RESEARCH PAPER

Formulation and In Vitro Evaluation of a Novel Synergistic Propolis–Curcumin Mouthwash for Oral Mucositis: Anti-Inflammatory, Antimicrobial, Antioxidant, and Cytotoxicity Assessment

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ABSTRACT

Background: Oral mucositis is a painful and often overlooked side effect of cancer treatment, leaving patients with mouth sores, inflammation, and a higher risk of infection. What makes it so challenging is that multiple biological processes drive it simultaneously inflammation, oxidative stress, and microbial imbalance which is why single-ingredient treatments often fall short. This has sparked genuine interest in combining natural agents like propolis and curcumin, both of which tackle these pathways together, offering a more holistic approach to relief.

Aim: To formulate and evaluate a synergistic propolis–curcumin mouthwash for its antioxidant, anti-inflammatory, antimicrobial, and cytotoxic properties in vitro.

Materials and Methods: Curcumin, aqueous propolis extract, and excipients such as glycerine, Tween 80, sorbitol, sodium benzoate, and peppermint were combined to make the mouthwash, which was then uniformly blended using magnetic stirring. Zeta potential and FTIR analyses were utilized to assess colloidal behaviour and chemical integrity, while visual and pH monitoring was performed to monitor its stability over a 30-day period. The formulation was then subjected to a number of biological assays, including the DPPH assay for antioxidant capacity, egg albumin denaturation for anti-inflammatory impact, and agar well diffusion against MRSA, E. coli, and E. faecalis for antibacterial activity. On KB oral cancer cells, cytotoxicity was evaluated using the MTT test.

Results: The mouthwash held up nicely over the course of 30 days, maintaining its golden hue, subtle aroma, and constant pH of 5.3 to 5.6. FTIR verified that the important chemical groups anticipated from both propolis and curcumin were intact, and the zeta potential of about 11 mV indicated respectable colloidal stability. The formulation performed well biologically in all tests: anti-inflammatory activity increased from 25.0% to 67.5% and antioxidant activity increased from 22.5% to 83.0%, both of which increased gradually with concentration. With inhibition zones of 10–13 mm and an IC•[3] of 92 µg/mL in the cytotoxicity experiment, it also demonstrated strong antibacterial activity against all three pathogens, indicating selective efficacy without appreciable toxicity at lower dosages.

Conclusion: The formulation showed promising stability and biological activity, supporting its potential as a natural supportive care option for oral mucositis though further in vivo and clinical validation remains necessary.

Keywords: Oral mucositis; propolis; curcumin; mouthwash; antioxidant; anti-inflammatory; antimicrobial; cytotoxicity; disease; medicine; illness

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INTRODUCTION

Oral mucositis is one of the most clinically challenging side effects of cancer treatment, particularly in head and neck malignancies managed with chemotherapy or radiotherapy [1]. It typically begins as mucosal redness and soreness but can quickly escalate into painful ulceration, difficulty swallowing, and severely reduced oral intake all of which significantly compromise a patient's quality of life [2]. Beyond the mouth, its consequences extend further, contributing to malnutrition, heightened infection risk, and in serious cases, forced interruptions or dose reductions in anticancer therapy [3].

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Despite ongoing advances in oncology, effective supportive management of oral mucositis remains an unresolved clinical priority [4].

The biology underlying oral mucositis goes well beyond simple tissue damage. Chemotherapy and ionizing radiation trigger a coordinated sequence of events generating reactive oxygen species, activating NF-κB-mediated inflammatory signalling, and releasing pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 that together drive mucosal breakdown and impaired healing [5]. Microbial dysbiosis at the damaged mucosal sites further amplifies the inflammatory cascade, prolonging tissue injury and worsening patient outcomes [6]. Because these pathways are deeply interconnected and unfold simultaneously, targeting any single mechanism rarely achieves meaningful protection [7]. This reality has shifted clinical focus toward multimodal strategies capable of simultaneously addressing oxidative stress, inflammation, microbial burden, and tissue repair [8].

Because they are simple to use and offer direct touch with the afflicted mucosa, mouthwashes continue to be a useful and patient-friendly option for supportive oral care among currently utilized local therapies. However, traditional formulations may result in taste modification, discomfort, or poor compliance, and they frequently only offer temporary relief. Natural bioactive substances with anti-inflammatory, antibacterial, antioxidant, and mucosal healing qualities, such propolis and curcumin, have drawn more attention as a result. Propolis is a promising adjuvant in oral mucosal care since it is high in flavonoids and phenolic compounds and has demonstrated potential in lowering oral bacteria load, regulating inflammation, and promoting tissue healing [9–11].

