Zebrafish models for the evaluation of essential oils (EOs): A comprehensive review

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REVIEW

Abstract

Essential oils (EOs) derived from crops, especially aromatic plants, have been well-acknowledged to provide several health benefits for humans. Zebrafish as an unprecedented tool has been widely used as an excellent vertebrate model in labs owing to its many useful characteristics. Its applications for rapidly and economically screening and identifying toxicity, safety, and bioactivity of EOs may serve to meet the rising demand for nutraceuticals, food supplements, and natural cosmetics. In this study, we summarized the research progress of zebrafish models in evaluating EOs. In addition, toxicity, safety, and various bioactivities of EOs were summarized using a wide variety of readily applicable zebrafish models covering antioxidant, anti-inflammatory, angiogenesis inhibition, neuromodulation, anesthesia, anti-melanogenesis, analgesic activities, etc. In conclusion, zebrafish is a valuable animal model for evaluating the bioactivity and safety of EOs, and using such a model may contribute to speeding up the identification of novel EOs with potential health functions and quality assessment, which in turn boosts the recognition of aromatic plants as important industrial crops and encourages a healthier way of life.

Keywords: Essential oils; Zebrafish; Toxicity; Safety; Bioactivity

Introduction

Aromatic plants have been used for thousands of years, resulting in a vast and comprehensive understanding of their properties and numerous applications as foods (Burdock and Carabin, 2009), perfumes (Mileva *et al.*, 2021), cosmetics (Sharmeen *et al.*, 2021), pharmaceuticals (Islam *et al.*, 2016), etc., which consequently became important industrial crops worldwide. Essential oils (EOs) as the main concentrated volatile components can

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be extracted from various aromatic plant sections such as leaves, stalks, flowers, bark, roots, seeds, fruits, and resins (Hanif *et al.*, 2019). These components include terpenes, alcohols, acids, esters, epoxides, aldehydes, ketones, amines, and sulfides (Sendra, 2016). In addition, the EOs of each aromatic plant has specific bioactive effects that correspond to their different ameliorative function. EOs have extensive bioactive spectrums, including antioxidant, anti-inflammatory, antimicrobial, analgesic, anti-plaque, respiratory, skin-emollient, anti-ulcer, anti-diabetic, anxiolytic, anti-seizure, and neuroprotective effects (da Fonsêca *et al.*, 2019; Đorđević *et al.*, 2007; Eddin *et al.*, 2021; Gandhi *et al.*, 2023; Li *et al.*, 2023; Silva *et al.*, 2003; Van Leeuwen *et al.*, 2011; Verallo-Rowell *et al.*, 2016; Zhang *et al.*, 2016). Many nutraceuticals (Campos *et al.*, 2019), food supplements (Matera *et al.*, 2023), and pharmaceutical prescriptions (Raut and Karuppayil, 2014) are explored and manufactured based on the bioactivities of EOs. Therefore, understanding and evaluating the bioactivity, toxicity, and safety of EOs are crucial for the development of industrial products containing EOs as raw materials. Currently, cell and mammalian models are commonly utilized to investigate the bioactivities of EOs (de Moraes Pultrini *et al.*, 2006; Manosroi *et al.*, 2006). However, as a mixture of distinct volatile components, EOs have relatively complex actions, which may raise difficulties for clarifying the complete functions of these bioactive substances from a cellular level alone. On the other hand, using mammalian models (e.g., mice or rats) to assess the bioactivity, toxicity, and safety of EOs is hampered by the time-consuming preparation of ethical certification for animal experiments, by the relatively lengthy analysis periods, and by the higher experimental costs. Moreover, there are currently more than 3,600 species of aromatic plants in the world, but only about one-ninth of them are developed and utilized adequately, the EOs of the other aromatic plants may have important industrial applications (Dezhi and Aili, 2008). Furthermore, the incorporation of EOs in the dietary supplement and cosmetic industries is a significant aspect of their ongoing shift toward environmentally friendly and sustainable products. However, it is crucial to acknowledge the safety concerns related to the utilization of EOs in the final product. Consequently, the implementation of more efficient quality control measures becomes imperative. Therefore, their bioactivity, toxicity, and safety need to be investigated urgently. In recent years, with the increased demands of the use of EOs in health products, it has become necessary to use a rapid, simple, visual, and systemic model organism for evaluation at a low cost to better understand the bioactivity, toxicity, and safety of EOs. The utilization of zebrafish model organisms has significant promise in addressing the existing challenges encountered in EOs research. First, the primary objectives of toxicological research are to expeditiously identify and characterize specific hazardous effects of EOs. Traditional methods of toxicity screening, which involve the use of rodents, dogs, and rabbits, are commonly associated with high costs and time requirements. Zebrafish embryos possess the capacity to effectively uptake minute molecular compounds at a rapid rate, potentially offering a valuable model for screening the toxicity of EOs. Furthermore, the application of transgenic zebrafish has promise in augmenting the examination of cells, tissues, organs, and biological processes, thereby improving the exploration of the biological functionalities of EOs. In conclusion, zebrafish is a highly suitable and refined model organism for effectively addressing the objectives and overcoming the obstacles encountered in the field of EOs research.

Zebrafish, also known by their scientific name *Danio rerio*, are small tropical fish (Arunachalam *et al.*, 2013). They are fertilized *ex vivo*, and can produce hundreds of embryos from a single reproductive pair (Zon and Peterson, 2005). The embryos are transparent, and the rapid development of zebrafish embryos enables the recording of the development of a vertebrate from a single cell through organogenesis to a zebrafish (Patton and Zon, 2001). In addition, it has low maintenance costs and occupies less space. The genomic sequence of this fish is readily modifiable, and the effects of mutations can be investigated with relative ease due to its rapid development (Gut *et al.*, 2017). As they share 70% of their genome with humans, zebrafish have become a suitable model organism (Howe *et al.*, 2013). The integration of advanced gene editing techniques and high-resolution in live imaging allows for the comprehensive examination and documentation of growth progression and disease states in zebrafish models with enhanced scientific precision and detail (Keller, 2013). In addition, this model has evolved into an alternative cell model for the study of diverse diseases (Novoa and Figueras, 2012). The results were consistent with *in vitro* investigations and more straightforward than *in vivo* tests by using mammalian models. Moreover, the zebrafish model is also investigated for toxicological research (Tanguay, 2018). The toxicity of natural compounds may be evaluated in zebrafish within several days by observing morphological changes in internal organs such as the heart (Qin *et al.*, 2021), brain, liver (Huo *et al.*, 2019), etc. This improves the screening process and the identification of key features for bioactive compounds. Therefore, the zebrafish has been recognized as an unparalleled model organism for promptly and inexpensively evaluating the bioactivity and safety of natural products in response to the increased need for the production of functional foods, nutraceuticals, and cosmetics.

A recent review summarized the applications of zebrafish models in the evaluations of the safety and the bioactivities of various natural products (Lin *et al.*, 2022); however,

only a small number of studies on EOs were included. Therefore, this review primarily compiled the literature regarding the use of zebrafish in EOs evaluation over the past two decades based on the public academic databases of Google Scholar, PubMed, and Scopus, using "essential oil", "volatile oil", "benzine" and "zebrafish" as keywords. The typical research flow for these investigations is shown in Figure 1. Sometimes, bioactivities of specific EOs are determined by its principal components. However, the overall activities cannot be easily attributed to any single component, as the existence of a combination of various molecules may significantly modify the activity. Therefore, in this review, we focused mainly on zebrafish models for evaluating the bioactivities concerning the overall composition of EOs. The activities summarized in this article include anti-angiogenic, anti-inflammatory, anti-oxidative, anesthetic, neuroprotective, anti-osteoporosis, and anti-melanogenesis, and the assessment of toxicity and safety of EOs using zebrafish models were also summarized (Figure 2). This review can prompt researchers to give more attention to zebrafish models, allowing for more establishments and more applications of useful zebrafish models for the evaluation on EOs.

