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BIOMEDICAL PROPERTIES AND APPLICATIONS OF CHITOSAN DERIVATIVES

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Abstract. In this era, there is a global concern in the use of bioactive molecules such as chitosan in the field of antimicrobial and antioxidant benefits. Because of its biodegradability, biological compatibility, antimicrobial, antioxidants activity, and high safety, chitosan may be applied to in a large number of applications. It could exist in many forms, such as fibers, gels, films, sponges, nanoparticles, and beads. Additionally, further uses for chitosan and its byproducts, including wound healing products, wastewater treatment, and cosmetics, have also been highlighted. This analysis aligns with our earlier review that presented data collected through 2023 and compiles the most recent results regarding the antibacterial, antifungal, and antioxidant properties of chitosan derivatives.

Keywords: Chitosan, chitosan derivatives, antimicrobial, antibacterial activity, antifungal activity.

Introduction. The interest in chitosan, a biodegradable, non-antigenic, non-toxic, and biocompatible natural polymer derived from chitin, is because of chitosan's several health- beneficial effects including highly antioxidant and antimicrobial activities [1]. In the Scopus database, up to 17,000 citations on this chitosan are found. These high citation numbers reflect a particular concern with the properties and uses of chitosan [2]. Chitosan has various applications in the biomedical industry. Also, plenty of studies have shown that it has a potent antioxidant and antimicrobial activities, so that it's usable either alone or combined with other polymers as an antimicrobial agent [3]. Chitosan is composed of beta1-4 linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit), which randomly distributed inside the polymer. Natural cationic polymers include chitosan, while the majority of polysaccharides either neutral or have an anionic charge. This chitosan property allows for creating multilayer structures or electrostatic complexes with other synthetic polymers or negatively charged natural [4]. Chitosan also has numerous biological activities, for instance, antimicrobial [5–9], antitumor [10], and antioxidant [9,11] proprieties. The degree of decetylation and the molecular weight of chitosan have a major impact on its biological activity. [12]. Chitosan is widely used in several biomedical and biological applications, including drug carriers [13], water treatment [14], as well as a tissue engineering scaffold.[15]. Due to its unique biological activities, chitosan has gained considerable attention in recent decades. The purpose of this review paper is to present current knowledge on the applications of chitosan and its derivatives' natural characteristics in pharmaceutical and medical fields. Additionally, results from recent publications will be used to characterize the biological

activities, antioxidant properties, and antibacterial properties of chitosan and its derivatives.

We hope that this review will provide the researcher with the required guidance based on a large amount of important and up-to-date information gathered in a single manuscript to help improve the good properties of chitosan and increase the range of uses for chitosan.

Chitosan. Every year, there is a growing interest in polymeric materials. One of the most abundant biomaterials, chitosan has numerous uses in industry, agriculture, biomedicine, and pharmaceuticals. Chitosan is a popular issue that is mentioned in a lot of scientific papers. This polysaccharide's appeal is mostly due to its desired qualities, which include hypoallergenic, biodegradable, and nontoxic characteristics. Its appropriate use depends on numerous modifiable factors. The degree of deacetylation (DD), degree of substitution (DS), length and location of a substituent in the glucosamine units of chitosan, pH, and molecular weight (MW) all affect the final biological activity of chitosan.

D-glucosamine and N-acetyl-D-glucosamine units connected by -1,4-glycosidic connections form the linear polysaccharide known as chitosan. It is generated from the naturally occurring polymer chitin. Less than 50% of the N-acetylglucosamine units in its structure are from the alkaline deacetylation of chitin. Four crystalline polymorphs can be formed by long chains of chitosan: three hydrated and one anhydrous[1]. Three reactive groups—a free amino group and main and secondary hydroxyl groups at C6 and C3—are the foundation of chitosan's reactivity. Quaternization, acylation, tosylation, Schiff base production, O-carboxymethylation, N-carboxyalkylation, N-succinylation, and graft copolymerization are among the most common chemical modifications. In both neutral and alkaline pH ranges, original chitosan is insoluble. Free amino groups are protonated and the polysaccharide becomes soluble at pH values less than 7. Its solubility can be increased across a broad pH range with the right chemical changes.

