Engineering 3 (2017) 731–737

Contents lists available at ScienceDirect

Engineering

journal homepage: www.elsevier.com/locate/eng

Research
Animal Nutrition and Feed Science—Review

Research Progress in the Application of Chinese Herbal Medicines in Aquaculture: A Review

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ARTICLE INFO

Article history:
Received 5 December 2016
Revised 26 April 2017
Accepted 9 May 2017
Available online 26 May 2017

Keywords:
Aquaculture
Chinese herbal medicines
Fish farming
Research progress

ABSTRACT

Due to increasing safety concerns regarding human consumption of fish products, an increasing number of medicinal chemicals are prohibited from use in aquaculture. As a result, Chinese herbal medicines are being increasingly used, coining the use of the term "green medicine." Research shows that Chinese herbal medicines have many beneficial effects on fish, including growth promotion, enhancement of disease resistance, and improvement in meat quality. Many effective ingredients have been discovered in Chinese herbal medicines, which function to promote feed intake, improve meat flavor, and increase digestive enzyme activity. They also regulate and participate in processes that improve the specific and non-specific immunity of fish; however, the composition of Chinese herbal medicines is very complex and it is often difficult to identify the effective ingredients. This article reviews the latest research and application progress in Chinese herbal medicines regarding growth and feed utilization, immunity and disease resistance, and the meat quality of cultured fish. It also discusses research on the chemical constituents of classical Chinese medicinal herbs and problems with the application of Chinese herbal medicines in fish culture. This article concludes by proposing that future studies on Chinese herbal medicines should focus on how to cheaply refine and extract the effective ingredients in classical Chinese medicinal herbs, as well as how to use them efficiently in aquaculture.

1. Introduction

Chinese herbal medicines have been used in China for thousands of years and are generally recognized as being natural and safe [1]. These herbs contain many effective ingredients, including polysaccharides, alkaloids, flavonoids, volatile oils, organic acids, and tannins, as well as nutrients such as amino acids, carbohydrates, minerals, and vitamins [2]. Studies have shown that these effective ingredients can increase appetite [3,4], promote metabolism, accelerate the synthesis of proteins [5,6], increase enzyme activity [7], improve immunity [8,9], increase disease resistance [10], and enhance meat quality [11,12] (Fig. 1).

Chinese herbal medicines have long been applied as attractants, growth promoters [13], antibacterial agents, and immunoprophylactic agents in aquaculture [14], and are considered to be effective alternatives to antibiotics, chemicals, vaccines, and other synthetic compounds [15]. In recent years, many studies have been conducted on the further application of Chinese medicinal herbs because of their advantages of being natural, innocuous, easy to prepare, and inexpensive, and because they have few side effects for either the fish or the environment [16]. Chinese herbal medicines can be administered as a whole plant, as parts of a plant (leaf, root, or

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http://dx.doi.org/10.1016/J.ENG.2017.03.017

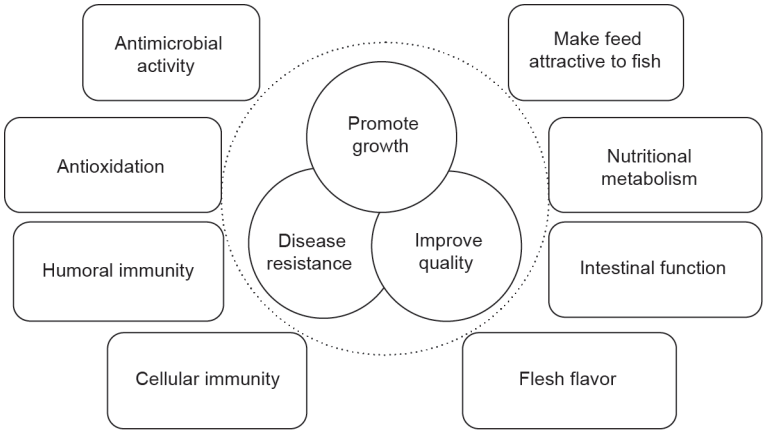


Fig. 1. Roles and main action mechanisms of Chinese herbal medicines when used on fish.

seed), or as extract compounds used either alone or in combination with other feed additives; they can be applied in the feed or via the immersion of fish in treated water [17]. However, the chemical constituents of Chinese herbal medicines are very complex and their biological activities are not always consistent. Additional investigations are needed to reveal the chemical structures and functional mechanisms of Chinese herbal medicines [18,19]. This study expounds on research progress in the extraction and purification of the effective ingredients of Chinese herbal medicine, introduces the chemical structures of some of the main effective ingredients in Chinese herbal medicines, and reviews the effects of Chinese herbal medicines on fish culture.

2. Studies on the chemical constituents of classical Chinese herbal medicines

The chemical constituents of Chinese herbal medicines are complex, diverse, and found in trace amounts, so it is very difficult to analyze and identify the effective ingredients in Chinese herbal medicines. More and more Chinese herbal medicines have been studied in detail and their key effective ingredients have been identified with the development of new analytical technologies (Table 1).

