

ANTIMICROBIAL ACTIVITY OF CHITOSAN-VITAMIN E-NANOEMULSION

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ABSTRACT

Chitosan is considered one of the most abundant polysaccharide in the world. Therefore, it has several applications in the food and pharmaceutical industries. However, the structure of chitosan may limit its solubility and bioavailability. The current study aimed to improve the physicochemical properties of chitosan by combining it with vitamin E nanoemulsion (CH-NE-vitE). The structural, thermal stability, and physical differences between chitosan and its new derivative were analyzed using the scanning electron microscope (SEM), Fourier transform infrared (FTIR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, and differential scanning calorimetry (DSC). Furthermore, the antimicrobial activities of CH-NE-vitE against *Staphylococcus aureus*, *Candida krusei* and *Enterobacter hormaechei* were examined using disc and well diffusion methods in addition to the determination of the minimum inhibitory concentration (MIC). The morphology changes in the treated microbes were visualized using the SEM. The characterization of CH-NE-vitE exhibited noticeable changes in the chitosan physical properties and chemical structure including increased solubility, interaction rates, and stability. The new derivative has inhibited the growth of both *S. aureus*, and *C. krusei* while promoting the growth of *E. hormaechei*. The minimum inhibitory concentrations against the *S. aureus*, and *C. krusei* were 1.563 mg/mL and 3.125 mg/mL, respectively. The produced CH-NE-vitE can be used in drug delivery, dermal products, and food packaging.

Keywords: Minimum inhibitory concentration; *Staphylococcus aureus*; *Candida krusei*; *Enterobacter hormaechei*; Polysaccharide

INTRODUCTION

Over the years, scientists have been searching and testing many different substances to treat pathogens around the world. It took a long time with multiple processes to find out the best choice depending on its efficiency, plenty, and safety. Nowadays, people are more attentive to medical sources that are more natural and less processed. One group of these environmentally friendly choices is the polysaccharides group and their derivatives. Mostly, they are used with other ingredients due to their ability to bind with bioactive agents that will increase activity duration. That is why more studies should be implemented for chitosan, which is a natural polysaccharide that can be extracted by processing chitin (Ways et al., 2018). Chitosan has been proven to be an antimicrobial agent that can be used for many applications including tissue engineering, drug delivery, and packaging material. However, chitosan has a complex structure with a high molecular weight that should be reduced by various techniques including hydrolysis by physical, enzymatic, and chemical processes.

Chitosan is an amino polysaccharide that has taken place in science due to its biological activities and special properties. These special properties include biocompatibility, low toxicity, and biodegradability. It can be derived from many sources since it is the second most plentiful polysaccharide in nature. For instance, it can be found in the exoskeleton of crabs, insects, shrimps, and lobsters. Clearly, chitosan can be obtained from these organisms by obtaining chitin that will go through many processes like deproteinization and demineralization of chitin (Singh et al., 2021). In other words, the acetyl group (COCH₃) is removed from the chitin that has been obtained from the exoskeleton of crustaceans, during a process known as deacetylation (Younes et al., 2015). As a result, the chitosan can be represented using the general formula as C₆H₁₁O₄N, since its monomers are composed of 2 free hydroxyl groups with one primary amine (Goy et al., 2016). Many applications and fields depend on chitosan nowadays, such as activating plant defenses, preserving food, and the cosmetics industry. There were many articles about antimicrobial actions which proved their efficiency against a vast number of pathogens. However, the physicochemical properties of chitosan affect the antipathogen's action and other activities greatly, which means that chitosan properties should be improved to increase its effectiveness. One of the major drawbacks of chitosan is its low solubility in water and basic solutions. Chitosan has to be solubilized in the acidic solution to be protonated and get solubilized in the aqueous solutions. Other factors that can affect the solubility of chitosan can

be polymer crystallinity, temperature, and degree of acetylation (Romanazzi et al., 2009).

Many research studies attempted to increase chitosan solubility by deacetylation, quaternization, and depolarization. Chitosan, commercially sold as flakes or even powder, can be dissolved in acidic conditions resulting in the protonation of the amine group in the glucosamine monomer. For chitosan deacetylation and demineralization, a basic solution is added for about 0.5 to 72 h at 65-100 °C. The prolonged heating may cause a reduction in the molecular weight and acetylation of chitosan (Ke et al., 2021). The mechanisms of chitosan actions against fungi and bacteria were extensively investigated which included the effect of the chitosan physicochemical characteristics, structure, and reactive hydroxyl groups as well as the surrounding environmental conditions. It has been found that chitosan actions against microbes can be classified according to the targeting range of the antimicrobials to intracellular impacts, extracellular impacts, or even both. Generally, chitosan cannot enter both the cell wall and the cell membrane due to its high molecular weight. However, it can work as a barrier to prevent cells from taking nutrients, change cell permeability, and act as a chelator of important ingredients, which are all considered extracellular antimicrobial actions. On the other hand, chitosan with less molecular weight can affect microbes internally, including RNA, mitochondrial function, and protein synthesis. Also, chitosan effects depend on the targeted microorganism type.

