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In Vitro evaluation of free radical scavenging activity of chitosan

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Abstract

To investigate antioxidant potency of chitosan in various established in vitro systems, such as superoxide (O2-)/hydroxyl (-OH) radicals scavenging, DPPH and reducing power. The reducing capability ranged from of 15.47 % to 19.00% at varying concentration (0.125 to 1.0mg/ml). The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging potential of chitosan ranged from 28.37% to 38.03% at varying concentrations (0.125 to 1.0mg/ml). Hydroxyl radicals scavenging activity of chitosan (0.125- 1.0 mg/ml) was ranging from 12.20% to 40.10%. Superoxide anion radical was ranging from 15.20% to 32.10% for the concentration between 0.125-1.0 mg/ml. overall, chitosan was good in antioxidant activity, and may be used as a source of antioxidants, as a possible food supplement or ingredient in the pharmaceutical industry. Finally, the scavenging rate and reducing power of chitosan increased with their increasing concentration.

Key-Words: Antioxidants, Chitosan

Introduction

Oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems. ROS major sources of primary catalysts that initiate oxidation in vivo and in vitro [1, 2]. These ROS creates oxidative stress which results in numerous diseases and disorders such as cancer, cardiovascular disease neural disorder, Alzheimer's disease, mild cognitive impairment, Parkinson disease, Alcohol induced liver disease, ulcerative colitis, aging and atherosclerosis [3-6].Nature has played an instrumental role in providing effective therapeutic entities. The historical relationship betweenmankind and the sea is usually for travelling, trading and as a nutritional source. Ocean cover more than 70% of the earth'ssurface but represents 95% of the biosphere (Ellis 2001). Chitin, found in the shell of crustaceans, the cuticles of insects, and the cell walls of fungi, is the second abundant biopolymer in the nature (Knorr 1984).Chitosan, a copolymer of (1→4)-2-acetamido-2-deoxy-*β*-D-glucan and (1→4)-2-amino-2-deoxy-*β*-D-glucan, has been receiving great attention as novel functional material for its excellent biological properties such as biodegradation, immunological, antioxidant and antibacterial activities [7-9].

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The antioxidant activity of chitosan and its derivatives has been reported [10–14]. Xie proposed the role of NH2 group in chitosan in the process of free radical scavenging [15]. And then it was proved by the researches of Lin and Kim [16–18].The objective of this study was to assess the antioxidant activities of chitosan.

Material and Methods

Drugs and chemicals

Chitosan was obtained as a gift from M/s. apex laboratories, Chennai, all of the other chemicals and reagents were obtained from Sigma Aldrich, Mumbai.

Scavenging ability on 1, 1-diphenyl 1-2-picryl hydroxyl radicals (DPPH)

The scavenging effect of chitosan on DPPH radical was examined using the modified method described earlier by Shimada et al [19]. Each chitosan sample (0.125-1.0mg/ml) in 0.2% acetic acid solution was mixed with 1ml of methanolic solution containing DPPH radicals, results/resulted in a final concentration of 1.0mM DPPH. The mixture was shaken vigorously and left to stand for 30min in the dark and the absorbance was then measured at 517nm against a blank. Ascorbic acid and BHA were used as standard. The scavenging ability was calculated as follows:

Scavenging ability (%) = [ΔA517 of control-ΔA517 of sample) /ΔA517 of control] X100.

Hydroxyl radical assay

The rate of reaction of hydroxyl radical with a putative antioxidant was determined by the deoxyribosemethod ofHalliwell et al [20]. The reaction mixture containing chitosan (0.125, 0.25, 0.5, 1.0 mg/ml) was incubated with deoxyribose (3.75 mM), H2O2 (1 mM), FeCl3 (100 lM), EDTA (100 lM) and ascorbic acid (100 lM) in potassium phosphate buffer (20 mM, pH 7.4) for 60 min at 37·C. The reaction was terminated by adding 1 ml of TBA (1% w/v) and 1 ml of TCA (2% w/v) and then heating the tubes for 15 min in a boiling water bath. After cooling, the absorbance of the mixture was measured at 535 nm against reagent blank. Ascorbic acid was used for comparison. The hydroxyl radical scavenging activity was calculated as following:

Scavenging effect (%) = [(A sample-A blank)]/[(A control-A blank)] X100

Where A_{blank} presents the absorbance of the blank (distilled water, instead of the chitosan) and $A_{control}$ presents the absorbance of the control (distilled water, instead of H2O2). Each test replicated three times.

