The Toothpaste's Containing Coffee bean Skin Demonstrated the Specific Physicochemical Properties, Inhibited the Growth of Streptococcus mutans and Increased the Viability of Neutrophil

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Abstract

Coffee bean skin contains amino acids, proteins, flavonoids, catechins equivalent to the seeds and leaves, which is thought to be used as toothpaste. As a basic ingredient of toothpaste, coffee bean skin must have good quality.

This study to analyze the Physicochemical properties (organoleptic, pH, homogeneity, spreadability, viscosity), the inhibition to S. mutans and cell viability of toothpaste's contained Robusta coffee bean skin.

Concentrations of toothpaste were 5%, 10%, 15%. Organoleptic test observed shape, color and odor, pH used a pH meter, viscosity test used vicotester VT 03. Spreadability test by measuring the diameter of toothpaste that was smear on the glass. Inhibition was carried out by measuring the diameter of the barriers against S. mutans. Cell viability used neutrophil cells. The cells were placed in 24-well microtiter plate, treated according to group. Viability was stained with Trypan Blue. Cells were counted under an inverted microscope with magnification 400 times from an average of 4 fields of view. The data obtained were ANOVA followed by LSD test and descriptive. Toothpaste preparation was cream colored, soft texture, homogeneous, smells of coffee, has a pH of 7 in accordance with the pH requirements for toothpaste preparations according to SNI 12-3524-1995, namely 4.5-10.5. Inhibition and cell viability tests showed significant differences, but not significant between 15% concentration with commercial toothpaste (ANOVA and LSD analysis, 0.05<p).

Toothpaste's contain coffee bean skin has physico-chemical properties, inhibited to S. mutans and increase cell viability equivalent to commercial toothpaste.

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Introduction

Brushing your teeth with toothpaste is important to prevent dental and oral infections. Toothpaste serves to clean teeth, reduce plaque formation, prevent caries, clean and polish tooth surfaces, eliminate or reduce bad breath, give a fresh taste to the mouth and maintain oral health. Toothpaste products will continue to change their chemical composition due to producer competition. Toothpaste contains antibacterial properties of various substances with different abilities to inhibit the growth of germs in the oral cavity, as well as a number of ingredients with specific purposes to solve certain problems. The many choices of toothpaste with various ingredients make it difficult for patients to choose the right toothpaste[1,2]. On the other hand, toothpaste products on the market can cause side effects such as canker sores, allergies, fluorosis, thyroid disorders to cancer. Some whitening products have been reported to have a pH as low as 4.0, while others have been reported to have a pH of 7.5. It has been reported that the higher the peroxide concentration, the more acidic the pH of the bleaching product.26 Some office whitening products containing 35% hydrogen peroxide may have a low pH[3]. The use of natural ingredients such as coffee bean husks is expected to minimize side effects.

Coffee bean skin contains amino acids, proteins, flavonoids, catechins equivalent to

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seeds and leaves (results of previous research). The skin of coffee beans like coffee beans contains an antioxidant, Trigonelline which is useful for protecting teeth. Although coffee drinkers generally have a slightly black tooth color due to these substances, this compound will protect teeth against S. mutans[4,5]. On the other hand, coffee does not interfere with the results after teeth whitening, so there is no need for dietary restrictions[6]. Previously we also researched about coffee plants, including: Steeping from green and black robusta coffee beans increase viability of Peripheral Blood Mononuclear Cells (PBMC) and salivary leukocytes which is induced by streptococcus mutans,Robusta Coffee Beans (Coffea canephora) Decrease IL-1α (Interleukin-1α) Expression and Increase the Number of Fibroblasts in Healing Process in Dental Pulp in Wistar Rat, the effect of steeping robusta coffee beans on monocytes:expression of IL-1β and TNF-α against streptococcus mutans[7-9]. Meanwhile, as a basic ingredient for toothpaste, coffee bean rind must have qualities regarding physical properties and biocompatibility that are equivalent to or better than toothpastes on the market. Therefore, this study aimed to analyze the quality (organoleptic, pH, homogeneity, spreadability, viscosity), microorganism inhibition and cell viability of Robusta coffee bean skin toothpaste.

Materials and methods

Research tools include: mortar and stamper, pH meter, rotary evaporator, a set of macerator tools, viscometer, digital caliper, autoclave, petri dish, incubator, ose needle, pH indicator paper, pH meter, glass object, microtiter plate, inverted microscope, micro pipette. The ingredients used were DMSO, Robusta coffee bean skin, 96% ethanol, menthol, calcium carbonate, sodium CMC, NaCL, sodium benzoate, sodium lauryl sulfate, saccharin, sorbitol, NA media, Streptococcus mutans isolate, Histopaque (Sigma), ficolhipaque (Sigma), HBSS (Hank's Balanced Salt Solution/Gibco), fungizone (Sigma), Trypan Blue, heparin, RPMI (Sigma).

