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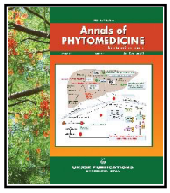

Review Article : Open Access

Recent advances in therapeutic potential of phytochemicals in cancer research through Zebrafish (*Danio rerio*) F. Hamilton: A promising experimental model

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Abstract

Zebrafish has been used as promising model organism for scientific investigation and analysis of various facets of oncology including metastasis, cancer growth, angiogenesis, tumor growth and drug response. This experimental animal model has been utilized to study the effects of phytochemicals in various areas, including cancer research, neuropharmacology, cardiovascular health, and inflammation. This review article highlights and reviewed the effects of phytochemicals through zebrafish as model organism for investigating their anticancer potential. These phytochemicals were included kimcuongin, murralongin, murrayone murracarpin, vincristine, polyphyllin VII, betulin, honokiol, indirubin, laminarin, resveratrol, curcumin, quercetin, *etc*. *In vivo* studies conducted in recent years on zebrafish were taken into account and reports revealed that these compounds exhibited anti-cancerous activity and some were not shown detectable toxicity even at high concentrations, suggesting they may have low toxicity profiles and could be potential candidates for further development as therapeutic agents. Additionally, di-indolylmethane (DIM), another compound found in *Glycosmis ovoidea*, has also been found to be non-toxic. GME, a compound derived from *Gnetum montanum*, has shown antitumor activity against SW480 cells in laboratory dishe/test tubes and in living organisms studies, revealing potential as a colon cancer treatment. Future research could be intensified with focal point on identifying and testing novel phytomedicines in zebrafish xenograft and transgenic zebrafish models to expand the knowledge on anticancer potential and will uncover new leads for drug development.

1. Introduction

Phytochemicals, natural compounds found in plants, have gained prominence in cancer research for their potential therapeutic benefits. These bioactive substances, such as flavonoids, polyphenols, and alkaloids, exhibit anticancer properties by influencing various cellular processes (Elhan and Iffat , 2021; Prairna *et al*., 2022). It explores the role of phytochemicals used in oncology experimentation, underlining their diverse mechanisms of action and potential applications as promising agents in the prevention and treatment of cancer (Srinivasan and Murali, 2022; Bhuneshwari and Sury, 2022).

Zebrafish as an experimental model organism is taken due to their unique characteristics, such as external fertilization, rapid development, transparent embryos, and regenerative abilities, which make them highly suitable for studying various biological processes (Figure 1). Since then, zebrafish have been extensively used in a wide range of research areas, including developmental biology, genetics, neuroscience, cancer research, cardiovascular research, drug discovery, and regenerative medicine.

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Zebrafish offers several advantages as a model organism, including their small size, high reproductive rate, ease of genetic manipulation, and similarity to human biology in many aspects. Zebrafish research has provided valuable insights into many fundamental biological processes and has led to numerous discoveries with implications for human health and disease. For anticancer drugs, zebrafish have been used to assess the efficacy and safety of various anticancer drugs, such as doxorubicin, paclitaxel, cisplatin and tyrosine kinase inhibitors. Zebrafish models have provided insights into the mechanisms of action, drug resistance, and potential side effects of these drugs.

Thus, this organism has been recognized as pivotal model in biomedical exploration due to compact size and straightforward upkeep. Their genetic resemblance to human genome and its transparent nature while taking observations of developmental processes, makes this organism very useful in phytochemical screening and drug development as per the recommendations for of zebrafish captive culture of animal husbandry (Alestrom *et al*., 2019; Lieggi *et al*., 2020; Howell and Cheyne, 2019). The procedure commences by emphasizing how zebrafish have emerged as a vital force in deciphering to amplify role of plant based secondary metabolites in cancer research (Chen *et al*., 2021; Hason *et al*., 2019), anti-infective drugs, and developmental drugs (Rosa *et al*., 2022).