Zebrafish Models for Toxicity and Safety Evaluation of EOs

Toxicity Evaluation of EOs Using the Zebrafish Model

Zebrafish permits the evaluation of chemical toxicity throughout the entire vertebrate developmental period (Jeong *et al.*, 2015), from embryo to adult, resulting in a comprehensive assessment of teratogenicity, tissue and organ injury, and mortality (Lira *et al.*, 2020; Zhong *et al.*, 2019). Currently, zebrafish are widely used to assess the toxicity of bioactive compounds including EOs. Alpha-Pinene is a terpene-class organic compound found in the EOs of numerous plants (Allenspach and Steuer, 2021). In an experiment, the toxicity of alpha-pinene was defined by the LC_{50} (Median Lethal Concentration) of 441.360 mg/L and EC_{50} (Median Effective Concentration) values of 367.795 mg/L, respectively, using embryos of wild type (AB) zebrafish. In addition, it was found that the teratogenicity of alpha-pinene in zebrafish embryos was observed until the excessive doses (320 or 640 mg/L, 72 hours) (Şişman and Ceylan, 2023) (Table 1). Volatile compound in EOs is frequently formulated into various formulations, such as nano-emulsions (Campolo *et al.*, 2020), microspheres (Benavides *et al.*, 2016), or micelles (Wang *et al.*, 2019), in order to improve their water solubility, stability, and bioavailability. The toxicity of these volatile compound formulations can also be evaluated using the zebrafish model. In a study, the toxicity of perillyl alcohol nano-emulsion was evaluated using adult wild type (AB) zebrafish, exposing that the zebrafish gills can be severely damaged in small concentration (25μg/L), and significant hepatic pathological alterations and even death occurred with the increase of concentrations (25μg/L) for 48 hours (Tavares Carvalho, 2016) (Table 1). In addition, transgenic zebrafish, particularly which are fluorescently labeled in cells (e.g., neutrophils) (Lieschke and Currie, 2007), tissues (e.g., vascular tissue) (Kamei *et al.*, 2010), and organs (e.g., liver) (Cheng *et al.*, 2022), are ideal for investigating bioactivity and toxicity of

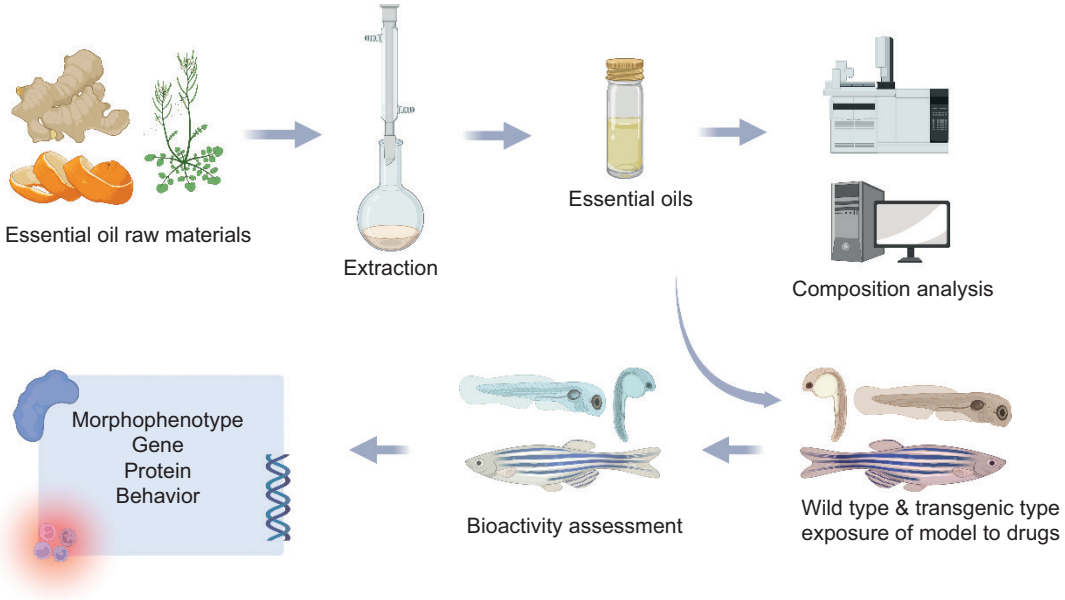

Figure 1. The typical research flow for EOs investigations using the zebrafish model. This encompasses the processes of isolating and extracting EOs, analyzing their chemical profile, evaluating their toxicity and safety, and assessing their diverse biological functions.

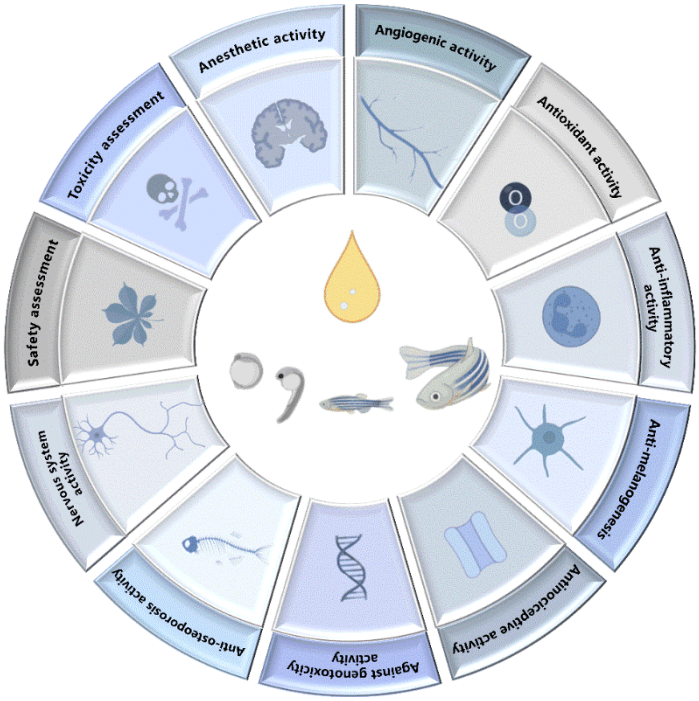

Figure 2. Available Zebrafish Models for Evaluating EOs. The central portion of the diagram illustrates the relevant zebrafish models corresponding to various developmental phases, while the outer ring provides a concise overview of the activities associated with EOs.

chemical compounds because their transparent embryonic and larval bodies enable their comprehensive and dynamic evaluation under the fluorescence microscope (Zhao *et al.*, 2015). Lodovichi *et al.* used transgenic zebrafish (*Tg* (Myl7: EGFP); *Tg* (flk1: GFP)) as well as the wild type (AB) species to determine the toxicity of thymoquinone micelles (Lodovichi *et al.*, 2022). The results revealed that the hatching rates of wild type (AB) and *Tg* (Myl7: EGFP) zebrafish lines treated with this thymoquinone micelles decreased significantly. Meanwhile, embryos displayed a dose-dependent increase in axis-tail curvature and cardiac edema, and transgenic lines displayed greater sensitivity. By the fishline of *Tg* (flk1: GFP), the thymoquinone micelles did not significantly alter the morphology of blood vessels (Table 1). More studies concentrated on the activities of the overall EO compositions were extracted from different plants. In these studies, the chemical composition of EOs is typically analyzed using Gas chromatography–mass spectrometry (GC-MS) techniques, and then wild-type or transgenic zebrafish embryos and larvae are used to evaluate the toxicity of EOs using a variety of evaluation indicators, such as teratogenicity, lethality, hatch rate, blood flow, heart rate etc. (Kim *et al.*, 2017; Mektrirat *et al.*, 2020; Thitinarongwate *et al.*, 2021) (Table 1). Interestingly, Rashan *et al.* assessed the systematic toxicity profile, including acute toxicity, genotoxicity, neuromuscular toxicity, cardiotoxicity, and hepatotoxicity, of frankincense EOs extracted from *Boswellia sacra* using wild type and three transgenic zebrafish (*Tg*(Cmlc2: GFP), *Tg*(fabp10: RFP), and *Tg*(ela31: EGFP) (Rashan *et al.*, 2023). The results indicated that frankincense EOs were not deleterious to zebrafish at the concentrations (≤300 μg/mL) tested. Moreover, Serifi *et al.* evaluated the potential carcinogenic toxicity (lateral line neuromas) of EOs extracted from *Pistacia lentiscus* Var. *chia* using zebrafish larvae (Serifi *et al.*, 2019). This is because lateral line neuromas contain sensory hair cells that can influence the swimming behavior of zebrafish, as well as their extraordinary sensitivity to environmental compounds (Ghysen and Dambly-Chaudiere, 2004). After 4 days of exposure to various concentrations of EOs, zebrafish larvae were stained with acetylated tubulin or FM1-43 and observed under the microscope for lateral line neuromas and hair cells, respectively. The results indicated that zebrafish mortality did not increase until reaching an excessive dose of 200 ppm (4 days) of EOs, which affected the lateral line neuromas and hair cells of zebrafish larvae. In addition, dozens of gene expression of zebrafish after EO administration were investigated and the results indicated further impact of these EOs on the immune system of zebrafish. These studies demonstrate that zebrafish is an effective model organism for assessing the toxicity of EOs.

Table 1. Toxicity Evaluation of EOs Using a Zebrafish Model.

