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Effect Of Guava Leaf Extract And Tea Tree Oil As Disinfectants On The Surface Roughness Of Soft Liner Incorporated With Titanium Dioxide Nanoparticles – An Invitro Study.

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Abstract:
Background: *Soft liners are used in removable prosthodontics to improve patient comfort. Their surface properties may be affected by disinfecting agents. Incorporation of titanium dioxide (TiO_2) nanoparticles may influence these properties. This in vitro study evaluated the effect of guava leaf extract and tea tree oil as disinfectants on the surface roughness of TiO_2 nanoparticle-incorporated soft liner.*
Materials and Methods: *Thirty specimens of TiO_2 nanoparticle-incorporated soft liner were divided into three groups (n = 10): distilled water, tea tree oil, and guava leaf extract. Surface roughness was assessed after immersion. The groups were compared using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, p-value < 0.05 was considered statistically significant.*
Results*: The guava leaf extract group showed the lowest mean surface roughness (1.508 ± 0.182), followed by tea tree oil (2.300 ± 0.363) and distilled water (2.612 ± 0.339). ANOVA showed a statistically significant difference among the groups (F = 34.733, p < 0.001). Tukey's test showed significantly lower surface roughness with guava leaf extract compared with distilled water and tea tree oil (p < 0.001), while the difference between distilled water and tea tree oil was not significant (p = 0.075).*
Conclusion: *Guava leaf extract demonstrated a significantly greater reduction in surface roughness of TiO_2 nanoparticle-incorporated soft liner compared with tea tree oil and distilled water.*
Key Word: *Soft liner; Titanium dioxide nanoparticles; Guava leaf extract; Tea tree oil; Surface roughness.*

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I. Introduction

Soft denture liners are resilient materials used on the intaglio surface of dentures to provide cushioning, improve patient comfort, and distribute functional stresses over the underlying oral tissues.[1],[2] They are particularly beneficial for patients who cannot tolerate the stresses exerted by dentures on the underlying mucosa. However, soft liners have several physical and microbiological limitations, including susceptibility to surface deterioration and microbial colonization. Colonization of denture surfaces, particularly by *Candida albicans*, is associated with denture stomatitis and may compromise the clinical performance of these materials.[1],[2],[3],[19],[20]

Surface roughness is an important property of soft denture liners because surface irregularities may facilitate microbial adhesion and biofilm accumulation. With prolonged use, soft lining materials may undergo changes such as hardening and increased surface irregularity, potentially increasing microbial colonization and the risk of denture-induced stomatitis.[6] Furthermore, commonly used denture disinfectants may adversely affect the physical and chemical properties of soft liners. Therefore, an ideal disinfecting agent should provide effective antimicrobial activity while maintaining the surface characteristics of the lining material.[5],[6]

Titanium dioxide (TiO_2) nanoparticles have attracted interest as functional additives in dental biomaterials because of their potential antimicrobial properties and ability to modify the physical characteristics of polymeric materials. Experimental studies have demonstrated that incorporation of TiO_2 nanoparticles into soft liners can influence surface properties and may reduce microbial growth under appropriate experimental conditions.[1],[2],[3],[4] However, the effect of nanoparticle incorporation and subsequent exposure to disinfecting solutions on the surface characteristics of soft liners requires further investigation.

The increasing interest in natural products has encouraged the evaluation of plant-derived substances as potential alternatives or adjuncts to conventional chemical disinfectants.[7]Tea tree oil (TTO), derived from *Melaleuca alternifolia*, possesses antimicrobial and antifungal properties. Previous in vitro investigations have demonstrated that TTO incorporated into denture soft liners significantly reduced the growth of *C. albicans*, with antifungal activity maintained following prolonged water storage.[20]Another investigation also demonstrated significant reduction in *C. albicans* growth following exposure of soft-lined denture specimens to TTO.[19] These findings suggest that TTO may have potential for use in denture hygiene and management of denture-associated microbial colonization.

Guava leaf (*Psidium guajava* L.) extract is another plant-derived material with potential disinfectant activity.[8],[13],[15],[18]Guava leaves contain tannins and other polyphenolic compounds that have been associated with antimicrobial activity. In a recent experimental study, guava leaf extract demonstrated the ability to reduce microbial counts on test surfaces, with a 40% extract producing an 83.56% reduction in total plate count. The authors concluded that guava leaves have potential for further development as a natural disinfectant and antiseptic.[9],[10],[11],[12],[14],[16],[17]

Although previous studies have investigated the antimicrobial potential of TiO_2-modified soft liners and the antifungal properties of TTO, limited evidence is available regarding the effect of **guava leaf extract and tea tree oil as disinfectants on the surface roughness of TiO_2 nanoparticle-incorporated soft liners**. Since surface roughness is clinically relevant to microbial accumulation and the longevity of resilient lining materials, evaluating the effect of these natural disinfecting agents on this property is important.

