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Potency of Herbal Extracts for the Treatment of Streptococcus Infection in Tilapia.

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Abstract: *The most common strategy to fight aquaculture disease is the use of antibiotics regardless of pathogens involved. However, such usage has been reported to have adverse effects. Natural herbs have gained prominence in research as a means of treatment and control of Streptococcus infections in tilapia. Immunostimulants from plants have become increasingly important as research focuses on environmental sustainability. This paper reviews the use of immunostimulantsand treatments for S. iniae (Thymus vulgaris, Rosmarinusofficinalis, Trigoneliafoenum-graecum, Cuminumcyminum, Pimentadioica), S. agalactiae (Andrographispaniculata, Helichrysumplicatum, Murrayakoenigii), and Streptococcus sp (Syzygiumaromaticum, Vinca minor, Nupharlutea, Cantellaasiatica, Citrus microcarpa, Morindacitrifolia) infections in tilapia. Detailed antimicrobial test for the use of herbs against Streptococcus has been reported for only two herbs: S. aromaticum and A. paniculata with the latter producing the highest efficacy (95%) in O. niloticus as well as overall efficacy. This paper has reviewed various herbs as effective treatment or preventive agent against Streptococcus infection in tilapia. steam distilled Syzium aromatic as reported has the best MIC value (30.0 µg/ml) followed closely by aqueous extract of Andrographispaniculata with 31.25 µg/ml. Considering inhibition zones, Rosmarinusofficinalis extracted with ethyl acetate gave the best inhibition zone of 37.5 mm followed by the aqueous extract of Andrographispaniculata (27.5 mm).*

Keywords: *Immunostimulants, Treatment, Herbs, Streptococcus, Tilapia*

1. INTRODUCTION

World per capita fish consumption has increased by 94% between the 1960's and 2012 [1]. Globalization has helped a great deal in spreading supply of fish between the continents and ensuring that consumption increased with increasing awareness on the benefits of fish as opposed to red meat. Aquaculture production in the world has grown by about 33% from the production level of 4.99 x 10^7tonnes in 2007 to 6.66 x 10^7 tonnes in 2012 with acontribution of 42% to total fish production in 2012 [1]. Asia with a contribution of 88% leads in world aquaculture production with a heavy production figure from China and Africa contributes 2% [1].

Intensification and increased density of aquaculture organisms is the bedrock of increased production. This is accompanied by risk of bacterial and other microbial attacks leading to disease outbreaks. Bacterial activity in pond systems remain inevitable but a balance of symbiotic bacteria and cultured organisms is desirable. Micro-organisms present in the ponds follow pond water quality dynamics with increasing nitrifying bacteria in elevated ammonia conditions [2].

[3]opined that the outbreak of *S. agalaciae* in Hybrid Tilapia in cages in Malaysia is traceable to increase in cage numbers in excess of carrying capacity hence greater cage density and susceptibility to the bacteria. *Streptococcus agalactiae* has been reported as extremely pathogenic with mortalities reaching 100% [4].

The use of immunostimulants has increased due to increased incidence of pathogenicity in fish farms, hatcheries and aquaculture facilities [5]. The control of these pathogens via synthetic chemicals and antibiotics has become rampant. There is fear of development of resistance to antibiotics by bacterial pathogens. Ten phenotypes of antibiotic resistant bacteria have been isolated from fish with many exhibiting multi drug resistance [6]. Isolates of *E. coli* from gills and the body surface of tilapia have been found to be resistant to ampicillin, tetracycline, and sulfametoxazol-trimetoprim with greater multi-drug resistance being observed for strains on the skin than gills [7]. Furthermore, a bottleneck that tends to increase drug resistance lies in the fact that only a few chemoterapeutants are approved for use on food fish due to concerns of bioaccumulation in both the food fish and the environment [5].

