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BIOCHEMICAL COMPOSITION OF CUTTLE FISH *SEPIA PRABAHARI* INK AND ITS BIOACTIVE PROPERTIES *IN-VITRO*

S. Shiva Shakthi [1], S. P. Archana [1], C. Arulvasu [*1] and N. Neethiselvan [2]

Department of Zoology [1], University of Madras, Guindy Campus, Chennai 600025, Tamil Nadu, India
Department of Fishing Technology and Fisheries Engineering [2], Fisheries College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Thoothukudi, Tamil Nadu, India

Keywords:

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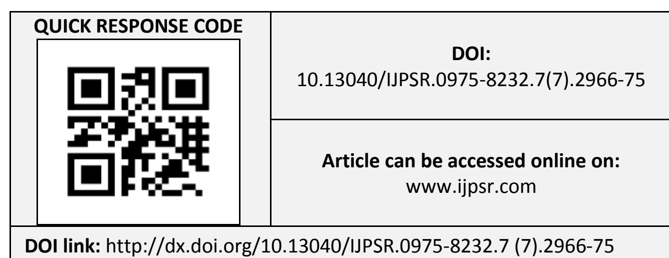
Correspondence to Author:
Dr. C. Arulvasu

Assistant Professor
Department of Zoology, University of Madras, Guindy Campus, Chennai - 600025, Tamil Nadu, India

Email: carulvasu@gmail.com

ABSTRACT: The present study describes the biochemical, antioxidant and anticancer properties of the crude and melanin free ink of *Sepia prabahari* which is rich in both organic and inorganic components. The quantitative analysis define the presence of protein (1.16 – 1.49 mg/ml), lipid (0.25 – 1.42 mg/ml), carbohydrates (0.01 – 1.14 mg/ml), ash (0.4 & 0.1%) and moisture (93.33%) content. The native PAGE profile showed distinct bands of protein with molecular weight ranging from 3.5 to 205 kDa. The ink is composed of different amino acids, lipids and sugars which was presented by TLC. The ink revealed good antioxidant activity, thereby lowering or retarding the initiation of lipid oxidation process. The ink was screened for its anticancer activity against human breast adenocarcinoma cell line (MCF 7) and it exhibited a strong cytotoxicity by inhibiting the cell growth. The results of comet assay revealed the DNA damage in the cells treated with ink which is a hallmark of apoptosis. Thus the ink, especially the melanin free ink is known to possess significant antioxidant activity and potent anti-proliferative effect. The present study suggests that the melanin free ink was richer in biochemical properties than the crude ink.

INTRODUCTION: Natural products have been the most productive source of leads for the development of drugs [1]. The modern tools of chemistry and biology now allow the scientists to detail the exact nature of biological effects of natural compounds on the human body, as well as to uncover possible synergy, which holds much promise for the development of new therapies against many devastating diseases [2]. Despite great advances in rational drug design, in which new medicines are synthesized based on knowledge of specific molecular targets, most prescribed medicines are derived from, or patterned after natural compounds from animal [3, 4].



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Venoms and toxins from animals like snakes, spiders, scorpions and insects are extremely potent because they often have very specific interactions with a macromolecular target in the body and have been used as lead compounds in the development of novel drugs [5].

In this perspective the marine organisms form a prominent component of the oceanic population, which significantly contribute to the production of cosmeceutical and pharmaceutical molecules with biologically efficient moieties [6]. Cephalopods, the largest group among molluscs are exclusively marine, intelligent and possess many medicinal properties [7, 8]. Inking by the cephalopods has long been recognized as an adaptive response to predation and physical threat by means of a combination of mechanisms that include chemical deterrence, sensory disruption and phago-mmicry [9]. Cephalopod ink is an alarming substance that confuses predators and alerts con specifics for the

presence of danger [10]. Sepia, commonly called cuttlefish, whose ink consists of melanin granules in a viscous colourless medium. The melanin pigment is manufactured in the mature cells of the ink gland, a highly specialized organ situated at the bottom of ink sac and deputed to continuous production of ink [9]. Melanin isolated from ink sac has been proposed as a standard for natural eumelanin. The cuttlefish ink finds wide application in homeopathic medicine and *Sepia officinalis* ink is used to treat hormonal imbalances especially in women [11]. Boiron Sepia 3X is Boiron's Single Medicine series and is most commonly used as a homeopathic remedy for mood swings [12]. It is also used to treat kidney stone, gonorrhoea and it also act as anti-oxidant, anti-radiation, antiretroviral, antibacterial and anticancer agents. Ink from *S.inermis* were tested against Moloney murine leukaemia virus reverse transcriptase (MMLVRT), and the ink showed strong inhibition of MMLVRT [13].

