ORIGINAL ARTICLE

Nickel and chromium ions release from stainless steel orthodontic bracket immersed in green betel leaf extract: an in vitro study

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ABSTRACT

Introduction: Corrosion on stainless steel brackets can occur as a result of using green betel leaves in the oral cavity because they contain a protein that functions as an electrolyte medium, affecting the release of nickel and chromium ions as an early sign of corrosion. The purpose of this study was to analyze the amount of nickel and chromium ions released from a stainless steel orthodontic bracket due to immersion in 25% green betel leaf extract. **Methods**: The type of research used was laboratory-experimental with a post-test control group design. Tests were carried out on 27 samples, which were divided into three test groups. Brackets were soaked in 25% green betel leaf extract, artificial saliva, and a mixture of 25% green betel leaf extract and artificial saliva for 48 hours and measured using the Atomic Absorption Spectroscopy (AAS). Data analysis in this study using the Shapiro-Wilk normality test and the homogeneity test using the Levene test. The normality and homogeneity test results were fulfilled, thereby permitting the continuation of the analysis with parametric tests, namely the one-way ANOVA test for nickel ions, followed by the Post Hoc Tukey test for nickel ions and the independent T-test for chromium ions. **Results**: The group soaked in 25% green betel leaf extract showed the highest nickel ion release (16.37 mg/kg), while the highest chromium ion release (18.03 mg/kg) was observed in the group exposed to the mixture of 25% green betel leaf extract and artificial saliva. One-way ANOVA results revealed a significant effect of soaking in green betel leaf extract on nickel ion release ($p=0.001$), confirmed by Tukey's Post Hoc test ($p=0.001$). The T-test indicated significant differences in chromium ion release ($p=0.001$). **Conclusion**: Green betel leaf extract has an effect on the release of nickel and chromium ions in stainless steel orthodontic brackets.

KEYWORDS
Orthodontic bracket, Stainless steel, Nickel, Chromium, Green betel leaf.

INTRODUCTION

Orthodontic treatment aims to avoid tissue damage, both aesthetically and somatically. In dentistry, there are several things that must be considered, such as prevention, interception, and correction of malocclusion and all other abnormalities in the dentofacial region. Orthodontic appliances used to treat malocclusion consist of 2 types, namely removable orthodontic appliances and fixed orthodontic appliances.[1,2] A fixed orthodontic appliance is an appliance that is attached to the tooth and cannot be removed by the patient himself.

Based on their ability to generate force, the components that make up the fixed orthodontic appliance system consist of two types: active components and passive components.[3] The main component of fixed orthodontic treatment is the bracket. Tooth movement can occur depending on the wires and springs attached to the bracket.[4] The bracket is a component that functions as a handle that transmits force from the active component to the teeth. Brackets have one or more slots that accept archwire.[5] The most commonly used bracket slot dimensions are 0.018 x 0.025 inches and 0.022 x 0.028 inches. A wider 0.022-inch slot width will produce lighter orthodontic forces than a 0.018-inch slot and can provide more comfortable orthodontic forces at the start of treatment.[6]

Stainless steel is the standard material used in orthodontic brackets. The type of stainless steel commonly used is AISI (American Iron and Steel Institute) Type 316L 'marine grade' stainless steel, which is an austenitic chromium-nickel steel with a composition of about 63–70% iron, 16–18% chromium, 10–14% nickel, and 2-3% molybdenum.[5] Stainless steel brackets have excellent anticorrosive properties, but they can corrode if they are exposed for a long period of time—months or even years—to potentially hazardous physical and chemical substances in the oral cavity.[7]

Corrosion is the process of losing some metal elements. Physical changes to the metal, such as discoloration and roughened surfaces, as well as chemical changes brought on by the release of metal ions known as corrosion products, are all indications of corrosion.[8] Nickel, manganese, iron, chromium, and copper are the corrosion products generated by stainless steel brackets. Due to their detrimental consequences, the release of nickel and chromium ions is among these corrosion products that is most extensively researched.[9]

Nickel gives the bracket good properties for formability, hardness, and heat resistance, while chromium is useful for increasing the resistance of stainless steel brackets to corrosion due to the formation of an oxide layer that is very attached to the surface and prevents further reaction with the metal below the surface.[9,10] The release of these metal ions can cause adverse biological effects both locally and systemically. Locally, the ions released can affect oral tissues by inhibiting the activity of enzymes or mitochondria and damaging DNA.[11]