In 2001, ultrasound technology was used to extract alkaloids, flavonoids, anthraquinone, and polysaccharides from Chinese medicinal herbs [20]. In 2004, supercritical fluid chromatography became an effective method for analyzing large molecular ingredients such as phospholipids, triglycerides, and carotenoids. Supercritical fluid technology has the advantage of high selectivity, which allows it to precisely analyze the effective ingredients in Chinese medicinal herbs when combined with technologies such as gas chromatography, high-performance liquid chromatography (HPLC), and gas chromatography/mass spectrometry [21]. In 2005, the development of capillary electrophoresis technology, along with the development of other analytical technologies, provided a method to separate the trace effective ingredients in Chinese herbal medicines [22].

In 2006, molecular distillation technology was applied to extract the volatile oils of *Atractylodes macrocephala*, *Allium sativum*, *Angelica sinensis*, and *Forsythia suspensa* [23]. In 2008, membrane extraction technology, high-speed counter-current chromatography separation technology, and molecular imprinting separation technology were used for herb chemical analysis [24]. In 2009, researchers found that cellulase could improve the extraction rate of polysaccharides [25]. Fourier-transform ion cyclotron resonance mass spectrometry/sequential mass spectrometry technology combined with HPLC was later used to obtain accurate high-quality data. An accurate mass obtained by high-resolution mass spectrometry, comprised of multistage mass spectrometry fragmentation information and mass spectrometry, was used to analyze and identify the ingredients of *Epimedium brevicornu* [26]. In 2012, ultraviolet spectrophotometry was used to identify and quantify the effective ingredients in Chinese medicinal herbs, and the concentrations of various polysaccharides, alkaloids, flavonoids, and volatile oils were determined [27]. In 2013, researchers used imidazole-based ionic liquid as the extractant to obtain quercetin and kaempferol from *Cedrela sinensis* and *Rosae sinensis* using a microwave extraction method [28].

In 2014, three principal components and the corresponding principal component equations were obtained by principal component analysis from 12 Chinese medicinal herbs. The results showed that principal component analysis could be used for a comprehensive evaluation of the quality of Chinese herbal medicines [29]. In 2015, magnetite (Fe_3O_4) magnetic nanoparticles were used as an intermediate carrier for the separation of active agents extracted from *Scutellaria baicalensis* and *Glycyrrhiza uralensis*, thus establishing a new method for the separation and purification of Chinese medicinal herbs [30].

Table 1
Chinese herbal medicines applied in aquaculture, and chemical structures of their main effective ingredients.

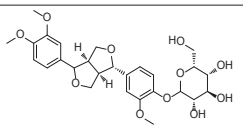
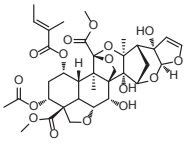
Herb name	Effective ingredient	Molecular formula	Structure
Forsythia suspensa	Phillyrin	$C_{27}H_{34}O_{11}$	
Azadirachta indica	Azadirachtin A	$C_{35}H_{44}O_{16}$	

Table 1 (*continued*)

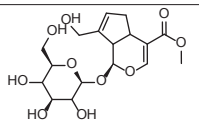
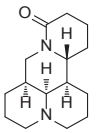
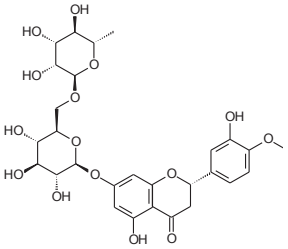
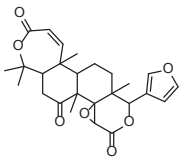
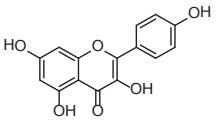
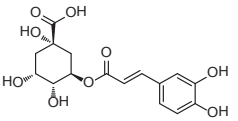
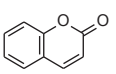
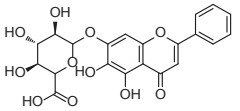
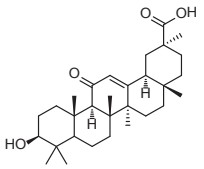
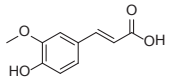
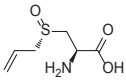
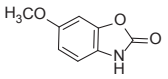
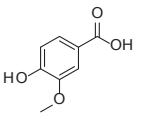
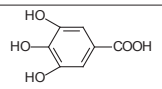
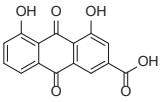
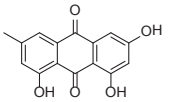
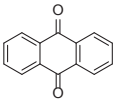
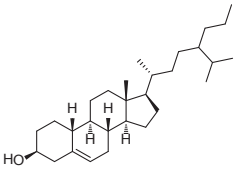
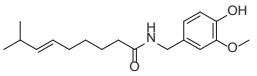
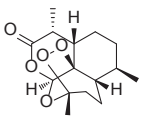
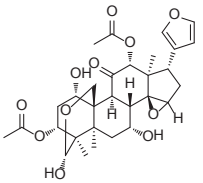
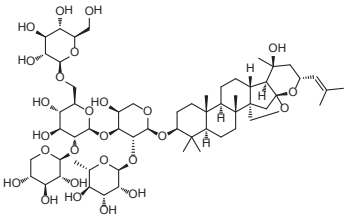
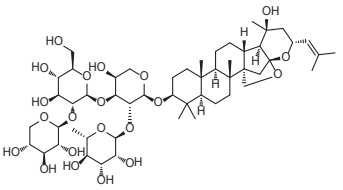
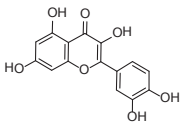
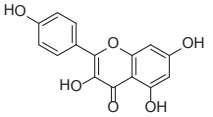
Herb name	Effective ingredient	Molecular formula	Structure
Gardenia jasminoides Ellis	Geniposide	$C_{17}H_{24}O_{10}$	
Sophora flavescens	Matrine	$C_{15}H_{24}N_2O$	
Citrus aurantium L.	Hesperidin	$C_{28}H_{34}O_{15}$	
Phellodendron chinense Schneid	Obacunone	$C_{26}H_{30}O_7$	
*Rhizoma Kaempferi*ae	Kaempferol	$C_{15}H_{10}O_6$	
Eucommia ulmoides	Chlorogenic acid	$C_{16}H_{18}O_9$	
*Angelica dahuri*ca	Coumarin	$C_9H_6O_2$	
Scutellaria baicalensis	Baicalin	$C_{21}H_{18}O_{11}$	
Glycyrrhiza uralensis	Glycyrrhetic acid	$C_{30}H_{46}O_4$	
Angelica sinensis	Ferulic acid	$C_{10}H_{10}O_4$	
Allium sativum	Alliin	$C_6H_{11}NO_3S$	
Rhizoma Phragmitis	Coixol	$C_8H_7NO_3$	
	Vanillic acid	$C_8H_8O_4$	