Ink, which is discarded as by-product, could be used as the best source for natural antioxidant after melanin removal [14]. *Sepia prabahari* is native of the Indian Ocean and found abundantly in Indian Coast and Gulf of Mannar at the depth ranging up to 100m [15]. The present study aims at the estimation of biochemical components in ink of *S. prabahari* with and without melanin and to evaluate its biomedical applications.

MATERIALS AND METHODS:

Chemicals:
All the chemicals used were analytical grade purchased from Sigma–Aldrich (St. Louis, MO, USA). Reagent grade chemicals where purchased from Merck (Darmstadt, Germany). Human breast adenocarcinoma cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune, India.

Collection and identification of experimental animal:
The specimens of *Sepia prabahari* were collected from Tuticorinbay, South East coast line of Tamilnadu, India during January and February of 2014 and identified [15]. The animals were preserved in ice and were brought to the laboratory within 24 hours of capture.

Preparation of ink sample:
The collected animals were held ventrally and the posterior end of the animal was squeezed to eject ink. The ink was collected and stored at -20°C [16]. The crude ink sample was divided into 2 parts and one part of it was subjected to centrifugation at 13,000 rpm for 45 minutes at 4°C to remove the melanin pigment [11]. The supernatant obtained was referred to as 'melanin-free ink' and was used for further analyses. The extracted ink samples both crude and melanin free ink were lyophilized, weighed and stored at 4°C until use.

Determination of moisture content:
The moisture content in the samples were determined by the method of Association of Official Analytical Chemists (AOAC) [17]. The difference in weight between the wet sample and the lyophilized samples were calculated and expressed as percentage moisture content of the sample.

Determination of ash content:
The ash content was determined by the method of AOAC [17]. The samples were weighed into clean dry beakers and placed at 100ºC for 24 hours in the hot air oven. The samples were transferred to crucibles in the muffle furnace and incinerated until it was free of black carbon particles and turn into white in colour. The temperature was maintained at 550ºC for about 5 hours. The samples were cooled, weighed and the percentage of ash content was determined.

Quantitative analysis of the ink:
The analysis were carried out on four samples namely, crude ink solid (CIS), crude ink liquid (CIL), melanin free ink solid (MFS) and melanin free ink liquid (MFL).

Determination of Proteins:
The total amount of protein in the samples were determined by the method of Bradford[18]. The samples were mixed with 5 ml of Bradford reagent. Protein standard (BSA) solution was also prepared and absorbance was read at 595 nm using Shimadzu 160 UV-VIS double beam spectrophotometer. Standard curve was prepared and the amount of protein in the ink samples of *S. prabahari* were calculated.

Determination of lipids:

The total amounts of lipids in the samples were estimated by sulpho-phospho-vanillin method as described by Barnes and Black stock [19]. The standard olive oil was taken in different concentrations and the ink samples were heated for 10 minutes in a boiling water bath, cooled, and an aliquot of each sample was placed in a dry tube. A blank contained concentrated sulphuric acid. To each was added 6 ml of phospho-vanillin reagent and kept at room temperature for 15 minutes. The absorbance was read at 546 nm in UV visible spectrophotometer.

Determination of total carbohydrate:

The total carbohydrate in the ink samples were quantified by anthrone method as described by Roe et al.[20].The weighed ink samples were taken into a boiling tube and were hydrolysed by keeping in water bath for 3 hours with 2.5 N HCl and cooled. After centrifugation the supernatant was collected and aliquots were taken for analysis. Glucose solution was taken as standard and 2.5 N HCl with distilled water served as blank. Then anthrone reagent was added and heated, cooled and absorbance was read at 630 nm.

Thin layer chromatography for Amino acids:

The thin layer chromatography of amino acids of the samples were analyzed by the method of Saeger and Slabaugh [21]. The standards were amino acids like alanine, glycine and asparagine. The ink samples were applied as a small spot on the silica coated readymade TLC plate with the help of capillary tube at 1.5 cm from the bottom. The plates were held vertical in a glass tank with Butanol/Acetic acid/Water (80:20:20 v/v) as solvent. The run was continued for about 2-3 hours and later the plate was dried. Finally the plates were sprayed with 0.1 % ninhydrin in acetone and kept in oven at 110ºC for 5 minutes. The R_f values were calculated.

