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Antimicrobial Activity of Eel Mucus: A Review

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***Abstract*— Epidermal fish mucus contains a wide range of bioactive metabolites involved with the defence mechanism. This review explores the potential of eel mucus extract for its antagonistic potential against common pathogenic microbes, which are commonly implicated in foodborne and human infections. The ability to adhere and invade the host cell and disarm the growth of other pathogenic microbes will also be discussed. Modes of action for eel mucus, including the antibacterial and antifungal properties of the bioactive metabolites, shall also be explored. Thus, this overview represents the potent bioactivities of mucus extracted from eel, which could be further explored as an alternative to antibiotics or synthetic drug agents.**

***Keywords*— Eel mucus; pathogenic microbes; antimicrobial activity; bioactive metabolites.**

I. INTRODUCTION

The eel is an elongated fish which looks more like a snake than regular fish (Fig. 1). Most of the species are finless, with a slippery and thick texture covering the whole body [1]. They have protruded gills, two rows of small mandibular teeth, and a pair of eyes covered with a thin layer of film [2]. Some species of eel inhabit tropical and sub-tropical water; however, most of the species lives in freshwaters such as coastal rivers, brooks, muddy areas such as marshes, ponds, and rice paddy field since those areas contain more nutrients and foods that are sufficient for the development of the eels [3]. Eel uniquely adapts to the respiration system where they breathe atmospheric air. However, at the same time, they live in low oxygen areas such as muddy areas by burrowing themselves and settling in muddy areas for a few months [2].

According to Shibuya et al. (2019), aquatic inhabitants are more susceptible to infections compared to their terrestrial counterparts due to the continuously exposed to a wide variety of microorganisms, including bacteria, viruses, and parasites, present in water bodies. Terrestrial animals may also encounter pathogens, but they generally have more control over their exposure and can avoid contaminated areas [4]. Therefore, the skin plays a crucial role by producing the first line of defence against any harm, such as pathogenic microorganisms or parasites. The eel's skin has a slippery and slimy texture covering the whole body through viscous colloids called mucus or mucin. Mucus is a glycoprotein secreted by the goblet cell

in the epidermal, which contains numerous bioactive peptides and molecules [5]. It could be a physical and biochemical barriers against pathogenic microorganisms and environmental toxins. Santoso et al. (2020) stated that mucus secretion by the epidermal cell is continuously secreted to form anti-biofilm agents that could prevent the invasion from any potential infectious microbes or parasites by trapping the microbes from entering the body. Several factors could trigger mucus production, including polluted environments, pathogen infections, food deficiency and immune system deprivation. Besides, the mucus secreted by eel is expressed in stressful conditions and is commonly used as a biomarker to study the toxicity level of the environment in certain areas [6]. When eels are exposed to pollutants or contaminants in their habitat, they may produce more mucus in an attempt to shield themselves. Therefore, the eel mucus can serve as a sensitive indicator of environmental health. By measuring the amount of mucus produced or analyzing its composition, scientists can gain insights into the presence and intensity of environmental stressors, including pollutants, heavy metals, and other toxins.

Fig. 1 Asian Swamp Eel *(Monopterus albus)* (Source: Stock Photos 2015; Credit: Sakdinon).

The eel mucus possesses antimicrobial activity with bioactive components, including lysozyme, immunoglobulin, complement protein, lectins, proteases, phosphatase, antibacterial proteins, and antimicrobial peptides (AMPs) [6]. These bioactive peptides exhibit antimicrobial properties, such as antibacterial and antifungal activity, which can invade the pathogenic cell membrane and disrupt their cell system. Other uses for mucin peptides include metabolic regulation of the eel, which has previously been shown to modulate the immune response and possess antioxidative, antifungal and antimicrobial activities [7]. With a wide range of uses, this review paper will focus on the antimicrobial activity of these bioactive molecules. In addition, we will review the processing and extraction of these bioactive compounds directly involved with antimicrobial activities. Finally, the mechanism of actions of bioactive compounds against some medically significant pathogens will be elaborated.

II. ANTIMICROBIAL ACTIVITY OF EEL MUCUS

The continual rise of antibiotic-resistant pathogens is a significant challenge in the medical field. This problem is particularly pronounced when they resist first- or second-generation antibiotics. When a microbe develops resistance to a broad range of antibiotics, the need for a third-generation or narrow-range antibiotic can concur a higher medical cost. Also, it may induce more toxicity towards the patients. In 2017, World Health Organization (WHO) urged scientists to seek an alternative solution to combat the antimicrobial resistance challenge. A continual efforts have since then exacerbated the search for antibiotic alternatives. Some antibiotic alternatives include the search for novel natural therapeutic remedies based on the unique mode of action of the bioactive molecules from the natural sources [8]. Nature-sourced compounds are believed to exhibit antimicrobial properties, which could help to scavenge any pathogenic microorganisms that can harm to human health. Furthermore, natural bioactive molecules also have low cytotoxicity levels, which is safer to use than synthetic drugs. According to Hedmon et al. (2018), eel mucus exhibits antimicrobial activities, making it a promising antimicrobial agent to combat pathogenic microorganisms [9].

