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Screening of Antimicrobial Activity from *Sepia lycidas*

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Abstract:

The kisslip cuttlefish can be found mainly within the Indo-West Pacific, at depths ranging from 15–100 meters. Additionally, this species exhibits many diverse, complex reproductive behaviors; for example, courting, mating displays, and mate competition. Other interesting behaviors includes their feeding and hunting methods, which entails turning towards a preferred direction to "jump on" and engulf their prey of small fish and crustaceans. Sepia lycidas has many human uses and is important in the economy of many Southeast Asian countries, especially since they are often eaten for their high nutritional value.

Keywords — **Squid fish, squid ink, squid microbial effects.**

I. INTRODUCTION

Sepia lycidas, commonly known as the kisslip cuttlefish, is a species of cuttlefish within the genus Sepia .They are also classified under the family Sepidae, which encompasses some of the most commonly known and recognized cuttlefish., this species of cuttlefish is most closely related to Sepia aculeata, Sepia esculenta, and Sepia_pharaonis. This species is typically reddish brown to purple in color, with patches and stripes present on their dorsal mantle. On average, they grow to be about 38 cm in length and weigh 5 kg at maximum[1].

They are also currently being studied as an alternative source of collagen for human use, since their thick outer skin direction to "jump on" and engulf their prey of small fish and crustaceans. Sepia lycidas has many human uses and is important in the economy of many Southeast Asian countries, especially since they are often eaten for their high nutritional value. They are also currently being studied as an alternative source of collagen for human use, since their thick outer skin contain high levels of collagen that goes to waste when they are eaten or caught as by catch[2], [3].

Figure: 1 Sepia lycidas

Cuttlebone, which is usually ellipsoid in shape, is located within the dorsal mantle of the cuttlefish to provide individuals with many benefits like protection, support, and buoyancgre. Sepia lycidas is a common species of large cuttlefish, and will grow to have an average mantle size of 38 cm and a maximum weight of 5 kg. In general, cuttlefish have binocular vision and are able to assess the exact distance to a target with acuracy.

Their coloring ranges from reddish brown to purple, and individuals have scattered ocellate patches and lightened stripes along their dorsal mantles. Additionally, this species has small suckers, all similar in size to one another, and has a hectocotylus present on their left ventral arm. Sepia lycidas can typically be found at depths ranging from 15–100 meters; however, they do migrate to shallow coastal areas from April to July for both the mating and breeding season. Specifically within the South China Sea, the kisslip cuttlefish resides in habitats that are covered with shells, gravel, sand, and seaweed, which is similar to both Sepia pharaonis and Sepia latimanus. Studies have shown a morphological asymmetry present in the cuttlebone curvature within the kisslip cuttlefish. The cuttlebone may be more convex, or more developed, on one side than it is the other, which causes the cuttlefish to exhibit preferences towards it's right or left side when hunting prey. For example, the cuttlefish will typically turn towards its right side when hunting, if the cuttlebone is more convex on the right side than the left[1], [3], [4]Sepia lycidas has a life cycle that is similar to other cuttlefish species, beginning with embryos hatching into a planktonic stage after about 25 days of incubation. Hatching times are vulnerable to changes in environmental conditions and can vary depending on light, temperature, and salinity. They continue to grow and develop through this planktonic stage until they are fully grown, benthic adults; however, juveniles will still show similar behaviors to benthic adults like burying themselves in the sand soon after hatching. Males reach sexual maturity at approximately 150 days after hatching, while females take longer to fully mature. Both males and females will usually die shortly after their spawning and brooding behaviors have ceased.

The kiss lip cuttlefish feeds primarily on fish and crustaceans, especially shrimp, which they engulf by "jumping" on their selected prey. Additionally, studies have found that they exhibit a turning behavior when hunting, and will turn their bodies either clockwise or counterclockwise before engulfing their prey, which is an example of behavioral dimorphism within the species[5]. Sepia lycidas have become very economically important in Southeast Asia, especially in Japan and Hong Kong since they are commonly eaten due to their high nutritional value. The way that cuttlefish are caught is dependent on the season, but they can be caught by hook, by using live cuttlefish lures, or through bycatch. Additionally, they have the opportunity to become an important species for commercial aquaculture because of their high food conversion, rapid population growth rates, and increasing growth in market value. Outside of aquaculture, this species is often used as a model organism and has been studied extensively as a potential alternative source of collagen, which humans use in cosmetics, biomedical materials, and foods Large amounts of collagen can be obtained from the cuttlefish's thick outer skin, so if the thermal stability can be improved then this species will be a good source of collagen and will help to minimize the pollution and odor impacts caused by treating the cuttlefish skins as waste[6], [7], [8].