Superoxide anion radical scavenging assay

The superoxide scavenging ability of chitosan was assessed by the method of Xing. The reaction mixture, containingchitosan (0.125, 0.25, 0.5, 1.0 mg/ml), PMS (30 lM), NADH (338 lM) and NBT (72 lM) in phosphate buffer (0.1 M, pH 7.4), was incubated at room temperature for 5 min and the absorbance was read at 560 nm(Shimadzu UV–Vis Spectrophotometers) against a blank. Ascorbic acid was used for comparison. Each test replicated three times.

Scavenging effect (%) = (1-A sample 560nm/A control 560 nm) X 100.

Reducing power

Reducing power was determined according to the method of Oyaizu[21]. Each chitosan sample (0.125 - 1.0mg/ml) was mixed with 2.5 ml of phosphate buffer (0.2M, PH-6.6) and 2.5 ml of potassium ferric cyanide (1%). Reaction mixture was kept in a water bath at 50◦C for 20 minutes. After incubation, 2.5ml of trichloroacetic acid (10%) was added and centrifuged at 3,000 rpm for 10 minutes. From the upper layer, 2.5ml solution was mixed with 2.5ml of distilled water at 0.5ml of ferric chloride (0.1%). Absorbance of all the solution was measured at 700nm. Increased absorbance indicated increased reducing power. Ascorbic acid was used for comparison.

Results and Discussion

Scavenging ability on 1, 1-diphenyl 1-2-picryl hydroxyl radicals (DPPH):

The scavenging ability of chitosan on DPPH radicals was reported at 38.03% at 1mg/ml.Ascorbic acid and BHA showed moderate to high scavenging abilities of 45.10% and 49.03% respectively. Thus the antioxidant activity of chitosan was found to be a moderate scavenger for DPPH radicals. The DPPH radical scavenging potential of chitosan ranged from 28.37% to 38.03 at varying concentrations (0.125 to 1mg/ml). Ascorbic acid and BHA were used on standard (Fig. 1).

Hydroxyl radical scavenging activity:

The chitosan had obvious scavenging activity and exhibited a concentration-dependent inhibition of deoxyribose oxidation. Moreover, it had Scavenging activity on hydroxyl radical compared with ascorbic acid (Fig. 2). Scavenging effect of chitosan was 27.90% at concentration of 0.5 mg/ml, whereas scavenging effect of ascorbic acid was 34.00% at same concentration. Hydroxyl radicals scavenging activity of chitosan (0.125- 1 mg/ml) was found to be 12.20 % to 40.10%. Ascorbic acid was used as standard.

Superoxide anion radical scavenging activity:

The inhibitory effect of the chitosan on superoxide anion radical was ranging from 15.20% to 32.10% for the concentration between 0.125-1 mg/ml (Fig. 3). However, the scavenging effect of Ascorbic acid (0.125- 1 mg/ml) was found to be higher than the chitosan and the range was from 19.00% to 45.00%. Ascorbic acid was used as standard.

Reducing power:

The reducing capability of chitosan was assessed based on the measurement of Fe3+-Fe2+ transformation. At 0.125 mg/ml of chitosan showed slight reducing power of 15.47%. The reducing power of chitosan was correlating well with increasing concentration. But values remained lower than ascorbic acid 34.50% at 0.125 mg/ml (Fig. 4). The reducing capability ranged from of 15.47 % to19.00% at varying concentration (0.125 to 1mg/ml). Ascorbic acid was used as standard.

Yen *et al.* [22] reported that crab chitosan showed moderate to high antioxidant activities of 58.3-70.2% at 1mg/ml and high antioxidant activities of 79.9-85.2% at 10mg/ml. In addition, the antioxidant activities of crab chitosan C60, C90 and C120 correlated with their N-deacetylation times. In the present investigation, chitosan showed consistent antioxidant activity with increased concentration.