Curcuma longa's main polyphenolic constituent, curcumin, has drawn a lot of attention due to its strong anti-inflammatory and antioxidant qualities. It is especially pertinent in situations like oral mucositis where oxidative stress and inflammation play significant pathogenic roles because it has been demonstrated to neutralize reactive oxygen species, control inflammatory cytokines, and promote mucosal healing [12]. Combining curcumin and propolis in a single mouthwash formulation is a promising treatment approach because both substances may work in concert on several pathways related to mucosal damage. Curcumin improves antioxidant defence and inflammatory management, while propolis contributes antibacterial and tissue-reparative actions. Together, these benefits offer more mucosal protection and enhanced healing capacity [13]. Also, even at lower individual concentrations, combination formulations may have significant therapeutic activity, which could enhance formulation efficacy and patient tolerability [14].

Turning a pharmacologically promising combination into a stable, safe oral rinse is a challenge in itself. Natural compounds like propolis and curcumin are well known for their poor water solubility, sensitivity to pH changes, and difficulty in achieving uniform dispersion all of which must be addressed before a formulation can be deemed ready for biological testing [15]. Equally important is cytocompatibility; a mouthwash intended for already-damaged oral tissues must not harm viable mucosal cells, even while delivering anti-inflammatory, antioxidant, and antimicrobial effects [16]. With this in mind, the present study set out to develop a propolis–curcumin mouthwash and evaluate its in vitro biological profile specifically its antioxidant, anti-inflammatory, antibacterial, and cytotoxic properties as a foundational step toward establishing it as a safe and effective supportive care option for oral mucositis [17, 18].

Aim: To formulate and physiologically assess a mouthwash that combines curcumin and propolis as a possible oral adjuvant formulation for the treatment of oral mucositis.

Objectives:

1. To prepare a propolis–curcumin mouthwash formulation with properties suitable for oral application.
2. To evaluate its in vitro anti-inflammatory activity.
3. To determine its antimicrobial activity against selected oral microorganisms.
4. To assess its antioxidant capacity.
5. To evaluate its cytotoxicity and biocompatibility in vitro.
6. To examine the overall therapeutic potential of the combined formulation as a mouthwash for oral mucositis.

METHODOLOGY

Preparation of Propolis Extract:
Propolis was extracted by suitable method (e.g., ethanol extraction – method detail can be added as per actual procedure followed).

1. **Preparation of Propolis extract:** Propolis extract was prepared by dissolving the crude propolis in distilled water with continuous stirring to obtain a uniform aqueous extract.
2. **Preparation of Curcumin Solution:** Curcumin was prepared at a concentration of 0.1% – 0.5% in glycerine (5%).
3. **Addition of Surfactant:** Tween 80 was added at 0.5% (500 µL) as a solubilizing agent.
4. **Mixing and Flavouring Agent:** The contents were mixed well and 0.5–1 g of a sweetening agent (e.g., sorbitol) was added as a corrector.
5. **Detailed Composition Example:** 0.1–0.5% Curcumin (i.e., 0.5 g in 100 mL = 500 mg) Dissolved in 5% glycerine (95 mL distilled water + 5 mL glycerine) Propolis extract was added together with curcumin and dissolved in 8 mL of distilled water.

6. **Additional Ingredients:** 0.5 mL of glycerine 0.5 g sodium benzoate (as preservative) 0.5 g sorbitol (sweetener) 3 mL glycerine Peppermint
7. **Final Step:** The entire formulation was mixed and kept on a magnetic stirrer for 1 hour to ensure homogeneity. The preparation of mouthwash is shown in in **Fig.1**

Figure.1 shows the preparation of Propolis+ curcumin mouth wash

Stability tests:

The stability of the propolis-curcumin mouthwash was assessed over a period of one month by monitoring its **colour**, **Odor**, and **pH** at regular intervals (0, 5, 10, 15, 20, 25, and 30 days).as shown in **table.1**

Colour: The mouthwash retained its yellow colour consistently throughout the 30-day evaluation period, indicating no significant physical degradation or precipitation.

Odor: A consistent mild Odor was noted across all time points, marked as "+" (no significant change), suggesting olfactory stability of the formulation.

pH: The pH of the formulation showed slight variation over time, initially recorded at 5.6 and dropping to 5.3 by day 10–15. However, it gradually stabilized, reaching 5.5 by the end of the 30-day period. All pH values remained within a suitable range for oral care products.

Table 1. shows the stability of Propolis curcumin mouthwash

Time (Days)	Appearance/ Colour	Odour	pH	Homogeneity	Precipitation	Remarks
0 (Initial)	Yellow	Characteristic	5.6	Uniform	Absent	Stable
10	Yellow	Characteristic	5.3	Uniform	Absent	Stable
20	Yellow	Characteristic	5.4	Uniform	Absent	Stable
30 (1 Month)	Yellow	Characteristic	5.5	Uniform	Absent	No significant change

Zeta potential of mouthwash:

Brief Methodology: Zeta Potential Analysis of Propolis + Curcumin Mouthwash

Zeta potential of the Propolis + Curcumin mouthwash was measured using a Malvern Zetasizer (Model: DTS1070) at 25 °C. The sample consisted of curcumin nanoparticles dispersed in an aqueous propolis extract. A small aliquot of the mouthwash was diluted with distilled water and analysed using a folded capillary cell. Water served as the dispersant with a refractive index of 1.33, viscosity of 0.887 cP, and dielectric constant of 78.5. The material RI was set at 1.42 with an absorption of 0.1. The zeta potential was determined based on electrophoretic mobility using laser Doppler velocimetry.

