DESIGN AND BIOLOGICAL EVALUATION OF BIOAVAILABLE POTENT AND SELECTIVE HISTAMINE INHIBITOR FOR INFLAMMATION FROM PUMPKIN PULP

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Abstract

Inflammation is a critical biological response to harmful stimuli, playing a central role in various diseases. Histamine, a biogenic amine produced by the decarboxylation of histidine, is a key mediator in inflammatory and allergic reactions. The inhibition of histamine activity offers a potential therapeutic approach for managing inflammation. This study focuses on the design and biological evaluation of bioavailable, potent, and selective histamine inhibitors derived from pumpkin pulp (Cucurbita maxima). The research commenced with the collection and authentication of pumpkin pulp, followed by the extraction and isolation of bioactive compounds using ethanol as a solvent. Phytochemical screening revealed the presence of flavonoids, polyphenols, and other bioactive compounds. Selected compounds with high binding affinity were synthesized and characterized using techniques such as NMR and FTIR spectroscopy. The synthesized compounds were evaluated for their histamine inhibition activity through in vitro assays, demonstrating significant inhibitory effects. This study successfully identifies and characterizes novel histamine inhibitors from pumpkin pulp, highlighting their potential as therapeutic agents for inflammation. The findings provide a foundation for future research and development of plant-based anti-inflammatory drugs.

Keywords: Histamine inhibitors, Pumpkin pulp, Inflammation, Bioavailability, Phytochemical screening

Introduction

Inflammation is the body's response to harmful stimuli and is essential for healing. Histamine plays a key role by increasing vascular permeability, inducing vasodilation, and attracting immune cells. However, chronic inflammation can lead to diseases like allergies, asthma, rheumatoid arthritis, and cardiovascular diseases, making the development of effective anti-inflammatory agents important **[1]**. Current treatments, such as NSAIDs and corticosteroids, have adverse side effects, especially with long-term use. This has led to interest in natural products for their anti-inflammatory compounds and favorable safety profiles. Pumpkin pulp (Cucurbita maxima) is rich in phytochemicals, including flavonoids, polyphenols, and vitamins, and has shown health benefits. Studies suggest that pumpkin pulp extracts have anti-

inflammatory properties, but the specific mechanisms and compounds are not well understood. Notably, research on pumpkin-derived selective histamine inhibitors is limited **[2-3]**. This study aims to design and evaluate bioavailable, potent, and selective histamine inhibitors from pumpkin pulp. By targeting histamine, a key inflammation mediator, we hope to offer a new therapeutic approach for inflammatory diseases. The study includes extraction and characterization of bioactive compounds from pumpkin pulp, in silico screening, synthesis, and biological evaluation of their anti-inflammatory activity. **[4-5]**

We hypothesize that pumpkin pulp contains compounds with significant histamine inhibitory activity that can be developed into effective anti-inflammatory agents. The research objectives are to

(1) identify and isolate bioactive compounds from pumpkin pulp,
(2) evaluate their histamine inhibitory activity in vitro and in vivo, and
(3) assess their bioavailability and safety.By exploring pumpkin pulp's natural therapeutic

potential, this study aims to develop safer and more effective anti-inflammatory treatments, addressing current therapy limitations and expanding natural product-based drug options.

Materials and Methods

1. Collection and Authentication of Plant Material

Pumpkin (Cucurbita maxima) specimens were collected from local farms in the eastern region of Uttar Pradesh, India. The plants were identified and authenticated by a botanist at the Department of Botany, University of Allahabad. Voucher specimens were deposited in the herbarium for future reference. The pumpkins were washed, peeled, and the seeds removed. The pulp was then freeze-dried and stored at -25°C until further use.

