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Original Article

Formulation of a Chitosan-Based Membrane Incorporating Citrus Limon Pericarpium Extracts and Evaluation of its Physical and Biological Characteristics: An *in vitro* Study

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ABSTRACT

Introduction: Periodontal disease is a prevalent oral health condition characterized by inflammation of the periodontium ultimately leading to tooth loss. **Aim and Objectives:** This study aims to develop a novel chitosan membrane with lemon peel extract incorporated for the treatment of periodontal disease. **Materials and Methods:** The chitosan-lemon peel membrane was fabricated by phase inversion method and was characterized by FTIR, XRD. Tensile strength, anti-inflammatory, antimicrobial, and biodegradability tests were conducted to assess its periodontal applications. **Results:** The FTIR analysis confirmed the presence of stability-related functional groups. Tensile strength was 10.31 MPa. A balanced crystalline-amorphous structure was shown by XRD. Contact angle was at 70.33°. Broad spectrum antimicrobial and anti-inflammatory action at 250 µg/mL was noted. Size-dependent biodegradability was present. **Conclusion:** With significant antibacterial and anti-inflammatory activity and an appropriate degradation profile, the chitosan-lemon peel membrane showed potential for periodontal pocket delivery. However, further cytotoxicity tests and clinical trials are warranted.

Keywords: Chitosan, Citrus Fruits, Lemon Peel, Local Drug Delivery, Periodontal Disease.

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INTRODUCTION

Periodontal disease is a multifactorial, common oral health affliction, encompassing a range of inflammatory conditions impacting the supportive structures of the teeth ultimately leading to tooth loss and compromising quality of life. Additionally, it exhibits a bidirectional relationship with systemic health (Heitz and Lang, 2013; Kishen *et al*., 2023; Könönen *et al*., 2019; George *et al*., 2025). Periodontal tissue homeostasis typically relies on a symbiotic balance between the host immune system and microbial interactions. A dysregulated microbial balance leads to a dysbiotic state. This, in turn, causes inflammation and initiates periodontal disease (Hajishengallis *et al*., 2020). The release of inflammatory byproducts into the Gingival Crevicular Fluid (GCF) exacerbates dysbiosis by providing nutrients to specific microbial species within the biofilm community. Scaling and root planing have been recognized as the gold standard treatment for periodontitis despite several drawbacks, such as difficulty in reaching interdental and furcation areas, which creates an environment for further bacterial colonization and subsequent disease recurrence. Even though systemic antimicrobial medication has reduced this effect by both systemically acting and reaching therapeutic crevicular levels, antibiotic resistance has become a significant problem as a result of treatment (Chatterjee and Ramamurthy, 2024). As a result, local drug administration into the periodontal pocket without any systemic absorption has shown promise in mitigating the negative consequences of conventional treatment (Bhansali, 2014; Drisko, 2001; Thamaraiselvan and Jayakumar, 2024).

Chitosan - an amino-polysaccharide obtained from the deacetylation of chitin in crustaceans exoskeletons is a copolymer of D-glucosamine and N-acetyl glucosamine. It is utilized in dentistry due to its proven antibacterial efficacy against both Gram-positive and Gram-negative bacteria, regenerative qualities, biocompatibility, flexibility and mucoadhesive nature (Elieh-Ali-Komi and Hamblin, 2016; Paradowska-Stolarz *et al*., 2023). It also helps promote neovascularisation and possess anti-inflammatory activity (Komara *et al*., 2025). Its osseoconductive property helps improve periodontal intra-bony defects (Al-Agooz *et al*., 2025). The FDA has recognized it for its biological activity.

Lemon, a member of the *Rutaceae* family, is extensively cultivated, with India producing 3548.4 million tons annually. The peel, which constitutes 50-65% of the fruit's weight, contains antibacterial

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compounds (8-geranyloxypsolaren, 5-geranyloxypsolaren, 5-geranyloxy-7-methoxycoumarin, phlorin), along with antioxidants (ascorbic acid, flavonoids), anti-inflammatory agents (eriotrocin), and angiogenic components (narirutin, hesperidin). Additionally, pectin in lemon peel is utilized in wound dressings and drug delivery (Athanasiadis *et al*., 2024; Demir *et al*., 2021; Miyake and Hiramitsu, 2011) It exerts its antimicrobial property by compromising the integrity of microbial cell membrane (Sahraei *et al*., 2025). It is active against periodontal pathogens notably red complex micro-organisms (Bacińska *et al*., 2025). Additionally, biofilm adherence to the tooth surface is prevented by inhibition of cell-cell communication (Khanem *et al*., 2025).