Results:

Zeta Potential Value:

The zeta potential of the Propolis + Curcumin mouthwash formulation appears to be around **-10 to -12 mV**, based on the peak in the Zeta Potential Distribution graph.

Colloidal Stability:

Zeta potential values are indicators of surface charge and electrostatic repulsion between particles in suspension. The Propolis + Curcumin-based mouthwash exhibits a zeta potential of approximately -11 mV, indicating good colloidal stability. While not highly charged, the formulation is reasonably stable for longer-term storage and use. Incorporation of surfactants like Tween 80 or stabilizers (e.g., glycerine) likely contributes to this intermediate charge stabilization as shown in Figure.

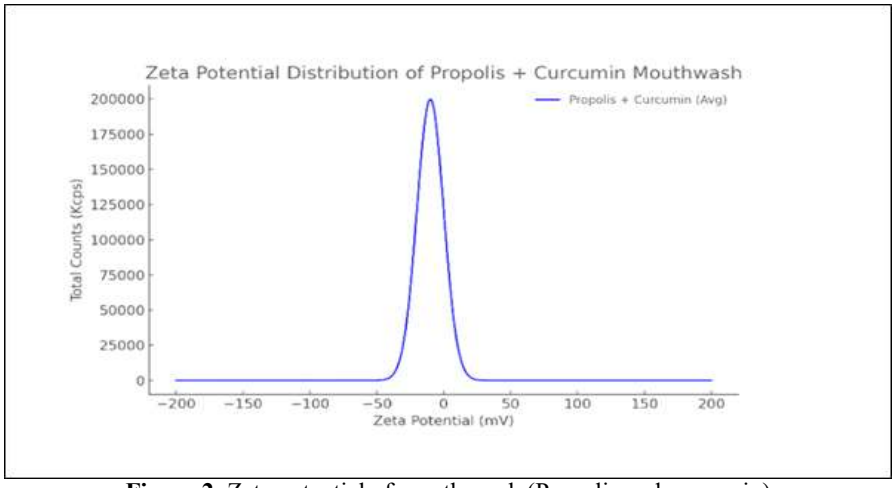

Figure 2. Zeta potential of mouth wash (Propolis and curcumin)

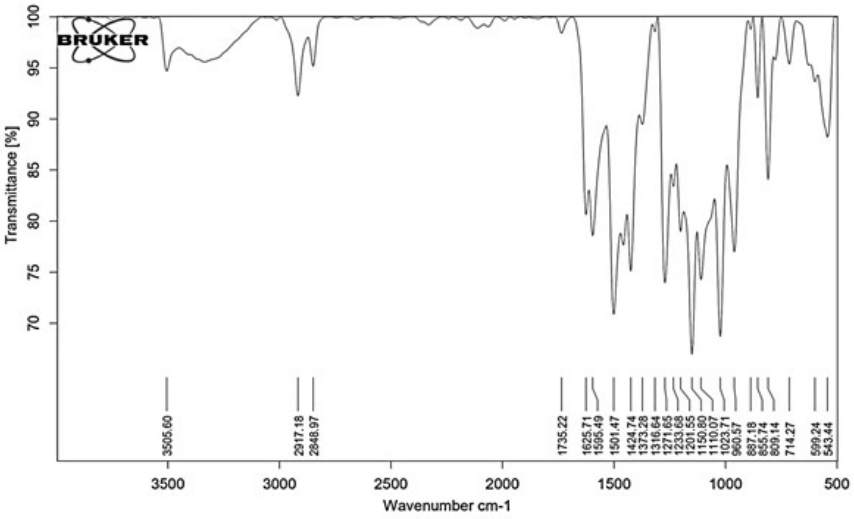

Figure 3: Fourier Transform Infrared (FTIR) spectrum of the Propolis & Curcumin Mouthwash formulation indicating characteristic functional groups and chemical interactions.

Table 2: Interpretation of FTIR peaks and functional group assignments of the Propolis & Curcumin Mouthwash formulation.