Chitosan derivatives Chemical alteration can be used to enhance the biological and physicochemical properties of chitosan. One of chitosan's primary disadvantages is its poor solubility in water and common organic solvents, which chemical changes can overcome. Moreover, adding other moieties to the polymer chain can enhance chitosan's other characteristics and increase its suitability for usage in medicinal applications. [17].

Chitosan derivatives can be synthesized using a variety of techniques, including chemical grafting, enzymatic processes, and direct modification [19]. It can be difficult to carry out a multistep organic synthesis of complicated compounds successfully. The primary worry is that specific functional groups may obstruct the reaction and produce undesirable products. Protective groups can be used to temporarily hide the functional groups that would otherwise conflict in order to avoid this problem. [22]. It is possible to add and remove protective groups from a molecule without affecting the final result. An excellent protective group needs to fulfill certain conditions, such as reacting.

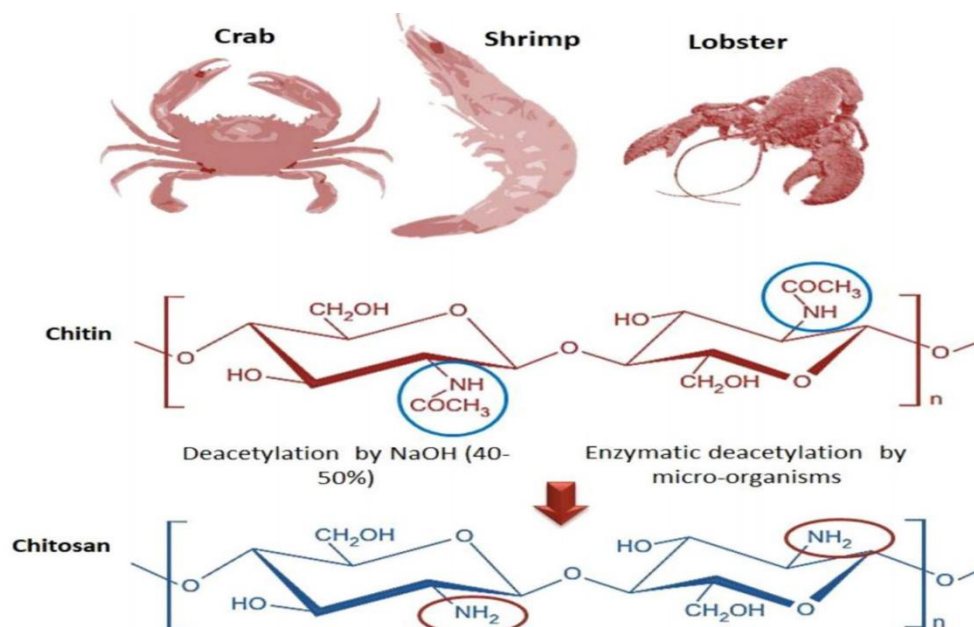


Figure 1. Commercial sources and chemical structure of chitin and chitosan.

Certain requirements must be met by an excellent protective group, such as preferentially interacting with the desired functional group to produce a stable and protected substrate. In order to produce a product with a satisfactory yield, a suitable protective group should also be efficiently and selectively removed. [23]. Three nucleophilic functional groups are present in chitosan: a primary-O.H. group at the C-6 position, a secondary-O.H. group at the C-3 position, and an -NH₂ group at the C-2 position. To achieve new moieties on the amino functionality of chitosan, it is desired to apply protective groups on the highly reactive hydroxyl moieties. Hydroxyl protective groups are commonly involved in organic chemical reactions; therefore, there are large amounts of hydroxyl protective methods available. The triphenylmethyl group can only be introduced at the primary-O.H. group of chitosan, leaving the secondary -O.H. group unprotected. Additionally, the procedure consists of three synthesis steps at 100 °C [24]. An alternative option for protecting alcohol is silyl ether protective groups. Silyl ethers, such as trimethylsilyl (TMS) and tert-butyldimethylsilyl (TBDMS) ethers, are readily formed from hydroxyl groups. Silyl ethers are simple to add to the molecule and are also easy to cleave. Because TBDMS ethers are stable and sterically hindered, they are promising protective groups. Compared to TMS ethers, TBDMS groups are approximately 104 times more stable against hydrolysis. Although TBDMS ethers are rapidly eliminated in mildly acidic or strongly alkaline environments, they remain stable in aqueous base media [23]. TBDMS-Cl combined with imidazole is how TBDMS protection is accomplished in synthesis. Rúnarsson [19] developed a synthetic strategy to protect the hydroxyl groups of chitosan using TBDMS protective groups. In the procedure, the TBDMS moiety is introduced to the mesylate salt of chitosan in one step to obtain a 100% 3,6-O-TBDMS protected chitosan.