Given the vast production and peel waste, lemon peel shows potential for recycling in medicinal applications, including a specialized membrane for periodontal therapy. Leveraging the proven optimal properties of Chitosan and lemon peel in treating periodontal disease, this study focused on development of a Chitosan membrane incorporating these diverse components of lemon peel and assessment of its physical and biological characteristics.

MATERIALS AND METHODS

Preparation of lemon peel extract

Lemon peels were gathered, and the white inner portion of the fruit was manually separated. Subsequently, the peels were sliced into smaller segments and sun-dried. Once dried, the pieces were ground into a coarse powder using a mixer blender, sifted through a sieve, and preserved in an airtight container.

A mixture of 10 g of the obtained lemon powder is combined with 100 mL of double-distilled water and stirred overnight at a temperature of 60°C. Following this, the stirred solution is filtered through a Will-Waltman filter number 1. The resulting filtrate yields the lemon peel extract.

Membrane fabrication process

Phase inversion method

A solution containing 10 g of chitosan is prepared by mixing it with a solution comprising 98 mL of water and 2 mL of nitric acid. Two portions of this mixture are distilled with eight parts of water. Subsequently, 0.5 g of prepared lemon peel extract is added to the resulting solution. The mixture of lemon peel extract and chitosan-based solution is then incubated in steam for 3 hr at 40°C. Once dissolved, the solution is poured into a petri dish and allowed to dry, forming a membrane.

FTIR

The Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) method was used to examine the functional groups and chemical interactions present in the chitosan-lemon peel membrane. A Perkin Elmer Spectrum IR Spectrometer (Version 10.7.2, United States) was used to conduct the analysis. The membrane sample measuring 5×5 mm was positioned directly onto the ATR crystal under constant pressure to ensure optimal contact. Characteristic peaks that corresponded to particular functional groups of the components of lemon peel and chitosan were found in the recorded spectra.

XRD

X-ray Diffraction (XRD) was used to examine the crystalline structure and phase composition of the chitosan-lemon peel membrane. A Bruker D8 Advance Powder X-ray Diffractometer was used to scan the samples over a 20 range in order to get the diffraction pattern. The degree of crystallinity of lemon peel into the chitosan matrix was ascertained by analyzing the recorded diffraction patterns.

Tensile strength testing

The tensile strength was assessed with the Instron Universal Testing Machine E-3000. The small portion of the prepared membrane was cut into rectangular strips of 1×5 cm. These strips were inserted into the tensile grips. The crosshead movement started the test, which went on until the membrane broke or reached its maximal elongation.

Contact angle testing

The small section of 5×5 mm of the fabricated Chitosan-Lemon peel membrane was cut and positioned at the contact angle goniometer (Ossila Goniometer). A tiny liquid droplet was sprayed onto the membrane's surface. This image is captured by the goniometer's CCD sensor and sent to the program to determine the angle produced at the liquid-solid interface.

Anti-inflammatory assay

Bovine serum albumin served as the protein source, with a concentration of 5 µg/mL utilized. The experiment was replicated in triplicate, with lemon peel extract concentrations at ratios of 20:40:16 parts. These ratios were mixed with the protein, dissolved, and subsequently centrifuged. Protein lysis was assessed post-centrifugation using Ultraviolet light spectrometry at 570 nm. The protein denaturation was compared with 1 mg salicylic acid as control.

Anti-microbial assay

The disk diffusion method is a widely employed technique for assessing the antimicrobial efficacy. The surface of an agar plate is initially inoculated by uniformly spreading a volume of the microbial inoculum across the agar surface. Subsequently, a sterile cork borer or tip was aseptically used to create a well with a diameter ranging from 6 to 8 mm. Following this, a volume of the lemon peel membrane of 1×1×1 cm and 25 µg/mL of crude extract as test group and 1:1 ratio of Antibiotics Erythromycin and Amoxicillin (ABS) was introduced into the well. The agar

plates were then incubated under conditions appropriate for the test microorganisms *(Staphylococcus aureus (S. aureus), Klebsiella pneumoniae (K. pneumoniae), Enterococcus faecalis (E. faecalis), Escherichia coli (E. coli))*.

