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Original Article

In Vitro Release Kinetics of Total Phenolics and Flavonoids from Commercial Propolis-based Toothpaste and Mouthwash

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Abstract

Background and objectives: Propolis contains polyphenolic compounds with antimicrobial, antioxidant and anti-inflammatory activities, supporting its potential application for oral care formulations. However, the product performance may depend on both active materials content and its release kinetics. This study investigated the *in vitro* release kinetics of total phenolics and flavonoids from commercial propolis-based toothpaste and mouthwash.

Methods: Release was assessed over eight hours in artificial saliva to simulate oral conditions. Released total phenolics and flavonoids were quantified by colorimetric assays with gallic acid and quercetin calibration curves, respectively. Release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models to characterize release behavior and compare the two products.

Results: Toothpaste exhibited greater cumulative phenolics release than mouthwash throughout the study period, achieving 41.45 ± 0.7% versus 36.9 ± 1.2% at 480 minutes. Flavonoids release was much lower, reaching 2.75 ± 0.2% for mouthwash and 0.27 ± 0.1% for toothpaste. Among the basic kinetic models, the Higuchi model provided the best fit for both products (correlation coefficient (R^2) = 0.9223–0.9778), suggesting diffusion-related release. Korsmeyer–Peppas modelling also showed high R^2 values (0.9743-0.9820), with release exponent values consistent with predominantly diffusion-related release behavior.

Conclusions: Toothpaste released more phenolics, while mouthwash exhibited higher flavonoids release, with diffusion-related processes contributing to release in both formulations. These findings suggest formulation-related differences in phenolics and flavonoids release under simulated oral conditions which may provide useful information for the future formulation development of propolis-based oral care products.

Introduction

Propolis, also known as 'bee glue,' is a complex resinous material produced by bees and used as a protective sealer to repair cracks and holes in beehives. It is a mixture of beeswax, bee saliva, and plant exudates collected from tree buds, sap flows, or other vegetal sources which are gathered by bees during visits to different kinds of plants.[1-3] Propolis is particularly rich in phenolic acids, flavonoids and other polyphenolic ingredients which have been associated with biological activities such as antibacterial, anti-inflammatory, antioxidant and wound healing effects.[4-11] Therefore, propolis is a promising candidate for oral health applications. Recently, propolis has been included in commercial oral care products such as toothpaste and mouthwash to improve oral hygiene and support the management of microbial and inflammatory conditions in the oral cavity.[12-16]

The presence of biologically active compounds in a product does not guarantee its biological activity. In phytochemical-based formulations, the release of compounds from the dosage form and their subsequent availability at the therapeutic target(s) are often more relevant than the total compounds concentrations. Conventional quantification of total phenolics and flavonoids evaluates the amount of compounds present in the formulation but does not reflect the fraction that can be released under physiological conditions or become available for interaction with oral tissues. Con-

Keywords: Propolis; Mouthwash; Toothpaste; Total phenolics; Total flavonoids; Release kinetics; *In vitro* release.
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sequently, two formulations containing similar levels of polyphenolic compounds may exhibit different release behaviors and ultimately different biological performances. This issue is particularly important in oral care products, where the availability of bioactive compounds may be influenced by factors such as short exposure time of the product in the oral cavity, dilution by saliva and formulation properties such as viscosity and pH.[17] Therefore, *in vitro* release data can provide additional information beyond total polyphenolic content by estimating the extent and rate at which bioactive compounds become available under simulated oral conditions. Such information may support formulation optimisation and improve understanding of the potential performance of propolis-based oral care products.

Previous studies of propolis-based oral-care preparations have primarily evaluated chemical profiles, antimicrobial activity, and clinical outcomes rather than release kinetics.[9,11,13,18-20] Propolis-based toothpaste and mouthwash have different formulation matrices and may therefore exhibit different release behaviors. Generally, toothpaste is a semisolid dosage form with higher viscosity, whereas mouthwash is a liquid formulation with lower viscosity; these differences in physical state and formulation composition may influence the release behavior of incorporated compounds.[21]

Accordingly, an extended *in vitro* release study can characterize whether compounds remain releasable beyond the immediate product application period. Therefore, the present study aimed to characterize and compare the *in vitro* release kinetics of total phenolics and flavonoids from commercial propolis-based toothpaste and mouthwash over eight hours.

Materials and methods

Materials

The propolis mouthwash and toothpaste products were developed by Nature's Laboratory Ltd. under the BEEVITAL® brand in Whitby, UK. Mouthwash (batch number B102662) and toothpaste (batch number B101160) were used as supplied by the manufacturer for the release study.

Chemicals and reagents

Gallic acid (GA), quercetin (Q), sodium carbonate (Na_2CO_3), potassium acetate ($C_2H_3KO_2$), aluminum nitrate ($Al(NO_3)_3$), Folin–Ciocalteu reagent, potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), sodium bicarbonate ($NaHCO_3$), calcium chloride ($CaCl_2$) were purchased from Sigma-Aldrich. The solvents used were methanol (Fisher Scientific, UK) and deionized water of analytical grade.

Preparation of artificial saliva

Briefly, to prepare one liter of artificial saliva (pH 6.8) 0.680 g of KH_2PO_4, 1.130 g KCl, 0.420 g of $NaHCO_3$ and 0.150 g of $CaCl_2$ were dissolved in deionized water by sonication for 30 minutes at room temperature as described in the cited literature with some modifications.[22]

In vitro release study

The release of polyphenolic contents of propolis was evaluated by placing 1 g of toothpaste and 1 mL of mouthwash into a container containing 4 mL of artificial saliva to simulate the oral cavity environment. This sample-to-medium ratio was selected to provide a practical release system and generate measurable analyte concentrations within the calibration range of the ultraviolet (UV) method in the limited medium volume used, rather than directly replicating clinical dosing. The toothpaste was directly mixed with artificial saliva and formed a slurry under shaking conditions without the use of a support membrane.

