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Antioxidant activity of *Loligo duvauceli* ink extract

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Abstract
Among the marine organisms the cephalopod constitute a large amount and they are well known for their defense mechanisms by jetting escape movement by changing their colour. The main class of cephalopod include squid and cuttle fish. The ink sac of *Loligo duvauceli* generated as a byproduct during processing industry and it has low market value and cause environmental pollution. The present study was carried out to evaluate the antioxidant activity of *Loligo duvauceli* ink in methanol extract and partially purified form by catalase activity, hydroxyl radical scavenging activity and reducing power assay. The Ic 50 value also determined compared to methanol extract the partially purified form shows the higher Ic50 value, this revealed that the antioxidant activity was lower in partially purified form compared to methanol extract.

Keywords: *Loligo duvauceli,* antioxidant activity, catalase, hydroxyl radical, reducing power assay

1. Introduction

In our body as a result of numerous physiological and biochemical process may produce oxygen centered free radicals and other reactive oxygen species, these free radicals can start chain reactions it can cause damage or death to cell. Antioxidants are the molecule that inhibits the oxidation of other molecules, antioxidants terminates these chain reactions by removing free radical intermediates and other oxidative reactions.[1, 2] Antioxidants are often reducing agents such as thiols, poly phenols and ascorbic acids. In food science antioxidants can be defined as a substance in foods when present at low concentration compared to those of an oxidizable substrate significantly prevents or decreases the adverse effects of reactive species such as reactive oxygen and nitrogen species or normal physiological functions in human[3].

The antioxidants are classified into two based on the functions, they are primary and secondary, the another classification based on enzymatic and non enz ymatic antioxidants. [4.5] The primary antioxidants react with lipid radicals and convert them in to more stable products, they are mainly phenol in structure, antioxidant vitamins, minerals, Flavonoid, catechins, carotenoids, β carotene, lycopene are included in this group [6]. The secondary antioxidants compounds that perform the function of capturing free radicals and stopping the chain reactions. Another classification of antioxidants as enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants are endogenously produced and it includes enzyme cofactors, low molecular weight molecule. Whereas non-enzymatic compounds are obtained through our diet.

Marine pharmacology is a developing new disciple which explores the searching the marine environment for bio active potential pharmaceuticals,screening of marine compounds and wide range of activities such as antiviral, antibacterial, antiparasitic, antitumor have been reported. Compounds from marine world developed as an option in cosmetics, pharmaceuticals and food industries.

The cephalopod constitutes in large amount among the marine organisms the squid and cuttle fish include the main class of cephalopods. In India the cephalopod resources are utilized mainly for export. The *Loligo duvauceli* the common name is squid, the main chemical component of their ink is melanin and mucous. Various studies reported that the molecule which are presents in mollusks and cephalopod possess antioxidant activity In present study the antioxidant activity in *Loigo duvauceli* ink in its and methanol extract form and partially purified form by catalase activity, hydroxyl radical activity and reducing power assay were investigated.

2. Materials and Methods

Loligo duvauceli were collected in fresh condition just after their landing by small trawlers from Beypore and brought in to laboratory cleaned and washed with fresh water to remove impurities. The ink glands were dissected and ink was collected by gently squeezing the gland and the ink was air dried.

2.1 Extraction and Purification

Crude extraction of *Loligo duvauceli* ink done using solvents, 25ml of the ink was extracted with 75ml of methanol in sterile glass bottles by parallel extraction method. [9] inks was mixed with solvents using sterile glass rod and refrigerated at 4ºc for 72 hours for crude extraction, each preparation was filtered. Crude extracts were collected weighed and were sterilized by exposure under uv light for 2hours. 5mg of extract was mixed in a sterile nutrient broth and was incubated for 2 hours which was placed on to nutrient agar for checking sterility of extracts were stored at 4ºc in brown bottles. The crude ink of *Loligo*

duvauceli was partially purified by ammonium sulfate precipitation and dialysis [10]. Ammonium sulfate (20-60%(w/v)saturation) was added to the supernatant fluid at 4ºc which had been obtained after sonication and was adjusted to pH 7.0 with 50mM sodium phosphate buffer. Everything was then left to stand overnight at 4ºc. The precipitate was centrifuged at 15000g for 30 min at4ºc and dissolved in 50mM sodium phosphate buffer (pH7.0) containing 3mM NaN3 and then dialysate Precipitation was induced with 3 volumes of acetones which had been cooled to -10ºc. After 2h the precipitate was centrifuged off at 12000g for 30 min, as above and then driedat4ºc for 24h.