Essential Oils (from)	Model	Findings	References
Alpha-pinene	Zebrafish embryo Wild type, AB	LC_{50}: 441.360 mg/L; 72 hours EC_{50}: 367.795 mg/L; 72 hours Teratogenic doses: 320 mg/L, cardiac edema, 48 hpf; absence of somite, ≤48 hpf, lordosis, yolk sac deformity, tail abnormality, and eye shrinkage, ≤72 hpf Safe concentrations: 20 and 40 mg/L, virtually no embryotoxicity and teratogenicity	Şişman and Ceylan (2023)
Perillyl alcohol nano-emulsion	Adult zebrafish Wild type, AB	LC_{50}: 33.4 µg/L; 48 hours Lethal doses: 50 and 125 µg/L, 100% of mortality, ≤3 hours Liver damage doses: 25, 35, and 50 µg/L Gills damage doses: 25 and 35 µg/L	Tavares Carvalho (2016)
Thymoquinone micelles	Zebrafish embryo Wild type, AB Tg (myl7: EGFP) Tg (flk1: GFP)	Low hatch rates: 1µM, 53 hpf Axis-tail curvature: 1µM, 27 hpf Cardiac edema: 0.3µM, 72 hpf Blood vessels: No significant change, Tg (flk1: GFP)	Lodovichi *et al.* (2022)
Trachyspermum ammi L., Apiaceae	Zebrafish embryo Wild type, AB	Twenty chemicals including thymol and β-cymene were identified by GC-MS. LC_{50}: 17.8 µg/mL, 96 hours Low hatch rates: 15 and 30 µg/mL, 96 hpf High mortality rates: 15 and 30 µg/mL, 96 hpf Heartbeat rates reduction: heartbeat rates decreased significantly after exposure to 15 and 30 µg/mL EOs by 70±5.2 bpm and 50±5.2 bpm, respectively, 60 hpf Morphological changes: yolk sac edema, opaque yolk, craniofacial malformation, and axial curvature, >5µg/mL, 96 hpf Apoptosis doses: 30 µg/mL, 48 hpf	Kim *et al.* (2017)
Zingiber cassumunar Roxb.	Zebrafish embryo Wild type, AB	Eleven chemicals including sabinene, 1,2-dimethyl-6-nitroindolizine, terpinen-4-ol, and γ-terpinene were identified by GC-MS. Embryotoxicity: ≥100 µg/mL, 96 hours Lethal doses (100%): 100 µg/mL, 96 hours Teratogenic doses: ≥10 µg/mL, 24 hours	Mektrirat *et al.* (2020)
Pogostemon cablin (Blanco) Benth.	Zebrafish embryo Wild type, AB	LC_{50}: 120 ppm, 96 hours	Wijaya (2020)
Eugenia caryophyllata Thunb.	Zebrafish larvae Wild type, AB	LC_{50}: 18.2 ± 5.52 mg/L, 96 hours	Doleželová *et al.* (2011)
P. lentiscus Var. *chia*	Zebrafish larvae Wild type, AB	Thirteen chemicals including α-pinene, myrcene, and β-pinene were identified by GC-MS. Nontoxic reaction to lateral line neuromasts: 10–20 ppm, 4 days. Lethal doses: 100–200 ppm, 4 days.	Serifi *et al.* (2019)
Zingiber ottensii Valeton	Zebrafish embryo/ larvae Wild type, AB	GC-MS analysis revealed that the EOs were predominantly composed of terpenoids, which consisted mainly of 21 monocyclic monoterpenoids and 7 sesquiterpenes. The most abundant compound was zerumbone (24.73%), which was followed by terpinen-4-ol, sabinene, and β-pinene. LC_{50}: 1.003 µg/mL, 96 hpf Lethal doses: 1.95 µg/mL, 96 hpf or 3.91 µg/mL, 72 hpf Heart rate reduction: 0.49–3.91 µg/mL, 72 hpf Low hatch rates: 0.49–3.91 µg/mL, 120 hpf Teratogenic doses: 0.49–3.91 µg/mL, 120 hpf, pericardial sac edema, coagulation, dented tail, poor reabsorption of the yolk sac, malformation of the yolk sac, and spinal curvature.	Thitinarongwate *et al.* (2021)
E. caryophyllata Thunb.	Zebrafish embryo/ larvae Wild type, AB	LC_{50}: 18.8±5.52 mg/L, juvenile zebrafish, 96 hours LC_{50}: 15.64±3.30 mg/L, zebrafish embryonic stage, 168 hours	Mácová *et al.* (2008)

(continues)

Table 1. Continued.

Essential Oils (from)	Model	Findings	References
Boswellia sacra	Zebrafish embryo/ larvae Wild type, AB Tg (cmlc2: GFP), Tg (fabp10: RFP), Tg (ela31: EGFP)	Twenty-three chemicals including α-pinene (79.59%), followed by δ-3-careen (9.94%), camphene (3.23%), and β-pinene (2.39%) were identified by GC-MS. Mortality(5%): 1000 μg/mL, 96 hours Teratogenicity: 1000 μg/mL, 96 hours No neuromuscular toxicity (3 hours), genotoxicity (96 hours), and hepatotoxicity (32 hours) were found at 300 μg/mL, and there was no obvious abnormality in related observation indexes. There was no cardiotoxicity (4 hours) at 100 μg/mL and no abnormality.	Rashan *et al.* (2023)
Ocimum basilicum	Zebrafish embryo Wild type, AB	Lethal doses: 200 μL/L, 96 hpf Delayed hatching doses: ≥100 μL/L Cardiotoxicity: ≥100 μL/L, 96 dpf, pericardial edema, blood congestion, and un-looped heart Teratogenic doses: ≥100 μL/L, 96 dpf Heart rate reduction: ≥100 μL/L, 96 dpf	Capparucci *et al.* (2022)
Leonurus japonicus Houtt.	Zebrafish embryo Tg(flk1, EGFP) sunitinib-induced injury model	TC_{50}: 1.67 ± 0.23 μg/mL, 2 hpf Low hatch rates: ≥12.5μg/mL, 24 hpf Cardiotoxicity: ≥6.25 μg/mL, 24 hpf, cardiac congestion, pericardial enlargement, and pericardial bleeding Heart rate reduction: ≥6.25 μg/mL, at 2 hpf; ≥25 μg/mL, at 48 hpf Teratogenic doses: ≥6.25 μg/mL, 2 hpf Lethal doses: ≥6.25 μg/mL, 2 hpf	He *et al.* (2018)
Artemisia argyi H. Lév. and Vaniot, *Artemisia verlotiorum* LaMotte	Zebrafish embryo Wild type, AB	Nontoxic dose and hatching rates 100%: 10 μg/mL, 36 hpf	Wang *et al.* (2023b)

Note: The research incorporated in this analysis provides a comprehensive overview of the utilization of zebrafish models in investigating the acute toxicity of EOs, encompassing teratogenesis, mortality, low hatchability, and particular organ toxicity. LC_{50}, half lethal concentration; EC_{50}, drug half effective concentration; TC_{50}, half toxic concentration.

Safety Evaluation of EOs Using the Zebrafish Model

Zebrafish as a nontarget organism is often used for detecting safe levels of environmental pollutants and is considered as an indicator of potential toxicity to human and animal health (Ullah *et al.*, 2018). Bioactive compounds extracted from plants that are typically used to improve the life quality must be prior to passing safety tests. Many EOs were found to possess superior repellent (Nerio *et al.*, 2010), antibacterial, and antiviral activities (Najar *et al.*, 2021). By zebrafish, the safety dose range of EOs prior to these bioactivity studies can be evaluated. For instance, EOs were found to have larvicidal and repellent properties. Here, LC_{50} is the most significant evaluation index. If the LC_{50} value of EOs at biologically active concentrations for mosquitoes is lower than the LC_{50} value for zebrafish, the effective repellent dose of EOs may be harmless to nontarget organisms and the environment (Baskar *et al.*, 2018; Luz *et al.*, 2020; Moura *et al.*, 2021). In the article on antibacterial and antiviral activities, in addition to evaluating the lethal effect of EOs, physiological changes in the heart and tail of zebrafish were evaluated in order to better comprehend the safety of EOs (Akermi *et al.*, 2022; Haddad *et al.*, 2019; Piasecki *et al.*, 2021). These studies demonstrate conclusively that the zebrafish is an excellent model organism for evaluating the biosafety of EOs and their products.

Zebrafish Models for Bioactivity Evaluation of EOs

Zebrafish Models for the Evaluation of Anti-oxidant and Anti-inflammatory Activity

Some compounds, including 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) (Ko *et al.*, 2014), metronidazole (MTZ) (Chen *et al.*, 2016), and hydrogen peroxide (H_2O_2) (Geun Lee *et al.*, 2022), can be used to induce oxidative stress models in zebrafish embryos or larvae. Commonly, these zebrafish models are often used to test the antioxidant strength of potential chemicals or extracts, such as EOs. Changes in biomarkers after exposure to oxidative inducers are typically used to assess a natural product's antioxidant efficacy. These biomarkers include reactive oxygen species (ROS) (Raguraman *et al.*, 2019), reactive superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), lipid peroxidation,

Table 2. Safety Evaluation of EOs Using Zebrafish Model.