Three independently prepared release tubes (n = 3) were used for each formulation. Samples were incubated in polypropylene tubes in a shaking incubator (Corning LSE™ Benchtop Shaking Incubator, Corning Inc., USA) set to 50 rpm and 37°C. A sample of 500 µL was withdrawn immediately after mixing (0 min), and at 5, 15, 30, 60, 180, 300, 480 min and replaced with an equal volume of fresh artificial saliva.[23] The same release tubes were sampled repeatedly throughout the study period, generating repeated measurements over time for each experimental unit. Withdrawn samples were analyzed directly without processing for their phenolic and flavonoid content by *Folin–Ciocalteu* method and Jurd and Geissmann colorimetric method, respectively, as per the cited literature.[24] Where required, the completed reaction mixtures were diluted before UV absorbance measurement to bring the readings within the respective calibration curves ranges. The cumulative release of polyphenolic content of samples was measured over a period of 8 h.[23]

Determination of total phenolics and flavonoids in samples

The total phenolic content was determined using the Folin–Ciocalteu method as described previously.[4,24] The sample (30 µL) was mixed with 150 µL of distilled water and 15 µL of Folin–Ciocalteu reagent (used as provided by supplier) for 6 minutes. 45 µL Na_2CO_3 (10% w/v) was added, and the final volume was adjusted to 300 µL with distilled water. The solutions were then stored in the dark for 2 hours at room temperature. The calibration curve of GA was constructed at 750 nm wavelength using a UV plate reader (FlexStation® 3, Molecular Devices, USA) operated with SoftMax® Pro software (version 5.4.5). Results were expressed as milligrams of GA equivalent (mg gallic acid equivalents (GAE)).

The cumulative release percentage of phenolic content (Pr) from the products was calculated using the following equation

$$P_r(\%) = \frac{V_0 \times P_n + V_r \times \sum_{i=1}^{n-1} P_i}{P_{\text{total}}} \times 100$$

where, V_0 and V_r are the initial volume of the artificial saliva media and the volume of removed at sampling, respectively, and P_n is the phenolic content in the sample taken at time point n. $\sum_{i=1}^{n-1} P_i$ is the sum of phenolic contents measured in all previous samples including the amount lost in aliquots and P_{total} is the total phenolic content initially present in the tested formulation.[23]

The total flavonoid content was determined using the Jurd and Geissmann colorimetric method as described previously.[4,24] Then, 6 µL of $C_2H_3KO_2$ (1 mol/L) and 6 µL $Al(NO_3)_3$ (10% w/w) were added to 30 µL of the sample, and the final volume was adjusted to 300 µL with methanol. The solutions were then stored in the dark for 40 minutes at room temperature. The calibration curve of Q was constructed at 400 nm wavelength using a UV plate reader (FlexStation® 3, Molecular Devices, USA) operated with SoftMax® Pro software (version 5.4.5). Results were expressed as

milligrams of Q equivalent (mg quercetin equivalents (QE)).

The cumulative release percentage of flavonoids (Fr) from the products was calculated using the following equation

$$F_r(\%) = \frac{V_0 \times F_n + V_r \times \sum_{i=1}^{n-1} F_i}{F_{\text{total}}} \times 100$$

where, V_0 and V_r are the initial volume of the artificial saliva media and the volume removed at sampling, respectively, and F_n is the flavonoid content in the sample taken at time point n. $\sum_{i=1}^{n-1} F_i$ is the sum of flavonoids content from measured in all previous samples including the amount lost in aliquots and F_{total} is the total flavonoid content initially present in the tested formulation.[23]

The cumulative release percentages were calculated relative to the theoretical total phenolic and flavonoid contents initially present in each formulation. Both formulations were prepared using the same weight of raw propolis and contained initial total phenolic and flavonoid contents of 21.636 mg GAE and 621.8 mg QE, respectively, per gram of toothpaste and per millilitre of mouthwash. This theoretical initial amount was calculated by multiplying the total phenolics and flavonoid content per gram of raw propolis by the mass of raw propolis (determined as per cited literature[3,4,24]) incorporated into each tested portion. The calculated theoretical initial phenolic and flavonoid contents in the tested portions were used as the respective denominators for calculating the cumulative release percentages. These percentages therefore correspond to release normalised to theoretical initial contents, rather than experimentally measured fractions of total phenolics or flavonoids of each formulation.

Kinetics of polyphenolics release

The cumulative release percentage data were fitted with the following kinetic models to characterize release behavior of the polyphenolic content from the products[23]:

- Zero-order kinetic model:

$$C_t = C_0 + K_0 t$$

- First-order kinetic model

$$\log(100 - C_t) = \log 100 - \frac{K_1 t}{2.303}$$

- Higuchi kinetic model

$$C_t = K_{\text{HC}}(t^{1/2})$$

where C_0 is the initial polyphenolic amount, C_t is the cumulative percentage of polyphenolic compounds released at time t and K_0, K_1 and K_{HC} are constants of polyphenolic release for zero-order, first-order, and Higuchi kinetic models, respectively.

- Korsmeyer–Peppas kinetic model

$$\frac{C_t}{C_\infty} = K_{\text{KP}} \cdot t^n$$

where $\frac{C_t}{C_\infty}$ is the fraction of polyphenolic compounds released at time t, K_{KP} is the release rate constant, and n is the release exponent.

Statistical analysis

Each release study experiment was performed in triplicate using three samples obtained from the same commercial product batch, and quantitative data were expressed as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism version 9.1.2. Differences in cumulative release percentages between mouthwash and toothpaste formulations over time were evaluated using repeated-measures two-way analysis of variance (ANOVA). Where significant effects were observed, Šídák's multiple comparisons test was used to compare formulations at individual time points. Statistical significance was set at $P < 0.05$. For the repeated-measures two-way ANOVA, each release tube was treated as the experimental unit, with time considered as the within-subject factor and formulation as the between-subject factor.

Results

Calibration curve of GA and Q

The calibration curve of GA was prepared using standard solutions in the range of 1-5 µg/mL. The plot of concentration versus absorbance (Fig. 1) exhibited good linearity with the regression equation y = 0.0241x + 0.0386 and correlation coefficient R^2 = 0.9948. The data showed good linearity over the tested concentration range for total phenolic quantification.

The calibration curve of Q was prepared using standard solutions in the range of 0.78-12.5 µg/mL. The plot of concentration versus absorbance (Fig. 2) exhibited good linearity with the regression equation y = 0.0025x + 0.0511 and correlation coefficient R^2 = 0.9974. The data showed good linearity over the tested concentration range for total flavonoid quantification.

Release profiles of samples over 8 h

The cumulative release profiles of phenolics and flavonoids from mouthwash and toothpaste products over 480 min are shown in Table 1 and Figures 3 and 4. Both formulations showed time-dependent release kinetics.

Toothpaste showed higher release of phenolics (Fig. 3) compared to mouthwash at all time points covered in the study, achieving (41.45 ± 0.7%) release compared to (36.9 ± 1.2%) at 480 minutes. This higher final release indicates greater apparent phenolic release from toothpaste under the experimental conditions.

On the other hand, flavonoid release (Fig. 4) remained much lower than phenolic release within both products, with mouthwash attaining (2.75 ± 0.2%) and toothpaste (0.27 ± 0.1%) at 480 min. The approximately 10-fold difference between mouthwash and toothpaste indicates a marked formulation-dependent difference in apparent flavonoid release.

