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# Effect of *Spondias mombin L.* extract on the wettability, roughness, color and morphology of bovine enamel

Franciara Maria Gomes Alves[1], Thaís Oliveira Cordeiro[1], Ana Margarida dos Santos Melo[1], Larissa Sgarbosa de Araújo Matuda[2], Daniela Lopes da Silva Amorieli[2], Joselucia da Nóbrega Dias[1], Boniek Castillo Dutra Borges[1], Ruthineia Diógenes Alves Uchôa Lins[1,*]

[1] Departamento of Dentistry, Federal University of Rio Grande do Norte (UFRN), Natal/RN, Brazil.

[2] University of West Paulista (UNOESTE), São Paulo/SP, Brazil.

**Corresponding author:**
Ruthineia Diógenes Alves Uchôa Lins
Federal University of Rio Grande do Norte – Departamento of Dentistry - Av. Sen. Salgado Filho, 1787 – Lagoa Nova, 59056-000 – Natal – Brazil.
Phone: +55 84 32154100 –
Mobile: +55 84 994013056
Email: aruthineia@gmail.com

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Although *Spondias mombin L.* extract has an excellent antimicrobial effect against oral microorganisms, it should be clarified how it affects enamel surface properties. **Aim:** To evaluate the color change, wettability/contact angle, surface roughness and morphology of bovine enamel submitted to the *Spondias mombin L.* extract. **Methods:** Thirty bovine teeth were distributed into the following groups: 0.12% chlorhexidine digluconate, 1:32 *Spondias mombin* L. extract and distilled water. Color change (CC) was evaluated after immerging specimens into the solutions for 14 days. Surface roughness (Ra) was measured using a roughness meter; wettability/contact angles (CA) were determined by the sessile drop method, and scanning electron microscopy images were obtained to characterize the morphology (SMA). The pH of the solutions was evaluated using a pHmeter. The Ra, CA, and CC data were parametric (Kolmogorov-Smirnov; $p>0.05$). Two-way ANOVA (for Ra and CA) and one-way ANOVA (for CC) with Tukey's posthoc tests at a significance level of 5% were used. SMA was analyzed descriptively. **Results:** The *Spondias mombin L.* extract revealed an acidic pH, and when in contact with the bovine teeth, it increased the wettability, but it did not cause statistically significant differences in the Ra. *Spondias mombin L.* extract caused the highest color change. The SEM images showed differences in the specimens' surface submitted to the extract compared to the other groups. **Conclusion:** *Spondias mombin L.* extract provided negative effects on bovine enamel's surface, including a high color change and a more wettable substrate.

**Keywords:** Phytotherapy. Anacardiaceae. Mouthwashes. Dental enamel. Surface properties.

## Introduction

Clinical evidence shows that dental biofilm control is essential in preventive dentistry and directly reflects individuals' oral health. The materials used for this purpose in dentistry attempt to maintain the dental surface's natural properties, including mineral composition, hardness, smoothness, translucency and low surface free energy/wettability[1-3]. The use of mouthwashes, along with the mechanical control of the biofilm, has also shown substantial ability to prevent biofilm from growing. Although 0.12% chlorhexidine digluconate is considered the gold standard in mouthwash, it promotes adverse effects such as staining of teeth, changes in gustation, and irritation of the mucosa[1,4,5]. Therefore, there is a need for scientific studies that evaluate herbal medicines' potential as an alternative of interest for future use in the control of dental biofilm, preventing and treating oral biofilm diseases[6].

The species *Spondias mombin* has been gaining notoriety among the currently studied plant extracts. In Brazil, this plant is mainly found in the North and Northeast regions. However, this species is also found in several parts of the world, such as Peru, Venezuela, Bolivia, Mexico, and western India[7]. Its leaves present, among other characteristics, components with antimicrobial and antioxidant properties, such as flavonoids, saponins, tannins, and phenolic compounds - those last ones are also associated with antiviral and antitumor activities[8]. Those phytochemicals have been constantly associated with effects against oral microorganisms, including Streptococcus mutans. A recent study showed that the hydroethanolic extract of *Spondias mombin L.* has an antimicrobial activity similar to Chlorhexidine 0.12% against oral bacteria of the genus Streptococcus, also showing an anti-adherent effect on Streptococcus mutans[9]. Besides, anti-inflammatory effects and properties arising from the extract of *Spondias mombin L.*, associated mainly with the tannin components, have also been observed[6,10-16].

