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THE EFFECT OF *Musa acuminata* AND *Ocimum basilicum* MIXED EXTRACTS TO THE SURFACE HARDNESS OF BIOACTIVE COMPOSITE RESIN

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One of the key elements of oral health is oral hygienne maintenance.[1] The majority of oral health problems are dental caries and periodontal diseases.[2] According to the Basic Health Research (RISKESDAS) in 2018, it was stated that the prevalence of dental and oral health problems reached 57.6%, with 94.7% of people have tooth brushing behavior, only 2.8% who did it properly.[3] Tooth brushing should be cleaned all of the tooth surfaces. However, tooth brushing alone is not enough to prevent periodontal disease, dental caries, and also reduce the prevalence oral health problem. There is a need in additional ways to control plaque on a tooth surface that cannot be reached with the toothbrush. In addition of tooth brushing, mouthwash is widely used to maintain hygiene.[4]

Mouthwash is a substances that used in the oral cavity with the aim of inhibiting plaque-forming bacteria on the teeth, eliminating bad breath, preventing dental caries, and having a therapeutic effect on the oral cavity.[5,6] The composition is based on water, salts, preservatives, antimicrobial agents and alcohol.[6] Alcohol contained in mouthwash has antiseptic content, may prevent the accumulation of plaque and inhibit bacteria's growth in the oral cavity. The use of mouthwash which containing alcohol is not recommended due to adverse effects for some users, such as burning sensation in the oral cavity, increased risk of ulcer and malignancy in the oral cavity.[7] Herbal mouthwash can be used to

minimize the side effect of using mouthwash containing alcohol. Herbal mouthwash that can be produced presently is is a mouthwash made from Mauli Banana and Basil Leaf extracts.[8]

Mauli banana (*Musa acuminata*) is one of the typical plants that derived from South Kalimantan. Every part of mauli banana is packed with nutrition and health benefits, especially the stem of banana which is known to have antiseptic and antioxidant effects.[9] It has been proven in the study of Apriasari et al (2014) that methanol extracts of mauli banana stem at concentration of 6.25%; 12.5%; and 25% have bacteriostatic effects on *Streptococcus mutans*, concentration of 25% have the highest antibacterial activity and not toxic to the cells.[10]

Moreover, the basil leaf (*Ocimum basilicum* L.) is the plant that also has antibacterial ability. The plant is easily obtained due to its growth that spread almost throughout Indonesia.[11,12] Basil leaf has bioactive compounds that act as antioxidants, antimicrobials, antiviral, antihypertensive, and anti-inflammatory agents.[13] Essential oils as one of the active ingredients on basil leaf has the highest content named Linalool. Linalool is similar to terpenoid alcohol.[14,15] The study of Marlindayanti et al (2017) reported the basil leaf extract with a concentration of 20% may reduce plaque accumulation and increase the pH of the oral cavity if used as herbal mouthwash.[16]

Many studies reported the use of mouthwash daily may affects the mechanical properties of the dental restoration material. Composite resins have been widely used as dental restoration for decades. In the recent years, a great research and development of composite resins delivered new class of composite resins, so-called bioactive restorative resin. The material exerts a bioactive role in the prevention, remineralization and restoration of active carious lesions.[17,18] Mouthwash influences the durability of composite resin. The durability of composite resin restorations may decrease due to resin degradation. The effect is found to be related to the degree of acidity (pH) and alcohol content. Furthermore, low pH content in mouthwash affects solubility sorption, surface degradation, and also surface hardness as mechanical properties of the composite resin.[19,20] The objective of the present study was to determine the effect of mauli banana (*Musa acuminata*) and basil leaf (*Ocimum basilicum.)* extracts against the surface hardness of the bioactive composite resin.