SD values were generally low relative to the corresponding mean values, indicating acceptable reproducibility of the measurements (n = 3). The repeated-measures two-way ANOVA indicated significant effects of formulation, time, and their interaction on both release datasets. For the total flavonoid content, significant effects were observed for time, formulation, and the time × formulation interaction (all $P < 0.0001$). Similarly,

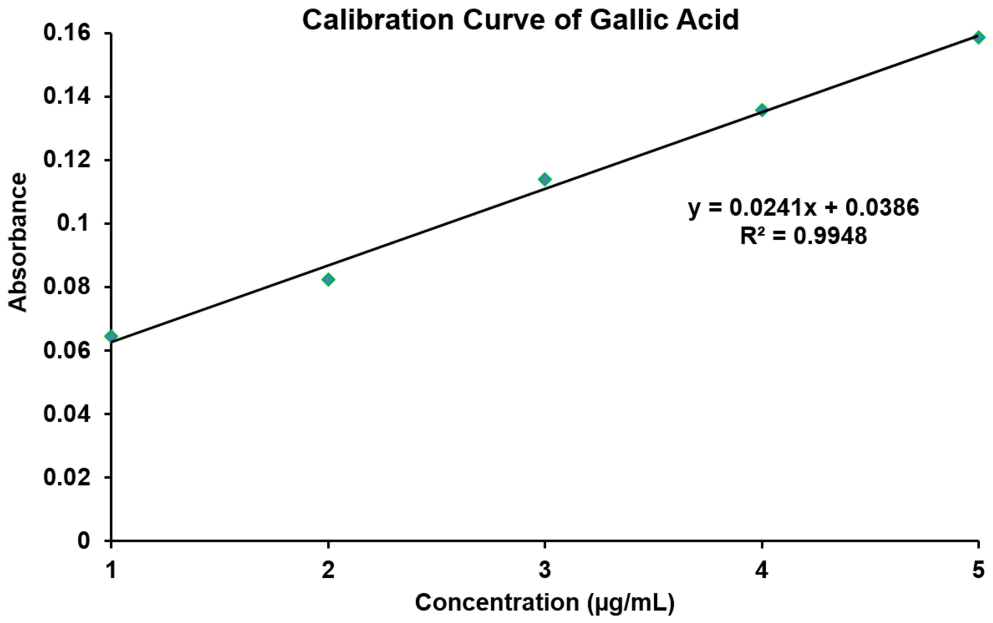


Fig. 1. Calibration curve of gallic acid.

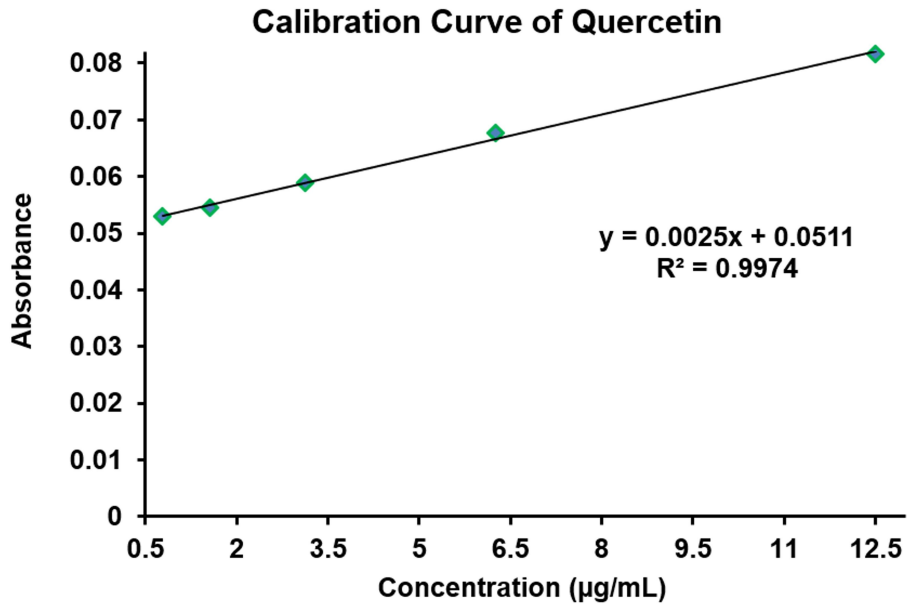


Fig. 2. Calibration curve of quercetin.

for the total phenolic content, significant effects were observed for time and formulation (both $P < 0.0001$), as well as for the time × formulation interaction ($P = 0.0002$). These results suggest that the release profiles of mouthwash and toothpaste differ significantly.

Following repeated-measures two-way ANOVA, Šídák's multiple comparisons test showed no significant differences in total phenolic content release between formulations at 0, 5, and 15 min ($P > 0.05$). However, significantly higher phenolics release from toothpaste was observed at 30 min ($P < 0.05$). This

Table 1. Cumulative release of phenolics and flavonoids from mouthwash and toothpaste over 480 min (mean ± SD)

	Phenolics					Flavonoids				
Time (min)	Mouthwash (%)	Mouthwash (mg GAE/mL)	Toothpaste (%)	Toothpaste (mg GAE/g)	Significance	Mouthwash (%)	Mouthwash (mg QE/mL)	Toothpaste (%)	Toothpaste (mg QE/g)	Significance
0	2.8 ± 0.21	0.60 ± 0.05	3.21 ± 0.8	0.69 ± 0.2	ns	0.29 ± 0.08	1.80 ± 0.48	0.01 ± 0.01	0.06 ± 0.05	*
5	7.5 ± 0.10	1.61 ± 0.02	9.15± 2.0	1.98 ± 0.4	ns	0.74 ± 0.14	4.60 ± 0.86	0.03 ± 0.02	0.19 ± 0.11	****
15	11.8 ± 0.31	2.56 ± 0.07	14.51± 1.4	3.14 ± 0.3	ns	1.11± 0.20	6.90 ± 1.21	0.04± 0.01	0.25 ± 0.04	****
30	15.6 ± 0.50	3.38 ± 0.11	19.38± 0.9	4.19 ± 0.2	*	1.44 ± 0.25	8.95 ±1.58	0.08 ± 0.01	0.50 ± 0.06	****
60	19.0 ± 0.78	4.11 ± 0.17	25.07± 1.4	5.42 ± 0.3	***	1.85 ± 0.13	11.50 ± 0.82	0.13 ± 0.09	0.81 ± 0.57	****
180	22.5 ± 0.88	4.88 ± 0.19	31.06± 2.0	6.72 ± 0.4	****	2.22 ± 0.07	13.80 ± 0.46	0.17 ± 0.08	1.06 ± 0.47	****
300	30.0 ± 0.91	6.49 ± 0.20	37.22± 2.5	8.05 ± 0.5	***	2.52 ± 0.12	15.67 ± 0.75	0.25 ± 0.06	1.55 ± 0.39	****
480	36.9 ± 1.22	7.98 ± 0.26	41.45 ± 0.7	8.97 ± 0.2	**	2.75 ± 0.19	17.10 ± 1.18	0.27 ± 0.09	1.68 ± 0.57	****

Values are presented as mean ± SD (n = 3). Statistical analysis was performed using repeated-measures two-way ANOVA followed by Šídák's multiple comparisons test. Significance refers to comparisons between mouthwash and toothpaste at the corresponding time point. ns = not significant ($P \geq 0.05$); *$P < 0.05$, **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. ANOVA, analysis of variance; GAE, gallic acid equivalents; MW, mouthwash; QE, quercetin equivalents;SD, standard deviation.

