the water-soluble ash is calculated by subtracting the weight of the water-insoluble ash from the total ash value. The percentage of water-soluble ash is then determined in relation to the air-dried sample value.

Extractive Value

Five grams of coarsely ground, air-dried medication were macerated in a closed flask for 24 hours, with frequent shaking during the first six hours and then left to stand for 18 hours. The solvent used was a mixture of ethanol, water, and chloroform. The mixture was quickly filtered to prevent alcohol loss. Twenty-five milliliters of the filtrate were dried at 105°C in a tared shallow dish. The amount of extractive soluble in alcohol was calculated based on the air-dried drug sample. The extractive value represents the quantity of soluble components extracted by the specific solvent.

$$\text{Extractive value (\%)} = \frac{weight\ of\ residue}{weight\ of\ dry\ powder} \text{ x } 100$$

Determination of swelling index

One gram of precisely weighed powder was added to a 50 mL measuring cylinder containing water. The mixture was set aside for 24 hours, with periodic shaking. The volume of the sample was then measured after this time.

The phytochemical screening of the extract:

Phytochemical	Test	Method	Observation/Remark
Glycosides	Anthrone Test	Combine the extract with a small amount of anthrone in a watch glass. Add one drop of concentrated H_2SO_4 and heat slowly over a water bath to form a paste.	Dark green color indicates the presence of glycosides.
	Fehling's Test A	Warm the sample with 200 mg and 5 mL of diluted H_2SO_4 on a water bath. Filter the acid extract, neutralize with 5% NaOH, add 0.1 mL of Fehling's solutions A and B, and heat for two minutes.	Note the amount of red precipitate formed.
	Fehling's Test B	Warm 200 mg of the sample with 5 mL of water instead of H_2SO_4. Add the same amount of NaOH, then add Fehling's solutions A and B and heat for two minutes.	Compare the amount of red precipitate; larger in Test A indicates glycosides.
Flavonoids	Shinoda's Test	Mix 5 mL of 90% alcohol, 0.5 g of magnesium turnings, and concentrated HCl into the extract. Boil for a few minutes.	Pink or red color indicates the presence of flavonoids.
Alkaloids	Dragendorff's Test	Add a few drops of acetic acid and Dragendorff's reagent to the extract and shake well.	Orange-red precipitate indicates the presence of alkaloids.
	Mayer's Test	Mix a few drops of Mayer's reagent and diluted hydrochloric	White precipitate indicates the presence of alkaloids.

		acid with the extract.	
	Wagner's Test	Add a few drops of Wagner's reagent along the sides of a test tube containing a few milliliters of filtrate.	Reddish-brown precipitate indicates the presence of alkaloids.

Animal

Adult Wistar albino rats, weighing 150–200 grams, were housed in polypropylene cages (50 cm x 30 cm x 25 cm). Following Behringer's (1973) protocol, the animals were acclimatized to the room conditions to ensure ethical treatment. The cages and animal area were kept clean and well-ventilated, with daily removal of excreta and leftover food. The room temperature was maintained at 26 ± 2°C, and rats were fed food pellets from the Poultry Research Station in Chennai, with unlimited access to clean drinking water.

Assessment of in vitro anti-inflammatory activity

Inhibition of albumin denaturation

The inhibition of albumin denaturation method was adapted to investigate the anti-inflammatory properties of pumpkin. The test extract was mixed with a 1% aqueous solution of bovine serum albumin, and the pH was adjusted with a small amount of 1 N HCl. The mixture was heated to 51°C for 20 minutes, then allowed to cool to 37°C for an additional 20 minutes. The turbidity of the sample was measured at 660 nm using a UV-visible spectrophotometer after cooling. To ensure accuracy, the experiment was performed in triplicate:

$$\textbf{Percentage inhibition} = (\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$$

Heat induced Haemolysis

To further evaluate the anti-inflammatory effect, a reaction mixture consisting of 1 mL of a 10% RBC suspension and 1 mL of the test material at concentrations ranging from 100 to 500 µg/mL was prepared. The drug extract solution was added to the test sample tubes, while saline was

used as a control. Diclofenac was administered as a standard medication. After incubating the tubes at 56°C for 30 minutes in a water bath, they were cooled with running tap water. The tubes were then centrifuged for five minutes at 2500 rpm.