There are several factors that affect the antimicrobial activity of chitosan. First, the protonation of chitosan enhances its solubility and its permeation to microbial cells. The second factor is the chitosan molecular weight that affects them differently depending on the microbe type including pore sizes, polysaccharides, proteins, and cell wall layers. The third factor is the deacetylation degree of chitosan which influences both the temperature and time of the chemical process. Generally, increasing temperature and process period will result in a high deacetylation degree. Higher deacetylation degrees lead to a more positive charge than lower one even if both are in the same acidic conditions. This means that the high deacetylation degree improves antimicrobial activity because it raises the electrostatic interactions (Ardean et al., 2021; Ke et al., 2021).

For bacteria, the difference between gram-positive and gram-negative is due to the cell wall structure, mainly the thicker peptidoglycan layer in gram-positive can affect the susceptibility to chitosan. Gram-negative bacteria are enriched in peptidoglycan and have a more negative charge due to the attachment of phosphorylated groups with lipopolysaccharide. Therefore, cationic chitosan will

bind strongly with negatively charged surfaces when the conditions are acidic or have a pH less than 6.5 (Ke et al., 2021; Yan et al., 2021). The gram-positive bacteria are less sensitive to chitosan due to the presence of teichoic acid with phosphate groups which are negatively charged. Thus, the chitosan resistance of *Staphylococcus aureus* can be eliminated by the cancellation of the teichoic acid biosynthesis pathway. Some chitosan oligomers can penetrate the thick cell wall of gram-positive bacteria to affect protein and DNA/RNA synthesis by inhibiting the DNA transcription. Chitosan has antifungal actions due to its ability to interact with cell membranes or the cell walls of the fungus. However, the chitosan minimum inhibitory concentration depends on the type of targeted fungus, solvent pH, and molecular weight and deacetylation degree of chitosan. One of the important factors is the unsaturated fatty acid components on the cell membrane which increase negative charge because of better membrane fluidity, so better chitosan susceptibility. On the other hand, chitosan has intracellular antifungal activities including inhabiting protein, DNA/RNA synthesis, and destroying mitochondrial activity (Alburquerque et al., 2010).

Generally, scientists are searching for more solutions to increase chitosan's solubility through adding different reagents, vitamins, oils, and other solvents. In the current research, the nanoemulsion technique was applied to produce chitosan derivatives. Nanoemulsion is a colloidal system consisting of immiscible liquids combined with the mean of surfactants. It contains dispersed droplets with a size of about 20-500 nm. Small size nano-droplets improve substance properties like tunable rheology and robust stability. Therefore, it is usually used in the biomedical applications such as pharmaceutical formulations (Gupta and Xie, 2018).

A recent review article highlighted the developed physical, chemical and mechanical properties of various fabricated chitosan nanoemulsions that hold promising potential in the food, domestic and pharmaceutical applications (Chaudhary et al., 2022). With the aim to improve the beneficial properties of the chitosan, the lipid soluble agent, vitamin E, was solubilized in the nanoemulsion. Vitamin E helps in preventing skin damage, neurological disorders, infertility, and cardiovascular diseases. It regulates enzyme activity, gene expression, cellular signaling, and cell proliferation. It inhibits the coagulation of platelets (Niki, 2015). Nanoemulsions are colloidal systems that consist of immiscible liquids homogenized with the aid of the surfactants. Nanoemulsions, produced by combining various essential oils with the aqueous solutions and the mixed surfactants of tween 80/span 20, exhibited valuable antitumor activities against different types of cancers (Al-Mutairi and Alkhatib, 2022; Bayoumi and Alkhatib, 2022; Bawadud and Alkhatib, 2023). Usually, non-ionic surfactant with several repeats of polyethylene glycol, such as tween 20, are selected in the formation of the emulsification systems applied in pharmaceutical formulations due to their non-toxic and biocompatible behavior (Sahoo et al., 2014). The anionic surfactants, e.g. sodium lauryl sulfate, were massively used in the domestic and pharmaceutical products due to their amphiphilic properties that promote their ability to reduce the surface tension between two immiscible liquids (Takei et al., 1985).

In the current study, chitosan was mixed with the nanoemulsion based on vitamin E oil to enhance its biocompatibility and physical properties. In particular, the effect of vitamin E nanoemulsion on the structure and antimicrobial activity of chitosan was investigated. The mixture of both chitosan and vitamin E nanoemulsion was tested against three microorganisms. First, *Staphylococcus aureus* is harmful gram-positive bacteria (Gulzar et al., 2018). Second, *Enterobacter hormaechei* is gram-negative infectious bacteria (Wang et al., 2020). The last is *Candida krusei* which is a pathogenic fungus (Pfaller et al., 2008).

