Role of phytobiotics in relieving the impacts of Aeromonas hydrophila infection on aquatic animals: A mini-review

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Aeromonas hydrophila is a ubiquitous bacterium with various hosts that causes mass mortality in farm-raised fish species and significant economic losses. The current antibiotic treatment is ineffective in controlling this bacterium infection in aquaculture species. Therefore, an evaluation of potential phytobiotics is needed to find an alternative antimicrobial agent to reduce the over-reliance on antibiotics in aquaculture and safeguard public and environmental health. Furthermore, the rise in antibiotic resistance cases among pathogenic bacteria indicates an urgent need for new fish and shellfish health management solutions. In this context, phytobiotics applications in aquaculture can be defined as any medicinal plant-based antimicrobial agent used in fish and shellfish species against MAS and the combination of phytobiotics with other antimicrobial and therapeutic agents against MAS.

KEYWORDS
motile aeromonad septicemia (MAS), plant extract, anti-bacterial activity, innate immunity, disease resistance, synergistic

Introduction

The aquaculture industry is essential in sustaining more than 1 billion population worldwide who depends on fish as their primary source of protein (1, 2). This fast-growing industry (3, 4) recorded an all-time high production, with a total live weight of 114.5 million tons (5). As aquaculture intensifies, diseases have become a major
constraint for the industry, i.e., Motile Aeromonad Septicemia (MAS) caused by *Aeromonas hydrophila*. *A. hydrophila* can be found in various environments such as marine, freshwater, brackish water, water supplies, and incredibly abundant during the warmer seasons (6). The hosts of *A. hydrophila* are vast, ranging from freshwater to marine aquatic species (7). This bacterium was also reported to be coinfected with epizootic ulcerative syndrome (EUS) (8). Thus, this highly virulent microorganism may cause mass mortality of aquaculture species on farm sites (9).

Aquatic animals infected by MAS exhibit symptoms such as cloacal hemorrhage (10), ascites (11), gastroenteritic hemorrhage (12), and septicemia ulceration on the skin (13). Furthermore, the *A. hydrophila* strain will determine the appearance of the symptoms in the infected fish (14). Serine protease (ser), cytotoxins and aerolysin (aer) are the common virulence factors that contribute to *A. hydrophila* pathogenicity (15). In addition, lipase (lip), cytotoxic enterotoxins (act, alt, ast), polar flagella (fla), DNases (exu), type III secretion system (ascV), cholesterol acyltransferase (gcaT), and elastase (ahyB) have been associated with *A. hydrophila* pathogenicity (16–18). These virulence factors, in combination with other factors, will lead to the pathogenicity of *A. hydrophila* (19).

Traditionally, fish farmers use antibiotics as prevention and treatment for aquaculture species health management. However, the misuse and overexploitation of antibiotics have led to increasing antibiotic resistance cases among pathogenic bacteria from aquaculture sites (20). For example, *A. hydrophila*, isolated from Nile tilapia, *Oreochromis niloticus*, was resistant to ampicillin and amoxicillin (21). Therefore, fish farmers have no choice but to increase antibiotic dosages to treat diseases in aquaculture. A study suggests a combination of two different antibiotics, thiamphenicol, and florfenicol, were effective at lower dosages against *A. hydrophila* infection in Nile tilapia (22). Consequently, studies are ongoing to find alternative treatments such as vaccination programs (23) and phytobiotics applications (24–28). Various studies have revealed the potential of phytobiotics as an alternative to antibiotics, thus, minimizing antibiotics usage in aquaculture. Phytobiotics contain bioactive compounds such as alkaloids, sterol, flavonoids, saponins, and tannins that possess bactericidal properties and stimulatory effects (29). For example, miswak, and *Salvadora persica*, contain broad spectrum bactericidal compounds such as benzyl isothiocyanate, salvadourea, salvadoreine (29), and vitamin C that can promote healing and tissue repair (30).

This review summarized the impacts of MAS caused by *A. hydrophila* in aquaculture, phytobiotics preparation for aquaculture uses, the antibiotic activity of medicinal herbs in aquaculture, phytobiotics-activated innate immunity in aquaculture species, phytobiotics enhanced tolerance of aquaculture species toward MAS caused by *A. hydrophila* and phytobiotics in combination with other antimicrobial and therapeutic agents against MAS caused by *A. hydrophila*.

