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Studies on antioxidant properties of starfish *Luidia maculata* (Muller & Troschel, 1842) off Parangipettai, Southeast coast of India

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PEER REVIEW

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Comments

This is a valuable research work in which authors have demonstrated the antioxidant activity of *L. maculata*. The activity was assessed based on fractions collected from crude ethanol extract. *L. maculata* was found to be of promising *in vitro* antioxidant potentials.
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ABSTRACT

Objective: To investigate the antioxidant properties of starfish *Luidia maculata*.
Methods: The crude ethanol extract was partially purified using liquid-liquid partition and size exclusion chromatography. The antioxidant properties of partially purified fractions were determined using the following methods: determination of total phenolic content, DPPH radical scavenging assay, hydrogen peroxide radical scavenging assay and reducing power assay.
Results: Among the five fractions, collected fraction 3, 4 and 5 exhibited antioxidant potential. Total phenolic content was high (360 μg GAE/g) in fraction 3. In DPPH assay, fraction 3 showed the highest IC_{50} value 0.27 mg/mL and hydrogen peroxide scavenging assay showed the highest IC_{50} value of 0.07 mg/mL in fraction 5. Whereas, fraction 3 showed the highest absorbance (0.3) in reducing power assay when compared to fraction 4 and 5.
Conclusions: In conclusion, the present study shows the extract of starfish *Luidia maculata* (F3) possesses good antioxidant activity.

KEYWORDS
Antioxidant, Total phenolic content, Starfish

1. Introduction

Antioxidants are the substance which protects our body from damages caused by free radicals produced during normal physiological processes. Antioxidants are considered as important nutraceuticals on account of many health benefits[1]. Free radicals contain unpaired electrons in their outer orbit which readily reacts with protein, lipid, DNA and other biomolecules that consequently causing damage to the cell structure and interfering with normal metabolism. The accumulation of free radicals in our body is thought to be the cause of many diseases like cancer, atherosclerosis, Alzheimer's disease, Parkinson's disease, diabetes, neurodegenerative and cardiovascular disease[2,3]. Antioxidants are produced in our body as the first line of defense to fight against the free radicals. The common antioxidants are vitamins, enzymes such as superoxide dismutase, glutathione peroxidase, catalases and some phytochemicals. Antioxidants act as free radical scavengers by preventing and repairing the damages caused by reactive oxygen species, and therefore antioxidants can enhance the immune defense and lower the risk of cancer and degenerative diseases[4]. Due to the adverse side effects of synthetic antioxidants, searching for natural and effective antioxidants has become crucial[5]. Research on natural antioxidant is now an intensive field due to their safety, nutritional and therapeutic values. In the search of natural antioxidants, a little attention has been paid to marine

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invertebrates. A number of studies have shown that the peptides derived from various marine protein hydrolysates such as fish, blue mussel, conger eel, microalgae and squid acted as potential antioxidants[6]. The ethyl acetate extract of green mussel *Perna viridis* shows promising results on antioxidant activity[7].

Echinoderms are the impressive sources of bioactive compounds from marine realm. They are subdivided in five classes, including crinoids (sea lilies and feather stars), asteroids (starfishes), ophiuroids (brittle stars), echinoids (sea urchins and sand dollars) and holothuroideas (sea cucumbers). Starfishes are exclusively marine invertebrate belong to the class for Asteroidea, phylum Echinodermata. Steroidal glycosides are the predominant compound present in starfish and are responsible for its general toxicity. More than 500 polyhydroxylated steroid compounds were described from different starfish species[8]. These glycosides possess many bioactivities like cytotoxic, haemolytic, antimicrobial, expectorant, antitussive, antiasthmatic, analgesic and other activities. Other than steroidal glycosides, starfish contains various bioactive compounds like cerebrosides, polysaccharides and gangliosides[9,10]. Previous studies revealed the antioxidant activity of some echinoderms like sea urchins and sea cucumbers. The enzymatic hydrolysate and polyhydroxylated naphthoquinone pigments of purple sea urchin *Strongylocentrotus nudus* showed promising antioxidant activities[11,12]. Polyhydroxylated naphthoquinone pigments from shells of purple sea urchin *Anthocidaris crassispina* possess antioxidant property[13]. The extract isolated from different body parts of green sea urchin *Strongylocentrotus droebachiensis* shows interesting antioxidant activity[14]. The main objective of the work is to investigate the antioxidant properties of the starfish *Luidia maculata* (*L. maculata*). Luidiidae is a widespread species in coastal waters all over the world. Partially purified fractions of ethanol extract of *L. maculata* were investigated for antioxidant activity.

