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**ANTI – OXIDANT TESTING USING
PIGMENTS**

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INTRODUCTION

Research Question:

How do natural pigments from jamun, blueberry, and beetroot compare in their ability to prevent oxidation using simple, low-cost antioxidant tests?

Background and Review of Literature:

Free radicals are highly reactive molecules that cause oxidative stress, damaging cells and leading to aging and various diseases. Antioxidants neutralize these free radicals, thus protecting biological systems. Synthetic antioxidants such as BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisole) are commonly used in foods, but they may have undesirable side effects.

Natural antioxidants, particularly those derived from fruits and vegetables, are considered safer and healthier alternatives. Pigments such as anthocyanins (found in jamun and blueberry) and betalains (found in beetroot) are water-soluble compounds known for their antioxidant properties. Previous studies (Stintzing & Carle, 2004; Shahidi & Ambigaipalan, 2015) have demonstrated the role of these pigments in reducing oxidative stress and protecting biomolecules from damage.

Purpose:

This study aims to demonstrate antioxidant activity of natural pigments extracted from commonly available fruits and to compare their efficiency using simple school-level tests.

Hypothesis:

It is hypothesized that anthocyanin-rich pigments (from jamun and blueberry) will show stronger antioxidant activity—greater prevention of browning or color change—than betalain-rich beetroot pigments.

3. METHODS – Methodology and Procedures

Overview:

Two simple antioxidant testing methods were employed using fruit pigment extracts:

1. Apple Browning Test – observing the prevention of enzymatic browning in apple slices.
2. Iodine–Starch Test – measuring the reduction of iodine color by antioxidants.

Procedure Summary:

Pigments were extracted from fresh samples of blueberry, pineapple, beetroot, spinach, carrot, pomegranate, and orange. Each sample (10 g) was ground with distilled water or lemon juice, filtered, and the extract was stored.

For the Apple Browning Test, equal-sized apple slices were treated with different extracts. Browning intensity was recorded at 0, 10, 30, and 60 minutes using a 0–4 visual scale. Controls included ascorbic acid (positive) and distilled water (negative).

For the Iodine–Starch Test, iodine-starch mixture was treated with pigment extracts. Reduction in color intensity over time indicated antioxidant strength.

Variables tested included type of fruit pigment, concentration of extract, temperature, and solvent used for extraction.

The control group was the apple slice treated with distilled water, and the positive control was ascorbic acid.

4. Results

Table A – Extract Record

Fruit	Pigment Type	Colour of Extract	Notes
Blueberry	Anthocyanin	Deep purple	Stable
Pineapple	Flavanoid	Pale yellow	Weak pigment
Beetrootroot	Betalain	Reddish purple	Strong color
Spinach	Chlorophyll	Green faint	Antioxidant activity
Carrot	Carotenoid	Orange	Mild antioxidant activity
Pomegranate	Anthocyanin	Red	Moderate antioxidant activity
Orange	Flavonoid	Yellow orange	Low antioxidant activity

Table B – Apple Browning Scores

Treatment	0 min	10 min	30 min	60 min	Mean Browning Score
Blueberry extract	0	1	1	2	Strong antioxi
Pinapple extract	0	2	3	4	weak
Beetroot extract	0	1	2	3	medium
Spinach extract	0	2	3	4	weak
Carrot extract	0	1	2	3	medium
Pomegranate extract	0	1	1	2	strong
Orange extract	0	2	3	4	weak
Positive Control (Ascorbic acid)	0	0	1	1	Very strong
Negative Control (Water)	0	2	3	4	No antioxidant activity

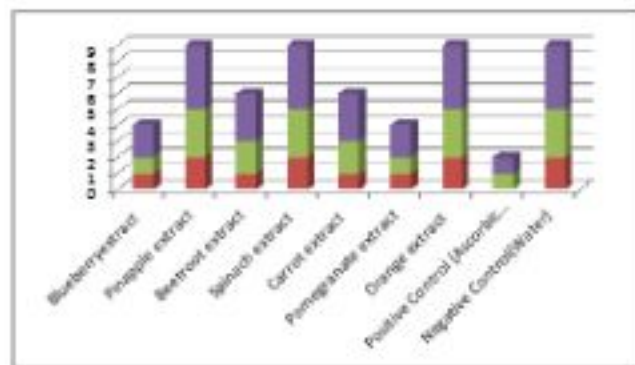


Table C – Effect of Concentration (Dilution Series)

