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Assessment of Nitric oxide scavenging activity and Total antioxidant activity of Chitosan extracted from carapace of freshwater edible crab *Sartoriana spinigera*

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Abstract: Antioxidants are produced by the living body to scavenge the free radicals. But due to increased exposure to undesirable factors such as pollutants, smoking, unbalanced diet, the body needs additional supplementary antioxidants. The study was conducted to observe antioxidative property in one such natural supplement that is chitosan. Chitosan is a cationic polysaccharide produced by N-deacetylation of chitin found in exoskeleton of arthropoda, especially crustacea. In this experiment, Chitosan was extracted from *Sartoriana spinigera* which is a freshwater edible crab of Jharkhand and its Nitric oxide scavenging activity and total antioxidant activity was studied. Percent of scavenging activity of Chitosan against Nitric oxide anion was found to be 21.95 %, 35.49%, 46.90%, and 66.04% at 0.5 mg/ml, mg/ml, 5 mg/ml and 10 mg/ml concentration respectively. Percent of total antioxidant activity of Chitosan was found to be 29.73 %, 33.78%, 50%, and 62.16 % at 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml concentration respectively.

Key words: ***Sartoriana spinigera,*** **exoskeleton, chitosan, antioxidants.**

INTRODUCTION

As metabolic processes take place in the human body for the purpose of oxidation, there is simultaneous formation of free radicals. Apart from *in vivo* production, free radicals also accumulate in the body as a result of anthropogenic activities, cigarette smoking, automobile gas exhaust and x-rays exposure. Free radicals are harmful to the body. The instability and reactivity of free radicals due to the lone electron in the outer shell can cause them to attack specific biomolecules in the body such as proteins and lipids[1]. Presence of abnormal amount of free radicals in the body leads to oxidative stress. To maintain normalcy, there is a balance between the quantity of free radicals and antioxidants that are produced by the body to scavenge free radicals and inhibit their deleterious effects in the body.

Nitric oxide and reactive nitrogen species (RNS) are free radicals that are derived from the interaction of NO with oxygen or reactive oxygen species[2]. Chronic exposure to nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis[3]. For estimation of total antioxidant potential, various scientists use different assay using DPPH, ABTS, Phosphomolyb denum complex and reducing power[4]. Due to increased concentration of free radicals in the body, additional supplements of antioxidants are needed to be consumed. Various medicinal plants and natural extracts have proved to have antioxidant properties against nitric oxide.

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However, natural antioxidants of animal origin are not acknowledged as much. Chitosan is one such natural source of antioxidants that is of animal origin. Chitosan is a linear polysaccharide formed by deacetylation of chitin. Chitosan is composed of two units: 80% of deacetylated unit-ß 1-4 linked D glucosamine and 20% acetylated unit-ß 1-4, N-acetyl-D-glucosamine. In this research, chitosan has been extracted from a freshwater crab *Sartoriana spinigera* abundantly found in Jharkhand, India. It is not only a delicacy, but is also of great ethnobiological significance and is used by the tribal people of Jharkhand against ailments such as bacterial infection, gastrointestinal problems.

The aim of this study was to extract Chitosan from carapace of *Sartoriana spinigera* and assess its Nitric oxide scavenging activity and Total antioxidant activity by phosphomolybdenum method.

MATERIALS AND METHODS

1. *Characterization of raw materials:*

Fresh specimen of freshwater crab *Sartoriana spinigera* were purchased from local market of Ranchi, Jharkhand. The specimens were sent to Zoological survey of India (ZSI), Kolkata for identification of species. The species were acclimatized for 7 days in laboratory of P.G. Department of Zoology, Ranchi University. The carapace was obtained.

2. *Extraction of chitosan:*

Extraction of Chitosan from the carapace was done following Takiguchi[5,6].

2.1 Demineralization:

20 g of carapace powder was soaked in 300 ml of 1N HCl for 24 hours. The residue obtained was washed with distilled water to obtain a neutral pH. It was dried and weighed.

2.2 Deproteinization:

Demineralized powder was added in 300 ml of 4% NaOH at 80^{o}C in hot water bath for 24 hours with 3 intervals. The residue obtained was washed with distilled water to obtain a neutral pH. It was again dried and weighed. Chitin was obtained after deproteinization.

2.3 Deacetylation:

Chitin was then added in 300ml of 50% NaOH and heated at 100^{O}C in hot water bath for 18 hours with 3 intervals. After every interval, NaOH was replaced by fresh quantity and residue washed with distilled water. The final residue obtained was brought into neutral pH. It was dried and weighed. The powder obtained was chitosan.

