EFFECTIVITY OF MULTIFUNCTION HERBAL TOOTHPASTE CONTAINING BAY LEAF (*EUGENIA POLYANTHA WIGHT)* EXTRACT AS EXTRINSIC STAIN REMOVAL ON TEETH AND DENTURE

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ABSTRACT

Background: *White teeth colour influence aesthetical appearance which leads to self-confidence and quality of life. Extrinsic stain is the most common aetiology of teeth discoloration, which caused by widely consumed products such as tea, coffee, and tobacco. It also formed on denture and cause negative impacts. Commercial extrinsic stain removal toothpastes only indicated for teeth and contain chemical active agent with undesirable effects. Aim of this study is to determine effectivity of bay leaf (Eugenia polyantha Wight) extract as toothpaste active agent for extrinsic stain removal on teeth and dentures.*
Method: *Pictures of teeth, artificial teeth, and acrylic resin base taken before experiment, after stain induction (using 25g of tea in 100ml of 100^{o}C water for 7 days, tea solution replaced every 24 hours), and after brushing. Toothpastes used for brushing were no active agent basic formulation toothpaste, bay leaf extract toothpastes at 5%, 10%, 15% concentrations, and commercial stain removal toothpaste as positive control. Specimens were brushed using automatic brushing machine for 70 seconds/surface, using 250g load, and speed 5 movements/sec. Colour index analysis of specimens' pictures were computerized and analysed using CIELAB method to obtain L-value of extrinsic stain removal. Data analysis performed using one-way ANOVA (α=0.05).*
Result: *There is effect of toothpaste containing bay leaf extract 15% to remove extrinsic stain both on teeth, artificial teeth, and acrylic resin base according to one-way ANOVA.*
Conclusion: *Bay leaf extract is effective and potential to be used as toothpaste active agent for extrinsic stain removal on teeth and denture.*

INTRODUCTION

Tooth colour gives a great impact to general appearance. White, clean, and bright teeth bring satisfaction and positive psychological effect. Survey was carried out to subjects aged 20-30 years old at several public health services, and stated that 95% of subjects declared white and clean teeth increase self-confidence[1]. This was in line with another survey stated that teeth colour is the main reason of people satisfaction to their appearance[2].

Substantiality of tooth colour causes tooth discoloration becomes clinical and aesthetical problems which leads in reducing quality of life[3]. Nevertheless, teeth are susceptible to discoloration, either intrinsic or extrinsic discoloration. Between those two types of discoloration, extrinsic discoloration is the most common case found in clinical practice[4]. It is caused by stain formed outside the teeth, so it is also called extrinsic stain, which become majority cause of teeth discoloration because the aetiology is chromogen or colour

substance from favourite products consumed by almost every people, such as tea, coffee, wine, and tobacco[5]. These products usually contain chromogen, such as tannin that adheres directly to the outer surface of tooth enamel or deposited into plaque or pellicle on tooth surface[6]. Extrinsic discoloration also happened on denture surface, both on the artificial teeth and denture base, especially acrylic resin denture which is mostly used for denture material[7]. The chromogen attached to denture surface through denture biofilm and/or microporosities of the denture[8,9].

This problem of discoloration, typically extrinsic discoloration on teeth and denture makes the requirement of extrinsic stain removal and/or whitening products increasing dramatically in the past decade. Various types of teeth and denture cleansers to remove extrinsic stain have been commercially on market, some of them are sold as 'over the counter products' without professional involvement in their application. Products available are vary, such as toothpaste, tablet, and liquid as teeth and denture cleansers[10]. Studies stated that toothpaste brushing is the best-known method to control biofilm compare to other mechanical and/or chemical methods. It is the most common daily home oral care product to maintain dental and gingival health. Toothpastes have to give maximum cleaning with minimum side effect, but some toothpaste chemical ingredients can harm dental and oral tissues, such as tooth wear, discomfort feeling, or the more severe, cytotoxic effect[10,11]. Toothpaste brushing is also commonly used to clean denture because it is considered as a simple, inexpensive, and effective method.[11] However, when this method is incorrectly performed, it will increase surface roughness of acrylic resin denture, later facilitates microorganism, organic matter, and stain attachment.[8] Another method to clean a denture is immersion in denture cleansers containing chemical active agents such as alkaline peroxides, alkaline hypochlorite, acids, enzymes, and disinfectants. These denture cleansers provide antibacterial and antifungal properties. Meanwhile, consequence of using these chemical cleansers is they may cause changes in physical and mechanical properties of acrylic resin denture because of these chemical ingredients' penetration into acrylic resin.[10]