### Impacts of MAS caused by *A. hydrophila* in aquaculture

Disease outbreak is a major constraint to the growth of the aquaculture industry. One of the sources of diseases is *A. hydrophila*, an opportunistic pathogen associated with secondary infection (31) and outbreaks (see Table 1). Fish infected with MAS will exhibit symptoms such as lose appetite, skin ulcerations, pale gills, swollen abdomen, and abnormal swimming pattern. Antibiotics such as oxytetracycline and terramycin were commonly used for treatment. *A. hydrophila* can cause MAS that has detrimental impacts on aquaculture, such as devastating fish farms, causing economic losses, and pose a threat to public health and environmental safety. This disease was first reported by Llobrera and Gacutan (32) in snakehead, catfish, carp and goby in Laguna de Bay, Philippines, where the infected species exhibited lesions and necrotic ulcers. Many studies reported that MAS was responsible for the mass mortality of aquaculture species. For instance, a mass mortality of Channel catfish, *Ictalurus punctatus*, in commercial fish farms in the Southeastern USA (31). Furthermore, the total loss of this outbreak in commercial fish farms amounted to millions of USD, leading to numerous fish farm closures (33).

The MAS is also reported to alter the appearance of an aquaculture species, contributing to the losses in market value. For example, Dierckens et al. (35) claimed that the Fairy shrimp, *Branchinecta gigas* (Lynch), infected with MAS appeared black, causing a substantial price drop. Furthermore, the risk of mass mortality among aquaculture species due to this disease forced fish farmers to harvest and sell their products in the market immediately to reduce losses. Moreover, disease control via antibiotics administration without adequately assessing the effectiveness of the treatments contributes to the infiltration of residues in the environment and microflora breakdown in the area (46), besides posing a threat to public health and the environment.

### Phytobiotics vs. commercially developed vaccines against MAS

Fish vaccination was developed more than 50 years ago (41) and reported to be effective in disease control. The vaccines in aquaculture can be administered via immersion or intraperitoneal injection. In addition, live vaccines were reported to be highly effective in stimulating vigorous antibody activity in fish when administered orally or by immersion. An ideal vaccine is safe for the fish, environmentally friendly, cost-effective, user friendly, and highly effective with little or no side effects (41). In a recent study, fish vaccination has been improved by dissolving microneedle patches. Yun et al. (42) developed this novel vaccination administration to prevent *A. hydrophila* infection in fish. Dissolving microneedle patches
TABLE 1 Impacts of MAS in aquaculture.

| Aquatic species                  | Impacts                                          | Prophylactic measures                                                                 | Location       | References |
|----------------------------------|--------------------------------------------------|----------------------------------------------------------------------------------------|----------------|------------|
| Freshwater Murray cod, Maccullochella peeli | Tail rot disease, mass mortality                  | Cephalosporin, chloromycetin, glycopeptides, macrolides, nitrofurans and penicillin drugs | Shanghai, China | (34)       |
| Channel catfish, Ictalurus punctatus | Mottle Aemonad Septicemia (MAS)                  | Probiotic, vaccination                                                                  | Southeastern USA | (31)       |
| Catfish, Clarias gariepinus       | 80–100% mortality in 2 weeks                     | Maintain good water quality for prevention of MAS infection                              | Surabaya, Indonesia | (35)       |
| Freshwater cultured whiteleg shrimp, Litopenaeus vannamei | Emerging causative agent; mass mortality of shrimp | Combination florfenicol and Punica granatum                                              | Fengxian, Shanghai, China | (36)       |
| Nile tilapia Oreochromis niloticus | Mass mortality                                    | Not mentioned                                                                           | São Paulo, Brazil | (37)       |
| Nile tilapia, O. niloticus        | High mortality                                    | Maintain good water quality for prevention of MAS infection                              | Egypt           | (38)       |
| Nile tilapia, O. niloticus        | 35–50% mortality; A. hydrophila carrying the antibiotic resistance gene | Ciprofloxacin                                                                            | Egypt           | (21)       |

were more effective and can be an alternative to injection in fish vaccination. Currently, the commercial vaccine (Alphaject Panga 2) is used against MAS, in stripped catfish farming in Vietnam (41). However, there are several issues with using the vaccine in aquaculture. High cost and labor intensive are two major factors to be considered by the fish farmer where these factors will hinder vaccines applied extensively in aquaculture.

