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Original article

Effect of preservative sodium benzoate on the stability of bilimbi fruit (*averrhoa bilimbi l.*) leaf extract and corrosion rate of stainless steel wire

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ABSTRACT

Introduction: Tannin in bilimbi leaves is a natural corrosion inhibitor for orthodontic wires. The research investigated the stability of bilimbi leaf extract when combined with sodium benzoate. **Objective**: This study aimed to evaluate the effect of sodium benzoate on the stability of bilimbi leaf extract and the corrosion rate of stainless steel orthodontic wires throughout 0, 21, and 42 days. **Methods:** Thirty-two samples of 0.7 mm diameter stainless steel wire were divided into control (K) and treatment groups (P). Initial weight measurements were obtained using an analytical balance, followed by daily immersion in bilimbi leaf extract with or without preservatives for 9 minutes and 36 seconds each day for 42 days. The stability of the extract was assessed using spectrophotometry, and the corrosion rate on days 0, 21, and 42 was analyzed using Two-way ANOVA with a confidence level of 95%, followed by Post hoc LSD. Results: Significant differences were observed in all test groups ($p<0.05$). The P0 group exhibited the highest extract stability, followed by K0, P21, K21, P42, and K42. The lowest corrosion rate was observed in the K0 and P0 groups, followed by P21, K21, P42, and K42. **Conclusion:** Sodium benzoate effectively delays the decline in the stability of bilimbi leaf extract and enhances the corrosion rate of stainless steel orthodontic wires on days 0, 21, and 42.

Keywords: Bilimbi leaf extract; corrosion inhibitor; corrosion rate; extract stability; sodium benzoate

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INTRODUCTION

Orthodontic treatment aims to rectify dental, jaw, and facial irregularities using orthodontic devices. Stainless steel wire is a frequently utilized component in orthodontic appliances in clinics owing to its favorable mechanical attributes, effective corrosion resistance facilitated by a protective chromium oxide layer that inhibits oxygen contact, and its cost-effectiveness.[1]

Orthodontic wires may undergo corrosion in the oral cavity due to conducive environmental factors such as masticatory pressure, saliva with a pH range of 6.8-7.2, the presence of microorganisms and enzymes, and acidic conditions resulting from the intake of food and beverages. This corrosion process leads to degradation and eventual deterioration of orthodontic wires.[2,3,4]

Corrosion poses a risk to patients as it can release mutagenic, cytotoxic, and allergenic nickel and chromium ions, posing health hazards. While the complete elimination of corrosion in orthodontic wires is challenging, prevention can be achieved by employing corrosion inhibitors. Organic corrosion inhibitors are favored for their eco-friendliness and absence of heavy metals or toxic substances. Common organic compounds that produce such inhibitors include gelatin, tannin, thiourea, and phenol.[5]

Bilimbi fruit (Averrhoa bilimbi L.) is commonly utilized in traditional Indonesian medicine.[6] Bilimbi leaves are known to contain a variety of compounds, including tannins, saponins, sulfur glucose, formic acid, peroxides, flavonoids, and triterpenoids.[7] The tannin content in bilimbi leaves is reported to be 10.92%.[8] Tannins act as corrosion inhibitors by enhancing the average pH stability of hydroxide and amphoteric oxide layers or repairing any damage to these layers on metal surfaces.[5] A concentration of 10% bilimbi leaf extract was found to be the most effective in reducing the corrosion rate.[9] For the next step of the study, product stability must be solved. Product stability refers to the capacity of a product to retain its original physical, chemical, microbiological, and pharmacokinetic attributes from the time of production throughout its storage duration. Various factors can influence product stability, such as the proliferation of microorganisms, alterations in the effectiveness of preservatives, and environmental conditions like temperature, humidity, air exposure, and light exposure.[10,11] Additionally, storage time is crucial in determining product stability, as it reflects the product's ability to maintain quality until a specific period or expiration date.[10] The stability of bilimbi leaf extract (Averrhoa bilimbi L.) can be measured using a UV-Vis spectrophotometer with a wavelength of 743 nm.[12]

Testing a drug by the Indonesian Ministry of Health (Depkes RI) on day 0 is a standard procedure for assessing the drug's resistance, while the standard evaluation for liquid pharmaceutical preparations is conducted at room temperature (15-30 °C) every week for 6 weeks or 42 days. Testing of bilimbi leaf extract (Averrhoa bilmbi L.) on day 42 is conducted to determine whether preservatives significantly maintain the level of active ingredients to prevent rapid degradation.[13]

