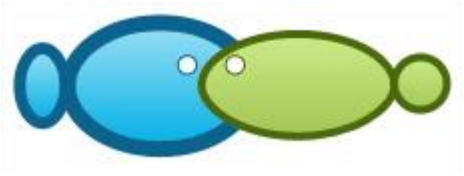

Bioactive compounds identification of octopus (*Octopus* sp.) ink extract as candidates for aquaculture immunostimulants: a preliminary study

Rangga I. Affandi, Fariq Azhar, Andre R. Scabra, Nuri Muahiddah, Septiana Dwiyanti, Marta A. Saputra, Damai Diniariwisan, Thoy B. C. Rahmadani, Yuliana Asri, Bagus D. H. Setyono

Aquaculture Study Program, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram, Mataram, West Nusa Tenggara, Indonesia. Corresponding author: R. I. Affandi, ranggaidrisaffandi@unram.ac.id

Abstract. Fish disease is still one of the obstacles in aquaculture that can cause a decrease in fish production levels. Therefore, it is necessary to find natural ingredients that contain bioactive compounds that are suitable as immunostimulants to prevent fish diseases, one of which is octopus (*Octopus* sp.) ink. Octopus ink is waste from fishery products that are not utilized or are directly discarded. However, its utilization in the field of fisheries, especially aquaculture, is still lacking. Other cephalopod inks that have been utilized in aquaculture are squid ink and cuttlefish ink. The purpose of this study was to investigate the content of bioactive compounds from octopus ink extract as a candidate for immunostimulants in fish. The method used is a quasi-experiment, namely testing bioactive compounds in octopus ink extract, then the test results are processed descriptively. The initial stage is the manufacture of octopus ink extract. Furthermore, antioxidant tests, phytochemical tests, and Fourier Transform Infra-Red (FTIR) were carried out. The results showed that octopus ink extract had strong antioxidant activity with an IC_{50} value of 94.4661 ppm. Octopus ink extract also proved to contain alkaloids, saponins, phenols, and steroids based on the results of phytochemical and FTIR tests. These compounds have many roles, such as immunostimulants, antibacterials, antivirals, antiparasitics, antifungals, antioxidants, and many more. Octopus ink has the potential to be used as an immunostimulant in the field of aquaculture with the need for further research.
Key Words: antioxidant, fisheries waste, phytochemical.

Introduction. Fish farming is also known as aquaculture, a fishery activity that produces aquatic biota (organisms) in a controlled environment with the aim of making a profit (Sutiani et al 2020). This has given rise to a desire in the community to develop aquaculture businesses, referring to the increasing market demand. The problems often faced in aquaculture are diseases that can cause a decrease in fish production levels (Affandi & Setyono 2023), both in terms of quantity and quality of production (Muahiddah et al 2023). Therefore, it is necessary to find natural ingredients that contain bioactive compounds so that they are suitable as immunostimulants to prevent fish disease.

Indonesia is the country with the third largest aquaculture production in the world after China and India. According to FAO (2022) in 2020 Indonesia was able to produce 12.152 million tons of fishery products and is projected to increase in 2030 to 13.678 million tons. Then the fishery production from the aquaculture sector is 5.227 million tons and is projected to also increase in 2030 to 6.598 million tons. In addition to the high level of fishery production, the demand for fishery products in Indonesia is also quite large because it continues to increase every year so that it can result in a lot of fishery waste produced from the remains of production carried out by the Indonesian people.

One type of waste produced from fisheries production is octopus (*Octopus* sp.) ink which is produced from the remaining octopus consumption by the community, which has a negative impact on the environment if not managed properly. Not many efforts have

been made to utilize octopus ink waste in aquaculture. Vennila et al (2011) found that octopus ink has antifungal activity on *Aspergillus fumigatus* and *Fusarium* sp. In a literature review study conducted by Affandi et al (2023), several compounds found in octopus ink were identified and summarized, including melanin (Moustafa & Awaad 2016; Besednova et al 2017; Hossain et al 2019; Shazwani & Rabeta 2020; Hernández-Zazueta et al 2021a, b), amino acids such as taurine and glutamate (Derby 2014), taurine, aspartic acid, glutamic acid, alanine, and lysine (Nair et al 2011), and alkaloids (Kalor et al 2019). In general, there has been little to no application of octopus ink in aquaculture, particularly as an immunostimulant.

In fish health management, disease prevention strategies can be carried out in various ways such as the use of antibiotics from chemicals and immunostimulants from natural ingredients. The use of antibiotics from chemicals has negative impacts such as the accumulation of residues in fish tissue and the emergence of drug-resistant pathogens. Therefore, it is necessary to prevent fish diseases with natural immunostimulants (Affandi et al 2019). Provision of good immunostimulants must pay attention to the optimal dose and frequency of administration. High doses of immunostimulants can suppress defense mechanisms, while low doses are less effective in increasing the immune response. Frequency and continuous administration of immunostimulants are needed to provide greater immune capacity to achieve optimal protection (Febriani et al 2013). Immunostimulants can be given orally, by immersion, or by injection (Muahiddah et al 2022).

The content of bioactive compounds in octopus ink still needs to be studied further. Further research is needed so that the potential of octopus ink extract as an immunostimulant for aquatic organisms can be known. Therefore, the purpose of this study was to determine the content of bioactive compounds in octopus ink extract which can later be used to prevent the emergence of diseases in fish as an immunostimulant so that aquaculture production becomes optimal.

Material and Method

Description of the study sites. This research was conducted from March to October 2024. Octopus ink extraction was conducted at the Fish Health Laboratory, Aquaculture Study Program, Faculty of Agriculture, University of Mataram and bioactive compounds identification of octopus ink extract was conducted at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram, Mataram, West Nusa Tenggara, Indonesia.