The use of eel mucus as a remedy has dated since ancient times. Traditionally, eel mucus is used to treat wounds, and skin burns as it has therapeutic properties that help to heal. During these times, medicine can be expensive; thus, inexpensive treatment, including burns and wounds, takes the form of natural remedies [10]. For example, mucus secreted from eel (*Anguilla bengalensis*) has been used to treat anaemia, burn injury, and weak immune systems, as reported by Hedmon et al. (2018) [9]. Previous work by Omardien et al. (2016) acknowledged that the mucus extracted from eel emits antimicrobial properties due to the bioactive compounds in the mucus [11]. The bioactive molecules responsible for antimicrobial properties include antimicrobial peptides (AMPs), lysozyme, proteases and immunoglobulin. These biologically active peptides could act as therapeutic agents, demonstrating a much broader spectrum of therapeutic activity than amphibian AMPs by testing against human and fish pathogens [12]. A list of antimicrobial properties of eel mucus extracts on tested microorganisms, methods used and results from previous studies are reviewed as shown in TABLE I.

A. Antibacterial Activity

According to Ebran et al. (2000), antibacterial activity is the ability of bioactive peptides in natural sources to invade pathogens and disrupt the cell as a defence mechanism [17]. The eel mucus is the first line of the defence system in fish. Najafian & Babji (2012) added that mucus contains a broad spectrum of antimicrobial peptides, such as enzymes and proteins, which can help kill any pathogen or parasite that tries to invade their bodies [7]. Bragadeesw & Thangaraj (2011) showed the antibacterial properties of eel mucus using the standard disc diffusion method and serial dilution assay which the purpose was to screen and observes the diameter of inhibition zones when the crude, aqueous and methanolic extracted eel mucus (*Anguilla anguilla*) are tested against Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus, Salmonella typhi, Salmonella paratyphi, Klebsiella oxytoca, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteous mirabilis, Escherichia coli, Proteous vulgaris* and *Vibrio* spp [18]. From the study, the highest diameter of the clear zone of inhibition is 10mm when 30µl crude extract is tested against *S. paratyphi*. The innate antimicrobial agents, such as lectin and cysteine protease in the

eel mucus extracts, penetrate the tested pathogens' cell walls to inhibit their growth. Fig. 2 shows the diameter of clear inhibition zones by the three eel mucus extracts of *Anguilla anguilla* against shrimp culture pond pathogen.

Hilles et al. (2019) and Liang et al. (2011) reported two factors that influenced the diameter of the inhibition zone: the types of mucus extraction and the types of tested bacteria [13, 16]. Firstly, they extracted the eel mucus into several solutions such as methanol, acidic, crude and aqueous extracts. They stated that this method must be done to activate the bioactive peptides in the eel mucus and to study which extraction is more soluble in the peptides so that these bioactive molecules can penetrate and inhibit the bacterial cell. Next, bacteria are grouped into two types which are Gram-positive and Gram-negative. The significant difference between these two bacteria is the cell wall. Balouiri et al. (2016) state that the Gram-positive cell wall is thicker than the Gram-negative because of the peptidoglycan content [19]. These differences influenced the response of the bacteria to the antimicrobial agents in the extracted eel mucus. The previous study conducted by Patel et al. (2020) explained that most Gram-negative bacteria are easier to be lysed by the antibacterial peptides in the mucus than Gram-positive bacteria due to the thickness of the cell wall [20].

TABLE I. ANTIMICROBIAL ACTIVITIES OF EEL MUCUS TESTED AGAINST PATHOGENIC MICROORGANISMS.

Extracts	**Method**	**Microorganism tested**	**Results**	**Eel species**	**References**
Aqueous extract and methanol extract were tested against oral pathogens.	Microdilution method using a sterile 96-well plate	Gram-negative bacteria: • *Klebsiella pneumonia* • *Pseudomonas aeruginosa* Gram-positive bacteria: • *Enterococcus faecalis* • *Streptococcus pyogenes* • *Streptococcus mutans* Fungi: • *Candida albicans*	The methanolic extract had higher antimicrobial activities than the aqueous extract, with the highest inhibition of 82% at 1000µg/ml concentration.	*Monopterus albus*	[13]
Aqueous extract.	Disc diffusion assay	*Salmonella typhi*	The aqueous extract can inhibit the growth of *S. typhi* bacteria in the concentration of 100%. The minimum inhibitory concentration is 12.5%.	*Anguilla* spp.	[14]
Phosphate buffered solution (PBS) extracts, crude extracts and aqueous extracts.	Disc diffusion assay	*Fungi:* • *Candida albicans* • *Candida krusei* • *Cryptococcus neoformans* • *Fusarium* spp.	Aqueous extracts were observed to yield antifungal activity out of three extractions, while PBS extracts and crude extracts failed to produce any positive result.	*Monopterus albus*	[15]
Acetone extract and acidic extract (10% acetic acid).	Inhibition zone assay	Gram-negative bacteria: • *Edwardsiella tarda* • *Aeromonas* sp. • *Aeromonas hydrophila* Gram-positive bacteria: • *Micrococcus luteus*	The acidic extracts of eel mucus exhibited stronger antibacterial activities against *E. tarda*, *Aeromonas* sp., *A. hydrophila* and *M. luteus* than acetone extracts, with a mean of 3.375 mm.	*Anguilla japonica*	[16]