MATERIALS AND METHODS

Materials

Chitosan, tween20, sodium lauryl sulfate, vitamin E, sodium hydroxide, and glacial acetic acid were purchased from Sigma Aldrich (USA). All of the materials required for the antimicrobial evaluation were provided by the Natural & Medical Sciences Research Center at the University of Nizwa (Nizwa, Sultanate of Oman).

Preparation of chitosan

Chitosan in the form of powder (0.5 g) was dissolved in 100 mL of 2.5 % (w/v) glacial acetic acid. Then, the chitosan solution was added to 200 mL of 1M NaOH solution.

Synthesis of chitosan nanoemulsion-based vitamin E

Nanoemulsion-based vitamin E was prepared by mixing 1 mL of vitamin E oil with 0.6 g of sodium lauryl sulfate, 0.4 mL of tween 20, and 1 mL of distilled water. The resultant nanoemulsion was mixed with the prepared chitosan solution followed by freeze drying for 72 h to produce the chitosan nanoemulsion-based vitamin E powder (CH-NE-vitE).

Characterization of chitosan nanoemulsion-based vitamin E

Scanning electron microscope (SEM)

The morphology of the synthesized CH-NE-vitE powder was characterized using the SEM which is capable of viewing morphology and microstructure of material by exposing it to electron beam (Abdullah and Mohammed, 2019). Both of chitosan and CH-NE-vitE were placed on stubs by the stereomicroscope to be covered with gold molecules which helps in emitting the secondary electrons that have been hit by the primary electron beam.

Fourier transform infrared spectroscopy (FTIR)

FTIR is used to determine the functional groups by obtaining the infrared spectrum of emissions, and absorption for different physical states of substance (Sharma et al., 2018). The CH-NE-vitE powder was set directly above the attenuated total reflectance crystal and closed properly. Then, the spectrum was analyzed as described elsewhere (Nandiyanto et al., 2019).

Nuclear Magnetic Resonance spectroscopy (NMR)

NMR spectroscopy is an analytical technique for observing the molecular structure, content and purity of a sample. It depends on the reactions between material and electromagnetic radiation to determine the local magnetic fields around the atomic nuclei of the individual material (Pilgrim, 2016). CH-NE-vitE was placed in a probe to be exposed into a magnetic field that was emitted by superconducting magnet. Then, the electric current was created strongly in the probe coils, which resulted in a second oscillating magnetic field. Therefore, the macroscopic magnetization rotated about 90° into the horizontal plane. Then, the net macroscopic magnetization returned to the vertical plane by processing around the magnetic field after excitation, resulting again with the current in the probe coil, but weaker than the first one. This is known as Free Induction Decay phenomenon. NMR Spectrum was analyzed as described elsewhere (Singh and Singh, 2022).

Differential Scanning Calorimetry (DSC)

DSC is a mechanism for measuring thermodynamics of liquid or solid phase transitions that can be either endothermic or exothermic (Biliaderis, 1983). The samples CH-NE-vitE and chitosan were weighed by microbalance after they were poured in the crucibles. Then, the covers of the crucibles were fixed by pressing with crucible sealing press. Next, both sample and standard were put inside the DSC, which was connected to the computer to specify combustion rate, initial and final temperatures.

Antimicrobial activity of chitosan nanoemulsion-based vitamin E

Preparation of the media

The media for bacteria were prepared by dissolving 3.9 g of Mueller Hinton Agar (MHA) in 100 mL of distilled water depending on the ratio which was mentioned in the bottle Muller Hinton powder. The media for fungi were prepared by dissolving the same amount as above for bacteria (3.9 g MHA + 100 mL distilled water), but 5 µg methylene blue powder was added too. Then, both media bottles were sterilized by autoclave at 121°C before it was poured in petri dishes placed in the biosafety cabinet. After pouring the agar, the petri dishes were locked by the paraffin.

Susceptibility tests using the inhibition zone

There are two common methods for testing susceptibility by inhibition zone: Agar well diffusion and agar disk diffusion methods. The main difference between them is the way of how the antibiotics are applied into the agar (King et al., 2008). In particular, the agar well diffusion method employ creating a hole in the agar by sterile cork borer and then filling it with the antimicrobial agent, while agar disk diffusion method utilizes sterilized filter paper disk to be placed above the agar and then directly filling it with the antibiotic using micropipette. After preparation of petri dishes, culture plates of *Staphylococcus aureus* (gram positive), *Enterobacter hormaechei* (gram negative), and *Candida krusei* (yeast) were inoculated using sterilized loops which were emerged in saline that was compared to a turbidity standard (0.5). Then, sterilized swab was used for picking up colonies of desired microorganisms, which were then streaked in the agar plates. The conducted experiments were performed five times through using the agar disk diffusion method for three times and the mixed methods of both agar disc and well diffusion methods for two times.