Octopus (Octopus sp.) ink extraction. The extraction of octopus ink commenced with the careful collection of ink from octopus specimens. The ink utilized in this process was sourced as a byproduct from an octopus processing factory located in Mataram City, West Nusa Tenggara, Indonesia. The collection procedure began with a vertical incision along the mantle to expose the ink sac. Tweezers were employed to extract the ink sac with precision, minimizing the risk of rupture. Subsequently, the extracted ink sac was placed into a designated container. To obtain the ink, the sac was incised using scissors and manually compressed to release the black pigment. The collected ink was then transferred into a sterile bottle and stored in a refrigerator to preserve its quality. The extraction process adhered to the methodology outlined by Affandi et al (2019), with certain modifications. The maceration technique was employed for extraction, which involved the immersion of octopus ink in a solvent. The ink-to-solvent ratio utilized was 1:3. Methanol was selected as the solvent due to its universal solubility properties. The measured quantities of octopus ink and methanol were transferred into an Erlenmeyer flask and subjected to homogenization using a shaker for 1.5 hours. Following this, the mouth of the Erlenmeyer flask was sealed with cotton and aluminum foil, which was secured with a binding material. The flask was then stored at a temperature of 4°C for a duration of seven days. After the completion of the maceration period, solvent evaporation was conducted using a rotary evaporator set at a rotational speed of 62 rpm for three hours.

Ethical approval. All animal experimentation and rearing were handled according to the animal welfare procedures, under the national accreditation no. SNI 6941:2017 of the Republic of Indonesia.

Antioxidant test. In the antioxidant test, 2 different concentrations of octopus ink extract were used, namely 100 and 50 ppm. A total of 0.5 mL of octopus ink extract with a concentration of 100 and 50 ppm was added to 1.5 mL of DPPH solution and vortexed for 2 seconds. The change of purple color to yellow indicates the efficiency of free radical scavengers. The absorbance was measured on a UV-Vis spectrophotometer (Shimadzu; Japan) with a wavelength of 517 nm after incubation for 30 minutes. Then the comparison with vitamin C p.a (Pro Analyst) as a standard was observed. Free radical scavenging activity (percent inhibition) was calculated as the percentage of DPPH color reduction using the formula (Wulan et al 2019):

% inhibition = [(control absorbance − sample absorbance) / control absorbance] x 100

The percent inhibition value is the ability of a compound to reduce free radicals. If a linear equation has been obtained in the form of the equation y = a + bx, then continue by calculating the IC_{50} value. The IC_{50} value is determined by the formula (Maitulung et al 2022):

$$IC_{50} = (50 - a) / b$$

where: a = intercept (from the equation y = a + bx);
b = regression coefficient or slope (from the equation y = a + bx).

Phytochemical test. Phytochemical tests are conducted to determine the presence of certain phytochemicals or secondary metabolites in a sample. Phytochemical identification or screening is a preliminary test carried out to determine the presence of both primary and secondary metabolites in an extract (Qomaliyah et al 2023). Phytochemical tests include tests for flavonoids, alkaloids, saponins, tannins, terpenoids, phenols, and steroids.

Flavonoids test. As much as 1 mL of octopus ink extract was put into a test tube, then added with magnesium powder and 2-4 drops of concentrated HCl. Then the mixture was shaken. The formation of orange to red color indicates the presence of flavonoids (Rahayu et al 2015).

Alkaloids test. A volume of 4 mL of octopus ink extract was mixed using 3 mL of methanol and 5 mL of ammonia, then the mixture was filtered. Furthermore, 2 mL of HCl solution was added to the filtrate and shaken. The results obtained were then put into 4 test tubes each containing 5 drops. Tube 1 contains a blank solution, while tubes 2, 3, and 4 will be mixed with 1 drop of Mayer, Wagner, Dragendorff reagent in each tube. Positive results in this test were indicated by each solution having white, brown or orange deposits (Oktavia & Sutoyo 2021).

Saponins test. Identification of the presence of saponin compounds was done by pouring 1 mL of octopus ink extract into a test tube, adding 10 mL of hot water, cooling and then shaking for 10 seconds until a stable foam was formed, then adding 1 drop of HCl through the wall of the test tube. Foam that did not disappear when added 1 drop of HCl indicated the presence of saponin in the sample (Nugraha et al 2024).

Tannins test. As much as 1 mL of octopus ink extract was added with 10 drops of $FeCl_3$ 1%. The change in solution to blackish blue or blackish green indicated that the sample was positive for containing tannin compounds (Maulida et al 2020).

Terpenoids test. The terpenoid test was carried out by taking 1 mL of octopus ink extract sample then adding Liebermen-Burchard reagent consisting of 2 mL of chloroform, 10

drops of acetic anhydride, and 3 drops of concentrated sulfuric acid. After that, it was shaken slowly and left for a few minutes then the changes that occur were observed. A positive test result indicating the presence of terpenoids is if a red or purple color appeared and a brownish ring was formed (Azalia et al 2023).

Phenols test. As much as 3 mL of octopus ink extract was added with 2 drops of $FeCl_3$ 5%. Samples containing phenol were indicated by the formation of green or yellow color (La Basy et al 2023).

Steroids test. The test was done by taking 2 mL of octopus ink extract. After that, 3 drops of concentrated HCl and 1 drop of concentrated H_2SO_4 were added. If a green color is formed, the sample is positive for containing steroids (Ergina et al 2014).

Fourier Transform Infra-Red (FTIR). Measurement of octopus ink extract was carried out using Fourier Transform Infra-Red (FTIR) spectrophotometer (Shimadzu; Japan) with deuterated triglycine sulphate (DTGS) detector. The sample was dropped on KBr pellets, then dried and analyzed using infrared spectroscopy (FTIR) with a wave range of 4000-400 cm^{-1}. This analysis was carried out to identify functional groups contained in octopus ink extract. FTIR works is that first the substance to be measured is identified, in the form of atoms or molecules. Infrared rays that act as a source of light are divided into two beams, one being passed through the sample and the other through the comparator. Then the substance passes through the chopper. After passing through a prism or grating, the beam will fall on the detector and be converted into an electrical signal which is then recorded by the recorder. Furthermore, an amplifier is needed if the signal produced is very weak. The standard used is Background (BKG). In a FTIR (Fourier Transform Infrared) test, BKG refers to a baseline spectrum recorded before analyzing a sample. This background spectrum accounts for any environmental interferences, such as CO_2, H_2O vapor, and instrument-specific noise, ensuring that only the sample's infrared absorption is measured. It helps improve accuracy by subtracting unwanted signals from the final spectrum (Sanjiwani et al 2020).

