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Research Article

ANTIOXIDANT PROPERTIES OF *PORTUNUS SANGUINOLENTUS* CHITOSAN FABRICATES CRAB SHELL

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ABSTRACT

The chitosan got from the chitin is the second most bottomless polysaccharide delivered after cellulose. It is the fundamental auxiliary segment of the exoskeletons of creatures like shellfish and is found in the cell dividers of growths moreover. Chitosan is delivered primarily from the crab shell squander. The present work is gone for the extraction of chitin from three spotted crab shell *Portunus sanguinolentus* and transformation of chitin into chitosan. The methanolic concentrate of the crab shell of *P. sanguinolentus* was subjected to standard subjective examination and the in-vitro cancer prevention agent movement was assessed by the assurance of aggregate cell reinforcement limit, 1.1-diphenyl-2 picrylhydrazyl (DPPH) radical searching action, Antioxidant action, and Nitric oxide potential. The examinations uncovered that the methanol concentrate of Co could productively rummage the free radicals in a dosage-dependent way. The outcomes were contrasted and the standard cancer prevention agent ascorbic corrosive. Along these lines, additional research might be justified to think about dynamic mixes of *P. sanguinolentus* that give the cancer prevention agent movement. The discoveries displayed here might have suggestions in the populace illness avoidance field.

Keywords: *Portunus sanguinolentus*, DPPH, Total antioxidant, Nitric oxide scavenge, Methanol.

INTRODUCTION

Chitin is one of the most to a great extent accessible inexhaustible modest biopolymer acquired from marine birthplace (Synowiecki & Al Khateeb, 1997). It is biocompatible, biodegradable (Amano & Ito, 1978) and bio-absorbable, with antibacterial and wound mending properties and furthermore with negligible immunogenicity. In this manner, there were a few examinations being directed to uncover its biomedical utilization of chitin. Fittingly, an extremely broad determination of projects in different territories, for example, sustenance building, item examine, microbiology, horticulture, wastewater treatment, tissue designing, bionanotechnology and sedate conveyance have just been accounted for late, chitin and its subsidiaries have just been dissected as presumably of utilization pharmaceutical and were regularly found to indicate negligible harmfulness (van der Lubben *et al*., 2001). Pharmacology and therapeutics to develop various drugs synthesis build new remedies for various diseases (Tamizhazhagan & Pugazhendy, 2017). Asia has abundant species of medicinal and aromatic plants and traditional medicines have practiced in Asia since ancient times. India has made use of medicinal plants to cure ailments of thousands of years (Tamizhazhagan *et al*., 2017a). Quality can be defined as the status of a drug that is determined by identity, purity, content and another chemical, physical, or biological properties, or by the manufacturing processes (Tamizhazhagan *et al*., 2017b). Antioxidant enzymes make up the first line of protection alongside oxidative constant worry and smash up caused by free radicals (Tamizhazhagan & Pugazhendy, 2017).

The crabs rank third after shrimps and lobsters for their regarded fish delicacy and furthermore the estimation of fishery they bolster. The crab fishery in India is quick creating and there is a huge degree for the crab meat because of its delicacy and wholesome extravagance. The crab meat contains a rich measure of protein, vitamins A and D, minerals, glycogen and free amino acids. The

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greater part of the marine crabs happening along the Indian coasts is having a place with the family portunidae. The monetarily vital portunid crabs found along Parangipettai drift are *Scylla serrata, S. tranquebarica, Portunus sanguinolentus, P. pelagicus, Podophthalamus vigil, C. feriata, C. lucifera, C. natator, C. granulata and C. truncata* (John Samuel *et al*., 2004; Varadharajan *et al*., 2009).

Normally happening cancer prevention agent substances additionally require security testing. Alerts with respect to a presumption of security of common cancer prevention agents have been more than once prompted since the reality cell reinforcement originates from a characteristic source does not demonstrate its accepted wellbeing (Hattori *et al*., 1998). Oxidative pressure is associated with the pathology of growth, arteriosclerosis, jungle fever and rheumatoid joint inflammation and could assume a part in neurodegenerative infection and maturing forms (Aruoma, 1997). The increase in free amino acid level suggests that tissues damage probably due to the increased proteolytic activity under toxic stress (Pichaimani *et al*., 2017). The antioxidant GSH is tripeptide-γ-glutamyl-cysteineglycine or reduced glutathione. In living organisms GSH plays an important role in normal cell function (Jayalakshmi *et al*., 2017).

