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ROBERTA JANAINA SOARES MENDES

AVALIAÇÃO DAS PROPRIEDADES FÍSICO-QUÍMICAS DE UM SISTEMA ADESIVO AUTOCONDICIONANTE CONTENDO PRODUTOS NATURAIS

EVALUATION OF PHYSICOCHEMICAL PROPERTIES OF A SELF-ETCHING ADHESIVE SYSTEM CONTAINING NATURAL PRODUCTS

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Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Mestra em Odontologia, na área de Odontopediatria.

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Orientadora: Aline Rogéria Freire de Castilho

Coorientadora: Regina Maria Puppin Rontani

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Provérbios 3:7

RESUMO

Os adesivos autocondicionantes foram introduzidos no mercado com o intuito de obter adesão adequada preparando simultaneamente a dentina. Apesar das significativas melhorias dos sistemas adesivos, a interface dentina-resina continua sendo a área mais fraca das restaurações resinosas. Assim, devido a necessidade em realizar adesão adequada e previsível do material restaurador à estrutura dentária, o presente estudo teve por objetivo avaliar as propriedades físico-químicas de um sistema adesivo universal (Single Bond, 3M™ ESPE) dopado com compostos de origem natural. Para a caracterização físico-química do sistema adesivo, a concentração dos compostos naturais quercetina e *trans,trans*-farnesol (*tt*-farnesol) foram determinadas, individualmente, com base na atividade oxidante que foi obtida por meio do ensaio do teste de DPPH, que se baseia na eliminação do radical livre estável 1,1-difenil-2-picrilhidrazil (DPPH·), com concentrações entre 0,24 mg/mL até 5 mg/mL, para ambos os compostos testados – quercetina e *tt*-farnesol. Após verificar qual a mínima concentração que os compostos quercetina e *tt*-farnesol apresentaram atividade antioxidante, 0,24 mg/mL e 1.43 mg/mL, respectivamente, estes foram inseridos ao sistema adesivo e confeccionados os espécimes (n= 5) correspondendo ao grupo experimental (GE) com dimensões de 7 mm x 1 mm. O sistema adesivo sem os compostos quercetina e *tt*-farnesol foi utilizado como grupo controle (GC) com dimensões de 7 mm x 1 mm. Os espécimes foram submetidos aos ensaios de sorção, solubilidade, dureza Knoop e porcentagem de amolecimento. A sorção (n = 5) e solubilidade (n = 5) se baseou na pesagem dos espécimes para obtenção, conforme determina ISO 4049:2019, das massas m1, m2 e m3, além do volume do espécime. Em seguida, foi feita a obtenção da dureza inicial (n = 5), e para que fosse obtida a porcentagem de amolecimento os espécimes foram imersos em etanol a 75% por sete dias e novas medidas de dureza foram feitas, para que fosse feita avaliação da Porcentagem de Amolecimento por meio da fórmula % Amolecimento = 100 – [(KHN2 x 100) / KHN1]. Os dados de sorção, solubilidade e da porcentagem de amolecimento foram submetidos ao Teste t de Student ($\alpha = 5\%$) enquanto os dados de dureza Knoop foram submetidos ao teste de Mann-Whitney ($\alpha = 5\%$). Ambos os compostos quercetina e *tt*-farnesol apresentaram atividade antioxidante (85,5% e 82%, respectivamente). A sorção de água foi similar nos GE e GC ($p > 0,05$) mas houve aumento significativo da solubilidade do GE. O GE apresentou menor dureza e maior porcentagem de amolecimento quando comparado ao GC. Conclui-se que a incorporação dos compostos quercetina e *tt*-farnesol modificou as propriedades físico-químicas do sistema adesivo.

Palavras-chave: Agente de Adesão Dentinária; Quercetina; Farnesol; Adsorção; Solubilidade; Dureza; Atividade Antioxidante

ABSTRACT

Self-etching adhesives were introduced to the market to obtain adequate support while preparing dentin. Despite significant improvements in adhesive systems, the dentin-resin interface remains the weakest area of resin restorations. Thus, due to the need to perform adequate and predictable support of the restorative material to the dental structure, the present study aimed to evaluate the physical-chemical properties of a universal adhesive system (Single Bond, 3M™ ESPE) doped with compounds of natural origin. For the physicochemical characterization of the adhesive system, the concentration of the natural compounds quercetin and trans-farnesol (tt-farnesol) was determined individually based on the oxidizing activity obtained by means using test assay, which is based on the elimination of stable free radical 1,1-diphenyl-2-picrilhydrazil (DPPH·), with concentrations between 0.24 mg/mL to 5 mg/mL, for both compounds tested – quercetin and tt-farnesol. After verifying the minimum concentration that the quercetin and tt-farnesol compounds presented antioxidant activity, 0.24 mg/mL and 1.43 mg/mL, respectively, these were inserted into the adhesive system and the specimens were made (n= 5) corresponding to the experimental group (EG) with dimensions of 7 mm x 1 mm. The adhesive system without the compounds quercetin and tt-farnesol was used as the control group (the CG) with dimensions of 7 mm x 1 mm. The specimens of the groups that would be evaluated were submitted to sorption, solubility, Knoop hardness, and softening percentage tests. The sorption (n = 5) and solubility (n = 5) were based on the weighing of the specimens to obtain, as determined by ISO 4049:2019, of the masses m1, m2, and m3, in addition to the specimen volume. Then, the initial hardness (n = 5) was obtained and to obtain the final hardness, the specimens were immersed in 75% ethanol for seven days and new hardness measurements were made, so that the Softening Percentage was evaluated using the formula % Softening = 100 – [(KHN2 x 100) / KHN1]. The data of sorption, solubility, and softening percentage data mitted to the student's t-test (α = 5%) while the Knoop hardness data were submitted to the Mann-Whitney test (α = 5%). Both quercetin and tt-farnesol showed antioxidant activity (85.5% and 82%, respectively). Water sorption was similar in EG and CG ($p>0.05$) but there was a significant increase in the EG solubility. The EG presented lower hardness and percentage of softening when compared to CG. It was concluded that the incorporation of quercetin and tt-farnesol compounds modified the physical-chemical properties of the adhesive system.

Keywords: Dentinal Adhering Agent; Quercetin; Farnesol; Adsorption; Solubility; Hardness; Antioxidant Activity

SUMÁRIO

1 INTRODUÇÃO

O conhecimento da Odontologia Baseada em Evidências tem direcionado o manejo da cárie dentária para uma abordagem médico-biológica, o que implica em uma intervenção mais conservadora da lesão cariosa (INNES et al., 2019). No entanto, o substrato remanescente na cavidade após a remoção seletiva do tecido cariado difere substancialmente da dentina hígida em aspectos químicos e morfológicos (MAZZONI et al., 2015). Estas mudanças impactam diretamente na qualidade da adesão à dentina (TJÄDERHANE et al., 2013), ocasionando falhas e trocas sucessivas das restaurações adesivas ao longo do tempo (YANG et al., 2017).

Claramente, a qualidade do processo de adesão em dentina desmineralizada é drasticamente reduzida devido ao aumento da porosidade da camada de dentina intertubular causada pela perda mineral e a persistência de cristais de cálcio resistentes ao ácido nos espaços intratubulares, que são capazes de impedir a penetração de monômeros resinosos através dos túbulos dentinários (BRESCHI et al., 2018). Em outras palavras, os monômeros resinosos enfrentam mais dificuldade na via de permeação entre as fibras de colágeno, causando um acúmulo de água próximo à interface adesiva que permite a degradação hidrolítica do polímero formado, resultando, assim, na diminuição da resistência de união (TJÄDERHANE et al., 2013). Além disso, sob a influência de tensões térmicas e mecânicas, os espaços interfibrilares sofrem tensão excessiva levando ao aprisionamento de ar e formação de lacunas ao longo de toda a extensão de penetração, causando a falha da interface resina/dentina (BRESCHI et al., 2018). Adicionalmente, as limitações nesse complexo processo de união contribuem para a formação de defeitos na camada híbrida, invariavelmente fadadas ao fracasso após função prolongada (STAPE et al., 2018; YANG et al., 2017).

