DIET DETECTIVE

Are spices going to reduce inflammation?

BY,

A. MOHAMED HAASIQ

(Grade -8)

Sana Model School.

Guide teacher: - Survath Jabeen.

Table of contents: -

ABSTRACT: -

Autoimmune diseases are health conditions that happen when your immune system attacks your body instead of defending it. If you have an autoimmune disease, your immune system is more active than it should be. Because there aren't invaders to attack, your immune system turns on your body and damages healthy tissue.

Autoimmune diseases are chronic conditions. This means if you have an autoimmune disease, you'll probably have to manage it and the symptoms it causes for the rest of your life.

A key feature of these conditions is chronic inflammation, a prolonged immune response that can cause tissue damage throughout the body. While genetic and hormonal factors play a role, diet is a significant environmental factor that can either promote or reduce inflammation. For instance, certain foods like those high in refined sugar and salt are known to be pro-inflammatory, while foods rich in antioxidants and specific spices like ginger and turmeric are considered anti-inflammatory.

This research aimed to know which common spice extracts with antiinflammatory or enzymatic properties

can reduce inflammation in the body due to auto immune disease using simple biological models. Two assays were designed: (1) **Egg-albumin heat denaturation** to simulate protein damage during inflammation, and (2) **Yeast fermentation under hydrogen peroxide stress** to simulate oxidative stress at the cellular level.

INTRODUCTION: -

What are Auto immune diseases?

Auto immune diseases are conditions in which your immune system mistakenly damages healthy cells in your body. Usually, your immune system is like your body's built-in security system. It automatically detects substances that shouldn't be in your body (like viruses, bacteria or toxins) and sends out white blood cells to eliminate them before they can damage your body or make you sick.

However, if you have an autoimmune disease, your immune system mistakes parts of your body, such as your joints or skin, as foreign. It releases proteins called autoantibodies that attack healthy cells.

What are the types of Auto immune disease?

There are more than 100 different autoimmune diseases. Some of them are Rheumatoid arthritis (RA), Lupus, Myositis, Sjögren's syndrome, Psoriasis, Psoriatic arthritis, Dermatomyositis, Type 1 diabetes, Addison's disease, Hashimoto's thyroiditis, Graves' disease.

What is cause of inflammation in Auto immune disease?

In Autoimmune diseases, inflammation arises when the immune system mistakenly identifies the body's own cells and tissues as foreign invaders. This misdirected immune response activates lymphocytes and triggers the release of pro-inflammatory mediators such as cytokines. These signals recruit additional immune cells, leading to redness, swelling, heat, and pain at the affected site. Unlike normal inflammation, which resolves once the threat is removed, autoimmune inflammation is chronic because the immune system continues to target self-tissues. Over time, this persistent inflammatory process causes progressive tissue damage and contributes to the symptoms and complications associated with autoimmune disorders.

Spices with potential anti inflammatory properties: -

1. **Turmeric (*Curcuma longa*) →** Curcumin is one of the best-studied natural anti-inflammatories; inhibits NF-κB, COX-2, and pro-inflammatory cytokines.
2. **Ginger (*Zingiber officinale*) →** Gingerols, shogaols reduce prostaglandin and cytokine production; useful in arthritis, gut inflammation.
3. **Cinnamon (*Cinnamomum spp*.) →** Cinnamaldehyde, polyphenols lower TNF-α, IL-6, NF-κB signaling.
4. **Garlic (*Allium sativum*)** → Allicin, diallyl sulfides modulate immune and inflammatory pathways.
5. **Black pepper (*Piper nigrum*)** → Piperine downregulates pro-inflammatory mediators, enhances bioavailability of curcumin.

Reason for conducting the two tests: -

Egg albumin assay: -

- During inflammation in the human body, **proteins get denatured** (lose their structure) due to heat and oxidative stress.
- The **egg albumin (egg white)** behaves similarly — it becomes **cloudy or coagulated** when heated.
- If a spice extract can **reduce this coagulation (less cloudiness)**, it suggests that it may **protect proteins from damage**, showing **anti-inflammatory potential**.

Yeast assay: -

- **Yeast cells** are living systems that produce carbon dioxide (CO_2) during fermentation.
- When exposed to **hydrogen peroxide (H_2O_2)**, they experience **oxidative stress** (similar to what happens in inflamed tissues).
- If a spice extract helps the yeast **keep fermenting and producing CO_2**, it means it is **protecting the cells from oxidative damage** — showing **anti oxidant** and **anti-inflammatory potential**.

