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Original Research Article

Cellular Response of Chitosan *Loligo sp* to Changes in Cell Metabolism of *Enterococcus faecalis*

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ABSTRACT

Root canal treatment aims to eliminate infection and prevent recontamination, especially against *Enterococcus faecalis* (*E. faecalis*) bacteria resistant to antibiotics and disinfectants. Squid bone (*Loligo Sp)*, containing chitosan with antibacterial and antioxidant properties, is potentially effective in killing *E. faecalis* and can be used as a natural ingredient in root canal care. This study evaluated the characteristics and effects of chitosan *Loligo Sp* in increasing toxicity and decreasing the viability and release of Nitric Oxide in *E. faecalis cells.* Chitosan characteristics were assessed using FTIR and GC-MS. The toxicity and viability of *E. faecalis* cells challenged with chitosan were evaluated with an MTT assay, bacterial cell morphology was examined by a microscope, and bacterial NO value was assessed using FTIR**.** This study shows that chitosan from squid bones has characteristics that support its use as an antibacterial agent. A chitosan concentration of 10% indicates more significant toxicity against *E. faecalis* cells, reaching 85%, approximating the positive chlorhexidine (CHX) control value of 90% toxicity. Chitosan significantly decreased the viability *of E. faecalis* at a concentration of 10%, reducing its viability by 27.8% (p=0.013). In addition, chitosan also inhibits the release of Nitric Oxide by *E. faecalis*, with the highest inhibition of 80% at a concentration of 10% (p=0.001). Chitosan extracted from squid bones effectively increases toxicity and inhibits *E. faecalis's* viability and Nitric Oxide release from the bacteria, showing strong potential as an antimicrobial agent.

Keywords: *Enterococcus faecalis*, Chitosan *Loligo Sp*, Nitric Oxide, Root canal, Toxicity and viability.

Introduction

Endodontic treatment aims to remove infected pulp tissue, eliminate pathogenic bacteria, and prevent recontamination of the tooth's root canal.[1] One of the biggest challenges in endodontic care is the existence of *E. faecalis*. These anaerobic, facultative Gram-positive bacteria resist various disinfection agents and antibiotics.[2] These bacteria can survive extreme environmental conditions such as nutrient scarcity and high temperatures. They are the leading cause of root canal maintenance failure due to their virulence and ability to form biofilms that are difficult to overcome.[3] Chitosan extracted from squid bones (*Loligo Sp*) has promising antibacterial and antioxidant properties, including its ability to damage cell walls and interfere with the metabolism of *E. faecalis*, making it a superior candidate in endodontic care.[4] Chitosan can also inhibit the release of Nitric Oxide (NO).[5] It is an essential molecule in the inflammatory process, thus affecting the viability and growth of *bacteria*.[6] However, there have been no reports specifically of the role of chitosan in inhibiting NO *E. faecalis*.[7]

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Previous research reported that chitosan is more effective in overcoming root canal infections that are resistant to synthetic agents such as Chlorhexidine (CHX) and Sodium hypochlorite (NaOCl), with the advantages of biocompatibility, non-toxic properties, and environmental friendliness.[8] While CHX does not dissolve necrotic tissue, it risks causing bacterial resistance, tooth staining, and allergic reactions. NaOCl is toxic and has a pungent odor, so chitosan is safer.[9] Also, chitosan effectively prevents biofilm formation and supports tissue healing[10], making it a safer, more effective, and sustainable antibacterial alternative to root canal care compared to synthetic agents.[11] Previous research has shown that chitosan *Loligo Sp* has effective antibacterial potential against *E. faecalis* and *S. mutans*, which cause root canal infections.[12] With a stable pH, chitosan damages bacterial cell membranes, inhibits biofilm formation, and increases cell membrane permeability, all leading to bacterial death.[13] Chitosan also has antioxidant properties that aid tissue healing and show lower bacterial resistance than synthetic agents such as CHX and NaOCl.[14] Chitosan is a safer and more effective alternative in endodontic treatment with its biocompatible and environmentally friendly properties. This study highlights the potential of chitosan derived from squid bone (*Loligo Sp*) as a natural antibacterial agent, particularly effective against *E. faecalis*, a major pathogen in resistant root canal infections. Bioactivity analysis revealed that chitosan disrupts bacterial cell membranes, inhibits essential enzymes, prevents biofilm formation, and reduces the production of Nitric Oxide (NO), a key molecule in bacterial defense mechanisms. The research methodologies used, including Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatography-Mass Spectrometry (GC-MS), MTT Assay, and NO release analysis, provide comprehensive insights into the chemical properties and bioactivities of chitosan, elucidating its mechanisms in suppressing bacterial growth.