Antioxidant activity of chitosan was 28.37-38.03% at 0.125 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 stipes*was 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 Halliwell*et al.*[20] have employed this system to assess the biological activity of various natural plant-derived biomolecules. 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 with the 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

Based on this study, Chitosan show significant antioxidant activity.Antioxidative properties of the various chitin and chitosan extracts are of great interest in food industry, since their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants by natural ones. Owing to its excellent protective features exhibited in antioxidant activity tests, the chitin and chitosan extracts from the crustacean species could be concluded as a natural source that can be freely used in the food industry. This study identifies opportunities to develop value added products from crustacean processing by-products with biological activity such as antioxidant properties.

References

1. Chew AL, Jessica JJA, Sasidharan S. Antioxidant and antibacterial activity of different parts of Leucasaspera. Asian Pac J Trop Biomed. 2012;2:176-180.
2. Dolai N, Karmakar, I, Sureshkumar RB, Kar B, Bala A, Haldar PK. Free radical scavenging activity and Castanoprisindica in mediating hepatoprotective activity of carbon tetrachloride intoxicated rats. Asian Pac J Trop Biomed.2012;2:S242-S251.
3. Shahwan D, Raza MA. Antioxidant potential and phenolic extracts and Mimusopselergi.Asian Pac. J.Trop Biomed. 2012; 2:547-550.
4. Thambiraj J, Paulsamy S, Sevukaperumal R. Evaluation of in vitro antioxidant activity in the traditional medicinal shrub of western districts of Tamilnadu, India,

AcalyphafruiticosaForssk. (Euphorbiaceae).AsianPac.J.Trop.Biomed.2012; 5127-5-130.
5. Sen S, Chakrabarty R, Sridhar C, Reddy YSR. Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. IntJ.PharmaceuticalSci Rev Res.2010; 3:91-100.
6. Rajkumar V, Guha C, Kumar RA. Antioxidant and anti-neoplastic activities of Picrorhizakurroaextracts.FoodChemToxicol2011; 49:363-369.
7. Kofuji K, Qian CJ, Nishimura M, Sugiyama I, Murata Y, Kawashima S (2005) EurPolym J 41:2784–2791.
8. Qi LF, Xu ZR, Jiang X, Hu CH, Zou XF (2004) Carbohydr Res 339:2693–2700.
9. Li WJ, Jiang X, Xue PH, Chen SM (2002) Chin Sci Bull 11:887– 889.
10. Zhao HR, Wang K, Zhao Y, Pan LQ (2002) Biomaterials 23:4459– 4462.
11. Guo ZY, Xing RE, Liu S, Yu HH, Wang PB, Li CP, Li PC (2005) Bioorg Med ChemLett 15:4600–4603.
12. Kogan G, Skorik YA, Zintnanova I, Krizkova L, Durackova Z, Gomes CAR, Yatluk YG, Krajcovic J (2004) ToxicolAppl Pharm, 201:303–310.
13. Sun T, Xie WM, Xu PX (2004) CarbohydrPolym 57:379–382.
14. Huang RH, Mendis E, Kim SK (2005) Int J BiolMacromol 36(1/2):120–127.
15. Xie WM, Xu PX, Liu Q (2001) Bioorg Med ChemLett 11:1699– 1701.
16. Lin HY, Chou CC (2004) Food Res Int 37:883–889.
17. Huang RH, Rajapakse N, Kim SK (2006) CarbohydrPolym 63:122–129.
18. Je JY, Kim SK (2006) Bioorg Med Chemdoi: 10.1016/j.bmc.2006.05.016.
19. Shimada K, Fujikawa K, Yahara K, and Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in Cyclodextrin emulsion. J. Agric. 1992; 40: 945−948.
20. Halliwell B, Gutteridge JM, and Aruoma OI. The deoxyribose method: a simple ‘‘test-tube’’ assay for determination of rate constants for reactions of hydroxyl radicals. Analytical Biochemistry. 1987; 165, 215–219.
21. Oyaizu M. Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Japn. J. Nutr. 1986; 44: 307−315.
22. Yen M, Yang J, and Mau J. Antioxidant properties of chitosan from crab shells. Carbohydrate Polymers. 2008; 74, 840-844.
23. Brand-Williams W, Cuvelier ME, and Berset C. Use of a free radical method to evaluate antioxidant activity, Food Science and Technology.1995; 28: 25-30.
24. Yamaguchi T, Takamura H, Matoba T, and Terao J. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. Bio sci Biotechnol Biochem, 1998; 62: 1201-1204.
25. Yen MT, Tseng YH, Li RC, and Mau JL. Antioxidant properties of fungal chitosan from shiitake stipes. LWT – Food Science and Technology. 2007; 40, 255–261.
26. Bloknina O, Virolainen E, and Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: A review. Annals ofBotany. 2003; 91, 179–194.
27. Dahl MK, and Richardson T. Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and amino acid. Journal of Dairy Science. 1978; 61, 400–407.
28. Smith C, Halliwell B, and Aroma OI. Protection by albumin against the pro-oxidant actions of phenolic dietary components. Food Chem. Toxicol. 1992; 6: 483-489.
29. Feng T, Du Y, Li J, and Kennedy JF. Enhancement of antioxidant activity of chitosan by irradiation.CarbohydrPolym. 2008; 73, 126-132.

