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Zebrafish as a tool for the discovery of anticonvulsant compounds from botanical constituents

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ABSTRACT

Epilepsy affects about 65 million people in the world, which makes this disease a public health problem. In addition to the incidence of recurrent seizures, this neurological condition also culminates in cognitive, psychological, behavioral, and social consequences to the patients. Epilepsy treatment is based on the use of drugs that aim to inhibit repetitive neuronal discharges, and consequently, the recurrence of seizures. However, despite the large number of antiepileptic drugs currently available, about 30–40% of patients with epilepsy do not respond satisfactorily to treatments. Therefore, the investigation of new therapeutic alternatives for epilepsy becomes relevant, especially the search for new compounds with anticonvulsant properties. The therapeutic potential of plant-derived bioactive compounds has been a target for alternative treatments for epilepsy. The use of animal models for drug screening, such as zebrafish, contributes to a better understanding of the mechanisms involved in seizures and for investigating methods and alternative treatments to decrease seizure incidence. The sensitivity of zebrafish to chemoconvulsants and its use in genetic approaches reinforces the contribution of this animal to epilepsy research. Moreover, we summarize advances in zebrafish-based studies that focus on plant-derived bioactive compounds with potential antiseizure properties, contributing to the screening of new drugs for epilepsy treatment.

1. Introduction

Epilepsy is a chronic neurological disease characterized by recurrent seizures. This neurological manifestation can culminate in neurobiological, cognitive, psychological, and social consequences to the patients (Fisher et al., 2014; Scheffer et al., 2017). It has been estimated that around 65 million people are affected by this disease in the world (Moshé et al., 2015). In developed countries, the prevalence is approximately 700 to 100.000 habitants, a number that can be twice greater in underdeveloped countries, making epilepsy a public health problem (Thurman et al., 2011).

Seizures are clinical manifestations characterized by the sudden and transient occurrence of signs and symptoms caused by synchronous abnormal and excessive neuronal activity (Scheffer et al., 2017). These discharges may involve only one cerebral hemisphere (focal type) or neural networks distributed bilaterally. In this case, both hemispheres show hyperactivity (general type). According to the brain area affected, the seizure can generate cognitive, sensory, motor, autonomic, and psychic changes (Baxendale and Thompson, 2016). The seizures manifestation may be provoked (e.g., by traumatic brain injury, infection), triggered by an acute insult in the central nervous system (CNS), or spontaneous (underlying epilepsy), related to external stimuli (e.g., by flashes of light, ringing tones) (Da Silva et al., 2020).

The basis for epilepsy treatment consists of eliminating seizures or reduce their frequency (White et al., 2007). Antiepileptic drugs (AEDs) aim to control seizures, seeking maximum tolerability and minimum undesirable side effects. These drugs may act to reduce repetitive neuronal discharges through the intensification of inhibitory synaptic neurotransmission, inhibition of excitatory synaptic neurotransmission, or modulation of voltage-gated ion channels (Czapinski et al., 2005; Rogawski and Löscher, 2004; White et al., 2007). Drugs commonly used in epilepsy treatments are those referred to as first-generation drugs (phenytoin, valproate, benzodiazepines, carbamazepine, ethosuximide, phenobarbital, and primidone) and new anticonvulsant drugs, referred to as second or third generation drugs (vigabatrin, gabapentin, felbamate, lamotrigine, oxcarbazepine, tiagabine, and topiramate) (Sankaraneni and Lachhwani, 2015).

In the past few decades, there has been a considerable advance in the

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treatment of epilepsy, but the search for drugs with better efficacy and tolerability is still needed (Löscher and Schmidt, 2011). The AEDs currently available guarantee effective seizure suppression. However, about 30–40% of patients who have epilepsy are refractory to pharmacological treatments (Kwan and Brodie, 2000). Therefore, the development of new approaches for epilepsy treatment has been intensifying.

The therapeutic potential of some plants and their bioactive compounds has been investigated as new antiseizure compounds (Manchishi, 2017; Sucher and Carles, 2015; Zhu et al., 2014). Herbal medicine therapies may yield new treatment options for patients whose seizures are uncontrolled and may represent inexpensive treatments for individuals worldwide with untreated epilepsy. Thus, there is a need for alternative animal models that can aid the investigation of epilepsy and provide *in vivo* drug screening possibilities, such as zebrafish, for systematically evaluating the efficacy and safety of these products. The use of medicinal plants for disease treatments is still a challenge, especially about epilepsy, given the poor knowledge and understanding of herb mechanisms *in vivo*, showing the importance of more clinical and preclinical studies. Therefore, this review will highlight recent studies using zebrafish as an animal model to search for plant-derived bioactive compounds with antiseizure properties.

2. Why zebrafish has been considered a suitable model organism for studying epilepsy and drug discovery?

Zebrafish (*Danio rerio*) is rapidly emerging as a powerful vertebrate model organism in the neuroscience field due to its characteristics and advantages. High fertility rate, external reproduction and fertilization, translucent eggs and embryos, and fast development allow the use of this fish as an animal model at different stages of life (Dahm and Geisler, 2006; Shin and Fishman, 2002). The general organization of the CNS of zebrafish, such as neuroanatomical characteristics, cell morphology, and neuronal circuits, are similar to those of mammals (Sager et al., 2010). The neurodevelopmental processes may be observed in translucent eggs and embryos, which is important for studying neurological disorders provoked by prenatal insults and teratogenic compounds (Martinez et al., 2018). This teleost has an evolutionarily conserved genome compared to humans, well-characterized behavior repertoire, and high sensitivity to pharmacological manipulations. Zebrafish fast responses against chemical compounds provide the development of models in research of translational neuroscience and drug discovery, even at early developmental stages (Howe et al., 2013; Kalueff et al., 2013; Stewart et al., 2014). Therefore, zebrafish is a model organism suitable for assessing the biological effects of substances that modulate brain function and the study of molecular mechanisms involved in epilepsy. Besides, this species has been used for screening potential drug candidates for epilepsy treatment (Fontana et al., 2018; Kalueff et al., 2014; Mezzomo et al., 2018).