On the other hand, phytobiotics are plants or plant-based bioactive compounds beneficial for farmers, animals and humans. Examples of phytobiotics are essential oil, legumes, herbs, fruits and vegetables, alkaloids, carotenoids, and phenolic compounds (43, 44). These phytobiotics are widely used in aquaculture feed additives to enhance the immune system and protect aquatic animals against MAS. Since phytobiotics are abundant and inexpensive, fish farmers utilized this treatment as a vaccine replacement in aquaculture health management.

Phytobiotics preparation for aquaculture uses

There are several methods to prepare phytobiotics for aquaculture uses, such as aqueous extracts, methanol extract, and powder form. Methanol is a universal solvent used to extract polar compounds, especially in plants (45), whereas aqueous extracts derive non-polar compounds in phytobiotics. Several studies utilize methanol as a polar aqueous solvent for non-polar extraction in phytobiotics preparation. For example, Wei et al. (46) and Zhou et al. (36) compared the efficacy of bacterial inhibition between methanolic and aqueous extracts. Most studies used methanol in phytobiotics preparation since this solvent was found effectively derives all the properties in the sample (45), Sheikhlar et al. (47), Lee et al. (20), Sheikhlar et al. (48), Bao et al. (49), and Rashmeei et al. (50). The extracts were then used as feed additives in the feeding trials.

Phytobiotics can also be prepared in powder form. For example, Thanikachalam et al. (51) prepared dried garlic peel powder before being incorporated into fish feed for a feeding trial. Similarly, phytobiotics are prepared as feed additives using Rosemary leaf (52), Psidium guajava L. leaf (53, 54), and Spirulina (Arthrospira platensis) (55). Furthermore, some phytobiotics can be commercially available such as tapioca (56), curcumin (57), origanum essential oil (38), and thyme, red thyme and pepper rosemary essential oil (58). Recently, studies have utilized the polysaccharide derived from Pistacia vera hulls (59) or fermentation in preparing phytobiotics (60).

Antibacterial activity of medicinal herbs in aquaculture

Despite the rapid action of antibiotics in controlling a disease outbreak, the residues can disrupt the natural microflora by seeping into the surrounding sediment and water bodies (61). Furthermore, antibiotics such as oxolinic acid, flumequine, oxytetracycline, and sulfadiazine are stable for up to 3 months in soil (62). Thus, there is a dire need for an alternative antimicrobial agent for aquaculture uses.

Medicinal herbs are extensively used as a treatment for human diseases. The whole plant or part of the herbaceous plant, such as twigs, roots, stem, flower, and fruit, are subjected to the extraction process for bioactive compound derivation (63).
Bioactive compounds include terpenoids, tannins, alkaloids, and flavonoids, which possess antibacterial properties (64). Furthermore, numerous studies have revealed the potential of medicinal herbs for aquaculture uses; thus, researchers have developed new and improved approaches for antibacterial discoveries from plants. For instance, antibacterial properties were characterized in polysaccharides derived from macroalgae, Chaetomorpha aerea (65) and nanoscale silver particles of butter fruit, Persea americana (66). These studies highlighted future research prospects for plant-based polysaccharide-derived compounds and nano-synthetic substances.

Based on the literature, many medicinal herbs were reported to demonstrate antibacterial properties against A. hydrophila. For instance, Murraya koenigii, Pandanus odoratissimus (46), Colocasia esculenta (67), and Euphorbia hirta (48) inhibited the growth of A. hydrophila (Table 2). These medicinal herbs contain bioactive compounds, such as carbazole alkaloids (68), phenolic compounds (69), polypeptides (70), and alkaloids (71), that was responsible for the antibacterial activities. Moreover, recent studies showed that pomegranate, Punica granatum (Peel), Prunus mume (fruit), Fructus toosendan (fruit), Artemisia argyi (leaves), Polygonum aviculare (leaves), Cephalanoplos segetum (leaves), and Artemisia capillaries (leaves) demonstrated the ability to inhibit A. hydrophila activities (36). Similarly, Piper betle, Piper sarmentosum, and Piper nigrum demonstrated inhibitory activities, as reported by Anjur et al. (72).

