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Antimicrobial activity of toothpaste containing coffee pulp and silver skin and its effect on tooth hardness and roughness

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ABSTRACT

Coffee Pulp and silver skin contain compounds that alleged as anti-microbials, so they might be used as toothpaste. We made toothpaste from ethanol extract of *Caffea robusta* pulp and silver skin, then concentration were adjusted to 20; 30; 40 and 50%. Furthermore, they were tested for antibacterial activity against *Staphylococcus aureus, Streptococcus sanguis* and *Candida albicans* and its effect on tooth hardness and surface roughness. Antibacterial test used paper disc diffusion method, the parameter was the diameter of inhibition zone. Analysis of tooth hardness and surface roughness were conducted on extracted tooth (maxillary first premolars) after being brushing, using Rockwell Hardness Testing Machine (Mitutoyo, Brazil) and Surface Roughness Tester (Roughness Tester TR 220, Dongguan, China), respectively. Data were analyzed using ANOVA and LSD. Results showed that toothpaste containing coffee pulp and silver skin, both have demonstrated strong antibacterial activity against *S. mutans, S. aureus, S. sanguis* and *C. albicans.* There was no effect of the application of its toothpaste on tooth hardness and surface roughness. Conclusion, Toothpaste containing pulp and silver skin of *Caffea robusta* has strong inhibition against *S. aureus, S. sanguis, C. albicans* and does not affect the roughness and hardness of the tooth surface in vitro. So, pulp and silver skin of *Caffea robusta* can used as ingredient of toothpaste, either alone or in combination with two.

Key words: *Caffea robusta*; *C. albicans*; coffee pulp; *S*; *mutans*; *S*; *aureus*; *S. sanguis*.

1 INTRODUCTION

The pulp, also known as the mesocarp, or mucilago, is the fleshy part of the fruit between the parchment and the skin. Other literature divides it into inner mesocarp or mucilago and outer mesocarp which is called pulp per se. Popularly, however, pulp is the exocarp, that is, the part of the mesocarp that is removed during the pulping process, and is a by-product of 55% of the whole berry. Most of the coffee pulp is produced when it is wet processing. The siverskin or perisperm or spermoderm, is the outer layer of the seed, consisting of sclerenchyma cells. Silverskin is thought to play a role in the accumulation and transport of biochemical compounds from the pericarp to the endosperm. (Figure 1) (Ameca et al., 2018; Farah, 2019). Coffee Pulp and silverskin contain bioactive compound including caffeine, phenol, chlorogenic acid, trigonelline and amino acids which are thought to have function as antimicrobials. Several studies reported that trigonelline can inhibit the growth of *S. mutans* (Nuhu, 2014; Anjani et al., 2020; Dewanti et al., 2019; Rante et al., 2021; Dewanti; Ristya, 2022). Our previous study show that the coffee silverskin contain polyphenols (393.0166 ± 85.5224 mg GAE/g and flavonoids (0.788592 ± 0.114787 mb QE) /g) (Dewanti; Ristya, 2022). In addition, coffee silver skin contains fiber, bioactive molecules, Calcium, high Potassium (Martuscelli et al., 2021). Calcium, Potassium, and Magnesium are the most abundant mineral elements (Gottstein et al., 2021). Those chemical compounds of coffee pulp and silverskin are thought to have benefit for making toothpaste.

Previosly, we had already made toothpaste with contain ethanol extract coffee Pulp and silverskin, each with concentrations of 5; 10 and 15%, and were test for organoleptic analysis, physical properties (pH, spreadability, viscosity, inhibition of *S. mutans*, neutrophil viability). The result showed that a concentration of 15% (both pulp and silverskin), demonstrated a pH, homogeneity, viscosity which meet to SNI (Indonesia Nasional Standart), good dispersion. This parameters are equivalent to parameter commercial toothpaste (Dewanti; Ristya, 2022). Base on that results, we wanted to know what about higher concentrations (20; 30; 40 and 50%). In addition, toothpaste must not damage the surface of the teeth or affect the hardness and roughness. Because if the hardness of the teeth decreases and the tooth surface gets rougher, it will make it easier for dental caries to occur. Meanwhile, tooth hardness can be affected by enamel remineralization. Tooth enamel remineralization can be affected by Calcium and phosphate. Calcium and Phosphate will be backfilled to strengthen teeth. The purposed of this study were to analyse antimicrobial activity of toothpaste containing pulp and silverskin of *Caffea robusta* and its effect on tooth hardness and roughness.

