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Research Article

Role of phytochemicals in farmed fish reproductive performance: A review

Sepehrfar D.[1]; Sudagar M.[1*]; Paknejad H.[1]; Yousefi Siahkalroodi S.[2*]; Norouzitallab P.[3]

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Abstract

Researchers have used phytochemicals as sustainable and valuable additives to improve reproduction traits and stimulate the immune system in farm-raised fish species followed by minimizing environmental and public health risks. In this regard, phytochemicals are plant-based bioactive compounds and are known as secondary metabolites. They contain various compounds with a remarkable potential to influence the reproductive performance of aquatic animals. Phytochemicals offer a promising approach to regulate reproductive performance and improve productivity in intensive aquaculture. According to the growing demand for eco-friendly solutions, phytochemicals reduce oxidative stress and enhance disease resistance in different farm-raised fish species. They have various bioactive compounds with unique physiological effects, especially on the reproductive performance of aquatic animals. However, the exact mode of action of these additives on aquatic reproduction traits is not yet well-documented. Overall, phytochemicals can be used as a practical alternative to synthetic additives and have the potential to promote sustainability in different aquaculture sectors around the world based on the availability and affordability of medicinal plants in the region. Polyphenols, flavonoids, isoflavones, anthocyanins, phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, and glucosinolates are well-known phytochemical molecules with innumerable properties in the host such as antibacterial, antiviral, antioxidant, and anti-inflammatory activities. These compounds are commonly administered orally in aquatic animals. Notably, plant extracts containing bioactive compounds like saponins and flavonoids play a vital role in inducing sexual inversion and fertility disorders. However, the concentration of phenolic compounds varies depending on the herbal genetics (genus, species, cultivar, and genotype), plant extraction methods, environmental conditions, and aquatic animal maturation status. Most studies declared that phytochemicals have a profound effect on fish reproduction due to their various bioactive compounds. They can also induce sexual maturation by interacting with hormone receptors, promote gonadal development by stimulating gonadotropin production, improve sperm quality through antioxidant properties, modulate hormone levels, and support reproductive behavior. However, the effects vary based on fish and plant species, dosage, and exposure duration. Further research is needed to optimize the use of phytochemicals in improving reproductive performance in farmed fish.

Keywords: Herb extract, Bioactive compounds, Oxidative stress, Fish reproduction, Aquaculture

1-Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
2-Department of Biology, Faculty of Biological Sciences, Varamin-Pishva Branch, Islamic Azad University, Pishva, Iran.
3-Department of Animal Nutrition and Management, Faculty of Veterinary Medicine and Animal Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden.
*Co-corresponding authors' Email: Sudagar2015@gmail.com; Siamak.yousefi1@gmail.com

Introduction

Medicinal plants consist of bioactive compounds such as alkaloids, flavonoids, phenols, and terpenoids, which are commonly used in aquaculture to harness their therapeutic properties. In this context, *Allium sativum*, *Azadirachta indica*, *Echinacea purpurea*, *Aloe vera*, *Curcuma longa*, *Zingiber officinale*, and *Punica granatum* are some examples of commonly used herbs in aquaculture industry (Mariappan *et al*., 2023). These plants offer antimicrobial, antiparasitic, immunostimulant, and wound-healing properties. However, their use requires an understanding of dosage, administration routes, and potential interactions (Dawood *et al*., 2018). Ongoing research aims to explore the full potential of medicinal plants in promoting the health status of aquaculture species.

In recent years, improving growth performance and artificial reproduction in aquaculture have been considered by numerous investigators. There is an increasing demand for intensive aquaculture, however, these production systems cause an increase in the organic load that disrupts the water quality in the environment. On the other hand, poor water quality can be stressful for fish and lead to low survival rates (Das, 2018). To control and prevent diseases and stress, over and miss-use of antibiotics can suppress the host's immune system and provide resistance against pathogenic microorganisms. From this point of view, the use of phytochemicals and their effect on the reproductive performance of aquatic animals is vital (Dien *et al*., 2023).

Phytochemicals are naturally derived by plants and contain diverse bioactive compounds. They have been also studied for their potential effects on the sexual maturation and reproduction of fish. These compounds have shown promise in improving reproductive performance in farmed fish species by stimulating the onset of sexual maturation in fish. Certain compounds, such as plant sterols and phytoestrogens, can mimic or modulate the effects of endogenous hormones involved in reproductive processes (Petrine and Del Bianco-Borges, 2021). By interacting with hormone receptors, phytochemicals can promote the development of reproductive organs and the initiation of gametogenesis. They have been shown to modulate the growth and development of gonads in fish and can stimulate the production of gonadotropins, which are responsible for regulating gonadal functions. By promoting the proliferation of germ cells and increasing the synthesis of sex steroids, phytochemicals can enhance gonadal development and ameliorate reproductive performance. Phytochemicals have been also investigated for their positive effects on sperm quality in fish (Ahmadifar *et al*., 2021). In this regard, polyphenols and flavonoids, possess antioxidant properties that can protect sperm cells from oxidative stress and DNA damage. By reducing oxidative damage and improving sperm motility and viability, phytochemicals can increase

fertilization success and overall reproductive performance in fish and shellfish. Phytochemicals can influence the production and release of reproductive hormones in fish. For example, some compounds can inhibit the production of gonadotropin-releasing hormone (GnRH), which regulates the synthesis and release of gonadotropins (Tvrda *et al*., 2019). By modulating hormone levels, phytochemicals can affect the timing and progression of reproductive processes, including spawning and gamete production. Also, they can positively affect reproductive behavior in fish. Some compounds can act as pheromones or attractants, stimulating courtship and spawning behaviors. Phytochemicals can enhance reproductive interactions and finally successful fertilization in host by mimicking natural cues (Balestrini *et al*., 2021).

It is important to note that the effects of phytochemicals on fish reproduction traits can vary depending on the fish/plant species, environmental conditions as well as herbal dosage and duration of exposure (Ahmadifar *et al*., 2021). Further research is needed to fully understand the mechanisms of action and optimize the use of phytochemicals for improving reproductive performance in different aquaculture organisms.

Broodstock management

Full control of sexual maturity and spawning to produce high-quality seeds is a basic requirement for successful aquatic production (Cheruiyot and Adhiaya, 2023). Fish are mainly affected by a wide range of human artifacts, including habitat loss, pollution, invasive species, and over-exploitation. In addition, climate change, especially the consequences of global warming, can affect fish at all levels of the biological organization, from the individual to the population level, and affect physiological and ecological processes in various direct and indirect ways (Upadhyay, 2020).