Furthermore, the resultant compound showed good solubility in ethanol (EtOH), dichloromethane (DCM), and N-methyl-pyrrolidone (NMP) [25, 26]. Applying protecting groups on the amino group of chitosan is desired in order to obtain novel moieties on the hydroxyl functionality of the molecule. The phthaloyl group, which uses phthalic anhydride to modify chitosan both O- and N-modified, is an instance of an amine-protecting group. Nevertheless, by include water in the reaction mixture, the O-phthaloylation can be prevented [27].

Chitosan antimicrobial activity. Chitosan has robust antibacterial efficacy against Gram-positive, Gram-negative bacteria in addition to fungi [3,5–6]. The antibacterial characteristics of chitosan rely on essential factors, including pathogen type, pH of the media, structural attributes, (specifically the DA, M.W.), source, and concentration of chitosan [28–30]. A key component of chitosan's antibacterial action is the media's pH. Below pH 6.5, chitosan demonstrates antibacterial action. Probably, this is attributable to the presence of a large number of amino groups on the polymer, where the positively charged $-NH_3^+$ groups increase the binding to negatively charged membrane constituents from pathogenic bacteria [29,31].

Chitosan antibacterial activity. Chitosan bactericidal activity is also affected by the kind of microbe. Additionally, the polymer chitosan exhibits antimicrobial activity against both Gram-positive and Gram-negative bacteria, including *Salmonella typhimurium*, *E. coli*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Vibrio parahaemolyticus*, *Enterobacter aerogenes*, and *Vibrio cholera* [29, 30]. Gram-positive bacteria include *Bacillus cereus*, *S. aureus*, *Bacillus megaterium*, *Lactobacillus plantarum*, *Listeria monocytogenes*, *Lactobacillus brevis*, and *Lactobacillus bulgaricus*. As a result, chitosan is thought to have broad antibacterial action; however, there is some disagreement about whether this makes it more effective against Gram-negative bacteria or Grampositive bacteria. Contrary to what some writers have claimed, chitosan has been shown to have more bactericidal action against Gram-negative bacteria [32, 33]. The DA value affects the Chitosan's antibacterial activity via determining the quantity of amino groups that are free (positively charged), which support such action. Accordingly, the antimicrobial activity is predicted to as the DA falls, increasing [5,34,35]. The studied pathogens determine the optimal M.W. value for chitosan, which is generally more active than COS [29, 36]. Chitosan sources may be fungus or marine. When comparing the chitosan's activity from various sources, marine chitosan was found to have higher activity than *Rhizopus oryzae* fungus chitosan [30]. However another study found that shiitake mushroom crude fungal chitosan has more potent more antibacterial power than chitosan from crustaceans [37]. The mode of action may have different possible mechanisms. Earlier studies indicate that chitosan has antibacterial properties, nevertheless, the precise process is not entirely known, and several factors influence activity, as stated above [5]. Additionally, chitosan's antibacterial qualities must be related to animated edible bundling [38]. Biofilms are formed primarily of chitosan, which has a long-term expression potential for sustenance. Products with antimicrobial coatings on fish, cereals, fruits, and vegetables prevent microbes from

growing, as shown by chitosan experiments, which operate as a barrier to improve the nutritional value and texture of the food [39, 40]. Eatable biopolymer films may also serve as a protective barrier and provide insight on how transporters can enhance the quality of food by claiming bioactive mixtures. Different antimicrobial agents, such as bacteriocins, natural acids, proteins, plant extracts, antibiotics, fungicides, and chelating agents (EDTA) on reducing food waste employing harmful microorganisms, can be combined with polymeric bioactive films microbes and lengthen the shelf life [41]. Biodegradable edible films made of chitosan could be utilized in product packaging in addition to the end product. Additionally, bioactive films are characterized by their perfect mechanical qualities, which form a shell that maintains those qualities and the presence of nutrition within [42]. The proposed antimicrobial mechanism of chitosan is illustrated in fig. in below.

