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Enhancing the antibacterial activity of thyme Essential oil emulsion through green decoration of β-Cyclodextrin with AgNPs for Hygienic cotton Fabric

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Abstract

Producing antibacterial textile using β cyclodextrin were successfully prepared, β cyclodextrin consider as a one agent emulsifier for thyme oil and reducing agent for silver nanoparticles, Produced emulsion and Silver nanoparticles (AgNPs) were examined and characterized. The obtained results confirmed the synthesis of thyme emulsion oil as microspheres has been appeared between 5-8 µm using 1:4 (emulsifier: β cyclodextrin) under optical microscope. While, producing spherical AgNPs using β-cyclodextrin with particle size 5-15 nm which analytically confirmed by showing characteristic peaks belongs to AgNPs. Incorporation binary thyme oil emulsions and AgNPs previously prepared by β cyclodextrin, showed deposition of emulsion appeared on the surface of fabrics as a coated film compared with blank cotton fabric, the pretreated samples were characterized using Polar microspore, Transmission Electron Microscope (TEM) and X-ray diffraction (XRD). Physical properties of Treated cotton fabrics properties as morphology were examined using Scanning Electron Microscopy/Energy Dispersive Spectroscopy (SEM-EDS). Besides antimicrobial activity were also examined against most popular infectious bacteria (E-coli).under SEM, while EDX indicated the prepsence of AgNPs in sample treated with thyme emulsion/ β-CD/AgNPs which proves the successful inclusion of AgNPs. Treated fabric using emulsion/ β-CD/AgNPs showed high antibacterial activity towards E. Coli and S. aureus that candidate produced fabrics treated thyme emulsion/ β-CD/AgNPs for antibacterial and medical applications.

Keywords: Antibacterial textile; β cyclodextrin; thyme emulsion; Medical finishing; Smart fibers.

1. Introduction

Antibacterial emulsion oil is a type of oil-based product that contains antibacterial properties [1-3]. It is typically made by mixing essential oils or components with a carrier oil or emulsifier to create a stable blend that can be applied topically or used in diffusers. The antibacterial properties in these oils are believed to come from the presence of compounds such as terpenes, phenolic, and ketones [4]. They are often used in natural products to help fight the growth of bacteria, viruses, and fungi that can cause infections and other health problems [5]. In general, most of essential oils had owned antimicrobial activity against a wide range of bacteria and fungi. Thyme oil has been traditionally used for its antimicrobial activity due to the presence of thymol, carvacrol, p-cymene, γ-terpinene, and linalool [6].
Thyme oil is formulated in an emulsion, it increases the surface area and facilitates the dispersion of oil droplets in the aqueous phase, leading to better accessibility and activity of the oil components[7]. Studies have shown that thyme oil emulsion has a broad spectrum of antibacterial activity against Gram-positive bacteria [8]. Thyme oil emulsion has also been found to have antifungal activity against Candida albicans and Aspergillus niger. The antibacterial activity of thyme oil emulsion is attributed to the disruption of bacterial cell membranes and inhibition of enzyme activity [9]. Thymol and carvacrol have been shown to disrupt the cell membrane of bacteria, leading to leakage of intracellular contents and ultimately cell death [10]. Additionally, thyme oil emulsion has been found to inhibit bacterial enzymes involved in important metabolic pathways, further contributing to its antimicrobial activity. Overall, thyme oil emulsion has strong antibacterial and antifungal activity, making it a promising natural alternative to traditional antimicrobial agents. It is important to note, however, that thyme oil may cause irritation or

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[11]. Unlike Gram +ve bacteria, Gram -ve bacteria have a significant resistance to thyme oil [12].

To overcome this tackle and to enhance the antibacterial activity of thyme oil emulsion, this study offered a novel way to stabilize thyme oil emulsion using biopolymer decorated nanoparticles in particular, cyclodextrin decorated silver nanoparticles. Cyclodextrins are cyclic oligosaccharides containing glucose units linked by alpha-1,4 glycosidic bonds [13]. They are commonly used as encapsulating agents for hydrophobic compounds in various pharmaceutical, cosmetic, and food applications. Cyclodextrins can form inclusion complexes with hydrophobic compounds, increasing their solubility, stability, bioavailability, and sensory attributes [14].

