

Original Article

Phytochemical-Driven Colorimetric Sensing Platform for Early Screening of Oral Squamous Cell Carcinoma: A Proof-of-Concept Study

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Oral squamous cell carcinoma (OSCC) carries a disproportionate disease burden in low-resource settings, where conventional diagnostic tools remain largely inaccessible. Saliva provides a non-invasive and readily obtainable medium for early pathology screening. Natural phytochemicals, including curcumin and anthocyanins, display well-characterized pH-responsive colorimetric behaviour, making them suitable candidates for integration into paper-based biosensing platforms. This study presents a proof-of-concept dual-coated phytochemical strip, combining curcumin and anthocyanins on a pH-sensitive cellulose substrate, designed for rapid salivary screening in oral oncology settings. Curcumin was obtained from turmeric rhizomes via ethanol maceration, and anthocyanins were isolated from red cabbage using aqueous acidified extraction. A dual-layer strip was constructed by applying curcumin on one face and anthocyanins on the reverse face of commercial pH paper. Parallel control strips were fabricated on neutral filter paper. Unstimulated saliva was collected from 20 participants (10 healthy controls and 10 clinically confirmed OSCC patients). Colorimetric responses were captured within 30 seconds and quantified using RGB-derived ΔE metrics. Structural and physicochemical characterisation involved UV-Vis spectroscopy, FTIR spectroscopy, DSC-TGA, and SEM-EDX analyses. Between-group comparisons were conducted using independent samples t-tests ($p < 0.05$). The dual-coated strip produced distinct, visually discriminable chromogenic transitions: yellow-violet under normal saliva and orange-bluish green under cancer saliva. UV-Vis data confirmed bathochromic shifts consistent with pH-responsive dye behaviour. FTIR indicated the presence of phytochemical functional groups on the substrate, albeit with acknowledged potential for signal overlap from the matrix. DSC-TGA revealed altered thermal stability upon cancer saliva exposure. SEM-EDX showed increased surface roughness and higher ionic signal intensities in cancer-exposed strips. Independent t-tests confirmed highly significant between-group differences across all measured parameters ($p < 0.001$). This proof-of-concept dual phytochemical strip offers a low-cost, eco-friendly, and biocompatible approach to first-line oral pathology screening using saliva. Its rapid response and ease of use highlight its potential utility in point-of-care settings, particularly in under-resourced communities. Large-scale, multi-centre validation is required before clinical deployment can be considered.

Introduction

The global incidence of oral cancer continues to climb, with a disproportionate impact observed in low- and middle-income countries where early detection strategies remain insufficiently developed. Despite notable advances in cancer diagnostics, the early-stage identifica-

tion of oral malignancies persists as a clinical challenge, largely owing to the absence of affordable, non-invasive, and accessible point-of-care tools [1]. Saliva, a complex biofluid produced within the oral cavity, represents a promising diagnostic medium because it is readily collectable and carries a wealth of biochemical information. Among its measurable parameters, salivary pH is an important physiological indicator

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that reflects both local and systemic perturbations associated with disease. Growing evidence indicates that patients with oral malignancies tend to exhibit elevated salivary pH, attributable to microbiome alterations, shifts in metabolic activity, and disrupted glandular function - changes that, though subtle, lend themselves to exploitation by pH-responsive sensing materials [2,3].

Conventional pH measurement approaches used in clinical practice - including electronic meters, litmus-based indicators, and electrochemical probes - frequently demand calibration, periodic maintenance, or costly instrumentation [4,5]. Their inaccessibility in rural or resource-limited environments, precisely the settings where oral cancer prevalence tends to be highest, underscores the demand for simpler and lower-cost alternatives. Paper-based colorimetric sensors employing natural pH indicators have emerged as an attractive option: they require no external power, are disposable, and produce visually interpretable results. These properties align well with the requirements of decentralised healthcare diagnostics.

Among natural pH-sensitive compounds, curcumin and anthocyanins have attracted sustained interest in analytical chemistry. Curcumin, the principal chromophore in turmeric (*Curcuma longa*), transitions from yellow in acidic environments to red-orange under alkaline conditions [6,7]. It possesses well-documented antioxidant, anti-inflammatory, and antimicrobial properties and has an extensive history of safe human consumption. Anthocyanins, extracted from red cabbage (*Brassica oleracea*), undergo broad pH-dependent chromatic transitions: red in acidic environments, purple at near-neutral pH, and green to yellow under alkaline conditions [8,9]. Their water solubility, non-toxicity, and amenability to green extraction methods make them well-suited for integration into biosensing applications.

Standalone application of natural dyes on inert substrates often yields limited sensitivity or colour contrast because of inadequate buffering capacity [10]. Commercial pH paper, by contrast, is pre-impregnated with indicator dyes and buffer systems engineered to produce rapid, high-contrast responses upon aqueous contact. Combining the intrinsic pH sensitivity of curcumin and anthocyanins with the enhanced performance characteristics of a buffered cellulosic substrate offers a route toward a dual-layered, biofunctionalized oral strip capable of registering the subtle salivary pH shifts associated with oral pathology. The dual-coating design, in which each face of the pH paper carries a different natural dye, provides a degree of multiplexed sensing that extends the effective detection range and improves visual discrimination.