Thin layer chromatography for Carbohydrates:

The thin layer chromatography of carbohydrates of the samples were analyzed by following the method of Halina and Jolanta[22]. The standards were glucose, galactose and lactose in isopropanol. The TLC plates with the spot of samples were placed in the N-Butanol/Acetic acid/Water (75:25:6 v/v) solvent. The run was continued until the solvent front reaches 1-2 cm from the top of the plates and dried. The plates were sprayed with aniline-diphenylamine, kept in oven at 110ºC for 5 minutes and the R_f values were calculated.

Thin layer chromatography for Lipids:

The thin layer chromatography of lipids of the samples were analyzed by following the method of Payne [23]. The standards used were. The TLC plate with the spots of samples and standard (olive oil and cholesterol) were first placed in chloroform/methanol/water (14:6:1 v/v) solvent and later in propanol/ammonia solution in the ratio (39:11). After complete run the plates were dried and exposed to iodine vapour. The bands appear dark brown in color in light brown background for which the R_f values were calculated.

Protein profile analysis:

The samples were analyzed by Native-PAGE to determine the protein profile according to the method of Maurer [24]. The gel plates were assembled and reagents were prepared for casting the gel. The percentage of running gel and stacking gel was 7% and 3% respectively. After polymerization, 5 µl of ink samples were mixed thoroughly with a drop of bromophenol blue and loaded in the wells. The electrophoretic apparatus was attached to power supply unit and once the run was complete the gel was stained. After destaining, the bands were visualized using an illuminator (Bio-rad gel documentation) and photographed.

Determination of antioxidant activity:

DPPH radical scavenging activity was determined as per the method of Blois [25] and modified by Binsan et al.[26]. The ink samples were prepared in concentrations of 100-500 µg/ml and 0.1 mM of DPPH in 95 % (v/v) ethanol was added to the sample. Ascorbic acid was taken as the reference standard and in the blank deionized water was used instead of the sample. The mixture was allowed to stand at room temperature in the dark for 30 minutes. The absorbance of the resulting solution was measured at 517nm using a spectrophotometer. The antioxidant activity of the ink samples were expressed as IC_{50} and compared with standard.

Evaluation of anticancer activity:

MTT Assay: The human breast adenocarcinoma cell line MCF 7 were plated in 96 well plate (1 x 10^5/well) with DMEM medium containing 10 % FBS. The cytotoxic effect of ink samples was assessed by MTT (3-(4, 5- dimethylthiazol-2yl)-2, 5- diphenyltetrazolium bromide) method of Mosmann [27]. The cells were incubated for 24 hours under 5% CO_2, 95% O_2, 37°C, allowing the cells to attach to the bottom of the well. The cells were treated with medium containing different concentration (100 to 500µg/ml) of the ink samples for 24 hours incubation. Control cultures were maintained without/absence of ink samples. After 24hours 10µl of 5 mg/ml MTT solution was added to each well and the cultures were further incubated for 4 hours at 37ºCand then 100 µl of DMSO was added. The absorbance was measured at 570 nm for each well and growth inhibition rate was calculated.

Cytomorphological Examination:

MCF-7 cells was trypsinized, 5 ml of growth medium was added to cell suspension and was placed in the sterilized petri plate. After 2 days the monolayer of cells formed was observed under Inverted microscope and photographed.

Comet Assay:

Comet assay was performed to determine the degree of DNA damage induced by ink. MCF 7 cells were exposed at different concentration of *S. prabahari* ink for 24 h and washed with phosphate buffer saline (PBS). The cells suspension was mixed with 75 µl of 0.5% low melting agarose (LMA) at 39ºC and spread on a fully frosted microscopic slide pre-coated with 200 µl of 1% LMA and then immersed in lytic solution (2.5 M NaCl, 10 mM Na-EDTA, 10 mM Tris–base and 0.1% Trion X-100, 10% DMSO; the last two compounds were added fresh, pH 10) for 1 h at 4ºC. The slides were then placed in a gel electrophoresis apparatus (containing 300mM NaOH and 10 mM Na-EDTA, pH 13) for 40 min to allow unwinding of DNA and the alkali labile damage. An electrical field (3000 mA, 25 V) was applied for 20 min at 4ºC to draw the negatively charged DNA towards anode. After electrophoresis, slides were washed thrice for 5 min at 4ºC in neutralizing buffer (0.4 M Tris, pH 7.5), followed by staining with75 µl of propidium iodide (40 lg/mL) and then the slides were examined using fluorescence microscope. The DNA contents in the head and tail were quantified by using CASP software.