Data analysis. The data analysis was conducted using descriptive methods, which involve summarizing and interpreting data. Additionally, a linear regression equation was generated to identify relationships between variables and trends within the dataset. The results were then evaluated in relation to existing findings. A comparison was made with published literature for further insights.

Results

Antioxidant test. The quantitative measurement of antioxidant levels was conducted using a UV-Vis spectrophotometer operated at a wavelength of 517 nm. The absorbance value and antioxidant levels of octopus ink extract can be seen in Table 1.

Table 1

Antioxidant content of octopus ink extract

Sample	*Concentration (ppm)*	*DPPH absorbance*	*Sample absorbance*	*Antioxidant content (%)*
Octopus ink extract	50	0.586	0.333	43.17
Octopus ink extract	100	0.586	0.288	50.85

Based on Table 1, it can be seen that the higher concentration will make the lower absorbance value and the higher antioxidant content (percent inhibition). This data was then used to create a linear regression equation, as shown in Figure 1.

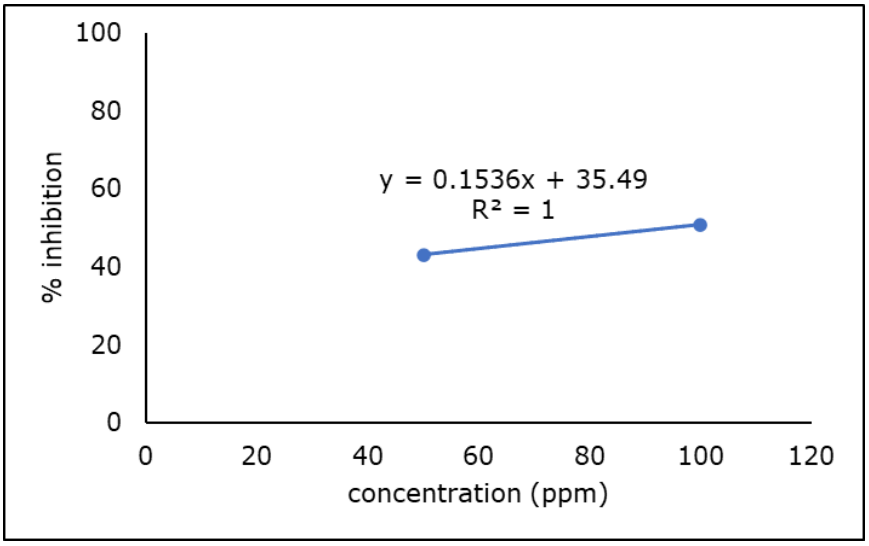

Figure 1. Linear regression equation of antioxidant content of octopus ink extract.

Figure 1 shows the relationship between concentration (ppm) and percent inhibition in octopus ink extract which produces a linear regression equation y = 0.1536x + 35.49 with an R^2 value of 1. The higher concentration will make the bigger percent inhibition. The percent inhibition value is the ability of a compound to reduce free radicals. From the linear equation above (y = 0.1536x + 35.49), the IC_{50} value can be calculated in Table 2.

Table 2

IC_{50} of octopus ink extract

Sample	*IC_{50} (ppm)*	*Antioxidant activity*
Octopus ink extract	94.4661	Strong

Phytochemical test. The results of phytochemical tests on octopus ink extract can reveal the types of bioactive compounds contained in it, as presented in Table 3.

Table 3

Phytochemical test results of octopus ink extract

Types of Compounds	*Result*
Flavonoids	-
Alkaloids	+
Saponins	+
Tannins	-
Terpenoids	-
Phenols	+
Steroids	+

Based on the results of phytochemical tests of octopus ink extract in Table 3, it shows that there are bioactive compounds, namely alkaloids, saponins, phenols, and steroids.

Fourier Transform Infra-Red (FTIR). Octopus ink extract was identified for its bioactive compound content using FTIR in the wave number range of 4000-400 cm^{-1}. The results obtained can be seen in Figure 2.

Based on the results of the FTIR test of the octopus ink extract above, it is known that there are 24 peak areas which include 3908.96 cm^{-1}; 3889.02 cm^{-1}; 3757.97 cm^{-1}; 3434.15 cm^{-1}; 3014.52 cm^{-1}; 2957.2 cm^{-1}; 2925.6 cm^{-1}; 2854.28 cm^{-1}; 2345.77 cm^{-1}; 2100.24 cm^{-1}; 1632.21 cm^{-1}; 1519.06 cm^{-1}; 1465.79 cm^{-1}; 1406.87 cm^{-1}; 1339.11 cm^{-1};

1214.82 cm^{-1}; 1085.92 cm^{-1}; 1048.39 cm^{-1}; 971.53 cm^{-1}; 932.6 cm^{-1}; 899.84 cm^{-1}; 719.62 cm^{-1}; 596.71 cm^{-1}; and 537.44 cm^{-1}.

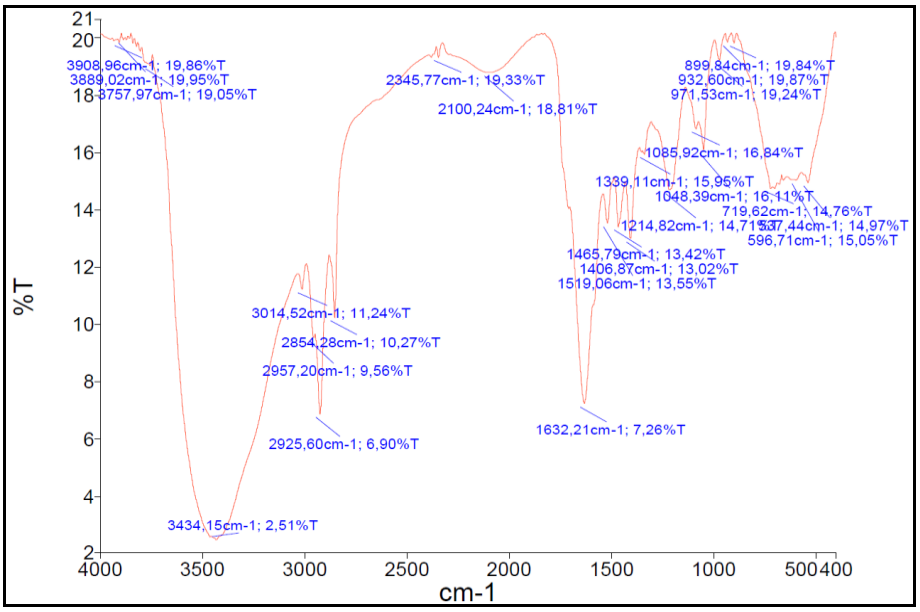

Figure 2. FTIR test results of octopus ink extract.

