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ARTICLE

IN VITRO BACTERIAL ADHERENCE ON TEETH SUBMITTED TO WHITENING WITH *MUSA PARADISIACA*.

Adherencia bacteriana *in vitro* en dientes sometidos a blanqueamiento con *Musa paradisiaca*.

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ABSTRACT:

Objetive: To compare *in vitro* bacterial adherence on teeth submitted to whitening with 50% ethanolic extract of Musa paradisiaca and 35% hydrogen peroxide.

Material and Methods: The study was experimental and used 18 premolars that were grouped into: G1 (control), G2 (50% ethanol extract of Musa paradisiaca) and G3 (35% hydrogen peroxide). The teeth were then exposed to a Streptococcus mutans culture for 24 hours, followed by centrifugation in thioglycolate broth. A culture on trypticase soy agar was done with a 1 in 100 dilution, and after 48 hours colony forming units (CFU) were counted. Statistical analysis was performed using the ANOVA test, complemented by the Bonferroni post-hoc.

Results: Bacterial adherence was 77x105 CFU/ml in Group 3 using 35% hydrogen peroxide, 40x105 CFU/ml in Group 2 using 50% ethanol extract of Musa paradisiaca, and 89x104 CFU/ml in Group 1 (control). The difference between the three groups was significant (p=0.000).

Conclusion: Both whitening methods cause bacterial adherence to the tooth surface, although to a lower degree with Musa paradisiaca.

KEYWORDS:

Tooth bleaching; Plant extracts; Musa paradisiaca; hydrogen peroxide; Bacterial adherence; Streptococcus mutans.

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RESUMEN:

Objetivo: Comparar la adherencia bacteriana *in vitro* en dientes sometidos a blanqueamiento con extracto etanólico de Musa paradisiaca al 50% y con peróxido de hidrógeno al 35%.

Material y Métodos: Comparar la adherencia bacteriana in vitro en dientes sometidos a blanqueamiento con extracto etanólico de Musa paradisiaca al 50% y con peróxido de hidrógeno al 35%.

Resultados: La adherencia bacteriana fue de 77x105 UFC/ml con el peróxido de hidrógeno al 35%, de $40x10^5$ UFC/ml con el extracto etanólico de *Musa paradisiaca* al 50% y de 89x104 UFC/ml con el control. La diferencia fue significativa entre los tres grupos (p=0.000).

Conclusión: Ambos métodos de blanqueamiento causan adherencia bacteriana en la superficie dental, siendo menor con Musa paradisiaca.

PALABRAS CLAVE:

Blanqueamiento de dientes; extractos vegetales; musa paradisiaca; peróxido de hidrógeno; adherencia bacteriana; Streptococcus mutans.

INTRODUCTION.

The color of the teeth is the most important factor of the smile, thus becoming a great aesthetic concern.[1] It is reported that 66.8% of patients are dissatisfied with the color of their teeth,[2] and strongly wish to undergo teeth whitening.[3] Teeth whitening is defined as any means to increase the whiteness of a tooth.[4] According to the American Academy of Cosmetic Dentistry (AACD), its popularity has increased over 300% between 2002 and 2007 and these numbers continue to grow by around 25% per year.[5]

In-office whitening is the most popular method, which uses 35% hydrogen peroxide (H_2O_2),[6,7] a relatively unstable compound of low molecular weight, with a high oxidizing agent.[8-10] It breaks down into free hydroxyl radicals that break the carbon double bonds of organic molecules into single bonds, which ends up lightening the color of the tooth.[11] It has been reported that it alters the morphology, roughness, microhardness and composition of the enamel,[12,13] as well as the bacterial adherence to the tooth surface, finding controversial results.[14-18]

Bacterial adherence is due to the diversity of microorganisms of the oral microbiota. The most studied has been *Streptococcus mutans*, a facultative anaerobic, *Grampositive coccus*.[16] The adhesins of its cell wall adhere to the acquired salivary film through specific receptors and then produce polysaccharides from sucrose, which allows bacterial colonization.[17] Some people believe that all these effects could be avoided by using natural resources as whitening agents.[19] This could explain the increasing number of studies that evaluate the whitening effectiveness of these natural substances[20,21] and their effect on morphology,[22-24] roughness,[22,25,26] microhardness[22,27,28] and composition[20,29,30] of the enamel. These do-it-yourself (DIY) methods represent an alternative to conventional whitening that promotes the use of vegetables and fruits, such as bananas.