Table 1 (*continued*)

Herb name	Effective ingredient	Molecular formula	Structure
Rhus chinensis Mill.	Gallic acid	$C_7H_6O_5$	
Rheum officinale	Rhein	$C_{15}H_8O_6$	
	Emodin	$C_{15}H_{10}O_5$	
Euphorbia humifusa	Anthraquinone	$C_{14}H_8O_2$	
Acalypha australis	Sitosterol	$C_{29}H_{50}O$	
Capsicum frutescens L.	Capsaicin	$C_{18}H_{27}NO_3$	
Artemisia carvifolia	Arteannuin	$C_{15}H_{22}O_5$	
Melia azedarach	Methyl kulonate	$C_{30}H_{38}O_{11}$	
Semen Ziziphi Spinosae	Jujuboside A	$C_{58}H_{94}O_{26}$	
	Jujuboside B	$C_{52}H_{84}O_{21}$	
Toona sinensis	Thiophene	C_4H_4S	
Rosa chinensis	Quercetin	$C_{15}H_{10}O_7$	
	Kaempferol	$C_{15}H_{10}O_6$	

3. Effects of Chinese herbal medicines on growth and feed utilization in fish

Some Chinese medicinal herbs can improve the palatability of feed by stimulating the taste buds of cultured fish, and therefore promote feed intake. For example, *Syzygium aromaticum* and *Pericarpium Citri Reticulatae* can significantly promote the food intake of the common carp, *Cyprinus carpio*, when added to a basic diet in a proportion of 0.2% [31]. Goldfish, *Carassius auratus*, are distinctly attracted by *Astragalus membranaceus*, *Dioscorea opposita*, *Crataegus pinnatifida*, *Lycium chinense*, *Pericarpium Citri Reticulatae*, and *Cortex Phellodendri.* Amur sturgeon, *Acipenser schrenckii*, are attracted by feed containing berberine and kaempferol [16]. In addition, 0.4% (w/w) water extractions of *Pericarpium Citri Reticulatae*, *Crataegus pinnatifida*, *Syzygium aromaticum*, *Eucommia ulmoides*, and *Schisandra chinensis* play important roles in promoting feed consumption in koi, *Cyprinus carpio* [32].

Some researchers consider that the free amino acids, nucleotides, and alkaloids in preserved *Pericarpium Citri Reticulatae* may be the cause of its attractiveness to fish [14]. Most scholars believe that Chinese herbal medicines have a unique smell and taste (i.e., *Cinnamomum tamala*, *Angelica sinensis*, *Crataegus pinnatifida*, *Illicium verum* Hook F, etc.), which play an important role in stimulating feed intake [33]. In addition, Chinese herbal medicines can stimulate the secretion of digestive juices and promote intestinal motility, which may also explain the increased feed intake from their use [13].