The utilization of herbal or natural ingredients as medicines and alternative of chemical ingredients has developed over the past three decades because it has potential therapeutic effects, such as antibacterial, antifungal, and etc.[12] These herbs are widely available around us, which can be obtained from various kinds of plants, but many of them currently untested and their use are either less observed or have not been observed before.[13] Bay leaf *(Eugenia polyantha Wight)* is an example of herbal plant which usually purposed as cooking spices. It is easily found in markets, spice shops, or grow freely in yards or gardens[14]. Even tough, bay leaf contains chemical contents with antibacterial and antioxidant effects, such as eugenol and citral[15,16,17]. 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[18]. It can also penetrate into the micro-porosity space of acrylic resin and damaging the carbonyl chain thereby releasing the chromogen bond with acrylic resin material[19]. Several researches conducted to determine the use of bay leaf in dentistry. Ramadhania[20] stated that various concentrations of bay leaf extract (5%, 10%, 15%, and 20%) are able to inhibit *Streptococcus sp* because of bay leaf chemical contents. Five percent, 10%, and 20% bay leaf infusion concentration were capable to inhibit bacterial and fungal growth, such as *Candida*

albicans[21]. It was implied that bay leaf is potential to be used for oral care products including denture cleanser[22].

Due to high demand of extrinsic stain removal to eliminate extrinsic stain on teeth and denture as good as giving pleasant appearance, leads bay leaf to become a potential choice for toothpaste active agent that provides maximum cleaning ability and safer effect. The aim of this study is to determine effectivity of bay leaf *(Eugenia polyantha Wight)* extract as toothpaste active agent for extrinsic stain removal on teeth and denture.

METHODS

Ethical aspect

This quasi-experimental study has been ethically approved by Ethics and Advocacy Unit, Faculty of Dentistry, Universitas Gadjah Mada (No.00799/KKEP/FKG-UGM/EC/2016).

Groups and treatment

The specimens of this study were 25 post-extraction human permanent central incisors, 25 artificial teeth (YUWEI™, A3 Shade), and 25 heat polymerization acrylic resin denture base (20x20x10 mm), which were divided into 3 experimental groups and 2 control groups. On the experimental groups, the specimens will be brushed using 3 bay leaf extract toothpastes (5%, 10%, and 15% concentration). The control groups used were toothpaste without active agent as negative control toothpaste and commercial stain-removal toothpaste (Pepsodent™ Whitening) as positive control toothpaste. The number of specimens for each group were 5 teeth, 5 artificial teeth, and 5 acrylic resin denture bases.

Bay leaf collection and determination

The fresh leaves of the bay plant were taken from the upper third of the stem originated from Bantul, Daerah Istimewa Yogyakarta. This herb has been authenticated as *Eugenia polyantha Wight* species by Biology Pharmacy Department, Faculty of Pharmacy, Universitas Gadjah Mada.

Extraction of bay leaf

Bay leaf with initial weight of 1500grams were extracted by maceration method. These leaves were washed under running water, then dried in a 45^0C drying cabinet for 48 hours. Then, it was cut into small pieces and mashed using a blender until became powder. Next, immersed in 70% ethanol solvent for 3x24 hours at room temperature[23]. Then evaporation process was carried out by vacuum distillation, followed by the thickening process with rotary evaporator in the temperature of 60^0C-70^0C, then using waterbath until the thick extract was obtained as much as 100g with 100% extract concentration[24]. The product of the process were three herbal plant extracts with brown colour, thick consistency, and a distinctive smell.