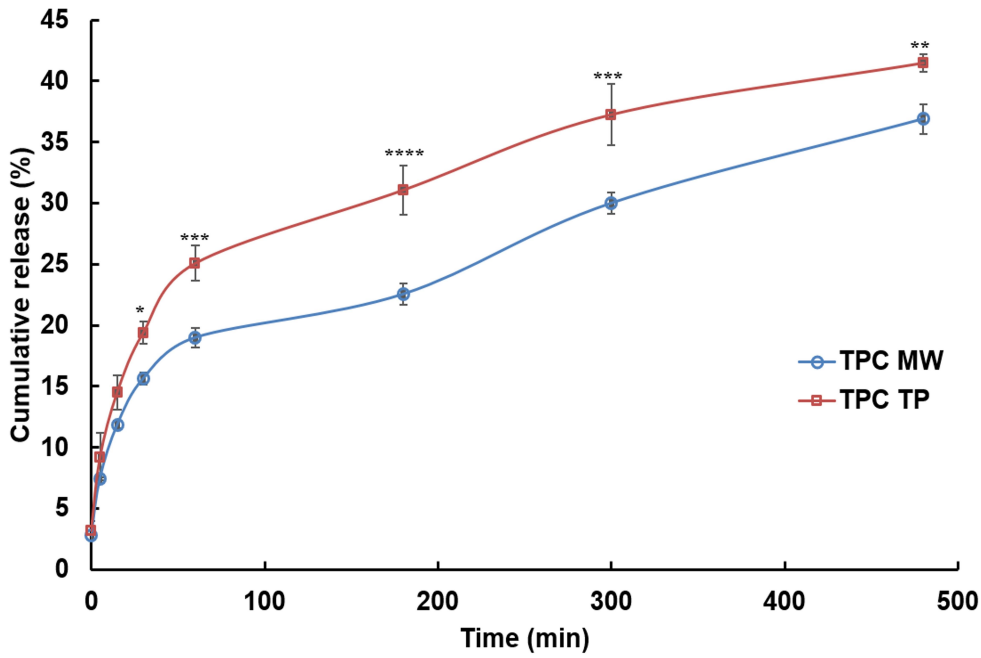


Fig. 3. Cumulative release percentage of phenolic released from mouthwash (MW) and toothpaste (TP) after 480 min in artificial saliva. Results are the mean of total phenolic content (TPC) ± SD. Data are presented as mean ± SD ($n = 3$). Statistical analysis was performed using repeated-measures two-way ANOVA followed by Šídák's multiple comparisons test. Significant differences between formulations at corresponding time points are indicated as * $P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$. ANOVA, analysis of variance; MW, mouthwash; SD, standard deviation; TP, toothpaste; TPC, total phenolic content.

difference in phenolic release between two products become more distinct at later time points with $P < 0.001$ at 60 and 300 min, $P < 0.0001$ at 180 min, and $P < 0.01$ at 480 min. For total flavonoids, significantly higher flavonoid release from mouthwash than toothpaste was observed at 0 min ($P < 0.05$). This difference increased over time with highly significant differences observed at all subsequent time points ($P < 0.0001$). These findings further support formulation-dependent differences in the release behavior of phenolics and flavonoids.

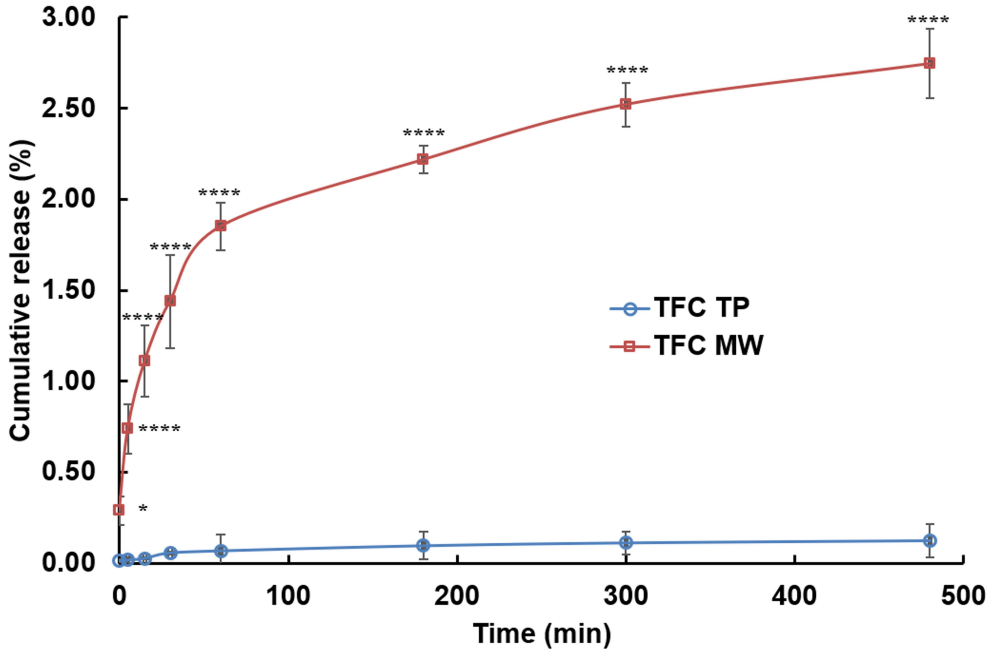


Fig. 4. Cumulative release percentage of flavonoid released from mouthwash (MW) and toothpaste (TP) after 480 min in artificial saliva. Results are the mean of total flavonoid content (TFC) ± SD. Data are presented as mean ± SD (n = 3). Statistical analysis was performed using repeated-measures two-way ANOVA followed by Šídák's multiple comparisons test. Significant differences between formulations at corresponding time points are indicated as *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. ANOVA, analysis of variance; MW, mouthwash; SD, standard deviation; TFC, total flavonoid content; TP, toothpaste.

