Seaweed extracts as antimicrobial agents in aquaculture

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Abstract

In the last 20 years, there has been an increasing interest in using various seaweed extracts as prophylactic and/or therapeutic agents in aquaculture. Up until now, most studies on the direct antimicrobial effect of seaweeds have taken place in various parts of Asia, particularly in India. All groups of seaweeds exhibit significant antimicrobial properties against many infectious agents of fish and shrimp, but the genera that appear to exhibit a broader range of antibacterial properties are *Asparagopsis* spp. (red seaweed) and *Sargassum* spp. (brown seaweed). The activity, can be affected by many factors and the method of extraction is one of the most important ones, as the extracts that are produced using organic solvents appear more efficient. In fish, almost all published information on bacterial pathogens comes from in vitro screenings, where extracts of different seaweed species were tested against many bacterial species. On the other hand, in shrimp, the studies have been focusing on the antimicrobial effects of seaweed extracts mainly against many *Vibrio* species. Regarding the viral pathogens, in fish there is only one published study on fish viruses (IHNV and IPNV), while in shrimp there are many studies on WSSV. There are only two published studies on fish parasites (*I. hoferi* and *Neobendenia* spp.) and no studies on pathogenic fish and shrimp fungi. Interestingly, there are no published studies on salmons and carps, the main fish species that are extensively farmed. When the antimicrobial properties were studied in vivo, the seaweed extracts were either incorporated directly in the feeds (dry or live), or added directly into the water in which the fish and shrimp were reared. In the last case, the water-soluble antimicrobial seaweed substances affected the communication between the bacterial pathogens, rather than their growth. The development of parasites was also affected. In addition, one study indicated that short-term immersion of shrimp in seaweed extracts appeared to have a therapeutic effect against *Vibrio parahaemolyticus*. On the other hand, incorporation of the extracts into the feeds appeared to be an effective delivery method for the prevention and treatment of different infectious diseases. Up until now there are no complete studies on the pharmacodynamics and pharmacokinetics of seaweed extracts in fish or shrimp. However, the findings indicate that they can reduce the bacterial load within the tissues. Another issue that has not been examined yet is the applicability of using these extracts on a commercial scale. Currently, the increased extraction cost inhibits the extensive use of these extracts. Other methodologies, such the production of synthetic analogues with similar properties, may decrease the production cost. Based on the published studies, seaweed

extracts exhibit promising antimicrobial properties, but further research is needed before the complete potential of seaweed extracts is assessed.

Keywords Seaweed, antimicrobial, fish, shrimp, aquaculture

Introduction

With an average annual growth rate of 8.9 % since 1970, aquaculture is considered to be the fastest growing food-producing sector in the world and accounts for about 36 % of the global fish supply and almost 60% of the global shrimp supply (FAO, 2014). In terms of quantity, farming of cyprinids dominates the aquaculture production, with 25.4 million T, while the production of salmonids and crustaceans (shrimp and prawns) contributes with 3.2 and 4.3 million T respectively (FAO, 2014). Diseases, either infectious or non-infectious, are important limiting factors that affect the production volume and consequently the production cost. In 2006, for instance, for a global production of 1.6 million T of salmon, the cost for sea lice treatments was estimated at 305 million € (Costello, 2009). It has been estimated that in Norway, the top salmonid producer in the world, the cost of sea lice control is about 0.19 € kg^{-1} of salmon (Costello, 2009). Furthermore, it was estimated that in 2010, over 77 million USD were spent in Norway on fish diseases management, including the implementation of legislation and support to surveillance and control programmes (The Fish Site, 2010).

The development of many vaccines, mainly against fish pathogens and the use of various antimicrobial agents have reduced the impact of many diseases. However, there is currently an increasing demand for more environment-friendly disease control schemes and many researchers have examined alternative approaches. Among these approaches, the use of various natural products that derive from different living organisms, such as plants (e.g. essential oils), animals (e.g. chitozan) and seaweeds has received a lot of attention (Romero et al., 2012).

Seaweeds, also known as macroalgae, are photosynthetic multicellular aquatic organisms that can be found in almost every aquatic environment, in all geographical areas. Humans had realized their important value as early as 14,000 years ago (Dillehay, et al., 2008). The first reports of seaweeds growing on ropes used for fish farming came from Japan, about 400 years ago (Buchholz et al., 2012). A more systematic culture started in the 1950's, in order to meet the increasing demand for seaweeds as food and mostly as sources of polymers. In 2012, over 21 million tons of seaweeds were produced, over 96 % of which were cultured in Asia (FAO, 2014).

Many studies, on different seaweed species have confirmed their nutritional value. In particular, seaweeds are low in calories, have high content of dietary fibers, are a good source of polyunsaturated fatty acids DHA and EPA and may contain proteins up to 44% dry matter with an amino acid profile of interest (Holdt and Kraan, 2011). The red and the green

seaweeds are generally rich in carbohydrates, whereas the brown seaweeds are generally richer in soluble fiber and iodine (Gupta and Abu-Ghannam 2011a). In some cases some essential amino acids might be limiting, as for example tryptophan, while the concentration of other amino acids, like taurine, can be high particularly in red algae (Dawczynski et al., 2007). In addition to their nutritional value, seaweeds exhibit interesting pharmacological properties, such as antioxidant, anti-inflammatory, antimicrobial and even anticancer properties (El Gamal, 2010; Gupta and Abu-Ghannam 2011a; Gupta and Abu-Ghannam 2011b; Holdt and Kraan 2011; Mohamed et al., 2012). The active compounds include polysaccharides (e.g. fucoidan), various phytochemicals (e.g. phlorotannins), carotenoids, minerals, peptides and lipids (Gupta and Abu-Ghannam 2011b; Holdt and Kraan 2011). It is worth mentioning that some of these compounds, as for example phlorotannins, are not found in terrestrial plants.

The present review focuses on published studies on the direct antimicrobial properties of seaweeds and their extracts against various pathogens of farmed fish and shrimp. Many of these extracts also exhibit significant immunostimulatory (Caipang et al., 2011) and antioxidant properties (Kang et al., 2013; Wijesinghe et al., 2014), which can enhance the resistance and immune response against many infectious agents, but these will not be discussed in the present review.

Control of infectious diseases in aquaculture

In contrast to terrestrial farmed animals, most of the fish species that are farmed today have been recently domesticated from wild populations and thus they are still not well adapted to the conditions that exist in farms (Kibenge et al., 2012). Many of these conditions, such as crowding, regularly handling, improper water quality parameters and the use of artificial commercial feeds, can cause various degrees of stress to fish, which in turn can make them more vulnerable to all infectious diseases (Huntingford et al., 2006). As a rule, the most common infectious diseases that are observed in farmed aquatic animals are those associated with bacterial pathogens (about 50%), followed by the viral, the parasitic and finally the fungal diseases (McLoughlin, 2006). Differences, depending on the species and country, may exist. For instance, in farmed salmonids bacterial diseases are not considered a major problem, compared to the losses caused by viral agents, but in marine fish species bacterial diseases are far more important in terms of financial loss and frequency (Johansen et al., 2011).The control of the infectious diseases that affect the farmed aquatic animals relies on

the use of effective prophylactic as well as therapeutic measures. Numerous studies have demonstrated that the extensive use of various chemotherapeutants used for the treatment of the parasitic, bacterial and fungal diseases in aquaculture have serious impacts on the environment and increase the health risks for both humans and animals (Burridge et al., 2010). It is well established for instance, that the extensive use of various chemicals induces a strong selective pressure on the pathogens, resulting in the appearance of multi-resistant strains. Subsequently, through the horizontal exchange of genetic material that occurs between bacterial species this resistance, which is an important virulence factor for many pathogens, is transferred to other pathogens. Furthermore, the resistance to the antimicrobial agents that is developed in animal bacterial pathogens can be also transferred to human pathogens (Martinez, 2009).

