

***Reynoutria elliptica* extract based denture cleansing materials: Antimicrobial efficacy, biocompatibility, and impact on surface properties**

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This study investigated the antimicrobial efficacy, biocompatibility, and surface effects of a denture cleanser containing *Reynoutria elliptica* extract. The cleanser was prepared with extract concentrations of 0, 100, and 150 µg/mL combined with 1% coco betaine as a natural surfactant. Antimicrobial efficacy against *Candida albicans* was tested, along with evaluations of surface microhardness, roughness, color stability, and solubility of acrylic resin after immersion. Biocompatibility was assessed using an agar diffusion assay and cytotoxicity tests. Results showed that the denture cleanser exhibited significant antimicrobial activity without altering surface properties or causing cytotoxic effects. These findings highlight the potential of denture cleansers formulated with *R. elliptica* extract to preserve the structural integrity and aesthetics of denture base materials while providing effective antimicrobial protection. This study offers valuable insights for developing natural, biocompatible denture cleansers for safe and effective dental care.

Keywords: Antimicrobial efficacy, Biocompatibility, *Candida albicans*, Denture cleanser, *Reynoutria elliptica*

INTRODUCTION

Maintaining a healthy oral environment is essential for denture users and requires effective care and maintenance. The accumulation of oral biofilms on dentures is a major concern as it has been associated with denture stomatitis and can contribute to more severe systemic diseases, particularly in elderly patients¹. Poor denture hygiene can result in the accumulation of plaque and calculus, leading to halitosis and aesthetic problems. In addition, rough denture surfaces are more prone to plaque accumulation than smoother surfaces^{2,3}.

Both mechanical and chemical methods are used to remove food particles and plaque from dentures. Mechanical methods include brushing with denture-specific cleansers or toothpaste and the use of ultrasonic cleaning devices^{4,5}. Chemical methods include the use of agents such as alkaline peroxide, sodium hypochlorite, disinfectants, and enzymes. These chemical approaches are particularly recommended for elderly patients or patients with disabilities who may have difficulty effectively brushing their dentures as the chemicals can reach areas inaccessible to brushes^{6,7}. Commercially available denture cleansers often contain alkaline hypochlorites, which effectively remove stains and dissolve organic matter, but can also cause adverse effects, such as eye and skin irritation, metal corrosion, and bleaching of the denture base resin^{8,9}.

Candida albicans, a common pathogen responsible for oral mucosal infections, often affects immunocompromised individuals by adhering to oral epithelial tissues or dentures, leading to opportunistic infections, such as oral candidiasis. The treatment of these infections typically involves antifungal therapy, including both topical and systemic medications, as well

as improved oral hygiene^{10,11}. The effective prevention of oral diseases depends on controlling biofilm formation in the oral environment. Previous studies have explored the use of agents such as chlorhexidine, sodium lauryl sulfate, and triclosan to prevent and treat oral diseases, whereas antibiotics, such as ampicillin, erythromycin, and penicillin, are commonly used to inhibit microbial growth^{12–16}. Although chlorhexidine gluconate is effective in treating denture stomatitis caused by *Candida* species, its use is limited owing to the potential for staining with prolonged daily use¹⁷.

Overuse of synthetic antibacterial agents can disrupt the balance of the oral microbiome by eliminating both beneficial and pathogenic microbes, raising concerns about unintended changes in oral health^{18–20}. Therefore, a more conservative approach to antifungal use is recommended to maintain host health while controlling harmful pathogens. Considering these concerns, recent studies have increasingly focused on natural remedies with fewer side effects that are suitable for long-term use, specifically investigating their antibacterial and anti-inflammatory properties^{21–24}. Among these natural remedies are perennial plants belonging to the Polygonaceae family that have traditionally been used in East Asian medicine for their properties of promoting blood circulation, relieving pain, and treating various ailments. In particular, *Reynoutria elliptica* has demonstrated promising antimicrobial activity against *Helicobacter pylori* and potential inhibitory effects against *Streptococcus mutans*, which are key contributors to the prevention of dental caries. The phenolic compounds present in the *R. elliptica* extract, including polyphenols and flavonoids, are known to exhibit antimicrobial properties through the inhibition of microbial growth. Studies have indicated

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that the antimicrobial activity is proportional to the concentration of these compounds, which disrupt microbial cell membranes and inhibit enzyme activity^{25–29}. Therefore, it is suggested that the polyphenolic and flavonoid components in *R. elliptica* could inhibit *S. mutans* and *C. albicans*, suppressing their pathogenic activities in the oral cavity. However, scientific studies comparing the antimicrobial efficacy, biocompatibility, and effects on denture surfaces of experimental denture cleansers containing various concentrations of naturally derived *R. elliptica* extracts with those of conventional denture cleansers are currently lacking.