This study reports the fabrication and characterisation of a novel pH-responsive oral strip employing red cabbage-derived anthocyanins on one face and turmeric-derived curcumin on the other. The strip is designed to respond rapidly and visually to salivary pH differences, enabling preliminary discrimination between normal and pathologically altered samples. The fabrication process is intentionally simple, reproducible, and environmentally benign, relying on ethanol and water-based extraction methods without synthetic chemicals or toxic solvents. The strip was evaluated against saliva samples from healthy individuals and from patients with a confirmed diagnosis of OSCC.

It is important to note from the outset that alkaline salivary pH is not exclusive to OSCC. Severe periodontal disease, chronic gingivitis, and certain forms of oral microbiomic dysbiosis are similarly associated with elevated pH values. The strip described here is therefore best understood as a first-line screening tool for oral pathology rather than a definitive OSCC diagnostic test. A positive colorimetric result should prompt further clinical evaluation, including periodontal assessment and, where clinically warranted, histopathological biopsy.

Materials and Methods

The primary materials used in this study included commercial universal pH paper (Whatman, pH range 1–14) and neutral filter paper (Whatman Grade 1), the latter serving as an inert, non-buffered control substrate. Fresh turmeric rhizomes (*Curcuma longa*) and red cabbage heads (*Brassica oleracea*) were sourced from local markets for natural pH indicator extraction. Ethanol (analytical grade), glacial acetic acid, and distilled water served as solvents and reagents. All consumables were pre-sterilised.

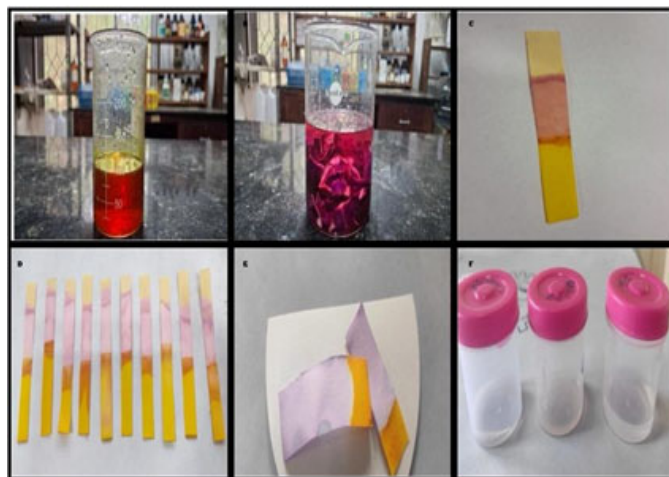


Figure 1: a) Curcumin extract from turmeric, b) Anthocyanin extract from red cabbage, c) Fabricated pH strip, d) Tray dried, e) Control neutral filter paper, f) Saliva collection

Analytical instruments included a UV-Visible spectrophotometer, an ATR-FTIR spectrometer, a simultaneous DSC-TGA system, and a scanning electron microscope with EDX capability.

Extraction of curcumin from turmeric

Curcumin was extracted by solvent maceration. Fresh turmeric rhizomes were washed with distilled water, sliced, and dried in a hot-air oven at 50°C for 48 hours, then ground to a fine powder. Ten grams of the powder were transferred to a 250 mL conical flask and macerated in 100 mL of 95% ethanol with continuous magnetic stirring at room temperature for 24 hours. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator at 40°C to yield a semi-solid curcumin-rich paste. This extract was stored in amber vials at 4°C in the dark until use [11] (figure 1a).

Extraction of anthocyanins from red cabbage

Anthocyanins were obtained by aqueous acidified extraction. Approximately 100 grams of chopped red cabbage leaves were boiled in 200 mL of distilled water acidified with 1% (v/v) glacial acetic acid under constant stirring for 30 minutes. Acidification preserved the flavylum cation structure of the anthocyanins. After cooling, the extract was filtered to remove plant residues, and the resulting deep purple filtrate was stored at 4°C. No further concentration was performed in order to preserve the native pigment structure [12] (figure 1b).

Fabrication of dual-coated pH paper strips

Universal pH paper was cut into 2 x 5 cm rectangles. Curcumin extract was applied uniformly to one face of each strip using a fine brush and dried at ambient temperature for 12 hours in the dark. Once the curcumin layer was fully dried, anthocyanin extract was applied to the opposite face using a separate brush to prevent cross-contamination. Strips were dried under the same conditions and stored in airtight containers with silica gel desiccants. The dual-coating design provided complementary pH sensitivity across a broader detection range than would be achievable with a single indicator (figure 1c, 1d).

Fabrication of control strips using neutral filter paper

Control strips were prepared on Whatman Grade 1 filter paper using the same coating and drying procedures described above. Each filter paper strip was coated with a single dye on one side only. These strips served to assess the standalone performance of natural dyes in the absence of the pre-buffered pH paper chemistry, isolating the contribution of substrate selection to sensor performance (figure 1e).