Musa paradisiaca (banana) belongs to the Musaceae family.[32,33] It is a tall and robust herb and its fruit is a soft and fleshy berry.[33,34] Although the literature regarding its effectiveness as a whitening agent is limited, it has been previously reported[13,35-39] to be similar to 35% hydrogen peroxide.[38] This could be explained by the presence of saponins,[34] salicylic acid, potassium, manganese and magnesium[13] in the peel of its fruit. On the other hand, it has been reported to have antimicrobial[40] and inhibitory activity

against *Streptococcus mutans*[41,42] because of the presence of flavonoids in its composition.[43] As the popularity of in-office whitening treatments using 35% hydrogen peroxide grows, their safety has been questioned because of their effects on the tooth surface reported in the literature, such as increased bacterial adherence.

Despite the lack of consensus on the results found in the literature, this calls our attention because a greater bacterial adherence could suggest morphological and roughness changes in the tooth surface, thus increasing susceptibility to dental caries and periodontal diseases. In view of the increasing concern regarding the use and abuse of in-office whitening agents, the use of natural resources as whitening agents has aroused the interest of both the population and the scientific community.

In view of this, the initiative arose to look for a new alternative treatment option made from a natural product such as Musa paradisiaca banana, which has a whitening effect similar to hydrogen peroxide, a lower price, an easier manipulation, a better accessibility, and antimicrobial properties that help reduce bacterial adherence, therefore the purpose of this study is to compare the adherence of Streptococcus mutans on teeth submitted to DIY-whitening using 50% Musa paradisiaca ethanol extract and to in-office whitening using 35% hydrogen peroxide (H_2O_2).

The present work evaluates the effect of teeth whitening, using Musa paradisiaca on bacterial adherence, having limited literature on the subject. This *in vitro* study seeks to provide basic information for the elaboration of natural whitening products based on a very frequently consumed fruit. Future studies are suggested to investigate its effect using other solvents and concentrations, as well as other species of the oral microbiota as test microorganisms.

MATERIALS AND METHODS.

This study was experimental *in vitro* and was carried out in the Laboratory of Molecular Microbiology and Biotechnology of the Faculty of Health Sciences of the Antenor Orrego Private University in La Libertad, Peru and in the Laboratory of Pharmacognosy of the Faculty of Pharmacy and Biochemistry of the National University of Trujillo, Peru, following the biosafety and use of the laboratory guidelines.[44]

The sample consisted of 18 premolars. Their surface was cleaned with rubber cups and prophylactic paste and then stored at 4°C in distilled water. The whitening agents used were 50% ethanol extract of Musa paradisiaca and 35% hydrogen peroxide.

The plant material was collected from an orchard of the Campiña de Moche, located at 8°C 08' 56.9" S and 79° 00' 25.1" W, at 35 m a.s.l., Moche, Trujillo, La Libertad, Peru. The plant was identified in the Antenor Orrego Herbarium (HAO) of the Natural and Cultural History Museum of the Antenor Orrego Private University of Trujillo as Musa Paradisiaca L. S. Leiva & U. Pantigozo. After drying and mounting, it was officially deposited in the HAO Herbarium with the number 20130, receiving the respective certificate.

To obtain the 50% ethanol extract of Musa paradisiaca, the mesocarp of the fruit was mechanically extracted until a weight of 5g was obtained. Maceration was used as the extraction technique. The material was placed in 100 ml of 50% alcohol and incubated for 7 days. Later, it was submitted to reflux using a water bath for 10 minutes. It was left to cool down and then filtered using Whatman grade 40 filter paper. The extract was stored in a sealed bottle and refrigerated at 4°C - 8°C (Figure 1).

Phytochemical screening was done for the identification of its metabolites, indicating the presence of alkaloids, reducing sugars, flavonoids, saponins and amino acids. The following protocols were used for the whitening treatments. In Group 1, teeth were preserved in sterile distilled water at 37°C. In Group 2, they were immersed for 6 h in 20 ml of 50% ethanol extract of *Musa paradisiaca* (Figure 2).

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In Group 3, 35% hydrogen peroxide was applied to their vestibular coronary surface for 15 min in two sessions. Between each session they were washed with distilled water and dried with absorbent paper. At the end of each treatment, teeth were kept in distilled water at 37°C for 24 h and then sterilized at 121°C for 60 min. For the bacteriological procedures, the strain of *Streptococcus mutans* ATCC 25175 was used. This was cultured on trypticase soy blood agar and incubated at 37°C under microanaerobic conditions for 24 h (Figure 3A).