2. Materials and methods

2.1. Chemicals and reagents

Folins phenol reagent and 1, 1-diphenyl 2-picrylhydrazyl (DPPH), ascorbic acid, gallic acid and hydrogen peroxide were purchased from Sigma Chemical Co. All other reagents and solvents used in this experiment were of analytical grade.

2.2. Collection

The starfish *L. maculata* was collected from Mudasalodai fish landing center, Southeast coast of India and brought to the laboratory with seawater in fresh condition and immediately air-dried for further analysis.

2.3. Extraction and purification

For the extraction, 1 kg of dried starfishes was kept in 3 L of 70% ethanol for 5 d. The solvent was then removed after squeezing the starfish and filtered through Whatman No. 1 filter paper. The solvent was evaporated at low pressure by using a rotary evaporator (Lark Innovative, model LICB-7) at 35 °C. The resultant crude was finally dried in vacuum desiccators and stored at 4 °C for further use[15].

The ethanol extract of the starfish *L. maculata* (5 g) was dissolved in distilled water and partitioned using cyclohexane and *n*-butanol. The *n*-BuOH extracts were evaporated under reduced pressure to give glassy material (1.5 g). The *n*-BuOH extract was chromatographed on Sephadex G-25 column (14.6 ×1.3) using MeOH: H_2O (2:1) as eluant and 5 fractions were collected at the flow rate of 15 mL/h.

2.4. Antioxidant activity

2.4.1. Determination of total phenols

Total phenolic content was assessed by using the Folin-Ciocalteu phenol reagent according to the modified methods of Mamelona *et al*[16]. A total of 0.1 mL of extracts (100 μg/mL) was transferred into the test tubes and their volumes were to 0.5 mL with distilled water. Then 0.25 mL of Folin-Ciocalteau reagent (1:10 v/v) and 1.25 mL of 20% aqueous sodium carbonate solution were added to the extracts and vortexed. The absorbance of blue colored mixtures were recorded after 40 min at 725 nm against a blank containing the same mixture except Folin-Ciocalteu reagent replaced with distilled water. The total phenolic contents were expressed as gallic acid equivalents in milligrams per gram of extract, using a standard curve generated with 200-1 200 μg of gallic acid.

2.4.2. DPPH radical scavenging assay

DPPH radical scavenging assay was carried out following the methodology of Peralta *et al*[17]. A total of 0.1 mL of different concentrations of extracts were taken in different tubes, 2.5 mL of 0.1 mmol/L methanolic solution of DPPH was added to the tubes and shaken vigorously. And 0.1 mL of ascorbic acid (10 mg/mL) was used as standard and 0.1 mL of methanol was used as blank. All the tubes were incubated at room temperature for 30 min and the absorbance was measured using spectrophotometer at 517 nm. The percentage radical scavenging activity was calculated using the following formula:

%radical scavenging=(Control OD-Sample OD/Control OD)× 100

2.4.3. Hydrogen peroxide radical scavenging activity

The hydrogen peroxide scavenging ability of the fractions was determined according to the method of Gulcin *et al*[18]. A solution of hydrogen peroxide (40 mmol/L) was prepared in phosphate buffer. Then 1 mL of extracts was added to 2 mL of 40 mmol/L hydrogen peroxide. The absorbance value of the

reaction mixture was recorded at 230 nm. A blank solution contained phosphate buffer saline without hydrogen peroxide and the standard contains 1 mL of ascorbic acid (10 mg/mL) instead of extract.