The mixed agar well and disc diffusion methods were utilized five times. The petri dishes were divided into 6 parts at which each sample was tested using both disk and diffusion methods. The first sample was prepared by mixing 0.01 g of pure chitosan powder with 1 mL of 0.5 % acetic acid which was labeled as pure while the second sample was made through diluting the first sample by half (diluted ½

pure chitosan + 0.5 % acetic acid). The third sample was prepared by dissolving 0.01 g of CH-NE-vitE in 1 mL of 0.5 % acetic acid. All these samples were mixed and stored in vials.

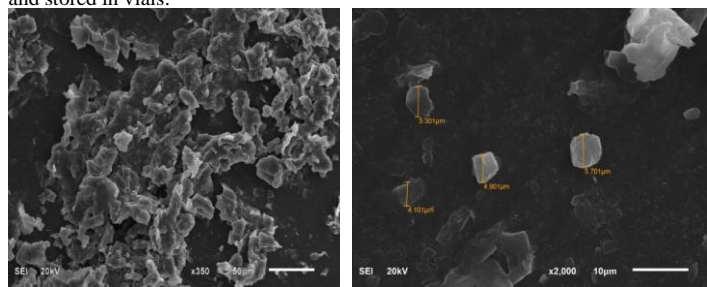


Figure 2 Scanning electron microscope images of chitosan with vitamin E nanoemulsion (CH-NE-vitE).

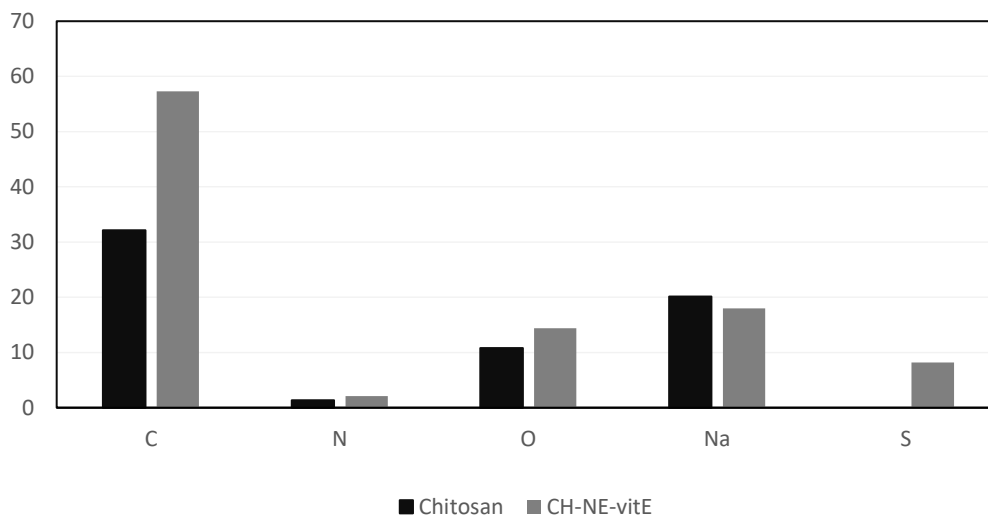


Figure 3 Comparison of energy dispersive X-ray spectroscopy (EDS) analysis of the SEM images between chitosan and CH-NE-vitE.

Structural change of microorganism under SEM

A 5 μL of both *S. aureus* and *C. krusei* were added into 0.5 mL normal saline (0.8 % NaCl). Then, 50 μL of CH-NE-vitE at 2 concentrations (MIC, and its previous dilution) were added. After mixing the antibiotics with the tested microorganisms, the tubes were incubated for 24 h. Next, they were run for SEM dehydration and coating.

RESULTS AND DISCUSSION

Characterization of chitosan

Scanning Electron Microscope (SEM)