Zebrafish is an efficient experimental model for the study of epilepsy, especially with the emerging development of different chemically-induced seizure models and genetic epilepsy models (Gawel et al., 2020b). Seizures induced by chemical agents, such as pentylenetetrazole (PTZ), kainic acid, picrotoxin, and domoic acid allow the evaluation of potential anticonvulsant drugs and ictogenesis studies. Classical chemoconvulsants used in rodent models, such as antagonists of gamma-amino-butyric acid receptor had their convulsive effects established in zebrafish (Baraban et al., 2005). PTZ is the most used chemoconvulsant, promoting seizure-like behavior in zebrafish larvae and adults. PTZ-induced seizure model in zebrafish is characterized by progressive behavioral changes, which can be identified in three distinct stages: increased swimming activity (stage I), fast swimming and in circles (stage II), and loss of posture and immobility for 1–3 s (stage III), which is the indication of tonic-clonic seizures (Baraban et al., 2005). In addition, this compound increases *c-fos* expression, reduces neurogenesis, and decreases *bdnf* expression in zebrafish (Baraban et al., 2005; Pineda et al., 2011).

Seizures induced by chemical agents demonstrate similar characteristics to episodes that occur in humans, as neuronal hyperexcitability and progressive behavioral manifestations, such as erratic swimming, hyperactivity, loss of posture, and electrical discharges in the CNS (Da Silva et al., 2020; Baraban et al., 2005).

Furthermore, the use of chemical agents leads to increased expression of *bdnf* (brain-derived neurotrophic factor) and *c-fos* during the development of seizures. Thus, these markers of neural activity are important to check seizure manifestations. The *c-fos* gene is a proto-oncogene that is rapidly activated in response to cellular stimuli, such as neuronal damage. Its basal concentration is low in neural cells; however, in the presence of some stimulus, such as seizures, its expression increases (de Melo et al., 2017; Malhi et al., 2014). Compounds capable of restoring normal levels of *c-fos* and *bdnf* can be great candidates for anticonvulsant drugs. Besides, larvae and adult zebrafish develop electroencephalographic patterns corresponding to the ictal and inter-ictal phases of seizures when exposed to the PTZ (Afrikanova et al., 2013; Baraban et al., 2005; Pineda et al., 2011). Proinflammatory cytokines and inflammatory mediators also play a role in the pathophysiology of epilepsy since they can be upregulated after seizures, suggesting that the inflammatory response may be an important point to verify during seizures (Barbalho et al., 2016).

In zebrafish, chemical agent-induced seizure models offer satisfactory results in identifying causal mechanisms, behavioral characteristics, and responses to drug treatment. Acute chemical induction is one of the easiest ways to screen a wide variety of compounds to assess their anticonvulsant properties. The behavioral seizure manifestations are simple to identify in zebrafish and they are very similar to seizure patterns seen in rodents (Gawel et al., 2020b; Stewart et al., 2012). In a few minutes, PTZ promotes a seizure-like behavior in zebrafish larvae and adults; however, behavioral responses to this agent are affected by sex, weight, and water temperature (Menezes and Da Silva, 2017). Although behavioral assessment of zebrafish allows direct and rapid identification of seizure responses, it is important to consider that this type of assessment is not sufficient to guarantee the expected excessive brain activity of a seizure (Da Silva et al., 2020). Thus, it is necessary to show more details in the study that complement behavioral data, such as recording of abnormal brain activity as well as markers that indicate neuronal alterations, such as *c-fos*, for example (Afrikanova et al., 2013; Baxendale et al., 2012; Kim et al., 2010).

Zebrafish is an excellent model organism for investigating genes associated with epilepsy, including mutants that show spontaneous seizures or hypersensitivity to proconvulsant drugs (Hortopan et al., 2010; Teng et al., 2010). Genetic models of epilepsy may contribute to a better understanding of the mechanisms of pathogenesis, including the identification of underlying processes associated with this disease (Grone et al., 2016). Mutant fish strains can be used to test the susceptibility of seizures to proconvulsant agents, select antiepileptic drug candidates, assess epileptiform discharges and brain activity to correlate genotype with behavioral manifestation (Griffin et al., 2016). Furthermore, these approaches can be combined with pharmacological and behavioral strategies that induce epileptic phenotypes, contributing to the identification of potential new targeted therapies.

Genome editing technologies allow the generation of various genomic modifications in cultured cells and animal models, including zebrafish. Clustered Regularly Interspaced Short Palindromic (CRISPR)/CRISPR-Associated Protein 9 (CRISPR/Cas9) technology has evolved rapidly and allowed the creation of loss-of-function mutants or those mimic patient mutations, being generated quickly and at a low cost (Da Silva et al., 2020; Jao et al., 2013). Mutations in the autosomal dominant gene STXBP1 are associated with epileptic encephalopathies, Lennox-Gastaut, and Dravet syndromes. Thus, one study used CRISPR/Cas9 to edit a gene that generated stable mutant strains for *stxbp1a* and *stxbp1b* in zebrafish. Homozygous stxbp1b mutants showed epileptic seizures associated with normal mobility, heart rate, metabolism, gross morphology, and mild hyperpigmentation (Da Silva et al.,

2020; Grone et al., 2016). Another study also used CRISPR/Cas9 to generate *aldh7a1* zebrafish mutants with phenotypes, including epileptiform electrographic activity rescued by pyridoxine and pyridoxal 50-phosphate resembling affected individuals with pyridoxine-dependent epilepsy (Da Silva et al., 2020; Pena et al., 2017; Zabinyakov et al., 2017). Other Genome Editing Technologies methods besides CRISPR/cas9 are also widely used, such as morpholinos, for example. One study found that the morpholino-induced knock-down of the 1a epilepsy susceptibility gene (*lgi1a*) caused a seizure-like phenotype in zebrafish larvae (Stewart et al., 2012; Teng et al., 2010). The inhibition of potassium voltage-gated KCNQ3 channels using antisense morpholino oligonucleotides affects the excitability regulation of central neurons, causing seizures behaviors in freely swimming zebrafish larvae (Chege et al., 2012; Stewart et al., 2012).