In vitro degradability test

To assess the *in vitro* degradability of membrane samples, a degradation medium was prepared using Phosphate Buffered Saline (PBS) at pH 7.4. Membrane samples of 2 cm, 4 cm and 6 cm were taken and placed in sterile containers with labeled information, and a measured volume of degradation medium was added to cover them completely. The samples were incubated at 37°C (Scigenics Biotech incubator) and gently agitated periodically to ensure uniform degradation. At predetermined intervals, samples were removed, rinsed with distilled water to eliminate loosely attached degradation products, and dried with a lint-free tissue. The initial mass of each membrane sample was recorded before degradation, and after each interval, the samples were weighed again to compare with the initial mass.

STATISTICAL ANALYSIS

The SPSS Statistics 27 (IBM Corp.) was used to conduct statistical analysis. Statistical significance was designated as a p-value of less than 0.05. One-way ANOVA and *post hoc* tests were employed to compare the groups.

RESULTS

Harnessing the benefits of Chitosan and lemon peel components, a novel Chitosan based membrane infused with lemon peel extract was developed by phase inversion method. The developed membrane is depicted in Figure 1.

FTIR

Figure 2 depicts the FTIR analysis of the Chitosan-lemon peel membrane. A noticeable peak at 1723 cm^{-1} suggests C=O stretching, most likely from ester or carboxylic acid groups in lemon peel, whereas peaks at 2930.5 cm^{-1} and 2864.1 cm^{-1} show C-H stretching from aliphatic chains. Peaks at 1465.7 cm^{-1} and 1367 cm^{-1} show CH_2 and CH_3 bending, whereas C-N stretching, which is a feature of the amine groups in chitosan, is shown at 1264.2 cm^{-1} and 1239.6 cm^{-1}. The presence of polysaccharides is supported by the C-O stretching vibrations detected at 1194.2 cm^{-1}, 1104.4 cm^{-1}, and 1064.6 cm^{-1}. The fingerprint region (730.1-451.99 cm^{-1}) highlights the composite's potential for applications requiring mechanical strength.

XRD

The XRD analysis pattern is portrayed in Figure 3. The chitosan-lemon peel membrane's XRD investigation shows a balanced structure with 49.8% amorphous and 50.2% crystalline phases. The crystalline structure of chitosan and potential contributions from lemon peel components are suggested by the peaks at 21.46°, 23.57°, and 29.38°.

Tensile strength

The tensile strength as measured for the developed membrane is given in Figure 4. The force-displacement curve of the chitosan-lemon peel membrane shows a typical stress-strain behavior with an initial elastic phase followed by plastic deformation. It reaches a maximum force of 14.46 N before failing at a displacement of 2.2 mm. A maximum tensile strength of 10.31 MPa, a tensile stress at break of 6.26 MPa, and a tensile strain at break of 5.48% are important mechanical characteristics that show moderate elasticity and mild ductility.

Contact angle

Figure 5 displays the image of the contact angle formed between liquid-membrane interface. With an average contact angle of 70.33°, the chitosan-lemon peel membrane exhibits moderate hydrophilicity.

Anti-inflammatory assay

The graph in Figure 6 shows the anti-inflammatory activity of the developed membrane at concentrations of 150 µg/mL, 200 µg/mL, 250 µg/mL and 1 mg Salicylic acid. Anti-inflammatory activity was high at concentration of 250 µg/mL of lemon peel (1.65) extract which exhibited more anti-inflammatory activity when compared to 1 mg of salicylic acid control (1.62).

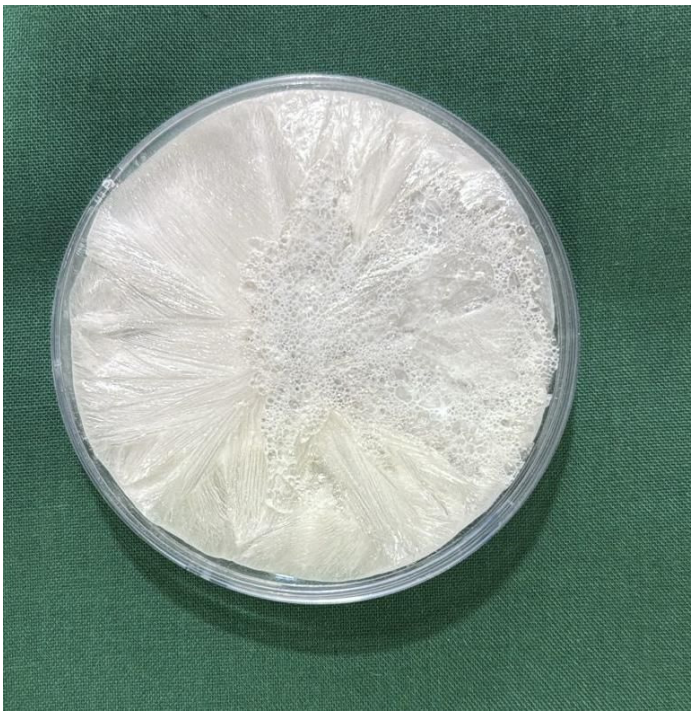


Figure 1: Chitosan based lemon peel membrane.