In aquaculture, the main routes of administration of the various chemotherapeutants are either via medicated feeds or by immersion. Both of these methods can have a direct impact on a wide range of bacterial species that live in the aquatic environment. In both cases, it is very difficult to control the leaching of the active substances to the immediate environment (Heuer et al., 2009) and thus residues of many antimicrobials are often found in the sediment under the fish and shellfish farms (Petersen et al., 2002; Romero et al., 2012). Miranda and Zemelman (2002) studied the presence of oxytetracycline-resistant bacteria in the environment of Chilean salmon farms and found that the number of oxytetracycline-resistant bacteria was significantly increased in the effluent water. The presence of these resistant bacteria was associated with previous treatments that took place in the farms. These findings are of great significance as many *in vitro* studies have already demonstrated the transferability of antibiotic resistance genes between fish or shrimp and human pathogens (Heuer et al., 2009). Moreover, the use of the various chemotherapeutants, including the antibiotics, has negative effects on many functions of the fish immune system. Romero et al. (2012) in their review on the use of antibiotics in aquaculture noted that treatment with oxytetracycline and oxolinic acid could induce significant immunosuppression in many fish species, while a less pronounced effect was observed after a treatment with florfenicol. All these findings stress therefore the urgency to minimize the use of any chemotherapeutant in aquaculture and indeed many countries have already developed strict legislations concerning their uses.

This necessity to reduce the use of chemicals is an important issue not only in aquaculture but in the whole animal farming industry. According to a report by World Human Organization (WHO, 2011) the implementation of effective biosecurity measures, the development of new vaccines, the use of prebiotics and probiotics, and good hygiene and

management practices are quite important for the control of many infectious diseases in both terrestrial and aquatic animal farming and can lead to a significant reduction in the use of antibiotics in animal farming. Furthermore, new legislations that would regulate and monitor the use of antibiotics should be implemented, while the use of antibiotics as growth promoters should be banned worldwide. Only qualified people, preferably veterinarians, should be responsible for monitoring the use of all chemicals used in animal farming. Experience from the terrestrial animal husbandry indicates that indeed strict legislations that require reduced use of antibiotics do not necessary result in increased costs to the farmers, as for example a survey in swine farms in Denmark has demonstrated (Aarestrup et al., 2010).

There is however a significant variation between countries concerning the use of chemotherapeutants, which may reflect the diverse degree of awareness of each society for environmental issues. This results in heterogeneity between the legislations in effect, in aquaculture producing countries. For example, Burridge et al., (2010) reported that the amount of antibiotics used in salmon farming between 2007 and 2008 in Chile and Norway, the two main salmon producing countries, was a few hundred metric tons in Chile and less than a metric ton in Norway. Furthermore, in many countries fish and shellfish farmers use increased amounts of various antimicrobial substances, even on a daily basis, as a preventive measure (Heuer et al., 2009).

As societies become more aware of the negative effects of the various treatments that are employed today in the control of the infectious diseases in aquaculture, various alternative approaches have been suggested. These include the use of probiotics to enhance the immune response of fish and shellfish, the use of bacteriophages against bacterial pathogens and the use of various natural products, such as essential oils, as antimicrobial agents (Romero et al., 2012). Among them, seaweeds have also been examined as potential sources of antimicrobial substances (Gupta and Abu-Ghannam, 2011b).

Seaweeds versus fish and shrimp pathogens

The dietary value of seaweeds, as potential substitutes for fishmeal, or as binding agents, has been extensively studied and the findings indicate that seaweed-based diets can be used for the farming of many aquatic organisms, such as fish, shrimp, sea urchins and abalones (Bindu and Sobha, 2004; Henry, 2012). Seaweeds have relatively simple cultivation methods and can grow fast. It is also possible to control the production of some of their bioactive extracts through the manipulation of the cultivation conditions (Plaza et al., 2008). Recent

studies have focused on culture systems integrating seaweed with fish or shrimp production. In these Integrated Multitrophic Aquaculture Systems (IMTA), the seaweeds play an important role first as biofilters and secondly as a source of biomass (Barrington et al., 2009). Seaweeds receive the nutrient-rich waste water from the fish or shellfish and use it for their growth. In this way, they can reduce the negative environmental impacts of fish farming through the removal of the waste materials (mainly N and P) that are released from the animals in the farms. The produced seaweed biomass adds market value to the production system as they can later be used in food, or pharmaceutical industry (Al-Hafedh et al., 2012).

The antimicrobial properties of seaweed extracts against many human and terrestrial animal pathogens are known since the end of the 19th century (Genovese et al., 2012). These antimicrobial properties can be affected by many factors, such as the habitats, the cultivation method, the growth stage of seaweeds, the season and the method used for the extraction of the bioactive components (Karthikaidevi et al., 2009; Govindasamy et al., 2011). For example, Osman et al. (2012), after screening many seaweed species against *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus spp.* and *Escherichia coli*, found that green seaweeds and particularly *Ulva fasciata*, tended to exhibit higher antimicrobial activity. This was more pronounced when the green seaweeds were collected in winter. On the other hand, Salvador et al. 2007, found that red seaweeds exhibited higher antimicrobial properties against many bacterial species, particularly the seaweeds which were collected in autumn. Regarding the method of extraction, organic solvents generally tend to be more efficient for the extraction of the active substances than water (Abu-Ghannam and Rajauria, 2013) and fractioned seaweed extracts appear more effective compared to crude (Radhika et al., 2014). One important characteristic of seaweeds that may pose a health risk is that they are prone to absorb heavy metals from their surrounding environment, especially if they are located in particularly polluted areas (Bailey et al., 1999). Furthermore, they may contain substances, such as kainoids, aplysiatoxins and polycavernosides, which may be toxic to humans and animals (Smit, 2004). For example, significant ichthyotoxic effects have also been reported by De Lara-Isassi et al. (2000), who used *Carassius auratus* to assess the toxicity of over 70 seaweed species. They concluded that Rhodophyta tended to be more toxic, while Chlorophyta appeared to be the least toxic. In some cases, the seaweed extracts can be toxic to certain fish and shellfish species, even at sub-antimicrobial concentrations (Mata et al., 2013).

In farmed fish, most studies on the antimicrobial properties of seaweeds have focused on various bacterial pathogens (14 out of the 17 presented in this review), while fewer studies exist on viral and parasitic pathogens (1 and 2 respectively out of the 17 presented in this

review). On the other hand, in farmed shrimp, the studies focused mainly on various pathogenic vibrios and the White Spot Syndrome Virus. Interestingly, although there are *in vitro* studies in the literature that demonstrate the antifungal activities of many seaweed extracts against human pathogenic fungi, such as *Aspergillus* spp. and *Candida albicans* (Plaza et al., 2010; Omar et al., 2012), there are no similar studies on the main pathogenic fish or shrimp fungi.

Despite the numerous studies on the antimicrobial effects of seaweed extracts against fish and shrimp pathogens, there is still limited information on the exact mechanism of action for most of these extracts. The reason is that although an assessment of any antimicrobial substance, as in the case of seaweed extracts, should include an initial *in vitro* screening followed by an *in vitro* study (Figure 1), most studies on the antimicrobial effects of seaweeds in fish and shrimp are either only *in vitro* or only *in vivo.* For example, 8 out of the 39 studies on seaweeds versus fish and shrimp pathogens discussed in this review included both *in vitro* and *in vivo* assays (Table 1 and 2). Furthermore, none of the eight studies on the White Spot Syndrome Virus included any preliminary *in vitro* study. Thus, it is not always clear if the observed protective result is either due to the direct antimicrobial effect, or due to immunostimulation, or the synergic effect.