In light of this knowledge, this in vitro study aimed to evaluate the effect of the *Spondias mombin L.* extract on the wettability, color change, surface roughness and morphology of bovine enamel compared with 0.12% chlorhexidine digluconate and distilled water. The null hypothesis tested was that there would be no differences among the solutions concerning all properties analyzed.

## Methods

### Specimens' preparation

Specimens of bovine teeth (n=30) collected from the Nellore animal breed with a mean age of 36 months were used from the meat industry. Fractured and irregular teeth were excluded after visual inspection. Initially, the bovine teeth were cleaned with pumice and water, with the aid of a rubber cup in a slow-speed handpiece and stored in distilled water. Subsequently, a transversal section was made, dividing the root and coronal portions, 2mm below the cementoenamel junction, having as reference the labial surface. The crowns were separated from the roots using a diamond disc (KG Sorensen, Cotia, SP, Brazil) in a straight handpiece. It was then made

longitudinal sections in the mesial, distal, and incisal surfaces, obtaining specimens (10mm x 10mm) from the labial enamel's flatter part. Specimens with cracks were excluded[17]. Finally, the specimens were stored in distilled water at room temperature until the moment of the immersion protocol.

### Obtaining the *Spondias mombin L.* extract

The sample of the collected raw vegetable material was deposited in the UFRN herbarium and identified by a botanist, with the number of exsiccate 12252. Spondias mombin's collected leaves were dried at room temperature for two weeks. From 100 g of the *Spondias mombin L.*, an extract was prepared by maceration, leaving it in contact with ethanol and distilled water (80:20, v/v) for seven days and subsequent lyophilization. After this period, the hydroethanolic extract was filtered, and the organic solvent was eliminated in a rotatory evaporator (TE210, Tecnal, Piracicaba, Brazil) under a vacuum at 45° C.

The hydroethanolic extract of *Spondias mombin L.* used in this research had a 1:32 dilution = 31.25 (mg/mL). This concentration was defined based on Lima et al.[8] (2017), which found that *Spondias mombin L.* extract showed antimicrobial activity superior to 0.12% chlorhexidine digluconate only from that concentration.

### Determination of pH

Ten mL of the tested solutions were analyzed in a pH meter (LUCA-210-E, LUCADEMA, São José do Rio Preto, Brazil), calibrated with phosphate and acetate buffer solutions, pH 7.0 and 4.0, respectively, at 25° C. After the extract preparation, the solutions were performed in triplicate. The pH of distilled water, 0.12% chlorhexidine digluconate, and *Spondias mombin L.* extract were, respectively, 7.0, 6.5, and 2.96.

### Immersion protocol and analyzed variables

The specimens (n=30) were randomly allocated into 3 groups: CLX - 0.12% chlorhexidine digluconate (n=10); DW - distilled water (n=10); and DE - diluted *Spondias mombin L.* extract (n=10). The following dependent variables were tested: wettability/contact angles (CA), surface roughness (Ra), color change (CC) and surface micromorphology (SMF). For CA and Ra, solution (CLX, DW, and DE) and time (24 h and 14 days) were the independent variables. For CC and SMF, solution was the independent variable. Figure 1 presents a schematic representation of the methods.

The immersion protocol used for all solutions followed the CLX manufacturer's (Periomax©, Iodontosul, Porto Alegre, RX, Brazil). Each specimen was positioned individually inside a flask containing 10mL of the specific solution, which was disturbed for 1min twice a day, with 12 hours intervals, for 14 days. In the interval between immersions, the specimens were stored in 1.5mL of artificial saliva (Farmafórmula, Natal, Brazil. Composition: Single syrup [Nipagin, Nipazol, Sugar, Water] - 20%; Glycerin - 10%; Carboxymethyl Cellulose Gel [CMC] - 2.5 to 8%; Cherry Flavorant - 0.1%. pH = 7.0)[17,18]. The solutions and artificial saliva were renewed after each sample submission period. Then, the specimens were dried at room temperature for 24 hours before the analyses[4].