Objective: -

To know which among the common spice extracts with properties that can reduce inflammation in the body due to auto immune disease using simple biological models.

Statement of Problem :-

Observing my mother's struggle with inflammation-related symptoms due to hyperthyroidism inspired this study to explore natural, accessible ways to reduce inflammation. Chronic inflammation is a key factor that worsens autoimmune conditions such as **hyperthyroidism**, where the body's immune system attacks its own thyroid gland, leading to hormonal imbalances and tissue damage.

Modern anti-inflammatory drugs often cause side effects when used long-term, making **natural plant-based alternatives** an attractive area of research. Common culinary spices—such as turmeric, ginger, garlic, cinnamon, and black pepper—are known to contain bioactive compounds with antioxidant and anti-inflammatory properties.

This experiment aims to determine which among these spice extracts shows the most effective **anti-inflammatory and antioxidant potential** using simple biological models, such as **egg albumin denaturation** (for protein protection) and **yeast oxidative stress assays** (for antioxidant protection). The goal is to identify natural dietary options that could help support the management of inflammation associated with autoimmune diseases like hyperthyroidism.

Research questions: -

1. Which spice extract has the highest anti inflammatory properties?

Hypothesis: -

Does turmeric prove to have higher Anti-inflammatory properties than others due to presence of curcumin?

Variables: -

Independent Variable:

- Type of treatment/extract applied: water (vehicle control), ginger, turmeric, garlic, cinnamon, black pepper,
- **Dependent Variables:**
- Egg Albumin Assay (protein damage model):
    - Cloudiness score (0–5 scale)
    - Mass of precipitate (g)
- Yeast Assay (oxidative stress model):
    - Balloon circumference (cm) over time.
    - Foam score.

Materials Required: -

Extract Preparation

- Fresh ginger, turmeric powder, cinnamon, garlic, black pepper.
- Distilled water (for controls and dilutions)
- Mortar and pestle or blender (for crushing/mashing extracts)
- Filter paper, coffee filters, or fine sieve
- Measuring beakers, graduated cylinders, pipettes

Egg Albumin Assay

- Fresh eggs (egg white)
- Test tubes or small transparent cups (clear plastic or glass)
- Water bath or hot plate with thermometer (~70 °C)
- Timer
- Pipettes or droppers
- white background + camera (for scoring cloudiness)
- Filter paper and balance

Yeast Oxidative Stress Assay

- Active dry yeast
- Granulated sugar
- Warm water (30–35 °C)
- Hydrogen peroxide (3% pharmacy grade, diluted to ~0.1–0.3%)
- Small bottles, test tubes, or conical flasks

- Balloons (to capture CO_2 gas)
- Rubber bands or tape (to secure balloons)
- Ruler (to measure balloon circumference)

Procedure:-

- The plant components were ground using a mechanical blender.
- The powdered areal components were placed in a separate, airtight container.
- The extract was dissolved in distilled water and left for 48 hrs
- The mixture was heated until the water evaporated.
- A spatula was used to scratch the dried powder once the water had evaporated.
- Finally, vials were used to keep the aqueous plant powder dry until future use.
- One gram of the aqueous plant extract powder was dissolved in **10mL of distilled water** to obtain a **stock concentration of 100 mg/mL**.

- From this stock, **three working concentrations** were prepared:

  I. **1% w/v** → used for **Replicate 1**
  II. **1.5% w/v** → used for **Replicate 2**
  III. **2% w/v** → used for **Replicate 3**

- For each replicate, **1mL of the respective working solution** was taken into test tubes to conduct the assay.

Egg Albumin solution: -

Making egg-albumin solution using a fresh hen's egg properly involves carefully cracking an egg and removing the yolk, adding 100mL of distilled water to the egg white, and stirring thoroughly. The water should be cold when making the solution or else it will coagulate.

Egg Albumin Assay: -

Sample Preparation: -

- Label tubes (Room-temp control, Heated control, Ginger, Garlic, Cinnamon, Black Pepper, Turmeric).
- Add 5mL egg albumin solution to each tube.
- Controls: -
    - Only egg albumin sol in a test tube placed in hot bath. (Control A)
    - Only egg albumin sol in a test tube without placing in hot bath. (Room-temperature control) (Control B)
- Add 1mL different extracts in each test tube.
- Mix gently.

Heat Treatment: -

- Place all tubes except room-temperature control in a 70 °C water bath for 5 minutes.
- Remove and cool to room temperature.