Bacterial pathogens

The main identified active antibacterial compounds found in seaweeds are: fatty acids, lipophilic and phenolic compounds, lectins, acetogenins, terpenes, alkaloids, polyphenolics, isoprenoid metabolites and hydrogen peroxide (Mohamed et al., 2012). In general, these substances can a) attack the bacterial cell walls and the cell membranes, which results in an extensive release of intracellular substances or/and disruption of the uptake and transportation of substances, as for example various phlorotannins (Hierholtzer et al., 2012) b) reduce the protein and nucleic acid synthesis in the bacterial cells (Cai et al., 2014) and c) inhibit respiration (Cai et al., 2014). Phlorotannins, as many other terrestrial tannins do, may also form complexes with some extracellular bacterial enzymes (Stern et al., 1996), thus reducing their effects. In most cases, the effects are dose dependent.

An area that has received a lot of attention is the effect of seaweeds and particularly some of their metabolites, on the quorum sensing mechanism, by which bacterial cells communicate between each other. This process, which depends on the population density, involves the production of certain substances, such as peptides, or lactones, which are then released into

the extracellular environment. When the concentration of these substances increases beyond a certain level they are then detected by specific receptors, located in the bacterial cell membranes, or cytoplasms. This in turn regulates the expression of certain genes. Many Gram positive and negative bacteria use this process to collectively regulate many processes, such as bioluminescence, formation of biofilms and the production of various virulence factors (Manefield et al. 2001; Rutherford and Bussler 2012). Active substances released from seaweeds, such as furanones, can disrupt this process, thus affecting the virulence of many pathogenic bacteria, as for example the virulence of many pathogenic *Vibrio* species (Defoirdt et al., 2006) (Figure 2). Because of these properties and particularly the effect on the biofilm formation, seaweed extracts have also been studied as antifouling agents in aquaculture (Jha et al., 2013). It is worth mentioning that an important advantage of such quorum sensing inhibitors, is that they do not induce strong selection pressure on the bacteria, as antibiotics do (Dobretsov et al., 2009).

Numerous studies have focused on the study of the direct antibacterial (either bactericidal or bacteriostatic) properties of seaweed extracts against human bacterial pathogens, such as: *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Clostridium* spp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus* spp., *Salmonella typhimurium*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Vibrio cholerae* (Vairappan and Suzuki, 2000; Vairappan et al., 2001; Xu et al., 2003; Christobel et al., 2011; Vijayabaskar and Shiyamala 2011; Ganeshamurthy et al., 2012; Marudhupandi and Kumar 2013; Saritha et al., 2013). In most cases, only *in vitro* assays were used to establish the antibacterial activities, such as disk diffusion or tube dilution methods.

Most of the bacterial species that can cause diseases in fish and shrimp are quite ubiquitous in the aquatic environment, as for example many members of the genus *Aeromonas* and the various pathogenic *Vibrio* species, such as *V. anguillarum* (also known as *Listonella anguillarum*), *V. alginolyticus* and *V. harveyi* (Genovese et al., 2012; Cavalo et al., 2013). Some of these bacteria, such as some pathogenic *Vibrio* species, can affect both fish and shrimp and in many cases the manifestation and the progress of the associated diseases are affected by the presence of various stressful conditions. In comparison to human bacterial pathogens, fewer studies have been conducted to identify the antibacterial potential of seaweed metabolites against these pathogens.

Comparisons between the different studies on the antibacterial properties of seaweeds against fish and shrimp are difficult, as different experimental protocols were used and particularly in relation to the extraction methods. However, it is worth noticing that in only 5

out of the 28 studies on fish and shrimp bacterial pathogens, water was used for the extraction (Table 1). Although none of the three groups of seaweeds appears to be significantly more effective, as different species belonging to all groups are effective against many bacterial pathogens, *Asparagopsis* spp. (red seaweed) and *Sargassum* spp. (brown seaweed) appear to exhibit a broader range of antibacterial properties (Table 3). Interestingly, most studies were conducted in Asia (mainly India), while considerably fewer in other parts of the world, which can be associated with the extensive use of seaweed in the human diet in this area.

Fish bacterial pathogens

Antibacterial activities of seaweed extracts have been found against many Gram positive and Gram negative fish pathogenic bacteria, as many *in vitro* screenings have indicated (Table 3): many pathogenic *Vibrio* species, *Aeromonas hydrophila* and *A. salmonicida*, *Edwarsiella tarda*, *Renibacterium salmoninarum*, *Photobacterium damselae* sbsp *piscicida*, *Pseudomonas anguilliseptica*, *Streptococcus iniae* and *Yersinia ruckeri* (Vairappan and Suzuki, 2000; Bansemir et al., 2004; 2006; Dubber and Harder 2008; Ganeshamurthy et al., 2012; Genovese et al., 2012; Rebecca et al., 2012; Singh et al., 2012; Cavallo et al., 2013; Maheswaran et al., 2013; Mata et al., 2013; Radhika et al., 2014).

Few of these studies investigated the potential of using seaweeds to control bacterial pathogens in the aquatic environment (Figure 2). Lu et al. (2008) demonstrated the antimicrobial properties of *Ulva clathrata* in a series of experiments. In one experiment in particular, they added *V. anguillarum* in tanks containing cultures of the seaweed (10 g fresh algae L^{-1}). The seaweed significantly reduced the growth of the bacterium in the water. However, the study did not include any experiment with fish and thus the applicability of these findings was not assessed. Mata et al. (2013) examined both *in vitro* and *in vivo* the antibacterial effect of the aqueous extracts bromoform and dibromoacetic acid from the red seaweed *Asparagopsis taxiformis* against the fish pathogen *Streptococcus iniae.* In that study, the extracts were added into the water containing barramundi (*Lates calcarifer*) fingerlings already infected with *Streptococcus iniae.* The findings indicated that addition of approximately 28 μg L^{-1} bromoform and 5 μg L^{-1} dibromoacetic acid could delay the growth of the bacterium in the water, but did not affect significantly the mortalities caused by *Streptococcus iniae.* This study however examined the activity of the extracts after the infection, while the possible prophylactic effect prior to infection was not investigated.

Addition of higher concentration of the extracts was more effective against the pathogen, but also induced mortality in the fish.

Shrimp bacterial pathogens

Almost all studies related to the antibacterial effects of seaweed extracts against shrimp pathogenic bacteria have focused on the bacterial genus *Vibrio* spp., as this represents the main bacterial group that can induce significant mortalities in shrimp farming (Defoirdt et al., 2006; Baleta et al., 2011; Selvin et al., 2011; Dashtiannasab et al., 2012; Manilal et al., 2012; Cavalo et al., 2013; Silva et al., 2013; Sivakumar et al., 2014; Thanigaivel et al., 2014). When *in vivo* studies were carried out, the extracts were delivered to the shrimp mainly through enriched *Artemia*, or medicated dry feeds. In one study, the extracts were added into the water that contained infected shrimp (Thanigaivel et al., 2014).