A formação da união dentina-adesivo é realizada predominantemente por retenção micromecânica devido a penetração da resina e emaranhamento entre as fibrilas de colágeno expostas na dentina, parcial ou totalmente, desmineralizada (BRESCHI et al., 2018) após utilização de ácidos ou monômeros resinosos ácidos (HE et al., 2019). De fato, é impossível durante o processo adesivo, deslocar completamente a água extrafibrilar de uma matriz de colágeno desmineralizada e, assim, promover completa infiltração na rede de colágeno, pois o espaço intermolecular limitado das fibrilas de colágeno torna difícil acomodar as moléculas de monômeros resinosos (CAO et al., 2015; HE et al., 2019; NIU et al., 2014).

Além disso, enzimas dentinárias endógenas, como as metaloproteinases da matriz (MMPs) e cisteína-catepsinas, são ativadas durante o processo de cárie e de adesão e iniciam a degradação da matriz de colágeno (HASHIMOTO; BRESCHI, 2003), denotando a necessidade de estratégias para a preservação da camada híbrida, incluindo a modulação da atividade enzimática endógena (CAO et al., 2015; JINDAL; BHAT, 2020).

Dentre os inibidores de MMPs, uma das alternativas mais conhecidas para a inibição da atividade colagenolítica endógena e estabilização da interface adesiva é o uso de digluconato de clorexidina: 1) incorporado ao agente condicionante, 2) incorporado na composição do sistema adesivo ou 3) como pré-tratamento da dentina após o condicionamento ácido (SACRAMENTO et a., 2012; MONTAGNER et al., 2014). Apesar de inibir a atividade de MMPs e cisteína-catepsinas, a ligação da clorexidina é um mecanismo reversível eletrostático e instável ao longo do tempo à lixiviação e união desta aos sítios aniônicos em mineralizações e desmineralizações dentinárias (BRESCHI et al., 2018), sem um consenso sobre a maneira ideal de aplicação do antimicrobiano. Ácido etilenodiaminotetracético (EDTA) e compostos de amônio quaternário são igualmente citados na literatura, porém não existem vantagens imediatas na resistência de união após o uso destas substâncias (MONTAGNER et al., 2014), enquanto que o glutaraldeído, apesar de promover aumento da resistência do colágeno, também forma uma barreira mecânica que impede a difusão tecidual sem inibir completamente a atividade das MMPs na região mais profunda da camada híbrida (ZHOU et al., 2019)

A utilização de agentes chamados *cross-linkings*, tais quais clorexidina, glutaraldeído, proantocianidina, quercetina entre outros (GOTTI et al., 2015; ZHOU et a., 2019; HONG et al., 2022), é uma alternativa clinicamente interessante para aumentar a estabilidade da união resina-dentina, pois os *cross-linkings* são inespecíficos em relação ao tipo de MMPs, são capazes de reduzir a degradação da interface adesiva, por meio da inibição da enzima colagenolítica endógena (bloqueiam o sítio de clivagem da enzima) e aumento da resistência à degradação do colágeno, ou indiretamente, pela eliminação da água entre as fibrilas de colágeno expostas, prevenindo assim, a degradação da matriz (PORTO et al., 2018; SCHEFFEL et al., 2014).

Considerados fontes abundantes de importantes propriedades farmacológicas, os produtos de origem natural e seus derivados são alternativas interessantes e amplamente explorados na indústria farmacêutica em busca de protocolos terapêuticos seguros e eficazes (CASTILHO; PARDI; MURATA, 2007; FERNANDO et al., 2017).

Dentre os compostos conhecidos, a quercetina ($C_{15}H_{10}O_7$), um flavonoide, é frequentemente utilizado na indústria alimentícia, presente em alimentos e bebidas como brócolis, cebola, maçã, pimentão, limão, uva, vinho (Alizadeh, Ebrahimzadeh, 2021). A quercetina tem expressiva atividade antimicrobiana além de exercer função antioxidante, capaz de contribuir com a inibição da formação de oxigênio reativo mediador do estresse oxidativo e atuar como supressor da expressão das proteínas da matriz extracelular MMP-9 e MMP-2 (ANGELLOTTI et al., 2020; DÁVILA-SÁNCHEZ et al., 2020;), contribuindo desta forma, para o aumento da resistência de união à dentina (YANG et al., 2017).

O *trans,trans*-farnesol ($C_{15}H_{26}O$), por sua vez, é um sesquiterpeno de 15 carbonos extraído de frutas cítricas, de ampla ação farmacológica devido a associação de potente atividade antimicrobiana (ANDRÉ et al., 2021), baixa toxicidade e importante atividade anti-inflamatória (DE ARAÚJO DELMONDES et al., 2019), além de potencial antimicrobiano e anticárie (DE CASTILHO et al., 2019; LEYVA DEL RIO et al., 2020). Em adição, o estudo feito por Khan e Sultana, 2011 revelou a capacidade do *tt*-farnesol de atenuar a atividade enzimática da 1,2-dimetil-hidrazina, reduzindo estresse oxidativo e migração de células inflamatórias (LEYVA DEL RIO et al., 2020).

Com base nos fundamentos descritos, os efeitos farmacológicos dos agentes bioativos quercetina e *tt*-farnesol fundamentam o uso destes na busca por terapias seguras e eficazes, sendo que a incorporação destes compostos em sistema adesivo pode melhorar substancialmente a composição bioquímica e microestrutura da matriz de dentina desmineralizada (BRESCHI et al., 2018; TJÄDERHANE et al., 2013). Por serem moléculas hidrofóbicas, dificultando a penetração do material a dentina úmida, faz-se necessário a utilização de um solvente que permita o deslocamento de água nos túbulos dentinários e assim, proporcione boa penetração dos monômeros na rede de colágeno da dentina desmineralizada, para reexpandir a rede de colágeno colapsada e fortalecer a união por meio do selamento da interface adesiva (VAN LANDUYT et al., 2007). Os sistemas adesivos universais têm a vantagem de um sistema de ligação por meio de uma etapa simplificada e menos sensível à técnica pois fornece uma interface estável ligada ao substrato dentário, unindo-se quimicamente à estrutura do dente, preservando a hidroxiapatita do dente, o que é importante para a durabilidade da ligação (GIANNINI et al., 2015) e tornando-o potencialmente benéfico para a integração de propriedades antioxidantes e antibacterianas quando em contato com os tecidos dentais (ZHOU et al., 2019).

Observando os possíveis benefícios para os tecidos dentinários dos compostos naturais quercetina e *tt*-farnesol, notou-se a necessidade de avaliação da junção desses compostos aos materiais restauradores, iniciando com acréscimo ao sistema adesivo simplificado que apresenta facilidades clínicas, mas necessidades de melhorias em suas propriedades. Assim, entendendo que o sucesso da restauração adesiva está diretamente ligado a qualidade da adesão ao substrato, nossa hipótese é que o sistema adesivo dopado com bioativos com múltiplas funções biológicas, é capaz de manter suas propriedades físico-químicas, possibilitando o uso clínico, a fim de melhorar a camada híbrida.

2 ARTIGO Evaluation of physicochemical properties of a universal adhesive system containing natural products

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ABSTRACT

This study aimed to evaluate the physicochemical properties of a universal adhesive system (Single Bond, 3M™ ESPE) doped with natural compounds. For the physicochemical characterization of the adhesive system, the concentration of the natural compounds quercetin and *trans,trans*-farnesol (*tt*-farnesol) was determined based on the antioxidant activity of both compounds using the DPPH assay with concentrations ranging from 0.24 mg/mL to 5 mg/ml for both compounds. Next, specimens (n=5; 7 mm x 1 mm) of an adhesive system containing both quercetin and *tt*-farnesol at concentrations of 1.43 mg/mL and 0.24 mg/mL, respectively (EG) were prepared. The adhesive system without quercetin and *tt*-farnesol was used as a control (CG). The specimens were submitted to sorption, solubility, Knoop hardness, and softening percentage assays. After obtaining the initial hardness, the specimens were immersed in 75% ethanol for seven days and new hardness measurements were taken. Data on sorption, solubility, and the percentage of softening were submitted to Student's t-test (α=5%) while the data on Knoop hardness was submitted to the Mann-Whitney test (α=5%). Both quercetin and *tt*-farnesol exhibited antioxidant activity (85.5% and 82%, respectively). Water sorption was similar in EG and CG (p>0,05) but GE had a significantly higher solubility after storage. EG showed lower hardness and higher softening when compared to CG. In conclusion, the incorporation of quercetin and *tt*-farnesol modified the physicochemical properties of the adhesive system.