2. SIGNIFICANCE OF STREPTOCOCCUS INFECTIONS IN FISH

Streptococcus infections of fish can be lethal although not common with a possibility of zonosis by certain species of streptococci [8, 9,&10]. *Streptococcus iniae*has been reported to cause infection in elderly people who handle fresh fish in Asia with predisposing factors such as those mentioned by [11] that include environment, season and immunity being responsible for infection which usually occurs via cuts during fish processing [12]. A clone of *S. iniae* has been identified to cause disease in fish and man as well [8, 12]. *Oreochromisniloticus*, an exotic species that is common in Thailand and Indonesia has been affected by infections of both *S. agalatiae*and *S. iniae* between 2001 and 2003 [13] and stocking density has been identified as the significant factor contributing to Tilapia mortality where infections with *S. iniae* occur [14]. Tilapia that are affected by Strepcoccus are unsightly and unmarketable due to spine displacement, distended abdomen, ocularhaemorrhage and 'pop-eye'. In terms of geographical spread, three world regions have been identified as those affected by streptococcus infection of tilapia: North America, the middle East and Asia-Pacific with at least 27 species of fish being documented to be affected by the pathogen [9].

3. TREATMENT AND CONTROL WITH ANTIBIOTICS

Control of Streptococcus has been carried out using antibiotics with vaccination also been reported to reduce infection rates [12]. Florfenicol, an antibiotic which is analogous to thiamfenicol was used in the sunshine bass at a dose of 10mg.kg^{-1}fish.day^{-1} to control *S. iniae* with 43% difference in mortality between challenged-treated and challenged-untreated fish [15]. [16]also reported the use of Florfenicol at a dose in the range of 0.5 to 1.0µg/ml in Tilapia to control *S. iniae* with emphasis on the safety of the antibiotic. Interestingly, the efficacy of florfenicol increased with increase in dose and in both cases, treatment was administered via feed. This implies that the complete elimination of *S. iniae* in fish can only be done with very high dose of antibiotics. [17], reported the use of 25, 50, 75 and 100 mg oxytetracycline per kg of fish per day with 90% and 100% elimination of the pathogen in the 75 and 100 mg.kg^{-1}fish.day^{-1} doses respectively. However, amoxicillin was found to eradicate the bacteria in blue Tilapia at 10, 30 and 80mg/kg of fish with pathogen free survivors emanating from the challenged fish [18]. Dietary manipulation in the control of microbes in the gut leading to immune response has gained prominence in fish research as derived from mammalian studies [19].

4. ENVIRONMENTAL CONCERNS

[20]maintained that streptococcus can be treated with antibiotics with high efficacy but legal issues plague their use since withdrawal period tends to be longer than time taken for re-infection to occur hence marketability is hampered. [21]pointed out that antibiotics should be used after exhaustive analysis has revealed the presence of bacteria as a disease agent. This is to forestall the risk of increased resistance by bacterial strains. Furthermore, [22] observed that information regarding the effect of residual antibiotics on humans is incomplete. [23]maintained that bacteria belonging to the same genera that have pathogenic effect on both fish and humans are particularly of importance considering the development of antibiotic resistance. Similarly, [24], pointed out that the use of non biodegradable antibiotics in the aquaculture sector creates a reservoir of these drugs that keep acting over a long period and therefore leads to development of antibiotic resistance by pathogenic bacteria.

Environmental friendliness and sustainability are key factors that will drive viability and profitability of aquaculture in the future and even now [25]. Ethical concerns about discharge of waste water and its polluting effects on wetlands and water bodies, indiscriminate use of antibiotics and chemicals in aquaculture and discharge of water with these chemicals into the environment are pertinent issues. Metabolites from fish ponds are often discharged in the waste waters and increased organic loading can cause depletion of oxygen in receiving waters [26]. The question arises: Is aquaculture the key to increasing fish production in view of declining wild fish stocks or it is the lock that will permanently shut out aquatic biodiversity?

Natural herbs have gained prominence in research as a means of treatment and control of *Streptococcus* infections in fish. This is an environmentally friendly approach although phyto-toxicity must be considered. The use of herbal remedies as alternatives to synthetic antimicrobial agents is a move towards user friendliness and avoidance of side effects [27].Immunostimulants from plants have become increasingly important as research focuses on environmental sustainability. Extensive review of plant derived immunostimulants against *Aeromonashydrophila*and Trichodina parasitic infections was given by [28]. Due to the scanty nature of information on the use of herbs in the prevention, treatment and control of *Streptococcus* infections in fish, a synthesis of available literature is given in Table 1 below.

5. Phyto-chemicals and potency

Medicinal herbs contain phytochemicals that are a source of antimicrobial activity and are also detrimental to feeding and utilization of feed. Toxicity of several phytochemicals is a function of the structure of the organism's digestive tract as well as physiology [43, 44].

