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# Potential Effect of Bay Leaf (*Syzygium polyanthum* [Wight] Walp.) Essential Oil for Herbal Toothpaste Active Agent

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## ABSTRACT

Bay leaf is a herbal plant containing essential oil with antioxidant activity. Antioxidant effects give bay leaf essential oil the ability as a toothpaste active agent. This research aim is to evaluate the chemical composition and antioxidant activity of bay leaf essential oil to find out its potential as a toothpaste active agent. The Bay leaf used in this research was taken from Lendah, Kulon Progo in the evening. Steam-hydrodistillation is conducted and chemical composition is analyzed using the GC-MS method. The Antioxidant activity test is conducted with DPPH and FRAP methods. Toothpastes were formulated with three concentrations of bay leaf essential oil (0.125%, 0.25%, 0.5%). A stain prevention test using formulated toothpaste, no active agent toothpaste (negative control), and commercial toothpaste (positive control) was conducted on 20 bovine teeth to obtain the value of colour change (ΔE) before and after the experiment, then analysed using one-way ANOVA parametric test (CI 95%). The chemical composition of bay leaf essential oil detected using GC-MS showed 29 compounds. The highest percentages are cis-4-decenal (37.87%), Decanal (16.73%), and octanal (16.63%). IC50 value from DPPH and FRAP method are 2.079µg/mL and 3.277µg/mL. One-way ANOVA test showed there was an effect from bay leaf essential oil as stain prevention toothpaste active agent. Bay leaf essential oils toothpaste has no significant difference in ΔE value to positive control toothpaste. The conclusion of this research is bay leaf essential oil contains aldehyde compounds in high percentages which provides a very high antioxidant effect. Bay leaf essential oil is the potential to be used as a toothpaste active agent.

**Keywords :** bay leaf; essential oil; DPPH; FRAP; toothpaste

## INTRODUCTION

Herbal plants have been developed for a long time around the world as active agents in various products. The use of herbal plant is because it is more affordable, easy to find, and safer than chemical active agents, also for its low toxicity (Galor and Benzie, 2011). Bay leaf (*Syzygium polyanthum)* is a plant from the *Myrtaceae* family, and is a herbal plant that is spread in various regions of Indonesia (Silalahi, 2017). This plant can be found in yards or plantations which are managed for various needs. Every year, the bay leaf supply in Indonesia reached 6,790 tons (Pribadi, 2009). Bay leaf is mostly utilized in the health sector, including as anti-inflammation, analgesic, and antidiabetic because of its high antioxidant properties. The utilization of bay leaf in dentistry is still minimal. Previous researches on the use of bay leaf in dentistry are bay leaf ethanol extract as an antibacterial toothpaste (Saputri et al., 2020), bay leaf infusion as mouthwash, and *denture cleanser* (Sumono and Wulan, 2008). Ethanol extract of the bay leaf with a 15% concentration is effective as extrinsic stain removal toothpaste active agent (Annisa et al., 2022).

Toothpaste is one of the most commonly used preventive materials (Rezaie et al., 2020). Toothpaste is formulated for several functions, such as tooth stain removal and inhibition, because of the high demand for aesthetics. Tooth stain gives a big impact on someone's appearance and leads to aesthetical dissatisfaction and reduces self-confidence, especially extrinsic tooth stain (Annisa et al., 2022). The tooth is prone to this kind of stain because the extrinsic stain is formed by chromogen from favourite products such as foods, beverages, and tobacco. One of the most common chromogens that induced extrinsic stain is tannin which acts as an organic chromogen (Cho, 2020).

Based on the phytochemical analysis, one of the contents of the bay leaf is an essential oil (Istiqomah et al., 2020). The utilization of essential oil in dentistry is an active agent of mouthwash or

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toothpaste because of its antimicrobial and antioxidant effects (Vranic et al., 2014). Bay leaf essential oil contains aldehyde (82.074%) and *hydrocarbon volatile compounds* (13.061%). This aldehyde compound has a role in antimicrobial, and high-activity antioxidant (Hamad et al., 2017). Bay leaf essential oil has a very high antioxidant activity with an IC50 value of less than 50 µg/mL (Wilapangga and Sari, 2018; Umaru et al., 2020). The high antioxidant activity provides potential use for the essential oil to be formulated as an active agent of toothpaste (Karadaglioglu et al., 2019). Previous research by Marquillas et al (2019) stated that antioxidant compounds can remove and inhibit tannin-induced tooth stains by reductant agent mechanism. This research aims to evaluate chemical composition, antioxidant activity using DPPH and FRAP methods, and stain prevention ability of bay leaf essential oil to find out the potential use of bay leaf essential oil as an active agent of toothpaste.

