The potentials of medicinal plant extracts as bio-antimicrobials in aquaculture

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Abstract: Fish is one of the cheapest and promising sources of animal protein; people can easily digest 93.2% and 93.7% of fish protein and fat, respectively. Stressors including overcrowding, high or sudden changes of temperature, handling, low dissolved oxygen, poor nutritional status and fungal or parasitic damage of the epidermis, contribute to physiological changes and heighten susceptibility to infection. The uncontrolled and repeated use of antibiotics to treat bacterial infections has in some cases led to the development of antibiotic-resistant pathogens. For fish disease control, immunostimulants and adjuvants used in fish vaccines are able to act as an alternative to antimicrobial agents like antibiotics used in fish culture. Medicinal plants as immunostimulants can be used not only against diseases but also as growth promoters, stress resistance boosters and preventatives of infections. Plants are rich in a wide variety of secondary metabolites of phytochemical constituents such as tannins, alkaloids, flavonoids, saponin, glycosides, phenolics, polysaccharides, proteoglycans, polysaccharides and volatiles oils which act against different diseases. Studies showed that the incorporation of medicinal plants in the diets of fish stimulated the immune system of fish and enhanced their disease resistance properties. In the present article, the use of some medicinal plants as immunostimulants or phyto-antibacterial agents either injection or dietary administration against fish diseases were discussed.

Keywords: Medicinal plants; Diseases; Fish; Phytochemicals; Immunostimulants.

Introduction

Aquaculture has become a key component of the animal health industry, due to the continued expansion of cultured fish and shellfish species (Kolkovski and Kolkovski 2011). Aquaculture is the fastest growing industry around the world with about 80 million tones being produced annually (Kolkovski and Kolkovski 2011). Aquaculture is therefore an emerging industrial sector which requires continued research with scientific, technical developments, and innovations (Alicia et al. 2005). Bacteria, the major group of pathogens, pose one of the most significant threats to successful fish production throughout the world (Rahman et al. 2009). Bacterial diseases are responsible for heavy mortalities in both culture and wild fishes throughout the world and most of the causative microorganisms are naturally occurring opportunistic pathogens which invade the tissue of a fish host, thereby rendered such susceptible to infection (Rahman et al. 2009). Among all other bacteria, Aeromonad, Pseudomonad and Edwardsiella tarda are the major bacterial fish pathogens which are widely distributed in aquatic organisms in nature (Rahman et al. 2009). The appearance and development of disease is the result of the interaction among pathogen, host and environment (Alicia et al. 2005) hence the understanding of this interaction will go a long way to assist in disease control.

It is well understood that harmful microbes, nutritional disorders and poor water quality or environmental disorders cause diseases which in turn have been major obstacles to aquaculture worldwide (Kumar and Aanatharaja 2007). Stressors including overcrowding, high or sudden changes of temperature, handling, low dissolved oxygen, poor nutritional status and fungal or parasitic damage of the epidermis, contribute to physiological changes and heighten susceptibility to infection (Bastardo et al. 2012). Attempts to control or prevent such devastating outbreaks using conventional antimicrobials and

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other chemotherapeutants have been generally unsuccessful (Jadhav et al. 2006). The uncontrolled and repeated uses of antibiotics to treat bacterial infections have in some cases led to the development of antibiotic-resistant pathogens (Flores et al. 2003; Food and Agriculture Organization 2006). Considering the potential threat of diseases on human and animal health, issues associated with the use of antibiotics, disease management should therefore concentrate on environmental-friendly, preventative methods such as the use of plants as antibacterial agents and immunostimulants.

Using immunostimulants seems to be an attractive alternative to control fish diseases and enhancement of growth (Raa 1996; Secombes 1994). In fish, several chemical immunostimulants such as levamisole (Siwicki et al. 1990), chitin (Sakai et al. 1992; Esteban et al. 2001), lactoferrin (Sakai et al. 1993), Nisin (Villamil et al. 2003), recombinant transferrin (Stafford et al. 2004), modified carbohydrate (Mishra et al. 2006), b-glucan (Das et al. 2009; EL–Boshy et al. 2010), chitosan (Geng et al. 2011), and various kinds of probiotics (Chiu et al. 2010; Harikrishnan et al. 2010; Harikrishnan et al. 2011) have been reported. These substances play a promising role in aquaculture by enhancing the resistance of cultured fish against diseases. The effect of such immunostimulant depends on various factors such as time, dosage, method of administration and the physiological condition of fish. A large number of plants have been used in traditional medicine for the treatment and the control of several diseases (Chakrabarti et al. 2012). Recent studies showed that the incorporation of medicinal plants in the diets of fish stimulated the immune system of fish and enhanced their disease resistance (Chakrabarti et al. 2012). This review attempts to re-emphasise the use of some known medicinal plants against fish diseases and the importance to the development of aquaculture in Africa.