The stability of bilimbi leaf extract (Averrhoa bilmbi L.) is known to decline from days 0, 21, to 42.[14] The decrease in the quality of the extract can be prevented by adding preservatives. Preservatives can provide protection to the product from fermentation processes and acidic environments caused by microbial decomposition.[15] High concentrations of the extract result in shorter product storage periods.[16] Adding preservatives to bilimbi leaf extract (Averrhoa bilimbi L.) extends storage time and enhances stability. The preservative added to bilimbi leaf extract is sodium benzoate. Sodium benzoate, a salt form of benzoic acid, is commonly used due to its better solubility compared to its acidic form. Sodium benzoate is typically used to preserve foods and beverages such as pickled vegetables, other vegetable products, fruit juices, soft drinks, and semi-finished products.[17] The permitted concentration of sodium benzoate in Indonesia is 1 g/kg for maximum food ingredients according to Regulation No. 36 of the Indonesian National Agency of Drug and Food Control (BPOM) in 2013,[18] and 600 mg for soft drinks according to Regulation No. 722 of the Indonesian Ministry of Health in 1988.[19] A concentration of 0.1% sodium benzoate is sufficient to preserve and work optimally at pH 4.5 or below.[20] This study was conducted to determine the effect of adding sodium benzoate preservative and the duration of extract storage on days 0, 21, and 42 on the stability of bilimbi leaf extract (Averrhoa bilimbi L.) and the corrosion rate of stainless steel orthodontic wires.

MATERIAL AND METHODS

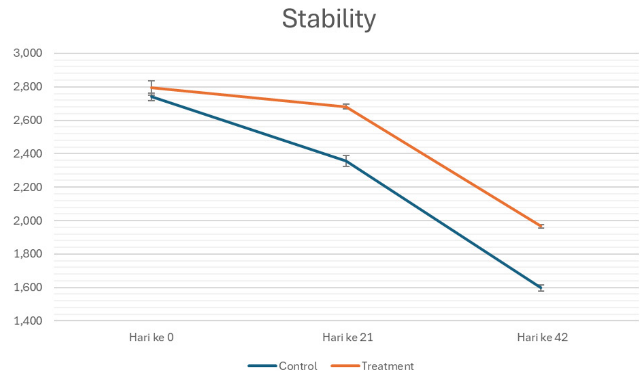
The study utilized 32 samples of stainless steel orthodontic wire measuring 0.7 mm in diameter. These wires were each cut into 3 cm lengths and bent into a U shape. Of these, 16 samples were soaked in extract without preservatives, and another 16 were treated with preservatives, all for varying durations up to 42 days. Prior to treatment, the initial weight of each wire was recorded. Additionally, all 32 wire samples were immersed in artificial saliva for the entire 42-day duration, with daily replacement of the saliva.

Furthermore, each stainless steel orthodontic wire was submerged in 10 ml of bilimbi leaf extract at a 10% concentration for precisely 9 minutes and 36 seconds. The corrosion rate of the wires was subsequently assessed using a specific formula. [21]

$$CR = \frac{Wo - W}{At}$$

Information :
CR = Corrosion rate (g/cm2d)
Wo = Initial weight of metal (grams)
W = Final weight of metal (grams)
A = Surface area of corroded metal (cm^2)
t = Treatment duration (d)

Table 1. Mean ± Standard Deviation absorbance value of starfruit leaf extract for control group on days 0, 21, and 42



Sample	Stability (Starfruit Leaf Extract)-		
	7	21	4 42
Control	2.740± 0.024	57± 0.034	1.598± 0.022
Treatment	2.794± 0.044	2.681± 0.014	1.966± 0.011

Table 2. Shapiro wilk normality test results of starfruit leaf extract (Averrhoa bilimbi L.) stability

Sample	n	Stability (Starfruit Leaf Extract) Day-		
		0	21	42
Control	4	.660*	.892*	.376*
Treatment	4	.071*	.519*	.441*

Note: significant (*) (p > 0.05)

Table 3. Homogeneity test result for the stability of starfruit leaf extract (Averrhoa bilimbi L.)