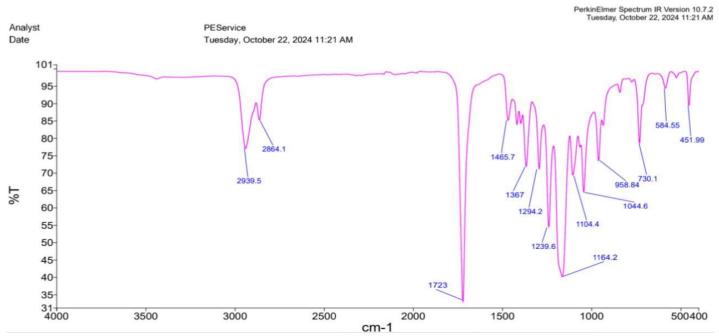


Figure 2: FTIR results of Chitosan-lemon peel membrane.

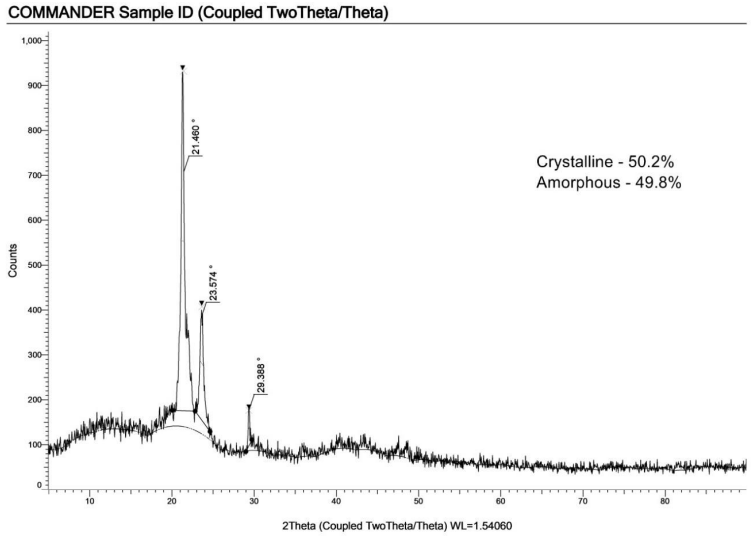
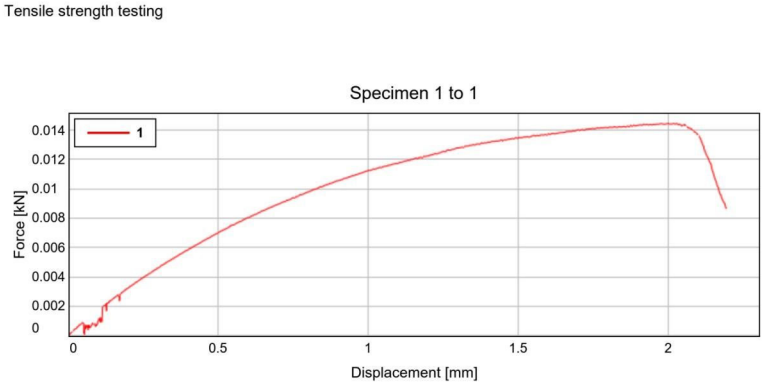


Figure 3: X-ray Diffraction analysis pattern of the Chitosan-lemon peel membrane.



	Maximum Force [N]	Tensile stress at Tensile strength [MPa]	Tensile strain (Displacement) at Break (Standard) [%]
1	14.46	10.31	5.48

	Specimen label	Tensile stress at Break (Standard) [MPa]
1	chitosan lemon peel	6.26

Figure 4: Force-displacement curve of Chitosan-lemon peel membrane.