### Medicinal plants in fish feed

Some medicinal plants/herbs had been evaluated experimentally in fish by various researchers, some with the use of specific part of the plant using varying the concentration/inclusion rate. Some of the recent ones were listed in the Table 1.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name</th>
<th>Part of the plant used</th>
<th>Concentration/inclusion rate</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allium cepa</td>
<td>Onion</td>
<td>Bulb</td>
<td>0, 0.5, 1.0, 1.5 and 2.0%</td>
<td>Bello et al. 2012a, Bello et al. 2012b</td>
</tr>
<tr>
<td>Tetracarpidium conophorum</td>
<td>Walnut</td>
<td>Leaves</td>
<td>0, 0.5, 1.0, 1.5 and 2.0%</td>
<td>Bello et al. 2012a, Bello et al. 2012b</td>
</tr>
<tr>
<td>Allium sativum</td>
<td>Garlic</td>
<td>Bulb</td>
<td>0.10, 20, 30, 40/100g/kg</td>
<td>Shalaby et al. 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bulb</td>
<td>0, 0.5 and 1.0/100g/kg</td>
<td>Nya and Austin 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2, 4, 6 and 8mg/ml</td>
<td>Muniruzzanian and Chowdhury 2004</td>
</tr>
<tr>
<td>Tetraselmis chuii</td>
<td>Microalgae</td>
<td>Whole</td>
<td>0, 100g/kg diet</td>
<td>Cerezuela et al. 2012</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>Microalgae</td>
<td>Whole</td>
<td>100g/kg diet Synergy (100g/kg</td>
<td>Cerezuela et al. 2012</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T. C + 10⁷cfu/g B.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>subtillis)</td>
<td></td>
</tr>
<tr>
<td>Euglena viridis</td>
<td>Microalgae</td>
<td>Whole</td>
<td>0, 0.1, 0.5 and 1.0/100g/kg</td>
<td>Das et al. 2009</td>
</tr>
<tr>
<td>Andrographis paniculata</td>
<td>Nees</td>
<td>Leaves and shoots</td>
<td>0, 500, 1000, 2000 and 3000mg/kg</td>
<td>Prasad and Mukthiraj 2011</td>
</tr>
<tr>
<td>Loniceria japonica</td>
<td>Honey suckle</td>
<td>Leaves</td>
<td>1.0%</td>
<td>Yin et al. 2008</td>
</tr>
<tr>
<td>Gynodermia lucidum</td>
<td>lacquered</td>
<td>Leaves</td>
<td>1.0%</td>
<td>Yin et al. 2008</td>
</tr>
<tr>
<td>C. gigentia</td>
<td>akand</td>
<td>Leaf</td>
<td>Synergy (0.5% L. J + 0.5% G.</td>
<td>Muniruzzanian and Chowdhury 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>L)</td>
<td></td>
</tr>
<tr>
<td>C. gigentia + A. Indica</td>
<td>Akand + neem</td>
<td>Leaf</td>
<td>2, 4, 6 and 8mg/ml</td>
<td>Muniruzzanian and Chowdhury 2004</td>
</tr>
<tr>
<td>Curcuma longa</td>
<td>Turmeric</td>
<td>Bulb/rhizomes</td>
<td>2, 4, 6 and 8mg/ml</td>
<td>Muniruzzanian and Chowdhury 2004</td>
</tr>
<tr>
<td>Viscum album</td>
<td>mistletoe</td>
<td>Leaf, trunk and fruits</td>
<td>0, 10, 50 and 200mg/kg</td>
<td>Park and Choi 2012</td>
</tr>
<tr>
<td>Ficus benghalensis</td>
<td>-</td>
<td>Root</td>
<td>5g/kg diet</td>
<td>Verma et al. 2012</td>
</tr>
</tbody>
</table>
Efficacy of medicinal plants acting against fish diseases

