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In Vitro evaluation of free radical scavenging activity of chitosan

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Abstract

Examining chitosan's antioxidant potential using well-established in vitro systems including DPPH and superoxide (O2-)/hydroxyl (-OH) radical scavenging. At concentrations between 0.125 and 1.0mg/ml, the reducing capacity varied from 15.47% to 19.00%. Concentrations of chitosan (0.125–1.0mg/ml) exhibited a scavenging capability for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals ranging from 28.37% to 38.03%. Chitosan's (0.125-1.0 mg/ml) ability to scavenge hydroxyl radicals varied between 12.20% and 40.10%. The concentration of the superoxide anion radical varied from 15.20% to 32.10% between 0.125 and 1.0 mg/ml. Overall, chitosan showed promising antioxidant action, suggesting its application as a dietary supplement or medicinal component. Last but not least, chitosan's ability to scavenge and to reduce rose as its concentration did.

Key-Words: Antioxidants, Chitosan

INTRODUCTION

Inadequate regulation of reactive oxygen species production and endogenous antioxidant defenses leads to oxidative stress. In vivo and in vitro, reactive oxygen species (ROS) are the principal catalysts that begin oxidation [1, 2]. Cancer, cardiovascular disease, neurological dysfunction, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol-induced liver disease, ulcerative colitis, aging, and atherosclerosis are only some of the illnesses and disorders that may be caused by ROS [3-6].The contribution of nature in delivering viable therapeutic entities cannot be overstated. Historically, people have relied on the ocean for transportation, commerce, and food. More over 70% of Earth's surface is covered by water, yet it is home to 95% of all life (Ellis, 2001). Chitin is the second most common biopolymer and may be found in crab shells, insect cuticles, and fungal cell walls (Knorr 1984).The biodegradable, immunological, antioxidant, and antibacterial characteristics of chitosan, a copolymer comprising (14)-2-acetamido-2- deoxy--D-glucan and (14)-2-amino-2-deoxy--D-glucan [7-9], have garnered it widespread interest as a new functional material

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.There is evidence that chitosan and its derivatives have antioxidant properties [10-14]. Xie postulated that the chitosan's NH2 group played an important part in free radical scavenging [15]. Then, studies by Lin and Kim [16-18] confirmed the theory.The researchers set out to determine how effective chitosan was as an antioxidant.Substances and TechniquesChemicals and medicationsAll other chemicals and reagents were purchased from Sigma Aldrich in Mumbai, whereas chitosan was kindly donated by M/s. apex laboratories in Chennai. capacity to remove DPPH (di-2-picrylhydrazyl) radicals from the body.Using a modified version of the approach previously reported by Shimada et al [19], we tested chitosan for its ability to scavenge the DPPH radical. Each 0.125-1.0mg/ml chitosan sample in 0.2% acetic acid solution was combined with 1ml of DPPH radical-containing methanolic solution, yielding a final DPPH concentration of 1.0mM. After 30 minutes of sitting undisturbed in the dark, the absorbance of the combination was measured at 517 nm against a blank. In this experiment, ascorbic acid and BHA served as controls. The ability to scavenge was determined by using the formula:

Capacity to recycle (in percentages) = (A517 of control - A517 of sample) / (A517 of control) X 100. Analysis of Hydroxyl Radicals

A putative antioxidant's ability to slow the hydroxyl radical's progress was measured using the deoxyribosemethod of Halliwell et al. [20]. Chitosan (0.125, 0.25, 0.5, and 1.0 mg/ml) was mixed with deoxyribose (3.75 mM), hydrogen peroxide (1 mM), iron chloride (100 lM), ethylenediaminetetraacetic acid (100 lM), and ascorbic acid (100 lM) in a potassium phosphate buffer (20 mM, pH 7.4) for 60 minutes at 37 degrees Celsius. After 15 minutes in a boiling water bath, the reaction was stopped by adding 1 ml of TBA (1% w/v) and 1 ml of TCA (2% w/v). The mixture was allowed to cool, and then its absorbance was measured at 535 nm against a reagent blank. To make a comparison, ascorbic acid was utilized. Scavenging activity against hydroxyl radicals was determined using the following formula:

Relative frequency of occurrence (%) = (A sample-A blank)The control-blank syntax reads: /[(A control-A blank)] X100The absorbance of distilled water in place of chitosan (the blank) and H2O2 (the control) are respectively represented by Ablank and Acontrol. There were three sets of tests for each set.Assay for the Removal of Superoxide Anion RadicalsChitosan's capacity to scavenge superoxide was measured using the Xing technique. Chitosan (0.125, 0.25, 0.5, and 1.0 mg/ml), PMS (30 lM), NADH (338 lM), and NBT (72 lM) make up the reactants in the reaction mixture.After incubating for 5 minutes at room temperature in phosphate buffer (0.1 M, pH 7.4), the absorbance was measured using Shimadzu UV-Vis Spectrophotometers at 560 nm. To make a comparison, ascorbic acid was utilized. There were three sets of tests for each set.The percentage of scavenging is calculated as 100 x (1-A sample 560nm/A control 560 nm).decreasing strengthThe Oyaizu[21] technique was used to calculate the reducing power. Each sample of chitosan (0.125-1.0mg/ml) was combined with a phosphate buffer (0.2M, PH-6.6) and potassium ferric cyanide (1%), totaling a volume of 5.0 ml. In a water bath, the reaction mixture was maintained at50 degrees Celsius for 20 minutes. 2.5ml of 10% trichloroacetic acid was added after incubation, and the mixture was centrifuged.10 minutes at 3000 rpm. Beginning with the surface,The 0.1 percent ferric chloride solution was diluted with distilled water to a volume of 2.5 milliliters. The whole solution's absorbance was evaluated at a wavelength of 700nm. Increased The higher the absorbance, the more powerful the reduction. To make a comparison, ascorbic acid was utilized.

Discussion and Results

Power to neutralize DPPH free radicals: 1, 1-diphenyl-2-picrylhydrazyl.It has been shown that at 1mg/ml, chitosan is 38.03% effective in scavenging DPPH radicals.Both ascorbic acid and butylated hydroxyanisole (BHA) have scavenging efficacies between 45 and 49 percent. Therefore, chitosan's antioxidant activity is a middling scavenger of DPPH radicals. Chitosan's ability to scavenge DPPH radicals varied from 28.37% at 0.125 mg/ml to 38.03 at 1 mg/ml. On the gold standard, ascorbic acid and BHA were employed (Fig. 1). Scavenging of hydroxyl radicals:Deoxyribose oxidation was inhibited by the chitosan, and the effect was dose-dependent. In addition, it was more effective than ascorbic acid in scavenging hydroxyl radicals (Fig. 2). At a dosage of 0.5 mg/ml, the scavenging efficacy of chitosan was 27.90%, whereas that of ascorbic acid was 34.00%. Chitosan's (0.125-1 mg/ml) scavenging ability against hydroxyl radicals was measured to be between 12.20% and 40.10%. The gold standard was ascorbic acid.

Scavenging of superoxide anion radicals:

Concentrations of chitosan between 0.125 and 1 mg/ml inhibited the superoxide anion radical by between 15.20% and 32.10%, as seen in Fig. 3. Ascorbic acid (0.125-1 mg/ml) was shown to have a greater scavenging effect than chitosan, with a range of 19.00% to 45.00%. The gold standard was ascorbic acid.

Lessening the punch:

The capacity of chitosan to reduce was evaluated by monitoring its ability to convert ferric iron (Fe3+) to ferric iron (Fe2+). The reducing power of chitosan was minimal at 0.125 mg/ml, measuring 15.47%. The ability of chitosan to attenuate was inversely proportional to its concentration. At 0.125 mg/ml, results were still under ascorbic acid's 34.50% (Fig. 4). At concentrations between 0.125 and 1mg/ml, the reducing capacity varied from 15.47% to 19.00%. The gold standard was ascorbic acid.

Yen et al. [22] found that crab chitosan has antioxidant activity between 58.3 and 70.2% at 1mg/ml and between 79.9 and 85.2% at 10mg/ml. Furthermore, there was a correlation between the N-deacetylation durations and antioxidant properties of C60, C90, and C120 crab chitosan. In this study, chitosan's antioxidant activity was shown to be rather constant across different concentrations. Antioxidant activity of chitosan was 28.37-38.03% at0.125 to 1mg/ml.One important mechanism of antioxidant involves the scavenging of hydrogen radicals. DPPH has a hydrogen free radical and shows a characteristic absorption at 517nm [23]. After encountering the proton-radical scavengers, the purple color of the DPPH solution fades rapidly [24]. In this study, DPPH was used to determine the proton-scavenging activity of the various disaccharide chitosan derivatives.Yen et al. [22] reported that fungal chitosan scavenged DPPH radicals by 28.4-53.5% at 10mg/ml, obviously chitosan from crab shells and shiitake stipeswas also not an effective scavenger for DPPH radicals. Yen et al.[25] reported the scavenging ability of crab chitosan C60 on DPPH radicals was 28.4% at 10mg/ml, whereas these of other crab chitosan were in the range of 46.4-52.3%. The scavenging ability of chitosan was 38.03% at 1mg/ml. However, at 1mg/ml, BHA and Ascorbic acid showed scavenging abilities of 49.03% and 45.10% respectively.Superoxide anion is a reduced form of molecular oxygen created by receiving one electron. It is an initial free radical formed from mitochondrial electron transport system [26]. Superoxide anion radicals are produced by a number of cellular reactions, including various enzyme systems, such as lipoxygeneases, peroxidase, NADPH oxidase and xanthine oxidase. They play an important role in the formation of other cell damaging free radicals, such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems. In the present study a superoxide radical scavenging assay was based on the capacity of chitosan to inhibit the reduction of nitro blue tetrazolium (NBT). Significant scavenging of superoxide radical was evident at all the tested concentration of chitosan. At 0.5mg/ml, the scavenging

percentage of chitosanagainst superoxide radical was 46.17 %.