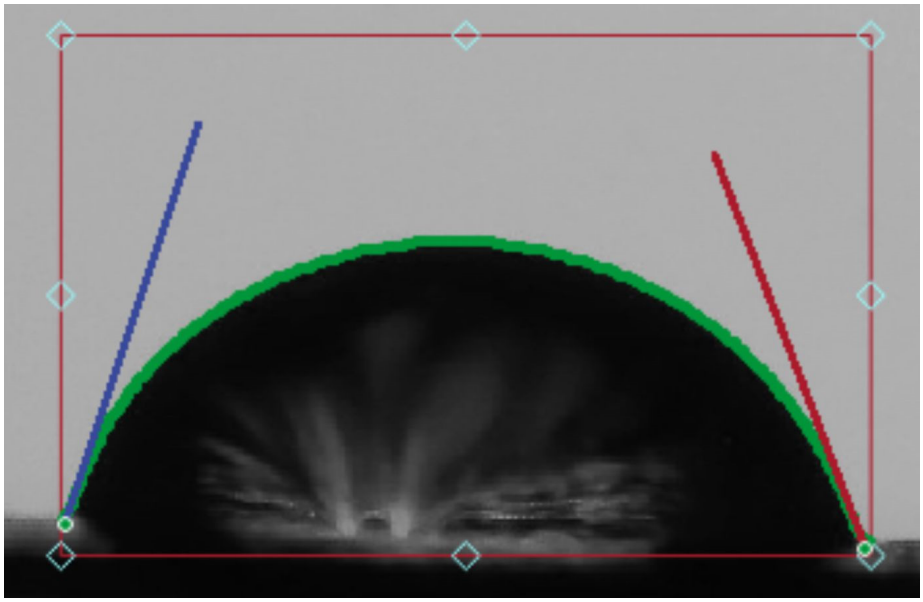


Figure 5: Measurement of contact angle.

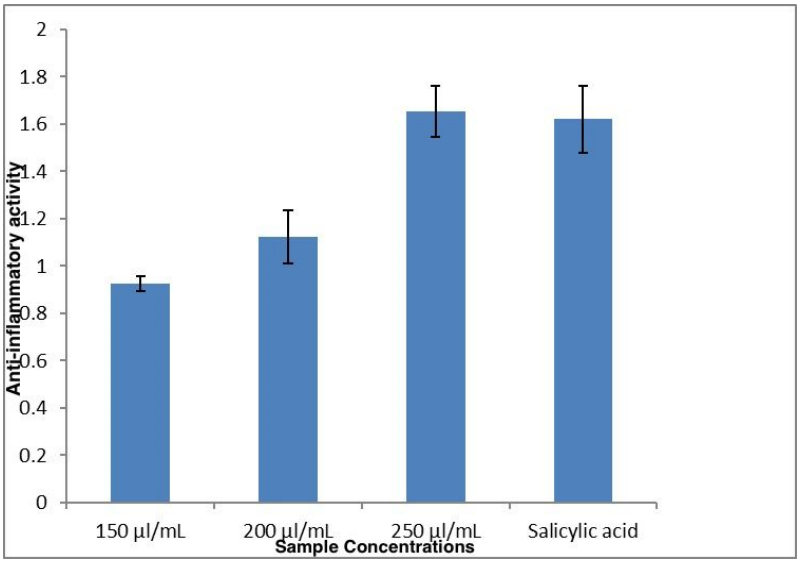


Figure 6: Graph showing anti-inflammatory activity at different concentrations.

Anti-microbial assay

Figure 7 shows the Minimum Inhibitory Concentration (MIC) achieved by the Chitosan - lemon peel membrane against *Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecalis, Escherichia coli.* Table 1 shows the dimensions of MIC. Minimum inhibitory concentration was higher in the chitosan-lemon peel membrane (12 mm) when compared to control antibiotics (11 mm) and crude extract (9 mm) against *S. aureus*. The MIC was also higher against *K. pneumoniae* (13 mm), *E. coli* (13 mm) and *E. faecalis* (13 mm) when compared to the control (10 mm, 11 mm, 12 mm). However crude extract did not show any inhibitory concentration against *E. coli*.

Degradability assay

Figure 8 portrays the percentage of membrane degradation for every sample size at the end of the incubation period. The control group showed increased degradation (nearly 55%). The 4 mm and 6 mm samples declined less from 6.8 mg to 5.3 mg and 10.2 mg to 7.4 mg (21% and 25%, respectively), whereas the 2 mm samples lost the most weight from 3.4 mg to 3 mg (8%), at an incubation period of 7 hr.

