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REVIEW



## Recent development in zebrafish model for bioactivity and safety evaluation of natural products

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### ABSTRACT

The zebrafish is a species of freshwater fish, popular in aquariums and laboratories. Several advantageous features have facilitated zebrafish to be extensively utilized as a valuable vertebrate model in the lab. It has been well-recognized that natural products possess multiple health benefits for humans. With the increasing demand for natural products in the development of functional foods, nutraceuticals, and natural cosmetics, the zebrafish has emerged as an unprecedented tool for rapidly and economically screening and identifying safe and effective substances from natural products. This review first summarized the key factors for the management of zebrafish in the laboratory, followed by highlighting the current progress on the establishment and applications of zebrafish models in the bioactivity evaluation of natural products. In addition, the zebrafish models used for assessing the potential toxicity or health risks of natural products were involved as well. Overall, this review indicates that zebrafish are promising animal models for the bioactivity and safety evaluation of natural products, and zebrafish models can accelerate the discovery of novel natural products with potential health functions.

### KEYWORDS

Zebrafish; animal model; bioactive compounds; health benefits; safety assessment

### 1. Introduction

The zebrafish (*Danio rerio*), is a member of the cyprinid fishes, widely distributed in freshwater habitats. Zebrafish are popular in aquariums and for scientific research because of their distinctive characteristics (Caro et al. 2016; Lawrence 2007). First of all, zebrafish are small, with adults usually not exceeding 3 – 4 cm in length, while embryos and larvae can fit in 96-well plates. The tiny body size allows a large number of zebrafish to be placed in a small space, and only requires a low amount of substances to be assessed. Additionally, zebrafish are prolific breeders, and spawning in weekly intervals may produce up to hundreds of fertilized eggs. The large clutch size makes it possible to collect thousands of embryos to evaluate an array of bioactive compounds. Besides, zebrafish are transparent in their early developmental stages, thereby enabling visualization of a variety of complex dynamic processes *in vivo*. Furthermore, zebrafish are well-suited to genetic manipulations, since the whole genome sequence of zebrafish has been reported (Howe et al. 2013). Rapid establishment of genetic manipulation zebrafish lines has become possible with the progress

of genome editing technologies, such as clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9, transcription activator-like effector nucleases (TALEN), and zinc finger nucleases (ZFN) (Albadri, Bene, and Revenu 2017; Y. Zhang et al. 2017; Zu et al. 2013). Moreover, zebrafish and humans have many similarities in genetics, anatomy, and physiology, making them an ideal model for studying the health benefits and diseases of humans (Howe et al. 2013). Last but not least, zebrafish are cheap and quite hardy in captivity, which makes them economically affordable and technically feasible in most laboratories. All these advantageous features have facilitated zebrafish to be extensively utilized as a valuable *in vivo* model in different research fields.

With the increasing demanding for natural products, such as microalgae (Barros de Medeiros et al. 2021), phenolic compounds (Cristina et al. 2017), probiotics (Siciliano et al. 2021), and omega-3 fatty acids (Gumus and Gharibzahedi 2021), in the development of functional food, nutraceuticals, and natural cosmetics, the zebrafish has emerged and been validated to be an unprecedented tool for rapidly and economically investigating the bioactivities and

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safety of natural products. A recent review discussed the modeling of developmental, mental, and metabolic disorders in zebrafish mainly from the genetic aspect (Choi et al. 2021). In addition, a recent review summarized the neurotoxicity of aquatic contaminants to zebrafish (Fitzgerald et al. 2021). However, few reviews summarize the establishment of zebrafish models and their applications in the evaluation of various bioactivities of natural products. Aiming to better understand the zebrafish models and their applications in the evaluation of natural product bioactivities, this review paper mainly collected the literature about the exploitation of zebrafish for natural product evaluation in the last five years, based on the databases of Web of Science Core Collection, PubMed, and Scopus. In this review, the key factors for the management of zebrafish in the laboratory and the possible approaches to delivering a precise dose of substances or insoluble substances to zebrafish are discussed. Besides, various zebrafish models for evaluating the bioactivities of natural products are summarized and discussed. The bioactivities discussed in this article include antioxidation, anti-inflammation, neuroprotection, cardioprotection, anti-cancer, regulation of metabolic disorders, intestinal protection, renal protection, anti-osteoporosis, and anti-melanogenesis. In addition, safety assessment by using zebrafish models is also involved in this article. Therefore, this review can attract more attention to zebrafish models, facilitating more useful zebrafish models to be introduced or established for the evaluation and development of functional foods, nutraceuticals, and natural cosmetics.

## 2. Management of zebrafish in the laboratory

In the following part, the key factors for the regular care and management of zebrafish in the laboratory, including environmental requiring, feeding, breeding, larval rearing, as well as the natural product delivery methods in zebrafish models are briefly summarized and discussed.

### 2.1. Environmental requiring

Zebrafish are easy to be cultivated, and can tolerate a wide range of environmental factors, facilitating it to be extensively used in different fields (Tsang et al. 2017). Although zebrafish have a good performance in environmental tolerance, it is necessary to provide an optimal living environment for the management of zebrafish in the laboratory. According to Tsang et al. (2017), the temperature of 28 °C, lighting condition of 14: 10 hours (light: dark), and pH of 7–8 are preferred.

### 2.2. Feeding

It is necessary to provide a balanced and complete diet to meet the nutritional requirements of zebrafish in the laboratory. In most cases, the adult zebrafish can be fed twice a day using commercially formulated diets with 300/400 microns in size or live brine shrimps (*Artemia nauplii*) hatched in the laboratory or the combination of both

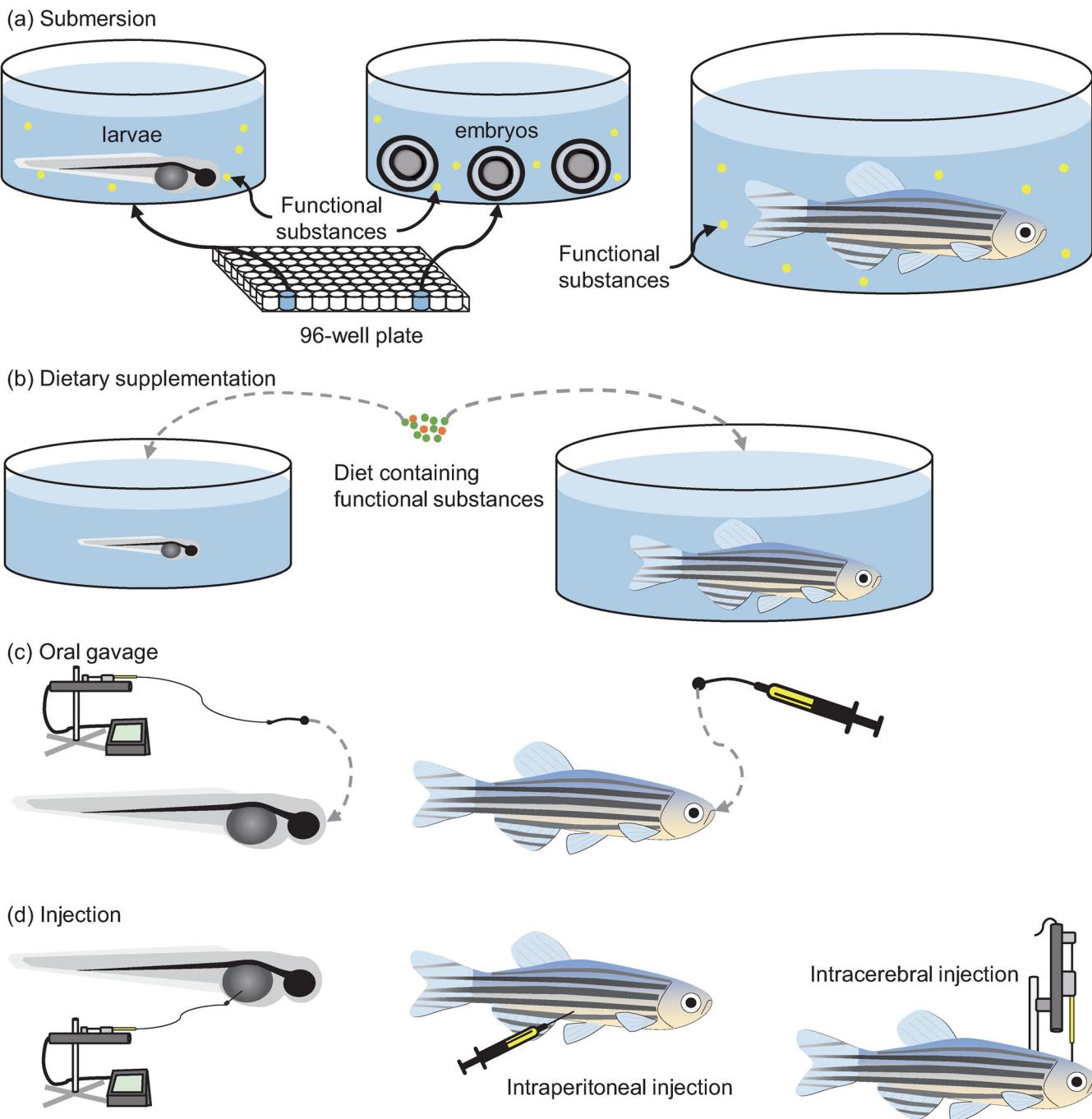
(Avdesh et al. 2012). Usually, food accounting for 3–5% of body weight per day is recommended for feeding zebrafish in the laboratory (Lawrence et al. 2012). Overfeeding should be avoided, as it can adversely influence the water quality, such as increasing nitrate contents and suddenly shifting the pH, thus affecting the viability and breeding of zebrafish (Tsang et al. 2017). In addition, the food residues and feces should be removed timely and the fish tanks need to be cleaned regularly.

### 2.3. Breeding

There are numerous factors to be considered in zebrafish breeding, including biotic factors and abiotic environmental parameters (Kolb, Hildebrandt, and Lawrence 2018; Tsang et al. 2017). For instance, the mating behavior is influenced by the sex ratio and the number of breeders, as well as the breeding chamber volume, while the spawning and egg production can be affected by different factors, including the age, size, and health status of the breeders, the interval that breeders are used for spawning, the light cycle, the temperature, the diet, and so on. Adult zebrafish are sexually dimorphic, and males can be distinguished from females by their brighter color and slender body shape, while females generally have a larger underbelly and slightly rounded body shape (Avdesh et al. 2012). It has been believed that setting up an extra male for breeding, such as two males with one female or three males with two females, would improve the efficiency of fertilization, however, it was further proposed that the use of males should be reduced because of the interference of male-male aggressive behaviors (Tsang et al. 2017). Zebrafish can reach sexual maturity at the age of two months (Lawrence et al. 2012) or a little earlier (Dabrowski and Miller 2018), and are able to maintain the reproductive activity throughout most of their life cycle, but zebrafish are rarely used as breeders in the laboratory if they are too young or too old (Tsang et al. 2017). Moreover, the spawning frequency is also an important consideration of zebrafish breeding, and once a week is thought to be optimal (Kurtzman et al. 2010). Too frequent spawning may reduce the quality and quantity of eggs, but too scant spawning may cause egg reabsorption and even blunt reproductive activity (Tsang et al. 2017). Furthermore, the photoperiod is quite important for zebrafish reproduction since they initiate the mating behavior at the onset of light after a period of total darkness, with spawning shortly thereafter (Avdesh et al. 2012). After breeding, the eggs can be gently collected by the plastic pipette, washed by the deionized water, and then transferred into a clean Petri dish or other containers filled with water or embryo medium. Notably, the unfertilized eggs should be removed promptly to reduce the risk of infection.

### 2.4. Larval rearing

Fertilized eggs can be kept at a constant temperature of  $28 \pm 0.5$  °C for 2–3 days until the larvae are hatched, and a lower temperature would prolong the development of



**Figure 1.** Natural product-delivery methods in zebrafish. (a) Submersion in water with compounds dissolved. (b) Feeding a diet with compounds supplemented. (c) Oral gavage with compound solutions after anesthesia. (d) Injection of compound solutions after anesthesia.

embryos. The larval zebrafish display a low level of spontaneous activity at the first two days after hatching. During this time, the yolk-sac reserves are sufficient to meet their nutritional demands without additional feeding. Then, the yolk is quickly exhausted, and the diets must be introduced. Live diets, such as rotifers and paramecium, or commercially formulated diets with 100 microns in size, are recommended food for young larval zebrafish, and then the food can be slowly replaced by 200 microns, and then 300/400 microns (Avdesh et al. 2012). Compared to the adults, diets for larval zebrafish need to be presented more frequently with a smaller amount each time. It usually takes 2–4 months for the zebrafish to grow up to maturity, but the time may be

shortened to 45 days under certain rearing conditions (e.g., water salinity, algal turbidity, illumination, and food availability) (Dabrowski and Miller 2018).

## 2.5. Delivering ways of natural compounds

The most common way is to add these compounds directly into the medium or water in which the zebrafish embryos, larvae, or adults are raised (Figure 1a) (Caro et al. 2016). However, it is impossible to evaluate some insoluble substances and difficult to determine the precise dosage in this way. There are some alternative techniques available to solve the problems (Figure 1b–d). Encapsulation and dietary

supplementation can be used for the delivery of insoluble substances. In previous studies, cryptolepine from *Cryptolepis sanguinolenta* (Mante et al. 2021), puerarin (Chen et al. 2019), and baicalin (Song et al. 2020) were encapsulated to increase their solubility before administration to zebrafish. In addition, insoluble substances were reported to be incorporated into the diet to make pellets with a diameter of 5 mm (Santos et al. 2020). Oral gavage and injection can be used for the delivery of a precise dosage of solution. With regard to oral gavage, for example, a cut needle tip (22 gauge) attached to a flexible tubing was used to inject a precise dose of the solution to the intestinal tract of anesthetized adult fish (Collymore, Rasmussen, and Tolwani 2013). The extension of the tip was stopped after it passed through the gills, and then the solution was slowly injected. By using this method, 88% of the fishes were uneventfully recovered. Injection can also be used for the gavage of compounds. For example, before injection, zebrafish were kept in a Petri dish with antibiotics and tricaine at 37°C until the fishes got anesthetized (Yan et al. 2020). After that, they were placed on a stage under microscopy and in their cavity (between the last two pleural ribs) were inserted by a needle (22 gauge) to inject the solution, and the injected fish were then transferred into a new Petri dish with antibiotics at 37°C. Due to a certain degree of technical difficulty, these techniques have been seldom used for the evaluation of natural products up to now.

