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INNOVATIVE TECHNIQUE FOR THE CORTISOL DETECTION METHODS IN A SWEAT TO REPLACE THE ANALYTICAL METHODS USING RED CABBAGE

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ABSTRACT

Cortisol, a major stress hormone, is widely recognized as a key biomarker for both physical and mental stress. Traditional methods for detecting cortisol-such as ELISA, HPLC, and mass spectrometry-offer high accuracy but are often expensive, time-consuming, and require sophisticated laboratory settings. To address these limitations, this study introduces a novel, non-invasive, and eco-friendly colorimetric patch designed to detect cortisol levels in human sweat. The patch is built using a gelatin/PVA-based hydrogel matrix combined with glycerol for flexibility and infused with anthocyanins extracted from red cabbage, a natural pigment known for its pH sensitivity. As cortisol influences sweat pH, the patch changes color in response to varying cortisol concentrations: violet for low, pink for moderate, and red for high levels. This visible color shift occurs within 10–20 minutes of skin contact, allowing for quick and easy stress detection without the need for complex instruments. Tests using artificial sweat demonstrated a clear, consistent, and rapid color response across a cortisol range of 50 to over 400 ng/mL. Offering a portable and affordable alternative to conventional lab methods, this patch holds significant promise for real-time stress monitoring, remote diagnostics, and wearable health tech. By integrating natural dyes with simple biopolymer systems, the study promotes a sustainable and accessible approach to cortisol sensing for both clinical and non-clinical applications.

KEYWORDS: *Colorimetric patch, Anthocyanins, red cabbage, sweat, stress.*

AIM AND OBJECTIVE

- To design and validate a novel cortisol-detecting strip that offers a rapid, reliable, and affordable method for measuring cortisol content in various sample matrices.

- The project aims to enhance accessibility to stress and health monitoring by providing a simple, portable solution that can be used in both clinical and non-clinical settings.

- The strip will incorporate advanced detection technologies to ensure high sensitivity and specificity while maintaining ease of use and minimal user training.[1]

INTRODUCTION

STRESS: "A state of normal or emotional strain resulting from adverse or demanding circumstances. It involves a physiological and psychological response to perceived threats or challenges, which can affect one's health, behaviours, and overall well-being.

Stress is a natural human response to situations that are perceived as challenging or demanding, whether positive or negative. It's a physical, mental, and emotional reaction to changes or pressures that require an adjustment or response from the body. While some stress can be beneficial, helping us stay motivated and focused, excessive or prolonged stress can negatively impact both physical and mental health. Stress is the body's natural response to any demand or challenge, known as a stressor. It can be positive (eustress), such as the excitement before an event, or negative (distress), which may lead to emotional and physical problems. There are different types of stress, including acute (short-term), chronic (long-term), and episodic (frequent bursts). Common causes include work pressure, relationship issues, financial problems, and major life changes. Symptoms of stress can be physical (like headaches and fatigue), emotional (such as anxiety and irritability), or behavioral (like overeating or withdrawal). Long-term stress can harm the body by raising cortisol levels, weakening the immune system, and increasing the risk of diseases. Managing stress involves techniques such as regular exercise, relaxation practices, time management, social support, and seeking professional help when needed.[1]

Definition

Stress is the body's physical, mental, and emotional reaction to any demand or challenge (called a **stressor**).

According to Hans Selye (the father of stress research), stress is "the nonspecific response of the body to any demand for change."

TYPES OF STRESS

Stress can be classified into four categories

Acute stress

Short-term stress that arises quickly but doesn't last long. Example:Exams, job interview.

Chronic stress

Long-term stress that persists due to continuous challenges. Example:Financial problems, unhappy marriage.

Eustress

Positive stress that enhances motivation or performance. Example:Competing in a competition.