Therefore, the present in vitro study was undertaken to evaluate and compare the effect of guava leaf extract and tea tree oil solutions on the surface roughness of TiO_2 nanoparticle-incorporated soft liner.

II. Material And Methods

Study Design:

An in vitro experimental study was conducted to evaluate and compare the effect of 1% Tea tree oil (TTO) and 1% Guava leaf extract solutions on the surface roughness of 1% Titanium dioxide (TiO_2) nanoparticle-incorporated GC Soft-Liner following immersion for 7 days. Distilled water was used as the control medium.

Specimen Preparation:

GC Soft-Liner was used as the soft lining material. Titanium dioxide (TiO_2) nanoparticles were incorporated into the monomer at a concentration of 1%. The material was prepared according to the experimental protocol, and specimens measuring **10 mm in diameter and 2 mm in thickness** were fabricated.

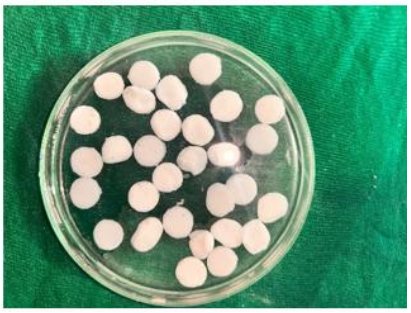


Fig 1: Acrylic disk of 2mm thickness
Fig 2: Putty moulds for smple preparation
Fig 3: Soft liner samples incorporated with TiO2 and 10mm diameter

Fig 4: Commercially available Guava Leaf Extract and Tea Tree Oil

A total of 30 specimens were prepared and randomly divided into three groups (n = 10) according to the immersion medium:

- **Group I – Tea tree oil group (TTO):** Specimens immersed in 1% TTO solution.
- **Group II – Guava leaf extract group (GLE):** Specimens immersed in 1% guava leaf extract solution.
- **Group III – Control group:** Specimens immersed in distilled water.

Preparation of Disinfecting Solutions:

A **1% v/v tea tree oil solution** was prepared to a final volume of 100 mL. A **1% w/v guava leaf extract solution** was prepared using guava leaf extract powder and made up to a final volume of 100 mL. 100mL Distilled water was used as the control medium.

Immersion Procedure:

The specimens were immersed completely in their respective solutions for **7 days**. Each specimen was maintained in its assigned immersion medium throughout the experimental period. Following the 7-day immersion period, the specimens were removed from the solutions and evaluated for surface roughness.

Fig 5: Immersion of TiO2 incorporated Soft Liner in Distilled Water, Tea Tree Oil and Guava Leaf Extract

Evaluation of Surface Roughness:

Surface roughness was evaluated using a **Surf Test surface roughness measuring instrument** following the 7-day immersion period. The surface roughness values obtained for each specimen were recorded for statistical analysis.

Fig 6: Surf Test surface roughness measuring instrument

III. Results

Statistical analysis:

The surface roughness measurements were expressed as mean ± standard deviation. The mean surface roughness values among the three groups were compared using **one-way analysis of variance (ANOVA)**. Tukey's post hoc test was subsequently performed for pairwise comparison between groups. A p-value < 0.05 was considered statistically significant.

Table 1. Comparison of Surface Roughness of Soft Liner Incorporated with Titanium Dioxide Nanoparticles after Immersion in Different Media (One-way ANOVA)

Group	Immersion Medium	n	Mean ± SD	F value	p value
Group 1	Distilled Water	10	2.612 ± 0.339	34.733	<0.001
Group 2	Tea Tree Oil	10	2.300 ± 0.363		
Group 3	Guava Leaf Extract	10	1.508 ± 0.182		