DISCUSSION

A novel generation of barrier membranes with enhanced characteristics is currently in the developmental stages. Criteria such as stability, easy tissue handling, biocompatibility, non-toxicity, biodegradability, and sustained drug release are

essential for effective localized drug delivery systems. Both membranes and films have the potential to fulfill these criteria adequately (Alqahtani *et al.*, 2023; Bajpai and Rajasekar, 2022; Paradowska-Stolarz *et al.*, 2023). Repurposing waste materials into valuable biomaterials has emerged as a key strategy for both energy conservation and waste management. In this regard, food waste presents an opportunity for beneficial applications in medicine, with fruit peels garnering particular attention for their potential health benefits. Utilizing the advantageous properties of both chitosan and lemon peel, a chitosan-based membrane was formulated by incorporating lemon peel extract and its physical and biological properties, including anti-inflammatory and antimicrobial effects, were investigated.

The FTIR analysis of the chitosan-lemon peel membrane supports the membrane's composite character and functional diversity by identifying unique functional groups that are provided by both chitosan and lemon peel. The retention of both biopolymer components is shown by peaks linked to the amine groups of chitosan (1264.2 cm^{-1} and 1239.6 cm^{-1}) and the presence of polysaccharides (1194.2 cm^{-1}, 1104.4 cm^{-1}, 1064.6 cm^{-1}). All of these molecular characteristics point to the membrane's mechanical qualities (Dai *et al.*, 2011; Indrani *et al.*, 2017). In a study done by Qui, Luquing *et al.* where the prepared Chitosan membrane showed approximately similar - CH and - CO stretching vibrations at a peak of 2909.41 cm^{-1} - 2357.14 cm^{-1} and 1625.649 cm^{-1} (Cui *et al.*, 2018). The C-O stretching vibrations have also observed approximately similar peaks at 11600 cm^{-1}-1750 cm^{-1} in a study done by Meydanju *et al.* with a lemon peel powder membrane (Meydanju *et al.*, 2022). The membrane exhibited a maximum tensile strength of 10.31 MPa, a tensile stress at break of 6.26 MPa, and a tensile strain at break of 5.48% show moderate elasticity and mild ductility. This value also correlates with a study done by Qurashi *et al.* on a Chitosan membrane blended with PVP with a tensile strength of 10.58 MPa (Qurashi *et al.*, 1992). Although the strength of a plain Chitosan film can reach up to 15 MPa (Cui *et al.*, 2018). A membrane with a tensile strength of 1 to 20 MPa has been observed to favor periodontal regeneration (Wang *et al.*, 2023). This well-balanced mechanical profile provides strength and flexibility to this polymer-based membrane thus facilitating easy moldability and adaptability of changing environment of periodontal tissues and strong enough to offer structural support. The force-displacement curve's initial elastic phase and plastic deformation further demonstrate that the membrane can sustain applied forces for a predetermined amount of time before failing, which is beneficial for applications needing durability and mechanical integrity. Crystalline peaks at 21.46°, 23.57°, and 29.38° are also on par with the observed crystalline peak at 22.8% of the lemon peel membrane in a study by Meydanju *et*