2 MATERIAL AND METHODS

2.1 Preparation of Extracts of coffee Pulp and silver skin

Extract pulp and silverskin of *Caffea robusta* were made by aerating until dry (900 grams), pulverizing it using a blender

and then filtering it using a sieve to get the powder. 200 g of pulp and silverskin powder was weighed, then put into a 1000 mL Erlenmeyer. Maceration was carried out using 96% ethanol 700 mL, stirred for 2-3 minutes and closed. Ultrasonication was carried out for 1 hour and filtered with filter paper. Dregs/residue added 96% ethanol 350mL. The stirring 2-3 minutes and closed. Ultrasonic 1 hour and filtered. Dregs/residue added 96% ethanol 350mL. The stirring 2-3 minutes and closed. Ultrasonic 1 hour and filtered. Dregs/residue added 96% ethanol 350mL. The stirring 2-3 minutes and closed. Filter until the residue/dregs were dry. Concentration of the filtrate using a rotary evaporator at 50 ºC 120 rpm until there was no ethanol dripping in the condensation. Extract concentration using an oven (water bath) at 50 ºC to constant weight. After that, the extract was weighed.

2.2 Preparation of Toothpaste

Toothpaste was made with the following composition. The composition of coffee bean skin toothpaste is as follows.

Making pulp and silverskin toothpaste in the following way. CMC (Carboxyl Methyl Cellulose) Na was weighed. Meanwhile 20 mL of hot aquadest was added to the mortar. CMC Na sprinkled over hot aquadest, wait 5 to 10 minutes until it swells, stir until homogeneous. Calcium carbonate, Magnesium Carbonate, Sodium Bicarbonate are weighed, then put into the mortar and stirred until homogeneous. Remove the material from the mortar. Sodium saccharin is put into the mortar, crushed until smooth, removed. All ingredients are stirred until homogeneous. Then added sorbitol, stirred. Sodium saccharin was added, stirred until homogeneous. Sodium Benzoate, Sodium Lauryl sulfate are weighed and the remaining distilled water is added, stirred until dissolved, then added to the previous mixture, stirred until homogeneous and becomes a paste. Add menthol which has been dissolved in ethanol, stir until it becomes a paste. So, we made toothpaste which we separated between coffee pulp and silverskin, each concentration is 20; 30; 40 and 50%.

2.3 Analysis of antibacterial activity

The method used is disc diffusion, by measuring the diameter of the resistance against, *S. aureus*, *S. sanguis*, *C. albicans* (pure isolate cultured in the Bioscience Dental Hospital University of Jember). The microorganisms were standardized with standard Mc. Farland 0.5 and obtained a

Figure 1: Coffee pulp (A), coffee silverskin (B).

Table 1: Toothpaste composition of fruit peel and coffee bean peel.

Material name	Function	Formulas (%)
Na CMC	paste base	1
Sorbitol	humectants	20
Menthol	Aroma	0.5
Sodium Benzoate	Preservative	0.5
Sodium Lauryl Sulfate	Surfactant	2
Sodium Saccharin	Sweetener	0.12
Calcium carbonate	abrasive	20
Magnesium Carbonate	abrasive	10
Ethanol 96%	Solvent	1
Distilled water ad	Solvent	100

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 35-37 b ºC in an incubator, then observed the area of bacterial growth inhibition using a digital caliper. Classified the Zone of Inhibition (ZOI): >20 mm (very strong); 10-20 mm (strong), 5-10 mm (medium); and < 5 no response (David; Stout, 1971; Hudzicki, 2016; Ouchari et al., 2019).

2.4 Analysis of tooth hardness and surface roughness

This study used permanent maxillary first premolars that had been removed that were free of caries, fracture, and restorations and were recommended by the ethical commission of the Faculty of Dentistry, University of Jember (1883/UN25.8/KEPK/DL/2023). Surface roughness was measured before and after treatment, while tooth hardness was carried out after treatment. Each sample was soaked in artificial saliva for 24 hours, brushed using an electric toothbrush. While toothbrush time is based on research by Pribadi, Cecilia and Aprodita, (2017) 30 seconds per day for two weeks (14 days). The sample that has been brushed is left for 60 seconds, rinsed with running water. Then the sample is soaked in artificial saliva until the next brushing. Roughness measurement with Surface Roughness Tester (Roughness Tester TR 220, Dongguan, China), hardness with Rockwell Hardness Testing Machine (Mitutoyo, Brazil).