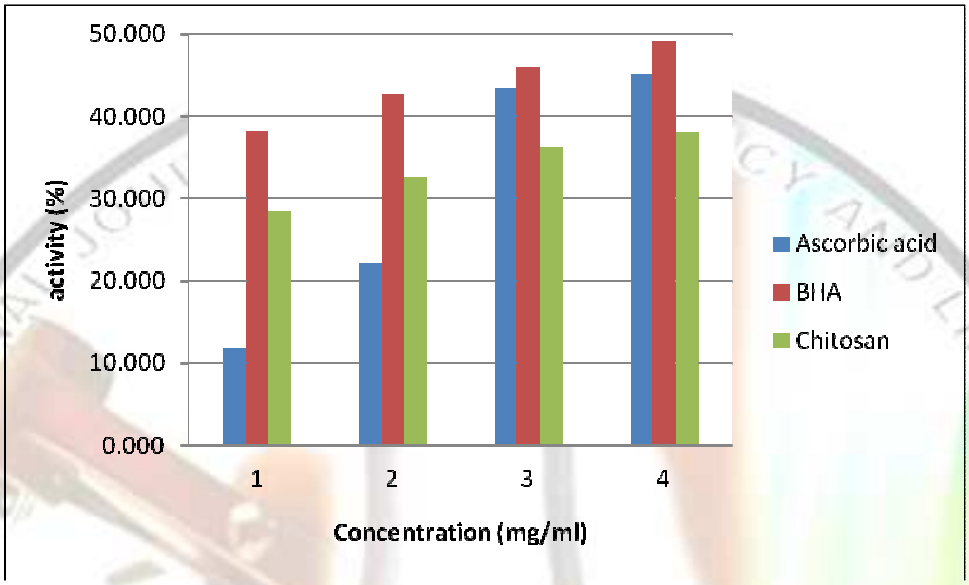

Fig. 1: Scavenging activities of the chitosan, ascorbic acid and BHA on DPPH radical. Each test replicated three times

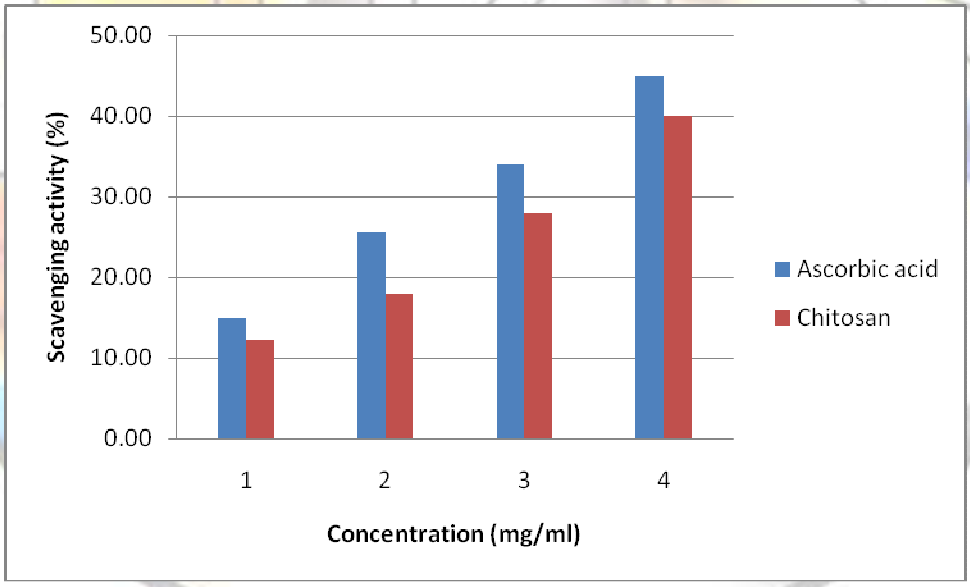

Fig.2: Scavenging effects of the chitosan and ascorbic acid on hydroxyl radicals. Each test replicated three times