Saliva collection and ethical compliance

Unstimulated whole saliva was collected from 20 adult participants: 10 healthy volunteers (normal-saliva group, NS) and 10 individuals with treatment-naïve, clinically confirmed OSCC (cancer-saliva group, CS), staged I–III (figure 1f). Ethical approval was obtained from the Institutional Review Board. All participants provided written informed consent in their preferred language, and the study was conducted in accordance with the Declaration of Helsinki (2013 revision) and ICH-GCP (E6 R2) standards.

Participants in the NS group were free of oral lesions, systemic illness, and recent antibiotic use. Exclusion criteria for both groups encompassed pregnancy, known salivary gland disorders, smoking within 12 hours, and alcohol consumption within 24 hours before sample collection. Participants abstained from food, beverages (except water), and oral hygiene products for 60–90 minutes prior to sampling. Saliva was collected by passive drool into sterile 2 mL microcentrifuge tubes kept on ice. A minimum of 5 mL was collected within 10 minutes. Samples were processed within 15 minutes or stored at 4°C for up to 4 hours; for extended analysis, samples were centrifuged (4,000 g, 4°C, 10 minutes), and the supernatant was aliquoted, flash-frozen in liquid nitrogen, and stored at -80°C for up to four weeks (one freeze–thaw cycle maximum) [13–15].

For strip testing, 200 μ L of saliva was applied to the centre of the dual-coated strip via micropipette. Colorimetric changes were documented by standardised digital photography within 1 minute under consistent lighting. All sample handling was conducted under biosafety level-II conditions.

UV-visible spectroscopy

Absorbance spectra of curcumin and anthocyanin extracts, diluted in ethanol and acidified water respectively, were recorded from 200–800 nm using a double-beam UV-Vis spectrophotometer. Peak positions for curcumin (~420–430 nm) and anthocyanins (~520–540 nm) were monitored to verify compound identity and stability. Spectral comparisons before and after saliva exposure were performed to assess pH-responsive bathochromic or hypsochromic shifts.

Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra were recorded in ATR mode from 4000 cm^{-1} to 400 cm^{-1} . Spectra of coated strips were compared against an uncoated pH paper baseline to attempt isolation of phytochemical-associated signals from the cellulose and buffer salt matrix. Characteristic curcumin bands (C=C stretching ~1625 cm^{-1} , phenolic O–H stretching ~3300 cm^{-1} and anthocyanin bands (C=O stretching ~1650 cm^{-1} ; C=C skeletal vibrations ~1400 cm^{-1}) were used as reference markers.

Differential scanning calorimetry–thermogravimetric analysis (DSC-TGA)

Thermal characterisation was conducted using simultaneous DSC-TGA. Approximately 10 mg of each sample was placed in aluminium crucibles and heated under a nitrogen atmosphere from 30°C to 600°C at 10°C/min. DSC data identified enthalpic transitions, while TGA provided mass loss profiles. Results were compared across untreated strips, normal saliva-exposed strips, and cancer saliva-exposed strips.

Scanning electron microscopy and energy-dispersive x-ray spectroscopy (SEM-EDX)

Surface morphology and elemental composition were evaluated using SEM-EDX. Samples were sputter-coated with gold prior to imaging. SEM was used to characterise surface texture and dye distribution; EDX provided elemental profiles. All SEM images include high-contrast scale bars (5 μm at 5,000 $\times$ magnification). It should be noted that EDX-detected ionic changes (Na, K) in saliva-exposed pH paper strips may partly reflect mobilisation of pre-existing buffer salts from the commercial pH paper substrate upon aqueous contact, and not solely salivary ionic content. This limitation is discussed in the discussion section.

Colorimetric evaluation and image analysis

High-resolution digital photography was used to document colorimetric responses under controlled lighting. RGB values extracted from strip images using ImageJ were converted to ΔE (CIELAB colour difference) to quantify chromatic change between normal and cancer saliva-exposed strips and between substrate types.

Statistical analysis

Statistical analysis was conducted using SPSS v25.0. Independent samples t-tests and Levene's test for equality of variances were applied to compare ΔE , surface roughness, and ionic concentrations (sodium, potassium) between groups. A p-value below 0.05 was considered statistically significant.

Results

Visual and colorimetric response to saliva

Upon contact with saliva, the dual-coated pH paper strips produced distinct and reproducible colour transitions within 30 seconds. In the presence of normal saliva (pH ~6.5–6.8), the curcumin-coated face (NS-Yellow) displayed a yellow-orange hue consistent with the dye's acidic-range coloration, while the anthocyanin-coated face (NS-White) appeared violet to reddish-purple. When cancer saliva (pH ~7.5–8.0) was applied, the curcumin face (CS-Yellow) shifted to reddish-orange and the anthocyanin face (CS-White) changed to a bluish-green, reflecting both dyes' alkaline-state spectral profiles. The buffering agents within the pH paper substrate appeared to accelerate proton exchange, enhancing colour contrast and response kinetics (figure 2). Control strips using neutral filter paper also registered colour transitions, but with reduced contrast and slower kinetics, confirming the importance of substrate chemistry in determining sensor performance.