This study investigated the potential of *R. elliptica* extract as a natural antimicrobial agent for use in denture cleansers. We focused on evaluating its antimicrobial activity against *C. albicans* strains and assessing its biocompatibility and impact on denture surface integrity. The objective of this study was to provide comprehensive preliminary data to support the inclusion of *R. elliptica* extract in denture cleanser formulations. The null hypothesis was that denture cleansers containing varying concentrations of *R. elliptica* extract would not 1) significantly alter the antimicrobial and biocompatibility properties or 2) affect the surface properties or dissolution of the denture material. We found that the denture cleanser containing *R. elliptica* extract effectively preserved the structural integrity and aesthetics of denture base materials while providing antimicrobial benefits.

MATERIALS AND METHODS

Preparation of experimental denture cleansers

R. elliptica was obtained from a herbal market (Hanyakjae market, Seoul, South Korea), with plant material originating from North Gyeongsang Province, South Korea. A total of 500 g of *R. elliptica* was finely ground and soaked in 5 L of 70% methanol solution. The mixture was subjected to extraction for 48 h at a controlled temperature of 25±1°C. After extraction, the solution was filtered through a Whatman #2 filter paper (Whatman, Maidstone, UK). The filtrate was concentrated and evaporated under reduced pressure using a vacuum evaporator (EYELA, Tokyo, Japan). To obtain a dry solid extract, the concentrated solution was first frozen at –20°C for 12 h and then freeze dried at –55°C for 48 h using a freeze dryer (Ilshin Lab, Gyeonggi-do, Korea). The resulting freeze-dried extract was finely pulverized using a mortar and pestle. The powdered extract was dissolved in dimethyl sulfoxide (DMSO; Amresco, Solon, OH, USA) to prepare stock solutions at concentrations of 0, 100, and 150 µg/mL. This dissolution process was performed at 25±1°C with continuous stirring using a magnetic stirrer for 24 h.

Experimental denture cleansers were formulated by incorporating each *R. elliptica* extract solution into a 1% solution of cocamidopropyl betaine (coco betaine), which served as a natural surfactant. This mixing process was also performed at 25±1°C with magnetic stirring for 24 h. The final concentrations of the extract in the

experimental denture cleansers were 100 µg/mL (RE100) and 150 µg/mL (RE150). A control denture cleanser without the *R. elliptica* extract (RE0) was prepared for comparison. In addition, a commercial denture cleanser, Polident (Stafford-Miller, Waterford, Ireland), was used as a commercial control (CC).

Evaluation of antimicrobial activity against *C. albicans*

1. Inhibition zone assay

To evaluate the antimicrobial activity of experimental and control denture cleansers, *C. albicans* (KCTC; 7965/ATCC 10231) was obtained from the Korean Collection for Type Cultures (KCTC; Jeollabuk-do, South Korea). *C. albicans* was grown in yeast mold (YM) medium (Becton Dickinson and Co., Franklin Lakes, NJ, USA) and incubated at 37±1°C for 24 h under aerobic conditions. For the inhibition zone assay, 100 µL of *C. albicans* suspension containing approximately 3×10⁵ cells/mL was spread evenly on YM agar plates. Sterile paper disks (10-mm diameter; 1-mm thickness) were impregnated with 20 µL of the control and experimental solutions and then placed on the inoculated agar plates. The plates were then incubated at 37±1°C for 24 h. The diameters of the inhibition zones around the paper disks were measured using Vernier calipers (Mitutoyo, Kawasaki, Japan) with an accuracy of 0.01 mm. Each inhibition zone assay was performed independently with six replicates. Data were presented as mean values and standard deviations.

2. Live/Dead staining procedure

A Teflon mold (diameter: 10.0 mm; height: 1.0 mm) was placed on a polyester film and then on a microscope slide (Paul Marienfeld, Bad Mergentheim, Germany). Autopolymerizing acrylic resin (Jet Denture Repair, Lang Dental, Wheeling, IL, USA) was prepared by mixing the powder and liquid components according to the manufacturer's instructions until the mixture reached a dough-like consistency. This mixture was then carefully packed into a Teflon mold to prevent the formation of air bubbles. A second polyester film was placed over the filled mold and covered with another microscope slide. Pressure was applied with a clamp for 1 min to remove any excess material and ensure uniform thickness. The resin was allowed to polymerize for 30 min at 25±1°C. After polymerization, the disk-shaped specimens were gently removed from the mold, and any excess material was smoothed off using 320-grit abrasive paper. Polymerized acrylic resin disks were prepared with dimensions of 10.0±0.1 mm in diameter and 1.0±0.1 mm in height.

