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THE COMPHREHENSIVE REVIEW OF OCTOPUS VULGARIS INK

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ABSTRACT

The inking behavior of *Octopus vulgaris*, oleoid cephalopods—squid and cuttlefish is the most distinctive and defining features of them. The planktonic period at the para-larval stage accounts for about 10–15% of *O. vulgaris* (9–15 month) life cycle and their sexual maturity occur in a few months later. Unused marine food items, such the ink from cephalopods, may contain bioactive substances that have positive health effects. The present review stated its possible anti-microbial, anti-cancer, anti-oxidant properties, anti-bacterial, anti-fungal, gastric acid inhibitor activity, anti-Schistosomal activity of the chemical ink made from *Octopus vulgaris* by in-vitro and in-vivo studies. It was also found that, ink obtained from O. vulgaris may be used in the development of formulations to treat various diseased conditions.

KEYWORDS: *Octopus Vulgaris*, Life Cycle, Ink Production, Biological Properties.

INTRODUCTION

Octopus vulgaris is also an important food source in many countries.[1] About 800 extant species of cephalopods, including octopuses and their close cousins' cuttlefishes, squids, and nautiloids, have been described in data. Octopus species are commercially available as nutritious seafood.[2] One mollusk species of significant ecological and commercial valued on a global scale was the common octopus. Although populations within its distribution range have been shown to differ genetically and morphologically, it was primarily found in the Mediterranean Sea and central-eastern Atlantic waters.[3] Cephalopods utilized the ink sac, a reservoir organ for black ink made primarily of eumelanin, to trick and protect themselves

from predator assaults.[4] There are numerous macro and micro molecules in the ink that have anti-inflammatory and anti-cancer properties.[5]

The *O. vulgaris* ink, a natural occurred from marine substances, may contain biological elements that can provided beneficial health advantages such as cancer prevention and antioxidant activity.[6] Due to some reports, there are 18 million people who have died from cancer globally. Cancer diseases in the organs such as in the stomach (5.72%), then the colon has (10.23%), (7.06%), lung (11.58%), and breast (11.55%) (IARC, 2018).[7] Presenting the current state of knowledge regarding cephalopod ink's production, natural functions, such as, chemical components and their roles in these functions, and human use, including the creation of medications and therapeutic applications, was the aim of this review.[8] A greater understanding of cephalopod ecology and life history aspects is a priority because the exploitation of cephalopods has increased recently, particularly in Europe, due to the decreased of other fishing resources.[9]

Life cycle of *octopus vulgaris*

According to the *O. vulgaris*, a female are ready to deposit the spawn then they hide in the dens and place the eggs on cluster on walls. After those females take care of eggs alone, for the 25 to 65 days they take care of the eggs during the life cycle. But they have the short duration of life cycle up to 12 to 18 months. Due to high amount of food conversion for the octopus their body weight increases up to 13%. Their habitats vary according to their life stage, para-larvae and selement stage. Part of the zooplankton was made up of creatures during the para-larvae stage, which lasts from hatching to about 30 to 40 days.[10] In the selement stage, these animals gradually change to benthonic life. Throughout their maturation, this may take up to five months, the female remains with the eggs, constantly protecting and nurturing them. In this final stage of its existence, the female octopus digests its own muscles instead of feeding. The female consequently perishes soon after the eggs hatch. Prior to settling on the sediment, the translucent hatchlings, known as para-larvae, which are 1-2 mm in size, go through a weeks-long planktonic phase that occurs primarily in shallow waters.[11]

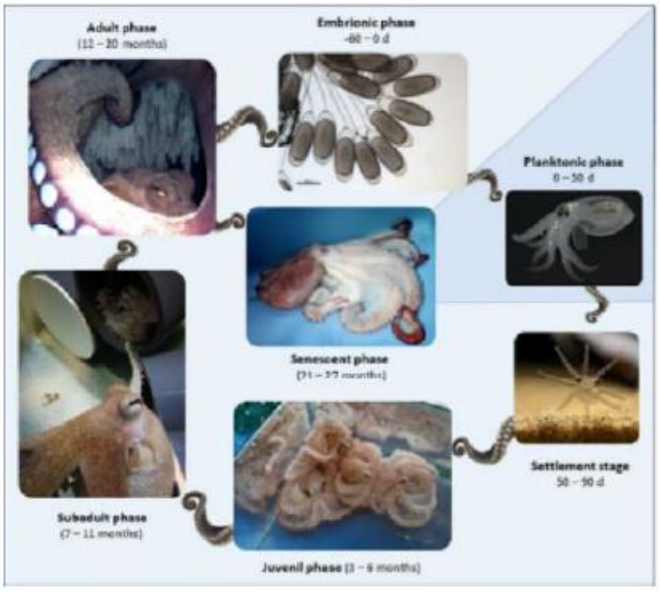

Fig. 1: Life cycle of *Octopus vulgaris.*

Composition of *octopus vulgaris* ink

One of the distinct and unique characteristics of Coleoid cephalopods, which include octopuses, cuttlefish, and squid, is their inking behavior. The ink contains a significant amount of melanin along with proteins, lipids, glycosaminoglycans, and different metals such as copper and cadmium.[12][13] Melanin granules are suspended in a viscous, colorless substance to form cephalopod ink. The digestive tract's ink gland cells degenerate in the mantle cavity and release their contents into the ink sac, which served as an ink reservoir. The glutamate/nitric oxide/cGMP signaling pathway was found in the ink gland appears to control the ink's synthesis and ejection.[12] Ink extracted has also been found to contain specific amino acids, including glycine, glutamic acid and aspartic acid.[14]