Studies show that Chinese herbal medicines can improve the growth of fish because of the effects of their bioactive substances that can promote metabolism, enhance the synthesis of protein, and activate digestive enzymes. A 10.4%–32.3% growth rate increase over the control group was found in *Sciaenops ocellatus* as a result of feeding *Scutellaria baicalensis* and *Poria cocos* by 2% proportion in total in diet [34]. Another study showed that the lignin, baicalin, polysaccharides, and berberine in some Chinese herbal medicines (i.e., *Forsythia suspensa*, *Coptis chinensis*, and *Poria cocos*) can act as growth-promoting agents in the Pacific white shrimp, *Litopenaeus vannamei* [35]. The growth rate of the ricefield eel, *Monopterus albus*, was remarkably increased by adding *Poria cocos* to the feed [36]. *Codonopsis pilosula* and *Glycyrrhiza uralensis* significantly improved growth and protein utilization by 33.6% in catfish, *Silurus asotus* [37]. A mixture of *Scutellaria baicalensis*, *Astragalus membranaceus, Angelica sinensis*, *Codonopsis pilosula, Lonicera japonica*, *Acanthopanax senticosus*, and dried *Pericarpium Citri Reticulatae* in feed can significantly improve the growth of darkbarbel catfish, *Pelteobagrus vachelli*, with 1% being the optimum inclusion rate found in the experimentally [38]. Feeding grass carp, *Ctenopharyngodon idellus*, a mixture of allicin and *Pericarpium Citri Reticulatae* was also shown to result in a 31% faster growth rate at a dosage of 0.3% (w/w). *Eucommia ulmoides* leaf may also improve the growth of grass carp [39]. Another study showed that *Houttuynia cordata*, *Lonicera japonica*, *Glycyrrhiza uralensis*, and *Poria cocos* can improve the growth of grass carp—a finding that was attributed to an increase in lysozyme activity [12]. *Crataegus pinnatifida*, *Eucommia ulmoides*, *Cuminum cyminum*, and *Cinnamomum cassia* improve the growth of the ricefield eel, *Monopterus albus*, because they improve the digestibility of nutrients by increasing the activity of protease by 61.33% [7]. Improving intestinal function and promoting nutritional metabolism are important roles in Chinese herbal medicines [40]. Supplementing the diet of tilapia, *Oreochromis mossambicus*, with 1000–1500 mg·kg^{-1} of *Astragalus membranaceus* polysaccharide can improve the length of the intestinal villus, crypt depth, and thickness of muscular and quantity of intestinal mucous cells, as well as increasing the number of intraepithelial lymphocytes [41]. Adding dried *Pericarpium Citri Reticulatae*, *Astragalus membranaceus*, and *Angelica sinensis* at 0.5% (w/w) to feed can promote intestinal villus growth and improve the liver cytoplasmic density of the Chinese perch, *Siniperca chuatsi* [42]. Adding 1 g·kg^{-1} of *Eriobotrya japonica* polysaccharide to the diet can significantly increase the intestinal villous length and muscle layer thickness of the digestive tract in goldfish [5]. Adding a 2% proportion of *Crataegus pinnatifida* to the feed of crucian carp was observed to increase the apparent digestibility of protein by 10.2% and the intestinal protease activity by 25.68% [6].

4. Effect of Chinese herbal medicines on immunity and disease resistance in fish

Many Chinese medicinal herbs act as antibacterial and bactericidal agents, which can strengthen immunity and enhance the disease resistance of an animal [43,44]. Lysozyme, alkaline phosphatase, serum protein, and superoxide dismutase are important non-specific immunological indexes of fish; these indicators can be used to study the effects of Chinese herbal medicines on immunity. Effective ingredients in Chinese herbal medicines such as polysaccharides, saponins, alkaloids, anthracene, essential oils, and organic acids were found to dramatically improve these immunological indexes in fish. It was also found that Chinese herbal medicines can enhance the body's immune response to antigens [45,46].

The spleen is a peripheral immune organ, and is the place where immune cells proliferate in response to an antigen. The development of the immune organs is closely related to the immune status of the body [47]. For fish, the body surface mucosal layer is an important barrier that aids resistance to pathogens, thus not only protecting the body from being eroded by parasites, fungi, bacteria, and other harmful organisms, but also resulting in skin with a normal permeability [48].

Astragalus membranaceus contains many effective ingredients that affect the humoral and cellular immunity of fish, and improve lymphokine-activated immunity and the activity of immunological regulatory factors [49]. *Codonopsis pilosula* has tonic and anti-inflammatory effects that can moderate the stress response of the tilapia, *Oreochromis mossambicus*, to their environment and strengthen their disease resistance [50]. *Angelica sinensis* has beneficial effects in the regulation of blood rheology as well as providing anti-inflammatory, anti-tumor, and antibacterial immunity; oral administration of a dietary dosage of 50 mg·kg^{-1} of *Angelica sinensis* polysaccharide can increase blood leukocytes and leukocyte phagocytosis in the orange-spotted grouper, *Epinephelus coioides* [51].

Five species of Chinese medicinal herbs—*Forsythia suspensa*, *Polyporus umbellatus, Scutellaria baicalensis*, *Poria cocos*, and *Coptis chinensis*—were fed to the red drum, *Sciaenops ocellatus*, and were found to increase the leucocyte phagocytic percentage and the activity of lysozyme by 5.89% and 138.7 IU·mL^{-1}, respectively, after 28 days of feeding [52]. Chinese herbal medicine mixtures containing *Achyranthes bidentata*, *Isatis indigotica*, *Glycyrrhiza uralensis*, dried *Pericarpium Citri Reticulatae*, *Cinnamomum cassia*, *Hordeum vulgare*, and *Massa Medicata Fermentata* were found to enhance lysozyme and antiproteinase activities and to increase the nitroblue tetrazolium (NBT)-positive cells of turbot, *Scophthalmus maximus*, when 2% (w/w) herb powder was added to the basal feed [53]. Yellow catfish, *Pelteobagrus fulvidraco*, juveniles were fed herbal mixtures of *Scutellaria baicalensis*, *Poria cocos*, *Isatis tinctoria*, *Astragalus membranaceus*, *Rheum officinale, Glycyrrhiza uralensis*, and *Lonicera acuminate*, and significant increases in growth, phagocytic activity of leukocytes, and lysozyme activity were observed at a 1% (w/w) addition to the diet [54]. Another study showed that a Chinese herbal medicine mixture containing *Ephedra sinica*, *Sophora flavescens*, *Scutellaria baicalensis*, and *Rhus chinensis* Mill. could effectively enhance the phagocytic activity of leukocytes and the lysozyme

