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Physicochemical Characterisation and Quercetin Content of Chitosan–TPP Nanoparticles Loaded with Sappan Wood (*Caesalpinia sappan* L.) Extract: A Preliminary Study Towards Anti-Plaque Mouthwash Development

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ABSTRACT

Dental plaque, a biofilm in which *Streptococcus mutans* plays a major role, is a key factor in dental caries and periodontal disease. Chlorhexidine-based mouthwashes are effective for plaque control, but their long-term use is associated with side effects, which has encouraged interest in herbal alternatives. Sappan wood (*Caesalpinia sappan* L.) contains bioactive compounds, including quercetin, for which antioxidant, anti-inflammatory and antibacterial activities have been reported; however, quercetin has low aqueous solubility and poor bioavailability. This preliminary study aimed to prepare chitosan–sodium tripolyphosphate (TPP) nanoparticles loaded with sappan wood ethanol extract and to characterise their particle size distribution and quercetin content as a first step towards a herbal anti-plaque mouthwash. Nanoparticles were prepared by ionic gelation, particle size was measured by dynamic light scattering (DLS), and quercetin content was determined by UV–Vis spectrophotometry against a quercetin calibration curve. The nanoparticles had a mean particle size (Dav) of 39.39 nm and a polydispersity index (PDI) of 0.1708. The volume-based percentiles were D10 = 17.77 nm, D50 = 29.59 nm and D90 = 49.21 nm, indicating that about 90% of the particle volume was below 50 nm. Quercetin concentrations in the measured solutions were 8.6681 and 8.7596 µg/mL in duplicate determinations, corresponding to 0.449% and 0.454% w/w of the dried nanoparticle powder (mean 0.452% w/w; relative percent difference 1.02%). The calibration curve was linear (R^2 = 0.99906). These results show that the formulation produced particles in the nanometre range with a relatively narrow size distribution and a measurable quercetin content in a single batch. Antibacterial and antibiofilm activity, zeta potential, morphology, encapsulation efficiency and storage stability were not assessed; these data, together with a fully validated, quercetin-specific analytical method, are required before the formulation can be considered for anti-plaque mouthwash use.

Keywords: Chitosan nanoparticles; Dynamic light scattering; Mouthwash; Sappan wood; Quercetin

INTRODUCTION

Dental and oral diseases associated with plaque, particularly those caused by bacterial biofilm formation, remain a global public health problem as they contribute to gingivitis, periodontitis and caries, which impact quality of life and healthcare costs (Mohanta et al. 2023). Chemical mouthwashes such as chlorhexidine have long been the standard for plaque control, but long-term use can cause side effects such as tooth discolouration, mucosal irritation, and disruption of the oral microbial ecosystem (Sedighi et al. 2024). This has prompted the search for safe yet effective natural alternatives, one of which is the use of sappan wood (*Caesalpinia sappan* L.), a Southeast Asian plant rich in bioactive compounds with antioxidant, anti-inflammatory, and antibacterial properties (Vij et al. 2023). Recent reviews confirm that sappan wood contains important metabolites such as brazilin, brazilein, protosappanin, and flavonoids, including quercetin, which have potential applications in oral health (Vij et al. 2023). Quercetin is known to have antibacterial, antioxidant, and anti-inflammatory properties that are relevant for suppressing the growth of *Streptococcus mutans* and inhibiting the formation of dental biofilm (Aggarwal et al. 2025). However, quercetin has disadvantages in the form of low water solubility, poor stability, and limited bioavailability, which limits its use in oral products (Zhao et

al. 2025). In this regard, nanotechnology can improve the physicochemical properties of quercetin through the formation of nanoparticles that increase solubility, stability, and penetration into target tissues (Yin et al. 2025).