Breeding in aquaculture animals

Successful artificial propagation is one of the key issues in the aquaculture industry. It involves the successful development and maturation of gonads followed by healthy genital system. The growth and development of the gonads depends on various external and internal factors, continues with the creation of secondary sexual traits and eventually lead to the production of gametes that are responsible for transmitting the genetic and environmental information to the next generation. The ultimate goal of all these processes is to preserve the population and increase the survival chance of the species (Yildirim *et al*., 2020). In terms of aquaculture, closing the life cycle of fish must be completely closed in captivity and independent of wild sources such as eggs, larvae, juveniles or breeders (Yue *et al*., 2023). Continuous control of reproduction from year to year in successive generations of fish kept and bred in captivity. It's an essential prerequisite for domesticating fish. One of the basic requirements for the reproduction of fish in captivity is to

know the biology of natural reproduction of each species. Of course, climatic and genetic differences between different populations can accelerate or delay sexual maturation. Usually, there is an inverse relationship between growth rate and the age of puberty, and the higher the growth rate, the lower the age of puberty (Valenzuela *et al*., 2019).

One of the appropriate criteria to evaluate the reproductive readiness of fish is the gonadosomatic index, because the increase in the weight of the testicles and ovaries indicates the production and storage of gametes. The growth of the gonads and the fish reaching the maximum gonadosomatic index requires complete sexual maturity, which is influenced by two There are external and internal factors (Flores *et al*., 2019). External factors include various environmental variables such as temperature, light, water flow and seasonal rainfall. These environmental factors play a stimulating role for the function of internal reproductive factors. Water temperature and photoperiod or photoperiods are two important and effective environmental factors on sexual maturation, and the most important internal factor affecting sexual maturation is gonadotropin hormones secreted from the pituitary gland (Dwyer and Quinton, 2019).

This effect is exerted by different hormones. When the hypothalamus is stimulated by environmental factors, it secretes gonadotropin-releasing hormone or GnRH, whose target tissue is the pituitary gland. Pituitary under the influence of GnRH hormone, gonadotropin hormone or GTH secretion. It is divided into two types: GTH I and GTH II. In fact, these two hormones promote the reproduction process by cooperating with each other (Suzuki *et al*., 2023). The way these hormones work is that they stimulate the sex glands to secrete steroid hormones. There are different types of steroid hormones and each one plays a specific role in the sexual maturation process. Androgenic hormones such as testosterone and 11-ketotestosterone play the main role in male sexual maturation (Zhang *et al*., 2020). It has been proven that the amount of secretion of these hormones increases significantly during the reproduction season and at the same time as the water temperature decreases, which is the main stimulus for the growth of gonads. Also, in female fish, the hormone 17beta estradiol is the main driver of oocyte growth (Chen *et al*., 2021).

The main hindrance to hatchery production is variable and usually low larval survival. Several factors contribute to this problem: low brood quality due to genetic degradation and lack of knowledge about brood feeding, lack of larvae, disease outbreaks caused by bacterial proliferation, and unstable microbial environment in the intensive larval rearing system (Lobanov *et al*., 2023).

One of the major problems encountered in aquaculture is the inconsistent supply of quality and suitable seeds that can be solved through systematic breeding programs. Providing better breeding reserves for

the aquaculture industry and developing safe and productive operations are expected to lead to more stable fish production and better incomes for farmers (Manzano *et al*., 2023). One of the most important risks of aquaculture is the occurrence of infectious diseases in aquaculture and if the appropriate environmental factors are provided for the activity of pathogens, the possibility of infectious diseases in aquaculture is very likely (Amian *et al*., 2020).

The role of stress on reproduction

Seed supply problems are one of the most important obstacles to aquaculture development and this issue is particularly significant for many farmed species, as their production heavily relies on the collection of broodstocks or seeds from wild populations (Shikuku *et al*., 2021). To establish a successful aquaculture operation, it is necessary to start with a sufficient number of healthy and genetically diverse seeds or juveniles. These seeds serve as the foundation for the entire production cycle, from grow-out to market. Some of the key problems associated with seed supply include (Ahmad *et al*., 2021):

1. Insufficient supply: In many cases, the demand for seeds surpasses the available supply from wild sources. This leads to a shortage of seeds, limiting the expansion of aquaculture operations.
2. Unsustainable collection: Over-harvesting of seeds from wild populations can have detrimental effects on the natural ecosystems. It can disrupt the balance of the ecosystem, decrease the population size of targeted species, and even lead to their extinction in extreme cases.
3. Genetic diversity: Capturing seeds from wild populations may result in a loss of genetic diversity, as only a subset of individuals is typically caught. This can reduce the resilience and adaptability of farmed populations to environmental changes and diseases.
4. Disease transmission: Wild-caught seeds may carry diseases or parasites that can be transmitted to farmed populations, leading to high mortality rates and economic losses.

To address seed supply problems in aquaculture, alternative approaches have been developed. These include the establishment of hatcheries for controlled breeding and production of seeds, genetic selection, and improvement to enhance desirable traits in farmed species, domestication, and selective breeding to reduce reliance on wild populations, and conservation efforts to protect wild populations and supplement them with hatchery-produced seeds (Hossain *et al*., 2022).

In conclusion, seed supply problems pose a significant obstacle to the development of aquaculture. To overcome these challenges, the industry is increasingly focusing on hatchery production, selective breeding, conservation measures, and sustainable practices to ensure a stable and sustainable seed supply for the growth of aquaculture.

In recent studies, stress has emerged as a prominent factor contributing to the challenges encountered in artificial fish reproduction. Stress, in this context, is often triggered by external adversarial factors or the specific conditions that give rise to it. It is important to note that the manifestation of stress-induced changes can significantly disrupt the stability of the body's internal environment, subsequently affecting the functioning of various tissues and organs (Ahmadifar *et al*., 2021).

These physiological changes represent adaptive responses to the presence of stressors and, as such, share common characteristics, mechanisms, and purposes. While the extent or magnitude of these physiological responses may vary, they generally serve the function of preparing the organism to cope with the stressor in question. Consequently, understanding and mitigating stress in the context of artificial fish reproduction is pivotal to improve success rates of this critical activity in aquaculture practices (Cooke *et al*., 2023).

