SANA
Model School

HARNESSING THE BIOCATALYTIC POTENTIAL OF ALOE VERA AS AN ECO-FRIENDLY ALTERNATIVE TO SYNTHETIC CATALYSTS

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A. INTRODUCTION

This research lies at the intersection of Biochemistry, Plant Biotechnology, and Green Chemistry. It investigates whether Aloe vera, a plant known for its medicinal and cosmetic uses, also possesses enzymatic properties that qualify it as a natural biocatalyst.

As industries seek eco-friendly alternatives to synthetic catalysts, plant-based enzymes are emerging as promising alternatives for applications in food technology, pharmaceuticals, biofuels, and environmental remediation. This study evaluates the presence and activity of two essential enzymes, catalase and amylase, within Aloe vera, using fundamental biochemical experiments.

A positive result could position Aloe vera as a low-cost, renewable, and accessible enzymatic resource, contributing to more sustainable and bio-based industrial processes.

B. PROBLEM STATEMENT AND BACKGROUND:

A biocatalyst is a naturally occurring substance, typically an enzyme, that accelerates chemical reactions without being consumed in the process. Owing to their high specificity, efficiency, and environmental compatibility, enzymes are central to modern biotechnological advancements.

While microbial enzymes are well-established, plant-derived enzymes are underexplored yet promising. Small reference, hints the enzymatic activity in Aloe vera, but thorough research is lacking, especially for commonly utilized enzymes such as:

- **Catalase**, which breaks down hydrogen peroxide (H_2O_2) into water and oxygen.
- **Amylase**, which hydrolyzes starch into simple sugars.

This study aims to fill that knowledge gap by testing for the presence of these enzymes in Aloe vera extracts, potentially unlocking new pathways for its application beyond traditional uses.

C. OBJECTIVE:

- **Research Problem:**

Can Aloe vera be classified as a natural biocatalyst based on the activity of catalase and amylase enzymes?

- **Research Question:**

Does Aloe vera contain functional and catalytically active enzymes that can be identified through standard biochemical reactions?

- **Relevance:**

Finding the enzyme presence in Aloe vera could lead to scalable, green alternatives to synthetic catalysts in industrial and environmental applications.

- **Experimental Approach:**

1. Extraction of Aloe vera gel.
2. Conducting a Catalase Test (hydrogen peroxide breakdown).
3. Conducting an Amylase Test (iodine-starch color fade analysis).

- **Variables:**

- **Independent Variable**: Presence of *Aloe vera* extract.
- **Dependent Variables**: Bubble formation (catalase); color change (amylase).
- **Controlled Variables**: Volume/concentration of H_2O_2, starch, iodine, temperature, reaction time.

- **Controls:**
  - **Catalase Test**: H_2O_2 without extract.
  - **Amylase Test**: Starch-iodine mixture without extract.

D. HYPOTHESIS:

If Aloe vera contains catalase and amylase, then:

- It will produce oxygen bubbles when exposed to hydrogen peroxide.
- It will cause fading of the blue-black color in an iodine-starch solution over time.

These results would confirm Aloe vera's potential as a plant-based biocatalyst.

E. PROCEDURE:

Materials Required:

- Fresh Aloe vera leaf
- Distilled water
- Blender / Knife
- Filter paper or cloth of any kind
- 3% Hydrogen peroxide
- Iodine solution
- 1% Starch solution
- Test tubes / Beakers / Measuring cylinders

- Pipettes / Droppers
- Timer / Stopwatch
- Heating source
- Thermometer
- Safety gloves and goggles

Preparation of Extracts:

1. A fresh Aloe vera leaf shall be cleaned, and the inner gel extracted.

2. The gel shall be blended with distilled water to prepare an extract.
3. The solution shall be filtered using a muslin cloth or any other thin cloth to obtain a clear liquid.

FILTERING THE ALOE EXTRACT

PURE ALOE EXTRACT

Two types of Aloe vera extracts shall be prepared:

- **Normal Extract**: Used without further treatment.
- **Heated Extract**: Heated in a stove/heating source ~80°C for 5 minutes to denature enzymes, then cooled to room temperature.