No model reproduces all the criteria of human epilepsy; however, induced-seizure models or genetic models of epilepsy provide good resources for the investigation of the mechanisms underlying epilepsy. Seizures induced by chemical agents are an easy way to screen a wide variety of compounds to verify their anticonvulsant properties (Da Silva et al., 2020; Pisera-Fuster et al., 2017). On the other hand, epilepsy models are based on genetic manipulation techniques that reflect the pathogenesis and characteristics of different human epilepsies (Copmans et al., 2017).

According to the aim of the study, chemical-induced seizure models and genetic models of epilepsy are available in zebrafish. This demonstrates the increasing use of zebrafish models to study the genetic and pharmacological modulation of epilepsy-related phenotypes. The advances in the zebrafish research field contribute to using this model as a valuable tool for studying a wide variety of neurological disorders and *in vivo* drug screening (Khan et al., 2017). Zebrafish emerge as an important model to high-throughput genetic and chemical screens to identify genetic pathways and molecule candidates to epilepsy treatments.

3. Plant-derived bioactive compounds as an alternative for epilepsy treatment

Botanical constituents have been used to treat seizures for thousands of years in diversified cultures. Evidence suggests that herbal therapies were used to treat seizures as early as 6.000 BC in India, with the origin of the Ayurveda (Schachter, 2009). Traditional Medicine systems are characterized by a complex and unique organization of diagnostics and therapeutics, in which the practitioners use methods that include mixtures of herbal extracts and other natural products to reduce symptoms or cure disease (Schachter, 2008).

Currently, therapies that include herbs in treatment are the most common approach to complementary and alternative medicine, which plays an essential role in the therapy to control seizures or complications caused by AEDs (Liu et al., 2017; Schachter, 2009). This alternative medicine is a group of diverse medical and health care systems, practices, and products that are not generally considered part of conventional medicine, which includes the use of substances found in nature, such as herbal extracts and isolated compounds from plants, for example. Many herbals that are used in complementary and alternative medicine have been investigated for their potential antiseizure effects (Sucher and Carles, 2015). Plants extract and its main constituents have demonstrated anticonvulsant properties in various experimental models, many times with effects that are similar to that of synthetic drugs, but showing fewer side effects (Schachter et al., 2009).

4. Botanical species with anticonvulsant properties

In the following sections, various studies using plants and their isolated compounds are described. A summary of the anticonvulsant compounds is presented in Table 1.

4.1. *Berberis sibirica*

Berberis sibirica is a representative plant of the Berberidaceae family and it is known to contain a variety of isoquinoline alkaloids, such as protoberberines (Kukula-Koch et al., 2016).

Berberine is an isoquinoline alkaloid of the protoberberine type found in the roots, rhizomes, and stem bark of *Berberis* sp. (Imenshahidi and Hosseinzadeh, 2016). This alkaloid has a low intestinal absorption rate after oral administration and is rapidly metabolized in the liver (Wang et al., 2017). This molecule can penetrate the blood-brain barrier and accumulate in the hippocampus (Lin and Zhang, 2018).

A study using PTZ-induced seizures in zebrafish larvae investigated the correlation between the anticonvulsant effect of berberine and the *stx1b* gene, given that mutations in this gene are related to the onset of fever-associated epilepsy syndromes (Zheng et al., 2018). As a result, berberine reduced locomotion and abnormal movements in larvae at 7 days post fertilization (dpf) subjected to seizures induced with 4 mM PTZ. Berberine also decreased the hyperactivity response caused by PTZ exposure in the light stimulation condition. The increase of the *c-fos* levels provoked by PTZ was inhibited by berberine, and this alkaloid promoted *stx1b* gene overexpression in PTZ-treated larvae, which was confirmed by an increase in Syntaxin 1B (STX1B) protein levels. Therefore, due to its properties, berberine might be able to suppress epilepsy-like seizures through the upregulation of *stx1b* gene expression. Seizures induced by PTZ may be partially mediated by STX1B deficiency. Therefore, adequate levels of this protein can decrease the hyperexcitation locomotion induced by PTZ in zebrafish. Thus, berberine can suppress PTZ-induced seizures by raising STX1B levels. STX1B attenuation is related to epileptic seizures induced by PTZ, and that gene might be a promising protein marker for the screening of new anticonvulsant compounds.

Another study showed that berberine and its synthetic derivatives (hydrophilic berberrubine and hydrophobicethyl-2-(9-demethoxyberberine bromide-9-yl) hydroxyacetate) increased the seizure onset latency and suppressed the seizure-like behavior after 15 mM PTZ treatment in 7 dpf zebrafish larvae. Animals also showed recovery on *c-fos* expression and neuronal discharges during seizures. Therefore, the findings suggest that berberine and its synthetic derivatives attenuate PTZ-induced seizures and potentially protect zebrafish from the occurrence of further seizures (Zhang et al., 2020).

As the berberine, palmatine is a protoberberine alkaloid also isolated from the methanolic extract of *Berberis sibirica* root that demonstrated to be a potential anticonvulsant compound (Gawel et al., 2020a). Palmatine exerted antiseizure activity in 6 dpf zebrafish larvae exposed to 20 mM PTZ and decreased *c-fos* and *bdnf* levels in PTZ-treated animals. This compound also decreased the hyperlocomotion induced by PTZ exposure. In addition, the combination of palmatine and berberine potentiates the decrease of PTZ-induced hyperlocomotion, suggesting the combination of these compounds may have a higher therapeutic value than isolated.

4.2. *Cannabis sativa*

Cannabidiol, Δ9-tetrahydrocannabinol, and cannabinol are constituents present in *Cannabis sativa* extract, classified as a group of molecules that act on cannabinoid receptors (Gaston and Friedman, 2017). Regarding the effects of cannabinoids on the development of seizures, a study investigated the effects of Δ9-tetrahydrocannabinol and cannabidiol on zebrafish PTZ-induced seizure model and a genetic model (*GABRA1*−/−) (Samarut et al., 2019). Both compounds were effective in reducing hyperactivity linked to seizures in zebrafish. However, when combined, Δ9-tetrahydrocannabinol and cannabidiol presented a synergistic effect that was more effective against the genetic model. At the same time, individual exposure may be more effective against the chemically-induced model (Samarut et al., 2019).