Sepia lycidas is a warm-ocean demersal Cephalopoda that inhabits offshore waters at a depth of approximate 15-100 m, and is mainly distributed from the Indian Ocean to the western Pacific, and few research studies have been reported on this species. In order to enrich theoretical knowledge for the artificial breeding of S. lycidas, the fine structure of reproductive system in male and female S. lycidas was observed by anatomical and histological methods. The results showed that the male reproductive system consists of nine parts, i. e.: testis, sperm duct, mucilaginous gland, ejaculatory apparatus gland, middle tunic gland, outer tunic gland, hardening gland, finishing gland, and spermatophoric sac. The sperm duct could be further divided into anterior, middle (ampullae), and posterior part. The mature spermatozoon produced in the testis and transported by the sperm duct, formed sperm masses in the mucilaginous and ejaculatory apparatus gland, then arranged in a new regular in the middle and outer tunic gland, and turned hard in the hardening gland, and formed mature spematophores in the finishing gland. The mature spematophores stored in the spermatophoric sac, and ejaculated outside by batches in the mating seasons. The female reproductive system consists of five parts, i. e.: ovary, oviduct, oviduct gland, a pair of nidamental gland, and a pair of subaltern nidamental gland. Oogenesis is asynchronous, and the oocyte matures by batches, forms a tertiary egg envelope by joint action of the oviduct gland, nidamental gland, and subaltern nidamental gland. The male and female reproduction system of S. lycidas is adapted to its manner of fertilization and spawning.The Journal of Basic & Applied[9].

Unfortunately due to overexploitation and insufficient recruitment, kiss lip cuttlefish populations have declined dramatically in more recent years. This has been caused more specifically by fishing gear, bycatch, and overwintering of both adults and juveniles. The species is currently listed on the IUCN Red List as “Data Deficient” because more information is needed on the species to determine an accurate conservation status. In recent years, the kiss lip cuttlefish has been studied to expand knowledge of Sepiidae species’ phylogenetic relationships and species geography. It was found that Sepia lycidas have a genomic composition and order that’s very similar to most other invertebrates, and, when compared 37 other cuttlefish species, they are most closely related to Sepia aculeata, Sepia esculenta, and Sepia pharaonis. Overall, the kisslip cuttlefish’s currently determined phylogeny is similar to its traditional taxonomy[10].

Invasive pulmonary aspergillosis (IPA) is a life-threatening disease in immunocompromised patients that requires aggressive therapy. Because of the widespread use of antibiotics, corticosteroids, antitumor drugs, and immunosuppressive drugs, the morbidity of IPA is currently increasing. The ink secretion of sepia lycidas species was identified as one of the novel sources of bioactive compounds[11].

So the present study designed to investigate the antifungal and antioxidant effects of Sepia lycidas ink extract against IPA in mice. Eighty neutropenic infected mice were randomly assigned into four main groups (20 mice/group). The 1st group was treated with saline, neutropenic infected, the 2nd group was treated with ink (200 mg/kg) and the 3rd group was treated with amphotericin B (150 mg/kg) and the 4th group was treated with ink plus amphotericin B (Ink 200 mg/kg and AMB 150 mg/kg)[12]. Treatment was started at 24 h after fungal inoculation and was administered for 3 consecutive days. The present study demonstrated good in vitro and in vivo antifungal activity of IE against Aspergillus fumigatus. Compared with IPA group; IE-treated, AMB-treated, and AMB + IE-treated animals had a 67.80%, 83.41%, and 72.68% reduction in the pulmonary fungal burden, respectively. Treatment with IE and/or AMB for one and three days significantly decreased MDA and increased GSH and SOD levels in the lung tissues as compared with the infected untreated group. In conclusion, the results of our in vivo and in vitro studies demonstrate that IE has therapeutic effect against invasive pulmonary aspergillosis via reducing oxidative stress. Because of the widespread use of antibiotics, corticosteroids, antitumor drugs, and immunosuppressive drugs, the morbidity of IPA is currently increasing. Emerging evidence suggests that marine natural products continue to be a major source of nutraceutical and functional foods and as a source material for the development of drugs[13].

The cuttlefish Sepia lycidas relies for defense on the ejection of a dark ink in order to create a dark, diffuse cloud which can obscure the predator’s view, allowing the cephalopod to make a rapid retreat by jetting away. Sepia ink consists of a suspension of melanin granules in a viscous colorless medium [14]. Most of the studies concerning antimicrobial activity include specific compartments like egg masses, hemolymph or whole body extracts of sepia lycidas [15]. Sepia lycidas not only exhibit the anti-microbial activity, but also constitute many classes of bioactive compounds which include antitumor, antileukemic and antiviral activities [16],

Squid ink is made up of many compounds and nutrients. It gets its dark color from a natural pigment called melanin, the same thing that gives color to your hair and skin.