Xenografting in zebrafish is a valuable research tool that allows scientists to study various biological processes, including cancer,

regeneration, development, immunology, and drug discovery, in a live organism with high genetic similarity to humans. Targeting stem-like self-renewal with the dietary supplement curcumin reduces cell migration and bulk formation in the zebrafish embryos, indicating that these effects are associated with the stem-like cell content of the mammosphere-derived cells. This zebrafish xenograft model provides a rapid, easy, and inexpensive assay to invade cancer stem-like cells *in vivo* and may be used for studying cancer stem cell biology and evaluating the efficacy of novel anticancer treatment strategies.

Neuropharmaceuticals: Zebrafish have been used to study the effects of neuropharma-ceuticals, including drugs used for treating neurological disorders such as epilepsy, Alzheimer's disease, and Parkinson's disease. Zebrafish models have allowed researchers to assess the effects of these drugs on neuronal development, behavior, and neurotransmitter pathways. Cardiac drugs: Zebrafish have been used to study the effects of cardiac drugs, such as beta and calcium channel blockers, and anti-arrhythmic drugs, on cardiac development, function, and regeneration. Zebrafish models have provided valuable insights into the molecular mechanisms underlying cardiac diseases and the effects of various drugs on the heart.

Anti-inflammatory drugs: Zebrafish have been used to study the effects of anti-inflammatory drugs, including nonsteroidal anti-inflammatory drugs and corticosteroids, on inflammation and immune responses.