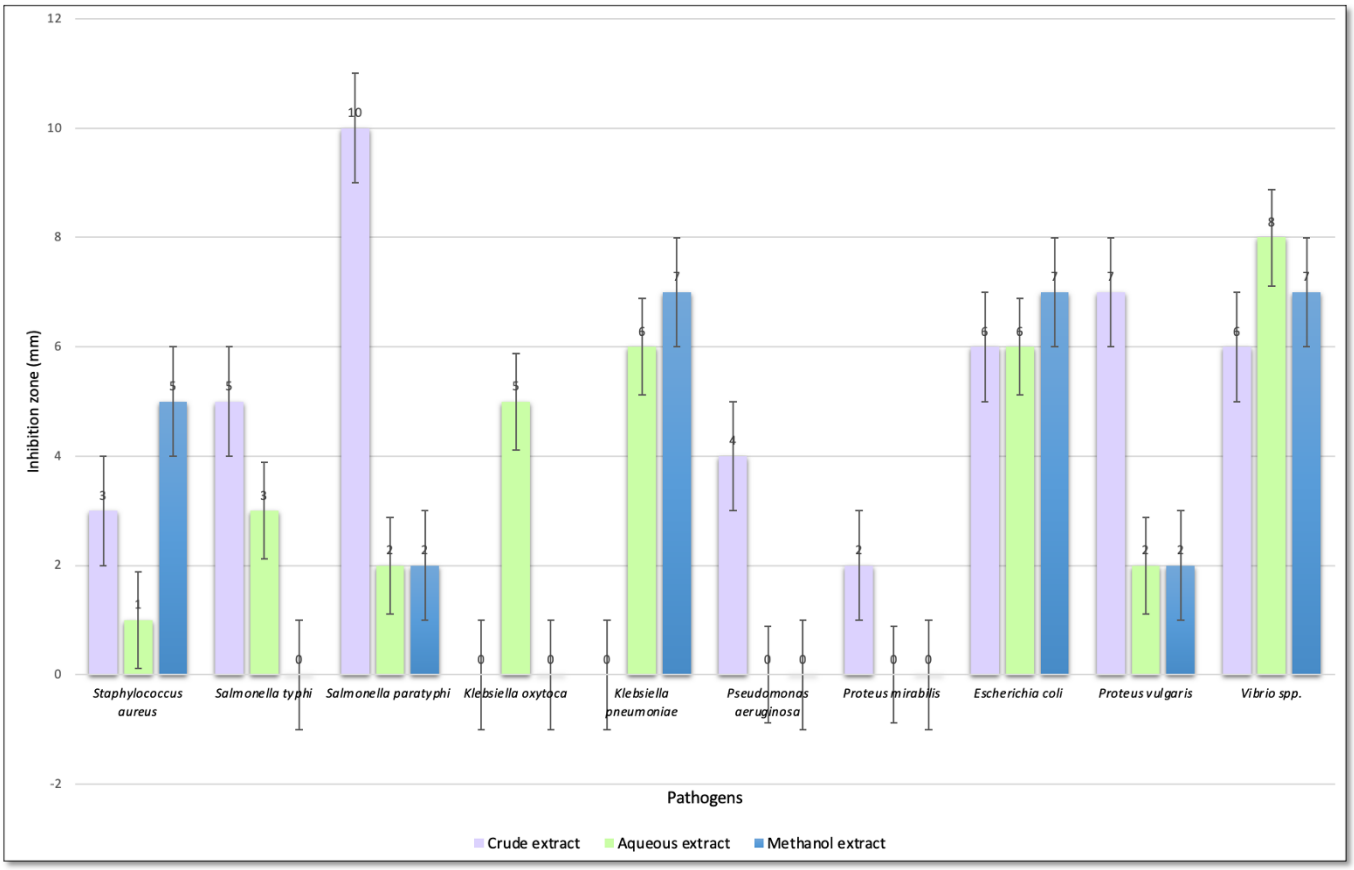


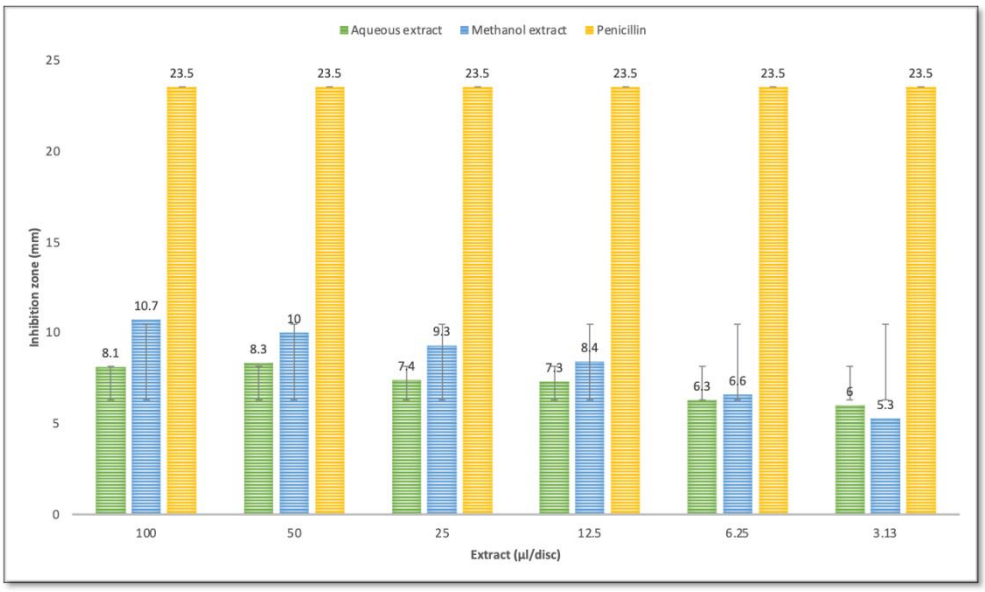
Fig. 2 Antibacterial activity of three extracts of eel mucus against shrimp culture pond pathogens [18].

Collective analysis of the antibacterial activities between mucus extracts from different eel species was also discussed. Research on the antibacterial activity of mucus extracted from *Monopterus albus* species was conducted by Hilles et al. (2019) [13]. In this study, the eel mucus was extracted into two types of extractions: aqueous extracts and methanol extracts. These two extracts were tested against Gram-positive and Gram-negative bacteria which are *E. coli* and *S. aureus*, using the disc diffusion method. This test was carried out to observe whether the two extracts exhibit antibacterial activity against the tested pathogenic bacteria based on the diameter of inhibition zones with penicillin as the positive control.

From Fig. 3 and Fig. 4, Hilles et al. (2019) explained that methanol extract had shown higher antibacterial activity than aqueous extract in *Monopterus albus* species based on the larger clear inhibition zones 10.7 mm and 8.1 mm in 100 µl/disc, respectively. The authors reported that the bioactive peptides in the eel mucus are more soluble in methanol extracts [13]. On top of that, Yeong Wei et al. (2010) added that the lower