Bioactivity	Essential Oils (from)	Model	Findings	References
Larvicidal and repellent activity	*Atalantia monophylla* Linn.	Adult zebrafish (Wild type, AB)	Forty chemicals including eugenol, sabinene, 1,2-dimethoxy-4-(2-methoxyethenyl) benzene, and beta-asarone were identified by GC-MS. Nontoxic doses: ≤100 mg/L up to 96 hours. LC_{50}: 69.59 mg/L after 96 hours.	Baskar *et al.* (2018)
	Mesosphaerum suaveolens (L.) Kuntze	Adult zebrafish (Wild type, AB)	The GC-MS analysis of EOs samples performed at different times during a year, showed qualitative and quantitative variations in the chemical composition. The 1,8-Cineole is the major compound in all seasons. LC_{50}: 126.2 ± 7.98 μg/mL, rainy; 94.6 ± 6.65 μg/mL, intermediary; 116.1 ± 4.74 μg/mL, dry, 48 hours.	Luz *et al.* (2020)
	Siparuna guianensis Aublet	Zebrafish embryo (Wild type, AB)	Eighteen different constituents including monoterpene β-myrcene (39.16%), sesquiterpenes epicurzerenone (16.02%), and β-copaene (9.33%) were identified by GC-MS. Hatching rates above 50%: 0.507 mg/cm^3, 96 hours LC_{50}: 0.936 mg/cm^3, 96 hours EC_{50}: altered yolk sac, 0.374 mg/cm^3; cardiac edema 0.283 mg/cm^3, 96 hours; the toxic reaction was reflected in the abnormal swimming behavior of zebrafish.	Moura *et al.* (2021)
Antibacterial activity	*Cymbopogon* Species (*C. nardus,* *C. citratus,* C *winterianus,* *C. flexuosus,* *C. schoenanthus,* *C. martinii,* *C. giganteus*)	Zebrafish embryo (Wild type, AB)	The chemical constituents of nineteen EOs from seven different citronella plants were analyzed. Five different chemotypes were determined by GC/MS and TLC analysis, including citronellal, geraniol, and citral (neral + geranial). Among the EOs tested at maximum tolerated concentration, except for two kinds of nontoxic, others showed different degrees of cardiotoxicity and developmental toxicity. (EOs: 0.04–0.46 mg/mL and 0.004–0.0046 mg/mL, 72 hours)	Piasecki *et al.* (2021)
	Cupressus sempervirens	Zebrafish embryo (Wild type, AB)	Twenty-seven chemicals including α-pinene (38.47%), δ-3-carene (25.14%), D-limonene (5.84%), and citronellal (5.33%) were identified by GC-MS. LC_{50}: 6.6 μg/mL, continuously examined every 24 hours.	Akermi *et al.* (2022)
Antiviral activity	*Ayapana triplinervis*	Adult zebrafish (Wild type, AB)	GC-MS analysis of EOs showed that the thymohydroquinone dimethyl ether (THQ) is the main component in this EO. At the antiviral effective concentration, THQ injection in zebrafish does not lead to any signs of stress and does not impact fish survival, demonstrating the absence of acute toxicity for THQ (0.15 mg/g of body weight, 6 days).	Haddad *et al.* (2019)

Note: The present analysis encompasses studies that offer a thorough examination of the application of zebrafish models in the investigation of safe concentrations of EOs for repellant, antibacterial, and antiviral purposes. LC_{50}, half lethal concentration; EC_{50}, drug half effective concentration

heart rate, survival rate, etc., in zebrafish (Chen *et al.*, 2021a; Hoseinifar *et al.*, 2023). Wang *et al.* induced oxidative stress in wild-type zebrafish using H_2O_2 (Wang *et al.*, 2023a). Results from *in vivo* investigations showed that zebrafish treated with EOs, which were produced from *Kaempferia galanga* L., had an enhanced survival rate and heart rate, and that ROS production and lipid peroxidation had been suppressed. Zebrafish treated with EOs also showed reductions in MDA levels and enhancements in SOD, CAT, and GSH-Px activities. He *et al.* (2020b) induced oxidative stress in zebrafish using MTZ and evaluated EOs derived from *Thymus quinquecostatus* celak, observing comparable regulatory activity (He *et al.*, 2020b). Importantly, by detecting the expression of oxidative stress-related genes in zebrafish, the authors summarized that antioxidant activity of these EOs may be related to the activation of the Keap1/Nrf2 pathway.

Zebrafish inflammation models induced by tailfin amputation have been successfully established in order to test the anti-inflammatory compounds (He *et al.*, 2020a). Natural products' anti-inflammatory efficacy may be measured by their ability to modulate neutrophil migration at the site of zebrafish caudal fin damage (Xiong *et al.*, 2022). Santos *et al.* (2022) discovered that EOs derived from *S. guianensis* Aublet decreased the migration of

neutrophils to the fin injury in zebrafish, which explains the EOs' potent anti-inflammatory activity (Santos *et al.*, 2022). Due to the fact that antioxidants (e.g., EOs) prevent inflammatory responses in living organisms, the aforementioned antioxidant indicators are investigated in anti-inflammatory activity of these EOs. And, the antioxidant enzyme test demonstrated that these EOs mitigated oxidative stress. In addition, the tailfin amputation model of zebrafish was also used to study the anti-inflammatory activity of EOs derived from *T. vulgaris* (Polednik *et al.*, 2018). Next, Meng *et al.* (2022) induced enteritis in wild-type zebrafish using oxazolone. *In vivo* studies demonstrated that *A. vulgaris*-derived EOs effectively alleviated enteritis in zebrafish by ameliorating intestinal histopathological damage, decreasing intestinal oxidative stress, restoring intestinal immune function, and altering the expression levels of IL-1, IL-10, and genes in the MyD88/TRAF6/NF-B pathway (Meng *et al.*, 2022). In addition, these EOs had substantially greater therapeutic effects on male zebrafish enteritis than on female zebrafish enteritis. Furthermore, the anti-inflammatory effectiveness of EOs nanoemulsions produced from *Rosmarinus officinalis* L. was assessed by observing the effects on the histopathological and cellular features of numerous zebrafish organs, including the gills, liver, kidney, and intestine (Borges *et al.*, 2018).

Zebrafish Models for the Evaluation of Angiogenesis Inhibitory (or Promotion) Activity

It is widely acknowledged that modulation of angiogenesis is an attractive therapeutic strategy for the treatment of a wide range of human diseases, such as cancer, heart diseases, and inflammation (Carmeliet and Jain, 2000; Fiedler and Augustin, 2006). The activity of EOs to modulate angiogenesis has been linked to their ability to combat these diseases, notably cancer (Bostancioglu *et al.*, 2012). Due to its small size and transparent body during embryonic and larval development, zebrafish is an ideal model for studying EOs-induced alterations in vascular structure. In wild-type zebrafish embryo or larvae, the degree of development of the dorsal longitudinal anastomotic vessel (DLAV) (Anegundi and Pancharatna, 2017), dorsal aorta (DA), posterior cardinal vein (PCV), and intersegmental vessel (ISV) can be used as an indicator of the anti-angiogenic activity of EOs (Zhao *et al.*, 2014). When a particular concentration of EOs was added, the growth of these vessels, particularly ISV, was significantly inhibited (Paramasivam *et al.*, 2012). It should be emphasized that red blood cell staining is useful for observing the variation of these blood vessels in zebrafish of the wild type (Yeh *et al.*, 2011). Nonetheless, transgenic zebrafish allow for more direct and practical observation of vascular structure. To examine the impact of substances on blood vessel development *in vivo*, zebrafish embryos or larvae from transgenic lines like *Tg* (fli1: EGFP) or *Tg* (flk1: EGFP) are a great choice (Elsayed *et al.*, 2020; Liao *et al.*, 2021). Transgenic zebrafish are useful for studying the effects of substances on angiogenic blood vessels in growing fish because their blood vessels continuously exhibit green fluorescence. Thus, the green fluorescence could be used to detect the existence or absence of blood vessels, and a quantitative evaluation could be performed by observing and comparing the same blood vessels in untreated control zebrafish (Zhong *et al.*, 2012). For instance, on these transgenic zebrafish, the anti-angiogenic activity of EOs derived from numerous plants was evaluated. Blood vessels of zebrafish after EOs treatment, primarily ISVs, can exhibit a decrease in the intensity and quantity of green fluorescence compared to those of control zebrafish, indicating that these EOs inhibit angiogenesis, and the expression level of some related genes can be used to further understand the mechanism of the anti-angiogenesis effect of EOs (Yue *et al.*, 2015). In addition, Zhou *et al.* (2021) found that the intensity and quantity of green fluorescence of ISV were considerably decreased when sunitinib was employed to produce damage to the ISV of Zebrafish *Tg* (flk1: EGFP) (Zhou *et al.*, 2021). EOs derived from *Perilla Frutescens* were shown to dramatically boost green fluorescence of ISV, suggesting that these EOs have a role in promoting angiogenesis (Zhou *et al.*, 2021).

Zebrafish Models for the Evaluation of Neuro-modulatory Activity

EOs may be useful in alleviating the symptoms of different nervous system disorders, which has led to a worldwide uptick in the use of aromatherapy for the treatment of insomnia, depression, anxiety, and some cognitive impairments (Lizarraga-Valderrama, 2021). Over the past few years, mounting research has revealed that EOs ingestion exerts measurable bioactivity, and that at therapeutic concentrations, EOs appear to be risk-free of the side effects that are frequent in many pharmaceutical products (Edris, 2007). However, additional research is needed to confirm their effectiveness in neuroprotection, and zebrafish are a great model to test the efficacy of EOs against neurological disorders.

Anxiety and depression can be studied using zebrafish as a model because of the fish's predictable behavioral response to anxiogenic and anxiolytic chemicals (Kalueff *et al.*, 2014). Commonly used behavioral assays for gauging anxiolytic and antidepressant effects in zebrafish include the light/dark and novel tank tests (Stewart *et al.*, 2012; Stewart *et al.*, 2014). The light/dark chamber was made to use zebrafish's dislike of brightly lit areas and their natural curiosity in new places as a measure of their anxiety (Araujo *et al.*, 2012). The device is made up of a tank that is split in half by a room in the middle (Haigis *et al.*, 2022). About half of the device is made of white materials, and the other half is made of black materials that don't reflect light. Fish generally like dark areas and

Table 3. Evaluation of Antioxidant and Anti-inflammatory Activity Using a Zebrafish Model.