Observation / Measurement: -

- Assign a cloudiness score (0–5):
    - 0 = clear, 5 = fully cloudy/opaque.

Yeast assay: -

- Extract Preparation

Pilot Test

- Tested extract volumes 2ml in 15mL yeast starter without H_2O_2.
- Add 3 drops of H2O2

Yeast Starter

- Dissolve 5g sugar in 100mL warm water (30–35 °C).
- Add 5g dry yeast, mixed, rest until foamy (5–10 min).
- Experimental Setup
- 15mL yeast starter per testtube.
- Add 2ml extract.
- Added H_2O_2 to stress groups

- Treatments:
    - Positive control (no H_2O_2)
    - Negative control (H_2O_2 + water)
    - Each extract + H_2O_2

Assay Procedure:-

- Bottles capped with balloons to capture CO_2.
- Measured balloon circumference at:
    - Every 10 min (first hour)
- Recorded observations (foam, color change).
- Repeat the experiment with different concentration of extract.

Experimental pictures: -

Extracts: -

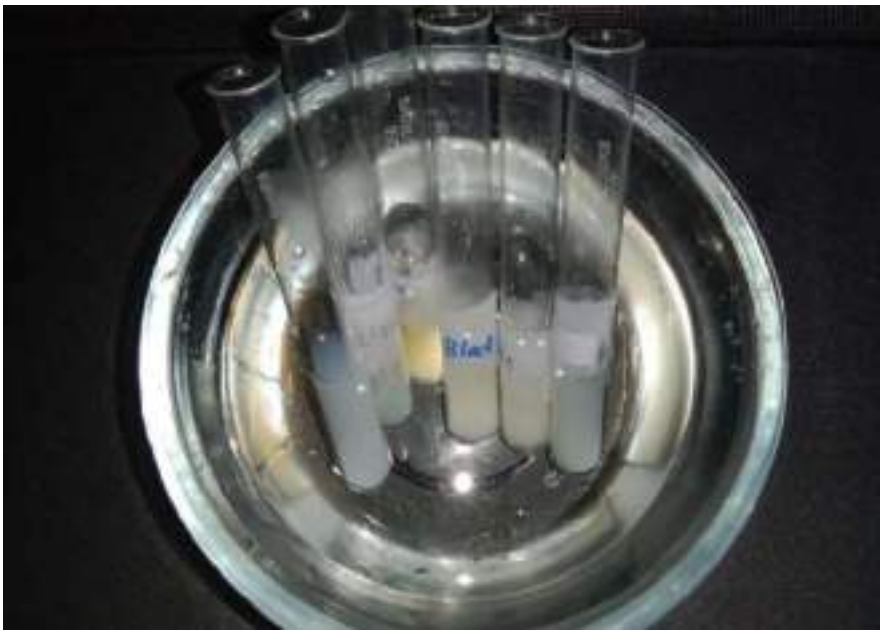
Diluted: -

Egg solution: -

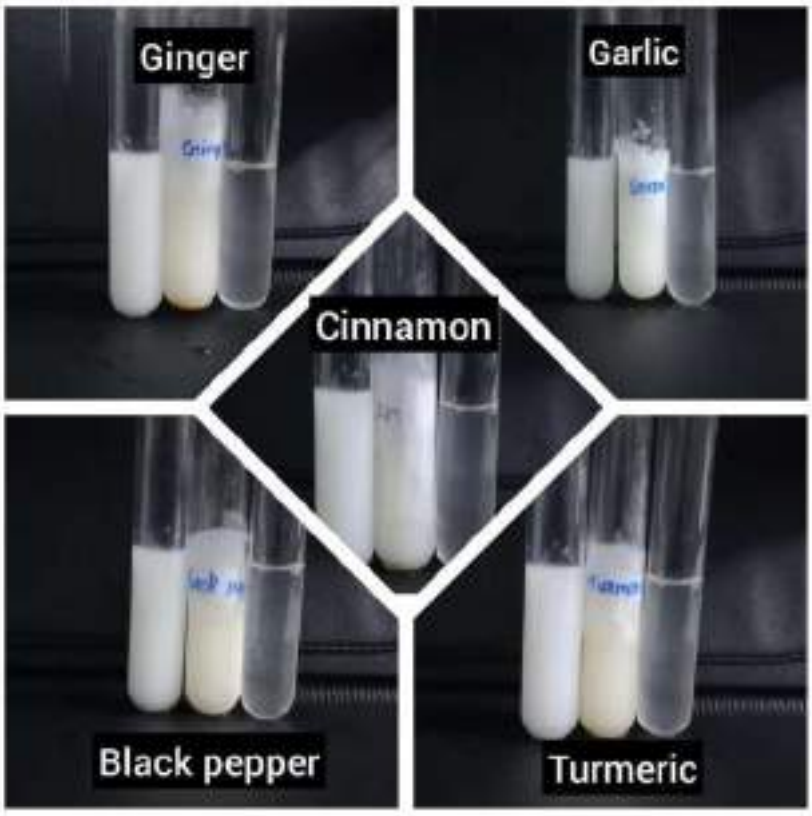
Egg solution + Extract: -

Controls for egg albumin assay: -

Testtubes placed in water bath: -

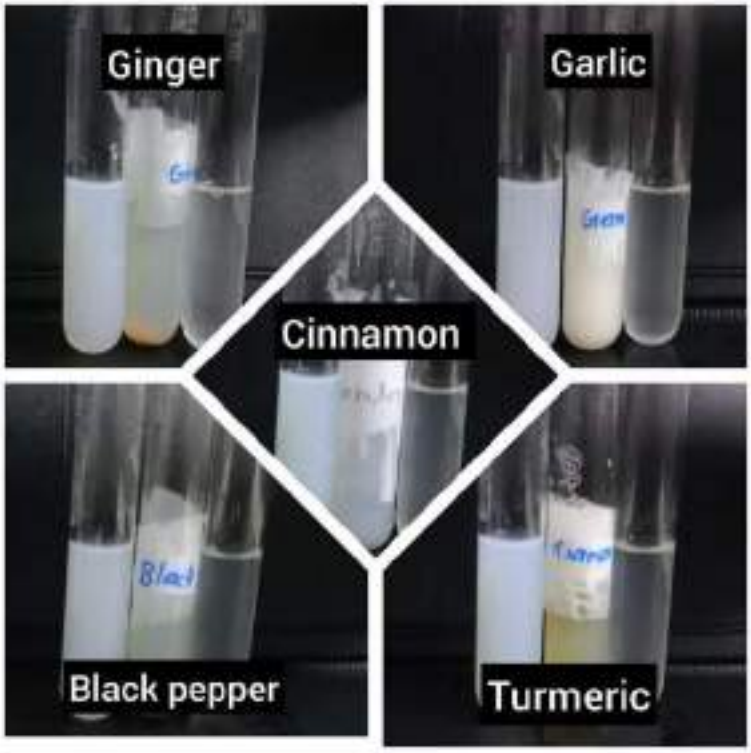


Replicate 1: -

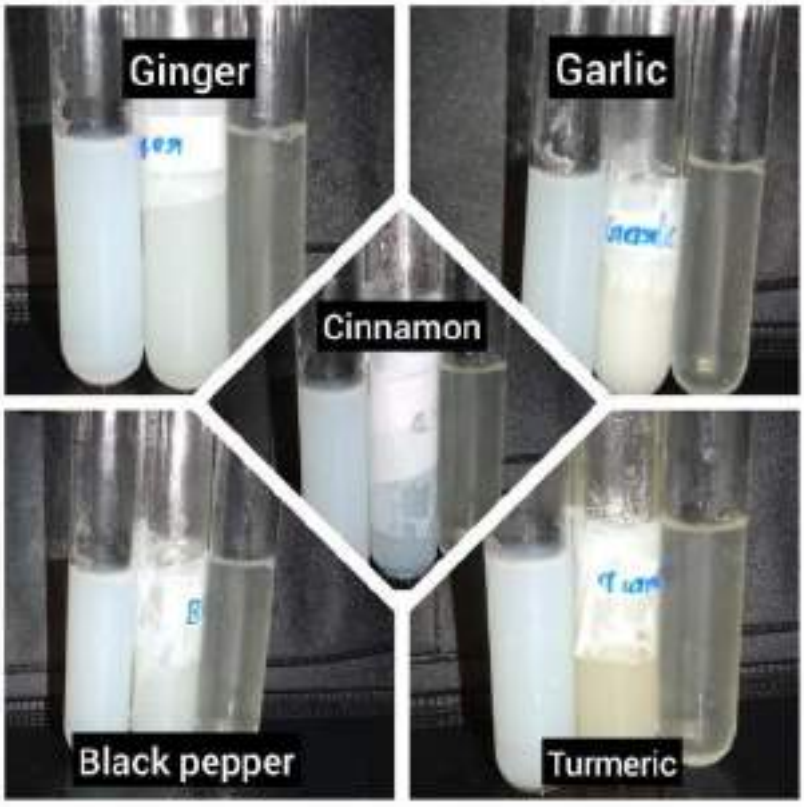


Left to Right (Control A, Extract test tube, Control B)

