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REVIEW

Medicinal and therapeutic properties of cephalopod ink: a short review

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Abstract

Cephalopoda is under the phylum of Mollusca with several important marine oceanic habitats. They are mostly well-known for their different uses such as food ingredients and nutraceutical benefits. Among all the different habitats of this class, different species of squid and cuttlefish often containing various nutraceutical properties along with the use as a food item. Surprisingly, the ink of these different species of cephalopods also contains different nutraceutical properties which are thrown away as by-product by most of the processing industries and consumers. There are different studies which focused on these properties obtained from the ink of these cephalopods. These studies clearly showed that this ink is a great source for decreasing various health problems and can be used widely in both pharmaceuticals and food industries. The aim of this review is to focus on its potentials and make it an evident towards the future uses of cephalopod ink.

1. Introduction

Cephalopods live in the marine environment and the number of cephalopod species is relatively low (approximately 700) compared to the number of other species; however, they are widely distributed in different marine habitats (Nair and Sherief, 2010; Derby, 2014). Nautiloidea (nautilus) and Coleoidea are the two major groups that represent cephalopods (Derby 2014), which include squids, cuttlefish, octopus and nautilus (Nair and Sherief, 2010). Among those, squid is the major constituent of the Cephalopoda class (Normal *et al.,* 2002). Currently, squid and cuttlefish are an important fishery product all over the world, especially in Southeast Asian countries (Hoque *et al.*, 2010). Cephalopods have historically been used as human food, especially among Greeks and Egyptians, in different ways (Sundaram, 2009).

Primarily in India, cephalopods were used in the dried form (Rao, 1954; Vijayakumaran, 1984). In the Philippines, cephalopods are first boiled in vinegar and are then fried in oil and spices (Voss, 1963). Squids are incorporated with fish stew in Britain to increase taste (Cornell and Handy, 1982). The Japanese have mastered the use of different varieties of cephalopods in various dishes (Silas *et al.*, 1985). Moreover, other varieties of cephalopods are popular as food dishes food such as pickles (Sundaram, 2009).

During the processing of squids and cuttlefish, the viscera and ink sac containing the ink are considered by-products and are potential threats for creating serious ecological problems as well as environmental pollution without proper management. These by-products can be a potential source of bioactive compounds and have been proven to be an alternative medicine with a wide range of therapeutic applications. The utilization of these by-products will benefit the processing industry as well as reduce serious ecological problems and pollution (McConnell *et al.,* 1993; Karim *et al.,* 2016).

Most cephalopods, excluding nautiloid, have ink sacs and thus produce ink (Hanlon and Messenger, 1996). Cephalopod species that live in low light, including deep sea areas, produce and use ink (Bush *et al.,* 2007). Even when cephalopods are small and young, ink sacs can be present and produce ink (Boletzky, 1987). In Japan, squid ink has traditional applications in food products (Nishimoto *et al.,* 1980). In addition, it is believed that 'Ika-shiokara,' which is a cured cuttlefish meat produced in Japan exhibits anti-septic effect when cuttlefish ink added to it (Takai *et al.,* 1993). Cephalopod ink has already been reported for its various

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therapeutic values (Takai *et al.,* 1992). The crude ink extracts from various species of cephalopods have been studied for their antimicrobial, preservative, antioxidant, anti-cancer, antiretroviral and many other properties (Mochizuki, 1979; Takai *et al.*, 1992; Takaya *et al.*, 1994; Sasaki *et al.,* 1997; Rajaganapathi *et al.*, 2000; Russo *et al.,* 2003; Sadok *et al.*, 2004; Yang *et al.*, 2005; Lei *et al.,* 2007; Girija *et al.,* 2008). These properties make cephalopod ink attractive. In this review, we attempt to combine all these properties to determine the potential prospects of using cephalopod ink in different ways.

2. Cephalopods ink and its components

Cephalopod ink is a natural substance discharged by cephalopods from their ink sac when they confront enemies and try to escape from predators (Ortonne *et al.,* 1981; Lei *et al.,* 2007). The release of dark ink is used as a defensive means to avoid enemies and risks (Liu *et al.,* 2011; Nicomrat and Tharajak, 2015). The ejected ink helps cephalopods to confuse predators and sends a signal to other cephalopods about the danger (Lucero *et al.,*1994).