The artificial reproduction of fish can be notably susceptible to a range of stressors, encompassing chemical, physical, and biological factors. Chemical stressors often manifest as alterations in the water's chemical parameters within the breeding facility. Physical stressors can involve various manipulations, impacts, fluctuations in ambient light, and the transportation and movement of reproductively active fish. Biological stressors come into play when for example the opposite sex is absent near the reproductive fish or when predatory fish share the same tank, potentially creating an intimidating environment (Milla *et al*., 2021).

It is worth noting that the manifestation of stress in fish reproduction can lead to a slowing down or cessation of reproductive activities and even minimal stress levels can affect the production or reproductive behavior of the fish (Zhang *et al*. 2021). This stems from the fact that in times of stress, some of the energy that should be expended on anabolic and reproductive activities should be used by the animal to fight and cope with stress. As a result, energy "investment" in the reproductive sector is reduced. Successful reproduction in fish depends more on the stability of environmental conditions than on the work of other organs of the body, and therefore any change in conditions may directly stop reproduction. Many species of fish are unable to reproduce in altered or imposed conditions without being under stress, and even for some species may even mature completely in captive gonads, but their reproduction is not possible without inducing ovulation artificially (Ahmadifar *et al*., 2021).

How the performance of stress on reproduction

Environmental stressors, especially nutrition, can affect sexual maturity, gamete quantity and quality, growth, and ultimately fertility (Castañeda-Cortés and Fernandino, 2020). Most stressors have an impact on neuroendocrine cascades, which involve the immediate

release of catecholamines and activation of the hypothalamic-pituitary-interrenal (HPI[1]) axis (Alfonso *et al*., 2021). The intensity, duration, and type of stressors as well as the species and stage of sexual maturity can lead to completely different responses. Depending on the developmental stage, stressors can accelerate or delay reproduction, which affects atresia, growth, and ovulation through physiologically variable responses. Most reproductive events are controlled by FSH [2]and LH [3]gonadotropins, and there are conflicting reports of the effect of stress on gonadotropins and sex hormones (Alix *et al*., 2020).

The underlying mechanism by which stress disrupts reproductive functions appears to be multifactorial. These include changes in the hypothalamic-pituitary-ovarian axis: gonadotropin secretion, follicular growth, steroid production, and corpus luteum function (Beroukhim *et al*., 2022). Evidence suggests that stress-induced impairment of oocyte growth ability involves changes in oocyte transcripts. Examining the molecular response to environmental stressors may help us understand the phenomenon of infertility and enable the development of new approaches to overcome these effects (Roth, 2018).

Mitochondrial function in relation to reproduction

Stress-induced impairment of oocyte growth ability refers to the negative impact of stress on the development and growth of oocytes, which are immature eggs. Oocytes play a crucial role in reproduction, as they eventually mature into eggs that can be fertilized by sperm. One of the mechanisms through which stress affects oocyte growth ability is by inducing changes in mitochondrial function. Mitochondria are known as the "powerhouses" of cells, as they generate energy in the form of adenosine triphosphate (ATP). They also have other important roles, including regulating cell metabolism, calcium signaling, and apoptosis. Under stress conditions, such as exposure to environmental toxins, heat, or oxidative stress, mitochondria can become dysfunctional. This can lead to reduced ATP production and an imbalance in cellular energy metabolism. As a result, oocytes may not receive an adequate energy supply for their growth and development. Mitochondrial dysfunction can also lead to the production of reactive oxygen species (ROS[4]), which are highly reactive molecules that can cause damage to cellular components, including DNA, proteins, and lipids. Accumulation of ROS can lead to oxidative stress, which is known to impair oocyte growth and development (Zia *et al*., 2022).

[1] Hypothalamic-Pituitary-Interrenal
[2] Follicle-Stimulation Hormone
[3] Luteinizing hormone
[4] Reactive Oxygen Species

Furthermore, stress-induced changes in mitochondrial function can affect other important processes in oocytes, such as meiotic maturation, cytoplasmic maturation, and fertilization potential. These processes are essential for the successful development of oocytes into viable embryos. Overall, stress-induced impairment of oocyte growth ability involves alterations in mitochondrial function, which can disrupt energy production, induce oxidative stress, and negatively impact various processes essential for oocyte development and fertilization. Understanding these mechanisms can help in developing strategies to mitigate the adverse effects of stress on reproductive health (Roth, 2018).

Heat-stress and reproduction

Global climate change is a phenomenon characterized by the increase in Earth's average temperature due to the accumulation of greenhouse gases in the atmosphere. This increase in temperature is predicted to have significant impacts on marine and coastal organisms. One of the major consequences of global climate change is the increase in heat stress. As temperatures rise, marine and coastal organisms are exposed to higher temperatures for longer durations. Heat stress can directly affect the growth, development, and reproductive performance of these organisms. Reproductive function is particularly sensitive to heat stress. In many marine organisms, such as fish, mollusks, and crustaceans, reproductive success is dependent on the proper functioning of their gonads. The gonads are responsible for producing gametes (eggs and sperm) and facilitating fertilization. However, under heat stress conditions, the growth and development of gonads are impeded. High temperatures can disrupt the delicate balance of hormones and enzymes required for normal gonad development. This disruption can result in fertility failure, where organisms are unable to produce viable gametes or successfully reproduce (Ghulam Mohyuddin *et al*., 2022).

Furthermore, the effects of heat stress on reproductive function may not be limited to the current generation. Studies have shown that heat stress can cause epigenetic changes, which are modifications to the DNA that can be inherited by future generations. These changes can alter gene expression patterns and potentially lead to fertility problems in subsequent generations. In addition to reproductive function, heat stress can also impact the growth and development of marine and coastal organisms. Higher temperatures can affect metabolic rates, nutrient uptake, and energy allocation, leading to reduced growth rates and smaller body sizes. These changes can have cascading effects on the overall health and survival of populations (Murray *et al*., 2022).

Overall, global climate change and the associated increase in heat stress pose significant challenges for marine and coastal organisms. The impairment of reproductive function, growth, and development can have far-reaching consequences for these organisms and

their ability to adapt and persist in changing environments. Understanding and mitigating these impacts is crucial for the conservation and management of marine ecosystems (Nash *et al*., 2019).