Discussion. Octopus ink extract with a concentration of 50 ppm has an antioxidant content of 43.17% and a concentration of 100 ppm has an antioxidant content of 50.85%. Based on these results, it can be seen that octopus ink extract has an inhibition concentration 50 (IC_{50}) value of 94.4661 ppm which is included in strong antioxidant activity. According to Sukweenadhi et al (2020), a compound is classified as a very strong antioxidant if the IC_{50} value is < 50 ppm, a strong antioxidant if the IC_{50} value is between 50 and 100 ppm, a moderate antioxidant if the IC_{50} value is between 101 and 150 ppm, and a weak antioxidant if the IC_{50} value is > 150 ppm.

Antioxidant compounds maintain immune cell function against homeostatic disorders caused by oxidative stress. Oxidative stress is a major aspect that contributes to high mortality associated with immune system dysregulation and causes several diseases (Hajian 2015). Antioxidant compounds play an important role in the body's defense against free radicals. Free radicals are produced during various endogenous and exogenous processes. Excessive free radical production can damage macromolecules such as nucleic acids, proteins, and lipids. This causes tissue damage in various chronic and degenerative diseases (Martemucci et al 2022). Antioxidants play a role in preventing oxidative damage to cells and tissues that can trigger the development of various diseases (Kozlov et al 2024).

The results of this study are the same as the results of several previous studies that detected the presence of alkaloids, saponins, phenols, and steroids compounds in *Loligo duvauceli* squid ink extract (Utami et al 2021; Sukmiwati et al 2023). Firmino et al (2021) stated that bioactive compounds have a positive effect on the growth and health of fish in aquaculture activities. Wang et al (2023) added that bioactive compounds derived from natural ingredients can regulate the immune system of fish, especially the multiplication of immune cells and have antioxidant properties.

The ink secreted by octopus is generally avoided by predators, particularly fish, due to its alkaloid content. Alkaloids are naturally occurring chemical compounds that contain nitrogen atoms and exhibit basic properties. Alkaloids are reported to have therapeutic and biological activities. Sukmiwati et al (2023) have shown that alkaloids have many types of biological activities such as antimicrobial, antioxidant, anticancer, anti-inflammatory, and antiviral activities. Alkaloids have the ability to increase non-specific immune activity and can be used as immunostimulants related to their role in preventing disease (Affandi et al 2019).

Saponins are referred to as natural biological response modifiers because of strong experimental evidence of their innate ability to alter the body's reaction to allergies, viruses, and carcinogens. Saponins exhibit antiallergic, anti-inflammatory, antimicrobial, and anticancer activities. Saponins have the property of precipitating and thickening red blood cells and also have the property of binding cholesterol and hemolytic activity (Jeyasanta & Patterson 2020). Subryana et al (2020) added that saponins can function as immunostimulant agents in aquaculture.

Phenolic bioactive compounds were also detected in *Octopus* ink extract. Phenolic compounds have polar properties, so they can be identified in polar solvents such as methanol. The presence of phenols indicates that they determine antioxidant activity in the extract. The content of phenolic compounds in a material can reduce free radicals by binding Fe ions and counteracting the enzymatic system that plays a role in the formation of free radicals such as cyclooxygenase, monooxygenase, or xanthine oxidase. Phenolic compounds are one of the main compounds that support antioxidant activity in a sample. Phenol content has a positive relationship with antioxidant activity as a free radical inhibitor (Jeyasanta & Patterson 2020). The use of phenolic compounds can improve the aquaculture industry, especially in terms of controlling and preventing disease, and is also considered a safe choice, especially when compared to antibiotics and other antimicrobials. Because of its benefits and functions, this compound can be beneficial for the aquaculture industry, such as immunostimulant agents, antioxidants, antibacterials, antivirals, antifungals, and antiprotozoa (Naiel et al 2023).

Other bioactive compounds detected are steroids. Steroid compounds are thought to have effects on increasing body stamina (aphrodisiac) and anti-inflammatory. Steroids isolated from animals and plants have high medicinal functions (Jeyasanta & Patterson 2020). Steroids promote various activities such as anti-stress, increased growth, appetite stimulation, immunostimulation, aphrodisiac, and antimicrobial (Shahbazi & Bolhassani 2016). Steroid compounds play a role in stimulating the body's defenses or as immunostimulators (Rozik et al 2021).

Immunostimulants, also known as immunostimulators are substances that activate the immune system of an organism for the prevention of disease and the enhancement of the body's natural resistance to various viral and bacterial infections. In general, immunostimulants induce the synthesis of specific antibodies and cytokines to fight disease attacks. The two main groups of immunostimulants are: a) specific immunostimulants that act as antigens to stimulate the immune response (eg vaccines), and b) non-specific immunostimulants without antigenic properties that enhance the immune response to other antigens (eg adjuvants and non-specific immunostimulants). Immunostimulants activate various elements of the organism's immune system. Immunostimulants develop non-specific immunotherapy and immunoprevention by stimulating the main components of the immune system such as phagocytosis, properdin and complement systems, protective secretory IgA antibodies, release of α- and γ-interferons, T and B lymphocytes, synthesis of specific antibodies and cytokines, and synthesis of pulmonary surfactant (Shahbazi & Bolhassani 2016).