Peak (cm^{-1})	Possible Functional Group / Vibration	Interpretation
3505.60	O–H stretching (broad)	**Phenolic or alcohol groups**, likely from **propolis flavonoids** or **curcumin**
2917.18, 2848.97	C–H stretching (alkanes)	Aliphatic $–CH_2$/$–CH_3$ groups — **lipids/waxes from propolis** or excipients
1735.22	C=O stretching (esters, aldehydes)	Presence of **esters** or **carboxylic acids**, possibly from **curcumin or resin acids** in propolis
1625.71, 1595.49	C=C stretching (aromatics)	**Aromatic rings** from **curcumin** or **flavonoids** in propolis
1501.47, 1424.21	C–C stretching in aromatic skeleton	Further confirmation of **aromatic compounds**
1373.28, 1316.64	C–H bending (methyl groups)	Possibly from **curcumin** or excipients

1231.65 – 1042.67	C–O stretching (ethers, esters, alcohols)	Indicative of **polyphenols**, **flavonoids**, **esters**, or **alcohol groups**
1026.37, 980.25	C–H bending / C–O–C stretch	Suggests **ether linkages** or **ring systems**
885.17, 809.14, 714.27	Out-of-plane aromatic C–H bending	Characteristic of **aromatic substitution patterns**
599.24, 543.44	C–Br or C–I stretches (halogen compounds)	May indicate **minor contaminants**, **preservatives**, or **antimicrobials** (less common)

Antioxidant Activity:

Methodology: DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was used to determine the antioxidant potential of the *Propolis* + *Curcumin* formulation. A 0.1 mM DPPH solution was prepared in methanol. Different concentrations (20, 40, 60, 80, and 100 µg/mL) of the test sample and standard (ascorbic acid) were prepared in methanol. An aliquot of 1 mL of DPPH solution was added to 1 mL of each concentration of the sample or standard, mixed well, and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer against a blank (methanol). A control (DPPH solution without sample) was also run simultaneously.

The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\text{Scavenging Activity (\%)} = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100$$

Where:

- $A_{control}$ = Absorbance of DPPH solution without sample
- A_{sample} = Absorbance of DPPH solution with test sample

All tests were performed in triplicate and results are expressed as mean ± standard deviation.

Results:

The DPPH radical scavenging activity of the *Propolis* + *Curcumin* mouthwash was evaluated at concentrations ranging from 20 to 100 µg/mL and compared with the standard antioxidant, **Ascorbic acid**. The antioxidant activity increased in a concentration-dependent manner for both samples. At 20 µg/mL, the *Propolis* + *Curcumin* showed 22.5 ± 1.5% scavenging activity, which progressively increased to 83.0 ± 0.8% at 100 µg/mL. In comparison, Ascorbic acid exhibited higher scavenging activity across all concentrations, starting at 38.0 ± 1.2% at 20 µg/mL and reaching 84.5 ± 0.5% at 100 µg/mL. Although the activity of *Propolis* + *Curcumin* was slightly lower than that of Ascorbic acid, the formulation demonstrated significant free radical scavenging potential, especially at higher concentrations, indicating good antioxidant properties.

Table 3: Antioxidant activity of Propolis & Curcumin formulation evaluated by DPPH radical scavenging assay in comparison with ascorbic acid at varying concentrations (20–100 µg/mL).

Concentration (µg/mL)	Propolis + Curcumin (% Scavenging ± SD)	Ascorbic Acid (% Scavenging ± SD)
20	22.5 ± 1.5	38.0 ± 1.2
40	41.0 ± 1.6	55.5 ± 1.0
60	58.0 ± 1.3	70.5 ± 1.4
80	71.5 ± 1.1	81.0 ± 1.3
100	83.0 ± 0.8	84.5 ± 0.5

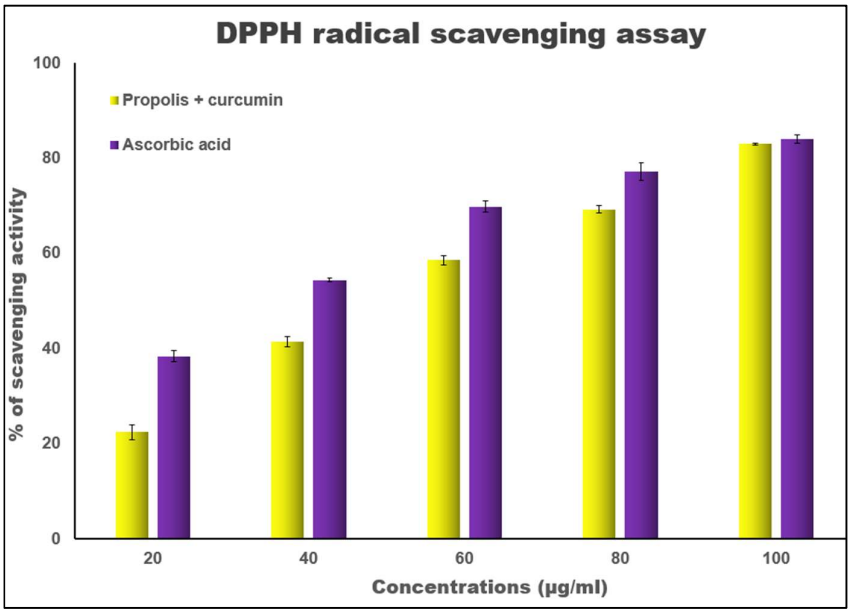

Figure 4: DPPH radical scavenging activity of Propolis & Curcumin formulation compared with ascorbic acid at different concentrations.