%radical scavenging=(Control OD−Sample OD/Control OD)× 100

2.4.4. Reducing power assay

The reducing power of the extract was measured using the methodology of Zhu *et al*[19]. A total of 1 mL of sample was mixed with 1 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 1 mL of potassium ferric cyanide (1%). Following incubation at 50 °C for 20 min, 1 mL of trichloroacetic acid (10%) was added to the mixture, vortexed and centrifuged at 2000 r/min for 10 min. The upper layer of solution (2 mL) was collected and mixed with 2 mL of deionized water and 0.3 mL of ferric chloride (0.1%). Following incubation at room temperature for 10 min, the absorbance of the mixture was measured at 700 nm. Higher absorbance indicated greater reductive potential. Gallic acid was used as a positive control. All the experiments were carried out in triplicate.

2.5. Statistical analysis

All the experiments were carried out in triplicate. The data were recorded as mean±SD.

3. Results

3.1. Determination of total phenolic content

The phenolic content in fraction 3, 4 and 5 was (360.00 ±40.00), (116.00±25.17) and (87.00±30.55) μg gallic acid equivalents per gram of extract respectively (Figure 1).

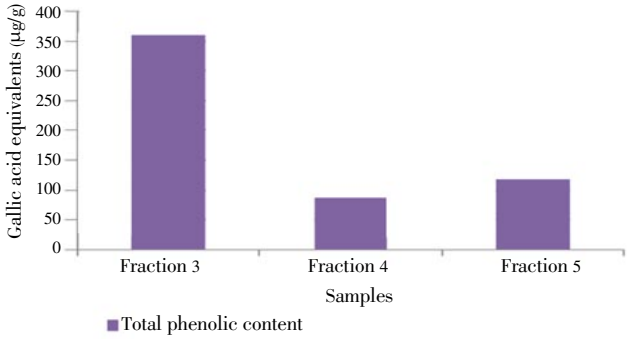

Figure 1. Total phenolic content in *L. maculata*.

3.2. DPPH radical scavenging assay

In DPPH radical scavenging assay, fraction 3 showed the highest radical scavenging activity of (19.60±0.25) at 100 μg/mL concentrations and the lowest (4.10±0.25) activity was observed at 20 μg/mL concentrations. The radical scavenging effect was directly proportional to concentration of the extract (Figure 2).

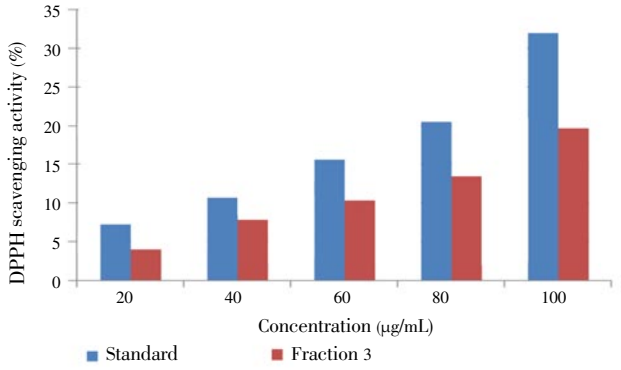

Figure 2. DPPH scavenging activity of starfish *L. maculata*.

3.3. Hydrogen peroxide radical scavenging activity

In hydrogen peroxide radical scavenging assay, fraction 5 showed (70.20±0.15) percentage of radical scavenging activity at 100 μg/mL whereas (12.60±0.20) percentage at 20 μg/mL concentration. The scavenging activity was dose dependent (Figure 3).

