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Assessment of free radical scavenging activity from ethanolic extracts of arms and suckers of marine cephalopod, *Uroteuthis sibogae*

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Abstract

The present study assessed the free radical scavenging capacity of the ethanolic extracts of arms and suckers of marine cephalopod *Uroteuthis sibogae*. The free radical scavenging properties of 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, Superoxide anion radical scavenging activity and Nitric oxide radical scavenging activity at five different concentrations was assessed. The inhibition was compared with respective synthetic antioxidants. Free radical scavenging at the highest concentration inhibition yield for DPPH was 51.72 %, Superoxide anion radical scavenging activity 60.92 % and Nitric oxide radical scavenging activity 53.03 % was reported. The results from the present study will be helpful to develop nutraceutical supplements obtained from the bioactive compounds of the arms and suckers the cephalopod species taken for the study. This would provide pharmacophore leads from molluscan species in combating free radical chain formation and being a source of target for degenerative and life style modified disease management.

Keywords: *Uroteuthis sibogae*, arms and suckers extract, Free radical scavenging activity

Introduction

Globally, the value and demand for cephalopod fisheries are constantly increasing. There aren't many studies, that attempt to explain the size and reach of the world's cephalopod trade. The percentage of the world's landings in capture fisheries that come from cephalopod fisheries has been progressively rising in recent decades. The global demand for squid fisheries as an export food source is rising tremendously ^[1].

Numerous marine compounds have chemical structures that result in unique pharmacological characteristics and strong biological activity ^[2]. The pharmaceutical industry has effectively created a number of bioactive metabolites that have been identified through various discovery initiatives ^[3]. Marine invertebrates are a remarkable source for novel bioactive chemicals and have been identified as a source of potential anti-microbial, anti-inflammatory, and anti-tumor medications since they rely entirely on innate immune pathways for host defence ^[4].

Apart from their nutritional value and nutritional advantages, marine molluscs, which belong to the Phylum Mollusca, are the most prominent group in charge of finding many natural products among marine invertebrates that have been used as a major source to develop therapeutic drugs ^[5].

Materials and Methods

- **Collection of samples:** The cephalopod species *Uroteuthis sibogae* were collected from Kasimedu fish landing centre during the month of March 2024. The whole animal specimens were selected based on same size and physical appearance. About two kg of the squid specimen were conveyed to the laboratory in insulated boxes with dry ice to maintain freshness of the animal. Using running tap water, the sand, mucus and debris were rinsed out of the body parts thoroughly. The skin, ink gland, pen and the tentacles was precisely removed and discarded. The arms along with their suckers was washed with distilled water. The tissues were further processed for preparation of ethanolic extract.
- **Identification and Authentication of the species:** Whole animal specimen transported to the laboratory and were identified using FAO identification keys as described ^[6].

International databases and authorised taxonomic references were used to identify the specimens morphologically and anatomically (FAO, 1984). The cephalopods were authenticated by Zoological Survey of India, Marine Biological Regional Centre, Santhome, Chennai. Authentication ID: F.No 4-49/2024-2025/Tech/426 dated 13/05/2024.

Processing of samples

Preparation of ethanolic extract: The arms and suckers was removed using sterile forceps and washed with distilled water and cut into small pieces. Using 10 ml of ethanol, 10g of the tissue were homogenized by using mortar and pestle following aseptic techniques. To the homogenate 100 ml ethanol was added and incubated for 72 hours at room temperature before further processing.

Concentration of the extract: This ethanolic extract was centrifuged at 10000 rpm for 10 min using an analytical centrifuge. The resulting supernatant was gathered and vacuum-concentrated at 30 °C in a Buchi rotary evaporator. The extract was filtered through Whatman No. 1 filter paper after the solvents were concentrated in a rotating evaporator with lowered pressure. The extracted contents were transferred to petri plates and allowed to evaporate to form a dark brown, sticky, viscous residue. Further, analysis was conducted using the crude residue.