Traifalgar et al. (2009) examined and demonstrated the overall protective effect of fucoidan extracted from *Undaria pinnatifida* against *Vibrio harveyi* in post-larvae black tiger shrimp (*Penaeus monodon*). In that study, the shrimp that were fed with 500 - 2000 mg kg^{-1} body weight for one month exhibited significantly lower mortality when infected artificially with the bacterial pathogen. Interestingly, the shrimp that were fed with the medicated feeds also exhibited improved growth performance. Selvin et al. (2011) confirmed the protective effect of *Ulva fasciata* extracts after feeding black tiger shrimp post-larvae with medicated feed for 2 weeks. Subsequently, they challenged the shrimp with four pathogens, namely *Vibrio fischeri, V. harveyi, V. alginolyticus* and *Aeromonas* spp. The group of shrimp fed with 1 g kg^{-1} seaweed extract exhibited significantly lower mortality. Similarly, Manilal et al. (2012) examined the protective and therapeutic effect of ethyl acetate partitioned fraction of *Asparagopsis* spp. in black tiger shrimp post-larvae. For this, they fed the shrimp for 3 weeks and then challenged them with lethal doses of *Vibrio harveyi, V. alginolyticus, V. parahaemolyticus* and *Photobacterium damselae.* In this study, the authors examined the therapeutic effect as the shrimp were also fed with the medicated feed after the infection. Shrimp fed with 850 and 1150 mg kg^{-1} exhibited significantly increased survival rate. In all the above studies, the exact mode of action of the extracts was not determined.

In a some studies, the authors attempted to explain the protective effect of the extracts only through their immunostimulatory properties. For example, Sirirustananun et al. (2011), studied the immunostimulatory effect of hot-water extract of *Gracilaria tenuistipitata* by feeding white shrimp (*Litopenaeus vannamei*) with 0.5, 1.0, and 2.0 g kg^{-1} dry diet for 14

days, before challenging them with *V. alginolyticus* and White Spot Syndrome Virus. The extracts induced a significant immunostimmulatory effect and increased survival rates. However, the study did not include any *in vitro* antibacterial assays, to indicate any possible direct antibacterial effect, which could also play an important role.

Kanjana et al. (2011) studied both *in vitro* and *in vivo* the protective role of some solvent extracts of the red seaweed *Gracilaria fisheri* against *Vibrio harveyi*. After an initial screening using a disc-diffusion assay, the authors used only the ethanol extracts for further *in vivo* studies. For the *in vivo* study, the authors fed the shrimp with enriched *Artemia salina* instars II (either with 0.5 or 1.0 mg mL^{-1}) for two weeks and then they artificially infected shrimp postlarvae with the bacterial pathogens. The results indicated both an antibacterial as well as an immunostimulatory effect (i.e. increased total haemocyte and granulocyte counts, increased phenoloxidase (PO) and superoxide dismutase (SOD) activities and increased super oxide anion production). Immanuel et al. (2004) also studied *in vitro* and *in vivo* the protective role of some seaweeds extracts against the shrimp pathogen *Vibrio parahaemolyticus* by feeding *Penaeus indicus* post-larvae with *Artemia franciscana* preadults enriched with 400 mg L^{-1} of butanolic extracts from *Ulva lactuca* and *Sargassum wightii*. In this study, the authors maintained the shrimp in water containing the pathogen for 30 days, while fed them with the seaweed extract enriched *Artemia*. Interestingly, they found that the extract that exhibited the highest inhibition zone in the initial *in vitro* screening, also induced reduced bacterial load in the internal organs of the infected shrimp and increased the survival rate.

Thanigaivel et al. (2014) conducted a study which has demonstrated the potential of using seaweed extracts as alternatives to antibiotics. The authors examined the antioxidant and antibacterial properties of an ethanol extract from the green seaweed *Chaetomorpha antennina*. Regarding the antibacterial properties, the authors first infected *Penaeus monodon* (mean weight 12 g) with *V. parahaemolyticus* and then treated the diseased shrimp by immersing them into water containing 250 mg L^{-1} of the seaweed extract for 12 – 48 h. This treatment resulted in 98% of survival of the treated shrimp. In addition, i.m. injection of 25 μL of the extract per shrimp protected the animals when they were subsequently infected by the bacterial pathogen. This is the first report that shows the therapeutic effect of a short-term administration of seaweed extracts.

A recent study by Sivakumar et al. (2014) demonstrated possible mechanisms that could explain the antimicrobial properties of *Ulva fasciata* against the pathogen *Vibrio harveyi*. Thus, they demonstrated that solvent seaweed extracts reduced the phospholipase, proteolysis, lipolysis and thermonuclease activities of treated bacteria. The study included also an

immersion challenge trial, in which *P. monodon* postlarvae were maintained in water containing *Vibrio harveyi* for 30 days. Addition of 200 μg mL^{-1} of extracts into the water resulted in significantly reduced mortality.

Defoirdt et al. (2006) examined the antibacterial effect of halogenated furanone extracted from the red seaweed *Delisea pulchra* against the shrimp bacterial pathogens *Vibrio campbellii*, *V. harveyi*, and *V. parahaemolyticus.* They reported that this natural product at the concentration of 20 mg L^{-1} could protect *in vivo* the brine shrimp *Artemia franciscana* against these bacterial pathogens, although the substance did not have any effect on the growth rate of the pathogens in the water. Higher concentrations were toxic to *Artemia*. The authors concluded that the protective effect was probably due to the disruption of the quorum sensing mechanism, as assessed by inhibition of bioluminescence, although a possible interaction between furanone and the shrimps was not excluded. Earlier, Manefield et al. (2000) had found that there is a link between bioluminescence and toxin production in *V. harveyi* and that the furanone that Defoirdt et al. (2006) also used could decrease the production of toxin by the bacterium. They also observed a protective effect in *P. monodon*, when they injected intramuscularly the animals with furanone-treated *V. harveyi* cultures. Rasch et al. (2004) examined the potential of using a synthetic halogenated furanone at significantly lower concentration (2.5 μg L^{-1}) to minimize the mortality caused by *Vibrio anguillarum* in rainbow trout (*Oncorhynchus mykiss*). Although no natural seaweed extracts were used, the use of synthetic furanone decreased the mortality caused by the bacterial pathogen, probably through the disruption of the quorum sensing mechanism. As in the study by Defoirdt et al.(2006), no effect of the synthetic furanone were observed on the growth, the survival, the respiratory activity and the motility of the bacterium.

Viral pathogens

Currently no antiviral drugs are used in aquaculture and thus the study of any substance with antiviral properties that can be used against fish or shellfish viruses is of great importance. The strategies that are currently used in aquaculture to control viral diseases rely on the use of effective vaccines (mostly in fish farming) and the development of lines of animals resistant to certain diseases through selective breeding (Kibenge et al., 2012). In shrimp farming, oral administration of immunostimulants has been suggested as a particularly promising method against viral pathogens (Sivagnanavelmurugan et al., 2012), as vaccination is a rather experimental control method (Sudheer et al., 2012).

The antiviral properties of seaweed extracts against human viruses are well reported. Various water-soluble extracts from red, brown and green seaweeds and particularly sulfated polysaccharides, exhibit antiviral properties against many viruses, such as the herpes simplex viruses (Saha et al., 2012; Son et al., 2013), the Japanese encephalitis virus (flavivirus) (Chiu et al., 2012) and the influenza virus (Jiao et al., 2012). The antiviral activities against human viruses have been assessed mainly by *in vitro* studies, on cell lines, but also by *in vivo* studies, using experimental animals (e.g. mice). These studies have shown that the extracts can suppress the replication of the viruses, and delay the manifestation of the disease symptoms, increasing the survival rates of the infected animals. The active substances found in seaweed extracts include among others: sulfoglycolipids, carrageenans and fucoidans (Mohamed et al., 2012). The mode of action depends on the substance but also on the virus. For instance, many sulfated polysaccharides may bind to the surface of the viruses (mainly enveloped viruses), or to virus receptors on the host cell surface, thus interfering with the attachment and the adsorption of the viruses to the host cells (Wang et al., 2012). Some carrageenans can also exhibit postbinding inhibitory effects, affecting the intracellular stages of the infection (Buck et al., 2006), and particularly the virus transcription and replication (Wang et al., 2012). Factors that may affect the antiviral properties of the sulfated polysaccharides include the sugar composition, the main chain length, the sulfation level and the sulfate pattern (Jiao et al., 2012). Phlorotannins from the brown seaweed *Ecklonia cava* were also found to exhibit inhibitory effect on HIV-1 reverse transcriptase and proteases (Ahn et al., 2004).