Percentage inhibition = (Abs control - Abs sample) × 100 / Abs control

Acute toxicity studies

The rat paw edema technique with carrageenan was used to assess the anti-inflammatory efficacy of the formulations. A 0.1 mL injection of a 1% carrageenan suspension was given to the right hind paws of the rats, effectively predicting anti-inflammatory activity with minimal instrumentation.

Anti-inflammatory activity

Carrageenan induced rat paw edema model

The rat paw edema technique induced by carrageenan was employed to evaluate the anti-inflammatory efficacy of the formulations. A 0.1 mL injection of a 1% carrageenan suspension was administered to the right hind paws of the rats. This method effectively predicts anti-inflammatory activity with minimal instrumentation.

Table : Groups and dose in Carrageenan induced rat paw edema method

S.NO	Group	Gel composition and dose received
1	Control group	Received 2g (gel base only)
2	Treated group Formulation low dose	Received 150 mg/kg drug extract
3	Treated Group Formulation high dose	Received 200 mg/kg drug extract
4	Standard drug group	Received 2g standard diclofenac

RESULTS

Physical Test of Crude Drugs

The organoleptic properties of pumpkin peel extract were evaluated, revealing a solid or semi-solid consistency and a greenish color, indicating chlorophyll and plant pigments. It had an earthy aroma and a distinct flavor, highlighting its unique sensory profile. These characteristics offer valuable insights for potential applications in culinary, cosmetic, or pharmaceutical formulation.

Table: The Organoleptic properties of the plant extract were evaluated for appearance, colour and taste.

Crude drugs	**Physical Test**			
	Nature	**Colour**	**Odour**	**Taste**
Pumpkin peel Extract	Solid, or Semi-solid	Greenish	It smell earthy	Distinct flavor

Extractive Values

The extractive values of pumpkin peel extract were determined for ethanol and aqueous solutions, revealing approximately 12% w/w for ethanol and 15% w/w for aqueous extraction.

Table : The Extractive Values of the plant extract were evaluated for ethanol and aqueous solutions.

Crude drugs	**Ethanol % w/w**	**Aqueous % w/w**
Pumpkin peel Extract	12	15

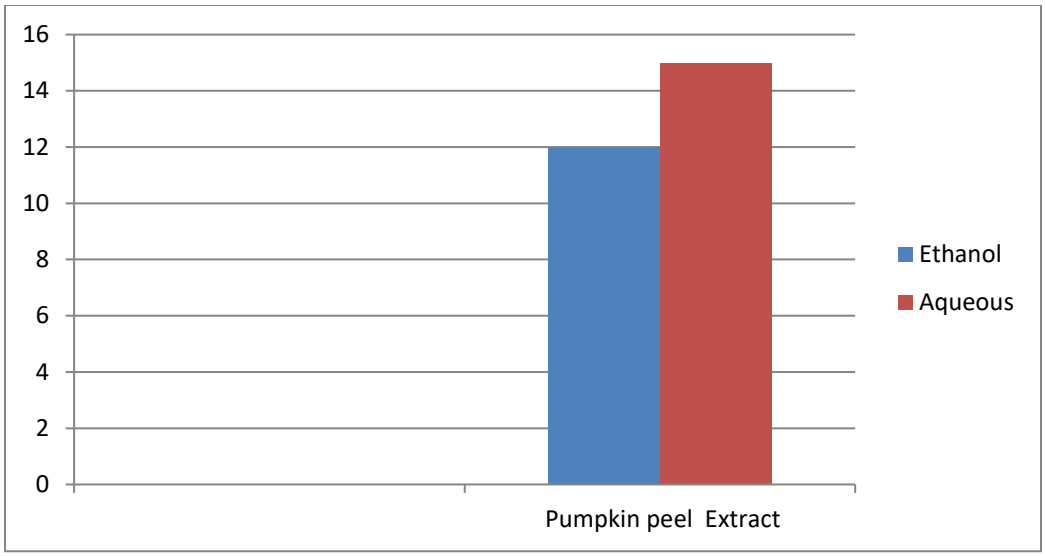

Fig: The Extractive Values of the plant extract were evaluated for ethanol and aqueous solutions.