In emulsion preparation, cyclodextrins can be used as emulsifying agents or as encapsulating agents for oils and other hydrophobic ingredients [15]. Cyclodextrins can form inclusion complexes with oils and other hydrophobic compounds, reducing their volatility and improving their stability in emulsions [16]. Cyclodextrins can also enhance the emulsifying properties of other emulsifiers, improving the stability and texture of the emulsion. Examples of cyclodextrins used in emulsion preparation include beta-cyclodextrin, hydroxypropyl-beta-cyclodextrin, and randomly methylated-beta-cyclodextrin [17]. Cyclodextrin is a type of cyclic oligosaccharide that is commonly used as a stabilizing agent in the preparation of silver nanoparticles [11]. Silver nanoparticles are effective antimicrobial agents due to their ability to release ions that can interact with bacterial cell walls and disrupt their function. When preparing silver nanoparticles, cyclodextrin can be used as a stabilizing agent to prevent the aggregation of the nanoparticles and control their size and shape. Cyclodextrin forms inclusion complexes with the silver ions, creating a stable environment for the formation of the nanoparticles. One study demonstrated the use of beta-cyclodextrin as a stabilizing agent for the preparation of silver nanoparticles[18]. The silver nanoparticles also exhibited strong antimicrobial activity against Gram-negative and Gram-positive bacteria, likely due to the controlled size and morphology of the nanoparticles [19, 20].

Antibacterial cotton fabrics can be produced using emulsions and silver nanoparticles [21]. Emulsions can be employed to disperse silver nanoparticles homogeneously in cotton fabric, increasing the surface area for contact with bacteria and providing a more uniform distribution of antimicrobial activity. To produce antibacterial cotton fabrics, silver nanoparticles can be added to an emulsion containing a surfactant and applied to the cotton fibers using a variety of techniques, such as padding or spraying [22-24].

Overall, the use of emulsions and silver nanoparticles can be an effective and eco-friendly approach for producing antibacterial cotton fabrics that can be used in various applications, including medical textiles, sportswear, and home furnishings. From this point of view there are no reports indicating using cyclodextrin as dual action for emulsifier for thyme oil and reducing agent to AgNPs. The current work also reporting the producing antibacterial fabric using binary mixture of cyclodextrin/thyme oil emulsion/AgNPs. Characterizations of emulsion and AgNPs produced using cyclodextrin were examined using Polar microspore, TEM and XRD. Physical properties of treated cotton fabrics properties as morphology were examined using SEM-EDS. Besides, the antimicrobial activity was also examined against most popular infectious bacteria Gram +ve and Gram -ve bacteria.

2. Experimental

Materials

β-Cyclodextrin (β-CD) and $AgNO_3$ were purchased from Sigma Aldrich. Sodium hydroxide, Sodium Chloride, and double distilled water were of laboratory grade chemicals. Thyme essential oil was provided and used as received from local market (Harraaz Co., Cairo, Egypt), Cotton fabric, supplied by El-Nasr Company for spinning weaving and Dyeing El-Mahallah El-Kubra, Egypt.

Methods

Preparation of AgNPs.

AgNPs were prepared though chemical reduction method using β-CD as reducing and stabilizing agent. In brief, 0.5 β-CD was dissolved in warm 100 ml distilled water and then 0.1 mm of $AgNO_3$ was added and pH was adjusted to 10. Reaction continued under stirring for 30 min. at 60 ^{o}C until color turns to dark yellowish brown [25].

Preparation of thyme O/W emulsion

Thyme oil-in-water emulsion of ratio 30:70 was fabricated and stabilized by the use of β-CD. In 10 ml vial, β-CD was dissolved in warm distilled water containing 0.1% NaCl and then the thyme oil was gently added and mixed using vortex for a while. The latter mixture was sonicated for 20 sec. using with an ultrasonic device with a dipping titanium probe close to the surface. Various series of thyme oil: β-CD were employed to investigate the most stabilized and effective formulation for emulsion preparation. Typically, different β-CD to oil ratios (1:1, 1:2, 1:3 and 1:4) were applied [26].

Preparation of thyme emulsion decorated AgNPs. An equivalent amount of AgNPs solution (based on β-

CD) was used for preparation of thyme O/W emulsion decorated AgNPs [26].

Cotton fabric, separately, in 100mL of thyme emulsion stabilized β-CD with and without decorated AgNPs for 30 min. at ambient temperature. Then, the loaded cotton fabrics were squeezed using a pad-dry-curing machine to finally obtain dried cotton fabrics loaded emulsion. The treated with thyme emulsion Bleached, cleaned and dried cotton samples (30 cm X 30 cm) were immersed applied pressure was adjusted to reach cotton weight uptake up to 100% of the initial weight before the treatment.

Characterizations

Optical microscope

The formed thyme emulsion droplets were monitored on Olympus optical microscopes with digital camera.