Currently there is only one study that indicates a possible protective effect of seaweed extracts against fish viruses (Infectious Hemopoietic Necrosis Virus and Infectious Pancreatic Necrosis Virus), while there are many studies on White Spot Syndrome Virus of shrimp. In contrast to bacterial pathogens, both water and organic solvents were used for the extraction (Table 2). The seaweed species that exhibited the antiviral activity were: for WSSV: red seaweeds: *Gracilaria tenuistipitata*, brown seaweeds: *Sargassum* spp. and *Cladosiphon okamuranus*, green seaweeds: *Acrosiphonia orientalis* and for IHNV and IPNV the red seaweed *Polysiphonia morrowii* (Table 3). All studies discussed in the present review took place in Asia, probably because there is an increased interest to develop effective control strategies against WSSV, as no effective vaccines are yet available for the shrimp industry.

Fish viral pathogens

458 Kim et al. (2011) used cell-based assay to assess the antiviral properties of the red alga
459 *Polysiphonia morrowii*. They found that the 80% (v/v) methanolic extract had significant
460 antiviral activity against two important fish viruses, the Infectious Hematopoietic Necrosis
461 Virus (IHNV - family Rhabdoviridae) and the Infectious Pancreatic Necrosis Virus (IPNV -
462 family Birnaviridae). Although, the study was *in vitro* and the authors did not provide any
463 evidence on the mechanism of action of these extracts on the viruses, the results indicate the
464 potential of using seaweed extracts against these viruses.
465
466 *Shrimp viral pathogens*
467
468 The White Spot Syndrome Virus (WSSV - family Nimaviridae) is the major pathogen
469 affecting the shrimp production worldwide. WSSV can induce up to 100 % mortality within a
470 few days, particularly at larval and juvenile stages. Various authors studied therefore the
471 antiviral properties of the seaweed extracts in particular against the WSSV by administrated
472 the extracts to shrimp either via enriched *Artemia* nauplii (Immanuel et al., 2010; Immanuel et
473 al., 2012; Sivagnanavelmurugan et al., 2012), or through medicated feeds (Chotigeat et al.,
474 2004; Manilal et al., 2009). Based on these studies, the effective concentration of extracts that
475 can be used to enrich *Artemia* ranges from 400 – 750 mg L^{-1}, while the shrimp should be fed
476 for about 20 days prior I order to acquire protection against the virus. On the other hand,
477 medicated feeds were efficient when the seaweed extracts were added at a concentration of
478 250-500 mg kg^{-1} body weight. The active components were found to be polysaccharides, in
479 particular fucoidans and sodium alginates (Takahashi et al., 1998; Chotigeat et al., 2004;
480 Manilal et al., 2009; Immanuel et al., 2012; Sivagnanavelmurugan et al., 2012). Chotigeat et
481 al., (2004) examined in particular the prophylactic and therapeutic effect of crude fucoidan
482 extracted from *Sargassum polycystum* against WSSV. Black tiger shrimps of different sizes
483 were fed with medicated feed 4 days prior to and ten days after an experimental infection. The
484 results showed that crude fucoidan at the concentration of 400 mg kg^{-1} of body weight day^{-1}
485 increased significantly the survival rate, while at the same time increased the phagocytic
486 activity of the shrimp haemocytes. Similar results were obtained in an earlier study by
487 Takahashi et al. (1998) who fed kuruma shrimp (*Penaeus japonicus*) with fucoidan extracted
488 from the brown seaweed *Cladosiphon okamuranus*, at the concentration of 100 mg kg^{-1} of
489 body weight day^{-1}.
490 In another study by Balasubramanian et al. (2006), the extracts, after their extraction by
491 either water or organic solvents, were first mixed with suspensions of WSSV in order to de-

activate the virus. Subsequently, the treated viral preparations were injected intramuscularly into marine shrimp (*Penaeus indicus*) and freshwater crab (*Paratelphusa hydrodomous*). Aqueous extracts of *Sargassum weightii* at a concentration of 3 mg per animal resulted in significantly less mortality in the infected animals.

In all the above studies on WSSV, the mechanisms explaining the antiviral action of these seaweed extracts were not determined. However, apart from the immunostimulatory effects, a direct antiviral effect of the extracts similar to that observed in other viruses, cannot be excluded as a study by Rudtanatip et al. (2014) indicates. These authors reported that sulfated galactans isolated from the red seaweed *Gracilaria fisheri* attached to certain sites on the viral envelope and hence inhibited the attachment of the viruses to the host cells.

Parasitic pathogens

The antiparasitic properties of many seaweed extracts have been studied on a wide range of human parasites, such as protozoa, (e.g. *Plasmodium* spp. and *Trichomonas* spp.) (Moo-Puc et al., 2008; Vonthron-Sénécheau et al., 2011), helminthes (e.g. *Ascaris* spp.) (Higa and Kuniyosh, 2000) and insects (e.g. mosquito larvae) (Bianco et al., 2013). The mechanism of action varies according to the extracts and the parasites. Thus, the extracts can either interfere with the binding of the parasites to the target host cells and the subsequent invasion (Patel 2012), or have a direct toxic effect on the parasites. For example, Moo-Puc et al. (2008) demonstrated the direct antiprotozoan activity of organic extracts derived from many seaweed species against *Trichomonas vaginalis* trophozoites, while Bianco et al. (2013) reported significant larvicidal activity of the red seaweed *Laurencia dendroidea* organic extracts against the larval stages of the mosquito *Aedes aegypti*. Despite the many studies on human parasites, the information on the antiparasitic properties of seaweeds against fish parasites is limited, while there are no published studies on shrimp parasites.

Hutson et al. (2012) examined the effect of aqueous extracts from two seaweeds *Ulva* spp. and *Asparagopsis taxiformis* on the parasitism of barramundi (*Lates calcarifer*) by the monogenean ectoparasite *Neobenedenia* spp. The extracts, at the concentration of 1/100 v/v, mainly affected the initial stages of the cycle of the parasites. In particular, they inhibited the embryonic development, delayed the time of first and last hatching and reduced the hatching success rate of the parasite. The *A. taxiformis* extracts appeared substantially more effective. Both extracts however had no significant effect on the survival of the attached adult parasites, or the infection success of oncomiracidia. The authors suggested that these extracts could be

particularly effective in either closed or integrated farming systems, if these seaweed species are co-cultivated along with the fish. There was however no assessment of the applicability of this method under farming conditions.

Ghany and Alla (2008) reported that when Nile tilapias (*Oreochromis niloticus)* experimentally infected with the protozoan fish endoparasite *Ichthyophonus hoferi* exhibited reduced mortality when fed post-infection with extracts from the seaweed *Fucus vesiculosus* (2 g Kg^{-1} body weight) for three months. It should be noted though that the study did not provide adequate information on the characteristics of the extracts, or how they were produced.

Conclusions and future priorities

Aquaculture is a growing industry and infectious diseases constitute one of the main limiting factors, affecting the production volume and cost. Assessment of the exact effects of the microbial diseases on the aquaculture production is very difficult, as there are direct and indirect effects. Stressful conditions can also compromise the immune system of fish and shellfish and subsequently reduce their response to any infectious agent (Huntingford et al., 2006).