## METHODOLOGY

### Materials

Bay leaves were obtained from Lendah, Kulon Progo, DIY. For antioxidant test, in DPPH method used *2,2-diphenyl-1-picryl-hydrazyl, ascorbic acid* from Sigma Aldrich Co. (St. Louis, USA), and *ethanol* as solvent from Merck (Darmstadt, Germany). For the FRAP test, used phosphate buffer (200mM, pH 6.6), potassium ferricyanate, *trichloroacetic acid*, iron (III) chloride, and *ascorbic acid*.

### Methods

### Ethical Aspect

Ethical Eligibility is obtained from the Research Ethics Commission, Faculty of Dentistry, Gadjah Mada University (No.00778/KKEP/FKG-UGM/EC/2021).

### Sample Preparation

Bay leaf sample was obtained from Lendah, Kulon Progo, DIY, which was taken on September 2021 in the afternoon, around 4 p.m. The criteria of bay leaf taken are the leaves on the old part (one-third of the lower part), have a dark green colour, which comes from trees with a trunk diameter of ≥10 cm. The whole bay leaves which had no traces of animals were cut with a *stainless* knife.

Before the steam-hydro distillation process, bay leaves were aerated in a room that was not exposed to direct sunlight for 3 days until the dried bay leaf is obtained. The dryness level of the bay leaf is indicated by the destruction of the bay leaf when crushed roughly. Whole dried bay leaves were crushed before steam-hydro distillation was conducted.

### Bay Leaf steam-hydro distillation

The distillation process was conducted in the Pharmacognosy-Phytochemistry Laboratory, Department of Pharmaceutical Biology, Faculty of Pharmacy UGM. The distiller used has a capacity of 1kg sample with a separator between the water and the sample. The distillation process was conducted for 4 hours starting from the first drop of essential oil appearing in the separator. The essential oil obtained from each distillation was stored in a dark bottle. The calculation of essential oil yield was conducted with the formula:

$$Yield\ (\%) = \frac{mass\ of\ essential\ oil\ obtained}{initial\ sample\ mass}\ x\ 100\%$$

### Gas Chromatography-Mass Spectroscopy (GC-MS) Test

One hundred microliters of bay leaf essential oil were dissolved in hexane to obtain a level of 1%. Essential oil solution in hexane is injected into gas chromatography. The result obtained compared to library Wiley7 to identify the compound. Compound concentration was calculated by integrating the chromatography peak area.

### Antioxidant Activity Evaluation

Antioxidant activity test was conducted with DPPH radical scavenging assay and Ferric Reduction Antioxidant Power (FRAP) method.

### Antioxidant activity test with DPPH method

The stock of DPPH solution was made of 10g DPPH in 25mL ethanol. The prepared blank solution, sample solution to be tested, and ascorbic acid solution as a positive control. For 200µL of DPPH solution each was mixed with bay leaf essential oil to be tested (concentration 0.31%, 0.63%, 1.25%, 2.50%) and ascorbic acid, then added ethanol until the volume of 1mL. This mixture was incubated in a dark room for 30 minutes and the absorbance reading was conducted with a spectrophotometer at 517 nm. The percentage of DPPH radical inhibition was calculated with the formula:

$$\%\ Inhibition = \frac{\text{A blank} - \text{A sample}}{\text{A blank}}\ x\ 100$$

with $A_{blank}$ is negative control absorbance and $A_{Sample}$ is essential oil absorbance or reference antioxidant. IC50 (*inhibitory concentration 50)* value, is the number of essential oil concentrations

needed to reduce 50% DPPH radical initial concentration, calculated graphically with linear regression [% *inhibition* = f (essential oil concentration)].