activity of the yellow catfish at a concentration of 400–800 mg·kg^{-1} [55]. The phagocytic activity of leukocytes, number of NBT-positive cells in the blood, and activity of superoxide dismutase, catalase, and lysozyme in the serum of tilapia, *Oreochromis mossambica*, were dramatically improved when the fish were fed a mixture of *Astragalus membranaceus*, *Codonopsis pilosula*, *Atractylodes macrocephala*, *Rheum palmatum*, *Isatis tinctoria*, *Scutellaria baicalensis*, and *Chrysanthemum indicum* [51]. A mixture of *Acanthopanax senticosus, Lycium chinese, Lonicera japonica*, and *Astragalus membranaceus* was fed to mirror carp, *Cyprinus carpio* var. *specularis*, juveniles, which increased the number of leukocytes, enhanced phagocytosis in the reticuloendothelial cells, and promoted the production of interferons, antibodies, and lymphocytes [56]. In cultured perch, the activities of superoxide dismutase, lysozyme, and catalase in the serum, spleen, liver, and kidney increased significantly when the fish were fed a mixture of Chinese herbal medicines at 1% and 2% proportion in feed [57]. In addition, some Chinese herbal medicines act to excite the adrenal cortical pituitary and induce hormone-like or gonadotropin-like effects in fish [45].

Persicaria hydropiper, *Rheum palmatum*, and *Diospyros ebenum* have strong inhibitory effects on *Aeromonas hydrophila*, which may cause hematosepsis on fish [58]. *Allium sativum* and *Rheum palmatum* have therapeutic effects on the skin diseases of bighead carp, *Aristichthys nobilis*, and silver carp, *Hypophthalmichthys molitrix*. *Euphorbia humifusa* and *Azadirachta indica* have significant effects on gill disease in bighead carp and common carp, while *Polygonum orientale* and *Acalypha australis* have obvious effects on mouth disease in silver carp and bighead carp [59]. Bathing fish with extract of *Zingiber officinale*, *Capsicum annuum*, and *Artemisia carvifolia* at concentrations of 5 mg·L^{-1}, 2.5 mg·L^{-1}, and 1 mg·L^{-1}, respectively, had dramatic effects on *Ichthyophthirius* disease; the insecticidal rate reached 100% in 24 h [60]. Azadirachtin has a positive correlation with the killing effect of *Trichodina*; an insecticide rate of more than 90% was obtained at a concentration of 0.8 mg·L^{-1} [10]. *Sophora flavescens*, *Melia azedarach*, *Nicotiana tabacum*, and *Stemona japonica* were found to effectively prevent *Trichodina* and other diseases [46]. A concentration of 2.75 g·L^{-1} of *Azadirachta indica* and 5.67 g·L^{-1} of *Sophora flavescens* was found to be optimal for killing *Chilodonella cyprinid*, with no insects surviving after 48 h [61].

5. Effects of Chinese herbal medicines on meat quality of cultured fish

Chinese herbal medicines can also be used to improve meat quality through improvements in the taste or color of fish flesh. The flavor of fish flesh is affected by its chemical composition. Amino acids, unsaturated fatty acids, aldehydes, and phenolics in muscle are important components that affect the taste of fish. *Allium sativum*, *Eucommia ulmoides*, and *Hippophae rhamnoides* are usually used as flavor agents; a total content of 60 mg·kg^{-1} in the diet can increase crude protein and flavor amino acids percentages in muscle as compared with the control group [62].

Feeding a 0.5% (w/w) mixture of *Poria cocos*, *Paeonia lactiflora*, *Herba Houttuyniae*, and *Rheum offcinale* has been shown to increase the crude protein and fatty acid content in the muscle of the Amur sturgeon, *Acipenser schrenckii* [11]. *Astragalus membranaceus*, *Angelica sinensis*, *Flos lonicerae*, and *Isatis indigotica* have been shown to significantly increase the inosinic acid concentration in the muscle of the Nile tilapia, *Tilapia nilotica*, while improving its flavor [63]. A mixture of *Scutellaria baicalensis*, *Poria cocos*, *Isatis indigotica*, *Astragalus membranaceus*, *Rheum palmatum*, *Glycyrrhiza uralensis*, *Lonicera japonica*, and *Eucommia ulmoides* added to the feed of common carp in 1% (w/w) proportion was found to increase the concentration of flavor by producing amino acids such as glutamate and aspartate in muscle [64].