Keywords: Dentinal Adhering Agent; Quercetin; Farnesol; Adsorption; Solubility; Hardness; Antioxidant Activity

INTRODUCTION

Monomers are critical to the formation of a reticulated matrix on dentin bonding systems as they are designed to provide chemical and physical stability of the resin-dentin interface (1,2). The resistance, durability, and bonding with both resin-enamel and resin-dentin protect the interface of restoration margins against penetration of bacteria that could allow the development of secondary caries which would require the early replacement of the restoration, thus causing the unnecessary removal of healthy dental structure with the extension of cavitary preparation to create retentive areas (2).

Besides the research efforts to improve long-lasting adhesive restorations, resin-dentin bonding is less durable than resin-enamel bonding due to dentin's heterogeneous structure and composition (2). Also, there are no effective alternatives to prevent hydrolytic degradation of dentin bonding, although various substances such as crosslinking agents are used to reduce collagen degradation (3,4). Collagen from demineralized dentin completely wrapped in adhesive represents the ideal hybrid layer, but this ideal structure has not yet been achieved in vivo (3,4,5,6). This failure is due, in part, to the difference in the depth of the adhesive infiltration and the depth of the demineralized dentin collagen (6). Diverse studies have focused on matrix metalloproteinase (MMP) inhibitors to decrease the degradation process of collagen fibrils within the hybrid layer (1,4,5). Likewise, the protein cross-linking agents induce conformational changes in the structure of MMPs (1,4).

Dentin/adhesive sealing quality is affected by the hybrid layer, clinical substrate (which features heterogeneous substrate, caries-affected dentin, and sclerotic dentin), adhesive composition, operator technique, saliva contamination, and patient characteristics (7). Bringing those topics together, the bonding plays a central role in the resin-based composite restoration (2) but the longevity of resin restorations is still clinically unsatisfactory. Thus, new bonding strategies must be considered to minimize collagen degradation at the resin-dentin interface. Among those strategies can be a vast range of natural compounds whose pharmacological properties would support their use as safe and effective alternatives to reach the longevity of resin-dentin bonding (5). The inclusion of bioactive compounds acts simultaneously on several mechanisms associated with the reduction of degradation of the resin-tooth interface (5).

Quercetin ($C_{15}H_{10}O_7$) is a natural crosslinking agent predominant in propolis, with a potential role in the modulation of the activity of MMP-2 and MMP-9 (6). Quercetin can form

a cross-linking bond with collagen, which reduces the formation of water channels, resists the attack of collagenase, and strengthens stability (6,8). As quercetin is a hydrophobic molecule (6), in theory, it would facilitate the penetration of the adhesive system into the wet dentin and could strengthen the bond between resin and dentin by sealing the adhesive interface, after repelling water from the dentinal tubules (9). Another propolis-extracted compound with broad pharmacological properties is the *trans,trans*-farnesol (*tt*-farnesol; $C_{15}H_{26}O$) (10). Given the antimicrobial activity potential of *tt*-farnesol (10,11) and the antioxidant activity of quercetin (6,7), the incorporation of both compounds into a self-etching adhesive system would likely enhance the durability of resin restorations by the biochemical composition, microstructure, and function of the demineralized organic matrix, mainly in areas of biofilm accumulation such as marginal interface (12).

Among dental restorative materials, adhesive systems are the ideal materials to present adequate antioxidant and physical-chemical properties due to the intimate contact with dental hard tissues, since the low sorption and solubility help in the adequate maintenance of the properties and characteristics of the polymer and the hardness in identifying the material's resistance to penetration or abrasion (1,4,7). The development of more effective bonding strategies is needed, but the physicochemical properties of adhesive systems could be influenced by the incorporation of the compounds. Therefore, this study aimed to evaluate a self-etching adhesive system doped with quercetin and *tt*-farnesol through the water sorption, solubility, microhardness, and softening percentage. The hypothesis is that the adhesive system doped with compounds with multiple biological functions can maintain its initial physicochemical properties.

MATERIALS AND METHODS

For the physicochemical characterization of the experimental adhesive system, the natural compounds quercetin and *tt*-farnesol and a universal adhesive system (EG; Single Bond Universal, 3M ESPE, Sumaré, SP, Brazil) were used. The adhesive system without compounds was used as a control group (CG). Details of the compounds and chemical composition of the adhesive system are shown in Table 1 while Figure 1 outlines the design of the study.

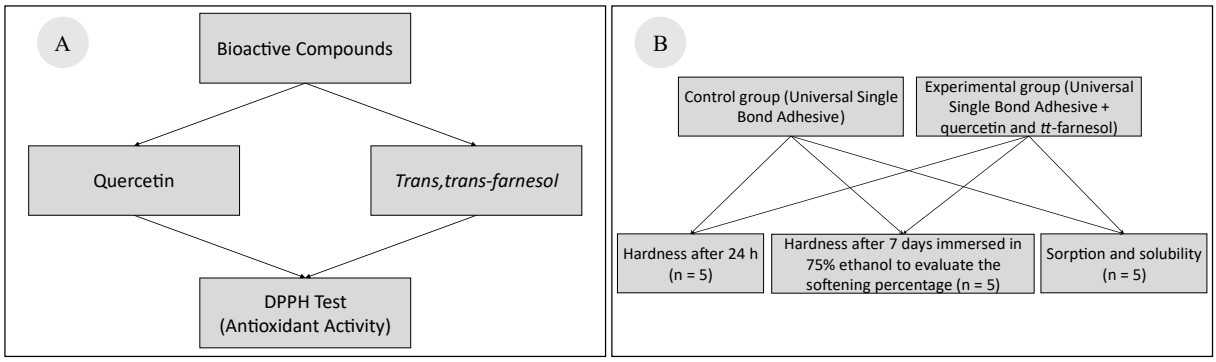

Figure 1. Flowchart of the experimental design used in this study. A - Preliminary study: Natural compounds that were submitted to the DPPH assay; B - Physicochemical characterization: Groups and assays. Source: Authors.

Table 1. Name, composition, CAS registry number, molecular weight, brand and batch of compounds, and adhesive system used in the study.

Product Name	**Composition**	**CAS Number**	**Molecular Weight**	**Molecular Formula**	**Brand**	**Batch**
Quercetin *	-	849061-97-8	302.24	$C_{15}H_{10}O_7$	Sigma-Aldrich	LRAB 7760
Tran,trans -farnesol*	-	106-28-5	222.37	$C_{15}H_{26}O$	Sigma-Aldrich	BCCC 6806
Single Bond Universal **	2-hydroxyethyl methacrylate, Bisphenol A dimethacrylate dimethacrylate dimethyl (BisGMA), 2-PROPENOIC ACID, 2-METHYL-, REACTION PRODUCTS WITH 1,10- DECANEDIOL AND PHOSPHORUS OXIDE (P2O5), Ethanol, Water, Silica treated from silane, Acrylic copolymer	-	-	-	3M™ ESPE	41266 41269 41278 41279 41282

	and itaconic acid, camphor quinone, DIMETHYLAMINOBENZOAT (-4), 2-dimethylaminoethyl methacrylate, 2,6-Di-terc-butyl-p-cresol

*Sigma Aldrich (www.sigma-aldrich.com)

**Material Safety Data Sheet information.

DPPH radical scavenging assay

To determine the compound concentrations to be incorporated into the adhesive system in a preliminary study, the DPPH (2,2-diphenyl-1-picrilhydrazil; Sigma-Aldrich) assay was performed. DPPH is a simple and fast assay that turns purple color into yellow when free radicals are neutralized by the antioxidants (13). The antioxidant activity of the compounds quercetin and *tt*-farnesol was evaluated by capturing the DPPH radical. First, an ethanolic solution of DPPH at 0.6 mM was prepared. Then, stock solutions of the quercetin and *tt*-farnesol at 5mg/mL were prepared by dissolving the compounds in 20 % DMSO (dimethyl sulfoxide; Sigma-Aldrich) (14). Aliquots of 100 µl of DPPH, were placed in a microplate of 96 wells, in triplicate. Wells with DPPH and ascorbic acid (5 mg/mL; Sigma-Aldrich) and 20 % DMSO were used as control.