antibacterial activity of aqueous extract could be due to the low presence of bioactive molecules in the extract. Hence, the methanol extract showed higher clear inhibition zones than the aqueous extract [21]. Furthermore, the researchers added that the tested strain bacteria might be related to the inhibition properties of mucus extract since *E. coli* is Gram-negative while *S. aureus* is Gram-positive. Gram-negative bacteria have thinner cell walls than Gram-positive bacteria. Hence, this test reported that *E. coli* showed higher inhibition zones than *S. aureus* since the mucus extracts can penetrate more easily on *E. coli* cell walls than *S. aureus* [13].

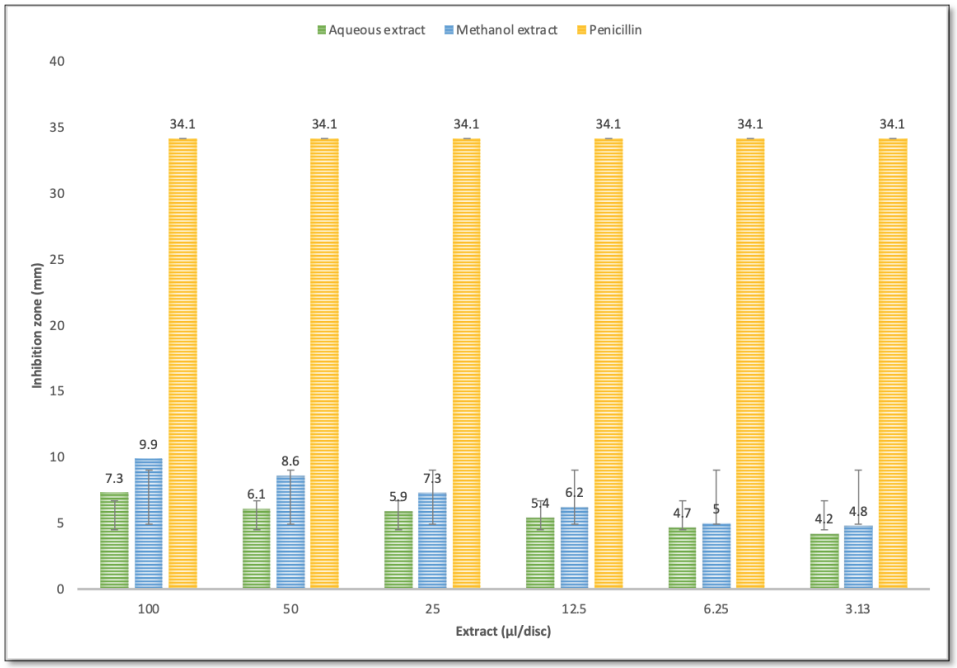
Nurtamin et al. (2016) reported that eel mucus also can be used to treat typhoid fever [14]. According to Tee (2002), typhoid fever is a contagious illness that can spread quickly through contaminated foods and polluted water caused by *Salmonella typhi* [22]. In Fig. 5, Nurtamin et al. (2016) reported that the aqueous extract collected from *Anguilla* spp. was tested against *S. typhi.* The results showed the formation of clear inhibition zones around the disc, indicating antibacterial properties in the eel mucus. The diameter of clear inhibition zones gradually decreases as the concentration of crude mucus extract decreases [14].