**Ferric Reduction Antioxidant Power (FRAP) Test**

A number of bay leaf essential oils were tested (concentration 1.25%, 2.5%, 5%) mixed with 0.5 mL phosphate buffer (200mM, pH 6.6) and 0.5 mL potassium ferricyanate (1%). This mixture and blank were incubated at a temperature of 50°C for 30 minutes. *Trichloroacetic acid* (10%) of 0.5mL was added to the incubated mixture and centrifuged (600rpm for 10 minutes). The supernatant of 1mL was obtained, added with 1 mL of water and 0.2 mL of iron (III) chloride (0.1% $FeCl_3$), and then an absorbance reading was conducted with a spectrophotometer at 700 nm. *Ascorbic acid* (0.1M) was used as a positive control. The higher the measured absorbance, the higher the ability of the reducing substance. The inhibition percentage calculation formula is

$$\%\ Inhibition = \frac{\text{A sample} - \text{A blank}}{\text{A sample}}\ x\ 100$$

with $A_{blank}$ is negative control absorbance, and $A_{Sample}$ is essential oil absorbance or reference antioxidant. Inhibition percentage value and essential oil concentration were conducted through linear regression analysis to obtain a linear regression equation then the IC50 value was calculated.

**Toothpaste formulation**

Toothpastes were made using three concentrations of bay leaf essential oil (0.125%, 0.25%, 0.5%) and no active agent toothpaste as negative control. Commercial toothpaste is used as a positive control. Bay leaf essential oil and negative control toothpaste were formulated in gel form. The compositions of bay leaf essential oil toothpaste are Carbopol 940, triethanolamine, sodium benzoate, glycerine, tween 80, water, and bay leaf essential oil. Negative control toothpaste was made with the same compositions but without bay leaf essential oil.

**Tooth stain prevention test**

Twenty bovine teeth are used as tested subjects to evaluate tooth stain prevention with five kinds of toothpaste so that each toothpaste is used to prevent stains on four bovine teeth. A tooth stain prevention test is conducted by the CIELAB method to evaluate colour change before and after the experiment. Firstly, baseline initial colour is obtained from all teeth before the experiment to get the initial colour value ($L_i$, $a_i$, $b_i$) using chromameter. All teeth were brushed using five kinds of toothpaste (three kinds of toothpaste of bay leaf essential oil, negative control, and positive control) by an automatic toothbrushing machine at a controlled speed, movement, and weight. After brushing, the teeth were immersed in tea solution to induce tooth stain for 3.5 hours. Finally, the teeth are measured again by chromameter to obtain the final colour value ($L_f$, $a_{f,}$ $b_f$). The initial and final L, a, b values from all teeth are calculated to attain colour change value (ΔE) by the formula:

$$\Delta E = \sqrt{\{(L_f\text{-}L_i)^2 + (a_f\text{-}a_i)^2 + (b_f\text{-}b_i)^2\}}$$

**Data analysis**

Phytochemical data was analysed using mass spectroscopy fragmentation from GC-MS that is compared to the Wiley7 database. Antioxidant activity obtained from DPPH and FRAP is analysed using linear regression and IC50 is calculated. The Colour change value (ΔE) from the tooth stain prevention test is analysed using one-way ANOVA parametric test with a confidence interval of 95%.

## RESULT AND DISCUSSION

The calculation of essential oil obtained from essential oil volume is multiplied by essential oil density, which is 0.9098 $^{g}/_{mL}$ (Istiqomah et al., 2020), so that essential oil density is 3.6mLx0.9098$^{g}/_{mL}$ = 3.27g.

$$Yield\ (\%) = \frac{mass\ of\ essential\ oil\ obtained}{initial\ sample\ mass}\ x\ 100\%$$
$$= \frac{3.27}{15}\ x\ 100\% = 0.021\%$$

Bay leaf essential oil yield obtained from this research is 0,021%. The yielded result is by bay leaf essential oil on previous researches summarized by Istiqomah et al., (2020), which is 0.017%-0.023%.

Bay leaf essential oil obtained approached the maximum yield value. There are several things which influenced the percentage of essential oil yield which are sampling time, treatment before the distillation process, and the distillation method used. In this research, bay leaf sample was taken in the evening. According to Istiqomah et al. (2020), the distillation of bay leaf which is taken in the evening produced more essential oils compared to bay leaf which is taken in the morning, because the water content of bay leaf which is taken in the evening is the lowest. Treatment before the distillation process was bay leaf shade drying method in the room which was not exposed to