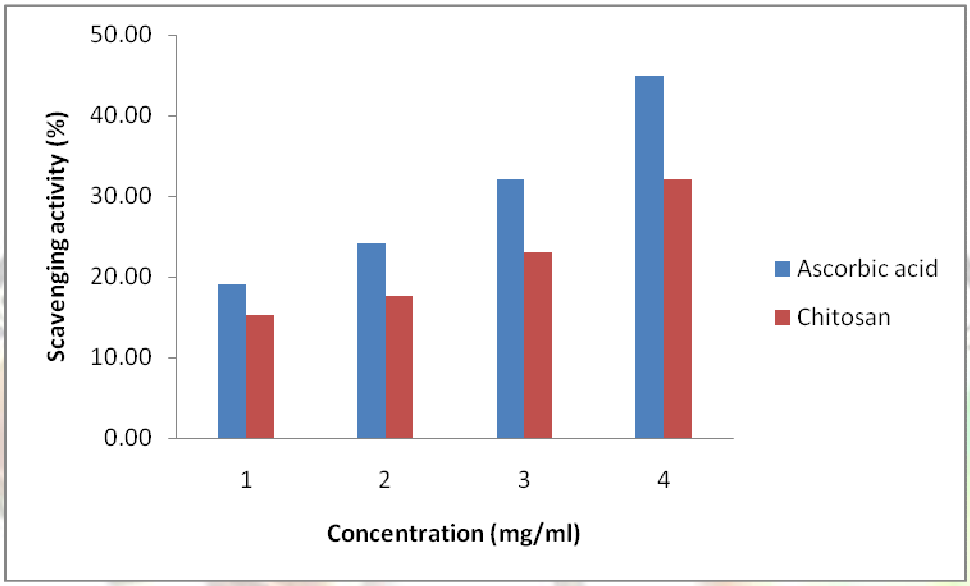

Fig. 3: Scavenging effects of the chitosan and ascorbic acid on Superoxide anion radicals. Each test replicated threetimes

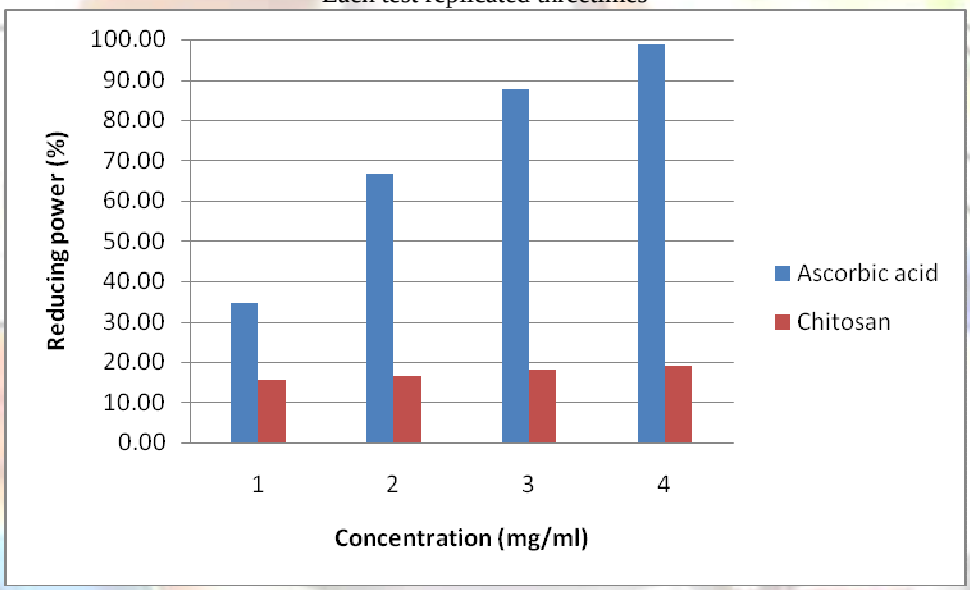

Fig. 4: Reducing power of the chitosan ascorbic acid