Sterilized acrylic resin disks were placed in individual wells of a 24-well plate (SPL Life Sciences, Gyeonggi-do, Korea). Each disk was inoculated with 100 µL of *C. albicans* suspension, which was carefully applied to the surface of the sample. The plates were then incubated at 37±1°C for 24 h to allow microbial adhesion. After incubation, the disks were gently rinsed with distilled water for 10 s to remove the loosely adherent microorganisms. The acrylic resin disks

exposed to *C. albicans* were transferred to a new 24-well plate with the inoculated surface facing upward. Next, 100 µL of the control or experimental denture cleanser was applied to the surface of each acrylic resin disk. The plates were incubated again for 24 h at 37±1°C. After the second incubation period, the samples were rinsed with distilled water for 10 s.

The viability of *C. albicans* on the surface of the acrylic resin disks was assessed using the LIVE/DEAD FungaLight Yeast Viability Kit (Molecular Probes, Eugene, OR, USA) according to the manufacturer's protocol. A mixture of equal volumes of propidium iodide and Syto 9 dye was prepared, and 3 µL of this mixture was added to 1 mL of phosphate-buffered saline (PBS). Each acrylic resin disk, still containing adherent *C. albicans*, was then immersed in this staining solution for 20 min at 37±1°C in a dark environment. Stained *C. albicans* cells on the acrylic resin disks were observed using a confocal laser scanning microscope (CLSM; LSM880, Carl Zeiss, Thornwood, NY, USA). Viable microorganisms fluoresced green, whereas non-viable microorganisms fluoresced red.

Application of experimental denture cleansers

A Teflon mold (diameter: 10.0 mm; height: 1.0 mm) was placed on the polyester film, which was then placed on a microscope slide. The acrylic resin disk specimens were prepared as per the same method described for the live/dead staining. The acrylic resin disk specimens were fully immersed in 2 mL of the control or experimental denture cleanser contained in individual wells of a 24-well plate. The immersion process was carried out for 7 and 30 day-periods at a constant temperature of 25±1°C. Control and experimental denture cleansers were refreshed daily. After immersion, the surface of the acrylic resin disk was evaluated.

Surface characterization of acrylic resin disks treated with experimental denture cleansers

The microhardness of the acrylic resin disk specimens was determined by measuring the Vickers microhardness using a microhardness tester (DMH-2; Matsuzawa Seiki, Tokyo, Japan) with a load of 50 g applied for 10 s. Indentations were made with a diamond indenter. Two random measurements were performed on the surface of each sample, and the average value was recorded as the surface microhardness.

The surface roughness of the acrylic resin disk specimens was characterized using optical surface profilometry (Contour GT, Bruker, Tucson, AZ, USA). The roughness parameter, *Ra*, was quantified. Measurements were performed at 10× magnification with a scan area of 231×173 µm.

Color differences were assessed using a spectrophotometer (CM-3500d, Minolta, Kyoto, Japan), with each measurement preceded by standard calibration using a white calibration plate. The *L**, *a**, and *b** values of the samples were determined, representing the lightness, redness-greenness, and yellowness-blueness, respectively, within the CIE Lab* color space.

To evaluate the color change induced by immersion in the control and experimental denture cleansers, color measurements were conducted at multiple points on each sample. The resulting data were averaged to ensure accurate and comprehensive color assessment. The overall color difference (ΔE^*) between samples before and after treatment was calculated using the following equation:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Each surface characterization test was independently performed with six replicates. Data were presented as mean values and standard deviations.

Solubility of acrylic resin disks treated with experimental denture cleansers

A Teflon mold (diameter: 20.0 mm, height: 1.5 mm) was placed on the polyester film, which was then placed on a microscope slide. The acrylic resin disk specimens were prepared as per the same method described for the live/dead staining. Solubility tests were performed according to a previously established protocol³⁰. Disk-shaped specimens were first placed in a desiccator for 24 h to achieve consistent drying. The mass of each sample was then measured. To determine the volume, the diameter was measured at three evenly spaced points and the thickness was measured at one central and four peripheral points with an accuracy of 0.01 mm. Following the initial measurements, each sample was immersed in 10 mL of control and experimental denture cleanser solution maintained at 25±1°C for a duration of 7 days. After the immersion period, the samples were thoroughly rinsed and then dried in a 37°C oven for 24 h until a stable mass was achieved, defined as a mass change of less than 0.2 mg per hour. The final mass of the samples was recorded and the solubility was calculated based on the changes in mass and volume before and after treatment. Each solubility test was performed independently with six replicates. Data were presented as mean values and standard deviations.

Analysis of polyphenols and flavonoids

For the polyphenol assay, 50 µL of the control or experimental denture cleanser was mixed with 650 µL of distilled water, followed by the addition of 50 µL of Folin–Denis reagent. After allowing the mixture to react for 3 min, 100 µL of a 10% Na₂CO₃ solution and 150 µL of distilled water were added to achieve a final volume of 1 mL. The mixture was incubated for 1 h, after which the absorbance was measured at 725 nm using an ultraviolet-visible spectrophotometer (X-ma 1200 Spectrophotometer; Human, Seoul, Korea). A calibration curve was generated using gallic acid (Sigma-Aldrich, St. Louis, MO, USA) at concentrations of 20, 40, 60, 80, and 100 µg/mL, allowing the total polyphenol content in the samples to be expressed in micrograms of gallic acid equivalents.