Exposure to PTZ produces a behavioral response characterized by

Table 1
Summary of compounds with anticonvulsant properties.

Botanical species	Constituents	Zebrafish stage	Zebrafish model	Molecular and cellular outcomes	Behavioral outcomes	Reference
Berberis sp.	Berberine	7 dpf	4 mM PTZ	↓ *c-fos* level	↓ Abnormal movements ↓ Hyperactivity	Zheng et al. (2018).
	Berberine	7 dpf	15 mM PTZ	↓ *c-fos* level	↑ Seizure onset latency ↓ Abnormal movements	Zhang et al. (2020).
	Palmatine	6 dpf	20 mM PTZ	↓ *c-fos* level ↓ *bdnf* level	↓ Hyperactivity	Gawel et al. (2020a).
Cannabis sativa	Δ9-tetra-hydrocannabinol	5 dpf	2.5 mM PTZ or 5 mM PTZ	No data	↓ Hyperactivity ↓ Hyperactivity	Samarut et al. (2019)
	Δ9-tetra-hydrocannabinol	5 dpf	GABRA1 Knockout	No data		
	Cannabidiol	5 dpf	2.5 mM PTZ or 5	No data	↓ Hyperactivity	Samarut et al. (2019)
	Cannabidiol	5 dpf	mM PTZ GABRA1 Knockout	No data	↓ Hyperactivity	
Citrus aurantium	Extract	Adult	3 mg/mL PTZ	No data	↑ Seizure onset latency	Rosa-Falero et al. (2014).
	Hesperidin	7 dpf	8 mM PTZ	↓ *c-fos* level ↑ *bdnf* level	↓ Hyperactivity ↑ Seizure onset latency	Sharma et al. (2021).
Cryptolepis sanguinolenta	Cryptolepine nanoparticle	Adult	170 mg/kg PTZ	No data	↑ Seizure onset latency ↓ Seizure score	Mante et al. (2021).
Curcuma longa	Micronized curcumin	7 dpf	3 mM PTZ	No data	↑ Seizure onset latency ↓ Occurrence of tonic-clonic seizures	Bertoncello et al. (2018).
	Turmeric oil	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Orellana-Paucar et al. (2012).
	Ar-, α, β-turmerone	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Orellana-Paucar et al. (2012).
	α-atlantone	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Orellana-Paucar et al. (2012).
Cynanchum otophyllum	Otophylloside F	6 dpf	10 mM PTZ	No data	↓ Hyperactivity	Li et al. (2015).
	Otophylloside B	6 dpf	10 mM PTZ	No data	↓ Hyperactivity	Li et al. (2015).
	Rostratamine	6 dpf	10 mM PTZ	No data	↓ Hyperactivity	Li et al. (2015).
	Otophylloside N	6 dpf	10 mM PTZ	↓ Apoptosis and neuronal activation ↓ *c-fos* level	↓ Abnormal movements ↓ Hyperactivity	Sheng et al. (2016).
Cyperus articulatus	Extract	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Brillatz et al. (2020a).
	Mustakon	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Brillatz et al. (2020a).
	Cyperotundone	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Brillatz et al. (2020a).
	1,2-dehydro-α-cyperone	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Brillatz et al. (2020a).
	Sesquichamaenol	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Brillatz et al. (2020a).
Gastrodia elata	Gasstrodin	7 dpf	15 mM PTZ	↓ *c-fos* level ↑ Oxidative defenses	↑ Seizure onset latency	Jin et al. (2018).
Garcinia oligantha	Oliganthin H	7 dpf	10 mM PTZ	↓ *c-fos* level ↓ *npas4a* level ↓ *pyya* level ↓ *bdnf* level	↓ Seizure activity ↓ Seizure duration ↓ Abnormal movements ↓ Velocity	Gong et al. (2020).
Helleborus odorus	Extract	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Brillatz et al. (2020b).
	Furostanol saponin	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Brillatz et al. (2020b).
	Bufadienolide hellebrin	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Brillatz et al. (2020b).
Indigofera arrecta	Extract	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Aourz et al. (2019).
	Indirubin	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Aourz et al. (2019).
Magnolia officionalis Table 1 (continued)	Extract	6 dpf	40 mM PTZ or 1 mM EKP	No data	↓Seizure activity ↓ Hyperactivity	Li et al. (2020).
	Magnolol	6 dpf	40 mM PTZ or 1 mM EKP	No data	↓Seizure activity ↓ Hyperactivity	Li et al. (2020).
	Honokiol	6 dpf	40 mM PTZ or 1 mM EKP	No data	↓Seizure activity ↓ Hyperactivity	Li et al. (2020).
Orthosiphon stamineus	Extract	Adult	170 mg/kg PTZ	No data	↓ Abnormal movements ↓ Hyperactivity	Choo et al. (2018).
Peucedanum alsaticum	Extract	6 dpf	40 mM PTZ	No data	↓ Hyperactivity	Kozioł et al. (2019).
	Lucidafuranocoumarin A	6 dpf	40 mM PTZ	No data	↓ Hyperactivity	Kozioł et al. (2019).
	Bergamottin	6 dpf	40 mM PTZ	No data	↓ Hyperactivity	Kozioł et al. (2019).
Piper nigrum	Piperine nanoparticles	7 dpf	5 mM PTZ	No data	↓ Hyperactivity	Ren et al. (2019).
Piper methysticum	Extract	Adult	170 mg/kg PTZ	↓ *bdnf* level	↓Seizure scores ↑ Seizure onset latency	Jaiswal et al. (2020).
Solanum torvum	Extract	7 dpf	20 mM PTZ	No data	↓ Hyperactivity	Challal et al. (2014).
Valeriana officinalis	Extract	Adult	0.1–20 mg/ml PTZ	No data	↑ Seizure onset latency	Torres-Hernández et al. (2015b).
	Valerenic acid	Adult	0.1–20 mg/ml PTZ	No data	↑ Seizure onset latency	Torres-Hernández et al. (2015b).
Zingber purpureum	Extract	6 dpf	20 mM PTZ	No data	↓ Hyperactivity	Brillatz et al. (2020c).
No data	Micronized resveratrol	7 dpf	3 mM PTZ	No data	↑ Seizure onset latency ↓ Occurrence of tonic-clonic seizures	Decui et al. (2020).
No data	Pterostilbene	4 dpf	20 mM PTZ	No data	↓Seizure duration	Nieoczym et al. (2019).
No data	α-linolenic acid	7 dpf	8 mM PTZ	↓ *c-fos* level	↓ Hyperactivity	Kumari et al. (2019).
No data	Methylated flavonoids	Adult	40 mM PTZ	No data	↓ Hyperactivity	Koirala et al. (2016).

hyperlocomotion and clonic activity that may culminate in loss of posture (tonic-clonic seizure). These behavioral parameters indicate a seizure-like behavior that provides a measure that may assess the potential anticonvulsant properties of the compounds. The genetic model produces spontaneous intense seizures characterized by loss of posture, indicating tonic-clonic seizures.