Cephalopod ink consists of a suspension of melanin granules in a viscous colourless medium. In the mantle cavity, the ink gland cells of the digestive tract degenerate and shed their content into the ink sac, which is used as a reservoir for the ink. The production and ejection of the ink seem to be regulated by the glutamate/ nitric oxide/cGMP signaling pathway located in the ink gland (Palumbo *et al.,* 1997; Palumbo *et al.,* 2000; Liu *et al.,* 2011). In addition to a large amount of melanin, the ink also contains proteins, lipids, glycosaminoglycans and various metals (Copper, Cadmium). (Lei *et al.,* 2007; Liu *et al.,* 2011; Zhong *et al.,* 2009). It also contains a variety of melanogenic enzymes, including tyrosine, which is a dopachrome-rearranging enzyme (Palumbo *et al.,* 1998).

3. Different medicinal and therapeutic properties of cephalopod ink

3.1 Antimicrobial activity

Different studies have been conducted to determine the antimicrobial activities of different squid inks. Nirmale *et al.* (2002), suggested that the freeze-dried and precipitated ink of the Indian squid *Loligo duvauceli* has good antibacterial effects. It mostly showed strong antibacterial effects against gram-negative bacteria, *Salmonella* spp. *Escherichia coli*, *Vibrio cholerae, V. parahaemolyticus* and *Pseudomonas* spp. However, the effects of the gram-positive bacteria *Staphylococcus* spp. and *Micrococcus* spp. are weaker than the effects against gram-negative bacteria. Giriji *et al.* (2011), reported on a novel antimicrobial protein, Lolduvin-s, which was isolated from the ink of Indian squid (*Loligo duvauceli)* and showed promising antibacterial and antifungal activities against different pathogens. Girija *et al.* (2014), revealed that squid (*L. duvauceli*) ink extract has antibacterial potential against dental caries pathogens. It has also been reported that squid ink has good antibacterial properties against extended spectrum beta-lactamase (ESBL)-producing strains of *E. coli* and *Klebsiella pneumonia* (Girija *et al.,* 2012). Karim *et al.* (2016) suggested that squids treated with 0.25% squid ink showed very low growth of bacteria during storage at 4°C. Nicomrat and Tharajak (2015) found that squid (*L. duvauceli)* and soft cuttlefish (*Sepioteuthis lessoniana*) ink have strong antimicrobial activity against biofilms causing microorganisms.

Mochizouki (1979), reported that cuttlefish ink has an inhibitory effect on *Staphylococcus aureus* and has antiseptic properties. The ink of pharaoh cuttlefish, *Sepia pharaonic*, has antibacterial effects against human pathogens such as *Pseudomonas aeruginosa, Staphylococcus epidermidis, K. pneumonia* and *E. coli*. In these cases, researchers have found that crude ink extracted in hexane and column-purified ink extracted in diethyl ether show maximum inhibitory effects against these pathogens (Nithya *et al.,* 2011). Vennila *et al.* (2010), reported that cuttlefish (*Sepia aculeate*) ink and squid (*L. duvauceli*) ink have antifungal effects against *Fusarium* spp. and *Aspergillus fumigates*. Diaz and Thilaga (2016), revealed that crude and partially purified ink extracts of squid (*L. duvauceli*) and cuttlefish (*Sepia pharaonis*) have antibacterial effects against eight different bacterial strains. Table 1 represents a brief information about different in research on antimicrobial properties of cephalopod ink. Ink from different species of cephalopods had been used for studying antimicrobial properties of the ink. Various pathogenic bacteria and microorganisms are used in these studies, a list of that microorganisms also included in Table 1. In most of the studies, different kinds of ink samples showed prominent antimicrobial activities against most of the pathogenic bacteria which made cephalopod ink a very good antimicrobial agent and thus it became an object of attraction among researchers.

3.2 Anti-cancer activity

Squid and cuttlefish ink has the potential to act as anticancer agents, and this is based on *in vitro* studies of cancer cell line. The anti-cancer effects of squid and cuttlefish ink occur through the initiation of apoptosis and are affiliated with different chemicals of ink (Derby, 2014).