Subsequently, a culture sample was collected and then diluted in thioglycolate broth contained in a test tube, which was shaken until reaching a turbidity equivalent to McFarland 0.5. Teeth were placed in test tubes with 5 ml of thioglycolate broth and 0.1 ml of the diluted strain and incubated for 24 h (Figure 3B).

After that, teeth were resuspended in fresh thioglycolate broth in test tubes, which were centrifuged at 3000 rpm for 10 minutes in order to detach the bacteria adhered to the entire surface of each tooth surface. For the preparation of the 1 in 100 dilution, 0.1 ml was taken of the liquid obtained previously and then cultured in TSA Agar plates, which were labeled with its respective whitening agent and repetition number and later incubated for 48 h. To determine the bacterial adherence to the entire surface of each tooth specimen, examination of each plate was performed through macroscopic observation using a magnifying glass. Previously the researcher had been trained on manual cell counting (Figure 4). The number of CFU/ml was calculated mathematically.

RESULTS.

Bacterial adherence to the tooth surface was greater in the Group 1 using 35% hydrogen peroxide with a mean of $77x10^5 \pm 58x10^4$ CFU/ml followed by the Group 2 using 50% ethanol extract of Musa paradisiaca with a mean of $40x10^5 \pm 67x10^4$ CFU/ml. (Table 1)

The analysis of variance (ANOVA) test shows that there is a significant difference between the three groups evaluated (p=0.000) (Table 2).

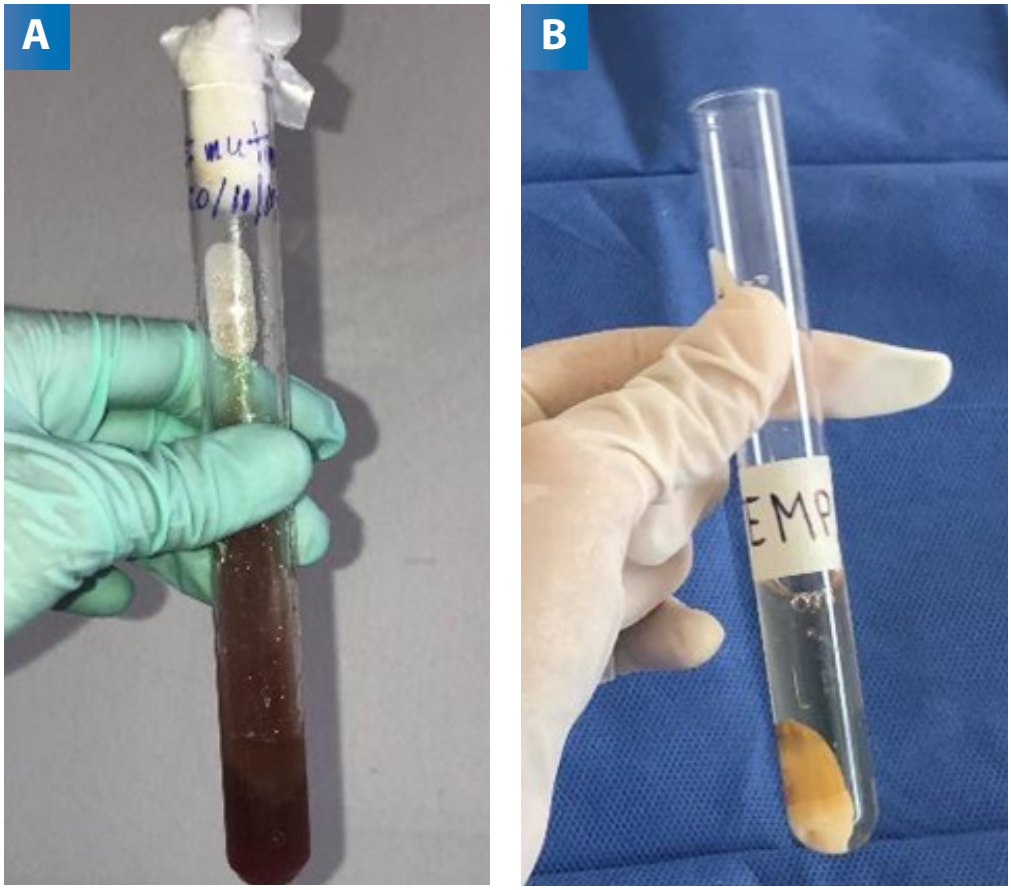
After the ANOVA test, the Post Hoc Bonferroni test was performed to compare the means of two groups, finding a statistically significant difference between each pair of groups (p<0.05) (Table 3).