Tannin, saponin, phytate, oxalate, flavonids and limonene can affect feed digestion or metabolism by acting as standalone biochemicals or in combination with other body chemicals as well as other inherent phytochemicals in the herb [43, 45, 46]. Tanins alter protein utilization and digestion while phytatic acid and oxalate affect the uptake and utilization of minerals by animals [47]. Saponin has a bitter taste that deters feeding hence palatability is a factor to consider for herbs that do contain saponin [48].

However, potency of herbs as antimicrobial agents depends, to a large extent on these phytochemicals. The presence of terpenes and steroids in garlic and almond confers on these plants the ability to act as agents against bacterial and viral attacks [49]. Phytochemicals also act as antioxidants that promote health hence, immunity against bacteria and other pathogens. This exposition therefore has provided various herbs that have been researched as treatment or preventive agent against *Streptococcus* infection in fish. Temperature of storage for medicinal herb extracts has been discovered to affect the potency with time.

[32], reported 4 and 8 fold increase in MIC_{24h} for *Rosmarinusofficinalis*stored at 50°C for 3 and 4½ months respectively while cold storage (-20°C, 4°C) as well as normal temperature storage (25°C) followed by autoclaving only doubled the MIC_{24h}.

Potency of *R. officinalis* in the form of raw leaf and ethyl acetate extracts included in feed has been found to reduce mortality of tilapia challenged with *S. iniae* with mortality of oxytetracycline treated fish falling in between these two treatments [33]. Furthermore, whole cumin seed meal in the diet of tilapia at rates between 0.5% to 2.0% was discovered to have no effect on the fish growth with the inclusion rate of 1.0% producing the least mortality in fish challenged with *S. iniae* [35]. An inclusion level of 1.0% of three herbs: *Thymus vulgaris*, *Rosmarinusofficinalis* and *Trigonellafoenumgraecum* have been found to improve phagocytic activity, haematocrit, red blood cells, white blood cells, neutrophil and monocyte counts in tilapia (*O. mossambicus*) with *T. foenumgraecum* creating elevated levels of plasma myeloperoxidase and lysozyme activity [30].

Table 1: Various Herbal extracts or whole herbs that have been reported for control and treatment of *Streptococcus* in fish.

Plant	Part used	Dose	Mode administered	Inhibition Zone	MIC	Extraction Method	Strep spp.	Fish spp.	Effect	Efficacy	Reference
Thymus vulgaris	Leaf	1.0%	Feed	-	-	-	S. iniae	O. mossambicusFry	Immunostimulant	86%	[29]
	Leaf	1.0%	Feed	-	-	-	S. iniae	O. mossambicus	Immunostimulant	78%	[30]
	Leaf	-	-	14.0mm	0.6 mg/ml	Aqueous	S. agalactiae	Oreochromissp. (Red Hybrid)	Antimicrobial test	-	[31]
Trigoneliafoenumgraecum	Leaf	1.0%	Feed	-	-	-	S. iniae	O. mossambicusFry	Immunostimulant	86%	[29]
	Leaf	1.0%	Feed	-	-	-	S. iniae	O. mossambicus	Immunostimulant	69%	[30]
Rosmarinusofficinalis	Leaf	8.0%	Feed	17.0 mm	-	Ethyl acetate	S. agalactiae	Oreochromissp.	Treatment	56%	[32]
	Leaf	1.0%	Feed	-	-	-	S. iniae	O. mossambicusFry	Immunostimulant	83%	[29]
	Leaf	1.0%	Feed	-	-	-	S. iniae	O. mossambicus	Immunostimulant	73%	[30]
	Leaf	5.6%	Feed	-	-	-	S. iniae	Oreochromissp.	Treatment	72%	[33]
	Leaf	4.0%	Feed	37.5 mm	-	Ethyl acetate	S. iniae	Oreochromissp.	Treatment	76%	
Cuminumcyminum	Seed	1.14%	Feed	-	-	Whole seed meal	S. iniae	O. massambicus (1st Feeding)	Immunostimulant Treatment	84%	[34]