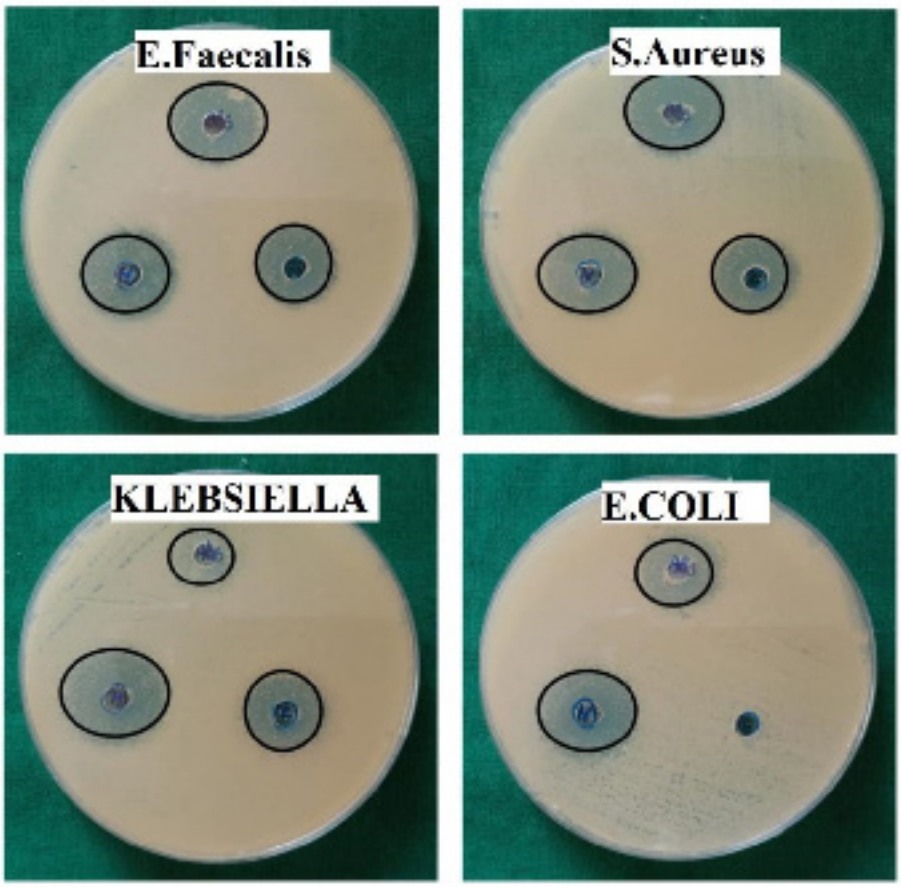


Figure 7: MIC achieved by Chitosan-lemon peel membrane (N), Crude Extracts (CE) of lemon peel powder and Amoxicillin (ABS).

Table 1: Dimensions of MIC.

Microorganism	Antibiotics (ABS)	Crude Extract (CE)	Chitosan–Lemon Peel Membrane (N)
E. faecalis	12	10	13
S. aureus	11	9	12
K. pneumoniae	10	11	13
E. coli	11	0	13

Minimum Inhibitory Concentration (MIC) dimensions (mm) of Chitosan–lemon peel membrane (N) compared with Crude Extract (CE) and standard antibiotics (ABS).

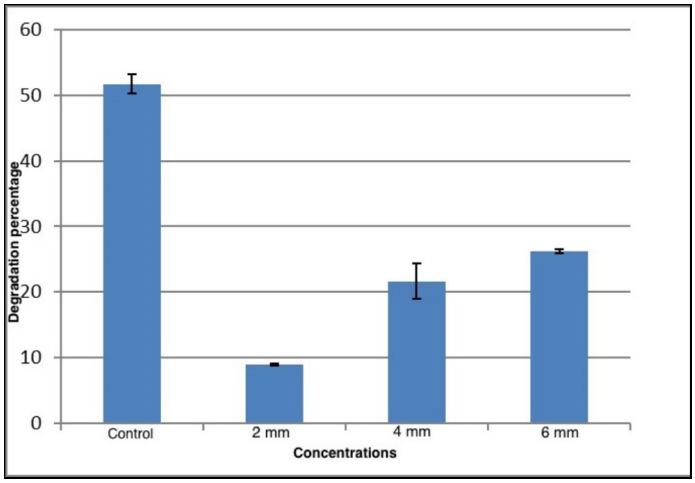


Figure 8: Degradation percentage of Chitosan-lemon peel membrane at varying concentrations.

al. (2022). Whereas for a Chitosan membrane infused with PVP, the crystalline peak was observed at 10.2% (Qurashi *et al.*, 1992). The well-balanced crystalline and amorphous phase imparts the membrane both mechanical strength and flexibility, thus facilitating tissue interaction and durability which is appropriate for promoting tissue regeneration, for serving as a bacterial barrier, and accelerating periodontal healing mostly due to synergistic effects of Chitosan and lemon peel components (Dai *et al.*, 2011). An average contact angle of 70.33º, indicating moderate hydrophilicity and rough surface suggests suitability for fibroblast and osteoblast migration and adhesion to the defective site. This cellular migration and adhesion are crucial for reattachment and regeneration in periodontal defects (Shah *et al.*, 2019).