MATERIAL AND METHODS

The study has passed the ethical clearance test published by the Faculty of Dentistry, Lambung Mangkurat University No.106 / KEPKG-FKGULM / EC / I / 2019. The specimens were fabricated with bioactive composite resin (Activa ™ Bioactive Restorative, Pulpdent), with 6 treatment groups and each group consisted of 5 specimens. 30 specimens with 10 mm of diameter and 2 mm of thickness based on ISO 4049 (2000) were prepared using plastic mold. The mold was placed on the top of glass slab and filled with bioactive resin until there was a slight excess, then condensed with Composite Filling Instrument. Matrix strip were placed on the surface and covered with glass slide on the top and gently pressed to obtain a smooth surface and to remove the material excess. All specimens were light-activated with a light cure LED unit for 20 s with an irradiation intensity of 800 mW/cm^2, subsequently stored in incubator for 24 h at 37ºC. After the period, the specimens were immersed for 7 days at 37ºC in a mixed solution of Mauli banana stem and basil leaf extracts with concentrations of 25% (group 1), 50% (group 2), 75% (group 3), and 100% (group 4). The controls used were Chlorhexidine gluconate 0.2% or CHX (group 5) and aquadest (group 6). The pH of all groups was recorded using a digital pH meter.

Mauli banana stem and basil leaf extracts were made using maceration method. Mauli banana stem and basil leaf extracts with concentration of 100% was made by immersing simplicia of Mauli banana stems for 5x24 hours while the basil leaf for 3x24 hours in 70% ethanol solvent. Maserat was filtered and evaporated using a rotary evaporator with a heating temperature of 40ºC and repeated until 100% extract was obtained. Mixed solution of Mauli banana stem extracts and basil leaf extracts with 100% concentration was a combination with a volume ratio of 1: 1 of 25% mauli banana stem extracts and 10% basil leaf extracts. A mixed solution of 100% mauli banana stem and basil leaf extract was further diluted using distilled water with concentrations of 25%, 50%, and 75% respectively.

Bioactive resins were carefully rinsed with aquadest, air dried and subsequently submitted to surface hardness test using the Vickers Microhardness Tester (Buehler Hight Quality Micro Hardness Tester model MM 0054, Japan). All the specimens were measured by given an indentation load of 100 gf for 15 seconds and the average diagonal length of the indentation results was calculated to obtain a surface hardness value.

Calculation of surface hardness with the Vickers Microhardness Test was using the following formula of the surface hardness value (VHN): [21,22]

$$VHN = \frac{(1.854)F}{d^2} \qquad d = \frac{d1 + d2}{2}$$

where F was the load applied in Newtons (gram), d was the diagonal indentation length of the two diagonals (µm), and 1.854 was the Vickers constant. Statistical analysis was using the One-way ANOVA test with a confidence level of 95% (α = 0.05), followed by Post Hoc Bonferroni analysis to determine the significance value.

RESULTS

The mean of surface hardness values of tested materials which immersed in mauli banana stem and basil leaf extracts with concentration of 25%, 50%. 75% and 100% were summarized in Table 1.

Table 1. Mean of Surface Hardness Value of all tested groups.

Groups	Mean ± Standard Deviation (VHN)
Mauli &basil extract (25%)	38,20 ± 2,58
Mauli & basil extract (50%)	41,40 ± 3,84
Mauli & basil extract (75%)	40,40 ± 3,55
Mauli & basil extract (100%)	49,95 ± 4,61
CHX	46,59 ± 1,10
AQUADEST	43,98 ± 3,82

For the mixed solution of mauli banana and basil leaf extract groups, the surface hardness values increased along with its concentration, except the 75% mauli banana and basil leaf extract. Based on table 1, the highest mean of surface hardness value of bioactive resin was on immersion of 100% mauli banana stem and basil leaf extracts (49.95 ± 4.61 VHN), while the concentration of 25% had the lowest surface hardness value (38.20 ± 2.58 VHN). The surface hardness values from the specimens immersed in CHX were higher than those immersed in the 25%, 50% and 75% mixed solution of Mauli banana and basil leaf extract groups and aquadest.