Statistical analysis:

Data obtained in the present study was subjected to standard statistical analysis by using statistical package for social sciences (SPSS) with 95% confidence level to find out the variation between the experimental sets and with the control.

RESULTS:

Determination of Moisture and ash content:

The percentage of moisture present in both the ink samples with and without melanin was 93.33%, which indicates the presence of high water content. The percentage of ash on the basis of wet weight was first calculated and the result for crude ink and melanin free ink were 0.4% and 0.1% respectively. The percentage of ash in the lyophilised crude ink and melanin free ink were 6% and 2% respectively.

Quantitative analysis of the ink:

The amount of protein, lipid and carbohydrates in crude ink solid (CIS), crude ink liquid (CIL) melanin free ink solid (MFS) and melanin free ink liquid (MFL) is given in **Table 1**. The quantity of proteins, lipids and carbohydrates had a slight variation within the lyophilized solid and liquid samples. There was also difference in the quantity of each between the crude and melanin free samples. On the whole the ink had a lower concentration of carbohydrates when compared to the proteins and lipids. There was also a greater difference in the quantity of lipids within solid and liquid samples. Proteins were the major components present in the crude and melanin free ink according to the results.

TABLE 1: BIOCHEMICAL COMPOSITION OF *S. PRABAHARI* INK

Samples	Proteins	Lipids	Carbohydrates
	Concentration (mg/ml)		
CIS	1.16 ± 0.23	1.36 ± 0.11	0.01 ± 0.06
CIL	1.43 ± 0.30	0.25 ± 0.20	0.03 ± 0.10
MFS	1.21 ± 0.28	1.42 ± 0.16	0.10 ± 0.09
MFL	1.49 ± 0.33	0.41 ± 0.24	0.14 ± 0.11

Thin layer chromatography:

In TLC of amino acids, the number of bands seen in CIS, CIL, MFS and MFL were 7, 6, 8 and 6 respectively (**Fig.1a**).

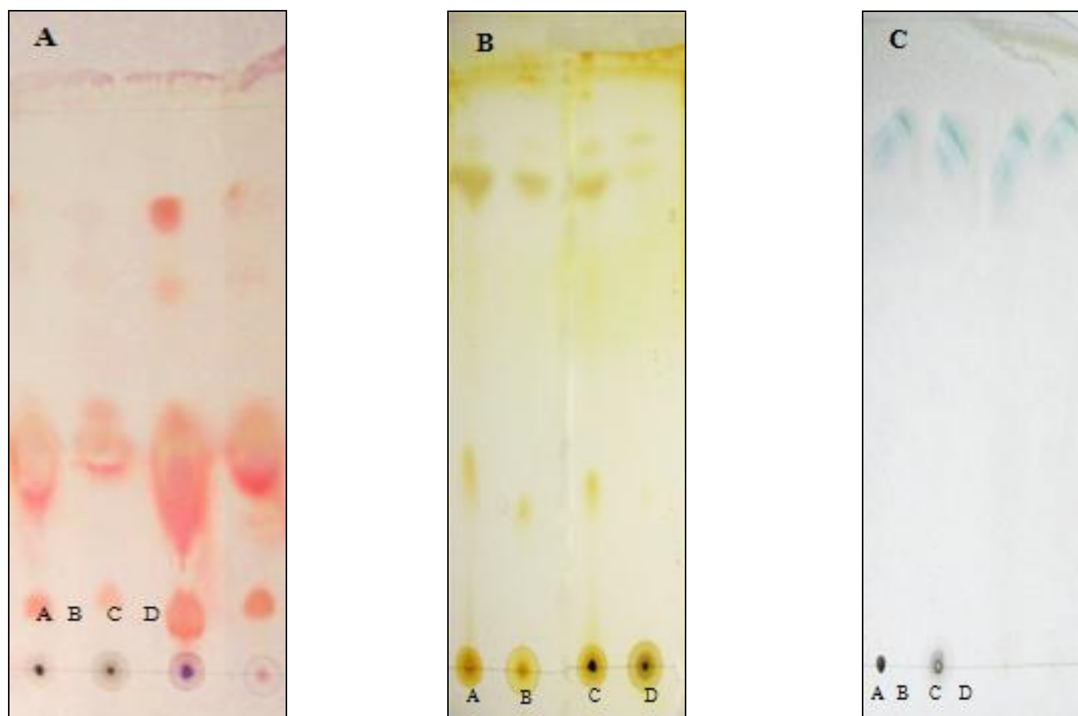

FIG. 1: TLC ANALYSIS OF *S. PRABAHARI* INK (A) AMINO ACIDS, (B) LIPIDS AND (C) CARBOHYDRATES: LANE - (A) CIS (B) CIL (C) MFS (D) MFL

The R_f value of various amino acid bands for the four ink samples were calculated. The amino acids found in the ink samples were tyrosine, arginine, histidine, glutamine, glycine, threonine, cysteine, valine, leucine, asparagine, methionine, isoleucine, lysine and glutamic acid (**Table 2**).