The compound concentration ranged from 5 mg/mL to 0.24 mg/mL for each antioxidant assay. The absorbance was measured at 517 nm, microplate reader ELISA (Eon ™, Bio-Tek Instruments, Wibrijk, Belgium) after 30 min of the addition of DPPH. The ability to reduce the DPPH radical was calculated using mean and standard deviation, to calculate the concentration capable of inhibiting 50 % of the DPPH. The percentage of antioxidant activity was evaluated using the following equation: % Antioxidant Activity = [(Ac - Aa)/Ac] x 100. (15)

Specimen preparation

After the determination of antioxidant activity by DPPH, the minimal antioxidant capacity of quercetin (0.24 mg/mL) and *tt*-farnesol (1.43 mg/mL) were used for the subsequent tests, which were sorption and solubility and microhardness and softening percentage.

Based on this, five discs (7 mm x 1 mm) of the adhesive system containing quercetin and *tt*-farnesol (EG) at concentrations of 0.24 mg/mL and 1.43 mg/mL, respectively, or not (CG) were prepared for each group and each essay using an elastomer mold (Silicone Printing Material by Condensation, Profile, Vigodent-Coltene, Rio de Janeiro, RJ, Brazil) according to ISO 4049:2019. For the specimen preparation of the control group, approximately 3 drops (90 µl) of the adhesive system were inserted in the elastomeric matrix, shaken for 20 s, with the aid of the triple syringe the solvent evaporated for 5 s, and then light-cured for 10 s. For the preparation of the specimen of the experimental group (adhesive system added with the compounds quercetin and *tt*-farnesol), approximately 3 drops (90 µl) were dripped into the elastomeric matrix, stirred for 20 s, with the aid of the triple syringe the solvent was evaporated for 5 s, and then light cured for 40 s.

Water sorption and solubility

Immediately after preparation, the specimens (n=5) were stored individually in an organizer with a partition and kept in a desiccator with silica gel at 37 ºC for 22 h. After that, the silica was changed, and specimens remained in the desiccator for another 2 h at 37 ºC. After 24 h in the desiccator, each specimen was weighed in analytical balance (BEL Engineering®, Piracicaba, São Paulo, Brazil) with an accuracy of 0.001 g. This weighing cycle was maintained and repeated until obtaining a constant mass (m1) of each disc referring to each group, using a variation ≤ 0.001 in the 24 h interval. The mass m1 was obtained after 13 days of weighing.

After obtaining the m1 mass, the diameter of specimens was measured, two measurements of diameter to remove the average later, and the thickness of the test body using a digital caliper (Mitutoyo, Tokyo, Japan) with an accuracy of 0.01 mm. From these values, the volume of the cylinder in mm^3 was calculated. After checking the volume of each disc, they were immersed individually in an organizer with a partition and each breakdown contained 4.66 mL of deionized water at 37 ºC for seven days, the decision of the final volume of water was calculated based on ISO 4049. After seven days, the water discs were removed and dried on absorbent paper for one minute then each specimen was weighed to obtain the m2, specimens were placed again in a desiccator containing silica in gel, and the procedures described to obtain the m1 mass were repeated to obtain a constant mass of m3. The constant m3 mass of specimens was taken after 10 days of weighing. The values in mg/mm^3 of water sorption (Wsp) and solubility (Wsl) were calculated based on the following equations: $Wsp = (m2-m3)/V$ and $Wsl = (m1-m3)/V$.

Hardness and Softening Percentage

To evaluate softening in a solvent, indirectly, the specimens of each control (CG) and experimental group (EG) were submitted to the Knoop microhardness evaluation test after 24 h (KHN1) of preparation of the specimens and after seven days of their immersion in 75 % ethanol (16) to evaluate the percentage of softening (KHN2). The specimens were prepared as described before and stored in the dark at 37 ºC for 24 h. After storage time, the surface hardness was measured using a Future Tech FM-100 microdurometer (Shimadzu, Tokyo, Japan) at a load of 25 gf for 10 s. Knoop hardness values were recorded. Then, the specimen was stored in 4 ml of 75 % ethanol solution, protected from light at 37 ºC for seven days. Five readings were taken for each specimen and the percentage of softening (% Softening) was calculated as follows: % Softening = 100 – [(KHN2 x 100) / KHN1].

Statistical analysis

The statistical data were subjected to analysis of normality using the following Shapiro-Wilk tests, and analysis of variance and homogeneity using Levene's tests and variance ratio. Student's t-test was used to assess sorption and solubility and to compare the degree of softening of specimens after being immersed in 75% ethanol. A non-parametric statistical test for independent specimens of Mann-Whitney was used for hardness analysis. The data on the antioxidant activity was analyzed descriptively to evaluate the ideal concentration and the DPPH test for the evaluation of antioxidant activity was evaluated using the ANOVA statistical test and Tukey's test for multiple comparisons. Jamovi 2.2.5 (Patreon, San Francisco, USA) was used for all statistical analyses. $P < 0.05$ was considered significant.

RESULTS

DPPH radical scavenging assay

Figure 2 shows that the compounds exhibited antioxidant activity like ascorbic acid (positive control). The highest value of antioxidant activity was observed for quercetin followed by *tt*-farnesol. It is observed that the percentage of absorption of the DPPH radical remained high for the compounds used in the study quercetin and tt-farnesol, equivalent to the positive control ascorbic acid and, as shown in figure 2B, the higher the absorbance, the lower the percentage of radical absorption DPPH (figure 2A) and no effect for antioxidant activity the compound is.

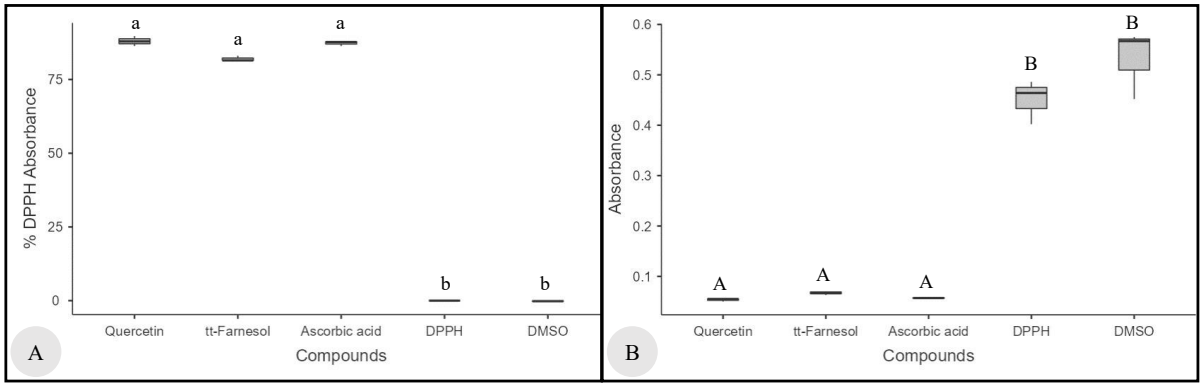

Figure 2. Evaluation of the antioxidant activity of the compounds quercetin and tt-farnesol about the control groups. A – percentage of DPPH radical scavenging; B - Absorbance measured at 517 nm. Different letters represent statistically significant differences between groups by ANOVA, followed by the post hoc Comparisons – Composite test ($p < 0.05$).

Quercetin presented an average of 85.8 % of free radical DPPH absorption. The compounds quercetin and *tt*-farnesol showed a percentage of DPPH radical capture greater than 50 % at different concentrations (Table 2).