UV-visible spectroscopic analysis

The ethanolic curcumin extract exhibited a primary absorption maximum at approximately 426 nm, consistent with $\pi \rightarrow \pi^*$ transitions of the conjugated diketone structure. Upon cancer saliva exposure, a bathochromic shift to approximately 432 nm was observed on the CS-Yellow strip, consistent with increased environmental polarity at alkaline pH. The anthocyanin extract in acidified aqueous solution produced a peak at approximately 530 nm. Following cancer saliva contact, the CS-White strip showed peak broadening with a shift toward 590 nm, indicative of conversion from the flavylium cation to quinoidal or chalcone base forms under alkaline conditions. Both spectral shifts confirmed the pH-responsive properties of the immobilised dyes (figure 3).

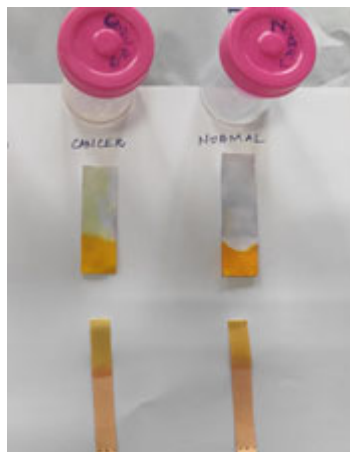


Figure 2: Visual colorimetric response in neutral filter paper and pH strips

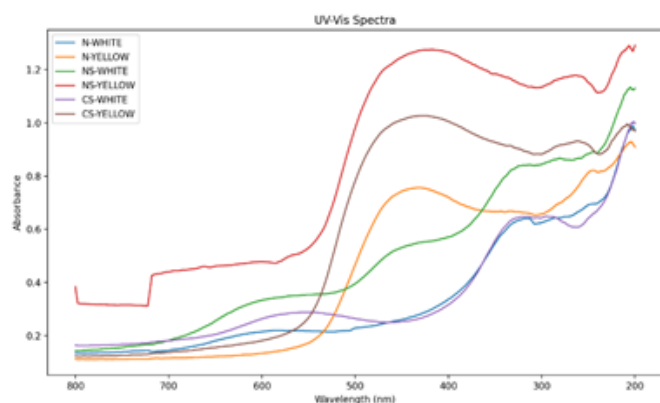


Figure 3: UV Vis Spectra of Biosensor Strip. UV-Vis absorption spectra of biosensor strips treated with normal (N), normal stimulated (NS), and cancer stimulated (CS) saliva under white and yellow light. Distinct absorbance patterns were observed, with increased absorbance in the 400–600 nm range for the cancer groups, especially in CS-Yellow, reflecting a higher chromophore concentration and biochemical complexity. The progressive shift in absorbance intensity supports optical detection of pathological saliva alterations.

The fluorescence emission profile of the curcumin-coated strip, excited at 420 nm, shows a single, pronounced band that rises steeply from 425 nm, reaches a maximum at 548 nm (4.25×10^6 a.u.), and then decays gradually toward 700 nm; the integrated area under the curve is 4.20×10^6 a.u. This broad green–orange band is characteristic of the δ – δ^* emissive transition of deprotonated curcumin, confirming successful dye deposition and an overall neutral to mildly basic micro-environment on the strip. The absence of secondary shoulders or short-wavelength peaks indicates minimal inner-filter or scattering artefacts. In contrast, the smooth monotonic fall-off beyond 600 nm suggests negligible re-absorption by red-cabbage anthocyanins on the opposite face. Taken together, these spectral features establish a reliable baseline against which saliva-induced quenching or bathochromic shifts can be compared for the diagnostic discrimination of normal versus oral cancer samples (figure 4).

Fourier transform infrared spectroscopy

FTIR analysis of the curcumin extract identified characteristic bands at

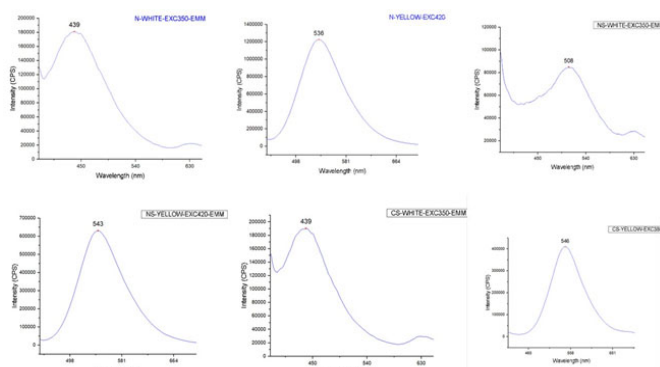


Figure 4: Fluorescence emission spectra of the biosensor strip (N-White, NS-White, CS-White, N-Yellow, NS-Yellow, CS-Yellow) showing baseline and saliva-induced changes under appropriate excitation wavelengths