Loss on Drying and Foreign Organic Matter

The loss on drying and foreign organic matter content of the pumpkin peel extract were evaluated as follows:

Table : Loss on Drying And Foreign Organic Matter

Crude drugs	**Loss on drying (% w/w)***	**Foreign matter (% w/w)***
Pumpkin peel Extract	8.25	1.25

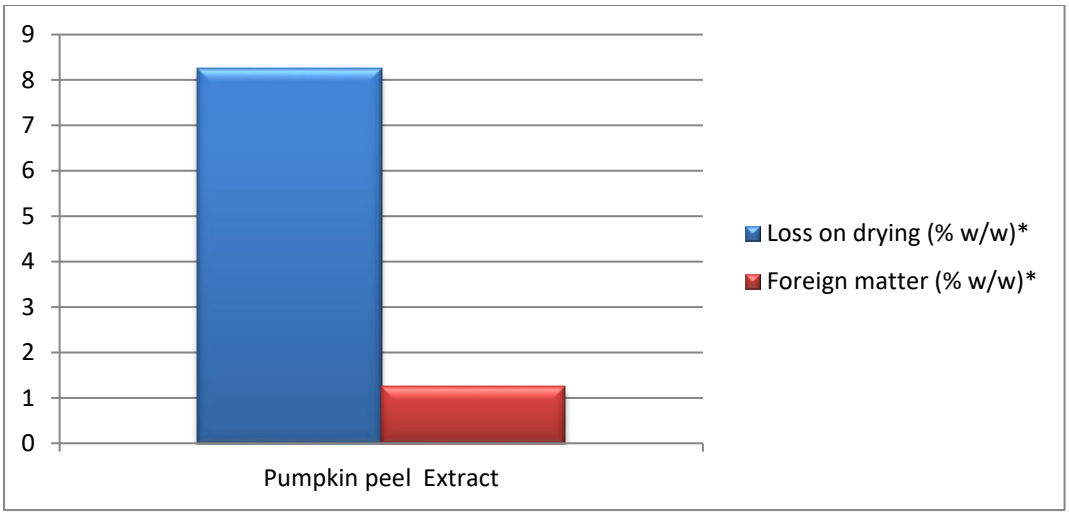

Fig: Loss on Drying and Foreign Organic Matter

Total Ash, Acid Insoluble Ash and Water Soluble Ash Values

In the evaluation of the pumpkin peel extract, significant parameters such as total ash, water-soluble ash, and acid-insoluble ash values were determined to ensure its quality and purity.

TABLE 5.4: Total Ash, Acid Insoluble Ash & Water Soluble Ash Values

Crude drugs	**Total ash value*** **% w/w**	**Water soluble ash*** **% w/w**	**Acid insoluble ash value*** **% w/w**
Pumpkin peel Extract	10.05	8.15	5.15