Figure 1. Preparation of 50% ethanol extract of *Musa paradisiaca*.

A: Reflux using a water bath. **B:** Filtration with Whatman grade 40 filter paper.

Figure 2. Exposure of teeth to 50% ethanol extract of *Musa paradisiaca*.

Figure 3. Bacteriological procedures with the strain of *Streptococcus mutans*.



A: Cultured strain of *Streptococcus mutans* ATCC 25175. **B:** Exposure of teeth to *Streptococcus mutans*.

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Figure 4. Petri plates of Colonies of *Streptococcus mutans* recovered from the teeth of the groups subjected to different whitening agents.

CFU/ml counts: A: Control. **B:** 50% ethanol extract of *Musa paradisiaca*. **C:** 35% hydrogen peroxide.

Table 1. Counts of bacterial adherence on tooth surface (CFU/mL).

GROUPS	N	MEAN	STANDARD DEVIATION
Group I – Control (distilled water)	6	$8.9x10^4$	$12x10^4$
Group II – 50% Musa paradisiaca ethanol extract	6	$40x10^5$	$67x10^4$
Group III – 35% hydrogen peroxide	6	$77x10^5$	$58x10^4$

Table 2. Analysis of Variance between the three groups.

SUM OF SQUARES		gl	ROOT MEAN SQUARE	F (ANOVA)	*p*-value
Inter-groups	139220640893333.300	two	69610320446666,600	262,646	0.000
Intra -groups	3975518146666.667	fifteen	265034543111.112		
Total	143196159040000.000	17			

Table 3. Significance level of comparison between whitening groups.

COMPARISON BETWEEN GROUPS		SIGNIFICANCE LEVEL (*p*-value= 0.05)
Group I - Control (distilled water)	Group II – 50% ethanol extract of *Musa paradisiaca*	0.000
Group I - Control (distilled water)	Group III – 35% hydrogen peroxide	0.000
Group II – 50% ethanol extract of *Musa paradisiaca*	Group III – 35% hydrogen peroxide	0.000

DISCUSSION.

Teeth whitening is one of the least aggressive modalities for the treatment of tooth discoloration.[11] There is a variety of tooth whitening methods, including professionally applied in-office whitening and DIY regimens.[22] The first is the most popular method and uses 35% hydrogen peroxide.[6,7]

There is controversy regarding its safety and possible adverse effects.[12] It has been reported to have an effect on the adherence of *Streptococcus mutans*, finding contradictory results.[14-18] On the other hand, the popularity of do-it-yourself (DIY) regimens has been on the rise.[45] *Musa paradisiaca* fruit has a peel rich in components that would explain its whitening action and its antimicrobial effect against *Streptococcus mutans*.[13,37,40,43]

The results obtained in the present study showed an increased bacterial adherence with both whitening methods, being greater with the in-office whitening using 35% hydrogen peroxide, followed by DIY whitening using 50% ethanol extract of Musa paradisiaca, and finally by the control group, obtaining a significant difference between the three groups.

The findings of this study about the effect of 35% hydrogen peroxide on increasing bacterial adherence to the tooth surface are in agreement with the results obtained by Hosoya *et al.*,[18] who observed an increased bacterial adherence after use 35% H_2O_2, by Al-Jubori *et al.*,[16] and Romero *et al.*,[17] who found that 7.5% hydrogen peroxide significantly increases bacterial adherence, and by Zheng *et al.*,[14] who found that 35% H_2O_2 increases the growth of biofilm since the third week after treatment. This may be due to the several alterations that occur in the enamel after being treated with hydrogen peroxide.[17]

Hydrogen peroxide can produce irregularities, which protect bacteria against shear forces, making the tooth more susceptible to bacterial adherence.[17,46] On the other hand, these findings disagree with Zheng *et al.*,[14] who observed a decrease in the growth of biofilm during the first [21] days, and with the results obtained by Ittatirut *et al.*,[15] who did not observe an increase in the biofilm formation when using 35% H_2O_2. This would be due to the continuous release of peroxide by whitening products, which would change the existing biological balance in the oral cavity. Likewise, it has been reported that the release of oxygen by hydrogen peroxide has an antibacterial effect and a mechanical action on dental plaque.[47]

An increase in bacterial adherence to the tooth surface submitted to DIY whitening using 50% ethanol extract of Musa paradisiaca was also found in the results. Since this is the first study that evaluates the effect of Musa paradisiaca on bacterial adherence to the tooth surface, there are no previous studies directly related, so comparisons were made with studies that evaluated the effects of fruits with components such as ascorbic acid and malic acid also present in *Musa paradisiaca* peel, which are associated with bacterial adherence to the tooth surface.

