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MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF *OCTOPUS AEGINA* AND *OCTOPUS DOLFUSII* IN GULF OF MANNAR COAST

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ABSTRACT: Antibacterial activity and protein content was studied in two species of octopus belonging to the family Octopoda. The highest zone of inhibition of about 34mm and 28mm were obtained against *Vibrio parahaemolyticus* and the protein content was 107.16µg/L and 136.20µg/L in *Octopus dolfusii* and *Octopus aegina*, respectively. SDS profile revealed bands of molecular weight of about 32.83 KDa to 72.36 KDa for both the species. Conclusively, the factors analyzed prove that the sample species play an important roles in chemical defensive mechanism against pathogens causing diseases in human and fishes. This study paves a way for further pharmaceutical research against pathogenic bacterial strains producing valuable drugs.

INTRODUCTION: Ocean offers a large biodiversity of flora and fauna which is estimated to be over 5,00,000 species which are more than double of the land species. This rich diversity of marine organisms assumes a great opportunity for the discovery of new bioactive substance. The marine environment comprises complex ecosystems and many of the organisms are known to possess bioactive compounds as a common means of self-defense or for the protection of eggs and embryos. Some organisms derive the chemistry from dietary sources, while others synthesize the compounds de novo [1].

From 1960s approximately 300 bioactive marine natural products were field for patent. Approximately, 6,500 bioactive compounds have been isolated from the marine organisms [2].

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In recent years, many bioactive compounds have been extracted, characterized and purified from many animals like bacteria, algae, dinoflagellates, tunicates, sponges, soft corals, bryozoans, cephalopods and echinoderms [3, 4].

Marine invertebrates offer a good source of potential antimicrobial drugs [5, 6, 7]. The word Mollusca comes from the Latin word mollus, meaning 'soft'. The phylum Mollusca includes animals that are usually soft bodied but have hard external shells of calcium carbonate [8, 9].

Cephalopoda Cuvier, 1797 is the third largest molluscan class (after gastropods and bivalves), and comprises more than 800 marine species, inhabiting a variety of ecosystems, ranging from coastal to abyssal depths. Some mollusks like cephalopods have evolved to having reduced, internalized shells, or to entirely losing their shells.

Cephalopods occur in all marine habits of the world like benthic-cryptic or burrowing in coral reefs, grass flats, sand, mud and rocks; epibenthic and pelagic in bays, seas and in the open ocean.

In nature, animals are provided with their own protective response against their predators, likewise marine mollusks are protected by their shells. Chemical defenses are used extensively by both shelled and non-shelled mollusks [10]. Cephalopods are famous for their defenses, from their fast jetting escape movements to changes in coloration that can be cryptic, disruptive or startling, to arm autotomy, to toxin venom and to inking.

Many studies on bioactive compounds from mollusks exhibiting antitumor, antibacterial and antiviral activities have been reported worldwide [11, 12, 13]. Antimicrobial peptides are important in the first line of the host defense system of many animal species [14]. Their value in innate immunity lies in their ability to function without either high specificity or memory. Moreover their small size makes them easy to synthesize without dedicated cells or tissues and they rapidly diffuse to the point of infection.

In the present investigation, an attempt has been made to screen the antimicrobial activity of the crude ethanolic extract of two species of Octopus namely *Octopus aegina* and *Octopus dolfusii* against some important human pathogens and fish pathogens and to study the protein profiles in these two species of octopus.

MATERIALS AND METHODS:

Sample collection and Identification: Octopus samples such as *Octopus aegina* and *Octopus dolfusii*, used in this study were obtained from Tuticorin Fishing harbor (10˚46' N Lat; 79˚50' E Long), situated in Southeast coast of India. The collected samples were brought to laboratory preserved with ice. The samples collected were studied by identification keys [15, 16, 17, 18, 19] and descriptions corroborated with examination of the collected specimens. The collected samples were dried using hot air oven and powdered, and the powdered samples were used for further studies.

Estimation of Protein concentration: Protein estimations were carried out by the method of [20] using BSA (Bovine Serum Albumin) as a standard.