Determination of free radical scavenging activity of ethanolic extracts of arms and suckers in *Uroteuthis sibogae*

2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity: DPPH radical scavenging activity was determined (7). 1.0 mL of the sample was added to 1.5 mL of 0.15 mM DPPH in 95% (v/v) ethanol was added to the sample. The mixture was mixed vigorously using a vortex mixer and allowed to stand at room temperature in the dark for 30 min. Antioxidants react with DPPH, and convert it to a yellow coloured compound, a-diphenyl-β-picryl hydrazine. The absorbance of the resulting solution was measured at 517 nm by UV spectrophotometer. The blank was prepared similarly using deionized water. A standard curve was prepared using butylated hydroxyl toluene as standard. This activity is given as the percent of DPPH

radical scavenged.

Superoxide anion scavenging assay: This was assayed by the nitroblue tetrazolium reduction method [8]. 1.2 mL of sodium pyrophosphate buffer, 0.1 mL of phenazine methosulphate and 0.3 mL of nitroblue tetrazolium, 0.2mL of the sample ethanolic extract and distilled water in a total volume of 2.8 mL constitute the assay mixture. The reaction was initiated by the addition of 0.2 mL NADH. The mixture was incubated at 30 °C for 90 sec and arrested by the addition of 1 ml of glacial acetic acid. The reaction mixture was shaken with 4 mL of n-butanol. The mixture was allowed to stand for 10 min and centrifuged. The intensity of the chromogen in the n-butanol layer was measured at 560 nm in a spectrophotometer. Percentage of free radical scavenging activity is expressed as 50% incubation of NBT reduction in one minute. Ascorbic acid was used as a standard

Nitric oxide radical scavenging activity: Radical scavenging was assayed by Griess reagent (9). For this measurement, the sample was dissolved in distilled water. Tubes were incubated at 29°C for three hours with varying concentrations (100–400µg/ml) of ethanol extract in sodium nitroprusside (5mM) in standard phosphate buffer saline (0.025M, pH 7.4). The control experiment was made in the same way but without the test chemicals and with the same amount of buffer. One millilitre of Griess reagent was added to the incubated samples after three hours. At 550 nm, the spectrophotometer recorded the absorbance of the colour formed when nitrite was diazotised with sulfanilamide and then coupled with naphthylethylenediamine hydrochloride. Ascorbic acid, which was used as a standard in contrast to ethanolic extract, underwent the same process. An inhibition percentage was computed.

Results

The present analysis showed that ethanolic extract from arms and suckers of *Uroteuthis sibogae* possess DPPH radical scavenging activity towards five concentrations at 200µl, 400µl, 600 µl, 800µl and 1000 µl. The increase in concentration increased the percentage radical scavenging activity (Table-1; Fig- 1).

Table 1: DPPH radical scavenging activity of ethanolic extract of arms and suckers of *Uroteuthis sibogae*

Concentration (µg/ml)	Test sample extract % radical scavenging activity	Butylated hydroxyl toluene - Standard % radical scavenging activity
200	16.66 ± 0.58	77.09 ± 0.59
400	22.81 ± 0.54	81.42 ± 0.62
600	31.06 ± 0.64	88.55 ± 0.57
800	36.88 ± 0.67	90.07 ± 0.83
1000	51.72 ± 0.65	97.69 ± 0.73
IC ₅₀	1.031	1.744

Values are average Mean ± SD of three (n = 3) independent analysis of the extract