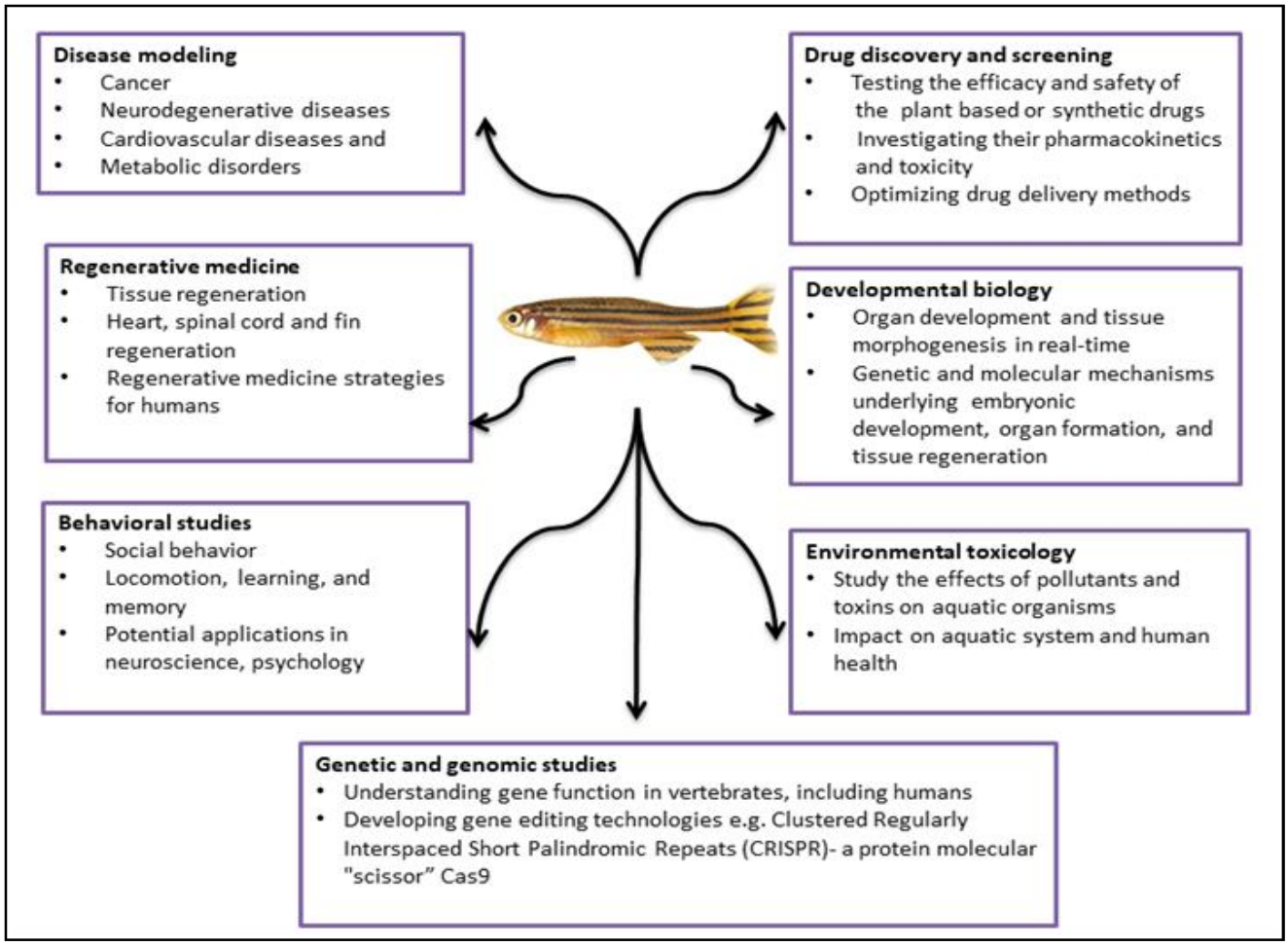

Figure 1: Fin to drug: A promising model for *in vivo* study and drug discovery.

Zebrafish models have allowed researchers to assess the effects of these drugs on inflammation related diseases, such as inflammatory bowel disease and rheumatoid arthritis. Zebrafish are a valuable model organism in metallodrug research (Karas *et al*., 2021). Similarly, both cytochalasin D and jasplakinolide (JAS) are natural compounds used in cell biology research to study actin dynamics and cellular processes such as cell shape, movement, and division. Cytochalasin D is a fungal toxin that disrupts actin filaments, while jasplakinolide is derived from the marine sponge jasplakinolidae and promotes actin filament formation. Both compounds are not approved for clinical use in humans but are commonly used as research tools in laboratory settings to investigate actin-dependent cellular events (Eom *et al*., 2022).

Antiinfective drugs: Zebrafish have been used to assess the efficacy and toxicity of different anti-infective drugs, such as antibiotics, antiviral drugs, and antifungal drugs. Zebrafish models have been used to screen and optimize drug candidates for infectious diseases, such as tuberculosis, malaria, and bacterial infections.

Developmental drugs: Zebrafish have been used to study the effects of developmental drugs, such as retinoids and teratogens, on embryonic development, organogenesis, and birth defects. Zebrafish

models have provided insights into the mechanisms of action and potential toxicities of these drugs during critical stages of development. These are just a few examples of the drugs that have been studied using zebrafish as a model organism. Zebrafish models offer unique advantages, such as their transparency during early development, rapid reproduction, and similarity to human physiology and genetics, which make them valuable tools in drug discovery and development research. CRISPR-Cas9 has promising applications in regenerative medicine research using zebrafish. They have remarkable regenerative capabilities, and CRISPR-Cas9 can be used to study the genes and mechanisms involved in tissue regeneration. Here, we reviewed effect of some phytochemicals that were screened for anti-cancerous activity using zebra fish as experimental model. The overall aim of this review is to summarize phytochemicals study *via* unique advantages of zebrafish in cancer research, drug discovery and development research, and how it can contribute to our knowledge and understanding of human health and disease in particularly focusing on cancer research using this organism as promising tool.

2. Plants used for anticancerous activity through zebrafish

Plants exhibited anticancerous activity and their potential evaluated through zebrafish studies listed Table 1 with their chemical structures shown in Figure 2.