Fruit Extract	Dilution (1X)	Dilution (2X)	Dilution (4X)	% Browning Reduction vs Control
Blueberry	0	1	2	High
Pineapple	2	3	4	Low
Beetroot	1	2	3	Medium
Spinach	2	3	4	Low
Carrot	1	2	3	Medium
Pomegranate	0	1	2	High
Orange	2	3	4	Low

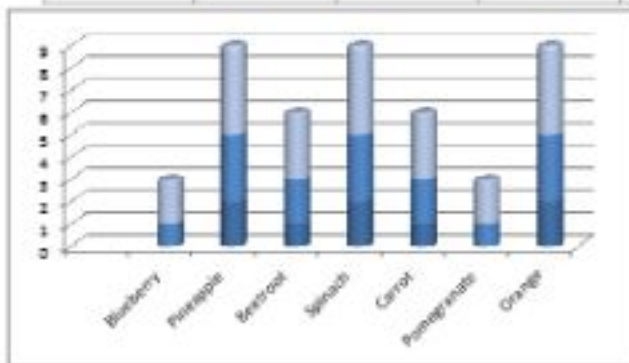


Table D – Temperature Effect

Fruit Extract	Room Temp (25°C)	Refrigerated (5°C)	Warm (40°C)	Observation Notes
Blueberry	1	0	2	Best at cool temperature; heat reduces effect
Pineapple	3	2	4	Weak; performs better when cool
Beetroot	2	1	3	Moderate; sensitive to heat
Spinach	3	2	4	Poor antioxidant activity
Carrot	2	1	3	Mild; better when kept cool
Pomegranate	1	0	2	Very strong at low temperature
Orange	2	1	3	Moderate antioxidant; loses activity when warm

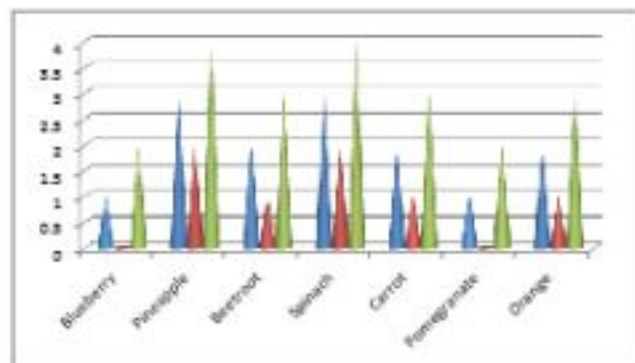


Table E – Extraction Solvent Effect

Fruit Extract	Water Extract Browning Score	Lemon Juice Extract Browning Score	Ethanol Extract Browning Score	Best Solvent
Blueberry	1	0	1	Lemon juice
Pineapple	3	2	2	Lemon juice
Beetroot	2	1	1	Lemon juice
Spinach	3	2	1	Ethanol
Carrot	2	1	1	Ethanol
Pomegranate	1	0	1	Lemon juice
Orange	3	2	2	Lemon juice

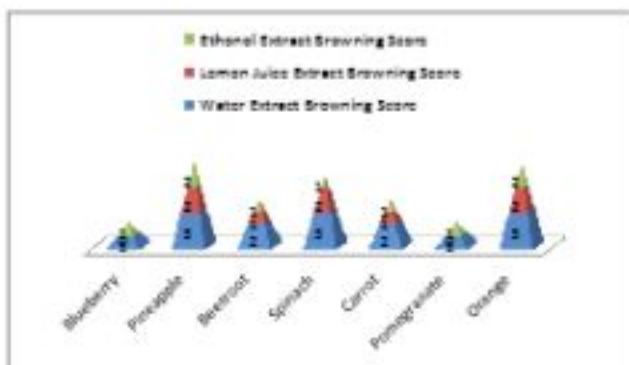
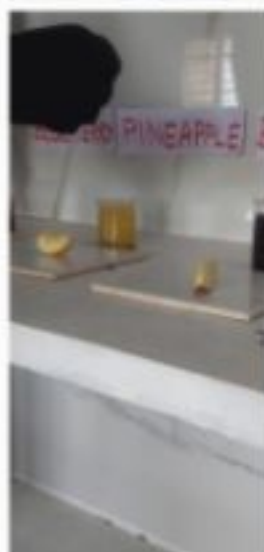