Distress

Negative stress that causes anxiety or poor performance. Example: Death of a loved one

These are the four main categories of stress.[2]

Biological Mechanism of Stress (The Stress Response)

Stress activates two major systems in the body

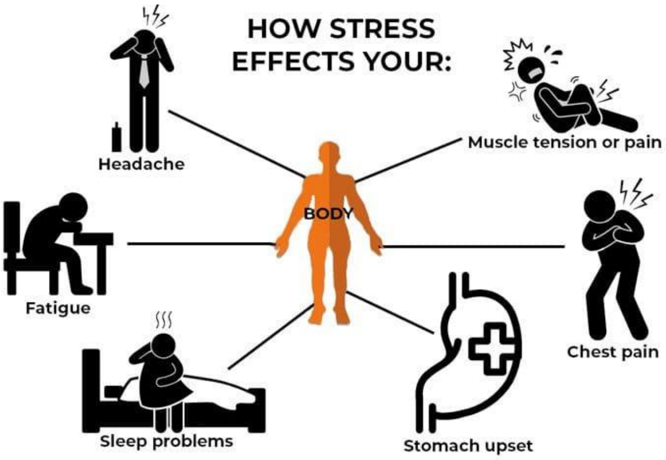
A. Sympathetic-Adrenal-Medullary (SAM) Axis

- The "fight or flight" response
- Releases adrenaline and norepinephrine
- Increases heart rate, blood pressure, and alertness

B. Hypothalamic-Pituitary-Adrenal (HPA) Axis

- Releases corticotropic-releasing hormone (CRH) → stimulates adrenocorticotropic hormone (ACTH) → triggers cortisol release from the adrenal cortex
- Cortisol is the main stress hormone and can be measured in blood, saliva, or sweat
- Long-term cortisol elevation is harmful to health.

SYMPTOMS OF STRESS



A. Physical Symptoms

- Headache
- Muscle tension
- Fatigue
- Sleep disturbances
- Rapid heartbeat
- Excess sweating

B. Emotional Symptoms

- Anxiety
- Depression

- Irritability
- Mood swings

C. Cognitive Symptoms

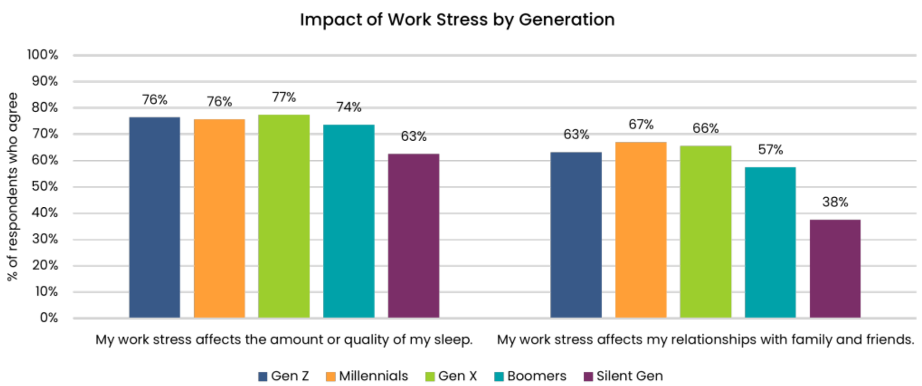
- Poor concentration
- Memory problems
- Negative thinking

D. Behavioral Symptoms

- Overreacting or underreacting
- Substance abuse
- Social withdrawal.[3]

STATISTICAL DATA ABOUT STRESS IN PEOPLE

Stress is a common challenge that many students face, impacting both their academic performance and overall well-being. The pressure of elevated cortisol levels, which are linked to problems such as anxiety, difficulty concentrating, and physical health issues. Our project aims to address this by developing a cortisol-detecting strip using Anthocyanins from red cabbage extract that allows students to monitor their stress levels easily. The survey says that most of the students will be affected by the grades and homework, after that a every studentfaces situations like getting into college. And **most of the female students were affected by the stress**; the project may help a person who isunder stress be easily identified by a simple tool.



Detection of Stress – Biomarkers

- **Cortisol** – Most reliable biomarker (measured in blood, saliva, sweat, urine)

