Role of *Eleutherine bulbosa* (Mill.) Urb. in shrimp and fish: a mini review

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Abstract. Medicinal plants have been commonly used in aquaculture to increase growth and immunity and control fish diseases as an effort to replace or as alternative to uses of chemical antibiotics and compounds. The use of medicinal plants in aquaculture provides safe and environmentally friendly compounds. Natural products of medicinal plants have been used in humans for thousands of years to treat disease. One of them is *Eleutherine bulbosa* (Mill.) Urb. This article describes the potential of *E. bulbosa* as an antibacterial, prebiotic, and antioxidant as well as its efficacy on aquatic organisms. Addition of extracts and powders of *E. bulbosa* through feed to white shrimp *Litopenaeus vannamei* was able to improve growth performance, immune response, and resistance against infection from pathogenic bacteria. Optimal use of *E. bulbosa* needs to pay attention to dose and size of shrimp cultured. So far, administration of *E. bulbosa* powder through feed was more effective and efficient than the use of *E. bulbosa* extract in a large scale. Research on *E. bulbosa* as an immunostimulant in fish is still limited. Further research on the use of *E. bulbosa* should be conducted to examine the effect of *E. bulbosa* on growth performance and fish health on a large scale aquaculture.

1. Introduction
The increasing demand for aquaculture production globally has led to intensification of aquaculture which is one of the main factors causing disease [1], [2]. Microbial diseases in the aquaculture industry have implications for serious economic losses, thus triggering the use of antibiotics for the treatment of diseases that can cause side effects on cultured organisms and the environment [3]. Use of immunostimulants for health management is a promising new development in aquaculture [4].

The use of medicinal plants as immunostimulants in aquaculture offers a significant advantage. They contain natural organic materials that do not pose a threat to fish health, environment, and human health as an alternative to use of antibiotics [5], [6], [7]. Medicinal plants contain a variety of nutrients and natural chemical compounds [8], which are very potential to be used in aquaculture because they are not only used as chemotherapy but also as food additives [9] and immunostimulants [10], [11], [12].

The use of herbal plants and their extracts has been investigated as part of an ongoing effort to develop safe food additives [13]. The affordable price and simple techniques required are driving factors of its use on a large scale aquaculture [10], [14], [15]. It is used in raw form, powder form, and extract form, and may be used in combination with probiotics [16].

*Eleutherine bulbosa* (Mill.) Urb. is one of local herbal plants known for its efficacy. In Indonesia, it is widely available in several regions with different names. *E. bulbosa* plants have good environmental
adaptations; they can grow in various types of climates and soil types; they can be propagated and harvested in a short time so that they are easily developed for industrial scale [17]. This provides a great opportunity to be developed in aquaculture.

2. The content and efficacy of E. bulbosa in vitro

E. bulbosa extract has antibacterial activity containing isoquinoline compounds [18], naphthalene [18], [19], [20] and phenolics, triterpenoids, flavonoids, saponins, quinone, steroids, and tannins [17], [21]. Secondary metabolite compounds of E. bulbosa extract were able to inhibit the growth of pathogenic aquatic organisms such as Vibrio harveyi [21], [22], Aeromonas hydrophila [22], V. parahaemolyticus [18], and Pseudomonas fluorescens [22], [23].

E. bulbosa also contains oligosaccharides such as FOS 10%, raffinose 7.5%, inulin 2.1%, and GOS 1%. Oligosaccharide extract of E. bulbosa was able to stimulate the growth of probiotic of Bacillus sp. NP5 and Pseudoalteromonas piscicida 1Ub and resistance to amylase enzymes and gastric acid in vitro test [24]. E. bulbosa is a potential source of prebiotics because it can increase the growth of gut microbiota [25].

In addition, E. bulbosa was reported to contain compounds from the xanthones, naphthalene, naphthoquinones, and anthraquinones groups that have antioxidant abilities, able to ward off free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) with IC50 of 1.48 g/mL [24]. E. bulbosa extracted with ethanol and water containing alkaloids, tannins, phenolics, flavonoids, triterpenoids, and steroids showed the ability to scavenge free radicals with IC50 values of 526 ppm for aqueous extracts and 112 ppm for ethanolic extracts [17].

3. Role of E. bulbosa for shrimp

The role of E. bulbosa (extract and powder) through feed on growth performance of white leg shrimp can be seen in Figure 1. The prebiotic content of E. bulbosa was able to enhance the total gut bacteria in white shrimp [26] and affected diversity of gut microbiota [27]. Addition of E. bulbosa extract and powder was capable to improve the health of the shrimp intestines. Perimeter ratio and microvilli density, protease, lipase, and amylase enzyme activity, digestibility and growth were higher than without E. bulbosa administration [27]. Thus, the oligosaccharide of E. bulbosa can be utilized by probiotic in the digestive tract of shrimp.