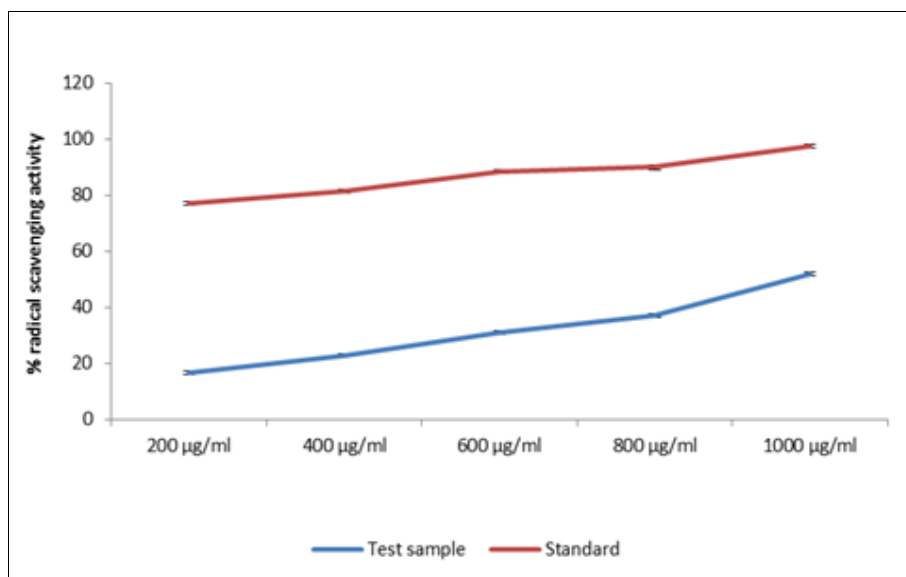


Fig 1: DPPH radical scavenging activity of ethanolic extract of arms and suckers of *Uroteuthis sibogae*

The present scavenging activity showed that ethanolic extract from *Uroteuthis sibogae* possessed antioxidant activity towards five concentrations at 20µl, 40µl, 60 µl,

80µl and 100µl which was concentration dependent. Ascorbic acid was used as standard (Table-2; Fig- 2).

Table 2: Superoxide anion radical scavenging activity of ethanol extract of arms and suckers of *Uroteuthis sibogae*

Concentration (µg/ml)	Test sample extract % radical scavenging activity	Ascorbic acid – Standard % radical scavenging activity
20	17.79 ± 0.61	47.90 ± 0.80
40	30.28 ± 0.80	55.34 ± 0.87
60	41.00 ± 0.62	57.70 ± 0.41
80	50.89 ± 0.34	67.22 ± 0.52
100	60.92 ± 0.47	84.25 ± 0.91
IC ₅₀	78.37	30.48

Values are average Mean ± SD of three (n = 3) independent analysis of the extract

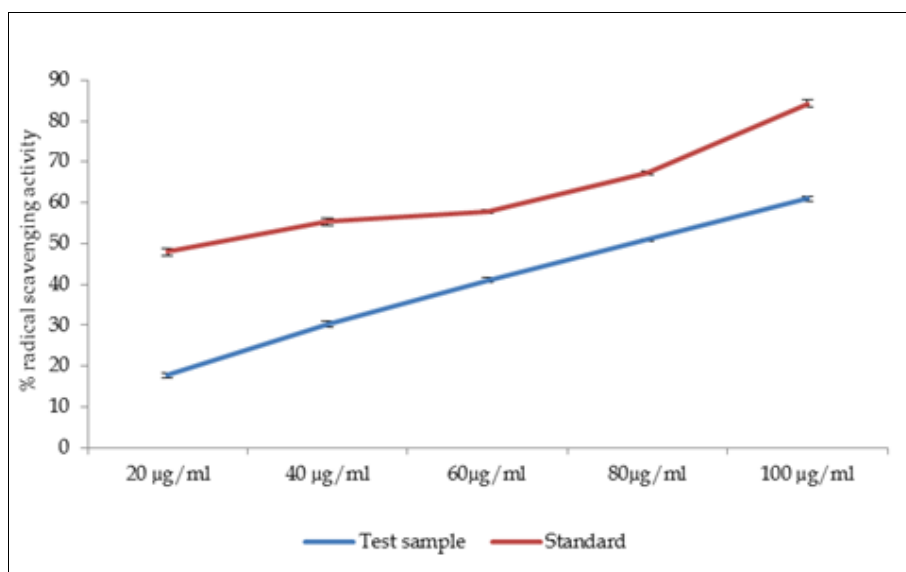


Fig 2: Superoxide anion radical scavenging activity of ethanolic extract of arms and suckers of *Uroteuthis sibogae*