Replicate 2: -



Replicate 3: -



Yeast starter: -

Yeast assay set up: -

Replicate 1

Replicate 2: -

Observation:-

Egg Albumin Assay: -

Treatment	Replicate 1(1%)	Replicate 2(1.5%)	Replicate 3(2%)	Mean Cloudiness	% Inhibition of Denaturation
Room-temp Control	0	0	0	0	100
Heated Control	5	5	5	5	0
Ginger Extract	3.5	4	2.5	3.33	33.33
Garlic Extract	2.5	4.5	5	4	20
Cinnamon Extract	5	1	3.5	3.16	36.66
Black Pepper Extract	4	3.5	3.5	3.66	26.66
Turmeric Extract	3	2	1.5	2.16	56.66

Cloudiness Score (0–5)

(Controls: Heated = 5, Room-temp = 0; therefore % Inhibition = ((5 − Mean Cloudiness)/5) × 100)

Yeast assay: -

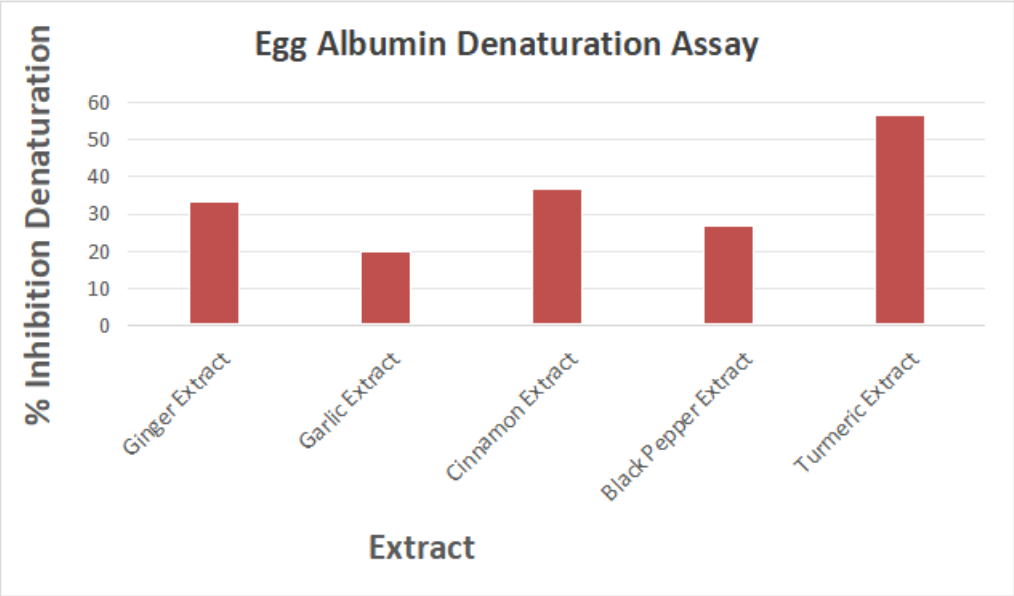
Balloon size: -

Treatment	Replicate 1 (1mL)			Replicate 2 (2mL)			Average (Mean)	Standard Deviation (SD)
	After 0 min	After 15:00 min	after 30:00 min	After 0 min	After 15:00min	After 30:00min	Mean Balloon Size (cm)	% Recovery vs Positive Control
Positive control	13	13	13.5	13	14	14	13.42	100
Negative control	4	5	7.5	8	10	10	7.42	0
Ginger Extract	10	9	12.5	11	13	12	11.25	63.83
Garlic Extract	10	12	12.5	10	12	12	11.42	66.67
Cinnamon Extract	7.5	12	12	9.5	11.5	12	10.75	55.5
Black Pepper	12	13	13.5	12	13	13	12.75	88.83
Turmeric Extract	11	11	13	11	12	13	11.83	73.50