Kinetics of flavonoids release

To interpret the release mechanism, cumulative percentage release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models as presented in Table 2 and Figures 5 and 6. Both formulations were best described by diffusion-based models, although the exact mechanism should be interpreted within the limits of an *in vitro* system.

For mouthwash, the release data revealed that Higuchi model provided the best fit among the basic kinetic models with R^2 = 0.9223, compared to the other basic kinetic models investigated (zero-order and first-order). These findings suggest that diffusion-related processes may contribute to flavonoid release. The Korsmeyer–Peppas model showed the highest overall fit (R^2 = 0.9750), with a release exponent of n = 0.2824, suggesting diffusion-related release behavior; however, conventional n-value thresholds are geometry-dependent and should be interpreted cautiously for the present system.[25,26]

Regarding toothpaste, both zero-order and first-order models exhibited moderate correlation coefficients (0.8862 and 0.8863, respectively), implying the limited suitability of these models for describing the release behavior. The Higuchi model showed the best fit among the basic kinetic models (R^2 = 0.9778), suggesting that diffusion-related processes contributed to flavonoid release. The Korsmeyer–Peppas model also provided a good fit (R^2 = 0.9743) with a release exponent of n = 0.4989. The value n = 0.4989 suggests diffusion-related release behavior; however, conventional Korsmeyer–Peppas thresholds are geometry-dependent, so mechanistic classification should be interpreted cautiously for the present system.[25,26]

Kinetics of phenolic release

For phenolic release, the kinetic model fitting results are shown in Table 3 and visualized in Figures 7 and 8.

The data suggest that diffusion contributed to the release of phenolics from both preparations, although the findings should be interpreted cautiously, given the limited scope of the *in vitro* model.

The Korsmeyer–Peppas model showed the highest R^2 values for both products (0.9801 for mouthwash and 0.9820 for toothpaste). Among the basic kinetic models (zero-order, first-order and Higuchi) investigated, the Higuchi model showed the best fit for both mouthwash (R^2 = 0.9723) and toothpaste (R^2 = 0.9527), suggesting that diffusion-related processes contributed to phenolic release from both formulations. Toothpaste showed a higher Higuchi constant than mouthwash (1.6987 *vs.* 1.447 %. min $^{-0.5}$) indicating a higher apparent phenolics release rate under the fitted model.

Discussion

The current study showed a time-dependent release of propolis-derived phenolics and flavonoids from the investigated commercial toothpaste and mouthwash preparations. However, both the extent and kinetics of release varied noticeably between the products. At 480 minutes, the highest percentage of phenolics release was detected in the toothpaste (41.45 ± 0.70%), while mouthwash showed 36.90 ± 1.22%. In contrast, the release of

Table 2. Kinetic models' parameters for percentage of flavonoid release from mouthwash and toothpaste

Formulation	Model	R^2	Release constant	Comment
Mouthwash	Zero-order	0.7421	0.0043 %/min	Poor fit
	First-order	0.7449	0.00005 min^{-1}	Poor fit
	Higuchi	0.9223	0.1079 %. $min^{-0.5}$	Best fit among basic models suggesting diffusion-controlled release from matrix system
	Korsmeyer-Peppas	0.975	K_{KP} = 0.0051, n = 0.2824	Highest R^2 with n<0.45, suggesting diffusion-related release behavior
Toothpaste	Zero-order	0.8862	0.0005 %/min	Moderate fit suggesting limited applicability to describe release kinetic
	First-order	0.8863	0.000005 min^{-1}	Moderate fit suggesting limited applicability to describe release kinetic
	Higuchi	0.9778	0.0126 %. $min^{-0.5}$	Best fit among all models, suggesting diffusion may contribute to release
	Korsmeyer-Peppas	0.9743	K_{KP} = 0.0001, n = 0.4989	Good fit; $n \approx 0.5$, suggesting diffusion-related release behavior, although mechanistic interpretation should be cautious because conventional Korsmeyer–Peppas thresholds are geometry-dependent

KKP, Korsmeyer–Peppas release constant.

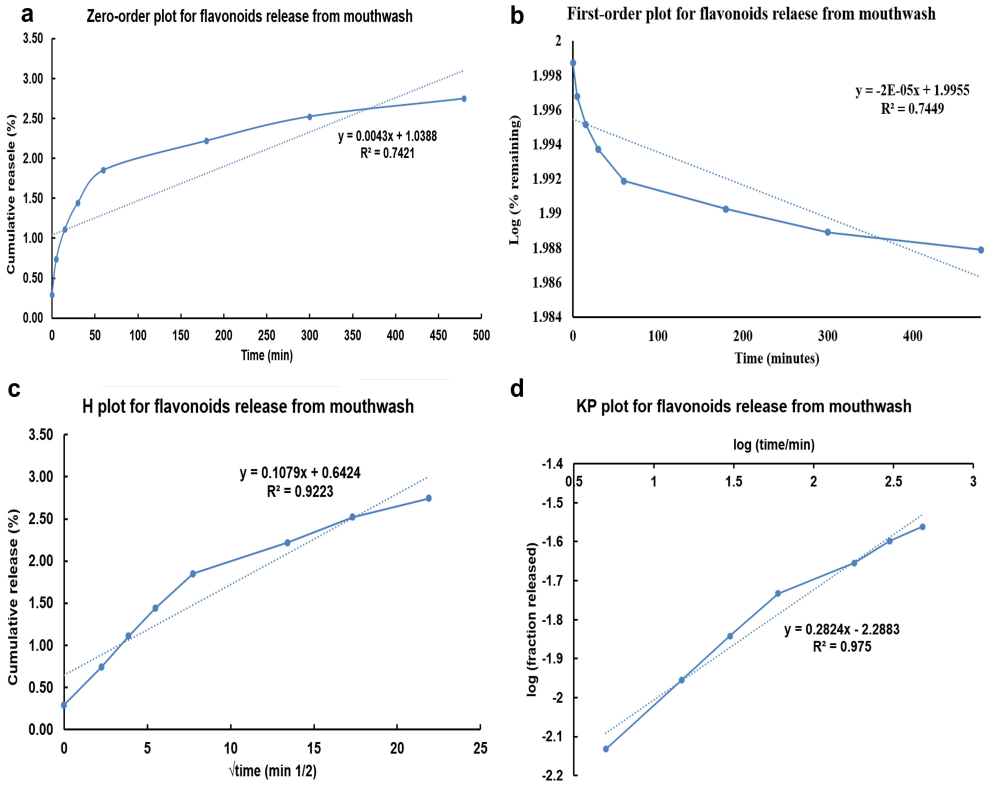


Fig. 5. Comparative release kinetics of flavonoids from mouthwash formulation evaluated using (a) zero-order, (b) first-order, (c) Higuchi and (d) Korsmeyer–Peppas models. Among the assessed models, the Higuchi and Korsmeyer–Peppas models showed the highest R^2 values, indicating that diffusion-related processes contributed to flavonoid release. KP, Korsmeyer–Peppas.