In oral health, one type of plant commonly used as a medicinal plant is green betel leaf (*Piper Betle L.*).[12] The betel plant has a length that reaches tens of meters. The shape of the betel leaf is flat, resembling a heart, and the stem is rather long. The leaf surface is green and smooth, has a sharp tip, grows alternately, is stalked, and gives off a distinctive aroma when squeezed.[13] Betel leaves contain essential oils that have antimicrobial activity. The essential oil contained in betel leaves is composed of several chemical components, such as beta-phenol, chavicol, and other phenolic compounds.[12]

Chavicol is the most common component found in betel leaves. Phenol compounds contained in betel leaf essential oil are strong antimicrobial and antifungal and effectively inhibit the growth of several types of bacteria.[14] Betel leaf can be used as a mouthwash that can eliminate bad breath because it contains an antiseptic that can kill bacteria.[12] In addition, betel leaf extract can also increase the amount of saliva, which will increase the amount of peroxidase, lysozyme, and antibodies that inhibit the growth of bacteria in the oral cavity.[15]

Based on the foregoing context, the research hypothesis was obtained, namely that green betel leaf extract has an effect on the release of nickel and chromium ions in stainless steel orthodontic brackets. This study showed the release of nickel and chromium ions in stainless steel orthodontic brackets immersed in green betel leaf extract with a greater amount of release than the release that occurred in the artificial saliva control group. Based on the results of previous studies, there are no study using 3 test solutions and using different ion release measuring instruments. The purpose of this study was to analyze the release of nickel and chromium ions that occur from stainless steel orthodontic brackets following immersion in green betel leaf extract.

METHODS

This type of research is laboratory experimental research, with a posttest control group design. The research began with the production of green betel leaf extract at the Herbal Laboratory of Yarsi University, followed by immersion in the bracket and calculating the amount of released nickel and chromium ions at the Testing and Certification Services Laboratory Unit, Bogor Agricultural University, in May-June 2022.

The research sample was a stainless steel maxillary Mini Roth 0.022-inch orthodontic bracket, immersed in 3 test groups, namely 25% green betel leaf extract, artificial saliva, and a mixture of green betel leaf extract and artificial saliva. The artificial saliva used was based on the composition of McDougall's artificial saliva with a pH of 6.8 consisting of potassium chloride, sodium chloride, sodium bicarbonate, di-sodium hydrogen phosphate heptahydrate, magnesium sulfate heptahydrate, and calcium chloride.

Based on the findings of sample calculations made using the Frederer method, it was found that each test group needed more than 8.5 samples to be considered valid, bringing it to 9 samples. Because there were three groups, the total number of samples was 27. The three groups consist of the artificial saliva group as the control group, the 25% green betel leaf extract group and a mixture of 25% green betel leaf extract and artificial saliva as the treatment group.

The research began with the extraction of 25% green betel leaf by maceration. 500 grams of dried green betel leaves were cut into small pieces, and then put in a container, and 96% ethanol solvent was added. The extraction process was carried out for 5 days in a place protected from light, and then the dregs were macerated for 2 days. The extract was distilled using a rotary evaporator to obtain a thick green betel leaf extract. After obtaining a thick extract, 2.5 grams of green betel leaf extract and 10 ml of distilled water were required to make green betel leaf extract at a 25% concentration.

After making 25% green betel leaf extract, a mixture of 25% green betel leaf extract and artificial saliva was made as the second treatment group. The test solution was prepared by mixing 5 ml of 25% green betel leaf extract with 5 ml of artificial saliva. After that, the samples were grouped, each consisting of nine maxillary stainless steel orthodontic brackets. The stainless steel brackets were soaked and stored in an incubator for 48 hours at 37 °C. After 48 hours, the bracket was separated from the test solution and continued with analysis using the Atomic Absorption Spectroscopy (AAS) to determine the amount of nickel and chromium ion release that occurred.

Data analysis was performed using the Shapiro-Wilk normality test and the homogeneity test using the Levene test. The normality and homogeneity test results were fulfilled, thereby permitting the continuation of the analysis with parametric tests, namely the one-way ANOVA test for nickel ions, followed by the Post Hoc Tukey test to determine which treatment groups provided differences in nickel ions and the independent T-test for chromium ions.