Analysing the antibacterial activity in eel mucus is crucial in the medical field and necessary in the food industry. The discovery of eel mucus to exhibit antibacterial properties can help to control the problem of food spoilage in food products caused by microorganisms and be an alternative for novel antimicrobial agents in the medical field.



Extract (µl/disc)	Aqueous extract	Methanol extract	Penicillin
100	8.1±0.33	10.7±0.17	23.5±0.21
50	8.3±0.11	10.0±0.09	23.5±0.21
25	7.4±0.19	9.3±0.34	23.5±0.21
12.5	7.3±0.45	8.4±0.03	23.5±0.21
6.25	6.3±0.07	6.6±0.46	23.5±0.21
3.13	6.0±0.02	5.3±0.45	23.5±0.21

Fig. 3 Antibacterial activity of eel skin mucus against *E. coli* using the disc diffusion method (mm). Results were expressed as mean ± SD (n=3) [13].



Extract (µl/disc)	Aqueous extract	Methanol extract	Penicillin
100	7.3±0.21	9.9±0.06	34.1±0.01
50	6.1±0.15	8.6±0.10	34.1±0.01
25	5.9±0.43	7.3±0.23	34.1±0.01
12.5	5.4±0.01	6.2±0.13	34.1±0.01
6.25	4.7±0.52	5.0±0.07	34.1±0.01
3.13	4.2±0.13	4.8±0.26	34.1±0.01

Fig. 4 Antibacterial activity of eel skin mucus against *S. aureus* using the disc diffusion method(mm). Results were expressed as mean ± SD (n=3) [13].

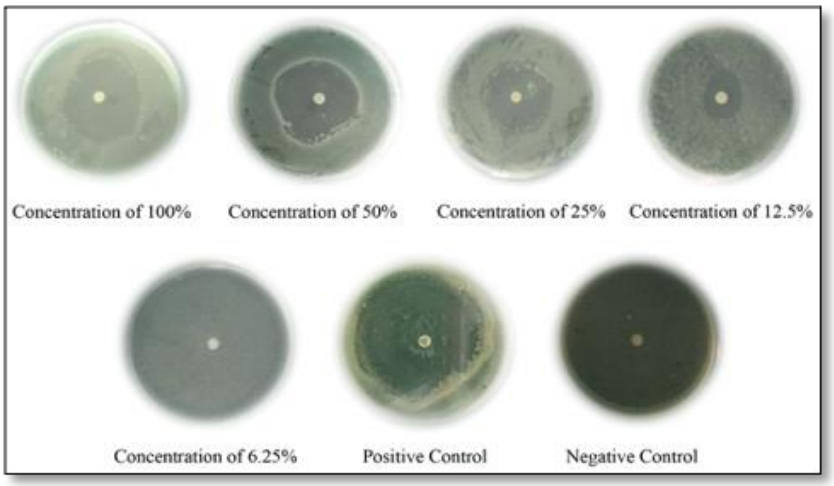


Fig. 5 Comparison of concentration of eel mucus extract of *Anguilla spp*. against *Salmonella typhi* [14].

B. Antifungal Activity

Food spoilage is one of the major losses to the food supply chain industries. Marshall & Warren (2018) reported that approximately 25% of postharvest-produced food was lost due to microbial spoilage. These spoilage microbes spoiled the food, and some could produce toxins and cause disease. Most diseases caused by foodborne pathogens are due to the ingestion of microbes in the food and produce toxins that, if consumed, can cause intoxication. Mould or fungi are the most common microbes associated with food intoxication due to their ability to colonise and produce toxins in food that is low in water activity and low moisture levels, such as grains, legumes and sugar [23]. Due to these issues, concentrated efforts and research have been established to combat these problems. More discoveries of novel antimicrobial peptides from natural sources were conducted, including antifungal peptides to control the growth of fungi and mould in food production and the medical field.