Table 2. Analysis of DPPH radical absorption percentage according to the concentration that presented the antioxidant activity of each compound

	Compounds	Concentration (mg/mL)	% of DPPH absorption
Mean	Quercetin	0.988	85.8
	tt-farnesol	0.988	36.3
Median	Quercetin	0.388	88.0
	tt-farnesol	0.388	20.6
Standard deviation	Quercetin	1.04	3.51
	tt-farnesol	1.04	44.3
Minimum	Quercetin	0.244	80.2
	tt-farnesol	0.244	-5.30
Maximum	Quercetin	2.62	88.3
	tt-farnesol	2.62	85.2
25th percentile	Quercetin	0.258	84.7
	tt-farnesol	0.258	-0.920
50th percentile	Quercetin	0.388	88.0
	tt-farnesol	0.388	20.6

75th percentile	Quercetin	1.43	88.1
	tt-farnesol	1.43	82.0

Based on the DPPH radical scavenging activity, the quercetin, and the *tt*-farnesol showed strong scavenging activity and were selected to be included in the adhesive system at their lowest concentrations of 0.24 mg/mL and 1.43 mg/mL, respectively.

Water sorption and solubility

Water sorption and solubility results are summarized in Table 3. Water sorption did not vary significantly between groups, but the solubility did ($p < 0.05$). The adhesive containing compounds (experimental group) presented greater water sorption and solubility than the control, evidencing that those specimens absorbed and retained much water from the external environment.

Table 3. Results of the evaluation of sorption and solubility (μg/mm³) of the control (CG) and experimental (EG) groups

	Group	Mean (SD)
Sorption	CG	132.3 (18.04)[a]
	EG	173 (12.7)[a]
Solubility	CG	34.4 (9.02)[b]
	EG	163 (11.8)[a]

CG - Control group. EG - Experimental group. Statistical differences between groups of materials are expressed by different superscript letters in columns Shapiro-Wilk, Levene's test, and Student's t-test

Hardness and Softening Percentage

As shown in Figure 3, the hardness and softening percentages showed better values in the control group, and the percentage of softening was higher in the experimental group, showing that the crosslinking density was changed after the addition of natural compounds.

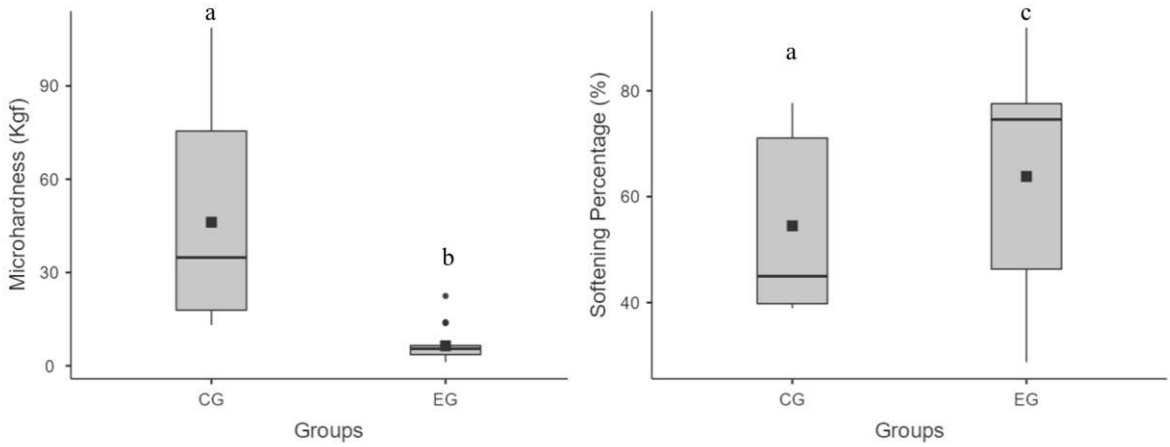

Figure 3. Hardness and softening Percentage of control (CG) and experimental (EG) groups. Distinct letters represent statistically significant differences between groups by Student's t-test for microhardness and Mann-Whitney for Softening Percentage.

DISCUSSION

The search for an ideal adhesive restoration has been a great challenge for researchers (2). Several attempts are being made to improve the antimicrobial activity and bonding of the adhesive systems, but their physicochemical properties could be compromised (10,17). Current efforts in exploring natural products could be an interesting alternative to developing a durable resin-dentin bonding using a biocompatible agent that promotes the suppression of extracellular matrix proteins (5,7,8,18). The adhesive system used in this study is called "Universal" and it represents the latest generation of dentin adhesive systems launched on the market. Universal adhesive systems promote a connection through a simplified step, with the advantage of forming a stable interface between the dental substrate and composite resin (1). Such an adhesive system can be used in a self-etching mode in one-step or two-step conditioning and rinsing mode without compromising the effectiveness of the adhesive material (10).

Quercetin and *tt*-farnesol were incorporated into the adhesive system based on their minimal antioxidant capacity found in the DPPH assay. This is a primary study to adjust the compound's concentration and their incorporation into the adhesive system, to improve the water sorption, solubility, hardness after 24 h, and the softening percentage. The idea of using compounds with antioxidant activity was to improve the bonding process by preventing the hydrolytic degradation of dentin bonds (19) but the hypothesis of the study was rejected.

Quercetin and *tt*-farnesol compromised the physicochemical properties of the adhesive system since the solubility and the softening percentage of the material were increased. During the specimen's preparation, delayed curing of the experimental adhesive system was observed, requiring more photoactivation time than recommended by the manufacturer. In total, it was necessary about 40 s of photoactivation of the specimens which means that the longer the curing, the worse permeability of the bonded interfaces (20). The negative effect of that delay in curing time was also reported by Del Rio et al (11), which added *tt*-farnesol to a universal adhesive system to evaluate their degree of conversion. Interestingly, the delaying photoactivation was not a concentration-dependent pattern (11). Mehmood et al (21) reported that high concentrations of quercetin might negatively influence the strength and the polymerization of the adhesive system. Likewise, Gotti et al (9) found that the addition of quercetin to a universal adhesive system did not compromise the integrity of the bonding, but greatly influenced polymerization (9).

It is noteworthy that the adhesive system used in the study is simplified and presents a high amount of solvent and hydrophilic monomers, thus, adhesive systems that have hydrophilic monomers are characterized by presenting polymerization below ideal, being important, observing the drying time of the air and curing time (22,23). Therefore, solvent-free adhesive systems have more complete polymerization (23). It can be assumed that the higher percentage of monomers and hydrophilic compounds, such as DMSO 20%, and the presence of water in simplified adhesives may compromise the polymerization reaction (23).

Immersion in 100% ethanol proved to be an appropriate method to classify and evaluate the softening percentage which indirectly shows results on the reduction of hardness after immersion in ethanol (16). However, it causes severe degradation of adhesive systems because there are more hydrophilic molecules but few inorganic particles in the adhesive system composition (16). Therefore, the immersion in 100% ethanol did not provide an evaluation of crosslinking density due to the loss of specimens after degradation. In addition, there is a reduction in the hardness of composites and adhesive systems after immersion in 100% ethanol (16). Since it was not possible to measure the hardness after immersion in 100% ethanol, the current study was performed using ethanol at 75% to evaluate the crosslinking density of the polymeric network (24).

The immersion of specimens in alcohol 75% allows us to evaluate, with better precision and without invalidity the use, the hardness of the surface, using softening in a solvent,

simulating the exposition to the chemicals in the oral condition (24). The mechanism of damage is attributed to the softening of the polymer matrix, being observed with maximum and not degrading effect on immersion in 75% ethanol (25). Thus, adding the natural compounds quercetin and *tt*-farnesol to the commercial adhesive system, possibly, decreased their degree of cure, that is, the conversion achieved during the reticulation reaction.

Contemporary adhesive solvents are water, acetone, and ethanol and the solvent composition is directly linked to their evaporation viability, the ability to replace the water present in the adhesive system, and the ability to promote the binding of these solvents (25,26). DMSO is classified as a class 3 solvent and has an amphiphilic characteristic, lower toxicity, and risk to human health (27). The choice of DMSO as a solvent for quercetin and *tt*-farnesol compounds was because solvents incorporated into dental adhesive systems play an important role in the bonding to hard tissues, as they can increase the spaces between fibrils, being a possible explanation for the increased penetration of the monomer, since collagen fibrils need to remain hydrated and DMSO is miscible in water (27,28). That characteristic is important because it suggests an interaction of DMSO with water, possibly improving the properties of wettability (28) and possibly allowing better solubility of compounds to the universal adhesive system due to its amphiphilic characteristic. Gotti et al, (9) observed that adhesive systems which had pure quercetin incorporated into them, without the aid of a solvent, presented a less homogeneous aspect, possibly affecting the solubilization of that antioxidant in the adhesive system (29).