	Seed	1.0%	Feed	-	-	Whole seed meal	S. iniae	O. mossambicus(Fingerling)	Immunostimulant	90%	[35]
Syzygiuma romaticum	Leaf	2.0%	Feed	20.20mm	30µg/ml	Steam distillation	Lactococcu sgarvieae	O. niloticus	Immunostimulant	90%	[36]
Pimentadio ica	Seed	10g/kg	Feed	-	-	-	S. iniae	O. mossambicusFry	Treatment	80%	[37]
Andrograp hispanicula ta	Leaf	8%	Feed	27.5mm	31.25µg/ml	Aqueous Extract	S. agalactiae	O. niloticus	Prophylaxis	95%	[38]
Helichrysu mpicatum	Leaf & Stem	-	-	13.88mm	-	Ethanol	S. agalactiae	-	Antimicrobial Tests	-	[39]
Vinca minor	Leaf	-	-	12.0mm	-	Ethanol	L. garvieae	-		-	
Nupharlute a	Leaf	-	-	10.75mm	-	Water	Enterococc us faecalis	-		-	
Murraya, koenigii	Leaf	-	-	9.30mm	0.39mg/ml	Methanol	S. agalactiae	-		-	[40]
Syzygiuma romaticum	Leaf	-	-	8.0mm	0.78mg/l	Methanol	S. aginosus	-		-	
Cantellaasi atica	Whole Plant	-	-	7.00mm	-	Methanol	Generic	Isolates from marine fish		-	[41]
Citrus microcarpa	Leaf	-	-	7.0mm	-	Methanol	Generic			-	
Morindacit rifolia	Fruit	-	-	7.0mm	-	Methanol & Aqueous	Generic			-	
Eugenia caryophyll us	Flower buds	-	-	16.0 mm	0.3mg/ml	Aqueous	S. agalactiae	-	Antimicrobial Test	-	[31]
Cinamomu mverum	Bark	10%	Feed	18.0mm	0.15mg/ml	Aqueous	S. agalactiae	Oreochromiss p. (Red hybrid)	immunostimulant	77%	[31]
Allium sativum	Bulb	-	-	10.0 mm	2.50 mg/ml	Aqueous	S. agalactiae	-	Antimicrobial Test	-	[31]
Moringaol eifera	Leaf	1.5%	Feed	13.1mm	0.6 mg/ml	Aqueous	S. agalactiae(Biotype 2)	Oreochromisni loticus	Immunostimulant	100	[42]

Inclusion of *C. verum*at a rate of 10% in diets improved survival of *Oreochromis* sp. exposed to *S. agalactiae* without affecting growth performance [31]. A disc diffusion assay of solvent extracts of *R. officinalis*against *S. iniae* via in-vitro tests on tilapia revealed efficacy of all extracts in inhibiting bacterial growth [33].

5. CONCLUSION

Pathogens are a common occurrence on fish farms and fish farmers and the bio-safety of products from aquaculture is of highest priority for consumers of aquatic foods derived from aquaculture. Phyto-chemicals are biodegradable, subtle on the environment and can be acquired at a relatively low cost.Focus on the use of plant extracts for the treatment and possible prevention of streptococcal infection in fish abound.

From the foregoing, steam distilled*Syzium aromatic* as reported has the best MIC value (30.0 µg/ml) followed closely by aqueous extract of *Andrographispaniculata* with 31.25 µg/ml. Considering inhibition zones, *Rosmarinusofficinalis*extracted with ethyl acetate gave the best inhibition zone of 37.5 mm followed by the aqueous extract of *Andrographispaniculata* (27.5 mm).

REFERENCES

[1] FAO (2014). *The State of World Fisheries and Aquaculture: Opportunities and Challenges*. Food and Agriculture Organization of the United Nations. Rome. 223pp

[2] Boyd CE, Gross A (1998) Use of probiotics for improving soil and water quality in aquaculture ponds. In Flegel TW (ed) *Advances in shrimp biotechnology*. National Center for Genetic Engineering and Biotechnology, Bangkok

[3] Najiah, M, Aqilah, N.I, Lee, K.L, Khairulbariyyah, Z, Mithun, S, Jalal, K.C.A, Shaharom-Harrison, F. and Nadirah, M. (2012). Massive Mortality Associated with Streptococcus agalactiae Infection in Cage-cultured Red Hybrid Tilapia Oreochromisniloticus in Como River, Kenyir Lake, Malaysia. Journal of Biological Sciences, 12: 438-442.