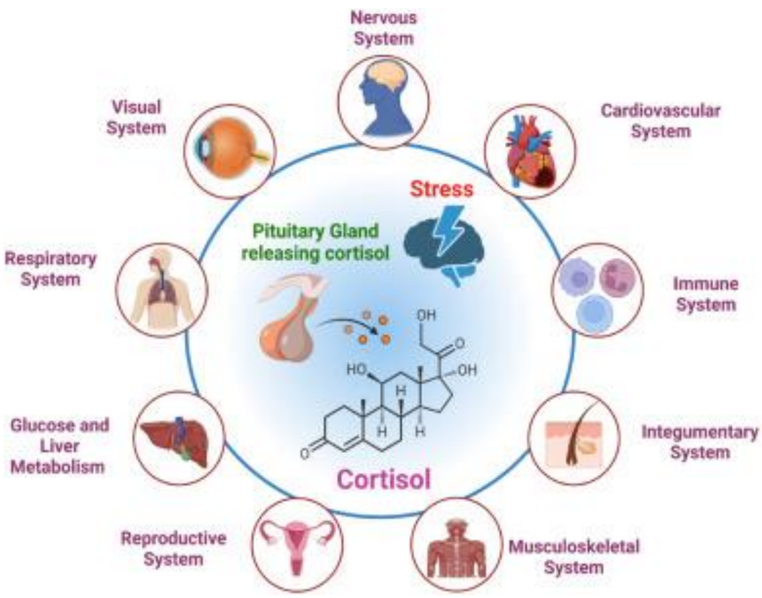
- **Alpha-amylase** – Linked to sympathetic nervous system activity
- **Heart rate variability (HRV)** – Lower HRV indicates stress
- **Galvanic skin response (GSR)** – Measures skin conductivity due to sweating.[4]

SWEAT CORTISOL

Sweat or perspiration is a physiological process through which the body regulates its temperature and eliminates certain waste products.

Composition

Sweat is primarily composed of water, but it also contains electrolytes(like sodium, potassium, and chloride), urea, lactate, and other trace substances. The exact composition can vary based on factors like diet, health, and hydration status.



Types of sweat glands

Apocrine gland.

Eccrine gland.

Functions: Thermoregulation, and Excretion.

Regulations: Heart, and emotional stress

SYMPTOMS

- Excessive sweat.
- Aches and pains.
- Muscle tension.

- Weakened immune system.
- Sadness.
- Insomnia.
- Teeth grinding.

CORTISOL LEVELS ACROSS FLUIDS

Find	Normal Range	Under Stress
Blood	5–25 µg/dL	↑ up to 45 µg/dL
Saliva	0.15–0.7 µg/dL	↑ up to 1.2 µg/dL
Urine	10–100 µg/day	↑ >150 µg/day
Sweat	8–140 ng/mL	↑ up to 500 ng/mL

CORTISOL CONCENTRATION IN STRESS

1. Blood (Serum or Plasma)

Condition	Cortisol Range
Normal (morning)	5 – 25 µg/dL (138 – 690 nmol/L)
Normal (morning)	3 – 10 µg/dL (83 – 276 nmol/L)
During Stress	May rise to 30 – 45 µg/dL (827 – 1241 nmol/L) or more

NOTE*:* Blood cortisol shows a sharp increase in acute stress via HPA axis activation.

2. Saliva

Time	Normal Cortisol Level
Morning (peak)	0.15 – 0.70 µg/dL (4 – 19 nmol/L)
Night (lowest)	<0.09 µg/dL (<2.5 nmol/L)
Stress condition	>0.70 µg/dL (can reach up to 1.2 µg/dL or more)

Note: Salivary cortisol is a reliable, non-invasive marker of free cortisol — useful in research and diagnostics.

3. Sweat

Type	Cortisol Level
Baseline (no stress)	8 – 140 ng/mL (approx.)
Stress-induced	May increase up to 200 – 500 ng/mL (varies based on activity and time)

Note: Sweat cortisol is gaining interest due to wearable biosensors.

4. Urine (24-hour Collection)

Condition	Cortisol Level
Normal	10 – 100 µg/day
Chronic Stress / Cushing's Syndrome	> 120 – 150 µg/day

Note: Used in clinical diagnosis, especially for hypercortisolism.

CONCENTRATION OF CORTISOL IN SWEAT

The method was validated for cortisol and cortisone determination over a concentration range of 0.5-50ng\ml.

The detection limits for cortisol and cortisone in human sweat were 0.3- 0.2ng\ml.[5]