The study aimed to assess the physicochemical properties and cellular responses of *Loligo Sp* chitosan, its toxicity, its impact on *E. faecalis* cell viability, and its ability to inhibit NO release, thus strengthening its potential for clinical applications in endodontics.

Material and Methods

This study used squid bones to produce chitosan and *E. faecalis* ATCC 29212 from the Oral Biology Laboratory, Faculty of Dentistry, Syiah Kuala University, Banda Aceh. The samples were divided into chitosan groups (10%, 5%, 2.5%, 1.25%, and 0.65%%), CHX positive control, and Phosphate Buffer Saline (PBS) negative control.

Bacteria Culture
The bacterial stock of *E. faecalis was* refreshed on Muller Hinton Agar (MHA) media and cultured in anaerobic jars at 37°C for 48 hours. One colony was cultured again in Brain heart infusion (BHI) liquid medium and then at 37°C for 48 h (Merck KGaA, Darmstadt, Germany). Then, it was resuspended and equalised to the Mc. Farland 0.5 (1.5 x 10^8 CFU/mL).[15]

Chitosan Preparation
Squid bone (*Loligo Sp*) were obtained from the sea area of Aceh, Ulee Lheu, Banda Aceh, Indonesia, with coordinates (5.556119962364292, 95.28599046385224). A total of 3 kg of squid bones were separated, washed, dried in the sun for five days, and ground to powder. The coarse powder was then sifted (100 mesh) for use in chitin insulation. The chitosan isolation process begins with demineralisation, where 100 g of squid bone powder is mixed with HCl 1 N (1:12 w/v) (Merck KGaA, Darmstadt, Germany), heated at 75°C for one hour, then filtered, washed until neutral, and dried for 24 hours at 75°C. In the deproteinisation stage, the demineralisation residue was mixed with NaOH 2 N (1:6 w/v) (Merck KGaA, Darmstadt, Germany), heated at 80°C for one hour, filtered, and dried. The resulting chitin was then converted to chitosan through deacetylation by boiling chitin in a 50% NaOH solution (1:5 w/v) (Merck KGaA, Darmstadt, Germany) at 90°C for 120 min. The results are filtered, washed until neutral, and dried for 24 hours at 80 °C.[16]

SEM-EDX
The chemical elements of chitosan were examined using SEM-EDX (Scanning Electron Microscope-Energy Dispersive X-ray Spectroscopy) (Thermo Fisher, Netherlands). The sample was placed in a vacuum chamber, and the height of the sample followed the calibration standard. The instrument was operated at 20 kV. The sample was slowly shifted to get the area to be photographed on the SEM screen. Brightness, contrast, and focus were set until an image was obtained to identify the chemical element. First, the area to be analyzed was determined. Next, data was taken by scanning on an EDX device and obtained within one second. The results obtained can be visualised on the EDX screen. The type and number of elements in the scan area were confirmed using EDX database software.[17]

Extration of Enterococus faecalis Cell
Enterococcus faecalis that had interacted with a chitosan solution in several different concentrations and controls incubated for 48 at 37 °C were then examined for cell morphology with Gram staining. Supernatants were taken and vortex ((Shimadzu, Japan) for 30 seconds, followed by adding 0.1 M HCl and incubating at 4 °C for 15 min. Centrifugation (Shimadzu, Japan) and repeated washing were carried out with PBS (Merck KGaA, Darmstadt, Germany), and 70% ethanol and the precipitate of the whole-cell extract *of E. faecalis was collected*.