Tyrosine, methionine and cysteine are the common amino acids found in the four samples. In TLC of lipids, there were 7 bands in CIS sample, 5 bands in CIL sample, 7 bands in MFS sample and 5 bands in MFL sample (**Fig. 1b**).

TABLE 2: R_F VALUES AND THE CORRESPONDING AMINO ACIDS, LIPIDS AND SUGARS PRESENT IN THE *S. PRABAHARI* INK

R_f Values	CIS	MFS	CIL	MIL
Amino Acids				
0.10	-	Arginine	-	-
0.14	Lysine	-	Lysine	Lysine
0.21	Aspargine	-	-	-
0.25	-	Glutamine	-	-
0.29	-	Threonine	Threonine	Threonine
0.32	-	-	Glutamic acid	-
0.36	Cysteine	Cysteine	-	Cysteine
0.40	Valine	Valine	-	-
0.47	Methionine	Methionine	Methionine	Methionine
0.55	Tyrosine	Tyrosine	Tyrosine	Tyrosine
0.80	-	-	-	Isoleucine
0.83	-	Leucine	Leucine	-
Lipids				
0.08	-	Ganglioside	-	-
0.10	Ganglioside	-	-	-
0.28	-		Lysolecithin	Lysolecithin
0.33	Sphingomyelin	Sphingomyelin	-	-
0.65	Phosphotidylethnolamine	-	-	-
	Phosphotidylethnolamine			
0.76	-	-	Phosphotidylethnolamine	Phosphotidylethnolamine

			-	-
0.80	Phosphotidylethnolamine	Phosphotidylethnolamine		
	-	-	Phosphotidylethnolamine	-
0.82			-	
	Pherosin	Phosphotidylethnolamine		Phosphotidylethnolamine
0.85	Kerasin	Pherosin	Pherosin	-
	-	Kerasin	Kerasin	Kerasin
0.92	-	-	-	Cholesterol
0.97		Free fatty acid	-	-
0.99				
1.00				
Carbohydrates				
0.84	Galactose	Galactose	Galactose	Galactose
0.86	Glucose	Glucose	Glucose	Glucose

The lipids found in the ink samples were gangliosides, sphingomyelin, phosphotidyl ethanolamine, pherosin, kerasin, cholesterol, lysolecithin and free fatty acids. Phosphotidyl ethanolamine and kerasin were found in all the four ink samples (**Table 2**).

In TLC of sugars, single band was obtained in all the four ink samples and the band appeared disc shaped greenish blue in color (**Fig. 1c**). The sugars present are galactose and glucose (**Table 2**).

Protein profile analysis:

The protein profile was determined by native-PAGE which showed distinct clear bands of molecular weight ranging from 3.5 to 205 kDa (**Fig. 2**). The total number of bands viewed was 13 in all the samples, indicating the presence of 13 different proteins.

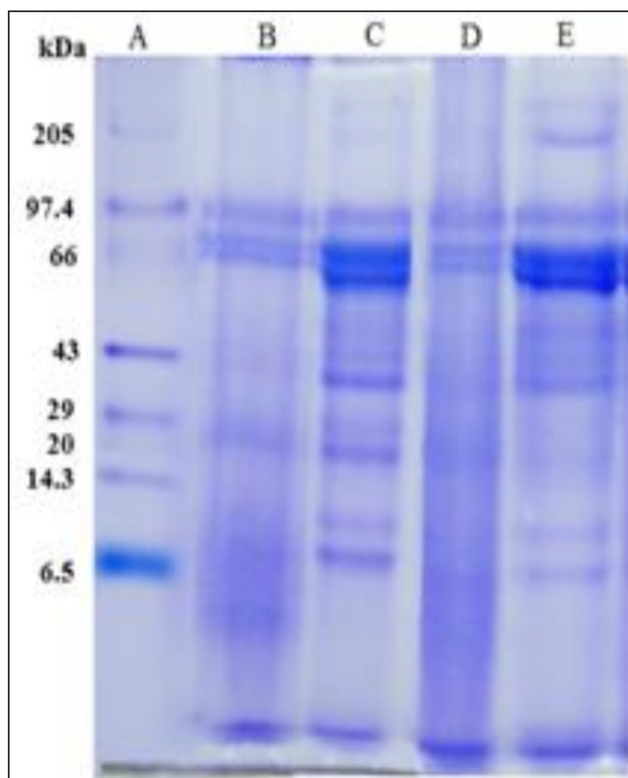

FIG. 2: NATIVE PAGE EVALUATION OF *S. PRABAHARI* INK; LANE A: MOLECULAR WEIGHT MARKER; B: CIS; C: CIL; D: MFS; E: MFL

Determination of antioxidant activity:

The antioxidant activity was slightly higher in melanin free ink than in crude ink sample (**Table 3**). The IC_{50} of the MFS sample was 148.58µg/ml which showed that it has slightly higher antioxidant activity compared to CIS, MFL and CIL with 150.37, 156.25, 159.20 µg/ml respectively.

TABLE 3: DPPH FREE RADICAL SCAVENGING EFFECT OF *S. PRABAHARI* INK

Concentration (µg/ml)	CIS	MFS	CIL	MFL
	% of Free radical scavenging activity			
200	66.5± 0.21	67.3± 0.28	62.8±0.16	64.0± 0.22
400	69.3± 0.28	70.6± 0.22	64.8± 0.21	69.7± 0.20
600	72.6± 0.20	73.8± 0.20	69.3± 0.19	71.4± 0.19
800	73.4± 0.30	75.1± 0.29	73.8 ± 0.24	74.2± 0.27
1000	75.5± 0.23	76.9± 0.27	75.1 ± 0.29	75.5±0.26
IC_{50}	150.3± 0.29	148.5± 0.30	159.2 ± 0.23	156.2± 0.19

Evaluation of anticancer activity:

In the MTT assay, the control cells had 100% cell viability. The MCF-7Human breast adenocarcinoma cells appeared polygonal in shape and the cell mat was very intact. The wells which were treated with the ink showed drastic changes in the viability of cells. Variations in the percentage of inhibition of cell growth with cell shrinkage and rupturing of cells were seen accordingly which lead t the aggregation of cells (**Fig. 3b**).

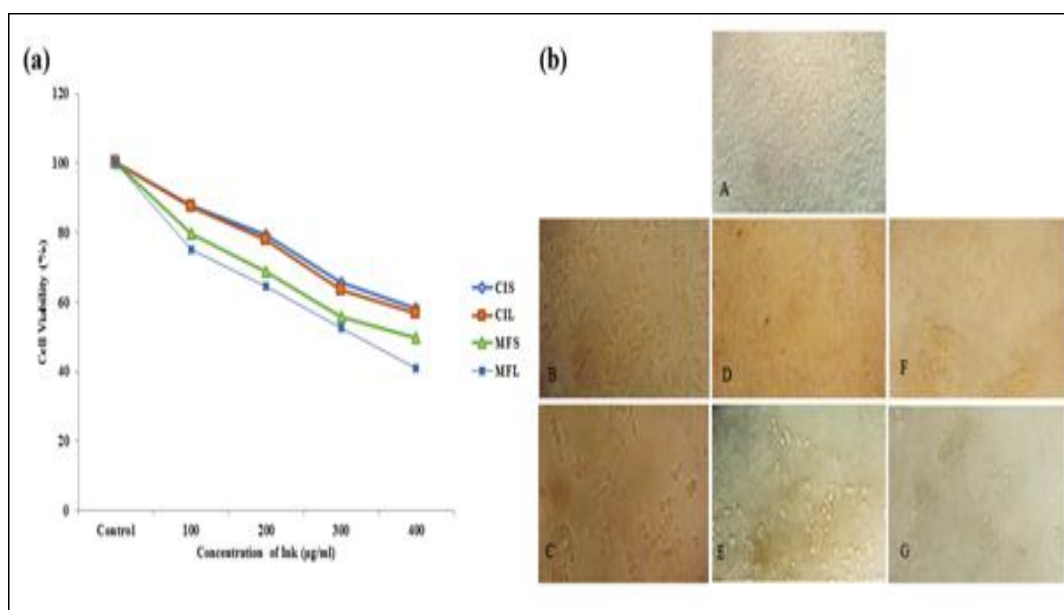

FIG. 3A: CELL VIABILITY OF MCF 7 CANCER CELLS FOR 24 HRS OF TREATMENT WITH INK OF *S. PRABAHARI* AT DIFFERENT CONCENTRATIONS. 3B. EFFECT OF *S. PRABAHARI* INK AT DIFFERENT CONCENTRATIONS AND MORPHOLOGY OF MCF 7 CANCER CELLS FOR 24 HRS OF TREATMENT. A: CONTROL CELLS; B & C: CELLS TREATED WITH 400 µG/ML OF CIS AND CIL; D & E: CELLS TREATED WITH 300µG/ML OF MFS AND MFL; F & G: CELLS TREATED WITH 400 µG/ML OF MFS AND MFL RESPECTIVELY (MAGNIFICATION 20X).