Seaweeds represent a group of aquatic organisms which is an important part of the marine food chain, as well as the human diet. In addition to their nutritional value, they also exhibit antimicrobial, immunostimulatory and antioxidant properties. In the last 20 years, there is an increasing interest in using various seaweed extracts as prophylactic and therapeutic agents in aquaculture.

Although there are fewer published studies on fish and shrimp pathogens compared to human and husbandry animal pathogens, the findings indicate that seaweeds can play an important role in the upcoming aquaculture sustainable practices.

There are few published studies, which included both *in vivo* and *in vitro* assessment of the direct antimicrobial properties of seaweeds. Regarding the fish pathogens, almost all published information comes from *in vitro* screenings, where extracts of different seaweed species were tested against many bacterial pathogens, while there is only one published study on fish viruses (IHNV and IPNV) and two on fish parasites (*I. hoferi* and *Neobendenia* spp.). Interestingly, there are no published studies on salmons and carps, which are extensively farmed. The studies on shrimp have focused on the antimicrobial effects of seaweed extracts mainly against many *Vibrio* species and WSSV. Although all the studies indicate the overall

positive effect of the extracts, they do not elucidate the exact mechanism of action and particularly within the animal tissues (Figure 1). Furthermore, although it is known that many seaweed extracts also exhibit immunostimulatory properties, which can contribute to the protective effect, in most studies these effects were never examined in parallel to the antimicrobial effects.

In general terms, all three groups of seaweeds (red, green and brown) exhibit antimicrobial properties, but the genera that appear to exhibit a broader range of activity are *Asparagopsis* spp (red). and *Sargassum* spp. (brown). It should be noted though, that comparison between species is difficult, as there are many factors that can affect the antimicrobial properties, and the same seaweed species may exhibit different properties depending on the season, or the geographical area.

The extraction method is also an important factor that can affect the efficacy of the produced extracts. In 27 out of 39 of the studies that are presented in this review, organic solvents were used for the extraction rather than water.

The modes of delivery of the active seaweed substances can either be through the water (released directly from the seaweeds, or added into it after their extraction), or through medicated feed (again after their extraction), as outlined in Figure 2. In the first case, mainly water-soluble substances of seaweeds can be released or added into the aquatic environment of the farmed fish and shrimp. These substances appear to affect the quorum sensing mechanism in bacteria with limited effects on the bacterial growth. When the extracts are added into the feeds (live or dry), they can act directly against the pathogens or by stimulating the immune system. In addition, there are no complete pharmacodynamic and pharmacokinetic studies, which can demonstrate the exact mode of action of any seaweed extract. This important issue should be included in future studies.

An important point that none of the published studies presented in our review has examined is the applicability of using any of these extracts on a commercial scale. The main issues related to this is the extraction cost and how the extracts can be delivered to fish or shrimp under the intensive farming conditions.

The production cost of seaweeds varies according to the country and it can be between € 160 and € 330 T^{-1} dry, in Asia and Europe respectively, but new seaweed culture techniques are expected to reduce this cost (Bruton et al. 2009). For the extraction of the active substances, there are a few methods that are available on a commercial scale and at the moment the cost of these methods is relatively high (Takahashi et al., 1998; Ibañez et al., 2012). The yield of the active substances extracted from seaweed is between less than 1 % up

to 40 % of the dry algal mass, depending on various factors, such the metabolite, seaweed species and season (Pereira and Costa-Lotufo, 2012). Possible solutions to the high production cost can be the production of synthetic seaweed active compounds, as some of them exhibit properties similar to the natural substance (Rasch et al. 2004; Defoirdt el., 2006), or the incorporation of the responsible seaweed genes into microorganism as Pereira et al., (2012) suggested. However, some of these techniques have many complex steps and can be applied only when the antimicrobial effect of the natural analogs is well demonstrated.

As discussed before, one mode of action is through the inhibition of the quorum sensing mechanism of the bacterial pathogens that exist in the water column, prior to infection. The active substances need to be constantly added into the water for long periods, as Rasch et al. (2004) did during their experimental challenges. Mata et al. (2013) examining the therapeutic effect of seaweed extracts also added the extracts to the water containing infected fish for a long period. In practice, this method can only be applied on land facilities, when fish are reared in small tanks and the water exchange rate is low (e.g. in hatcheries). In addition, the administration of therapeutics extracted from seaweed must be monitored continuously, as sudden increases of the concentration of the antimicrobial substance can be lethal (Rasch et al. 2004; Mata et al. 2013) and exposure periods must be as short as possible (Thanigaivel et al., 2014). More studies on short-term exposures are therefore required to confirm the efficacy of such treatments, particularly against parasitic pathogens.

The safest delivery method reported is through medicated feed, as the dose of the extract per animal treated can be calculated more accurately. This method applies to all farming systems and can decrease the bacterial load in the tissues (Immanuel et al. 2004). Thus, this method of delivery will probably be the most effective and applicable one. Nevertheless more studies investigating the effect seaweed extracts on pathogens are necessary to support this hypothesis.

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List of figures

Figure 1. A general scheme used in the assessment of antimicrobial activity of seaweed extracts or metabolites. The initial *in vitro* screening indicates the best candidates for the *in vivo* studies. This stage can include many assays, depending on the bioactive component and its potential application. The *in vivo* studies are designed in such a way so that the important information is collected by using the minimum number of animals. Based on all available information, the best method of administration of the tested extract is then proposed.

Figure 2. Modes of administration of the seaweed extracts in fish and shrimp farming.

Table 1 Assessment of the antimicrobial properties of seaweed extracts against fish pathogens.

	Seaweed genus/species	Extraction method	Fish species	*In vitro* assays	*In vivo* assays	Pathogen	Results
Bacterial	*Asparagopsis armata*[a] (red)	Organic solvents	−	Agar diffusion assay	−	*Vibrio anguillarum* *Pseudomonas anguilliseptica* *Aeromonas salmonicida* *Aeromonas hydrophila* *Yersinia ruckeri*	*In vitro* antibacterial activity
	Laurencia chondrioides[b] (red)	Organic solvents	−	Agar diffusion assay	−	*Vibrio anguillarum* *Pseudomonas anguilliseptica* *Aeromonas salmonicida* *Aeromonas hydrophila* *Yersinia ruckeri* *Photobacterium damselae* sbsp *piscicida*	*In vitro* antibacterial activity
	Mastocarpus stellatus[c] (red) *Ceramium rubrum*[c] (red) *Laminaria digitata*[c] (brown)	Organic solvents	−	Bacterial growth inhibition assay	−	*Aeromonas salmonicida* *Vibrio anguillarum* *Photobacterium damselae* subsp. *damselae* *Vibrio alginolyticus* *Yersinia ruckeri*	*In vitro* antibacterial activity
	Halimeda micronesia[d] (green)	Organic solvents	−	Agar well diffusion assay	−	*Aeromonas hydrophila* *Vibrio alginoticus* *V. parahaemolyticus* *Edwarsiella tarda*	*In vitro* antibacterial activity