Table I. Chemical Composition of Bay Leaf Essential Oil from GC-MS Analysis Result

| No. | Retention Time | %area | Compound Name | Hit Value |
|---|---|---|---|---|
| 1 | 4.011 | 4.08 | α-pinene | 99 |
| 2 | 4.798 | 16.63 | Octanal | 99 |
| 3 | 5.196 | 0.42 | 1-limonene | 97 |
| 4 | 6.198 | 0.23 | Nonanal | 96 |
| 5 | 7.554 | 37.87 | cis-4-Decenal | 95 |
| 6 | 7.692 | 16.73 | Decanal | 98 |
| 7 | 8.898 | 0.27 | vitispirane | 94 |
| 8 | 10.250 | 0.26 | α-Copaene | 94 |
| 9 | 10.534 | 0.26 | Dodecanal | 92 |
| 10 | 10.901 | 0.29 | trans-Caryophyllene | 93 |
| 11 | 11.172 | 0.19 | Dihydropseudoionone | 90 |
| 12 | 11.361 | 0.54 | α-Humulene | 97 |
| 13 | 11.616 | 0.39 | α-Gurjunene | 88 |
| 14 | 11.754 | 2.00 | 4,11-selinadiene | 93 |
| 15 | 11.806 | 1.97 | β-Selinene | 95 |
| 16 | 11.870 | 0.61 | Valencene | 86 |
| 17 | 11.911 | 1.58 | α-Selinene | 92 |
| 18 | 12.122 | 0.36 | γ-Cadinene | 93 |
| 19 | 12.224 | 2.33 | α-Panasinsene | 88 |
| 20 | 12.623 | 4.31 | Nerolidol | 95 |
| 21 | 13.274 | 0.67 | Selina-4(14),11-diene | 88 |
| 22 | 13.417 | 1.79 | Humulene oxide | 87 |
| 23 | 13.589 | 0.35 | Veridiflorol | 85 |
| 24 | 13.671 | 0.25 | Elemol | 83 |
| 25 | 13.748 | 0.51 | δ-Cadinene | 88 |
| 26 | 13.923 | 0.58 | Viridiflorol | 83 |
| 27 | 14.051 | 0.63 | Juniper camphor | 92 |
| 28 | 14.743 | 2.94 | Farnesol | 94 |
| 29 | 16.175 | 0.96 | Hexahydrofarnesyl acetone | 89 |

direct sunlight. The drying temperature was not too high when the shade drying process was conducted. It is quite good to reduce the water content in fresh leaves without destruct the active substance and volatile compounds (Luliana et al., 2016). The rough crushing process of bay leaf optimized the essential oil produced. This treatment caused the intracellular enzymes to break down leaf cells, which makes it easier for the essential oil to come out. The rough crush resulted in a leaf size which is not too small so that the leaves cells which stores the oil are not destructed (Khasanah et al., 2015). The distillation process using the steam-hydro distillation principle can increase the yield produced because in this principle the sample location which contains essential oil and carrier water is separated, so that water and essential oil do not evaporate at the same time. Compared to water distillation, sample essential oil will come out of the water and evaporate at the same time when the heating process is conducted, which causes many essential oils left in the water is evaporated and decreased the yield (Yuliarto et al., 2012).

Chemical composition evaluation of bay leaf essential oil was conducted with GC-MS (*Gas Chromatography-Mass Spectrometry),* which identified of total 29 compounds. The chemical compound composition identified is presented in Table I.

GC-MS analysis results in table I show that 29 components are dominated by the aldehyde compound from bay leaf essential oil. The highest aldehyde compound percentage contained in bay leaf essential oil are cis-4-Decenal (37.87%), Decanal (16.73%), and Octanal (16.63%). This is following previous research which explained that the main constituent of bay leaf essential oil obtained by steam is the aldehyde compound which is cis-4-Decenal (43.489%) (Hamad et al., 2017). In the research of Wartini et al. (2009) sit. Istiqomah et al. (2020) showed that the constituent of bay leaf essential oil obtained by steam is cis-4-Decenal (18.74%). Aldehyde compound