RESULTS

The results showed that the average values for the release of nickel and chromium ions that occurred in stainless steel orthodontic brackets were immersed in 3 test solutions: 25% green betel leaf extract, artificial saliva, and a mixture of green betel leaf extract and artificial saliva, as outlined in Table 1.

Table 1. Average amount of release of nickel and chromium ions after immersion in the three test groups

Groups	Nickel Ion		Chromium Ion	
	Mean (mg/kg)	St.dev	Mean (mg/kg)	St.dev
Artificial Saliva	1.95	0.059	-	-
Green Betel Leaf Extract + Saliva	3.78	0.061	18.03	0.074
Green Betel Leaf Extract 25%	16.37	0.067	4.13	0.068

According to Table 1, the average number of nickel ions in the control group, specifically artificial saliva, was recorded with the lowest average value of 1.95 mg/kg, and the average number of chromium ions could not be detected (<0.5). In the mixed group of 25% green betel leaf extract and artificial saliva, the average number of nickel and chromium ions was higher than the control group, with the number of nickel ions being 3.78 mg/kg and chromium ions being 18.03 mg/kg. The 25% green betel leaf extract group produced the highest average number of nickel ions, namely 16.37 mg/kg, while the average number of chromium ions had a lower average than the mixed group of 25% green betel leaf extract and artificial saliva, namely 4.13 mg/kg.

Table 2. Normality test using the Shapiro-Wilk test

Groups	Sig *Shapiro-Wilk Nickel Ion*	Sig *Shapiro-Wilk Chromium Ion*	Result
Artificial Saliva	0.999	-	Normal
Green Betel Leaf Extract + Saliva	0.688	0.468	Normal
Green Betel Leaf Extract 25%	0.915	0.725	Normal

Table 3. Levene's homogeneity test

Data	*Levene's Test*	
	Sig	Result
Amount of Nickel Ion	0.782	Homogen
Amount of Chromium Ion	0.798	Homogen

Based on the Shapiro-Wilk normality test, it was confirmed that the data on the number of nickel and chromium ions exhibited a normal distribution (sig > 0.05). Therefore, the test comparison proceeded with a homogeneity test. The data homogeneity test revealed that the number of nickel and chromium ions had a sig Levene Test value of > 0.05, indicating that the variety of data was homogeneous. The requirements for normality and homogeneity had been met, so to determine the effect of immersing green betel leaf extract on the release of nickel ions in stainless steel orthodontic brackets, the one-way ANOVA test was used.

Table 4. One-Way Anova test on nickel ion amounts

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1109.380	2	554.690	141322.318	0.001
Within Groups	0.094	24	0.004		
Total	1109.474	26			

The results of the one-way ANOVA test showed that the sig value of the F test was 0.001 (sig <0.05), which indicated that there was an effect of soaking green betel leaf extract on the release of nickel ions in stainless steel orthodontic brackets in the three treatment groups. To determine which treatment groups have a significant impact, the post-hoc Tukey test was subsequently utilized.

Table 5. Post-hoc Tukey Test results: number of nickel ions

Treatments		**Mean Difference**	**Std. Error**	**Sig**	**Results**
Artificial Saliva	Green Betel Leaf Extract + Saliva	-1.83000*	.02953	0.001**	Significant
	Green Betel Leaf Extract 25%	-14.42000*	.02953	0.001**	Significant
Green Betel Leaf Extract + Saliva	Green Betel Leaf Extract 25%	1.83000* -12.59000*	.02953 .02953	0.001**	Significant

** $p < 0.01$; * $p < 0.05$

Post hoc Tukey test results showed that there was a significant difference in the number of nickel ions in the treatment group (25% green betel leaf extract and a mixture of 25% green betel leaf extract and artificial saliva) compared to the control group (p = 0.001). Likewise, the two treatment groups (25% green betel leaf extract and a mixture of 25% green betel leaf extract and artificial saliva) also showed significant differences. Thus, it can be concluded that the administration of 25% green betel leaf extract resulted in the greatest release of nickel ions compared to the other groups.