3 RESULTS

Antibacteria activity of coffee pulp and silverskin on *S. aureus*, *S. sanguis* and *C. albicans* were presented on Figures 2, 3, 4. Meanwhile, the results of the tooth surface roughness test before and after brushing can be seen in the figure (Figure 5, 6). The results of the analysis of tooth hardness after brushing can be seen in Figure 7.

Figure 2 is the result of the presentation of the inhibition of coffee pulp and silverskin toothpaste against *S. aureus* for each treatment group. The greater the concentration, the greater the inhibitory power of coffee pulp toothpaste against *S. aureus*. The results of the ANOVA analysis of inhibition of coffee pulp and silverskin toothpaste against *S. aureus* ($p<0.05$), both there were a significant difference, after the different LSD test there were a significant difference between all groups. Coffee pulp toothpaste was stronger in inhibiting *S. aureus* than silverskin toothpaste.

Inhibition of coffee pulp and silverskin toothpaste against *S. sanguis* (Figure 3). The diagram showed that the inhibitory power of coffee pulp and silver skintoothpaste against S. *sanguis* increasing with higher doses. The results of the ANOVA analysis of inhibition of coffee pulp and silverskin toothpaste against *S. sanguis* ($p<0.05$), there were a significant difference, after the different LSD test there were a significant difference between all groups. Coffee pulp and silverskin toothpaste can inhibit the growth of *S. sanguis*, but coffee pulp has a stronger ability.

Inhibition of coffee pulp and silverskin toothpaste against *C. albicans* (figure 4), from the diagram it can be seen that the strength of the inhibitory power of coffee pulp and silverskin toothpaste against *C. albicans*, the higher the dose, the greater the inhibition. The results of the ANOVA analysis of inhibition of coffee pulp and silver skin toothpaste against *C. albicans* ($p<0.05$), there were a significant difference, after the different LSD test there were a significant difference between all groups. From the figure it can also be seen that coffee pulp toothpaste is stronger in inhibiting *C. albicans* than silverskin toothpaste, but the strength is not much different.

The surface roughness of the coffee bean skin before and after treatment treat with coffee bean skin, coffee fruit skin toothpaste (pictures 5 and 6). The results of the ANOVA analysis of the surface roughness of the coffee bean skin before and after treating with coffee bean skin toothpaste ($p<0.05$), there is a significant difference, after the different LSD test there is a significant difference between all groups.

Analysis of tooth hardness after brushing with coffee pulp and silverskin toothpaste (Figure 7). ANOVA analysis proved that of tooth hardness after brushing with coffee pulp and silverskin toothpaste ($p<0.05$), there was a significant difference, after the different LSD test there was a significant difference between all groups.

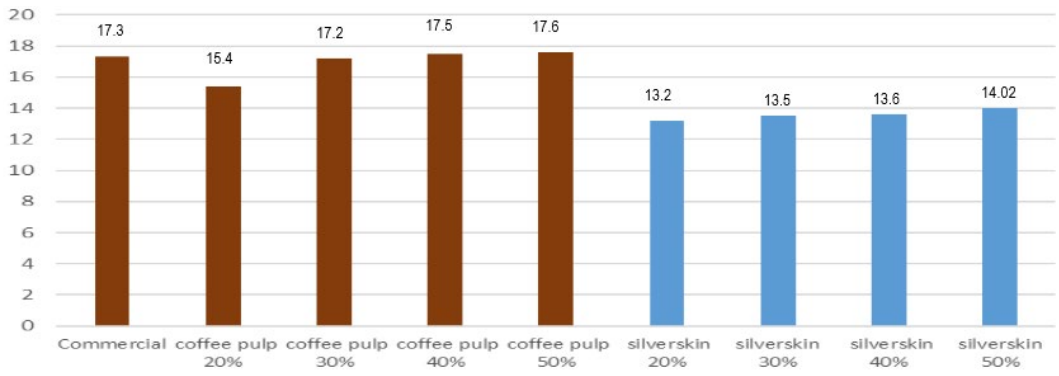

Figure 2: Inhibition of coffee pulp and silverskin toothpaste against *S. aureus*.