2.2 Determination of antioxidant activity by catalase activity method

The activity of catalase was assayed following the method described by Pari and Latha (2004) [11]. The percentage inhibition was evaluated following decrease in absorbance at 620nm. The reaction mixture consisted of 0.4 ml of hydrogen peroxide (0.2M), 1ml of 0.01M phosphate buffer (pH 7.0) and 0.1ml of sample. The reaction of the mixture was stopped by adding 2ml of dichromate-acetic acid reagent (5% $K_2Cr_2O_7$ prepared in glacial acetic acid).The changes in the absorbance was measured at 620nm and recorded.

2.3 Determination of antioxidant activity by hydroxyl radical scavenging method

Deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium. The reaction mixture containing $FeCl_3$ (100 µM), EDTA (104 µM),H_2O_2 (1 mM) and 2-deoxy- D-ribose (2.8 mM) were mixed with or without squid ink at various concentrations (10-50 µg) in 1 ml final reaction volume made with potassium phosphate buffer (20 mM, pH 7.4) and incubated for 1 hr at 37°C. The mixture was heated at95ºC in water bath for 15 min followed by the addition of 1 ml each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). Finally the reaction mixture was cooled on ice and centrifuged at 5000 rpm for 15 min. Absorbance of supernatant was measured at 532 nm. The % hydroxyl radical Scavenging activity of test sample was determined accordingly in comparison with negative control, Ascorbic acid was taken as the positive control.

2.4 Determination of antioxidant activity by reducing power assay

The reducing power of the sample was evaluated according to the method of Yen and Chen (1995) [12]. A volume of 1.0 ml of the squid ink prepared and BHT, Vitamin C and Vitamin E (0 - 5.0mg/ml) were mixed individually to the mixture containing 2.5ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of potassium ferri cyanide ($K_3Fe(CN)_6$) (1%w/v). The resulting mixture was incubated at 50°C for 20 min, followed by the addition of 2.5ml of trichloroacetic acid (10% w/v), which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with 2.5ml of distilled water and 0.5 ml of ferrous chloride (0.1%, w/v). The absorbance was measured at 700 nm against a blank sample.

3. Result and Discussion

The results of antioxidant activity were determined by catalase activity method the changes in absorbance were measured at 620nm and recorded the Percentage inhibition was calculated using the equation:

% catalase inhibition = [(normal activity – inhibited activity) / (normal activity)] × 100%.

The catalase activity of methanol extract of *Loligo duvauceli* ink was given in (table 1) and (fig1), the catalase activity of partially purified form was given in (table2) and (fig 2).In hydroxyl scavenging activity the percentage of radical activity was measured in methanol extract and partially purified form of *Loligo duvauceli* ink which is expressed in (table3 and 4),the Ic50 value for methanol extract form was recorded as 33.33(µg/ml) where as in partially purified form Ic 50 value recorded as 40(µg/ml) (fig3 and 4)

The reducing power assay of *Loligo duvauceli* ink in methanol extract and partially form also evaluated which is expressed in (table 5 and 6) and fig (5 and 6) the activity was found higher in methanol extract compared to partially purified form.

Table 1: Catalase activity of methanol extract of *Loligo duvauceli*ink

Concentration (µg/ml)	Inhibition (%)	
	Catalase	*Loligo duvauceli* ink methanol extract
20	44 ± 0.00	38 ± 0.02
40	50 ± 0.00	44 ± 0.01
60	60 ± 0.01	52 ± 0.01
80	69± 0.01	61 ± 0.02
100	74 ± 0.02	66 ± 0.00

Table 2: Catalase activity of partially purified *Loligo duvauceli* ink

Concentration (µg/ml)	Inhibition (%)	
	Catalase	Squid ink(Purified)
20	44 ± 0.00	33 ± 0.00
40	50 ± 0.00	42 ± 0.00
60	60 ± 0.01	49 ± 0.01
80	69± 0.01	58 ± 0.00
100	74 ± 0.02	62 ± 0.01