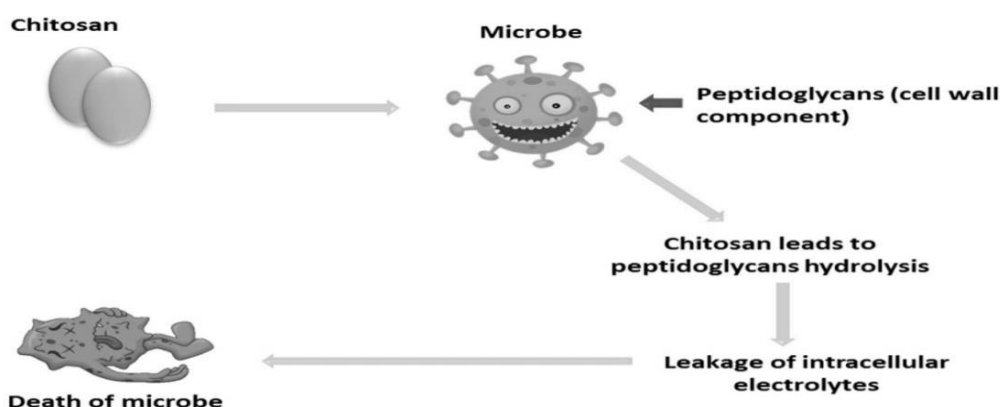
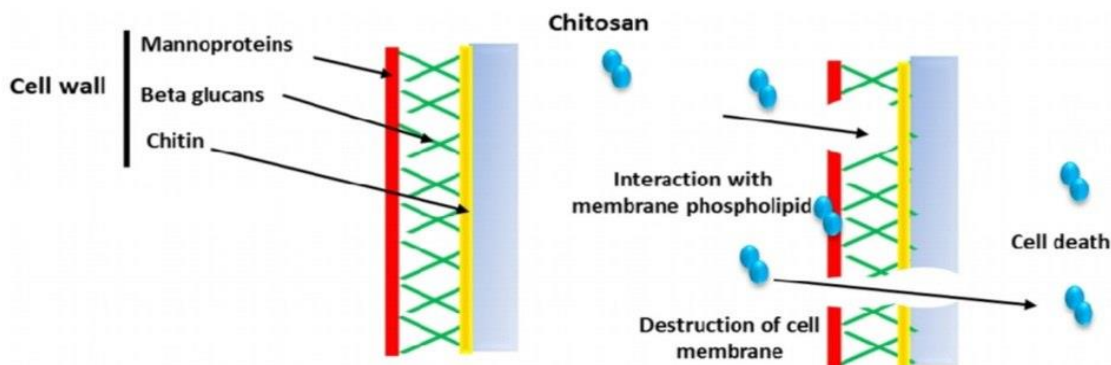


Figure 2. The proposed antimicrobial mechanism of chitosan.

Chitosan antifungal activity. Chitosan exhibits antifungal properties against yeasts and molds, including *Fusarium oxysporum*, *Botrytis cinerea*, *Rhizoctonia solani*, *Candida lambica*, *Phomopsis asparagi*. These properties could be owned to be fungistatic to a greater extent than fungicidal, spore germination, inhibiting growth, as well as tube lengthening [43]. The structure of the cell wall, which directly impedes growth, determines the mode of action. Chitosan is also expected to function more rapidly on More fungus than bacteria [5].

Certain compounds of chitosan have reportedly have potent antifungal properties compared to chitosan and probably fungicidal effect. In this regard, Li et al. [44] stated that two derivatives of quaternary chitosan referred to as 4-(5-chloro-2-hydroxybenzylidene amino)-pyridine(CHPACS) and 4-(5-bromo-2-hydroxybenzylideneamino)-pyridine (BHPACS) showed up to 100% rate of inhibition for *Cladosporium cucumerinum* and *Monilinia fructicola*. Also, Tan et al. [45] described the potential using quaternary chitosan derivatives phosphonium groups as antifungal agents. In particular biocomposites of multi-walled carbon nanotubes with modified chitosan derivatives showed a very potent antifungal activity against *Cryptococcus neoformans* [46]. More recently, [47] demonstrated that a series of derivatives of chitosan of fixed contents of

hydrophobic groups and diethylaminoethyl but different molecular weight showed superior antifungal properties against *Aspergillus flavus*. The proposed antifungal mechanism of chitosan is illustrated in below.



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