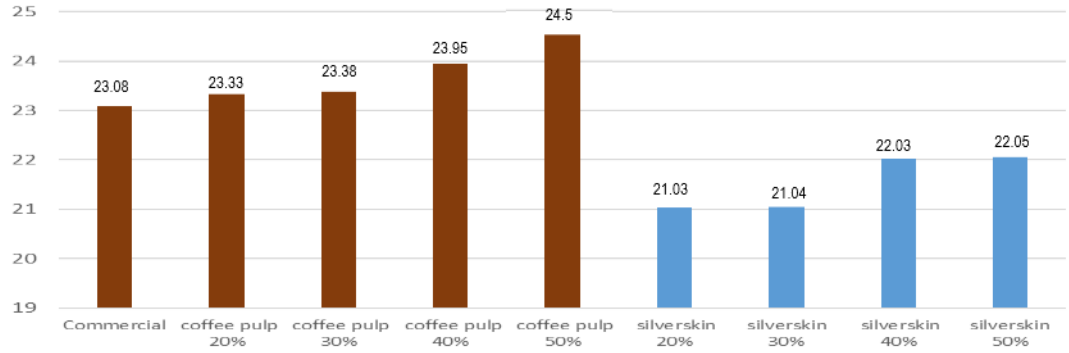

Figure 3: Inhibition of coffee pulp and silver skin toothpaste against *S. sanguis*.

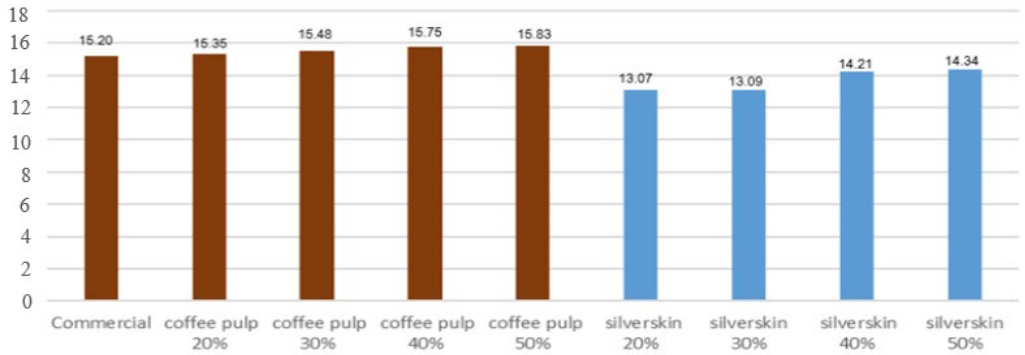

Figure 4: Inhibition of coffee pulp and silverskin toothpaste against *C. albicans*.

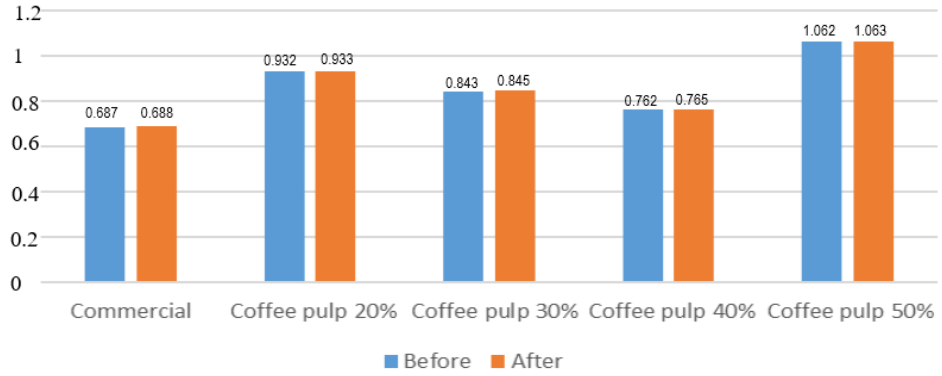

Figure 5: The surface roughness of the tooth before and after being treated with toothpaste contain coffee pulp.