Although superoxide is a relatively weak oxidant, it decomposes to form stronger reactive oxidative species, such as single oxygen and hydroxyl radicals, which initiate peroxidation of lipids [27]. In the present study, chitosan effectively scavenged superoxide in a concentration dependent manner. Further, superoxides are also known to indirectly initiate lipid peroxidation as a result of H2O2. Hydroxyl radical scavenging activity of chitosan was obtained in the deoxyribose system. In this system, chitosan exhibited a concentration-dependent inhibition of deoxyribose oxidation. Earlier, numerous workers Halliwellet al.[20] have employed this system to assess the biological activity of various natural plant-derivedbiomolecules. Smith et al. [28] reported that molecules that can inhibit deoxyribose degradation are those that can chelate iron ions and ender them inactive or poorly active in a Fenton reaction. In the present study, in another assay system, we found chitosan had moderate chelating effect. So, it is likely that the chelating effect of chitosan on metal ions may not be responsible for the inhibition of deoxyribose oxidation.The reducing power of different molecular weights of γ-ray treated chitosan has determined by potassium ferricyanidereduction method showed that low molecular weight γ-ray treatedchitosan exhibited high reducing power and the reducing powerincreased withthe increases of chitosan concentration [29]. In the present study, the reducing power of chitosan was19.00% at 1mg/ml. However,ascorbic acid showed reducing power of 98.70 at 1mg/mlrespectively. It shows that chitosan wasnot effective in reducing power. It seems that reducingg powers ofchitosan also correlated with their N-deacetylation times [25].

Conclusion

This research suggests that Chitosan has powerful antioxidant properties.The food industry has taken a keen interest in the antioxidant qualities of chitin and chitosan extracts due to the emerging trend toward replacing synthetic antioxidants with natural additions. Crustacean chitin and chitosan extracts fared well in tests of antioxidant activity, leading researchers to conclude that they are a safe and sustainable natural resource for use in the food sector. Crustacean processing by-products having biological activity, such as antioxidant qualities, are the focus of this research.

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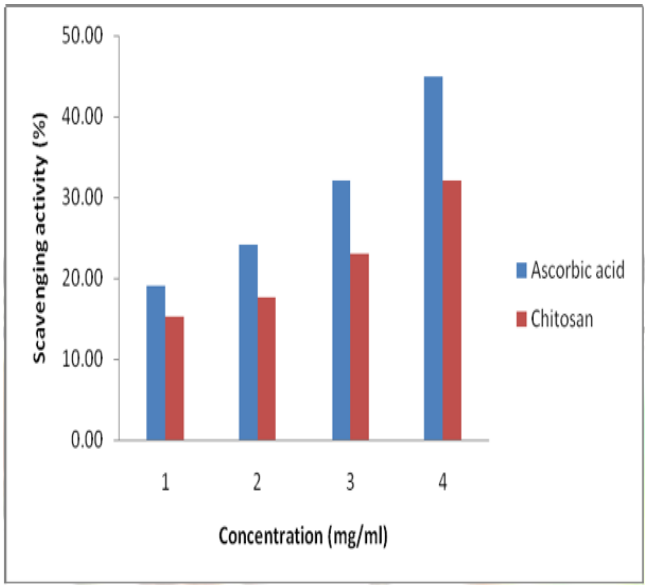

Fig. 3: Scavenging effects of the chitosan and ascorbic acid on Superoxide anion radicals. Each test replicated threetimes

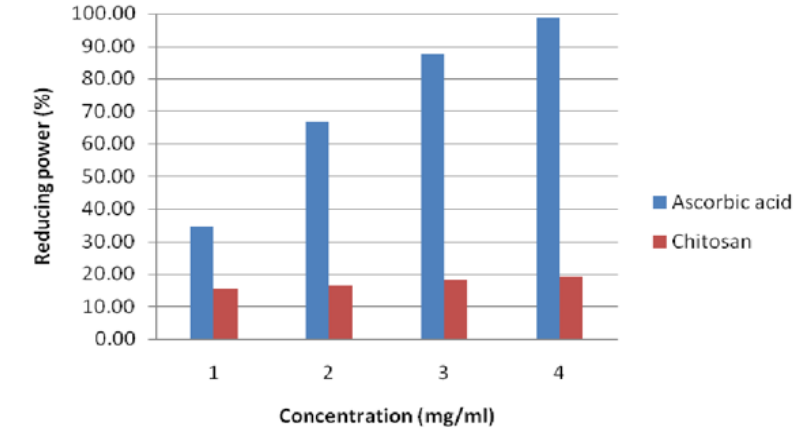

Fig. 4: Reducing power of the chitosan ascorbic acid