The melanin free ink liquid sample showed the lowest percentage of cell viability when compared to solid samples (**Fig. 3a**). The CIS and CIL samples had IC_{50} value at the concentration of 400 µg/ml, the values were 58% and 56% respectively. The IC_{50} value of MFS and MFL were 55% and 52% respectively which was observed at the concentration of 300µg/ml.

Comet Assay:

The results of DNA damage on the cells treated with ink of *S. Prabahari* are shown in **Fig.4**. The appearance of comet tails corresponding with the induction of DNA damage were visible. MCF 7 cells treated with MFS and MFL at maximum concertation showed increased extant of DNA damage. The span of the comet tail was ten and six times that of the control while, MFS and MFL at IC_{50} concentration shows four and two time of span of tail, respectively, compared to control cells. DNA damage was not observed, as the halo surrounding cell nuclei was clearly visible in control cells.

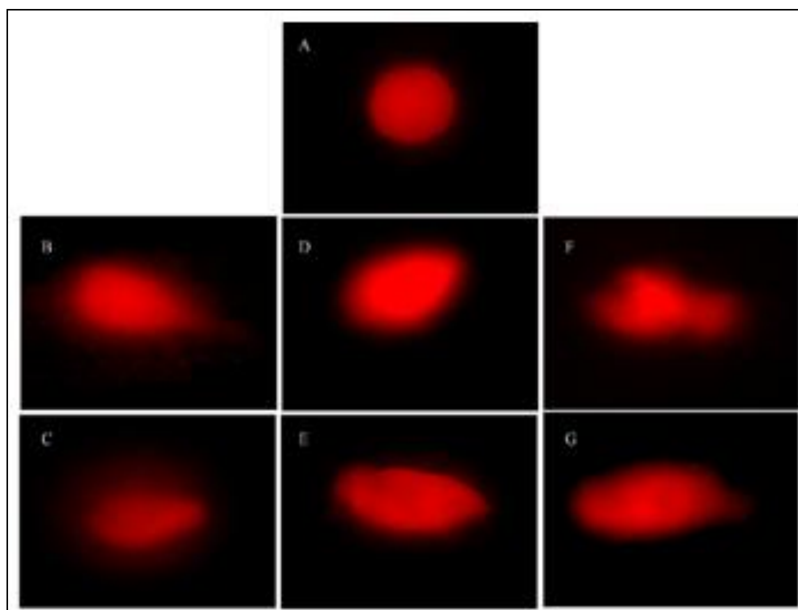

FIG. 4: ANALYSIS OF APOPTOTIC INDUCING EFFECT OF *S. PRABAHARI*ON MCF 7 CANCER CELLS FOR 24 HRS OF TREATMENT ASSESSED BY COMET ASSAY.A: CONTROL CELLS; B & C: CELLS TREATED WITH 400 µG/ML OF CIS AND CIL; D & E: CELLS TREATED WITH 300 µG/ML OF MFS AND MFL; F & G: CELLS TREATED WITH 400 µG/ML OF MFS AND MFL RESPECTIVELY

DISCUSSION: Among all cephalopods, sepiaink consists of many useful constituents and has wide biological applications. Attempts are being made to utilize these defense materials to produce bioactive substances such as antibacterial, anticancer, antiviral and antioxidative agent [11]. According to Neethiselvan and Venkatramani [15] a new species, *Sepia prabahari* was identified but no further details were reported. Thus with the available background about *S. Prabahari* and the properties of sepia ink, the present study was designed to evaluate the biochemical properties, analyze the protein profile and to determine its antioxidant and anticancer properties.