In addition to improving growth performance, supplementation of E. bulbosa extract and powder was also able to increase immune response and resistance to V. parahaemolyticus infection (Figure 1). Supplementation of extract and powder through feed can increase cellular immune responses such as total hemocyte count (THC) and granular or semi granular cells. In addition to increasing the cellular immune responses, it also enhances humoral immune responses such as phenol oxidase (PO), prophenoloxidase (proPO), lipopolysaccharide and -1,3-glucan binding protein (LGBP), and peroxinetin (PE) [26]. The high immune response of shrimp by E. bulbosa addition was the effect of its bioactive compounds [28]. According to [29], eleutherinol was a specific compound in E. bulbosa capable of stimulating T helper cells. Meanwhile, iso-eleutherin and eleutherin compounds in the E. bulbosa was capable to increase the immune responses of humoral like interleukin (IL2) and interferon (IFNγ) which activation of T helpers. Besides that, the high of diversity and total gut microbiota in the white leg shrimp with addition of E. bulbosa might also influenced the immune response through pattern recognition receptors which plays a critical role in recognizing foreign bodies [28].
4. Role of E. bulbosa for fish

Research on use of E. bulbosa as an immunostimulant in fish was still limited. In addition to inhibiting pathogenic bacteria in fish in vitro, supplementation of E. bulbosa extract to juvenile Carp (Cyprinus carpio) by immersion was to affect the hematology of fish and inhibit infection from V. harveyi with the best dose obtained at 80 ppm [22]. The supplementation of E. bulbosa extract through feeding to striped catfish Pangasianodon hypophthalmus increased digestive enzymes, growth performance, and immunity of fish with the best dose obtained at 30 g/kg feed [30]. The results of this study also showed that the administration of E. bulbosa extract was affected by the dose. According to [31], use of optimal dose of medicinal plants to increase the fish's immune system was very important to understand to avoid risk of immunosuppression. Further research is needed to examine the effect of E. bulbosa on growth performance and fish health.

5. Optimal dose of E. bulbosa in shrimp

Effects of immunostimulants on cultured organisms can be influenced by the source of the immunostimulant, route of administration, dose, species of cultured organism, and duration of administration [32]. Utilization of E. bulbosa through feed on white shrimp could affect diversity of gut microbiota [27] and growth performance [27], [33], as well as ability to stimulate immune responses [26] affected by dose. Similar dose (12.5 g/kg feed) was able to work simultaneously to increase growth performance and stimulate immune response of shrimp. However, use of high doses of E. bulbosa could suppress immune response. Some use of high doses of other medicinal plants could also suppress immune response in fish [14]. According to Hong et al. [29], eleutherin and iso-eleutherin compounds at a concentration of 10 M after incubation for 24 hours increased the formation of T helper. However, incubation for 48 hours could inhibit formation of helper T cells. Extract dose of 2.5 mg/mL (if converted into powder at 25 g/kg) in vitro test could suppress growth of probiotics Bacillus sp. NP5 and P. piscicida 1Ub [14]. Likewise, the results of intestinal microbial diversity test...
showed that a high dose of powder administration of 25 g/kg of feed gave a lower diversity of shrimp gut microbiota [27].

Thus, the use of a precise dosage may provide optimal results. In addition, several studies related to use of medicinal plants or prebiotics, frequency of administration also had an influence against growth performance and immune responses. So, further research is needed to explore the use of *E. bulbosa* for aquaculture.

### 6. *Eleutherine bulbosa* extract and powder on shrimp

The content of *E. bulbosa* is very potential for aquaculture organisms. However, the application of *E. bulbosa* in the form of extract requires extraction costs. Utilization of medicinal plants in the form of extracts in aquaculture requires expensive extraction costs and until now a cheaper extraction method has not been found [34]. The use of powder certainly provides convenience in the production method. It only requires simple equipment so that it can easily be applied in aquaculture.

As previous studies have demonstrated the use of *E. bulbosa* powder through feed at a dose of 12.5 g/kg and extract at a dose of 1.25 g/kg feed (if converted to powder equivalent to 12.5 g/kg) gave different results. The addition of *E. bulbosa* in the form of powder through feed gave more optimal result against growth performance [27], expression of immunity-related genes, as well as several other immune response parameters compared to *E. bulbosa* extract [26]. Use of extracts was influenced by the extraction process, and the type of solvent. Meanwhile, the use of powder, if used directly, still had complex nutrition, so that the opportunity to be utilized was also greater. Despite the possibility of self-extracting aquatic organisms through their digestive tract, the results obtained from the use of powder provide better growth performance and immune response.

According to [35], polyketide compounds such as Eleutherinol and Isoeleuthoside C in *E. bulbosa* were enzymatically hydrolyzed first and then re-extracted with ethanol. According to [19], the compound extraction process of several compounds in *E. bulbosa* such as Eleuthinone A, Eleuthraquinone A, and Eleuthraquinone B was influenced by the type of extraction solvent. Thus, the application of *E. bulbosa* powder for rearing white shrimp for aquaculture was highly recommended compared to the use of extracts.

### 7. Conclusion

The supplementation of extract and powder of *E. bulbosa* on white shrimp could increase gut health parameters (such as digestive enzyme, digestibility, and intestinal microvilli), growth performance, immune response, and resistance to *V. parahaemolyticus* infection. The supplementation of *E. bulbosa* extract on fish was increased digestive enzymes, growth performance, and immunity. The use of *E. bulbosa* in aquaculture organisms was influenced by the dose. Utilization application of powder was more effective and efficient than the use of extract at a large scale. However, further research is needed on application for larval stage aquatic organisms. The use of extracts was likely to be more effective than powders.

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