2.1 *Glycosmis ovoidea* Pierre

These compounds kimcuongin, murralongin, murrayone and murracarpin, derived from *Glycosmis ovoidea* Pierre is a plant species belonging to the Rutaceae family. It is commonly known as "Egg-Shaped Glycosmis" or "Thai Orangeberry" and is native to Southeast Asia, including countries such as Thailand, Malaysia, and Indonesia. G. *ovoidea* is a small tree or shrub that can grow up to 3-5 meters in height. It has simple, alternate, glossy leaves with entire margins and produces small, fragrant flowers. The fruit of *G. ovoidea* is a round or ovoid berry that is usually orange or reddish in color when mature. In traditional medicine, various parts of *G. ovoidea* are used for their potential medicinal properties, such as being used as a febrifuge, tonic, or for treating wounds and digestive ailments. Additionally, some studies have reported the presence of bioactive compounds in *G. ovoidea*, such as flavonoids, alkaloids, and terpenoids, which may possess antioxidant, anti-inflammatory, and anticancer properties. However, further research is needed to fully understand the potential medicinal properties of *G. ovoidea* and its effectiveness in various health conditions.

In vivo studies conducted on zebrafish (*Danio rerio*) revealed that compounds kimcuongin, murralongin, murrayone and murracarpin, derived from *G. ovoidea*, did not show any detectable toxicity even at concentrations as high as 50 M. This suggests that these compounds may have low toxicity profiles and could be potential candidates for further development as therapeutic agent (Blanco Carcache *et al*., 2022). Similarly, di-indolylmethane (DIM) was found to be non-toxic (Shilpa *et al*., 2022).

NF-B mediated ABC (ATP-binding cassette), a transport proteins, involved in the active transport of various molecules across cellular membranes at the cost of ATP hydrolysis. ABC transporters have been reported in many organisms, including humans, and are associated with multidrug resistance (MDR) in cancer cells, where they can efflux anticancer drugs from cells, leading to reduced drug efficacy (Sun *et al*., 2022).

2.2 *Gnetum montanum*

It is a species of climbing shrub of Gnetaceae family, commonly known as "Mountain Jointfir" or "Mountain Gnetum" and is native to several countries in Southeast Asia, including Thailand, Malaysia, Indonesia, and the Philippines. *G. montanum* has male and female reproductive organs are borne on separate plants, is a dioecious plant having large, heart-shaped leaves with prominent veins and can grow up to 10 meters in length. It has been used in traditional medicine in some indigenous communities for various purposes. In some cultures, the leaves of *G. montanum* are used as a poultice for treating skin ailments, and the stem bark is used for its potential medicinal properties, such as being used as an antipyretic, diuretic, and wound healer. Some reports have revealed that bioactive compounds in *G. montanum,* including alkaloids, flavonoids, and stilbenoids, which may possess antioxidant, anti-inflammatory, and other pharmacological activities. In addition to its traditional medicinal uses, *G. montanum* is also known for its culinary use. The young leaves and shoots of *G. montanum* are sometimes used as a leafy vegetable in some local cuisines, and the seeds are sometimes consumed as a food source due to their high protein and fat content. It is worth noting that while *G. montanum* has been used in traditional medicine and is consumed as food in some cultures, scientific research on its medicinal properties and safety profile is limited. GME showed *in vitro* and *in vivo* antitumor activity against SW480 cells by inducing apoptosis, causing cell cycle arrest, and attenuating AKT signaling, suggesting potential as a colon cancer treatment. The term "xenograft model of Zebrafish" refers to the transplantation of human or other non-zebrafish cells into zebrafish for research purposes. This type of model is used to study the behavior, growth, and response of transplanted cells in a living zebrafish organism. Xenograft models are commonly used in cancer research to investigate tumor growth, metastasis, and drug response in an *in vivo* system.