2.4 Characterization of chitosan:

Average degree of deacetylation of chitosan extracted from carapace of *Sartoriana spinigera* was found to be 78.53±2.03% following Brugnerotto *et al.*[7] FT-IR study for showed absorbance bands of chitosan extracted from *Sartoriana spinigera* showed seven peaks at 891.11cm^{-1},1029.99 cm^{-1}, 1427.32 cm^{-1}, 1577.77 cm^{-1},1662.64 cm^{-1}, 2885.51 cm^{-1} & 3259.70 cm^{-1}, that were similar to the standard chitosan for comparison[8].

3. *Antioxidative activity:*

3.1 Nitric oxide scavenging activity

Nitric oxide scavenging activity was estimated following Kumar *et al*[9]. The experiment of superoxide scavenging activity was conducted in Biogenics Laboratory, Hubli, Karnataka. Various concentrations (0.5mg/ml, 1 mg/ml, 5 mg/ml and 10 mg/ml) of samples and Butylated Hydroxy Anisole (BHA) were taken in different test tubes and made up to 3ml with 0.1M phosphate buffer (pH 7.2). Sodium Nitroprusside (5mM) prepared in buffered saline (pH 7.2) was added (1 ml) to each tube. The reaction mixture was incubated for 30 min at RT. A control without the test compound, but with an equivalent amount of methanol was maintained. After 30 min, 1.5 ml of above solution was mixed with 1.5 ml of Griess reagent (1% Sulphanilamide, 2% phosphoric acid and 0.1% N-1-Naphthyl Ethylenediamine Dihydro chloride). The absorbance of the samples was measured at 546 nm. Nitric oxide radical scavenging activity was calculated using the following formula:

$$\% \text{ NO radical scavenging activity} = \frac{(\text{control OD} - \text{sample OD})}{\text{control OD}} \times 100$$

3.2 Total antioxidant activity

Total antioxidant activity by phosphomolybdenum method was estimated following Prieto *et al*[10]. Various concentrations of chitosan (50 µg/ml, 100 µg/ml, 200 µg/ml and 400µg/ml) were mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate and 4mM Ammonium molybdate)**.** The tubes containing reaction solution were incubated at 95^{O}C for 90 minutes**.** Absorbance of the solution was measured at 695 nm using a UV-VIS SPECTROPHOTOMETER against blank after

cooling at room temperature. Methanol was used as blank. Ascorbic acid was used as standard.

STATISTICAL ANALYSIS

Correlation coefficient was calculated and regression graph were prepared to study the dependence of variables on independent variables. Student's t test between % scavenging activity of chitosan and standard was calculated.

RESULT AND DISCUSSION

Table 1: Scavenging activity of Standard (BHA) and Test (Chitosan) in different concentrations against Nitric oxide free radical

Sl. No.	Concentration (mg)	Standard – BHA (%)	Test - Chitosan (%)
1.	0.5	100	21.95
2.	1	100	35.49
3.	5	100	46.90
4.	10	100	66.04

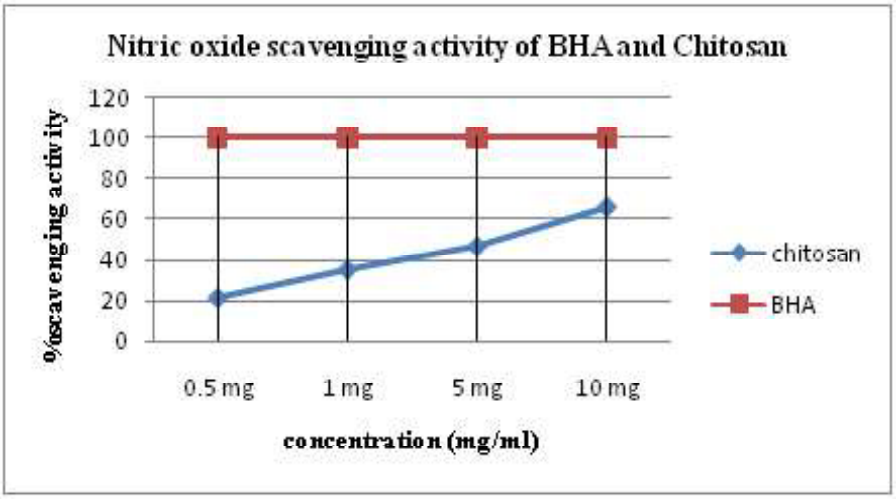

Fig 1: Scavenging activity of different concentration of Chitosan and BHA on Nitric oxide free radical

