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Synthesis, characterization and antimicrobial evaluation of two aromatic chitosan Schiff base derivatives

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ABSTRACT

Recently, there have been significant scientific interests to scientists for the chemical modifications of chitosan to increase its applications. The main objective of this study was to prepare two aromatic chitosan Schiff bases (I and II) via coupling with 4-chloro benzaldehyde and benzophenone respectively for improvement the antimicrobial property of chitosan. The chemical structures of the prepared Schiff bases verified through FT-IR, TGA and DSC. However, degrees of substitution were estimated using potentiometric analysis, and they were 7.9% and 4.17% for Schiff bases (I and II) respectively. Antimicrobial activities evaluation were conducted against three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* sp.), two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and *Candida albicans* strain. The antimicrobial activities of the new derivatives increased significantly more than chitosan in most microorganisms. The minimum inhibitory concentration (MIC) of Schiff base (I) with concentration (50 μg/ml) exhibited the highest activity against *C. Albicans* with growth inhibition up to 27.42%. While, 50 μg/ml of Schiff base (II) showed high activity against *E. coli*, *Salmonella* sp., *S. aeureus* and *B. cereus* more than chitosan. The results clearly suggested that the new Schiff bases could be applied as antimicrobial wound dressing agents to ameliorate wound healing.

1. Introduction

Chitosan (deacetylated form of chitin) is one of the most common polymers found in nature, with chitin second only to cellulose regarding natural abundance [1,2]. Both chitin and chitosan found in the shells of crustaceans and insects and certain other organisms, including many fungi, algae, and yeast. Chitosan is biopolymers composed of *N*-acetylated glucosamine and glucosamine units linked by β-(1-4)glycosidic bonds. Chitosan has been exhibiting various promising biological activities, including antimicrobial, antitumor, and hemostatic activity, and the acceleration of wound healing [3,4]. The Unique properties of chitosan have driven the researchers to apply it in promising biomedical applications such as tissue engineering, wound dressing, gene and drug delivery, etc. [5–9].

The antimicrobial activity of chiton saccharides is a function of several structural factors such as their degree of deacetylation (free amine groups), and their molecular weight. However, the environmental conditions can also have a significant effect such as the pH presence of some ions such as Ca^{2+}, Mg^{2+} ion [10–12]. It was reported that the antimicrobial activity of chitosan has been linked to the glucosamine amino group so, the antimicrobial activity increase with decreasing pH [13–15]. Indeed, the reported

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antibacterial activities of chitosan saccharides showed a broad range of activity depending on the properties of the used material and the type of assay. The antimicrobial features of chitosan have not standardized, and, therefore, it is difficult to compare results from one study to another [16,17]. Mutations of microorganisms are leading the scientists to increase antimicrobial activity of chitosan via modification of chemical structures. Chitosan has three reactive groups, *i.e.*, primary (C-6) and secondary (C-3) hydroxyl groups on each repeated unit, and the amino groups (C-2) on each deacetylated unit. Therefore, these reactive groups of chitosan are readily subjected to chemical modifications to alter its mechanical and physical properties. Also, the coupling of free amine groups on chitosan with the carbonyl group in aldehydes and ketones was very easy and common reaction to produce Schiff bases along polymer backbone (—RC═N—). Several chitosan Schiff bases have been prepared and published as chelating agents, antimicrobial and antioxidant materials, etc. [18–22].

Although chitosan was established in the various literatures as antimicrobial biopolymer, the continuous mutations of bacteria to resist and minimize action of antibiotics have driven scientists to develop and improve antimicrobial materials. This study concerned with synthesis and characterization of new derivatives of chitosan Schiff bases. Moreover, the antimicrobial activities of the obtained materials were evaluated to be used in the biomedical applications.

2. Materials and methods

2.1. Materials

Shrimp shells collected from wastes of seafood restaurants in Alexandria. 4-Chlorobenzaldehyde (purity 97% M.W., 140.57) obtained from Sigma-Aldrich (Germany). Benzophenone (purity 99 M.W., 182.22) obtained from Sigma-Aldrich (Germany), acetic acid (purity 99.8%), hydrochloric acid (purity 37%) and sodium hydroxide pellets (purity 99–100%) were purchased from Sigma-Aldrich (Germany). Sulfuric acid (Purity 98%, M.W., 96) purchased from Sigma-Aldrich (Germany) and Ethanol (Purity 99.9%, M.W.46.07) from International Co. for Supp. & Med. Industries (Egypt).