flavonoids was much lower in both products, with cumulative release percentage values of 2.75 ± 0.19% for mouthwash and 0.27 ± 0.09% for toothpaste at 480 min. These findings suggest that propolis-derived phenolics were released more readily than the flavonoids under the conditions of the present study, and greater phenolics release was observed for the toothpaste formula-

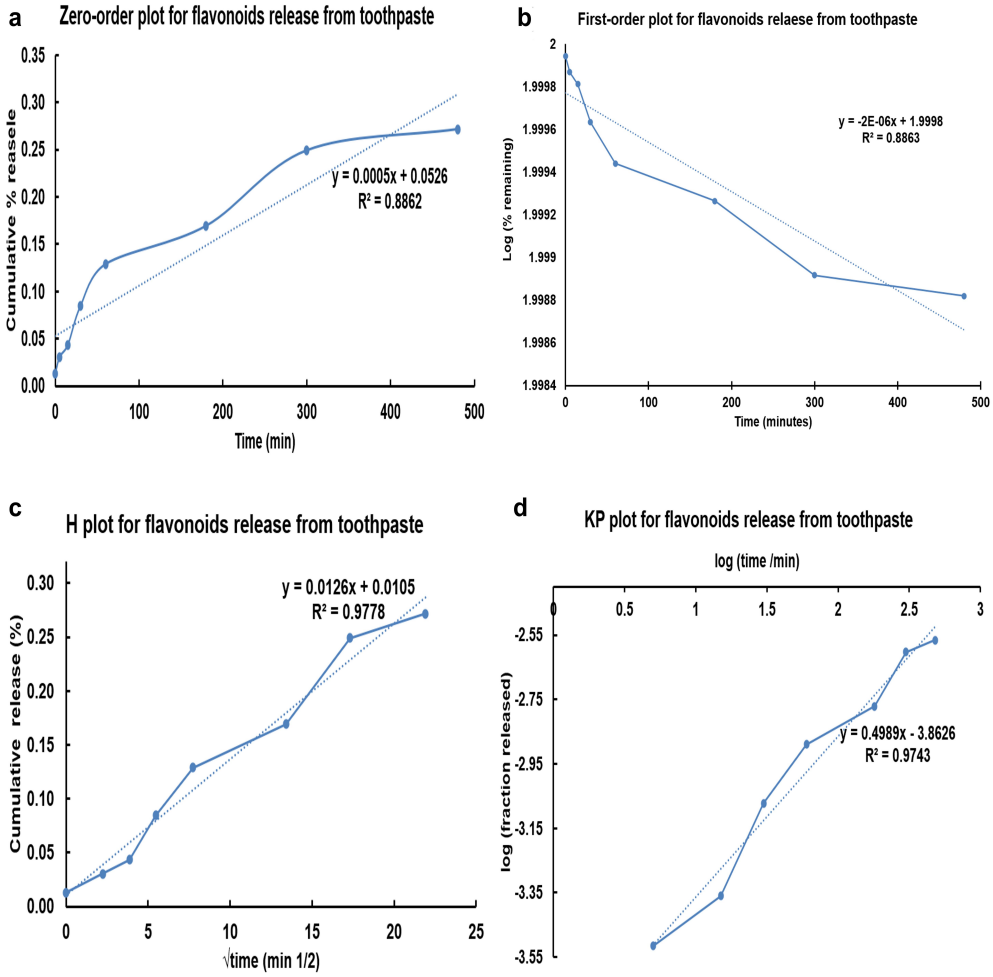


Fig. 6. Comparative release kinetics of flavonoids from toothpaste formulation evaluated using (a) zero-order, (b) first-order, (c) Higuchi and (d) Korsmeyer–Peppas models. Among the assessed models, the Higuchi and Korsmeyer–Peppas models showed the highest R^2 values, indicating that diffusion-related processes contributed to flavonoid release. KP, Korsmeyer–Peppas.

tion.

The release-model analyses suggested diffusion-related behavior in both products. The Higuchi model provided the best fit among the basic kinetic models for both phenolics and flavonoids release. The Korsmeyer–Peppas *n* values were also consistent with diffusion-related release behavior[25]; however, conventional thresholds for classifying Fickian and anomalous transport are geometry-dependent and should therefore be interpreted cautiously for the present release system.[25,26]

Although both products showed similar overall release behaviors, the toothpaste exhibited higher diffusion constants for phenolics release, suggesting a faster release rate than that of the mouthwash. These results suggest that the formulation matrix may affect not only cumulative amount released, but also the rate at which active compounds become available in the release medium.

The detected variations in the release kinetics between toothpaste and mouthwash may be related to the formulation composition and the hydrophobic nature of propolis.[27] Propolis is composed of several hydrophobic constituents with limited aqueous solubility.[28] Toothpaste is a semisolid preparation that typically contains excipients such as surfactants that can improve the dispersion and apparent solubility of hydrophobic compounds in aqueous media.[21] In addition, these additives form a structured matrix that can trap propolis constituents and protect them from rapid release.[21] Upon mixing toothpaste with artificial saliva, the formulation excipients particularly surfactants may facilitate partitioning of phenolics into the aqueous release medium, potentially contributing to the higher apparent release from toothpaste formulation. This interpretation is consistent with the higher Higuchi model rate constant observed for the toothpaste (1.6987 %. $min^{-0.5}$) compared to the mouthwash (1.447 %. $min^{-0.5}$), suggesting a higher apparent rate of phenolics release under the experimental conditions. These results may reflect greater apparent availability of phenolics from the toothpaste in the aqueous release medium. In contrast, mouthwash is a liquid dispersion in which propolis is solubilized by alcohol, surfactants

Table 3. Kinetic models' parameters for percentage of phenolic release from mouthwash and toothpaste

Formulation	Model	R^2	Release constant	Comment
Mouthwash	Zero-order	0.8735	0.0609 %. min^{-1}	Moderate fit, suggesting limited suitability for describing the release profile
	First-order	0.9113	0.0007 min^{-1}	Higher R^2 value compared to zero-order model indicates a better fit, suggesting that the release may follow concentration-dependent kinetic rather than a constant release rate
	Higuchi	0.9723	1.447 %. $min^{-0.5}$	Best fit among the basic models, suggesting diffusion may contribute to release
	Korsmeyer-Peppas	0.9801	K_{KP} = 0.0475, n = 0.3252	Highest R^2 with $n < 0.45$, suggesting diffusion-related release behavior
Toothpaste	Zero-order	0.7969	0.069 %. min^{-1}	Poor fit
	First-order	0.8492	0.001 min^{-1}	Poor fit
	Higuchi	0.9527	1.6987 %. $min^{-0.5}$	Best fit among the basic models, suggesting diffusion may contribute to release
	Korsmeyer-Peppas	0.9820	K_{KP} = 0.0600, n = 0.3224	Highest R^2 with $n < 0.45$, suggesting diffusion-related release behavior

KKP, Korsmeyer–Peppas release constant.

or other solubilizers.[21] However, when the mouthwash was mixed with artificial saliva, it became diluted which may have reduced the concentration of solubilizers. This may have decreased the solubilization of some hydrophobic propolis compounds, thereby limiting the apparent release of phenolic compounds into the aqueous medium. Nonetheless, these interpretations should be considered cautiously because the present study did not directly evaluate the physicochemical behaviors of individual propolis constituents.