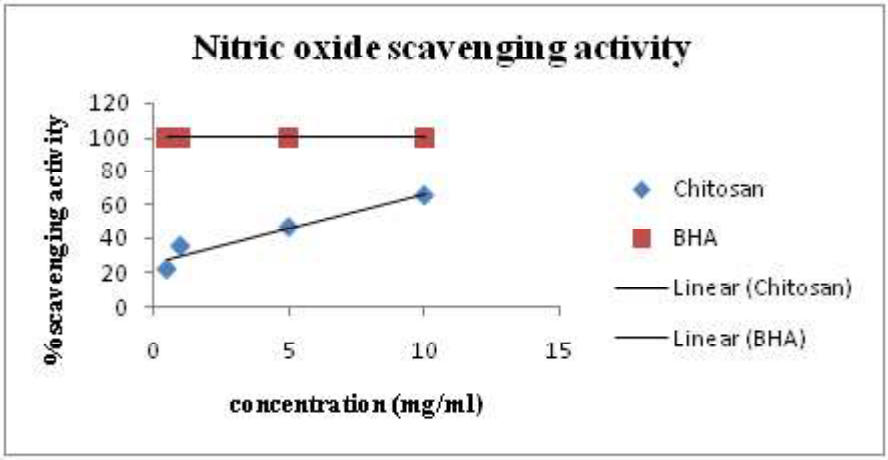

Fig 2: Graph representing correlation between concentration of BHA and chitosan and percent of nitric oxide scavenging activity

In the present study (Table:- 1) showed the percent scavenging activity of BHA against Nitric oxide anion was 100 %, 100%, 100% and 100% at concentration of 0.5 mg/ml, 1 mg/ml, 5 mg/ml and 10 mg/ml respectively. Percent of scavenging activity of Chitosan against Nitric oxide anion was found to be 21.95 %, 35.49%, 46.90%, and 66.04% at 0.5 mg/ml, 1 mg/ml, 5 mg/ml and 10 mg/ml concentration respectively.

Chitosan concentration showed positive correlation with its percent scavenging activity, indicating that as concentration of chitosan increased, its scavenging activity against Nitric oxide free radical also increased with the slope value b=4.09 (R^2= 0.96) (Fig:- 2).

The result of present study showed that chitosan extracted from carapace of *Sartoriana spinigera* exhibits antioxidant activity against Nitric oxide free radical.

No work on Nitric oxide scavenging activity of chitosan has been reported till date. However, studies on antioxidant activity of Balofloxacin and Prulifloxacin was conducted by Ranka[11] which showed that percent inhibition of NO with balofloxacin at 10mg/ml, 20 mg/ml, 30 mg/ml and 40 mg/ml was 34.49%, 49.09%, 60.42% and 73.14% respectively, whereas, inhibition of NO with Prulifloxacin at 10mg/ml, 20 mg/ml, 30 mg/ml and 40 mg/ml was 40.94%, 53.09%, 66.25% and 88.06% respectively. In vitro nitric oxide scavenging activity of three Bangladeshi medicinal plants were studied by Parul[12], and it was observed that *Phyllunthus freternus* showed percent scavenging at 50 µg/mL, 100 µg/mL and 200 µg/mL as 51.00±0.011 %, 60.16 ± 0.015% and 60.807± 0.005% respectively against nitric oxide. Leaves of *Triumpetta rhomboidae* at 50 µg/mL, 100 µg/mL and 200 µg/mL showed percent scavenging activity of 30.21± 0.008 %, 50.92± 0.013% and 53.942±0.005% respectively. Barks of *Casuarina littorea* showed nitric oxide scavenging activity as 29.008± 0.013%, 41.418±0.010% and 54.017± 0.006% at 50 µg/mL, 100 µg/mL and 200 µg/mL respectively.

Table 2: Total antioxidant activity of Standard (Ascorbic acid) and Test (Chitosan) in different concentrations

Sl.No.	Concentration (µg/mL)	Standard Ascorbic acid (%)	Test Chitosan (%)
1.	50	30.86	29.73
2.	100	41.97	33.78
3.	200	60.49	50
4.	400	79.01	62.16

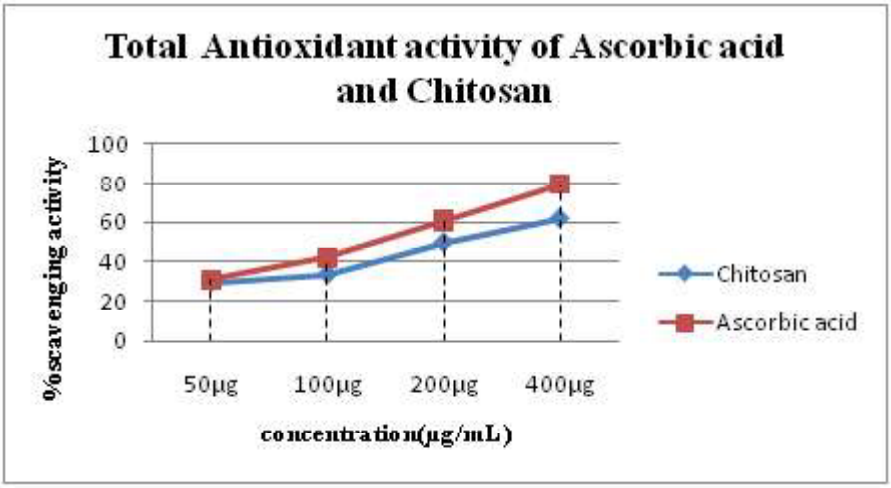

Fig 3: Total antioxidant capacity of different concentration of Chitosan and Ascorbic acid