[4] Ferguson, H.W, Morales, J.A. and Ostland, V.E. (1994). Streptococcus in aquarium fish. *Diseases of Aquatic Animals* 19: 1-6.

[5] Maqsood, S, Singh, P, Samoon, M.H. and Munir, K. (2011). Emerging role of immunostimulants in combating the disease outbreak in aquaculture. *IntAquat Res* (2011) 3: 147-163

[6] Karki, H, Mustafa, A, LaMaster, A. and Dhawale, S. (2013). Antibiotic Resistant Bacteria in the Gut of Hatchery-reared Tilapia and Coho Salmon. *Universal Journal of Miucrobiology Researc*h 1(3): 43-46.

[7] Rocha, R, Leite, L.O, de Sousa, O.V. and Vieira, R.H.S. (2014). Antimicrobial Susceptibility of Escherichia coli Isolated from Fresh-Marketed Nile Tilapia (*Oreochromisniloticus*). Journal of Pathogens Volume 2014 (2014), Article ID 756539, 5 pages http://dx.doi.org/10.1155/2014/756539

[8] Weinstein, M.R, Litt, M, Kertesz, D.A, Wyper, P, Rose, D, Coulter, M, McGeer, A, Facklam, R, Ostach, C, Willey, B.M, Borczyk, A. and Low, D.E. (1997). Invasive infections due to a fish pathogen, Streptococcus iniae. S. iniae Study Group. The New England Journal of Medicine 337(9): 589-594.

[9] Agnew, W. and Barnes, A.C. (2007). *Streptococcus iniae*: An aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination. *Veterinary Microbiology* 122: 1–15.

[10] Yanong, R.P.E. and Francis-Floyd, R. (2009). Streptococcal Infections of Fish. Circular 57, The Fisheries and Aquatic Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Available at: http://edis.ifas.ufl.edu/fa057

[11] Novotny, L, Dvorska, L, Lorencova, A, Beran, V. and Pavlik, I. (2004). Fish: a potential source of bacterial pathogens for human beings. VeterinarniMedicina 49(9): 343-358.

[12] Baiano, J.C.F. and Barnes, A.C. (2009). Towards Control of *Streptococcus iniae*. *Emerging Infectious Diseases* 15(12): 1891-1896.

[13] Yuasa, K, Kamaishi, T, Hatai, K, Bahnnan, M. and Borisuthpeth, P. (2008). Two cases of streptococcal infections of cultured tilapia in Asia, pp. 259-268. In: Bondad-Reantaso, M.G, Mohan, C.V, Crumlish, M. and Subasinghe, R.P. (Eds.). Diseases in Asian Aquaculture VI. Fish Health Section, Asian Fisheries Society, Manila, Philippines. 505 pp.

[14] Shoemaker, C.A, Evans, J.J. and Klesius, P.H. (2000). Density and dose: factors affecting mortality of Streptococcus iniae infected tilapia (*Oreochromisniloticus*). Aquaculture 188: 229 - 235.

[15] Bowker, J.D, Ostland, V.E, Carty, D. and Bowman, M.P. (2010). Effectiveness of aquaflor (50% florfenicol) to control mortality associated with *Streptococcus iniae* in freshwater-reared sub-adult sunshine bass. *Journal of Aquatic Animal Health* 22(4): 254-265.

[16] Gaunt, P.S, Endris, R, McGinnis, A, Baumgartner, W, Camus, A, Steadman, J, Sweeney, D. and Sun, F. (2010). Determination of florfenicol dose rate in feed for control of mortality in Nile tilapia infected with *Streptococcus iniae*. *Journal of Aquatic Animal Health* 22(3): 158-166.

[17] Darwish, A.M, Rawles, S.D, and Griffin, B.R. (2002). Laboratory Efficacy of Oxytetracycline for the Control of *Streptococcus iniae* Infection in Blue Tilapia. *Journal of Aquatic Animal Health* 14(3): 184-190.

[18] Darwish, A.M. and Hobbs, M.S. (2005). Laboratory Efficacy of Amoxicillin for the Control of *Streptococcus iniae* Infection in Blue Tilapia. *Journal of Aquatic Animal Health* 17(2): 197-202

[19] Ringø, E, Olsen, R, Jensen, I, Romero, J. and Lauzon, H. (2014). Application of vaccines and dietary supplements in aquaculture: possibilities and challenges. Reviews in Fish Biology & Fisheries 24(4): 1005–1032.