### Phytobiotics activated the innate immunity of aquaculture species

Antibiotics are essential for aquaculture species health management (40, 75, 76). Nevertheless, antibiotic action is not limited to the target bacteria but also other microorganisms in aquatic animals and the environment (77). Moreover, antibiotic residues in the environment contribute to the development of resistance genes in the microorganisms (78). Vivekanandhan et al. (79) reported that almost 99% of A. hydrophila isolated from seafood (fish and prawn) in the wet market of South India were resistant to novobiocin, bacitracin, rifampicin, and mexiticillin. Meanwhile, De Silva et al. (80) have revealed that all A. hydrophila isolated from Yesso scallop, Patinopecten yessoensis were resistant to ampicillin, colistin, vancomycin, and cephalothin. Therefore, phytobiotics offer an alternative solution in aquaculture species health management to overcome antibiotic resistance.

Phytobiotics can also activate the innate immunity of aquaculture species to stimulate disease resistance. Many studies have proven the potential of phytobiotics as an alternative antimicrobial agent for aquaculture uses. For instance, Boswellia serrata resin extract was reported can be phytobiotic to enhance the immune system of Nile tilapia and Oreochromis niloticus against Staphylococcus aureus infection (81). In the study, B. serrata resin extract can activate the innate immune system of O. niloticus via the immune response assay, disease resistance assay and growth performance experiment. Similar findings were reported on Spopora flavescens (82), peppermint (Mentha piperita) (83), Aloe vera powder (84), Spirulina platensis (85), citrus lemon peel essential oil (86). The mentioned phytobiotics can improve aquaculture species’ growth performance and activate their innate immune system against diseases.

Phytobiotics also stimulate mucus production on fish skin, which acts as a non-specific immunity. Fish skin mucus serves as a primary physical defense against various pathogens. A recent study showed that feeding Siberian sturgeon (Acipenser baeri) with barley fruit extract for 56 days caused a remarkable improvement in the skin mucus bactericidal activity against A. hydrophila, where the bacterial inhibition zone diameter is higher compared to the fish fed with unsupplemented diet (control group) (87). Another phytobiotics mechanism is through immune cells improvement and macrophages activation, as reported by Chen et al. (88), where Salvia miltiorrhiza polysaccharide exhibited the ability to modulate the disease resistance of sturgeon against A. hydrophila infection. In addition, phytobiotics from plant secondary metabolites such as saponin, essential oils, phenolic compounds, polysaccharides, and polypeptides demonstrated the potential to treat the bacterial infection with low toxicity (89). The phytobiotics can also act as active site modulators, enzymes as catalytic sites, receptors and proteins for disease treatments (90).

### Phytobiotics enhanced aquaculture species tolerance against MAS

Existing studies indicated that fish farmers utilize antibiotics, chemicals, phytobiotics, probiotics, prebiotics, yeast extract, vaccine, and disinfectants to control MAS caused by Aeromonas hydrophila in commercial farms (91). Nonetheless, these treatments have been proven unsustainable in preventing MAS caused by A. hydrophila. This catastrophic bacterial disease is responsible for the mass mortality of aquaculture species and significant economic losses. Nevertheless, excessive antibiotic usage will increase antibiotic resistance among pathogenic bacteria, leading to its inefficacy in aquaculture disease control. Therefore, fish farmers must be given more options to use other antimicrobial agents instead of over-relying on antibiotics. Recent studies showed promising findings on phytobiotics in controlling A. hydrophila infection in aquaculture species. The phytobiotics were used as a feed additive given together with feed to aquaculture species for some time can activate and enhance the immune system of aquaculture species against A. hydrophila. For example, Zhang et al. (92) revealed that 1–2% of Flos populi extract incorporated in feed and given to Goldfish (Carassius auratus) for 45 consecutive days could enhance medicated fish growth, antioxidative status, non-specific immunity, and...
disease resistance to A. hydrophila infection (Table 3). Many other studies have shown a similar trend of findings in the literature. Therefore, there is no doubt phytobiotics can enhance the tolerance of aquaculture species against Motile Aeromonas Septicemia.