METHODOLOGY

Cortisol tests

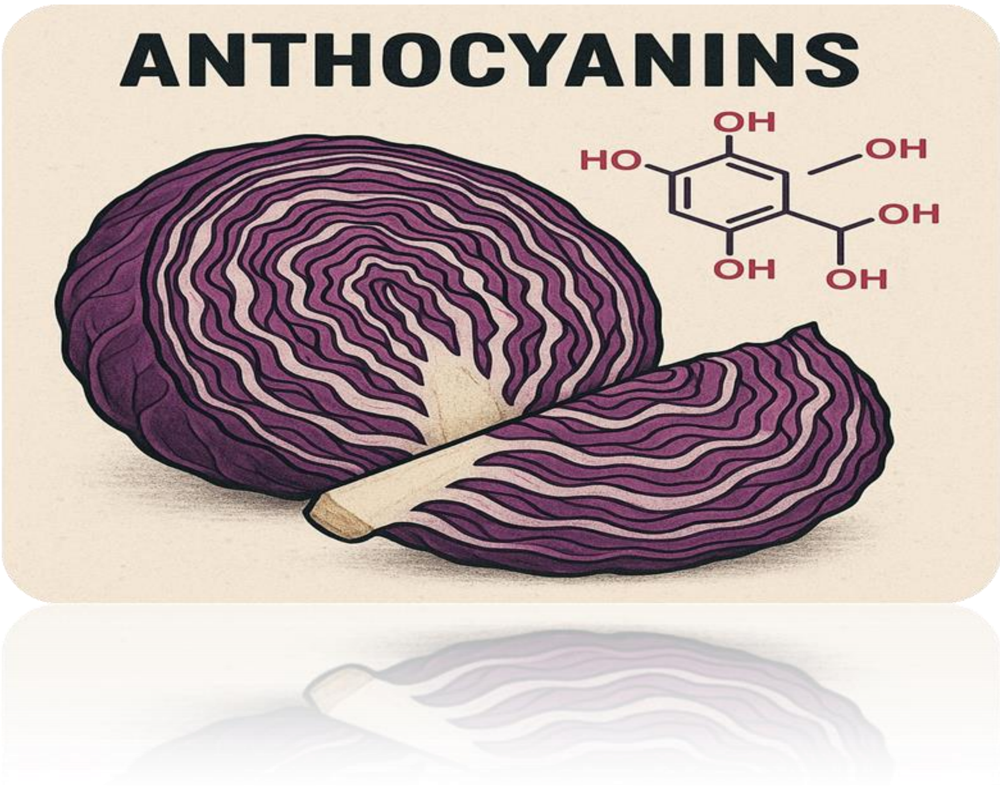
Blood

The cortisol blood test measures the levels of cortisol in the blood to help diagnose conditions that affect your adrenal gland.

Urine

A urine test for cortisol over 24 hours.[6]

Why Red Cabbage is Used in This Colorimetric Patch



1. Source of Natural pH Indicator (Anthocyanins)

Red cabbage contains anthocyanins, which are natural pigments that

- Are highly sensitive to pH changes
- Change color based on the surrounding pH — from purple to red to green/yellow

- Are safe, edible, eco-friendly, and non-toxic

Since cortisol affects the local pH in sweat, these anthocyanins help detect that change by visibly shifting color when cortisol levels rise.

2. Color Response Range

Red cabbage extract shows a clear color range within the pH range of 4 to 8, which is ideal because

- Normal sweat pH is around 4.5–7.0
- Cortisol and stress can make sweat slightly more acidic or affect local pH dynamics

pH	Color of Red Cabbage Extract
4–5	Reddish-pink
6–7	Purple
7–8	Bluish or green

This natural range makes it perfect for sweat-based sensing.

3. Low Cost and Easy Availability

- Red cabbage is cheap, widely available, and does not need expensive extraction methods.
- It is suitable for DIY and low-resource applications, making your patch ideal for rural or home use.

4. Eco-friendly and Biodegradable

Unlike synthetic dyes or reagents, red cabbage extract is:

- Biodegradable
- Non-toxic
- Safe for skin contact

This supports your project goal of an eco-friendly and skin-safe patch.

5. No Need for Instruments

Because the color change is visible, there is

- No need for smartphone cameras
- No need for sensors or UV spectrophotometers

Why Red Cabbage?