Table 2. Significance value of bioactive resin's surface hardness value.

Group	P value					
	25 %	50 %	75 %	100 %	CHX	Aquadest
25%	—	1,00	1,00	0,00*	0,21	0,12
50%	—	—	1,00	0,01*	1,00	0,38
75%	—	—	—	0,03*	1,00	0,13
100%	—	—	—	—	0,17	1,00
CHX	—	—	—	—	—	1,00
aquadest	—	—	—	—	—	—

abbreviation: *Post Hoc Bonferroni test*
*= significant (p<0,05)

Statistical analysis of data showed significant difference (p <0.05) in hardness values between 100% with 25%, 50% and 75% mixed solution. In comparison to the control group (aquadest), there was no siginificant difference (p >0.05) between specimens immersed in CHX and the mixed solution of mauli banana and basil leaf extracts in all concentration.

DISCUSSION

Daily used of mouthwash may cause many adverse effects in oral tissue and restorations.[23] This condition leads people to start using herbal-based mouthwash as an alternative. Herbal that can be used as a mouthwash are Mauli banana stem and Basil leaf extract. Information about the effect of herbal-based mouthwash toward the surface hardness of composite resin is still not widely available in any literature.[24] The exposure of composite resin against various oral conditions demands composite resin to exhibit significant durability. The durability of composite resin can be determined by its properties, such as the surface hardness. Hardness can be defined as the resistance of a material to penetration or indentation and related to its ability to abrade or be abraded by opposing dental structures or materials.[23,25] Therefore, any chemical softening on the surface of resin composite which resulting from the use of mouthwash would affect the clinical durability of the resin restoration.[23] Decrease in the composite resin hardness may result in premature failure of a composite resin restoration and subsequently lead to further replacement of restoration.[26]

In the present study, the surface hardness value increased along with the solution concentration, except 75% mixed solution of mauli and basil leaf extract. The highest surface hardness value found in

the 100% mixed solution of mauli banana and basil leaf extracts and significantly different with 25%, 50% and 75%. This result is related to the higher the concentration of the mixed solution of mauli and basil stem extracts, the higher the active substances contained in it. The most active substances in mauli banana stem extract are phenol-derived compounds namely tannin, flavonoids and saponins, while the most active substances in basil leaf extract are dominated by essential oils containing terpenoid alcohol in the form of linalool.[10,15] In addition, increasing the amount of substances in the mixed solution of mauli banana stem and basil leaf extracts result in higher viscosity of solution. Viscosity is affected by particle size, particle count, and concentration. The greater the concentration of the solution, the greater the viscosity of the solution.[19,27] A solution with high viscosity is difficult to penetrate into the Bioactive Glass (BG) particles in the bioactive composite resin. Therefore, even the 100 % mixed solution of mauli and basil leaf extract had the lowest pH among all group, due to its viscosity, could not alter the hardness. On the contrary, it kept the value of surface hardness.[27] It can be seen in the highest surface hardness value which belong to 100% mixed solution of mauli and basil stem extracts and followed by CHX group and aquadest group.

Based on the pH level, the present study result was not in agreement with Research by Poggio et al (2012), which stated that composite resins exposed to acidic solutions with different degrees of acidity (pH) could affect the surface hardness of composite resins.[25] In the present study, it was found that the higher the concentration of the mixed solution of mauli and basil stem extracts, the lower pH of solution (concentration of 25% has a pH 5.73; 50% has a pH 5.21; 75% have a pH 5.05; and 100% has a pH 4.95, respectively) which is more acidic when compared to aquadest. The lower the pH, the more acidic it is, and the higher the pH, the more alkaline it is going to be. However, the lower pH of immersion solution exhibits the higher surface hardness value. This is due to the viscosity that considered play dominant role. Therefore, pH level may not influence the hardness. In theory, the pH of the tested solution could be one of the factors that may result in composite resin degradation. The study of Miranda et al (2011) stated that the low pH of solution may induce phenomena of sorption and hygroscopic expansion, due to the production of methacrylic acid as the result of the degradation process of the enzymatic hydrolysis.[28]