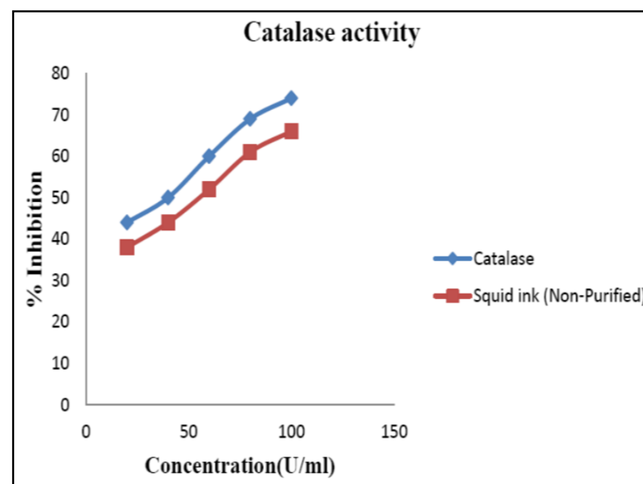

Fig 1: Catalase activity of methanol extract of *Loligo duvauceli* ink:

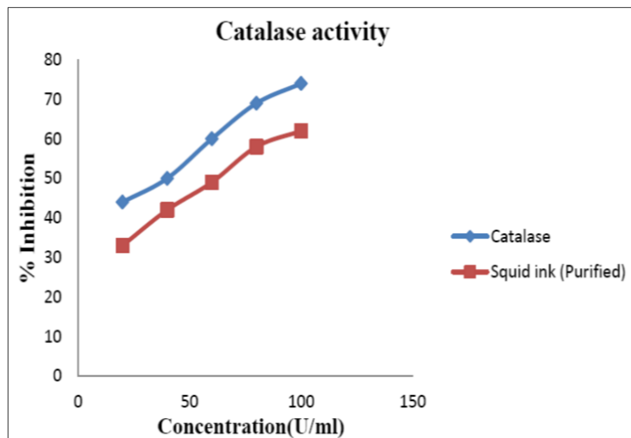

Fig 2: Catalase activity of partially purified form of *Loligo Duvauceli nk*

Table 3: Hydroxyl radical scavenging activity of methanol extract of *Loligo duvauceli* ink

Concentration (µg/ml)	Inhibition (%)	
	Ascorbic acid	*Loligo duvauceli* ink methanol extract
20	39 ± 0.01	37 ± 0.03
40	45 ± 0.02	40 ± 0.03
60	53 ± 0.02	49 ± 0.03
80	59 ± 0.01	54 ± 0.00
100	65 ± 0.02	60 ± 0.02

Table 4: Hydroxyl radical scavenging activity of partially purified form of *Loligo duvauceli* ink

Concentration (µg/ml)	Inhibition (%)	
	Ascorbic acid	*Loligo duvauceli* ink partially purified form
20	39 ± 0.01	20 ± 0.05
40	45 ± 0.02	29 ± 0.03
60	53 ± 0.02	38 ± 0.00
80	59 ± 0.01	53 ± 0.02
100	65 ± 0.02	59 ± 0.01

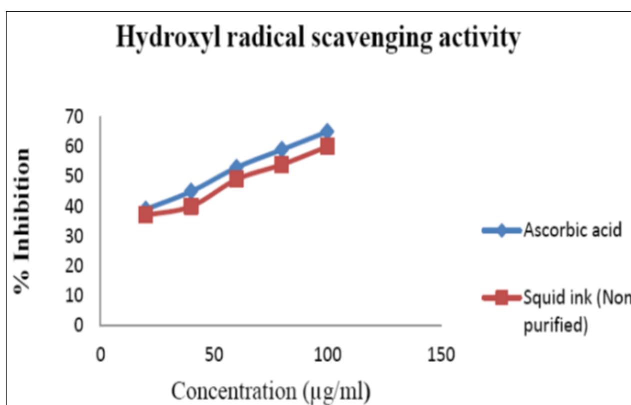

Fig 3: Hydroxyl radical scavenging activity of methanol extract of *Loligo duvauceli* ink:

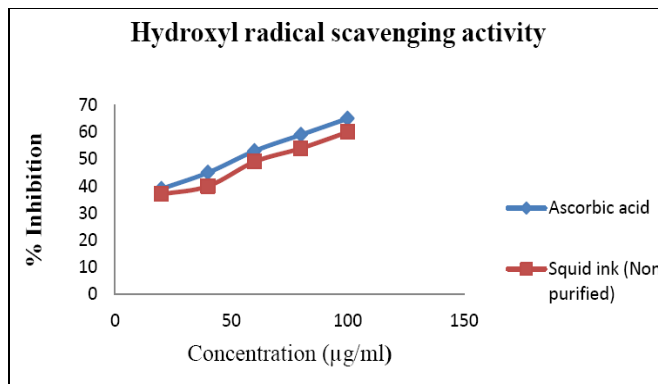

Fig 4: Hydroxyl radical scavenging activity of partially purified form of *Loligo duvauceli* ink:

Table 5: Reducing power assay of methanol extract of *Loligo duvauceli* ink

Concentration (µg/ml)	Inhibition (%)	
	BHT	*Loligo duvauceli* ink methanol extract form
20	0.285 ± 0.002	0.268 ±0.000
40	0.317 ± 0.005	0.299 ± 0.001
60	0.365 ± 0.003	0.322 ± 0.001
80	0.397 ± 0.001	0.346 ± 0.001
100	0.421 ± 0.002	0.364 ± 0.000

Table 6: Reducing power assay of partially purified form of *Loligo duvauceli* ink**:**

Concentration (µg/ml)	Inhibition (%)	
	BHT	*Loligo duvauceli* ink patially purified form
20	0.285 ± 0.002	0.244 ±0.003
40	0.317 ± 0.005	0.292 ± 0.002
60	0.365 ± 0.003	0.305 ± 0.003
80	0.397 ± 0.001	0.336 ± 0.002
100	0.421 ± 0.002	0.351 ± 0.002

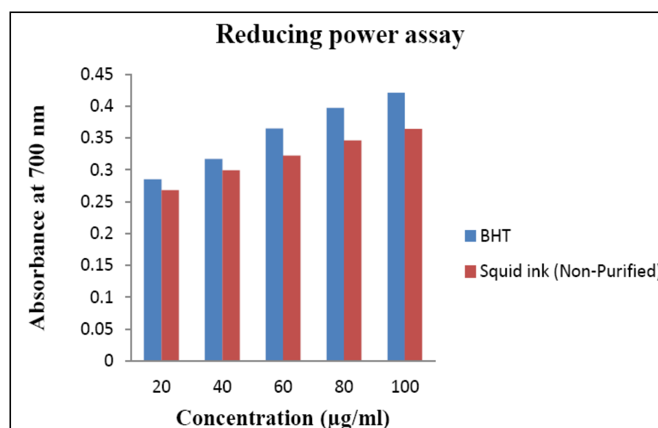

Fig 5: Reducing power assay of methanol extract of *Loligo duvauceli* ink:

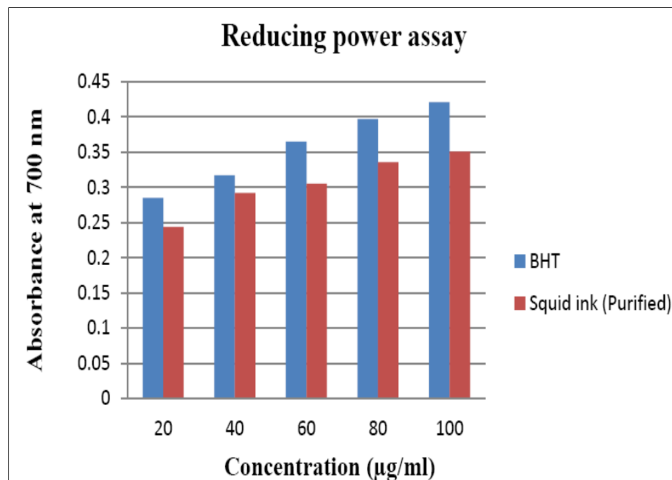

Fig 6: Reducing power assay partially purified form of *Loligo duvauceli* ink:

4. Conclusion

In the present study, the *Loligo duvauceli* ink methanol extract and partially purified form were tested for their antioxidant activities by catalase activity, hydroxyl radical scavenging activity and reducing power assay. Extractions are conducted by parallel extractions using methanol as solvent and purification done by ammonium sulfate precipitation and dialysis. This study concludes that by stating the use of squid ink will have been considerable attention in relation with oxidative stress and cell damage cancer therapy and longetivity.

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6. References

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