The genetic model mimics the more severe nature of seizures than the chemical model. It is evidenced when the concentration of Δ9-tetrahydrocannabinol and cannabidiol that was needed to oppose the GABRA1−/− seizures was higher than the concentration that was effective on PTZ-induced seizures. On the other hand, the combination of these compounds decreased the need for a higher level of Δ9-tetrahydrocannabinol and cannabidiol to oppose the GABRA1−/− seizures (Samarut et al., 2019).

4.3. *Citrus aurantium*

Citrus aurantium is popularly known as bitter orange, and it is native to southeast Asia (Carvalho-Freitas and Costa, 2002). A study characterized the composition of *Citrus aurantium* leaf extract and identified constituents as hesperidin, neohesperidin, and neohesperidin dihydrochalcone, which are essential bioactive compounds from this plant. As a result, the exposure to *Citrus aurantium* extract increased the latency to the onset of a seizure in adult zebrafish exposed to PTZ (Rosa-Falero et al., 2014). The authors tested the extract of other citrus genus members for anticonvulsant properties, but only *Citrus aurantium* showed this property.

Hesperidin is a flavonoid glycoside found in large quantities in orange peel, and it proved to be an important candidate for anticonvulsant compounds. Hesperidin increased the seizure latency and reduced hyperactive responses of 7 dpf zebrafish larvae exposed to 8 mM PTZ. Elevated levels of *bdnf* with a decrease in *c-fos* expression were also observed in the 1h-hesperidin treatment following PTZ exposure (Sharma et al., 2021). In addition, the *in silico* computational analysis showed the observed anticonvulsant effect through interaction with the central CREB–BDNF pathway (Sharma et al., 2021).

4.4. *Cryptolepis sanguinolenta*

Cryptolepine is the principal alkaloid from *Cryptolepis sanguinolenta*, a West African shrub. A study investigated the anticonvulsant properties of cryptolepine solid lipid nanoparticles to verify whether this type of formulation would increase its blood-brain barrier permeability properties and potentially improve its anticonvulsant activity in the adult zebrafish PTZ-induced seizures model. Animals received 170 mg/kg PTZ administered intraperitoneally, 10 min after cryptolepine pretreatment. The cryptolepine solid lipid nanoparticles group showed an increased antiseizure effect when compared to animals exposed to cryptolepine only. This may be associated with increased bioavailability of cryptolepine to the target site formulated as solid lipid nanoparticles. The anticonvulsant properties of cryptolepine solid lipid nanoparticles are evident by the decrease in mean seizure score and increased latency to onset of the seizures (Mante et al., 2021).

The solid lipid nanoparticles method enhances cryptolepine blood-brain barrier permeability and contributes to improving its anticonvulsant property. Nanoparticles are an important strategy for the use of anticonvulsant compounds with poor aqueous solubility.

4.5. *Curcuma longa*

Turmeric (*Curcuma longa*) is a plant of the Zingiberaceae family, originally from South Asia (Ahmad et al., 2020). Turmeric chemical composition is constituted by the presence of turmeric oil, rich in oxygenated sesquiterpenoids (ar-,α,β-turmerone, and α-atlantone), and curcominoids (curcumin, monodemethoxycurcumin, and bisdemethoxycurcumin) (An et al., 2020).

Curcumin, the main bioactive compound of turmeric, has been extensively investigated due to its potential therapeutic effect on seizures. However, the bioavailability of this compound is low, which requires the development of tools that potentiate the effectiveness of curcumin, such as the micronization technique (Aguiar et al., 2016). Micronized curcumin treatment showed an anticonvulsive effect in larval and adult zebrafish exposed 30 min before to 3 mM PTZ. This compound demonstrated similar effects to classic AEDs since valproate and micronized curcumin reduced the occurrence of tonic-clonic seizures in both larvae and adult animals (Bertoncello et al., 2018). Furthermore, other turmeric constituents also demonstrated efficacy in suppressing seizures induced by 20 mM PTZ in 7 dpf zebrafish larvae. Turmeric oil, ar-,α,β-turmerone, and α-atlantone exhibit anticonvulsant properties, which indicate that these compounds may be considered anticonvulsant drug candidates (Orellana-Paucar et al., 2012).

4.6. *Cynanchum otophyllum*

Cynanchum otophyllum from the Asclepiadaceae family is an endemic herb of southwest China. A study evaluated 19 compounds from roots of *Cynanchum otophyllum* in the changes on the locomotor activity of 6 dpf zebrafish larvae exposed to 10 mM PTZ. The results showed that three compounds (otophylloside F, otophylloside B, rostratamine 3-O-β-D-oleandropyranosyl-(1 → 4)-β-D-cymaropyranosyl-(1 → 4)-β-D-cymaropyranoside) exert a significant anticonvulsant effect, besides that, these compounds do not promote any neurotoxic and sedative effects or alteration on locomotor activity (Li et al., 2015). The potential anticonvulsant properties of Otophylloside N, a steroidal glycoside isolated from *Cynanchum otophyllum,* treatment was also evaluated. This compound reduced the fast swimming induced by PTZ exposure in zebrafish larvae. Besides, Otophylloside N attenuated PTZ-induced apoptosis and neuronal activation in zebrafish (Sheng et al., 2016).

These studies contribute to the investigation of the neuroprotective effects of *Cynanchum otophyllum* constituents, which might be candidates for novel antiepileptic drugs.