Levene Statistic	Significant
1.778	.168*

Note: significant (*) (p > 0.05)

The stability test of bilimbi leaf extract involved eight samples divided into four control group samples (bilimbi leaf extract with a 10% concentration) and four treatment group samples (bilimbi leaf extract with a 10% concentration and sodium benzoate), each subjected to prolonged storage periods of 0, 21, and 42 days on stainless steel orthodontic wire. Absorbance values of the samples from both groups were measured using a UV-Vis spectrophotometer at a wavelength of 743 nm. The measurements were conducted initially on the 0th day of the study for the untreated extract group and subsequently on the 21st and 42nd days. The results were then compared to determine any changes in the stability of the bilimbi leaf extract.

Data pertaining to the extract stability and corrosion rate underwent statistical analysis using SPSS, beginning with Cohen's Kappa test, followed by the Saphiro-Wilk normality test and Levene's homogeneity test. Subsequently, the two-way ANOVA test was performed to identify significant differences, which were further analyzed using a post hoc LSD test.

RESULTS

Bilimbi leaf extract was irradiated with a spectrophotometer UV-Vis to determine the number of active substance molecules to obtain

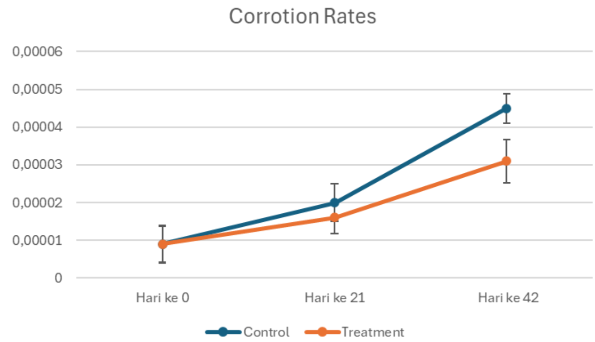
Table 4. Test results of the effect of sodium benzoate on the starfruit leaf extract stability using two-way ANOVA test

	Sum of Squares	Df	Square Mean	F	Sig.
Group	.312	1	.312	423.332	.000*
Day	4.122	2	2.061	2793.048	.000*
Day*Group (Interaction)	.102	2	.051	69.211	.000*
Error	.013	18	.001		
Total	4.549	23			

Table 5. Post Hoc LSD test results on the effect of sodium benzoate on extract stability

Group	Group
C0	C21*; C42*; T0*; T21*; T42*
C21	C0*; C42*; T0*; T21*; T42*
C42	C0*; C21*; T0*; T21*; T42*
T0	C0*; C21*; C42*; T21*; T42*
T21	C0*; C21*; C42*;T0*; T42*
T42	C0*; C21*; C42: T0*; T21*

Table 6. Mean ± standard deviation of stainless steel orthodontic wire corrosion rates on days 0, 21, and 42



Group	Mean±Standard Deviation of Stainless Steel Corrotion Rate		
	0	21	42
Control	$0.9 \times 10^6 \pm 0.48 \times 10^7$	$0.2 \times 10^4 \pm 0.5 \times 10^5$	$0.45 \times 10^4 \pm 0.4 \times 10^5$
Treatment	$0.9 \times 10^5 \pm 0.5 \times 10^5$	$0.16 \times 10^4 \pm 0.4 \times 10^5$	$0.000031 \pm 0.6 \times 10^5$

Note : Value in units of g/cm2d

Table 7. Shapiro wilk normality test results of stainless steel orthodontic wire corrosion rates

Sample	N	Corrosion Rate Day-		
		0	21	42
Control	16	.085*	.109*	.145*
Treatment	16	.057*	.180*	.308*

Note: significant (*) ($p > 0.05$)

Tabel 8. Hasil Uji HomogenitasLaju Korosi Kawat Ortodonti *Stainless*

Levene Statistic	**Signifikansi**
0.305	.905*

Note: significant (*) ($p > 0.05$)

stability data. The results of the stability data of the control group and the treatment group are in Table 1. The control and treatment groups were normally distributed ($p > 0.05$) and homogeneous ($p > 0.05$), using Shapiro-Wilk normality test and Levene's respectively (Table 2 and 3), followed by tested with a two-way ANOVA, and LSD. Significant differences were observed in all test groups ($p < 0.05$). The stability was higher in the extract group and in all intra days group. The stability is decreasing on day 12 through day 42. The P0 group exhibited the highest extract stability, followed by K0, P21, K21, P42, and K42. The lowest corrosion rate was observed in the K0 and P0 groups, followed by P21, K21, P42, and K42.