Toothpaste formulation

Bay leaf extract was formulated as active agent for three different experimental toothpastes with various concentration (5%, 10%, and 15% bay leaf extract content). Negative control toothpaste was also formulated using same formula as bay leaf extract toothpaste, but without active agent. Formulation of toothpastes was following the composition by Akotakar[25], which was shown in table 1.

Table 1. Composition of toothpastes

Ingredients	Weight (gram/100grams)			
	F1	F2	F3	F4 (Negative control)
Calcium carbonate	50	50	50	50
Sodium lauryl sulphate	2.5	2.5	2.5	2.5
Glycerine	20	20	20	20
Gum arabicum	1	1	1	1
Saccharine	0.1	0.1	0.1	0.1
Carmine	0.25	0.25	0.25	0.25
Bay leaf extract	5	10	15	0
Aquadest	21.15	16.15	11.15	26.15

Calcium carbonate, sodium lauryl sulphate, gum arabicum, saccharine, glycerine, and carmine were weighed and taken in mortar. Next, they were mixed with aquadest and added slowly into mortar containing bay leaf extract with three different weights to obtain 5%, 10%, and 15% bay leaf extract concentration. Then, each solution was triturated well until a paste consistency is formed[25]. Negative control toothpaste was made using same steps, but without addition of bay leaf extract.

Specimen preparation

The specimens of experimental and control groups (25 post-extraction human permanent central incisors, 25 artificial teeth, and 25 heat polymerization 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.

Pictures of specimens taking

There are three times of specimen pictures taking; pre-treatment, after extrinsic stain induction, and after brushing. These pictures taken from a static position, with specimen to lens distance was 1.5cm for all treatment and control groups. Lighting used was 5watt lamps (Philips®) from 3 sides (right, middle, and left), with 1mm blue filter[26]. Pictures were taken using CCD camera with 8mm lens, shutter speed 8, aperture 8f, ISO 200[27].

Extrinsic stain induction

All specimens were induced by extrinsic stain which was done by immersing them in tea solution made from 2 grams of black tea powder in pouch within 100ml of 100^0C boiled water. The tea solution was waited 10 minutes before immersion. The immersion process was done for 7 days and changed every 24 hours[28]. After stain induction process, the pictures of all specimens were taken.

Brushing with experimental and control toothpastes

Later, the specimens were brushed using experimental groups toothpastes and control groups toothpastes. The toothpastes used were diluted in distilled water at a ratio of 1:3 by weight. The dilution was done in order to allow the toothpastes injected into the automatic toothbrushing machine. Brushing process used an automatic toothbrushing machine at the weight of 250grams, and 5motions/second speed[1]. Toothbrush (Oral-B® Extra Soft) was attached to the machine. The brushing process was done for 70 seconds on each surface, which was equivalent to proper recommended tooth brushing frequency for extrinsic stain removal: twice a day, with a duration of 2-3 minutes for 14 days[29]. After the specimens have been brushed, after brushing pictures were taken.

Extrinsic stain removal evaluation and data analysis

The procedure followed by colour index analysis using CIELAB method. The CIELAB method is a method made by the International Agency CIE to produce a colour standard of different equipment. This method has been computerized in the Image-J program, where originally the RGB *(Red Green Blue)* image 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 picture[30,31]. This study only uses the L-value because it shows a black and white differences, namely the tendency of extrinsic stain formation to extrinsic stain removal. Then the L-value of extrinsic stain removal is obtained by finding out the difference of L-value after extrinsic stain induction and L-value after brushing with experimental and control toothpastes. The L-value of extrinsic stain removal is analysed by One-way ANOVA parametric test followed by the Post-hoc LSD test with a 95% of confidence level.

RESULT

The CIELAB method colour analysis with Image-J program is used to obtain L-value of extrinsic stain removal based on the difference of picture after extrinsic stain induction and after brushing with toothpastes on teeth, artificial teeth, and acrylic resin denture base. Mean and standard deviation of L-value extrinsic stain removal by negative control toothpaste, experimental toothpastes, and positive control toothpaste are presented on Table 2.