6. Problems in the application of Chinese herbal medicines in aquaculture

At present, there are some problems with the wide application of Chinese herbal medicines in aquaculture. Firstly, because the effective ingredients in Chinese medicinal herbs are numerous, it is difficult to identify all the effective ingredients. The bioavailability of effective ingredients in plants is affected by the stage of growth and by the location where the herbal plants are grown; therefore, it is difficult to determine the accurate utility dosage of Chinese medicinal herbs. As a result, further research is required on how to control the quality of Chinese medicinal herbs [65,66]. Secondly, difficulties arise in applying Chinese herbal medicines to feed or water under commercial conditions. It is difficult to determine how to refine herbal products and cheaply extract the effective ingredients, especially when there are few effective ingredients present in Chinese medicinal herbs [67]. Thirdly, very few studies have been conducted to determine the functional mechanisms of Chinese herbal medicines. Until recently, research on Chinese medicinal herbs has mainly focused on fish performance, leaving the mechanism of action unclear for most herbs. Furthermore, traditional Chinese medical theory has mainly been developed with respect to humans, and thus cannot explain the true mechanisms of herb activity on aquatic animals, because fish have different mechanisms for the metabolism and immunization of these products [46].

In conclusion, existing studies have clarified the positive role of Chinese herbal medicines in growth, immunity, and disease resistance in aquaculture. Inadequate research on the functional mechanisms and on the differences in the quality of herbal plants causes uncertainty in their application. In the future, studies on Chinese herbal medicine should mainly focus on how to cheaply refine and extract the effective ingredients in classical Chinese medicinal herbs. Studies on the functional mechanisms of these effective ingredients should also be conducted.

Compliance with ethics guidelines

Hongyu Pu, Xiaoyu Li, Qingbo Du, Hao Cui, and Yongping Xu declare that they have no conflict of interest or financial conflicts to disclose.

References

[1] Zhang T, editor. Chinese material medica. Beijing: Higher Education Press; 2010. Chinese.
[2] Wang L, Wu L. Application and problems of Chinese herbal medicines in aquaculture. China Feed 2005;(2):28–9. Chinese.
[3] Zhu G, Bai D, Li Y, Ma J, Wu X, Ning B. The primary study of 12 Chinese herbs as feed attractants on the part of *Scophthalmus maximus*. J Anhui Agric Sci 2010;38(32):18233–4. Chinese.
[4] Bai D, Wang F, Guo Y, Zhu G, Mao H, Sun J, et al. Primary study of Chinese herbs as feed attractants in *Tinca*. J Tianjin Agric Univ 2009;16(1):5–8. Chinese.
[5] Liu C. Effect of loquat polysaccharide on digestion characteristics and growth of goldfish. Fujian Agri Sci Technol 2016;(5):3–6. Chinese.
[6] Qiu X, Zhou H, Hua X, Liu X, Cao D. The effect of dietary Chinese herb additives on growth and digestibility of allogynogenetic crucian carp. J Xinyang Teach College 2004;26(6): 551–5. Chinese.
[7] Li J, Hu Y, Xun Z, Dai Z, Xiao T, Mao P, et al. Effect of Chinese herbal medicines on eel growth, non-specific immunity and intestinal digestive enzyme activity. Feed Ind 2013;34(16):26–30. Chinese.
[8] Kirubakaran CJW, Alexander CP, Michael RD. Enhancement of non-specific immune responses and disease resistance on oral administration of *Nyctanthes arbortristis* seed extract in *Oreochromis mossambicus*. Aquacult Res 2010;41(11):1630–9.
[9] Harikrishnan R, Balasundaram C, Heo MS. Review: Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. Aquaculture 2011;317(1–4):1–15.
[10] Wu W, Zhu X. Toxic effects on fishes and application on the parasitic diseases controlled by azadirachtin. Chinese J Pesticide Sci 2003;5(2):85–9. Chinese.
[11] Ma L, Peng X, Li D. Effect of dietary medicinal herb compound additives on nutrition composition and texture profile of Amur sturgeon (*Acipenser schrenckii*). J