2. Extraction of Pumpkin Pulp

The freeze-dried pumpkin pulp was ground into a fine powder. A 100 g sample of the powdered pulp was subjected to extraction using 500 mL of ethanol in a Soxhlet extractor for 24 hours. The ethanol extract was filtered and concentrated under reduced pressure at 45°C using a rotary evaporator to yield a viscous extract. This crude extract was further partitioned using solvents of increasing polarity: n-hexane, ethyl acetate, and water. The resultant fractions were dried and weighed, yielding hexane (1.5 g), ethyl acetate (0.6 g), and aqueous (0.2 g) fractions **[6-8]**

3. Method of Preliminary Phytochemical Screening

The preliminary phytochemical screening of the pumpkin pulp extract aimed to identify the presence of various bioactive compounds using qualitative tests. The pumpkin pulp was processed into an extract, which was then subjected to a series of standard phytochemical assays. **Alkaloids** were tested using Wagner’s reagent. A reddish-brown precipitate indicated

their presence. For **flavonoids**, two tests were conducted: the Lead Acetate Test, where a yellow precipitate formed upon adding lead acetate solution, and the Alkaline Reagent Test, where an intense yellow color that turned colourless upon acid addition confirmed flavonoids. **Tannins** were identified using the Ferric Chloride Test, which produced a dark green or blue-black color upon the addition of ferric chloride solution. **Saponins** were detected by the Foam Test; vigorous shaking with distilled water resulted in stable foam formation. **Terpenoids** were tested using the Salkowski Test, where the addition of chloroform and concentrated sulfuric acid to the extract produced a reddish-brown interface, indicating terpenoids. Lastly, **phenolics** were confirmed using the Ferric Chloride Test, where a blue or green color developed upon adding ferric chloride solution. These qualitative tests demonstrated the presence of various bioactive compounds in the pumpkin pulp extract, including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolics. The results provided a foundational understanding of the phytochemical constituents of pumpkin pulp, guiding further isolation and characterization of specific bioactive compounds with potential histamine inhibitory and anti-inflammatory activities. This preliminary screening was crucial in validating the therapeutic potential of pumpkin pulp, supporting its use in the development of natural anti-inflammatory agents.**[9-10]**

4. Identification of Active Compounds

The bioactive compounds in the fractions were identified using several analytical techniques:

- **Nuclear Magnetic Resonance (NMR) Spectroscopy**: The structural elucidation of isolated compounds was performed using a Bruker DRX-300 NMR Spectrometer with DMSO-d6 as the solvent. Proton (1H) and carbon (13C) NMR spectra were recorded and analyzed.
- **Infrared (IR) Spectroscopy**: A PerkinElmer IR Spectrometer was used to identify functional groups present in the compounds. The IR spectra provided characteristic absorption bands corresponding to different functional groups.
- **High-Performance Liquid Chromatography (HPLC) with Diode Array Detection and Mass Spectrometry (HPLC-DAD-ESI/MS)**: This technique was utilized to profile and quantify the phenolic content of the pumpkin pulp extract. Compounds were identified by comparing retention times and mass spectra to those of known standards.

5. Quantitative estimation of Phenol and Flavonoid

Polyphenols and flavonoids are classes of compounds found abundantly in plants, known for their antioxidant properties and potential health benefits. Quantifying these compounds in plant extracts is crucial for assessing their nutritional and medicinal value. Two common methods used for this purpose are the Folin-Ciocalteau assay for total polyphenol content (TPC) and the quercetin calibration curve method for total flavonoid content (TFC).**[10]**

- To determine TPC using the Folin-Ciocalteau assay, a sample extract undergoes a series of steps. Initially, 0.1 mL of the extract is mixed with an equivalent amount of Folin-

Ciocalteau reagent. After a brief incubation, 0.8 mL of a 5% sodium carbonate solution is added, followed by a 40-minute incubation at 40°C. The resulting solution's absorbance is measured at 740 nm using a spectrophotometer. Gallic acid serves as the reference standard for creating a calibration curve, enabling the calculation of TPC in milligrams of gallic acid equivalents (GAE) per gram of dry weight. The formula used for calculating TPC (CTP) is CTP = C × V / w, where C is the concentration of polyphenols, V is the volume of the extraction medium, and w is the dry weight of the plant material.

- Similarly, the determination of TFC involves another calibration curve, this time constructed with quercetin as the standard. In this method, 100 µL of the sample extract is mixed with 860 µL of 35% aqueous methanol and 40 µL of a 5% AlCl3 solution in 0.5 M CH3COONa. After incubation, the absorbance of the resulting solution is measured at 415 nm. The concentration of flavonoids (CTFn) is then calculated using the formula CTFn = C × V / w, where C is the concentration of flavonoids, V is the volume of the extraction medium, and w is the dry weight of the plant material. TFC is expressed in milligrams of quercetin equivalents (QE) per gram of dry weight.
- Both assays provide quantitative data essential for comparing the polyphenol and flavonoid contents of different plant extracts. These compounds are of significant interest due to their potential health benefits, including antioxidant, anti-inflammatory, and anti-carcinogenic properties. Researchers and food scientists utilize these methods to evaluate the nutritional quality and functional properties of various plant-based foods and herbal medicines.