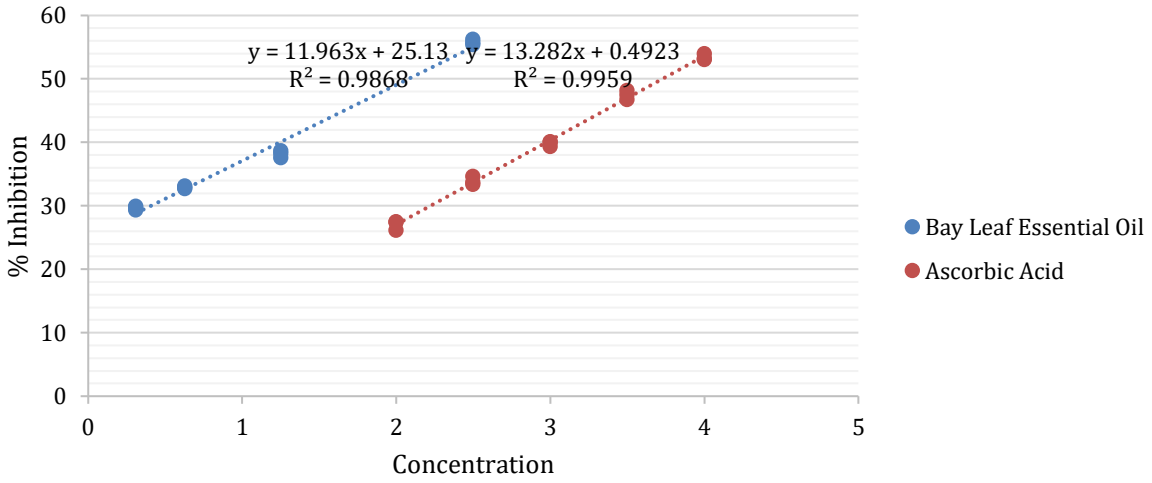


Figure 1. Concentration and Inhibition Percentage of Bay Leaf Essential Oil and Ascorbic Acid tested with DPPH Methods

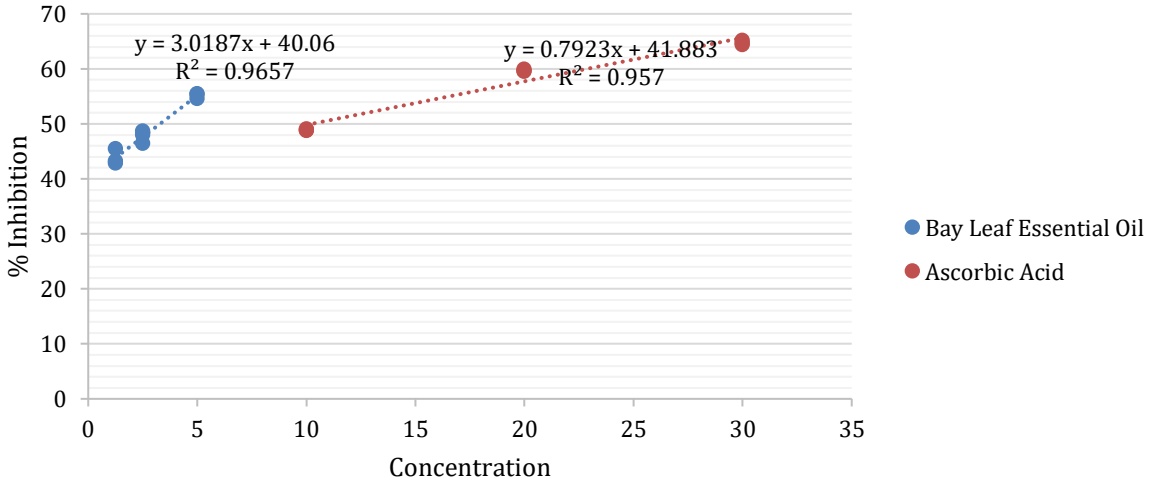


Figure 2. Concentration and Inhibition Percentage of Bay Leaf Essential Oil and Ascorbic Acid tested with FRAP Methods

percentage is higher than the previous research which used the steam distillation method. The difference in this percentage of which is caused by different distillation methods, wherewith the presence of solution in steam distillation method such as n-hexane caused aldehyde compound to evaporate or be destructed caused by oxidation so that the percentage is lower (Wartini et al., 2009 sit. Istiqomah 2020). The different places for obtaining the bay leaf and different treatments before the distillation process also determine the percentage difference of essential oil obtained (Hamad et al., 2017).

The antioxidant activity test was conducted using DPPH and FRAP methods. The concentration and inhibition percentage of bay leaf essential oil and ascorbic acid obtained from DPPH and FRAP methods are presented in figure 1 and figure 2.