Other stressors

Successful reproduction in fish depends on the complex control mechanism of light and endocrine systems as well as many environmental variables. Water temperature and daily photoperiod are the most important of these factors, food frequency, water quality, social environment, physical suitability of the spawning bed, etc. may each be decisive factors. Also, oxidative stress leads to DNA damage, a decrease in the number of eggs, and an increase in the number of apoptotic eggs (Awasthi *et al*., 2018). Therefore, it is observed that there are many preconditions for fish reproduction and the success or failure of reproduction depends to a large extent on these preconditions (Chen *et al*., 2020).

Gonad maturity

Generally, quality control of gametes is an important issue for the aquaculture industry. Management of fish reproduction depends on the use of the best breeders. Decreased quality and quantity of sex cells (sperm and eggs) can impair reproduction in fish and thus affect the population of the species (Valdebenito *et al*., 2015).

Egg quality

Egg quality in fish is essential for successful breeding and fertilization. An important factor in egg quality is proper yolk formation, as the growth and development during the larval stages of various commercially valuable fish species depend on the composition of nutrients and availability in the yolk sac (Chen *et al*., 2023). These maternal nutrients include proteins, carbohydrates, lipids, vitamins, minerals, and ions that are transported from the liver to the ovaries by lipoprotein particles, including vitellogenin. Yolk composition may be affected by nutrition status as described in some fish species such as *Anguilla japonica*, *Anguilla australis*, *Oncorhynchus clarkii*, *Oryzias latipes*, and *Euthynnus lineatus* (Reading *et al*., 2018). In addition, some other maternal factors that may affect egg quality are stored in the egg, such as gene transcription, which guides early embryonic development. Improper regulation of gene or protein expression may lead to poor egg quality and failure within a few hours after fertilization (Cheung *et al*., 2019). This transcription of genes such as apoc1 on lipoprotein metabolism, Cathepsin_Z gene on low egg quality, ccnb3, ccne2, and cycB on reproduction may provide important markers because their expression levels may be used to screen living organisms for possible spawning success. In addition to these inherent factors, stress may lead to ovarian atresia or fertility failure and can affect fish behavior, fertility, and ovulation rate. Finally, aging after ovulation may occur when the eggs are too mature and the fish do not spawn on time, leading to low fertility (Reading *et al*., 2018).

Sperm quality

Fish sperm quality assessment is useful for assessing the outcome of reproduction and successful fertilization. Sperm motility parameters such as sperm velocity are a key feature of performance when helping fish reproduction. Apart from motility, concentration, volume, pH, and osmolality of sperm plasma are also often evaluated and are the main indicators of sperm quality in fish. However, other parameters also determine sperm fertilization potential, including DNA fusion, membrane stability, mitochondrial status, and enzymatic activity. Measuring all of these parameters in fish sperm provides complex information about male fertility and helps improve embryo maintenance protocols as well as gamete transfer and fertilization processes (Kowalski and Cejko, 2019).

Reproduction for restocking

Preserving endangered species of fish and effectively managing invasive species poses a substantial global challenge. Large rivers around the world have faced extensive degradation due to various human activities, such as hydropower generation, flood control measures, water supply systems, and navigation channels. The proliferation of dams has exacerbated these issues, resulting in adverse ecological consequences for local fish populations. This multifaceted issue is of utmost importance for both environmental conservation and economic reasons (Terêncio *et al*., 2021). The construction and operation of hydropower dams can have significant consequences for fish habitats. Dams alter the flow of rivers, obstruct fish migration routes, and modify water temperature and sediment transport, all of which affect fish populations. Balancing the need for clean energy with the preservation of aquatic biodiversity is an ongoing challenge (Costea *et al*., 2021). Flood management strategies often involve channelization and levees, which can disrupt natural river processes. This can lead to the loss of critical fish spawning and rearing habitats. Sustainable flood control measures that consider the needs of both human communities and aquatic life are essential (Hohensinner *et al*., 2018). The diversion of river water for human consumption and the creation of navigation channels can further harm fish habitats. Finding ways to minimize the impact on aquatic ecosystems while meeting the needs of various stakeholders is crucial (Alhakami *et al*., 2022). The proliferation of dams worldwide, driven by the increasing demand for water and energy resources, presents a considerable challenge. Each new dam must be carefully assessed for its environmental impacts, and existing dams should be retrofitted with fish-friendly infrastructure where possible (Kondolf and Yi, 2022).

In general, the conservation of endangered fish species is vital for maintaining biodiversity and preserving fragile ecosystems. As large river systems are often home to unique and endangered species, the negative impacts of human interventions can lead

to their decline. Implementing protective measures and habitat restoration projects are essential to safeguard these vulnerable fish populations. In addition, restocking programs have been applied to secure the existence of the species (Hohensinner *et al*., 2018). In recent decades, due to changes in hydrological conditions due to the construction of dams, spawning activities of Chinese carp species such as *Hypophthalmichthys molitrix*, *Mylopharyngodon piceus*, *Aristichthys nobilis*, and *Ctenopharyngodon idella* have been severely affected. Reproductive management, including spawning, fertilization, spawning, and embryonic development, can be an effective measure to maintain the population of Chinese carp (Chen *et al*., 2021). The spawning ground is very important in the reproduction of commercially valuable species. It is not yet clear why some species are unable to spawn naturally, and for this reason, it is usually attributed to the presence of unknown chronic stressors or the absence of an unknown natural environmental sign (Eissa and Wang, 2016).

The role of phytochemicals

This paper declared the importance of using dietary phytochemicals to improve the reproductive performance of broodstock in aquaculture industry to enhance the quality of eggs and sperm and ultimately, improve fertility. Although using plant-based proteins in aquafeed can negatively affect the sexual development and reproduction of fish due to the presence of anti-nutritional substances (de Jager, 2019), it was previously reported that herbal hormones and bioactive compounds can improve reproduction in fish based on certain concentrations/exposure time in aquaculture industry. Furthermore, herbal supplements containing bioactive compounds can have various effects on aquatic reproductive performance. Phenolic compounds, known for their antioxidant activity, can reduce and control stress in aquatic animals. They can also have weak estrogenic effects, affecting ovarian growth and reproductive functions in mammalians. As there are serious concerns about using synthetic drugs, there is an emerging interest in using medicinal plants to boost fertility in the aquaculture industry. Some physiological responses and pathways related to the role of phytochemicals are presented in Figure 1 (Ahmadifar *et al*., 2021).