Octopus ink production

The primary ink gland was a part of the ink sac, which makes black ink was based on melanin. It secretes this substance into the ink, Sac lumen before being released by a duct into the hindgut closed to the anus. Concurrent with the ink sac's secretion, the only mucus gland near it, secretes and releases mucus.[10] The ink gland's glutamate/nitric oxide/cGMP signaling pathway appears to control the ink's synthesis and ejection.[13]

Biological properties of *octopus vulgaris* ink extract

Anti-microbial activity

The causes for many human infections are micro-organisms. The melanin fraction, aqueous and organic extracts of octopus ink was found to possess antimicrobial activity against a range of micro-organisms. But constituents responsible for its antimicrobial activity are not disclosed in the literature.[8]

Anti- oxidant activity

The Premalignant lesions' growth inhibition and regression could be achieved by a range of antioxidants. According to research on Broiler chicks, crude squid ink and extracts have antioxidant properties. Ink extracts used as antioxidants in the food industry, however, restricted by the presence of the black pigment melanin.[15] Polysaccharides in cephalopods have also been shown to possess antioxidant properties.[16] For example, melanin, a naturally occurring substance was founded in octopus' ink, functions as a reducing or antioxidant agent, stabilizing the ink's inherent components and halting oxidation.[6]

Antioxidants are regarded as key characteristics of biological compounds, which are employed to counteract oxidative stress. The total antioxidant capacity (TAC) and the ability to scavenge free radicals (DPPH) were evaluated. The assessment of antioxidant activity was conducted through the DPPH test and the phosphor-molybdenum assay.[16]

Table 1: Anti- oxidant activity of *O. Vulgaris* ink.

Sample	Total anti-oxidant capacity (mg AAE/g dry extract)	DPPH IC50 (µg/ml)3
Ink extract	159.33 ± 4.16	56.24 ± 2.41
Ascorbic acid	-	7.46 ± 1.35

Thus research was found that the ink of *Octopus vulgaris* exhibited a total antioxidant capacity of 159.33 mg AAE/g of dry extract, indicating its ability to convert molybdenum (VI) to molybdenum (V). Additionally, when compared to ascorbic acid, which served as the reference compound with an LC_{50} value of 7.46±1.35µg/ml, the ink also showed effectiveness in scavenging DPPH free radicals, with an LC_{50} value of 56.24±2041µg/ml.[16]

Anti-cancer activity

The anticancer activity of octopus ink was proved by various studies. According to *in vitro* investigations of several cell types and cell lines, cephalopod ink may have anticancer

properties. Apoptosis is the main ingredient that is induced to provide the effect, and various compounds present in the ink are frequently linked to this [8]. Ozopromide (OPC) is a novel compound that can be isolated from *O. vulgaris* ink extracts that was found in previous studies. The ozopromide has effective anti-proliferative and pro-apoptotic activities in a few cancer cell line studies, that includes the prostate (22Rv1), lung adeno-carcinoma (A549) and epithelioid cervix adeno-carcinoma (He La) cells. However, the test results provide the greatest effects observed on A549 cells. The structural characterization of OPC was verified by C-/H-NMR, FTIR, and COSY2D. Through these cell lines studied on various tissues that can show the action of ozopromide, which prevented the human malignant cells from growing in the cervix (He La), prostate (22Rv1), lung (A549), and breast (MDA-MB-231).[5]

The *Octopus vulgaris* ink also has anti-proliferative effects. Although DM did not have an inhibitory impact below 100 μg/mL in the human breast cancer cell line (MDA-MB-231), it has some disadvantages like that, showing the reduced effect of anti-proliferative action among the octopus ink extracts on colorectal adeno-carcinoma cell lines (HT-29 and HCT116).[7] In the presence of normal Baby Hamster Kidney Fibroblasts (BHK) cell lines, the anticancer properties of the ink were tested using the Michigan Cancer Foundation-7 human hepato-cellular carcinoma (HCT), human liver cancer cell line (HepG2), and human breast cancer cell line (MCF-7).[16] The *Octopus vulgaris* ink, also has antitumor activity.[17] By this Among cephalopods, the common octopus is a well-known model species and had effective anti-cancer activity.[18]

Anti-bacterial activity

According to this review of *O. vulgaris*, ink has antibacterial properties against gram-positive bacteria and gram-negative bacteria. The gram-positive bacteria like *Streptococcus faecalis*, *staphylococcus aureus* and B*acillus subtilis*. The gram-negative bacteria's include Escherichia coli, Pseudomonas *aeruginosa* and *Neisseria gonorrhoeae*.[12]

Table 2: Anti-bacterial activity of *O. vulgaris.*

Name of cephalopods	Mode of ink preparation	Microorganism	Results
Octopus vulgaris	An octopus crude ink aqueous extract was utilized.	1. Fusarium spp. (mold) 2.Aspergillus fumigates (mold) 3.Pseudomonas aeruginosa (G +) 4.Staphylococcus aureus	It shows the strong anti-bacterial activity.