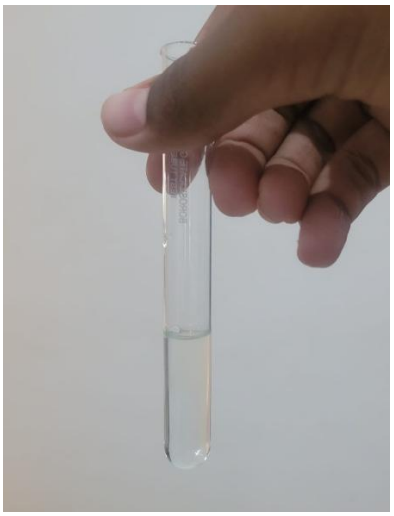
Catalase Activity Test (Foam Height Test)

1. Three test tubes shall be prepared, each containing 5 mL of 3% hydrogen peroxide.

Hydrogen Peroxide

2. Add the following to each tube:
    - Tube 1(Control): No extract (distilled water only).
    - Tube 2: 5 mL Normal Aloe vera extract.
    - Tube 3: 5 mL Heated Aloe vera extract.

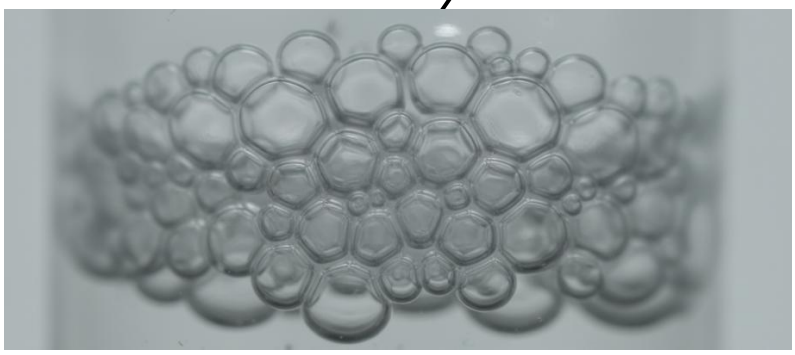
3. Observe and measure the maximum foam height formed over 2–3 minutes using a ruler.
4. Repeat tests for consistency and record values.



Fresh Aloe Extract

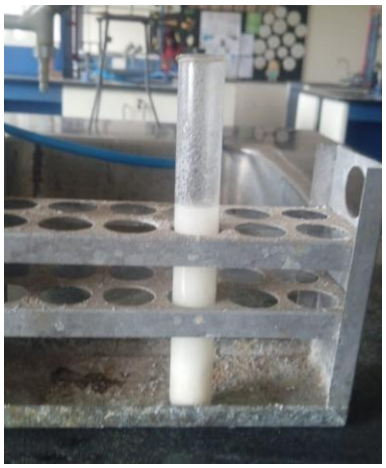
Adding extract to Hydrogen Peroxide

O2 Bubble Formation



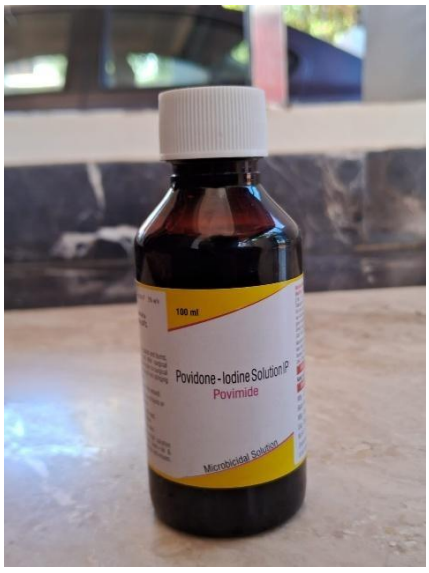
Amylase Activity Test (Iodine-Starch Color Fade Comparison)

1. Mix 2 mL of 1% starch solution with 2 mL of each Aloe vera extract (normal and heated) in separate test tubes.



Starch Solution

2. Add 2–3 drops of iodine solution to each of the test tubes.



Iodine

3. Include a control sample with starch and iodine but no Aloe extract.
4. Also include a sample with starch and using heated aloe extract.
5. Observe and compare the rate and extent of color fading over 3 Trials
6. Record changes in color intensity using visual scoring.

Presence of starch

Solution Along with Aloe extract

Fading of the Blue-Black color

F. RISKS AND SAFETY:

- Handle hydrogen peroxide with gloves and goggles to prevent irritation.
- Dispose of chemical waste responsibly.
- Perform all experiments in a well-ventilated lab with appropriate supervision.

G. RESULTS:

When hydrogen peroxide was added to the fresh Aloe vera extract, foam and oxygen bubbles formed, indicating the presence of the catalase enzyme acting as a biocatalyst.