4.7. *Cyperus articulatus*

Cyperus articulatus is a native herb from Amazonia, but this plant is also found in Africa, where a decoction of its rhizomes is traditionally used to treat epilepsy (Rakotonirina et al., 2001).

A study investigated the activity of various sesquiterpene compounds from a hexane extract of *Cyperus articulatus* dried rhizomes. This extract showed stronger anticonvulsant activity and reduced seizures in 7 dpf zebrafish larvae exposed to 20 mM PTZ. Due to its properties, the extract had its constituents isolated and tested. As a result, four sesquiterpenoids were identified as cyperotundone, mustakon, 1,2-dehydro-α-cyperone, and sesquichamaenol, which protected zebrafish larvae against PTZ-induced seizures (Brillatz et al., 2020a).

The identification and bioactivity characterization of the antiseizure extract compounds allow a better chemical evaluation of the isolated compound in an animal model, discarding the influence of the other compounds present in the extract.

4.8. *Embelia ribes*

Embelin is a benzoquinone alkaloid, the main compound found in red berry fruits of the *Embelia ribes* species (Lu et al., 2016). The physical and chemical properties of embelin are favorable to its therapeutic potential, and its ability to penetrate the blood-brain barrier makes this compound a candidate for the treatment of CNS diseases (Kundap et al., 2017).

Embelin retarded seizures manifestation and reduced epilepsy-induced memory alterations in adult zebrafish submitted to kindling induced by 80 mg/kg PTZ for 10 days. A repeated small dose of PTZ for various days produces a chronic epilepsy-like condition in zebrafish, a

method that may be applied to understand the underlying mechanism of epileptogenesis. Moreover, embelin treatment also demonstrated an anti-inflammatory effect via downregulation of inflammatory markers, and immunohistochemistry data suggested that embelin promoted neuronal protection in animals (Kundap et al., 2019b). Besides, embelin was capable of reducing epileptic seizures and improved the cognitive function of the zebrafish against acute PTZ-induced seizures (Kundap et al., 2019a). These studies suggest that embelin could be a potential compound for epilepsy treatment and memory dysfunction.

4.9. *Gastrodia elata*

Gastrodin is the main phenolic glucoside compound derived from *Gastrodia elata* (Liu et al., 2018). A study investigated the effects of gastrodin pretreatment on PTZ-induced seizures in 7 dpf zebrafish larvae and its properties underlying mechanism related to antioxidative defense. Animals received gastrodin treatment 24h before 15 mM PTZ, and seizures were induced until stage III. As a result, gastrodin showed decreased seizures and increased the latency period to the onset of seizures stages II and III. In addition, a suppression in *c-fos* expression was also detected in gastrodin pretreatment (Jin et al., 2018).

This compound prevented oxidative damage promoted by PTZ-induced seizures (Jin et al., 2018). Seizure activity may result in reactive oxygen species production via NADPH oxidase activation (Kovac et al., 2014). Animals treated with NADPH oxidase inhibitors decreased this enzyme activity, reducing reactive oxygen species generation produced by PTZ exposure (Jin et al., 2018).

Thus, these findings suggested that seizure caused reactive oxygen species accumulation by activating NADPH oxidase in larval zebrafish. Therefore, indicating that gastrodin potentially protects from further seizures by modulating oxidative stress.

4.10. *Garcinia oligantha*

Garcinia oligantha is a shrub distributed mainly in dense forests of China, and it is one of the sources of natural xanthone derivatives, arising interest in its potential pharmacological activities (Tang et al., 2019).

Three derivatives from leaf extracts of *Garcinia oligantha* (oliganthin H, oliganthin I, and oliganthin N) had the antiseizure activity evaluated in larval zebrafish assays. After compounds pretreatment for 6h, 7 dpf zebrafish larvae were submitted to 10 mM PTZ exposure. Results showed that oliganthin H was effective against PTZ-induced seizures. It reduced seizure activity, velocity, seizure duration, and the number of bursts in zebrafish larvae. In addition, PTZ treatment elicited a transcription of a specific set of genes encoding transcription factors, such as *c-fos*, *npas4a*, *pyya*, and *bdnf*. Pretreatment of oliganthin H was capable of restored induction of these genes, demonstrating its potent anticonvulsant effect (Gong et al., 2020).

4.11. *Helleborus odorus*

Helleborus odorus is a native plant from Greece widely used during antiquity and physicians usually prepared herbal wines and decoctions to treat mental disorders (Brillatz et al., 2020b).

The root methanolic extract of *Helleborus odorus* demonstrated significant antiseizure properties through reducing locomotor activity induced by 20 mM PTZ in 7 dpf zebrafish larvae. In addition, the bioassay-guided fractionation of the methanolic extract of *Helleborus odorus* resulted in the isolation of furostanol saponin and the bufadienolide hellebrin, which both compounds were effective to decrease the PTZ-induced locomotor activity in zebrafish larvae (Brillatz et al., 2020b).

4.12. *Indigofera arrecta*

Indigofera arrecta is a plant that belongs to the Fabaceae family, and it is known for its traditional use for the treatment of epilepsy in Congo (Hu et al., 2008).

The extract of the leaves of *Indigofera arrecta* showed potent anticonvulsant activity in zebrafish larvae, then the secondary bioactive metabolites of this plant extract were isolated and identified. The bioassay-guided fractionation of the extract resulted in the identification of the compound indirubin. Zebrafish larvae at 7 dpf were exposed to the extract of *Indigofera arrecta* or indirubin for 18h before 20 mM PTZ treatment. The results showed that indirubin reduced larval locomotor activity induced by PTZ. In addition, the electroencephalographic analysis showed that indirubin decreased the number of interictal and ictal-like spikes and the total cumulative duration of epileptiform activity in zebrafish larvae (Aourz et al., 2019).

4.13. *Magnolia officinalis*

Magnolia officinalis is a species of the Magnoliaceae family mainly distributed in east and southeast Asia. Herbal preparations of its bark are typically used as decoctions and the main compounds found in its composition are magnolol and honokiol (Schifano et al., 2017).