	Essential Oils (from)	Model	Findings	References
Antioxidant effect	*K. galanga* L.	Zebrafish embryo (Wild type, AB), H_2O_2-induced oxidative stress model	Twenty-eight chemicals including *trans* ethyl p-methoxycinnamate (32.01%), n-pentadecane (29.14%), and *trans* ethyl cinnamate (19.50%) were identified by GC-MS. Improving survival rates; recovering heartbeat rates; reducing ROS fluorescence intensity, apoptotic cells, lipid peroxidation, and MDA level; improving CAT, GSH-Px, and SOD dose-dependently (EOs, pretreatment 0.4, 0.8, and 1.6 mg/mL, 1 hour; incubated with H_2O_2, 2 mM, 24 hours)	Wang *et al.* (2023)
	T. quinquecostatus celak.	Zebrafish larvae (Wild type, AB) and Tg(krt4:NTR-hKikGR)^{cy17}, dihydrochloride, metronidazole-induced oxidative model	One hundred and thirty chemicals including 1,8-cineole, terpinen-4-ol, linalool, and γ-terpinene were identified by GC-MS. LC_{10}: 20 μg/mL, 48 hpf Increasing epidermal fluorescent spots, SOD, and CAT; reducing ROS fluorescence intensity and MDA level; downregulating the expression of Keap1; upregulating the expression level of Nrf2, Sod1, Cat, and Hmox1 (EOs, 5, 10 and 20 μg/mL, 24 hours, Tg(krt4:NTR-hKikGR)^{cy17})	He *et al.* (2020)
Anti-inflammatory effect	*S. guianensis*	Zebrafish embryo, larvae (Wild type, AB), tailfin injury model, H_2O_2-induced oxidative stress model	Forty-one chemicals including β-myrcene, germacrene-D, bicyclogermacrene, α-muurolol, and siparunone were identified by GC-MS. Inhibiting the recruitment of neutrophils dose-dependently (EOs, 12.5–0.39 μg/mL, pretreated for 2 hours, continue processing for 6 hours after modeling); promoting caudal fin regeneration dose-dependently (EOs, 12.5-0.39 μg/mL, 72 hpf) Biochemical parameters assay, at 96 hpf: CAT↓, SOD↑, GST↓ EOs showed no protective effect against cell apoptosis and 12.5 μg/mL had a cardiotoxic effect.	Santos *et al.* (2022)
	Thymus vulgaris L.	Zebrafish embryo (Wild type, AB), tailfin injury model	Fifteen chemicals including thymol and p-cymene were identified by GC-MS. Reducing neutrophils infiltration (EOs, 0.001% (v/v), 0.0005% (v/v), 72 hpf, pretreated for 2 hours, continue processing for 6 hours after modeling)	Polednik *et al.* (2018)
	Artemisia vulgaris	Adult zebrafish (Wild type, AB), oxazolone-induced enteritis model	Alleviating enteritis; improving the intestinal histopathological damage dose- and time-dependently; changing the expression levels of IL-1β, IL-10, and genes in MyD88/TRAF6/NF-κB pathway; reducing acid mucin; increasing occludin and ZO-1 mRNA expression (EOs, 20, 40, and 80 μg/g, for 3 and 6 days) Biochemical parameters assay of the intestine: ROS↓, SOD↑, MDA↓, CAT↑, ACP↑, AKP↑, MPO↓ in both male and female zebrafish EOs had more significant therapeutic effects on the enteritis of male zebrafish than that of female zebrafish.	Meng *et al.* (2022)
	R. officinalis L.	Adult zebrafish (Wild type, AB), carrageenan-induced edema model	The EOs were submitted to the analysis-coupled GC–MS, which highlighted 1,8-cineol and camphor as major compounds. Inhibiting inflammatory edema; relieving histopathological change of gill, kidney, and liver (EOs, 498 μg/kg, 5 hours)	Borges *et al.* (2018)

Note: This summary provides an overview of research that has assessed the effectiveness of EOs in terms of their antioxidant and anti-inflammatory properties utilizing both wild-type and transgenic zebrafish as experimental models. "↑" or "↓" elucidate the regulatory impact of EOs on oxidation indicators. "↑", upregulating; "↓", downregulating.

Table 4. Evaluation of Angiogenic Activity Using a Zebrafish Model.

	Essential Oils (from)	Model	Findings	References
Anti-angiogenic effect	Thymoquinone from *Nigella sativa*	Zebrafish embryo (Wild type, AB)	Lethal dose: ≥5μM, 48 hours Inhibiting intersegmental vessel formation; downregulating the expression of VEGF-A mRNA dose-dependently. (EOs, 4μM, 48 hours)	Paramasivam *et al.* (2012)
	n-butylidenephthalide from *Radix Angelica sinensis*	Zebrafish embryo (Wild type, AB)	Fifty-one chemicals including n-butylidenephthalide were identified by GC-MS. Inhibiting intersegmental vessel formation (EOs, 0.01 μg/mL, initiated at the 13 somite stage (ss), observation at 72 hpf)	Yeh *et al.* (2011)
	Moringa oleifera and *Moringa peregrina* seeds	Zebrafish embryo (Wild type, AB/ Tuebingen TAB-14), Tg(fli1,EGFP)	The chemical compositions and fatty acid compositions of the two essential oils were analyzed by LC-MS/MS and GC, 13 fatty acids including oleic acid and palmitic acid were identified. LD_{50}: M. oleifera, 21.24 ± 0.44μg/mL; M. peregrina, 25.11 ± 0.547 μg/mL, 12 hours Perturbing intersegmental and sub-intestinal vessels formation (EOs, 10–20 μg/mL, 3 dpf)	Elsayed *et al.* (2020)
	Furanodiene	Zebrafish embryo Tg(fli1, EGFP)	Inhibiting intersegmental vessel formation (EOs, 10 μM, the effect of using 50 μM was more obvious, 72 hours)	Zhong *et al.* (2012)
	Aromatic-turmerone from *Curcuma longa* Linn	Zebrafish embryo Tg(fli1, EGFP)	Inhibiting subintestinal vessel formation (EOs, 12.5 and 25 μg/mL, 48 hours) Downregulating the expression of angiogenic genes Ang-2 and Tie-2 (EOs, 25 μg/mL, 24 hours)	Yue *et al.* (2015)
	Ferula akitschkensis	Zebrafish embryo Tg(VEGFR2, GFP)	Forty-one chemicals including tricyclo [4.4.0.0(2,7)] dec-3-ene-3-methanol, 1-methyl-8-(1methylethyl), and adamantane, 2-hydroperoxy-2(2-oxiranyl) were identified by GC-MS. Inhibiting intersegmental and inferior intestinal vessels formation dose-dependently (EOs, 37.5, 18.75, and 9.375 μg/mL, 14 hours and 48 hours)	Han *et al.* (2022)
	Curcuma phaeocaulis Valeton	Zebrafish embryo (Wild type, AB), Tg(flk1, GFP)	LC_{50} (vinegar-processed products): 67.315 μg/mL, 12 hours Inhibiting intersegmental vessel formation (EOs, 20 μg/mL, 12 hours)	Liao *et al.* (2021)
	Kelussia odoratissima and *A. sinensis*	Zebrafish embryo, larvae Tg(fli1, EGFP), Tg (ins, GFP-NTR)	Fifty-two chemicals including Z/E-ligustilide were identified by GC-MS. Eleven compounds were common to both EOs. Blocking intersegmental vessels outgrowth formation; inducing pancreatic beta cells regeneration (EOs from *A. sinensis*) (EOs, embryo, 7.81 μg/mL; 18 hours; larvae, 1.95 μg/mL, 2 days)	Rezaei *et al.* (2023)
Pro-angiogenic effect	*P. frutescens* (L.) Britt. and perillaldehyde	Zebrafish embryo Tg(flk1, EGFP), sunitinib-induced injury model	Promoting intersegmental vessel formation dose-dependently (EOs, from 1.25 to 20 μg/mL, 48 hpf) (Perillaldehyde, from 3.13 to 50 μM, 48 hpf)	Zhou *et al.* (2021)

Note: This summary provides an overview of research that has assessed the effectiveness of EOs in terms of their angiogenesis inhibitory (or promotion) properties utilizing both wild-type and transgenic zebrafish as experimental models. LD_{50}, half lethal dose; LC_{50}, half lethal concentration.

stay away from bright ones (Stewart *et al.*, 2011). The time it takes to leave the center chamber and the number of changes between compartments can be used to figure out how well EOs treat anxiety and depression. For instance, a light/dark test has been used to assess the anxiolytic activity of EOs derived from *E. caryophyllata* Thunb. and *C. citratus* (DC.) Stapf (da Silva Campelo *et al.*, 2023; Mendes Hacke *et al.*, 2020). Zebrafish spent substantially

more time in the light compartment after being treated with these EOs, suggesting that they alleviate anxiety. Latency time and the number of midline crossings between the light and dark compartments in zebrafish are not significantly affected by these EOs, showing that there are no significant changes to the sensory systems or locomotor activity of zebrafish. In addition, the novel tank test works on a similar concept to the light/dark test. The novel tank test relies on the zebrafish's natural tendency to seek shelter after being placed in an unfamiliar tank (Stewart *et al.*, 2012). Until they feel safe enough to go to the top of the tank, they prefer to hang around at the bottom. Each fish is given its own section of the tank for the duration of the test (about five to six minutes) and the tank is effectively split into two equal halves by a horizontal line. People track how long it takes for the fish to reach the top half of the tank, how often they cross to the upper half, how erratically they swim, and how long they spend frozen. Anxiolytic substances can reduce latency and encourage exploration of the tank's top levels (Farias-Cea *et al.*, 2023). Using a novel tank assay, the anxiolytic activity of EOs derived from *Lippia alba* and *Aloysia triphylla* was evaluated (Junior *et al.*, 2018). Moreover, the anxiolytic activity of EOs in a zebrafish model was also evaluated using an open-field test and electroencephalogram power spectrum (Ly *et al.*, 2023; Nguyen *et al.*, 2022; Nonato *et al.*, 2023; Szaszkiewicz *et al.*, 2021).