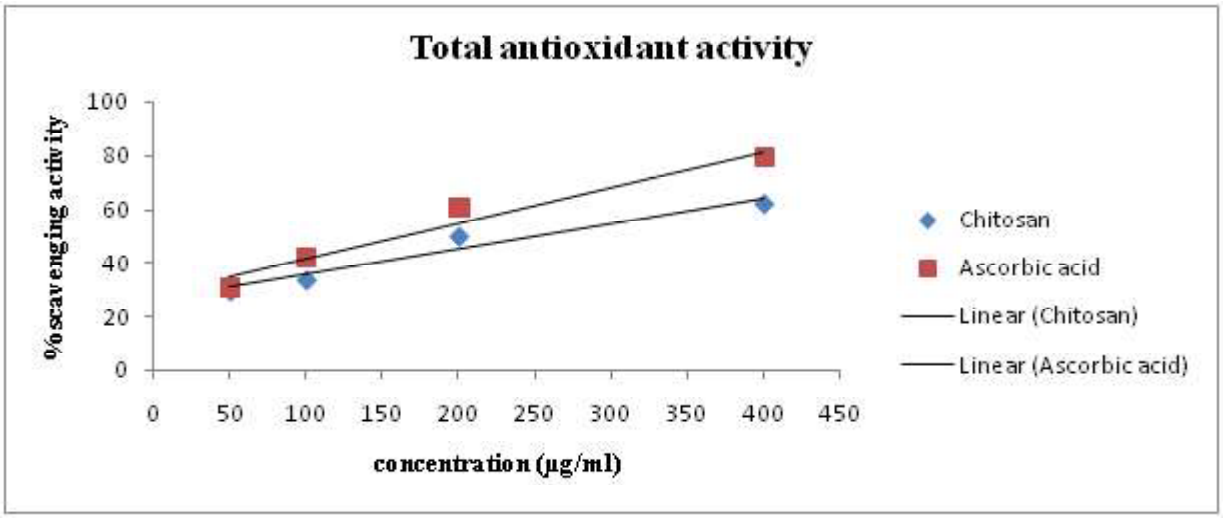

Fig 4: Graph representing Concentration of Ascorbic acid and chitosan used and their percent scavenging activity.

In the present study Table 2 showed the percent of total scavenging activity of Ascorbic acid was 30.86%, 41.97%, 60.49% and 79.01% at concentration of 50 µg/ml, 100 µg/ml , 200 µg/ml and 400 µg/ml respectively. Percent of total antioxidant activity of Chitosan was found to be 29.73 %, 33.78%, 50%, and 62.16 % at 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml concentration respectively.

Chitosan concentration showed positive correlation with its percent scavenging activity, indicating that as concentration of chitosan increased, its Total antioxidant activity also increased with the slope value b=0.094 (R^2= 0.97) (Fig:-4).

Aliyu[13] reported that the total antioxidant capacity of chitosan (TAC) was based on reduction of Mo(VI) to Mo(V) and subsequent formation of green phosphate/ Mo(V) complex at acidic pH.

Most of the studies on calculating Total antioxidant capacity have been done on plant extracts. Aliyu[13] observed that n-butanyl root extract of *Anchomanes difformis* possessed significant total antioxidant capacity equivalent to 90mg/g ascorbic acid at 500 and 1000 µg/mL concentration. Elkhamlichi[14] reported that ethyl acetate extract of the seeds of *Calycotoma villosa* also possessed significant total antioxidant capacity equivalent to 157.6 mg/g ascorbic acid at higher concentration (100 µg/mL).

The antioxidant capacity of chitosan extracted from carapace of *Sartoriana spinigera* against four free radicals showed that chitosan is highly efficient in scavenging the free radicals. Thus, chitosan can be encouraged to be used as a natural antioxidant in pharmaceutical industry.

CONCLUSION

The demand of supplement antioxidants has now bent towards an approach to nature and natural sources. In this study, Chitosan extracted from carapace of *Sartoriana spinigera* proved to have Total antioxidant activity as well as Nitric oxide scavenging activity. This study encourages the use of natural sources of antioxidants such as Chitosan to potentially replace synthetic drugs in the pharmaceutical market.

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