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Evaluation of herbal gel toothpaste formulated using bay leaf essential oil on physicochemical characteristics and extrinsic stain removal

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ABSTRACT

Introduction: Bay (*Syzygium polyanthum*) leaf may be used as an extrinsic stain-removing agent in toothpaste. This study assesses the formula of bay leaf essential oil toothpaste in terms of physicochemical and extrinsic stain removal properties.
Methods: Using a gel composition with varying quantities of bay leaf essential oil (0%, 0.125%, 0.25%, and 0.5% v/v), tubes of toothpaste were formulated. Commercial stain-removal toothpaste was used as a positive control. Five toothpastes were evaluated based on their organoleptic properties, pH, moisture content, foaming ability, abrasiveness, spreadability, gritty matter, and homogeneity. Extrinsic stain removal evaluation was carried out using 20 bovine teeth that were split into five groups of toothpaste (n = 4). The specimens were tea-stained and submitted to simulated brushing. The colour difference (ΔE) was analysed to evaluate extrinsic stain removal using the parameter of CIE-Lab (*Commision Internacional de l'Eclairage L*a*b*). Using a chromameter, the L*a*b value of the teeth before and after brushing was used to measure the ΔE value. Data were analysed through one-way ANOVA with a confidence level of 95%.
Results: All tubes of toothpaste tested met acceptable physicochemical parameter standards. The bay leaf essential oil toothpaste with 0.25% and 0.5% v/v concentrations, as well as commercial toothpaste, produced better ΔE values than the 0% v/v group. There were no statistically significant differences between bay leaf essential oil at 0.5% v/v and commercial toothpaste ($P > 0.05$).
Conclusion: Results demonstrated that bay leaf essential oil toothpaste had an extrinsic stain removal effect equivalent to positive control and promoted good physicochemical characteristics.

Implication for health policy/practice/research/medical education:
Toothpaste with bay leaf essential oil might be a breakthrough in oral hygiene products for teeth whitening by removing surface stains. This study provides new data on the development of bay leaf as an active agent to replace chemical active agents and abrasives in toothpaste with acceptable efficacy.
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Introduction

Tooth discoloration reduces aesthetic appearance and self-confidence leading to a diminished quality of life (1). Discoloration or stain is classified into two groups, namely the intrinsic and extrinsic ones, with extrinsic stain having a higher prevalence of 43.5% (2). Extrinsic stain is caused by chromogens such as tannins in dietary products, including coffee, tea, and wine (3). This type of staining increases the surface roughness of teeth, facilitating debris, plaque, and microbial adhesion, which begins dental and oral problems (4). Its impact can lead to an increase in demand for teeth whitening and stain-removing agents (3).

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Several teeth whitening and stain removal products are currently available on the market most of which use abrasive materials and chemical active agents. However, the use of these products has been associated with different side effects, such as tooth sensitivity, enamel and dentin wear, oral mucosal irritation, and allergic reactions (5). Herbal plants are being used as active agents, because they have good biocompatibility and minimal side effects (6).

The herbal plant bay (*Syzygium polyanthum)* leaf contains essential oil with potential therapeutic effects such as antibacterial and antioxidant activities. This plant grows in many areas of the world, specifically in Southeast Asia, including Indonesia (7). Bay leaf is frequently used as an herb and spice, as well as a medicine to treat diarrhoea. However, studies of the bay leaf in dentistry are limited to its antibacterial properties, using its extract or infusion. There is still no dental product available from the bay leaf as an active agent, regardless of its high availability and potential therapeutic effects. The essential oil of this plant has shown capability as an antimicrobial against *Streptococcus mutans* and high strength antioxidant activity, making it possible to work as an active agent in dentistry products (8). In our previous research, bay leaf extract was used as an active ingredient in toothpaste to eliminate in-vitro tea stains from extracted human teeth and dentures. The positive results demonstrated that bay leaf extract is an excellent agent for removing extrinsic stains from teeth and dentures (9). The physicochemical characteristics of toothpaste have not been studied due to specific limits.

The use of toothpaste includes a certain formulation of materials and a combination of some active agents. This leads to a careful formulation and several evaluations. Therefore, this study aims to establish the best formula for bay leaf essential oil toothpaste and evaluate its physicochemical characteristics compared to commercial ones. It also evaluates the effect of various concentrations of bay leaf essential oil in the toothpaste formula on removing extrinsically stained teeth in vitro.