Table 1. Antimicrobial properties of cephalopods ink

Name of cephalopods	Mode of ink preparation	Microorganism	Results	References
Squid *(L. duvauceli)* Cuttlefish (*S. pharaonis*)	• Fridge dried crude ink • Partially purified ink by ammonium sulphate	• *S. aureus* (ATCC 25923) • *B. subtilis (*MTCC 441*)* • *P. aeruginosa (*ATCC 27853*)* • *A. hydrophila,* • *S. pyogenes,* • *V. fischeri* • *K. pneumonia (*ATCC 15380*)* • *E. coli (*ATCC 25922) • *C. albicans* (MTCC 227*)* (Fungal strain)	• 100 µL crude ink concentration of both squid and cuttlefish had good activity against all sample microorganism. • 20 µL concentration had good activity only against *V. fischeri* (squid ink) and *K. pneumonia, A. hydrophila* (cuttlefish ink). • Only two fractions (30% and 40%) of partially purified squid ink and one fraction (80%) partially purified cuttlefish ink exhibited activity.	Diaz and Thilaga (2016)
Squid (*L. duvauceli)* Soft cuttlefish (*Sepioteuthis lessoniana*)	• Crude ink pretreated with different temperature (Room temp, 40, 60, 80, 100 ˚C) for 15 minutes	• *E. coli* (ATCC 338849) • *S. aureus* (ATCC 12600) • *B. subtilis (*D83357) • *P. aeruginosa* (ATCC 14886) • *A. fumigatus* (Af293) (Fungus) • *S. cerevisiae* (ATCC 204508) (Yeast)	• Both squid and cuttlefish ink had good activity, but squid ink had more than the cuttlefish ink. • Ink pretreatment from Room temperature to 60 ℃ had more antimicrobial activity than high temperature treated ink.	Nicomrat *et al.* (2015)
Squid	• Crude ink extracted with different solvents (Acetone, Ether, Butanol, Hexane, Ethanol, Methanol, Chloroform, Ethyl acetate)	• *E. coli* (ESBL strain) • *K. pneumonia* (ESBL strain)	• Hexane extract of squid ink showed good antibacterial activity against both organisms. •	Giriji *et al.* (2011)
Indian squid (*L. duvauceli)*	• Crude ink extracted with different solvents (hexane, ethyl acetate, acetone, diethyl ether and chloroform)	• *L. acidophilus* • *S. mutans* • *A. viscosus* • *C. albicans* • (All bacterial pathogens are isolated from carious dentine)	• Only hexane extract of crude squid ink showed good antibacterial properties against all these dentine pathogens. •	Girija *et al*., (2014)
Indian squid (*L. duvauceli*)	• Antimicrobial protein named Lolduvin-S isolated from squid ink and subjected for antimicrobial test.	• *E. coli* (ESBL strain) • *K. pneumonia* (ESBL strain) • *S. aureus* (methicillin-resistant) • *C. albicans* (Amphotericin B resistant)	• Isolated protein from squid ink showed significant activity against all the organisms.	Girija *et al.,* (2011)
Indian squid (*L. duvauceli*)	• Squid ink sample was produced by different method like fridge drying, vacuum drying and precipitation at different pH	• *Salmonella spp.* • *Pseudomonas spp.* • *V. cholerae* • *E. coli* • *V. parahaemolyticus* • *Staphylococcus spp.* • *Micrococcus spp.* • *P. leiognathi*	• Fridge dried and precipitated ink show good activity against gram-positive bacteria. • No activity against gram-negative bacteria.	Nirmale *et al.* (2002)

Table 1 (Continued). Antimicrobial properties of cephalopods ink

Name of cephalopods	Mode of ink preparation	Microorganism	Results	References
Squid (*L. duvauceli*)	• Aqueous extract of crude ink of squid, cuttlefish, and octopus were used.	• *Fusarium* spp. (mold) • *Aspergillus fumigates* (mold) • *Pseudomonas aeruginosa* (G +) • *Staphylococcus aureus* (G +) • *V. cholerae* (G-) • *Salmonella enterica* serovar Paratyphi (G -) • *Shigella boydii* (G -) • *Shigella dysenteriae* (G -) • *K. pneumonia* (G -)	• Squid, cuttlefish and octopus ink showed good antifungal activity. • But these ink samples did not show good antibacterial activity against the test pathogens.	Vennila *et al.* (2010)
Cuttlefish (*Sepia aculeate*)				
Octopus (*Octopus vulgaris*)				
Cuttlefish (*S. pharaonis*)	• Crude ink extracted by acetone, chloroform, butanol, hexane • Partial purification of ink was also done by normal phase silica gel column chromatography.	• *Escherichia coli* • *Citrobacter spp.* • *K. pneumonia* • *S. epidermidis* • *P. aeruginosa*	• Crude ink extract in hexane and column purified ink extract in diethyl ether showed the highest activity against all test microorganisms.	Nithya *et al.* (2011)