Epilepsy is a significant neurological disorder. The induction of seizures in zebrafish by pentylenetetrazole (PTZ) is a standard experimental model for the discovery of new anticonvulsant agents (Jin *et al.*, 2020). Recently, it was found that EOs have antiepileptic activity using this model. The EOs' anti-epileptic effectiveness was determined by comparing the zebrafish locomotor activity in the EOs treatment group with that of the PTZ-induced zebrafish epilepsy model group (Bezerra *et al.*, 2023; Orellana-Paucar *et al.*, 2012). The expression of genes involved in these processes was also identified to better understand how EOs exert their antiepileptic effects (Orellana-Paucar *et al.*, 2013).

Many EOs' neuroprotective properties have been studied in zebrafish models using the scopolamine-induced memory impairment paradigm, which may mimic Alzheimer's disease symptoms (Boiangiu *et al.*, 2022; Capatina *et al.*, 2020a; Capatina *et al.*, 2020b; Todirascu-Ciornea *et al.*, 2019). T-maze and Y-maze tests may be used to evaluate zebrafish spatial memory (Benvenutti *et al.*, 2021). Measurements of antioxidant enzyme activity and gene expression in redox-related signaling pathways might inform future investigations into the mechanisms by which these EOs exert their anti-neurodegenerative effects (Boiangiu *et al.*, 2023; Brinza *et al.*, 2023; Capatina *et al.*, 2021; Sharma *et al.*, 2022). In addition, trimethyltin or aluminum trichloride-induced neurodegeneration model in zebrafish was also used to evaluate the neuro-modulatory activity of EOs and their products (Chen *et al.*, 2021b; More and Pawar, 2023).

Zebrafish Models for the Evaluation of Anesthetic Activity

Clove oil, the main component of which is eugenol (over 70% of clove oil's composition) (Chaieb *et al.*, 2007), is extracted from the clove plant *Syzygium aromaticum*, *Eugenia aromatica,* or *Eugenia caryophylatta* (Selles *et al.*, 2020), and is used worldwide as a moderate topical analgesic for toothache, headache, and joint discomfort in humans (Ayushi *et al.*, 2020), as well as a food flavoring agent (Nurdjannah and Bermawie, 2012). Clove oil has been successfully used to immobilize and suppress the sensory systems of fish. In a study on zebrafish larvae (30 days old), Grush *et al.* (2004) investigated the anesthetic effects of clove oil with MS-222 (Ethyl 3-aminobenzoate methanesulfonate, a popular fish anesthetic agent) (Grush *et al.*, 2004). Unlike MS-222, clove oil was effective in inducing anesthesia quickly and at low doses. Clove oil prolonged fish recovery durations relative to those seen with MS-222 at similar doses. These results imply that clove oil has the potential to be a useful anesthetic for zebrafish, with advantages over MS-222 such as reduced cost, reduced dose, increased safety, and maybe reduced fatality rates. In another study, zebrafish of varying ages (embryos, 1 day; larvae, 5 days; adult, 9–11 months) were used to investigate the anesthetic activity of clove oil and to compare it to that of MS-222 and benzocaine (Ehrlich *et al.*, 2019). The results of this study indicate that clove oil possesses potentially superior anesthetic activity to MS-222 and benzocaine in the embryonic and larval stages of zebrafish. In addition, Davis *et al.* (2015) discovered that adult zebrafish anesthetized with clove oil were able to collect more serum and had lower cortisol levels than those anesthetized with an equivalent dose of MS-222 (Davis *et al.*, 2015). Clove oil's anesthetic effect can also be assessed by observing zebrafish behavior. Sánchez-Vázquez *et al.* (2011) found that adult zebrafish exposed to higher concentrations of clove oil exhibited less swimming behavior, and this shift in behavior follows a diurnal rhythm (Sánchez-Vázquez *et al.*, 2011). In addition, aversion to MS-222 and clove oil was compared using a conditioned place avoidance paradigm (i.e., light/dark test) by Wong *et al.*, 2014. Each anesthetic was presented to zebrafish in their light/dark preference at first. Zebrafish exposed to MS-222 avoided spending time on their favored side; however, this aversion was mitigated with clove oil. These data imply that clove oil, rather than MS-222, may be the superior anesthetic for zebrafish. Moreover, the bioavailability of clove oil is restricted because of its volatility, chemical

Table 5. Evaluation of Nervous System Activity Using a Zebrafish Model.

	Essential Oils (from)	Model	Findings	References
Anti-anxiety effect	*L. alba* and *A. triphylla*	Adult zebrafish (Wild type, AB)	The main chemical compositions of the EOs of *L. alba* leaves include linalool, eucalyptol, α-citral, and the EOs of *A. triphylla* leaves including α-citral, e-carveol, and limonene. Attenuating anxiety-like locomotive behavior in novel tank test without preference behavior (EOs, 150 μL/L *L. alba*; 100 μL/L *A. triphylla*, filmed for 6 min) Cortisol analysis: 15 min, the EOs of *A. triphylla* was stressful and did not attenuate cortisol increase after stress, *L. alba* EOs attenuated the cortisol response to stress and was not stressful.	Junior *et al.* (2018)
	C. citratus (DC.) stapf	Adult zebrafish (Wild type, AB), flumazenil-induced $GABA_A$ receptor inhibition model	Relieving anxiety-like locomotive behavior in light–dark preference test; regulating GABAergic system; citral and geraniol have synergistic effect on anti-anxiety effect. (EOs, 10 mg/L, 10 min)	Mendes Hacke *et al.* (2020)
	Limonene, β-Myrcene, Linalool	Adult zebrafish (Wild type, AB)	Relieving anxiety-like locomotive behavior in open field and novel object approach test; linalool groups demonstrated only minor alterations in locomotion. (limonene doses from 0.25% to 0.75%; linalool doses from 0.0001% to 0.00125%; β-myrcene doses from 0.001% to 0.1%, 10 min) Repeating exposure causes neuroadaptations or metabolic tolerance, resulting in a negligible effect on behavior. (Limonene 0.39% or β-myrcene 0.0083%, 7 days)	Szaszkiewicz *et al.* (2021)
	Anthemis nobilis L. (roman chamomile) and *Citrus reticulata* Blanco (tangerine)	Adult zebrafish (Wild type, AB)	The GC-MS analysis proved that EOs of *A. nobilis* mainly contain pentadecyl-3-methyl-2-butenoate, hexadecyl-3-methyl-2-butenoate, 1-piperidinol, and *trans*-1-ethyl-3-methyl-cyclopentane; the main chemical constituents of EO from *C. reticulata* are limonene and γ-terpinene. Improving anxiety-like locomotor impairment in light–dark test (EOs, 10 mg/L, 20 min) Tangerine EOs showed a tendency to reduce anxiety, but it was not statistically significant.	Silveira *et al.* (2022)
	Cinnamaldehyde of *Cinnamomum cassia*	Adult zebrafish (Wild type, AB), MK-801-induced anxiety-like model	Twenty-seven chemicals including (E)-cinnamaldehyde and (Z)-cinnamaldehyde were identified by GC-MS. Recovering changes in the EEG power spectrum (Cinnamaldehyde, 5 mg/L, 30 min) Electroencephalogram(EEG)	Nguyen *et al.* (2022)
	Gamisachil-tang, Guibi-tang, Sihogayonggolmoryeo-tang, Danchisoyosan, Sihosogansan, Soyosan	Adult zebrafish (Wild type, AB), MK-801-induced anxiety-like model	EOs from Gamisachil-tang and Sihosogansan reversed the changes in the EEG signals and decreased the theta/beta and delta/beta ratios. EOs from Gamisachil-tang, Danchisoyosan, Sihosogansan, and Soyosan regulated the EEG power spectrum. (EOs, 10 mg/L, 30 min) Electroencephalogram(EEG)	Ly *et al.* (2023)
	Lippia alba, Lippia sidoides, Lippia gracilis	Adult zebrafish (Wild type, AB), flumazenil-induced $GABA_A$ receptor inhibition model	Attenuating anxiety-like locomotive behavior in open field and light/dark tests; regulating GABAergic system (LaEO, LsEO, 4 mg/kg; LgEO, 40 mg/kg, 30 min)	Nonato *et al.* (2023)
	E. caryophyllata Thunb.	Adult zebrafish (Wild type, AB), flumazenil-induced $GABA_A$ receptor inhibition model	Relieving anxiety-like locomotive behavior in open field and light/dark tests; regulating GABAergic system (EOs, eugenol and nanoemulsion; 4, 12 and 20 mg/kg, 30 min)	da Silva Campelo *et al.* (2023)

(*continues*)

Table 5. Continued.