The pure chitosan morphology was very different from CH-NE-vitE one. The SEM image of pure chitosan displayed a network made of smooth thin fibers, wide pores, and non-uniform surface (Figure 1). The average size of the pores is $(11.29 \pm 3.90) \mu\text{m}$, and the coefficient of variation is 34.38 % implying random distribution of the pore sizes. In contrast, the SEM image of CH-NE-vitE exhibited thick layers, spherical particles, non-uniform surface, and sharp edges (Figure 2). The average droplet size of the sphere is $(5.001 \pm 0.68) \mu\text{m}$. The coefficient of variation percentage is 13.66 % indicating a homogenous distribution. Accordingly, the addition of vitamin E nanoemulsion to chitosan reduced the pore sizes, increased the continuity, and thickened the form which was similar to what was observed in the SEM images of the pyridylurea-chitosan derivatives synthesized by Zhang et al. (2019) and SEM images of chitosan-s-triazinyl-bis(2-aminomethylpyridine) and chitosan-s-triazinyl-bis(8-oxyquinoline) derivatives synthesized by Al-Rasheed et al. (2020). The Energy Dispersive Spectroscopy analysis of the SEM images revealed that the CH-NE-vitE has increased elements including C, N, O, and S, except Na element has relatively decreased when compared to pure chitosan (Figure 3). The remarkable increase in the carbon relative to the other elements may stem from the addition of vitamin E, which contains 29 carbon atoms. The new appearance of the sulfur in CH-NE-vitE is attributed to the presence of sodium lauryl sulfate in the nanoemulsion.

Determination of the minimum inhibitory concentration (MIC)

The determination of the MIC was implemented by serial dilution for the stock solution of 0.2 g/mL of CH-NE-vitE dissolved in 0.5 % acetic acid. Notifying that the chosen concentration was based on the well-known density of the chitosan (0.20 -0.4 g/m) (Ramawat and Mérellon, 2015). In particular, the first tube (1/2) contained 500 μL of the stock solution diluted in 500 μL of 0.5 % acetic acid followed by micro pipetting 500 μL of the sample from the first tube (1/2) to be added to the second tube (1/4) that was diluted with 500 μL of 0.5 % acetic acid and so forth until a dilution factor of 1/2048 in the 11th tube is obtained.

All tested microorganisms were cultured in nutrient agar, each one in two petri dishes to test all the 11 dilutions using agar well diffusion method, and the 12th place was for the standard antibiotic. The standard antibiotic filter paper selected according to the used microorganism: gentamicin (10 μg) for *E. hormaechei* (gram negative), ciprofloxacin (10 μg) for *S. aureus* (gram positive), and amphotericin B (3.2 mg/mL) for *C. krusei* (yeast). After leaving *S. aureus* and *E. hormaechei* for 24h, and *C. krusei* for 48h, the inhibition zones were measured and the MIC was calculated by multiplying the last inhibitory dilution by the chitosan density.

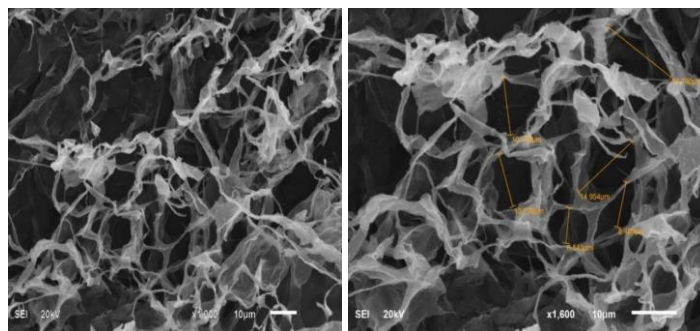


Figure 1 Scanning electron microscopic images of pure chitosan dissolved in 2.5% glacial acetic acid and 1M NaOH.

Fourier transform infrared Spectroscopy (FTIR)

FTIR measurements were carried out to recognize the functional groups within CH-NE-vitE. The FTIR spectrum of CH-NE-vitE, shown in Figure 4, exhibited the peak IR band observed at 3455.64 cm^{-1} as a characteristic of the O-H bonds stretching modes for the O-H groups possibly of chitosan, vitamin E, and tween 20. The peaks at 2917.09 , 2850.78 , and 1467.27 cm^{-1} represent the vibrations of C-H bonds included in chitosan, vitamin E and tween 20. The band at 1734.32 cm^{-1} refers to C=O bonds from tween 20 with the transmittance more than 90%. The strong peaks at 1248.46 , 1218.73 and 1083.18 cm^{-1} correspond to C-N bonds that almost belong to chitosan. Another peak that might be resulted from combinations of the components is 1560.74 cm^{-1} for N-H. According to Queiroz et al. (2015), the FTIR spectrum of the pure chitosan revealed high amounts of C-N and N-H bonds which stem from the presence of amine group (NH_2). In addition, C-C and C-H are common bonds in the chitosan structure while the O-H can be the resultant of hydroxyl group.