Asparagopsis taxiformis[e] (red)	Organic solvents	−	Agar diffusion assay	−	*Aeromonas salmonicida* *Photobacterium damselae* subsp *damselae* *Photobacterium damselae* subsp *piscicida* *Vibrio alginolyticus* *Vibrio harveyi* *Vibrio parahaemolyticus* *Vibrio vulnificus*	*In vitro* antibacterial activity
Ulva spp.[f] (green)	Organic solvents	−	Agar well diffusion assay	−	*Aeromonas hydrophila* *Edwarsiella tarda*	*In vitro* antibacterial activity
Padina gymnospora[g] (brown) *Padina tetrastomatica*[g] (brown) *Sargassum wightii*[g] (brown) *Turbinaria ornata*[g] (brown)	Organic solvents	−	Disk diffusion assay Minimum inhibitory concentrations	−	*Edwardsiella tarda* *Vibrio alginolyticus* *Aeromonas hydrophila* *Renibacterium salmoninarum*	*In vitro* antibacterial activity
Gracilaria dura[h] (red) *Gracilaria gracilis*[h] (red) *Gracilariopsis longissima*[h] (red) *Chaetomorpha linum*[h] (green) *Cladophora rupestris*[h] (green) *Ulva prolifera*[h] (green)	Organic solvents	−	Disk diffusion assay	−	*Vibrio ordalii* *Vibrio salmonicida* *Vibrio alginolyticus* *Vibrio vulnificus*	*In vitro* antibacterial activity

Gracilaria corticata[i] (red) *Caulerpa racemosa*[i] (green) *Caulerpa sertularioides*[i] (green) *Chaetomorpha antennina*[i] (green) *Padina gymnospora*[i] (brown) *Sargassum wightii*[i] (green)	Organic solvents	–	Agar well diffusion assay	–	*Vibrio parahaemolyticus* *Aeromonas hydrophila*	*In vitro* antibacterial activity
Hypnea musciformis[j] (red) *Gracilaria corticata*[j] (red) *Ulva fasciata*[j] (green) *Codium tomentosum*[j] (green) *Sargassum wightii*[j] (brown) *Dictyota dichotoma*[j] (brown) *Padina tetrastromatica*[j] (brown)	Water	_	Disk diffusion assay	_	*Vibrio alginolyticus* *Vibrio fischeri* *Vibrio harveyi*	*In vitro* antibacterial activity
Ulva clathrata[k] (green)	Water	_	Addition of bacterial suspension in seaweed cultures	_	*Vibrio anguillarum*	Inhibition of bacterial growth in the water
Ulva reticulata[l] (green)	Organic solvents	_	Minimum inhibitory concentrations Enumeration of bacteria on the surface of seaweed	_	*Aeromonas hydrophila* *Vibrio alginolyticus* *Vibrio parahaemolyticus*	*In vitro* antibacterial activity Decrease in number of bacterial colonies
Padina tetrastomatica[m] (brown) *Stoechospermum marginatum*[m] (brown) *Ulva fasciata*[m] (green)	Organic solvents	_	Agar well diffusion method	_	*Aeromonas hydrophila*	*In vitro* antibacterial activity

	Asparagopsis taxiformis[n] (red)	Water	*Lates calcarifer*	Solid media antagonism assay Broth dilution assay	Immersion challenge followed by administration of the extract through the water	*Streptococcus iniae*	Delay of the growth of the bacterium in the water Not significant reduction in the mortality rate
Viral	*Polysiphonia morrowii*[o] (red)	Organic solvents	–	Cytotoxicity assay Cytopathic effect reduction assay Plaque reduction assay Cytotoxicity assay.	–	Infectious Hematopoietic Necrosis Virus Infectious Pancreatic Necrosis Virus	*In vitro* antiviral activity
	Fucus vesiculosus[p] (brown)	–	*Oreochromis niloticus*	–	Feeding trial using naturally infected fish	*Ichthyophonus hoferi*	Reduced mortality
Parasitic	*Ulva* spp.[q] (green) *Asparagopsis taxiformis*[q] (red)	Water	*Lates calcarifer*	Immersion treatment of various developmental stages of the parasites.	Immersion treatment of infected fish	*Neobenedenia* spp.	Inhibition of the embryonic development, increase in the time of first and last hatch and reduced hatching success of the parasite

[a] Bansemir et al. (2006); [b] Bansemir et al. (2004); [c] Dubber and Harder (2008); [d] Ganeshamurthy et al. (2012); [e] Genovese et al. (2012); [f] Rebecca et al. (2012); [g] Singh et al. (2012); [h] Cavallo et al. (2013); [i] Maheswaran et al. (2013), [j] Christobel et al. (2011) [k] Lu et al. (2008); [l] Vairappan and Suzuki (2000); [m] Radhika et al. (2014), [n] Mata et al. (2013); [o] Kim et al. (2011); [p] El Ghany and Alla (2008); [q] Hutson et al. (2013)

Table 2 Assessment of the antimicrobial properties of seaweed extracts against shrimp pathogens.

	Seaweed genus/species	Extraction method	Shrimp species	*In vitro* assays	*In vivo* assays	Pathogen	Results
Bacterial	*Undaria pinnatifida*[a] (brown)	Organic solvents	*Penaeus monodon*	−	Feeding trial and immersion challenge	*Vibrio harveyi*	Reduced mortality
	Ulva fasciata[b] (green)	Organic solvents	*Penaeus monodon*	−	Feeding trial and injection challenge	*Vibrio alginolyticus* *V. harveyi* *Aeromonas spp.*	Reduced mortality
	Asparagopsis spp.[c] (red)	Organic solvents	*Penaeus monodon*	−	Feeding trial and injection challenge	*Vibrio harveyi* *Vibrio alginolyticus* *Vibrio parahaemolyticus* *Photobacterium damsela*	Reduced mortality
	Gracilaria tenuistipitata[d] (red)	Water	*Litopenaeus vannamei*	−	Feeding trial and injection challenge	*Vibrio alginolyticus*	Reduced mortality
	Gracilaria fisheri[e] (red)	Organic solvents	*Penaeus monodon*	Disk diffusion assay Minimum inhibitory concentrations	Safety test for the seaweed ethanol extract Enrichment of *Artemia salina* Immersion challenge of shrimp postlarvae and juveniles	*Vibrio harveyi*	*In vitro* antibacterial effect Reduced mortality

Ulva lactuca[f] (green) *Sargassum wightii*[f] (brown)	Organic solvents	*Penaeus indicus*	Disk diffusion assay	Enrichment of *A. salina* Immersion challenge of shrimp juveniles	*Vibrio parahaemolyticus*	*In vitro* antibacterial effect Reduced bacterial load in the internal organs Reduced mortality
Delisea pulchra[g] (red) Synthetic furanone[g]	Organic solvents	*Artemia franciscana*	Growth inhibition of furanone in liquid growth medium and water (plate counts) Disruption of AI-2 quorum sensing by synthetic furanone	Addition of the extract into the water and challenge tests	*Vibrio harveyi* *Vibrio campbellii* *Vibrio parahaemolyticus*	Disruption of the quorum sensing mechanism
Sargassum polycystum[h] (brown)	Water	*Penaeus monodon*	Agar diffusion assay Minimum inhibitory concentrations	Feeding trial and incubation challenge	*Vibrio harveyi*	Reduced mortality
Ulva fasciata[i] (green)	Organic solvents	*Penaeus monodon*	Agar well diffusion assay Minimum inhibitory concentrations Effect on virulence factors	Immersion challenge	*Vibrio harveyi*	*In vitro* antibacterial effect Reduced activity of many virulence factors Reduced mortality