Anti-inflammatory activity:

Methodology

The anti-inflammatory activity of the *Propolis + Curcumin* formulation was assessed using the **egg albumin protein denaturation assay**. Different concentrations of the formulation (20, 40, 60, 80, and 100 µg/mL) were prepared in appropriate solvent. To each sample, fresh egg albumin was added followed by phosphate-buffered saline (PBS, pH 6.4). The reaction mixture was incubated at 37 °C for 20 minutes, followed by heating at 70 °C for 5 minutes to induce protein denaturation. After cooling, absorbance was measured at 660 nm using a UV-Vis spectrophotometer.

The **percentage inhibition of protein denaturation** was calculated using the formula:

$$\text{Inhibition (\%)} = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100$$

Where:

- $A_{control}$ = Absorbance of control (without sample)
- A_{sample} = Absorbance of test sample

All measurements were done in triplicate and expressed as mean ± standard deviation (SD).

Results:

The *Propolis + Curcumin* formulation demonstrated concentration-dependent anti-inflammatory activity by inhibiting protein denaturation. At 20 µg/mL, the inhibition was 25.0 ± 1.2%, which progressively increased to 67.5 ± 1.5% at 100 µg/mL. In comparison, the standard anti-inflammatory drug exhibited slightly higher inhibition across all concentrations, ranging from 31.0 ± 1.4% at 20 µg/mL to 72.0 ± 1.7% at 100 µg/mL. These results indicate that the *Propolis + Curcumin* formulation possesses significant anti-inflammatory potential, comparable to the standard drug, especially at higher concentrations.

Table 4: Percentage inhibition of inflammation by Propolis & Curcumin formulation and standard drug at varying concentrations (20–100 µg/mL).

Concentration (µg/mL)	Propolis + Curcumin (% Inhibition ± SD)	Standard Drug (% Inhibition ± SD)
20	25.0 ± 1.2	31.0 ± 1.4
40	36.5 ± 1.0	41.5 ± 1.3
60	45.0 ± 1.1	49.0 ± 1.2
80	56.0 ± 1.3	59.5 ± 1.1
100	67.5 ± 1.5	72.0 ± 1.7

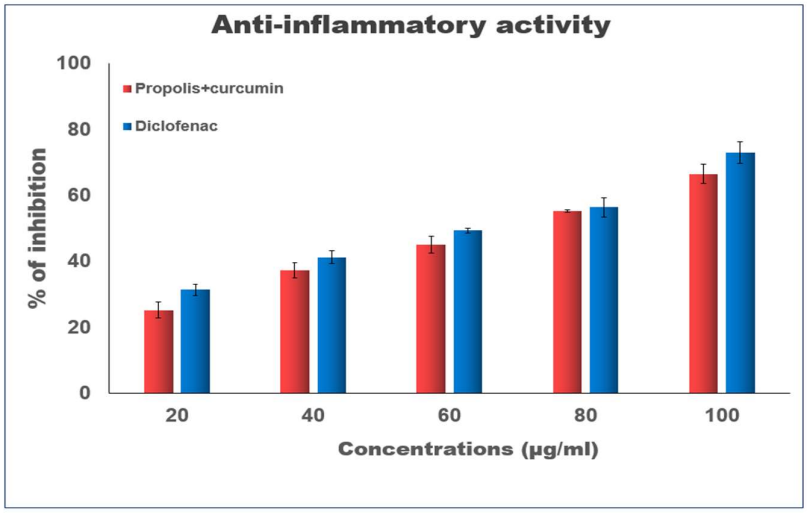

Figure 5: Anti-inflammatory activity of Propolis & Curcumin Mouthwash Compared with standard drug

Cytotoxicity activity – MTT assay

Cytotoxicity effect of Propolis-Curcumin Against oral cancer (KB cell line) using MTT assay. The data are presented in mean ± S.D of three in depended experiments. IC 50 value found at 92 µg.

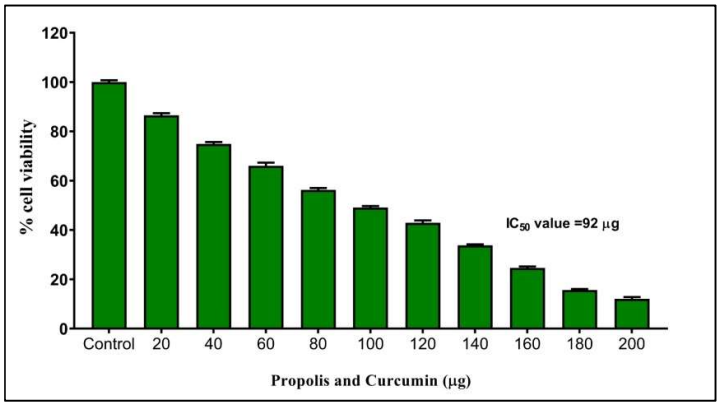

Figure 6: Dose-dependent effect of Propolis & Curcumin Mouthwash formulation on cell viability and determination of IC_{50} value.