The part of cancer prevention agents has gotten expanded consideration amid the previous decade. Be that as it may, the utilization of such cancer prevention agents has potential wellbeing risk (Park *et al*., 2004). In this way, as of late, interests have been produced for seeking powerful normal cancer prevention agents, since they can shield human body from free radical and retard the advance of numerous endless sicknesses. The hemolymph, in many scavengers, is a dry liquid containing an expansive number of cells or hemocytes and different natural and inorganic constituents. Around 90% of hemolymph is water, which decides its aggregate volume. The hemolymph fills in as a washing medium for different tissues and organs as they do not have an epithelial coating of a genuine coelom. Accordingly, the hemolymph shapes the gathering spot of both the crude materials required and the results of different physiological exercises of the body. Since the hemolymph isn't specifically associated with the outside condition any adjustment in it can be taken as a measure of the physiological condition of the inward condition of the in-place creature. The haemolymph of shellfish have intense antimicrobial peptide indicated different cluster against a few human pathogens (Arul Prakash *et al*., 2011) and assumes a part in have resistance reaction including self or non-self-acknowledgment, cell to cell correspondence, superoxide anion action, melanisation, phagocytosis, cytotoxicity and exemplification (Sahoo *et al*., 2005).

Current scenario regular items from marine examples have a wide range of natural exercises and various restorative applications incorporate antiviral, antibacterial, and antitumor. Cyclic and direct peptides found from marine creatures have expanded our insight about new intense cytotoxic, antimicrobial, particle channels particular blockers, and numerous different properties with novel compound structures related to unique instruments of pharmacological movement (Zheng *et al.*, 2011). There is an expanding enthusiasm for cancer prevention agents, especially in those of free radicals in different maladies. These obsessive and clinical foundations have provoked to research novel and intense cancer prevention agent peptides from crab which are at last of restorative utilize. Henceforth the present examination was meant to distinguish the cancer prevention agent action of hemolymph from delicate and hard-shelled crabs of *Charybdis luciferase*. Biomaterials are those non-living materials utilized as a part of the restorative, biomedical and different fields, meaning to communicate with the organic framework (Wang & Chio, 1998).

A large number of these materials, for example, chitosan, are utilized as successful choices for the substitution of tissues, including bone tissue since they don't present dangers of illness transmission or invulnerable dismissal, and also boundless accessibility and minimal effort. A standout amongst the most vital fields of utilizations of common mixes it is prescription. Such materials would have a few favorable circumstances over-engineered ones. Materials got from nature, have been appeared to yield speedier recuperating with less contradiction in individuals.

Chitosan is framed by deacetylation of chitin. Chitin is a normally happening polysaccharide found in the exoskeleton of shellfish, for example, crabs (Blumenthal & Roseman, 1957). Chitosan advances cross-linkage with different cross-connecting operators, for example, glutaraldehyde, sodium tripolyphosphate (TPP), genipin and so forth. In the past investigation, we utilized copper as another metal particle and complexed it with chitosan. Cu (II) particles could be effectively adsorbed on the chitosan surface at pH 6. As of late, the superb antibacterial property of copper against gram +ve and ¯ve microscopic organisms and cytotoxicity potential against numerous disease cells have been accounted for (Koleva *et al*., 2002). Despite the fact that copper has been appeared to have anticancer movement in certain disease cells, there is no examination on its ecacy in osteosarcoma. Chitin and its subordinates including D-glucosamine-carboxymethyl chitin and Dihydroxypropyl chitin have safe tweaking (immunomodulatory) effects and can influence intrinsic and greedy resistance which prompts increment cell movement and the discharge of cytokines and chemokines (Wanasundara & Shahidi, 1998).

Chitosan additionally happens normally in a few microorganisms, for example, parasites and yeast and is viewed as the biggest biomaterial after cellulose as far as usage and dissemination. Its concoction name is 2-amino-2-deoxy-b-D-glucopyranose and its recipe is ($C_6H_{11}O_4N$). The advancement of business applications for chitosan has advanced. Chitin and chitosan are delivered monetarily in India, Poland, Japan, Thailand, USA, Norway and Australia (Ghorbel *et al.*, 2011). The present study was carried isolated compound chitosan enhanced fabricated have many oxidative functions were the focus.