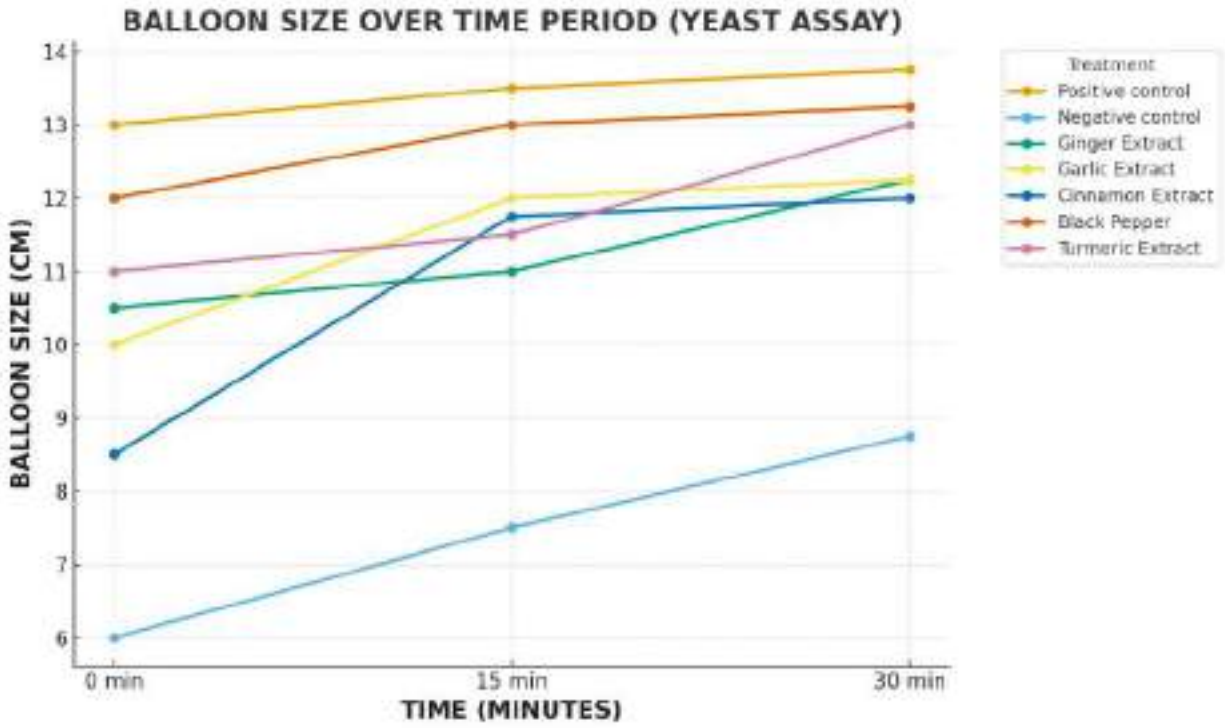
% Recovery = (Sample - Negative Control) / (Positive Control - Negative Control) × 100

Data Analysis: -

Egg Albumin Assay: -



Yeast assay: -



Results and Discussion:-

The study evaluated the anti-inflammatory potential of common spice extracts using two simple biological models.

Egg Albumin Assay: -

1. Control Comparisons

- Room-temperature Control (0 cloudiness, 100% inhibition): Represents no protein denaturation — egg albumin remains clear.
- Heated Control (5 cloudiness, 0% inhibition):
  Indicates maximum denaturation of egg proteins due to heat, producing full cloudiness.
- **Interpretation of Cloudiness:**
  - Cloudiness results from coagulation of egg albumin proteins due to heat.
  - Substances that reduce cloudiness are effectively preventing or minimizing this denaturation, indicating anti-inflammatory or protein-stabilizing potential.
- **Extract Efficacy:**
  - Turmeric extract exhibited the highest protection (56.66% inhibition), suggesting strong antioxidant and anti-inflammatory effects—likely due to curcumin, which stabilizes proteins and prevents oxidative damage.
  - Cinnamon and Ginger also showed moderate protective activity, probably from phenolic compounds and antioxidants.

- Black Pepper (piperine) showed mild protection, which may synergize with turmeric in combination studies.
- Garlic extract was least effective in preventing denaturation under these conditions.

- **Overall Trend:**
  - Effectiveness order:
    Turmeric > Cinnamon > Ginger > Black Pepper > Garlic
  - This suggests that Turmeric has the strongest potential to prevent protein denaturation and may therefore be the most effective natural anti-inflammatory agent among those tested.

Yeast Assay: -

1. Control Comparisons

- Positive Control:
  Represented normal, healthy yeast activity without stress — similar to a non-inflamed biological condition.
- Negative Control:
  Showed very low CO_2 production, indicating strong oxidative damage and cellular stress — comparable to an inflamed state where normal metabolic activity is suppressed.