### **3. Zebrafish models for evaluating the bioactivities of natural products**

Natural product extracts or purified compounds exhibit a broad spectrum of bioactivities, such as antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, and anti-obesity effects (Gandhi et al. 2020; Mao et al. 2019; Meng et al. 2020; Tang et al. 2019). In recent years, a variety of zebrafish models have been used for the bioactivity evaluation of natural products. The current zebrafish models employed to evaluate the bioactivities of natural products are summarized in Figure 2, which are discussed below in detail.

#### **3.1. Zebrafish models for the evaluation of antioxidant capacity**

The zebrafish has been extensively used as an *in vivo* model to screen the bioactive components from natural sources with antioxidant potential in recent years, and related studies are summarized in Table 1. The oxidative stress models of zebrafish embryos or larvae can be induced by different chemicals, such as 2,2'-azobis(2-methylpropionamidine)dihydrochloride (AAPH), hydrogen peroxide ( $H_2O_2$ ), and *tert*-butyl hydroperoxide (*t*-BHP), and can also be induced by electromagnetic radiation, such as ultraviolet B (UVB) and gamma-ray. These zebrafish models are commonly applied to evaluate the antioxidant capacity of food functional compounds or extracts. After the exposure of oxidative inducers, the changes of biomarkers, such as reactive oxygen species (ROS), nitric oxide (NO), lipid peroxidation, antioxidant enzyme activities, malondialdehyde (MDA), heart-beating

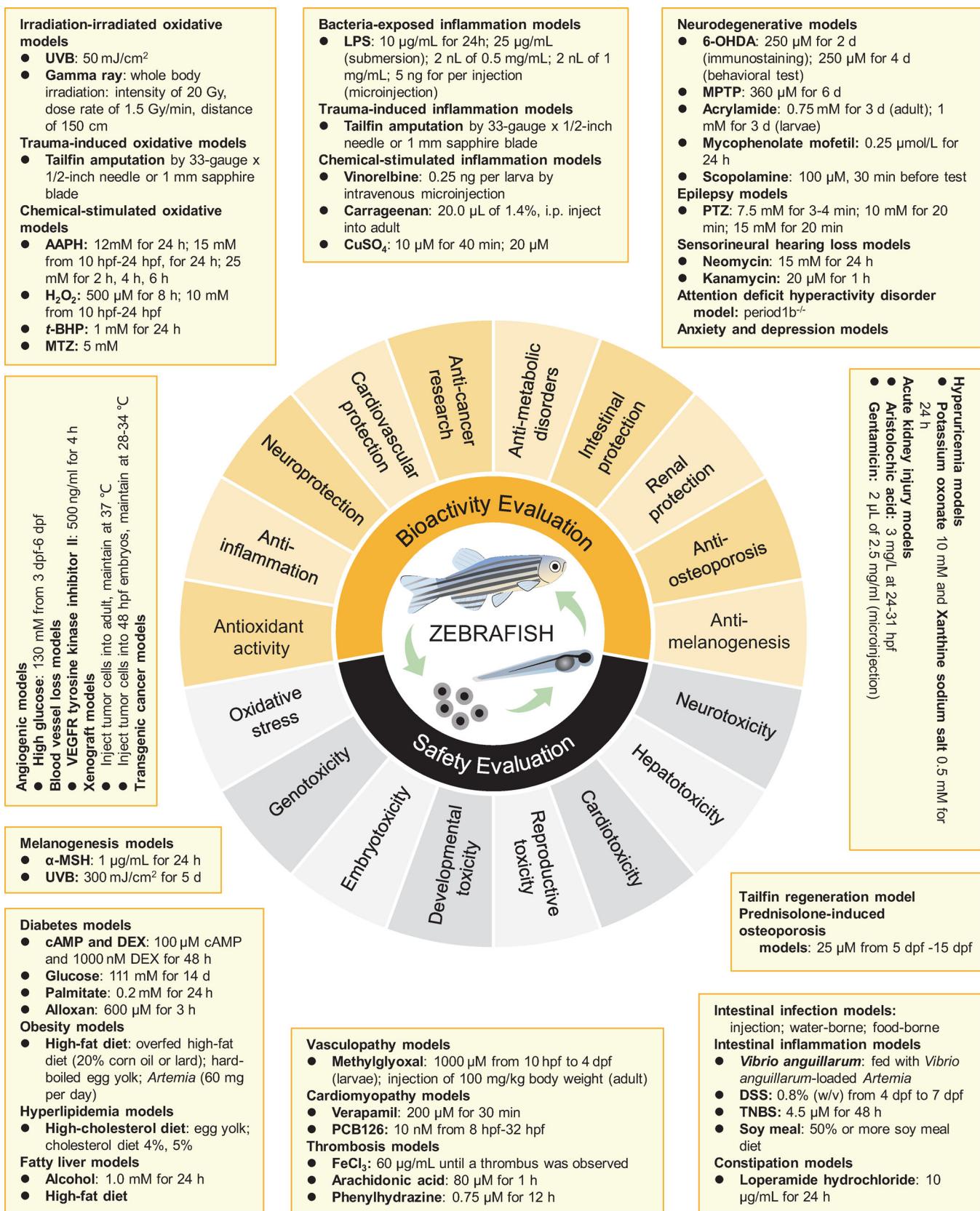
rate, cell death, and survival rate, are generally used to evaluate the antioxidant capabilities of natural product (Carrillo et al. 2016; Carrillo, Guzmán, and Vilcacundo 2017; T. He et al. 2020; Oh et al. 2020).

Several other oxidative stress models of zebrafish have also been developed. A recent study investigated the antioxidant activities of water extracts of *Antirhea borbonica* in erythrocytes by creating a methylglyoxal (MGO)-mediated glycoxidative damage model of adult zebrafish through injecting with 2.5  $\mu$ L of MGO (100 mg/kg of body weight), and the intracellular ROS levels of the red blood cells were evaluated by flow cytometry assay using a 2'-7'dichlorofluorescin diacetate probe (Delveaux et al. 2020). Another study determined the *in vivo* antioxidant activities of anthocyanins extracted from *Titanicus* edible flowers by using a caudal fin injury-induced oxidative stress model of zebrafish larvae, and the CellROX-Orange assay was performed to measure the wound-induced ROS accumulation, while a transgenic zebrafish line, Tg(mpeg1: EGFP), was used in this research for macrophage imaging (Chensom et al. 2020). Another transgenic zebrafish model, Tg(krt4: NTR-hKikGR)<sup>cy17</sup>, was introduced to evaluate the *in vivo* antioxidant capacity of the thyme oils by counting fluorescence spots on the epidermal cells of the transgenic fish, which overexpressed the NTR-hKikGR fusion protein driven by the skin-specific krt4 promoter, and the number of fluorescence spots on the skin cells was significantly decreased under the oxidative stress induced by the treatment of metronidazole (MTZ) (T. He et al. 2020). The *in vivo* antioxidant activity from soybean was also evaluated in MTZ-induced Tg(krt4: NTR-hKikGR)<sup>cy17</sup> larval zebrafish as well (X. B. Li et al. 2020). In addition, zebrafish embryos under normal physiological conditions were also used to assess the antioxidant capacity of *Hypericum hookerianum* aqueous extracts in a liposome system by investigating the mRNA expression of antioxidant genes, such as superoxide dismutase (SOD) and catalase (CAT) (Pradeep et al. 2019).

#### **3.2. Zebrafish models for the evaluation of anti-inflammatory property**

A variety of zebrafish inflammation models induced by different chemicals or amputation of the tailfin have been successfully established to screen anti-inflammatory compounds from natural products, such as polysaccharides, lipids, triterpenoids, diterpenoids, isoflavone, ginsenoside, chlorogenic acid, cajaninstilbene acid, caseahomopene A, and tuberatolide B, which have been discovered to have potential anti-inflammatory abilities based on zebrafish models (Table 1).

The lipopolysaccharide (LPS)-stimulated zebrafish inflammation model has been widely used for *in vivo* anti-inflammatory research in recent years. The model is established through the exposure of zebrafish embryos to LPS or by microinjection of LPS in the yolk of zebrafish larvae. LPS-induced changes in NO formation, ROS production, cell death, heart-beating rate, survival rate, as well as the expression of anti-inflammatory genes, are key biomarkers to reflect the potent anti-inflammatory properties. Besides,



**Figure 2.** Application of zebrafish models in bioactivity and safety evaluation of natural products. Zebrafish models for the bioactivity evaluation are highlighted in boxes with a concise description of the modeling approach. Abbreviations: AAPH, 2,2'-azobis(2-methylpropionamidine)dihydrochloride; t-BHP, *tert*-butyl hydroperoxide; MTZ, metronidazole; UVB, ultraviolet B; LPS, lipopolysaccharide; 6-OHDA, 6-hydroxydopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PTZ, pentylenetetrazole; VEGFR, vascular endothelial growth factor receptor; cAMP, cyclic adenosine monophosphate; DEX, dexamethasone; PCB126, 3,3',4,4'-pentachlorobiphenyl; DSS, dextran sodium sulfate; TNBS, 2, 4, 6-trinitrobenzene sulfonic acid;  $\alpha$ -MSH,  $\alpha$ -melanocyte-stimulating hormone.

**Table 1.** Application of zebrafish models in the bioactivity evaluation of natural products.

| Natural products                       | Extracts/Compounds             | Models   | Main Effects and Mechanisms  | References   |
|--|--------------------------------|--|--|--|
| Antioxidant activity                   |                                |  |  |  |
| <i>Argentine Patagonia barberry</i>    | Ethanol extracts               | AAPH-induced zebrafish embryos   | Reducing ROS production  | Boeri et al. (2020)                                      |
| <i>Undaria pinnatifida sporophylls</i> | Fucoidan                       | AAPH-induced zebrafish larvae  | Reducing cell death, ROS generation, and lipid peroxidation production   | Oh et al. (2020)   |
| <i>Sargassum horneri</i>               | (-)Loliolide                   | AAPH-induced zebrafish embryos   | Reducing ROS level and heartbeat rate; improving survival rate   | H. S. Kim et al. (2020)                                  |
| <i>Thymus quinquecostatus Celak</i>    | Thyme oils                     | AAPH-induced zebrafish larvae; MTZ-induced Tg(krt4: NTR-hKikGR) <sup>cY17</sup> zebrafish larvae | Reducing ROS production and lipid peroxidation; enhancing endogenous antioxidant system by activating Keap1/Nrf2 pathway | T. He et al. (2020)                                      |
| <i>Sargassum fulvellum</i>             | Polysaccharides                | AAPH-stimulated zebrafish larvae   | Improving survival rate; reducing heart rate, ROS production, cell death, and lipid peroxidation levels                  | L. Wang et al. (2019)                                    |
| <i>Hippocampus abdominalis</i>         | Tripeptide (AGD)               | AAPH-induced zebrafish embryos   | Reducing ROS level dose-dependently  | H. S. Kim et al. (2019)                                  |
| Velvet antler                          | Tetrapeptide (WDVK)            | AAPH-induced zebrafish embryos   | Inhibiting the generation of ROS   | Ding et al. (2019)                                       |
| <i>Amaranthus caudatus</i>             | Hydrolyzed protein             | AAPH-induced zebrafish embryos; H <sub>2</sub> O <sub>2</sub> -induced zebrafish larvae          | Reducing ROS production and lipid peroxidation   | Vilcacundo et al. (2018)                                 |
| Tea waste                              | Polyphenols                    | H <sub>2</sub> O <sub>2</sub> -induced in zebrafish embryos                                      | Improving survival rate  | Gao et al. (2020)  |
| <i>Hizikia fusiforme</i>               | Fucoidan                       | H <sub>2</sub> O <sub>2</sub> -stimulated zebrafish  | Improving survival rate; reducing heart rate, ROS production, cell death, and lipid peroxidation                         | Wang, Jayawardena, et al. (2020)                         |
| <i>Codium fragile</i>                  | Sulfated polysaccharides       | H <sub>2</sub> O <sub>2</sub> -mediated oxidative stress   | Improving survival rate; normalizing heartbeat; reducing ROS, cell death, and lipid peroxidation dose-dependently        | Wang, Oh, et al. (2020)                                  |
| <i>Ligusticum chuanxiong hort</i>      | Polysaccharide                 | H <sub>2</sub> O <sub>2</sub> -induced zebrafish embryos   | Reducing ROS generation and cell death   | L. Wang et al. (2019)                                    |
| <i>Arthrospera platensis</i>           | Peptide (VH12)                 | H <sub>2</sub> O <sub>2</sub> -induced in zebrafish embryos                                      | Reducing intracellular ROS level   | Sannasimuthu and Arockiaraj (2019)                       |
| <i>Hizikia fusiforme</i>               | Polysaccharides                | H <sub>2</sub> O <sub>2</sub> -induced zebrafish larvae  | Improving survival rate; reducing heart rate, ROS generation, and cell death   | L. Wang et al. (2018)                                    |
| Sri Lankan seaweed                     | Enzymatic and water extracts   | H <sub>2</sub> O <sub>2</sub> -induced in zebrafish embryos                                      | Improving survival rate; reducing heart rate, ROS generation, and cell death   | I. P. S. Fernando et al. (2018)                          |
| <i>Chenopodium quinoa Willd.</i>       | Quinoa protein concentrate     | H <sub>2</sub> O <sub>2</sub> -induced zebrafish larvae  | Inhibiting lipid peroxidation  | Vilcacundo et al. (2017)                                 |
| Milk proteins                          | Native and heated hydrolysates | H <sub>2</sub> O <sub>2</sub> -induced zebrafish larvae  | Inhibiting lipid peroxidation and TBARS <i>in vivo</i>   | Carrillo, Guzmán, and Vilcacundo (2017)                  |
| Hen egg lysozyme                       | Antioxidant peptides           | H <sub>2</sub> O <sub>2</sub> -induced zebrafish larvae  | Inhibiting lipid peroxidation  | Carrillo et al. (2016)                                   |
| <i>Viviparus contectus</i>             | Extract                        | t-BHP-induced zebrafish embryos  | Reducing ROS production  | Y. S. Kim, E. K. Kim, X. Dong, W. B. Shin, et al. (2019) |
| <i>Lindera glauca</i>                  | Blume stem extracts            | t-BHP-induced zebrafish embryos  | Increasing antioxidant related gene expressions and enzyme activities  | Y. S. Kim, E. K. Kim, X. Dong, J. S. Park, et al. (2019) |
| Soybeans, shrimp heads, and egg yolks  | Lipids                         | MTZ-induced Tg(krt4: NTR-hKikGR) <sup>cY17</sup> zebrafish larvae                                | Increasing fluorescent spot  | X. B. Li et al. (2020)                                   |
| <i>Antirhea borbonica</i>              | Aqueous extract                | Methylglyoxal-mediated glycoxidative damages   | Restoring membrane deformability; reducing oxidative stress and eryptosis  | Delveaux et al. (2020)                                   |
| <i>Saccharina japonica</i> against     | Fucoidan                       | UVB-irradiated zebrafish   | Reducing cell death, intracellular ROS level, NO production, lipid peroxidation in a dose-dependent manner               | Su et al. (2020)   |
| <i>Ishige okamurae</i>                 | Diphlorethohydroxy-carmalol    | UVB-irradiated zebrafish   | Reducing cell death, lipid peroxidation, ROS, and inflammatory response  | W. X. Wang et al. (2019)                                 |
| <i>Loliolus beka</i> gray meat         | Taurine                        | Gamma ray-irradiated zebrafish embryos   | Diminishing the production of ROS and NO; improving survival rate  | W. Lee et al. (2019)                                     |
| <i>Ishige okamurae</i>                 | Polysaccharide                 | Gamma ray-irradiated oxidative stress  | Reducing cell death and the production of ROS and NO   | Lee et al. (2017)  |
| <i>Titanbiculus</i>                    | Anthocyanins                   | Caudal fin of wounded Tg(mpeg1: EGFP) zebrafish  | Suppressing the generation of ROS  | Chensom et al. (2020)                                    |
| <i>Hypericum hookerianum</i>           | Aqueous extracts               | Zebrafish embryos  | Up-regulating the expression of antioxidant genes  | Pradeep et al. (2019)                                    |
| Anti-inflammatory activity             |                                |  |  |  |
| <i>Ajuga pantantha</i>                 | Diterpenoids                   | LPS-exposed zebrafish embryos  | Reducing NO and ROS levels   | W. P. Liu et al. (2020)                                  |
| <i>Casearia kurzii</i>                 | Caseahomopene A                |  |  | An et al. (2020)   |

