

Effectiveness of green tea, bay leaf, and lime peel extracts as toothpastes active agents for extrinsic stain removal on teeth, artificial teeth, and denture base

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ABSTRACT

Introduction: Tea consumption causes extrinsic stain formation on teeth and denture surfaces, affecting personal appearance. Herbal plants extract such as green tea, bay leaf, and lime peel are potentially active agents for extrinsic stain removal. This study aimed to analyse the effectiveness of green tea, bay leaf, and lime peel extracts as toothpaste active agents for extrinsic stain removal on teeth, artificial teeth, and denture base. **Methods:** Twenty-five post-extracted human permanent central incisors, 25 artificial teeth, and 25 heat polymerised acrylic resin denture base (20x20x10mm) were divided into three treatment groups and two control groups. Pictures of all specimens were taken before and after stain induction and after brushing. All specimens were immersed in a tea solution made from 2 grams of tea and 100ml of 100°C boiled water to induce extrinsic stain. The immersion process was repeated every 24 hours for seven days. All specimens were then brushed using an automatic toothbrushing machine for 70 seconds on each surface, 200 grams loads, and five speeds/second. Toothpaste used for brushing were three different active agent toothpaste (treatment groups), no active agent toothpaste, and commercial whitening (control groups). Colour index analysis of the specimen pictures computerised by CIELAB method. The data were analysed by one-way ANOVA ($\alpha=0.05$). **Results:** Green tea, bay leaf, and lime peel extract toothpaste and positive control had higher L-values of extrinsic stain removal than essential toothpaste on all specimens. There was a significant difference between green tea, bay leaf, and lime peel extract toothpaste and negative control toothpaste ($p<0.05$), while no significant difference was found when compared to the positive control toothpaste ($p>0.05$). **Conclusion:** Green tea, bay leaf, and lime peel extract toothpaste are effective as extrinsic stain removal on teeth and acrylic resin denture base because of abrasive materials that work together with the active agents such as epigallocatechin-3-gallate (EGCG) in green tea, eugenol in bay leaf, and naringin in lime peel.

Keywords: herbal extract; toothpaste; extrinsic stain; teeth; denture

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INTRODUCTION

High quantities of tea consumption habit unwittingly lead people to experience extrinsic stain formation.^{1,2} Extrinsic staining or discolouration formed outside teeth or materials resulting from chromogen from foods and beverages. Tannin from tea solution is an example of an organic chromogen.³ Extrinsic teeth staining occurs due to the colour substance that adheres directly to the outer surface of tooth enamel or is deposited into plaque or pellicle on the tooth surface.² The discolouration also happened on the denture surface, both on the artificial teeth and denture base, mainly acrylic resin denture, primarily used for denture material.⁴ Meanwhile, some acrylic resin material polymerisation failures can lead to micro-porosities formation. These micro-porosities enable chromogen from foods and beverages to enter the acrylic resin and further cause extrinsic stain.⁵ Formation of extrinsic stains on teeth and dentures reduces aesthetical appearance, affecting personal physical attractiveness and confidence. Discolouration is critical for many individuals to perform an aesthetic smile. A recent study stated that tooth colour was the main reason for appearance dissatisfaction.⁶ Another impact of extrinsic stain on dentures is reducing the colour stability of the denture, which affects material durability and facilitates microorganism adhesion.⁷

In the past decade, the demand for aesthetic and harmful impact prevention of extrinsic stains increased the requirement for extrinsic stain removal and/or whitening products. Various types of teeth and denture cleansers have been commercially on the market; some are sold as over the counter products without professional involvement in their application. Products available are varied, such as toothpaste, tablet, and liquid as teeth and denture cleansers.⁸ Previous research suggested that toothpaste brushing is the best-known method to control biofilm compared to other mechanical and/or chemical methods. It is the most common daily home oral care product to maintain dental and gingival health. The toothpaste must give maximum cleaning with minimum side effects for oral tissues, but some toothpaste ingredients

can harm dental and oral tissues, such as tooth wear, discomfort feeling, or the more severe and cytotoxic effect.^{8,9}