Before making toothpaste, the coffee bean skin was screened for phytochemicals using the TLC method (Thin Layer Chromatography for flavonoids, polyphenols, triterpenoids, alkaloids, anthraquinones),

1. Making Coffee Bean Skin Toothpaste

Sodium CMC is sprinkled over hot water as much as 20 times the amount of Na CMC, let stand for 30 minutes, then grind until homogeneous. Menthol is dissolved in ethanol. The extract was diluted with ethanol, added sorbitol and menthol solution, added Na CMC dispersion, stirred until homogeneous. Added calcium carbonate, sodium benzoate, sodium lauryl sulfate and saccharin, stirred until toothpaste is formed. Coffee bean skin toothpaste used with concentrations of 5%, 10%, 15%.

2. Organoleptic Test

Organoleptic test observed shape, color and odor.

3. pH measurement

The paste preparation (1 gram) was dissolved with 10 ml of distilled water and measured using an OHAUSS pH meter.

4. Viscosity test

Viscosity test using vicotester VT 03.

5. Spreadability test

Spreading power by measuring the diameter of toothpaste smeared on a glass (1 gram), covered with a glass and then given a load of 200 g.

6. Inhibitory Test.

The method used is disc diffusion, by measuring the diameter of the resistance against, S. mutans (pure isolate cultured in the Biomedical Lab, Faculty of Dentistry, University of Jember). The microorganisms were standardized with standard Mc. Farland 0.5 and obtained a concentration of 1.5x108CFU/mL. A paper disk (6mm) was inserted into the sample, then placed in a petri dish containing the solidified media. Incubated for 24 hours at a temperature of 35ºC-37ºC in an incubator, then observed the area of bacterial growth inhibition using a digital caliper (Balouir et al., 2016). David and Stout (1971) in Ouchari et al., 2019, classified the zone of inhibition (ZOI): >20 mm (very strong); 10-20 mm (strong), 5-10 mm (medium); and <5 mm (no response).

7. Cell viability test

Peripheral blood collection of healthy people as much as 6 cc is then mixed with anticoagulant (heparin). Layer 3 ml of histopaque on the falcon, 3 ml of ficoll on top of histopaque, 6 ml of blood on it carefully. ficoll-hypaque centrifugation, take the monocyte layer, add 1:1

HBSS and pipette. Centrifugation, add HBSS, fungizone and penstripe Cells were placed on a 96-well microtiter plate 8 x 105 cells/well for 45 minutes at 37°C and washed 4 times with medium. Cells were placed in 24-well microtiter plates, treated with S. mutans (100µl/well) + toothpaste (5%, 10%, 15%), except C- (S. mutans only), then incubated for 5 hours. was stained with Trypan Blue. Live cells (in white) were counted under an inverted microscope with magnification 400 times from an average of 4 fields of view.

The data obtained were ANOVA followed by LSD test and descriptive.

Results

Analysis of the levels of polyphenols (393.0166 ± 85.5224 mg GAE/g and flavonoids (0.788592 ± 0.114787 mb QE/g) using 425 nm wavelength spectrophotometry with comparison of Quercetin (flavonoid) and gallic acid (polyphenol).

The results of the physicochemical test: the preparation of coffee bean skin toothpaste has a soft, homogeneous texture, smells of coffee, has a pH of 7, in accordance with the pH requirements for toothpaste preparations according to SNI 12-3524-1995, namely 4.5-10.5. All toothpastes are homogeneous. The viscosity of the coffee bean skin toothpaste is 200 dPa, according to the standard (200-500 dPa's). The spreadability that meets the standards is 15% (5) coffee bean husk toothpaste, while 5% (4) and 10% (4.5) gels are good between 5 to 7 cm.

sample name	Organoleptic
Coffee bean skin Toothpaste 5 %	Shape: Toothpaste Color: light brown Smell: Mint coffee scent
Coffee bean skin Toothpaste 10 %	Shape: Toothpaste Color: Brown Smell: Coffee, mint
Coffee bean skin Toothpaste 15 %	Shape: Toothpaste Color: dark brown Smell: Aroma of coffee, mint
Commercial toothpaste	Shape: Toothpaste White color Smell: Mint

Table 1. Organoleptic of Coffee bean skin.

Inhibition against S. mutans, there was a significant difference from the Anova analysis and LSD test (p 0.05). All groups can inhibit S. mutans except C-. The largest diameter was found at C+ followed by T1, T2 and T3, categorized as having a strong inhibitory power (>10). The higher the concentration of coffee bean rind toothpaste, the larger the diameter of the barrier (figure 1).