(continued)

**Table 1.** Continued.

| Natural products                       | Extracts/Compounds               | Models   | Main Effects and Mechanisms   | References   |
|--|----------------------------------|--|---|--|
| <i>Saccharina japonica</i>             | Fucoidan                         | LPS-exposed zebrafish embryos  | Inhibiting NO and ROS formation in a dose-dependent manner  |  |
| <i>Lantana camara</i>                  | Triterpenoids                    | LPS-exposed zebrafish embryos  | Reducing the cell death rate, NO, and ROS production  | Ni et al. (2020)   |
| <i>Sargassum horneri</i>               | Fucoidan                         | LPS-exposed zebrafish embryos  | Reducing NO and ROS levels  | P. Wu et al. (2020)                                      |
| <i>Sargassum horneri</i>               | Sulfated polysaccharide          | LPS-exposed zebrafish embryos  | Down-regulating heart-beating rate, cell death, NO, and ROS levels  | Sanjeewa et al. (2019)                                   |
| Sri Lankan seaweed                     | Enzymatic and water extracts     | LPS-exposed zebrafish embryos  | Reducing LPS-induced toxicity, cell death, and NO levels  | Sanjeewa et al. (2018)                                   |
| Sea buckthorn-based beverage matrix    | <i>Lactobacillus rhamnosus</i>   | LPS-microinjected zebrafish larvae   | Reducing NO production  | I. P. S. Fernando et al. (2018)                          |
| <i>Sargassum macrocarpum</i>           | Tuberatolide B                   | LPS-microinjected zebrafish larvae   | Alleviating colonic damage; declining colonic expression of TNF- $\alpha$ and IL-1 $\beta$                                | Sireswar, Biswas, and Dey (2020)                         |
| <i>Chimonanthus nitens</i> Oliv        | Ethanol extracts of leaves       | LPS-microinjected zebrafish larvae   | Enhancing survival rate; inhibiting NO production and mRNA expression of inducible NO synthase                            | Y. S. Kim, E. K. Kim, X. Dong, W. B. Shin, et al. (2019) |
| <i>Schisandra chinensis</i> Baillon    | RPG-OM-30E                       | LPS-microinjected zebrafish larvae; Tailfin wounding in transgenic zebrafish Tg(mpx:EGFP) <sup>i114</sup> larvae   | Inhibiting the recruitment of neutrophils; reducing mRNA expression of TNF- $\alpha$ , IL-6, but not IL-1 $\beta$         | Q. Sun et al. (2017)                                     |
| Kudingcha                              | Chlorogenic acid                 | Tg(mpx:EGFP) transgenic zebrafish  | Decreasing the number of neutrophils migrating to the wound area; reducing mRNA expression of IL-1 $\beta$ , but not IL-6 | Yang et al. (2019)                                       |
| <i>Panax ginseng</i>                   | Ginsenoside Rg1                  | Tailfin cutting in wild type, gr <sup>s357</sup> mutant, and double transgenic Tg(mpx:GFP) <sup>i114</sup> /mpeg1:mCherry -F <sup>umsf001</sup> ) zebrafish larvae | Promoting neutrophil reverse migration via phosphorylation of ERK and AKT   | W. Zhang et al. (2020)                                   |
| <i>Cajanus cajan</i> L.                | Cajaninstilbene acid             | Tailfin cutting in double transgenic Tg(Coronin-EGFP/Lyc-dsRed) zebrafish larvae   | Attenuating neutrophilic inflammation at the amputation site; no effect on tissue regeneration                            | Huang et al. (2016)                                      |
| <i>Clerodendrum cyrtophyllum</i> turcz | Ethanol extract                  | CuSO <sub>4</sub> -induced inflammation in zebrafish   | Inhibiting the migration of neutrophils and macrophages   | Nguyen et al. (2020)                                     |
| Soybeans, shrimp heads, and egg yolks  | Lipid                            | CuSO <sub>4</sub> -stimulated inflammation in Tg(Lyz:EGFP) zebrafish larvae  | Decreasing the expression of inflammatory genes, pro- and anti-inflammatory cytokines                                     | X. B. Li et al. (2020)                                   |
| <i>Millettia pachycarpa</i>            | Isoflavone                       | CuSO <sub>4</sub> -stimulated inflammation   | Reducing macrophage migration   | Tu et al. (2020)   |
| <i>Katsuwonus pelamis</i>              | Peptide                          | CuSO <sub>4</sub> -stimulated inflammation in transgenic zebrafish larvae  | Alleviating the state of CuSO <sub>4</sub> -stimulated inflammation   | Z. G. Wang et al. (2019)                                 |
| <i>Ganoderma lucidum</i>               | Screening immunoactive compounds | Vinorelbine-induced neutropenia or macrophage deficiency models in Tg(mpx:GFP) zebrafish larvae  | Alleviating neutrophil granulocyte aggregation  | Z. H. Li et al. (2020)                                   |
| <i>Dendrobium devonianum</i>           | Polysaccharide                   | Vinorelbine-induced neutropenia or macrophage deficiency model   | Alleviating vinorelbine-induced neutropenia or macrophage deficiency; enhancing the phagocytic function of macrophages    | da Silva et al. (2020)                                   |
| <i>Annona muricata</i> L.              | Fruit bar                        | Carrageenan-induced inflammation in adult zebrafish  | Activating macrophages mainly through TLR4  | Pradeep et al. (2019)                                    |
| <i>Hypericum hookerianum</i>           | Aqueous extracts                 | Zebrafish embryos  | Preventing carrageenan-induced abdominal edema  | Jevtic et al. (2017)                                     |
| <i>Cucumis sativus</i>                 | Leaf extract                     | Zebrafish embryos  | Up-regulating the expression of anti-inflammatory genes   | Abidar et al. (2020)                                     |
| Neuroprotective effect                 |                                  |  | Inhibiting the generation of encephalitogenic cells   |  |
| <i>Ceratonia siliqua</i> L.            | Aqueous extract from leaves      | 6-OHDA zebrafish model   | Presenting positive antioxidant and anti-AChE activities; improving cognitive function                                    | M. Q. Li et al. (2018)                                   |
| —                                      | Acteoside                        | 6-OHDA-induced neural damage in zebrafish model  | Preventing movement disorders and dopaminergic neuron death; up-regulating antioxidant enzymes by                         |  |

(continued)

**Table 1.** Continued.

| Natural products                 | Extracts/Compounds                   | Models   | Main Effects and Mechanisms  | References                                      |
|----------------------------------|--------------------------------------|--|--|---|
| <i>Palythoa caribaeorum</i>      | Pcshk3 (a novel toxic peptide)       | 6-OHDA-induced neurotoxicity   | activating the Nrf2/ARE signaling pathway  |   |
| <i>Eucommia ulmoides</i> Oliver  | Extracts from leaves /phenolic acids | MPTP-modeled PD  | Improving locomotive behavior<br>Reversing the loss of dopaminergic neurons and neural vasculature; reducing apoptotic cells; relieving locomotor impairments by activating autophagy                  | Liao et al. (2018)<br>S. S. Zhang et al. (2020) |
| —                                | Pinostrobin                          | MPTP-induced PD zebrafish  | Attenuating the loss of dopaminergic neurons; improving behavior deficiency  | C. W. Li et al. (2018)                          |
| <i>Pueraria lobata</i>           | Puerarin                             | Unchallenged zebrafish   | Encapsulated puerarin was repaid absorbed  | Chen et al. (2019)                              |
| <i>Rosmarinus officinalis</i> L. | Essential oil                        | Scopolamine-induced dementia   | Reversing anxiety, memory impairment, and brain oxidative stress; reducing brain ache activity   | Capatina et al. (2020)                          |
| <i>Garcinia indica</i>           | Garcinol                             | Acrylamide-induced cognitive defects   | Improving behavioral defects, oxidative injury, neuroinflammation, undesirable APP processing, tau hyperphosphorylation  | Sharma and Kang (2020)                          |
| <i>Juglans regia</i> L.          | Antioxidant peptide                  | Mycophenolate mofetil-induced nerve injury   | Inhibiting caspases 3/7 and 8 activities; improving brain-derived neurotrophic factor expression   | M. C. Liu et al. (2019)                         |
| <i>Artocarpus altilis</i>        | Protein fraction                     | Zebrafish anxiety model  | Reversing anxiety behavior via serotonergic system   | Gonçalves et al. (2020)                         |
| <i>Hylocereus polyrhizus</i>     | Extracts from pulp and peel          | Zebrafish anxiety model  | Exhibiting anxiolytic effect via GABAergic system  | Lira et al. (2020)                              |
| Soybean                          | Isoflavones                          | Zebrafish models of anxiety and depression   | Producing anxiolytic and antidepressant effects  | de Melo et al. (2020)                           |
| <i>Cyperus articulatus</i> L.    | Solvents extracts from rhizomes      | PTZ-induced seizures   | Hexane extract exhibit the highest anti-seizure activity   | Brillatz et al. (2020)                          |
| <i>Berberis sibirica</i>         | Palmatine                            | PTZ-induced seizures   | Exerting anti-seizure activity; decreasing c-fos and bdnf levels; decreasing hyperlocomotion dose-dependently  | Gawel, Kukula-Koch, et al. (2020)               |
| Berberis                         | Berberine                            | PTZ-induced seizures   | Attenuating seizures and modulate anti-inflammatory effect to protect further seizures   | B. Y. Zhang et al. (2020)                       |
| <i>Semen pharbitidis</i>         | Pharbitin                            | PTZ-induced seizures in zebrafish  | Reducing PTZ-induced seizures  | M. X. Liu et al. (2019)                         |
| <i>Cryptolepis sanguinolenta</i> | Cryptolepine                         | PTZ-induced seizures   | Cryptolepine of entrapped form exerted a better anti-seizure effect than that of free from   | Mante et al. (2021)                             |
| <i>Sesamum indicum</i> L.        | Sesamin                              | Kanamycin-induced hair cell damage   | Increasing in kinocilia numbers, neuromasts and otic cells; recovery of neuromast  | Y. H. Kim et al. (2020)                         |
| <i>Persea americana</i>          | Oil extract                          | Neomycin-induced otic cell damage  | Recovery of otic hair cells  | Nam et al. (2019)                               |
| <i>Persea americana</i>          | Juglanin and (+)-lyoniresinol        | Neomycin-induced hair cell damage  | Cell regeneration in neomycin-damaged hair cell  | Park et al. (2019)                              |
| <i>Angelica sinensis</i>         | Ferulic acid                         | Neomycin-induced ototoxicity in transgenic zebrafish Tg(pvalb3b:TagGFP)                | Protecting against hair cell loss; decreasing intracellular ROS production and TUNAL reactions   | Ju et al. (2017)                                |
| —                                | Sodium selenite                      | Neomycin-induced hair cell damage in transgenic zebrafish Tg(Brn3C:EGFP)               | Protecting against hair cell loss of neuromasts; reducing apoptosis; preventing ultrastructural changes  | Chang et al. (2016)                             |
| <i>Citrus tachibana</i>          | Water extract                        | Adult and larval zebrafish   | Increasing overall food intake, body weight, and plasma triglycerides  | Yamada et al. (2020)                            |
| <i>Flemingia philippinensis</i>  | Auriculasin                          | Attention deficit hyperactivity disorder model: period1b <sup>-/-</sup> zebrafish      | Reducing hyperactivity; increasing melatonin and dopamine content  | T. Y. Wang et al. (2018)                        |
| Cardiovascular protection        | Resveratrol                          | Zinc oxide-induced vascular structural abnormalities during embryonic fish development | Inhibiting cardiac morphological and functional damage via the suppression of ROS increase, prevention of mitochondrial membrane potential dysfunction, and counteraction with cell apoptosis/necrosis | Giordo et al. (2020)                            |
| —                                | Secoisolariciresinol                 | 3,3',4,4',5-pentachlorobiphenyl-induced pericardial edema in zebrafish embryos         | Inhibiting the formation of pericardial edema  | Tokunaga, Woodin, and Stegeman (2016)           |