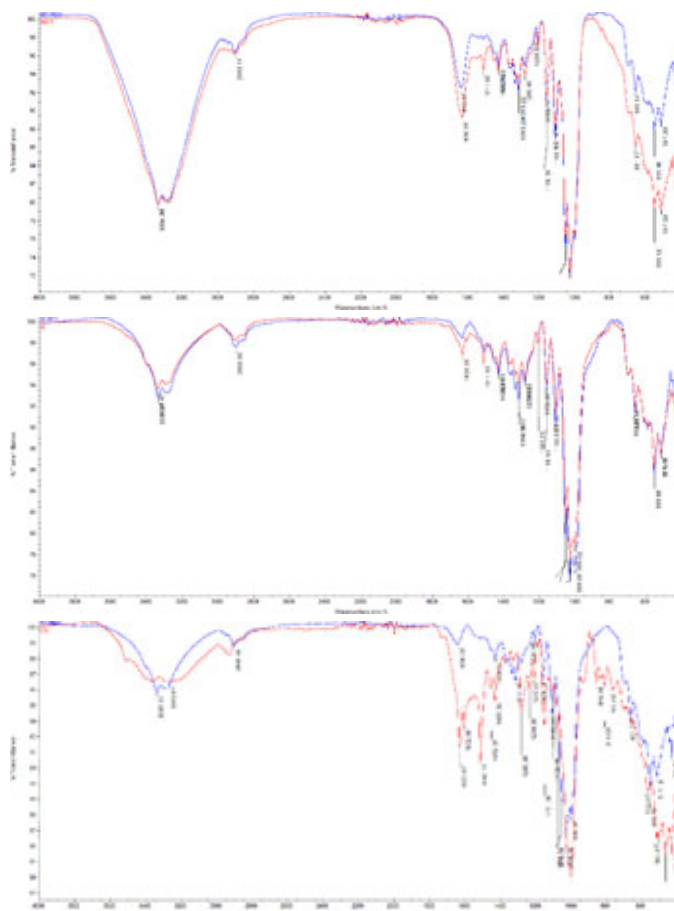


Figure 5: Fourier Transform Infrared (FTIR) spectra of the fabricated biosensor strip (N)-A, strip coated with normal saliva (NS)-B, and strip coated with cancer saliva (CS)-C were recorded to assess chemical interactions and functional group modifications

1627 cm^{-1} (aromatic C=C stretching), 1509 cm^{-1} (C=O stretching, α -diketone), and 1274 cm^{-1} (C–O stretching). Anthocyanin-coated samples showed bands around 3300 cm^{-1} (O–H stretching), 1650 cm^{-1} (conjugated C=O stretching), and 1400 cm^{-1} (C=C skeletal vibrations) (figure 5).

Peaks observed in the coated strips - particularly phenolic O–H stretching (~ 3300 – 3400 cm^{-1}) and aromatic C=C stretching (~ 1625 cm^{-1}) - were elevated relative to the uncoated pH paper baseline, suggesting the presence of immobilised phytochemical residues on the substrate. However, it should be noted that commercial pH paper contains pre-impregnated synthetic indicator dyes and buffer salts whose IR signatures overlap with those of the natural dye coatings. Definitive assignment of individual peaks to phytochemical species cannot therefore be made without rigorous baseline subtraction and differential spectroscopy. The functional group assignments reported here should be understood as indicative of phytochemical presence rather than exclusive attribution, and the results are best viewed in the context of the complementary spectral and morphological data presented.

Thermal analysis (DSC–TGA)

Thermal characterisation of biofunctionalised dual-indicator strips

The thermal behaviour of dual-coated strips was evaluated under three conditions: untreated control strips, normal saliva-exposed strips, and cancer saliva-exposed strips. These analyses aimed to assess the influence of biological fluid composition on the enthalpic transitions and degradation profiles of the sensor system.

Untreated strips (control samples)

The unexposed dual-indicator strip (N-t) displayed a single endothermic event at 42.29°C with an associated enthalpy of 435.6 J/g. TGA recorded 12.08% mass loss, primarily attributable to evaporation of loosely bound moisture and volatile components from the dye coatings. This serves as the thermal baseline for the biofunctionalised cellulosic system.

Effect of normal saliva on thermal properties

Exposure to normal saliva shifted the endothermic peak on the turmeric-coated face (NS-t) slightly to 43.47°C, with a reduced enthalpy of 304.4 J/g and marginally increased mass loss of 13.18%. These changes are consistent with partial interaction between salivary biomolecules and curcumin, though the precise molecular basis of this interaction warrants further investigation. The anthocyanin-coated face (NS-r) produced two distinct thermal events at 23.80°C and 38.75°C, with markedly elevated enthalpy (972.8 J/g) and mass loss (24.29%), suggesting energetically complex interactions between anthocyanins and salivary constituents such as mucins or electrolytes.

Thermal response of cancer saliva-exposed strips

DSC-TGA thermograms of biosensor strips under normal (N), normal saliva-exposed (NS), and cancer saliva-exposed (CS) conditions, showing heat flow and weight loss profiles across temperature ranges (figure 6).