FTIR Spectrum
The chitosan nano functional cluster was evaluated using Fourier Transform Infrared (FTIR) with a transmission spectrum at a wave number of 4000 cm^{-1} - 400 cm^{-1} (Shimadzu, Japan). The sample was placed on a transparent infrared prism surface with a higher refractive index than the sample (1.39). The light was directed through a prism and reflected, and then the intensity and absorption spectrum were recorded. This FTIR analysis assessed nitric oxide deformation in chitosan solution-affected *E. faecalis* cells with an ATR-FTIR mode approach, focusing on the wavelength range of 1540-1560 cm^{-1} for identifying Nitric Oxide deformation that was transferred by RESolution (Shimadzu) for generating and interpreting FTIR spectra with a focus on clarity and detail.

GC-MS Analysis
The chemical compounds of Chitosan *Loligo Sp* were examined by GC-MS analysis (QP2010PLUS), with G.C column melting (2010), coated with polymethyl silicon (Shimadzu, Japan). The following conditions were set: 80–200 °C and flow rates of 5 °C/min and 200 °C for 20 min. FID temperature 300 °C, injection temperature 220 °C, nitrogen-carrying gas at a flow rate of 1 mL/min, separation ratio 1:75. The pressure was set at 116.9 kPa. The column length was 30 m with a diameter of 0.25 mm and a flow rate of 50 mL/min.

MTT Assay
A total of 20 µL of bacterial medium was transferred into a 96-well plate, then 30 µL of bacterial suspension was added and adhered to at 37 °C for three hours. Next, a chitosan solution of 100 µL each was added and adapted for 24 hours. The well plates were treated with MTT (4,5-Dimethyitliiazol-2-yl, 2,5-diphenyItetrazolium bromide assay) Assay (Abcam plc, Boston, USA) reagent. The supernatant was removed from each well and washed once with PBS. Each well was filled with 50 µL of MTT solution and incubated for 3 hours at 37°C. Then, 100 µL of acidified isopropanol 0.04 N was added to each well and placed on an orbital shaker at 50 rpm for one hour. The MTT test results were read with a 550 nm Elisa reader, and the percentage of live cells was computed from Equation 1:

$$\text{Percentage of live cells} = \frac{Mean\ Absorbance\ of\ sample}{Mean\ Absorbance\ of\ Negative\ Control\ (PBS)}\ X\ 100\%$$

Statistical analysis
The significance of toxicity and viability properties and the inhibition of Nitric oxide E. faecalis by chitosan was assessed with One-way ANOVA with a significance of $p<0.05$.

Results and Discussion

Fourier Transform Infrared Spectroscopic analysis of *Loligo Sp* chitosan aims to identify and analyze the functional groups in the extracted chitosan (Figure 1). FTIR is a technique used to obtain the infrared spectrum from the absorption or transmission of solid, liquid, or gaseous samples, which are then analyzed to determine their chemical composition and molecular structure.[18] The infrared spectrum of chitosan extracted from *Loligo* Sp bone shows several characteristic peaks indicating the presence of major functional groups in the chitosan. Strong peaks around 3400-3200 cm^{-1} indicate the presence of hydroxyl (-OH) and amine (-NH2) groups, characteristic of the primary and secondary amine groups in chitosan. In addition, the weak band at around 2900 cm^{-1} is due to the aliphatic C-H stretch. A peak around 1650-1550 cm^{-1} indicates a C=O stretch vibration of the amide, indicating the presence of a portion of an undeacetylated acetyl group in the chitosan chain.[19] The other peaks around 1150-1020 cm^{-1} are related to the C-O-C group stretching vibration of the glycosidic bond, which is an integral part of the chitosan structure.[20] From the FTIR spectrum, it can be concluded that chitosan produced from squid bones contains an amine group (NH2) that is important for biomedical applications, especially in endodontics dentistry. The presence of peaks indicating an acetyl group that has not been fully deacetylated suggests that this chitosan has a structure corresponding to that of natural chitosan. The strong hydroxyl group also shows the potential of chitosan in forming hydrogen bonds, which is essential in various biological applications.[21] Overall, the FTIR results show that the extraction process of chitosan from squid bones was successful, with structures and characteristics suitable for use in biomedical applications.