Various plants and herbs had found their use in aquaculture and most are found more in the tropics, one of such is Onion bulb which belongs to the family of Alliaceae: cepa group (common onion), proliferum group and aggregatum group. *Allium cepa* is a pungent herb that protects against infection, relaxes spasms, used as expectorants, diuretic and has been reported to reduce blood pressure, clotting and blood sugar levels (Deni 1996). Alongside with walnut (*Tetracarpidium conophorum* or *pluknetia conophora*) which belongs to the family Euphorbiaceae, a bitter, astringent herb that has been used as expectorant, laxative and anti-cancer agent, with ability to dissolves kidney stone (Deni 1996) has been reported to have antimicrobial activities against some pathogenic bacteria of interest in aquaculture (*P. aeruginosa*, *B. subtilis*, *P. fluorescens*, *S. aureus*, *E. coli*, *S. typhi*). The ethanolic extract of walnut leaves had the the widest zone of inhibition with *S. aureus* (13.5mm), followed by *P. aeruginosa* (12mm) and *B. subtilis* (12mm) while the least zone of inhibition was obtained in *S. typhi* (10mm) and *E. coli* did not show zone of inhibition. With onions, *B. subtilis* (12mm) had the widest zone of inhibition, followed by *P. fluorescens* (11mm), *S. aureus* (11mm), while the least was obtained from *E. coli* (9mm) (Bello et al. 2013).

*In vivo* studies also revealed decreased values in the bacterial load of treated groups (onion bulb and walnut leaves) as the levels of inclusion (0.5%, 1.0%, 1.5% and 2.0%) increased and as the months increased (Bello et al. 2012a). In this study, enterobacteriacea load in skin, liver, gills and intestine of *C. gariepinus* fed *A. cepa* and *T. conophorum* were lower than the control with significant decrease (P<0.05) at 4, 8 and 12 weeks post administration. The decrease in bacterial load in fish and water as observed in that study has been associated with the presence of antimicrobial properties in walnut leaf and onion bulb hence their inclusion as a replacement or additive in fish feed could possibly aid productivity in aquaculture. The use of plant products in aquaculture industry has been reported to be safe since they are highly biodegradable and do not have any side effects (Blumenthal et al. 2000) such as drug resistance as observed with synthetic antibiotics. Apart from the antibacterial effect *in vivo*, Bello et al. 2012b also reported the growth enhancing properties of onion bulb and walnut leaf residues – based diets with an increase in the body weight gain in fish fed on onion bulb and walnut leaf supplemented diets.

This property was attributed to the improved digestive activity which enhanced the synthesis of vitamins, cofactors and enzymatic activity. It was also speculated that it could be due to the presence of growth stimulants or constituents in onion bulb (flavonoids and thiosulfimates) and walnut leaves (alkaloids and tannins) as reported by Azu and Onyeagba 2007 and Kumar and Anantharaja 2007. These properties was evaluated by investigating the gut morphometric changes and the plant extracts was found to enhanced the absorptive area by increase in villus height and width so also the cryptal depth. (Bello et al. 2012b). Apart from onion bulb and walnut leaves, other researchers also reported the antibacterial, immunostimulating and growth enhancing properties of some medicinal plants that include:

_Eclipta alba_ (false daisy, bhringaraja) which belong to the family of compositae. It is a bitter/sweet herb that has a tonic effects on the circulatory, nervous and digestive systems. Christybapita et al. 2007 observed the immunostimulatory effect of aqueous extract (AqE) of *Eclipta alba* leaf (oral administration as feed supplement) in tilapia fish, *Oreochromis mossambicus*. It was noted that the *E. alba* extract enhances non-specific immune responses and disease resistance of *O. mossambicus* against _Aeromonas hydrophila_ infection.

_Ginger_ (*Zingiber officinale*) belong to the family of zingiberaceae, it is a sweet pungent, aromatic, warming herb was also found by Yin et al. 2008 when administered orally to increase the phagocytic capability of cells in rainbow trout (fish), while the extracts of 4 Chinese herbs (*Rheum officinale*, *Andrographis paniculata*, *Isatis indigotica* and *Lonicera japonica*) increased the phagocytosis of white blood cells of *Carp, Cyprinus carpio*. The
immunostimulant effects of the dietary intake of 3 plants (Viscum album, Urtica dioica and Zingiber officinale) extracts on rainbow trout (Oncorhynchus mykiss) (Dügenci et al. 2003).

Azadirachta indica (Neem) tree which belong to the family of meliaceae has a bitter, tonic herb that acts as antipyretic and antiinflammatory agent (Deni 1996) The leaves of this plant had been shown to contain nimbin, azadirachtin and meliantriol which possess insecticidal and antiviral properties (Chitmanat et al. 2005). The insecticidal and antibacterial effect has been explored in aquaculture (Biswas et al. 2002, Winkaler et al. 2007, Abdul Kader Mydeen and Hanifia 2011) with very little information on the use of its antiviral properties in fish. Ravikumar et al. 2011 studied that among 15 coastal medicinal plants/ parts of plants, A. indica, Cinnamomum verum and Eupatorium odoratum exhibited excellent antibacterial activity against 10 bacterial pathogens from diseased ornamental fishes. The antimicrobial activity of Aqueous extract of 3 medicinal plants: A. indica (leaf), Solanum torvum (Sundakai fruit coat) and Curcuma longa (rhizome) against the in vitro growth of A. hydrophila, isolated from infected fresh-water fish, Channa striatus (Abdul Kader Mydeen and Hanifia 2011).