For the determination of flavonoid content, 1 mL of diethylene glycol was added to 100 µL of the experimental

denture cleanser, followed by the addition of 100 μ L of 1 N NaOH. The mixture was incubated at 37°C for 1 h, and the absorbance was measured at 420 nm using the same spectrophotometer. Naringin (Sigma-Aldrich) was used to create a standard curve at concentrations of 20, 40, 60, 80, and 100 μ g/mL, which was then used to quantify the flavonoid content in the samples. All measurements for polyphenols and flavonoids were conducted in six independent experiments.

Biocompatibility of experimental denture cleansers

The cytotoxicity of the experimental denture cleansers was evaluated using an agar diffusion assay according to the ISO 10993-5:2009³¹⁾ and ISO 7405:2018³²⁾ standards. L929 mouse fibroblasts were selected for the biocompatibility assessment owing to their established use in cytotoxicity testing protocols. For the assay, cell suspensions were prepared by seeding 10 mL of a suspension containing 2.5×10^5 cells/mL in 100 mm diameter cell culture dishes (SPL, Pocheon-Si, Korea). The cultures were incubated for 24 h at $37 \pm 1^\circ\text{C}$ in a humidified incubator with 5% CO_2 to allow the cells to adhere and form a monolayer. After initial incubation, the culture medium was carefully removed and replaced with 10 mL freshly prepared agar medium containing $2 \times$ RPMI 1640 (Sigma, Ayrshire, UK) mixed with 1.8% agarose. The agar medium was allowed to solidify at $25 \pm 1^\circ\text{C}$ to form a stable gel over the cell monolayer. After the agar solidified, 10 mL of neutral red solution (0.01% in PBS; Sigma-Aldrich) was added to each dish and incubated for 20 min in the dark to stain the viable cells. After the staining period, excess neutral red solution was gently removed by rinsing with PBS. Sterile paper disks (10 mm diameter, 1 mm thickness) were impregnated with 50 μ L of the experimental denture cleanser and carefully placed on the surface of the agar. Positive controls (PCs), consisting of latex sheets, and negative controls, consisting of sterile paper disks containing 50 μ L of distilled water, were included in each dish to compare analysis under the same conditions. The disks were then incubated for 24 h under identical environmental conditions.

After incubation, the extent of discoloration and cell lysis was evaluated using light microscopy. Decolorization was scored and graded according to the following scale: 0, no detectable discoloration; 1, discoloration limited to the area directly under the sample; 2, discoloration extending up to 5 mm from the sample; 3, discoloration extending up to 10 mm from the sample; 4, discoloration extending beyond 10 mm from the sample; and 5, complete discoloration of the culture. Cell lysis, an indication of compromised membrane integrity, was quantified using the following scoring system: 0, no detectable cell lysis; 1, <20% cell lysis; 2, 20%–40% cell lysis; 3, 40%–60% cell lysis; 4, 60%–80% cell lysis; and 5, >80% cell lysis. All measurements for agar diffusion assay were conducted in six independent experiments.

Statistical analysis

All data from the control and experimental denture cleanser groups were analyzed using one-way analysis of variance with PASW, version 18.0 (IBM, Armonk, NY, USA), to assess interactions across different concentrations of *R. elliptica* extract. To identify significant differences between these concentrations, *post-hoc* analyses were performed using Tukey's multiple comparison test, with the significance level set at 0.05.

RESULTS

Antimicrobial properties

Antimicrobial activity against *C. albicans* was evaluated using an inhibition zone assay, as illustrated in Figs. 1a and b. After 24 h of incubation, the inhibition zones observed in the RE100 and RE150 groups were significantly larger than those observed in the RE0 group ($p < 0.05$). In addition, no significant difference was found between the inhibition zones of the RE100 and RE150 groups compared with that of the CC group ($p > 0.05$).

The viability of *C. albicans* was further assessed using fluorescence staining, with the results presented in Fig. 1c. The RE0 group exhibited a higher number of viable *C. albicans*, as indicated by green fluorescence. In contrast, the RE150 group showed a reduced number of viable fungi, with a corresponding increase in the proportion of dead fungi, as indicated by the red fluorescence. The RE150 group showed a pattern of *C. albicans* viability similar to that observed in the CC group.

Surface characterization and solubility of acrylic resin disks treated with experimental denture cleansers

The surface microhardness of the acrylic resin disks was evaluated after 7 and 30 days of immersion in the control and experimental denture cleansers (Fig. 2a). For both immersion periods, no significant differences in the microhardness were observed between the control and experimental groups ($p > 0.05$). In addition, increasing the concentration of *R. elliptica* extract did not significantly affect the surface microhardness of the acrylic resin disks ($p > 0.05$).