6. Analysis of anti-inflammatory activity

In the study of pumpkin pulp's anti-inflammatory properties, researchers employed a method based on the prevention of protein denaturation, focusing on its potential therapeutic benefits. Protein denaturation refers to the alteration of a protein's structure, rendering it dysfunctional. In this case, bovine serum albumin (BSA) served as the model protein to assess how pumpkin pulp, specifically its oil, could mitigate this process. The experimental procedure began by preparing a reaction mixture containing BSA solution and pumpkin pulp. The pH of the mixture was adjusted to ensure optimal conditions for the inflammatory conditions in biological systems.After the specified incubation period, the absorbance of both the control sample (without pumpkin seed oil) and the test sample (with pumpkin seed oil) was measured using a spectrophotometer set at a wavelength of 660 nm. The absorbance readings reflect the extent of protein denaturation in each sample: higher absorbance indicates greater denaturation.

Where:

- Ac is the absorbance of the control sample (without pumpkin pulp).
- As are the absorbance of the test sample (with pumpkin pulp).

By comparing these absorbance values, researchers were able to quantify the extent to which the pumpkin pulp inhibited BSA denaturation. A higher percentage of inhibition indicates stronger anti-inflammatory activity, as it suggests that the components of pumpkin pulp, likely

bioactive compounds such as antioxidants and anti-inflammatory agents, effectively protected BSA from structural damage induced by inflammatory processes.

RESULT AND DISCUSSION

The percentage yields of the fractionated re-extracts from the 50% ethanolic extract of pumpkin pulp were determined following solvent extraction procedures. Initially, 100 grams of pumpkin pulp was dissolved in 500 mL of 50% ethanol to produce the crude extract. Subsequent fractionation involved sequential solvent extractions: hexane, ethyl acetate, and aqueous layers were obtained and concentrated individually. The yields for each fraction were calculated based on the initial weight of pumpkin pulp used. The hexane fraction yielded 1.5 grams, representing 8.82% of the original extract. The ethyl acetate fraction yielded 0.6 grams, accounting for 3.45%, while the aqueous fraction yielded 0.2 grams, constituting 1.11% of the original extract. These yields indicate the efficiency of the solvent extraction process in isolating different compounds from the pumpkin pulp, with varying degrees of solubility in hexane, ethyl acetate, and aqueous solvents.