2. Interpretation of Balloon Size: -

- Balloon size represents the amount of CO_2 produced during yeast fermentation.
- Larger balloon size → higher yeast activity → stronger antioxidant and anti-inflammatory potential.
- Smaller balloon size → inhibited yeast activity → weaker protective effect against oxidative stress.

- Indicates the ability of extracts to protect cells from oxidative and inflammatory damage.

3. Extract efficacy: -

- **Black Pepper Extract:**
  Produced the highest balloon inflation among the extracts, showing that it protected yeast cells from oxidative stress. This reflects a strong anti-inflammatory effect, as it maintained normal cellular function despite stress conditions.
- **Turmeric Extract:**
  Ranked second in performance, displaying significant protection against oxidative damage. Its bioactive compound **curcumin** likely reduced stress and inflammation, confirming its **potent anti-inflammatory nature**.
- **Garlic Extract:**
  Showed moderate anti-inflammatory activity, helping yeast recover partially from oxidative damage through sulfur-based antioxidants like allicin.
- **Ginger Extract:**
  Displayed balanced anti-inflammatory potential, keeping yeast function close to the healthy control, suggesting effective but not enhanced protection.
- **Cinnamon Extract:**
  Was the least effective, with reduced balloon size, indicating lower anti-inflammatory capacity and possible mild inhibition of yeast activity.

Overall Interpretation (both Assays)

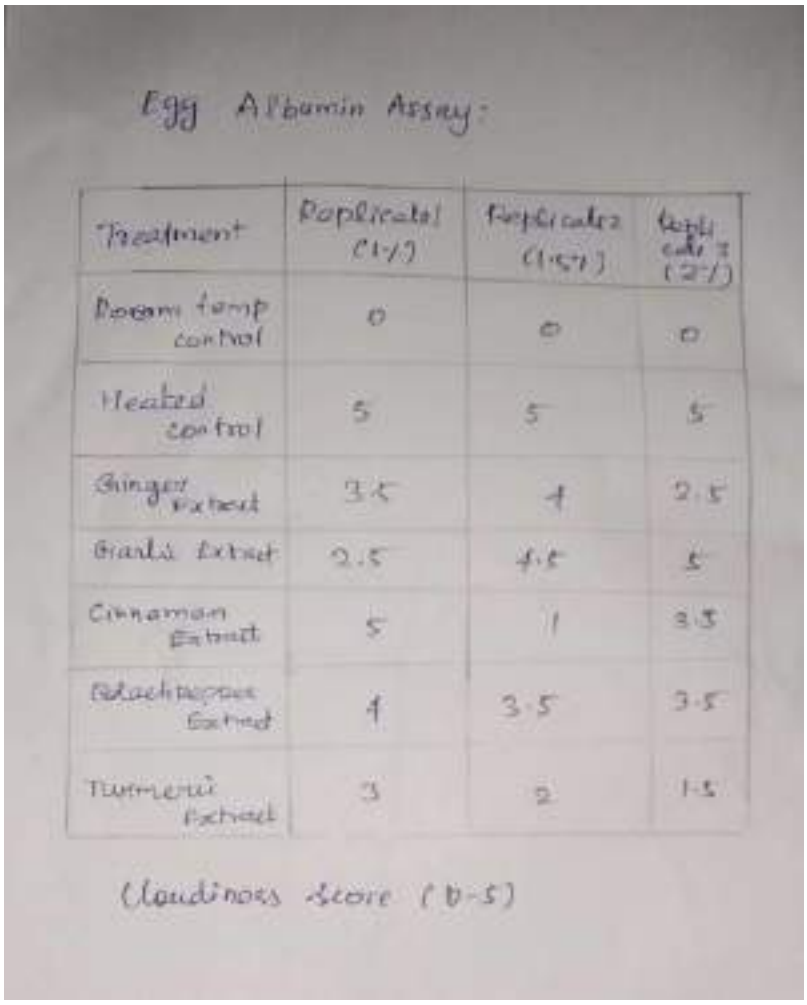
- Turmeric Extract demonstrated anti-inflammatory activity, making it the most effective spice in protecting biological systems from oxidative and inflammatory damage.