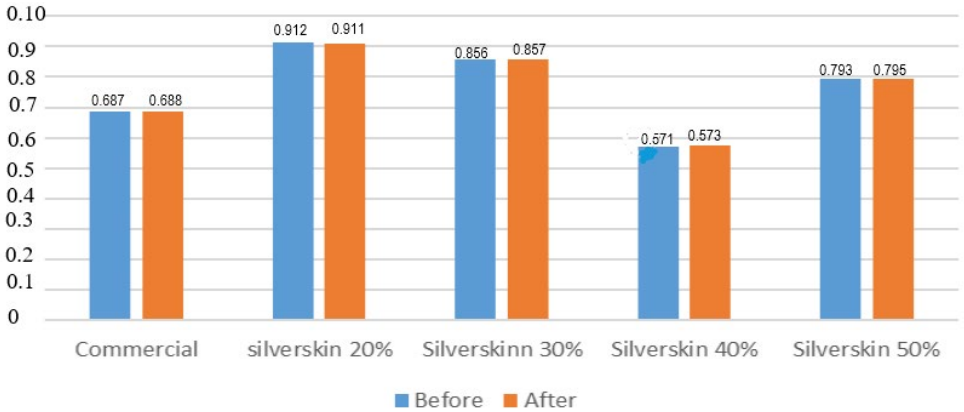

Figure 6: The surface roughness of before and after treated with silverskin toothpaste.

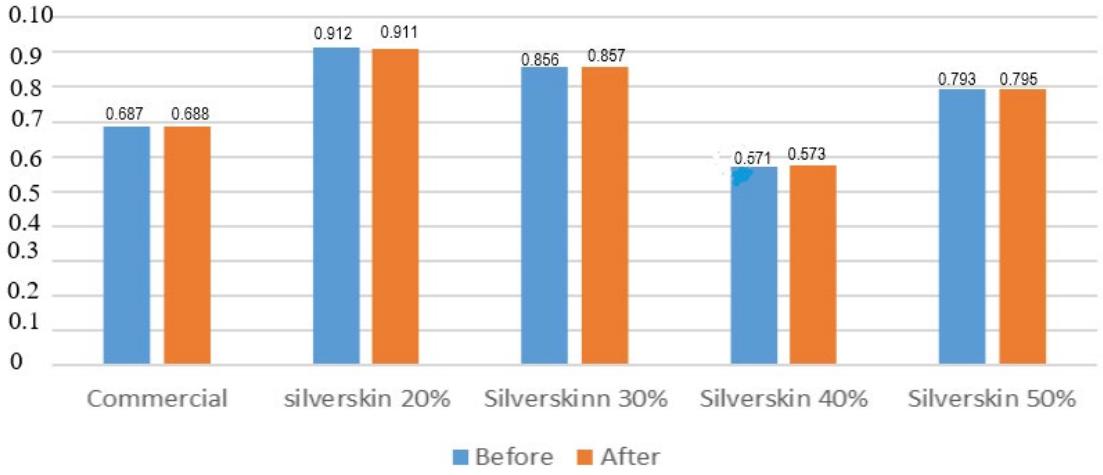

Figure 7: Analysis of tooth hardness after brushing with coffee pulp and silverskin toothpaste.

4 DISCUSSION

The results of the study proved that coffee pulp and silverskin from *caffea robusta* had strong inhibition against *S. aureus, S. sanguis* and *C. albicans*. This is presumably because of the content such as polyphenols, flavonoids, alkaloid content in coffee including caffeine and trigonellin. Polyphenols have the potential to protect against oxidative stress which prevents cell damage and works to damage bacterial cell walls (Dewanti et al., 2019). Caffeine is a purine alkaloid, can inhibit esterase enzymes along with DNA and RNA polymerase, inhibits cellular respiration, plays a role in DNA intercalation, causes damage and lysis (Dewanti; Ristya, 2022; Dewanti et al., 2022). Alkaloid compounds have a base group that contains nitrogen which will react with amino acid compounds that make up the bacterial cell wall and bacterial DNA, so that the cell wall layer is not formed completely and causes cell death. Alkaloid compounds having a base group containing nitrogen will react with amino acid compounds that make up the bacterial cell wall and bacterial DNA, so that the cell wall layer is not formed completely and causes cell death (Dahliati; Teruna; Jasril, 2014). d-phenylalanine, d-proline, and d-tyrosine isomers are thought to be active in inhibiting S. aureus biofilm formation. Amino acids prevent the localization of cell–cell adhesion proteins, thereby inhibiting the formation of aggregates necessary for biofilm development (Hochbaum et al., 2011). On the other hand, amino acids are the main source of nutrition for fungi, so the mechanisms by which amino acids may be exploited to identify novel drug targets and review potential hurdles to bring this approach into clinical practice (Mccarthy; Walsh, 2018).