The solvents dissolve the monomers and carry them inside the tooth structure, facilitating adequate infiltration into the exposed collagen network with adhesive monomers (17). It is also reported that a high concentration of DMSO as treatment can improve immediate and long-term bond strength (30). However, the amount of DMSO could be crucial to the physicochemical properties of the adhesive system. As found by Salim Al-Ani, et al (31) higher amounts of DMSO can impair the chemical and mechanical properties, regardless of the composition of the adhesive monomer. In that study, the incorporation of 5% or more of DMSO increased the degree of conversion of monomers, demonstrating that the increasing concentrations of DMSO resulted in a slight increase in cell viability (31). However, a higher degree of conversion does not necessarily imply an improvement in the quality of the polymer, which may compromise the quality of the polymer network, because the more crosslinking, the more resistant to hydrolytic degradation compared to those with lower crosslinking density (32). As well as the increase in the hydrophilic component, the DMSO facilitates the infiltration

of the adhesive, however, there are disadvantages to the water sorption property that weakens the polymer, plasticizing it and promoting the chemical hydrolysis of the adhesive (33).

The findings presented here are preliminary and, although quercetin and *tt*-farnesol incorporated into adhesive systems in other concentrations could open perspectives to enhance the durability of adhesive restorations (34), long-term studies are needed to comprehensively understand the behavior and longevity of the resin-dentin interface of those natural products-based adhesive systems.

Based on these findings, other assumptions could be explored. At first, the association of the compounds individually to the self-etching adhesive system as well as the evaluation of other material properties such as degree of conversion, bond strength, and short and long-term release of compounds from adhesive systems. Besides, it would be interesting to verify the clinical significance of those variations in physical-chemical and mechanical properties, since in the oral cavity the sum of thermal, mechanical, and chemical stimuli occurs concomitantly and repeatedly. Also at another time, the possibility of using a different solvent to dissolve the compounds before their incorporation into the adhesive system or even some different concentrations of the DMSO could be useful.

CONCLUSION

Based on the findings of this study, the addition of natural compounds modified important physicochemical properties of the adhesive system.

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3 CONCLUSÃO

- Os compostos quercetina e *tt*-farnesol apresentaram atividade antioxidante com base no teste de DPPH.

- A incorporação dos compostos quercetina e *tt*-farnesol alterou a sorção de água do sistema adesivo autocondicionante.

- A incorporação dos compostos quercetina e *tt*-farnesol aumentou a solubilidade e alterou a porcentagem de amolecimento do sistema adesivo, diminuindo a dureza superficial do sistema adesivo.

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APÊNDICE

Apêndice 1 – Etapas do projeto de pesquisa

Preparo das soluções para avaliação de atividade antioxidante

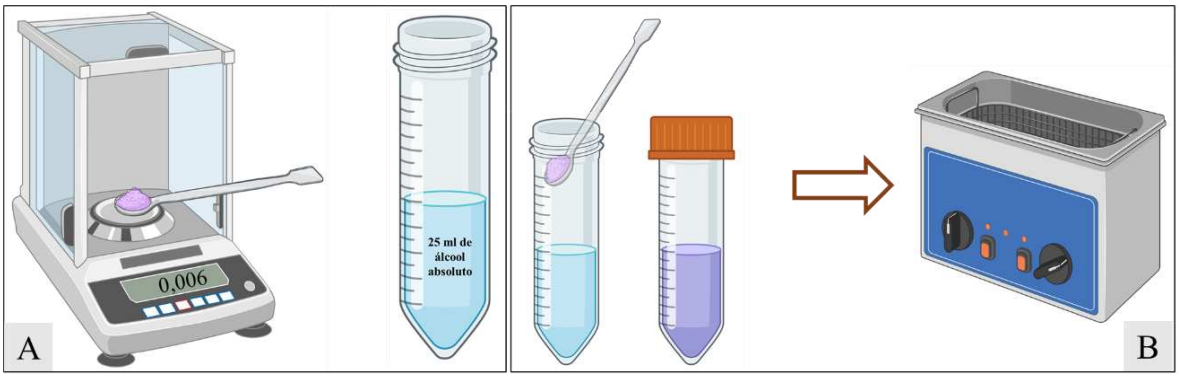

Figura 4 Preparo da solução de DPPH. A - Pesagem do pó de DPPH; B - Inserir em álcool absoluto e levar para lavadora ultrassônica

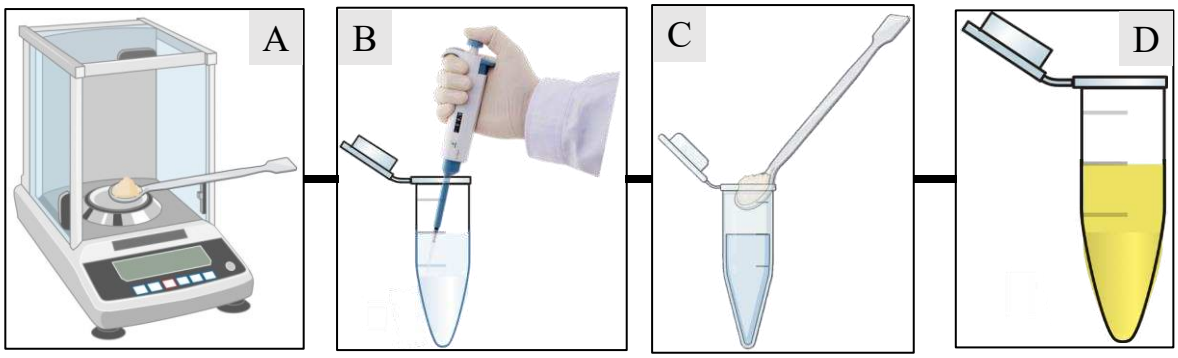

Figura 5 Preparo da solução de quercetina. A - Pesagem do composto quercetina; B - Medida de 1 ml de DMSO em microtubo eppendorf; C - Diluição do composto; D - Solução pronta para o teste de DPPH; E – Solução levada para a lavadora ultrassônica