Phytobiotics enhance the fish's immune system by increasing the presence of immune markers such as immunoglobulin M (IgM), nitric oxide and lysozyme. For example, Abdellatief et al. (108) revealed that a combination of sage (Salvia officinalis) and Spirulina platensis (Arthrospira platensis) can boost the immune system of Nile tilapia by increasing their immune response against Pseudomonas aeruginosa. Furthermore, phytobiotics, in the form of antioxidants such as phenols and polyphenols, are beneficial for fish health by improving the immune system of aquatic animals against A. hydrophila (109). For instance, Naiel et al. (52) revealed that 10 g/kg rosemary (Salvia rosmarinus) leaves enhanced Nile tilapia’s immune system and increased their disease resistance against A. hydrophila. Moreover, the phytobiotic promoted lysozyme and serum catalase activity in fish against MAS.

Overall, phytobiotics with low dose and shorter administration duration are the best candidate as antimicrobial agent to against MAS. Based on literature survey, phytobiotics such as polysaccharide of Ficus carica (106), essential oil of thyme, red thyme and pepper rosemary (58) and methanolic extract of Pepperomia pellucida (leaves) (20) were effective in controlling MAS infection as the duration of administration is shorter compared to other phytobiotics. Furthermore, low dose is needed for the mentioned phytobiotics. Further studies need to be carried out in the near future to reveal potential bioactive compounds that present in Gracilaria persica (107), Salvadoria persica (29), Andrographis paniculata (105), Curcumin (57), Morinda oleifera (60), and many more. The future study findings are important to understand the mechanisms of the bioactive compounds in mitigating A. hydrophila impacts on aquatic animals.

**Phytobiotics combined with other antimicrobial agents/therapeutic agents against MAS**

The emergence of antibiotic resistance among pathogenic bacteria from aquaculture sites revealed that antibiotics alone are not a sustainable antimicrobial agent (110). Therefore, researchers have proposed alternative solutions, such as phytobiotics. Several studies have also reported on the synergistic effects between phytobiotics and other supplements such as boron (94), probiotics (111, 112), Spirulina (113), and antibiotic (36) to relieve the impacts of A. hydrophila in aquaculture species (Table 4). Thus, these combinations are promising antimicrobial agents for cost-effective treatments in aquaculture disease management.

**Conclusion and recommendation**

In summary, MAS is a catastrophic disease in the aquaculture industry that can lead to significant economic losses and environmental and public health hazards. Antibiotics
### TABLE 3 Medicinal herbs used to mitigate A. hydrophila impacts on aquatic animals.