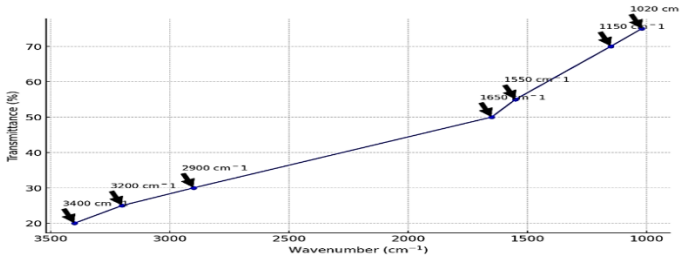

Figure 1: FTIR graph showing the spectrum of chitosan of *Loligo Sp*. This graph shows the relationship between the number of waves (in cm^{-1}) and the transmittance (%), with markers at the prominent peaks indicating the presence of critical functional groups in the chitosan.

Table 1 provides an overview of the chemical composition of chitosan analyzed by EDX. Elemental content values were determined based on the area below the peak at a specific energy position in the spectrum, which can be calculated using EDX analysis software. In the EDX analysis, the dominant elements detected in chitosan extracted from squid bones were Carbon (C) and Oxygen (O), reflecting the organic structure of chitosan. Nitrogen (N) indicates the presence of amine groups, which are essential components in the structure of chitosan and play a role in various biochemical functions. Calcium (Ca) and Phosphorus (P) are thought to be derived from the squid bone minerals that remain after extraction, indicating that the original minerals have not been completely removed during purification. Sodium (Na) is likely derived from salt residues or contaminants from the refining process. In the EDX spectrum, Carbon (C) was detected in the range of 0-1 keV with high intensity due to the high Carbon content in chitosan. Oxygen (O) was detected in the 0.5-1.5 keV range and Nitrogen (N) around 0.3-0.4 keV. Calcium (Ca) appears in the spectrum between 3-4 keV, indicating mineral residues from squid bones. Phosphorus (P) was seen at 2-2.2 keV, with varying intensities, while Sodium (Na) was detected in the range of 1-1.1 keV (Figure 2). These peaks provide information on the chemical composition of chitosan, which is essential for understanding its physicochemical properties and relevance in biomedical and pharmaceutical applications.[22]

Table 1: Results SEM-EDX of chitosan *Loligo Sp* showing some elements and percentage composition.

No	Chemical Element	Symbol	Content (%)
1	Carbon	C	42.5
2	Oxygen	Or	35.3
3	Nitrogen	N	14.2
4	Calcium	Ca	5.8
5	Phosphorus	P	1.4
6	Sodium	On	0.8

The chemical elements in chitosan, such as carbon (C), oxygen (O), nitrogen (N), calcium (Ca), phosphorus (P), and sodium (Na), play an essential role in inhibiting the development of *E. faecalis*.[23] Carbon and oxygen form polymer chains that interact with bacterial cell membranes disrupting stability and causing cell leakage.[24]

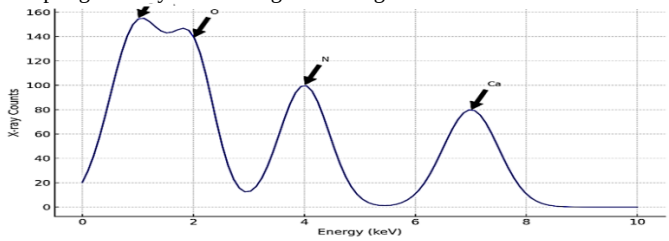

Figure 2: Spectrum graph of EDX analysis on chitosan *Loligo Sp*. This graph shows the distribution of X-rays at various energy levels (keV), with markers at critical peaks indicating the presence of elements such as Carbon (C), Oxygen (O), Nitrogen (N), and Calcium (Ca).