Nik Mohd Ikram et al. (2013) stated that antifungal activity is a study used to discover the ability of specific novel antimicrobial peptides to eradicate the growth of fungi by invading the fungal cell, triggering the whole fungal cell system and eventually killing them [15]. Cheung et al. (2015) added that mucus extracted from eels had been the main focus of many studies since eels live in close proximity to the microorganism. Hence, there are a lot of novel antimicrobial peptides as effective agents developed in the eel mucus under these intense environmental pressures [24]. Nik Mohd Ikram et al. (2013) conducted a screening test of antifungal activities in the eel mucus (*Monopterus albus*) against *C. albanicans, C. kriuser, C. neofarmans* and *Fusarium* spp. through the Kirby Bauer Disc Diffusion method to observe antifungal properties in the bioactive peptides in the mucus, as listed in TABLE II [15].

From TABLE II, the authors reported that the mucus extracted from *M. albus* species is divided first into three extracts which are crude extract, aqueous extract and Phosphate Buffer Saline (PBS) extract, before being tested against the fungi, which are *C. albicans, C. krusei, C. neoformans* and *Fusarium* spp. by which to observe the presence of antifungal properties in the mucus through the diameter of inhibition zones on the disc [15]. Based on the table, the authors stated that the eel mucus possesses antifungal properties due to the formation of a clear zone of inhibition, which indicates that the eel mucus inhibits the growth of tested fungi. Throughout hours of incubation (24h, 48h and 72h), the authors reported that the diameter of clear zones increased. However, the authors revealed that the antifungal peptide is activated and works efficiently in aqueous extract compared to PBS saline extract. This is expected since PBS extracts are a less polar compound which could result in little or no active antifungal agents. Based on the test conducted, the authors explained that antimicrobial activity correlated with extracts' polarity. Thus, mucus extracted using higher polarity solvents was more effective in scavenging radicals and worked well as microbial inhibitors than those obtained through less polar solvents [15].

TABLE II. ZONE OF INHIBITION (mm) OF WATER EXTRACT FROM THE MUCUS OF *Monopterus albus* ON FUNGI USING THE MOIST DISC ABSORPTION TECHNIQUE AFTER 24h, 48h and 72h INCUBATION [15].

Extract Concentration (mg/ml) / Fungi	Time (hour)	Serial 10-Fold Dilution				Pure Mucus	Negative Control	Positive Control
		1	0.1	0.01	0.001			
C.albanicans	24	9.5±0.5	9.0±1.0	8.5±1.5	9.0±1.0	-	-	25.0±0**
	48	8.5±1.5	8.5±1.5	8.5±1.5	8.5±1.5	-	-	30.0±0**
	72	8.5±1.5	8.5±1.5	8.5±1.5	8.5±1.5	-	-	30.0±0**
C.Kriuser	24	-	-	-	-	-	-	29.0±1.0**
	48	14.0±1.0	13.5±1.5	13.5±1.5	13.0±1.0	-	-	33.5±1.5**
	72	14.5±0.5	14.5±0.5	14.0±0	14.5±0.5	-	-	31.0±1.0**
C.neofarmans	24	No growth	No growth	No growth	No growth	No growth	No growth	No growth
	48	16.5±1.5	12.5±0.5	15.0±0	12.5±0.5	-	-	18.0±0*
	72	14.5±0.5	11.0±1.0	15.0±0	11.5±0.5	-	-	17.0±1.0
Fusarium spp	24	No growth	No growth	No growth	No growth	No growth	No growth	No growth
	48	13.5±1.5	10.0±0	19.5±0.5	20.0±0	-	-	17.0±0*
	72	15.5±0.5	10.0±0	19.5±1.5	20.0±0	-	-	18.0±1.0*

Note: The disc size was 6 mm; - no inhibitory effect, *Amphotericin-B,**Fluconazole; Negative control was distilled water or PBS

Adel et al. (2018) stated that the mode of action of antifungal activity by eel mucus was observed through the formation of pores on the fungal cell membrane under salt and energy-dependent environment or formation, which eventually inhibited the germination of conidia on eel skin [25]. These antifungal properties within the mucus could be utilised to formulate new drugs to treat infectious fungal diseases caused by pathogenic microorganisms or may be beneficial in aquaculture, human health problems, agriculture, medical, and food industries.

III. BIOACTIVE COMPOUND ANALYSIS OF EEL MUCUS

According to Suzuki et al. (2000), eel mucus comprises glycoprotein, lectin, lysozyme and immunoglobulin as an outer barrier against invading toxins, pathogens and parasites living within the same habitat of eels. Besides, the eel skin mucus is known to have high haemagglutinin activity. Haemagglutinin activity is an action of a specific bioactive peptide which binds with compatible carbohydrates or proteins of foreign cells to form a clump. The authors revealed that lectins are one of the components of eel mucus, which can agglutinate carbohydrates, protein, or cells of foreign materials from penetrating the body or colonising the skin surface [26]. According to Shiomi et al. (1989), lectin is a family member of protein and glycoprotein that is neither antibodies nor enzymes which could recognise specific carbohydrate structures and agglutinate foreign pathogenic cells by the mechanism of binding to cell-surface glycoconjugates [27]. Muramoto et al. (1999) added that lectin is also involved in modulation between cells, cell-matrix interaction, and induction of intracellular signals, including immune response and cell growth [28]. TABLE III lists bioactive compounds found in the eel mucus and their characteristics based on previous studies.