The current findings align with those of previous studies that reported the dependency of propolis constituents release on the formulation type, particularly in semisolid preparations such as gels, creams and mucoadhesive formulations.[29,30] Previous semisolid propolis formulations have shown matrix-dependent release behavior; a mucoadhesive gel study reported good Higuchi fitting, whereas another semisolid system identified polymer-chain relaxation as an important contributor to release.[29,30] Collectively, these observations suggest that formulation composition may be a critical factor in determining the availability of propolis compounds, rather than the concentration of propolis in the product.

In contrast, most studies investigating propolis in oral health applications have focused on its antimicrobial, antibiofilm and anti-inflammatory activities rather than release kinetics.[10,31-35] Previous studies of propolis-based oral-care preparations have primarily evaluated biological or clinical outcomes, while the amount of active compounds released from the formulations has generally not been quantified.[9,11,13,18-20] The current findings provide additional information regarding the release behavior of commercially available propolis-based oral care products and suggest that formulation design may affect the availability of released compounds.

The early stage release profile is particularly relevant to practical oral use conditions. Within the first 60 min, both products exhibited rapid phenolic release, with toothpaste reaching 25.07 ± 1.40% cumulative release and mouthwash reaching 19.00 ± 0.78%. Similarly, flavonoid release increased rapidly during the initial phase before reaching a slower release rate. Although actual oral exposure times are considerably shorter than the duration of *in vitro* experiments, these findings suggest that a substantial proportion of releasable compounds becomes available shortly after application. Nevertheless, the extent to which this early release translates into *in vivo* exposure remains uncertain and may depend on salivary clearance, product retention, and interactions with oral tissues.

The recorded release kinetics are relevant when considered alongside the realistic oral contact times of the products. The tooth brushing process typically takes around 1–2 minutes, while rinsing with mouthwash generally occurs for approximately 30–60 seconds.[36,37] Although release was measured over 8 hours *in vitro*, the prolonged release observed from toothpaste indicates continued release within the experimental system; whether such release persists after oral application requires *in vivo* confirmation. However, these interpretations should be considered tentative because the *in vitro* model does not fully replicate the complex physiological conditions of the oral cavity. Therefore, the practical relevance of the observed release profiles may depend on the extent to which the released compounds remain associated with the oral surfaces following product use.

The present results may also provide useful insights for formulation optimization. Increasing the hydrophilicity of the formulation base could potentially improve the aqueous release of propolis compounds, particularly if flavonoids release is limited. Additionally, rational selection of excipients such as surfactants may enhance partitioning of hydrophobic compounds into the aqueous phase without adversely affecting product's stability or sensory attributes.[21,38] Regarding propolis hydrophobic constituents, alternative solubilization approaches, such as cyclodextrins,

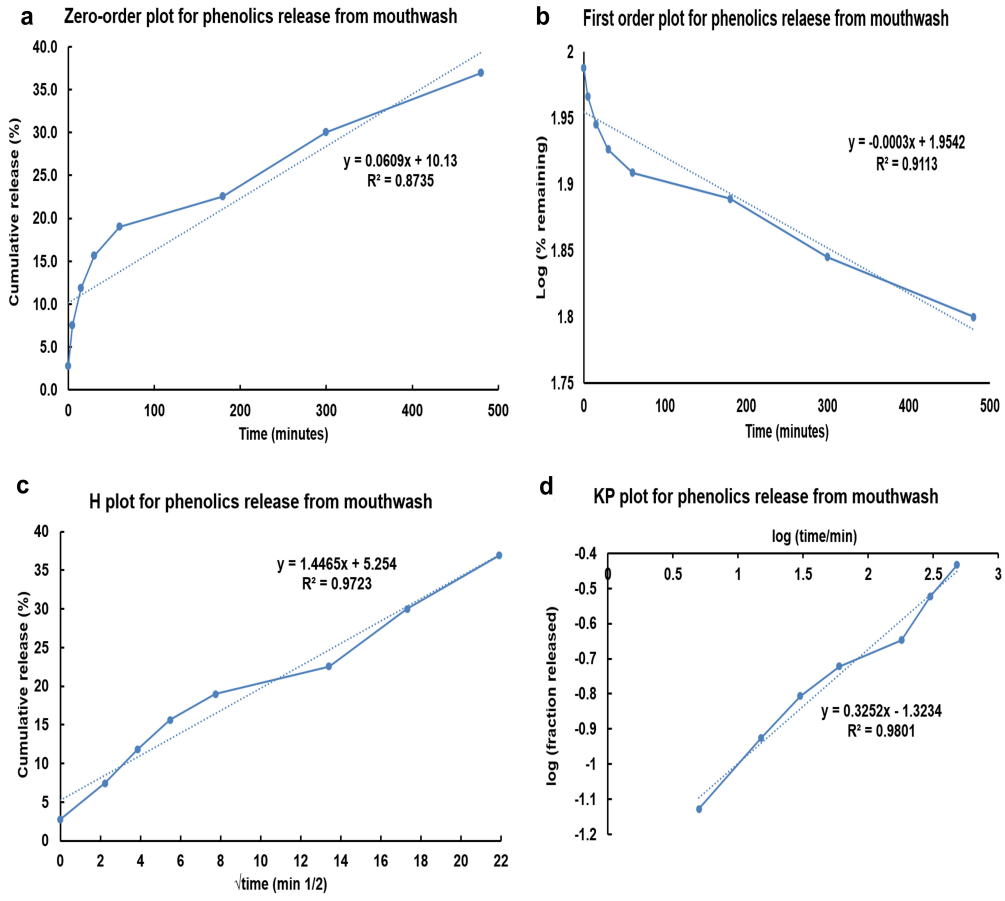


Fig. 7. Comparative release kinetics of phenolics from mouthwash formulation. Among the assessed models, the Higuchi and Korsmeyer–Peppas models showed the highest R^2 values, indicating that diffusion-related processes contributed to phenolic release. KP, Korsmeyer–Peppas.

could also be explored to maintain solubility following contact with saliva.[39] Such formulation strategies may increase the amount of bioactive compounds available for interaction with oral tissues; however, any effect on efficacy would require biological or clinical validation.