The group of bioactive composite resin that was immersed in aquadest had lower surface hardness value than the group that immersed in the 100% mixed solution of mauli banana stem and basil leaf extracts. It is assumed that the decrease in the surface hardness was due to a chemical softening of the bioactive resin matrix by water molecule.[29] In accordance to the research of Porto et al (2014), water absorption occurs through diffusion of water into a composite resin which may result in an increase in mass through the accumulation of water molecules in the micro space of the bioactive composite resin, therefore, disintegrate the filler and matrix of the bioactive composite resin and result in degradation. [30] Water molecules, namely H^+ and OH^- ions, elicited from water are believed to be factors that causing the softening of the resin composite material surface.[29]

The results of the study seem to be inversely proportional to the theories that described about pH level and alcohol contain in linalool. Handayani 2016 stated that alcohol could penetrate into the polymer and cause the release of unreacted monomers that result in damage to the polymer chain. [31] The low pH of alcohol-containing mouthwash catalyzes the ester group from dimethacrylate monomer which present in the composite. This is followed by hydrolytic degradation of the composite material. [26] Alcohol and phenol have a hydroxyl group (-OH) that binds to the aromatic ring found in a solution of the mauli banana stem and basil leaf extract. The hydroxyl group (-OH) of the active substance in the mixed solution may undergo molecular breakdown to form H+ and C_6H_5O- or phenolic acid.[31] This acid causes breakage of the siloxane Si-O-Si bond which is a composite resin binder into Si-OH. Group (-OH) plays an important role as a link between polymer chains with one another (cross link). Therefore, it will strengthen between layers or chains in bioactive resins. If the bonding of the group (-OH) in the composite resin is stronger, then the surface hardness of the composite resin will also be stronger.[32]

Apart from the active substance of the mixed solution of mauli banana stem extract and basil leaf extract, the content of bioactive glass (BG) also influences the mechanical properties of the bioactive composite resin. The presence of BG in bioactive composite resin establish the resin surface hardness even higher when exposed to a solution. When BG is exposed to water, BG may undergo the formation of hydroxyapatite (HA). In the initial process, hydrogen ions from the solution may release sodium and calcium ions, therefore Si-O-Si bond will be damaged. Silica will bind to the OH group and form a Si-OH bond. When the Si-OH group settles, the silica-rich layer is repolymerized, attracting calcium and phosphate ions to precipitate in the form

of Amorphous Calcium Phosphate (ACP), which then crystallizes to HA. [27] This is thought to be able to preserves the structural bond of bioactive resin, therefore the surface hardness is maintained.

Dental professionals frequently search the most esthetic material for dental restoration with the highest wear resistance properties. However, its performance depends on the environment in which they are placed and worked on. Mouthwash and contact to the restorations, influencing on its physical and mechanical properties due to its composition.[6] Clinically, the mouthwash effects on resin composites may be different according to some factors, such as acquired biofilm, beverages, oral care products and food habits, which cannot be reproduced *in vitro*. Those factors may interfere with the physical and mechanical properties of the materials, influencing the durability of resin composite. [28]

The limitations of this in vitro test are related to the fact that the conditions at the time of the study were not in accordance with the actual state of the oral cavity. However, this study was managed to approach the actual state of immersion in saline solution and stored in incubator at 37 which is the normal temperature of the oral cavity. On the basis of the results and despite the limitations of this study, it seems reasonable to conclude that immersion of bioactive resin composite in the mixed solution of Mauli banana stem (*Musa acuminata*) and Basil leaf (*Ocimum basilicum*) extracts not alters the composite resin surface hardness compared to chlorhexidine and aquadest.

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