II. MATERIAL AND METHODS

SAMPLE COLLECTION:

Collection and sample preparation Sample with physical appearance was collected from perangipettai south east coast which is coastal area and inhabitants of large number of flora and fauna. The squid was identified as sepia lycidas which is commonly known as kiss lip or cuttlefish. Freshly obtained squids were dissected and ink glands were manually removed from the viscera. The ink glands were then placed in clean plastic containers before placing them in the blast freezer. The frozen ink glands later entered the freeze dry to drain out the water (lyophilization process) and retain the compound in the sample. The squid ink is kept in an airtight plastic container. The container was then covered with aluminum foil to prevent any light to penetrate through. The samples were stored in a freezer at -20°C before further analysis.

III. SAMPLE PREPARATION

Sample was extracted with modification according to the method by Ikram et al. (2009) where the extract was obtained by mixing 0.5 g of sample with 50 ml of 70% ethanol (v/v) in conical flask wrapped with aluminum foil to prevent any loss of the solvent as well as to avoid direct light from penetrating the extract. The mixture was then shaking in an orbital shaker (Lab companion, Model SI600R) for overnight at 160 rpm and 27°C. The mixture was then centrifuged in a centrifugal (Kubota, Model 4000) at 2500 rpm for 30 min to obtain a clear solution. These steps were repeated using distilled water.

IV. ANTIMICROBIAL ACTIVITY

Take Muller Hinton agar , conical flask, whattman paper, Organism like (Bacillus ,E.coli,candida, pseudomonas, streptococcus) ,petriplate, micropipette, sepia lycidas.(Squid ink)

Prepare the desirable amount of muller hinton agar and sterilize it. Pour the media into the petriplate and allow it to solidify. Inoculate the different type if organism like bacillus, E.coli, candida, pseudomonas by streaking method. Put the disk in the petriplate and mark it as blank, standard, sepia lycidas (squid ink) sample. Incubate the petriplate at 37°c for 24hrs.

V. RESULT

ANTIMICROBIAL ACTIVITY:

All the colonies grown on luminescent agar were small, transparent and luminescent. The total bacterial count varied between 1.17 x 106 and 1.26 x 106 cfu/ml of ink. Since the light organ was not separated from the ink gland, there is a possibility that the count of total bacteria includes those in the light organ. All the bacteria isolated from ink were identified as Sepia lycidas. It appears that squids are specific in their selectivity of symbionts associated with ink sac, but it is not clear why only one type of luminescent bacteria inhabits the light organ. The bacteria Sepia lycidas are documented to inhabit inshore waters below 40-m depth from where the squids are caught. It could be the dominance of the particular luminous bacteria in the surrounding water or could be transferred from the parent.

Table 1: Antimicrobial activity

Organisms	Zone of Inhibition (mm)	
Bacterial strains	pH 4.0	pH 11
Pseudomonas aeruginosa	22	32
Bacillus subtilis	23	28
Candida albicans	19	25
E. coli	15	19
Staphylococcus aureus	13	13

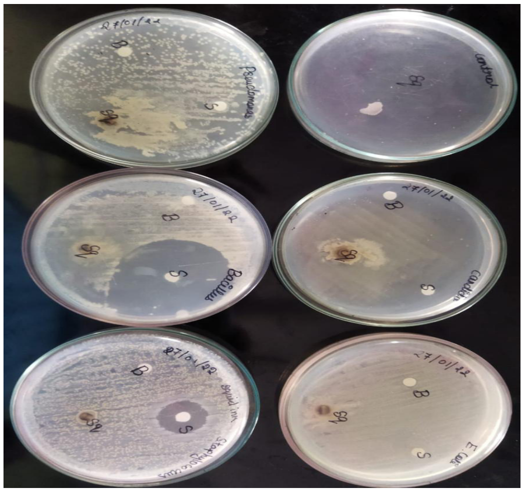

Figure 2: zone of inhibition

VI. DISCUSSION

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. Most cephalopods, excluding nautiloid, have ink sacs and thus produce ink.

Cephalopod species that live in low light, including deep sea areas, produce and use ink. Even when cephalopods are small and young, ink sacs can be present and produce ink. Cephalopod ink has already been reported for its various therapeutic values. The crude ink extracts from various species of cephalopods have been studied for their antimicrobial, preservative, antioxidant, anti-cancer, antiretroviral and many other properties. 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.

VII. 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 squid 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.

ACKNOWLEDGEMENT


We would like thank to **ANUSHA N Research Scholar** from **BIOTECHNOLOGY, PRIST UNIVERSITY, and PONDICHERRY** who worked with us and gave her full dedication and sincerity for work. We would like to appreciate her work and her article writing work.

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