Strips exposed to cancer saliva exhibited altered thermal profiles. On the curcumin-coated face (CS-t), the transition was recorded at 36.80°C with an enthalpy of 438.4 J/g and a mass loss of 11.26%. The earlier onset temperature tentatively suggests that cancer-associated salivary constituents - possibly reactive metabolites, proteolytic enzymes, or altered ionic species - may reduce the energy threshold required for structural reorganisation of the curcumin matrix; however, this interpretation is mechanistically speculative and would require molecular-level validation in subsequent work.

The anthocyanin-coated face (CS-r) retained a biphasic transition pattern at 24.03°C and 42.25°C, though with a substantially reduced enthalpy of 432.0 J/g and mass loss of 13.82%. This reduction compared to normal saliva-exposed strips may reflect less favourable molecular interactions between anthocyanins and the altered biochemical environment of cancer saliva, possibly attributable to higher oxidative load, elevated pH, or the presence of atypical metabolic products. These observations are proposed as working hypotheses and should be tested in future mechanistic studies.

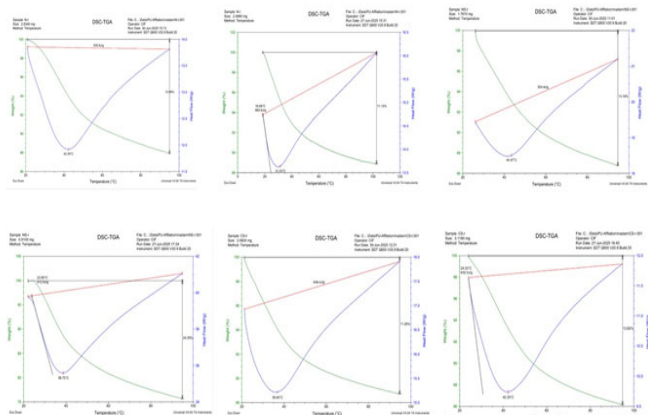


Figure 6: Differential Scanning Calorimetry – Thermogravimetric Analysis (DSC-TGA) thermograms of curcumin-anthocyanin infused biosensor strips treated with normal (N), normal stimulated (NS), and cancer stimulated (CS) saliva

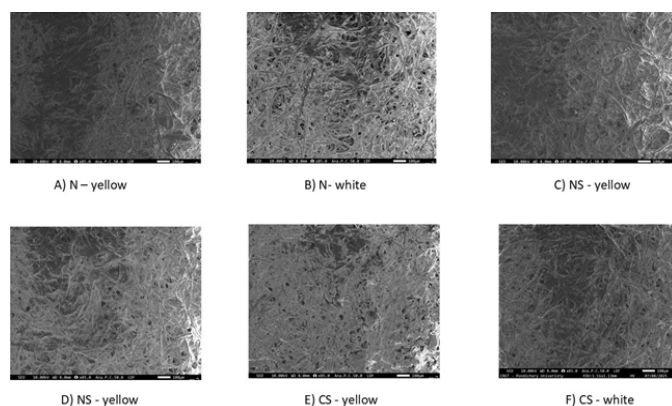


Figure 7: Scanning Electron Microscopy (SEM) images of biosensor strips coated with normal saliva (A–B), normal stimulated saliva (C–D), and cancer stimulated saliva (E–F) under different lighting conditions (yellow and white). Images were captured at a magnification of 5,000x

Collectively, the DSC-TGA data indicate that salivary composition measurably influences the thermal behaviour of the biofunctionalised strip surface, with cancer saliva consistently producing distinct thermal profiles relative to both untreated and normal saliva-exposed controls.

SEM morphology and EDX composition

SEM analysis revealed distinct morphological characteristics across coating types. The curcumin-coated surface showed granular, uneven structures consistent with crystalline aggregation during drying. The anthocyanin-coated surface appeared smoother, with fine fibrous coverage typical of aqueous extract deposition. Cancer saliva-exposed strips displayed more irregular and fragmented surface features, reflecting greater biochemical interaction (figure 7).

EDX spectra confirmed dominant carbon and oxygen signals in both coating types, with trace potassium and calcium on the anthocyanin-coated face originating from red cabbage extract. Saliva-exposed strips showed additional signals for sodium, phosphorus, and chlorine. Cancer saliva-treated strips exhibited higher Na and K intensities relative to normal saliva-treated controls (figure 8).

It is important to note, however, that commercial pH paper contains pre-impregnated buffer salts. Aqueous saliva exposure may mobilise these salts and contribute to the ionic signals detected by EDX. A parallel control study employing neutral filter paper exposed to both saliva types would be required to definitively attribute observed ionic elevations to salivary biochemical content rather than to substrate-derived artefact. This represents a recognised limitation of the present study and is recommended as a priority for future validation work.

Comparison with control filter paper strips

Control experiments on neutral filter paper confirmed that curcumin and anthocyanins can register pH-responsive colour changes in saliva when used alone. However, these responses were less vivid and slower in onset compared to pH paper-based strips. Curcumin shifted from pale yellow to orange-red, and anthocyanins from purple to green, though with lower colour saturation and reduced temporal stability. The buffering components within commercial pH paper appear to enhance proton exchange kinetics and stabilise dye equilibria, reinforcing the importance of substrate selection in paper-based biosensor design.