Various studies report that quercetin nanoparticle formulations can improve the efficiency of active substance delivery through modification of particle size and surface area (Yin et al. 2025). Quercetin nanoparticles of approximately 100 nm and low polydispersity are reported to have high encapsulation efficiency and better antioxidant activity compared to conventional forms (Zhao et al. 2025). A quercetin mouthwash formulation demonstrated significant antimicrobial potential against plaque bacteria and was assessed as comparable to conventional mouthwashes in *in vitro* tests (L et al. 2024). Another study showed that the use of quercetin liposomes with mint oil in mouthwash improved formulation stability and retention of active compounds in the oral cavity (Castangia et al. 2022). Biomedical reviews also emphasise that nano-quercetin overcomes the pharmacokinetic limitations of quercetin by increasing its solubility and stability, thereby supporting its application in oral therapy (Li et al. 2025). In oral biofilms, nanoparticle size is considered an important factor because smaller particles may penetrate the biofilm matrix and deliver antibiofilm agents more effectively (Lan et al. 2025). This is in line with reports that nanoparticle-based nanomedicine strategies can enhance biofilm eradication through pore penetration and high surface area (Blanco-Cabra et al. 2024).

Although various quercetin nanoparticle strategies have been developed, research integrating sappan wood extract-based nanoparticles specifically for anti-plaque mouthwash applications remains very limited (Vij et al. 2023). Most studies have only explored pure quercetin or other compounds such as brazilin from sappan wood, while comprehensive data on quercetin in nanoparticle formulations are not yet available (Pyun et al. 2022). Existing quercetin-based mouthwash formulations generally do not link particle size analysis with measurable quercetin levels, even though these parameters are important for assessing product stability and effectiveness (Castangia et al. 2022). In fact, biofilm research emphasises particle size and polydispersity as determinants of successful biofilm penetration and disruption (Lan et al. 2025). On the analytical side, although the HPLC-DAD/UV method has been validated for measuring quercetin levels in several matrices, its application specifically to nanoparticles loaded with sappan wood extract has not been widely implemented (Carvalho et al. 2023). Therefore, there is still a research gap regarding the relationship between the characterisation of sappan wood nanoparticle particle size and quercetin content as basic quality attributes that need to be established before antibiofilm activity can be meaningfully evaluated.

Based on this gap, the present preliminary study aimed to prepare chitosan–TPP nanoparticles loaded with sappan wood ethanol extract and to characterise two basic quality attributes: particle size distribution, measured by dynamic light scattering (DLS) using a particle size analyser, and quercetin content, determined by UV–Vis spectrophotometry. Antibacterial, antibiofilm and clinical effects were not evaluated. The study is intended to provide baseline physicochemical data for the subsequent development and testing of a nano-based herbal anti-plaque mouthwash.

METHODS

Materials and Identification

Sappan wood (*Caesalpinia sappan L.*) was obtained from the Among Tani market, Batu, East Java, Indonesia. Plant identification was carried out at the UPT Materia Medica Laboratory, Batu, Malang, to ensure the authenticity of the species. The chemicals used included 96% technical ethanol, propylene glycol, 10% dimethyl sulfoxide (DMSO), distilled water, chitosan, glacial acetic acid, and 0.4% sodium tripolyphosphate (TPP). Pure quercetin standard (≥98%) was used to prepare the calibration curve for quantitative analysis.

Sappan Wood Extraction

Dried sappan wood powder was extracted using the maceration method in 96% ethanol. The extract obtained was filtered, then evaporated using a rotary evaporator at a low temperature until a concentrated extract was obtained. The resulting thick extract was further dried to reduce the remaining solvent and stored in a tightly closed container before being formulated into nanoparticles.

Production of Nanoparticles from Sappan Wood Extract

Nanoparticles were prepared by ionic gelation. Chitosan (1 g) was dissolved in 100 mL of 1% (v/v) glacial acetic acid under magnetic stirring until completely dissolved, giving a 1% (w/v) chitosan solution. Separately, 500 mg of sappan wood ethanol extract was dissolved in a co-solvent mixture of 20 mL propylene glycol, 20 mL 70% ethanol, 20 mL 10% dimethyl sulfoxide (DMSO) and 100 mL distilled water (160 mL in total). Propylene glycol, ethanol and DMSO were used as co-solvents to dissolve the poorly water-soluble extract constituents, including quercetin, before contact with the aqueous chitosan phase; propylene glycol is also a humectant commonly used in mouthwash formulations. After homogenisation, 40 mL of the 1% chitosan solution was added, giving 0.4 g chitosan in 200 mL (0.2% w/v before TPP addition).