Diaz *et al.* (2014), evaluated the anti-cancer activity of squid (*L. duvauceli*) ink. Crude and partially purified squid ink was used on the Hep G2 cell line, and cell viability and cell proliferation assays were performed. They revealed having good anti-carcinogenic activity of partially purified *L. duvauceli* ink on the HepG2 cell line.

Takaya *et al.* (1994), reported on the antitumour peptidoglycan fraction of squid ink obtained from *Illex argentinus.* Tris-HCl buffer (pH 6.8) was used for the extraction and the fractionation of the ink to obtain the peptidoglycan fraction. Strong antitumour activity was found by applying it against Meth-A fibrosarcoma in BALB/c mice. As the fraction of squid ink has no direct cytotoxic effect against Meth-A cells, the stimulation of host-mediated responses may be the reason for the suppression of tumour growth. Sasaki *et al.* (1997), also studied the antitumour activity of the peptidoglycan fraction of squid (*I. argentinus*) ink, which was delipidated in acetone and used against Meth-A tumours from BALB/c mice, and they found 64% cure rate against Meth-A tumours. Later, Naraoka *et al.* (2000) showed that there are two different components that are responsible for the anti-tumour activity of squid ink. They suggested that illexin-peptidoglycan, tyrosinase and the complex of these two are responsible for the antitumour activity of squid ink, and the complexity of the two components showed the highest antitumour activity against Meth-A tumour cells of BALB/c mice. Zhong *et al.* (2009) worked on the protective effects of squid ink in chemotherapy. In the study, BALB/c mice were used as animal models, and injuries were induced by cyclophosphamide. This study showed positive results for the protection of the haemopoietic system from chemotherapeutic injury and suggested that it could be employed to develop cell protective drugs for use in clinical treatment of tumours.

Chen *et al.* (2010) reported that chemically sulphated polysaccharides (SIPs) isolated from squid (*Ommastrephes bartrami)* ink have good antitumour activity against HepG2 tumour cells. Both *in vitro* and *in vivo* studies provide substantial proof that SIPs have potential compounds for the prevention of tumour metastasis.

Senan *et al.* (2013a) suggested that ink extracts of different cuttlefish species, such as *Sepia pharaonis, Sepia aculeate, Sepiella inermis* and squid (*L. duvauceli*), that were delipidated in acetone and extracted by Tris-HCl buffer have potential antiproliferative effects against chick embryo fibroblast cells, which provides proof of the potential use of the ink extracts as an anticancer agent. Another study by Senan *et al.* (2013b) provided information about the anticancer activity of the purified C2 fraction of *S. pharaonis* ink against cervical cancer cell lines-HeLa and Caski. Fahmy and Soliman (2013) reported that cuttlefish (*Sepia officinalis*) ink extract has cytotoxic activity and can be used as promising anticancer drugs, which was

determined using the sulphorhodamine B (SRB) method against hepatocellular carcinoma (HepG2) cell line. Soliman *et al.* (2015) found that *S. officinalis* ink extract had anticancer effects against Ehrlich ascites carcinoma (EAC) in Swiss albino mice. He added that *Sepia* ink extract inhibits tumour growth in the ascites tumour model. Russo *et al.* (2003) applied melanin-free *S. officinalis* ink on various cell lines, including PC12 cells, and found that it was cytotoxic to the PC12 cell lines and could be used in future carcinogenic drugs.