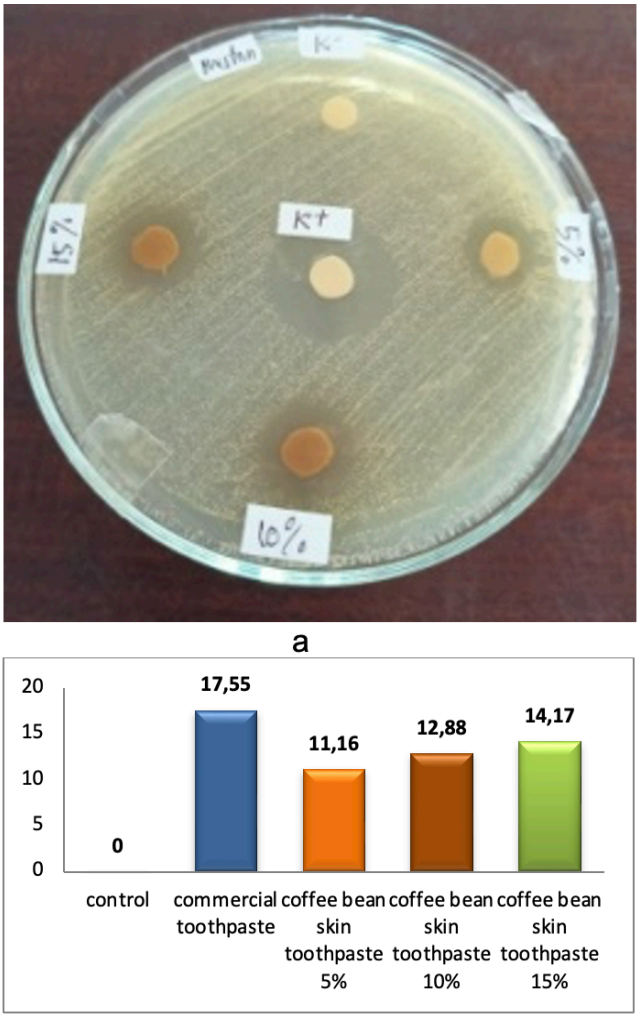

Figure 1. barrier zone of coffee bean skin toothpaste against S. mutans. The zone of inhibition (a), Bar chart (b).

The results of Anova and LSD analysis showed cell viability, C-(S. mutans) had more dead cells (dark in color), while C+(commercial toothpaste) had the most live cells (light/white in color). However, the number of live C+ cells with T1(Coffee bean skin Toothpaste 5 %), T2(Coffee bean skin Toothpaste 10 %), T3(Coffee bean skin Toothpaste 15 %) was not significantly different (Figures 2 and 3).

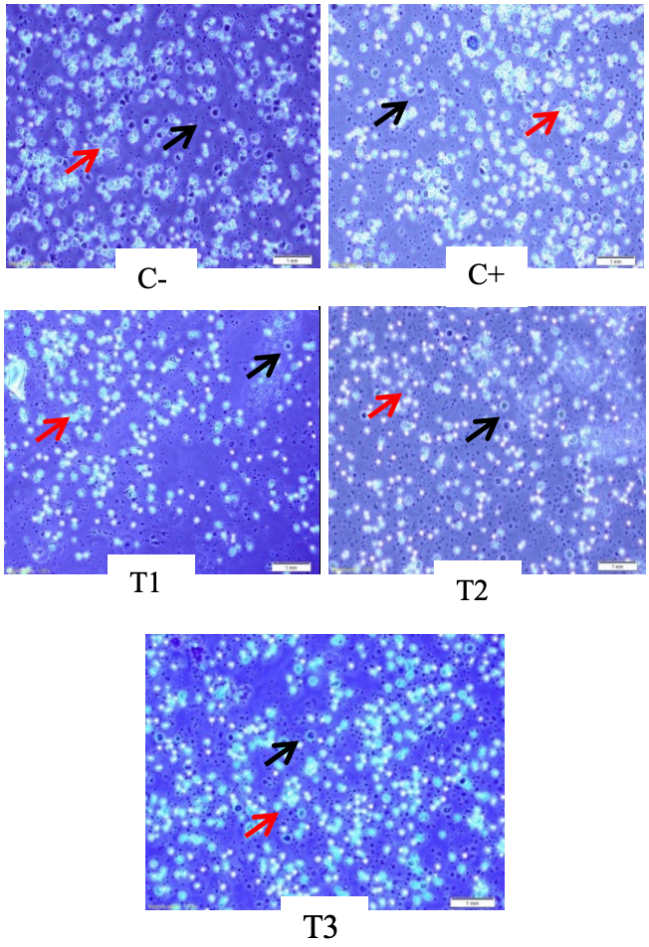

Figure 2. Cell viability was depicted with white cells (red arrows), while dead cells were dark (black arrows).