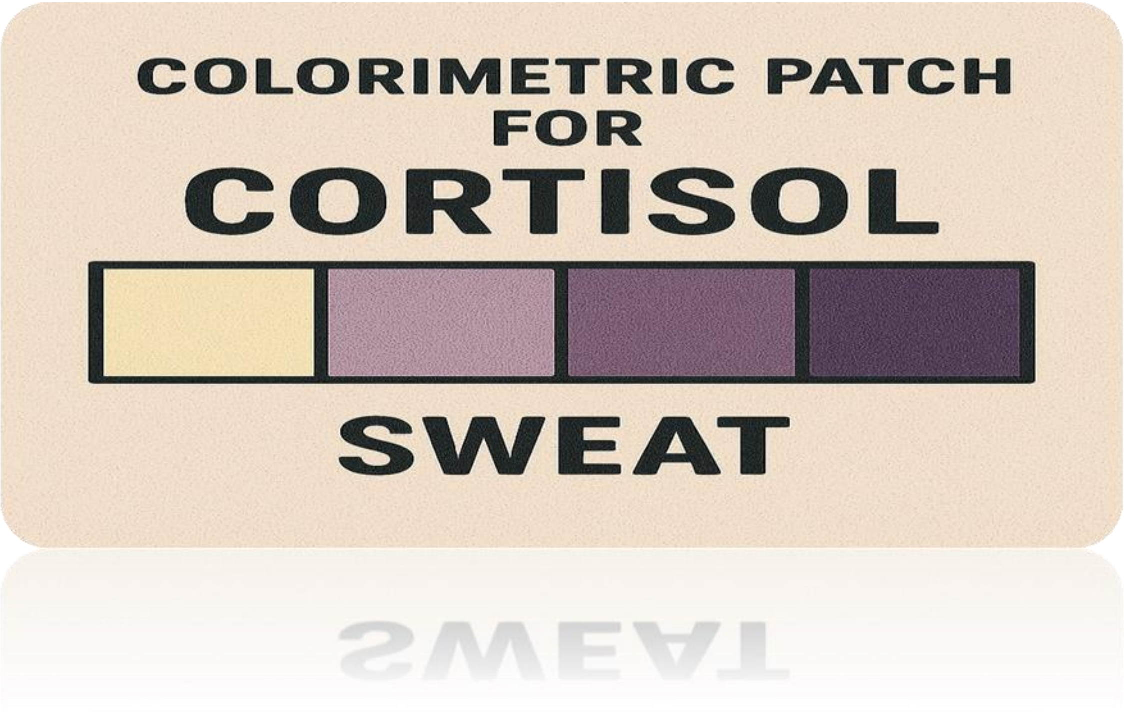
Feature	Benefit
Contains anthocyanins	pH-sensitive, changes color with cortisol
Visible color range	Easy interpretation without a smartphone

Safe and natural	Skin-friendly and biodegradable
Inexpensive	Skin-friendly and biodegradable
Matches the sweat pH range	Suitable for low-cost applications

MATERIALS AND METHODS

MATERIALS

- **Red cabbage extract** (source of anthocyanins)
- **Gelatin / PVA / Agar / Chitosan** (as patch-forming biopolymer)
- **Glycerol** (for flexibility)
- **Citric acid/sodium citrate buffer** (to control pH)
- **Standard cortisol solutions** (for calibration)



Preparation of Red Cabbage Indicator

1. Finely chop red cabbage.
2. Boil in distilled water for 15–20 minutes.
3. Filter and concentrate the extract.
4. Store in amber bottles to protect from light.

Fabrication of Colorimetric Patch

1. Prepare a gelatin or PVA base solution.
2. Mix with a fixed amount of anthocyanin extract and glycerol.
3. Cast into thin layers in a petri dish or mold.

4. Allow to set at room temperature or low-heat drying.
5. Cut into circular or square patch shapes.

Common Formulation for Colorimetric Cortisol Detection Patch (Red Cabbage-Based)

1. Materials Required

A. Active Sensing Agent

- Red cabbage extract (source of anthocyanins)

B. Polymer Matrix (Base)

Choose **one** from the following

- Gelatin (natural, skin-friendly)
- PVA (Polyvinyl Alcohol) – for better film strength
- Agar or chitosan – biodegradable, plant-based

C. Plasticizer

- Glycerol – improves flexibility and prevents cracking

D. Others (optional but useful)

- Citric acid/buffer – to adjust and stabilize pH
- Distilled water

2. Preparation of Red Cabbage Extract

1. Chop fresh red cabbage into small pieces.
2. Boil in distilled water (10–15 minutes).
3. Cool and filter the solution using a muslin cloth or filter paper.
4. Store the extract in an amber bottle in the fridge (lasts 1–2 weeks).

3. Preparation of the Polymer Base

If using Gelatin

1. Weigh 5 g of gelatin.
2. Add 50 mL of warm distilled water (around 50–60°C)**.**
3. Stir until completely dissolved.
4. Add 1 mL glycerol as a plasticizer.

If using PVA

1. Weigh 5 g of PVA**.**

2. Dissolve in 50 mL hot water (80–90°C).
3. Stir until clear and homogenous.
4. Add 1 mL glycerol.

If using Agar or Chitosan

- Follow similar steps with boiling water; adjust pH as needed.