Another plant that has been extensively studied include Garlic (Allium sativum) which belong to the family of liliaceae. A pungent, warming herb has been reported to inhibits bacterial growth, lowers fever, reduced blood pressure, cholesterol and blood sugar levels. It has been used as rejuvenatives, detoxicant and aphrodisiac in ayurvedic medicine (Deni1996). Studies abound on its medicinal and culinary purposes (Rahman et al. 2009). The bulbs which contained an acrid volatile oil (0.25%), propyl disulphide which is a powerful germicide (Anawer 2001). Garlic or onion has been mixed to the shrimp pellet and fed every day to protect the bacterial infection (Direkbusarakom 1992).

Some investigations revealed the growth promoting, antibacterial effect of garlic and enhancement of blood parameters, erythrocytes counts (RBC) and haemoglobin (Hb) content in fish fed on diets containing A. Sativum were higher than the control while the total plasma protein content was significantly higher in fish fed on diets containing A. Sativum (Shalaby et al. 2006). The lever enzymes: Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) decreased significantly with increasing levels of A. sativum. The combination containing A. sativum, A. indica and Curcuma longa (turmeric) in spawn had been shown to resist disease in Catla catla fish While the combination of Indian almond (Terminalia catappa) and garlic (Allium sativum) have been useful as an ectoparasiticial alternative in fish especially Trichodina sp. infections in tilapia (O. niloticus) fingerlings.

On the effect of medicinal plants on the oxidative stress parameters, various medicinal plants had been reported to increase the levels of superoxide anion production, lysozome, serum bactericidal activity, serum protein and albumin (P< 0.05) when challenged with Aeromonas hydrophila (Das et al. 2009). Herbal mixture containing (A. koreanum, G. Liralensis and P. Ginseng) was found also found to enhance the total protein, glucose, phagocytic, respiratory burst actitivities, complement and lysozome activities significantly in infected fish fed with supplementation diet from week 4 to 12. This suggested that the herbal mixture supplementation diet enhanced growth, blood chemical constituents, and non-specific immunity in Olive flounder against Streptococcus parauberis (Harikrishnan et al. 2011). However the possible role in disease resistance has not been accorded due attention in fish.

Another very important plant of interest is Aloe vera which belong to the family of Liliaceae/ Aloeaceae. It is an intensely bitter, pungative herb that have shown prospect as an antifungal, anti-inflammatory, healing promoting agent. It also has anthelmintic property. The growth promoting effect and disease resistant property was proven in gold fish Carassius auratus when experimentally challenged by A. hydrophila (Ahilan et al. 2010). Harikrishnan et al. 2010 reported that mixed herbal extracts supplementation diets restored the altered haematological parameters and triggered the innate immune system of goldfish (C. auratus) against A. hydrophila infection. This synergistic effect of herbs has been reported in other fishes, including Japanese flounder and Clarias.
gariepinus (Ahilan et al. 2010). A. vera has also been used found to a disease suppressing agent and showed antibacterial effect in juvenile rock fish. The growth increase in Labeo rohita fish fed with herbal supplemented diet was due to improved food utilization and high protein synthesis. The benefit of herbal growth promoters as an additive in the carp feed has also been the focus of many researchers.

Withania somnifera (winter cherry) is bitter – sweet, astringent, warming herb with a horse-like smell, it acts mainly on the reproductive and nervous systems. It has sedative, rejuvenative and aprodisiac effects. Sharma et al. 2010 observed the stimulatory effect of dietary doses of Withania somnifera (Ashwagandha) root on immunity and disease resistance against A. hydrophila infection in Indian major carp, L. rohita fingerlings. Kolkovski and Kolkovski 2011 also reported that some herbal extracts are very effective against gills and skin flukes like Benedenia seriolae. Nargis et al. 2011 seen the immunostimulant effects of the dietary intake of A. sativum and Vitex negundo extracts on fingerlings of L. rohita fish.