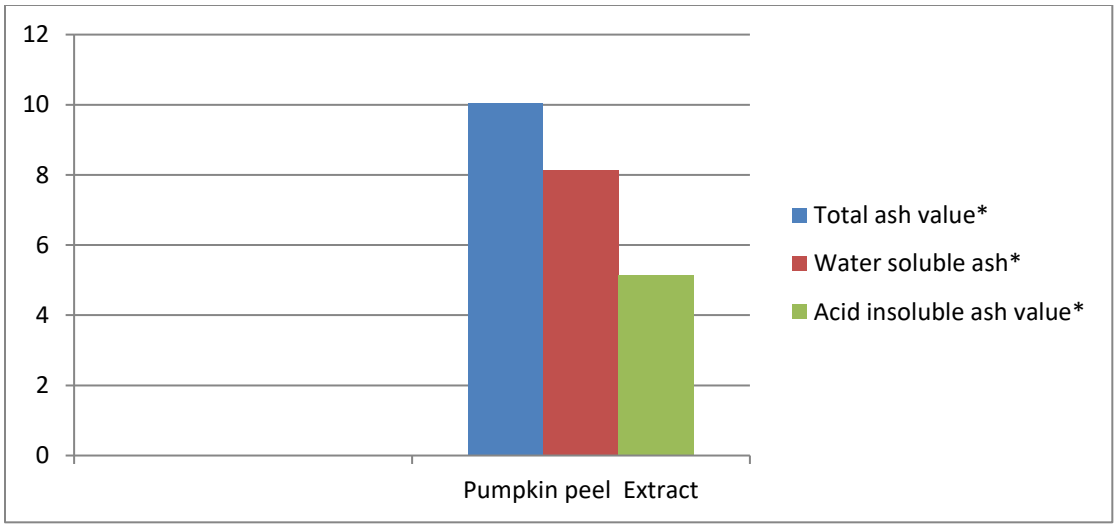

Fig: Total Ash, Acid Insoluble Ash and Water Soluble Ash Values

Phytochemical Screening

Phytochemical screening was conducted on the extract obtained from Pumpkin Peel Extract to identify the presence or absence of various classes of phytochemical compounds.

Table 5.4: Phytochemical screening for extract of leaves Extract

S.No	Chemical Tests	*Pumpkin peel Extract*
		Ethanol
1.	Tests for Steroids and Triterpenoids:	
	• Liebermann's Burchard Test	+
	• Salkowski Test	+
2.	Test for Saponins:	
	• Foam Test	-
3.	Tests for Alkaloids:	
	• Hager's Test	+
	• Mayer's Test	+
4.	Tests for Glycosides:	
	• Borntrager's Test	-
	• Keller Killiani Test	-
5.	Tests for Tannins and Phenolic compounds:	
	• Gelatin Test	-
	• Ferric Chloride Test	-
6.	Tests for Flavonoids:	
	• Ferric chloride Test	+
	• Alkaline reagent Test	+
7.	Tests for Proteins:	
	• Biuret Test	+
	• Xanthoproteic Test	+
8.	Test for Carbohydrates:	
	• Fehling Test	+

"+"Found

"-" Not Found

Pharmacological Screening

Inhibition of albumin denaturation

The study investigated the impact of ethanol drug extract on the inhibition of albumin denaturation, a process often associated with inflammation and various pathological conditions. The results, outlined in the table, demonstrate the efficacy of different concentrations of the ethanol drug extract in inhibiting albumin denaturation.

Table: Effect of Ethanol drug extract on inhibition of albumin denaturation

S. No	Sample	Concentration (µg/ml)	Absorbance at 660nm	% inhibition
1	Control	-	0.366	-
2	FOMRULATION -1	100	0.271	26.05
3	FOMRULATION -2	200	0.241	35.25
4	FOMRULATION -3	300	0.231	38.88
5	FOMRULATION -4	400	0.201	46.18
6	FOMRULATION -5	500	0.190	49.25
7	Diclofenac	100	0.130	65.08