Table 2. Mean and standard deviation of L-values extrinsic stain removal by toothpastes on the surfaces of the specimens

	Negative control	**Experimental toothpaste**			**Positive control**
	Basic toothpaste	**Bay Leaf extract 5%**	**Bay Leaf extract 10%**	**Bay Leaf extract 15%**	**Commercial toothpaste**
Teeth	1,82 ± 0,68	3,02± 1,45	6,20 ± 1,67	6,21± 1,44	8,07± 1,73
Artificial teeth	2,21± 1,49	3,71± 0,99	12,69± 1,58	12,60±2,85	11,59± 3,02
Acrylic resin denture base	1,21± 0,57	2,45± 0,56	5,82± 2,44	8,19± 1,90	8,87± 2,44

Table 2 shows that the three groups of experimental toothpastes have the L-value of extrinsic stain removal greater than L-value of extrinsic stain removal of basic toothpaste on the specimens. The Kolmogorov-Smirnov normality test and Levene's Test homogeneity test were carried out, and obtained the p-value eventually of p>0.05, that is, the data is normally distributed and showed a homogeneity of variance, so that one-way ANOVA parametric test can be continued. The result of the one-way ANOVA test is presented in the Table 3.

Table 3. Result of One-way ANOVA test

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	1093.972	14	78.141	23.567	0.000
Within Groups	198.939	60	3.316		
Total	1292.911	74			

One-way ANOVA test showed that there is significant difference of L-value extrinsic stain removal between groups of toothpaste on teeth, artificial teeth, and acrylic resin base ($p<0.05$). It shows that there is an effect of different toothpaste on extrinsic stain removal of the specimens.

After conducting the one-way ANOVA test, the LSD test was conducted to determine the effectiveness of experimental toothpastes by comparing experimental toothpastes and control toothpastes. A summary of the results of the LSD test is presented in the Table 4.

Table 4. The result of LSD test on experimental toothpastes and positive control toothpaste comparation

Toothpaste type	Toothpaste comparison	Specimen	p-value	Interpretation
Bay leaf 5%	Positive control	Teeth	0.001	Significant difference
Bay leaf 5%	Positive control	Artificial teeth	0.001	Significant difference
Bay leaf 5%	Positive control	Acrylic resin denture base	0.001	Significant difference
Bay leaf 10%	Positive control	Teeth	0.109	No significant difference
Bay leaf 10 %	Positive control	Artificial teeth	0.343	No significant difference
Bay leaf 10%	Positive control	Acrylic resin denture base	0.010	Significant difference
Bay leaf 15%	Positive control	Teeth	0.111	No significant difference
Bay leaf 15%	Positive control	Artificial teeth	0.385	No significant difference
Bay leaf 15%	Positive control	Acrylic resin denture base	0.554	No significant difference

Table 4 shows that there is no significant difference between L-value of extrinsic stain removal of bay leaf 15% toothpaste and positive control toothpaste on teeth, artificial teeth, and acrylic resin denture base. It means that the experimental toothpaste containing bay leaf extract 15% has the same ability as positive control toothpaste in removing extrinsic stain on all specimen surfaces. However, experimental toothpaste containing bay leaf extract 10% shows significant difference compared to positive control toothpaste on acrylic resin denture base extrinsic stain removal. Same result showed by experimental toothpaste containing bay leaf extract 5% both on teeth, artificial teeth, and acrylic resin denture base.

DISCUSSION

This study aims to determine the effectivity of bay leaf *(Eugenia polyantha Wight)* extract as toothpaste active agent for extrinsic stain removal on teeth, artificial teeth, and acrylic resin denture base. Bay leaf extract was formulated in various concentration (5%, 10%, and 15%). Result showed that there was an effect of bay leaf extracts toothpastes in removing extrinsic stain on three kinds of specimens, which 15% bay leaf extract concentration having effectiveness equal to the positive control toothpaste.

Extrinsic stain on teeth occurs due to negative charge of stain or chromogen molecules tend to bind directly to positive ion, for example the calcium ions (Ca^{2+}) on enamel[6]. Pellicle on teeth surface also facilitates chromogen attachment.