The results of the analysis of tooth surface roughness and hardness tests of coffee pulp and silverskin from *caffea robusta*, there was no significant difference. This means that the roughness and hardness of the tooth surface is not affected by brushing using toothpaste on the seed coat and skin of the Robusta coffee fruit. This is in accordance with the theory that toothpaste must not damage the surface of the teeth or affect the hardness and roughness of the teeth. Because if the hardness of the teeth decreases and the tooth surface gets rougher, it will make it easier for dental caries to occur. The level of abrasiveness of toothpaste (Radioactive or Relative Dentine Abrasiveness (RDA), is a standardized test by ISO (International Organization for Standardization). It is said that the higher the RDA value, the greater the potential for abrasion to enamel and dentin. According to the ADA, the RDA value in over 100 is categorized as very abrasive. The recommended safety limit is below 250. Medium RDA category (60-100) and high RDA (> 100) (Priyanto; Irene, 2021). Meanwhile, tooth hardness can be affected by enamel remineralization. Tooth enamel remineralization can be affected by Calcium and phosphate. Calcium and Phosphate will be backfilled to strengthen teeth. This is presumably due to the high content of Calcium and Phosphorus in the skin of the Robusta coffee bean (Gottstein et al., 2021). This is also supported by research on the ash content of coffee skin samples from the Jember, Banyuwangi, and Malang areas, respectively, which were 6.93, 11.88 and 5.6%. Ash content shows much mineral content (Wardhana; Eka; Ahmad, 2019). Calcium and Phosphorus stimulate remineralization. In addition, the presence of antioxidants in coffee pulp and silverskin can also reduce the occurrence of demineralization. Demineralization that occurs is often also associated with the release of oxygen radically by peroxide which can react with minerals and organic substances in tooth tissue. The formation of enamel micro-scratches on the surface of the teeth will affect the hardness of the teeth and cause the retention of microorganisms, making it easy for dental caries, periodontitis and candidiasis to occur.

Tooth surface roughness due to toothpaste can be caused by many factors, such as toothbrush bristles, compressive strength when brushing teeth and toothpaste. The toothpaste factor can be caused by the abrasive ingredients found in

toothpaste. In addition, the roughness of toothpaste also depends on the hardness of the particles, shape, size, range of distribution and concentration. Therefore, the abrasiveness must be moderate so as not to cause damage to hard and soft tissues (Pachaly; Roselaine, 2012; Pratiwi; Deviyanti; Syifa, 2019). This is evident from our research on pulp of *Caffea robusta* derived from Jember Regency, East Java Province of Indonesia which contains high levels of protein, amino acids and bioactive components (Dewanti; Ristya, 2022).

5 CONCLUSION

Toothpaste from pulp and silverskin of *Caffea robusta* has strong inhibition against *S. aureus, S. sanguis, C. albicans* and does not affect the roughness and hardness of the tooth surface in vitro. So, coffee pulp and silver skin *Caffea robusta* can used as ingredient of toothpaste, either alone or in combination with two.

6 AUTHORS CONTRIBUTION

IDR wrote the manuscripts and performed the experiment, IDS supervised the experiment and co-work the manuscript, RWE review and approved the final version of the work.

7 REFERENCES

AMECA, G. M. et al. Chemical composition and antioxidant capacity of coffee pulp. **Ciência e Agrotecnologia**, 42(3):307-313, 2018.

ANJANI, G. et al. Bioactive components and antibacterial activity in Robusta coffee leaves (coffea canephora). **International Journal of Pharmaceutical Research,** 12(3):1374-1382, 2020.

DAHLIATI.; TERUNA, H. Y.; JASRIL. Sintesis dan uji aktivasi antibakteri dari senyawa 1-FENIL-3-(1-NAFTIL)-5-(2-KLOROFENIL)-2-PIRAZOLIN. **JOM FMIPA**, 1(2):396-402, 2014.

