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Phyto chemical evalution and antioxidant activity of methanol extract of *Loligo duvauceli* Ink

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Abstract
The present study, the *Loligo duvauceli* ink which is generated as a byproduct in processing industry, the ink samples are screened for phytochemical evalution.Large number of compounds are present in the ink like phenol, flavonoid, protein,carbohydrate, aminoacids. Among the protein, flavonoids are present in higher amount where as sterols, terpenoids, saponins are absent in the *Loligo duvauceli* ink. In proteins phenols flavonoids, carbohydrate are quantitatively estimated in methanol extract and partially purified form and the antioxidant activity of Loligo duvauceli ink was done by ferric thiocyanate assay and thiobarbituric acid assay the results were compared using *Loligo duvauceli* ink by using water as a solvent.

Keywords: *Loligo duvauceli,* ferric thiocyanate assay, thiobarbituric acid assay

1. Introduction
The *Loligo duvauceli* which is a cephalopod in the class of Mollusca, they constitute most significant part in the marine environment. These cephalopod represents two larger groups namingly Nautilodea (nautilus) and Coleoidea [1]. The former groups do not produce ink but the later includes squids, octopuses, cattle fish they produce ink. The cephalopod uses these inks for their living in low light or dark conditions, such as including deep sea. These cephalopods have the ability to produce and release ink even at their small and young sizes. The ink of cephalopod is the secretions from two glands namingly ink sac and funnel organ. The ink sac with its ink gland produces a black ink mainly composed of melanin and the funnel organ is a mucous producing gland which is also part of the ink of cephalopod, inking by cephalopod has been recognized as a part of adaptive response to predation and various physical threats which include the combination of chemical deterrence, sensory disruption and phago mimicry [2]. The squid ink function as a warning signals or alarm substance that made confuses the predators and give alerts co specifics to the presence of danger.
Cuttle fish ink has wide application in the homeopathic medicine. The squid ink has wide application in food products in Japan [3]. In India the cephalopods are mainly exploited for export,in squid processing industry the ink sac is the major waste material and which causes the major environmental problem. The squid ink has to play various primary role in alternative medicine and large range of application in therapeutics [4]. The present study the primary phytochemical evalution of the methanol extract of *Loligo duvauceli* was conducted some important compounds like protein, flavnoid, phenols, present in the sample quantitatively estimated. The *Loligo duvauceli* ink was partially purified and the protein carbohydrate, phenol, flavanoids are quantitatively estimated. The radical scavenging activity is estimated by ferric thiocyanate assay and thiobarbituric acid test in methanol and water extract of loligo duvauceli ink.

2. Materials and methods
Loligo duvauceli were collected from Beypore fort by trawl catch, brought into the laboratory, cleaned and washed with fresh water and all the impurities are removed. The ink sacs were dissected and ink was collected by gently squeezing the gland with spatula. The ink was dried using hot air oven until it is free from water content using the dry ink powder the extract was prepared. The 25ml of squid ink was mixed with 75ml of methanol in sterile glass bottles by parallel extraction method. Ink was slowly mixed with methanol using a sterile glass road and refrigerated at 4ºc for 72 hrs. Each preparations was filtered and were concentrated under vacuum. Crude extracts were collected weighed and the sterility was checked under UV light for about 2 hours. The extract were stored at 4º in brown bottles. The *Loligo duvauceli* ink was partially purified by ammonium sulphate precipitation and dialysis. The preliminary phytochemical evaluations of methanol extractof *Loligo duvauceli* ink was carried out the following ways [5]

2.1 Tests for carbohydrate

Molisch's test: Dissolve small amount of methanol extract of *Loligo duvauceli* ink in 4ml distilled water and filtered. The filtrate was subjected Molisch's test formation of reddish brown ring indicate the presence of carbohydrate.

Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution the brown colour indicate the presence of carbohydrate.