Concurrent with bad oral hygiene condition and inability to remove chromogen, extrinsic stain is formed and will be more severe[32]. Tannin, as an example of chromogen from tea, has double bound molecule, such as carbonyl or aromatic bound. This kind of chromogen has high affinity to protein, so it can bind to pellicle molecule. Tannin absorbs visible light and reflects colour that is detected by eyes as yellow, brownish stain[6].

Formation of extrinsic stain on artificial teeth and acrylic resin denture base caused by tannin that is attached to polar compound of polymethyl methacrylate acrylic resin. Some factors that accommodate tannin attachment to polymethyl methacrylate are biofilm formation on denture, micro-porosity, and the increasing of acrylic resin surface roughness[33,27]. The attachment of this chromogen is supported by bad oral hygiene, especially in condition with presence of plaque, biofilm, and calculus. High affinity to protein exhibited in tannin enable this chromogen to penetrate into plaque and biofilm[6]. The presence of calculus supports the formation of extrinsic stain by which the calculus incorporates tannin into the calcium phosphate lattice[6,34].

Brushing with toothpaste is one of methods to remove extrinsic stain from teeth and denture[4]. In present study, brushing is carried out using automatic toothbrushing machine which can be set at static and controlled time, weight, and speed. Then, the picture after extrinsic stain induction and the picture after brushing were analysed using CIELAB method by Image-J colour analysis program to obtained the L (lightness) value of extrinsic stain removal and later undergo data analysis.

One-way ANOVA test result (Table 3) showed that there was an effect of different toothpastes used in this study to extrinsic stain removal on teeth, artificial teeth, and acrylic resin denture base ($p<0.05$). It was shown from the significant difference between types of toothpaste used in all three groups of specimens to the L-value of extrinsic stain removal. This significant difference is due to the different formulation of the toothpastes, especially the active agents and abrasives. Basic toothpaste as the negative control toothpaste was made by formulations from calcium carbonate, glycerol, SLS, carmine, saccharin, sodium benzoate, and aquadest[25]. There is no active agent added to basic toothpaste. The extrinsic stain removal of basic toothpaste comes from abrasive material calcium carbonate[35]. In the other side, experimental toothpastes were made with same formulation as basic toothpaste but were added bay leaf extract 5%, 10%, and 15% as active agent. The extrinsic stain removal commercial toothpaste as the positive control toothpaste has the composition of calcium carbonate, sorbitol, water, sodium lauryl sulphate (SLS), sodium silica, hydrated silicate, flavourings, 1.12% sodium mono-fluor-phosphate, 0.7% perlite, aluminium oxide, cellulose gum, sodium saccharin, potassium citrate, SMSM hydantoin, CI 74160, CI 77891. The positive control toothpaste contains four kinds of abrasives, such as calcium carbonate, sodium silica, aluminium oxide, and perlite[35,36].

The LSD test result shows there was no significant difference between extrinsic stain removal on teeth, artificial teeth, and acrylic resin denture base by experimental toothpaste containing 15% bay leaf extract compared to the positive control toothpaste ($p>0.05$). This shows that bay leaf extract 15% toothpaste has the same effectivity as positive control toothpaste to remove extrinsic stain on teeth and denture, even though bay leaf extract toothpaste only contains lower hardness abrasive such as calcium carbonate, compared to positive control toothpaste that contains higher abrasives[35, 36]. The ability of bay

leaf extract toothpaste to remove extrinsic stain effectively is due to the addition of bay leaf extract 15% as toothpaste active agent.

Abrasive materials are the main ingredients for removing plaque and stain on dental and denture surface. Many factors define the degree and ability of abrasive materials, one of them is hardness of the particles[37]. Calcium carbonate contained in basic toothpaste and experimental toothpastes has a hardness of 3mohs, whereas commercial toothpaste as positive control toothpaste contains higher abrasives in the form of calcium carbonate (3mohs hardness), sodium silica (6-7mohs hardness), aluminium oxide (9mohs hardness), and perlite (5.5mohs hardness)[38]. Excessive use of abrasives in toothpaste increases enamel surface roughness and further causes enamel and dentin wear[37,39]. This is associated by the abrasion mechanism of abrasive particles that induces scratches in enamel surface[35]. Abrasives increase denture surface roughness, which further increase bacteria, plaque, and fungi attachment[36]. Another impact of abrasives to denture is alteration of physical properties of denture materials, such as rapid discoloration of denture, decreasing the colour stability, and also disturbing aesthetical appearance because it enables more chromogens to adhere[11].