The FTIR test results show 24 peak areas, namely 3908.96 cm^{-1}; 3889.02 cm^{-1}; 3757.97 cm^{-1}; 3434.15 cm^{-1}; 3014.52 cm^{-1}; 2957.2 cm^{-1}; 2925.6 cm^{-1}; 2854.28 cm^{-1}; 2345.77 cm^{-1}; 2100.24 cm^{-1}; 1632.21 cm^{-1}; 1519.06 cm^{-1}; 1465.79 cm^{-1}; 1406.87 cm^{-1}; 1339.11 cm^{-1}; 1214.82 cm^{-1}; 1085.92 cm^{-1}; 1048.39 cm^{-1}; 971.53 cm^{-1}; 932.6 cm^{-1}; 899.84 cm^{-1}; 719.62 cm^{-1}; 596.71 cm^{-1}; and 537.44 cm^{-1}. The absorbance at these peaks can be classified as follows:

1. 3600-3900 cm^{-1} are the bond of C-H and O-H groups (Kayed et al 2024);
2. 3500-3370 cm^{-1} is the bond of N-H group (Affandi et al 2019);
3. 3200-2800 cm^{-1} is the bond of C-H group (Kayed et al 2024);
4. 2400-2100 cm^{-1} is the bond of C-O group (Kayed et al 2024);
5. 2000-1500 cm^{-1} are the bond of C=C and C=N in aromatic groups and C=O (COOH) double bonds in carboxylic groups (Nandiyanto et al 2019);
6. 1500-1200 cm^{-1} are the bond of C-H and O-H groups (Zaltariov 2021);
7. 1100-500 cm^{-1} are the bond of C-C and C=O groups (Nandiyanto et al 2023).

The FTIR results of octopus ink extract showed peaks at absorbance of 3908.96 cm^{-1}; 3889.02 cm^{-1}; and 3757.97 cm^{-1} which include C-H and O-H group bonds, indicating the presence of alkaloid compounds (Sahribulan & Pagarra 2022). The FTIR spectrum of octopus ink extract shows that octopus ink extract contains amine groups. This is indicated by the absorbance at 3434.15 cm^{-1} which is part of the N-H group which includes the amine group. Affandi et al (2019) stated that one of the compounds included in the amine group is alkaloids. According to Sari et al (2019), alkaloids contain nitrogen as part of a cyclic system and contain varying substituents such as amine, amide, phenol and methoxy groups so that alkaloids are semi-polar and can act as antibacterial compounds. The mechanism of action of this compound in antibacterial activity is by damaging cell metabolism so that bacterial growth can be inhibited. Andayani et al (2017) stated that bioactive alkaloids increase nonspecific immune activity and can be used as immunostimulants, especially in relation to the role of disease prevention. Alkaloids increase the number of macrophage phagocytic activities. Macrophages play a role in the innate and adaptive immune systems. In innate immunity, macrophages function as phagocytic cells that digest pathogens.

Saponins are characterized by the presence of -OH groups, C-O carbonyl groups, aromatic C=C rings, and a stretch of two C-H groups (Rizkita et al 2021). In this study, octopus ink extract was found to have absorbance at 3014.52 cm^{-1}; 2957.2 cm^{-1}; 2925.6 cm^{-1}; and 2854.28 cm^{-1} which have C-H bonds, and 2345.77 cm^{-1} and 2100.24 cm^{-1} which indicate the presence of C-O bonds, thus indicating the presence of saponin compounds. The antibacterial mechanism of saponins is associated with damage to cell walls and membranes (Pikhtirova et al 2023). In general, the antibacterial mechanism of action is by inhibiting bacterial replication and damaging the structural components of bacteria. Saponins work as antibacterials by denaturing proteins in bacterial cells. Saponins, similar to detergents, lower the surface tension of cells before damaging the bacterial cell membrane. Damaged cell membranes disrupt bacterial cells, and saponins diffuse into cells through the cytoplasmic membrane. This disrupts membrane stability and cytoplasmic leakage resulting in cell death (Mulia et al 2023).

In this study, the absorbance of octopus ink extract was found at 1632.21 cm^{-1} and 1519.06 cm^{-1} which has C=C, C=N, and C=O (COOH) bonds. Absorbance 1465.79 cm^{-1}; 1406.87 cm^{-1}; 1339.11 cm^{-1}; and 1214.82 cm^{-1} in this study found in octopus ink extract have C-H and O-H group bonds. Phenolic compounds are compounds that have C=C group (Billes & Mohammed-Ziegler 2007) and hydroxyl groups (-OH) (Diniyah & Lee 2020). In general, the mechanism of antibacterial action is by inhibiting bacterial replication and damaging the structural components of bacteria (Mulia et al 2023). Phenolic compounds are free radical removers and metal binders, and cause inhibition of various physiological activities of bacteria. Another mechanism of phenol as an antibacterial is denaturation of cell proteins and inhibition of bacterial nucleic acid synthesis (Widowati et al 2021).

The FTIR test of this study showed absorbance at 1085.92 cm^{-1}; 1048.39 cm^{-1}; 971.53 cm^{-1}; 932.6 cm^{-1}; 899.84 cm^{-1}; 719.62 cm^{-1}; 596.71 cm^{-1}; and 537.44 cm^{-1} from octopus ink extract. Steroid compounds have a carbonyl group C=O (Fasya et al 2019). In general, the antibacterial mechanism of action is by inhibiting bacterial replication and damaging the structural components of bacteria (Mulia et al 2023). The antibacterial mechanism of steroid compounds is to inhibit cell membrane function and form extracellular protein complex compounds. Steroid compounds cause damage to cell membranes, then release intracellular compounds (Widowati et al 2021).

In this study, it was found that octopus ink extract contained alkaloids, saponins, phenols, and steroids. Octopus ink extract also had strong antioxidant activity with an IC_{50} value of 94.4661 ppm. In the end, it can be seen that octopus ink extract can be used as an immunostimulant in aquaculture.