	Essential Oils (from)	Model	Findings	References
Anticonvulsant and Anti-epileptic effect	Bisabolene sesquiterpenoids of *Curcuma longa*	Zebrafish larvae Tg(fli1a: EGFP)y[1] strain, PTZ-induced seizure model	^{1}H-, ^{13}C-NMR spectra, and LC-MS results suggested that three of the eight active constituents of EOs identified have anticonvulsant activity, including α, β-turmerone, ar-turmerone and α-atlantone. Reducing both the number and the duration of ictal-like discharges and the cumulative duration of epileptiform discharges. (EOs, 10 μg/mL, pre-treated 1 hour, PTZ and EOs treated together for 15 min)	Orellana-Paucar *et al.* (2012)
	Ar-turmerone of *Curcuma longa*	Zebrafish larvae (Wild type, AB), PTZ-induced seizure model	Downregulating the expression of c-fos; upregulating the expression of brain-derived neurotrophic factor (bdnf) (Ar-turmerone, 46 μM, 1 hour)	Orellana-Paucar *et al.* (2013)
	Citrus sinensis Linn. Leaves	Zebrafish larvae (Wild type, AB), PTZ-induced seizure model	Twenty-five chemicals including sabinene, myrcene, α-pinene, and β-pinene were identified by GC-MS. Teratogenic dose: 2-4 μl/mL, 80 hours Inhibiting convulsive-like locomotive behavior concentration-dependently. (EOs doses from 0.5 to 1.5 μl/mL, 1 hour)	Prabahar (2018)
	Pyrrolone-fused benzosuberene of *Cedrus deodara*	Zebrafish larvae (Wild type, AB), PTZ-induced epileptic seizure model	Improving epilepsy symptoms; downregulating the expression of c-fos, PIK3CA, PIK3R1, AKT, mTOR, Rps6, and Rps6kb1. (PBS-8 and PBS-9, 1μM, 1 hour)	Tanwar *et al.* (2019)
	Cyperus articulates L.	Zebrafish larvae (Wild type, AB), PTZ-induced seizure model	The hexane extract was analyzed by HPLC, GC-MS, UHPLC-TOF-HRMS, NMR, and MPLC, and four antiseizure components were identified, including cyperotundone, mustakone, 1,2-dehydro-α-cyperone, and sesquichamaenol. Toxic dose: 50–200 μg/mL, 18 hours Relieving epileptic movements (EOs, 10, 30 μg/mL, 4, 13, 45, 135, and 460 μM for four pure compounds, 18 hours)	Brillatz *et al.* (2020)
	cis-jasmone	Adult zebrafish (Wild type, AB), PTZ-induced seizure model	Inhibiting convulsive-like locomotive behavior; modulating the GABAergic system (*cis*-jasmone, 0.1, 0.3 mg/mL, 1 hour)	Bezerra *et al.* (2023)
Neuroprotective effect	*Schinus terebinthifolius*	Adult zebrafish (Wild type, short-thin strain), scopolamine-induced memory deficits model	Twenty-seven chemicals including β-phellandrene, α-pinene, terpinen-4-ol, α-phellandrene, and β-pinene were identified by GC-MS. Improving anxiety-like locomotive behavior and memory in novel tank diving and Y-maze tests AChE↓, SOD↑, CAT↑, GPX↑, MDA↓ (EOs, 10, 25, and 50 μL/L, pre-treated 8 days)	Todirascu-Ciornea *et al.* (2019)
	R. officinalis L.	Adult zebrafish (wild-type, short-fin strain), scopolamine-induced memory deficits model	Seventy-seven chemicals including eucalyptol, α-pinene, camphor, and camphene were identified by GC-MS. Improving anxiety-like locomotive behavior and memory in novel tank diving and Y-maze tests AChE↓, SOD↑, CAT↑, GPX↑, MDA↓ (EOs, 25, 150, and 300 μL/L, 1 hour a day for 8 consecutive days)	Capatina *et al.* (2020)
	T. vulgaris L.	Adult zebrafish (wild-type, short-fin strain), scopolamine-induced memory impairment model	More than 70 components including thymol, p-cymene, and sesquiterpene β-caryophyllene were identified by GC-MS. Improving anxiety-like locomotive behavior and memory in novel tank diving, Y-maze, and novel object recognition tests AChE↓, SOD↑, GPX↑, GSH↑, MDA↓, protein carbonyl↓ (EOs, 25, 150, and 300 μL/L, treated 13 days)	Capatina *et al.* (2020b)

(*continues*)

Table 5. Continued.

Essential Oils (from)	Model	Findings	References
Origanum vulgare ssp. *Hirtum*	Adult zebrafish (wild-type, short-fin strain), scopolamine-induced cognitive impairments model	Fifty-four chemicals including thymol, p-cymene, and γ-terpinene were identified by GC-MS. Improving anxiety-like locomotive behavior and memory; modulating GABAergic system activity in novel tank diving, Y-maze, and novel object recognition tests. AChE↓, SOD↑, CAT↑, GPX↑, GSH↑, MDA↓, protein carbonyl↓ (EOs, 25, 150, and 300 μL/L, treated 13 days)	Capatina *et al.* (2021)
Origanum vulgare ssp. *Hirtum*	Adult zebrafish (Wild type, AB), scopolamine-induced memory impairment model	The EOs mix was perceived by the GC-MS being thymol, p-cymene, and terpinene. Improving anxiety-like locomotive behavior and memory in novel tank diving, Y-maze, and novel object recognition tests. AChE↓, SOD↑, CAT↑, GPX↑, GSH↑, MDA↓, protein carbonyl↓ (EOs, 1, 4, and 8 g/L, pretreatment 1 hour a day for 7 days)	Sharma *et al.* (2022)
Angelica purpurascens (Avé-Lall.) Gilli.	Adult zebrafish (wild type, short-fin strain), scopolamine-induced memory impairment model	Twelve chemicals including β-phellandrene, sabinene, α-pinene, germacrene-D, α-phellandrene, and p-cymene were identified by GC-MS. Improving anxiety-like locomotive behavior and memory; modulating GABAergic system activity in novel tank diving, Y-maze, and novel object recognition tests. AChE↓, SOD↑, CAT↑, GPX↑, MDA↓, carbonylated protein↓ (EOs, 25, 150 μL/L, once daily for 13 days)	Boiangiu *et al.* (2022)
Coriandrum sativum var. *microcarpum*	Adult zebrafish (Wild type, AB), scopolamine-induced memory impairment model	Ameliorating cognitive dysfunction and anxiety state in novel tank diving, Y-maze, and novel object recognition tests; adjusting cholinergic system; decreasing oxidative stress in the brain. AChE↓, SOD↑, CAT↑, GPX↑, MDA↓, carbonylated protein↓ (EOs, 25, 150, and 300 μL/L, 21 days)	Brinza *et al.* (2023)
Glaucosciadium cordifolium (Boiss.) Burtt & Davis	Adult zebrafish (Wild type, AB), scopolamine-induced memory impairment model	Twenty-two chemicals including limonene, α/β-pinene, p-cymene, and α-phellandrene were identified by GC-MS. Mitigating the memory deficits and the anxiety-like behavior in novel tank diving, Y-maze, and novel object recognition tests; adjusting cholinergic system; reducing the oxidative stress. AChE↓, SOD↑, CAT↑, GPX↑, MDA↓, carbonylated protein↓ (EOs, 25 and 150 μL/L, 17 days)	Boiangiu *et al.* (2023)

Note: This summary provides an overview of research that has assessed the effectiveness of EOs in terms of their neuro-modulatory properties (e.g. anti-anxiety effect, anticonvulsant and anti-epileptic effect, and neuroprotective effect) utilizing both wild-type and transgenic zebrafish as experimental models. "↑" or "↓" elucidate the regulatory impact of EOs on oxidation indicators. "↑", upregulating; "↓", downregulating.

instability, and hydrophobic nature. The utilization of nano-drug-loaded dosage forms has been contemplated. Effectively contrasting the distribution of different clove oil nanoformulations in various organs has been facilitated by zebrafish (Kheawfu *et al.*, 2022). And different nanoformulations of EOs extracted from *Alpinia galanga* and *Aeollanthus suaveolens* were also evaluated for organ distribution properties and anesthetic activity in zebrafish using comparable techniques and indicators (de Oliveira Ferraz *et al.*, 2020; Khumpirapang *et al.*, 2021). Furthermore, recovery time served as a crucial metric for assessing the anesthetic efficacy of various EOs such as anise oil, thyme oil, and mint oil (Seyidoglu and Yagcilar, 2020), and EOs derived from *Cuminum cyminum* (Khosravanizadeh *et al.*, 2020), *Acmella oleracea*, *Piper alatabaccum* (Leite *et al.*, 2022).

Table 6. Evaluation of Anesthetic Activity by Zebrafish Model.