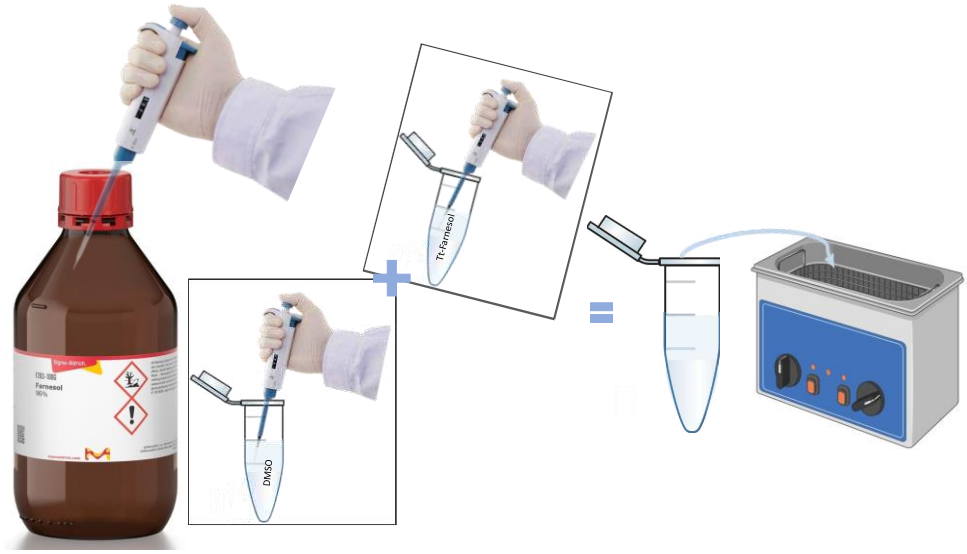

Figura 6 Preparo da solução de *tt*-farnesol

Teste de DPPH

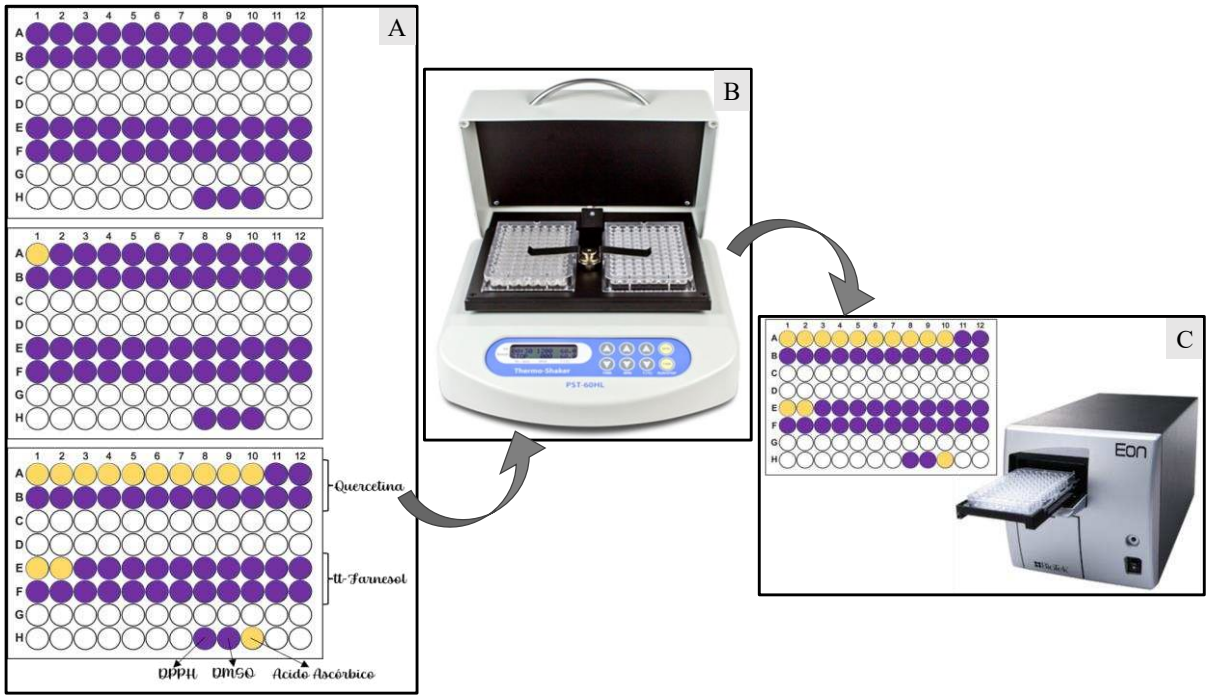

Figura 7 Teste de atividade antioxidante por meio do consumo do radical DPPH. A - Inserindo a solução de DPPH e em seguida a solução dos compostos que terão atividade antioxidante verificada; B - Agitador térmico de placas; C- Microplaca de 96 poços e Espectrofotômetro de microplaca Eon™ - Leitor Elisa

Ensaios físico-químicos do sistema adesivo

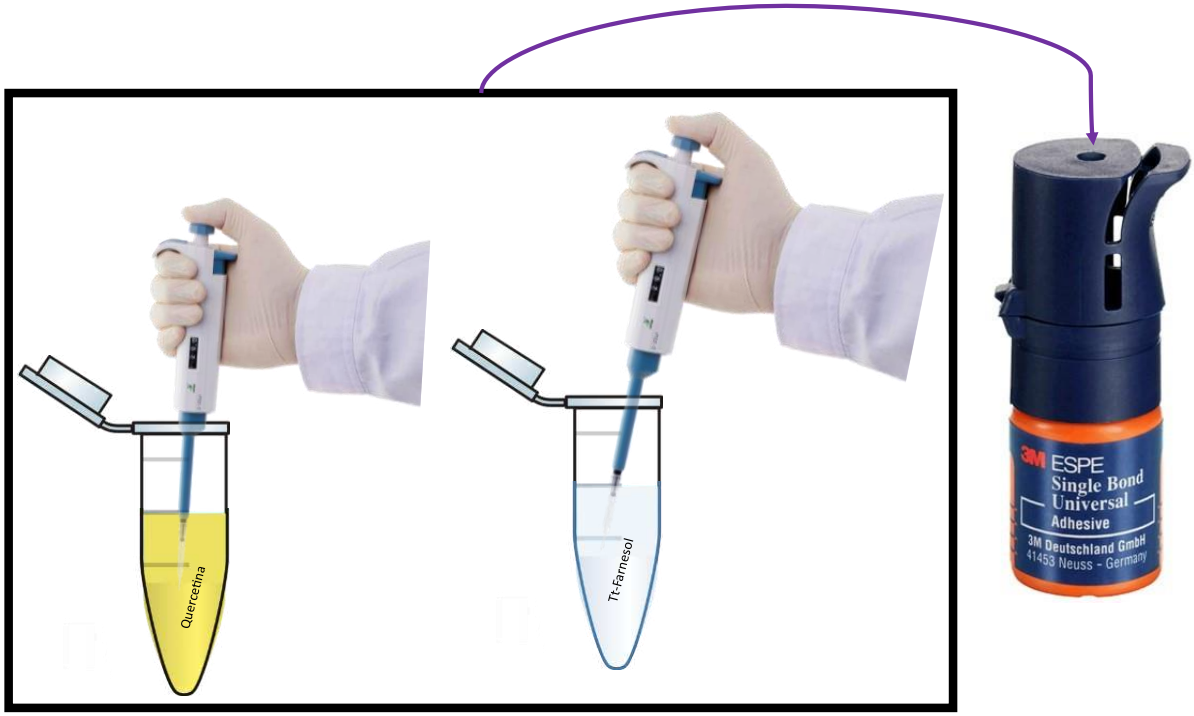

Figura 8 Adição dos compostos quercetina e *tt*-farnesol ao sistema adesivo comercial

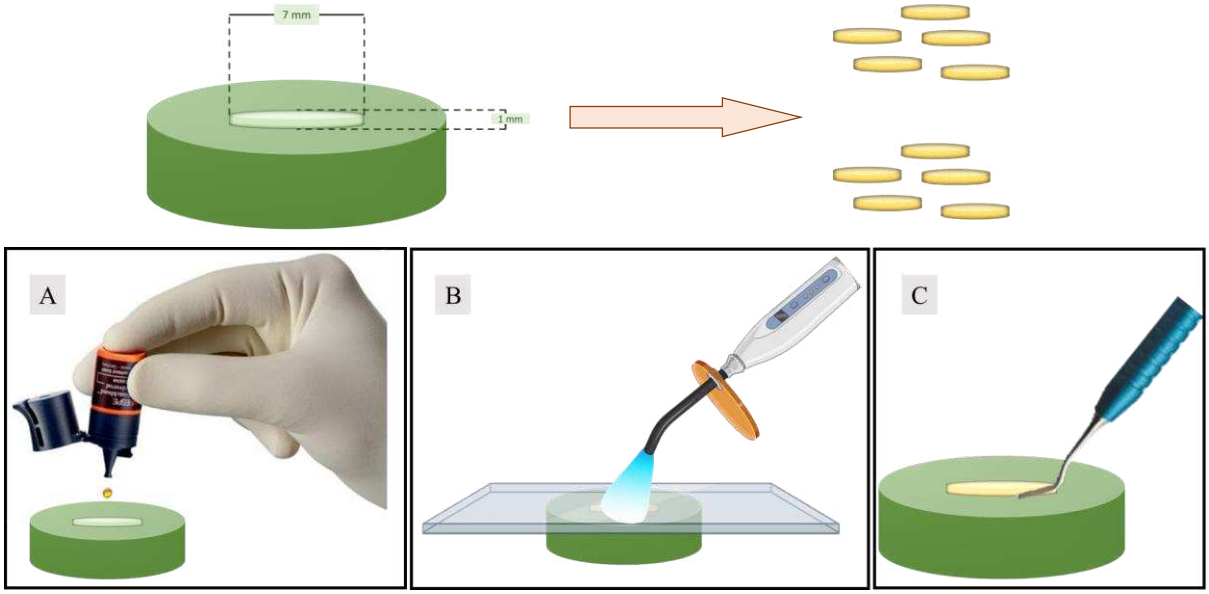

Figura 9 Preparo das amostras para posterior avaliação das propriedades físico-químicas do sistema adesivo. A – Gotejamento do sistema adesivo na matriz elastomérica; B – Fotopolimerização do sistema adesivo conforme recomendação do fabricante; C – Remoção das amostras com auxílio de espátula de inserção