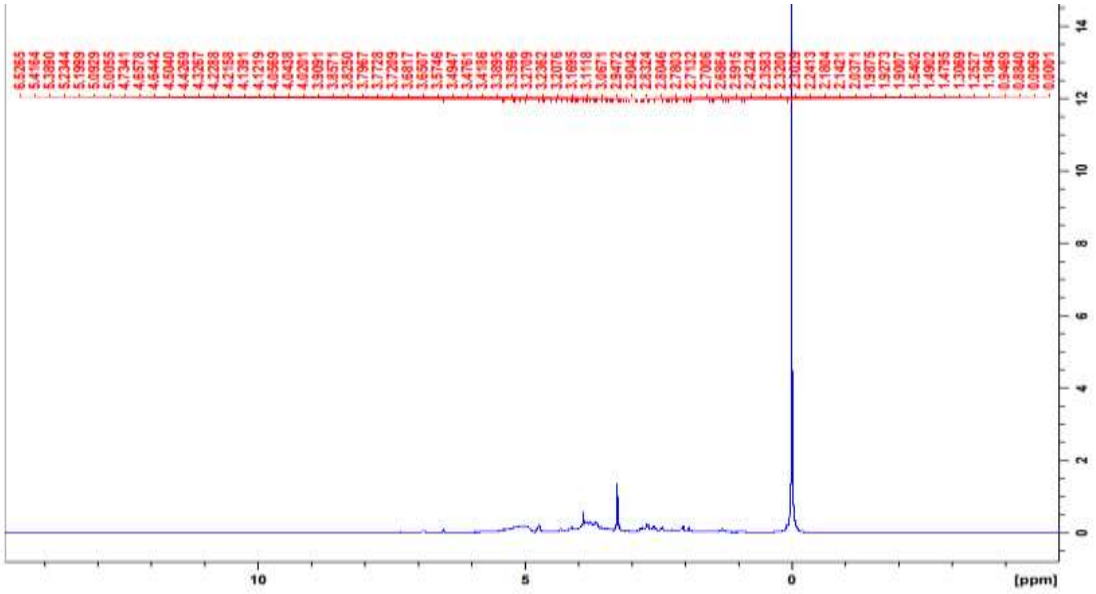
The phytochemical screening of the 50% ethanolic extract of the herbal sample revealed a rich composition of bioactive compounds through various solvent fractions. Initially, the crude extract appeared thick and brown, indicative of concentrated and possibly viscous material. The extraction process involved sequential solvent extraction using hexane and ethyl acetate after initial dissolution in distilled water, yielding distinct fractions. Phytochemical tests indicated that alkaloids were absent in all fractions, while phenolic compounds were detected in the 50% methanol extract and hexane fraction but absent in the ethyl acetate and aqueous fractions. Flavonoids, confirmed by positive results in Shinoda and ferric chloride tests, were consistently present across all fractions, highlighting their abundance in the herbal sample. Additionally, terpenoids were found only in the ethyl acetate fraction, as indicated by a positive Salkowski test, while tannins were predominantly present in the ethyl acetate fraction based on ferric chloride testing. Thin Layer Chromatography (TLC) corroborated the presence of flavonoids in all fractions, underscoring their prevalence and potential pharmacological significance in the herbal extract.

Spectral analysis

The characterization was done by NMR and IR

Nuclear magnetic resonance Spectral analysis

The Nuclear magnetic resonance spectroscopy for the synthesized compounds was recorded using Bruker DRX-300 NMR Spectrometer using DMSO-d6.



NMR spectra of 50 % ethanolic extract

1H NMR, δ ppm: 1.93 (S, 3H, OCH3), 2.01 (S, 3H, OCH3), 2.52 (S, 1H, OH), 2.67 (S, 1H, OH), 2.83 (S, 1H, OH), 4.68 (S, 1H, OH), 1.54 (s, 1H, Ar-H), 2.33 (s, 1H, Ar-H), 3.45 (s, 1H, Ar-H),3.83 (s, 1H, Ar-H), 4.21 (s, 1H, Ar-H),4.46 (s, 1H, Ar-H).

Infrared Spectral Analysis

The Infrared spectra for the synthesized compounds were noted using PerkinElmer FTIR Spectrometer using KBr