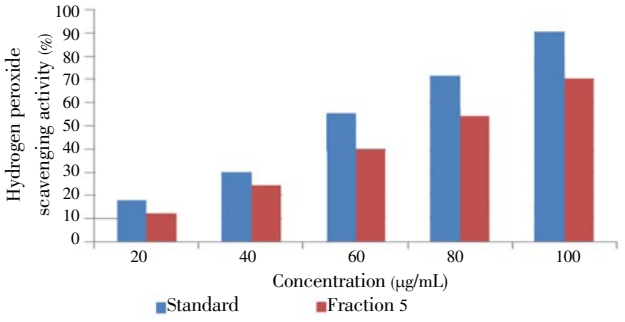

Figure 3. H_2O_2 scavenging activity of starfish *L. maculata*.

3.4. Reducing power assay

The optical density value of fraction 3 was (0.30±0.12) at 700 nm and the lowest optical density value (0.07±0.04) was observed in fraction 5 (Figure 4).

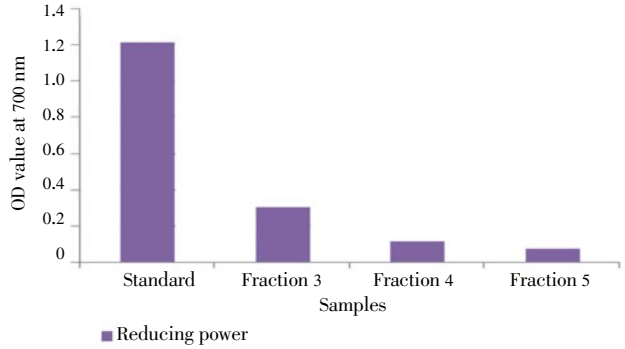

Figure 4. Reducing power of starfish *L. maculata*.

4. Discussion

Phenolic compounds are the naturally occurring antioxidants mostly present in plants and also in some marine invertebrates like sea cucumber, sea urchins, *etc*. At least 8000 different bioactive compounds are considered to be polyphenols. The total phenolic content of sea cucumber,

Curcumaria frondosa varied from (22.5±4.0) to (236.0±36.3) mg of GAE/100 g of dry weight depending on tissues and extracts. The digestive tract extract contains the highest phenolic content while gonad extract contains the lowest phenolic content[16]. Total phenolic content in aqueous extract of *Holothuria scabra*, *Holothuria leucospilota*, *Stichopus chloronotus* were (4.85±0.04), (9.70±0.09) and (8.27±0.04) GAE mg/g, respectively and in case of organic solvent extracts the phenolic content was (1.53±0.05), (2.91±0.10) and (1.66±0.11) GAE mg/g, respectively[20]. The total phenols in digestive tract of *Strongylocentrotus droebachiensis* varies from (25.7±6.0) to (74.4±29.4), whereas in gonad extract it varies from (14.4±4.3) to (30.6±12.3) mg GAE/100 g[14]. The highest phenolic content (360.00±40.00) μg GAE/g) was observed in fraction 3 and the lowest (87.00±30.55) μg GAE/g) in fraction 5. The results showed the reasonable amount of phenolic content in starfish *L. maculata* which can be a good source of antioxidant.

In case of DPPH assay, fraction 3 possesses better DPPH radical scavenging activity when compared to other fractions. The highest activity (19.60±0.25) was found in 100 μg/mL concentration which is compared to the DPPH radical scavenging activity of standard ascorbic acid (31.9±0.2) at 100 μg/mL concentration. The IC_{50} was calculated as 273 μg/mL. The result shows the dose dependent activity, *i.e.* the highest activity is observed in highest concentration. The polyhydroxylated naphoquinone pigments from spines of *Strongylocentrotus nudus* and shells of *Anthocidaris crassispina* exhibit good DPPH scavenging percentage with increasing amount of pigments[12,13]. The EC_{50} value of DPPH activity of different angular naphthopyrone compounds isolated from the crinoids *Oligometra serripina* varied from 22.5 μg/mL to 46.55 μg/mL[21]. The aqueous extract of *Stichopus chloronotus* shows the IC_{50} value equal to 2.13 mg/mL for DPPH scavenging activity[20]. The DPPH radical scavenging percentage of various peptide fractions from *Strongylocentrotus nudus* gonad extract differs from (20.64±0.64) to (69.04±0.81)[11]. Different solvent fractions of *Stichopus japonicus* show DPPH scavenging activity in the range between 30%-40%[22].