Diagnostic differentiation based on colorimetric response

The dual-coated strip consistently differentiated between normal and cancer saliva through visually discriminable colour combinations: yellow-low-violet for normal saliva and orange-blue-green for cancer saliva. Quantitative RGB-derived ΔE analysis confirmed a statistically significant difference in chromatic response between groups ($\Delta E > 20$ in most

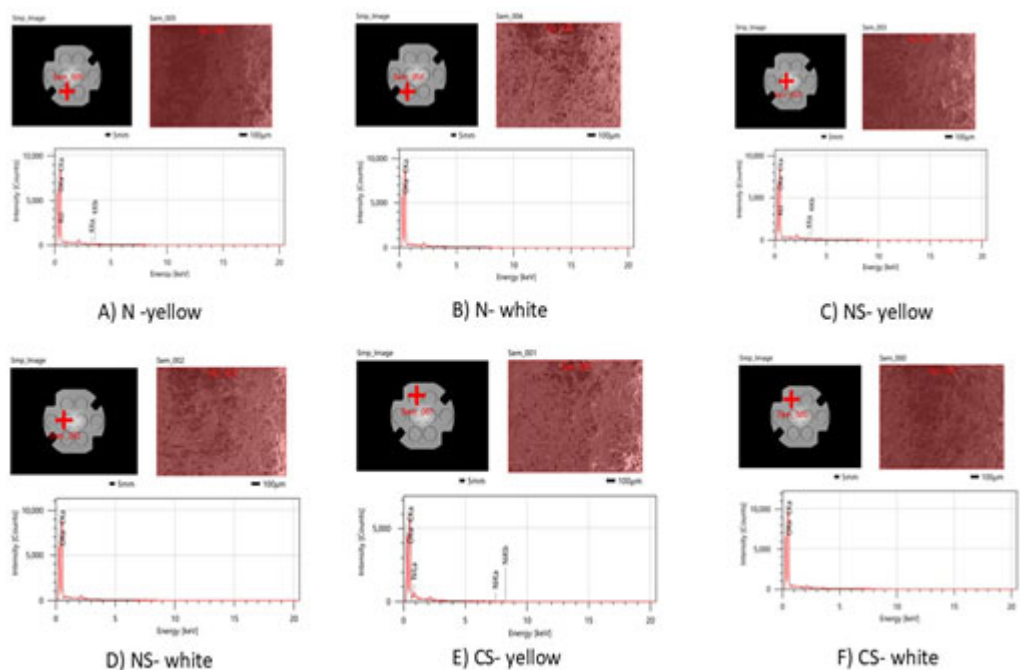


Figure 8: Energy-Dispersive X-ray Spectroscopy (EDX) elemental mapping and spectra from SEM scans of normal (A–B), normal stimulated (C–D), and cancer stimulated (E–F) saliva-treated biosensor strips. The red-highlighted points indicate the positions of the elemental scan. The spectra show distinct peaks for sodium (Na), potassium (K), nitrogen (N), and trace metallic elements. Cancer group samples exhibited elevated intensities for Na and K, correlating with ionic dysregulation in cancer saliva

cases). The dual-layer architecture provided complementary signals across a wider pH range and offered internal cross-verification between the two indicator faces.

Across all four parameters, Group 2 (cancer saliva) demonstrated significantly higher values than Group 1 (normal saliva), with highly significant between-group differences ($p < 0.001$) and 95% CIs not crossing zero. Cancer saliva exposure produced markedly elevated colour shifts ($\Delta E^* = 6.95 \pm 0.92$ vs. 3.37 ± 0.49), greater surface roughness ($0.812 \pm 0.042 \mu\text{m}$ vs. $0.387 \pm 0.045 \mu\text{m}$), and higher Na and K atomic percentages, with large effect sizes (Cohen's $d > 3$) across all measures.

Discussion

This study presents a dual-indicator colorimetric strip, constructed from curcumin and anthocyanins on a pre-buffered cellulosic substrate, as a

proof-of-concept first-line screening tool for detecting pathological salivary pH alterations. The results demonstrate that the strip consistently differentiates between normal and abnormal saliva through visually distinct chromogenic responses, corroborated by spectral, thermal, morphological, and elemental characterisation.

The chromogenic mechanism exploited in this work - pH-induced colour change in natural plant dyes - is well established in the food science and analytical chemistry literature. Similarly, the association between elevated salivary pH and oral malignancy has been previously documented [16-19]. The incremental contribution of the present work lies in combining a dual-dye system on a pre-buffered cellulosic substrate to create a multiplexed, point-of-care diagnostic format suitable for under-resourced clinical settings. This is a translational engineering advance rather than a fundamental scientific breakthrough, and the manuscript is framed accordingly.