(continued)

**Table 1.** Continued.

| Natural products  | Extracts/Compounds   | Models   | Main Effects and Mechanisms   | References  |                                       |
|---|--|--|---|---|---------------------------------------|
| <i>Palythoa caribaeorum</i>                               | A peptide precursor containing a ShK domain                | Zebrafish larvae   | Decreasing heart rate, stroke volume, cardiac output at low doses via the blockage of $K_{Ca}3.1$ ; inducing pericardial edema and blood accumulation at high doses       | Liao et al. (2018)  |                                       |
| <i>Artemisia annua</i> L.                                 | Artesunate   | Verapamil-induced zebrafish heart failure                                    | Exhibiting a cardioprotective effect when at low concentrations; inducing pericardial edema and circulation defects at high concentrations                                | Zheng et al. (2020)   |                                       |
| Brazilian green propolis                                  | Water extract  | High glucose-induced vasculopathy in zebrafish larvae                        | Inhibiting high glucose-induced structural abnormalities in hyaloid and cerebral vessels  | Saito et al. (2018)   |                                       |
| Fermented black garlic and its Amadori products           | —  | Chemical-induced vascular lesions in zebrafish                               | promoting angiogenesis; inhibiting thrombus formation   | X. M. Zhang et al. (2019)   |                                       |
| Citrus fruits   | Naringin and naringenin                                    | Chemical-induced blood vessel loss model in zebrafish                        | Inducing angiogenesis activity on the restoration of blood vessel loss; reversing VRI-induced down-regulation of flt1 mRNA expression                                     | L. Chen et al. (2018)   |                                       |
| <i>Gastrodiae Rhizoma</i>                                 | Ten bioactive compounds                                    | Zebrafish model  | Contributing to formation of new blood vessels  | M. Liu et al. (2020)  |                                       |
| <i>Gardenia Fructus</i>                                   | Geniposide, citric acid, and quinic acid                   | Arachidonic acid-induced thrombus  | Decreasing heart area; recovering the intensity of heart red blood cells; improving the thrombosis  | Shi et al. (2020)   |                                       |
| <i>Crataegus pinnatifida</i>                              | A sesquiterpenoid  | Ferric chloride-induced thrombus   | Increasing the time to form thrombocytes  | Gao et al. (2017)   |                                       |
| Hawthorn leaf   | Terpenes and flavones                                      | Ferric chloride-induced thrombus   | Increasing the time to form thrombocytes  | Gao et al. (2019)   |                                       |
| <i>Ginkgo biloba</i>                                      | Bilobalide and ginkgolides                                 | Zebrafish thrombosis model   | Increasing zebrafish heart red blood cells intensity  | Lu et al. (2018)  |                                       |
| <i>Salvia miltiorrhiza</i> , and <i>Panax notoginseng</i> | Rosmarinic acid, lithospermic acid, and salvianolic acid B | PHZ-induced zebrafish thrombosis   | Increasing cardiac erythrocytes, decreasing aggregation of erythrocyte  | Yin et al. (2020)   |                                       |
| Anti-cancer effect  |  |  |   |   |                                       |
| —   | Tetrandrine derivatives                                    | Zebrafish model  | Inhibiting the angiogenesis   | R. H. Zhang et al. (2020)   |                                       |
| <i>Moricandia sinaica</i> Boiss.                          | Methanol extract of the stem and leaves                    | Zebrafish embryos  | Inhibiting the formation of the inter-segmental, sub-intestinal vein, and dorsal longitudinal anastomotic veins formation in a dose-dependent manner                      | Farooq et al. (2020)  |                                       |
| <i>Casearia kurzii</i>                                    | Diterpenoids   | Zebrafish  | Inhibiting tumor proliferation and migration  | Liang et al. (2020)   |                                       |
| <i>Ishige okamurae</i>                                    | Ishophloroglucin A   | Transgenic zebrafish Tg(flk:EGFP) embryos; high glucose-induced angiogenesis | Suppressing vessel formation; down-regulating expression of VEGFR2 and downstream signaling molecule cascade  | Fernando et al. (2019)  |                                       |
| <i>Trigonella foenum graecum</i> L.                       | Ethyl iso-allocholate (A steroidal derivative)             | Zebrafish  | Inducing caspase-dependent apoptosis in cancer cells; reducing tumor growth, metastasis, and angiogenesis   | Thakur and Ahirwar (2019)   |                                       |
| <i>Ishige okamurae</i>                                    | Diphlorethohydroxycarmalol                                 | High glucose-induced angiogenesis in zebrafish embryos                       | Suppressing retinal vessel dilation and vessel formation; inhibiting expression of VEGFR2 and downstream signaling cascade  | K. H. N. Fernando et al. (2018)   |                                       |
| <i>Scutellaria baicalensis</i>                            | Baicalein derivatives                                      | Transgenic zebrafish Tg(fli1:EGFP)   | Inhibiting the angiogenesis   | X. Y. Jiang et al. (2018)   |                                       |
| —   | Protocatechuic acid and syringic acid                      | Transgenic Tg(fli1a:EGFP) y1-type zebrafish embryos                          | Down-regulating the angiogenesis-related signal transduction pathway of VEGF $\alpha$ -VEGFR2 or Ang2-Tie2; inhibiting on VEGF-induced migration of HUVEC and vasculature | Hu et al. (2018)  |                                       |
| Red wine  | Dried extract  | Transgenic zebrafish Tg(fli1:EGFP)y1   | Reducing intersegmental vessel formation  | H. Y. Sun et al. (2017)   |                                       |
| <i>Euphorbia pekinensis</i> Rupr.                         | Water extract  | Transgenic zebrafish Tg(flk:mCherry)   | Inhibiting the angiogenesis; increasing VEGFR3 expression, while decreasing other 23 genes including Met, VEGF $\alpha$ , Flt1  | W. T. Zhang et al. (2017)   |                                       |
| —   | <i>Garcinia xanthochymus</i>                               | Chlorogenic acid<br>Biflavonoids   | Zebrafish embryo<br>Zebrafish embryos   | Inhibiting the angiogenesis<br>Inhibiting the growth of subintestinal vessels; downregulating the | Lin et al. (2017)<br>Li et al. (2017) |

(continued)

**Table 1.** Continued.

| Natural products                                       | Extracts/Compounds                       | Models   | Main Effects and Mechanisms   | References   |
|--|--|--|---|--|
| Kudingcha  | Chlorogenic acid-enriched extract        | Tg(flk1:EGFP) zebrafish embryos  | expressions of Angpt2 and Tie2 genes<br>Inhibiting angiogenesis   | Zhong et al. (2017)  |
| <i>Choerospondias axillaris</i>                        | Proanthocyanidins                        | Transgenic zebrafish embryo  | Suppressing new blood vessel formation in a concentration-dependent manner  | Li et al. (2016)   |
| Brown seaweed  | Fucoidan                                 | Transgenic fish with hepatocellular carcinoma  | Decreasing the expression of lipogenic factors and enzymes, fibrosis, and cell cycle/proliferation markers; reducing liver cancer formation   | P. Wu et al. (2020)  |
| <i>Laminaria japonica</i>                              | Fucoidan                                 | Zebrafish xenograft assay<br>Transgenic zebrafish Tg(fli1:EGFP)                              | Blocking angiogenesis and micrometastasis   | Hsu et al. (2020)  |
| Brown algae<br><i>Fucus vesiculosus</i>                | Fucosterol<br>Fucoidan                   | Zebrafish xenograft model<br>Zebrafish xenograft assay<br>Transgenic zebrafish Tg(fli1:EGFP) | Decreasing tumor formation<br>Disrupting tumor formation and vascular development   | Bae, Lee, Song, et al. (2020)<br>Bae, Lee, Yang, et al. (2020) |
| <i>Artemisia asiatica</i>                              | Eupatilin                                | Zebrafish xenograft assay<br>Transgenic zebrafish Tg(fli1:EGFP)                              | Inhibiting tumorigenesis; suppressing angiogenesis  | Lee et al. (2020)  |
| <i>Klebsiella pneumoniae</i>                           | Microcin E492                            | Zebrafish larvae harboring xenografts of human cancer cells                                  | Reducing the tumor cell mass  | Varas et al. (2020)  |
| —<br>—   | Luteolin and quercetin<br>Propyl gallate | Zebrafish xenograft larvae<br>Zebrafish xenograft models                                     | Decreasing metastasis of tumor cells<br>Inducing cell apoptosis and increasing the number of necrotic cells in a time-and dose-dependent manner                                       | Fan et al. (2019)<br>Wei, Huang, and Chang (2019)              |
| <i>Rhizoma curcumae</i><br><i>Sinularia flexibills</i> | Furanodiene<br>Sandensolide              | Zebrafish xenograft model<br>Zebrafish xenograft model                                       | Reversing multiple drug resistance<br>Suppressing colony formation and growth of oral squamous cell carcinoma; inducing apoptosis and cell cycle arrest                               | Zhu et al. (2019)<br>Yu et al. (2018)                          |
| Propolis   | Caffeic acid phenethyl ester             | Zebrafish xenograft model  | Inhibiting the cell growth of oral squamous cell carcinoma  | Chung et al. (2017)  |
| Anti-metabolic disorders<br><i>Eriobotrya japonica</i> | Corosolic acid                           | cAMP and DEX-induced T2D zebrafish   | Reducing glycogen degradation and increasing glucose consumption by regulating insulin receptor signals and some key enzymes in carbon metabolism                                     | Xu et al. (2019)   |
| <i>Eysenhardtia polystachya</i>                        | Methanol/water bark extract              | Glucose-induced diabetic zebrafish   | Promoting pancreatic β-cell survival and insulin secretion  | Garcia Campoy et al. (2018)                                    |
| <i>Forsythia koreana</i> flowers                       | Lignans                                  | Alloxan-induced pancreatic islets damage in zebrafish  | Recovering alloxan-induced pancreatic islet damages   | Y. G. Lee et al. (2019)  |
| <i>Malva verticillata</i>                              | —  | Alloxan-induced pancreatic islets damage in zebrafish  | Synergistic effect of two major components  | Ko et al. (2019)   |
| <i>Malva verticillata</i>                              | Flavonoid 8-O-glucuronides               | Alloxan-induced pancreatic islets damage in zebrafish  | Improving pancreatic islet size   | Ko et al. (2018)   |
| <i>Polysiphonia japonica</i>                           | Crude extracts                           | Palmitate-induced β-cells damage in zebrafish embryos  | Preserving cell viability and insulin secretion   | Cha et al. (2018)  |
| Fermented loose tea                                    | Aqueous extract                          | High-fat diet-fed zebrafish  | Declining lipid level   | Y. Xiao, K. Zhong, et al. (2020)                               |
| Dark tea   | Theabrownins                             | High-fat (egg yolk) zebrafish model  | Hypolipidemic activity  | Y. Xiao, M. Y. Li, et al. (2020)                               |
| <i>Etlingera calophrys</i>                             | Ethanolic extract                        | High-fat diet-induced obese zebrafish  | Displaying optimum reduction of triglycerides and blood glucose; suppressing adipogenesis in visceral adipose tissue  | Idrus et al. (2020)  |
| Chinese bayberry                                       | Myricanol                                | High-fat diet-fed zebrafish  | Inhibiting lipid accumulation by suppressing adipogenic factors   | Shen et al. (2019)   |
| —  | Alginate oligosaccharide                 | High-fat diet-induced obese zebrafish  | Suppressing obesity and pathophysiological disorders by modulating lipid metabolism, suppressing inflammation, down-regulating apoptosis-related genes, and improving immune function | Tran et al. (2019)   |
| Mingshan Laochuancha green tea                         | Water extract                            | High-fat diet-fed zebrafish  | Reducing lipid level  | Xiao et al. (2019)   |
| <i>Citrus sinensis</i> Juice                           | Flavonoid-rich extract                   | Diet-induced obese zebrafish   |   | Montalbano et al. (2019)                                       |

(continued)