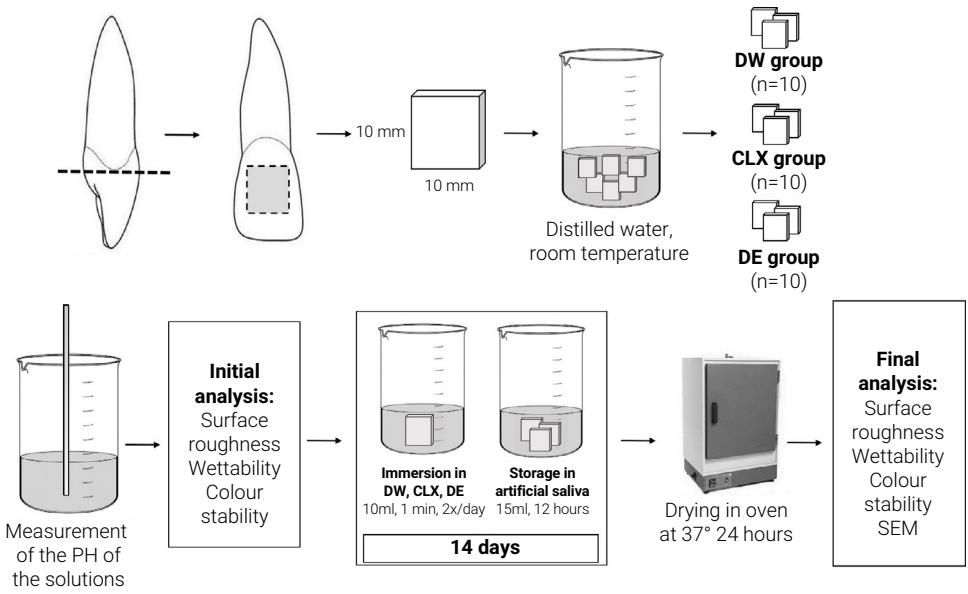


**Figure 1.** Schematic representation of the methods.

## Wettability/contact angles

Adapting the protocol described by Costa et al.[19] (2018), the wettability was evaluated using the sessile drop method. The contact angle between the dental surface and the liquid was determined. A drop of distilled water (5 µl) was released on the specimens' surface with the aid of an automatic micropipette over a distance of 20mm, in front of a photographic camera. The surface was positioned in the central and perpendicular part of the lens. The images were captured at a distance of 30cm, 5s after the drop was dumped. Subsequently, the contact angle was measured using the *Surftens 4.7 Automatic software* (OEG GmbH, Frankfurt, Oder, Germany), adapting its settings for "distilled water" and "4 (four) point analysis". Each image was analyzed in triplicate, and an average was obtained.

## Surface roughness assessment (Ra)

For Ra analysis, the Hommel Etamic W10 roughness meter (JENOPTIK Industrial Metrology Germany GmbH, Germany) was used, equipped with a diamond needle with a radius of 2 mm. The needle was moved at a constant speed of 0.5 mm/s with a load of 0.7 mN. The cut-off value was set at 0.25 mm. The average surface roughness (Ra) of different locations (parallel, oblique and perpendicular) was obtained[20,21].

## Color change analysis (CC)

To determinate the CC, the specimens' color was assessed at baseline (T0) and after 14 days (T14) of immersion in each solution. Four measurements were performed on enamel using a digital spectrophotometer (EasyShade, VitaZahnfabrik, Bad Säckingen, Germany), and an average value was obtained. The data recorded by the colorimeter were used to calculate the CIEDE2000 color change (ΔE00) according to the following equation:

$$\Delta E00 = \left[\left(\frac{\Delta L'}{K_L S_L}\right) + \left(\frac{\Delta C'}{K_C S_C}\right) + \left(\frac{\Delta H'}{K_H S_H}\right) + R_T\left(\frac{\Delta C'}{K_C S_C}\right)\left(\frac{\Delta H'}{K_H S_H}\right)\right]^{1/2}$$

The values of ΔL', ΔC', and ΔH' are the differences in lightness, chroma, and hue between T14 and T0. $S_L$, $S_C$, and $S_H$ are the weighting functions for the lightness, chroma, and hue components, respectively. $K_L$, $K_C$, and $K_H$ are the parametric factors to be adjusted according to different viewing parameters. In this study, $K_L$, $K_C$, and $K_H$ were set to 1. Color change considering T14-T0 was obtained with 50:50% perceptibility (PT = 0.81 ΔE00 units) and 50:50% acceptability (AT = 1.77 ΔE00 units) thresholds[22,23].