SDS PAGE: Electrophoresis of the crude extract was carried out by the method of [21] on 2-mm vertical gel consisted of 5% stacking gel mix, and main running gel mix of 30% acrylamide. The mixture was poured into the gel mold above the separating gel. The comb was inserted without any air bubble. After polymerization, the comb was removed without distorting the shape of the well. The gel plate was fixed in the electrophoretic apparatus and was filled with the electrode buffer without any air bubbles at the bottom of the gel. 30 µl of the crude samples were loaded to each of the wells along with the standard marker proteins. And the gel was run at 100V until the dye reaches the bottom of the gel.

When the tracking dye reached the bottom of the gel the power was stopped. The gel was carefully removed from the mold and immersed in staining solution overnight with uniform shaking at 37ºC. The gel was transferred to a suitable container containing the destaining solution and shaken gently and continuously.

The process was continued until the background of the gel appeared colorless. Standard molecular weight markers ranging from 25 to 116 kDa were used to determine the molecular weight of individual proteins. Molecular weight determination of an unknown protein by SDS PAGE was calculated using the formula,

Molecular weight determination of an unknown protein by SDS PAGE:

$$y = mx+b$$

Where, y = log MW; m = the slope (1.743); x = Rf (of unknown protein); b = the y-intercept (2.788)

Rf Calculation:

$$\frac{\text{Migration distance of protein}}{\text{Migration distance of dye front}}$$

Preparation of extracts for antibacterial activity: Cephalopods were brought to laboratory, body tissues were removed, cut into small pieces and homogenized and extracted with 90% ethanol at room temperature for about 24-48 h [22]. The extracts were centrifuged to collect the supernatant and it was concentrated under vacuum in a rotary evaporator (LARK, Model: VC-100A) at low temperature. The crude ethanol extract was assayed for anti-bacterial activity.

Microbial cultures: Five species of human pathogens; *V*ibrio *cholerae*, *Bacillus cereus*, *Proteus vulgaris*, *Escherichia coli*, and *S. dysenteriae*, were obtained from the Christian Medical College Hospital, Vellore. Five fish pathogens; *Vibrio harveyi*, *Vibrio sclintis*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio anguillarum* were obtained from Fisheries Department, Cochin. All the bacterial pathogens were grown on nutrient agar and maintained at 4°C.

Antibacterial Activity: Antibacterial activities of the extracts were analyzed using well diffusion technique. The wells with 5mm diameter were punched with a sterile cork borer on to the Muller Hinton agar plates that was previously inoculated with the bacterial cultures. The wells were filled with 50 µl of the extract. Plates were held in the refrigerator for 2 hours and then incubated at 37°C for 24 hours. The wells containing the solvent alone were used as negative control. Antibacterial activities were evaluated by measuring the zone of inhibition showed in millimetres [23].

RESULTS: The protein concentration of powdered samples of each species *Octopus aegina* and *Octopus dolfusii* was determined as 107.16µg/L and 136.20µg/L respectively. The electrophoretic profile of crude powdered sample of *Octopus* sp., *Octopus aegina* and *Octopus dolfusii* revealed prominent bands from 32.83 KDa to 72.36 KDa shown in **Figure 1** and proved that both the species were distinct. The antibacterial activity of ethanol extracts of two species *Octopus dolfusii* and *Octopus aegina* is presented in **figure 2**.

No activity was observed against four human pathogenic strains *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris* and *Shigella dysenteriae* .The highest inhibition zone of 23mm diameter was obtained against *Vibrio cholera* in extract of *Octopus dolfusii* and 12mm in extract of *Octopus aegina*. The activity of samples against fish pathogens were also studied, among the five bacterial strains, highest zone of inhibition of about 34mm and 28mm was obtained against *Vibrio parahaemolyticus* from the extract of *Octopus dolfusii* and *Octopus aegina*, respectively. Zone of inhibition against *Vibrio anguillarum* was about 27mm and 25mm of *O. dolfusii* and *O. aegina*, respectively followed by growth of inhibition zone of 22mm against *Vibrio alginolyticus* in the extracts of *Octopus dolfusii*. The same range of inhibition was obtained against *Vibrio harveyi* and *Vibrio alginolyticus* (21mm) in the extract of *Octopus aegina*, followed by same range of inhibition observed with the extract of *Octopus dolfusii* against strains of *Vibrio sclintis.*