**Table 1.** Continued.

| Natural products   | Extracts/Compounds                             | Models  | Main Effects and Mechanisms   | References                      |
|--|--|---|---|---------------------------------|
| Green tea  | Extract  | Diet-induced obese zebrafish  | Reducing adipocyte cell size, visceral adipose tissue, body weight, and body mass index; modulating some obesity-related genes  | Zang et al. (2019)              |
| <i>Panax ginseng</i>   | Ginsenoside Rg1                                | High-fat diet-induced obese zebrafish                                   | Ameliorating obese phenotypes by activating Wnt/beta-catenin and AMPK pathway signaling   | Koh et al. (2017)               |
| Wine lees  | Polyphenols                                    | Zebrafish embryos   | Decreasing lipid and triglycerides  | Caro et al. (2017)              |
| Ginsenoside Rb1 with <i>Cordyceps sinensis</i> and <i>Ascomycota</i> sp. | —  | Diet-induced zebrafish hyperlipidemia                                   | Modulating zebrafish lipid metabolism   | F. Li et al. (2020)             |
| —  | 2R,3R-Dihydromyricetin                         | Hyperlipidemia zebrafish  | Attenuating hypercholesterolemia by down-regulating cholesterol synthesis and assembly or secretion of lipoproteins as well as up-regulating cholesterol transport and efflux | Y. P. Wu et al. (2020)          |
| Cruciferous vegetables   | Indole-3-carbinol                              | High-cholesterol diet-fed zebrafish                                     | Lowering lipid level  | Jiang et al. (2019)             |
| Monascus-fermented rice product  | Screening of lipid-lowering compounds          | High-cholesterol diet-fed zebrafish                                     | Decreasing lipid deposition by inducing autophagy   | Liang et al. (2019)             |
| <i>Angelica keiskei</i>  | Isobavachalcone                                | High-cholesterol diet-fed zebrafish                                     | Ergosterol with optimum lipid-lowering activity   | Lee et al. (2018)               |
| <i>Pleurotus eryngii</i>   | Polysaccharide                                 | High-cholesterol diet-induced zebrafish hyperlipidemia                  | Decreasing intrahepatic fat deposits; rescuing liver steatosis  | Wei et al. (2018)               |
| <i>Sparassis crispa</i>  | —  | Diet-induced obese zebrafish  | Lowering lipid level during lipid absorption phase  | Matsuura et al. (2020)          |
| <i>Palmaria mollis</i>   | —  | Diet-induced obese zebrafish  | Suppressing body weight gain and ameliorating lipid accumulation in liver; up-regulating PPAR $\alpha$ pathway genes expression   | Nakayama et al. (2018)          |
| <i>Lactobacillus rhamnosus</i> GG  | —  | Alcohol-induced fatty liver   | Ameliorating dyslipidemia, hepatic steatosis, visceral adiposity  | Bruch-Bertani et al. (2020)     |
| <i>Lactobacillus plantarum</i>   | —  | Alcohol-induced liver injury  | Lowering hepatic lipid accumulation   | Y. P. Liu et al. (2019)         |
| Intestinal protection  |  |   |   |                                 |
| <i>Cladosiphon okamuranus</i>  | Fucoidan                                       | Larval and adult zebrafish  | Improving SOD, CAT, HO1, and gstk1 activities; activating Keap1/Nrf2/ARE signal pathway   | Ikeda-Ohtsubo et al. (2020)     |
| <i>Lobosphaera incisa</i>  | Omega-6 long-chain polyunsaturated fatty acids | Unchallenged zebrafish  | Increasing the abundance of Comamonadaceae and Rhizobiaceae and decreasing abundance of Enterobacteriaceae; decreasing IL-1 $\beta$   | Nayak et al. (2020)             |
| High-fat-saturated diet  | DHA  | High-fat diet-fed zebrafish   | Higher expression of cox-2 and lox-1; decreasing IL-10  | Arias-Jayo et al. (2019)        |
| Pea, soy, and wheat gluten protein                                       | —  | Zebrafish   | Decreasing the growth of <i>Pseudomonas</i> ; decreasing saturated fatty acid in body lipid   | Dhanasiri et al. (2020)         |
| <i>Bifidobacterium animalis</i> F1-7                                     | —  | Loperamide hydrochloride-induced constipation                           | Many intergenic regions and genes related to immunity and antioxidant defense system were differentially methylated   | Lu, Zhang, Liang, et al. (2019) |
| <i>Bifidobacterium longum</i> and <i>Bifidobacterium infantis</i>        | Heteropolysaccharides                          | Dextran sodium sulfate-induced enterocolitis                            | Promoting zebrafish intestinal peristalsis by regulating the genes related to serotonin synthesis and modulating serotonin release  | Llamas-Arriba et al. (2019)     |
| <i>Lactobacillus rhamnosus</i> GG  | —  | 2,4,6-Trinitrobenzene sulfonic acid-induced enterocolitis               | Decreasing larval mortality   | Sireswar and Dey (2019)         |
| Fermented <i>Dendrobium candidum</i>                                     | High content of polyphenols                    | Oxazolone-induced intestinal inflammation                               | Sea buckthorn matrix exhibited a higher protective effect than apple juice probably owing to its higher accumulation of phenolic compounds                                    | Gong et al. (2020)              |
| Orange juice   | High content of flavonoids                     | <i>Vibrio anguillarum</i> -induced enteritis                            | Mitigating the oxazolone-induced intestinal inflammation by regulating the host immune responses and the gut microbiota   | Cirmi et al. (2020)             |
| <i>Spirulina maxima</i>  | Pectin   | <i>Edwardsiella piscicida</i> and <i>Aeromonas hydrophila</i> infection | Inhibiting the inflammation of zebrafish intestinal mucosa  | Edirisinghe et al. (2019)       |
| <i>Amukkara chooram</i>  | —  | <i>Candida albicans</i> infection                                       | Modulating immune responses and improving performance   |                                 |

(continued)

**Table 1.** Continued.

| Natural products   | Extracts/Compounds  | Models  | Main Effects and Mechanisms  | References  |
|--|---|---|--|---|
| —  | Resveratrol   | <i>Salmonella typhimurium</i> and <i>Escherichia coli</i> infection               | Clearing biofilm formed by <i>Candida albicans</i> from intestinal epithelial cells and relieving tissue damages<br>Resveratrol increased the clearance of two bacteria stimulate via the stimulation of xenophagy | Rajamohamed and Siddharthan (2019)<br>Al Azzaz et al. (2019)                                  |
| Renal protection<br>Dopamine and <i>L</i> -mimosine<br>Yaocha  | —   | Cisplatin-induced ototoxicity and nephrotoxicity<br>Zebrafish hyperuricemia model | Otoprotective and nephroprotective effects<br>Reducing uric acid level   | Wertman et al. (2020)<br>Xiong et al. (2020)  |
| —  | Resveratrol and ursolic acid                                  | Aristolochic acid-induced nephrotoxicity  | Inhibiting kidney malformations; improving blood circulation; suppresses expression of pro-inflammatory genes  | Ding et al. (2015)  |
| Anti-osteoporosis<br><i>Sinomenii Caulis</i>   | Extracts  | Prednisolone-induced osteoporosis   | Promoting bone formation of cranial bones  | W.-J. Liu et al. (2020)   |
| <i>Dimocarpus longan</i> fruit<br><i>Epimedii Folium</i><br><i>Zingiber officinale</i>                   | —<br>Circinal-icaritin and icaritin<br>10-Gingerol            | Zebrafish embryos<br>Zebrafish embryos<br>Prednisolone-induced osteoporosis       | Enhancing bone mineralization<br>Suppressing bone reabsorption<br>Suppressing osteoclast-specific cathepsin K, mmp2, and mmp9  | Son et al. (2019)<br>J. Jiang et al. (2018)<br>Zang et al. (2021)                             |
| <i>Dunaliella salina</i>   | Peptide   | Zebrafish induced by Dexamethasone  | Promoting stained integral optical density   | Y. Chen et al. (2021)   |
| <i>Erzhi Pill</i>  | 72 active compounds   | Zebrafish induced by Dexamethasone  | Inhibiting the decrease of skull mineralization; Increasing osteoblastic differentiation   | Zhong et al. (2021)   |
| Fermenyed <i>Crassostrea gigas</i>   | Extracts  | Zebrafish Larvae and amputated adult fish   | Improving bone mineral density in larval zebrafish and promoting tail fin regeneration in adult zebrafish by activating Wnt/β-catenin-induced osteoblast differentiation   | Molagoda et al. (2019)  |
| Anti-melanogenic activity<br><i>Dalbergia pinnata</i>  | Essential oils  | Zebrafish embryos   | Reducing tyrosinase activity and melanin synthesis in a dose-dependent manner  | Zhou et al. (2020)  |
| Four lichen species<br><i>Inula britannica</i><br><i>Fomitopsis castanea</i> mycelia<br>American ginseng | Extracts<br>Inularin<br>Exopolysaccharides<br>Ginsenoside C-Y | Zebrafish larvae<br>Zebrafish embryos<br>Zebrafish embryos<br>Zebrafish embryos   | Depigmenting activities<br>Decreasing pigmentation<br>Decreasing the pigment density<br>Inhibiting melanin secretion and tyrosinase activity and decreased melanin content   | Malaspina et al. (2020)<br>Jang et al. (2020)<br>Jin et al. (2019)<br>X. Y. Liu et al. (2019) |
| <i>Coix lacryma</i>  | Bran oil  | Zebrafish embryos   | Reducing tyrosinase activity and melanin production dose-dependently   | Ting et al. (2019)  |
| <i>Piper longum</i>  | Piperlongumine  | Zebrafish embryos   | Showing potent antimelanogenic activity  | Jeon et al. (2019)  |
| Black ginseng  | Ginsenosides Rg5 and Rkl                                      | Zebrafish embryos   | Inhibiting melanin activity and tyrosinase levels  | Jin et al. (2018)   |
| <i>Morchella esculenta</i>   | Polysaccharide FMP-1  | Zebrafish larvae  | Reducing melanin contents and tyrosinase activities by inhibiting CREB and p38 pathways  | Cai et al. (2018)   |
| <i>Ligusticum sinense</i>  | (3S,3aR)-Neocnidilide   | Zebrafish embryos   | Demonstrating anti-pigmentation activity   | Cheng et al. (2018)   |
| <i>Hibiscus syriacus</i> L.  | Anthocyanins  | α-MSH-stimulated zebrafish larvae   | Attenuating pigmentation; inhibiting melanogenesis by activating the ERK pathway   | Karunaratne et al. (2019)   |
| <i>Ganoderma lucidum</i>   | Polysaccharide  | UVB-induced melanogenesis   | Antagonizing cAMP/PKA and ROS/MAPK pathways  | Hu et al. (2019)  |

Abbreviations: 6-OHDA, 6-hydroxydopamine; AMPK, adenosine monophosphate activated protein kinase; CAT, catalase; CREB, cAMP-response element-binding protein; ERK, extracellular signal regulated kinase; GABA, gamma-aminobutyric acid; HO1, heme oxygenase-1; IL, interleukin; LPS, lipopolysaccharide; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NO, nitric oxide; Nrf2, nuclear factor erythroid 2-related factor 2; PD, Parkinson's disease; PHZ, Phenylhydrazine; PTZ, Pentylenetetrazole; ROS, reactive oxygen species; SOD, superoxide dismutase; t-BHP, tert-butyl hydroperoxide; TALEN, transcription activator-like effector nucleases; TNF-α, tumor necrosis factor alpha; UVB, ultraviolet B; VEGFR, vascular endothelial growth factor receptor.

some chemical-induced zebrafish models have also been reported, such as copper sulfate ( $\text{CuSO}_4$ )-stimulated inflammation (Nguyen et al. 2020; Tu et al. 2020), vinorelbine-induced neutropenia, or macrophage deficiency (Z. H. Li et al. 2020; Wu et al. 2019), and carrageenan-induced abdominal edema (da Silva et al. 2020).

Several transgenic zebrafish lines have been generated for anti-inflammatory research, and they were mainly applied for the establishment of injury inflammation models induced by cutting the tailfin of the larvae (M. He et al. 2020; Huang et al. 2016; Yang et al. 2019), and some chemical-induced models have been reported as well (Z. H. Li et al. 2020; Z.

G. Wang et al. 2019). The zebrafish lines Tg(mpx:EGFP)<sup>i114</sup> and Tg(Lyz:EGFP), which express green fluorescent proteins (GFP) in neutrophils, were used for monitoring the neutrophils at inflammatory areas (Z. H. Li et al. 2020; Yang et al. 2019; W. Zhang et al. 2020). Two double-transgenic zebrafish lines that express different colors of fluorescence in neutrophils and macrophages were also developed, including the Tg(mpx:GFP<sup>i114</sup>/mpeg1:mCherry-F<sup>umsF001</sup>) line that expresses green fluorescence in neutrophils and expresses red fluorescence in macrophages (M. He et al. 2020), and the Tg(Coroninla-eGFP/Lyc-dsRed) line which has yellow fluorescent neutrophils and green fluorescent macrophages (Huang et al. 2016). In addition, a glucocorticoid receptor (GR) mutant zebrafish line, gr<sup>s357</sup>, was introduced to investigate the dependency of GR on the anti-inflammatory effects of ginsenoside Rg1 (M. He et al. 2020).

### **3.3. Zebrafish models for the evaluation of neuroprotection**

Several models of neurological disorders, such as neurodegenerative, nerve injury, anxiety and depression, seizure, sensorineural hearing loss, and attention deficit hyperactivity disorder (ADHD) models, have been established in zebrafish (Table 1).

#### **3.3.1. Neurodegenerative models**

Neurodegenerative diseases like Parkinson's disease (PD) and Alzheimer's disease (AD) affect millions of people all over the world (Barnhill, Murata, and Bronstein 2020). Due to the lack of a viable option to cure the patients, it is still urgent to explore safe and effective drugs for the prevention and treatment of neurodegenerative diseases. Zebrafish turned out to be an encouraging model for the discovery of natural products with anti-neurodegenerative potential (Wasel and Freeman 2020), which are summarized in Table 1.

PD occurs primarily from the loss of dopaminergic neurons in the substantia nigra. The most recognized experimental PD models induced by neurotoxins, such as 6-hydroxydopamine (6-OHDA) (M. Q. Li et al. 2018) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (C. W. Li et al. 2018), have been established in zebrafish. Two assays are commonly implemented to determine the neuroprotective effects of the natural products in zebrafish PD models. One is zebrafish anti-tyrosine hydroxylase (TH) whole-mount immunostaining, which evaluates the loss of dopaminergic neuronal populations (S. S. Zhang et al. 2020), the other is zebrafish locomotion assay, which records the locomotive behavior of zebrafish under an automated video tracking system (Liao et al. 2018). Zebrafish model can also be used to evaluate the bioavailability of anti-PD drugs. For example, puerarin, a naturally occurring compound in traditional Chinese medicine *Pueraria lobata*, can be used to treat PD, but its poor water solubility limits its utilization (Chen et al. 2019). The encapsulated puerarin with increased solubility was administrated to zebrafish and the rapid absorption of nanoparticles into circulation was observed with Förster resonance energy transfer (FRET) (Chen et al. 2019).