P<0.05* is considered statistically significant, SD: Standard Deviation

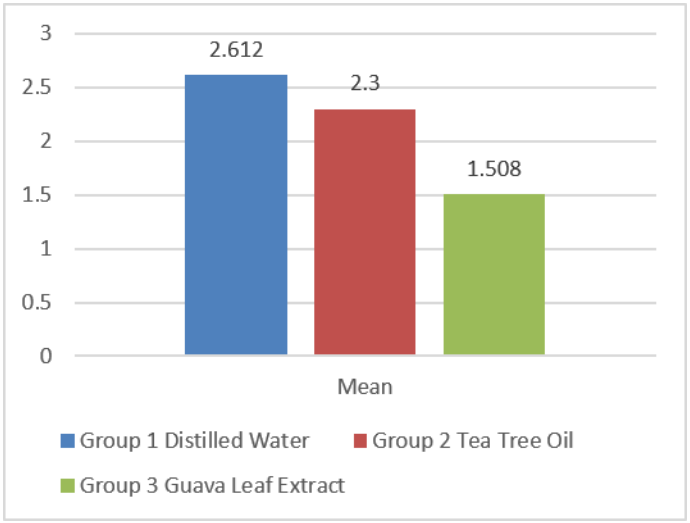
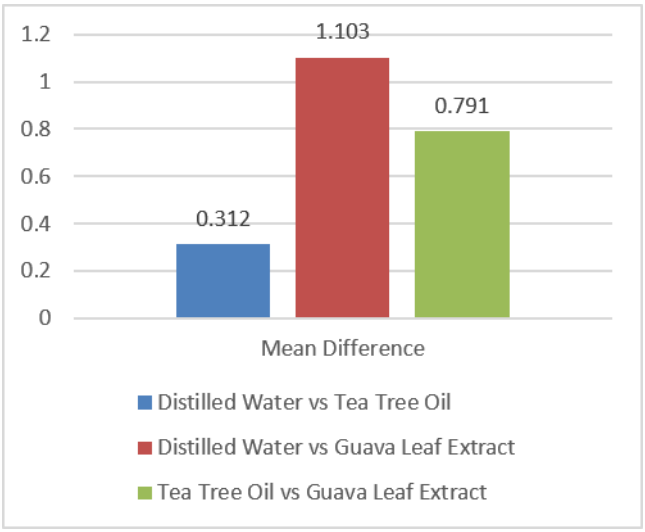


Table 2. Tukey's Post Hoc Comparison of Surface Roughness of TiO_2-Incorporated Soft Liner

Comparison	Mean Difference	p value
Distilled Water vs Tea Tree Oil	0.312	0.075
Distilled Water vs Guava Leaf Extract	1.103	<0.001
Tea Tree Oil vs Guava Leaf Extract	0.791	<0.001

P<0.05* is considered statistically significant



Interpretation

The surface roughness of titanium dioxide (TiO_2) nanoparticle-incorporated soft liners differed significantly following immersion in different media (Table 1). The Guava Leaf Extract (GLE) group demonstrated the lowest mean surface roughness (1.508 ± 0.182), followed by the Tea Tree Oil (TTO) group (2.300 ± 0.363), while the Distilled Water (DW) group exhibited the highest mean surface roughness (2.612 ± 0.339). One-way ANOVA revealed a statistically significant difference among the three groups ($F = 34.733$, $p < 0.001$). Tukey's post hoc analysis showed that the GLE group had significantly lower surface roughness than both the DW and TTO groups ($p < 0.001$ for both comparisons), whereas no statistically significant difference was observed between the DW and TTO groups ($p = 0.075$) (Table 2).

IV. Discussion

Surface roughness is an important physical characteristic of resilient denture lining materials because changes in the surface may influence their clinical performance and susceptibility to microbial accumulation.[6] The principal finding of the present study was that immersion medium significantly influenced the surface roughness of TiO_2-incorporated soft liner.

The mean surface roughness values obtained were 1.508 ± 0.182 for the guava leaf extract group, 2.300 ± 0.363 for the TTO group, and 2.612 ± 0.339 for the distilled-water group. Tukey's post hoc analysis

demonstrated that the guava leaf extract group had significantly lower surface roughness than both the distilled-water group and the TTO group ($p < 0.001$ for both comparisons). In contrast, the difference between the TTO and distilled-water groups was not statistically significant ($p = 0.075$). These findings indicate that the reduction in surface roughness observed in the present study was primarily associated with immersion in guava leaf extract rather than TTO.