In previous studies, Muramoto & Kamiya (1992) revealed that the bioactive compounds in eel mucus are Congerin 1 and 2. Congerin 1 and 2 are galectins isolated and purified only from conger eel (*Conger myriaster*) skin mucus, both of which possess beta-galactosidase-binding lectin with a subunit of 135 amino acid residues. Furthermore, Congerin 1 and 2 have the capacity to agglutinate rabbit, sheep and horse erythrocytes and even *Vibrio anguillarum*, a marine bacterium [29]. In general, characteristically, both Congerin 1 and 2 can withstand the low temperature of up to -20 °C during storage for a month. Additionally, Congerin 1 and 2 are stable over an extensive range of pH values between 5 and 11. The beta-galactosidase lectin, however, is reasonably heat stable at approximately 50°C to 60°C, with Congerin 1 being more heat stable than Congerin 2. Both Congerin 1 and 2 have acidic amino acid contents greater than alkali amino acid with a difference of around 24%, thus, explaining the ability of the eel mucus to agglutinate foreign cells. This is done by binding to the foreign substances' protein or carbohydrate structure, as the lectin's acidity influences the haemagglutinin activity [29].

Tasumi et al. (2002) revealed that Japanese eel (*Anguilla japonica*) lectin consists of AJL-1 and AJL-2, which are lactose-specific lectins isolated from *A. japonica*. Generally, AJL-1 is classified to the galectin family, characterised by its specific binding to the beta-galactosidase sugar - similar to Congerin, a lectin from Conger eel [30]. Meanwhile, Tsutsui et

al. (2016) added that AJL-2 belongs to the C-type lectin family. AJL-1 and AJL-2 possess the hemagglutinating activity induced by the lectin secreted from the thick and sticky mucus of Japanese eel (*A. japonica*) [31]. Tasumi et al. (2004) added that AJL-1 is a lectin that showed specificity for beta-galactosidase in a Ca^{+} -independent manner and pH stable across the broad range of approximately between pH 7 to 10 [32]. In addition, the haemagglutinin activity of AJL-1 is stable even under a high concentration of NaCl. The AJL-1 can agglutinate not only to beta-galactosidase specific sugar but also to agglutinate *Streptococcus* sp., trapping the pathogenic bacteria from penetrating the body. Hence, the listed physicochemical properties of AJL-1 show that AJL-1 is one of the mucus components responsible for protecting the eel from invading infectious microbes or parasites [33]. Meanwhile, AJL-2 is a C-type lectin with Ca^{+}-independent manner. In particular, the haemagglutinin activity of AJL-2 is not affected by the level of Ca^{+}, as it can survive in osmotic conditions even in an environment with a deficient Ca $level^{+}$. Tasumi et al. (2002) added that AJL-2 possesses the same ionic conditions as water in the surrounding habitat and is stable over a broad range of pH values, approximately 3 to 12. AJL-2 also induce agglutination activity and suppresses the growth of *E.coli* K12, which indicates that they participate in host defence [30].

Furthermore, Okamoto et al. (2009) revealed that multiple acidic cysteine protease inhibitors were discovered by the purification and isolation from skin mucus extract of Japanese eel, *Anguilla japonica*, known by the conventional name, Eel-CPI-2 and Eel-CPI-3 [34]. The authors stated that aquatic pathogens and parasites attempt to penetrate the host cell by colonising the skin surface and digesting the host tissue using various enzymes to acquire nutrients for their growth. Hence, eel skin mucus secretes these acidic protease inhibitors (Eel-CPI-2 and Eel-CPI-3) by inhibiting the enzymes of pathogenic microbes and parasites from colonising on the skin surface. These two acidic cysteine proteases have physicochemical and biological roles similar to the C-type lectin AJL-2. However, the acidic cysteine proteases do not show galectin activity by which the acidic cysteine protease inhibitors do not bind to specific sugars. Eel-CPI-2 and Eel-CPI-3 are also known as papain-like cysteine proteases. They share a similar sequence to the papain inhibitory site, where they inactivate the proteases released from pathogenic microbes and undergo lytic activity against them to prevent colonisation on the skin surface [34].