The foam height was much greater in the fresh extract than in the heated one, showing that heat denatured the enzyme. The control with only hydrogen peroxide showed no reaction, confirming that the bubbles were due to enzyme activity.

Similarly, when Aloe vera extract was mixed with starch solution, The color in the iodine test began to fade but remained light blue, indicating partial breakdown of starch. This shows that Aloe vera extract contains amylase with measurable but moderate activity. The reaction was slower than expected, possibly due to enzyme dilution or suboptimal temperature.

These results show that Aloe vera contains active enzymes such as catalase and amylase, which act as natural biocatalysts and are affected by temperature.

The chemical equation involved in the catalase test is:

$$H_2O_2\ (aq)\ (\text{with catalase}) \dashrightarrow H_2O(l) + O_2(g)$$

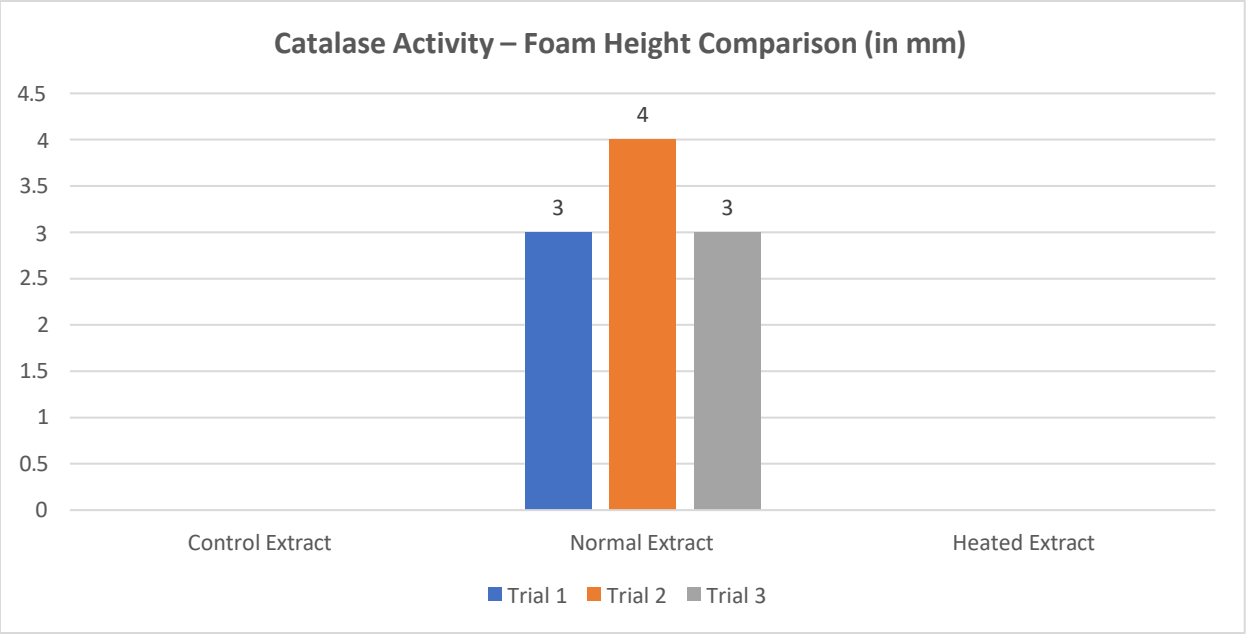
$$\text{Starch}(aq) + H_2O(l)(\text{with Amylase}) \dashrightarrow \text{Maltose} + \text{Dextrin}$$

Table 1: Catalase Activity – Foam Height Comparison

Sample Type	**Volume of H_2O_2**	**Volume of Extract**	**Foam Height(cm) Trial 1**	**Trial 2**	**Trial 3**
Control (no extract)	5 ml	2 ml	0	0	0
Normal extract	5 ml	2 ml	0.4	0.3	0.4
Heated Extract	5 ml	2 ml	0	0	0

Table 2: Amylase Activity – Color Fading Comparison

Sample Type	**Volume of starch**	**Volume of Extract**	**Observed color Trial 1**	**Trial 2**	**Trial 3**
Control (no extract)	5 ml	2 ml	Blue- black color	Blue-black color	Blue-black color
Normal extract	5 ml	2 ml	Light blue	Light blue	Light blue
Heated Extract	5 ml	2 ml	Blue- black color	Blue-black color	Blue-black color



Tools for Analysis:

- Foam height (in cm) will be measured with a scale.
- Color fading will be evaluated using a visual scale.
- Bar graphs will be used to compare catalase and amylase activity across the three conditions.
- Results will be expressed in terms of percentage activity loss in the heated extract compared to the normal extract.