Effect of guava leaf extract on surface roughness:

The most important observation of the present study was the significantly lower surface roughness following immersion in 1% guava leaf extract. The mean roughness value decreased to 1.508 ± 0.182 compared with 2.612 ± 0.339 in the distilled-water control, representing a mean difference of 1.103, which was statistically significant ($p < 0.001$). This is due to the phytochemical composition of guava leaves.[8],[9],[10] Guava leaves contain considerable amounts of tannins and polyphenolic compounds, these constituents have recognized antimicrobial properties and may act through several mechanisms, including disruption of microbial cell membranes, inhibition of cell-wall synthesis, interference with protein and enzyme activity, and inhibition of biofilm-related processes.[11],[12],[13],[14]

The findings are nevertheless consistent with the potential of guava leaf preparations as natural disinfecting agents.[15][16][17] Mahyuni et al.[18]reported antimicrobial and disinfectant potential of *Psidium guajava* leaves and attributed much of this activity to tannins and polyphenolic constituents. Their study demonstrated that guava leaf preparations could reduce microbial counts and suggested their potential development as natural disinfectant and antiseptic ingredients. Although their investigation evaluated microbial reduction rather than surface roughness, the findings provide biological support for the selection of guava leaf extract as an experimental disinfecting medium in the present study.

Effect of tea tree oil on surface roughness:

The TTO group demonstrated a mean surface roughness of 2.300 ± 0.363, which was lower than the distilled-water control value of 2.612 ± 0.339. However, the difference was not statistically significant (mean difference = 0.312; $p = 0.075$). Therefore, within the conditions of the present experiment, 1% TTO did not significantly alter the surface roughness of the TiO_2-incorporated soft liner compared with distilled water.

The absence of statistically significant difference between TTO and distilled water is noteworthy because previous studies have established the antifungal potential of TTO against microorganisms associated with denture stomatitis.[19],[20] Pachava et al.[20] reported that incorporation of TTO into denture soft liner significantly reduced *Candida albicans* growth and that the antifungal effect persisted during prolonged water storage. Similarly, Singhania et al.[19] reported significant inhibition of *C. albicans* associated with TTO-treated soft-liner specimens. Previous studies primarily assessed **antifungal activity or Candida colonization**, whereas the present study assessed **surface roughness after immersion**, the present study used **1% v/v TTO as an immersion solution for 7 days**. Consequently, differences in concentration, mode of delivery, exposure duration, formulation, and interaction with the polymer matrix may contribute to differences between studies.

Comparison between guava leaf extract and tea tree oil

A particularly important finding was the statistically significant difference between guava leaf extract and TTO. The guava leaf extract group demonstrated a mean surface roughness of 1.508 ± 0.182, whereas the TTO group demonstrated 2.300 ± 0.363, with a mean difference of 0.791 ($p < 0.001$). This indicates that, under the experimental conditions used in the present study, guava leaf extract was associated with a greater reduction in surface roughness than TTO.

This finding is particularly relevant because both agents were investigated as natural disinfecting media. Their chemical composition, concentration, vehicle, solubility, and interaction with the polymeric matrix may differ substantially. The present findings therefore highlight the need to evaluate not only the antimicrobial potential of natural disinfectants but also their effects on the physical properties of denture materials.

Possible influence of titanium dioxide nanoparticles:

The present study used soft liner incorporated with 1% TiO_2 nanoparticles. The incorporation of TiO_2 is relevant because previous research has demonstrated that TiO_2 nanoparticles can modify the physical and antimicrobial characteristics of soft denture liners. TiO_2 nanoparticles have been investigated for their antimicrobial properties, with proposed mechanisms involving the generation of reactive oxygen species and subsequent damage to microbial cell structures.[1],[2],[3],[4]

Dhanalakshmi et al.[1] reported reduced surface roughness in soft liners incorporated with TiO_2 compared with their respective control groups and suggested that fine nanoparticles may occupy surface irregularities, reduce surface defects, and improve surface homogeneity when appropriately dispersed within the polymer matrix. They have also reported reduced surface roughness in soft liners reinforced with zirconium dioxide nanoparticles.[5]

The discrepancy between the numerical reduction observed in the present study and the statistically significant effects reported in some previous studies may be related to differences in nanoparticle concentration, particle dispersion, soft-liner composition, specimen preparation, surface treatment, and testing protocols. Dhanalakshmi et al.[1] used substantially higher TiO_2 concentrations in their experimental groups, whereas the present investigation used 1% TiO_2 incorporated into the liner monomer. Therefore, the findings from higher-concentration studies cannot be directly extrapolated to the 1% concentration used in the present study.