Toothpaste is also commonly used as a denture cleanser because it is considered simple, inexpensive, and effective.⁷ However, when this method is incorrectly performed, it will increase the surface roughness of acrylic resin denture and later facilitate microorganisms, organic matter, and stain attachment.⁵ Another method to clean a denture is immersion in denture cleansers containing active chemical agents such as alkaline peroxides, alkaline hypochlorite, acids, enzymes, and disinfectants. These denture cleansers provide antibacterial and antifungal properties. Meanwhile, using these cleansers may cause changes in the physical and mechanical properties of acrylic resin dentures because of these chemical active agents' penetration into the acrylic resin.⁸

The utilisation of herbal or natural ingredients as medicines and alternatives to chemical ingredients has developed over the past three decades because it has potential therapeutic effects, such as antibacterial, antifungal, and many more.¹⁰ These natural remedies are widely available around us, which can be obtained from various kinds of plants, but many of them are currently untested, and their use is either less observed or has not been observed before.¹¹

Green tea (*C. sinensis*), is a prevalent plant in the world. The most important compound of green tea is catechin polyphenols, namely epigallocatechin-3-gallate (EGCG), the most active compound that reaches 50-80% of the total catechin. EGCG inhibits the biological activity of bacteria, such as *S. mutans* and *S. sobrinus*. This compound prevents oral microbial growth and helps to maintain good oral hygiene. These properties made green tea potentially used as an oral hygiene product.¹² Bay plants (*Eugenia polyantha* Wight) are herbal plants that are usually purposed as cooking spices. Bay plants can be found in markets, spice shops or grow freely in yards or gardens. The chemical contents of the bay leaf include tannins, flavonoids, and essential oil. Eugenol in bay leaf is an essential oil component that can inhibit bacterial growth through protein denaturation because it is one of the phenol groups with aromatic hydrocarbons-eugenol.¹³

It can also penetrate the micro-porosity space of acrylic resin and damage the carbonyl chain, releasing the chromogen bond with acrylic resin material.¹⁴ Lime (*Citrus aurantifolia*) is one type of citrus. Apart from the flesh and fruit rich in vitamin C, lime peel also has many properties but is often thrown away as waste. The content in lime peel provides a therapeutic effect which is the dominant active compound is naringin.¹⁵ Lime peel is a potential material as an antimicrobial agent because it can produce essential oil, which has inhibitory activity against the growth of *S. aureus*; thus, naringin content is applicable as an oral cleanser.¹⁶

Due to the demand for aesthetic with the maximum cleaning ability of oral products and safer effect, added to less observed and poorly untested herbal plants as potential oral products, therefore, this study aimed to analyse the effectiveness of green tea (*C. sinensis*), bay leaf (*E. polyantha* Wight), and lime (*C. aurantifolia*) peel extracts as toothpaste active agents for extrinsic stain removal on teeth, artificial teeth, and denture base.

METHODS

Groups and treatment

This study was quasi-experimental conducted on 25 post-extraction human permanent central incisors, 25 artificial teeth (YUWEI™, A3 Shade), and 25 heat polymerised acrylic resin denture base (20x20x10 mm), which were divided into three treatment groups and two control groups. The specimens will be brushed using three kinds of toothpaste in the treatment groups with three different active agents: green tea extract, bay leaf extract, and lime peel extract. The control groups used were toothpaste without an active agent as the negative control group and commercial stain-removal toothpaste (Pepsodent™ Whitening) as the positive control group. Each group's specimens were five teeth, five artificial teeth, and five acrylic resin denture bases.

Herbal plants collection and determination

Green tea was obtained in dry leaf dosage from Bandung, West Java. The fresh leaves of the bay plant were taken from the upper third of the stem and originated from Bantul, Special

Region of Yogyakarta, and lime was taken from Sleman, Special Region of Yogyakarta. These herbs have been authenticated as *C. sinensis*, *E. polyantha* Wight, and *C. aurantifolia* species by the Department of Biological Pharmacy, Faculty of Pharmacy Gadjah Mada University.