Guo-Fang *et al.* (2011) performed enzymatic hydrolysis of *S. officinalis* ink using trypsin enzyme and isolated an oligopeptide from hydrolysates, which have anticancer activities. These potential anticancer properties were proved when the isolated oligopeptide from *Sepia* ink hydrolysates were used against human prostate carcinoma cells (DU-145). Huang *et al.* (2012) reported that Sepia ink oligopeptide (SIO), which is a tripeptide extracted from *Sepia esculenta* ink, inhibits prostate cancer by inducing apoptosis. SIO was applied against three human prostate cell lines DU-145, PC-3 and LNCaP to prove the potency of SIO as an anti-carcinogenic agent.

Wang *et al.* (2008) applied sulphated *Sepiella maindroni* ink polysaccharide (SIP-SII) to human ovarian carcinoma cells SKOV3 and human umbilical vein vascular endothelial cells ECV304, and their results suggested that SIP-SII might oppress the migration and invasion of carcinoma cells via inhibition of matrix metalloproteinases-2 (MMP-2) proteolytic activity. Zong *et al.* (2013) found that SIP-SII has anti-metastatic and anti-angiogenic properties, which also result in the depression of the invasion and migration of carcinoma cells. Changlong *et al.* (1999) revealed that squid ink and its extracts might activate immunity of cells by stimulating natural killer cells and macrophages to kill tumour cells indirectly. Table 2 represents the different studies on anticancer properties of cephalopods ink. Most of the anticancer studies of cephalopods ink were done against different cell lines to measure the anticancer properties. The ink had been used in a different mode and good anticarcinogenic effect was found in almost all cases shown in Table 2.

3.3 Antioxidant activity

Squid and cuttlefish ink have antioxidant properties that reside in both the melanin and melanin-free fractions of the ink (Derby, 2014). Liu *et al.* (2011) studied the antioxidant activity of squid ink on growing broiler chickens by mixing ink in their diet. Total SOD (Superoxide dismutase) activity and MDA (malondialdehyde) content determination results showed strong antioxidant abilities of squid ink. Vate and Benjakul (2013) studied the melanin-free ink from the splendid squid *Loligo formosana.* They performed differently *in vitro* antioxidant tests, including DPPH radical scavenging activity, ABTS radical scavenging activity, ferric reducing antioxidant power (FRAP) and chelating activity towards Fe^{2+}, and found good antioxidant value in the melanin-free squid ink.

Sun *et al.* (2011) studied the polysaccharides extracted from squid ink by alkaline protease and tested for the antioxidant activities using DPPH, radical scavenging and a FRAP assay and found a high amount of antioxidant value from the extracted polysaccharides. Chen *et al.* (2007) removed the melanin from squid ink, and a free radical scavenging activity test was conducted on the removed melanin. They showed that squid melanin scavenge hydroxyl free radical remarkably which are indicators of the antioxidant properties of squid melanin. Lin and Chen (2005) also isolated melanin from cuttlefish (*Sepia*) ink and investigated the antioxidant properties, and they found high antioxidant value. Lei *et al.* (2008) also found antioxidant effects of melanin-Fe squid ink when they were using it as a treatment for iron deficiency anaemia (IDA) in rats.

Fahmy and Soliman (2013) investigated the antioxidant activity of cuttlefish (*S. officinalis*) ink extract using 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging and lipid peroxidation assays and measured promising antioxidant properties from the *Sepia* ink extract. Another study by Soliman *et al.* (2015) was conducted to compare the antioxidant and anti-cancer activities to 5-fluorouracil (5-Fu) *in vivo*, where Swiss albino mice were used as experimental animals, and they found good antioxidant properties.

An *in vivo* study was conducted by Saleh *et al.* (2015) and provided information on the complications of hepatic fibrosis associated with bile duct ligation (BDL) and the potential curative role of *S. officinalis* ink extract in hepatic damage induced by BDL. The result showed a significant reduction in oxidative stress, which proves the antioxidant activity of the ink extracts. Another *in vivo* study provided information that cuttlefish ink increases the antioxidant level in mice (Lei *et al.,* 2007). Studies on antioxidant properties of cephalopod inks are listed in Table 3. Both *in vitro* and *in vivo* studies had been done to determine the antioxidant properties of cephalopod ink. The mode of antioxidant studies is given in Table 3.