The ink was collected as per the procedure stated by Fiore et al.[16]. In order to widen the application of ink the present study aimed to investigate properties of both crude ink and melanin-free ink of *S. prabahari*. The ink was lyophilized for storage purpose and to avoid degradation of proteins. The sticky nature is due to the presence of mucus [9]. According to Zhenget al.[28] the ink of sepia not only consists of melanin but also rich in organic and inorganic components, our results were in line with his reports. The percentage of moisture was almost similar in both crude ink and melanin free ink. The ash content was higher in crude ink and lesser in melanin free ink. The difference in percentage may be due the melanin pigment composed of minerals like calcium, potassium, magnesium and iron [29]. Protein content was higher in melanin free ink and lyophilized samples than crude ink and liquid samples. The *S. prabahari* ink consists of a good amount of protein and this indicates the biological activity of the ink as reported earlier in other species of sepia [14]. The percentage of carbohydrate content is very less when compared to the other biochemical components of the ink, where the melanin free ink was rich in sugars than crude ink. The lyophilized ink sample had less carbohydrate but the liquid ink sample had high amount of carbohydrates. Our results were in correspondence with the reports of Mimura et al.[30].

The lipid content determined was in line with the previous reports, increased fat content was observed in melanin free ink and solid samples in comparison to crude ink and liquid samples. This shows that the biochemical composition of *S. prabahari* go in hand with the results reported for biochemical components of other species of sepia[31]. There are no reports or information before regarding the protein profile analysis of sepia ink. The molecular weight of proteins observed according to the protein marker was taken as the initial information regarding the molecular weight of proteins of *S. prabahari* ink. The amino acids that might occur in the four ink samples in large amounts were lysine, isoleucine, methionine,

glutamic acid and tyrosine. Our results were in harmony with that of the ink of *S. Officinalis* [32]. Tyrosine was found in the ink and aids in melanogenesis, (*i.e.*) the hydroxylation of tyrosine to dopa (3, 4-dihydroxyphenylalanine) and its oxidation to dopaquinone [33]. The ink had higher concentration of lipids like sphingomyelin and lysolecithin which are helpful in the process of apoptosis [34, 35]. The bands of the liquid sample slightly varied from the solid sample. In TLC of sugars, the only band appeared was greenish blue in colour and indicates the presence of primary sugars like glucose and galactose.

This also clearly shows that there is less amount of sugar content in the ink samples and was finalized with the data of Kokoureck [36]. Lipid oxidation is one of the major causes for deterioration of natural products for which several antioxidants are used [37]. The free radical scavenging activity was high in melanin free ink than the crude ink. The result indicated that melanin free ink was able to act as reducing agent which provided electron for stabilization as reported previously [14]. Additionally, some compounds in melanin free ink could chelate pro-oxidative metals, thereby lowering or retarding the initiation of lipid oxidation process [38].

The development of novel chemotherapeutic agents would play a key role in the treatment of refractory or relapsing cancer patients. The assay of *S.prabahari* ink against MCF-7 breast cancer cell lines revealed good anticancer activity. The melanin free ink had a higher rate of antiproliferative activity than the crude ink. In the comet assay, it was possible to quantify and to distinguish cells with different rates of DNA damage, thus the analysis of the average values of the scores for each treatment group was very important [28]. The comet assay has been established as a simple, rapid, cheap, flexible and, most importantly, sensitive method to detect DNA damage, which is also able to detect DNA damage in individual cells.

In this assay, cells are embedded in agarose, lysed in an alkaline buffer, and subjected to an electric current. Relaxed and broken DNA fragments stream further from the nucleus than intact DNA, so the extent of DNA damage can be measured by the length of the stream [29]. In the present study, the treatment of *S. prabahari* ink for 24h on MCF-7 cells caused an increase in DNA damage to the unchallenged control.Further studies are still needed to understand the various mechanisms regulating the anti-proliferative function and apoptosis inducing property of the cuttlefish ink. The results obtained were more homologous with the results published by Senan et al.[11].

The relation between oxidative stress and cancer has been assumed that ingestion of antioxidants is useful in preventing carcinogenesis [39]. So, if the novel cancer therapeutic drug has both antioxidant and anti-inflammatory properties, it can be a promising anticancer drug.

CONCLUSION: The present study reveals that the ink of *S. prabahari* is biochemically rich with good amount of water, minerals, proteins, lipids and carbohydrates. The major part of the work to be noted is that, the melanin free ink is composed of abundant biochemical components than crude ink. Moreover it possesses higher activity as the antioxidant and anticancer agents. It is obvious that a unique molecular mechanism is involved in the melanin free ink after the removal of melanin. The liquid samples had increased level of biochemical components other than lipids which signals the minute changes in the composition after freeze drying. Since the ink is a cheap and readily available waste material of processing industry, it will be meaningful to look at ink as a potential raw material for drug production through further studies.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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