n = 3, Values are expressed as ± SEM

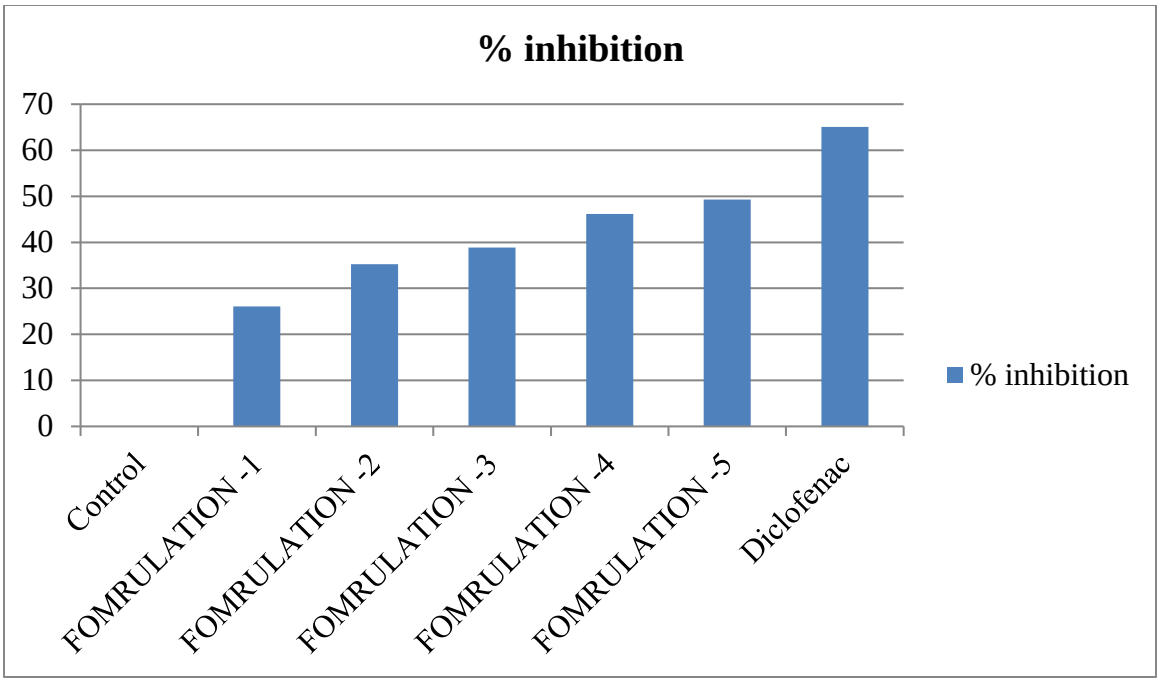

Fig: Effect of Ethanol drug extract on inhibition of albumin denaturation

Heat induced Haemolysis

The investigation assessed the influence of an ethanol drug extract on the heat-induced haemolysis of erythrocytes, a process relevant to understanding its potential therapeutic effects, particularly in conditions involving erythrocyte damage or hemolysis. The experimental setup involved subjecting erythrocytes to heat-induced stress and measuring the absorbance at 660nm, indicative of haemolysis, in both control and treated samples.

Table 5.6: Effect of Ethanol drug extract on heat induced haemolysis of erythrocyte

S. No	Sample	Concentration (µg/ml)	Absorbance at 660nm	% inhibition
1	Control	-	0.356	-
2	FOMRULATION -1	100	0.254	27.65
3	FOMRULATION -2	200	0.240	33.50
4	FOMRULATION	300	0.221	38.02

	-3			
5	FOMRULATION -4	400	0.210	40.05
6	FOMRULATION -5	500	0.202	44.05
7	Diclofenac	100	0.114	65.57