The surface roughness of the acrylic resin disks was also evaluated after 7 and 30 days of immersion in the control and experimental denture cleansers (Fig. 2b). No significant changes in surface roughness were observed between the control and experimental groups at any time point ($p > 0.05$). Similarly, variations in the concentration of *R. elliptica* extract had no significant effect on the surface roughness of the acrylic resin disks ($p > 0.05$).

The color stability of the acrylic resin disks was evaluated by measuring the ΔE^* values after 7 and 30 days of immersion in the experimental denture cleansers (Fig. 2c). The ΔE^* values for the experimental groups were not significantly different from those of the control group at either time point, indicating no significant color change ($p > 0.05$). Furthermore, increasing the concentration of *R. elliptica* extract had no significant

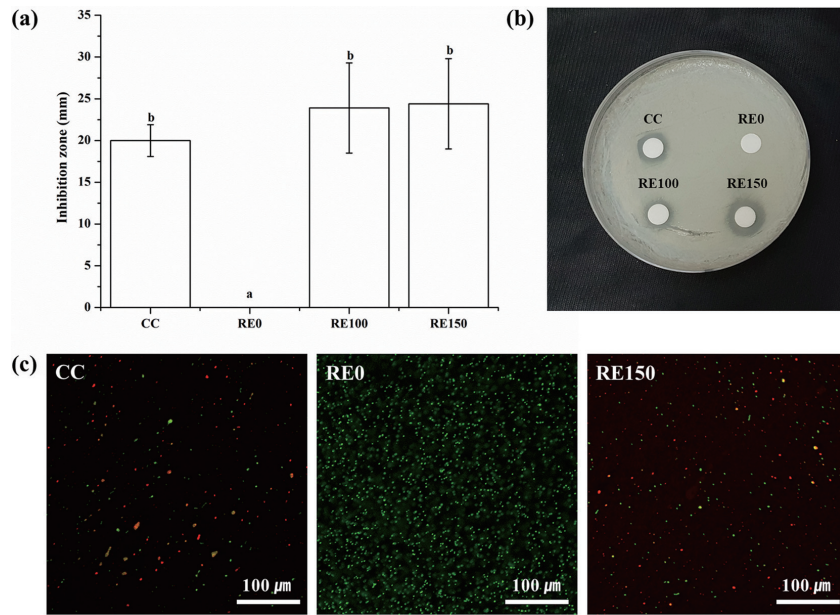


Fig. 1 Antimicrobial properties of control and experimental denture cleansers. (a) Diameter of inhibition zones on agar plates. Different lowercase letters above the bars indicate statistically significant differences between groups; (b) Representative agar plate showing inhibition zones for the commercial control (CC, positive control), cleanser without extract (RE0, negative control), and experimental cleansers containing *R. elliptica* extract (RE100, RE150); (c) Representative fluorescence microscopy images showing live/dead staining of *C. albicans* for the commercial control (CC, positive control), cleanser without extract (RE0, negative control), and experimental cleanser containing 150 µg/mL *R. elliptica* extract (RE150).