H. DISCUSSION:

From the above result, we can say that Aloe vera contains enzymes like Catalase, Amylase, and much more.

This result proves that these naturally occurring bio-catalysts can be used in various industries.

When I performed the catalase test, I was not able to observe any oxygen bubbles. Turns out the enzymes have become ineffective as it was not stored in a refrigerated environment from which we could say that THE EXTRACT MUST BE STORED IN A REFRIGERATED ENVIRONMENT.

The tools must also be thoroughly washed before using in order to prevent contamination and get accurate results.

I. APPLICATIONS:

Substitute for Chemical Catalysts: Can act as a natural and safe substitute for harsh chemical catalysts in simple biochemical processes.

Wastewater Treatment: Has potential use in eco-friendly wastewater treatment, where the **Catalase** enzyme helps break down peroxide residues and aids in detoxification.

Industrial De-sizing: The **Amylase** enzyme can be tested in the textile industry for processes like textile de-sizing.

Green Product Development: May contribute to the development of **green materials**, enzyme-based detergents, or organic processing techniques.

Food and Bio-based Processing: Applicable in food technology and bio-based food processing where mild enzymatic activity is required.

Pharmaceutical and Medical Use: Since Aloe vera is biocompatible, its enzymes can be useful in developing drug delivery systems, wound healing gels, or oral enzymatic supplements.

Sustainable Biotech Alternatives: Integrating the enzymes into advanced fields like **bioplastic degradation**, compost acceleration, or biosensor development aligns with global sustainability goals.

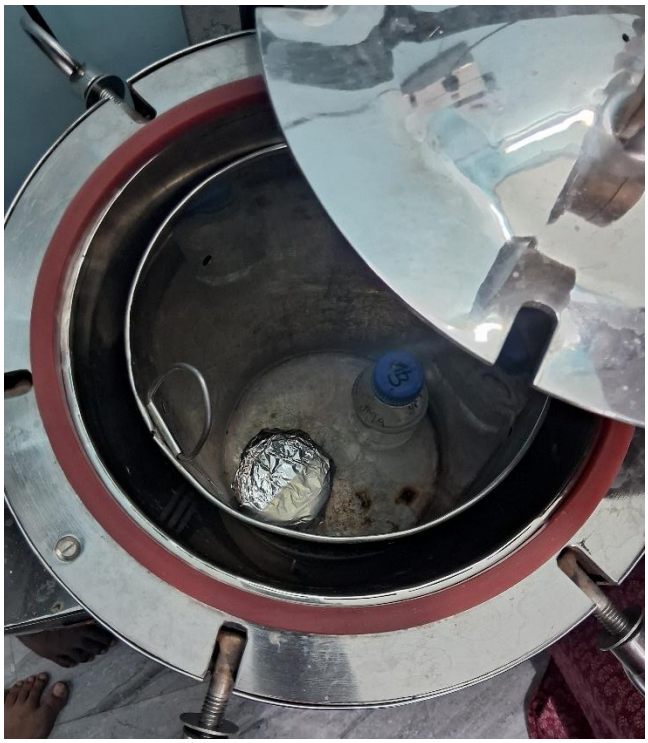
Educational Tool: Useful in educational experiments to demonstrate fundamental enzyme activity.

Aloe vera also has anti-bacterial and anti-fungal properties which prevents the growth of bacteria and fungi. Therefore, it can increase the shelf life of a particular product. In order to prove these properties, I performed Disc Diffusion method which is a test performed to prove the anti-microbial effects on organic products. The procedure for the test is as follows:

1. Prepare a Bacillus subtilis bacterial culture and spread it evenly on nutrient agar Petri dishes to create a uniform bacterial lawn. Divide each plate into three sections for testing the three samples: fresh aloe vera extract (S1) , heated aloe vera extract (S3), and sterile water(S2) as a negative control.
2. Next, take three sterile antibiotic discs and dip each disc into one of the samples. Carefully place each disc onto its respective section on the agar plate, ensuring full contact so the extract can diffuse properly.
3. Incubate the plates inverted at 36°C in an incubator for 18–24 hours. The inverted position prevents condensation from interfering with bacterial growth.
4. After incubation, observe the growth of bacteria around each disc. The section with fresh aloe vera extract shows a clear zone of inhibition, indicating that bacterial growth has been prevented. In contrast, the sections with heated extract and sterile water show normal bacterial growth with no inhibition.
5. These observations indicate that fresh aloe vera extract exhibits antibacterial activity against Bacillus subtilis, while heating reduces its effectiveness. The sterile water serves as a negative control, confirming that the observed inhibition is specifically due to the aloe vera extract.