Delisea pulchra[j] (red)	Organic solvents	*Penaeus monodon*	Inhibition of luminescence T1 toxin production	Toxicity of supernatant extracts from furanone-treated *V. harveyi* cultures assess by i.m. injection	*Vibrio harveyi*	Inhibition of luminescence and T1 toxin production Reduced mortality
Chaetomorpha antennina[k] (green)	Organic solvents	*Penaeus monodon*	Well diffusion method	Immersion treatment after i.m. and immersion challenge I.m injection of extract followed by infection	*Vibrio parahaemolyticus,*	*In vitro* antibacterial effect Therapeutic effect after challenge Improved histological picture after treatment with the extracts Protective effect of the i.m. injection of the extract
Padina gymnospora[l] (brown)	Organic solvents	–	Disk diffusion assay	–	*Vibrio parahaemolyticus, Vibrio brasiliensis, Vibrio xuii, Vibrio navarrensis*	*In vitro* antibacterial effect
Sargassum oligocystum[m] (brown)	Organic solvents	–	Disk diffusion method	–	*Vibrio alginolyticus, Vibrio parahaemolyticus Vibrio harveyi*	*In vitro* antibacterial effect
Sargassum latifoliumn (brown)	Organic solvents	–	Disk diffusion method	–	*Vibrio alginolyticus, Vibrio parahaemolyticus Vibrio harveyi*	*In vitro* antibacterial activity

Viral	*Sargassum wightii*[o] (brown)	Organic solvents		−	Enrichment of *Artemia* nauplii with fucoidan Immersion challenge	White Spot Syndrome Virus	Reduced mortality
	Sargassum wightii[p] (brown) *Sargassum duplicatum*[p] (brown)	Water	*Penaeus monodon*	−	Enrichment of *A. salina* Immersion challenge of shrimp postlarvae	White Spot Syndrome Virus	Reduced mortality
	Sargassum wightii[q] (brown)	Organic solvents	*Penaeus monodon*	−	Enrichment of *Artemia franciscana* nauplii Immersion challenge Viral load using nested PCR	White Spot Syndrome Virus	Reduced mortality
	Sargassum polycystum[h] (brown)	Water	*Penaeus monodon*	−	Feeding trial and immersion challenge	White Spot Syndrome Virus	Reduced mortality
	Acrosiphonia orientalis[r] (green)	Organic solvents	*Penaeus monodon*	−	Feeding trial and immersion challenge	White Spot Syndrome Virus	Reduced mortality
	Cladosiphon okamuranus[s] (brown)	−	*Penaeus japonicus*	−	Feeding trial and immersion challenge	White Spot Syndrome Virus	Reduced mortality
	Sargassum wightii[t] (brown)	Water	*Penaeus indicus Paratelphusa hydrodomous*	−	Determination of viral inactivation using i.m. injection of shrimp	White Spot Syndrome Virus	Reduced mortality
	Gracilaria tenuistipitata[d] (red)	Water	*Litopenaeus vannamei*	−	Feeding trial and injection challenge	White Spot Syndrome Virus	Reduced mortality

[a] Traifalgar et al. (2009); [b] Selvin et al. (2011); [c] Manilal et al. (2012); [d] Sirirustananun et al. (2011); [e] Kanjana et al. (2011); [f] Immanuel et al. (2004); [g] Defoirdt et al. (2006); [h] Chotigeat et al. (2004); [i] Sivakumar et al. (2014); [j] Manefield et al. (2000), [k] Thanigaivel et al. (2014), [l] Silva et al. (2013), [m] Baleta et al. (2011), [n] Dashtiannasab et al. (2012), [o] Sivagnanavelmurugan et al. (2012); [p] Immanuel et al. (2010); [q] Immanuel et al. (2012); [r] Manilal et al. (2009); [s] Takahashi et al. (1998); [t] Balasubramanian et al. (2006).

Table 3. Seaweed species tested against fish and shrimp pathogens. The table summarizes the findings presented in Tables 1 and 2 of this review.

Seaweed genus/pecies	Geographical area	Pathogen
Red seaweeds		
Asparagopsis armata	Atlantic, France	Vang, Pang, Asal, Ahyd, Yruc
Asparagopsis taxiformis	Italy	Valg, Vpar, Vhar, Vvul, Asal, Pdad, Pdap,
Asparagopsis taxiformis	Australia	Sini, Neo
Ceramium rubrum	North Sea	Asal, Valg, Yruc
Delisea pulchra	India	Vhar, Vcam, Vpar
Delisea pulchra	Australia	Vhar
Gracilaria corticata	India	Vpar, Ahyd, Valg, Vhar, Vfis
Gracilaria dura	Italy	Vord, Valg
Gracilaria fisheri	Thailand	Vhar
Gracilaria gracilis	Italy	Vsal
Gracilaria tenuistipitata	Taiwan	Valg, WSSV
Gracilariopsis longissima	Southern Italy	Valg, Vvul
Hypnea musciformis	India	Vhar, Vfis
Laurencia chondrioides	Gran Canaria	Vang, Pang, Asal, Ahyd, Yruc, Pdapi
Mastocarpus stellatus	North Sea	Asal, Vang
Polysiphonia morrowii	South Korea	IHNV, IPNV
Green seaweeds		
Acrosiphonia orientalis	India	WSSV
Caulerpa racemosa	India	Vpar, Ahyd
Caulerpa sertulrioides	India	Vpar, Ahyd
Chaetomorpha antennina	India	Vpar, Ahyd

Chaetomorpha linum	Southern Italy	Vvul, Vord
Chladophora rupestris	Southern Italy	Vvul, Vsal, Vord
Codium tomentosum	India	Valg, Vhar, Vfis
Halimeda micronesia	India	Valg, Vpar, Ahyd, Etar
Ulva clathrata	China	Vang
Ulva fasciata	India	Valg, Vhar, Vfis, Aero
Ulva prolifera	Southern Italy	Vord
Ulva lactuca	India	Vpara
Ulva reticulata	Malaysia	Valg, Vpar, Ahyd
Ulva spp.	Australia	Neo
Brown seaweeds		
Cladosiphon okamuranus	Japan*	WSSV
Dictyota dichotoma	India	Valg
Fucus vesiculosus	Egypt*	Icth
Laminaria digitata	North Sea	Vang, Pdada, Yruc
Padina gymnospora	India	Vpar, Ahyd, Valg,
Padina gymnospora	Brazil	Vpar, Vbra, Vxui, Vnav
Padina tetrastomatica	India	Valg, Vhar, Etar, Ahyd
Sargassum duplicatum	India	WSSV
Sargassum latifolium	Persian Gulf	Vpar, Valg, Vhar
Sargassum oligocystum	Philippines	Vpar, Valg, Vhar
Sargassum polycystum	Thailand	Vhar, WSSV
Sargassum wightii	India	Vpar, Ahyd, Valg, Vhar, Vfis, Rsal, WSSV
Stoechospermum marginatum	India	Ahyd
Undaria pinnatifida	Japan	Vhar
Turbinaria ornata	India	Rsal

Aero=*Aeromonas* spp., Ahyd=*Aeromonas hydrophila*, Asal=*Aeromonas salmonicida*, Etar=*Edwardsiella tarda*, Icth=*Ichthyophonus hoferi*, IHNV=Infectious Hemopoietic Necrosis Virus, IPNV=Infectious Pancreatic Necrosis Virus, Neo=*Neobenedenia* spp., Pang=*Pseudomonas anguilliseptica*, Pdad=*Photobacterium damselae* sbsp damselae, Pdap=*Photobacterium damselae* sbsp *piscicida*, Rsal=*Renibacterium salmoninarum*, Sini=*Streptococcus iniae*, Valg=*Vibrio alginolyticus*, Vang=*Vibrio anguillarum*, Vbra=*Vibrio brasiliensis*, Vcam=*Vibrio campelii*, Vfis=*Vibrio fischeri*, Vhar=*Vibrio harveyi*, Vord=*Vibrio ordalii*, Vpar=*Vibrio parahaemolyticus*, Vsal=*Vibrio salmonicida*, Vvul=*Vibrio vulnificus*, Vxui=*Vibrio xuii*, WSSV=White Spot Syndrome Virus, Yruc=*Yersinia ruckeri*, *Area where the study took place.

The relevant references are cited in Tables 1 and 2.