Zebrafish are a popular model organism for xenograft studies due to their small size, transparency, rapid development, and genetic similarities to humans. Xenograft models of Zebrafish offer a valuable tool for understanding cellular behavior and drug response in a living organism, providing insights into cancer biology and potential therapeutic approaches (Pan *et al*., 2022). SilPP7 exhibits anti-angiogenic and anti-metastatic activities in HCC, mediated by down regulation of NF-B/MMP-9/VEGF pathway, indicating its potential as a therapeutic agent for HCC management (Zhang *et al*., 2021).

NAN treatment in HCT116 and HT29 human CRC cells resulted in G1 phase cell cycle arrest, caspase-independent apoptosis, and significant downregulation of cyclin D1 levels. This downregulation was attributed to direct targeting of eEF1A by NAN, which partially decreased protein synthesis. Furthermore, NAN suppressed tumor growth in a zebrafish xenograft model (Hou *et al*., 2021).

2.3 *Cochinchina momordica* seed extract (CMSE)

It is *C. momordica*, scientifically known as *Momordica cochinchinensis*, is a species of perennial vine in the Cucurbitaceae family. It is commonly known by various names, including "Gac fruit," "Baby Jackfruit," "Spiny Bitter Gourd," or "Sweet Gourd." *C. momordica* is native to Southeast Asia, particularly in Vietnam and Thailand, and is also found in other countries in the region. It is known for its distinctive appearance, with a spiky or prickly outer covering and a bright red-orange fruit flesh inside. The fruit is typically

round or oblong in shape and has a sweet and mild flavor, often described as a combination of carrot and sweet potato. The fruit is rich in carotenoids, particularly beta-carotene, which gives it its vibrant color and has earned it a reputation as a nutrient-rich food. In addition to its culinary uses, *C. momordica* has been used in traditional medicine in some cultures. In Vietnamese and Thai traditional medicine, various parts of the plant, including the fruit, seeds, leaves, and roots, have been used for their potential medicinal properties. It has been used as a digestive aid, a tonic, and for its supposed anti-inflammatory and anticancer properties, among others. Some studies have also reported antioxidant and immunomodulatory activities associated with *C. momordica*.

Due to its potential medicinal properties and nutritional value, *C. momordica* has gained attention as a functional food and a natural source of bioactive compounds. Zebrafish embryos were exposed to varying doses of *C. momordica* seed extract from 2 dpf to 3 dpf in this study. CMSE led to dose-dependent cardiotoxicity in zebrafish, including pericardial edema, cardiac apoptosis, increased ROS production, reduced blood flow velocity, and downregulated expression of cardiac marker genes. These results indicate that CMSE may cause cardiotoxicity through mechanisms involving inflammation and oxidative stress (Du *et al*., 2021).

2.4 *Vinca rosea*

Vinca rosea, known as *Catharanthus roseus*, is a species of flowering plant in the Apocynaceae family. It is commonly known as "Madagascar periwinkle" or simply "vinca." *V. rosea* is native to Madagascar, widely cultivated and naturalized in many regions around the world. *V. rosea* is a small, evergreen perennial plant that typically grows up to 1 meter in height. It has glossy, dark green leaves and produces attractive flowers with five petals that come in various colors, including white, pink, and shades of purple. The flowers have a characteristic shape with a prominent central tube and are typically solitary or borne in small clusters.

It has been used in traditional medicine in some cultures for its potential medicinal properties. It contains various alkaloids, including vinblastine and vincristine, which have been found to exhibit anticancer activity and are used as chemotherapy drugs in the treatment of various types of cancer, such as leukemia and lymphoma.

These alkaloids interfere with the growth by inhibiting cell division and disrupting the formation of microtubules, which are essential for cell replication. In addition to its medicinal uses, *V. rosea* is also cultivated as an ornamental plant in gardens and landscapes due to its attractive flowers and glossy foliage. It is commonly used as a ground cover, in borders, or in containers. However, it's worth noting that all parts of *V. rosea*, including the leaves and stems, are considered toxic and should not be ingested by humans or animals without proper medical supervision, as they can cause adverse effects. As with any medicinal plant or herbal remedy, it is advised to consult a well-qualified healthcare professional or a knowledgeable practitioner before using *V. rosea* or its extracts for medicinal purposes, as it may interact with medications, have potential side effects, and require proper dosage and administration.