[20] Amal, M.N.A. and Zamri-Saad, M. (2011). Streptococcosis in Tilapia (*Oreochromisniloticus*): A Review. *Pertanika Journal of Tropical Agricultural Science* 34(2): 195–206.

[21] Yanong, R.P.E. (2013). Use of Antibiotics in Ornamental Fish Aquaculture. Circular 84, School of Forest Resiources and Conservation Program in Fisheries and Aquatic Sciences, UF/IFAS Extension. Available at: http://edis.ifas.ufl.edu/fa084

[22] Smith, P, Hiney, M.P. and Samuelesen, O.B. (1994). Bacterial resistance to antimicrobial agents used in fish farming: a critical evaluation of method and meaning. Annual review of fish diseases, 4: 273-313.

[23] Heuer, O.E, Kruse, H, Grave, K, Collignon, P, Karunasagar, I. and Angulo, F.J. (2009). Human Health Consequences of Use of Antimicrobial Agents in Aquaculture. *Clinical Infectious Diseases* 49(8): 1248-1253

[24] Cabello F.C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental microbiology* 8(7): 1137-1144

[25] Gegner, L. and Rinehart, L. (2009). Aquaculture Enterprises: Considerations and Strategies. A Publication of ATTRA-National Sustainable Agriculture Information Service. [Online]. Accessed 09-09-2013. 12 pp. https://knightowl.ncat.org/~basecamp/120472/attrapdfpubs/aquaculture_032910.pdf

[26] Cruz, P.M, Ibáñez, A.L, Hermosillo, O.A.M. and Saad, H.C.R. (2012). Use of Probiotics in Aquaculture. International Scholarly Research Notices *Microbiol*. 2012: 916845.

[27] Kamble, M.T, Chavan, B.R, Ataguba, G, Azpeitia, T, Medhe, S.V, Jain, S. and Jadhav, R.R. (2014). Application of *Moringaoleifera* For Development of Sustainable and Biosecure Aquaculture. *Proceeding of International Conference of Aquaculture Indonesia (ICAI) 2014. 86–95.*

[28] Murthy, K.S. and Kiran, B.R. (2013). Review on usage of medicinal plants in fish diseases. *International Journal of Pharma and Bio sciences* 4(3): (B) 975-986

[29] Yılmaz, S, Ergün, S. and Soytaş, N. (2013a). Herbal Supplements are Useful for Preventing Streptococcal Disease During First-Feeding of Tilapia Fry, *Oreochromismossambicus*. The Israeli Journal of Aquaculture-Bamidgeh, 65.2013.833, 5 pages

[30] Gültepe, N, Bilen, S, Yılmaz, ,Güroy, D. and Aydın, S. (2014). Effects of herbs and spice on health status of tilapia (*Oreochromismossambicus*) challenged with *Streptococcus iniae*. *ActaVeterinaria Brno* 83(2): 125-131.

[31] Alsaid, M, Daud, H, Bejo, S.K. and Abuseliana, A. (2010). Antimicrobial Activities of Some Culinary Spice Extracts against *Streptococcus agalactiae* and Its Prophylactic Uses to Prevent Streptococcal Infection in Red Hybrid Tilapia (*Oreochromis* sp.). *World Journal of Fish and Marine Sciences* 2(6): 532-538

[32] Zilberg, D, Tal, A, Froyman, N, Abutbul, S, Dudai, N. and Golan-Goldhirsh, A. (2010). Dried leaves of *Rosmarinusofficinalis* as a treatment for streptococosis in tilapia. Journal of Fish Diseases 33: 361–369.

[33] Abutbul, S, Golan-Goldhirsh, A, Barazani, O. and Zilberg, D. (2004). Use of *Rosmarinusofficinalis* as a treatment against *Streptococcus iniae* in tilapia (*Oreochromis*sp.). *Aquaculture* 238: 97–105.

[34] Yılmaz, S, Ergün, S. and Soytaş, N. (2013b). Dietary supplementation of cumin (*Cuminumcyminum*) preventing streptococcal disease during first-feeding of Mozambique tilapia (*Oreochromismossambicus*). Journal of BioScience and Biotechnology 2(2): 117-124.