The weight data of the wires were initially subjected to Cohen's Kappa test to assess the consistency of measurements between the two researchers. The results of Cohen's Kappa test for both control and treatment groups on days 0, 21, and 42 ranged from 0.40 to 0.60, indicating a fair to moderate level of consistency in wire weight data between the researchers. The corrosion rate measurements in both the control and treatment groups were then presented as the average corrosion rate of stainless steel orthodontic wires, as illustrated in Table 6.

Table 9. Test results of the effect of sodium benzoate on the stainless steel orthodontic wire corrosion rates using two-way ANOVA test

	Sum of Squares	Df	Square Mean	F	Sig.
group	8.075E-10	1	8.075E-10	35.665	.000
day	1.444E-8	2	7.222E-9	318.956	.000
Day*Group (Interaction)	8.036E-10	2	4.018E-10	17.746	.000
Error	2.038E-9	90	2.264E-11		
Total	1.809E-8	95			

Note: significant (*) (p>0.05)

Table 10. Post Hoc LSD test results on the effect of sodium benzoate on the stainless steel orthodontic wire corrosion rates

Group	Group
C0	C21*; C42*; T0*; T21*; T42*
C21	C0*; C42*; T0*; T21*; T42*
C42	C0*; C21*; T0*; T21*; T42*
T0	C0*;C21*;C42*;T21*; T42*
T21	C0*;C21*;C42*;T0*; T42*
T42	C0*;C21*;C42:T0*;T21*

Keterangan: K=Kelompok Kontrol; P=Kelompok Perlakuan; signifikan (*) (p<0.05)

The control and treatment groups were normally distributed (P > 0.05) and homogeneous (P > 0.05), using Shapiro-Wilk normality test and Levene's respectively (table 7 and 8), followed by tested with a two-way ANOVA, and LSD. Significant differences were observed in all test groups (p<0.05). The lowest corrosion rate was observed in the K0 and P0 groups, followed by P21, K21, P42, and K42 (table 9), The largest value of corrosion rate was found in the control group on day 42 while the smallest value is in day 0 the control group as well as in the treatment group.

DISCUSSION

Corrosion produces ions such as nickel and chromium which are harmful to the body because they can cause allergies and are toxic. Corrosion of stainless steel wire can be inhibited by applying a corrosion inhibitor. Organic inhibitors are an alternative to inorganic inhibitor because they have minimal side effects and not harmful to the environment.[22] One of the inhibitor that can be used is tannin which can be found in bilimbi (*Averrhoa bilimbi L.*) leaf that was proved to be effective in inhibiting the corrosion rate of stainless steel orthodontic wire with a concentration of 10%.[9] Bilimbi leaf extract as a corrosion inhibitor needs to be tested for stability to ensure the quality of the extract, its safety for the body, and the effectiveness of the resulting product during storage and use time (shelf life).[23,24]

Bilimbi leaf extract with a concentration of 10% experienced a significant decrease in stability on day 21,[14] so an additional preservative was needed to slow the decrease in stability. The preservative used in this study was sodium benzoate with a concentration of 0.1%. The choice of sodium benzoate is based on the effect it has on the body that is safe, stable, and easily soluble in water.[25,26] This study was conducted to determine the effect of sodium benzoate and storage time on days 0, 21, and 42 on the stability of bilimbi leaf extract and the corrosion rate of stainless steel orthodontic wires. The result of the study were carried out with a two way ANOVA test.