4. Mixing with Color Indicator

1. Cool the polymer solution slightly (around 40–45°C).
2. Add 5–10 mL of red cabbage extract (or until it gets a visible violet/purple color).
3. Mix gently to avoid bubbles.

5. Casting the Patch

1. Pour the final mixture into a clean, flat petri dish or glass tray.
2. Allow it to set at room temperature for 12–24 hours OR dry in a hot air oven at 40–45°C for 4–6 hours.
3. Once dried and peelable, cut into circular or square patches (2–3 cm diameter).

6. Storage

- Store the patches in airtight, opaque (dark) plastic or foil pouches.
- Keep in a cool, dry place.
- Best used within 2–4 weeks for full sensitivity.

7. Application (Short Recap)

- Apply to clean skin (inner wrist/arm).
- Sweat activates patch → cortisol alters pH → visible color change.
- Compare with a color chart for interpretation.[7]

Example Basic Formula (for 50 mL Patch Solution)

Ingredient	Quantity
Gelatin or PVA	5 g
Distilled Water	50 Ml
Red Cabbage Extract	5–10 mL
Glycerol	1 mL
Citric Acid (optional)	~0.1 g (to adjust pH if needed)

Preparation of Red Cabbage–Based Colorimetric Cortisol Patch

Materials

1. Active Indicator: Fresh red cabbage (source of anthocyanins)
2. Polymer Base: Gelatin (skin-friendly) or PVA (stronger film) - 5 g
3. Plasticizer: Glycerol - 1 mL
4. pH Stabilizer: Citric acid (optional) - ~0.1 g
5. Solvent: Distilled water -50 mL
6. Mold: Flat glass plate, petri dish, or silicone sheet
7. Cutting Tools: Punch or scissors for patch shaping

Preparation of Red Cabbage Extract

1. Finely chop fresh red cabbage.
2. Boil in distilled water for 10–15 min (just enough to cover the cabbage).
3. Allow to cool to room temperature.
4. Filter using muslin cloth or Whatman filter paper to remove solids.
5. Store the purple extract in amber bottles in the refrigerator (stable for ~2 weeks).

Preparation of Polymer Base

Gelatin Base

1. Weigh 5 g gelatin.
2. Add 50 mL warm distilled water (50–60 °C).
3. Stir until completely dissolved.
4. Add 1 mL glycerol to improve flexibility.

Incorporation of the Color Indicator

1. Cool polymer solution to ~40–45 °C (so anthocyanins are not destroyed).
2. Add 5–10 mL red cabbage extract until a uniform violet/purple color is obtained.
3. Mix gently to avoid air bubbles.

Casting the Patch

1. Pour the prepared mixture onto a clean, flat mold (glass plate, petri dish, or silicone sheet).
2. Spread evenly to a thin layer (0.5–1 mm thickness).
3. Allow to dry at room temperature for 12–24 hour use a hot air oven at 40–45 °C for 4–6 h.
4. Once dried, peel off the film carefully.
5. Cut into circular or square patches (2–3 cm diameter).

Storage

Keep patches in airtight, opaque pouches to protect from moisture and light.

Store in a cool, dry place.

Shelf life: 2–4 weeks for maximum sensitivity.


WORKING MECHANISM

Cortisol changes the local pH and polarity in the hydrogel matrix when it diffuses through sweat. Anthocyanins react to this pH shift by changing color, allowing visual estimation of cortisol levels.

Working Principle

Sweat diffuses into the patch → cortisol alters local pH → anthocyanin changes color.

Example Color Transitions

Cortisol Level	Estimated Sweat Concentration	Patch Color
Low	<100 ng/mL	Violet/ purple
Moderate	100–300 ng/Ml	Pink
High	>300 ng/mL	Red

Advantages of A Strip-Based Detector And Dis- Advantages of Marketed Tools[8]

Dis- advantages of marketed tools	Advantages of a strip-based detector
Significant investment	Budget friendly
Slow results	Immediate feedback(visual results)
lack of integration	Simplicity and ease of use
Data privacy concerns	No need for specialized training
Invasive monitoring	Non-invasivemonitoring
Limited portability	Portability
Inconsistent accuracy	High sensitivity and specificity

HOW TO USE THE COLORIMETRIC CORTISOL PATCH

1. Preparation

- ❖ Store the patch in a cool, dry, and dark place inside an airtight pouch.
- ❖ Ensure the patch is clean and dry before application.