MATERIALS AND METHODS

Preparation of extract

The *Portunus sanguinolentus* were collected from the fish market at Chidambaram (Lat. 11°.39'N and Long. 79º 69'E) near Vandigate. The skeleton was placed in Ziploc cover and frozen overnight and then subsequently cut into smaller pieces using a meat tenderizer. 100 g of crab shells were ground to get coarse powder and cleaned with water and 0.9% saline by blending the shell powder in a 500 ml measuring utensil with water for a couple of minutes. After this, the shell powders are separated off. This procedure is rehashed to the point when sand and different soils are evacuated. The cleaned crab shell (Figure 1) powders are dried overnight in the drying broiler at 80°C.

Figure1. *Portunus sanguinolentus* crab shell.

Total Antioxidant Activity

The total antioxidant activity of the fractions was determined according to the method of (Prieto *et al*., 1999). 0.3mL of each fraction was mixed with 3.0 mL of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate, and 4Mm ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min in a water bath. The absorbance of all the sample mixtures was measured at 695 nm. Ascorbic acid (100 μg/mL) was used as a standard control.

Determination of the Total Phenolics

The total phenolic content of fractions (bioactive compounds) was determined spectrophotometrically with Folin-Ciocalteu reagent, using a slightly modified method by Junaid *et al*., 2006). The extract of the fraction was mixed with Folin-Ciocalteu reagent (1: 1) and 4mL of sodium carbonate (1M) was added and allowed to stand for 15 minutes. The absorbance was read spectrophotometrically at 765 nm. A standard curve was plotted using different concentrations of gallic acid (standard, 0– 1000 μg/mL) and the total phenolic content of ethenol extract was estimated as μg gallic acid equivalents (GAE)/mg of extract. The reaction was conducted in triplicate, and the results were averaged.

Total Flavonoid content

The Flavonoid content was estimated using the spectrophotometric method (Junaid *et al*., 2006; Viarengo *et al*., 1997). The total flavonoid content was estimated as μg quercetin equivalents/mg of extract. Ethyl acetate extracts of *Portunus sanguinolentus* (250 µl) was mixed with 1.25 mL of distilled water and 75 µl of 5 % $NaNO_2$ solution. After 5 min, 150 µl of 10 % $AlCl_3$ was added. After 6 min, 500 µl of 1M NaOH and 275 µl of distilled water were added to prepare the mixture. The solution was mixed well & the absorbance was read at 510 nm using UV-VIS spectrophotometer.

DPPH Radical Scavenging Activity

The free radical scavenging activity of the metabolite, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method of (Kumaran, 2006) and the absorbance was measured at 517 nm spectrophotometrically (Elico SL 159, India). The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percent of DPPH radical scavenging activity was calculated as,

Inhibition (%) = (A – A0) / A × 100.

Where,

A is the absorbance of the control.

A0 is the absorbance of the metabolite/standard.

Nitric Oxide Radical Scavenging Activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined using the Griess Illosvoy reaction. 2ml of 10mM sodium nitroprusside in 0.5ml of phosphate buffer saline (pH 7.4) was mixed with 0.5ml of extract and standard (curcumin) at various concentrations and the mixture was incubated at 25°C for 150min. From the incubated mixture 0.5ml was taken out and added into 1ml of sulfanilic acid reagent and incubated for 5min. Finally, 1ml naphthyl ethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring absorbance at 540 nm using a spectrophotometer. Nitric oxide radical scavenging activity was calculated using the formula,

Inhibition (%) = (Ao – Ae) / Ao × 100.

Where,

Ao is the absorbance of the control.

Ae is the absorbance of the extract/standard.

RESULTS

Total Phenol and Flavonoid content were estimated using gallic acid and quercetin as standard references. The Stigmasterol Trimethylsilyl Ether produced 0.321 ± 0.001

mg gallic acid equivalents as phenol content and 0.014 ± 0.0002 mg quercetin equivalents as phenol and flavonoid content (Table 1).

Table 1. Total phenol and flavonoid content of Stigmasterol Trimethylsilyl Ether.

Compound Name	Total flavonoid content mg quercetin equivalent	Total phenol content mg Gallic acid equivalent
Stigmasterol Trimethylsilyl Ether	0.014 ± 0.002	0.321 ± 0.001

The value represents Mean ± SD.