- Black Pepper Extract showed exceptional antioxidant potential and moderate anti-inflammatory activity — it can also enhance turmeric's absorption and action (due to piperine).
- Garlic and Ginger Extracts exhibited moderate to good effects in both assays, suggesting supportive roles in reducing inflammation.
- Cinnamon Extract showed variable and weaker responses, indicating limited anti-inflammatory benefit at the tested concentrations.

Conclusion: -

- The combined findings from the Egg Albumin and Yeast Assays demonstrate that Turmeric extract is the most potent natural anti-inflammatory agent among the tested spices.
- The presence of curcumin contributes to its superior ability to protect proteins and cells from oxidative and inflammatory damage.
- Thus, the study supports the hypothesis that Turmeric possesses higher anti-inflammatory properties than other spices, primarily due to its curcumin content, making it a valuable natural compound for managing inflammation associated with oxidative stress and autoimmune conditions.

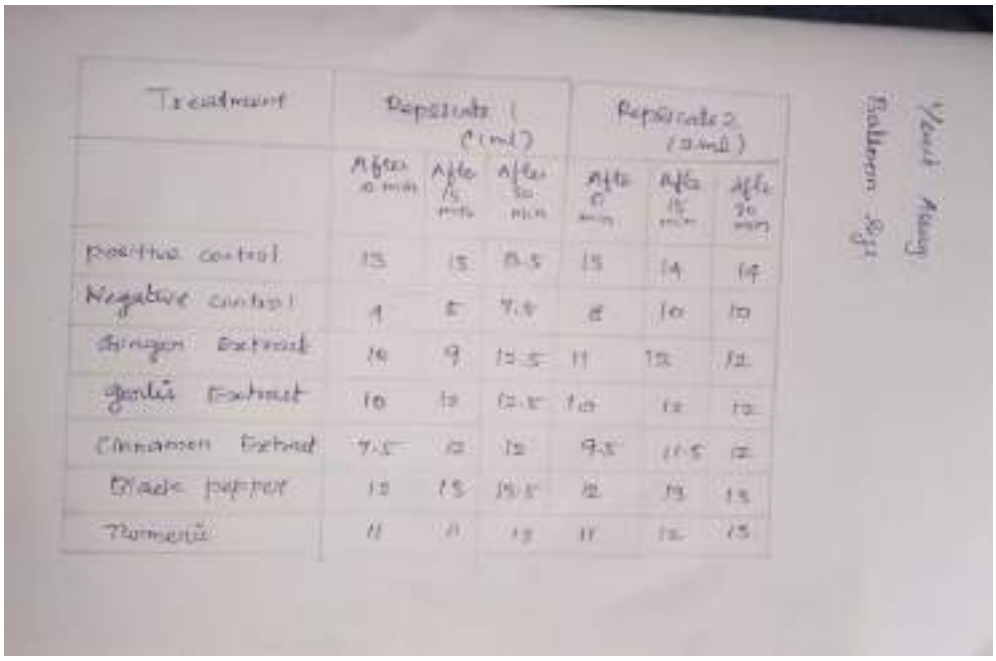
Log book: -



Egg Albumin Assay:

Treatment	Replicate1 (1%)	Replicates (1.5%)	Replicate 3 (2%)
Room temp control	0	0	0
Heated control	5	5	5
Ginger Extract	3.5	4	2.5
Garlic Extract	2.5	4.5	5
Cinnamon Extract	5	1	3.5
Black pepper Extract	4	3.5	3.5
Turmeric Extract	3	2	1.5

Cloudiness Score (0-5)



Treatment	Replicate 1 (1 ml)			Replicate 2 (2 ml)		
	After 10 min	After 15 min	After 30 min	After 10 min	After 15 min	After 30 min
positive control	13	13	13.5	13	14	14
Negative control	9	8	9.5	8	10	10
Ginger Extract	10	9	12.5	11	12	12
Garlic Extract	10	12	12.5	10	12	12
Cinnamon Extract	9.5	12	12	9.5	11.5	12
Black pepper	12	13	13.5	12	13	13
Turmeric	11	11	13	11	12	13

Yeast Assay
Balloon Exp.

Future Enhancement: -

- Repeat using ethanol or mixed-solvent extracts to enhance bioactive yield
- Quantify curcumin, gingerols, and piperine by HPLC

Bibiliography: -

- https://www.researchgate.net/publication/367167929_In_vitro_AntiInflammatory_Egg_Albumin_Denaturation_Assay_An_Enhanced_Approach
- https://www.healthline.com/health/autoimmune-disorders
- https://pmc.ncbi.nlm.nih.gov/articles/PMC6385872/