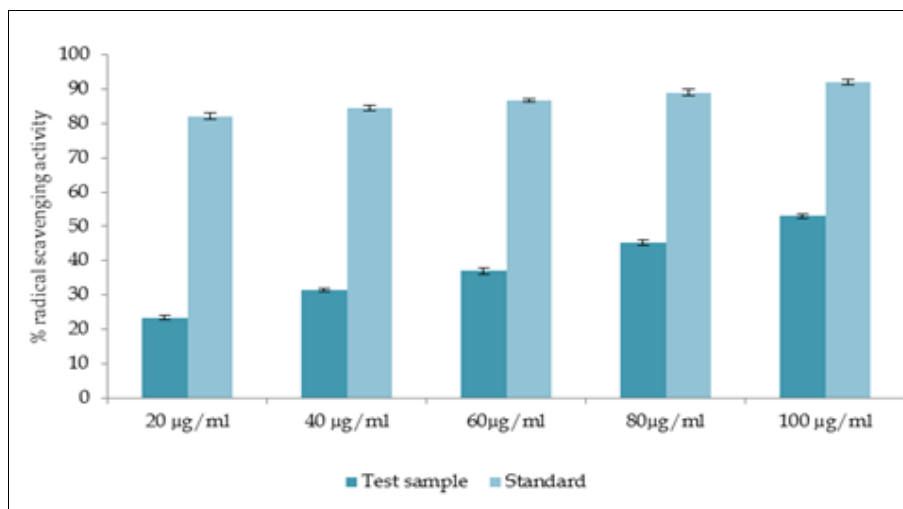
Ethanolic extract from *Uroteuthis sibogae* possessed free radical scavenging activity towards five concentrations at 20µl, 40µl, 60µl, 80µl and 100µl. The results obtained were

concentration dependent. Standard used was ascorbic acid to compare with the test extract (Table-3; Fig-3).

Table 3: Nitric oxide radical scavenging activity of ethanol extract of arms and suckers of *Uroteuthis sibogae*

Concentration ($\mu\text{g/ml}$)	Test sample extract % radical scavenging activity	Standard - Ascorbic acid % radical scavenging activity
20	23.35 ± 0.68	82.14 ± 0.81
40	31.52 ± 0.42	84.46 ± 0.79
60	37.04 ± 0.94	86.62 ± 0.43
80	45.16 ± 0.80	89.06 ± 0.96
100	53.03 ± 0.66	92.06 ± 0.85
IC ₅₀	92.82	294.742

Values are average Mean $\pm$ SD of three ($n = 3$) independent analysis of the extract

**Fig 3:** Nitric oxide radical scavenging activity of ethanol extract of arms and suckers of *Uroteuthis sibogae*

Discussion

It has been demonstrated that marine organisms are abundant in bioactive chemicals that improve human health and could lead to new developments in pharmacology [11]. Molluscs were found to include natural bioactive substances with particular kinds of activity, including peptides, sterols, terpenes, polypropionates, nitrogenous chemicals, macrolides, prostaglandins, fatty acid derivatives, and alkaloids [12].

Multiple mechanisms, including reductive capacity, radical scavenging activity, and the inhibition of free radical oxidation through the termination of radical chain reactions, chelation of transition metals, and stimulation of antioxidative enzymes, have been used to achieve the antioxidant activity [13]. Based on the IC₅₀ calculation, ethanolic extract exhibited more efficient and appreciable radical scavenging activity when compared with the respective synthetic antioxidant compounds.

According to the current study, *Uroteuthis sibogae* arms and suckers have a high DPPH scavenging capacity in ethanolic extract that is based on the concentration. A radical containing an odd electron, DPPH, combines with hydrogen that has been given by an antioxidant. The absorbance drops when the DPPH radical gains one additional electron [14]. Test samples' reactivity with a stable free radical is determined by the assay. The DPPH radical was scavenged by electron donation, which is why test samples reduced the absorbance of the radical. Ethanolic skin extracts of *Uroteuthis sibogae* have shown comparable results in terms of antioxidant potential and DPPH scavenging activity [15].

The result of the present study shows that crude extract of arms and suckers of *Uroteuthis sibogae* had the potential activity of scavenging superoxide anions. This indicates that the crude extract of tissue sample has a good source of

natural antioxidants. A direct nitric oxide free radical scavenging effect to reduce the quantity of nitrite produced from the breakdown of sodium nitroprusside may be the cause of the suppression of nitric oxide free radical release [16].

For the development of marine-derived bioactive chemicals to be successful as innovative medicines in the treatment or prevention of chronic diseases, new technologies and researchers are essential.

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