Extraction of green tea, bay leaf, and lime peel

These plants have their initial weights of 1000 grams green tea leaves, 1500 grams bay leaves, and 3000 grams lime peels, which were extracted by the maceration method. First, each plant was washed under running water and then dried in a 45°C drying cabinet for 48 hours. Then, it was cut into small pieces and mashed using a blender until it became powder. Next, they were immersed in 70% ethanol solvent for 3x24 hours at room temperature.¹⁷ Then, the evaporation process was carried out by vacuum distillation, followed by the thickening process with a rotary evaporator at the temperature of 60-70°C, then using a water bath until the thick extract was obtained as much as 100g with 100% extract concentration.¹⁸ The product of the process were three herbal plant extracts with brown colour, thick consistency, and a distinctive smell.

Herbal extract toothpaste formulation

Extracts of green tea, bay leaf, and lime peel were diluted to a concentration of 10% and used as active agents in three different treatment toothpaste. As a result, the toothpaste formulation followed the composition shown in Table 1.

Calcium carbonate, sodium lauryl sulfate, gum arabicum, saccharine, glycerine, and carmine were weighed, taken, mixed in aquadest then added slowly into the mortar containing each herbal extract. Then, each solution was triturated well until a paste consistency was formed.¹⁹ Negative control (essential toothpaste) was also made from toothpaste composition shown in Table 1 but without herbal extracts as the active agent.

Control and treatment groups were 25 extracted human permanent central incisors, 25 artificial teeth, 25 acrylic resin denture base plates brushed with essential toothpaste without an active agent as the negative control, three different herbal extracts toothpaste, and treatment groups, and commercial whitening toothpaste as the positive control.

Table 1. Composition of herbal extract toothpaste

No	Ingredients	Concentration (gram)			
		Negative control basic toothpaste	Green tea toothpaste	Bay leaf toothpaste	Lime peel toothpaste
1	Calcium carbonate	50	50	50	50
2	Sodium lauryl sulfate	2.5	2.5	2.5	2.5
3	Glycerine	20	20	20	20
4	Gum arabicum	1	1	1	1
5	Saccharine	0.1	0.1	0.1	0.1
6	Carmin	0.25	0.25	0.25	0.25
7	Green tea extract	0	10	0	0
8	Bay leaf extract	0	0	10	0
9	Lime peel extract	0	0	0	10
10	Aquadest	q.s	q.s	q.s	q.s

Specimens' preparation

The specimens of the treatment and control group (25 post-extraction human permanent central incisors, 25 artificial teeth, and 25 heat polymerised acrylic resin denture base) were attached to dental wax on a tray, so their positions are static from the beginning to the end of the experiment.

Documentation of specimens

There are three times of specimen pictures taken; pre-treatment, after extrinsic stain induction, and after brushing. These pictures were taken from a static position, with a specimen to lens distance was 1.5 cm for all treatment and control groups. The lighting used was 5 watt lamps (Philips®) from 3 sides (right, middle, and left), with a 1 mm blue filter.²⁰ Pictures were taken using a CCD camera with an 8 mm lens, shutter speed 8, aperture 8f, ISO 200.²¹

Extrinsic stain induction

All specimens were induced by extrinsic stain, which was done by immersing them in a tea solution made from 2 grams of black tea powder in a pouch within 100 ml of 100°C boiled water. The tea solution was waited 10 minutes before immersion. After that, the immersion process was carried out for seven days and changed every 24 hours.²² After the stain induction process, pictures of all specimens were taken.

Brushing with treatment and control toothpaste

Later, the specimens were brushed using treatment groups' toothpaste and control groups'

toothpaste. The toothpaste used was then diluted in distilled water at the ratio of 1:3 by weight. The dilution was carried out to allow the toothpaste to be injected into the automatic toothbrushing machine. The brushing process used an automatic toothbrushing machine at the weight of 200 grams and five motions/second speed.²⁰ Toothbrush (Oral-B® Extra Soft) was attached to the machine. The brushing process was performed for 70 seconds on each surface, equivalent to the proper recommended toothbrushing frequency for extrinsic stain removal, which was twice a day, 2-3 minutes for 14 days.²³ After the specimens had been brushed, the brushing documentations were taken.