Also, many plants like *Vitex agnus-castus* are used for their potential fertility-enhancing properties in humans and livestock. However, there is limited research available on the specific effects of *Vitex agnus-castus* on fish and shellfish species in terms of fertility enhancement. The effects of herbal supplements, on aquatic species can vary depending on the species itself, the dosage, and the specific physiological processes involved in reproduction. a study conducted on zebrafish (*Danio rerio*) showed that an extract of *Vitex agnus-castus* had a positive impact on the reproductive behavior and egg production of female fish. It was found

that the extract increased the number of eggs produced and improved the fertilization rate (Enayat Gholampour *et al*., 2020).

Also, While there is limited research specifically on the effects of *Punica granatum* on fish and shellfish fertility, studies on its bioactive compounds suggest potential positive impacts on reproductive performance. *P. granatum* contains several bioactive compounds, including Polyphenols, Flavonoids, and Tannins, which have been linked to antioxidant, anti-inflammatory, and antimicrobial properties. These properties may indirectly contribute to improved fertility by promoting overall health and reducing oxidative stress in fish and shellfish.

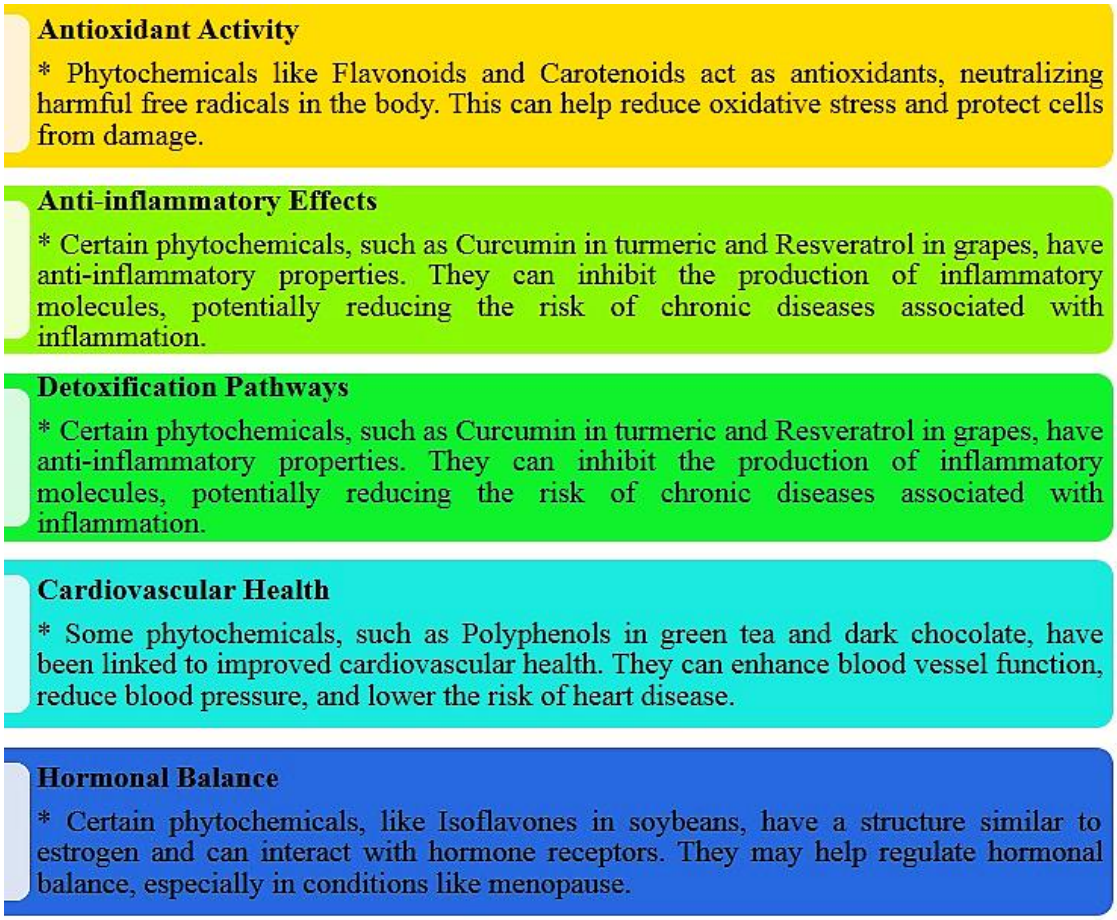

Figure 1: Some physiological responses and pathways related to the role of phytochemicals.

One study conducted on Nile tilapia (*Oreochromis niloticus*) investigated the effects of pomegranate peel extract on reproductive performance (EA Badrey *et al*., 2021). The results showed that the extract positively influenced gonadal development, ovarian maturation, and fecundity in female fish. It was suggested that the antioxidant activity of the extract played a role in enhancing reproductive parameters. In another study on Pacific white shrimp (*Litopenaeus vannamei*), the inclusion of pomegranate peel extract in the diet

was found to improve reproductive performance. The extract was shown to increase the number of spawned eggs, enhance larval survival, and improve the growth and survival of newly hatched larvae (Ismail *et al*., 2019).

The presence of these compounds in other plants such as *Aloe barbadensis* Miller has been confirmed. *Aloe vera* contains various bioactive compounds, including polysaccharides, vitamins (A, C, B, and E), minerals, and enzymes, which have been linked to antioxidant, anti-inflammatory, and immunomodulatory properties. These properties may indirectly contribute to improved fertility by promoting overall health and reducing oxidative stress in fish and shellfish and its gel is very spermatic and increases male fertility by increasing sperm quality (Yavas and Gultekin, 2020).

The study conducted on Nile tilapia fish (*Oreochromis niloticus*) investigated the effects of food supplements with *Aloe vera* gel extract on reproductive performance and the results showed that the extract had a positive effect on the growth of gonads, ovarian maturation and fertility in female fish (Abdel-Aziz *et al*., 2023).

Rubus idaeus L. also contains bioactive compounds, especially flavonoids such as anthocyanins and flavonols, phenolic acids (mostly alginic and gallic acids) and vitamin C, the synthesis of which is influenced by the fertilization system and puberty (Frías-Moreno *et al*., 2021).

Oxidase activity and oxidation lead to the breakdown of protein bonds, DNA damage, and liver disorders, but flavonoids are powerful antioxidant compounds that prevent the oxidation of fats and free radicals by inhibiting protein kinase production (Martemucci *et al*., 2022).