Transmission Electron Microscope (TEM)

TEM was used to assess the potential impact of the modification on the elemental and structural properties of the synthesized AgNPs nanoparticles, whether alone or over and within the fabric surface. The TEM analysis was done using JEOL-JEM-1200 (Japan).

X-ray diffraction (XRD)

XRD measurements were made using Philips X'Pert powder diffraction system (Philips Analytical, The Netherlands) equipped with a vertical goniometer in the Bragg-Brentano focusing geometry. The X-ray generator was operated at 40 kV and 50 mA, using the CuKα line at 1.54056 Å as the radiation source.

Scanning electron microscope and energy dispersive x-ray analysis (SEM and EDX).

SEM analysis was done using a scanning electron probe micro analyzer (JXA-840A, Japan). The specimens in the form of films were mounted on the specimen stabs and coated with thin film of gold by the sputtering method. The micrograph was taken at magnification of 1000 using (KV) accelerating voltage. The composition of selected nanoparticles was determined by energy dispersive X-ray (EDX) analysis using INCa (X Sight)

Antimicrobial Activity Test

Test disc diffusion method with some modification was used for screening test of the cotton fabric sample for antimicrobial activity. Nutrient agar for bacteria 0.1 ml of an appropriate dilution of the test culture was used. The treated fabric samples (1-cm diameter) were placed on the surface of the incubated plates at 35°C for 24 hours. Diameter of plates inhibition zone (mm) including the disc diameter was measured for each treatment[27].

3. Results and discussion

Thyme essential oil contains an antioxidant group known as thymol that enhances the immune system. Emulsification of such oils in aquoues media supports their medical applications for warding off bacteria and viruses. However, the statbility of O/W emulsion has been recently gained much more anxious attention. Herein, stabilization of Oil-in-Water based biopolymers for Pickering emulsion fabrication was achieved through utilizing of β-CD. The mechanism of O/W stabilization includes a complexation of aquoueos β-CD molecules with oil, resulting in a structure resembling where the active β-CD corresponds to the hydrophilic head whilest the lipophilic cavities of β-CD, in turn, acts as hydrophobic tail that can missible with oil phase.

The droplets of the as-formed O/W thyme emulsion stabilized β-CD can be visualized by an optical microscopy as represented in figure 1. It can be depicted from figure 1 that, the well stabilized emulsion droplets with uniform smaller average size lied around 1-2 µm were formed succssfully at different thyme oil concentrations indicating the efficacy of β-CD to stabilize the oil droplets even at higher ratio upto β-CD: oil, 1:4. However, some droplets with a bigger size were observed at range of 5-8 µm. This results reflect the effect of β-CD as a biosurfactant for stibelizing the O/W thyme emulsion through decreasing the water oil interface tension[27].

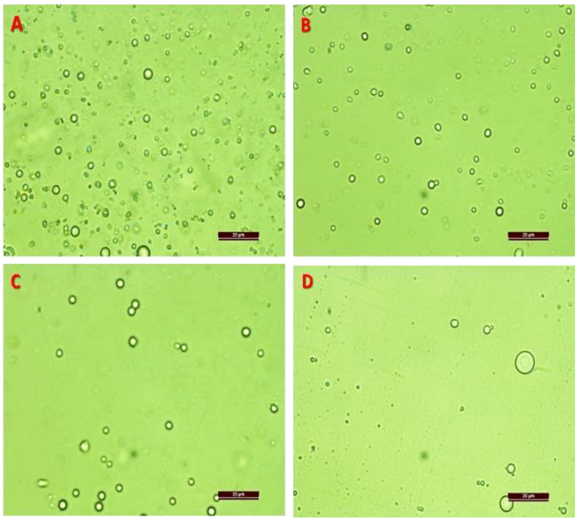

Figure 1: Optical microscopic image of O/W thyme emulsion stabilized β-CD at different β-CD : thyme oil ratios 1:1 (A), 1:2 (B), 1:3 (C) and 1:4 (D).

Preparation of AgNPs using β-CD

AgNPs as a unique precursor are usually used for their textural features especially antimicrobial activity [27,28]. Because the demand to develop a new emulsion with abroad antibacterial activity comprising Gram +ve and Gram -ve pathogens has recently become a necessity. In this regard, AgNPs were chemically prepared using β-CD as a reductant and stabilizing agent by the virtue of free alcholic OH groups that allocated at the backbone of β-CD at C6. TEM and XRD results (shown in figure 2) revealed the successful preparation of spherical AgNPs with smaller diameter size average ranged at 5 – 15 nm. The dispersion of AgNPs exhibited homogenous with poly dispersion affirming the powerful ability of β-CD to reduce and stabilize Ag^0 in small clusters with uniform shape and narrow size.