Essential Oils (from)	Model	Findings	References
Clove oil	Zebrafish larvae (Wild type, AB)	LC_{50}: 21 ppm, 96 hours Compared with MS-222(100ppm), Eugenol(60ppm) has faster anesthetic time and longer anesthetic effect.	Grush *et al.* (2004)
	Adult zebrafish (Wild type, AB)	LC_{50}: MS-222, 170.6 ± 7.4 mg/L (ML), 215.6 ± 3.9 mg/L (MD); eugenol, 70.3 ± 3.1 mg/L (ML), 104.9 ± 5.4 mg/L (MD), 15 min (middle of the light phase [ML], middle of the dark phase [MD])	Sánchez-Vázquez *et al.* (2011)
	Adult zebrafish, larvae, embryo(Wild type, AB)	a) Prolonged anesthesia: 24 hours, embryo; 2 hours, larvae Compared with MS-222 (200 ppm) and benzocaine (200 ppm), the anesthetic effect of clove oil (60 ppm for embryo; 90 ppm for larvae) and AQUI-S (active ingredient iso-eugenol, 40 ppm) lasts longer, although it takes a certain amount of anesthesia time. b) Short-term anesthesia: 15 min, adult Compared with MS-222 (200 ppm) and benzocaine (200 ppm), it takes longer for clove oil (100 ppm) and AQUI-S (150 ppm) to recover from anesthesia.	Ehrlich *et al.* (2019)
	Adult zebrafish (Wild type, AB)	Compared with other anesthetics (MS-222 (175mg/L), Etomidate (2 mg/L) and Propofol (5mg/L) combined with lidocaine (150 mg/L)), clove oil (45 mg/L) anesthesia time was faster and has no respiratory inhibitory effect.	Jorge *et al.* (2021)
	Adult zebrafish (Wild type, AB)	Compared with MS-222 (150 mg/L), zebrafish have less aversion to clove oil (55 mg/L) in light/dark box experiment.	Wong *et al.* (2014)
	Adult zebrafish (Wild type, AB), Tg(isl1-hsp70l:mRFP), Tg(UAS:GCaMPHS), Tg(5xUAS:RFP)	Greater amounts of serum could be collected and lower serum levels of cortisol were present in fish euthanized with clove oil (0.1%) compared with an equipotent dose of MS222 (7.8 mM).	Davis *et al.* (2015)
	Adult zebrafish (Wild type, AB)	Clove oil nanoformulations: 150 mg/L, 10 min Fish skin permeation: self-microemulsifying drug-delivery system, 18.17±0.64 μg/cm^2, increasing penetration flux. Distribution: Clove oil entered the body of the zebrafish through the gills and skin and then accumulated in the brain.	Kheawfu *et al.* (2022)
A. galanga	Adult zebrafish (Wild type, AB)	The GC-MS analysis showed that 1,8-cineole and 4-allylphenyl acetate were the main active compounds of EOs. Distribution: EOs entered the body of the zebrafish through the gills and skin and then accumulated in the brain. Determination of optimal dose for fish anesthesia: 300 mg/L Skin permeation fluxes: 1,8-cineole (EtOH-AGO), 0.58 μg/cm^2 min; Self-nanoemulsifying drug delivery system (SNEDDS-AGO-2), 2.46 μg/cm^2 min	Khumpirapang *et al.* (2021)
A. suaveolens Mart. Ex Spreng nano-emulsification	Adult zebrafish, embryo (Wild type, AB)	The GC-MS analysis exhibited β-farnesene, linalool, and massoialactone are the major components of the EO. Nano-emulsion enhanced the sedative effect of EOs (embryo, doses from 25 to 100 μg/m, 96 hours), and males were more sensitive to EOs (adult zebrafish, 125 mg/kg, 14 days).	de Oliveira Ferraz *et al.* (2020)
Anise, Thyme, Mint	Adult zebrafish (Wild type, AB)	Males were found to be more sensitive than female zebrafish to recovery times. (thyme: 1, 5, 10 mg/L, 20 mg/L; mint: 10, 20, 30 mg/L)	Seyidoglu and Yagcilar (2020)
C. cyminum	Adult zebrafish (Wild type, AB)	The lowest effective concentrations were based on the efficacy criteria of complete anesthesia induction within 180 s and recovery within 300 s. Under similar anesthesia effect, the dosage of essential oil was less. (EOs, 0.24 mL/L; 2-phenoxyethanol, 0.35 mL/L)	Khosravanizadeh *et al.* (2020)
A. oleracea and P. alatabaccum	Adult zebrafish (Wild type, AB)	Inducing deep anesthesia; no significant increase of nitrite or nitrate (NOx-) levels in both the encephalon and cephalic kidney tissues. (*A. oleracea* flowers, 2 mL/L; leaves, 3 mL/L; *P. alatabaccum*, 2.5 mL/L)	Leite *et al.* (2022)

Note: This summary provides an overview of research that has assessed the effectiveness of EOs, in particular clove oil, in terms of their anesthetic activity properties utilizing both wild-type and transgenic zebrafish as experimental models. LC50, half lethal concentration.

Zebrafish Models for the Evaluation of Other Bioactivities

It is often believed that the best place to find effective and safe depigmentation ingredients is in natural products. Natural compounds with anti-melanogenic effects have been screened extensively using zebrafish embryos and larvae as potent models (Chaita *et al.*, 2017). However, only a small number of studies using zebrafish models have examined the anti-melanogenic activity of EOs. During embryonic development, zebrafish will create melanin in their bodies; zebrafish exposed to EOs show a large decrease in this melanin, which might be indicative of an anti-melanogenic effect (Ho *et al.*, 2023; Kim *et al.*, 2023; Yan *et al.*, 2023; Zhou *et al.*, 2020). In addition, the anti-nociceptive effects of EOs on a zebrafish model of chemically induced pain were investigated (Batista *et al.*, 2021; Lima *et al.*, 2020). Moreover, the effects of EOs on anti-osteoporotic, anti-genotoxicity, and promotion of intestinal microbial homeostasis have been investigated in zebrafish models (Hasankhani *et al.*, 2023; Khalil *et al.*, 2011; Stephen, 2019). Furthermore, indicators including growth, liver histology, and antioxidant effects were used in the zebrafish model to assess the efficacy of EOs as natural dietary supplements (Ramos *et al.*, 2022; Silva *et al.*, 2022).

Limitation

As of now, there exist several constraints in the utilization of zebrafish models for EOs research. Zebrafish exhibit a lack of certain organs, including lungs and limbs, which poses challenges in administering compounds due to the persistence of chorion up to 48 hours post-fertilization (Kari *et al.*, 2007; Santos *et al.*, 2018). Nevertheless, these obstacles can be addressed using human or automated removal methods, as well as modern procedures like microinjection (Bauer *et al.*, 2021). Moreover, the scope of pharmacokinetic investigations in this species is constrained not only by challenges associated with the delivery of water-soluble substances and the utilization of solvents that may influence toxicological outcomes but also by deficiencies in comprehending the operational metabolic mechanisms in zebrafish embryos (Félix *et al.*, 2019). Furthermore, despite the identification of primary chemical constituents in EOs through various studies, a comprehensive understanding of the specific role played by these chemical components in the efficacy of EOs remains elusive. Consequently, additional study is required to address this knowledge gap. Moreover, the extent of zebrafish's absorption of EOs remains uncertain, as does the attribution of the observed effects to either the elevated concentrations of individual compounds or the synergistic interactions among the chemicals found in the EOs (Rubinstein, 2006). Subsequent investigations may direct their attention toward enhancing zebrafish models to depict distinct facets of human biology more accurately.

Conclusions

The present review primarily summarizes and discusses the zebrafish models that are most widely used in EOs assessment, as well as the construction of zebrafish models and associated evaluation indicators. Using zebrafish as a model organism is an efficient and cost-effective way to test and evaluate EOs for their health benefits. The toxicity, safety, and bioactivity of EOs can be studied using a number of readily applicable zebrafish models, including those generated by chemical induction and transgenic lines with fluorescent tagging. The following areas need greater investigation to make zebrafish models more useful and informative for the evaluation of EOs. First, to optimize its use in EOs' product development, the zebrafish model described in this review may be applied to more EOs assessment studies. Second, new zebrafish models are needed for analyzing the biological activities of EOs, as current studies do not cover all the important biological functions of EOs. The utilization of transgenic zebrafish lines that include specific genetic modifications, in conjunction with multidimensional imaging techniques, can be employed to examine the possible impacts of various EOs on zebrafish in future studies. Finally, the current study does not go far enough to fully grasp the regulation mechanisms of EOs in a zebrafish model. To examine the mechanism of action of EOs more thoroughly and systematically, not only restricted to cytokine and gene expression detection, advanced research tools should be applied, such as omics technology, etc. Overall, zebrafish modeling is an effective method for assessing EOs, which have the potential to advance the fields of functional foods, nutraceuticals, and cosmetics; raise awareness about the value of aromatic plants as important industrial crops; and improve healthy lifestyle.

Author Contributions

M.H. and M.S. designed the study. X.M., L.L., J.C. and R.S. carried out document and data analysis. L.W. and M.Y. drafted the manuscript. S.D. contributed to revisions of the manuscript. All authors read and approved the final manuscript.

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Consent for Publication

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Conflicts of Interest

All contributing authors declare no conflicts of interest.

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