2.2. Microorganisms

Five bacterial strains and one eukaryote strain were used for evaluating the antimicrobial activities of chitosan and its derivatives. The investigated microorganisms included three Gram-negative bacteria (*E. coli*, *P. aeruginosa* and *Salmonella* sp.) and two Gram-positive bacteria (*S. aureus* and *B. cereus*) as well as, *C. albicans* strain. The strains were refreshed through inoculating in LB broth (peptone 1%, yeast extract 0.5%, NaCl 1%, and pH 7 ± 0.2), and incubated overnight at 37 °C and 150 rpm in a rotary shaker.

2.3. Methods

2.3.1. Extraction of chitin from shrimp shells

According to the published procedure [23], the demineralization of shells was the main process for chitin preparation. In this step, the shells were dispersed in 5% (w/v) HCl at room temperature in the ratio of 1:14 (w/v) overnight. After 24 h, the shells were quite squishy and rinsed using water to remove acid and calcium chloride. The demineralized shells were treated with 5% (w/v) NaOH at room temperature for 24 h in the ratio of 12:1 (v/w). The residues were collected and washed to neutrality many times in running tap water and then; distilled water to obtain pure chitin.

2.3.2. Preparation of chitosan from chitin

Preparation of chitosan is simply deacetylation of chitin in alkaline medium. Removal of acetyl groups from the chitin was achieved using 50% (w/v) NaOH with a solid to solution ratio of 1:50 (w/v) at 100–120 °C for 12 h. The resultant chitosan washed to neutrality with distilled water; Fig. 1 [24].

2.3.3. Chitosan purification

According to the previous method [25], chitosan sample was dissolved in 2% (w/v) acetic acid and was left overnight. Then, the chitosan solution was filtrated using cheesecloth to remove contaminants and undissolved particles. Finally, chitosan was precipitated with 5% (w/v) NaOH, collected and washed with distilled water to remove the excess of alkali.

2.3.4. Preparation of chitosan Schiff base derivatives

According to our previous article [21], 1 g of chitosan was dissolved in 50 ml of 2% (w/v) acetic acid and stirred at room temperature for 6 h. 10 ml of ethanol contains (1.86 mM) of aldehyde or ketone (4-chloro benzaldehyde or benzophenone) were added drop wise to the solution. The mixture was kept under stirring for 6 h at 50 °C. The formation of a deep yellow gel referred to the formation of the chitosan Schiff base. The resulting product was precipitated in a solution of 5% sodium hydroxide. The precipitate was filtered and washed with water and ethanol several times to remove unreacted aldehyde or ketone. The products were filtered and dried in a vacuum oven at 60 °C overnight. The schematic diagram describes the proposed mechanistic pathway for synthesis of Schiff bases (I) and (II) is presented in Fig. 2.

2.3.5. Characterization

2.3.5.1. Fourier transform infrared spectrophotometer (FT-IR) analysis. The structures of the chitosan and chitosan derivatives were investigated by FT-IR spectroscopic analyzes using FT-IR (Model 8400 S, Shimadzu, Japan). Samples (2–10 mg) were mixed thoroughly with KBr. The sample was pressed into pills with a Specac compressor (Specac Inc., Smyrna, USA) and the absorbance of samples scanned from 500 to 4000 cm^{-1}. DD of chitosan material was calculated according to the following equation [26]:

$$NH2\% = 100 \times \left(\frac{1 - \left(\frac{A1655}{A3450} \right)}{1.33} \right)$$

where A is area of the peak.

2.3.5.2. Determination of degree of acetylation using potentiometric titration. 0.1 g of chitosan or chitosan derivatives was dissolved in 20 ml of 0.1 N HCl. The solution was kept under stirring overnight

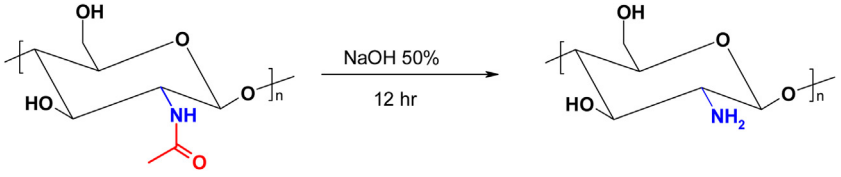

Fig. 1. Preparation of chitosan.