TABLE III. LIST OF BIOACTIVE COMPOUNDS IN EEL MUCUS

Fish	**Conger eel (*Conger myriaster*)**	**Japanese eel (*Anguilla japonica*)**		
Lectin name	Congerin 1 and 2	AJL-1	AJL-2	Eel-CPI-2 and Eel-CPI-3
Type of lectin	Galectin (the protein that binds specifically to beta-galactosidase sugars)	Galectin (the protein that binds specifically to beta-galactosidase sugars)	C-type lectin (carbohydrate-protein binding that is dependent on calcium)	Acidic cysteine protease inhibitors
Specific sugar	Beta-galactosidase sugar	Beta-galactosidase sugar	Lactose	Lactose
Agglutination of bacteria	• Rabbit, sheep and horse's blood • Marine bacterium (*Vibrio anguillarum*)	*Streptococcus* sp.	*E. coli* K12	*E. coli* K12
Other characteristics	• Stable over low temperatures and heat stable. • Both have acidic amino acid content greater than the alkali amino acid.	• Growth regression of *E. coli* • Ca^{+} independent activity	Ca^{+} independent activity	Share a similar sequence to the papain inhibitory site
References	[28], [29]	[30], [32], [33]	[31], [32], [33]	[34]

Subramanian et al. (2008) explained that the antimicrobial activities of eel mucus are determined by the solubility of the bioactive peptides in the mucus with the solvents [35]. Initially, the extracted eel mucus must first be dissolved in various solvents, including crude, aqueous, methanol and acidic, before being tested against tested pathogenic microbes. This is done to study which solvent can stimulate the bioactive mucus peptides to act as antimicrobial agents that can be expressed against the tested pathogens by efficiently binding to the protein or carbohydrate site of pathogens or parasites on the cell membrane and disrupting the innate mechanism of the pathogen from penetrating the host cell. Theoretically, Ikram and Ridzwan (2013) added that a strong polar solvent, such as aqueous or acidic extracts, could exhibit high antimicrobial agents compared to a less polar solvent, like crude extracts [15]. Then, Adel et al. (2018) explained that this is because mucus extracted using higher polarity solvents was more effective as bioactive peptides inhibitors than those extracted through less polar solvents [25]. Iea (2011) also added that highly soluble proteins have good dispersibility when it is mixed with high polar solvents as the effectiveness of their functionality is determined by the conformation and content of hydrophobic and hydrophilic in the bioactive molecules, pH, temperature and ionic strength between the bioactive peptides in the mucus and the solvent used for extractions [36].

IV. MECHANISM ACTION OF BIOACTIVE METABOLITES IN EEL MUCUS AGAINST THE PATHOGENIC TARGET CELL

Ebran et al. (2000) stated that the antimicrobial peptides in mucus are composed of glycoprotein, lysozymes, proteases and immunoglobulin, which these components considered necessary as an innate antimicrobial system in the skin mucus [17]. This is important in stressful conditions, i.e., invading microbial pathogens and parasites. Adel et al. (2018) stated that several mechanisms of action would stimulate the mucus's antimicrobial peptides when encountering pathogen microbes and parasites. These involve the destruction of the cytoplasmic membrane, creating the pore or channel or inhibiting the cell wall and nucleic acid of microbial invasion [25].

According to Shai (1999), the channel or pore formation on the cell membrane of pathogens was initiated by the glycoprotein's solubility in the solvents' high polarity. Next, the soluble glycoprotein in the solvents stimulated the antibacterial properties within themselves. Then, the hydrophobic surfaces of glycoprotein attached themselves to the complementary lipid core of the target cell, which is the pathogens' cell membrane. Then, the soluble bioactive molecules become permeable to the target membrane; thus, the pore or ion channel is formed. Finally, the antimicrobial agents could penetrate the cell membrane of the target cell through the formed pore or channel and, eventually, lyse the whole cell [37].

As for the inhibition of cell wall synthesis, Santoso et al. (2020) explained that it was initiated by lectin activation in the eel mucus. The haemagglutinin activity of lectin is stimulated when the microbial load invasion on the surface increases. The lectin binds to the compatible protein binding site at the target cell. Then, the lectin becomes permeable to the target membrane, thus, agglutinates the target cell colony and clustering them together into a clump before it is entirely discarded from the eel skin by replacing the old mucus with the new ones [6].

V. CONCLUSION

The eel mucus's many uses and modes of action have been demonstrated numerous times. Notably, this review highlights the broad-spectrum classes of bioactive metabolites in the eel mucus that have an antimicrobial potential against specific bacteria and fungi. The eel mucus extract possesses the ability to inhibit microbial invasion by affecting the viability of the pathogens' cell membrane through the formation of large pores and eventually scavenging the growth of the pathogens. This indicates that eel mucus is a potential antimicrobial agent as an alternative to preservatives, antibiotics, or other drug agents in the medical field as well as in the food industry. Though, more experimental groundwork is lacking, such as genetic expression, regulation, and structural-based mechanisms for the mode of action. Future study could focus working on this research to provide concrete proof of concept for the eel mucus's antimicrobial/antifungal and other health properties. Hence, it is recommended that more profound studies must be done to explore a wider spectrum of eel mucus activity and the safety of using it before it can be utilised maximally.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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