DAVID, W.; STOUT, T. R. Disc plate method of microbiological antibiotic assay. I. Factors influencing variability and error. **Applied Microbiology**, 22(4):659-665, 1971.

DEWANTI, I. D. A. R. et al. Robusta Coffee (*Coffeacanephora*) Decreasing IL-1α (Interleukin-1α) Expression and Increasing the Number of Fibroblast in Healing Process in Dental Pulp in Wistar Rats. **Journal of Mathematical and Fundamental Sciences**, 51(1):68-76, 2019.

DEWANTI, I. D. A. R. et al. The effect of steeping robusta coffee beans on Monocytes: Expression Of IL-1β and TNF-α Against Streptococcus mutans. **Coffee Science**, 14(4):477-483, 2019.

DEWANTI, I. D. A. R. et al. The 6th international conference on translational medicine and health sciences (ICTMHS). 28-29 October 2022. Undip Semarang, Indonesia. Available in: https://mkep.fk.undip.ac.id/events/the-6th-international-conference-on-translational-medicine-and-health-sciences-ictmhs/. Access in: July 31, 2023.

DEWANTI, I. D. A. R. et al. 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. **Journal of Dentomaxillofacial Science (J Dentomaxillofac Sci)**, 4(3):154-158, 2019.

DEWANTI, I. D. A. R.; RISTYA, W. E. Y. The toothpaste's containing coffee bean skin demonstrated the specific physicochemical properties, inhibited the growth of streptococcus mutans and increased the viability of neutrophil. **journal of international dental and medical research**, 5(1):66-71, 2022.

FARAH, A. **Coffee**: Production, quality and chemistry. The Royal Society of Chemistry. 2019. London. 836p.

GOTTSTEIN, V. et al. Coffee silver skin: Chemical characterization with special consideration of dietary fiber and heat-induced contaminants. **Foods**, 10:1705, 2021.

HOCHBAUM, A. et al. Inhibitory effects of D-Amino acids on staphylococcus aureus biofilm development. **Journal of Bacteriology**, 193(20):5616-5622, 2011.

HUDZICKI, J. Kirby-Bauer Disk Diffusion Susceptibility test protocol. **American society for microbiology ©** 2016.

MARTUSCELLI, M. et al. Characterization of coffee silver skin as potential food-safe ingredient. **Foods**, 10:1367, 2021.

MCCARTHY, M. W.; WALSH, T. J. Amino acid metabolism and transport mechanisms as potential antifungal targets. **International Journal of Molecular Sciences**, 19(909):1-12, 2018.

NUHU, A. Bioactive micronutrients in coffee: Recent analytical approaches for characterization and quantification. **ISRN Nutrition**, 384230:1-13, 2014.

OUCHARI, L. et al. Antimicrobial potential of actinomycetes isolated from the unexplored hot Merzouga desert and their taxonomic diversity. **Biology Open**, 8(2):bio035410, 2019.

PACHALY, R.; ROSELAINE, T. P. Analysis of surface roughness of human enamel exposed to bleaching agent and submitted to brushing. **Acta Odontol Latinoam**, 25(1):59-66, 2012.

PRATIWI, D.; DEVIYANTI.; SYIFA, A. Pengaruh sikat dan pasta gigi anak terhadap kekasaran permukaan SIK dan Kompomer. **JKGT**, 1(2):21-24, 2019.

PRIBADI, N.; CECILIA, G. J. L.; APRODITA, P. Y. Kekasaran PERMUKAAN resin komposit nanofiller setelah penyikatan dengan pasta gigi whitening dan nonwhitening. **ODONTO Dental Journal**, 4(2):73-78, 2017.

PRIYANTO, R.; IRENE, P. Studi laboratoris pengaruh pasta gigi charcoal dan whitening terhadap kekasaran permukaan enamel. E-Prodenta Journal of Dentistry, 5(1):414-423, 2021.

RANTE, H. et al. Antibacterial activity of robusta coffee (*Coffea robusta* L.) peel extract against human pathogen bacteria. **Journal of Experimental Biology and Agricultural Sciences,** 9:S264-S268, 2021.

WARDHANA, D.; EKA, R.; AHMAD, N. Characteristics of robusta coffee husk obtained from dry processing method of smallholder coffee plantation in east java. **Agritop**, 17(2):220-229, 2019.