Nitrogen, through positively charged amine groups, interacts with negatively charged bacterial cell walls, increases membrane permeability, causes cell lysis, and prevents biofilms' formation.[25] Calcium can interfere with the adhesion of biofilms, and phosphorus can affect bacterial DNA replication, while sodium can cause osmotic stress in bacterial cells.[26] The synergistic interaction between these elements strengthens the antibacterial properties of chitosan, making it an effective agent for inhibiting the growth and development of *E. faecalis*. The results of this study provide important insights into the chemical composition of chitosan extracted from squid bones (Figure 3), which contributes to understanding its potential applications in biomedical and pharmaceutical fields. GC-MS data showed the chemical composition of the *Loligo Sp chitosan (Table 2)*.

Table 2: Results GC-MS of chitosan *Loligo sp* showing retention times and relative percentage composition of some important constituents.

No	Chemical compound	Retention Time (minutes)	Relative Percentage (%)
1	Glucosamine	10.5	25.4
2	Acetic Acid	12.8	18.3
3	N-acetylglucosamine	14.3	15.7
4	Fatty Acids	16.7	10.9
5	Chitosamine	19.1	8.5
6	Hydrochloric Acid	20.3	7.2
7	N-acetylgalactosamine	22.5	6.1
8	Carbonates	24.0	5.6
9	Polymeric Chitosan	26.2	2.3

Glucosamine is one of the main compounds in chitosan, which is formed from chitin deacetylation and plays an essential role in its structure. Acetic acid, which is found due to deacetylation, may also be derived from the chitosan isolation process. N-acetylglucosamine indicates an acetyl group still left in the chitosan chain, indicating that the deacetylation process does not eliminate the acetyl group. In addition, fatty acid detection can indicate the presence of contaminants or residues from the original biological source, which may still be left after the purification process.

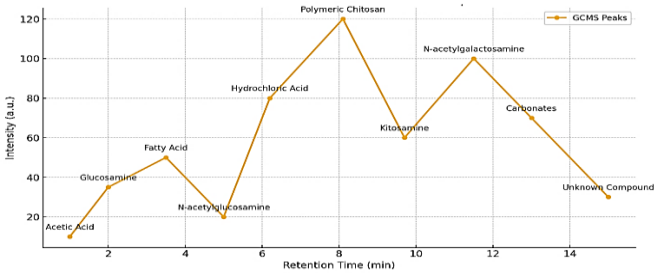

Figure 3: The GCMS graph represents the chemical profile of chitosan derived from *Loligo* sp. Each peak is labeled with the corresponding compound detected at specific retention times, including notable compounds such as Acetic Acid, Glucosamine, and Polymeric Chitosan.

Chitosamine, the primary compound of chitosan, was also identified, reinforcing the existence of the main structure of chitosan.[27] Compounds such as glucosamine, N-acetylglucosamine, fatty acids, chitosamine, hydrochloric acid, N-acetylgalactosamine, carbonate, and chitosan polymers work synergistically to inhibit the growth of *E.*

faecalis. Glucosamine and chitosan, with their positive charges, damage the bacterial cell wall through electrostatic interactions. In contrast, N-acetylglucosamine and chitosan polymers prevent the formation of biofilms, which are essential for bacterial survival.[28] Fatty acids and hydrochloric acids damage cell membranes and disrupt the osmotic balance, causing ion leakage and cell lysis. N-acetylgalactosamine inhibits cell wall synthesis, and carbonate affects bacterial metabolic processes by disrupting enzymes and ionic balance.[29] These mechanisms lead to metabolic disorders, stunted growth, and bacterial cell death in *E. faecalis*.