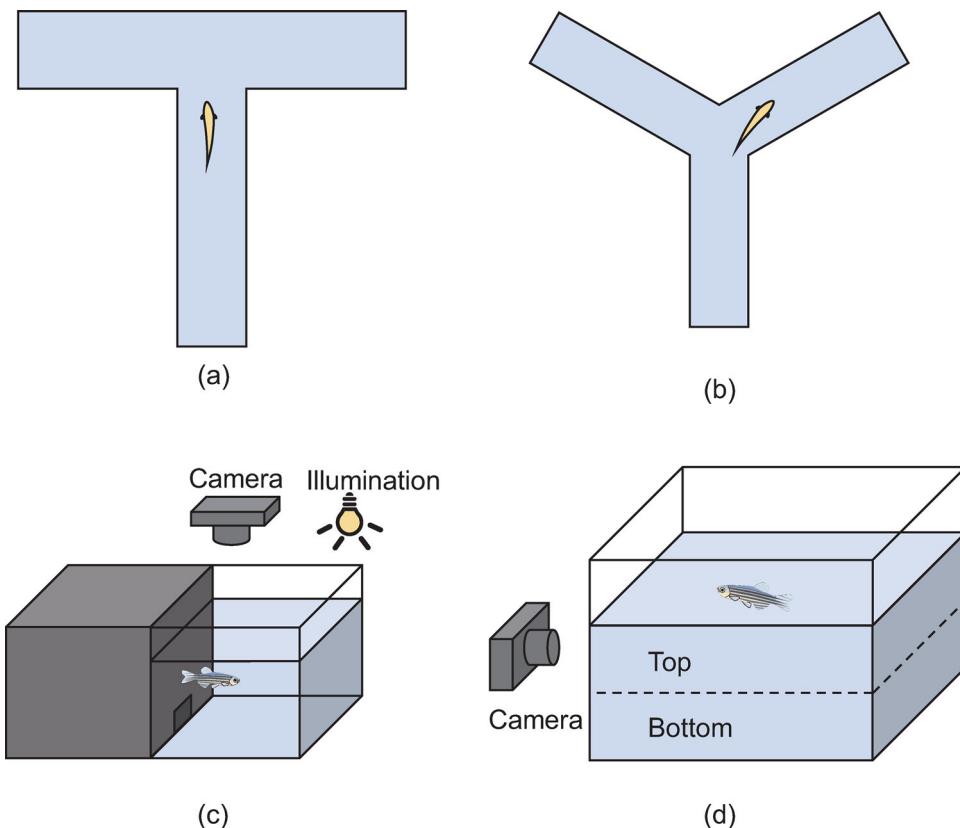
AD is a progressive neurodegenerative disorder characterized by memory loss and cognitive impairment. Zebrafish models, including scopolamine-induced dementia model (Capatina et al. 2020), acrylamide-induced cognitive defects model (Sharma and Kang 2020), and mycophenolate mofetil-induced nerve injury model (M. C. Liu et al. 2019), can mimic the symptoms or pathogenesis of AD, and they have been used to evaluate the neuroprotective effects of rosemary essential oil, garcinol, and antioxidant peptide from walnut, respectively. The spatial memory in zebrafish can be assessed by using the T-maze or Y-maze tasks (Figure 3a,b). The anti-neurodegenerative effects of natural products are considered to be associated with their antioxidant capacity, therefore, further mechanism exploration can target to measure the activity of antioxidant enzymes and the expression of genes in redox-related signaling pathways (Abidar et al. 2020; M. Q. Li et al. 2018).

#### **3.3.2. Anxiety and depression models**

Anxiety and depression are common psychiatric illnesses that affect the social capacity of humans. As an emerging animal model of anxiety and depression, zebrafish respond predictably to anxiogenic and anxiolytic drugs in behavior, and they are easy to be handled and inexpensive to be maintained, making it a suitable model to study disorders like anxiety, and depression (Stewart et al. 2015). The behavioral assays, light-dark test, and novel tank-diving test as shown in Figure 3c,d, are normally carried out to evaluate the anxiolytic and antidepressant activities in zebrafish (de Melo et al. 2020). Zebrafish prefer safe areas, like dark environments and the bottom of water tanks. Meanwhile, their instinct drives them to explore novel and potentially risky environments, which can trigger anxiety in zebrafish. Anxiogenic substances can increase the time zebrafish spend in safe areas, whereas the substances like breadfruit pulp protein fraction (Gonçalves et al. 2020), pitaya pulp and peel that contain phytolactams isomers, betanins isomers, and flavonoids (Lira et al. 2020), and isoflavone from germinated soybean (de Melo et al. 2020) can decrease the time (Table 1).

#### **3.3.3. Epilepsy models**

Epilepsy is one of the most common and serious neurological disorders. Zebrafish seizure model induced by pentylenetetrazole (PTZ), a noncompetitive antagonist of  $\gamma$ -aminobutyric acid receptors, is a well-recognized experimental model for screening and identifying anti-seizure drugs (Gawel, Langlois, et al. 2020). Using this model, compounds with pharmacological applications from traditional medical herbs, such as berberine from berberis (B. Y. Zhang et al. 2020) and palmatine from Berberidaceae (Gawel, Kukula-Koch, et al. 2020), were reported to exert anti-epileptic activity recently. Other natural products with anti-epileptic activity are summarized in Table 1. Moreover, an advanced high-throughput, neuroprotective drug-screening approach based on live zebrafish was established by combining whole-brain activity mapping with machine learning, which could build a functional classifier by associating the



**Figure 3.** Behavioral tests in zebrafish. Spatial memory in zebrafish was evaluated by T-maze task (a) or Y-maze task (b); Anxiolytic and antidepressant activities in zebrafish were assessed using light-dark test (c) or novel tank-diving test (d).

non-clinical compounds with known therapeutic drugs (Lin et al. 2018). In this study, the authors highlighted their successful prediction by validating the antiepileptic activity of predicted anti-seizure compounds in the PTZ-induced zebrafish seizure model. The bioavailability of free and encapsulated anti-seizure drugs could be compared using zebrafish models. *Cryptolepis sanguinolenta* is traditionally used to manage different disorders in West Africa, and cryptolepine, the major alkaloid in it, is not hydrophilic. In a previous study, cryptolepine and its solid-lipid nanoparticle formulation were tested on PTZ-induced zebrafish (Mante et al. 2021). Compared to free cryptolepine, solid-lipid nanoparticle formulation decreased seizure score and extended the latency to onset of seizures.

Collectively, zebrafish models have been established for PD, AD, ADHD, anxiety, and sensorineural hearing loss models, offering particular advantages to test the neuroprotective effects of natural products, and can facilitate to screen and discover novel natural products that have the potential to prevent and manage these disorders.

### 3.4. Zebrafish models for the evaluation of cardiovascular protection

Several recent reviews summarized different zebrafish models used in the cardiovascular system, e.g., cardiac regeneration models, chemical-induced models of cardiomyopathy or vasculopathy, and genetic mutation or modification models (Beffagna 2019; Giardoglou and Beis 2019; Zhao et al.

2019). Herein, the use of zebrafish models in the discovery of natural products with cardiovascular protective functions, including cardioprotective, vascular protective, and antithrombotic effects, is primarily discussed.

#### 3.4.1. Cardiomyopathy models

A recent study investigated the cardioprotective effect of resveratrol against oxidative damage induced by zinc oxide nanoparticle (ZnO NP) in the zebrafish model, and it was demonstrated that ZnO NP-induced zebrafish cardiac morphological and functional abnormalities during the embryonic development were notably counteracted by resveratrol intervention (Giordo et al. 2020). In another study, the 3,3',4,4',5-pentachlorobiphenyl (PCB126)-induced pericardial edema in zebrafish embryos was found to be inhibited by secoisolariciresinol, a natural lignan-type polyphenolic compound derived from the flaxseed, which indicated a rescuing effect of secoisolariciresinol on the cardiac morphological abnormalities (Tokunaga, Woodin, and Stegeman 2016). Interestingly, several natural products displayed a cardioprotective activity in low doses, but they revealed cardiotoxicity at high concentrations. For instance, by using two transgenic zebrafish lines, Tg(fli1:EGFP)y1 and Tg(CMLC2:GFP), it was found that PcsK3, an ShK-like peptide from *Palythoa caribaeorum*, accumulated in the yolk sac stripe and displayed a cardioprotective effect at doses less than the IC50, while accumulated in the blood and induced cardiac morphological abnormalities at doses higher than the IC50 (Liao et al. 2018). Moreover, artesunate, the bioactive compound

from a traditional Chinese medical herb *Artemisia annua* L. (Asteraceae), showed a cardioprotective effect on verapamil-induced zebrafish heart failure model at low concentrations, while it induced cardiotoxicity in larval zebrafish, such as pericardial edema and circulation defects, at high concentrations (Zheng et al. 2020).

### 3.4.2. Vasculopathy models

Due to its small size and transparent body in the early stage, zebrafish can be used as an ideal model for the study of vascular disorders by observing the changes in vascular structure. Zebrafish-based vasculopathy model induced by high glucose was used to study the vascular-protective effect of Brazilian green propolis water extract, and it attenuated high glucose-induced structural abnormalities in hyaloid and cerebral vessels of zebrafish larvae (Saito et al. 2018). In the study of the protective activity of resveratrol against ZnO NP-induced oxidative damage, the authors found that resveratrol also significantly counteracted the vascular morphological abnormality in zebrafish induced by ZnO NP, suggesting a vascular-protective activity of resveratrol (Giordo et al. 2020). Besides, fermented black garlic samples and their Amadori products showed a vascular-protective effect on zebrafish by rescuing chemical-induced vascular damage (X. M. Zhang et al. 2019). Naringin and naringenin isolated from the pericarp of citrus fruits were reported to exhibit pro-angiogenic activity on the restoration of vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitor II-induced blood vessel loss (L. Chen et al. 2018). Furthermore, based on the metabolomics approach, bioactive compounds (e.g., gastrodin, citric acid, parishin A, parishin C, parishin E, stigmasterol, and *p*-hydroxybenzyl alcohol) from *Gastrodiae Rhizoma* were identified to have pro-angiogenic activity in the zebrafish model, contributing to the formation of new blood vessels (M. Liu et al. 2020), and fermented black garlic and the Amadori products were found to promote angiogenesis as well (X. M. Zhang et al. 2019). Moreover, a transgenic zebrafish line, Tg(fli1:EGFP)y1, which is a model of intersegmental blood vessel insufficiency, was used to investigate the pro-angiogenic activities of *Rubia cordifolia* extracts (Y. Chen et al. 2018).

### 3.4.3. Thrombosis models

Thrombosis is a leading cause of death. Several zebrafish thrombosis models, including phenylhydrazine (PHZ)-induced thrombosis (Zhu et al. 2016), arachidonic acid-induced thrombus (Shi et al. 2020), and ferric chloride ( $\text{FeCl}_3$ )-induced thrombus (Gao et al. 2017, 2019), have been established either by microinjection or direct soaking, and applied for the discovery of safe and effective antithrombotic natural products. Antithrombotic efficacy is normally evaluated by staining the wild-type zebrafish larvae with O-dianisidine dye liquor, and then measuring the staining intensity of the heart red blood cells or observing the morphology of thrombus in caudal vein under a fluorescence microscope. Furthermore, there are also transgenic zebrafish lines, such as Tg(LCR:EGFP) and Tg(CD41:EGFP) that express GFP in erythrocytes and platelets,

respectively, which allow labeling erythrocytes and platelets, offering an opportunity to dynamically evaluate the antithrombotic activities of compounds (Li et al. 2021). Several antithrombotic compounds have been identified from natural plants, such as terpenoid glycosides, terpenes, and flavones from the leaf extracts of *Crataegus pinnatifida* (Gao et al. 2017, 2019), bilobalide and ginkgolides from extracts of *Ginkgo biloba* (Lu et al. 2018), rosmarinic acid, lithospermic acid, and salvianolic acid B from *Salvia miltiorrhiza* and *Panax notoginseng* (Yin et al. 2020), geniposide, citric acid, and quinic acid from the fructus of *Gardenia jasminoides* (Shi et al. 2020).

In general, several zebrafish models have been introduced to evaluate the cardiovascular protection of natural products, while further studies are needed to increase the use of available zebrafish models to expand the screening of cardiovascular-protective compounds.

## 3.5. Zebrafish models for the evaluation of anti-cancer effect

Zebrafish models are excellent tools for the discovery of anti-cancer preclinical drugs. Zebrafish have been used to identify natural products with potential anti-cancer activities by observing the developmental phenotypic defects of embryos after exposure to them, or by examining their anti-angiogenic effects (Chávez et al. 2016). Zebrafish can also be used as a patient avatar for the study of cancer biology or precise therapy by using transgenic cancer models or xenotransplantation of human cancer cells into zebrafish (Astell and Sieger 2020; Fazio et al. 2020; Usai et al. 2020).

### 3.5.1. Anti-angiogenic models

Angiogenesis is an essential process for the growth and metastasis of tumors, and it is a hallmark of cancer progression (Tulotta et al. 2016). Due to the high similarities in the development of vascular structure between zebrafish and humans, zebrafish embryos are used as a popular and valuable model to evaluate the anti-angiogenic effects of food components. The expression of red or green fluorescent proteins in endothelial cells of transgenic zebrafish lines, including Tg(flk:mCherry), Tg(flk1:EGFP), Tg(fli1:EGFP), and Tg(fill1a:EGFP)y1, makes it possible for visual observation of blood vessels and the evaluation of anti-angiogenic effects *in vivo*. Moreover, angiogenic models of zebrafish induced by high-glucose were also used to evaluate the anti-angiogenic effects of natural products (Fernando et al. 2019). A variety of angiogenesis inhibitors from natural products, such as baicalein derivatives, biflavonoids, chlorogenic acid, diphlorethoxyhydroxycarmalol, ethyl iso-allocholate, isophloroglucin A, eupatilin, fucoidan, luteolin, proanthocyanidins, protocatechuic acid, quercetin, syringic acid, and tetrandrine derivatives, have been discovered using these models (Table 1).