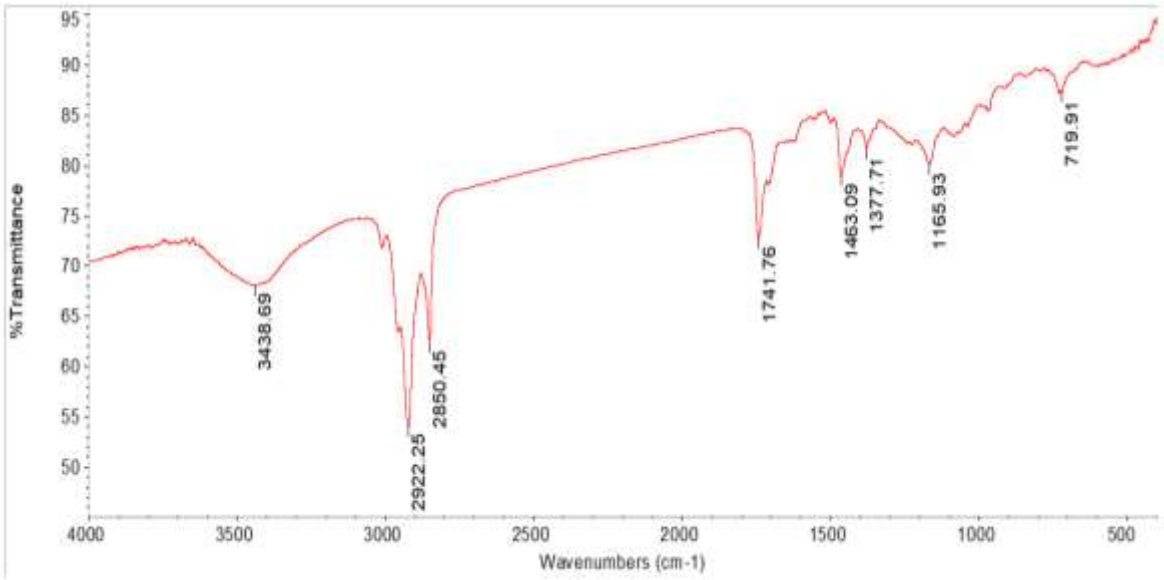


Fig .5.2: FTIR OF 50 % ethanolic extract

FTIR (KBr) cm-1: 1156.93 (ether –C-O), 1337 (C-N str), 1463 (-NO2), 1741 (Ar, C=C str), 1741 (C=N str), 2922 (Ali, C-H str), 3057 (Ar, C-H str), 3438.69 (N-H str)

Characterization of the Compounds Profile

Eight compounds were identified and categorised into three families: phenolic acids (peak 3) and flavonoids (peaks 2, 4, 5, 6, 7, and 8). A phenolic profile of the Pumpkin PULP 50% ethanol extract is shown in Figure 3, with a focus on the compounds identified. Peak 3 displayed a deprotonated ion [M-H]− at m/z 405 and a sizable MS2 fragment at m/z 281, in addition to hydroxybenzoic acid at m/z 137, CO2 loss, and glucosyl residue and CO2 loss at m/z 93. Though tentatively, peak 3 was determined to be 7 4-O-(60-O-glucosyl-4"-hydroxybenzoyl)-4-hydroxybenzyl alcohol. Additionally, by contrasting mass spectra, retention durations, and UV-vis at the maximum absorption (λmáx 280 nm) of the accessible Molecules 2022, 27, 8366,3 out of 15 standard compound Peak 1, identified as (-)-epicatechin at m/z 289, was confirmed to have [M-H]−.

FTIR analysis confirmed the presence of functional groups consistent with phenolic acids and flavonoids; HPLC-MS analysis identified specific compounds, including 7 4-O-(6-O-glucosyl-4"-hydroxybenzoyl)-4-hydroxybenzyl alcohol; further characterization of the compounds was carried out through spectral analyses; NMR analysis revealed a variety of chemical shifts indicative of the presence of phenolic and flavonoid compounds; and quantitative estimation of total phenolic and flavonoid content revealed higher levels in the 50% ethanolic extract compared to the aqueous extract. Gallic acid's total phenolic content was 4.23 mg/g in the aqueous extract and 7.78 mg/g in the 50% ethanolic extract. Quercetin's total flavonoid concentration was 2.208 mg/g in the aqueous extract and 3.155 mg/g in the 50% ethanolic extract.

The anti-inflammatory activity varied among the different extracts, with the ethyl acetate fraction showing the highest activity (72% to 85% inhibition), followed by the hexane fraction (62% to 75%), the crude ethanolic extract (52% to 63%), and the aqueous fraction (40% to 53%).

Quantitative Estimation of Total Phenolic and Flavonoid

The total phenolic content of the pumpkin pulp extract was determined to be 7.78 mg/g of gallic acid for the 50% alcoholic extract and 4.23 mg/g of gallic acid for the aqueous extract. Additionally, the total flavonoid content was found to be 3.155 mg/g of quercetin for the 50% alcoholic extract and 2.208 mg/g of quercetin for the aqueous extract.

Analysis of anti-inflammatory activity

Anti-Inflammatory Activity: The anti-inflammatory activity of pumpkin pulp was evaluated using various extracts. The results showed a concentration-dependent activity, with different extracts exhibiting varying degrees of anti-inflammatory effects. Specifically:

- The 50% ethanolic extract demonstrated anti-inflammatory activity ranging from 52% to 63%.
- The fractionated hexane extract showed activity between 62% and 75%.
- The fractionated ethyl acetate extract exhibited the highest activity, ranging from 72% to 85%.
- The fractionated aqueous extract displayed activity between 40% and 53%
- confirmed to have [M-H]−.