Various studies have been conducted on the nutrition of breeders and juveniles that reduce breeding problems by controlling over generations (Molés *et al*., 2020; Lavin *et al*., 2021; Thoré *et al*., 2021). This review focuses on sustainable approaches such as the use of phytochemicals to control stress and the health of broodstock, facilitate their breeding, and improve the health of their offspring.

Phytochemicals classification and definition

Phytochemicals are chemical compounds that are naturally occurring in plants, they generally have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens, or predators, and they play various biological roles and are classified into different categories based on their chemical structure and functions. In the context of fish, phytochemicals can be introduced based on their biological roles (Table 1) (Sutili *et al*., 2018):

It is important to note that while some phytochemicals can have beneficial effects on fish health, others can be toxic or have negative impacts. The specific biological roles of phytochemicals in fish can vary depending on the compound, dosage, and fish species (Ahmadifar *et al*., 2021).

Among the phytochemicals that have been mentioned as potential health benefits are polyphenols, flavonoids, isoflavones, anthocyanins, phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, and glucosinolates. Table 2 presents antioxidant-rich phytochemicals (Bose *et al*., 2020). The number of phytochemicals in nature may be more than 4,000 different species. From phytochemical extractions and analysis of phenolic characteristics in fruits, it has been found that the concentration of phenolic compounds varies depending on genetics (sex, species, cultivar, and genotype), environmental factors, and maturity status (Samaniego *et al*., 2020).

Without any doubt, broodstock nutrition is one of the most important factors in reproductive success, So, The effect of phytochemicals on broodstock nutrition and reproductive success can be significant.

Table 1: The classification of phytochemicals and their biological roles.

Phytochemicals	biological roles
Phenolics	Phenolic compounds, such as flavonoids and phenolic acids, are widely distributed in plants and have antioxidant properties. These compounds can scavenge free radicals and protect fish tissues from oxidative damage.
Terpenoids	Terpenoids are a diverse group of compounds that include carotenoids, steroids, and essential oils. Carotenoids, such as astaxanthin, are responsible for the vibrant colors observed in some fish species. They also act as antioxidants and have immune-enhancing properties.
Alkaloids	Alkaloids are nitrogen-containing compounds found in various plants. Some alkaloids, such as caffeine and theobromine, can act as stimulants in fish, affecting their behavior and metabolism.
Saponins	Saponins are glycosides that have detergent-like properties. They can form complexes with cholesterol in fish cell membranes, leading to increased membrane permeability. Saponins also have immune-modulating effects and may enhance the immune response in fish.
Tannins	Tannins are polyphenolic compounds that have astringent properties. They can bind to proteins and form complexes, which can affect the digestion and absorption of nutrients in fish.
Glycosides	Glycosides are compounds that contain a sugar molecule attached to a non-sugar moiety. Some glycosides, such as cardiac glycosides, can affect the cardiovascular system of fish by altering heart rate and contractility.
Organic acids	Organic acids, including citric acid and malic acid, are involved in various metabolic processes in fish. They can regulate pH levels, enhance nutrient absorption, and act as antimicrobial agents.

Phytochemicals can enhance the overall health and well-being of broodstock, leading to improved reproductive performance. Phytochemicals have several positive effects on broodstock nutrition: Phytochemicals with Antioxidant activity, neutralize harmful free radicals, improve overall health and reproductive success, enhance the immune system, increasing resistance to diseases and infections, Some phytochemicals act like hormones,

positively impacting reproductive hormones and broodstock reproductive success, and reduce inflammation, promoting a healthier environment for reproduction, Finally, certain phytochemicals enhance nutrient absorption, supporting overall health and reproductive success (Ahmadifar *et al*., 2021).

It is important to note that the specific effects of phytochemicals on broodstock nutrition and reproductive success may vary depending on the species, diet composition, and other factors. Additionally, the optimal dosage and combination of phytochemicals for broodstock nutrition need to be carefully determined through scientific research and trials. In summary, phytochemicals can positively influence broodstock nutrition and reproductive success through their antioxidant, immunomodulatory, hormonal, anti-inflammatory, and nutrient absorption-enhancing effects. Including phytochemical-rich ingredients in the diet of broodstock can be beneficial for their overall health and reproductive performance (Ghosi Mobaraki *et al*., 2020).

Table 2: Antioxidant-rich phytochemicals with their food sources and health benefits.

Phytochemicals	Sources	health benefits
Carotenoids	Carrots, Tomatoes, Parsley, Orange, Green Leafy, Vegetables, Chenopods, Fenugreek, Spinach, Cabbage, Radish, Turnips.	Antioxidants protect against uterine, prostate, colorectal, lung, and digestive tract cancers
Phytosterols	Vegetables, Nuts, Fruits, Seeds	Suppress the growth of diverse tumor cell lines via initiation of apoptosis and concomitant arrest of cells in the G1 phase of the cell cycle.
Limonoids	Citrus Fruits	Inhibiting phase 1 enzymes and inducing phase 2 detoxification enzymes in liver, provide protection to lung tissue. Detoxify enzymes.
Polyphenols (Flavonoids, Isoflavonoids, Anthocyanidins)	Fruits, Vegetables, Cereals, Beverages, Legumes, Chocolates, Oilseeds	Action against free radicals, free radicals mediated cellular signaling, inflammation, allergies, platelet aggregation, and hepatotoxins
Glucosinolates	Cruciferous Vegetables	Protection against cancer of colon, rectum, and stomach
Phytoestrogens	Legumes, Verrie, Whole Grains, Cereals, Red Wine, Peanuts, Red Grapes	Protection against bone loss and heart disease, cardiovascular disease, breast and uterine cancers
Terpenoids	Mosses, Liverworts, Algae, Lichens, Mushrooms	Antimicrobial, antiparasitic, antiviral, anti-allergic, anti-inflammatory, chemotherapeutic, antihyperglycemic, antispasmodic
Polysaccharides	Fruits And Vegetables	Antimicrobial, antiparasitic, antiviral, anti-allergic, anti-inflammatory, lowering serum, enhances defense mechanism
Saponins	Oats, Leaves, Flowers, and Green Fruits of Tomato	Protection against pathogens, antimicrobial, anti-inflammatory, and antiulcer agent

The roles of phytochemicals in reproductive function are as follows:

Gonad maturation

Several plants contain bioactive compounds with the potential to modify reproduction to improve efficiency and ultimately increase production. the compounds have been shown to affect the brain or gonads directly via a well-connected axis called the hypothalamic-pituitary-gonadal (HPG) axis where important peptide hormones such as gonadotropin-releasing hormone (GnRH) and gonadotropin-inhibiting hormone (GnIH) are secreted. The hypothalamic-pituitary-pituitary-gonadal axis (HPD) integrates a lot of information such as external environment, social stimuli from potential mating partners, and nutritional status to regulate the sequential processes of gonad maturation, spermatogenesis or oogenesis, reproductive behavior, and spawning (Mir and Bhat, 2021). In males and females, the production of FSH and LH, respectively, in the anterior pituitary is controlled by the secretion of gonadotropin-releasing hormone (GRH) from the hypothalamus, which is transmitted to the pituitary gland in the port system of the hypothalamic-pituitary gland. The feedback effects of testosterone, estrogen, and statin (made in the testes and ovaries in response to follicle-stimulating hormone stimulation) on the hypothalamus, along with LH and FSH levels in the anterior pituitary gland, are controlled in the bloodstream (Ghahvehchi-Hosseini *et al*., 2021). Plant extracts along with their bioactive compounds have been tested in many fish species to increase reproductive function and develop gonads and plants containing alkaloids have been reported to reduce the concentration of pituitary-gonadal hormones (Mir and Bhat, 2021).

In a study, *Moringa oleifera* improved the quality of *Clarias gariepinus* gland by 6% more than the control group and 8% better than *Carica* papaya (Munir *et al*., 2022). In another study, the potential of herbal plants and lactic acid bacteria to improve the quality of sperm of *Oreochromis niloticus* infected with mercury was evaluated. The results showed that supplementation of dietary supplements did not alter fish GSI, except for *A. sativum* supplements, which significantly reduced GSI and increased the motility and viability of fish sperm exposed to Hg. In fact, probiotic supplements and herbal plants can restore the quality of damaged sperm (motility and viability) of *O. niloticus* exposed to heavy metals mercury (Emon *et al*., 2023). a study was carried out to investigate the effects of the different inclusion levels of pawpaw seed meal on gonads of genetically improved farmed tilapia (GIFT). Based on this, seeds of pawpaw are an efficient agent of inducing sterility as they are destructive to ovary and testes tissues so dry seeds of pawpaw are recommended for use in controlling tilapia breeding (Christopher *et al*., 2021).

Egg maturity

Fertilization in most fish species depends on the production of high-quality eggs from females and sperm from males to have successful fertility. Factors such as the number of spawns, collection time, water quality, temperature, and type of feeding greatly impact fertility (Gazsi *et al*., 2021). During the follicular growth stages, the follicular fluid provides the necessary conditions for the growth and maturation of oocytes, and since the growth and maturation of oocytes are influenced by the composition of follicular fluid, any change in the biochemical composition of follicular fluid affects the quality of oocytes (Marchiani *et al*., 2020). The physalins in the extract of *Physalis alkekengi* have a steroid-like structure and can easily cross cell membranes and bind to steroid receptors in the cytoplasm, enter their receptor inside the nucleus, and attach to a portion of DNA, and in protein synthesis and Or the enzyme disrupts, and negatively affects pregnancy and ovulation (Mashayekh *et al*., 2020). Estrogen and progesterone are known to be vital hormones for maintaining pregnancy and blastocyst implantation in humans and other mammals. These two hormones are controlled by the gonadotropins (FSH and LH). Biological factors affect the levels of these hormones and can inhibit ovarian function (Mir and Bhat, 2021). Injection of *Matricaria recutita* extract due to the presence of phytoestrogen compounds increased the growth and maturation of oocytes in *Trichogaster trichopterus*. Soy genistein also prevents the destruction of oocytes during ovarian differentiation and reduces oocyte death (Ahmadniaye Motlagh *et al*., 2023). The hydroalcoholic extract of *Artemisia absinthium* with plant compounds, including carbohydrates, alkaloids, saponins, phytosterols, proteins, amino acids, tannins, phenolic compounds, flavonoids in high amounts reduces oogenesis by acting on the hormones of the pituitary gland and ovary, and therefore the consumption of high doses of the *Artemisia absinthium* plant has a detrimental effect on the female reproductive system and shows signs of infertility. In another study, the effect of alcoholic extract of *Artemisia annula* on rat ovary and testicular tissue was investigated and the results reported histological changes and malignant lesions on the testes and ovaries, including corpus luteum destruction, reduction and destruction of follicles, and connective tissue destruction (Slighoua *et al*., 2020).

Sperm motility

The most common cause of infertility in male is their inability to produce enough healthy, active, and motile sperm. The ability to conceive in men depends to a large extent on the number, quality, motility, and shape of the sperm, and a disorder in any of these factors can cause male infertility. Lack of testicular development and growth, diseases of the reproductive system, increased scrotal temperature, immune problems, endocrine disorders, lifestyle, environmental and nutritional factors are considered the main causes of male

infertility that negatively affect sperm parameters (Gholami-Ahangaran *et al*., 2021). Some chemical compounds in plants may improve the hormonal environment or spermatogenic processes in fish.

Petroselinum crispum leaves, due to their antioxidant properties, prevent the formation of free radicals and lipid peroxidation and prevent sperm cell damage by free radicals. Improves sperm quality and fertility parameters and increases gonadal pituitary hormones (Badr *et al*., 2021). There is evidence to suggest that ginger enhances semen quality by improving various sperm parameters, viability, motility, morphology, and DNA integrity. Based on research results in different species, ginger seems to have strong antioxidant properties (due to the presence of active phenolic compounds) and androgenic activity. Ginger enhances semen quality and increases sperm fertility by disrupting the production of free radicals, resolving oxidative chain reactions, reducing oxidative stress, and altering the levels of gonadotropin (LH, FSH) and sex hormones (such as testosterone). The antioxidant and androgenic properties of ginger cause sperm to integrate with the natural morphological structure (head, middle, and tail) and chromatin. The rate of DNA failure and damage to the mitochondrial genome in these cells is minimal, and they have the highest motility, the highest viability, and the best fertility. Therefore, the use of ginger significantly improves the biological parameters of sperm (number, overall motility, survival rate, and normal morphology) and also increases all specialized sperm fertility indices (Gholami-Ahangaran *et al*., 2021).