The highest hydrogen peroxide scavenging activity was observed in fraction 5, *i.e.* (70.20±0.15) at 100 μg/mL and the EC_{50} value was 73 μg/mL. The previous study has demonstrated the hydrogen peroxide scavenging activity of pigments sea urchin *Anthocidaris crassispina*[13]. The electron-donating potential of a compound, termed as reducing capacity, may serve as a significant indicator of its potential antioxidant activity[23]. Reduction of Fe^{3+} to Fe^{2+} is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action[24]. In case of reducing power assay, highest OD value was observed in fraction 3 (0.30±0.04). The previous study on enzyme hydrolysate from *Strongylocentrotus nudus* has demonstrated the absorbance that ranged between (0.289±0.007) to (0.731±0.005)[11]. The absorbance value of pigments from *Strongylocentrotus nudus* exhibited a dose dependent effect[12].

The result of the present study reveals the potential of starfish extract as an antioxidant. Among the three fractions tested, fraction 3 exhibits good antioxidant activity in all the four assays. Generally the epidemiological evidence reveals that the low antioxidant intake or low antioxidants level in blood increased the risk of cancer[25]. It is believed that antioxidants exert their protective effect by decreasing oxidative damage to DNA and by decreasing abnormal increases in cell division. Not only cancer but most of the diseases like cardiovascular disease, heart diseases, diabetes mellitus, *etc.* are consistently related to antioxidants. Consequently an antioxidant compound can act as an anticancer agent and also as potential a medicinal compound. This study concludes that the extract of starfish *L. maculata* possesses potent antioxidant property. The result also supports the previous studies on presence of steroidal glycosides and glucocerebrosides that may be responsible for the antioxidant activity. Further research on this aspect facilitates to find an excellent bioactive compound from starfish *L. maculata*.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Antioxidants are very important for human health. Free radicals contains unpaired electrons in their outer orbit which readily reacts with protein, lipid, DNA and other biomolecules consequently causing damage to the cell structure and interfering with normal metabolism. The accumulation of free radicals in our body is thought to be the cause of many diseases like cancer, atherosclerosis, Alzheimer's disease, Parkinson's disease, diabetes, neurodegenerative and cardiovascular disease.

Research frontiers

The present work deals with antioxidant properties of starfish *L. maculata*. Purified fractions were tested in four different *in vitro* antioxidant assays. Among them fraction 3 showed good antioxidant potential.

Related reports

Research on natural antioxidant is now an intensive field due to their safety, nutritional and therapeutic values.

Antioxidant activity of some echinoderms like sea urchins (*Anthocidaris crassispina*, *Strongylocentrotus nudus*) and sea cucumbers showed promising antioxidant activities.

Innovations and breakthroughs

In the present study, authors made an attempt to investigate the antioxidant properties of starfish *L. maculata* with DPPH radical scavenging assay, hydrogen peroxide radical scavenging assay and reducing power assay.

Applications

The starfishes contain rich source of steroidal compounds. In fish landing center the starfishes are consider to be bio-waste materials. In the present study these bio-waste materials were used for biological applications of antioxidant properties.

Peer review

This is a valuable research work in which authors have demonstrated the antioxidant activity of *L. maculata*. The activity was assessed based on fractions collected from crude ethanol extract. *L. maculata* was found to be of promising *in vitro* antioxidant potentials.

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