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 anti inflammatory 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.

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, ginger, turmeric, garlic, cinnamon, black pepper,

Dependent Variables:

- Egg Albumin Assay (protein damage model):
  - Cloudiness score (0–5 scale)
- Yeast Assay (oxidative stress model):
  - Balloon circumference (cm) over time.

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 H_2O_2

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:-

- Test tubes capped with balloons to capture CO_2.
- Measured balloon circumference at:
    - Every 15 min (first half an hour)
- Recorded observations. Repeat the experiment with different concentration of extract.
-

Data Analysis

Egg Albumin Assay:

- **Control Comparisons:**
    - **Control A (Heated)**: Maximum protein denaturation, 0% inhibition.
    - **Control B (Room Temperature)**: No denaturation, 100% inhibition.
    - **Interpretation**: Cloudiness = protein denaturation; less cloudiness = better anti-inflammatory protection.
- **Extract Efficacy**:

    - **Turmeric**: Strongest protection (56.66% inhibition), likely due to curcumin's antioxidant and anti-inflammatory effects.
    - **Cinnamon & Ginger**: Moderate activity, likely due to phenolic compounds and antioxidants.
    - **Black Pepper**: Mild protection, potentially enhancing turmeric's effects.
    - **Garlic**: Least effective in preventing denaturation.
- **Effectiveness Order**: Turmeric > Cinnamon > Ginger > Black Pepper > Garlic.

Yeast Assay:

- **Control Comparisons:**
    - **Positive Control**: Healthy yeast activity.
    - **Negative Control**: Low CO_2 production, indicating oxidative stress.
- **Interpretation**: Balloon size correlates with yeast activity — larger = better anti-inflammatory protection.
- **Extract Efficacy**:

    - **Black Pepper**: Best performance, highest balloon inflation, showing strong anti-inflammatory protection.
    - **Turmeric**: Second-best, confirming its anti-inflammatory effects via curcumin.
    - **Garlic**: Moderate activity, likely due to sulfur-based antioxidants like allicin.
    - **Ginger**: Balanced protection, similar to garlic.
    - **Cinnamon**: Least effective, with small balloon inflation, indicating weak anti-inflammatory activity.
- **Effectiveness Order**: Cinnamon < Ginger < Garlic < Turmeric < Black Pepper.

Overall Interpretation:

- **Turmeric**: Most potent anti-inflammatory agent.
- **Black Pepper**: Strong antioxidant and moderate anti-inflammatory; enhances turmeric's absorption.
- **Garlic & Ginger**: Moderate effects, supporting anti-inflammatory roles.
- **Cinnamon**: Least effective, with weaker anti-inflammatory benefits 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.
- Repeat using ethanol or mixed-solvent extracts to enhance bioactive yield
- Quantify curcumin, gingerols, and piperine by HPLC

Future Enhancements

- 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.