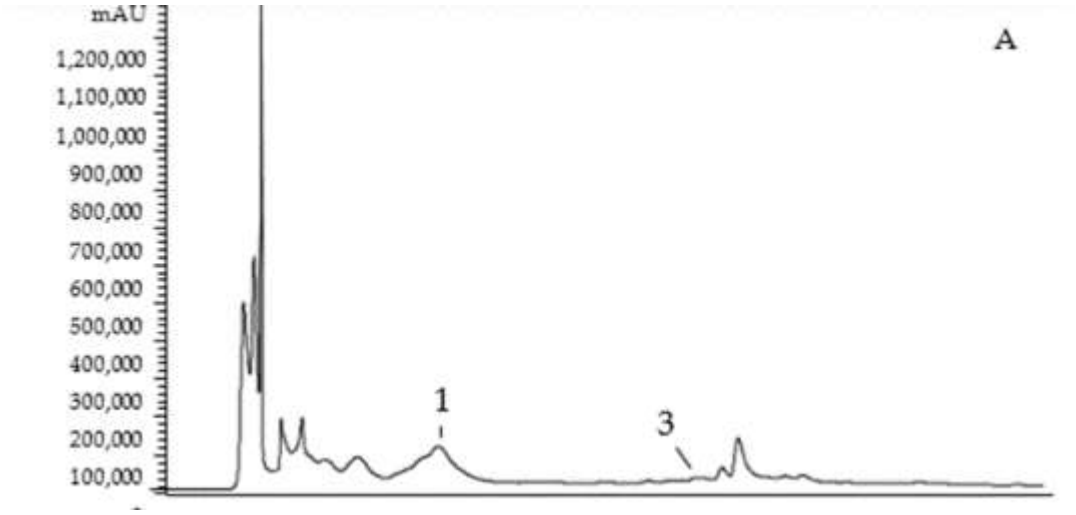
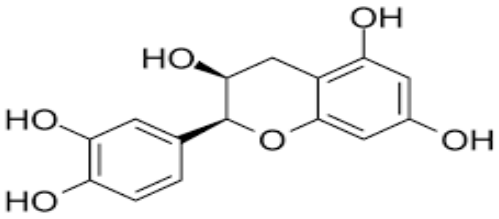


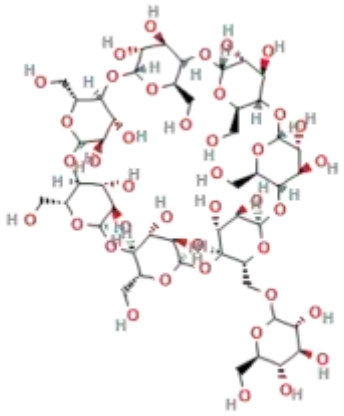
Fig. 5.3: Chromatogram, recorded at 280 nm

Table. 5.5: Compound characterized by HPLC -dad-ESI/MS in 50% aqueous extract

Peak		**Rt (min)**	**λmax (nm)**	**[M-H]− (m/z)**	**MS^2 (m/z)**	**Tentative Identification**
1		7.71	280	289	245(100), 205(45)	(-)-Epicatechin
3		14.73	263	405	281(100),137(12),93(5)	7 4-O-(60 -O-Glucosyl-4”-hydroxy benzoyl)-4-hydroxy benzyl alcohol



(-)-Epicatechin



7 4-O-(6-O-Glucosyl-4”-hydroxy benzoyl)-4-hydroxy benzyl alcohol

Total Phenolic and Flavonoid of pumpkin pulp extract

S.No.	Extracts	Total phenol content (mg/g of gallic acid)	Total flavonoids content (mg/g Quercetin)
01	50 % Alcoholic extract of pumpkin pulp extract	7.78	3.155
02	Aqueous extract of pumpkin pulp extract	4.23	2.208