Table 1: Independent *t*-test results comparing roughness, sodium, potassium, and colour between Group 1 and Group 2. Values are presented as mean $\pm$ standard deviation (SD). Mean difference, 95% confidence interval (CI), *t*-value, degrees of freedom (df), and *p*-value are shown. A *p*-value < 0.05 was considered statistically significant. Equal variances were assumed unless otherwise indicated by Levene's test

Parameter	Group 1 (n=10) Mean $\pm$ SD	Group 2 (n=10) Mean $\pm$ SD	Mean Diff.	95% CI	t (df)	p-value
Roughness	0.554 $\pm$ 0.032	0.835 $\pm$ 0.030	-0.281	-0.310 to -0.252	-20.16 (18)	<0.001
Sodium	0.121 $\pm$ 0.012	0.202 $\pm$ 0.023	-0.081	-0.098 to -0.064	-9.88 (18)	<0.001
Potassium	0.223 $\pm$ 0.015	0.327 $\pm$ 0.028	-0.104	-0.125 to -0.083	-10.51 (18)	<0.001
Colour (ΔE)	6.664 $\pm$ 0.069	7.592 $\pm$ 0.360	-0.928	-1.171 to -0.685	-8.02 (18)	<0.001

An important clinical caveat must be foregrounded: the pH-responsive chromogenic mechanism of this strip is not specific to OSCC. Alkaline salivary shifts are also associated with severe chronic periodontitis, necrotising ulcerative gingivitis, and oral microbiomic dysbiosis. A colour change on this strip should therefore be interpreted as indicative of a pathologically altered oral environment, and not as a definitive diagnosis of malignancy. Any positive result should prompt thorough clinical examination, periodontal assessment, and, where appropriate, histopathological biopsy. The strip is best positioned as an adjunctive first-line screening tool that flags patients for further evaluation, rather than as a standalone OSCC diagnostic test.

UV-Vis and fluorescence data confirmed bathochromic shifts and altered emission intensity in cancer-exposed strips, validating the retention of pH-sensitive optical properties after immobilisation [20-22]. The FTIR findings indicated the presence of phytochemical residues on the substrate surface; however, the spectral overlap with signals from the commercial pH paper's pre-impregnated synthetic indicators and buffer salts precludes unambiguous functional group assignment. Future studies should employ controlled differential spectroscopy using uncoated pH paper as a blank reference to isolate phytochemical contributions more precisely. This limitation does not undermine the functional performance of the strip, but it tempers the confidence with which specific molecular interactions can be inferred from FTIR data alone.

The DSC-TGA data revealed distinct thermal profiles between saliva-treated and untreated strips, and between normal and cancer saliva conditions. The mechanistic explanations proposed - involving enzymatic degradation, ionic complexation, or oxidative modification of dye matrices - are plausible in light of known salivary biochemistry [23,24] but remain speculative at the current level of evidence. These hypotheses are offered as working proposals to guide future mechanistic investigation rather than as established conclusions.

The EDX findings of elevated Na and K signals in cancer saliva-exposed strips are consistent with reports of electrolyte dysregulation in the cancer salivary environment [26,27]. However, a critical consideration is that commercial pH paper is pre-loaded with buffer salts that may be mobilised by aqueous salivary contact, artificially augmenting ionic signals. A definitive attribution of observed ionic changes to salivary content rather than substrate artefact requires a parallel EDX control study using neutral filter paper exposed to both saliva types. This is acknowledged as a methodological gap and is prioritised for future validation.

The dual-layered design of the strip provides both practical and analytical advantages. The two indicator faces independently register pH shifts, providing a form of internal redundancy and extending the effective chromatic detection range. Statistical analyses confirmed large-effect discriminative power (Cohen's $d > 3$) across all measured parameters, which is encouraging at a proof-of-concept stage.

Limitations and Future Directions

This study carries several recognised limitations. First, the sample size of $n=20$ is consistent with a pilot proof-of-concept investigation and does not permit generalisable clinical conclusions. Large-scale, multi-centre validation across diverse patient populations, incorporating patients with periodontal disease as confounders, is required before the strip can be considered for clinical deployment. Second, the EDX ionic attributions require a neutral paper control. Third, the FTIR interpretation is constrained by matrix spectral overlap. Fourth, long-term strip stability under varying storage conditions - particularly temperature and humidity - has not been formally evaluated. Future iterations of this platform could incorporate smartphone-based colorimetric quantification, multiplexed detection of molecular biomarkers (proteins, miRNAs), and integration into microfluidic formats to further improve diagnostic specificity and analytical depth.

Conclusion

This proof-of-concept study describes the fabrication and preliminary validation of a dual-coated phytochemical colorimetric strip for first-

line oral pathology screening using non-invasive saliva samples. By integrating curcumin and anthocyanins on a pH-sensitive cellulosic substrate, the strip delivers rapid, visually interpretable chromogenic responses that differ meaningfully between normal and pathologically altered saliva. Physicochemical characterisation confirmed the stability and pH-responsiveness of both dye coatings, and statistical analyses demonstrated significant between-group differences across colorimetric, morphological, and ionic parameters. The strip does not function as a definitive OSCC diagnostic test and cannot discriminate between malignancy and severe inflammatory oral conditions on the basis of pH alone; positive results require clinical corroboration and histopathological confirmation where indicated. Large-scale, multi-centre validation is required before clinical deployment. Within these limitations, the platform suggests promise as a low-cost, eco-friendly, and accessible screening adjunct for settings where conventional diagnostic infrastructure is limited.

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