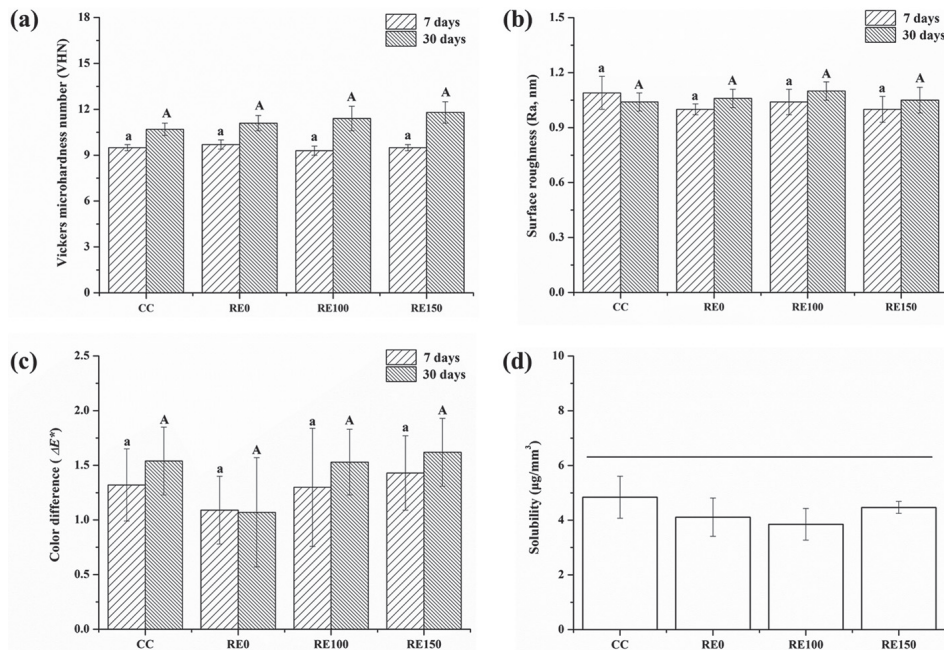


Fig. 2 Surface characterization and solubility of acrylic resin disks treated with control and experimental denture cleansers. (a) Vickers microhardness; (b) surface roughness; (c) color change (ΔE^*) after 7 and 30 days of immersion; (d) solubility of acrylic resin disks after immersion in control and experimental denture cleansers. Each bar represents the mean, with error bars indicating the standard deviation. Different lowercase letters (e.g., 'a', 'b') above the bars denote statistically significant differences between groups after 7 days of immersion ($p < 0.05$). Different uppercase letters (e.g., 'A', 'B') indicate statistically significant differences between groups after 30 days of immersion ($p < 0.05$). Horizontal bars above the groups represent no significant differences between them ($p > 0.05$).

effect on the ΔE^* values of the acrylic resin disks ($p>0.05$).

The solubility of the acrylic resin disks was evaluated after immersion in the control and experimental denture cleansers (Fig. 2d). Throughout the immersion period, no significant differences in solubility were observed between the control and experimental groups ($p>0.05$). In addition, the concentration of *R. elliptica* extract did not significantly affect the solubility of the acrylic resin disks ($p>0.05$).

Contents of polyphenols and flavonoids

Quantification of polyphenols and flavonoids in the control and experimental denture cleansers was performed, and the results are summarized in Table 1. Polyphenols and flavonoids were not detected in the CC and RE0 groups, but were present in all experimental groups. The RE100 group showed a polyphenol content of 38.9 ± 2.6 $\mu\text{g/mL}$ and a flavonoid content of 8.9 ± 2.1 $\mu\text{g/mL}$, while the RE150 group showed a higher polyphenol content of 52.5 ± 1.4 $\mu\text{g/mL}$ and a flavonoid content of 13.5 ± 2.2 $\mu\text{g/mL}$. Increasing the concentration of *R. elliptica* extract resulted in a statistically significant increase in the polyphenol and flavonoid contents ($p<0.05$).

Biocompatibility

The cytotoxicity of the control and experimental denture cleansers was evaluated using an agar diffusion assay.

The decolorization and lysis scores are presented in Table 2. No abnormalities or decolorization were observed in the cells directly beneath or surrounding the experimental groups. The cytotoxicity assessment indicated that none of the experimental groups exhibited significant cytotoxicity, as demonstrated by their response indices compared with the negative and positive controls. After 24 h of incubation, the decolorization zones in the PC group measured 2.45 ± 0.08 cm, which was significantly larger than that of those observed in the experimental groups ($p<0.05$) (Figs. 3a,

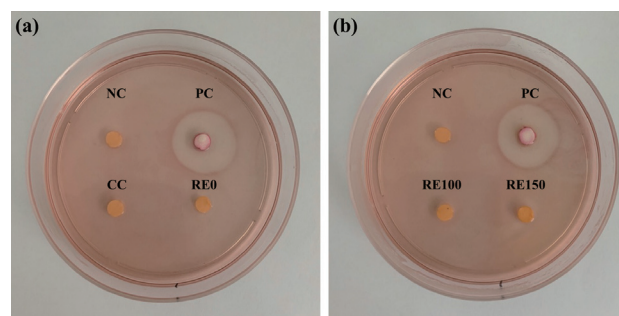


Fig. 3 Representative images from the agar diffusion assay.

(a) negative control (NC), positive control (PC), commercial control (CC), and RE0 on agar plates; (b) NC, PC, RE100, and RE150 on agar plates.

Table 1 Quantification of polyphenols and flavonoids in control and experimental denture cleansers

	Polyphenol content ($\mu\text{g/mL}$)	Flavonoid content ($\mu\text{g/mL}$)
CC	0 ^a	0 ^a
RE0	0 ^a	0 ^a
RE100	38.9 ± 2.6 ^b	8.9 ± 2.1 ^b
RE150	52.5 ± 1.4 ^c	13.5 ± 2.2 ^c

Superscript letters (a, b, c) indicate significant differences between the control and experimental groups for each component ($p<0.05$).

Different letters represent statistically significant differences. CC, commercial control; RE0, without *R. elliptica* extract; RE100, concentration of 100 $\mu\text{g/mL}$ of *R. elliptica*; RE150, concentration of 150 $\mu\text{g/mL}$ of *R. elliptica*.