Huazhong Agric Univ 2015;34(3):111–6. Chinese.
[12] Tan J, Deng Y, Cao Y, Yao Y, Ma C. Effects of Chinese herbal compound in feed on growth, muscle composition and immune-related enzyme activities of juvenile grass carp (*Ctenopharyngodon idellus*). Guangdong Agri Sci 2015;(10):109–3. Chinese.
[13] Luo S. Researches progress of application of attractant in aquaculture. Guizhou Agri Sci 2012;40(9):183–6. Chinese.
[14] Wang Y, Yang Y, Liu D. Research advance of application Chinese herbal medicine in feeding attractant of aquatic animal. China Feed 2010;(23):32–4. Chinese.
[15] Zhou X, Zhou H, Wang D, Shi L, Cao T. Current situation and prospects of the application of Chinese herbal medicine feed additives. Guizhou Agri Sci 2016;44(7):77–80. Chinese.
[16] Wang D, Liu Y, Xu S. Practical manual of aquatic animal feeds. Shanghai: Shanghai Scientific & Technical Publishers; 2004. Chinese.
[17] Chang J. Medicinal herbs: Drugs or dietary supplements? Biochem Pharmacol 2000;59(3):211–9.
[18] Chen L, Huan Z, Wang X, Xiao G, Hu Y, Qin Q, et al. Research progress of application of dietary Chinese herbal medicine additives in fish. Fish Sci 2014;(3):190–4. Chinese.
[19] Liu F. Application and perspective of Chinese herb medicines in protection and treatment of disease of aquatic animals. Modern Fish Inform 2003;(7):17–20. Chinese.
[20] Li H, Wu T. Application of ultrasonic technology in the extraction of Chinese herbal medicine components. Lishizhen Med Mater Med Res 2001;24(4):299–300. Chinese.
[21] Yan J, Tong S, Jin J. Application of supercritical fluid to analyze the effective components of TCM. Lishizhen Med Mater Med Res 2004;15(3):179–80. Chinese.
[22] Feng J. Study on capillary electrophoresis and its application in separation and analysis of Chinese traditional medicine ingredients [dissertation]. Nanning: Guangxi University; 2005. Chinese.
[23] Ju X, Sun T, Wang B, Zhao H. The applications of molecular distillation in separation and purification of Chinese herbals. J Tangshan Teach College 2006;28(2):35–7. Chinese.
[24] Zhu X, An X. Research progress on extraction and separation technology of effective components of Chinese herbal medicine. Guangzhou Chem Ind 2008;36(4):12–4. Chinese.
[25] Li F, Cai H, Yan Z, Guo W. Study on the extraction of *Rhizoma Phragmitis* polysaccharide by an enzymatic method. Sci Technol Food Ind 2009;4(96):156–9. Chinese.
[26] Zhang J. Application research on composition analysis and structural identification of Chinese herbal medicines [dissertation]. Beijing: Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences; 2009. Chinese.
[27] Lv H. Analyze the effective composition of Chinese herbal medicine and its effects on bowel function of rabbits [dissertation]. Fuzhou: Fujian Agriculture and Forestry University; 2012. Chinese.
[28] Liu X. Research and application of imidazolium-based ionic liquids for the separation and analysis of traditional Chinese medicine and protein [dissertation]. Changsha: Hunan University; 2013. Chinese.
[29] Pu L, Li H, Pu Y, Jiang H. Principal component analysis of trace elements in 12 commonly used Chinese herbal medicines. J Guangxi Normal Univ 2014;32(4):96–100. Chinese.
[30] Li R. Application of Fe_3O_4 magnetic nanoparticles on the separation of ingredients in traditional Chinese medicine [dissertation]. Changsha: Hunan Normal University; 2015. Chinese.
[31] Guo Y, Xing K, Chen C, Yang Y, Zhu G. Study on some Chinese herb medicines as feed attractants in carp (*Cyprinus carpio*). Fish Sci 2005;12(3):3–4. Chinese.
[32] Wang L, Ji Z, Hu Z. Study of eight kinds of Chinese herbs on koi attractant activity. Feed Res 2010;(1):66–7. Chinese.
[33] Zhang L, Zeng H, Zhang J, Ai Q, Mai K. A study on feeding attraction activity of Chinese herbal medicine for Japanese sea bass *Lateolabrax japonieus*. Mar Fish Res 2008;29(4):101–4. Chinese.
[34] Ma A, Yan M, Chang W, Xie Q, Chen S, Shan L. Effects of Chinese herbs on the growth and immune function in *Sciaenops ocellatus*. Mar Sci 2009;33(12):96–102. Chinese.
[35] Guo W, Li J. Effect of Chinese herbal medicine on growth and immune factors of *Litopenaeus vannamei*. Feed Ind 2005;26(6):6–10. Chinese.
[36] Ruan G, Yang D, Wang J. Effects of herbal feed additives on immunity and growth of *Monopterus albus*. Feed Ind 2005;26(24):34–6. Chinese.
[37] Han Q, Ma Y. The test of different herbal additives feeding catfish. Animal Breed Feed 2006;(12):54–6. Chinese.
[38] Tang L, Wang J, Chen Y, Yao J, An M. Effect of compound Chinese herbal medicines on growth of *Pelteobagrus vachelli*. Guizhou Agric Sci 2010;38(11):194–5. Chinese.
[39] Leng X, Meng X, Li J, Li X, Hua X. Preliminary study on *Eucommia* leaf on grass carp growth, serum non-specific immune index and meat quality. J Fish China 2008;2(3):434–40. Chinese.
[40] Kong X, Wu Y, Liao Y, Hou Z, Liu H, Yin F, et al. Effects of Chinese herbal ultra-fine powder as a dietary additive on growth performance, serum metabolites and intestinal health in early-weaned piglets. Livest Sci 2007;108(1–3):272–5.