4. Other properties of cephalopod ink

Squid (*L. duvauceli*) and cuttlefish (*Sepiella inermis*) ink have antiretroviral activities, which were reported by Rajaganpathi *et al.* (2000). Squid ink has anti-

Table 2. Anti-cancer properties of cephalopod ink

Name of Cephalopods	Mode of Ink used	Name of assay and cell line used for study	Findings and Future concerns	References
Squid (*L. duvauceli*)	• Fridge dried crude ink • Partially purified ink by ammonium sulphate	• MTT assay (Cell viability and cell proliferation assay) • HepG2 cell line	• Partially purified ink showed good anticarcinogenic activity against HepG2 cell line. • Can be used treating Hepatic cancer	Diaz *et al.* (2014)
Squid (*O. bartrami*)	• Gel filtrated melanin free ink sulfated by $PySO_3$	• MTT assay • The human hepatocellular liver carcinoma cell line (HepG2)	• Showed dose-dependent suppression of cell invasion and migration in HepG2. • Can be a potential candidate for the prevention of tumor metastasis.	Chen *et al.* (2010)
Cuttlefish (*S. pharaonis*)	• Crude ink delipidated with acetone then gel filtrated to get purified peptidoglycan fraction	• MTT assay • Comet assay • Cervical cancer cell lines (HeLa and Caski)	• Showed significant anticancer activity • Can be a potential drug for treating cervical cancer	Senan *et al.* (2013a)
Cuttlefish (S. *officinalis*)	• Crude ink aqueous extract was lyophilized	• Sulphorhodamine B (SRB) method • HepG2 cell line	• Results showed good cytotoxicity against the cell line which may lead it as a potential anticancer drug.	Fahmy *et al.* (2013)
Cuttlefish (*S. officinalis*)	• Crude ink	• MTT assay • Human glioblastoma cells U87	• Showed cytotoxicity against the cell line •	Ellouze *et al.* (2015)
Cuttlefish (*S. pharaonis*) Cuttlefish (*S. inermis*) Cuttlefish (*S. aculeata*) Squid (*L. duvauceli*)	• Crude ink delipidated with acetone and extracted with Tris-HCl followed by lyophilization	• Cell viability assay using Trypan Blue • Ethidium bromide /acridine orange staining • Chick embryo fibroblasts cells	• Ink of *Sepia pharaonis* showed highest antiproliferative activity • Found therapeutic potential of the ink as an anticancer agent.	Senan *et al.* (2013b)
Cuttlefish (*S. officinalis*)	• Melanin free cuttlefish ink	• Cell viability • PC12 cell line and Caco2 cell line	• Showed good result against the cell lines.	Russo *et al.* (2003)
Cuttlefish (*Sepia esculenta*)	• Oligopeptide isolated from hydrolysates of Sepia ink	• Cell viability using CCK-8 assay • Human prostate carcinoma cell, DU-145	• Gln-Pro-Lys peptide showed significant effect against DU-145 cell line. • Potential antitumor agent	Guo-Fang *et al.* (2011)
Cuttlefish (*Sepia mendroni*)	• Ink polysaccharide (SIP) isolated from the ink by enzymolysis, anion-exchange and gel-permeation chromatography.	• MTT assay • Cell viability analysis by trypan blue exclusion • Human ovarian carcinoma cell line SKOV3 and human umbilical vein vascular endothelial cell line (HUVEC) ECV304	• Findings from this study suggested good anticancer activity against human carcinoma cells.	Wang *et al.* (2008)
Cuttlefish (*S. mendroni*)	• Oligopeptide isolated from hydrolysates of Sepia ink	• Cell viability using CCK-8 assay • Human prostate carcinoma cell, DU-145, PC-3 and LNCaP cells	• Good anticarcinogenic properties found in the ink sample.	Huang *et al.* (2012)