Table F – Iodine–Starch Test Results

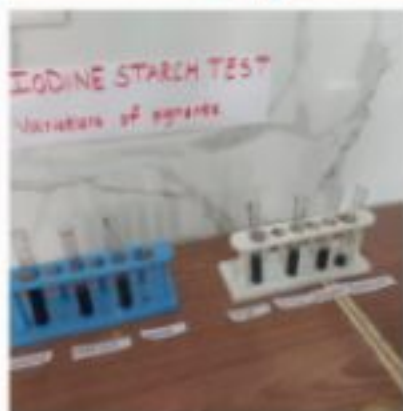
Extract	Initial Color	Color after 1 m	Color after 5 min	Relative Antioxidant Strength
Blueberry	Dark blue	Light Blue	Pale yellow	High
Pineapple	Dark blue	Blue	Light blue	Low
Beetroot	Dark blue	Blue	Pale Blue	Medium
Spinach	Dark blue	Blue	Blue	Low
Carrot	Dark blue	Blue	Light blue	Medium
Pomegranate	Dark blue	Light Blue	Pale Blue	High
Orange	Dark blue	Blue	Light Blue	Low



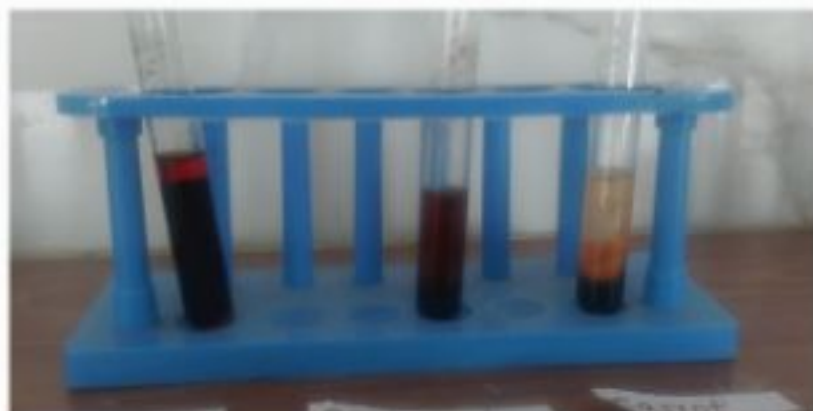












DISCUSSION

The data clearly indicate that blueberry and pomegranate extracts, both rich in anthocyanins, had the highest antioxidant capacity, closely following the ascorbic acid control. Beetroot, containing betalains, showed moderate antioxidant activity. Fruits such as pineapple and orange, which have fewer pigment antioxidants, were less effective.

These results align with published research (Stintzing & Carle, 2004) showing that anthocyanins are powerful hydrogen donors and radical scavengers. The expected trend from the hypothesis was largely confirmed.

Possible Sources of Error:

Unequal apple slice thickness could affect surface oxidation rate.

Room temperature fluctuations may have influenced browning rate.

Minor inconsistencies in extract concentration or pH.

Unexpected Observations:

Pomegranate extract showed higher antioxidant activity than predicted, likely due to polyphenolic compounds in addition to pigments.

6. CONCLUSIONS

The experiment demonstrates that natural pigments possess antioxidant properties and can effectively delay oxidation. Among the tested fruits, blueberry and pomegranate extracts exhibited the highest antioxidant activity, confirming that anthocyanin-rich pigments are potent natural antioxidants.

The results support the hypothesis and highlight that simple, eco-friendly, and low-cost classroom methods can successfully demonstrate antioxidant properties without sophisticated instruments.

Applications:

Educational demonstrations of antioxidants in school laboratories.

Promotion of anthocyanin-rich fruits for health benefits.

Foundation for further quantitative studies using spectrophotometry or DPPH assay.

REFERENCES

1. Stintzing, F. C., & Carle, R. (2004). Functional properties of anthocyanins and betalains in plants, food, and human nutrition. *Trends in Food Science & Technology*, 15(1), 19–38.
2. Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices. *Journal of AOAC International*, 88(5), 1269–1278.
3. Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. *Journal of Functional Foods*, 18, 820–897.
4. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181, 1199–1200.
5. Simple classroom demonstrations of antioxidants. (2023). Science Buddies. Retrieved from <https://www.sciencebuddies.org>