Table 6. Results of Independent T-Test

Groups	**Mean**	**Sig**	**Results**
Green Betel Leaf Extract 25%	4.13	0.001	Significant
Green Betel Leaf Extract + Saliva	18.03		

The independent t-test was used to examine the effect of soaking green betel leaf extract on the release of chromium ions in stainless steel orthodontic brackets. The results of the independent t-test showed that there was an increase in the release of chromium ions after being given a mixture of 25% green betel leaf extract and artificial saliva, with a significant difference (sig = 0.001).

DISCUSSION

Stainless steel orthodontic brackets are an important element of orthodontic appliances because they can determine the force of the braces and stay in the mouth for a long time. The presence of nickel and chromium content in stainless steel brackets will increase the bracket's resistance to corrosion.[8] Based on the results of the research that has been done, the amount of nickel ions contained in the bracket before immersion in the three test groups was 58184.63 mg/kg, while chromium ions were 20.40 mg/kg. After 48 hours of immersion, nickel and chromium ions were released from the brackets immersed in each of the test groups.

The release of nickel and chromium ions that occurred after immersion for 48 hours in 25% green betel leaf extract showed the highest average release of nickel ions, namely 16.37 mg/Kg and the average number of chromium ions, namely 4 .13 mg/Kg. In the mixed group of 25% extract and artificial saliva, there was a release of nickel ions with an average of 3.78 mg/Kg and the number of chromium ions showed the highest average result, namely 18.03 mg/Kg. In the artificial saliva control group there was also the release of the lowest average amount of nickel ions, namely 1.95 mg/Kg and chromium ions < 0.5 mg/Kg so that they could not be detected by Atomic Absorption Spectroscopy (AAS).

The results showed that the release of nickel and chromium ions occurred more in the 25% green betel leaf extract treatment group compared to the artificial saliva control group. The results of this study are in line with the research conducted by Dundu et al. (2017) regarding the release of metal ions (Ni, Cr, and Fe) that occur in orthodontic brackets due to immersion in a 50% betel leaf extract solution, aquabidest, artificial saliva, and sodium fluoride. Based on the results of that study, the highest release of metal ions occurred in the 50% green betel leaf extract solution group, and the smallest release of Ni and Fe ions was in the group immersed in saliva.[16] The difference in the release of nickel and chromium ions in the two test groups was due to differences in pH. green betel leaf extract and artificial saliva. Green betel leaves have acidic properties with a lower pH, which is 4.9, compared to the pH of artificial saliva, which is 6.8.

Green betel leaf is a medicinal plant that is rich in chemical compounds that have a variety of biological activities. Betel leaves contain water, protein, carbohydrates, minerals, fat, fiber, and 4.2% essential oils, which consist of chavicol, cavibetol (bethel phenol), and allylpyrocatecol (hydroxychavicol). Other compounds contained in it are allylpyrocatecol mono and diacetate, carvacrol, eugenol, eugenol methyl ether, p-cymene, cineol, caryophyllene, kadinen, estragol, terpenes, sesquiterpenes, phenylpropane, tannins, carotenes, thiamine, riboflavin, nicotianic acid, vitamin C, sugar, starch, and amino acids.[15,17,18] The content of protein and vitamin C contained in green betel leaves can affect the release of nickel and chromium ions because they can act as electrolyte media, which can trigger electrochemical reactions as a sign of corrosion.[16,19]

Corrosion can increase the bracket’s surface roughness, which causes an increase in the frictional force between the bracket and the archwire, so it is necessary to apply a greater force to overcome friction and produce physiological movement of the teeth.[20,21] Friction is a force of resistance to the movement of an object that moves in contact with another object. Friction in orthodontics can reduce the efficiency of orthodontic appliances. The magnitude of the force lost to overcome the amount of friction that occurs ranges from 12-60%, resulting in additional drag on the anchorage and reducing the speed of tooth movement.[22]

According to the results of this study, in the 25% green betel leaf extract treatment groups, nickel ion release was higher than its chromium ion release. The results of this study are in line with research conducted by Sumule et al. (2015) regarding the release of nickel and chromium ions in stainless steel orthodontic brackets immersed in carbonated drinks.[23] This may occur because the composition of the stainless steel orthodontic brackets used in this study contained more nickel ions than chromium ions. Stainless steel brackets with a higher nickel composition will release more nickel ions because nickel atoms are not tightly bound to other metal atoms, so the possibility of nickel being released from the stainless steel alloy increases.[24] However, this is inversely proportional to the results of research conducted by Bonde et al. (2016), which revealed that stainless steel orthodontic wires immersed in coconut water exhibited a higher release of chromium ions compared to nickel ions. This may occur due to the composition of stainless steel, which generally contains 18% chromium and 8% nickel. In addition, chromium also has a more negative electrode potential value than nickel, so chromium is more reactive and releases electrons more easily.[25]