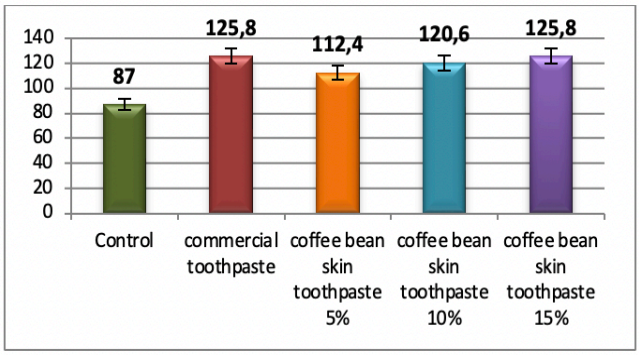

Figure 3. Bar chart of the cell viability of the coffee bean shell toothpaste.

Discussion

The preparation of coffee bean skin toothpaste is soft, homogeneous, has a distinctive coffee smell, has a pH of 7 in accordance with the pH requirements for toothpaste preparations according to SNI 12-3524-1995, namely 4.5-10.5, pH so that it does not irritate the oral mucosa. The smell of this coffee can be a special attraction, because in this world there are many coffee lovers, so we hope that many people will like it. Meanwhile, the dispersion power that most meets the standards similar to commercial toothpaste is coffee bean husk toothpaste with a concentration of 15%, meaning that the ability to spread toothpaste when applied to a toothbrush is good.

Coffee bean skin toothpaste with concentrations of 5%, 10%, 15%, and commercial toothpaste had a strong ability to inhibit S. mutans bacteria, although it was not as strong as commercial toothpaste. The higher the concentration of the coffee bean husk, the stronger the ability to inhibit the growth of S. mutans. This is presumably because of its bioactive content, the higher the concentration, the higher the bioactive content. Antioxidants (caffeic acid, chlorogenic acid, coumaric acid, sinapat acid, chlorogenic acid, flavonoids, melanoidin, faran, pitol and maltol) react directly with free radicals and form stable molecules. Chemical content of coffee such as flavonoids, xanthine, antioxidants, alkaloids, polyphenols act as anti-inflammatory, antibacterial; platelet aggregation inhibits the growth of Streptococcus mutans[7-11]. The content of alkaloids in coffee include caffeine and trigonelin. Caffeine is a purine alkaloid, has antimicrobial activity, which inhibits esterase enzymes along with DNA and RNA polymerase, inhibits cellular respiration, plays a role in DNA intercalation, causes damage and lysis[12-15]. Trigonelin which is a pyridine alkaloid can disrupt the stability of the bacterial cytoplasmic membrane which causes an imbalance in the metabolic function of bacteria so that the growth of bacteria inhibited[16]. Flavonoids are antioxidants that have antibacterial and antifungal effects because they contain phenol groups. Phenol groups can coagulate proteins, and reduce the surface tension of microbial cells[13.17]. In addition, the lipophilic nature of flavonoids interferes with microbial membranes, which will inhibit bacteria from forming a defense system[18]. Triterpenoids react with porins (trans membrane proteins) on the outer membrane of the bacterial cell wall, forming a strong polymeric bond, resulting in the destruction of the porin[7-9.19]. The ability to maintain cell viability by coffee bean rind toothpaste, especially at a concentration of 15%, is thought to be due to the presence of

flavonoids. Flavonoids activate Ca2+ in mitochondria which makes cells able to produce ATP so they can survive[20]. In addition, flavonoids have the function of preventing precipitation, damage to cell synthesis, protein denaturation, damage to cell metabolism, increasing the work of Transforming Growth Factor-beta 1 (TGF-1) which is the main factor to stimulate fibroblast proliferation. This antioxidant content is thought to work through suppression. on the production of NO (Nitric Oxide). This suppression mechanism can go through several possibilities. First, it may inhibit the action of the cytokine-induced NO synthase (iNOS) enzyme by controlling iNOS mRNA. Another possibility is to inhibit arginine transport through the CAT-2 mRNA (Cationic Amino Acid Transporter-2 mRNA) control mechanism. This can occur because it is suspected that monocyte cell damage is caused by the effects of nitric oxide (NO) free radicals. Usually NO can be produced by cells exposed to pathogens, which is caused by the formation of various cytokines and endotoxins of pathogenic bacteria that are damaging to cells. K attack pathogens, NO will be synthesized in large quantities. NO which is synthesized in large quantities will cause impaired function and cell proliferation (cytostatic)[21.22].

Conclusions

Toothpaste's contain coffee bean skin has physico-chemical properties, inhibited to S. mutans and increase cell viability equivalent to commercial toothpaste.

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Declaration of interest

There is no conflict of interest between all authors and all involved in the research or writing of articles.

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