The mixture was stirred for 10 min, after which 20 mL of 0.4% (w/v) sodium TPP solution (80 mg TPP) was added dropwise (approximately one drop every 3 s) under continuous stirring at 300 rpm. TPP acted as the ionic cross-linker: its negatively charged phosphate groups interact with the protonated amino groups of chitosan, and nanoparticle formation was indicated by the appearance of a homogeneous opalescent suspension. Stirring was continued for a further 15 min. The final suspension volume was 220 mL, giving final concentrations of 0.18% (w/v) chitosan, 0.036% (w/v) TPP (chitosan:TPP mass ratio 5:1), 2.27 mg/mL extract, 9.1% (v/v) propylene glycol, approximately 6.4% (v/v) ethanol, 0.91% (v/v) DMSO and

approximately 0.18% (v/v) acetic acid. The suspension was observed visually for five days for changes in colour, turbidity and sediment formation; this was a preliminary qualitative check only and not a formal storage stability study. The suspension was then dried in an oven at 50 °C for 24 h to obtain a dry nanoparticle powder, which was used for particle size and quercetin analyses. Because propylene glycol and DMSO have high boiling points, part of these co-solvents may remain in the dried powder; quercetin content is therefore expressed per mass of dried powder.

Particle Size Characterisation

Particle size and size distribution were measured by dynamic light scattering (DLS) using a particle size analyser (BIOBASE BK-802N). The dried nanoparticle powder was redispersed in distilled water and measured at 21 °C. The parameters recorded were mean particle size (Dav), polydispersity index (PDI) and the volume-based percentiles D10, D50 and D90. Zeta potential, particle morphology (TEM/SEM) and encapsulation efficiency were not determined in this preliminary study.

Analysis of Quercetin Levels

Quercetin in the nanoparticle powder was quantified by UV–Vis spectrophotometry. Approximately 1.2 g of powder (accurately weighed) was hydrolysed with acetone and made up to 100 mL. A 20 mL aliquot was extracted with ethyl acetate and the extract was made up to 50 mL. A 5 mL aliquot of the ethyl acetate extract was diluted to 12.5 mL, and its absorbance was read at the maximum absorption wavelength of quercetin. The quercetin concentration in the measured solution (C, µg/mL) was calculated from the calibration curve of the quercetin standard (≥98%), y = 0.0034908 + 0.044067212x, where y is absorbance and x is concentration (µg/mL). Quercetin content (% w/w) was calculated as [C × (12.5/5) × 50 × (100/20)] / (W × 10^6) × 100, where W is the sample mass (g). For example, for sample 1, 8.6681 µg/mL × 2.5 × 50 mL × 5 = 5,418 µg quercetin in 1.2060 g of powder, i.e. 0.449% w/w. Method performance was evaluated only by calibration linearity (coefficient of determination, R^2) and agreement between duplicate determinations; specificity, accuracy (recovery), intermediate precision, and limits of detection and quantification were not evaluated. Because UV–Vis absorbance may include contributions from other flavonoids and phenolic compounds in the extract and from formulation components, the results are interpreted as quercetin-equivalent content.

Data Analysis

Particle size and quercetin analyses were performed on a single nanoparticle batch. Quercetin was determined in duplicate, using two separately weighed portions of the same powder, and results are reported as individual values and the mean. Agreement between duplicates was expressed as the relative percent difference (RPD = $|x_1 - x_2|$ / mean × 100), with RPD < 2% taken as acceptable repeatability. Because only one batch and two determinations were analysed, batch-to-batch reproducibility could not be evaluated and the data are presented descriptively.

RESULTS AND DISCUSSION

Figure 1 shows the particle size distribution of the sappan wood extract nanoparticles (nano-sappan) measured by dynamic light scattering (BIOBASE BK-802N). The mean particle size (Dav) was 39.39 nm with a polydispersity index (PDI) of 0.1708. The percentile values were D10 = 17.77 nm, D50 = 29.59 nm, and D90 = 49.21 nm; thus, 80% of the particle volume lay between ~18 and ~49 nm (median ~29.6 nm), indicating a relatively narrow size distribution. Measurement conditions: dried powder redispersed in distilled water, 21 °C.