[41] Huang Y, Lin X, Wang Q, Xie J, Chen J, Zhao J, et al. Effects of *Astragalus* polysaccharide on structure of intestinal villus and intestinal immunocyte of tilapia. Chinese J Anim Nutri 2010;(1):108–16. Chinese.
[42] Lin J, Liu Q. Effects of Chinese herbal medicine on intestinal villus structure and hepatocyte characteristics of *Siniperca chuatsi*. China Anim Husb Veter Med Abst 2015;(11):218–9. Chinese.
[43] Gong J, Yin F, Hou Y, Yin Y. Review: Chinese herbs as alternatives to antibiotics in feed for swine and poultry production: Potential and challenges in application. Can J Anim Sci 2014;94:223–41.
[44] Kong X, Hu Y, Yin Y, Wu G, Rui R, Wang D, et al. Chinese herbal ingredients are effective immune stimulators for chickens infected with the Newcastle disease virus. Poult Sci 2006;85(12):2169–75.
[45] Ma G, Yu X, Zhong Q. Application prospects for the prevention and control of fish diseases by the use of traditional Chinese herbal medicines. Chinese J Trad Vet Sci 2006;(5):35–8. Chinese.
[46] Chai G, Liu G, Zhao J, Ji S. Application prospects of traditional Chinese medicine in aquaculture. Chinese J Vet Drug 1999;33(2):52–4. Chinese.
[47] Kong X, Wu G, Liao Y, Hou Z, Liu H, Yin F, et al. Dietary supplementation with Chinese herbal ultra-fine powder enhances cellular and humoral immunity in early weaned piglets. Livest Sci 2007;108(1–3):94–8.
[48] Li X, Ma C, Li Y, Jiang Z, Wang B, Yao Y, et al. Effect of Chinese herbal medicines on immunity of flounder *Paralichthys olivaceus*. J Northeast Agric Univ 2011;42(3):60–7. Chinese.
[49] Yang X, Liu X, Sun J. The clinical application of *Astragalus*. Jilin J Trad Chinese Med 2004;24(11):60. Chinese.
[50] Zhang Z, Lin X, Zhang W, Wang Q, Li H, Yao H, et al. Effects of a Chinese herbal compound on nonspecific immunity in blood of tilapia. Fish Sci 2011;30(1):1–5. Chinese.
[51] Wang Q, Zhao H, Liu Z, Chen C, Xing K. Effects of dietary *Angelica sinensis* on non-specific immunity of *Epinephelus coioides*. J Anhui Agri Sci 2011;39(22):13857–60. Chinese.
[52] Ma A, Yan M, Chang W, Xie Q, Chen S, Shan L. Effects of Chinese herbs on the growth and immune function in *Sciaenops ocellatus*. Mar Sci 2009;33(12):96–101. Chinese.
[53] Li H, Zhang T, Li Q. Effect of Chinese herbs on nonspecific immunity on turbot. J Dalian Ocean Univ 2013;28(2):115–20. Chinese.
[54] Zhang Y, Zheng J, Li W, Xiao L. Effects of Chinese herbal medicines on growth and nonspecific immunity in yellow catfish *Pelteobagrus fulvidraco*. Fish Sci 2010;29(4):225–8. Chinese.
[55] Sheng Z, Huang W, Zhang A, Yang H, Ma L, Xu X, et al. Effect of complex Chinese herbs on non-specific immune function and disease resistance in yellow catfish (*Pelteobagrus fulvidraco*) by oral administration. J Huazhong Agric Univ 2012;31(2):243–6. Chinese.
[56] Meng Z, Chen Y, Liu M. Effect of Chinese herbal compounds on nonspecific immunity of *Cyprinus carpio* var. *specularis* juvenile fish. J Northeast Agric Univ 2011;42(3):54–9. Chinese.
[57] Liang Y, Sun X, Sun Y, Su J, Shi D, Ma J. Effect of the compound Chinese herbal medicine on the antioxidant capacity and immune function of perch. J Anhui Agric Sci 2009;37(15):7026–9. Chinese.
[58] Zhang W, Gong Y, Jin S, Sun Z, Zhang L, Tong L, et al. Antimicrobial tests of Chinese herbal medicine and their mixtures on *Aeromonas hydrophila*. Prog Fish Sci 2012;33(1):114–21. Chinese.
[59] Yang J, Liu Y, Zou S, He Z, Yuan Z. Study on the disease prevention of silver carps bighead by Chinese herbal medicine. Hubei Agric Sci 2013;52(11):2618–20. Chinese.
[60] Lin Q, Xu B, Cai L. The effectiveness of ginger and pepper in the treatment of *Ichthyophthirius multifiliis* disease. Aquaculture 2005;26(2):40–1. Chinese.
[61] Shi H. Antiprotozoal effects of six Chinese medicinal herbs on *Chilodonella cyprini* and *Paralembus digitiformis* Kahl *in vitro*. Shandong Fish 2008;25(10):4–7. Chinese.
[62] Lin J, Zhu Q, Liang P, Qiu M. Effect of different additives on growth and meat quality of blunt snout bream (*Megalobrama amblycephala*). Fujian J Agri Sci 2015;30(7):636–41. Chinese.
[63] Tang J, Wu Z, Jian J, Lu Y, Tang Y. Effects of compound Chinese herbal medicine on the growth and muscle composition of tilapia. Feed Ind 2009;30(16):19–21. Chinese.
[64] Zhang Y, Cai B, Chen W. Effects of compound Chinese herbal medicine on growth performance and muscle quality of common carp. Sci Fish Farm 2012;(12):72–3. Chinese.
[65] Wang X, Zhang S, Wang D, Jiao J. Application and problems of Chinese herbal medicine in prevention and control of aquatic animal diseases. Sci Fish Farm 2016;(11):55–7. Chinese.
[66] Wu M. The application prospect of Chinese herbal medicine in aquaculture. J Anim Sci Veter Med 2008;27(2):64–5. Chinese.
[67] Zhou X, Zhou H, Wang D, Shi L, Cao T. Current situation and prospects of the application of Chinese herbal medicine feed additives. Guizhou Agri Sci 2016;44(7):77–80. Chinese.