Free radicals are having a lone pair of electron containing groups which produced harmful effects to the living beings by causing oxidation mechanism which results in the lethal effect. The antioxidants are the agent which prevent oxidation process being produced by this kind of free radical such as OH, O, NO, and Fe^{+}.

In total antioxidant activity **(**Figure 2), the Stigmasterol Trimethylsilyl Ether has produced dose dependent manner increasing activity in the range of 65.853 ± 0.001, 72.097 ± 0.001 and 78.341 ± 0.001 respectively (Table 2).

Stigmasterol Trimethylsilyl Ether exhibited a significant dose-dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid is presented in (Figure 3). Stigmasterol Trimethylsilyl Ether moderately inhibited nitric oxide in dose-dependent manner (Figure 4), with ascorbic acid.

Table 2. The total antioxidant activity of Stigmasterol Trimethylsilyl Ether

Assay list	Sample name	Total Anti-Oxidant Activity		
		100µg/ml	200µg/ml	300µg/ml
Total antioxidant	Stigmasterol Trimethylsilyl Ether	65.853 ± 0.001	72.097 ± 0.001	78.341 ± 0.001
	Vit – C	75.099 ± 1.0	82.621 ± 1.0	87.203 ± 0.57
DPPH Assay	Stigmasterol Trimethylsilyl Ether	60.53 ± 0.001	59.85 ± 0.001	58.57 ± 0.001
	Vit – C	59.92 ± 1.0	32.69 ± 1.0	37.75 ± 0.57
Nitric Oxide Radical Scavenging	Stigmasterol Trimethylsilyl Ether	79.34 ± 0.001	82.03 ± 0.001	83.19 ± 0.001
	Vit – C	23.09 ± 1.0	40.21 ± 1.0	56.03 ± 0.57

The value represents Mean ± SD.

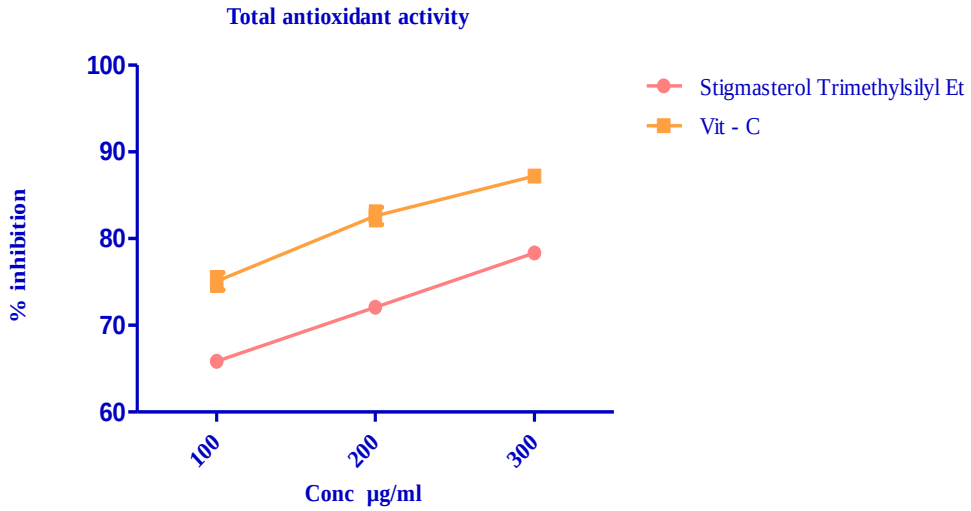

Figure 2. The Total antioxidant activity of Stigmasterol Trimethylsilyl Ether.