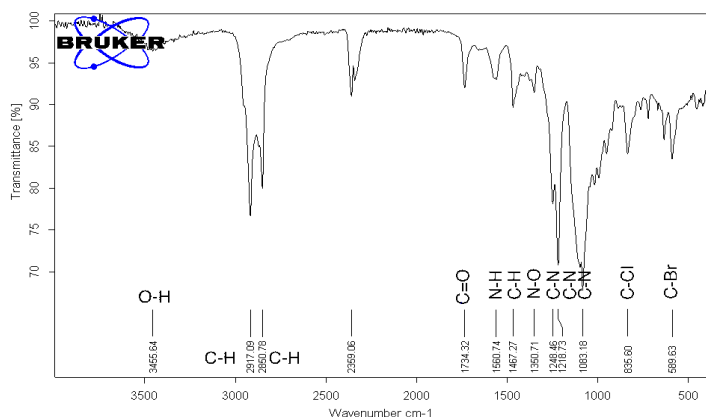


Figure 4 FTIR spectrum of CH-NE-vitE

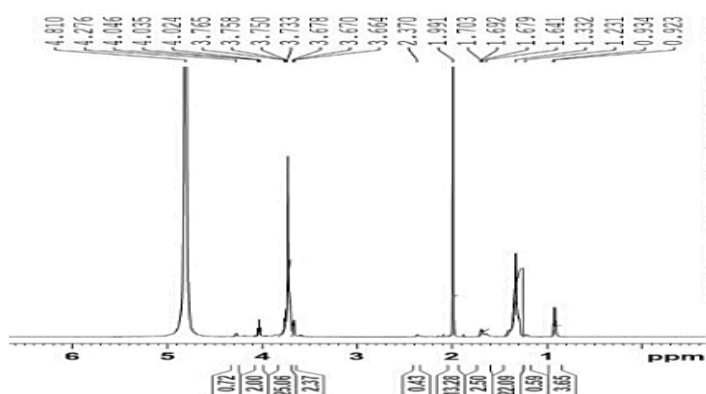


Figure 5 CH-NE-vitE NMR spectrum Nuclear Magnetic Resonance (NMR) Spectroscopy

The NMR spectrum revealed the formation of new bonds, and interactions between the components of the mixture (CH-NE-vitE) due to chemical shifting. By comparing the NMR spectrum of pure chitosan (Facchinatto et al., 2020), with the one of CH-NE-vitE (Figure 5), chemical shifting has occurred toward up field. Also, the increasing in the peaks heights indicates more hydrogen bonds formation. Bonds can be formed because of interaction between OH from tween20, sodium lauryl sulfate, or NH₂ of chitosan.

Differential Scanning Calorimetry (DSC) Spectroscopy

Pure chitosan shows endothermic reaction due to the structure of polymers that takes less heat to disintegrate as described by Ramasamy et al., (2014). In contrast, mixing chitosan with glacial acetic acid and NaOH changed the heat flow, so the endothermic reaction takes higher temperature and three different transitions (Figure 6). In contrast, CH-NE-vitE did not go through endothermic reactions only, but it goes through exothermic reactions which can be due to crystallization, cross-linking, and oxidation (Figure 7).

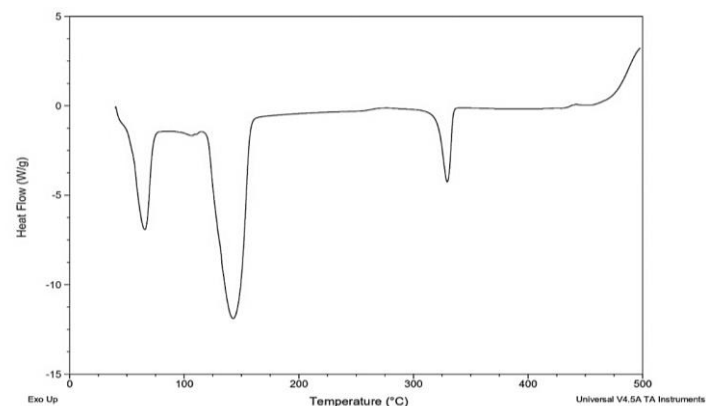


Figure 6 DSC spectrum of chitosan dissolved in 2.5% glacial acetic acid and 1M NaOH.

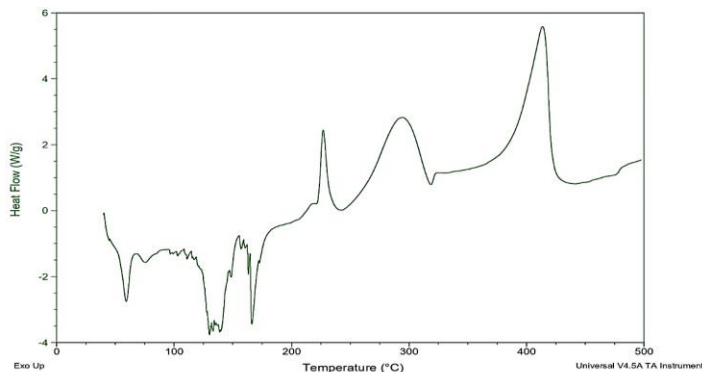


Figure 7 DSC spectrum of CH-NE-vitE.