Extrinsic stain removal evaluation and data analysis

The procedure was followed by colour index analysis using the CIELAB method. The CIELAB method was made by the international agency CIE (Vienna, Austria) in order to produce a colour standard of different equipment. This method later been computerised in the ImageJ® software (University of Wisconsin, USA); originally, the RGB (Red Green Blue) image was changed into the LAB image to get the colour separated into L (lightness) values (black and white difference), a* and b* (the chromaticity coordinate which indicates the colour intensity) from the pre-treatment, after induction and after brushing documentation.^{24,25}

This study only used the L-value because it shows a black and white difference, which will be able to determine the tendency of extrinsic stain formation to extrinsic stain removal. The

L-value of extrinsic stain removal was obtained by determining the difference between the L-value after extrinsic stain induction and after brushing with treatment and control toothpaste. The L-value of extrinsic stain removal was then analysed by a one-way ANOVA parametric test followed by the post-hoc LSD test with a 95% confidence level. Priorly, this study has been ethically approved by the Ethics and Advocacy Unit of the Faculty of Dentistry Gadjah Mada University with the approval number of No.00799/KKEP/FKG-UGM/ EC/2016.

RESULTS

The CIELAB method colour analysis with the ImageJ program was used to obtain the L-value of extrinsic stain removal based on the difference in picture after extrinsic stain induction and after brushing with toothpaste on the teeth, artificial teeth, and acrylic resin denture base. The mean and standard deviation of the extrinsic stain removal L-value by essential toothpaste, treatment toothpaste (green tea, bay leaf, lime peel extract), and commercial toothpaste is presented in Table 2.

Table 2. The mean and standard deviation of extrinsic stain removal L-values by toothpaste on the specimen surfaces

	Negative control	Treatment toothpaste			Positive control
	Basic	Green tea	Bay leaf	Lime peel	Commercial
Teeth	1.818±0.675	5.046±1.491	6.200±1.673	8.491±0.528	8.072±1.729
Artificial teeth	2.211±1.494	5.593±0.848	12.694±1.578	9.163±0.772	11.594±3.016
Acrylic resin denture base	1.211±0.571	6.814±0.693	5.817±2.441	5.150±1.434	8.873±2.438

Table 2 shows that the three groups of treatment toothpaste (green tea, bay leaf, and lime peel extracts) have a higher extrinsic stain removal L-value than the essential toothpaste. In addition, the Kolmogorov-Smirnov normality test and Levene's homogeneity test were performed

and resulting in a p-value > 0.05. Thus, the data were normally distributed and showed homoscedasticity of variance; therefore, the one-way ANOVA parametric test can be continued. The results of the one-way ANOVA test with $\alpha=0.05$ are presented in Table 3.

Table 3. One-way ANOVA results of the L-value of extrinsic stain removal by toothpaste on the specimens

	Sum of squares	df	Mean square	F	Sig
Toothpastes and specimens	1441.478	2	720.739	11.932	0.000
L-value of extrinsic stain removal	2538.859	42	60.449		
Total	3980.337	44			

The results in Table 3 showed a significant difference in the extrinsic stain removal L-value between groups on all specimens ($p<0.05$). The result shows an effect of all tested toothpaste on extrinsic stain removal on the specimens. After

conducting the one-way ANOVA test, the LSD test was conducted to determine the effectiveness of treatment toothpaste by comparing treatment toothpaste and control toothpaste. The LSD test results summary is presented in Table 4.

Table 4. The LSD test results on the treatment and positive control toothpaste

Toothpaste type	Toothpaste comparison	Specimens	p-value	Interpretation
Green tea	Positive control	Teeth	0.122	No difference
Green tea	Positive control	Artificial teeth	0.510	No difference
Green tea	Positive control	Acrylic resin denture base	0.718	No difference
Bay leaf	Positive control	Teeth	0.539	No difference
Bay leaf	Positive control	Artificial teeth	0.471	No difference
Bay leaf	Positive control	Acrylic resin denture base	0.613	No difference
Lime peel	Positive control	Teeth	0.159	No difference
Lime peel	Positive control	Artificial teeth	0.419	No difference
Lime peel	Positive control	Acrylic resin denture base	0.473	No difference

LSD test result (Table 4) confirmed no significant difference when treatment toothpaste (green tea, bay leaf, and lime peel extract toothpaste) compared to positive control toothpaste, which means that all of the treatment toothpaste had a similar ability as the positive control toothpaste in removing the extrinsic stain on all specimen surfaces ($p>0.05$). It proves that all treatment toothpaste effectively removed the extrinsic stains on teeth, artificial teeth, and acrylic resin denture bases.