| Species                     | Medicinal herbs                                                                 | Dose                  | Duration | References |
|-----------------------------|---------------------------------------------------------------------------------|-----------------------|----------|------------|
| Rohu, Labeo rohita          | Garlic, *Allium sativum*                                                        | 1–10 g per kg of fish | 10 days  | (93)       |
| Nile tilapia, *O. niloticus*| *Psidium guajava* (Dried leaf powder)                                            | 1 feed: 4–24 *Psidium guajava* (dried leaf powder) | 10 days  | (54)       |
| Nile tilapia, *O. niloticus*| Chinese medicinal herbs, *Astragalus membranaceus* and *Lonicera japonica*       | *Astragalus 0.1% + Lonicera 0.1%* | 28 days  | (94)       |
| African catfish, *Clarias gariepinus* | Powdered garlic peel                                                          | 0.5% incorporated in fish feed | 20 days  | (51)       |
| Goldfish, *Carassius auratus*| Mixed herbal extracts                                                            | 400–800 mg/kg diet    | 4 weeks  | (95)       |
| Nile tilapia, *O. niloticus*| *Green tea, Camellia sinensis L.*                                                | 0.5 g/kg diet         | 12 weeks | (96)       |
| Nile tilapia, *O. niloticus*| Cinnamon, *Cinnamomum zeylanicum*                                               | 1% g/kg diet          | 8 weeks  | (97)       |
| Nile tilapia, *O. niloticus*| *American ginseng, Panax quinquefolium*                                         | 1–5 g per kg diet     | 8 weeks  | (98)       |
| Nile tilapia, *O. niloticus*| Mistletoe, *Viscum album coloratum* powder                                     | 50 mg per kg diet     | 80 days  | (99)       |
| African catfish, *Clarias gariepinus* | Methanolic extract of *Morus alba* foliage                                   | 7 g per kg of feed    | 30 days  | (47)       |
| *O. niloticus* GIFT         | Chinese herbal mixture (*Astragalus, angelica, hawthorn, licorice and honeysuckle*) | 0.5–2%/kg diet        | 4 weeks  | (100)      |
| Labeo rohita                | *Psidium guajava L.* (leaves)                                                   | 0.5% of diet          | 60 days  | (53)       |
| *L. rohita* fingerling      | Tapoca                                                                           | C/N ratio 15          | 60 days  | (56)       |
| Crucian carp                | Polysaccharides of *Ficus carica, Radix isatidis, Schisandra chinensis*        | <0.8/kg diet          | 4 weeks  | (101)      |
| Catfish, *C. gariepinus*    | Methanol extract of *Euphorbia hirta* (aerial part)                              | 5 g/kg of fish        | 30 days  | (48)       |
| Silver catfish, *Rhamdia quelen* | *Aloysia triphylla* essential oil                                             | 2 ml/kg diet          | 21 days  | (102)      |
| Red hybrid tilapia,         | Methanolic extract of *Pepperomia pellucida* (leaves)                          | 25 ppm of feed        | 7 days   | (20)       |
| Oreochromis spp.            | *Spirulina, Arthrophyta platensis*                                             | 13.53 g/100 g diet    | 46 days  | (55)       |
| Gibel carp, *Carassius auratus* | gibelio var. CAS III                                                           | Tetra, *Cotinus coggygria* and mallow, *Malva syvestris* | Tetra 1,000 mg/kg diet and mallow 500 mg/kg diet | (103) |
| Gilthead sea bream, *Sparus aurata*; European sea bass, *Dicentrarchus labrax* | *Tetra, Cotonus coggygria* and mallow, *Malva syvestris* | Tetra 1,000 mg/kg diet and mallow 500 mg/kg diet  | (103) |
| Common carp, *Cyprinus carpio* L. | *Ginkgo biloba leaf extract*                                                   | 10 g/kg diet          | 8 weeks  | (49)       |
| *Clarias gariepinus*        | *Piper beetle, Psidium guajava, Tithonia diversifolia*                          | 8% per kg of fish feed| 28 days  | (104)      |
| Nile tilapia, *O. niloticus*| *Pistacia vera-derived polysaccharide* (hull)                                   | 5–10 g/kg of feed     | 60 days  | (59)       |
| Common carp, *Cyprinus carpio* L. | *Origanum essential oil*                                                        | 15 g/kg diet          | 8 weeks  | (38)       |
| Nile tilapia, *O. niloticus*| Rosemary leaf powder                                                            | 10 g/kg diet          | 14 days  | (52)       |
| Goldfish, *Carassius auratus*| *Chasteberry, Vitex agnus-castus extract*                                      | 15 g/kg diet          | 8 weeks  | (50)       |
| Goldfish, *Carassius auratus*| Fermented moringa, *Moringa oleifera* Lam                                    | 40% replacement of fish meal | 50 days  | (60)       |
| Grass carp.                 | Curcumin                                                                         | 438.20 mg/kg diet     | 60 days  | (57)       |
| *Ctenopharyngodon idella*   | *Andrographis paniculata*                                                       | 2% of fish body weight| 60 days  | (105)      |