Graphics in figure 1 and figure 2 showed an increase in the percentage of inhibition along with the increase in bay leaf essential oil concentration and ascorbic acid. The value of IC50 for each sample in each testing method is presented in table II.

Table II shows the IC50 value of bay leaf essential oil, with ascorbic acid as a comparison. IC50 value from antioxidant testing methods obtained results in the same range below 50µg/mL This shows bay leaf essential oil has a very high antioxidant activity. There is a difference in the

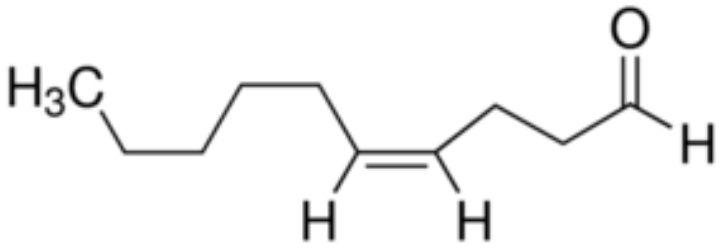


Figure 2. Chemical Structure of cis-4-decenal (Merck, 2022)

Table II. Value of IC50 bay leaf essential oil and ascorbic acid tested using DPPH and FRAP methods

| Test Compound | IC50 (µg/mL) Value | |
|---|---|---|
| | DPPH Method | FRAP Method |
| Bay leaf essential oil | 2.079±0.016 | 3.277±0.289 |
| Ascorbic Acid | 3.727±0.027 | 10.236±0.241 |

Mean and deviation standard of IC50 value (n=3 for DPPH and FRAP method)

Table III. Mean and standard deviation of ΔE value from tooth stain prevention test by five tested kinds of toothpaste

| Type of toothpaste | Mean and standard deviation of ΔE value |
|---|---|
| Negative control | 3,38±1,52 |
| Bay leaf essential oil 0.125% | 3,14±1,34 |
| Bay leaf essential oil 0.25% | 3,08±1,49 |
| Bay leaf essential oil 0.5% | 2,96±0,54 |
| Positive control | 2,23±0,64 |

IC50 value of bay leaf essential oil when it is tested with both methods, this is caused by the different reactions and responses to the tested compounds (Abdulrahman et al., 2019). The result of this research is following previous research, in which is IC50 value of bay leaf essential oil tested with the DPPH method is categorized as very strong, which is 18.42 µg/mL (Umaru et al., 2020); 19.97 µg/mL (Wilapangga and Sari, 2018).

The high antioxidant activity of bay leaf essential oil is caused by the high content of the aldehyde compound in the bay leaf essential oil (Hamad et al., 2017). *The radical scavenging* principle by antioxidants neutralizes free radicals by donating electrons so that it breaks the reaction chain caused by free radicals to react with other molecules (Lee et al., 2016). The aldehyde compound with the highest percentage in bay leaf essential oil is cis-4-decenal (Table III). This compound has an alkyl group ($CH_3$) which is an *electron-donating group* which has a role in activating the aromatic ring by increasing the electron density in the ring through the *inductive donating effect* (Hunt, 2015). This finding is synergistic with previous research which showed aldehyde compounds such as decanal and octanal that dominated essential oil isolated from sweet orange are proved to have the highest antioxidant activity compared to single isolated compounds, with IC50 value from the DPPH test being 66.10±0.23µg/mL (Liu et al., 2012). Another previous research (Dong et al., 2020) showed a high percentage of Decanal contained in the essential oil of sweet ginger gives high antioxidant activity evaluated by the DPPH and ABTS method, with a radical scavenging rate of more than 85%.

Tooth stain prevention test by five tested kinds of toothpaste shows colour change (ΔE) value before the experiment and after stain induction presented in table III.

Table III showed that there is decreasing in ΔE value with the increasing concentration of toothpaste. The lowest ΔE value was generated by positive control toothpaste, followed by bay leaf essential oil toothpaste at 0.5%. One-way ANOVA test results significance value p=0.714 (p>0.05). All toothpaste tested had no significant difference in ΔE value. The result showed that all toothpaste has the same ability to prevent tooth stain because the ΔE values are not different to positive control toothpaste statistically.