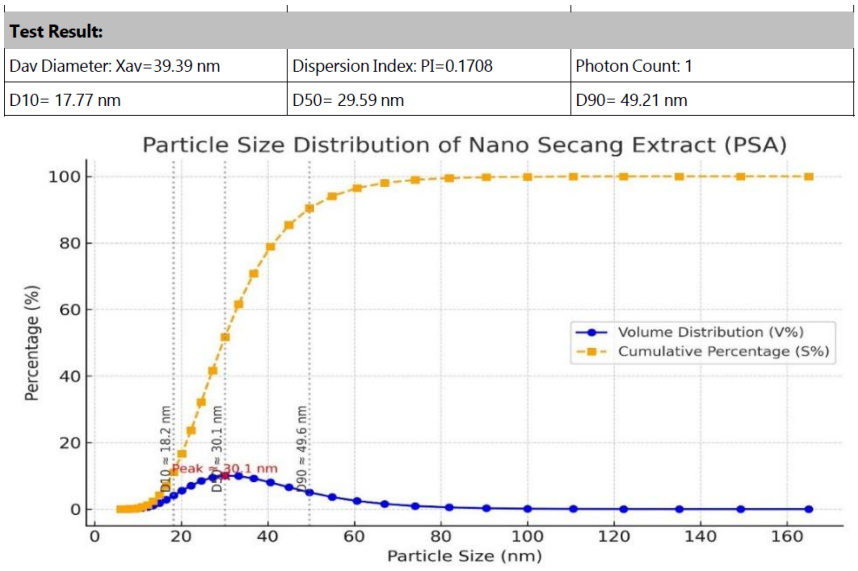


Figure 1. Particle size distribution of chitosan–TPP nanoparticles loaded with *Caesalpinia sappan* L. extract measured by dynamic light scattering

Table 1. Volume-based particle size distribution of chitosan–TPP nanoparticles loaded with *Caesalpinia sappan* L. extract (DLS)

Size	V%	S%	Size	V%	S%	Size	V%	S%	Size	V%	S%
6.06	0.00	0.00	14.92	1.82	4.21	36.75	9.21	70.82	90.47	0.27	99.76
6.70	0.01	0.01	16.50	2.81	7.02	40.62	8.02	78.84	100.00	0.13	99.89
7.41	0.01	0.02	18.23	4.08	11.09	44.89	6.56	85.40	110.53	0.06	99.96
8.19	0.04	0.06	20.15	5.54	16.64	49.62	5.03	90.43	122.17	0.03	99.98
9.05	0.08	0.14	22.28	7.07	23.70	54.84	3.62	94.05	135.03	0.01	99.99
10.00	0.17	0.31	24.62	8.46	32.17	60.62	2.45	96.50	149.25	0.00	100.00
11.05	0.34	0.65	27.21	9.51	41.67	67.00	1.55	98.05	164.96	0.00	100.00
12.22	0.63	1.28	30.08	10.02	51.70	74.06	0.92	98.98			
13.50	1.11	2.38	33.25	9.92	61.62	81.85	0.52	99.49			

Table 2. Quercetin content of the dried nanoparticle powder determined by UV–Vis spectrophotometry

Sample mass (g)	Hydrolysate volume, acetone (mL)	Aliquot extracted (mL)	Ethyl acetate extract volume (mL)	Aliquot pipetted (mL)	Final dilution volume (mL)	Absorbance	Concentration (µg/mL)	Quercetin content (% w/w)
1.2060	100.0	20.0	50.0	5.0	12.5	0.38547	8.6681	0.449
1.2063	100.0	20.0	50.0	5.0	12.5	0.38950	8.7596	0.454
Mean	—	—	—	—	—	—	—	0.452
RPD (%)	—	—	—	—	—	—	—	1.02

Note: Quercetin concentration was calculated from the calibration curve y = 0.0034908 + 0.044067212x (Multiple R = 0.99953; R^2 = 0.99906; adjusted R^2 = 0.99875), where y is absorbance and x is concentration (µg/mL). RPD = relative percent difference between duplicate determinations of the same batch (acceptance limit < 2%); it reflects analytical repeatability, not batch-to-batch reproducibility.