Table 3 shows the results of the chitosan toxicity test against *Enterococcus faecalis*. The higher the chitosan concentration, the greater the toxicity to *E. faecalis*, with a concentration of 10% indicating a toxicity of 85%, comparable to the positive control of CHX (90%). In contrast, negative control of PBS showed a toxicity of 10%, signalling that most cells remained alive. The one-way ANOVA test revealed a significant difference at a chitosan concentration of 2.5% (p=0.027), demonstrating the potential of chitosan as an effective antimicrobial agent, especially at higher concentrations, for clinical applications, such as in endodontic treatments.

Various studies support the role of chitosan as a toxic agent against bacteria through multiple mechanisms. Chitosan works by damaging bacterial cell walls through electrostatic interactions, leading to cytoplasmic leakage and cell death. [30] Chitosan also interferes with transporting nutrients and ions in cells, resulting in metabolic dysfunction and bacterial death.[28] Its toxic properties are also affected by its molecular weight and degree of deacetylation, where low-molecular-weight chitosan is more effective in inhibiting bacterial growth.[31] In addition, chitosan activity is more optimal at low pH, which increases its positive charge and strengthens its interaction with the bacterial cell wall.[32] These findings suggest that chitosan damages cell membranes and interferes with cell homeostasis, making it a powerful natural antibacterial agent against *Enterococcus faecalis* **(Fig. 4).**

Table 4 illustrates the cell viability of *E. faecalis* after being affected by chitosan extracted from squid bones. The test was carried out using the MTT Assay method at a wavelength of 550 nm, with several concentrations of chitosan (10%, 5%, 2.5%, 1.25%, and 0.65%) and lower cell viability indicating higher toxicity of chitosan to *E. faecalis*. At a chitosan concentration of 10%, the cell viability of *E. faecalis* was 27.8%, suggesting that most cells did not survive, comparable to the positive control (CHX) agent with a viability of 22.2%. In contrast, at lower chitosan concentrations, cell viability increased, with a concentration of 0.65% indicating the highest viability of 72.2%, showing that the effect of chitosan on cells decreased with decreased concentration. Negative control (PBS) showed the highest cell viability at 100%, indicating that the cell *E. faecalis* ultimately survived without the influence of chitosan. Calculation of *E. faecalis* live cells based on absorbance data from the MTT Assay showed that cell viability was calculated as a percentage of negative control (PBS), which was considered 100% viability. [33] This result aligns with that reported in Figure 4, where toxicity in *E. faecalis* cells caused changes in cell morphology as an indicator of damage to *E. faecalis* cells. Previous research reported that among the signs of toxic cells shown morphologically with a clumped shape with each other, the chain shape and septa between cells are no longer visible.[15]

Chitosan can chelate essential metal ions such as magnesium and calcium, which are important for bacterial metabolism and growth, including *E. faecalis*.[32] By binding to these ions, chitosan interferes with the function of enzymes and the structure of the bacterial cell wall, thereby inhibiting cell replication and metabolism, which can slow or stop the growth of bacteria.[32] Chitosan also increases the permeability of bacterial cell membranes, leading to osmotic imbalances.[34] Bacteria such as *E. faecalis* require strict osmotic regulation to maintain internal balance, and when chitosan causes changes in permeability, essential ions and molecules exit or enter the cell uncontrollably, disrupting homeostasis and ultimately leading to the death of bacterial cells.[35]

Table 3: T*oxicity* value of chitosan *Loligo Sp* against *E. faecalis* cells

Chitosan	N	Cell toxicity (550 nm)			**p-value*
		Mean	SD	Toxic (%)	
10%	3	0.25	0.02	85	
5%	3	0.35	0.03	75	
2.5%	3	0.45	0.04	65	0.027
1.25%	3	0.55	0.05	55	
0.65%	3	0.65	0.06	45	
C+ (CHX)	3	0.20	0.01	90	
C- (PBS)	3	0.90	0.07	10	

***** One Way ANOVA

Table 4: Viability of *E. faecalis* Cells after being affected by *Chitosan Loligo Sp*