Conclusions. Based on the results of data analysis in this study, it can be concluded that octopus ink extract has strong antioxidant activity with an IC_{50} value of 94.4661 ppm. Octopus ink extract also contains alkaloids, saponins, phenols, and steroids based on the results of phytochemical and FTIR tests. These compounds have many roles, such as

immunostimulants, antibacterials, antiviruses, antiparasitics, antifungals, antioxidants, and many more. Octopus ink has the potential to be used as an immunostimulant in the field of aquaculture with the need for further research.

Acknowledgements. The authors thank the Research and Community Service Institute of the University of Mataram for funding this research with grant number 1329/UN18.L1/PP/2024. Special thanks to the Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram for providing facilities and supports.

Conflict of interest. The authors declare that there is no conflict of interest.

References

Affandi R. I., Setyono B. D. H., 2023 [The potential of the kalmegh (*Andrographis paniculata*) as an immunostimulant in fish]. Jurnal Vokasi Ilmu-Ilmu Perikanan 4(1):131-141. [in Indonesian]

Affandi R. I., Fadjar M., Ekawati A. W., 2019 Active compounds on squid (*Loligo* sp.) ink extract powder as immunostimulant candidate to against shrimp disease. Research Journal of Life Science 6(3):150-161.

Affandi R. I., Fadjar M., Muahiddah N., Setyono B. D. H., 2023 [The potential of octopus (*Octopus* sp.) ink as an immunostimulant in whiteleg shrimp (*Litopenaeus vannamei*)]. Ganec Swara 17(1):318-325. [in Indonesian]

Andayani S., Fadjar M., Rahman M. F., 2017 Isolation and identification of jellyfish alkaloid (*Bougainvillia* sp.) as immunostimulant to profile of protein and fagocite activity of tiger grouper (*Epinephelus fuscoguttatus*). Aquacultura Indonesiana 18(2):67-71.

Azalia D., Rachmawati I., Zahira S., Andriyani F., Sanini T. M., Supriyatin, Aulya N. R., 2023 [Qualitative test of active compounds flavonoids and terpenoids on several plants of Fabaceae and Apocynaceae in TNGGP Bodogol area]. BIOMA: Jurnal Biologi Makassar 8(1):32-43. [in Indonesian]

Besednova N. N., Zaporozhets T. S., Kovalev N. N., Makarenkova I. D., Yakovlev Y. M., 2017 Cephalopods: the potential for their use in medicine. Russian Journal of Marine Biology 43(2):101-110.

Billes F., Mohammed-Ziegler I., 2007 Vibrational spectroscopy of phenols and phenolic polymers. Theory, experiment, and applications. Applied Spectroscopy Reviews 42: 369-441.

Derby C. D., 2014 Cephalopod ink: production, chemistry, functions and applications. Marine Drugs 12(5):2700-2730.

Diniyah N., Lee S. H., 2020 [Phenolic composition and antioxidant potential of legumes – a review]. Jurnal Agroteknologi 14(1):91-102. [in Indonesian]

Ergina, Nuryanti S., Pursitasari I. D., 2014 [Qualitative test of secondary metabolites compounds in palado leaves (*Agave angustifolia*) extracted with water and ethanol]. Jurnal Akademika Kimia 3(3):165-172. [in Indonesian]

FAO (Food and Agriculture Organization), 2022 In brief to the state of world fisheries and aquaculture 2022: towards blue transformation. FAO, Rome, 30 pp.

Fasya A. G., Purwantoro B., Ulya L. H., Ahmad M., 2019 [Antioxidant activity of steroid isolates from thin layer chromatography results of n-hexane fraction of *Hydrilla verticillata*]. Alchemy: Journal of Chemistry 8(1):23-34. [in Indonesian]

Febriani D., Sukenda, Nuryati S., 2013 [Kappa-carrageenan as immunostimulant to control infectious myonecrosis (IMN) disease in white shrimp *Litopenaeus vannamei*]. Jurnal Akuakultur Indonesia 12(1):70-78. [in Indonesian]

Firmino J. P., Galindo-Villegas J., Reyes-López F. E., Gisbert E., 2021 Phytogenic bioactive compounds shape fish mucosal immunity. Frontiers in Immunology 12: 695973.

Hajian S., 2015 Positive effect of antioxidants on immune system. Immunopathologia Persa 1(1):e02.

Hernández-Zazueta M. S., García-Romo J. S., Noguera-Artiaga L., Luzardo-Ocampo I., Carbonell-Barrachina Á. A., Taboada-Antelo P., Campos-Vega R., Rosas-Burgos E. C., Burboa-Zazueta M. G., Ezquerra-Brauer J. M., Martínez-Soto J. M., Santacruz-Ortega H. del C., Burgos-Hernández A., 2021a *Octopus vulgaris* ink extracts exhibit antioxidant, antimutagenic, cytoprotective, antiproliferative, and proapoptotic effects in selected human cancer cell lines. Journal of Food Science 86(2):587-601.

Hernández-Zazueta M. S., Luzardo-Ocampo I., García-Romo J. S., Noguera-Artiaga L., Carbonell-Barrachina Á. A., Taboada-Antelo P., Campos-Vega R., Rosas-Burgos E. C., Burboa-Zazueta M. G., Ezquerra-Brauer J. M., Burgos-Hernández A., 2021b Bioactive compounds from *Octopus vulgaris* ink extracts exerted anti-proliferative and anti-inflammatory effects *in vitro*. Food and Chemical Toxicology 151:112119.

Hossain M. P., Rabeta M. S., Azan T. H., 2019 Medicinal and therapeutic properties of cephalopod ink: a short review. Food Research 3(3):188-198.

Jeyasanta I., Patterson J., 2020 Bioactive properties of ink gland extract from squid *Loligo duvauceli*. Ecologia 10(1):9-19.

Kalor J. D., Simaremare E. S., Futwembun A., Wabiser G., Gunawan E., Yabansabra Y., 2019 Cytotoxic test of *Octopus cyanea* ink extract. Journal of Ecological Engineering 20(8):144-152.

Kayed K., Issa M., Al-ourabi H., 2024 The FTIR spectra of Ag/Ag_2O composites doped with silver nanoparticles. Journal of Experimental Nanoscience 19(1):2336227.

Kozlov A. V., Javadov S., Sommer N., 2024 Cellular ROS and antioxidants: physiological and pathological role. Antioxidants 13(5):602.