A lower ΔE value indicated an inconsiderable colour change of teeth before and after the experiment (Irfany et al., 2014). This study proved that bay leaf essential oil toothpaste can prevent tooth stains because it can prevent colour change after stain induction post-brushed with toothpaste as good as the positive control. The prevention of tooth stains by bay leaf essential oil toothpaste might be caused by the high antioxidant activity of bay leaf essential oil. Previous research by Marquillas et al (2019) showed tannin-induced extrinsic stain is removed by an antioxidant compound that acts as a reductant agent. This agent can degrade tannin as a stain inductor.

According to the present study, the tannin-induced tooth stain might be degraded by bay leaf essential oil before being able to bind to the tooth surface. As proved by antioxidant tests with DPPH and FRAP methods, bay leaf essential oil has very high activity as an antioxidant. An antioxidant will donor the electron so that it can reduce the compound containing free electrons (Irshad et al., 2012). Bay leaf essential oil has very high antioxidant activity and is potential to be used as a toothpaste active agent, but further research concerning formulation, physicochemical of bay leaf essential oil toothpaste, and another ability test of bay leaf essential oil toothpaste needs to be conducted.

## CONCLUSION

Bay leaf essential oil contains an aldehyde compound with the highest percentage of cis-4-decenal, which makes bay leaf essential oil has very high antioxidant activity proven by an antioxidant test with DPPH and FRAP methods. The high antioxidant activity shows the ability of the bay leaf in neutralizing free radicals as an electron donor so that bay leaf essential oil is the potential to be used as a toothpaste active agent.

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## REFERENCES

Abdulrahman, M.D., Nudin, H., Fatihah, N., Khandaker, M.M., Ali, M.A. & Mat N., 2019, 'In Vitro Biological Investigations on Syzygium polyanthum Cultivars', *IJAB.* 22, 1399-1406.

Annisa, M., Nuryanti, A., & Dewi, A. H., 2022, 'Effectivity of Multifunction Herbal Toothpaste Containing Bay Leaf (*Eugenia polyantha* Wight) Extract as Extrinsic Stain Removal on Teeth and Denture', *Odonto Dental Journal.* 9, 40-50.

Awaluddin, N. & Wahyuningsih, S., 2019, 'Penentuan Aktivitas Antioksidan Ekstrak Metanol Klika Anak Dara', *J Farm FKIK UINAM*. 2, 38–45.

Bansal, T. & Harpreet, K., 2016, 'Benefits of essential oil', *J. Chem. Pharm. Res.* 8, 143–149.

Chandra, K.A. & Proborini, W.D., 2018, 'Analisa Komposisi Minyak Atsiri Kulit Jeruk Manis Hasil Ekstraksi Metode Microwave Hydrodiffusion and Gravity Dengan GC-MS', *J Ilm Tek Sipil dan Tek Kim*. 3, 53-59.

Cho, M. J., 2020, 'The Tooth Whitening Effect of Toothpaste Containing High Cleaning Silica and Sodium Hexametaphosphate and the Preventive Effect of Staining by Coffee, Tea and Wine', *International Journal of Clinical Preventive Dentistry*. 1, 192–199.

Hamad, A., Mahardika, M. G. P., Yuliani, I., & Hartanti, D., 2017, 'Chemical constituents and antimicrobial activities of essential oils of Syzygium polyanthum and Syzygium aromaticum', *Rasayan Journal of Chemistry*, 10, 564–569.

Hsouna, A.B., Gargouri, M., Sayahi, N., Manif, W., Dhifi, W., Ben R, et al., 2018, 'Potential anti-inflammatory and antioxidant effects of Citrus aurantium essential oil against carbon tetrachloride-mediated hepatotoxicity: A biochemical, molecular and histopathological changes in adult rats', *Environ. Toxicol.* 34, 1–13.

Hudaib, M., Speroni, E., Di Pietra, A. M., & Cavrini, V., 2012, 'GC/MS evaluation of thyme (Thymus vulgaris L.) oil composition and variations during the vegetative cycle', *Journal of Pharmaceutical and Biomedical Analysis*, 29, 691–700.

Hunt, I., 2015, 'Reaction of Arenes, Electrophilic Aromatic Substitusion', *Chapter 12.* Available from: https://www.chem.ucalgary.ca/courses/351/Carey5th/Ch12/ch12-8d.html

Irfany Dharmautama, M., & Damayanti, I., 2014, 'Stabilitas warna basis akrilik gigi tiruan lepasan setelah pembersihan dengan ekstrak dan infusa bunga rosella', *Journal of Dentomaxillofacial Science.* 13, 38-43.