Table 2 Decolorization and cell lysis results of the agar diffusion test

Test materials	Decolorization index	Lysis index	Interpretation
NC	0	0	Non-cytotoxic
PC	4 ± 0	5 ± 0	Severely cytotoxic
CC	0	0	Non-cytotoxic
RE0	0	0	Non-cytotoxic
RE100	0	0	Non-cytotoxic
RE150	0	0	Non-cytotoxic

NC, negative control; PC, positive control; CC, commercial control; RE0, without *R. elliptica* extract; RE100, concentration of 100 $\mu\text{g/mL}$ of *R. elliptica*; RE150, concentration of 150 $\mu\text{g/mL}$ of *R. elliptica*.

b). In addition, there were no significant differences in the decolorization zones among the RE0, RE100, and RE150 groups.

DISCUSSION

Acrylic resins have long been used in prosthodontics, with continuous improvements contributing to improved physical properties and biocompatibility. These materials are primarily used in complete and partial dentures^{33,34}. For people with partial or complete edentulism who depend on removable dentures, maintaining oral hygiene, including the care of the remaining teeth, gingiva, and dentures, is essential for prolonging the life of the dentures. Therefore, the use of specialized oral hygiene products, such as denture cleansers, is strongly recommended^{9,35}. However, many commercially available denture cleansers are composed of synthetic chemicals that have been shown to alter the surface properties of denture base resins and may cause cytotoxic effects when in contact with oral tissues³⁶. This study was conducted to develop a new denture cleanser formulated with natural plant extracts from *R. elliptica* and evaluate its antimicrobial efficacy, biocompatibility, and effects on the physico-chemical properties and color stability of denture base resins.

To evaluate the effect of experimental denture cleansers containing *R. elliptica* extract on the growth of pathogenic microorganisms, *C. albicans*, a common causative microorganism of oral candidiasis, was cultured in the control and experimental groups. As shown in Fig. 1, the agar disk diffusion method was used to evaluate antifungal activity. The results showed that different experimental groups impregnated into paper disks produced clear inhibition zones around the disks, indicating the suppression of *C. albicans* growth. Notably, the RE150 group exhibited significantly higher antifungal activity than the RE0 group, as confirmed by the live/dead staining analysis. These results suggest that the antimicrobial efficacy of *R. elliptica* extract can be applied in denture cleansers, particularly in the prevention of oral candidiasis. The results of our study are consistent with those of previous studies on the use of *R. elliptica* extract in oral care products targeting *C. albicans*³⁷. In addition, the *R. elliptica* extract showed efficacy against several bacterial pathogens, including *S. mutans*, a key bacterium associated with dental caries³⁸. The antimicrobial activity of *R. elliptica* extract is primarily attributed to its high concentration of polyphenols and flavonoids, which are known to interfere with microbial processes, such as cell wall synthesis, membrane integrity, and oxidative balance, ultimately affecting microbial survival^{39–41}. These bioactive compounds have also been shown to directly inhibit microbial proliferation, providing a mechanistic basis for the antimicrobial effects observed in this study (Table 1).

Polyphenols are secondary plant metabolites that exhibit antioxidant and antibacterial properties. Our extraction analysis revealed that higher concentrations

of *R. elliptica* extract corresponded to higher levels of polyphenols and flavonoids. This is consistent with previous research indicating that the antimicrobial efficacy of plant extracts is closely related to their phenolic content^{40,41}. Moreover, the polyphenols and flavonoids in the extract may also alter microbial cell surface hydrophobicity and charge characteristics, thus decreasing microbial adherence and plaque formation potential^{42,43}. This mechanism provides additional insight into the reduced adhesive plaque observed with the experimental denture cleanser, although direct microstructural evidence was beyond the scope of this study. Therefore, it is reasonable to conclude that the polyphenols and flavonoids present in the *R. elliptica* extract used in this study contributed to the inhibition of *C. albicans* growth and its pathogenic potential in the oral cavity.

Effective denture cleaning methods and cleansers must preserve the integrity of denture base resins^{30,36}. Previous research has indicated that denture cleansers can affect the mechanical strength, color stability, and surface morphology of these materials^{44–46}. Improper mechanical cleaning can lead to resin abrasion and increased surface roughness, which, in turn, can promote plaque and calculus accumulation⁴⁷. Evaluation of the surface hardness of acrylic resins after exposure to denture cleansers is critical because the hardness reflects the ability of the material to withstand masticatory forces⁴⁸. A decrease in hardness could result in uneven stress distribution during mastication, whereas an increase in surface roughness could promote microbial adhesion, staining, and plaque accumulation^{49,50}. Based on previous findings³⁸, antimicrobial efficacy and biocompatibility were confirmed across concentrations of 50 to 200 µg/mL. In this study, we selected higher concentrations (100 and 150 µg/mL) to specifically investigate their potential impact on denture surface properties, hypothesizing minimal effects at lower concentrations. Results indicated that the denture cleanser containing *R. elliptica* extract at these concentrations did not negatively affect the hardness or roughness of the denture base resin (Figs. 2a, b). Traditional denture cleansers, such as alkaline hypochlorites, are known for their effectiveness in removing stains and organic debris. However, they can also cause adverse effects, including eye and skin irritation, metal corrosion, and bleaching of denture base resins^{8,9}. Chlorhexidine gluconate, which is often used to treat denture stomatitis caused by *Candida* species, is limited by its potential to cause staining⁵¹. In contrast, *R. elliptica* extract caused no discoloration of the denture base resin (Fig. 2c). Therefore, maintaining the aesthetic properties of acrylic resins is critical. This study investigated whether the experimental denture cleanser containing *R. elliptica* extracts could maintain the original color of acrylic resin. A high-precision spectrophotometer was used to quantitatively assess the color changes before and after treatment⁵². The results showed no significant color differences between the control and experimental