Adding antioxidants to the diet improves sperm health. Phytochemicals also act as neurotransmitters, regulate hormonal neuronal functions, stimulate the activity of sperm-producing tubules, and regulate androgen circulation levels. As a result, breeder performance can be enhanced by the use of phytochemical rejuvenators, which ultimately lead to the production of gametes and healthier offspring (Muhiardi *et al*., 2020). Also, *Crocus sativus* L. by strengthening the antioxidant defense system, in addition to reducing oxidative stress, can also increase the lifespan of sperm and the number of live sperm. In addition, the compounds in saffron stigma, in addition to having a direct effect on the steroidogenesis processes of Leydig cells and spermatogenesis, stimulate the production of FSH, LH, and testosterone by proliferating the epithelial cells of the seminiferous tubules and increasing the activity of Leydig cells. In this way, it increases the number of spermatocytes and spermatogenesis (Chorosho *et al*., 2023). The presence of antioxidants in *Matricaria chamomilla* extract is one of the possible mechanisms of the plant's effect on increasing sperm count. Free oxygen species reduce the motility and number of sperm through the peroxidation of sperm membrane lipids. Chamomile is rich in flavonoids and phenolic compounds that are effective antioxidants in neutralizing oxygenated radicals, thus protecting sperm cell

damage by free radicals and improving sperm quality and fertility parameters (Afrigan *et al*., 2019).

Reviewing the studies conducted in this field, it seems that the use of medicinal plants as immune stimulants is a suitable alternative to antibiotics, vaccines, and synthetic compounds.

Reproductive behavior and stress reduction

One of the effective factors in infertility is oxidative stress which is the result of an imbalance between reactive oxygen species and antioxidants in the body and can lead to sperm damage, deformity, and ultimately male infertility. Testicular tissue is very vulnerable to oxidative stress because it has high metabolic activity and a significant amount of highly unsaturated fatty acids. Free radicals disrupt various parts of the testicles, especially testicular germ cells, leading to atrophy of the testicles and reduced sperm production (Asadi *et al*., 2017). Oxidative stress is known to be a major cause of testicular dysfunction, therefore, many researchers have used antioxidant compounds to reduce fertility damage (Alahmar *et al*., 2021).

For example, olive leaf extract containing flavonoid and polyphenolic compounds in testicular oxidative stress in rats. Also, fenugreek seed extract reduced oxidative stress and testicular tissue damage and improved spermatogenesis in rats (Erfani Majd *et al*., 2021).

Inhibition of reproductive function by activating the stress response has been observed since ancient times. At the neuroendocrine level, several studies have focused on the interaction between corticotropin-releasing systems (CRF) and gonadotropin-releasing hormone (GnRH) systems in vertebrates. The greatest effect of stress on reproduction seems to be to change the amount of blood steroids that the reproductive cycle proceeds only with the presence of sufficient and necessary amounts of them. Manipulation stress reduces plasma testosterone levels and 17-beta-estradiol levels, which in turn lowers vitellogenin levels and ultimately reduces ovarian volume and size, and more importantly, the survival rate of fertilized eggs (because egg yolk is low) (Kriegsfeld *et al*., 2018). Many experiments have shown that cortisol released into the bloodstream as a result of stress is a major factor in reduced reproduction. This hormone also reduces the level of estradiol and vitlogin in the bloodstream and also reduces the size of the gonads. Some experts believe that cortisol does not stop the secretion of estradiol, but reduces its biological activity by stimulating the production of binding proteins (Genazzani *et al*., 2020). The antioxidant properties of extracts of some plants such as *H. rosa-sinensis* against oxidative stress can lead to enhanced fertility by changing the production of hormones necessary for spermatogenesis with its simultaneous effect on fertility (Abedi *et al*., 2020).

Health status

Many herbs are designed to improve health, but their mechanism of action is still unclear. *Moringa oleifera* leaves in

either the feed or water to promote good health which is vital in the growth process of fish, especially broodstock. Also, *C. papaya* leaf is recommended in the treatment of fish disease due to its boost in White Blood Cell (WBC) production (Hamid *et al*., 2022). The use of phytochemicals is considered an alternative to antibiotics and is useful for disease management and control in aquatic animals. Medicinal plants are an integral part of the cultural heritage of many people from different cultures and countries and therefore are closely related to maintaining good health. phytochemicals are biologically active plant compounds that are associated with a reduced risk of various chronic diseases due to their anti-inflammatory, antioxidant, and anti-free radicals (highly active and carcinogenic) effects (Mokhtari *et al*., 2021).

Research gaps and further investigations

There is limited research specifically on the dietary administration of phytochemicals to ameliorate reproductive performance in aquaculture species. However, based on general knowledge of phytochemical effects and their potential benefits, the following recommendations can be made for broodstock nutrition management: using a diverse range of polyphenols to maximize their antioxidant and anti-inflammatory effects including carotenoid-rich substances is recommended. Carotenoids are known for their antioxidant properties and can enhance reproductive success. Adding phytochemicals to broodstock diet can be considered a potential alternative for synthetic drugs to induce or inhibit spawning. The dosage recommendation of commercial herbal additives depends on the plant species, extraction methods, specific product features, and should be followed by the manufacturer's guidelines and recommendations. It is important to note that further research is needed to determine the optimal dietary doses of phytochemicals for different aquaculture species to achieve the best reproductive performance.

In today's world, the use of medicinal plants as a substitute for synthetic drugs to improve fertility is considered due to the support of the World Health Organization (WHO) for maintaining public health and reproductive health. Several studies have shown that some medicinal plants with fertility-enhancing properties in male by improving antioxidant activity can prevent the formation of free radicals and lipid peroxidation and reduce oxidative stress and prevent cellular damage in the sperm cells. They can also increase the number of testicular vessels, the lifespan, and the number of sperm, increase sperm quality, and protect germ cells. On the other hand, herbs and their derivatives can enhance the activity of different levels of the hypothalamic-pituitary-gonadal axis, the secretion of LH and testosterone. Using medicinal plants can effectively improve fertility (*e.g.* sperm survival and mortality), pituitary hormone levels, histological changes in the testes, and spermatid depletion in males. However, further clinical studies

are required in fish and shellfish. On the other hand, some medicinal plants inhibit the uptake of 5 alpha-reductase (a factor that converts testosterone to dihydrotestosterone) resulting in improving gonadotropins and testosterone, the affinity for sex-specific protein, thickness of the basement membrane, sperm motility and sperm viability, and reducing germinal epithelial cells. Investigations have been often focused on the effects of phytochemicals on human and animal models, however, there is scarce information regarding dietary phytochemicals on fish reproductive performance and further research is required in the future.

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