Materials and Methods

Materials

The effects of four experimental toothpaste formulations comprising 0% (negative control), 0.125%, 0.25%, and 0.5% v/v bay leaf essential oil were examined in this in vitro study. Subsequently, the toothpaste was compared to a positive control (Pepsodent Whitening®, Jakarta, Indonesia) on extrinsic stain removal. Bay leaf was collected from a bay leaf tree in Lendah, Kulon Progo, Yogyakarta, in September 2021. The plant was identified and authenticated by the Faculty of Pharmacy, Universitas Gadjah Mada, at the Pharmaceutical Biology Laboratory as *Syzygium polyanthum* (Wight) Walp., and a specimen was depositedthere (UGM/FA/13993/M/03.02). Bay leaves were pre-treated and underwent hydro-steam distillation to obtain essential oil. Bovine teeth used in this experiment were obtained from Mancasan Abattoir, Yogyakarta, Indonesia. The sample size of bovine teeth was estimated using the formula expressed by Daniel and Cross (10) with an α=0.05. Therefore, the overall number of samples was thought to be 20 (n=4 for each group of toothpaste).

Formulation of toothpaste

Experimental toothpaste was formulated in gel form with different concentrations of bay leaf essential oil using the formula in Table 1. The ingredients were weighed with digital scales and mixed in two separate stages. The first stage was gel formation followed by adding a mixture of oil-based ingredients, namely essential oil, glycerin, and tween 80 to the gel. Carbopol 940 was dispersed in warm aquadest (70°C; 30 mL) in a beaker glass placed on a magnetic stirrer (WiseStir®) set for 300 rpm, 70°C, and 30 minutes. Sodium benzoate dissolved in 5 mL of Aquadest was mixed with dispersed Carbopol 940 followed by the addition of triethanolamine. In a separate dish, tween 80, glycerin, and essential oil were mixed and stirred for 5 minutes, then put together in the gel base to reach homogeneity.

Physicochemical evaluation of toothpaste

The four experimental tubes of toothpaste containing different concentrations of bay leaf essential oil, namely 0% v/v as the negative control, 0.125% v/v, 0.25% v/v, and 0.5% v/v, as well as commercial toothpaste as the positive control, were analysed for physicochemical evaluation

Table 1. Experimental toothpaste formula

Ingredients	Kind of toothpaste			
	0%v/v	0.125%v/v	0.25%v/v	0.5%v/v
Bay leaf essential oil	0	0.125	0.25	0.5
Carbopol 940	1.5	1.5	1.5	1.5
Tween 80	1.5	1.5	1.5	1.5
Glycerin	1	1	1	1
Sodium benzoate	1	1	1	1
Triethanolamine	1.25	1.25	1.25	1.25
Aquades	93.75	93.625	93.5	93.25

following the ISO 11609 indicator. It also included additional indicators (11), such as organoleptic properties, moisture content, foaming ability, spreadability, pH, abrasiveness, gritty matter, and homogeneity.

Ten trained panelists completed laboratory-scale organoleptic tests as part of this investigation. They were chosen based on the following criteria (12): Not in a condition of illness (flu, coughs, mouth ulcers, etc) that would interfere with organoleptic testing and not colour-blind. The test was conducted by distributing a questionnaire to the panellists and asking them about the colour, taste, odour, and consistency of the toothpaste. For the hedonic organoleptic test, panelists were given a questionnaire requesting their likes and dislikes regarding the toothpaste on a scale (1–5): 1 (most despise), 2 (despise), 3 (neutral), 4 (adorable), and 5 (most adorable).

Sample preparation and baseline initial color measurement

The Ethics and Advocacy Unit, Faculty of Dentistry, Universitas Gadjah Mada, granted approval for this research (No. 00778/KKEP/FKG-UGM/EC/2021). A solution of 0.1% thymol (pH 7.0) was used to store all bovine tooth samples during the experiment. The preparation was finalised after the bovine teeth were fixed in dental wax to ensure that the surface was flat enough for colour measurement. The baseline colour of the bovine teeth was determined using a chromameter (Konica Minolta CR-400, Japan) to acquire the CIE-Lab, namely the *Commission Internationale de l'Éclairage* L*a*b colour space, written in L_1, a_1, and b_1. L* denotes luminance, with numbers ranging from 0 to 100 (dark to light). The red-green component is represented by the a* value, where positive values imply red and negative values suggest green. Positive and negative b* are represented by yellow and blue values, respectively.