A study tested various species of plants with potential anticonvulsant properties to identify compounds that could be important against pharmacoresistant epilepsies. Zebrafish larvae at 6 dpf were exposed to 40 mM PTZ or 1 mM ethylketopentenoate (EKP) to induce a seizure model with locomotor and brain hyperactivity. As a result, the authors identified six plant extracts effective in reduce PTZ-induced seizure movement, such *Anemarrhena asphodeloides*, *Bupleurum chinensis*, *Scutellaria baicalensis,* and *Magnolia officinalis*. The extract of *Magnolia officinalis* was effective in inhibited both PTZ- and EKP-induced seizure behavior. Thus, due to its properties, isolated compounds of *Magnolia officinalis* were also tested. Magnolol and honokiol displayed an effective antiseizure activity against PTZ and the EKP model (Li et al., 2020).

The EKP is a lipid-permeable inhibitor of glutamic acid decarboxylase that increases locomotor activity, induces epileptiform events, and increases synaptic activity-regulated *c-fos* expression in zebrafish larvae (Zhang et al., 2017). The EKP model is characterized by inadequate responses to several existing AEDs, emerging as a tool for studies of animal models of drug-resistant seizures. However, further studies are required to establish if this model represents any epilepsy syndrome.

4.14. *Orthosiphon stamineus*

Orthosiphon stamineus is an herb widely distributed in southeast Asia and China, and the proteins from its leaves may be potentially protective for CNS disorders, such as epilepsy. Pretreatment for 30 min with the proteins extracted from *Orthosiphon stamineus* leaves decreased seizure score and prolonged seizure onset time in adult zebrafish treated with 170 mg/kg PTZ via intraperitoneal injection. In addition, pretreatment also resulted in a swimming behavior comparable to that of the control group (Choo et al., 2018). Due to its anticonvulsive properties, proteins extracted from *Orthosiphon stamineus* leaves may be candidates for seizure control.

4.15. *Peucedanum alsaticum*

Herbs from the *Peucedanum* genus are commonly utilized in traditional medicine, in which their coumarin derivates are constituents with potential therapeutic actions for the treatment of neurological disorders (Vogl et al., 2011).

The potential anticonvulsant properties of *Peucedanum alsaticum* extract and its compounds were tested in the zebrafish PTZ-induced seizure model. Zebrafish larvae of 6 dpf were exposed to dichloromethane extract of *Peucedanum alsaticum*, and its isolated compounds,

bergamottin and lucidafuranocoumarin A. The results demonstrated that the *Peucedanum alsaticum* extract and the constituent bergamottin exhibited weak anti-seizure activity in zebrafish larvae exposed to PTZ. In contrast, the constituent lucidafuranocoumarin A was effective and decreased the hyperactivity caused by PTZ-induced seizures (Kozioł et al., 2019).

4.16. *Piper nigrum*

Piperine is an alkaloid, the main bioactive compound present in the fruits of the *Piper nigrum,* but also found in other species that belong to the Piperaceae family (Haq et al., 2020). These plants are from India, and their fruits are used as food condiments worldwide (Ren et al., 2019).

The potential anticonvulsant properties of piperine have been demonstrated in several studies with rodent models and clinical trials. In the zebrafish model, piperine nanoparticle treatment showed an anti-seizure effect on 7 dpf zebrafish larvae exposed to 5 mM PTZ. A significant reduction in total movements was observed when piperine nanoparticles were added to the PTZ medium (Ren et al., 2019).

Due to its poor aqueous solubility, treatment with piperine results in low bioavailability. The nanoparticle's method proved to be effective in increasing the bioavailability of this compound and presenting adequate protection against the convulsions induced by PTZ. Unformulated piperine was not effective in protecting against PTZ-induced seizures, even at high concentrations, when compared to piperine nanoparticles.

4.17. *Piper methysticum*

Piper methysticum is a native herb to the South Pacific islands, and root decoctions are consumed in South Pacific ceremonies (Garrett et al., 2003).

The anticonvulsive potential of *Piper methysticum* aqueous extract was reported in a study that investigated the properties of various stem tissues from this plant, using adult zebrafish exposed to 170 mg/kg PTZ, via intraperitoneal injection. The results indicated that adult zebrafish exposed to aqueous extract of stems without peel exhibited the seizure score and onset time significantly reduced when compared to the control group (Jaiswal et al., 2020). The effects of the *Piper methysticum* aqueous extract are comparable to the diazepam in terms of seizure intensity and onset time. Furthermore, the *bdnf* expression was reduced after extract treatment, which may be an important factor in the antiseizure properties (Jaiswal et al., 2020).

4.18. *Solanum torvum*

Solanum torvum is an herb found in South China and Southeast Asia, and its leaves decoctions are traditionally used to treat seizures. The main bioactive constituents of this plant are steroidal saponins.

Zebrafish larvae at 7 dpf were exposed to different extracts of *Solanum torvum* aerial parts for 18h before 20 mM PTZ treatment. As a result, the methanolic and aqueous extracts exhibited a reduction of PTZ-induced movement. In addition, six spirostane glycosylated triterpenes were isolated from extracts of *Solanum torvum* and had their biological activity tested. These compounds showed anticonvulsant activity in the zebrafish PTZ-induced seizure model through the reduction in the hyperlocomotion activity provoked by PTZ (Challal et al., 2014).

4.19. *Valeriana officinalis*

Valerian (*Valeriana officinalis*) is a herb native from Europe and North Asia, and valerenic acid is a sesquiterpenoid considered the main chemical constituent (Gao and Björk, 2000; Torres-Hernández et al., 2015a).

A study investigated the anticonvulsant properties of *Valeriana officinalis* extract and valerenic acid. The interaction of those preparations with the activity of AEDs was also investigated. Adult zebrafish pretreated for 1h to both valerenic acid and *Valeriana officinalis* extract had the period to the onset of the seizure increased when exposed to PTZ. Besides that, ethanolic *Valeriana officinalis* extract potentiated the anti-seizure effects of both phenytoin and clonazepam while the valerenic acid interacted synergistically only with clonazepam, increasing the latency period to seizures and improving animal survival (Torres-Hernández et al., 2015b).

The combination of multiple drugs, including natural products, may be used to obtain better seizure control, especially for refractory epilepsy. Studies investigating the synergic effects of these compounds are necessary for future epilepsy management.