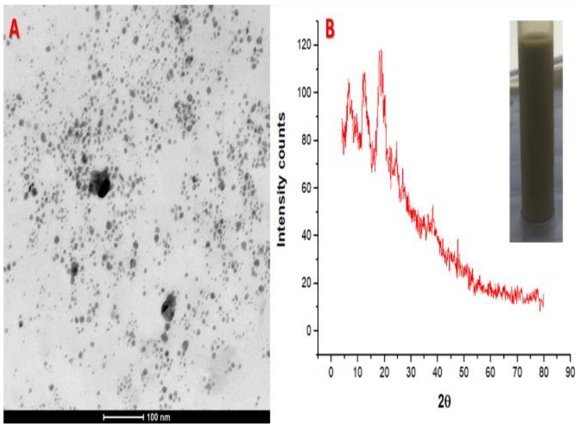

Figure 2: TEM (A) and XRD (B) of AgNPs prepared using β-CD (photo inside graph is the colloidal solution of AgNPs).

Apart from the diffraction peaks of crystal phase of β-CD which existed from 5^o-20^o , the crystallographic feature of AgNPs exhibited new diffractions peaks at 38.4^o, 46.7^o and 65.4^o that can be assigned to [111], [200] and [220] crystalline planes of Ag [20, 28]. These aforementioned findings emphasized the successful preparation of AgNPs by the dint of dual role of β-CD in alkaline medium. Preparation of O/W thyme emulsion decorated AgNPs.

The as-formed β-CD/AgNPs was used for stabilizing thyme oil using the same recipe instead of β-CD. The optical microscopic and photographic image of the prepared thyme O/W emulsion was shown in figure 3 as well as their stability for 7 days.

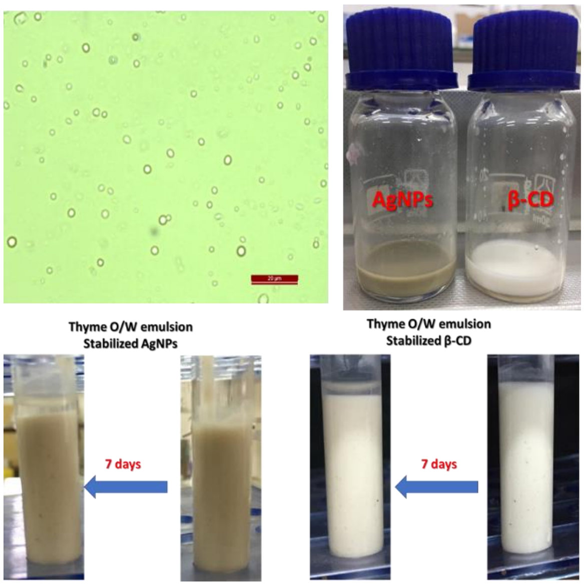

Figure 3: Optical microscopic image for thyme O/W emulsion stabilized AgNPs (left upper image), photographic image for thyme O/W emulsion stabilized β-CD and AgNPs (right upper image) and their stability after 7 days (down image).

Figure 3. represents the successful preparation of thyme O/W emulsion based on stabilizing effect of β-CD /AgNPs. The size and size distribution were the similar to that obtained from β-CD stabilzing emulsion, however, the color of AgNPs appeared in the emulsion appointed to the well distribution as appeared from the optical microscopic image. Both emulsions were storaged for 7 days to invistigate the stability against either coalescence or flocculation. As indicated visually from figure 3, both prepared emulsions are stable for 7 days which indicatives to the stabilizing efficiency of β-CD. Nevertheless, decoration of β-CD by modification with AgNPs increased the stability of emulsion where, the emulsion kept at the same level without down movement by the effect of gravity as observed from β-CD stabilized thyme O/W emulsion.

Cotton treated thyme emulsion stabilized β-CD/AgNPs.

Cotton fabrics were immersed separately in emulsion stabilized β-CD and β-CD/AgNPs and their surface morphologies were scanned by the use of SEM as clearly appeared from figure 4. As clearly indicated from figure 4, the deposition of emulsion appeared on the surface of fabrics as a coated film compared with blank cotton fabric. However, treatment with thyme emulsion/ β-CD/AgNPs exhibited a dense deposition which may related to their small and uniform droplet emlsion size.