Lowest zone of inhibition was observed against *Vibrio harveyi* of about 15mm in the extract of *Octopus dolfusii* and 18mm against *Vibrio sclintis* in extract of *Octopus aegina.* This proves that the both the species of Cephalopods in which the samples were extracted in ethanol were very active against the fish pathogens, the results were given in **Figure 2,** which will be highly preferable for aquaculture constraints to combat bacterial diseases.

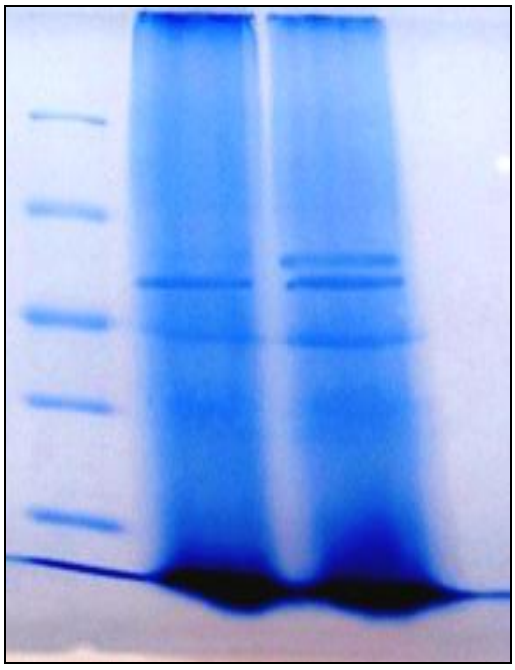

FIG. 1: SDS PAGE REPRESENTING SEPARATION OF PROTEINS IN 12% GEL

Lane 1: sample a Protein molecular weight marker; Lane 2: Sample b (*Octopus dolfusii*); Lane 3: Sample a (*Octopus aegina*)