Apêndice 2 – Imagens das amostras no ensaio de Dureza Knoop

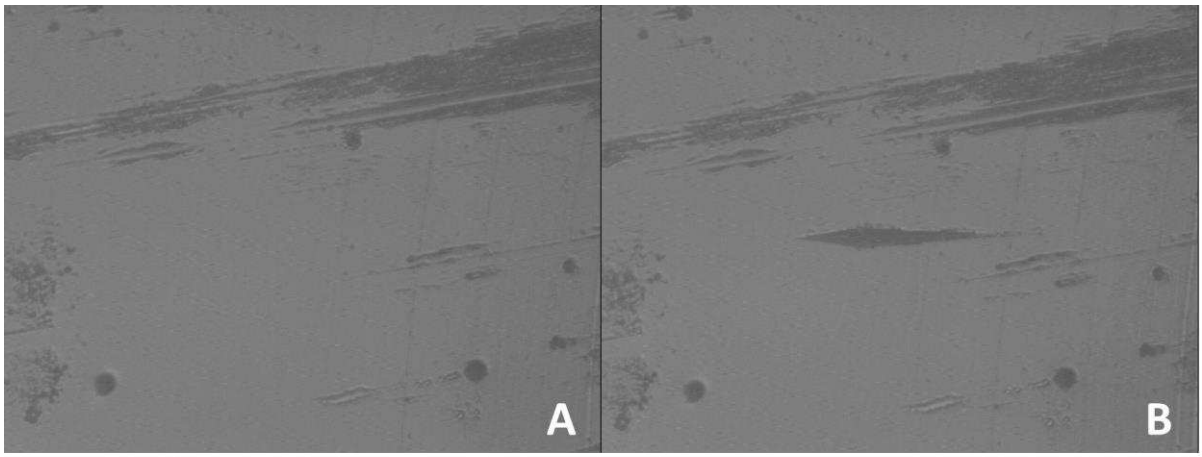

Figura 10 Imagens da microdureza do grupo controle após 24 h de preparo das amostras (Lens x10). A - Antes da indentação; B - Após a indentação

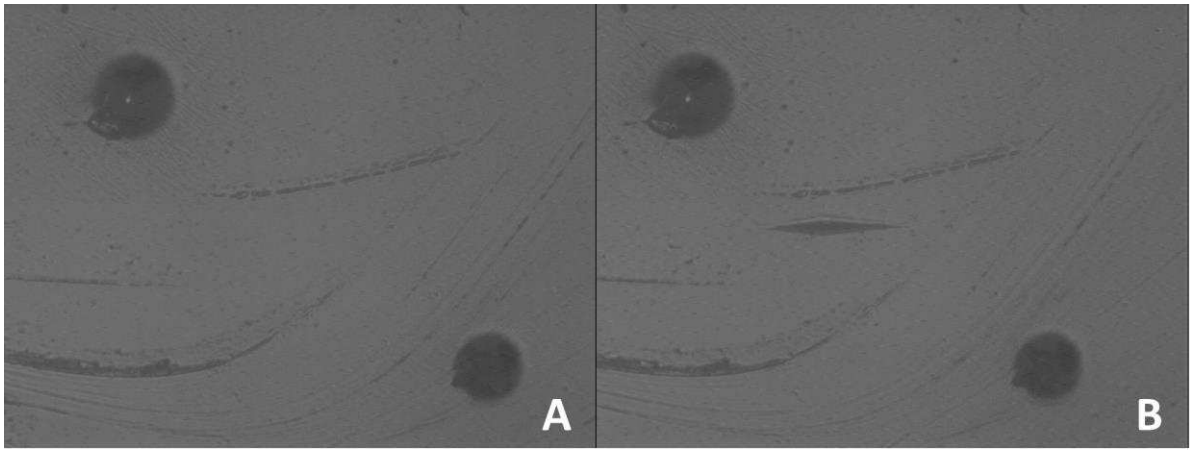

Figura 11 Imagens da microdureza do grupo controle após 7 dias imersos em álcool a 75% (Lens x10). A - Antes da indentação; B - Após a indentação

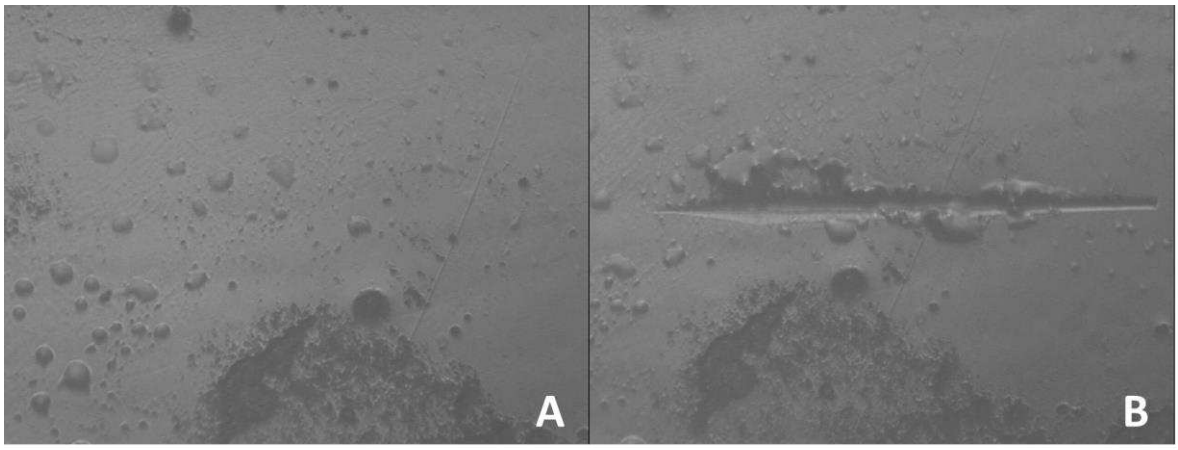

Figura 12 Imagens da microdureza do grupo experimental após 24 h de preparo das amostras (Lens x10). A - Antes da indentação; B - Após a indentação

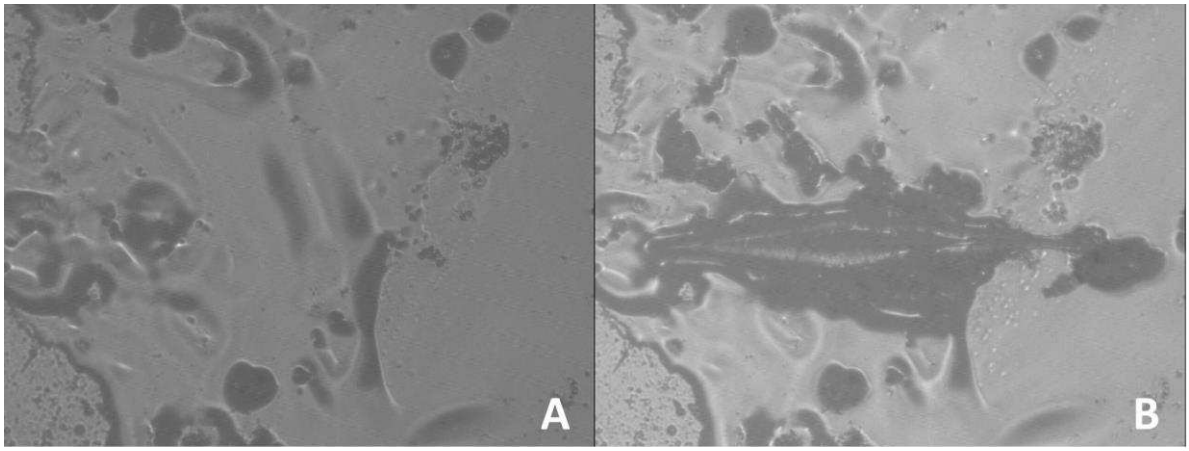

Figura 13 Imagens da microdureza do grupo experimental após 7 dias imersos em álcool a 75% (Lens x10). A - Antes da indentação; B - Após a indentação

ANEXOS

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