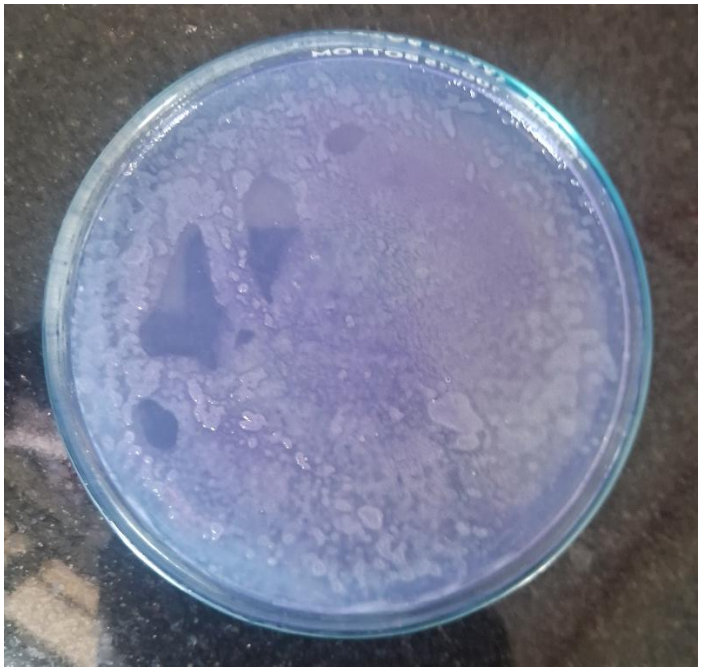
Make sure that all the tools are sterilized before use in order to prevent contamination, and also make sure that you handle the bacteria safely. Label all the parts in order to avoid mix up.

Agar Nutrition Broth



Auto clave

Placing the samples with bacteria



Bacteria colony

Filling cuvettes with heated aloe extract, fresh aloe extract.

Adding antibiotic disks to extracts

Placing the discs in the bacterial colony

Inhibition zones are formed around the discs

J. FUTURE SCOPE:

The confirmation of enzymatic activity in Aloe vera opens up multiple promising research and application avenues:

1. Enzyme Isolation and Purification

- Future studies can isolate and purify the specific catalase and amylase enzymes from Aloe vera to determine their molecular properties, optimum pH, temperature ranges, and kinetic parameters.

2. Industrial Application Trials

- Aloe vera-derived enzymes could be tested in:
    - Textile de-sizing (amylase)
    - Bio-bleaching and detoxification (catalase)
    - Wastewater treatment
    - Bio-based food processing

3. Medical and Pharmaceutical Integration

- Since Aloe vera is biocompatible, its enzymes might be useful in drug delivery systems, wound healing gels, or oral enzymatic supplements.

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5. Enzyme Stability and Shelf-Life Studies

- Investigating stability, storage conditions, and preservation techniques could help in developing Aloe vera-based commercial enzyme products.

6. Medical and Pharmaceutical Integration

- Since Aloe vera is biocompatible, its enzymes might be useful in drug delivery systems, wound healing gels, or oral enzymatic supplements.

7. Sustainable Biotech Alternatives

- Integrating Aloe vera enzymes into bioplastic degradation, compost acceleration, or biosensor development aligns with global sustainability goals.

8. Comparative Phytochemistry

- Extend the study to compare enzyme profiles across different Aloe species or other medicinal plants, expanding the catalog of plant-based biocatalysts.

K. CONCLUSION

This research demonstrates that Aloe vera possesses enzymatic activity, specifically catalase and amylase, validated through simple biochemical experiments. These findings support the hypothesis that Aloe vera can serve as a natural biocatalyst, paving the way for its use in environmentally friendly, plant-based industrial processes.

By building on this foundational work, future research can unlock Aloe vera's full potential—not only as a medicinal plant but also as a biotechnological resource contributing to a greener future.

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