TABLE 1: DETERMINATION OF RF VALUE FOR PROTEIN MARKER

S. No.	KDa	Log Mw	Rf
1	116	2.064	0.173611
2	66.2	1.82	0.347222
3	45	1.65	0.541667
4	35	1.544	0.694444
5	25	1.39	0.902778

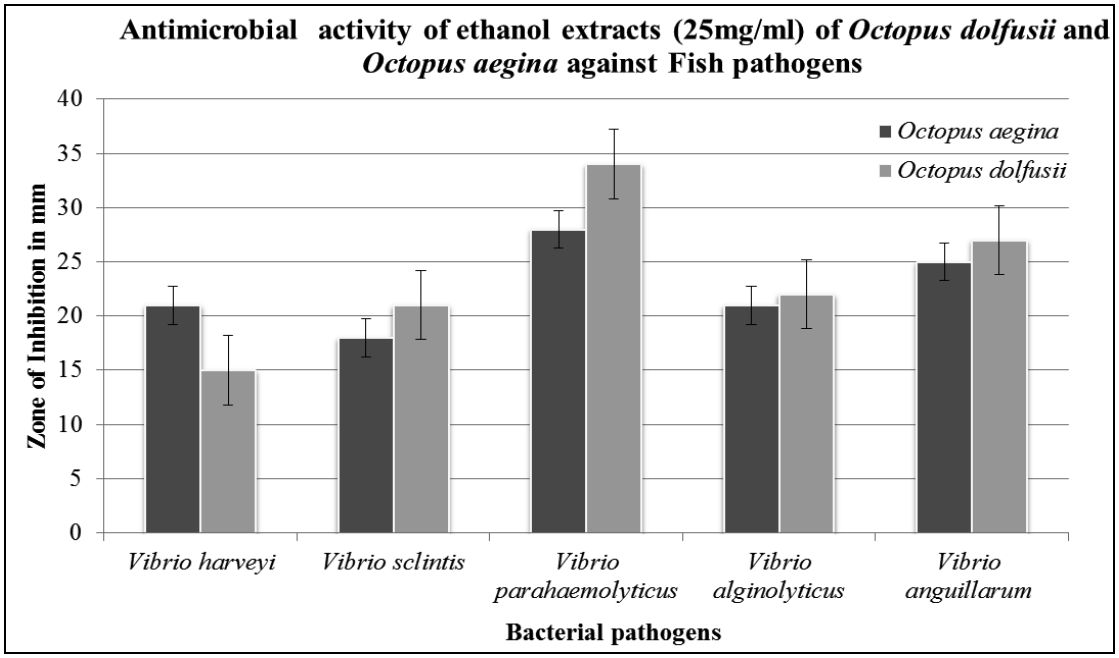

FIGURE 2: ANTIMICROBIAL ACTIVITY OF ETHANOL EXTRACTS (25MG/ML) OF *OCTOPUS DOLFUSII* AND *OCTOPUS AEGINA* AGAINST FISH PATHOGENS

DISCUSSION: In recent years, greater attention has been paid to study the bioactivity of natural products due to their potential pharmacological importance. Most of the antibacterial agents isolated from marine sources have not been active enough to compete with classical anti-microbials obtained from microorganisms [24].

However, majority of marine organisms are yet to be screened for discovering useful antibiotics. In order to explore the components and its effect on various living systems, we have accessed antimicrobial activity against Human and Fish pathogens and also studied their protein profiles.

Our results collaborates with results of [25] who has investigated the same species of Octopus with methanol which is a polar solvent and the study resulted as follows, with 100% concentration, the highest inhibition zone of 17mm was observed against *Escherichia coli* in *Octopus dolfusii* extract, 15mm against *Vibrio parahaemolyticus* in *Octopus aegina* extract, and lowest inhibition zone of 9mm against *Streptococcus pneumonia*e, *E. coli*, *Staphylococcus aureus*, *Streptococcus* sp., in *Octopus dolfusii*, O. *aegina* extract respectively.

The crude and purified sample of Glycosaminoglycans (GAGs) from *Euprymna berryi* showed activity against five pathogenic bacteria and four fungal strains [26].

The activity was higher in 100% concentration and lower in 25% concentration; but activity was absent in negative control. Antibacterial activity has been already reported in various mollusks like oyster *Crassosstrea virginica*, mussel *Mytilus edulis*, *Geukensia demissa*, muricid mollusks *Dicathias orbita* and sea hare *Dolabella auricularia* [27, 28, 29, 30, 31].

Antimicrobial peptides have been isolated from haemocytes of *Mytilus edulis* [32, 33] and *M. galloprovincialis* [34, 35, 36] and from the sea hare *Dolabella auricularia* [37]. Moderate antibacterial and antifungal activity were also reported from the extracts of various bivalve mollusks [38]. Broad spectrum of antibacterial activity has been reported for aqueous ink extract of the cephalopods *L. duvaceli* and *S. pharaonis* against nine human pathogens [39].

The protein concentration of *Octopus vulgaris* was estimated to about 144 µg/ml, and the molecular weight profiling exhibited electrophoretic form of protein bands from ink sample of *Octopus* sp., ranging from 82 to 248 KDa has been studied [40].

The current study was carried out from the powdered tissue sample of *Octopus aegina* and *Octopus dolfusii*, resulting with protein concentration of about 107.16µg/L and 136.20µg/L respectively, and molecular weight determination

done using SDS-PAGE, the protein bands existed from 32.83 KDa to 72.36 KDa for both the species, which proves the powdered tissue sample of both the species, were relatively less distinct to the protein concentration profiling of the ink sample of *Octopus vulgairs*.

To conclude, the present study has proved that the cephalopods, *Octopus aegina* and *Octopus dolfusii* has antimicrobial activities and are also likely to be rich in proteins and ethanol being a polar solvent is good in isolating bioactive compounds from mollusk. Further research on characterization and purification of the compounds could indicate the good source of antibacterial agents and also could replace the existing inadequate and cost effective antibiotics.

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