n = 3, Values are expressed as ± SEM

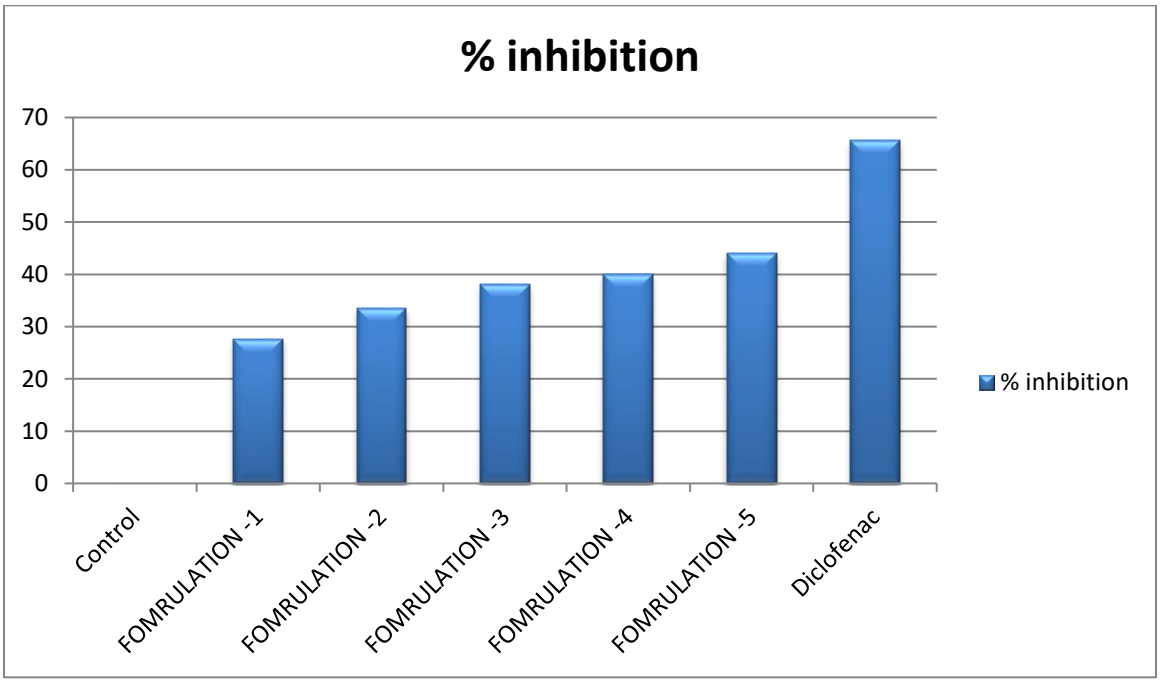

Fig: Effect of Ethanol drug extract on heat induced haemolysis of erythrocyte

In vivo study

Based on in vitro anti-infflamatory study of gel containing ethanol. low dose formulation and high dose formulation was selected containing methanol drug extract.

Carrageenan induced rat paw edema method

The investigation assessed the efficacy of a formulation in an anti-inflammatory study using a carrageenan-induced rat model. This model is commonly utilized to evaluate potential anti-inflammatory agents due to its relevance to human inflammatory conditions.

Table: The effect of formulation for anti-inflammatory study carrageenan induced rat model

S.No	Groups	Mean percentage increase in paw weight	% reduction of edema (1 hr)	% reduction of edema (2 hrs)	% reduction of edema (3 hrs)	% reduction of edema (4 hrs)
1	Control group	32.5	0	0	0	0.0
2	Treated group low dose	12.30	19.11	35.31	38.12	42.01
3	Treated Group high dose	14.42	21.65	22.34	33.42	40.40
4	Standard drug group	18.05	25.55	30.05	39.53	45.31

Acute toxicity studies

These results indicate that neither formulation induced any acute toxicity or adverse effects at the administered doses. Therefore, based on these findings, both the low and high dose formulations can be considered safe for further exploration and evaluation in preclinical and potentially clinical studies.

Table: Acute toxicity studies

S.No	**Treatment**	**Dose mg/kg**	**No. of animals**	**Mortality**			**Toxicity profile**
				7 Days	**14 days**	**21 days**	

1	**Low dose formulation**	**150**	**5**	**0**	**0**	**0**	**Safe**
2	**High dose formulation**	**200**	**5**	**0**	**0**	**0**	**Safe**

CONCLUSION

In conclusion, the pumpkin peel extracts exhibits promising sensory and extractive properties, indicating its potential for pharmaceutical and herbal applications. The anti-inflammatory studies revealed that the extract effectively inhibits albumin denaturation and erythrocyte hemolysis in a concentration-dependent manner, with significant reductions in edema observed in rat models. Additionally, acute toxicity tests demonstrated a favorable safety profile for both low and high-dose formulations, suggesting their viability for further preclinical and clinical investigations.

Acknowledgment

I would like to express my sincere gratitude to all those who contributed to this study.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study.

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