Extrinsic stain induction

Human unstimulated saliva was applied to bovine teeth for two minutes to activate acquired pellicles and enhance extrinsic stain production (13). Before use, a tea solution containing 2 grammes of tea and 100 mL of hot water was cooled for 10 minutes. Following this, the samples were submerged in a tea solution for a week (7 days), with the solution being replaced daily (9).

Brushing and final color measurement

Each stained sample of the bovine teeth was fixed in the centre of the brushing machine at the Faculty of Engineering, Universitas Gadjah Mada, and set to 200 g of weight with five movements per second for 70s. The Oral-B® extra soft toothbrush was used for each toothpaste application. The brushing duration was conducted according to Annisa et al (9), following the theory of extrinsic stain removal. This involved brushing teeth twice a day for 2 weeks with each session lasting for 3 minutes on average for 72 tooth surfaces. After bovine teeth were stained and brushed, the final colour measurement was conducted using the same protocol as the initial colour measurement to obtain the final L, a*, and b* values (L_2, a_2, b_2).

Color evaluation

The colour difference (ΔE) of bovine teeth before and after the experiment (the initial and final L*, a*, b* colour parameters) was calculated according to the following equation (3):

$$\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

Where:
ΔE: Colour difference of bovine teeth before and after experiment
L_1: Initial luminance (L* value) before experiment
L_2: Final luminance (L* value) after staining and brushing with toothpaste
a_1: Initial a* value before experiment
a_2: Final a* value after staining and brushing with toothpaste
b_1: Initial b* value before experiment
b_2: Final b* value after staining and brushing with toothpaste

Statistical analysis

The stain removal efficiency of five different tubes of toothpaste on stained bovine teeth was measured quantitatively by the value of colour difference (ΔE) and assessed using a one-way ANOVA. After that, we conducted a post hoc LSD with $P = 0.05$ functioning as the statistical significance threshold.

Results

Formulation of bay leaf essential oil toothpaste

Figure 1 shows the results of the formulation of bay leaf essential oil toothpaste (0.0%, 0.125%, 0.25%, and 0.5% v/v) and commercial toothpaste. The formulations were satisfactory in terms of colour, taste, smell, and consistency.

Physicochemical evaluation of toothpaste

Ten trained panelists completed the organoleptic evaluation, utilising descriptive and hedonic tests. The organoleptic characteristics, including the colour, flavour, aroma, and consistency of five tubes of toothpaste employed in this study are detailed in Table 2.

The results of an organoleptic test based on hedonic and descriptive criteria revealed variations among all of the tubes of toothpaste tested. As the concentration of bay leaf essential oil increased, the flavour, colour, and aroma of the toothpaste intensified. Comparing 0.5% v/v essential oil toothpaste to another bay leaf essential oil toothpaste based on hedonic criteria, 0.5% v/v essential oil toothpaste

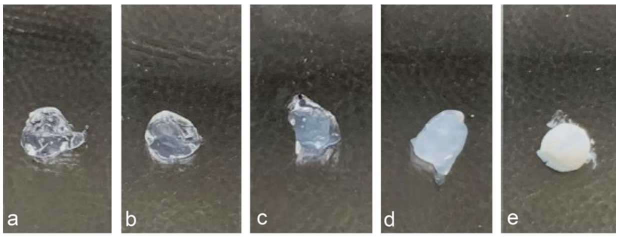

Figure 1. Visualization of (a) 0% v/v, (b) 0.125% v/v, (c) 0.25% v/v, (d) 0.5% v/v bay leaf essential oil, and (e) commercial toothpaste. The color of the toothpaste became increasingly noticeable as the concentration of bay leaf essential oil increased, with the 0.5% v/v bay leaf essential oil toothpaste having the most noticeable color compared to another bay leaf essential oil toothpaste.

had the most favourable colour, flavour, aroma, and consistency ratings. Other physicochemical evaluation results are displayed in Table 3.

Based on the foaming-ability physicochemical parameter, the experimental toothpaste produced less foam than the commercial ones, as illustrated in Figure 2. The lowest foaming ability is obtained with 0.5% v/v bay leaf essential oil toothpaste.

Other physicochemical parameters of gritty matter, such as spreadability, homogeneity, and abrasiveness, showed favourable results. The formula of the experimental toothpaste had no sharp or gritty matter and was also less abrasive than the commercial, as shown in a glass slide under a microscope with 40 times magnification in Figure 3.