Viscum album (mistletoe) belong to the family of viscaceae is a pungent, bitter- sweet warming herb that lowers blood pressure, stimulate the immune system, slows heart beat, relaxes sperms and has sedative, diuretic anti- cancer effects (Deni1996). The study of Park and Choi 2012 shown that the dietary supplemented mistletoe extract appeared to significantly elicit non-specific immune responses in terms of the respiratory burst activity, lysozyme activity, phagocytic activity, and ACH50 activity in tilapia. 50 mg mistletoe-treated group showed the highest survival rate compared to control and the challenged test suggests decreased mortality rate following experimental infection with A. Hydrophila. The dietary supplementation of an appropriate concentration of mistletoe extract has an enhancing effects on disease resistance of tilapia. Medicinal plants: ginger, nettle and mistletoe acts as an adjuvant therapy in rainbow trout through enhanced phagocytosis, cellular and humoral defense mechanisms against pathogens. The traditional Chinese medicines in yellow croaker elevated the non specific defense mechanism and increased the disease resistance of fish against bacterial pathogens.

Apart from challenge studies being used as a means to evaluates the usefulness of medicinal plants in aquaculture, haematology had also been widely used in clinical and pathological conditions in aquatic and terrestrial animals. Bello 2013 reported decreased in MCV, MCH and MCHC in fish treated with walnut leaf and onion bulb residues, post challenge while the values of lymphocytes recorded in post challenge periods were higher than the one obtained in pre- challenge and the control fish post challenge. It was also reported that increases in values of total protein, albumin, globulin level and albumin and globulin ratio of treated groups of post challenge test compared to pre – challenge and the control of post challenge are thought to be associated with a stronger innate immune response of fish. This suggests that walnut leaf and onion bulb residues could enhance non – specific immune response. This speculation need to be further elucidated using some modern techniques.

Achyranthes aspera was studied by Rao et al. 2006 who reported that Achyranthes aspera in the diet increased the non-specific immunity and significantly decreased mortality when L. rohita were experimentally infected with Aeromonas hydrophila, a bacterial pathogen hence Achyranthes can be used as a prophylactic to reduce mortalities associated with disease. Besides stimulating immunity, Achyranthes also enhanced the growth rate of the fish as well as increased serum protein, lysozyme, total serum protein and A/G ratio which are good indicators of health status. Results indicate that Achyranthes improves phagocytosis and killing activity by neutrophils and macrophages. It also enhances superoxide anion when fish fed with Achyranthes incorporated diets.

Some other plants that look promising in aquaculture includes Lonicera japonica which belongs to the family of caprifoliaceae. It has been reported to have anti- bacterial, diuretic, antipyretic and anti inflammatory properties. It has been shown to also relaxes spasms (Lee et al. 1998). It has been reported that Lonicera significantly increased blood neutrophil activity and promoted phagocytosis by the neutrophils in
bovine at the correct concentration (Hunn et al. 1992). Also *Ganoderma lucidum* which anti-allergenic, anti-viral and anti-bacterial effects (Deni 1996) and has been found to promote phagocytosis and stimulate proliferation of lymphocytes (Wang et al. 1997).

The combination of the extracts of these two Chinese herbs (*Lonicera japonica* and *Ganoderma lucidum*) in diets of tilapia fish, (*Oreochromis niloticus*) act as immunostimulants and appear to improve the immune status and disease resistance. when used alone or in combination increased the survival of fish after challenge with *Aeromonas hydrophila* (Yin et al. 2008). The results of the experiment conducted showed that both *Ganoderma* and *Lonicera* were able to enhanced phagocytosis and stimulates lysozyme activity after two weeks, but not respiratory burst activity of phagocytic blood cells, total protein or total immunoglobulin in plasma.

*Datural metel* (Thorn apple) belong to the family of solanaceae. It was studied by Ravikumar et al. 2010 who observed that the chloroform extract of *Datural metel* plant has wide range of antimicrobial activity against many fish pathogens. *D. metel* which collected from the Kanyakumari coast can be used as a putative antimicrobial drug in the aquaculture maintenance. The chloroform extract of *D. metel* can be effectively used as a potential antimicrobial agent to overcome the problem of mass mortality of ornamental fish in aquarium. Turker et al. 2009 also reported that the alcoholic and aqueous extracts of *Nuphar lutea*, *Nymphea alba*, *Stachys annua*, *Genista lydia*, *Vinca minor*, *Fragaria vesca*, *Filipendula ulmaria* and *Helichrysum plicatum* herbs of Bold (Turkey) have antibacterial activity against *A. hydrophila*, *Yersinia ruckeri*, *Lactococcus garvieae*, Str. agalactae and *Enterococcus faecalis* bacteria isolated from fish. This observation provides the aquaculturists with a promising management tool with the use of medicinal plants for control or treatment of fish diseases especially in resource poor settings.

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