The results obtained agree with Kwon *et al.*,[22] who observed changes in enamel microhardness and morphology after using a strawberry mixture; by Jung *et al.*,[20] who found decreased levels of calcium and phosphorus after using grapefruit and plum extracts; by Hartanto *et al.*,[27] who observed a decrease superficial microhardness since the third week after using a strawberry paste; and finally by Dewi29, who found decreased calcium levels after using an apple extract. All these effects on the enamel could be explained by the presence of substances such as ascorbic acid[48] and malic acid,[49] which can bind calcium to the enamel, causing porosities in the enamel crystals, and therefore, erosion, which could cause a rougher surface, thus facilitating bacterial adhesion.[28,30,47] We also found the presence of sucrose, glucose and fructose50. Streptococcus mutans produces glucans from sucrose to increase bacterial accumulation,[15] which would explain the increased bacterial adherence to the tooth surface. However, the results obtained disagree with Jung *et al.*,[20] who observed increased levels of calcium and phosphorus after using a

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grapefruit extract; and Asmawati *et al.*,[30] who did not find a significant difference in the levels of calcium, phosphorus, oxygen, potassium and sodium. On the other hand, there are studies that have evaluated the antibacterial effect of Musa paradisiaca peel against *Streptococcus mutans*. Sultan *et al.*,[41] observed an antimicrobial activity and an inhibitory effect of aqueous and methanolic extracts of Musa paradisiaca peel on the formation of *Streptococcus mutans* biofilm while Septiana[42] found that the extract of *Musa paradisiaca* L. banana peel is effective against the growth of *Streptococcus mutans*. This would be due to the presence of alkaloids, saponins and flavonoids, which are responsible for its antimicrobial action and its inhibitory activity.[42]

However, Mohsien,[51] who evaluated the anti-microbial effect of Musa paradisiaca peel against *Streptococcus mutans* did not observe changes when using Musa paradisiaca extract alone, but did observe a high antibacterial activity when using it in combination with Citrus lemon extract.

Hydrogen peroxide-based whitening agents can significantly influence bacterial adherence to the tooth surface. This should be taken into account in dental practice for the correct use of these whitening agents in the office. This explains why it is important for professionals to be informed about the safety of these procedures in order to be able to minimize their possible adverse effects. On the other hand, although 50% ethanol extract of *Musa paradisiaca* caused an increased bacterial adherence, this was lower in comparison to 35% hydrogen peroxide. Therefore, we consider 50% ethanol extract of Musa paradisiaca as a new treatment alternative based on a natural product with a whitening effect similar to 35% hydrogen peroxide but with an smaller effect on the increase of bacterial adherence to the tooth surface.

Since this study is *in vitro*, it has limitations such as not defining which whitening method causes a greater bacterial adherence to the dental surface *in vivo*. Other factors such as alternative storage solutions; exposure time, frequency, concentration and pH of the whitening agent, as well as salivary pH still need to be studied. As previous research is limited, the findings of this investigation will serve as the basis for future studies, using other solvents and concentrations, as well as other species of the oral microbiota as test microorganisms.

CONCLUSION.

Bacterial adherence to the tooth surface is lower using 50% ethanol extract of *Musa paradisiaca* in comparison to 35% hydrogen peroxide, however, further research is suggested to evaluate the effects of Musa paradisiaca on other properties of the enamel, such as its surface integrity.

Conflict of interests:

The authors declare no conflict of interest, no affiliation with any organization or entity, or economic interest in the topic developed in this manuscript.

Ethics approval:

The Manual of Biosafety in Molecular Microbiology and Biotechnology Laboratories (BSL-2) of the Antenor Orrego Private University was respected during the execution of this investigation, and the handling and disposal of the residues of the samples used were taken into account. Teeth were obtained from external private dental offices. The principles of the Declaration of Helsinki were not considered as it was an *in vitro* study.

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