### Surface Microorphology Analysis (SMA)

For the SMS, three specimens of each group were randomly selected (n=3) at T14. After 24h dry, the specimens were gold-sputtered, and images were recorded through a Scanning Electron Microscope (SEM-FEG, Zeiss Gemini, Germany). Images with 2000X magnification in the center of the sample were obtained and descriptively analyzed[19].

### Statistical analysis

Data analyses were performed through the Statistical Package for Social Sciences software (SPSS - IBM SPSS Statistics Subscription, version 25). The normality of the data was verified using the Kolmogorov-Smirnov test ($p>0.05$). The descriptive analysis presented the mean and standard deviation. The CA and RA were analyzed using two-way ANOVA/Tukey posthoc tests (solution *versus* time). For CC, one-way ANOVA/Tukey posthoc tests were used. The level of significance was set at 95% ($p<0.05$). The photomicrographs of the surface morphology were evaluated qualitatively.

## Results

### Wettability/Contact angles (CA)

There were statistically significant differences among solutions ($p>0.01$), times ($p<0.01$) and in the interaction of solutions *versus* times ($p<0.01$). Multiple comparisons are shown in Table 1. Regardless of the time, DE showed statistically decreased contact angles than DW and CLX. Only DE provided statistically lower contact angles at T14 compared to T0.

**Table 1.** Mean (standard deviation) of the contact angles according to the solution and time tested in this study.

| Solution | Time | |
|---|---|---|
| | Baseline (T0) | 14 days (T14) |
| Distilled water (DW) | 53.81 (3.46) Aa* | 48.36 (3.17) Aa |
| 0.12% chlorhexidine digloconate (CLX) | 56.86 (4.39) Aa | 54.95 (4.78) Aa |
| *Spondias mombin L.* extract (DE) | 51.36 (3.68) Ba | 39.26 (4.90) Bb |

*Different capital letters reveal statistically significant differences among the solutions for the same time ($p<0.05$). Different lowercase letters reveal statistically significant differences between times for the same solution ($p<0.05$).

## Surface roughness (RA)

There were no statistically significant differences among solutions neither between times (p>0.05). Table 2 presents detailed Ra values.

**Table 2.** Mean (standard deviation) of surface roughness (µm) according to the solution and time tested.

| Solution | Time | |
|---|---|---|
| | Baseline (T0) | 14 days (T14) |
| Distilled water (DW) | 2.12 (0.91) Aa* | 1.55 (0.87) Aa |
| 0.12% chlorhexidine digloconate (CLX) | 2.04 (1.04) Aa | 2.23 (0.91) Aa |
| *Spondias mombin L.* extract (DE) | 2.36 (0.82) Aa | 1.65 (0.41) Aa |

*Different capital letters reveal statistically significant differences among the solutions for the same time (p<0.05). Different lowercase letters reveal statistically significant differences between times for the same solution (p<0.05).

## Color change (CC)

There were statistically significant differences among the solutions (p<0.01). Multiple comparisons are shown in Table 3. DE showed a statistically higher CC than DW and CLX. Only DE presented ΔE00 units higher than the perceptibility (0.81) and acceptability (1.77) thresholds.

**Table 4.** Mean (standard deviation) of the color change (ΔE00) according to the solution tested.

| Solution | | |
|---|---|---|
| Distilled water (DW) | 0.12% chlorhexidine digluconate (CLX) | *Spondias mombin L.* extract (DE) |
| 0.74 (0.14) b* | 0.79 (0.38) b | (1.89) a |

*Different lowercase letters reveal statistically significant differences among the solutions (p <0.05).

## Surface micromorphology (SMA)

The images obtained (Figure 2) point that exposition to CLX promoted mineral-like precipitation compared with DW. However, specimens exposed to the DE showed characteristics of enamel dissolution.