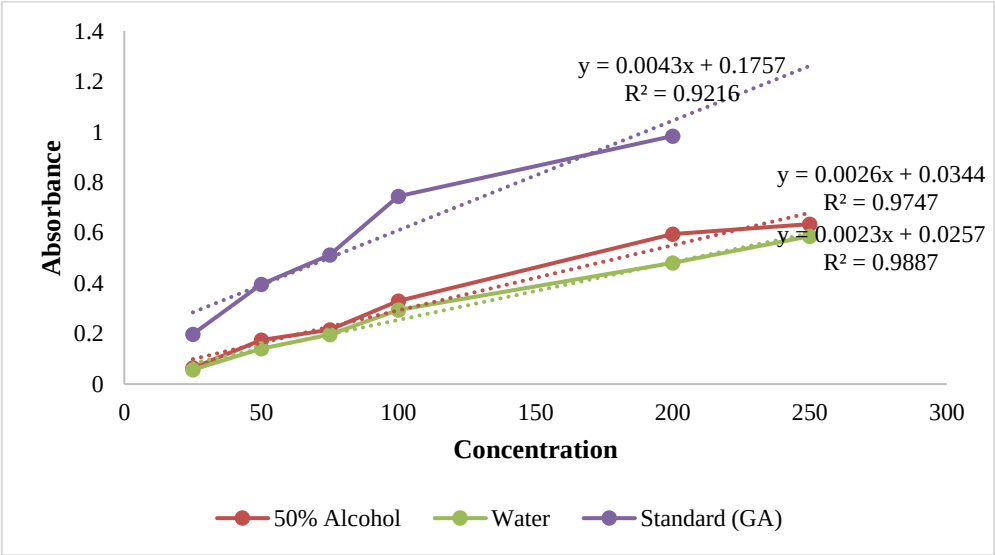
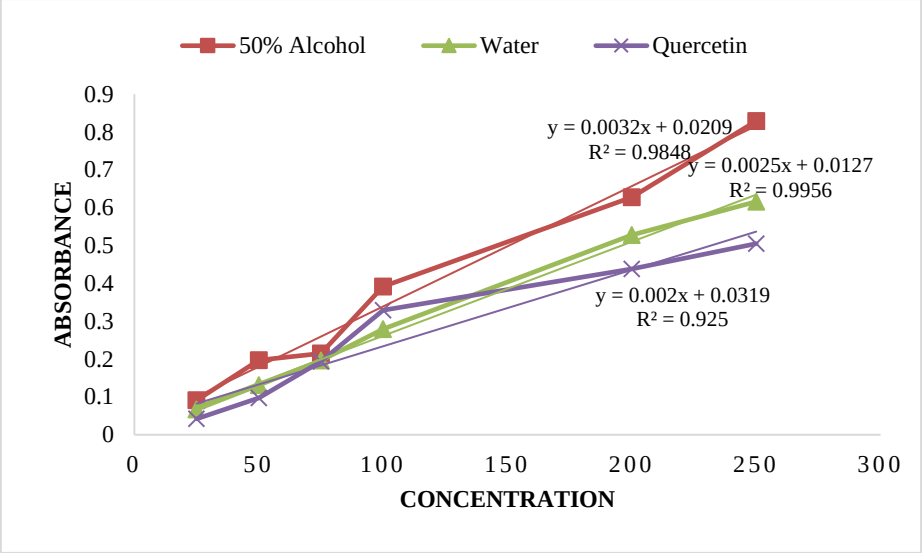
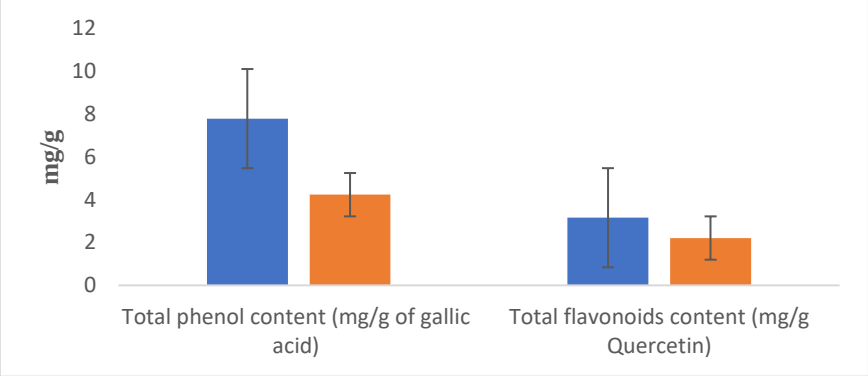
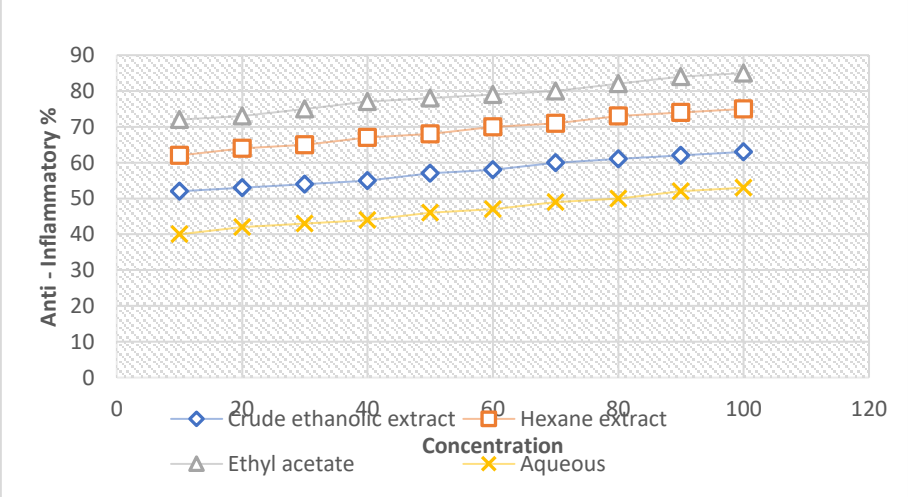