A product over time may experience changes realted to smell, appearance, taste, or decrease in the resulting benefits. Changes which are also decay occur due to contamination of microorganisms from the external environment and the growth of microorganisms in them. [27]

Bilimbi leaf extract can experience these changes so that it decreased tannin levels. The stability of a product can be determined by physical, chemical, microbiological condirions, therapeutic effects, and product toxicity.[10] Bilimbi leaf exctract in this study was chemically tested for a stability using spectrophotometry UV- Vis. The resulting light is absorbed by te tannin content in the extract at a certain wavelength which will be processed into absorbance values. The absorbance value produced by spectrophotometry UV-Vis then becomes the stability value data because the more molecules of the active substance that absorb light, the greater the absorbance value.[28]

The two-way ANOVA analysis's outcome concerning sodium benzoate's impact on bilimbi leaf extract stability indicated a significant disparity in absorbance values between the groups, signifying the effectiveness of sodium benzoate in enhancing stability. Post hoc LSD testing affirmed that sodium benzoate-treated bilimbi leaf extract demonstrated superior inhibition of stability decline compared to untreated extract over the 42 days. Notably, there was a notable difference in absorbance values between control and treatment groups on days 0, 21, and 42, highlighting the interaction between preservatives and storage duration. The LSD test findings aligned with the mean extract stability difference, indicating an increase over time, particularly evident in the treatment group with more concentrated extract coloration. Previous studies in anthocyanin and carotenoid pigments support this, emphasizing that higher color concentration correlates with increased pigment content and absorbance values. Extracts exhibiting a brownish-yellow hue in the treatment group demonstrated higher absorbance due to elevated tannin pigment levels. [29,30,31] Various factors, including temperature, light exposure, microbial contamination, and chemical processes such as oxidation and hydrolysis, likely influenced the decrease in extract stability at days 0, 21, and 42.[24]

In this experiment, bilimbi leaf extract was stored at room temperature, which, at 25°C, can become unstable due to environmental factors such as weather and sunlight exposure. [32] Elevated temperatures can lead to the degradation of tannins in the extract, resulting in reduced levels. [24] However, stability can be maintained by storing the extract at lower temperatures or by adding antioxidants to prevent the formation of free radicals.[33]

Light exposure also plays a role in extract stability, as it can accelerate the decomposition of extract compounds, leading to a decrease in active substance levels.[34] Extended exposure of extract to oxygen triggers oxidation and hydrolysis processes. Hydrolysis, for instance, splits water molecules into hydrogen and hydroxyl ions.[35] Oxygen's impact on the extract is twofold: firstly, it generates an excess of free radicals compared to the available antioxidants through oxidation reactions, and secondly, it disrupts hydroxyl groups. Consequently, the active ingredients undergo decomposition, and their ability to bind with metal ions to form chelate complexes diminishes, reducing effectiveness. [36,] Storing the extract at lower temperatures or adding preservatives is essential to mitigate these stability factors.[37]

In this study, sodium benzoate, an antimicrobial preservative, was used at a concentration of 0.1%.[38] Sodium benzoate was chosen for its safety profile and absence of teratogenic and carcinogenic effects.[39] Its mechanism of action involves inhibiting the growth of microorganisms by interfering with cell membrane permeability, microbial DNA, and intracellular enzymes.[40] The results indicated a significant difference between the extract with 0.1% sodium benzoate and the unpreserved extract, suggesting that higher concentrations of sodium benzoate minimize extract changes by inhibiting microbial growth.[41]

The two-way ANOVA analysis revealed a significant difference in the corrosion rate of stainless steel orthodontic wires due to the presence of sodium benzoate. The average corrosion rate increased from day 0 to day 42 in both groups, attributed to a decrease in tannin levels, impairing the extract's ability to inhibit wire corrosion effectively. The current studies align with the theory that declining active substance levels diminish product effectiveness.[40] The reduction in tannins within bilimbi leaf extract can compromise its capacity to deter the corrosion rate of stainless steel orthodontic wires. The post hoc LSD test, examining the interaction between sodium benzoate and storage time, further confirmed its impact on extract stability. Corrosion rate measurements in both groups corresponded to the post hoc LSD test results, indicating a significant difference between the control and treatment groups on days 0, 21, and 42. Extracts treated with preservatives exhibited superior stability to those without, as evidenced by higher absorbance in the treatment group. Thus, the research hypothesis concerning the influence of sodium benzoate and storage time on extract stability and wire corrosion rate on days 0, 21, and 42 has been substantiated, demonstrating decreased extract stability and increased wire corrosion over time in both groups.

CONCLUSION

The research concludes that adding sodium benzoate and adjusting storage time on days 0, 21, and 42 slows the decline in bilimbi leaf extract stability and reduces the corrosion rate of stainless steel orthodontic wires. Further exploration of starfruit leaf extract applications in orthodontics is necessary.

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