In present study, the experimental toothpaste removes extrinsic stain mechanically and chemically. The abrasives contained in toothpaste combine to toothbrushing method remove extrinsic stain from teeth and dentures mechanically. This mechanical action helps remove pellicle, plaque, and biofilm that facilitate extrinsic stain attachment to teeth and denture[6]. The chemical mechanism of extrinsic stain removal performed by bay leaf extract which mainly contains eugenol. It is phenolic compound that acts as hydrogen donors that release H^+ ions[40]. The hydrogen ion will be drawn to hydroxyapatite bound and tannin, generates electron conjugation, and alter the double bound of hydroxyapatite and tannin to form a simpler bound, finally decrease the tannin as causal pigment of discoloration[41]. 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 of eugenol active agent through a substitution reaction, resulting in extrinsic stain removal. The ability to perform substitution reaction affected by degree of acidity of compounds[42]. The degree of acidity of eugenol is lower than tannin[43], the lower the degree of acidity, the stronger the ability to attain stability with other ion such as hydroxyapatite ion[41,44]. These theories explain possible mechanisms of how extrinsic stain removed chemically by experimental toothpaste active agent.

The removal of extrinsic stain from artificial teeth and acrylic resin denture base occurred chemically by the polar compound of the eugenol active agent in experimental toothpastes that bind to polymethyl methacrylate of acrylic resin[45]. The presence of gaps between molecules in the inter-polymer chain of acrylic resins causes the polar compounds of the active agent enters and forms bond with the acrylic resin, thus untying the tannins from the acrylic resin. The bond between acrylic resin and the polar compound of this active agent occurs due to electrostatic forces[46].

The most effective concentration of bay leaf extract for extrinsic stain removal toothpaste active agent is 15%, which was shown by no significant difference to positive control toothpaste, whereas the 5% concentration having lower L-value of extrinsic stain removal that is significantly different to positive control toothpaste. It indicates that 5% bay leaf extract concentration is inadequate to give effective removal of extrinsic stain. The 10%

concentration of bay leaf extract also inadequate to remove extrinsic stain on acrylic resin denture base, as this material has higher degree of water solubility and microporosity compared to teeth and artificial teeth, so that chromogen accumulation and absorption is higher too[47,48], therefore higher concentration of bay leaf extract is needed.

It can be implied that the experimental bay leaf extract toothpaste with 15% concentration is potential as extrinsic stain removal and tend to be safer than positive control toothpaste by considering the abrasive material and natural active agent used in formulation of this toothpaste. Present study did not perform physicochemical evaluation of experimental toothpastes. Besides, the extraction of the bay leaf was raw extraction only, there was no chromatography test. Nevertheless, this present study gives a recent knowledge that bay leaf extract is effective as extrinsic stain removal on teeth and denture, which can be considered as herbal active agent toothpaste alternative for chemical active agent and high abrasives commercial toothpaste. Further research is needed regarding physicochemical evaluation of the toothpaste, chromatography test of the extract used to determine the main responsible active agent to remove extrinsic stain and its mechanism, also the side effects of using bay leaf extract toothpaste on living tissues.

CONCLUSION

Bay leaf extract is effective and potential to be used as toothpaste active agent because of chemical mechanism of eugenol contained in bay leaf extract that removes extrinsic stain on teeth, artificial teeth, and acrylic resin denture base. Variation of bay leaf extract concentration influence the effectivity of the toothpaste, which 15% concentration of bay leaf extract gives worthwhile extrinsic stain removal both on teeth and denture, hence it is considered as multifunction extrinsic stain removal toothpaste.

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