Table 1 shows the size distribution of nanoparticles loaded with sappan wood extract, ranging from 6.06 to 164.96 nm, with the highest volume concentrations at 30.08 nm (V% = 10.02%), 33.25 nm (V% = 9.92%), and 36.75 nm (V% = 9.21%). The median distribution (S% ≈ 50%) was achieved at a size of ~30 nm, while 90% of the particle volume was below 50 nm. Only a very small fraction of the particle volume (about 0.1%) lay above 100 nm. This pattern reflects a narrow distribution with a predominance of particles sized 30–37 nm, consistent with the low PDI (0.1708). However, DLS alone cannot confirm particle shape or morphological homogeneity, which would require TEM/SEM analysis.

Table 2 shows the quercetin content of the dried nanoparticle powder determined by UV–Vis spectrophotometry. The measured concentrations were 8.6681 and 8.7596 µg/mL, corresponding to 0.449% and 0.454% w/w (mean 0.452% w/w, or about 4.5 mg quercetin-equivalent per g of powder). The RPD between the duplicate determinations was 1.02%, below the 2% limit, indicating acceptable repeatability of the measurement.

The calibration curve (y = 0.0034908 + 0.044067212x; Multiple R = 0.99953; R^2 = 0.99906; adjusted R^2 = 0.99875) showed good linearity. Linearity alone, however, does not establish the validity of the method, and the absence of specificity, recovery and detection/quantification limit data is acknowledged as a limitation (see Discussion).

The detection of quercetin in the nanoparticle powder is consistent with previous reports that sappan wood contains flavonoids, including quercetin (Vij et al. 2023). Quercetin has been reported in other studies to have antibacterial and antibiofilm activity against oral pathogens, including *Streptococcus mutans* (Pourhajibagher et al. 2022; L et al. 2024). Whether the quercetin in the present formulation exerts such effects was not tested in this study.

Nanoparticle formulation may improve the solubility and stability of quercetin and, based on the literature, could facilitate its penetration into the plaque biofilm. These possibilities were not tested in the present study and are presented as hypotheses. The present data provide a basis for further work, including zeta potential, morphology, encapsulation efficiency and storage stability testing, antibacterial and antibiofilm testing against plaque-forming bacteria, and safety evaluation in the oral cavity.

The nanoparticle formulation showed two main findings: particles in the tens-of-nanometres range with a relatively narrow size distribution, and quercetin detected at similar levels in duplicate determinations. These findings describe the basic physicochemical quality of a single batch and do not demonstrate anti-plaque activity. Based on the literature, particle size, together with surface charge, may influence how particles interact with and penetrate the plaque biofilm matrix, and quercetin provides a pharmacological rationale for the formulation; both points remain hypotheses that require experimental testing.

In the literature, the correlation between nanoparticle size and biofilm penetration has been widely reported. Comprehensive studies in the field of microbiology show that nanoformulations, through their high surface area and dimensions below the pore threshold, are able to overcome limited diffusion in biofilms and increase the direct exposure of active agents to target microorganisms. The review emphasises that particle surface area and size are the most effective design levers for penetrating the polysaccharide matrix of biofilms and achieving meaningful eradication. In addition, experimental studies of particles measuring tens of

nanometres show better accumulation and penetration than larger sizes, underscoring the importance of maintaining a tight size range such as that observed in the present formulation. Recent nanomedicine perspectives also highlight that nano platforms ranging from liposomes, nanoemulsions, nanogels to inorganic frameworks offer opportunities to bypass biofilm barriers through multiscale interactions and regulated surface charges. The size profile obtained in this study is consistent with these design principles; however, whether the present nanoparticles actually penetrate plaque biofilm was not tested and remains a hypothesis (Mohanta et al. 2023; Xie et al. 2025; Yuan et al. 2022).

This relevance is further strengthened by evidence that quercetin—both free and encapsulated—can disrupt biofilm formation and reduce bacterial virulence. A recently reported nanoparticle formulation containing quercetin suppressed bacterial biofilm formation and mitigated oxidative stress in an invertebrate animal model, demonstrating that delivering quercetin in nanoform not only improves stability but also facilitates effective interaction with microbial communities. On this basis, it can be hypothesised that the present formulation, which places quercetin in a nano-sized and relatively homogeneous vehicle, may promote more uniform exposure of plaque biofilm to quercetin; this hypothesis requires direct testing (Sun et al. 2024).