Limitations

The current study has several limitations. First, the applied *in vitro* model cannot fully capture the dynamics of the oral cavity, including saliva flow, swallowing, dilution, enzymatic activity, and binding of the released compounds to biofilms or oral tissues. Therefore, the release data should be considered indicative and not directly translated to *in vivo* results. Secondly, the application of total phenolics and flavonoids measurements does not reflect individual active compounds; therefore, the detected release profiles over 8 hours could be attributed to compound-specific stability or degradation. Thirdly, the study covered only two commercial products; therefore, the findings should not be generalized across other propolis-based oral care preparations. Furthermore, formulation blanks and matrix controls were not applied in this study as propolis-free formulations of the investigated commercial products were not accessible due to proprietary formulation restrictions. Therefore, potential contribution of formulation excipients to the colorimetric measurements cannot be completely excluded.

Future investigations should address these limitations by complementing release studies with chromatographic methods such as liquid chromatography-mass spectrometry (LC-MS) to create a profile of individual propolis compounds and assess the stability of the released compounds over time. Additionally, the application of a release medium that better mimics saliva by adding substances such as mucins may enhance the physiological significance of the assay. Furthermore, the correlation of *in vitro* release data with biological assays of antimicrobial and antibiofilm activities would assist in establishing whether the released compounds contribute meaningfully to therapeutic activity. Such research would provide a stronger mechanistic and translational foundation for the design and assessment of propolis-based oral care products.

Conclusions

The current study demonstrated differences in *in vitro* release profiles of phenolics and flavonoids from the investigated propolis-based toothpaste and mouthwash formulations. Toothpaste showed higher cumulative phenolics release, whereas

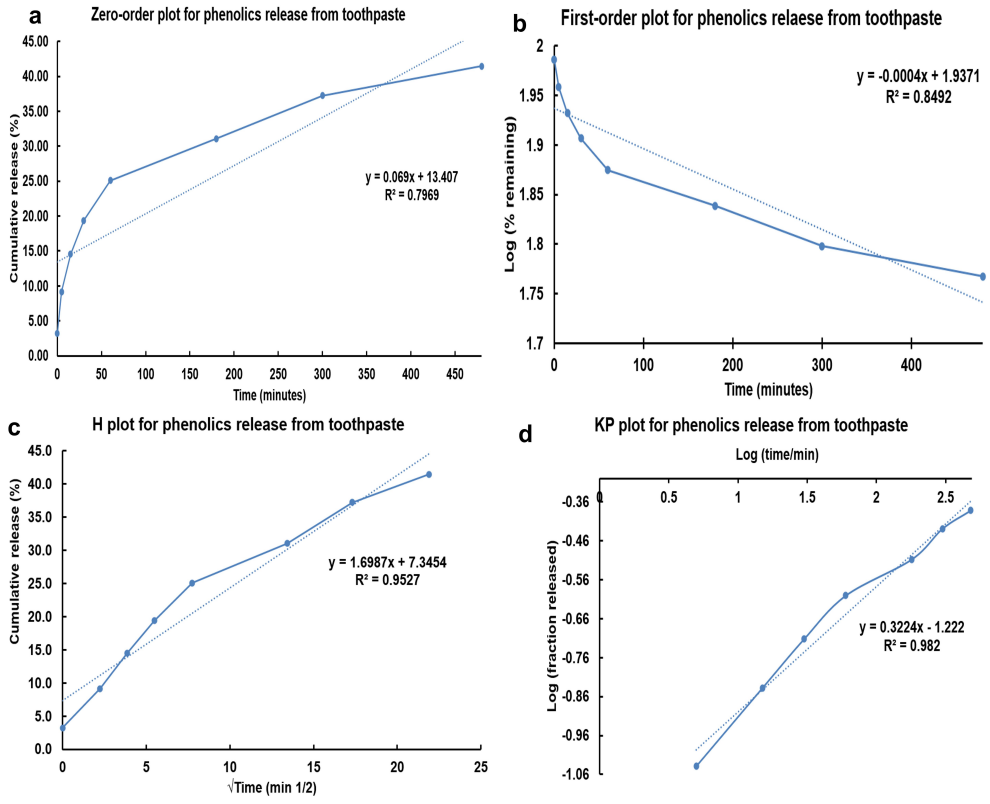


Fig. 8. Comparative release kinetics of phenolics from toothpaste formulation evaluated using (a) zero-order, (b) first-order, (c) Higuchi and (d) Korsmeyer–Peppas models. Among the assessed models, the Higuchi and Korsmeyer–Peppas models showed the highest R^2 values, indicating that diffusion-related processes contributed to phenolic release. KP, Korsmeyer–Peppas.

mouthwash exhibited higher flavonoid release under the experimental conditions. Phenolic and flavonoid compounds are of particular interest in oral health due to their antioxidant, anti-inflammatory and antimicrobial properties, which may contribute to maintaining oral health and protecting against microbial challenges. Release data were consistent with diffusion-related kinetic models, suggesting that formulation characteristics may influence both the amount and type of compounds released. The higher apparent phenolics release from toothpaste may reflect formulation-dependent solubilization and matrix effects, although these mechanisms were not directly measured. These findings contribute to the limited literature on the release behavior of propolis-derived compounds from commercial oral care products. However, the results should be interpreted within the limitations of the *in vitro* model and the evaluation of only two commercial formulations. Further studies are required to characterize the individual released compounds and establish the potential relationships between *in vitro* release behavior and biological or clinical performance.

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Conflict of interest

BK is a Quality Research Manager and JF is the Chief Executive Officer (CEO) at Nature's Laboratory Ltd., the manufacturer of the products investigated in this study. The products were supplied by the company. BK contributed to manuscript preparation and review. Nature's Laboratory Ltd. had no role in the study design, experimentation, data analysis, or interpretation of results. The remaining authors declare no competing interests.

Author contributions

Writing – original draft (GM, PS, BK), conceptualization (GM), study design (GM, PS), experimentation (GM, PS), data analysis (GM), visualization (GM), writing – review & editing (BK), supervision (BK, AP), resources (JF), and final proofreading of the manuscript (AP). All authors have approved the final version and publication of the manuscript.

Ethical statement

No ethics committee approval was required for this study.

Data sharing statement

The data supporting the findings of this study are included in this published article. Additional underlying data including individual replicate measurements and statistical analyses data are available from corresponding author upon reasonable request.

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