The results also showed that there was a release of nickel ions in the control group, which was a stainless steel orthodontic bracket that was immersed in artificial saliva. This is because saliva is an electrolyte that allows reactions to occur between metal ion brackets and saliva, resulting in electrochemical damage to the brackets.[19] Saliva contains bioactonate, chloride, potassium, sodium, nitrogen compounds, and protein. A mixture of high chlorides in saliva or intake of various foods and drinks with low pH can affect the release of metal ions into the oral cavity.[26] In addition, chloride ions can damage the oxide layer on the bracket surface and cause the release of metal ions such as nickel, iron, chromium, molybdenum, and titanium. Nickel ions have soluble properties in salivary fluid, so the length of time the bracket is in contact with salivary fluid can affect the release of metal ions.[27] In this study, the release of chromium ions in the control group of artificial saliva was not detected. This could happen because the amount of chromium ions released is below 0.5 mg/kg, which is the detection limit of the Atomic Absorption Spectroscopy used in this study.

According to the research findings, the tested stainless steel orthodontic bracket released nickel and chromium ions at levels that are still considered safe. The average daily intake of Ni from food sources is 200-300 µg and Cr is 280 µg, while the concentration of Ni in drinking water is generally below 20 µg/L and Cr is around 0.43 µg/L.[24] On the other hand, if nickel's absorption exceeds 2.5 µg/kg, it can cause allergic symptoms. This absorption rate is also lower than concentrated doses that can cause allergic reactions, namely 600–2500 µg.[28] Higher doses of chromium ion release can also induce side effects, such as insomnia or sleep disturbances, headaches, vomiting, and diarrhea.[29] In addition, the release of chromium ions can reduce corrosion resistance because chromium provides corrosion resistance.[27] Excessive nickel and chromium ion release over time will harm the body's health because they are heavy metals that can trigger type IV hypersensitivity reactions.[10]

The results of this study show that green betel leaf extract can cause the release of nickel and chromium ions in the tested stainless steel orthodontic brackets; however, the amount of chromium ion release that occurred in the artificial saliva control group was very small, so it could not be detected by the Atomic Absorption Spectroscopy (AAS). Consequently, researchers propose that future research on the release of nickel and chromium ions in green betel leaf extract should include increasing the number of samples and using different types of orthodontic brackets, as well as studying changes in the surface of the brackets after soaking to determine the type of corrosion that occurs on the brackets.

In this study, we only looked at the release of nickel and chromium ions that occurred when immersed in green betel leaf extract, artificial saliva, and a mixture of 25% green betel leaf and artificial saliva but did not examine the amount of corrosion that occurred. The release of nickel and chromium ions occurred in stainless steel orthodontic brackets immersed in three test groups, namely 25% green betel leaf extract, artificial saliva, and a mixture of green betel leaf extract and artificial saliva for 48 hours. The results obtained from the test indicated that the largest release of nickel ions occurred in the 25% green betel leaf extract group, namely 16.37 mg/kg, while the largest chromium ion release occurred in the mixed group of 25% green betel leaf extract and artificial saliva, namely 18.03 mg/kg. From the results of this test, the smallest release of nickel and chromium ions occurred in the artificial saliva group.

The limitation of the research is the amount of chromium ion release that occurred in the artificial saliva control group was very small, so it could not be detected by the Atomic Absorption Spectroscopy (AAS).

CONCLUSION

Green betel leaf extract has an effect on the release of nickel and chromium ions in stainless steel orthodontic brackets. The amount of nickel and chromium ions released by these stainless steel orthodontic brackets is still within the safe threshold because the maximum amount that might trigger an allergic reaction is between 600 and 2500 µg. The implication of this research is that excessive nickel and chromium ion release can have negative effects on health as well as increase the friction force, which in turn increases the pull on the anchoring and slows tooth movement.

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