		(G +) 5.Salmonella entericaserovar Paratyphi (G -)	

This table showed that octopus vulgaris, ink method of production and the results against the bacterial organisms.

Anti-fungal activity

From the aqueous extract of the octopus crude ink it has the some anti-fungal properties by the octopus mold activity.[13] The *Octopus vulgaris* ink have effective against the *Aspergillus flavis* and the yeast C*andida albicans*.[16] In the recent research that octopus has many bioactive compounds such as proteins, peptide and polyphenolic substances which contribute to the antimicrobial properties.[13]

Inhibitory activity of gastric juice secretion

The crude melanin was extracted from *Octopus vulgaris*, then eluted and lyophilized. The SDS polyacrylamide gel electrophoresis was utilized in this study, displaying the peak obtained through filtration using a Sephadex G-200 column with 0.1 m Na2HPO4 (pH 8.5) containing NaCl. They administered atropine sulfate at dosages ranging from 1 mg to 5 mg/kg to inhibit gastric juices in pylorus-ligated rats. The dose was subsequently increased to 10 mg/kg via the intra-peritoneal route. For assessing anti ulcerogenic activity, metiamide was found to reduce the ulcer index caused by aspirin at doses between 50 mg and 100 mg/kg. The three melanin samples displayed UV absorption at a wavelength of 280 nm. The chemical composition of the octopus consisted of 79.0% melanin pigment, 17.5% protein, and 1.7% sugar, which showed a single peak at pH 1.8 during iso-electric focusing.[19]

The abnormality of gastric acid secretion and utility are very common in many people. The *Octopus vulgaris* ink had proven to have inhibitory activity on gastric acid secretion. So the study was conducted on rodents like rats, it was discovered that octopus melanin from *Octopus vulgaris* ink bags inhibited gastric secretion. That melanin of *Octopus vulgaris* was composed of only high molecular fraction and was different from the squid melanin. Based on its inhibitory effects on gastric secretion, the melanin fraction demonstrated a strong inhibitory activity on ulcer formation in rats with pylorus ligation and ulcers induced by aspirin. It shared this effect with squid melanin.[19]

Anti- schistosomal activity

The anti-Schistosomal is a drug to kill the parasites like a worms that affects the liver, intestine etc., The current *in-vitro* study revealed that, ink produced by octopus had anti-schistosomal activity. The schistosomiasis is an endemic disease in tropical regions and it affects billions of the people worldwide. This present study results showed the effect on both wormicidal and larvicidal like, *S. mansoni* worms, miracide and cercariae, it also consequently decreased the transmission of schistosomiasis and control the disease.

Table 3: Anti- schistosomal activity of O. vulgaris.

Concentration (ppm)	LC_{10}	LC_{25}	LC_{50}	LC_{90}	Slope
B. Alexandrina	44.07	65.03	88.3	132.5	1.1
Schistosoma mansoniworms	4.7	14.01	24.2	43.7	1.2

The data presented on the Table-1 that illustrates the molluscicidal effectiveness at various concentrations of LC. A total of 20 distinct compounds were examined using GC-MS, revealing that one compound demonstrates molluscicidal activity with an LC_{50} of 88.3 mg/l against B. Alexandrina snails. Additionally, it exhibits a wormicidal effect on *S. mansoni* worms, showing an LC_{50} of 24.2 mg/L after a 24-hour exposure period. The GC-MS analysis was conducted with a Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS, utilizing a TG-5MS fused silica capillary column (30m, 0.251mm, 0.1mm film thickness) and a measurement setup employing electron ionization at 70 eV, with helium gas used to maintain a steady flow rate. The injector temperature was set at 280°C, while the oven temperature started at 50°C for 2 minutes before being increased to 150°C at a rate of 7°C per minute. The measurements of all the identified constituents were quantified by calculating a percent relative peak area.[16]

CONCLUSION

The review is concluded that, present findings on the therapeutic activity of *Octopus vulgaris* ink and its complex composition, like pigments, enzymes and bioactive compounds offer potential application in biotechnology, medicine and food industries. It is also concluded that, *Octopus vulgaris* ink exits antifungal, antibacterial, anticancer, antimicrobial, gastric acid inhibitory and anti-schistosomal activity. Based on this review, octopus ink become valuable resource in innovation and could be forwarded for pharmaceutical research and drug development to treat various diseases.

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