Betulin, a natural compound from birch trees, shows potential medicinal properties like anticancer, anti-inflammatory, and antiviral effects. Zebrafish embryos, with their transparency and ease of blood vessel visualization, serve as a valuable model for studying anti-angiogenesis, including mechanisms of action and off-target effects, aiding in the evaluation of anti-angiogenic therapies (John *et al*., 2021).

2.5 Honokiol and berberine

They are natural compounds with diverse medicinal properties. Honokiol from Magnolia species, and berberine, from various plants, have been studied for anti-inflammatory, antioxidant, anticancer effects (Shi *et al*., 2020).

2.5.1 Honokiol as anticancer agent

It is a bioactive compound isolated from the stem, bark and seed cones of different Magnolia species, such as *Magnolia officinalis* and *Magnolia grandiflora*. It has been used in traditional Chinese and Japanese medicine for centuries. Honokiol has been studied for its potential anti-inflammatory, antioxidant, anti-anxiety, and anticancer properties.

It has also been shown to have neuroprotective effects and may have potential therapeutic applications in neurodegenerative diseases such as Alzheimer's disease. Honokiol has been investigated for its potential use in various cancer types, including breast, lung, liver, prostate, and colorectal cancers, due to its ability to inhibit cancer cell growth, induce apoptosis (programmed cell death), and suppress tumor angiogenesis. The pharmacoinformatic approach for *in silico* study provides to decode the potential of novel honokiol derivatives as therapeutic agents against various types of breast cancer.

2.5.2 *Berberis, Hydrastis* and *Coptis*

Berberine is a natural alkaloid compound found in the roots, stems, and bark of several plant species, including *Berberis, Hydrastis* and *Coptis*. It has been used in traditional Chinese, Ayurvedic, and Native American medicine for its potential medicinal properties. Berberine has been studied for its potential anti-inflammatory, antioxidant, antidiabetic, and anticancer properties. Having potential benefits in managing diabetes, it may help regulate blood sugar levels and improve insulin sensitivity.

Berberine has also been investigated for its potential phytochemical to have anticancer properties, as it has been shown to inhibit growth of cancer cells, accentuate apoptosis thereby invade tumor and metastasis in various cancer types, including breast, prostate, liver, and colorectal cancers. Whereas, berberine has been reported to inhibit cancer cell growth by various means and mechanisms, including induction of apoptosis, inhibition of cell cycle progression, suppression of tumor angiogenesis, and inhibition of metastasis.

It has also been reported to regulate or harmonize multiple signaling routes involved in cancer cell proliferation and survival, such as AMPK, mTOR, and NF-κB pathways. Honokiol has been measured to inhibit cancer cell growth through various mechanisms, including induction of apoptosis, inhibition of cell cycle progression, suppression of tumor angiogenesis, and inhibition of metastasis. It has also been shown to modulate multiple signaling cascade associated with in cancer cell proliferation and survival, such as NF-κB, MAPK and PI3K/Akt routes.

2.6 Indirubin

Indirubin is a natural compound that is found in various plant species, including but not limited to *Isatis tinctoria* (woad), *Polygonum tinctorium* (Japanese indigo), and *Strobilanthes cusia* (Dyer's knotweed). These plants are known for their traditional medicinal uses and have been used in herbal remedies in different cultures for centuries. Indirubin has been extracted from these plants and studied for its potential medicinal properties, including its anticancer, anti-inflammatory, and neuroprotective effects. Further research is ongoing to explore the pharmacological properties and therapeutic potential of indirubin from different plant sources (Wang *et al*., 2020).