From a mechanistic perspective, quercetin plays a multifaceted role as a quorum sensing inhibitor in opportunistic pathogens, reducing the expression of virulence factors while also affecting the ability of microbes to form biofilms. Furthermore, quercetin can participate in combinatorial approaches, for example, conjugated with metals or combined in hybrid platforms that exhibit synergistic antimicrobial and immunomodulatory activities. Findings regarding silver-quercetin-based interfaces and azithromycin-quercetin-loaded sensitive micelles show growth inhibition, biofilm dissolution, and macrophage reprogramming towards a more resolutive phenotype. Although the context is not limited to the oral cavity, these cross-species anti-biofilm effects and modulation of the local immune response are relevant to the gums and tooth surfaces that are habitats for polymicrobial biofilms. Thus, the quercetin detected in the present formulation may be relevant not only as a marker compound but also as a potential contributor to anti-plaque activity through several biological targets, although this was not evaluated in the present study (Tran and Hadinoto 2021).

This correlation is also reflected in in vitro data on quercetin-based mouthwash formulations, which show inhibitory effects against oral pathogens and competitive performance compared to commercial herbal mouthwashes. Although these formulations do not yet utilise a nano platform, the results indicate that quercetin itself has baseline antimicrobial activity in the oral environment. Compared to the free quercetin approach, nano-delivery may add layers of advantage: increased solubility in the saliva environment, protection against oxidative degradation, and the possibility of more controlled release during rinsing. Taken together, the literature suggests that nano-delivery systems such as the present formulation could help overcome the important limitations of quercetin, namely solubility and stability, while retaining antimicrobial activity; however, this was not tested here and needs experimental confirmation (L et al. 2024).

Beyond its direct antibacterial effects, quercetin also demonstrates the capacity to modulate the oral tissue environment. Preclinical evidence shows that quercetin supplementation can inhibit excessive inflammatory responses and support a more symbiotic microbial ecology. In the context of anti-plaque, this aspect is important because the long-term success of biofilm control depends not only on killing bacteria, but also on restoring homeostasis in periodontal tissue. The practical implication is that mouthwash formulations with nano-scale quercetin could potentially offer a multi-target approach: disrupting biofilm, reducing inflammation, and supporting a more stable microenvironment (Laky et al. 2024; Mooney et al. 2021).

The duplicate quercetin determinations agreed closely (RPD 1.02%), but this reflects the repeatability of a single analytical procedure on one batch and is not sufficient to establish method validity or formulation consistency. For complex matrices such as extracts in nanoparticles, the use of validated HPLC-DAD-based methods is often recommended due to their robustness against matrix interference and their ability to separate analytes from co-eluting plant constituents. Several recent studies have optimised and validated HPLC methods for quercetin, including in nanoparticle systems, with a focus on selectivity, linearity, and traceability. Although the UV–Vis measurements in this study gave consistent duplicate results, the adoption of HPLC-DAD at an advanced stage will strengthen quality claims, facilitate inter-batch comparisons, and support future regulatory requirements for oral care products. The present values should therefore be regarded as preliminary quercetin-equivalent data that need to be confirmed with a specific, fully validated method (Carvalho et al. 2023; Kim et al. 2021).

From a formulation design perspective, the small particle size and narrow distribution in the present formulation may have consequences for performance during mouth rinsing. The literature suggests that particles measuring tens of nanometres are more likely to penetrate dense biofilm layers and avoid the diffusion retardation often experienced by larger particles. This relevance encompasses two aspects: first, access to the tooth-biofilm interface where key matrix-forming enzymes operate; second, increased exposure uniformity, thereby reducing the heterogeneity of the antibacterial response. Ultimately, when size is kept small and surface charge is regulated, the delivery of quercetin to enzymatic targets or to microcolonies within the biofilm may become more efficient. This is in line with the design of many modern anti-biofilm platforms that utilise organic polymers,

nanogels, and even functional inorganic materials to maximise interaction with the biofilm (Sheng et al. 2023; Yuan et al. 2022).

Recent evidence shows that combining quercetin with other materials, either as a cofactor or as a hybrid component, can produce enhanced anti-biofilm effects. Combination with metal oxides, for example, shows biofilm eradication in difficult-to-treat pathogens; while the use of porous structures or micropolar layers on biomaterial surfaces is able to integrate specific bactericidal effects with beneficial immune reprogramming. While the contexts are diverse (infected wounds, implant interfaces, hard tissues), the general principles drawn from these studies (local delivery, virulence reduction and immune modulation) provide a relevant roadmap for oral applications. In the development of mouthwashes, the choice of excipients and stabilisers compatible with the oral cavity will determine how well these principles can be translated (Rohatgi, Ganapathy, and Sathishkumar 2023).