Clinical significance

From a clinical perspective, maintaining a smooth soft-liner surface is desirable because surface irregularities may provide favorable sites for microbial retention and biofilm accumulation. The present study indicates that microorganisms can adhere to soft-liner surfaces and that increased surface roughness may contribute to microbial colonization. Therefore, the significantly lower surface roughness observed after guava leaf extract immersion may be considered a favorable material-related finding.

Nevertheless, it would be inappropriate to conclude from the present study alone that guava leaf extract will reduce denture stomatitis or microbial colonization clinically. The present investigation measured only surface roughness and did not directly evaluate *Candida albicans*, bacterial adhesion, biofilm formation, cytotoxicity, color stability, hardness, tensile strength, or dimensional changes. Further microbiological and clinical studies are therefore required before recommending guava leaf extract as a routine denture disinfectant.

Strengths of the study:

An important strength of the present investigation is the standardized comparison of two plant-derived disinfecting agents with a distilled-water control using the same TiO_2-incorporated soft-liner material. The specimens were standardized to 10 mm diameter and 2 mm thickness, and all groups were subjected to the same 7-day immersion period. The use of one-way ANOVA followed by Tukey's post hoc analysis allowed both overall and pairwise differences among the three immersion media to be evaluated.

Another strength is that the study evaluated natural disinfecting agents in relation to a specific physical property of a nanoparticle-modified soft liner. This study has investigated TTO predominantly in relation to antifungal activity, while evidence concerning guava leaf extract and the surface characteristics of TiO_2-modified soft liners is comparatively limited. The present study therefore contributes preliminary evidence regarding the effect of these agents on surface roughness.

Limitations:

The present study has several limitations.

First, it was an in vitro investigation and therefore cannot fully reproduce the complex oral environment, including fluctuations in temperature and pH, salivary enzymes, mechanical stresses, dietary exposure, and microbial biofilm development.

Second, only one concentration of TTO (1% v/v), one concentration of guava leaf extract (1% w/v), and one concentration of TiO_2 (1%) were evaluated. Therefore, the effect of concentration-dependent changes could not be determined.

Third, the immersion period was limited to 7 days. Although this provides useful information regarding short-term exposure, clinical dentures may remain in service for much longer periods and may undergo repeated cycles of disinfection.

Fourth, the present study assessed surface roughness as the principal outcome and did not directly evaluate antimicrobial efficacy. Therefore, the observed reduction in roughness with guava leaf extract cannot be interpreted as direct evidence of superior antimicrobial activity.

Finally, the present study did not investigate other clinically relevant properties such as surface hardness, tensile strength, tear strength, bond strength, color stability, water sorption, dimensional stability, or cytotoxicity. Future investigations should therefore evaluate multiple concentrations and longer immersion periods and should combine surface roughness assessment with microbiological, mechanical, chemical, and biological evaluations.

V. Summary

Surface roughness in soft denture liners promotes microbial biofilm growth and denture stomatitis. This study evaluated the physical impact of natural disinfectants on an advanced soft liner modified with 1% titanium dioxide (TiO_2) nanoparticles.

Methodology: Thirty standardized soft-liner specimens were divided into three groups (n = 10) and immersed for 7 days in 1% Guava Leaf Extract (GLE), 1% Tea Tree Oil (TTO), distilled water (control) respectively. Surface roughness was measured using a Surf Test instrument and analyzed via one-way ANOVA and Tukey's post hoc tests.

Key Results:

- Guava Leaf Extract maintained the smoothest surface with the lowest roughness values (1.508 ± 0.182).
- The control group showed the highest roughness (2.612 ± 0.339), followed closely by Tea Tree Oil (2.300 ± 0.363).
- GLE showed a highly significant advantage over both the control and TTO groups ($p < 0.001$).
- The difference between TTO and the control was not statistically significant ($p = 0.075$).

Therefore, the findings suggest that guava leaf extract may have potential as a natural immersion/disinfecting medium that is compatible with the surface characteristics of TiO_2-incorporated soft liner. However, because this study did not assess microbial activity or long-term clinical behavior, further laboratory and clinical research is required before translating these findings into clinical recommendations.

VI. Conclusion

Within the limitations of this in vitro study, immersion in **1% guava leaf extract resulted in significantly lower surface roughness** of 1% TiO_2 nanoparticle-incorporated GC Soft-Liner compared with 1% tea tree oil and distilled water. Although 1% tea tree oil demonstrated a numerically lower surface roughness than distilled water, the difference was not statistically significant. Therefore, **1% guava leaf extract may have potential as a natural disinfecting medium that preserves the surface characteristics of TiO_2 nanoparticle-incorporated soft liner**.

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