### 3.5.2. Transgenic cancer models

Due to the extensive homology with human cancer-related genes, convenience for genetic manipulation, and rapid development of advanced transgenic and genome editing

**Table 2.** Application of zebrafish models in safety evaluation of natural products.

| Toxicity type          | Measurements  | Food/Contaminant  | Toxic substance             | Toxicological study                   | Reference               |
|------------------------|---|---|-----------------------------|---------------------------------------|-------------------------|
| Oxidative stress       | The levels of catalase, superoxide dismutase, reactive oxygen species, glutathione-S-transferase, and malondialdehyde   | <i>Clerodendron cyrtophyllum</i> Turcz  | Ethanol extract from leaves | Exposure from 6 hpf to 5 dpf          | Nguyen et al. (2020)    |
| Genotoxicity           | DNA damage index, number of micronuclei and nuclear abnormalities   | Flavor ingredient   | 4-Ethylbenzaldehyde         | Exposure for 21 d                     | Bencsik et al. (2018)   |
| Embryotoxicity         | Survival rate/mortality, phenotypic and sublethal alterations, including hatching rate, spontaneous movement, pericardial edema, yolk edema, blood stasis, blood circulation, heartbeat, and notochord malformation rate  | <i>Polygonum multiflorum</i> Thunb.   | Extracts                    | Acute toxicity                        | Yang et al. (2018)      |
| Embryotoxicity         | Mortality rate; embryonic malformations (yolk-sac edema, spinal curvature, tail deformity, uninflated swim bladder and cardiac defects.) by using a stereoscope   | Cannabis  | Cannabinol                  | Exposure from 24 to 96 hpf            | Chousidis et al. (2020) |
| Embryotoxicity         | Survival rates, LC <sub>50</sub> , hatching rate, morphological deformities, heart rates, ROS assay   | <i>Atractylodes lancea</i> (Thunb) DC. (AL)                                       | Atractylodin and β-eudesmol | Exposure for 72 hpf                   | Tshering et al. (2021)  |
| Developmental toxicity | Morphological changes under stereomicroscope, hatching rates  | <i>Gardenia jasminoides</i> Ellis   | Genipin                     | Exposure for 72 hpf                   | Xia et al. (2021)       |
| Developmental toxicity | NOEC, LC <sub>50</sub> , EC <sub>50</sub> , teratogenicity, egg coagulation, mortality, hatching, yolk sac edema, pericardial edema, body length, and heartbeat   | <i>Clerodendron cyrtophyllum</i> Turcz  | Ethanol extract from leaves | Exposure from 6 hpf to 5 dpf          | Nguyen et al. (2021)    |
| Developmental toxicity | Morphological characteristics (coagulation of eggs, tail detachment, heart beat, and hatching rates)  | <i>Dodonaea viscosa</i>   | Methanolic crude extract    | Exposure for 96 h                     | Khan et al. (2021)      |
| Reproductive toxicity  | Parental fishes: fertility and histopathology of gonad, gonadosomatic index, hormones level, such as estradiol and testosterone, reproductive neuroendocrine-related genes expression; offsprings: number of eggs, fertilization rate, teratogenesis, lethality, and heart rate | <i>Endoplectura uchi</i> (Huber) Cuatrec.   | Extracts from barks         | Exposure for over 21 consecutive days | Hyacienth et al. (2020) |
| Cardiotoxicity         | Heart malformation rate, pericardial edema areas, sinus venosus-bulbus arteriosus distance, heart rate; expressions of cardiac development-related key transcriptional regulators   | Several plants  | α-asarone                   | Exposure from 24 to 192 hpf           | X. Shang et al. (2020)  |
| Cardiotoxicity         | Measurement of heart development and morphology in a temperature-controlled room; video recordings by using transmitted cold lighting (LED)   | Cannabis  | Cannabinol                  | Exposure for 48, 72, and 96 hpf       | Chousidis et al. (2020) |
| Hepatotoxicity         | Pathological section analysis; fluorescence imaging of liver; genes expression or transcriptome analysis  | <i>Tripterygium wilfordii</i> Hook.   | Triptolide                  | Exposure for 72 h                     | Huo et al. (2019)       |
| Hepatotoxicity         | Pathological section analysis; fluorescence imaging of liver; genes expression or transcriptome analysis  | Bioactive compound  | Aloe emodin                 | Exposure for 72 h                     | Quan et al. (2019)      |
| Hepatotoxicity         | Gene expression, caspase activity, triglyceride concentration, histology, and immunohistochemistry  | Food ingredient   | Palmitic acid               | High-fat diet for 2 wk and 4 wk       | Ding et al. (2018)      |
| Neurotoxicity          | Frequency of coiling behavior; expression of serotonin receptor 5-htr1ab and 5-htr1bd gene  | <i>Aconitum</i> spcies  | Aconitine                   | Exposure from 4 hpf to 96 hpf         | H. Chen et al. (2021)   |
| Neurotoxicity          | Locomotor activity via a video tracking system (ZebraBox)   | Ayahuasca prepared from <i>Banisteriopsis caapi</i> and <i>Psychotria viridis</i> | —                           | Exposure for 120 and 144 h            | Andrade et al. (2018)   |
| Neurotoxicity          | Basal locomotor activity; visual motor response; vibrational-evoked escape response   | Cannabis  | Cannabinol                  | Exposure from 24 to 96 hpf            | Chousidis et al. (2020) |

Abbreviations: EC<sub>50</sub>, median effective concentration; LC<sub>50</sub>, median lethal concentration; LED, light-emitting diode; NOEC, no observed effect concentration; ROS, reactive oxygen species.

technologies, various transgenic zebrafish have been generated and quickly developed as valuable *in vivo* models for cancer research (Raby et al. 2020a). Transgenic zebrafish cancer models can be created by microinjection at the one-

cell stage embryos or electroporation in adults with transgenesis vectors. Recent reviews summarized the common transgenic zebrafish models in cancer research, including the timeline of key developments, the advantages of using these

models, as well as the technical difficulties and limitations (Fazio et al. 2020; Raby et al. 2020a). Even though the transgenic zebrafish models have been established for a long time and a large number of transgenic models are available, few models have been used in the evaluation of the anti-cancer potential of natural products. Recently, it is noticed that the hepatocellular carcinoma models of transgenic zebrafish, including Tg(fabp10a:HBV-HBx-mCherry, myl7:EGFP)x Tg(fabp10a:src, myl7:EGFP), and Tg(fabp10a:HBV-HBx-mCherry, myl7:EGFP, p53<sup>-/+</sup>)xTg(fabp10a: src, myl7: EGFP, p53<sup>-/+</sup>), were used to investigate the anti-cancer effects of oligo-fucoidan (S. Y. Wu et al. 2020).

### 3.5.3. Xenograft cancer models

Zebrafish have many advantages, such as high fecundity, rapid breeding, transparency, low costs, and most importantly, the immature immune system in the early stage of development, making larval zebrafish to be an ideal xenograft cancer model without needing an immunocompromise (Leslie 2017). Since the first xenograft model was established in zebrafish larvae about 15 years ago, the xenotransplantation of tumor cells into larval zebrafish has been extensively reported in different cancers (Fazio et al. 2020). However, there are some inherent limitations in larval zebrafish xenograft cancer models, such as a short experimental time window, limited numbers of transplanted cells, and a low experimental temperature. Afterward, xenograft models using immunodeficient adult zebrafish as the recipients of cancer cells were further developed to compensate for these limitations (Yan et al. 2020). Moreover, zebrafish were recently applied to implant patient-derived cancer cells (Fior et al. 2017), and were rapidly emerged as a promising model for personalized medicine and precise cancer therapy (Raby et al. 2020b; J. Xiao et al. 2020). The tumor cells or patient-derived xenograft models were usually generated in larval zebrafish within 48 hours of post-fertilization by microinjection of labeled cancer cells at 34°C or in immunocompromised adult zebrafish by injection of the labeled cancer cells at 37°C. The delivery of drugs or tested compounds can be easily achieved by directly adding them into the water. By using the zebrafish xenograft cancer models, several natural products, including caffeic acid phenethyl ester, eupatilin, fucoidan, fucosterol, furanodiene, luteolin, microcin E492, osthole, propyl gallate, quercetin, and sandensolide, have been identified to possess strong anti-cancer properties, by decreasing tumor formation, reducing tumor cell proliferation, growth, and metastasis, and inducing apoptosis of cancer cells (Table 1).

In summary, zebrafish models are promising animal models for the large-scale screening of anti-cancer natural products, and can be used to discover novel anti-cancer compounds from natural sources in the future.

### 3.6. Zebrafish models for the evaluation of metabolic disorders

Diabetes, obesity, and associated metabolic disorders, such as insulin resistance, hyperglycemia, hypertension,

hyperlipidemia, and steatosis hepatitis, are becoming the major public health challenges across the world (Li et al. 2019; A. Shang et al. 2020). Plant-based diets are associated with lower risks of these complex metabolic disorders (Chen et al. 2020). Therefore, it is a need for appropriate models for studying these disorders and exploring effective treatments. The zebrafish show the potential to be an attractive animal model to seek efficacious natural products for the prevention and treatment of metabolic disorders (Salmi, Tan, and Cox 2019; Zang, Maddison, and Chen 2018).

#### 3.6.1. Diabetes models

Type 2 diabetes is the most common diabetes and is closely related to obesity (Magkos, Hjorth, and Astrup 2020). Zebrafish type 2 diabetes models can be established by overfeeding artemia or commercial diet-induced obesity, accompanied by a decrease in glucose tolerance and a rise in insulin resistance (Zang, Shimada, and Nishimura 2017). Other models induced by cyclic adenosine monophosphate (cAMP) combining with dexamethasone (Xu et al. 2019) and glucose (Garcia Campoy et al. 2018) were also created for the study of type 2 diabetes in zebrafish, by which corosolic acid from *Eriobotrya japonica* and the bark extracts of *Eysenhardtia polystachya* were discovered to have anti-diabetic effects. Type 1 diabetes is usually developed in children and occurs when the pancreas fails to produce insulin (Ilonen, Lempainen, and Veijola 2019). Two zebrafish models of type 1 diabetes were established by damaging the pancreatic islets and  $\beta$  cells by chemicals, such as alloxan or palmitate. Palmitate-induced damage model was used to evaluate the  $\beta$  cell protective effect of the red seaweed *Polysiphonia japonica* extract (Cha et al. 2018). Alloxan-induced pancreatic islet damage model was used to identify the anti-diabetic effect of flavonoid 8-O-glucuronide, which was isolated from the aerial parts of *Malva verticillata* (Ko et al. 2018). Moreover, the synergistic protective effect of two major compounds from *malva verticillata* on injured pancreatic islets was demonstrated in the same model (Ko et al. 2019). The fermented extract of *Forsythia koreana* flowers and four lignans isolated from it exhibited recovery effects on alloxan-damaged pancreatic islets in zebrafish (Y. G. Lee et al. 2019).

#### 3.6.2. Obesity models

Obesity is a complex medical condition characterized by excessive body fat accumulation that may increase the health risk of various associated diseases, such as diabetes, cancer, and cardiovascular diseases (A. Shang et al. 2020). It is a worldwide health epidemic caused by a combination of different factors. The changes in lifestyle, especially the diet, and the lack of physical exercise are considered the main causes of overweight or obesity currently (Birgani, Motamedi, and Kanaani 2019). Overconsumption of foods rich in fat and/or sugar can cause obesity. Accordingly, a series of diet-induced obese animals were developed as *in vivo* models for the study of obesity (Hariri and Thibault 2010). Overfeeding or feeding with a high-fat diet also causes fat

accumulation and obesity in zebrafish, and it has been applied to evaluate the anti-obesity effect of natural products like *Etlingera calophrys*, *Citrus sinensis*, and green tea (Idrus et al. 2020; Montalbano et al. 2019; Zang et al. 2019).

### 3.6.3. Fatty liver models

Fatty liver is another metabolic disorder characterized by abnormal fat accumulation in the liver. There are generally two types of fatty liver, alcoholic fatty liver, and nonalcoholic fatty liver. A high-fat diet is one of the major factors that contribute to nonalcoholic fatty liver disease. Natural products are reported to be good sources for the prevention and treatment of liver diseases (Meng et al. 2018a, 2018b). Thus, both alcohol-induced liver injury, and high-fat diet-induced obese zebrafish with hepatic steatosis are developed to discover the natural products with hepatoprotective effects. Supplementation with probiotic *Lactobacillus rhamnosus* GG presented a lower hepatic lipid accumulation in a zebrafish model of alcoholic liver disease, and the mechanism of the hepatoprotective effect was associated with the regulation of inflammatory response and gut permeability (Bruch-Bertani et al. 2020). Y. P. Liu et al. (2019) demonstrated the hepatoprotective effect of *Lactobacillus plantarum* by regulating the Keap1/Nrf2/ARE pathway in larval zebrafish with alcoholic liver injury. By using the diet-induced obese zebrafish model, Matsuura et al. (2020) revealed the protective effect of *Lactobacillus*-fermented cauliflower mushroom (*Sparassis crispa*) on diet-induced hepatic steatosis through the activation of  $\beta$ -oxidation, and Nakayama et al. (2018) demonstrated the hepatoprotective effect of red seaweed *Palmaria mollis* by inhibiting lipogenesis and activating  $\beta$ -oxidation. Moreover, a transgenic zebrafish line, Tg(L-FABP:EGFP) that expresses a GFP in the liver, was introduced to evaluate the protective effects of phospholipids, isolated from female squid gonads, on fatty liver diseases (Xia et al. 2020).

Overall, zebrafish models of metabolic disorders established by chemical induction or dietary alteration are efficient in evaluating the effectiveness of natural products in metabolic disorders. Taking advantage of these models can be a good supplement to accelerate the screening and evaluation process of natural products, even an alternative for functional food development. In addition, many zebrafish genetic manipulation models of metabolic disorders are currently available (Benchoula et al. 2019), and can be introduced to explore related molecular mechanisms of natural products.

## 3.7. Zebrafish models for the evaluation of intestinal protection

Zebrafish do not have a stomach, but they have a gut that, although histologically different, is functionally highly homologous to that of humans (Flores et al. 2019). As an attractive model, zebrafish has been widely applied in various aspects of intestinal protection assessment, such as regulating immune responses, modulating gut microbiota,

promoting intestinal peristalsis, and protecting against intestinal injury, inflammation, and infection.