This study has several limitations. First, the tests focused on the physicochemical characteristics and quantification of quercetin; there are no direct data on antibacterial or antibiofilm activity on major oral pathogens such as *Streptococcus mutans* or *Enterococcus faecalis*. However, evidence from studies of free quercetin, quercetin hydrogels, and quercetin-based mouthwash formulations has shown promising responses, so comparative testing between nano and non-nano forms in the oral environment is needed to assess the actual advantages of nanoparticles. Second, quercetin was quantified by UV–Vis spectrophotometry, which is prone to interference from other phenolic compounds in the extract and from formulation components. Only linearity (R^2) and duplicate agreement were assessed; specificity, accuracy/recovery, precision and limits of detection and quantification were not determined. The reported values should therefore be regarded as quercetin-equivalent content, and a validated, quercetin-specific method such as HPLC-DAD should be used in future work. Third, formulation stability under simulated saliva conditions, compatibility with common mouthwash ingredients (e.g., humectants and co-solvents), and organoleptic comfort were not evaluated, all of which are important parameters for translation from the laboratory to the product. Fourth, as long-term use of antimicrobial mouthwashes may affect microbial ecology, safety testing that takes into account the oral-gut microbiota and the risk of dysbiosis would be valuable, in line with findings that potent antibacterial agents can trigger changes in the composition of the systemic microbiota in certain scenarios (Carvalho et al. 2024; Kim et al. 2021; L et al. 2024).

In addition, the physicochemical characterisation was limited to DLS particle size. Zeta potential, morphology (TEM/SEM), encapsulation efficiency and quantitative storage stability were not measured, so the colloidal stability and homogeneity of the nanoparticles cannot be fully established; moreover, DLS was performed on redispersed dried powder, and particle size may differ from that in the freshly prepared suspension. Finally, all measurements were made on a single batch, with duplicate quercetin determinations, so batch-to-batch reproducibility was not assessed. Future studies should analyse at least three independently prepared batches, each in triplicate.

From a development perspective, future research could draw on lessons from modern nano platforms. In vitro testing that mimics the oral cavity—for example, multispecies biofilms on hydroxyapatite—will enable evaluation of biofilm formation inhibition, mature biofilm disruption capabilities, and effects on glucan and virulence enzyme production. In addition, measuring the kinetics of quercetin release under pH and ionic composition conditions that resemble saliva will help optimise the duration of mouthwash use and frequency of use. Mechanistically, focusing on the disruption of quorum sensing pathways and the inhibition of key biofilm enzymes can provide a causal understanding of how nano-sappan systems work. Previous experimental evidence suggests that quercetin can target virulence factors and cell-to-cell communication, so verification in the oral environment will strengthen the argument. At the translational stage, studies of oral epithelial cell cytotoxicity, mucosal irritation, and small animal testing to assess safety and effects on gingival inflammation will lay the groundwork for clinical trials (Pourhajibagher et al. 2022; Sedighi et al. 2024).

CONCLUSION

This preliminary study shows that chitosan–TPP nanoparticles loaded with sappan wood extract can be prepared by ionic gelation with a mean particle size of 39.39 nm, a PDI of 0.1708 and about 90% of the particle volume below 50 nm, and that the dried nanoparticle powder contains a measurable quercetin content (mean 0.452% w/w by UV–Vis spectrophotometry) with acceptable agreement between duplicate determinations. These findings describe the particle size characteristics and quercetin content of a single batch. They do not demonstrate antibacterial or anti-plaque efficacy, improved solubility or bioavailability of quercetin, long-term stability, or clinical suitability. Further studies are needed, including zeta potential, morphology, encapsulation efficiency and storage stability testing on multiple batches; quercetin quantification by a fully validated, specific method; antibacterial and antibiofilm testing against oral pathogens; and safety evaluation, before the formulation can be considered as an active ingredient in herbal anti-plaque mouthwash.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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