[35] Yılmaz,S, Ergün, S. and Türk, N. (2012). Effects of Cumin-Supplemented Diets on Growth and Disease (*Streptococcus iniae*) Resistance of Tilapia (*Oreochromismossambicus*). The Israeli Journal of Aquaculture-Bamidgeh, IJA_64.2012.768, 5 pages

[36] Rattanachaikunsopon, P. and Phumkhachorn, P. (2009a). Protective effect of clove oil–supplemented fish diets on experimental *Lactococcusgarvieae* infection in tilapia. Bioscience, Biotechnology and Biochemistry 73, 90294- 1–5.

[37] Yılmaz, S. and Ergün, S. (2014). Dietary Supplementation with Allspice *Pimentadioica*Reduces the Occurrence of Streptococcal Disease during First Feeding of Mozambique Tilapia Fry.Journal of Aquatic Animal Health, 26(3): 144-148

[38] Rattanachaikunsopon, P. and Phumkhachorn, P. (2009b).Prophylactic effect of *Andrographispaniculata* extracts against *Streptococcus agalactiae* infection in Nile tilapia (*Oreochromisniloticus*). Journal of Bioscience and Bioengineering 107(5): 579–582.

[39] Turker, H, Yildirim, A.B. and Karakas, F.P. (2009). Sensitivity of Bacteria Isolated from Fish to some Medicinal plants. Turkish Journal of Fisheries and Aquatic Sciences 9: 181-186.

[40] Najiah, M. Nadirah, M. Arief, Z. Zahrol, S. Tee, L.W. Ranzi, A.D. Amar, A.S.Laith, A.R. Mariam, M. Suzana, S. and Aida, R.J. (2011). Antibacterial Activity of Malaysian Edible Herbs Extracts on Fish Pathogenic Bacteria. Research Journal of Medicinal Plant, 5: 772-778.

[41] Wei, L.S, Musa, N, Sengm, C.T, Wee, W. and Shazili, N.A.M. (2008). Antimicrobial properties of tropical plants against 12 pathogenic bacteria isolated from aquatic organisms. African Journal of Biotechnology 7(13): 2275-2278.

[42] Kamble, M.T. (2012). *Immunomodulatory effect of herbal extract on tilapia (Oreochromisniloticus) infected by Streptococcus agalactiae Biotype 2.* Asian Institute of Technology, Thailand. M.Sc. Thesis 2012.

[43] Kumar, R. (1992). Anti-nutritional factors. The potential risks of toxicity and methods to alleviate them. In: Speedy, A. and Pugliese, P.(Eds). Legume trees and other fodder trees as protein sources for livestock. *Proceedings of the FAO Expert Consultation held at the Malaysian Agricultural Research and Development Institute (MARDI)* in Kuala Lumpur, Malaysia, 14–18 October 1991. FAO Animal Production and Health Paper 102. pp 145-160.

[44] Kumar, M.V. (2009). *Veterinary Toxicology*. New India Publishing Agency, New Delhi. 520pp.

[45] Makkar, H.P.S. (1993). Antinutritional factors in foods for livestock. In: Gill, M, Owen, E, Pollot, G.E,Lawrence, T.L.J. (Eds). *Animal Production in Developing Countries. Occasional publication No. 16.* British Society of Animal Production, pp. 69–85.

[46] Aganga, A.A. and Tshwenyane, S.O. (2003). Feeding Values and Anti-Nutritive Factors of Forage Tree Legumes. *Pakistan Journal of Nutrition* 2(3): 170-177.

[47] Francis, G, Makkar, H.P.S. and Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture* 199: 197–227.

[48] Oluremi, O.I.A, Ngi, J. and Andrew, I .A. (2007): Phytonutrients in citrus fruit peel meal and nutritional implication for livestock production. *Livestock Research for Rural Development. Volume 19, Article #89.* Retrieved August 23, 2014, from http://www.lrrd.org/lrrd19/7/olur19089.htm

[49] Takeoka, G, Dao, L, Teranishi, R, Wong, R, Flessa, S, Harden, L. and Edwards, R. (2000). Identification of three triterpenoids in almond hulls. Journal of Agriculture and Food Chemistry 48: 3437-3439.