Antimicrobial activity of chitosan and CH-NE-vitE

Susceptibility test of chitosan and CH-NE-vitE

The findings of the susceptibility test support the results of many research that chitosan can work as antimicrobial (Younes & Rinaudo, 2015). Even though, the first three runs did not show any inhibition zone except with the standard antibiotic (Figures 6 - 8). This can be due to the low concentration of chitosan, or other experimental mistakes. As shown in Figure 8, CH-NE-vitE presented more growth inhibition for both *S. aureus* and *C. krusei* by well diffusion method relative to the disk method whereas pure chitosan could not form any inhibition zone. The measured inhibition zones of CH-NE-vitE applied into *S. aureus* were 22 ± 1.5 mm and 10 ± 1.3 mm using well and disc diffusion methods, respectively. In contrast, the measured inhibition zones of CH-NE-vitE subjected to *C. krusei* were 17 ± 1.4 mm and 2 ± 0.65 mm using well and disc diffusion methods, respectively. The presence of lipopolysaccharide (LPS) increases the negativity of the gram-negative bacteria, which help it bind easily to the cationic chitosan on acidic conditions. Even though, many other experiments show that gram-negative are less susceptible to chitosan than gram-positive due to the presence of teichoic acid. Clearly, phosphate groups within teichoic acid increase the negativity of gram-positive. In fact, the antibacterial activity of chitosan cannot be explained by depending on the electrostatic reactions only, because it can be affected by many other factors including acidic medium, deacetylation degree, and solubility (Yan et al., 2021).

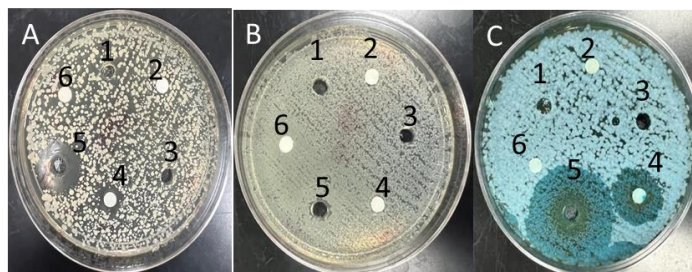


Figure 8 The antimicrobial activity against A: *S. aureus*, B: *E. hormaechei*, C: *C. Krusei*. The mixed agar well and disc diffusion methods were utilized five times. The petri dishes were divided into 6 parts at which each sample was tested using both disk and diffusion methods. The first sample (1, 2) was prepared by mixing 0.01 g of pure chitosan powder with 1 mL of 0.5 % acetic acid which was labeled as pure while the second sample (3, 4) was made through diluting the first sample by half (diluted ½ pure chitosan + 0.5 % acetic acid). The third sample (5, 6) was prepared by dissolving 0.01 g of CH-NE-vitE in 1 mL of 0.5 % acetic acid. All these samples were mixed and stored in vials.

Determination of the minimum inhibitory concentration (MIC)

The MIC determinations of CH-NE-vitE were more effective in *S. aureus* with 12 mm inhibition zone at 1/256 dilution (Figure 9). Therefore, the MIC was 1.563 mg/mL resulted from the multiplication of 1/256 by 0.4 g/mL. In contrast, the MIC for *C. krusei* was 3.125 mg/mL resulted from the multiplication of 1/128 by 0.4 g/mL (Figure 10). The relative lower values of MIC for CH-NE-vitE imply a great antimicrobial activity. A recent study reported that the combination of the essential oil nanoemulsion with the cross-linked chitosan derivative hydrogel displayed larger MIC (15 mg/mL) (Cai et al., 2023).

SEM characterization of the microorganism's morphology

The untreated *S. aureus* have rough spherical shapes that cluster smoothly like grapes with smooth surface (Licitra, 2013). In contrast, the untreated *C. Krusei* has elongated rod-like shape (Essayag et al., 1996). In the current study, as the

concentration of the antibiotic increased, the number of microorganisms has decreased for both *S. aureus* (Figures 11, 12), and *C. Krusei* (Figures 13, 14) and more intracellular spaces were displayed. The shape of the treated *S. aureus* did not change while the shape of *C. Krusei* was altered to exhibit more elongated and disrupted cells.



Figure 9 Measurement of MIC for CH-NE-vitE against *S. aureus*. The designated numbers 1 – 11 represent the serial dilutions from 1/2, 1/4, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048, alongside the 10 µg of the standard antibiotic ciprofloxacin (12).



Figure 10 Finding the MIC of CH-NE-vitE against *C. krusei*. The designated numbers 1 – 11 represent the serial dilutions from 1/2, 1/4, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048, alongside the 3.2 mg/mL of the standard antibiotic amphotericin B (12).