La Basy L., Dusra E., Mahulauw M. A. H., Sillehu S., Tombalissa S. R., 2023 [Antibacterial effectiveness test with different concentrations of squid (*Loligo* sp.) ink extract and seaweed (*Eucheuma cottonii*) extract in Buano village waters against *Streptococcus mutans* bacteria that cause dental caries]. Calory Journal: Medical Laboratory Journal 1(4):28-38. [in Indonesian]

Maitulung I., Maarisit W., Pareta D. N., Lengkey Y. K., 2022 [Antioxidant activity test of ethanol extract of manukan roots (*Rhinacanthus nasutus* (L) Kurz)]. Biofarmasetikal Tropis (The Tropical Journal of Biopharmaceutical) 5(2):127-134. [in Indonesian]

Martemucci G., Costagliola C., Mariano M., D'andrea L., Napolitano P., D'Alessandro A. G., 2022 Free radical properties, source and targets, antioxidant consumption and health. Oxygen 2(2):48-78.

Maulida S., Hakim A. R., Mohtar M. S., 2020 [Analysis of tanin content of ethanol extract of candlenelux (*Aleurites moluccana* (L.) Willd) stem bark using titrimetric method]. Journal of Pharmaceutical Care and Science 1(1):85-93. [in Indonesian]

Moustafa A. Y., Awaad A., 2016 Comparative histopathological and histochemical impacts induced by the posterior salivary gland and ink sac extracts of *Octopus vulgaris* in mice. The Journal of Basic and Applied Zoology 74:23-36.

Muahiddah N., Affandi R. I., Diamahesa W. A., 2022 The effect of immunostimulants from natural ingredients on vanamei shrimp (*Litopenaeus vannamei*) in increasing non-specific immunity to fight disease. Journal of Fish Health 2(2):90-96.

Muahiddah N., Azhar F., Marzuki M., Scabra A. R., Affandi R. I., Sumsanto M., Asri Y., Diamahesa W. A., Diniariwisan D., 2023 [Fish disease control counseling for tilapia farmers in tarpaulin ponds in Kramajaya village, West Lombok]. TEKIBA : Jurnal Teknologi dan Pengabdian Masyarakat 3(2):53-58. [in Indonesian]

Mulia D. S., Raicha R., Lunggani C. P., Erina S. N., Wuliandari J. R., Purbomartono C., Isnansetyo A., 2023 Antibacterial activity of ethanol extract of banana, cassava, and pineapple peels against a fish pathogen *Aeromonas hydrophila*. Biodiversitas 24(1): 481-485.

Naiel M. A. E., El-Kholy A. I., Negm S. S., Ghazanfar S., Shukry M., Zhang Z., Ahmadifar E., Abdel-Latif H. M. R., 2023 A mini-review on plant-derived phenolic compounds with particular emphasis on their possible applications and beneficial uses in aquaculture. Annals of Animal Science 23(4):971-977.

Nair J. R., Pillai D., Joseph S. M., Gomathi P., Senan P. V., Sherief P. M., 2011 Cephalopod research and bioactive substances. Indian Journal of Geo-Marine Sciences 40(1):13-27.

Nandiyanto A. B. D., Oktiani R., Ragadhita R., 2019 How to read and interpret FTIR spectroscope of organic material. Indonesian Journal of Science and Technology 4(1):97-118.
Nandiyanto A. B. D., Ragadhita R., Fiandini M., 2023 Interpretation of fourier transform infrared spectra (FTIR): a practical approach in the polymer/plastic thermal decomposition. Indonesian Journal of Science and Technology 8(1):113-126.
National Standardization Agency, 2017 [SNI 6941:2017 Frozen raw octopus]. National Standardization Agency, Jakarta, Indonesia, 7 pp. [in Indonesian]
Nugraha N. D., Sanjiwani N. M. S., Udayani N. N. W., 2024 [Phytochemical identification and determination of saponin compound content in the ethanol extract of butterfly pea flower (*Clitoria ternatea* L.)]. USADHA: Jurnal Integrasi Obat Tradisional 3(1):8-13. [in Indonesian]
Oktavia F. D., Sutoyo S., 2021 [Phytochemical screening, total flavonoid content, and antioxidant activity of ethanol extract of *Selaginella doederleinii*]. Jurnal Kimia Riset 6(2):141-153. [in Indonesian]
Pikhtirova A., Pecka-Kiełb E., Zigo F., 2023 Antimicrobial activity of saponin-containing plants: review. Journal of Dairy, Veterinary and Animal Research 12(2):121-127.
Qomaliyah E. N., Indriani N., Rohma A., Islamiyati R., 2023 [Phytochemical screening, total flavonoids and antioxidants of *Kalanchoe pinnata* Linn. leaves]. Current Biochemistry 10(1):1-10. [in Indonesian]
Rahayu S., Kurniasih N., Amalia V., 2015 [Extraction and identification of flavonoid compounds from red onion skin waste as a natural antioxidant]. al Kimiya: Jurnal Ilmu Kimia dan Terapan 2(1):1-8. [in Indonesian]
Rizkita A. D., Dewi S. A., Wibowo E. A. P., Maulana I., 2021 [Isolation and identification of saponin from leunca (*Solanium ningrum* L) extract by infrared spectrophotometry]. Jurnal Ilmiah Sains 21(2):166-169. [in Indonesian]
Rozik M., Maryani, Rosdiana, Rochman A., 2021 Non-specific immune response development of *Aeromonas hydrophila*-infected Nile tilapia *Oreochromis niloticus* with application of immunostimulant of saluang belum (*Luvunga sarmentosa*) extract. AACL Bioflux 14(1):536-545.
Sahribulan, Pagarra H., 2022 [Identification of functional groups of secondary metabolites compounds of ethanol extract of Java wood leaves *Lannea coromandelica*]. Jurnal Binomial 5(2):161-168. [in Indonesian]
Sanjiwani N. M. S., Paramitha D. A. I., Wibawa A. A. C., Ariawan I. M. D., Megawati F., Dewi N. W. T., Mariati N. P. A. M., Sudiarsa I. W., 2020 [Making aloe vera-based hair tonic and analysis with fourier transform infrared]. Widyadari 21(1):249-262. [in Indonesian]
Sari R. C., Wijayanti I., Agustini T. W., 2019 The effectiveness of melanin from squid ink (*Loligo* sp.) as antibacterial agent against *Escherichia coli* and *Listeria monocytogenes*. IOP Conference Series: Earth and Environmental Science 246(1): 012022.
Shahbazi S., Bolhassani A., 2016 Immunostimulants: types and functions. Journal of Medical Microbiology and Infectious Diseases 4(3-4):45-51.
Shazwani Z. A., Rabeta M. S., 2020 Enzymatic hydrolysis as an approach to produce alternative protein from cephalopods ink powder: a short review. Food Research 4(5):1383-1390.
Subryana N., Wardiyanto W., Susanti O., 2020 [The application of moringa leaf extract *Moringa oleifera* (Lam, 1785) to increase non specific immunity of tilapia *Oreochromis niloticus* (Linnaeus 1758) infected by *Aeromonas hydrophila*]. Journal of Aquaculture and Fish Health 9(3):194-203. [in Indonesian]
Sukmiwati M., Edison, Nuzul V., 2023 Antioxidant potential of squid ink (*Loligo duvauceli*). BIO Web of Conferences 74:02001.
Sukweenadhi J., Yunita O., Setiawan F., Kartini, Siagian M. T., Danduru N. P., Avanti C., 2020 Antioxidant activity screening of seven Indonesian herbal extract. Biodiversitas 21(5):2062-2067.