Chitosan	N	Cell toxicity (550 nm)			**p-value*
		Mean	SD	Toxic (%)	
10%	3	0.25	0.02	27.8	
5%	3	0.35	0.03	38.9	
2.5%	3	0.45	0.04	50.0	0.013
1.25%	3	0.55	0.05	61.1	
0.65%	3	0.65	0.06	72.2	
C+(CHX)	3	0.20	0.01	22.2	
C - (PBS)	3	0.90	0.07	100	

***** One Way ANOVA

The one-way ANOVA test at a chitosan concentration of 2.5% showed a statistically significant difference in cell viability compared to the other groups, with a p-value of 0.013. This indicates that chitosan effectively decreases the viability of *E. faecalis* cells at higher concentrations, making it a potential antimicrobial agent in clinical applications. Table 5 reports the results of measuring the average absorbance of the peak of NO identification using FTIR for various chitosan concentrations. Lower absorbance values indicate that chitosan was influential in inhibiting NO release by *Enterococcus faecalis*. The frequency of N-O stretching (1540-1560 cm^{-1}) and C-H bending (2850-2950 cm^{-1}) was identified in the FTIR spectrum, reflecting the presence of specific functional groups. The Percentage of inhibition was calculated based on the ability of chitosan to reduce NO release compared to negative control (PBS). At a chitosan concentration of 10%, the inhibitory power reached 80%, almost equivalent to the positive control (CHX), which showed an inhibitory power of 85%. This proves that at high concentrations, chitosan is very effective in inhibiting NO release by *E. faecalis*. Along with decreased concentration, the effectiveness of chitosan decreased, with the lowest concentration (0.65%) showing an inhibition of 40%. Negative control (PBS) showed no inhibitory effect, as seen from the high absorbance value and 0% inhibition. These results show that chitosan extracted from squid bones has the potential to be an effective agent to inhibit NO release by *E. faecalis*, with increased effectiveness as the concentration of chitosan ($p<0.05$) increases. Chitosan has also been reported to inhibit bacteria's NO release.[36] NO is a signaling molecule that plays a role in the bacteria's defense mechanism against adverse environmental conditions.[37] By inhibiting NO production, chitosan makes bacteria more susceptible to oxidative stress conditions, which accelerates bacterial death. It is crucial in treating root canal infections, where *E. faecalis* often resists adverse environmental conditions.[38]