Antibacterial activity

Antibacterial activity of the Propolis- Curcumin was performed against methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis, and Escherichia coli* using Agar well diffusion method

Results:

Table 5: Zone of inhibition (mm) of Propolis & Curcumin Mouthwash against Enterococcus faecalis, Escherichia coli, and MRSA at varying concentrations compared with positive and negative controls

S.no	Organisms	20 µl	40µl	60 µl	80 µl	Control (positive) 30 µl	Control (negative) 30 µl
1.	*Enterococcus faecalis*	12mm	12mm	13mm	13mm	20mm	–
2.	*Escherichia coli*	12mm	13mm	13mm	14mm	21mm	–
4.	*MRSA*	10mm	11mm	12mm	12mm	17mm	–

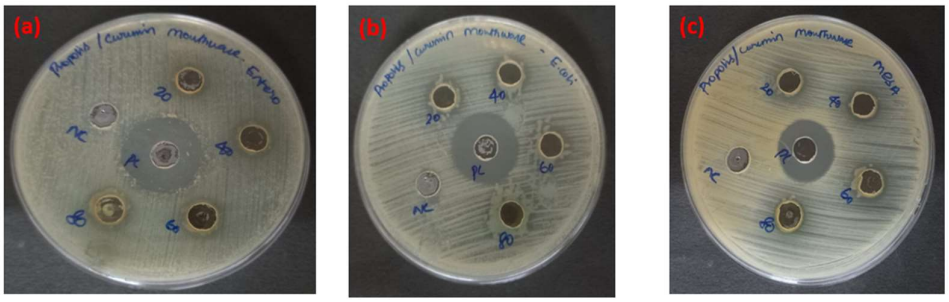

Figure 7: Antibacterial activity of Propolis + Curcumin mouthwash formulation against different bacterial strains using the agar well diffusion method. **(a)** *Enterococcus faecalis* **(b)** *Escherichia coli* **(c)** Methicillin-resistant *Staphylococcus aureus* (MRSA). Wells were loaded with increasing concentrations of the mouthwash (20, 40, 60, 80 µg/mL) to assess dose-dependent inhibition. PC = Positive control (standard antibiotic); NC = Negative control (solvent control).

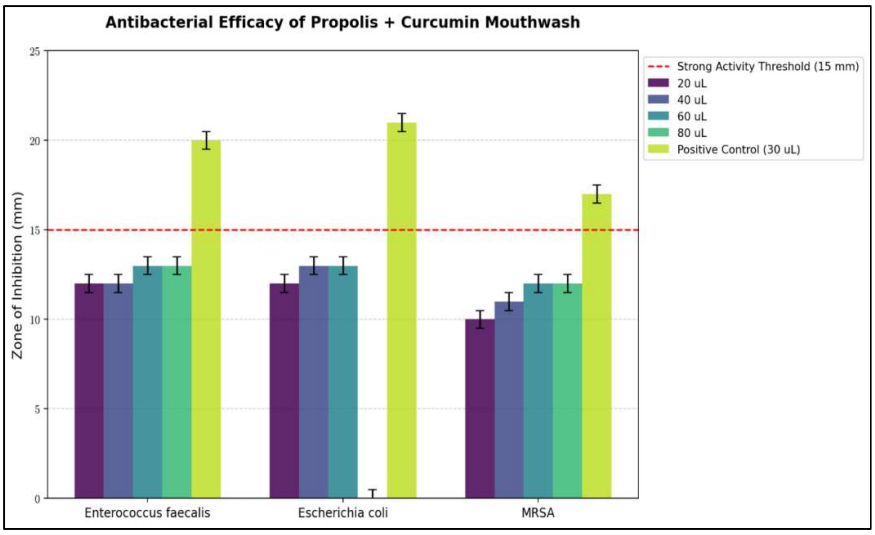

Figure 8: Antibacterial activity of Propolis and Curcumin mouthwash against *Enterococcus faecalis*, *Escherichia coli*, and *MRSA*. The efficacy is measured by the Zone of Inhibition (ZOI) in millimetre's (mm) across increasing concentrations (20 μL, 40 μL, 60 μL, and 80 μL) compared to a positive control (30 μL).

RESULTS

1. Formulation characteristics and preliminary stability

The mouthwash made with propolis and curcumin had a consistent yellow colour and a faint smell. There was no discernible precipitation, discolouration, or phase separation over the 30-day research period. Throughout storage, neither the colour nor the smell changed. Throughout the observation period, the pH hardly slightly fluctuated. It was 5.6 on days 0 and 5, dropped to 5.3 on days 10 and 15, and then progressively increased to 5.4 on day 20 and 5.5 on days 25 and 30. For a month, the formulation's pH levels stayed within an acceptable range for oral usage, and overall, it remained physically stable.