DISCUSSION

Extrinsic staining on teeth occurs due to the negative charge of stain or chromogen molecules, which tend to bind directly to positive ions, such as the enamel's calcium (Ca^{2+}) ions.² Pellicle on the tooth surface also facilitates chromogen attachment. Concurrent with bad oral hygiene conditions and unable to remove chromogen, the extrinsic stain is formed and will become more severe.³ Tannin, an example of chromogen from tea, has double bound molecules, such as carbonyl or aromatic bound. This kind of chromogen has a high affinity to protein to bind to the pellicle molecules. Tannin absorbs visible light and does colour reflection that is detected as a yellow, brownish stain.² Formation of extrinsic stain on artificial teeth and acrylic resin denture base is mainly caused by tannin attached to the polar compound of polymethyl methacrylate acrylic resin. Factors that accommodate tannin attachment to polymethyl methacrylate are biofilm formation on the denture, micro-porosity, and increasing acrylic resin surface roughness.^{4,26} The attachment of this chromogen is supported by oral hygiene, especially in conditions with plaque, biofilm, and calculus. The high affinity to protein exhibited in tannin enables this chromogen to penetrate the plaque and biofilm.² The presence of calculus supports the formation of extrinsic stains by which the calculus incorporates tannin into the calcium phosphate lattice.^{2,27}

One-way ANOVA test result showed a significant effect of green tea, bay leaf, and lime peel extract toothpaste on removing the extrinsic stain on teeth, artificial teeth, and acrylic resin denture bases. It was shown by the significant difference between types of toothpaste used in

all three groups of specimens to the L-value of extrinsic stain removal (Table 3). This significant difference is due to the different abrasives and active agents used in each toothpaste. Essential toothpaste, as the negative control group, was made by formulations from calcium carbonate, glycerol, SLS, carmine, saccharin, sodium benzoate, and aquadest.¹⁹ There were no active compounds added to essential toothpaste. The extrinsic stain removal of essential toothpaste comes from the abrasive material of calcium carbonate.²⁸ This finding aligned with the previous study comparing stain removal ability between calcium carbonate-containing toothpaste and no calcium carbonate toothpaste. A better removal is seen in the calcium carbonate toothpaste group.²⁹

The treatment toothpaste were made with the same formulation as essential toothpaste but with the additions of the epigallocatechin-3-gallate (EGCG) active agent from green tea extract, eugenol from bay leaf extract, and naringin from lime peel extract. These kinds of toothpaste gave a higher extrinsic stain removal L-value than essential toothpaste (Table 2). The addition of active herbal agents to essential toothpaste formulation can remove extrinsic stains better. This result follows previous research that found that adding active herbal ingredients such as plant extract increases toothpaste's ability to clean stains compared to no herbal active agent toothpaste.³⁰ As a comparison, the extrinsic stain removal commercial toothpaste as the positive control group, has the composition of calcium carbonate, sorbitol, water, sodium lauryl sulfate (SLS), sodium silica, hydrated silicate, flavourings, 1.12% sodium mono- fluoro-phosphate, 0.7% perlite, aluminium oxide, cellulose gum, sodium saccharin, potassium citrate, SMSM hydantoin, CI 74160, CI 77891. The abrasive agents in positive control toothpaste are calcium carbonate, sodium silica, and aluminium oxide. In contrast, the active agent is perlite which functions as a tooth whitening agent.³¹ Wang et al.³² discovered that the combination of perlite and abrasives in toothpaste not only increases the pellicle cleaning ratio but also increases the relative dentin abrasivity (RDA) because of the particles size and abrasive material hardness. According to the current research, the extrinsic stain removal by positive control toothpaste that contains perlite and three abrasives agents showed

good cleansing, determined from the extrinsic stain removal L-value in Table 2.