The anti-microbial assay indicate that the minimum inhibitory concentration of the newly developed lemon peel membrane surpassed that of conventional antibiotics which is comparable to other citrus fruit extracts like tomato, orange extracts and other anti-oxidant extracts like tea extracts (Hamdan *et al.*, 2024; Silla *et al.*, 2025). However, the lemon peel membrane surpassed garlic extracts which was an established antimicrobial and anti-inflammatory agent (Sunil *et al.*, 2025). The increased MIC against *Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecalis, Escherichia coli* indicates that the developed Chitosan-lemon peel membrane possesses antimicrobial activity against both Gram-positive and Gram-negative microorganisms. Furthermore, the anti-inflammatory properties of 250 µg/mL of this lemon peel membrane exceed those of 1 mg of salicylic acid. The anti-inflammatory property was also investigated in another study which showed significant anti-inflammatory effects at 230-325 µg/mL (El Aboubi *et al.*, 2022). The lemon peel membrane showed better anti-inflammatory properties when compared to boswellia essential oil (Obiștioiu *et al.*, 2023), α-mangostin membrane (Shahzad *et al.*, 2025). The anti-inflammatory action may be due to decrease in IL-1β, TNF- α and IL-6 levels - primary inflammatory cytokines of periodontitis thus making it a viable option for controlling periodontal inflammation (Zou *et al.*, 2018). The chitosan-lemon peel membrane degradability test shows a size-dependent degradation pattern, with smaller samples (2 mm and 4 mm) degrading more quickly than larger ones (6 mm sample). Since larger samples disintegrated more slowly, this suggests a regulated degradation profile, which may be useful in periodontal regeneration, where gradual disintegration of periodontal membranes offers structural support in the early stages of healing and breaks down as new tissue grows. The stability of the control sample supported the idea that the sample size could be changed to tune membrane deterioration.

The investigation into the membrane's physical, mechanical, anti-inflammatory and antimicrobial properties reveals promising potential for its use in the treatment of periodontitis as a local drug delivery system. The membrane's ability to promote anti-inflammatory and antibacterial activity furthers the possibility of using it as a dressing post periodontal surgery and in recession coverage procedures at the palatal donor site after graft harvesting to promote wound healing. Its cellular adhesive potential could also promote regeneration of intra-bony defects (Sah *et al.*, 2019). Further investigation into the cytotoxic potential of lemon peel membrane is essential to determine dose dependent relation to the periodontal ligament and alveolar bone and to determine its regeneration potential and prospective potential for periodontal wound healing. These findings should be correlated with clinical trials to assess the disease prognosis, clinical improvement of symptoms, and overall enhancement of patients' quality of life.

CONCLUSION

With its promising mechanical strength, anti-inflammatory, and antibacterial qualities, the chitosan-lemon peel membrane created in this study demonstrates potential as a localized drug delivery system for periodontitis. The regulated, size-dependent degradability of the membrane promotes progressive periodontal repair as well. Although these results are promising, more investigation is required to assess cytotoxicity to identify safe and efficient dosage ranges. Future clinical trials are necessary to demonstrate the membrane's efficacy and safety in order to establish it as a practical and long-lasting periodontal treatment modality with significant clinical benefits.

ACKNOWLEDGEMENT

The authors have no acknowledgements to declare.

ABBREVIATIONS

FDA: Food and Drug Administration; **GCF:** Gingival Crevicular Fluid; **ATR-FTIR:** Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy; **XRD:** X-ray Diffraction; **ABS:** Antibiotics erythromycin and amoxicillin; **N:** Chitosan-Lemon Peel Membrane; **CE:** Crude Extract of Lemon Peel Powder; **PBS:** Phosphate Buffered Saline; **MIC:** Minimum Inhibitory Concentration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

CONCEPTUALIZATION

Jennifer Jeyaruby, Sankari Malaiappan, Shaheb Ali. Data curation: Jennifer Jeyaruby, Sankari Malaiappan. Writing – Original draft: Jennifer Jeyaruby. Writing – Review and editing: All authors. Supervision: Sankari Malaiappan.

DATA AVAILABILITY STATEMENT

The datasets generated during the current study are available from the corresponding author on reasonable request.

ETHICAL APPROVAL

This study did not involve human or animal subjects or tissue; hence, ethical approval was not required.

SUMMARY

This study aimed to develop a novel Chitosan-based membrane incorporating lemon peel extracts and evaluate its physical and biological characteristics. The potential applications of this membrane include its use in the treatment of periodontal diseases, such as in local drug delivery, wound healing, periodontal dressing, and as an agent with anti-inflammatory and antimicrobial properties. This study presents significant outcomes, notably being the first to synthesize a chitosan-based membrane with incorporated lemon peel extracts. The combination of the beneficial properties of both chitosan and lemon peel resulted in a membrane with favorable physical characteristics, such as a tensile strength of 10.31 MPa and a contact angle of 70.33º, making it suitable for membrane use and tissue regeneration. Furthermore, the membrane exhibited strong anti-inflammatory activity, surpassing the control substance (Salicylic acid), and demonstrated effective antimicrobial properties against both Gram-positive and Gram-negative bacteria. This suggests that the developed Chitosan-lemon peel membrane holds promise for controlling inflammation and aiding in the restoration of dysbiotic states in gingivitis and periodontitis.

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