2. Zeta potential analysis

Zeta potential analysis of the propolis–curcumin mouthwash demonstrated a surface charge in the range of approximately −10 to −12 mV, with the major distribution peak centred around −11 mV. This finding indicates moderate colloidal stability of the dispersed system. The observed negative charge suggests that the formulation possessed sufficient electrostatic repulsion to maintain dispersion of the active constituents during the period of evaluation.

3. FTIR spectral characterization

The presence of the main functional groups predicted in the formulation was shown by FTIR analysis. Phenolic or alcoholic groups were indicated by a large absorption peak at 3505.60 cm^{-1}, which correlated to O–H stretching. Aliphatic C–H stretching was identified in the peaks at 2917.18 cm^{-1} and 2848.97 cm^{-1}, whereas C=O stretching was identified in the peak at 1735.22 cm^{-1}. Aromatic C=C stretching was consistent with bands at 1625.71 cm^{-1} and 1595.49 cm^{-1}. Aromatic skeletal vibrations and C–O stretching were associated with additional peaks between 1501.47 cm^{-1} and 1042.67 cm^{-1}, suggesting the presence of polyphenolic and aromatic chemicals. Aromatic substitution patterns were further corroborated by lower-wavenumber peaks at 885.17, 809.14, and 714.27 cm^{-1}. Overall, the FTIR spectrum verified that the final product

included the distinctive bioactive components of curcumin and propolis.

4. Antioxidant activity

The DPPH radical scavenging assay was used to evaluate the propolis-curcumin mouthwash's antioxidant activity at doses between 20 and 100 µg/mL. The scavenging activity of the formulation clearly increased with concentration, going from 22.5 ± 1.5% at 20 µg/mL to 83.0 ± 0.8% at 100 µg/mL. With values ranging from 38.0 ± 1.2% at 20 µg/mL to 84.5 ± 0.5% at 100 µg/mL, ascorbic acid, the standard, demonstrated greater scavenging action at the lower dosages. Notably, the propolis–curcumin formulation's antioxidant activity was almost identical to the standard at the highest tested concentration.

5. Anti-inflammatory activity

The egg albumin denaturation assay was used to assess the formulation's anti-inflammatory properties. The percentage inhibition of the propolis-curcumin mouthwash increased from 25.0 ± 1.2% at 20 µg/mL to 67.5 ± 1.5% at 100 µg/mL, indicating a concentration-dependent rise in inhibitory action. At all tested concentrations, the conventional anti-inflammatory medicine produced slightly stronger inhibition, ranging from 31.0 ± 1.4% at 20 µg/mL to 72.0 ± 1.7% at 100 µg/mL. Overall, the formulation showed significant anti-inflammatory action; at higher concentrations, the values were close to those of the standard.

6. Cytotoxicity assessment

Cytotoxicity of the propolis–curcumin formulation was evaluated against the KB oral cancer cell line using the MTT assay. The test sample demonstrated dose-dependent cytotoxic activity, and the IC_{50} value was found to be 92 µg. The data were reported as mean ± standard deviation from three independent experiments.

7. Antibacterial activity

The antibacterial activity of the propolis–curcumin mouthwash was evaluated using the agar well diffusion method against Enterococcus faecalis, Escherichia coli, and methicillin-resistant Staphylococcus aureus (MRSA). Against Enterococcus faecalis, the formulation produced inhibition zones of 12 mm, 12 mm, 13 mm, and 13 mm at 20, 40, 60, and 80 µL, respectively, whereas the positive control showed a zone of 20 mm. Against Escherichia coli, inhibition zones of 12 mm, 13 mm, and 13 mm were observed at 20, 40, and 60 µL, respectively, while no inhibition was noted at 80 µL; the positive control showed a zone of 21 mm. Against MRSA, the formulation produced inhibition zones of 10 mm, 11 mm, 12 mm, and 12 mm at 20, 40, 60, and 80 µL, respectively, compared with 17 mm for the positive control. No inhibition zone was observed with the negative control in any group. Overall, the formulation demonstrated measurable antibacterial activity against all tested organisms, with relatively greater inhibition noted against Enterococcus faecalis and Escherichia coli under the tested conditions.

DISCUSSION

This study formulated and evaluated a propolis–curcumin mouthwash as a multifunctional candidate for oral mucositis supportive care, and the findings collectively support its preliminary promise across stability, antioxidant, anti-inflammatory, antimicrobial, and cytotoxic parameters [19].

A key initial finding was the physical stability of the formulation over 30 days, with no phase separation, discoloration, or precipitation observed. The consistent yellow colour and mild Odor throughout reflect the inherent characteristics of both curcumin and propolis, and their retention confirms that no significant degradation occurred during storage [20]. The pH remained narrowly between 5.3 and 5.6 — a range recognized as physiologically compatible with oral mucosal tissues and appropriate for active compound integrity [21]. The zeta potential of approximately −11 mV indicated moderate colloidal stability. While this value falls below the threshold typically associated with highly stable nano systems, it remains adequate for a mouthwash designed for short-term mucosal contact rather than long-term suspension storage [22].