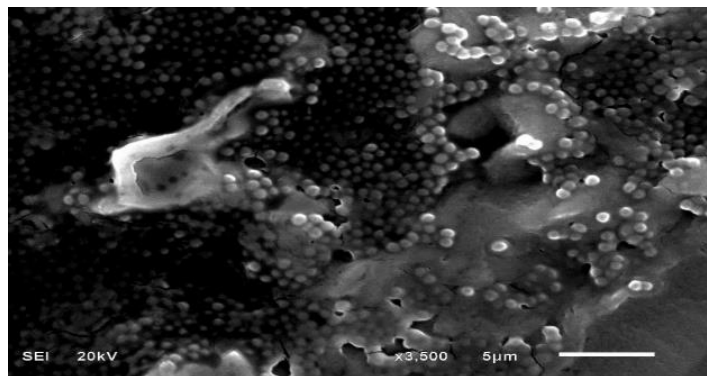


Figure 11 SEM images of *S. aureus* treated with 3.125 mg/mL CH-NE-vitE.

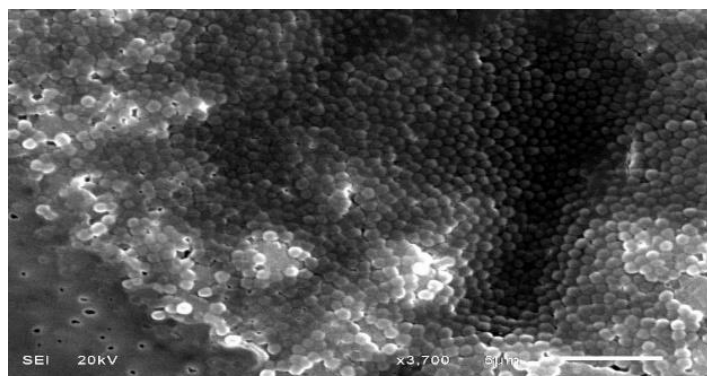


Figure 12 SEM images of *S. aureus* treated with 1.563 mg/mL CH-NE-vitE.

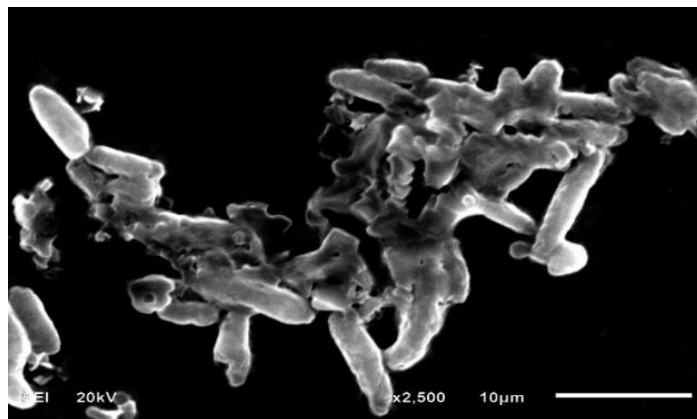


Figure 13 SEM images of *C. Krusei* treated with 6.25 mg/mL CH-NE-vitE.

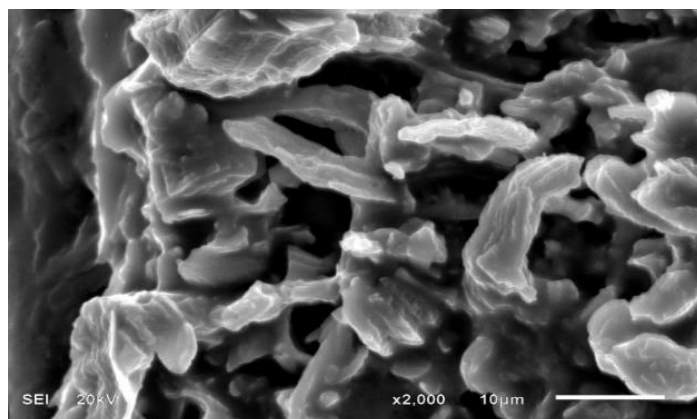


Figure 14 SEM images of *C. Krusei* treated with 3.125 mg/mL CH-NE-vitE.

CONCLUSION

We have developed the pure chitosan properties by the addition of vitamin E nanoemulsion, tween20, and sodium lauryl sulfate. It displayed antimicrobial activity against *S. aureus* and *C. krusei*, while promoting the growth of *E. hormaechei*. Vitamin E nanoemulsion increases the susceptibility and permeability of chitosan. According to the results of the current study, CH-NE-vitE has obvious antimicrobial actions, but these actions need to be tested for clinical and dermal studies that should be considered in future research.

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