Extrinsic stain removal evaluation

Table 4 shows the colour difference (ΔE) of both the bay leaf essential oil experimental and commercial toothpaste. The colour difference shows the extrinsic stain removal ability of the toothpastes.

As the concentration of bay leaf essential oil increased, the colour difference (ΔE) of bovine teeth before and after brushing with toothpaste decreased (Figure 4). The extrinsic stain removal ability of 0.5% v/v bay leaf essential oil toothpaste was approximately two times higher than the negative control and almost as high as the positive control. The colour difference (ΔE) was identified as 6.46±0.44, 6.49±0.43, and 13.51±3.77 for the positive control, 0.5% v/v essential oil toothpaste, and negative control.

According to the statistical analysis, various bay leaf essential oil concentrations influenced the extrinsic stain removal ability of toothpaste ($p=0.009$). Based on the results, the 0.5% v/v bay leaf toothpaste was substantially different from the negative control ($p= 0.002$) but not from the positive control toothpaste ($p= 0.987$) (Figure 4). This suggests that 0.5% v/v bay leaf essential oil toothpaste has the same extrinsic stain removal capability as the commercial one.

Table 2. Result of the organoleptic evaluation of experimental toothpaste and commercial toothpaste

Toothpaste	Criteria							
	Colour		Taste		Smell		Consistency	
	Hedonic	Descriptive	Hedonic	Descriptive	Hedonic	Descriptive	Hedonic	Descriptive
0% v/v Essential oil	2.8±0.42	Transparent	2.7±0.48	No taste	2.8±0.42	No smell	3.8±0.42	Smooth
0.125% v/v Essential oil	2.9±0.57	Transparent	2.7±0.48	A bit sweet	3.2±0.63	Aromatic	3.9±0.32	Smooth
0.25% v/v Essential oil	3.1±0.57	Opaquer	3.1±0.57	Sweet	3.2±0.42	Aromatic	4.1±0.32	Smooth
0.5% v/v Essential oil	4.1±0.32	Most opaque	3.5±0.71	Sweet	4.1±0.32	Aromatic	4.3±0.48	Smooth
Commercial	4.3±0.48	White	4.3±0.48	Mint	4.5±0.53	Menthol	4.4±0.52	Smooth
Standard for descriptive test		Appealing colour		Not bitter		Pleasant smell		Smooth

Table 3. Result of physicochemical evaluation of experimental toothpastes and commercial toothpaste

Toothpaste	pH	Foam (cm)	Moisture content (%)	Abrasiveness	Spreadability (cm)	Gritty matter	Homogeneity
0% v/v essential oil	6	0.7	43.8	Minimum	7.258±0.100	No	Good
0.125% v/v essential oil	6	0.5	44.0	Minimum	8.134±0.122	No	Good
0.25% v/v essential oil	6	0.5	53.0	Minimum	7.086±0.062	No	Good
0.5% v/v essential oil	6	0.4	46.6	Minimum	7.114±0.163	No	Good
Commercial	9	3.0	16.8	More scratch than bay leaf toothpaste	6.434±0.069	No	Good
Standard	pH 5.5-10.5	Max: 15 cm	Gel:35-55%, Pasta max: 39%	Minimum scratch	Max: 8.500	No gritty matter	Good

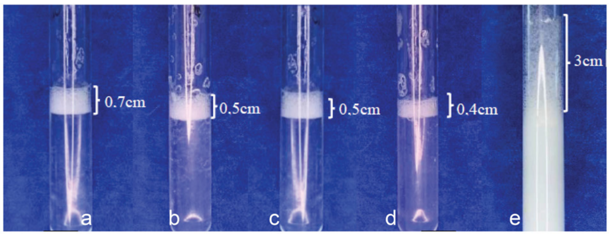

Figure 2. Foam ability of (a) 0% v/v, (b) 0.125% v/v, (c) 0.25% v/v, (d) 0.5% v/v bay leaf essential oil, and (e) commercial toothpaste with the commercial toothpaste having the highest foam compared to others.