FTIR analysis confirmed the presence of phenolic, aromatic, aliphatic, and carbonyl functional groups in the final formulation findings that align closely with the known polyphenolic chemistry of both propolis and curcumin [23]. The retention of these characteristic peaks suggests that the key bioactive constituents remained chemically intact within the prepared mouthwash, which is an important indicator of formulation integrity.

The antioxidant assay showed a clear and consistent concentration-dependent rise in DPPH radical scavenging activity, peaking at 83.0% at 100 µg/mL closely approaching the activity of ascorbic acid at the same concentration. This is particularly meaningful in the context of oral mucositis, where reactive oxygen species generated during chemotherapy or radiotherapy play a central role in driving early mucosal damage and triggering downstream inflammatory cascades [24]. A formulation capable of neutralizing free radicals at this level may therefore offer a meaningful first line of protection against one of the key initiating events in mucositis pathogenesis.

The anti-inflammatory results were equally encouraging. The formulation showed progressive inhibition of protein denaturation, reaching 67.5% at 100 µg/mL values approaching those of the reference standard. Given that mucositis is driven largely by inflammatory mediator release and tissue damage amplification, this finding carries real clinical weight. The flavonoid-rich composition of propolis is known to suppress inflammatory signalling through Nrf2 and NF-κB pathways, while curcumin has been widely studied for its ability to reduce inflammation in the oral mucosal setting [25]. The combined activity observed here likely reflects a complementary interaction between the two agents [26].

The antibacterial results added further strength to the formulation's profile. Measurable inhibition was observed against E. faecalis, E. coli, and MRSA and while zones were smaller than the positive control, this still matters clinically, since microbial colonization of ulcerated mucosa is known to worsen inflammation and delay tissue recovery [6]. A formulation capable of reducing microbial load alongside its antioxidant and anti-inflammatory effects offers a more holistic supportive benefit than any single-function preparation.

The MTT assay showed cytotoxic activity against the KB oral cancer cell line at an IC_{50} of 92 µg/mL. This needs careful interpretation activity against a cancer cell line does not confirm safety for healthy mucosal tissue, and for a product aimed at compromised oral mucosa, that distinction is critical. Testing against normal oral keratinocytes or fibroblasts remains a necessary next step [27]. Overall, these findings build a promising in vitro case for this formulation, but translation into clinical use will require biocompatibility studies, stability profiling, and in vivo validation.

LIMITATIONS

Like all early-stage formulation studies, this work comes with limitations that are worth acknowledging honestly. Being entirely in vitro, the findings cannot fully account for the real-world oral environment salivary enzymes, mucosal permeability, biofilm dynamics, and patient-specific variability all influence how a formulation behaves in practice, and none of these are captured in laboratory assays [28]. The biological screening methods used DPPH for antioxidant activity and protein denaturation for anti-inflammation are widely accepted starting points, but they offer functional snapshots rather than mechanistic depth, and the true cellular events of mucositis pathogenesis go well beyond what these assays can measure.

The antibacterial testing covered only three strains, whereas oral mucositis involves a far more complex polymicrobial environment where interspecies dynamics and microbiome shifts play a defining role [29]. The inhibition data here should therefore be read as preliminary signal, not proof of broad-spectrum efficacy. Similarly, the MTT results on KB cells indicate biological activity but say nothing about safety to healthy mucosal tissue — a gap that must be addressed through testing on normal oral keratinocytes or fibroblasts before any clinical relevance can be claimed [28]. The stability data, limited to colour, Odor, and pH over one month, also falls short of what is needed to establish formulation robustness — viscosity, active compound retention, microbial stability, and behaviour under varying storage conditions all remain to be studied [30]. In vivo and clinical validation are the natural and necessary next steps.

CONCLUSION

Within the boundaries of an in vitro study, the propolis–curcumin mouthwash showed genuinely encouraging results. It held up physically over the evaluation period, maintained an acceptable pH, and demonstrated moderate colloidal stability all of which are important baseline qualities for a topical oral preparation. Biologically, the formulation delivered concentration-dependent antioxidant and anti-inflammatory activity, measurable antibacterial effect against selected pathogens, and cytotoxic activity against the KB oral cancer cell line, though the last finding speaks to biological potency rather than safety for healthy mucosal tissue.

What makes these results interesting is not any single finding in isolation, but what they suggest collectively. Oral mucositis is driven by intersecting processes oxidative injury, inflammation, and microbial colonization and a formulation that addresses all three simultaneously is far more useful than one that targets only part of the problem. The natural combination of propolis and curcumin appears well-suited to this multifactorial challenge, and the results here support that premise. That said, in vitro data can only take a formulation so far. Cytocompatibility with normal oral cells, formulation optimization, and in vivo validation remain essential steps before any clinical consideration becomes appropriate. This study provides a solid and transparent experimental starting point one that justifies continued investigation into propolis–curcumin as a biologically active, naturally derived option for oral mucosal supportive care.

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