4.20. *Zingiber purpureum*

The rhizomes of *Zingiber purpureum* are a type of tropical ginger from Southeast Asia, commonly used as a spice (Akagi et al., 2015).

Zingiber purpureum was extracted by different types of solvents (hexane, ethyl acetate, methanol, and water), then 6 dpf zebrafish larvae were submitted to the extracts and exposed to 20 mM PTZ. As a result, the hexane extract showed the most robust antiseizure activity, reducing the hyperlocomotor activity induced by PTZ treatment (Brillatz et al., 2020c).

4.21. *Other compounds with anticonvulsant properties in the zebrafish model*

4.21.1. *Resveratrol*

Resveratrol is a polyphenolic compound of the stilbene family found mainly in grape skin and seeds and other types of plants, such as peanuts and berries (Galiniak et al., 2019). The capacity of resveratrol to cross the blood-brain barrier was demonstrated in animal models, such as mammals and invertebrates (Baur and Sinclair, 2006). The potential anticonvulsant properties of resveratrol have been investigated, and it seems to be an effective pharmacological alternative for suppressing seizure development (Pallàs, 2014).

The anticonvulsant effect of resveratrol on the PTZ-induced seizure model was investigated in zebrafish (Decui et al., 2020). Zebrafish larvae (at 7 dpf) were exposed to non-micronized and micronized resveratrol 30 min before PTZ (3 mM) exposure. Seizure analysis of the animals was based on stages (I, II, and III) and latency to reach each seizure episode. Micronized resveratrol exposure reduces the occurrence of tonic-clonic seizures (stage III), and the animals took longer to reach seizure stages I and III. In contrast, non-micronized resveratrol neither increased the latency of any seizure stages nor had effects on the occurrence of tonic-clonic seizures (stage III). Despite these promising pharmacological properties of resveratrol, the clinical use of this compound is limited due to its low bioavailability (Cottart et al., 2010). Therefore, micronization technology is a useful tool to increase the bioavailability of compounds (Aguiar et al., 2016).

The potential anticonvulsant properties of the pterostilbene, a natural dimethylated analog of resveratrol, were also investigated. This compound demonstrated anticonvulsant properties through the reduction of PTZ-induced locomotor activity in 4 dpf zebrafish larvae. Exposure to pterostilbene significantly reduced both the number and total duration of epileptiform discharges, confirming the anticonvulsant potential of this compound (Nieoczym et al., 2019).

4.21.2. *α-linolenic acid*

The α-linolenic acid is an essential omega-3 fatty acid, derived from plants, is present in leafy vegetables, flax, and chia seeds (Kim et al., 2014). The anticonvulsant properties of this compound were observed in a PTZ-induced seizure model in zebrafish. Fish embryos exposed to α-linolenic acid had a reduction in hyperactive response mediated by 8 mM PTZ in 7 dpf larvae, observed as a decrease in total distance traveled and speed. The exposure also increased the latency to the first

tonic-clonic seizure occurrence induced by PTZ, indicating an antiseizure effect. Furthermore, *c-fos* mRNA expression was also reduced in α-linolenic acid exposed larvae treated to PTZ (Kumari et al., 2019).

Reports have shown that an Omega-3 fatty acid-enriched diet may reduce seizures in epileptic patients (Schlanger et al., 2002). Thus, this study suggested α-linolenic acid as a potential dietary supplement to manage childhood epilepsy due to its anticonvulsant properties on zebrafish larvae.

4.21.3. *Methylated flavonoids*

A study investigated the potential of methylated flavonoids as a source for anticonvulsant drug discovery since the methylation of flavonoids could raise the biological activity by increasing compound bioavailability (Koirala et al., 2016). As a result, methylated flavanones naringenin 4′,7-dimethyl ether, and naringenin 7-O-methyl ether were highly effective against PTZ-induced seizures in larval zebrafish whereas naringenin, kaempferol, and kaempferide possess only a limited activity (Copmans et al., 2018).

5. Conclusion

This review showcased how the zebrafish may be used in the research of candidate compounds for epilepsy treatment. The investigation of new therapeutic alternatives for epilepsy becomes relevant, especially for refractory epilepsy.

Zebrafish emerges as a promising animal model in the CNS disease research field, providing a better understanding of etiology and possible treatments for these diseases. Whereas the zebrafish neuroanatomical characteristics are similar to those of mammals, and its neuronal pathways are conserved when compared to the other vertebrate models, this species becomes an important tool in neurobehavioral and neuropsychiatry studies. The development of genetic and molecular techniques contributes to the advancement in research and helps discover new compounds candidates for the treatment of epilepsy.

Although zebrafish has many advantages in the study and screening of plants with anticonvulsant potential, this model also has some limitations to be considered. There are some physiological differences between fish and humans, such as thermoregulation, which can affect the pharmacodynamics of the compounds tested. In addition, there is no way to directly convert the dose used in zebrafish to a clinical dose to be used in humans, requiring further studies that may involve data variability, reliability, and general reproducibility (Wang et al., 2021). Another point is that most studies used chemical substances to induce seizures. These chemoconvulsants do not reflect the pathogenesis and characteristics of epilepsy as in genetic models. Therefore, there is a need for studies including models of genetic manipulations as close to epilepsy as possible. However, mechanisms of seizure onset and the effects of pharmacological intervention therein can be investigated through the chemical-induced seizures models, as well as initial drug screens.

Plant-derived bioactive compounds provide an alternative for the treatment of epilepsy, whether in their potential anticonvulsant properties and a possible complement to the traditional treatments currently available. Although many plant compounds have low bioavailability, some methods such as micronization of the compounds and the use of nanoparticles may represent an important contribution an important contribution to the increase in the pharmacological properties of these chemicals, allowing these compounds to cross the blood-brain barrier more easily.

Therefore, research in this area becomes relevant, helping to identify compounds that are candidates for the treatment of epilepsy and to understand the beneficial effects of these compounds. In this way, the high sensitivity of zebrafish provides researchers with a useful model organism capable of identifying new anticonvulsant compounds, alternative molecular targets, and techniques that can contribute to future treatments for refractory epilepsy.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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