2.2 Tests for Phenol

The extract was spotted on a filter paper, a drop of phosphomolybdic acid reagent was added to the spot and then ammonia vapors was exposed, the blue colour in the spot indicate the presence of phenol.

2.3 Tests for Flavonoids

Shinoda test: To some methanol extract a piece of magnesium ribbon and 1ml of concentrated hydrochloric acid was added. A pink or red colouration of solution indicates the presence of flavonoid.

Lead acetate test: To 5 ml methanol extract 1ml of lead acetate was added, flocculent white precipitate indicate the presence of flavonoid.

2.4 Tests for Tannins

Braestmer's test: To a 2mlofmethanol extract 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey colourations of the solution indicate the presence of tannin.

2.5 Test for steroid/terpenoid

Liebermann-Buchardtest: To1 ml of extract, 1 ml chloroform, 2 to 3ml acetic anhydride and 1 to 2 drops of conc. sulphuric acid are added. Dark green colouration of the solution indicated the presence of steroid and dark pink or red colouration of the solution indicate the presence of terpenoids.

2.6 Tests for Alkaloids

Draggendorf's test: A drop of extract was spotted on a small piece of precoated TLC and plate was sprayed with modified dragendrof's reagent orange colouration of the spot indicates the presence of alkaloids.

Wagner's test: The extract was treated with few ml of Wagner's reagent. The reddish brown precipitation indicated the presence of alkaloids.

2.7 Tests for glycosides

Legal's test: Dissolve the extract in pyridine 2ml added sodium nitropruside solution and made alkaline with sodium hydroxide solution, pink to red colour solution indicate the presence of glycosides.

2.8 Tests for Saponins

Foam test: 1ml of extract was dilute with 20 ml of distilled water and shaken with a for graduated cylinder for 15 minutes. A1 cm layer of foam formation indicates the presence of saponins.

2.9 Test for Anthraquinones

Borntrager's test: Some of the extract was heated with 10% ferric chloride solution and 1 ml of conc. Hydrochloric acid. The extract was cooled, filtered and filtrates was shaken with diethyl ether. which is then extracted with strong ammonia, pink or red colouration of aqueous layer indicated the presence of anthraquinones.

2.10 Test for Aminoacids

Ninhydrin test: Dissolve a small quantity of extract few ml of water and 1 ml of ninhydrin reagent was added, blue colour indicate the presence of amino acid.

2.11 Tests for Fixed Oils and Fats

Press small quantity of the petroleum ether extract between two filter paper, oil stains on the paper indicate the presence of fixed oils.

2.12 Determination of Phenol Content

The phenol content was estimated by Folin's ciocalteu's reagent (FCR). Gallic acid was used as standard. The solution of extracts was diluted to 10 ml with distilled water and FCR reagent 1ml was added and mixed well and sodium carbonate was added, make up all the tubes in to desired quantity and incubated at room temperature for 90 minutes, after that read the absorbance value at 750 nm in UV spectrometer. The phenol content was determined by comparison with standard Gallic acid, and result were presented by milligram equivalence of Gallic acid The phenol content in partially purified form can also be estimated.

2.13 Estimation of Flavonoids

The flavonoids were estimated by aluminum chloride method by using standard quercetain. Different concentration of standards were taken, aluminum chloride was added, 1N potassium acetate was added and make up all the tubes in desired quantity and mix well and read the absorbance value at 500nm, the flavonioid content also estimated in partially purified form also.

2.14 Estimation of Protein

The protein content in methanol and partially purified form can be by using Folin ciocalteu reagent, BSA is used as standard, read the absorbance value against protein concentration. From the graph the protein content in *Loligo duvauceli* calculated.

2.15 Estimation of Carbohydrates

The carbohydrate content in methanol extract and partially purified form of *Loligo duvauceli* ink estimated by Anthrone method. The samples were taken and were diluted to 10ml using methanol and then mix well and incubate at room temperature the total carbohydrate content could be calculated by plotting concentration against absorbance value.