Irshad, M., Zafaryab, M., Singh, M., & Rizvi M.M.A., 2012, 'Comparative Analysis of the Antioxidant Activity of Cassia fistula Extracts', *Int J Med Chem.* 12, 1–6.

Istiqomah, H., & Ayuska, A., 2020, 'Karakterisasi Minyak Atsiri Daun Salam (Syzygium

polyanthum Wight) Asal Kalimantan Barat', *J Kim Khatulistiwa.* 1, 37–44.

Karadağlıoğlu, Ö.İ., Ulusoy, N., Başer, K.H.C., Hanoğlu, A., & Şık, İ., 2019, 'Antibacterial activities of herbal toothpastes combined with essential oils against streptococcus mutans', *Pathogens*. 8, 15-21.

Khasanah, L.U., 2015, 'Pengaruh Perlakuan Pendahuluan terhadap Karakteristik Mutu Minyak Atsiri Daun Jeruk Purut (Citrus hystrix DC)', *J Apl Teknol Pangan.* 4, 48–55.

Lee, C.Y., Nanah, C.N., Held, R.A., Clark, A.R., Huynh, U.G.T., Maraskine, M.C., et al., 2015, 'Effect of electron-donating groups on polyphenol-based antioxidant dendrimers', *Biochimie,* 111, 125–34.

Luliana, S., Purwanti, N.U. & Manihuruk, K.N., 2016, 'Pengaruh Cara Pengeringan Simplisia Daun Senggani (Melastoma malabathricum L.) Terhadap Aktivitas Antioksidan Menggunakan Metode DPPH (2,2-difenil-1-pikrilhidrazil)', *Pharm Sci Res.* 3, 20–29.

Marquillas, C.B., Procaccini, R., Malmagro, M.V. & Sánchez-Martín, M.J., 2020, 'Breaking the rules: tooth whitening by means of a reducing agent', *Clin Oral Investig.* 24, 2773–2779.

Pratiwi, F.R.N.I., 2016, 'Formulasi Sediaan Gel Pasta Gigi Minyak Atsiri Kemangi (Ocimum basilicum L.) dan Uji Aktivitas Antibakteri terhadap Bakteri *Streptococcus mutans*', *Thesis,* Universitas Muhammadiyah Surakarta.

Rezaie, H. R., Rizi, H. B., Khamseh, M. M. R., & Ochsner, A., 2020, *A Review on Dental Materials,* Springer, Switzerland.

Saputri, G.A.R., Chusniasih, D. & Putri, E.A., 2020, 'Formulasi Pasta Gigi Ekstrak Daun Salam (Syzygium polyantha wight) sebagai Penghambat Pertumbuhan Streptococcus mutans', *J Farm Malahayati.* 3, 66–79.

Silalahi, M., 2017, 'Syzygium polyanthum (Wight) Walp (Botani, Metabolit Sekunder dan Pemanfaatan)', *Jurnal Dinamika Pendidikan*, 10, 187–202.

Sumono, A. & Wulan, S. D, A., 2008, 'The use of bay leaf (Eugenia polyantha Wight) in dentistry', *Dent. J. (MKG)*, 41, 147-150.

Umaru, I. J., Umaru, K. I. & Umaru, H. A., 2020, 'Phytochemical screening, isolation, characterization of bioactive and biological activity of bungkang, (*Syzygium polyanthum*) root-bark essential oil', *Korean Journal of Food & Health Convergence*, 6, 5–21.

Vranic, E., Lacevic, A., Mehmedagic, A. & Uzunovic A., 2014, 'Formulation Ingredients for Toothpastes and Mouthwashes', *Bosnian Journal of Basic Medical Sciences,* 4, 51-58.

Wilapangga, A. & Sari, L.P., 2018, 'Analisis Fitokimia Dan Antioksidan Metode DPPH Ekstrak Metanol Daun Salam (*Eugenia polyantha)', IJOBB*. 2, 19–24.

Yuliarto, F.T., Khasanah, L.U. & Anandito, R.B.K., 2012, 'Pengaruh Ukuran Bahan dan Metode Destilasi terhadap Kualitas Minyak Atsiri Kulit Kayu Manis', *Jurnal Teknosains Pangan,* 1, 15-20.