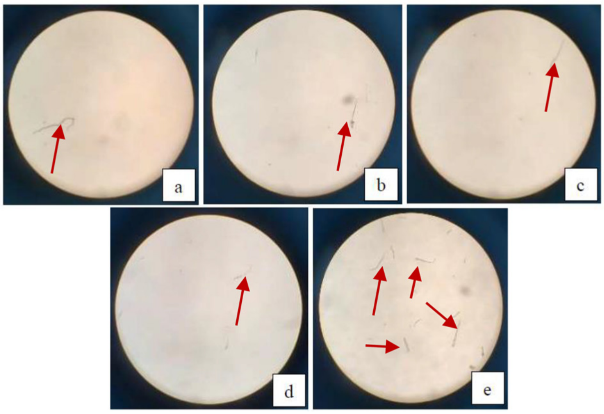

Figure 3. Abrasiveness of (a) 0% v/v, (b) 0.125% v/v, (c) 0.25% v/v, (d) 0.5% v/v bay leaf essential oil, and (e) commercial toothpaste, where the commercial toothpaste causing more scratches on the glass plate (the black lines pointed by red arrows) showed through microscope with 40 times magnification.

Discussion

Toothpaste formulation, including the different concentrations of essential oil and negative control requires several considerations for the types of material used, the percentage of the material, etc. In this formula, Carbopol 940 was selected as a gel base for its ability to form a homogeneously dispersed gel resistant to heat up to 104ºC, bacteria, and fungi. A 1.5% concentration of Carbopol 940 was recommended based on the former study to produce an acceptable gel formula for toothpaste with good viscosity, spreadability, stability, acceptability, and safety (14). Subsequently, triethanolamine was added to the formula as a thickening agent to make the gel consistent and transparent, and neutralise the acidity (14,15). Tween 80 in a 1.5% concentration was used as a surfactant to stabilise oil and water emulsions. This was because it had polar and non-polar groups that combined two dissimilar substances and was an anionic surfactant with low irritation potency (16,17). To maintain the humidity and viscosity of toothpaste, glycerin was used as the humectant due to its antibacterial effect and low toxicity (18). The preservative used was a low cytotoxicity preservative consisting of 1% sodium benzoate (19).

Table 4. Color difference (ΔE) resulted from all toothpaste evaluated (n=4)

Toothpaste	ΔE
0% v/v bay leaf essential oil (negative control)	13.51±3.77
0.125% v/v bay leaf essential oil	10.22±3.94
0.25% v/v bay leaf essential oil	7.27±2.56
0.5% v/v bay leaf essential oil	6.49±0.43
Positive control	6.46±0.44

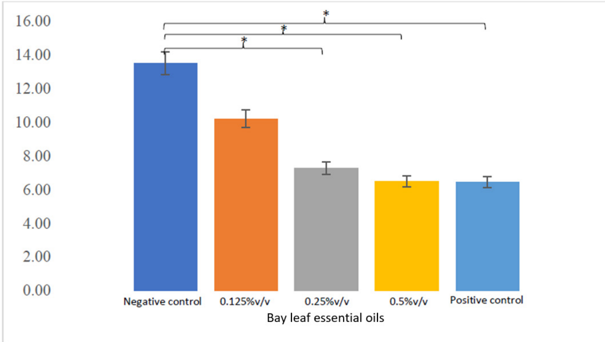

Figure 4. Decreasing of colour difference (ΔE) values of bovine teeth before and after brushing with the negative control, three different bay leaf essential oils (0.125%, 0.25%, and 0.5% v/v), and the positive control toothpastes (*$P<0.05$).

A commercial toothpaste was used for extrinsic stain removal as the positive control toothpaste. The composition of the commercial toothpaste was as follows: abrasive agents consisting of calcium carbonate, hydrated silicate, 0.7% perlite, aluminium oxide, sorbitol as a humectant, sodium lauryl sulphate (SLS) as a surfactant, flavouring, 1.12% sodium monofluorophosphate, gum cellulose thickening agent, sodium saccharine sweetener, potassium citrate, water, DMDM hydantoin preservatives, and the colouring agent (CI 74160 and CI 76891) (20,21).

The results of the physicochemical evaluations of the five toothpastes are shown in Table 2. It was discovered that the organoleptic parameters of the formulated bay leaf essential oil toothpaste were acceptable in terms of colour, taste, smell, and texture, despite the absence of colourant and flavouring. Meanwhile, the requirements of toothpaste based on organoleptic parameters are a clear and appealing colour, satisfying taste and smell, as well as good texture and consistency to be applied (22). According to the acidity (pH), the formulated toothpaste was at pH 6, which was within the acceptable and safe range of 4.5–10.5 (23). The foaming ability of all toothpaste used was within the standard range of less than 15 cm measured (24). Foaming ability is an indicator of the act of surfactant as a 'bridge' for hydrophobic and hydrophilic groups (25). This demonstrated that the bay leaf essential oil toothpaste had good performance without the use of SLS, and its addition did not relieve the surfactant ability.