Sutiani L., Bachtiar Y., Saleh A., 2020 [Model analysis of freshwater fish which is dominated by gurame fish (*Osphronemus gouramy*) in Sukawening village, Bogor, West Java]. Jurnal Pusat Inovasi Masyarakat 2(2):207-214. [in Indonesian]
Utami D. R., Irwan I., Agustina S., Karina S., Afriani S., 2021 Antibacterial activity of *Escherichia coli* from squid ink (*Loligo* sp.) n-hexane extracts. IOP Conference Series: Earth and Environmental Science 869(1):012033.
Vennila R., Kumar R. K. R., Kanchana S., Arumugam M., Balasubramanian T., 2011 Investigation of antimicrobial and plasma coagulation property of some molluscan ink extracts: gastropods and cephalopods. African Journal of Biochemistry Research 5(1):14-21.
Wang B., Thompson K. D., Wangkahart E., Yamkasem J., Bondad-Reantaso M. G., Tattiyapong P., Jian J., Surachetpong W., 2023 Strategies to enhance tilapia immunity to improve their health in aquaculture. Reviews in Aquaculture 15(1):41-56.
Widowati R., Handayani S., Al Fikri A. R., 2021 Phytochemical screening and antibacterial activities of senggani (*Melastoma malabathricum* L.) ethanolic extract leaves. Jurnal Ilmu Pertanian Indonesia 26(4):562-568.
Wulan, Yudistira A., Rotinsulu H., 2019 [Antioxidant activity test of ethanol extract of *Mimosa pudica* Linn. leaves using DPPH method]. Pharmacon 8(1):106-113. [in Indonesian]
Zaltariov M. F., 2021 FTIR investigation on crystallinity of hydroxypropyl methyl cellulose-based polymeric blends. Cellulose Chemistry and Technology 55(9-10): 981-988.

Received: 11 November 2024. Accepted: 12 January 2025. Published online: 25 February 2025.
Authors:
Rangga Idris Affandi, Aquaculture Study Program, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram, Pendidikan Street, No. 37, 83125 Mataram, West Nusa Tenggara, Indonesia, e-mail: ranggaidrisaffandi@unram.ac.id
Fariq Azhar, Aquaculture Study Program, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram, Pendidikan Street, No. 37, 83125 Mataram, West Nusa Tenggara, Indonesia, e-mail: fariqazhar@unram.ac.id
Andre Rachmat Scabra, Aquaculture Study Program, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram, Pendidikan Street, No. 37, 83125 Mataram, West Nusa Tenggara, Indonesia, e-mail: andrescabra@unram.ac.id
Nuri Muahiddah, Aquaculture Study Program, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram, Pendidikan Street, No. 37, 83125 Mataram, West Nusa Tenggara, Indonesia, e-mail: nurimuahiddah@unram.ac.id
Septiana Dwiyanti, Aquaculture Study Program, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram, Pendidikan Street, No. 37, 83125 Mataram, West Nusa Tenggara, Indonesia, e-mail: antiseptiana@unram.ac.id
Marta Ade Saputra, Aquaculture Study Program, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram, Pendidikan Street, No. 37, 83125 Mataram, West Nusa Tenggara, Indonesia, e-mail: martade@gmail.com
Damai Diniariwisan, Aquaculture Study Program, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram, Pendidikan Street, No. 37, 83125 Mataram, West Nusa Tenggara, Indonesia, e-mail: damaidiniari@unram.ac.id
Thoy Batun Citra Rahmadani, Aquaculture Study Program, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram, Pendidikan Street, No. 37, 83125 Mataram, West Nusa Tenggara, Indonesia, e-mail: citra@unram.ac.id
Yuliana Asri, Aquaculture Study Program, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram, Pendidikan Street, No. 37, 83125 Mataram, West Nusa Tenggara, Indonesia, e-mail: yulianaasri@unram.ac.id
Bagus Dwi Hari Setyono, Aquaculture Study Program, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram, Pendidikan Street, No. 37, 83125 Mataram, West Nusa Tenggara, Indonesia, e-mail: bagus.setyono@unram.ac.id

How to cite this article:
Affandi R. I., Azhar F., Scabra A. R., Muahiddah N., Dwiyanti S., Saputra M. A., Diniariwisan D., Rahmadani T. B. C., Asri Y., Setyono B. D. H., 2025 Bioactive compounds identification of octopus (*Octopus* sp.) ink extract as candidates for aquaculture immunostimulants: a preliminary study. AACL Bioflux 18(1):500-511.