### 3.7.1. Unchallenged models

Diet is one of the key factors affecting the health of the host, and gut microbiota is involved in this regulatory process (Cao et al. 2019). The unchallenged zebrafish is used as a valuable model to investigate the interactions among the diet, gut microbiota, and host health. Both the zebrafish larvae and adults were applied to evaluate the effects of *Cladosiphon okamuranus* fucoidan on microbiota composition and immune responses (Ikeda-Ohtsubo et al. 2020). The immune responses were evaluated by the expression of pro- and anti-inflammatory cytokine genes in larval and adult zebrafish, as well as live imaging of neutrophils and macrophages in larval zebrafish, while the microbiota compositions, whole microbiota of larval zebrafish, and gut microbiota of adult zebrafish, were analyzed by 16S rRNA gene sequencing (Ikeda-Ohtsubo et al. 2020). The expression of some genes related to immune responses and the diversity of gut microbiota were found to be increased in the zebrafish fed with commercial diets supplemented with microalgae rich in omega-6 long-chain polyunsaturated fatty acids compared to the non-supplemented controls (Nayak et al. 2020). Another study revealed that high-saturated fat feeding altered the composition of gut microbiota and changed the lipid profile and metabolism of the zebrafish, while supplementation with fish oil could limit the enrichment of *Pseudomonas* and ameliorate the effect of the high-saturated fat diet on the lipid profile (Arias-Jayo et al. 2019). Besides, a recent study demonstrated that a large number of intergenic regions and genes related to immunity and antioxidant defense system were differentially methylated in the mid-gut of zebrafish fed with diets containing pea, soy, and wheat gluten (Dhanasiri et al. 2020).

### 3.7.2. Constipation models

Gastrointestinal motility disorders are highly prevalent, and it is still challenging the treatment of these widespread conditions. The zebrafish model of constipation induced by loperamide hydrochloride has been constructed and applied for the screening of intestinal peristalsis-promoting probiotics with high retention capacity (Lu, Zhang, Yi, et al. 2019). By using this model, *Bifidobacterium animalis* F1-7 was screened out from 18 strains with the most effective effect on intestinal peristalsis promotion, and further studies revealed that *Bifidobacterium animalis* F1-7 promoted zebrafish intestinal peristalsis by regulating the genes related to serotonin synthesis and modulating serotonin release (Lu, Zhang, Liang, et al. 2019).

### 3.7.3. Inflammatory bowel models

Chemical-induced and genetic animal models of intestinal inflammation are commonly used for the study of human inflammatory bowel diseases (Mizoguchi et al. 2020). Deficiency or mutation in genes, such as class III PI3-kinase

(PIK3C3) (Zhao et al. 2018), macrophage stimulating 1 (MST1) (Witte et al. 2014), ubiquitin-like with PHD and ring finger domains 1 (UHRF1) (Marjoram et al. 2015), DNA methyltransferase 1 (DNMT1) (Marjoram et al. 2015), and CDP-diacylglycerol-inositol 3-phosphatidyltransferase (CDIPT) (Thakur and Ahirwar 2019), were reported to result in intestinal damage and inflammation in zebrafish, and they have been proposed to serve as disease models for the research of human inflammatory bowel diseases and the development of potential therapeutic approaches.

Chemical-induced intestinal inflammation has been established in zebrafish. Recently, larval zebrafish enterocolitis model induced by dextran sodium sulfate (DSS) was used to evaluate the potential beneficial effects of heteropolysaccharide-producing bifidobacteria on intestinal health (Llamas-Arriba et al. 2019). It was revealed that the intestinal protective effect of *Lactobacillus rhamnosus* GG, a beneficial bacterium, might be synergic with phenolic compounds from fruit products, such as sea buckthorn and apple juice, via a zebrafish enterocolitis model induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Sireswar, Biswas, and Dey 2020; Sireswar and Dey 2019). The fermented *Dendrobium candidum* containing a high content of polyphenols, was found to relieve the oxazolone-induced intestinal inflammation in zebrafish by regulating the host immune responses as well as the gut microbiota (Gong et al. 2020). In addition, fish are susceptible to bacterial infections that can lead to enteritis. Recent research demonstrated the intestinal protective effect of an orange juice extract particularly high in flavonoids on an adult zebrafish model of enteritis triggered by *Vibrio anguillarum* (Cirmi et al. 2020).

### 3.7.4. Infection models

Contaminated food and water-induced gastrointestinal infection remains a major disease burden worldwide. Studies revealed that probiotics and certain functional foods can be used as effective supplements to strengthen the digestive system and therefore reduce the pathogenicity of pathogens in several different zebrafish intestinal infection models (Al Azzaz et al. 2019; Edirisinghe et al. 2019; Rajamohamed and Siddharthan 2019). Mirabdollah Elahi et al. (2020) evaluated the intestinal protective effect of probiotic *Lactobacillus acidophilus* in zebrafish model of *Escherichia coli* O157: H7 infection in intestines and the histopathological results showed that adding *Lactobacillus acidophilus* into the zebrafish diet could reduce the *Escherichia coli* O157: H7 infection-induced tissue damage. Rajamohamed and Siddharthan (2019) found that *Amukkara chooram* had the potential to clear *Candida albicans* biofilm from intestinal epithelial cells and relieve tissue damages in zebrafish infection model. Pectin extracted from *Spirulina maxima* was identified to modulate immune responses and improve performance in larval and adult zebrafish against *Edwardsiella piscicida* and *Aeromonas hydrophila* infections (Edirisinghe et al. 2019). A transgenic GFP-Lc3 zebrafish model, which expresses a GFP and an autophagy marker LC-3, was used to demonstrate the role of resveratrol on autophagy-dependent intracellular

bacteria clearance in zebrafish infected with *Salmonella typhimurium* and *Escherichia coli* (Al Azzaz et al. 2019).

Altogether, zebrafish models for constipation, inflammation, and infection have been established, which can promote the understanding of intestinal disease mechanisms and provide particular advantages for screening natural substances for intestinal protection.

### 3.8. Zebrafish models for the evaluation of renal protection

As the nephron functional units of embryonic kidney are highly conserved between teleosts and mammals, zebrafish can be utilized as a useful animal model to study the pathogenesis of human kidney diseases and to develop therapeutic strategies such as screening of nephroprotective drugs (Outtandy et al. 2019). McKee and Wingert (2016) demonstrated to construct a zebrafish model of acute kidney injury by microinjection of nephrotoxin into the zebrafish embryo. The nephroprotective effects can be determined by measuring the glomerular filtration rate and nephrotoxin-induced kidney malformations. Moreover, Y. Zhang et al. (2019) established a larval zebrafish model of acute hyperuricemia by administration of potassium oxonate, a uricase inhibitor, and xanthine sodium salt, a uric acid synthesis precursor. With these models, zebrafish can offer a robust platform for high-throughput screening of natural products with nephroprotective effects or uric acid-lowering activities.

Despite this, only a few studies used zebrafish models to evaluate the protective effects of natural products on the kidney. Ding et al. (2015) demonstrated the nephroprotective effects of resveratrol and ursolic acid on a zebrafish nephrotoxicity model induced by aristolochic acid. Two transgenic zebrafish lines, Tg(wt1b:EGFP) and Tg(gata1:DsRed), were used to record the subtle changes of kidney and red blood cell circulation, respectively. Dopamine and L-mimosine were identified to have both otoprotective and nephroprotective effects by using a zebrafish model of cisplatin-induced ototoxicity and nephrotoxicity (Wertman et al. 2020). Yaocha, a complex natural product, was found to reduce the uric acid level in the zebrafish hyperuricemia model (Xiong et al. 2020).

### 3.9. Zebrafish models for the evaluation of anti-osteoporosis effects

Due to the highly conserved characters in bone formation and composition between human and bony fish, the zebrafish is utilized as a useful model for the study of human skeletal diseases, like osteoporosis (Witten et al. 2017). Zebrafish models are often used in conjunction with *in vitro* cell line models or more recognized osteoporosis models of ovariectomized rodents to assess the anti-osteoporosis effects of natural products. Two isomeric flavonoid aglycones from *Epimedii folium*, circinal-icaritin (CIT) and icaritin (IT), of which CIT was revealed to have stronger anti-osteoporosis effects by suppressing bone reabsorption in zebrafish embryos (J. Jiang et al. 2018). It was demonstrated that the

fruit extracts of *Dimocarpus longan* could enhance bone mineralization in zebrafish and prevent bone loss in osteoporotic rats by inhibiting RANKL-induced osteoclast differentiation (Son et al. 2019), and the extracts of fermented *Crassostrea gigas* could enhance bone mineral density in larval zebrafish and promote tail fin regeneration in adult zebrafish by activating Wnt/β-catenin-induced osteoblast differentiation (Molagoda et al. 2019). Recently, a zebrafish model of osteoporosis induced by prednisolone was established to demonstrate the anti-osteoporosis effects of the extracts of *Sinomenii caulis* (W.-J. Liu et al. 2020).

### **3.10. Zebrafish models for the evaluation of anti-melanogenesis effects**

Since some safety issues have been reported in the popular depigmenting agents (Burnett et al. 2010), such as kojic acid and some hydroquinones, discovering new molecules with anti-melanogenic activities is still in great demand in the cosmetic industry. Natural products are considered to be the major sources to discover safe and potent depigmentation compounds (Zaid and Ramahi 2019). Due to the advantages of the fast pigmentation process, rapid breeding, highly fecund, small size, easy handling, and early-stage transparency, zebrafish embryos and larvae have been used as powerful models for the large-scale screening of natural products possessing anti-melanogenic properties (Lajis 2018). The melanogenesis models of zebrafish larvae are established based on UVB-irradiation (Hu et al. 2019) or under the stimulation of α-melanocyte-stimulating hormone (α-MSH) (Karunaratne et al. 2019). The melanin contents and the tyrosine activity can be used as the key biological indicators to evaluate the anti-melanogenesis effects of the candidate compounds. As shown in Table 1, substances like polysaccharides from *Ganoderma lucidum* (Hu et al. 2019), ginsenosides from black ginseng (Jin et al. 2018) and American ginseng (X. Y. Liu et al. 2019), anthocyanins from *Hibiscus syriacus* L. (Karunaratne et al. 2019), piperlongumine from *Piper longum* (Jeon et al. 2019), and inularin from *Inula britannica* (Jang et al. 2020) were recently identified to possess anti-melanogenic activities based on zebrafish models.

## **4. Zebrafish models for safety evaluation**

Mammals are the best models for toxicological studies to predict possible health risks of substances to humans, but the evaluation is time-consuming and expensive. Although additional research is needed to further validate the safety of novel natural ingredients and additives in humans, zebrafish has been used as an emerging and excellent primary model for safety assessment of natural products by rapidly screening out harmful substances to reduce the unnecessary costs in mammal studies. A recent review summarized the use of zebrafish in food safety analysis, and highlighted the possible sources that may result in health risks, not only in the use of food ingredients, additives, preservatives, and transgenic foods, but also from food contaminant residues, such as mycotoxins, agrochemicals, pharmaceuticals, and heavy

metals (Bailone et al. 2019). Because of this, the use of zebrafish as a model for safety evaluation, including oxidative stress, genotoxicity, embryotoxicity, developmental toxicity, reproductive toxicity, cardiotoxicity, hepatotoxicity, and neurotoxicity is briefly summarized (Table 2). According to the exposure time, the toxicological studies can be divided into acute toxicity and chronic toxicity tests. Furthermore, data on zebrafish parameters, such as survival rate/mortality, hatching rate, growth retardation, teratogenicity, heartbeat, cardiac malformation rate, pericardial edema, pericardial edema, yolk sac edema, DNA damage, spontaneous movement, autonomous behavior, fertility, and histopathology, can be easily accessed. Therefore, zebrafish should also be valuable models for validating the safety of natural products.

## **5. Conclusions and perspectives**

This review primarily highlighted the commonly used zebrafish models in natural product evaluation. The regular care and management of laboratory zebrafish, the establishment of zebrafish models and related evaluation indicators, and the natural products evaluated by zebrafish models are mainly summarized and discussed in this paper. The exploitation of zebrafish as a tool provides a reliable and accessible strategy for rapidly and economically screening and evaluating natural products with potential health benefits for humans. Various zebrafish disease models, mainly the models by chemical induction and dietary alteration or transgenic lines with fluorescent labeling, are easy to be applied to observe the regulatory effects of natural products. Furthermore, the innovation and development of automated instruments and auxiliary analytical techniques have been widely reported to encourage the application of zebrafish as a powerful tool for high-throughput screening and evaluation of natural products with multiple bioactivities, which can contribute to the discovery of novel functional substances and drugs derived from natural sources.

In order to make zebrafish models more practical and more instructive for human health, the following aspects should be further explored. It is necessary to understand the regulatory mechanisms of natural products in zebrafish and the relationship of the mechanisms between zebrafish and humans. Genetic manipulation models, such as specific gene knockout, overexpression, or introduction of target genes, which contribute to studying the mechanisms, can play important roles in future exploitation. Currently, there is a lack of standardized and unified experimental operations, such as environmental conditions (where the laboratory zebrafish can be raised), techniques (how the models can be established or the natural products can be delivered), time (when the treatments can be started or terminated), and measurements (what indicators can be the best to reflect the results of effectiveness or toxicity). It would be valuable to create a database for model selection and establish a set of normative guidelines dedicated to their application. The evaluation of controlled release and efficacy of encapsulated natural substances in zebrafish should be paid attention to

in the future. Also, the bioavailability and bioaccessibility of natural products in zebrafish should be further studied. The limitations of zebrafish models for each disease or health issue and the possible methods to surmount them should be further studied. Advanced sensitive and precise technologies and intelligent and automated systems are still needed to meet the requirements of high-throughput evaluation and analysis, and affordable intelligent automated zebrafish systems for the husbandry of fish are also needed. Overall, the modeling of zebrafish is an efficient tool for the evaluation of natural products, which can play an important role in the development of functional foods, nutraceuticals, and cosmetics.

## Author contributions

F.J. Lin, F. Geng, and R.Y. Gan conceived the idea, F.J. Lin and H. Li wrote the manuscript, D.T. Wu, Q.G. Zhuang, H.B. Li, F. Geng, and R.Y. Gan edited and revised the manuscript. All authors have read and agreed to the final version of the manuscript.

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