(Continued)
TABLE 3 (Continued)

| Species                  | Medicinal herbs                                      | Dose                  | Duration | References |
|--------------------------|------------------------------------------------------|-----------------------|----------|------------|
| Nile tilapia, *O. niloticus* | Essential oil of thyme, red thyme and pepper rosemary | 1.2 mg per g of feed | 20 days  | (58)       |
| Nile tilapia, *O. niloticus* | Miswak, *Salvadora persica* powder                   | 2% of diet            | 8 weeks  | (29)       |
| Crucian carp             | *Ficus carica* polysaccharides                      | 0.4%/kg diet         | 4 weeks  | (100)      |
| Goldfish, *Carassius auratus* | *Flos populi* extract                              | 1-2 g/kg diet        | 45 days  | (92)       |
| Persian sturgeon, *Acipenser persicus* | *Gracilaria persica*                | 2.5 g/kg diet        | 8 weeks  | (107)      |

TABLE 4 Medicinal herbs and antimicrobial/therapeutic agents reduce the impacts of *A. hydrophila* on aquatic animals.

| Species                  | Medicinal herbs                                      | Supplements           | Dose                  | Duration | References |
|--------------------------|------------------------------------------------------|-----------------------|-----------------------|----------|------------|
| Nile tilapia, *O. niloticus* | Chinese medicinal herbs, *Astragalus membranaceus* and *Lonicera japonica* | Boron                 | *Astragalus membranaceus* | 0.1% + 0.05% boron; *Lonicera japonica* 0.1% + 0.05% boron | 4 weeks  | (94)       |
| Common carp, *Cyprinus carpio* L. | Triherbal extract                                      | Triherbal extract     | *Lactobacillus* | 200 mg/kg diet—triherbal extract; 0.1 g/kg diet—probiotic | 4 weeks  | (112)      |
| Nile tilapia, *O. niloticus* | Garlic powder                                        | Spirulina platensis powder | *Spirulina platensis* | Garlic—5 g/kg diet; *Spirulina* 10 g/kg diet | 8 weeks  | (113)      |
| Freshwater white leg shrimp, *Litopenaeus vannamei* | *Pomegranate, Punica granatum extract (peel)* | Florfenicol           | *Florfenicol* | 0.03 PPM | 7 days   | (36)       |
| Nile tilapia, *O. niloticus* | Thyme, Cinnamon                                       | Probiotic, *Bacillus subtilis* | *Bacillus subtilis* | Thyme, Cinnamon; 0.1 g/kg diet—probiotic | 8 weeks  | (111)      |

have been proven a short-term solution in managing bacterial disease in the aquaculture industry. Therefore, phytobiotics are a viable alternative for aquaculture species health management and for maintaining public health and environmental safety. Despite that, researchers must monitor phytobiotics toxicity as a prerequisite for aquaculture application. Numerous studies have demonstrated the potential of phytobiotics in activating the innate immune system and stimulating disease resistance of aquaculture species against MAS such as essential oil of thyme, red thyme and pepper rosemary whereas methanolic extraction was the best way in preparing phytobiotics. Besides, bioactive compound such as polysaccharide of *Ficus carica* can be effective phytobiotics. Furthermore, applying less ecological footprint antimicrobial agents like phytobiotics can gain consumer confidence in aquaculture products. Nevertheless, there is a gap between the scientific approach and practical use of phytobiotics in aquaculture that can be addressed through knowledge and technology transfer among aquaculturists. Moreover, phytobiotics application in aquaculture remains inconsistent in terms of dosage and duration. These factors are crucial in phytobiotics against MAS because different dosages and duration will lead to variable outcomes. Additionally, phytobiotics efficacy in controlling MAS depends on the source of phytobiotics and environmental conditions. Also, phytobiotics can be administered orally via incorporation in aquaculture feed, thus, a practical, non-stressful, and convenient administration of feed additives for various aquaculture species. Overall, phytobiotics are promising antimicrobial agents in controlling MAS. Most importantly, phytobiotics derived from agricultural wastes have been proven sustainable in aquaculture practices. Further studies need to be carried out in the near future to characterize bioactive compounds that present in phytobiotics and responsible to inhibitory activity against MAS. This is important to understand the mechanisms of the bioactive compounds. In addition, new potential phytobiotics can be explored from plant families such as Rutaceae, Fabaceae, and Moringaceae.
Author contributions

ZA: conceptualization, writing—review and editing, and writing—original draft. WW: writing—original draft and writing—review and editing. SM, HC, MH, MI and HV: writing—review and editing. KW: supervision and conceptualization. LS: project administration, writing—original draft, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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