Table 3. Antioxidant properties of cephalopod ink

Name of the cephalopods	Mode of ink	Types of experimental models for determining antioxidant activity	Methods of determining Antioxidant activity	Results	References
Cuttlefish	• Crude ink diluted and mixed with boiler diet in different concentration	• *In vivo* • Arbor Acres broiler chicken was used	• Total SOD (Superoxide dismutase) activity determination • MDA (malondialdehyde) content determination	• High dose of ink showed good SOD activity on broiler chicken serum • Also reduced MDA level significantly.	Liu *et al.* (2011)
Spendid Squid (*Loligo formosana*)	• Melanin free ink was used for different antioxidant test	• *In vitro* assays	• DPPH radical scavenging activity • ABTS radical scavenging activity • Ferric reducing antioxidant power • Chelating activity toward Fe^{2+}	• Good antioxidant properties were found *in vitro* assays. • High concentration of melanin free ink shows good antioxidant activity on different model systems for determining antioxidant activity	Vate and Benjakul (2013)
		• Different Model systems	• β-Carotene-linoleate model system • Lecithin liposome system • Fish mince model system		
Squid (*Ommastrephes bartrami*)	• Squid ink melanin-Fe mixed with diet	• *In vivo* • Early weaned male Wistar rats (4 weeks old) were used	• Total SOD (Superoxide dismutase) activity determination • GSH-Px (Glutathione peroxidase) activity determination • MDA (malondialdehyde) content determination	• SOD and GSH-Px activity increased while MDA content decreases in the serum of the rats.	Lei *et al.* (2008)
Cuttlefish (*S. officinalis*)	• Crude ink aqueous extract was lyophilized	• *In vitro*	• DPPH radical scavenging activity	• Significant reduction of oxidative stress was found on BDL group after treatment with sepia ink	Saleh *et al.* (2015)
		• *In vivo* (*Male albino rats*) • Divided into two groups the Sham-operated control and bile duct ligated (BDL) group	• Lipid peroxide level determination • GSH-Px (Glutathione peroxidase) activity • Total SOD activity • Glutathione-S-transferase (GST)		
Cuttlefish	• Ink was hydrolyzed by proteinase enzyme and vacuum dried prior to use	• *In vivo* • Healthy female ICR mice divided into five groups	• Total SOD activity	• Results showed increased antioxidant level in mice serum.	Lei *et al.* (2007)

Table 3 (Continued). Antioxidant properties of cephalopod ink

Name of the cephalopods	Mode of ink	Types of experimental models for determining antioxidant activity	Methods of determining Antioxidant activity	Results	References
Cuttlefish (*S. officinalis*)	• Crude ink aqueous extract was lyophilized	• *In vitro*	• FRAP assay • Lipid peroxidation assay	• Significant antioxidant activity found in cuttlefish ink	Fahmy *et al.* (2013)
Cuttlefish (*Sepia* spp.)	• Melanin isolated from the ink	• *In vitro*	• Ferric thiocyanate method • Ferric reducing antioxidant power (FRAP) • Chelating activity toward Fe^{2+}	• High melanin concentration showed good antioxidant activity	Lin and Chen (2005)

inflammatory effects, which were studied by Mimura *et al.* (1987). Low molecular weight melano proteins were obtained from *Ommastrephes bartrami* ink and studied for anti-inflammatory effects using carrageenan-induced rat paw oedema. In addition, Fahmy and Soliman (2013) also found anti-inflammatory effects of cuttlefish (*Sepia*) ink.

Kim *et al.* (2003) extracted and purified an angiotensin-converting enzyme from squid ink which can dilate blood vessels and lower blood pressure. According to Mimura *et al.* (1982) squid ink has strong anti-ulcerogenic properties. Melanin extracts constitute almost 90% of squid ink and could inhibit gastric secretion in rats. The molecular weight of melano protein that is contained in the active fractions might be responsible for the anti-ulcerogenic activity, by enhancing the glycoprotein activity of gastric mucosa. In addition to the above properties, *Sepia* ink also has anti-neoplastic properties (Soliman *et al.,* 2015) and plasma coagulation properties (Vennila *et al.,* 2010) which make it an attractive element among researchers.

5. Conclusion

Compared to synthetic products, natural bioactive products have the fewest side effects, and the marine environment can be a good source of natural bioactive products. Among different marine products, cephalopod ink is one of the best sources of bioactive products. Different studies which were demonstrated in this study provide information about the neutraceuticals properties of the cephalopods ink. This review also collaborates information about various modes of using the ink with methodology, which can be helpful for future researchers to conduct a new research. Functional and nutraceutical properties of cephalopods ink are mainly focused in this study so that awareness on using cephalopods ink can be built up. In the near future, it is hoped that this can reduce the wastage of the ink industrially. Proper consciousness on its variety of medicinal and therapeutic properties will make the ink an attractive object for preparing functional food and alternative medicine.

Conflict of interest statement

We declare that we have no conflict of interest.

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