2.16 Free radical scavenging activity by ferric thiocynate methd

The standard method was described by (Kikuzaki and Nakatani 1993) was used [6]. A mixture of 4ml of methanol extract of *Loligo duvauceli* ink in 4ml of absolute ethyl alcohol, 4.1mlof 1,5% of Linolenic acid in absolute ethyl alcohol 8.0ml of 0.005 M phosphate buffer p^H 7.0 and 3.9 ml of water was placed in an oven at 40°c in the dark. To 0.1ml of this solution 9.7 ml of 75 % ethanol and 0.1ml of 30% ammonium thiocyanate. After 3 minutes 0.01 ml of 0.002 M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance of red colour was read at 540nm each 24 hours until the day after the absorbance of control has reached its maximum. The reaction BHT and alpha tocopherol is used as a positive control while mixture without

the methanol and water extract used as negatives controls. Free radical scavenging activity in methanol and water extract of *Loligo duvauceli* ink also conducted by ferric thiocyanate method.

2.17 Thiobarbituric acid method

The method (Ottulengi 1959) [7] was referred. To 1ml of methanol and water extract of *Loligo duvauceli* ink solution add 2ml of 20% trichloro acetic acid and 2ml of 0.67 % 2-thiobarbituric acid was added and placed in a boiling water bath and cooled and centrifuged at 300rpm for 20 minutes, read the absorbance at552 nm. The free radical scavenging activity based on the final value of FTC method.

3. Results anddiscussion

The *Loligo duvauceli* methanol extract was preliminary screened to identify the major chemical class of compounds. The *Loligo duvauceli* ink was checked for mainly 18 class of compounds namely phenols,flavanoids, alkaloids, saponins, glycosides, proteins and other they are represented in table 1. Among the phytochemical evaluation proteins, flavones, phenols are found in large in Loligo duvauceli ink represented by +++ and the compounds which was present in average represented by ++ and compound which was present in small amount represented by + and the compound which was absent represented by – (negative sign).

Determination of phenol

The phenol content was estimated by Folin-ciocalteu reagent (FCR) by using Gallic acid as standard. The total phenol content was determined by comparison with standard calibration curve of Gallic acid, the total phenol content was expressed as milligram equivalence of Gallic acid and it was represented in table 2. The total phenol content in the methanol extract of *Loligo duvauceli* ink was found to be 0.008milligram equivalence of Gallic acid where as in the partially purified form the total phenol content is 2.65 milligram equivalence of Gallic acid.

Estimation of Flavonoids

The flavonoids are estimated by aluminum chloride method using standard quercertain. The flavonoid content was calculated by comparing with the standard quercertain curve, which was expressed as the percentage milligram equivalence of quercertain. The flavonoid in the methanol extract of Loligo duvauceli was found to be 0.00224 milligram equivalence of quercertain, where as in the partially purified form it is 1.323 milligram equivalence of quercertain and expressed in table 3.

Estimation of protein

The protein can be estimated by Folin-Ciocalteu reagent by using BSA as a standard, the protein are expressed as percentage of weight and for methanol extract which was found to be 22% and for partially purified it is 20% of dry weight and resented in table 4.

Estimation of carbohydrate

The carbohydrate was calculated by anthrone method, the total carbohydrate in methanol extract of Loligo duvauceli was found to be 17% of dry weight where as in partially purified form it was 20% of dry weight and expressed in table 5.

Determination of free radical scavenging activity by ferric thiocyanate method

Determination of free radical scavenging activity by thiobarbituric acid

4. Conclusion

In the present study the *Loligo duvauceli* ink the phytochemical evaluation, was conducted some compounds were present as highly active, some were average and some of the compounds were absent. Based on the preliminary screening some compounds were quantitatively estimated. The free radical scavenging activity of *Loligo duvauceli* ink was conducted by ferric thiocyanate and thiobarbituric acid method. This study concludes that the Loligo duvauceli inks will definitely considerable attention to radical and oxidative stress and cell damage.

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