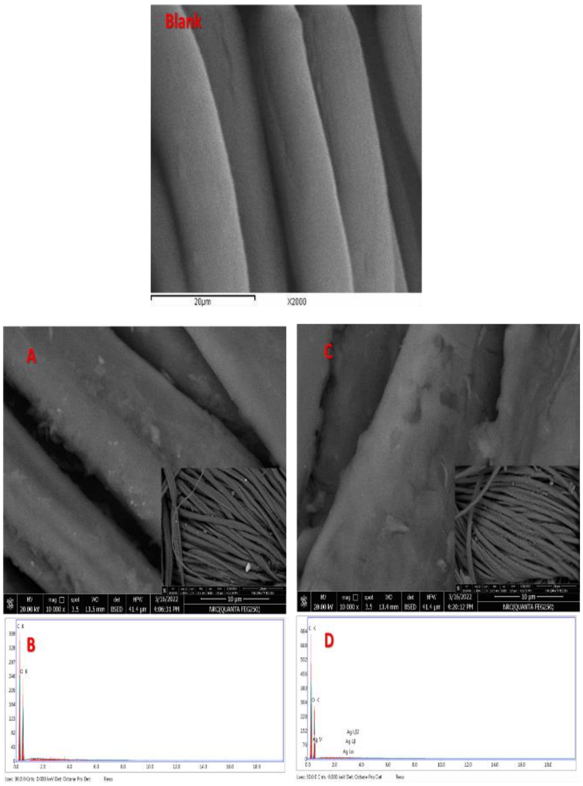

Figure 4: SEM and EDX for cotton fabrics treated thyme emulsion stabilized β-CD (A & B) and β-CD/AgNPs, (C & D).

Moreover, the EDX indicated the prepsence of AgNPs in sample treated with thyme emulsion/ β-CD/AgNPs which proves the successful inclusion of AgNPs in emulsion as well as in the treated cotton fabrics with 0.15 weight%.

Antibacterial activity of cotton fabric treated thyme emulsion.

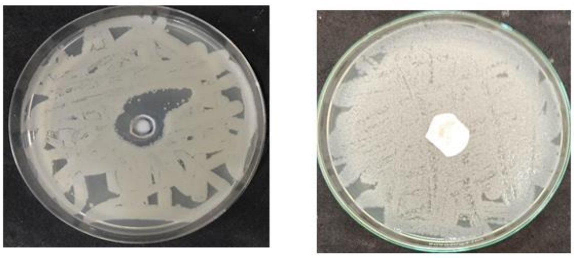

Figure 5: Antibacterial activity of cotton fabrics treated thyme emulsion stabilized β-CD against; Gram +ve Staphylococcus aureus (right picture) and Gram -ve Escherichia Coli (left picture).

Antibacterial activity of cotton fabrics treated thyme emulsion stabilized β-CD has been examined against two pathogenic bacteria E. coli and S. aureus, where the results revealed the activity against S. aureus with clear zone 22.0 mm as obviously appointed in figure 5. Therefore, the potential antibacterial activity of thyme emulsion was limited. On the other hand, the activity of cotton fabrics treated thyme emulsion stabilized β-CD/AgNPs showed high antibacterial activity towards E. Coli and S. aureus as seen in figure 6.

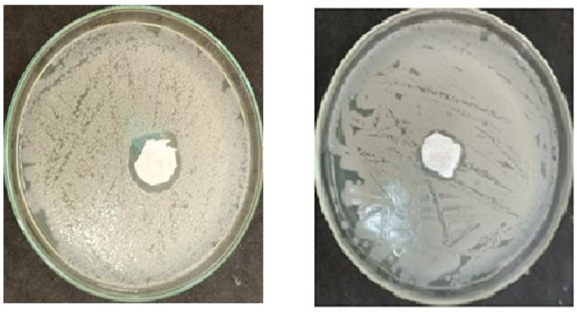

Figure 6: Antibacterial activity of cotton fabrics treated thyme emulsion stabilized β-CD/AgNPs against; Gram +ve Staphylococcus aureus (right picture) and Gram -ve Escherichia Coli (left picture).

4. Conclusion

Antibacterial cotton textiles producing using thyme oil emulsion/ β-CD/AgNPs. Produced emulsion and AgNPs characterized using Polar microspore, TEM and XRD. While physical properties of cotton treated emulsion/ β-CD/AgNPs were characterized using SEM-EDS. thyme emulsion oil as microsphere between 5-8 µm using 1:4 (emulsifier: β cyclodextrin), while TEM confirmed producing spherical AgNPs using β cyclodextrin with particle size 5-15 nm. Treated fabric incorporated emulsion appeared on the surface of fabrics as a coated film compared with blank cotton fabric. Treated fabric using emulsion/ β-CD/AgNPs showed high antibacterial activity towards E. Coli and S. aureus that candidate produced fabrics treated thyme emulsion/ β-CD/AgNPs for antibacterial and medical applications.

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