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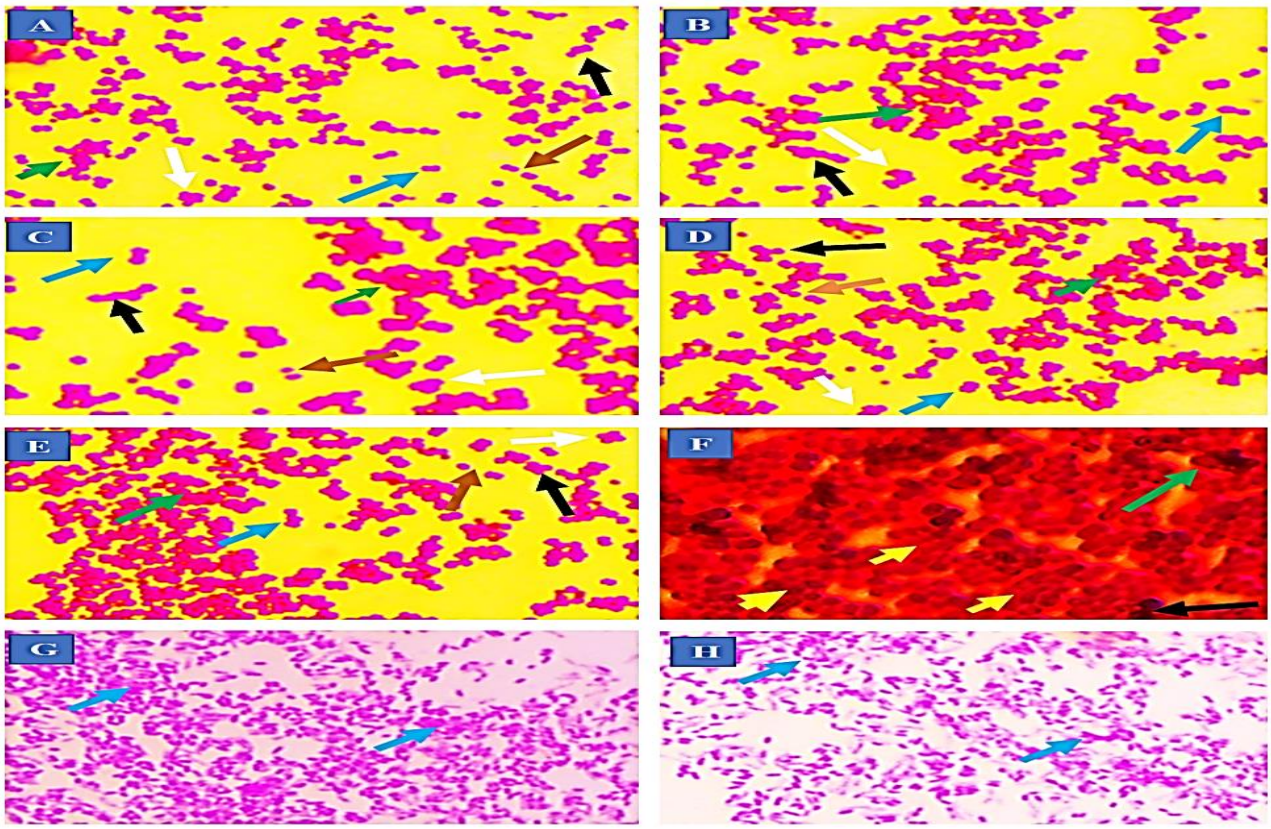

Figure 4: Morphology of *E. faecalis* cells due to the toxicity of chitosan from squid bones. A (10%), B (5%), C (2.5%), D (1.25%). E (0.65%), F (C+), G and G (C-). Blue Arrow (Normal cells that do not change), Green Arrow (Cells that undergo coagulation or fail to grow), Black Arrow (Cells that fail to undergo elongation of chain formation), White Arrow (lysis cells), Yellow Arrow (Cell death, loss of cell function), Brown Arrow (Septa separation between cells occurs). The image was obtained from gram coloring results with 400x magnification)

Table 5: FTIR results of inhibitory values of Nitric oxide of *E.* faecalis release affected by Chitosan *Loligo sp*

Chitosan Concentration	N	NO Peak, Wavelength (1540-1560 (cm^{-1})				Inhibitory (%)	*p-value
		Mean	SD	N-O Stretching	C-H Bending		
10%	3	0.30	0.02	1540-1560	2850-2950	80	
5%	3	0.40	0.03	1540-1560	2850-2950	70	
2.5%	3	0.50	0.04	1540-1560	2850-2950	60	
1.25%	3	0.60	0.05	1540-1560	2850-2950	50	0.001
0.65%	3	0.70	0.06	1540-1560	2850-2950	40	
C+(CHX)	3	0.25	0.01	1540-1560	2850-2950	85	
C- (PBS)	3	1.50	0.08	1540-1560	2850-2950	0	

***** One Way ANOVA

Conclusion

This study shows that chitosan *Loligo sp* has the potential to be an effective antibacterial agent against *Enterococcus faecalis*. It has essential functional groups and bioactive compounds that support its antibacterial and antioxidant properties. In addition, chitosan toxicity against *E. faecalis* is concentration-dependent, reaching up to 80% at a concentration of 10%. In addition, it effectively inhibits the release of Nitric Oxide (NO) and the viability of *E. faecalis cells*. The antibacterial properties of chitosan have great potential to be applied in endodontic treatments.

Conflict of interest

The authors declare no conflict of interest.

Author's Declaration

At this moment, the authors declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

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