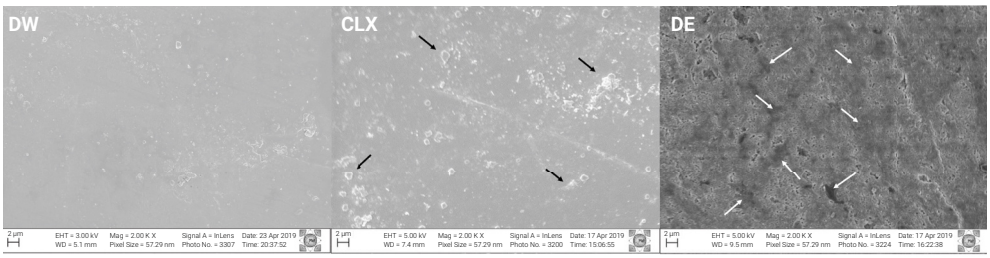


**Figure 2.** Scanning electron microscopy images of specimens exposed to distilled water (DW), 0.12% chlorhexidine digluconate (CLX), and *Spondias mombin L.* extract (DE). Mineral-like precipitation was observed in specimens exposed to CLX (arrows), while enamel dissolution was perceived in specimens exposed to DE (arrows).

## Discussion

The null hypothesis tested in this experiment - that there would be no differences among the solutions concerning all properties analyzed - was rejected since exposition to the *Spondias mombin L*. extract promoted enamel color and micromorphology changes.

Although the SEM images demonstrated possible changes in the specimens' surface from the *Spondias mombin L*. extract group, the surface roughness before and after immersion protocol did not indicate statistically significant changes in any of the groups. According to Field et al.[24] (2013), the assessment of surface roughness may not provide details on the surface texture, wear resistance and the ability to retain liquids, limiting this type of analysis. However, the analysis of surface roughness can indicate possible changes in the dental structure, especially when associated with other variables' analysis[25]. It is likely that an acidic pH presented by the *Spondias mombin L*. extract caused enamel dissolution and changes on enamel topography (Figure 2DE), which were not detected employing the roughness test.

Dantas et al.[26] (2015) defined wettability as the liquid's ability to wet a solid, exemplifying it as a drop of liquid resting on a solid surface which the liquid may or may not spread. Water is a polar liquid that tends to spread over a surface with high surface energy and form a drop in areas with low energy. Regarding surface wettability, the contact angle data indicate that the dental enamel has undergone significant changes when exposed to the extract of *Spondias mombin L*. A decrease in the contact angle was observed, which suggests an increase in the free surface energy and, consequently, in wettability.

According to Luz et al.[27] (2008), the two main factors that can affect the wetting behavior of a solid by a liquid are: topographic inhomogeneity, caused by surface roughness or porosity and chemical inhomogeneity, caused by the presence of contaminants on the solid surface. Likely, the acidic pH of the Spondias mombin L. extract promoted enamel dissolution, increasing porosity (Figure 2) and wettability.

Regarding color change, the specimens submitted to the *Spondias mombin L*. extract presented the highest color change compared to distilled water and 0.12% chlorhexidine digluconate. Since this extract has a brown color, an acidic pH, and contains alcohol, it presents greater potential to cause specimens darkness[23,28].

Comparatively, the CLX group showed no clinically noticeable color changes. However, chromatic changes on the dental surface exposed to CLX are related to its ability to precipitate pigments on the dental surface, whether from drinks or food[1,29,30]. The fact that the present study did not establish a contact of specimens with any drink or food may explain this result. Therefore, before clinical use of the Spondias mombin leaf extract, their dark color and acidic pH should be modified to avoid enamel damage and darkening.

As this work involved bovine enamel as the first substitute from human enamel, further investigations should perform similar evaluations using human enamel. Finally, it is worth mentioning that one of the limitations of *in vitro* studies is the reproduction of real conditions. Even when it is conducted as close as possible to a clinical

situation, laboratory conditions do not reproduce exact oral conditions, known for their extreme complexity.

Thus, further clinical trials should be designed to investigate the interaction between Spondias mombin L. extract and oral tissues.

## Conclusion

The *Spondias mombin L*. extract altered the micromorphology, promoted color change and a more wettable bovine enamel surface.

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