Fig. 5.4: Absorbance at (760 nm) of various concentrations (µg/mL) of standard (gallic Acid) and pumpkin pulp extract in total phenolics content



Absorbance at (760 nm) of various concentrations of pumpkin pulp extract (µg/mL) concerning Quercetin Equivalent





Anti-inflammatory activity of different extract of Pumpkin Pulp

Table Anti-Inflammatory Activity of Pumpkin Pulp

Concentration	Crude ethanolic extract	Hexane extract	Ethyl acetate	Aqueous
10	52	62	72	40
20	53	64	73	42
30	54	65	75	43
40	55	67	77	44
50	57	68	78	46
60	58	70	79	47
70	60	71	80	49
80	61	73	82	50
90	62	74	84	52
100	63	75	85	53

CONCLUSION

The investigation into the anti-inflammatory activity of pumpkin pulp aimed to elucidate the potential of this natural substance in mitigating inflammation, a crucial factor in many chronic diseases. The study evaluated various extracts of pumpkin pulp, focusing on their effectiveness in reducing inflammation.The results revealed that the anti-inflammatory activity of pumpkin pulp varied significantly across different extracts. The 50% ethanolic extract demonstrated moderate anti-inflammatory effects, with activity ranging from 52% to 63%. This indicates that the ethanolic extract can provide a reasonable degree of inflammation reduction, making it a viable option for moderate anti-inflammatory applications.

A more pronounced effect was observed with the fractionated hexane extract, which exhibited anti-inflammatory activity between 62% and 75%. This higher range suggests that the hexane extract contains more potent anti-inflammatory compounds or a higher concentration of active ingredients compared to the ethanolic extract. The hexane extract's efficacy highlights its potential for more robust anti-inflammatory treatments.However, the most significant anti-inflammatory effects were observed with the fractionated ethyl acetate extract. This extract displayed the highest activity, ranging from 72% to 85%. The superior performance of the ethyl acetate extract suggests that it may contain the most effective anti-inflammatory agents among the tested extracts. This extract's ability to achieve such high levels of activity indicates its potential as a powerful natural anti-inflammatory agent, which could be beneficial in treating various inflammatory conditions.

On the other hand, the fractionated aqueous extract showed the least anti-inflammatory activity, ranging from 40% to 53%. Although this extract still exhibited some degree of anti-inflammatory effect, its lower activity compared to the other extracts suggests that water-soluble compounds in pumpkin pulp are less effective in reducing inflammation. This finding

indicates that while the aqueous extract can contribute to anti-inflammatory effects, it may not be as potent as the other extracts, particularly the ethyl acetate and hexane extracts.

In conclusion, the study highlighted the significant anti-inflammatory potential of pumpkin pulp, with varying degrees of effectiveness across different extracts. The fractionated ethyl acetate extract emerged as the most potent, followed by the hexane extract and the 50% ethanolic extract. The aqueous extract, while still effective, demonstrated the lowest activity. These findings underscore the potential of pumpkin pulp, especially the ethyl acetate fraction, as a natural anti-inflammatory agent. Further research and development could optimize these extracts for therapeutic applications, offering a natural alternative for managing inflammation-related conditions.

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