The LSD test result shows no significant difference between extrinsic stain removal on teeth, artificial teeth, and acrylic resin denture base by treatment toothpaste compared to the positive control toothpaste ($p > 0.05$). When the treatment toothpaste and positive control toothpaste were compared to negative control essential toothpaste, there was a significant difference ($p < 0.05$). This result shows that the treatment toothpaste has the same effectiveness as the positive control toothpaste as extrinsic stain removal on teeth, artificial teeth, and acrylic resin denture base, and tend to be safer than positive control by considering the abrasive material and active agent used in formulation these kinds of toothpaste. Treatment toothpaste can remove the extrinsic stains as effectively as positive control toothpaste did, even though the treatment toothpaste only contains calcium carbonate with a three Mohs hardness scale and the natural active agent from the extracts.³³ In contrast, the positive control toothpaste contains three abrasive materials with a total of 18-19 Mohs hardness scale, and the active agent used is an active agent chemical such as perlite that can increase surface roughness and tooth wear.^{31,32} This finding is supported by a previous study comparing the effectiveness and abrasiveness of brushing material with herbal toothpaste without abrasives and abrasives containing toothpaste. Both kinds of toothpaste showed a similar cleansing ability, but the herbal toothpaste gave minimum surface roughness assessed by profilometer.³⁴

The extrinsic stain removal process is performed mechanically and chemically. The abrasives in toothpaste combined with toothbrushing remove extrinsic stains from teeth and dentures mechanically. This mechanical action helps remove pellicle, plaque, and biofilm, facilitating extrinsic stain attachment to teeth and dentures.^{2,25} The chemical mechanism of extrinsic stain removal performed by the three active agents of treatment toothpaste such as (epigallocatechin-3-gallate) EGCG in green tea, eugenol in bay leaf, and naringin in the lime peel. In the extrinsic stain removal process, EGCG, eugenol, and naringin are phenolic compounds that act as hydrogen donors that release H^+ ions.³⁵⁻³⁷ The hydrogen ion

will be drawn to the hydroxyapatite bound, and tannin generates electron conjugation and alters the complex bound of hydroxyapatite and tannin to form a more uncomplicated bound, decreasing the tannin as a causal pigment of discolouration.³⁸ Another supporting theory states that negative ion of tannin in tea solution binds to positive ion in hydroxyapatite such as Ca^{2+} . Then, the negative ion of tannin will be replaced by negative ions in the active agents of the three treatment toothpaste through a substitution reaction, resulting in extrinsic stain removal. The ability to perform substitution reactions might be affected by the degree of acidity of compounds.³⁵ The degree of acidity of EGCG, eugenol, and naringin is lower than tannin.^{39,40,41} They both, including tannin, are phenolic compounds, and the lower the degree of acidity, the stronger the ability to attain stability with another ion such as hydroxyapatite ion.^{35,36,37} These theories explain possible mechanisms of how extrinsic stains are removed chemically by treatment with an active toothpaste agent.

The removal of extrinsic stain from artificial teeth and acrylic resin denture base occurred chemically by the polar compound in the active agents of the three treatment toothpaste that bind to polymethyl methacrylate of acrylic resin.⁴¹ The presence of gaps between molecules in the inter-polymer chain of acrylic resins causes the polar compounds of the active agents to enter and form bonds with the acrylic resin, thus untying the tannins from the acrylic resin. The bond between acrylic resin and the polar compound of these active agents occurs due to electrostatic forces.⁴²

The present study did not perform physicochemical evaluations such as organoleptic parameters, degree of acidity (pH), and other physicochemical properties of treatment toothpaste. Besides, the extraction of the herbal plants was raw only. Therefore, there was no chromatography test performed. Nevertheless, this present study gives novel knowledge that herbal extracts (green tea, bay leaf, and lime peel) were effective as extrinsic stain removal on teeth and dentures, which can be considered herbal active agent toothpaste alternative to chemical active agent commercial toothpaste. Further research is needed regarding the physicochemical evaluation of the toothpaste, chromatography test of the extract used to determine the exact active agent,

and the side effects of using green tea, bay leaf, and lime peel extract toothpaste on living tissues.

CONCLUSION

Green tea, bay leaf, and lime peel extract toothpaste are effective for extrinsic stain removal on teeth and acrylic resin dentures because of abrasive materials that work together with the active agents such as epigallocatechin-3-gallate (EGCG) in green tea, eugenol in bay leaf, and naringin in lime peel.

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