groups, and no changes were visible to the naked eye. Therefore, *R. elliptica* extract did not adversely affect the color stability of the acrylic resin surface. Furthermore, the stability of the denture base resin in the oral cavity is essential because dissolution compromises the structural integrity of the denture^{53,54}. Our solubility test results showed no significant differences between the experimental and control groups (Fig. 2d), suggesting that the denture cleanser containing *R. elliptica* extract did not compromise the durability of the material.

Because denture cleansers can directly contact the mucosa, it is important to evaluate their cytotoxicity^{38,55}. The agar diffusion assay, commonly used to assess cytotoxicity, involves the direct application of materials to an agar plate, allowing visual confirmation of the affected cellular areas. Preliminary experiments confirmed that a concentration of *R. elliptica* extract >150 µg/mL significantly reduced cell viability³⁸. Accordingly, the concentration range for the test groups in this study was determined based on these findings. In this study, denture cleansers containing *R. elliptica* extract showed no cytotoxicity at any of the tested concentrations (Table 2; Fig. 3).

We investigated whether the incorporation of plant-derived *R. elliptica* extracts into denture cleansers affects their antimicrobial efficacy and surface properties. Despite the lower concentration of *R. elliptica* extract compared with other antimicrobial agents, it showed a significant inhibitory effect on the growth of microbes responsible for oral diseases. While this study focused on the antimicrobial effects of the denture cleanser containing *R. elliptica* extract specifically targeting the prevention of oral candidiasis caused by *C. albicans*, it is acknowledged that the findings may be strain-specific. To enhance the generalizability of the results, future studies should include a wider range of fungal strains. In addition, since this study focused on short-term effects, further research is needed to determine the optimal concentration of *R. elliptica* extract that maintains antimicrobial efficacy without compromising the stability of the denture base resin over a longer period of time. It is important that dental acrylic resin materials maintain mechanical strength over extended periods in the oral environment. While this study assessed surface properties such as hardness, roughness, and color stability after 7 and 30 days of exposure to the denture cleanser, these short intervals may not fully reflect the long-term interactions that occur during months or years of continuous clinical use.

Color stability was specifically evaluated because it is an important aesthetic factor directly related to patient satisfaction. Although changes in color can indirectly indicate potential alterations in surface properties, direct assessment of the microstructural changes (e.g., surface morphology) was not conducted in this study. Therefore, future research incorporating microstructural imaging techniques, such as scanning electron microscopy (SEM), is recommended to better correlate surface alterations with color stability data. Furthermore, critical mechanical properties such as flexural strength

and wear resistance were not evaluated in the current study. Given the clinical importance, future studies are strongly recommended to include longer immersion tests and comprehensive mechanical property assessments to more thoroughly establish clinical durability. Despite these limitations, this study successfully simulated the oral environment to evaluate the antimicrobial activity of the denture cleanser containing *R. elliptica* extract, and provided valuable insights into the antimicrobial properties of dental materials infused with plant extracts. This study demonstrated that a denture cleanser containing *R. elliptica* extract effectively inhibited the growth of *C. albicans*, confirming its potential as a natural antimicrobial agent. No significant differences in antimicrobial efficacy or biocompatibility were observed among the tested concentrations of the extract, supporting the null hypothesis that different concentrations would not alter these properties. In addition, the inclusion of the extract did not affect the surface properties or dissolution of the denture base resin, confirming the second null hypothesis.

CONCLUSIONS

This study demonstrated that a denture cleanser containing *R. elliptica* extract effectively inhibited the growth of *C. albicans*, highlighting its potential as a natural antimicrobial agent. The inclusion of the extract did not adversely affect the surface properties or dissolution of the denture base resin, confirming the compatibility of the cleanser with the material. These findings suggest that *R. elliptica* extract could be a promising component for denture cleansers, providing effective microbial control without compromising the integrity of the denture material or biocompatibility.

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