The moisture content of the five tubes of toothpaste was acceptable to reach a favourable spreadability and viscosity (22). Bay leaf essential oil toothpaste showed less abrasiveness compared to the positive control. This was because the formulated toothpaste did not contain any abrasive agents. Moreover, the side effects of sustainable use of abrasives were reported in many studies, which included email and dentin wear (10). This indicated that bay leaf essential oil toothpaste is safer than those containing abrasives.

The statistical analysis of variance revealed a significant difference (P = 0.009) among the effects of various bay leaf essential oil concentrations on toothpaste's capacity to remove extrinsic stains. The ΔE value represented the colour difference between the initial and final L, a*, b* colour parameters that were measured before staining and after brushing. In this study, the ΔE value was measured before staining and after brushing. A lower ΔE value showed better stain removal performance because there was no considerable difference before and after the experiment (26). This established that 0.5% v/v bay leaf essential oil toothpaste was effective in removing extrinsic stains compared to the positive control. This is evidenced in the mean and standard deviation of ΔE value, namely 6.49±0.43 and 6.46±0.44 for the bay leaf and commercial tubes of toothpaste, which were statistically insignificant between the two tubes of toothpaste on the LSD test ($p > 0.05$). This showed that the removal of extrinsic stains from teeth might be carried out by using bay leaf essential oil. Annisa et al (8), through a phytochemical analysis, discovered that bay leaf essential oil contained a high amount of aldehyde compounds (71.72%) consisting of *cis*-4-decenal, decanal, and nonanal with a high value of antioxidants tested using two methods, namely the DPPH and FRAP tests.

The extrinsic stain was established by the deposition of tannin on tooth pellicles through hydrogen bonds (27). Meanwhile, the high content of aldehyde in bay leaf essential oil inhibits tannin through two mechanisms: (a) The aldehyde in bay leaf essential oil has a suicidal effect. This substance binds to the active site of tannin, then breaks the hydrogen bond between tannin and the pellicles of teeth to remove the extrinsic stain (28), and (b) the high antioxidant activity of aldehyde, which plays an important role as a reductant agent to degrade tannin (29). According to Marquillas et al (29), an antioxidant agent has a visible whitening effect through a reduction reaction. Their study was using sodium metabisulfite (MBS) as an antioxidant agent to whiten teeth stained by tannin. However, compounds with high antioxidant activity made them reductant agents, which reduce, degrade, and make tannin colourless.

A former study also used essential oil as a mouthwash active agent to prevent black stains through its antimicrobial and antioxidant features against oral biofilm bacteria, which support extrinsic stain adhesion (3). Harfouch et al (30) used the essential oil of *Salvia officinalis* as a toothpaste active agent in order to prevent tooth decay. However, there are no previous studies formulating the essential oil of bay leaf as toothpaste and using it as an active agent to remove extrinsic stains. Therefore, the toothpaste formula with 0.5% v/v bay leaf essential oil has the same indistinguishable ability and physicochemical properties as commercial toothpaste to remove extrinsic stains. It also showed a tendency for improvement over commercial toothpaste because bay leaf essential oil toothpaste was not formulated using SLS or abrasive materials. Future investigations into *in vitro* cytotoxicity and *in vivo* evaluation are recommended to bring this toothpaste closer to being used as an alternative extrinsic stain removal method.

Conclusion

Despite its limitations, this study suggested that bay leaf can be beneficial to the dentistry field, especially by using its essential oil as a toothpaste active agent. The results showed that the formula of toothpaste with 0.5% v/v bay leaf essential oil was effective for extrinsic stain removal compared to commercial toothpaste and also obtained acceptable physicochemical characteristics.

Authors' contributions

MA: coming up with ideas, gathering data, contributing to methodology, analyzing and interpreting data, and writing the first draft manuscript. H: Methodology, analysing and interpreting data, study supervision, manuscript review. YBM: Methodology, analyzing and interpreting data, study supervision, and manuscript review.

Conflict of interests

There are no competing interests to declare.

Ethical considerations

The authors have strictly adhered to ethical standards for authorship, data collection, review, and analysis. This research has been approved by the Ethics and Advocation Unit, Faculty of Dentistry, Universitas Gadjah Mada (No. 00778/KKEP/FKG-UGM/EC/2021).

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