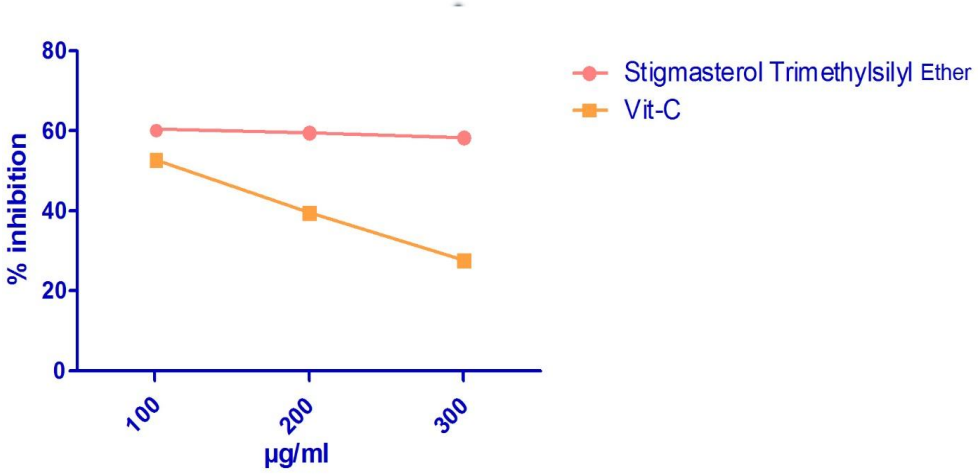

Figure 3. DPPH Scavenging activity (Each value represents Mean ± SD, n=3).

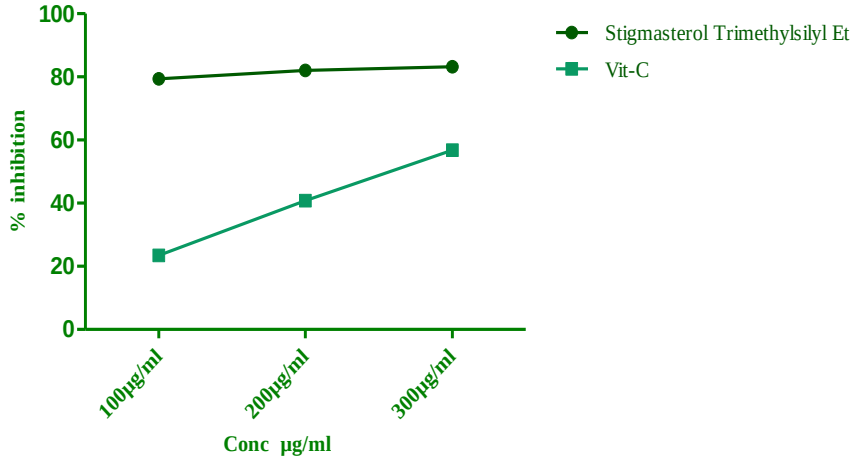

Figure 4. Nitric Oxide Radical Scavenging Activity (RSA).

DISCUSSION

Antioxidants due to their scavenging activity are useful for the management of those diseases. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts (Koleva *et al.*, 2002; Kumar *et al*., 2008). The therapeutic potential of natural medicinal plants as an antioxidant in reducing such free radical-induced tissue injury, suggests that many plants have antioxidant activities that can be therapeutically useful (Kanatt *et al*., 2007). India has made use of medicinal plants to cure ailments of thousands of years (Tamizhazhagan & Pugazhendy, 2017). Excellence defined as the status of a drug that is strongly minded by identity, purity, content and another chemical, physical, or biological properties, or by the developed processes (Tamizhazhagan *et al.*, 2017a). Antioxidant enzymes make up the first line of protection alongside oxidative steady worry and shatter up caused by free radicals (Tamizhazhagan *et al*., 2017a), compared to another source of laboratory animals like fish's vegetation and vertebrate animals to expand pharmacology and therapeutics various drugs synthesis build new remedies for various diseases (Tamizhazhagan *et al.*, 2017b). These differences might be due to their different antioxidant mechanisms or variations in their ability to scavenge free radicals. A fair correlation between total phenolic content and antioxidant activity was also observed. These observations clearly indicated a cross-linkage between phenolics and antioxidant activity. However, a large number of bio active compound groups are implicated for antioxidant activity (Tamizhazhagan *et al.*, 2017a). Ascorbic acid acts as a chain-breaking antioxidant impairs the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine. These differences might be due to their different antioxidant mechanisms or variations in their ability to scavenge free radicals. A fair correlation between total phenolic content and antioxidant activity was also observed.

CONCLUSION

These observations clearly indicated a cross-linkage between phenolics and antioxidant activity. However, a large number of bioactive compound groups are implicated for antioxidant activity. The results based on the related to other finding more efficacies have the chitosan fabricated freshwater crab of *Portunus sanguinolentus*.

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