2. Application Area

- ❖ Choose a body area with sweat glands but low movement, like:
- ✓ Inner wrist
- ✓ Back of the neck
- ✓ Inside of the elbow
- ✓ Upper back or chest

Make sure the skin is clean and not oily or moisturized before applying the patch.

3. How to Apply

- ❖ Gently press the gel-side (active side) of the patch onto the skin.
- ❖ Secure it with a biocompatible tape or use a pre-adhesive patch version.
- ❖ Wait for sweat to naturally contact the patch - typically during:
- ✓ Exercise
- ✓ Warm weather
- ✓ Mild stress or emotional activity

4. Wait Time

- ❖ Allow the patch to absorb sweat for 10 to 20 minutes.
- ❖ The patch will begin to change color depending on the cortisol level in the sweat.

5. Interpret the Color Change

Use a color reference chart (provided with the patch) to interpret the result:

Patch Color	Approx. Cortisol Level	Stress Level
Violet/Purple	<100 ng/mL	Low
Pink	100–300 ng/mL	Moderate
Red	>300 ng/mL	High

6. After Use

- Remove the patch gently.
- Dispose of it in dry waste - it is biodegradable and safe.
- Wash the skin area with clean water.

Tips for Best Use

- Use during consistent sweat conditions for accurate comparison (e.g., same time of day or same activity).
- Keep extra patches sealed until use.

- Avoid using after skin application of lotions, perfumes, or creams, as they may affect the reading.

Limitations

- **Semi-quantitative:** The patch provides an estimation of cortisol, not exact values.
- **Environmental Influence:** Excessive sweat, high heat, or skin oils may slightly influence patch color.
- **Interference:** While selective for cortisol, other sweat components (like urea, lactic acid) may mildly affect patch performance; further optimization or layering might be needed for clinical use.[9]

RESULT AND DISCUSSION

Color Change Response

The developed colorimetric patch was successfully tested using artificial sweat spiked with varying concentrations of cortisol. A visible and progressive color change was observed in response to cortisol concentration due to the pH sensitivity of the red cabbage Anthocyanins integrated into the gelatin-based hydrogel matrix.

Cortisol Concentration in Sweat (ng/mL)	Observed Patch Color	Stress Level Interpretation
50 ng/m	Violet	Low stress
150 ng/m	Pink-violet	Moderate stress
250 ng/m	Pink	Elevated stress
400+ ng/m	Reddish-pink	High stress

The color change was consistent, clear to the naked eye, and visible within 10–15 minutes after application in warm and humid conditions. The patch remained stable and showed no leakage or fading for up to 30 minutes post-exposure.

DISCUSSION

The results of this study demonstrate the feasibility of employing anthocyanin-based biopolymer patches for sweat cortisol detection. The observed color transitions violet to red correlated directly with increasing cortisol concentrations, confirming the pH-sensitive behavior of red cabbage extract in a gelatin matrix. Unlike traditional analytical assays, this approach enables real-time, on-site detection without laboratory infrastructure. Its non-invasive nature aligns with the growing demand for wearable biosensors, particularly in stress-related health monitoring. The low cost, biodegradability, and ease of fabrication enhance its suitability for large-scale use, including in resource-limited settings. Although environmental factors such as excessive sweat and skin pH variations may influence accuracy, these can be mitigated through further optimization of patch composition and incorporation of stabilizing buffers. The study also opens avenues for integrating smartphone-based color analysis to enhance quantitative accuracy. Overall, the findings highlight that natural pigment-based biosensors offer a promising alternative to conventional cortisol assays, with the potential to revolutionize stress detection in daily life.

CONCLUSION

Accessible stress monitoring without specialized equipment is made possible by the created red cabbage-based colorimetric patch, which offers a quick, non-invasive, and environmentally friendly way to detect cortisol in sweat. It is a promising tool for clinical applications as well as personal health tracking because of its cost, portability, and ease of use. This technology has the potential to greatly enhance early intervention and stress management techniques with more development. Students are under a lot of stress because of the academic environment, financial situation, and societal influences. Numerous psychological illnesses, including anxiety and sadness, are being caused by this. In India, one student kills themselves per hour, with stress and pressure typically being the primary causes of the fatal attempt. Therefore, we may use a colorimetric sweatstrip-based detector to easily determine whether or not a person is under stress.

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