2.7 Laminarin

Ovarian cancer's late-stage diagnosis and high mortality necessitate new treatments. Laminarin, a beta-1,3-glucan isolated from brown algae, exhibits anticancer effects by inhibiting ovarian cancer (OC) cell growth, inducing apoptosis, and regulating signaling pathways, presenting it as a potential novel suppressor for ovarian cancer (Bae *et al*., 2020).

2.8 Resveratrol

A natural compound found in plants like red grapes and peanuts, is studied for its potential medicinal properties including antioxidant, anti-inflammatory, anticancer, and cardioprotective effects (Eo and Kim, 2019).

2.9 Curcumin (CUR)

It had been reported to have similar effects as control when treated with alcohol confirming its role in hepatic steatosis using zebrafish model. Gynecological cancers present formidable challenges with high mortality, side effects, and drug resistance. Curcuma oil, derived from *Curcuma longa* and containing curcumin, emerges as a promising natural therapeutic agent. This study systematically reviews evidence supporting curcumin's efficacy in ovarian, cervical, and endometrial cancers. Results indicate its ability to inhibit cell growth, tumor formation, enhance chemotherapy effectiveness, and improve patients' quality of life. Despite promising preclinical findings, further research is needed to enhance bioavailability and conduct advanced experiments (Zhang *et al*., 2023).

Table 1: Phytochemicals shown anticancerous activity using zebrafish as experimental model

S.No.	Compound	Plant source	Activity	References
1.	Kimcuongin, Murralongin, Murrayoneand Murracarpin,	*Glycosmis ovoidea* Pierre	Breast cancer lines	Blanco Carcache *et al*., 2022
2.	Diindolylmethane DIM 1&4	Cruciferous vegetables	Breast cancer cells	Shilpa *et al*., 2022
3.	Vincristine (VCR)	*Vinca rosea*	Chemotherapy	Sun *et al*., 2022
4.	*G. montanum* extract (GME)	*Gnetum montanum* Markgr	Colon cancer cells SW480	Pan *et al*., 2022
5.	Polyphyllin VII (PP7)	*P. polyphylla*	Antiangiogenic and antimetastatic in hepatocellular carcinoma	Zhang *et al*., 2021
6.	Water and ethanol extract (CMSE)	*Cochinchina momordica* seeds (CMS)	Cardiotoxicity	Du *et al*., 2021
7.	Betulin	*Betula* spp.	Anticancer	John *et al*., 2021
8.	Honokiol	*Magnolia* species	Anti-inflammatory, antioxidant, anticancer effects	Shi *et al*., 2020
9.	Indirubin	*Isatis tinctoria, Polygonum tinctorium*	Anti-inflammatory, antioxidant, anticancer effects	Wang *et al*., 2020
10.	Laminarin	Brown algae	Ovarian cancer	Bae *et al*., 2020
11.	Resveratrol	Red grapes and peanuts	Anti-inflammatory, antioxidant, anticancer effects	Eo and Kim, 2019
12.	Curcumin	*Curcuma longa*	Anti-inflammatory, antioxidant, anticancer effects	Zhang *et al*., 2023
13.	Quercetin	*Hypericum attenuatum*	Anti-inflammatory, antioxidant, anticancer effects	Lin *et al.,* 2012

2.10 Quercetin

This flavonoid, isolated from *Hypericum attenuatum*, has been recently reported that the Keap1, a cytoplasmic protein involved in oxidative stress, Nrf2 and Antioxidant Response Element (ARE) system defends against oxidative stress, a major factor in aging and diseases. Nrf2 activation, a prospective therapeutic approach, involves disrupting protein-protein interaction. In this study, compounds were found to have significant effect as anticancer, antioxidant, and anti-inflammatory agents and were identified through *in silico* molecular docking in zebrafish, suggesting their potential as safer Nrf2 activators for various diseases (Raghunath *et al*., 2019) and its role in the inhibition of angiogenesis was studied *in vivo* on transgenic zebrafish (Lin *et a*., 2012).

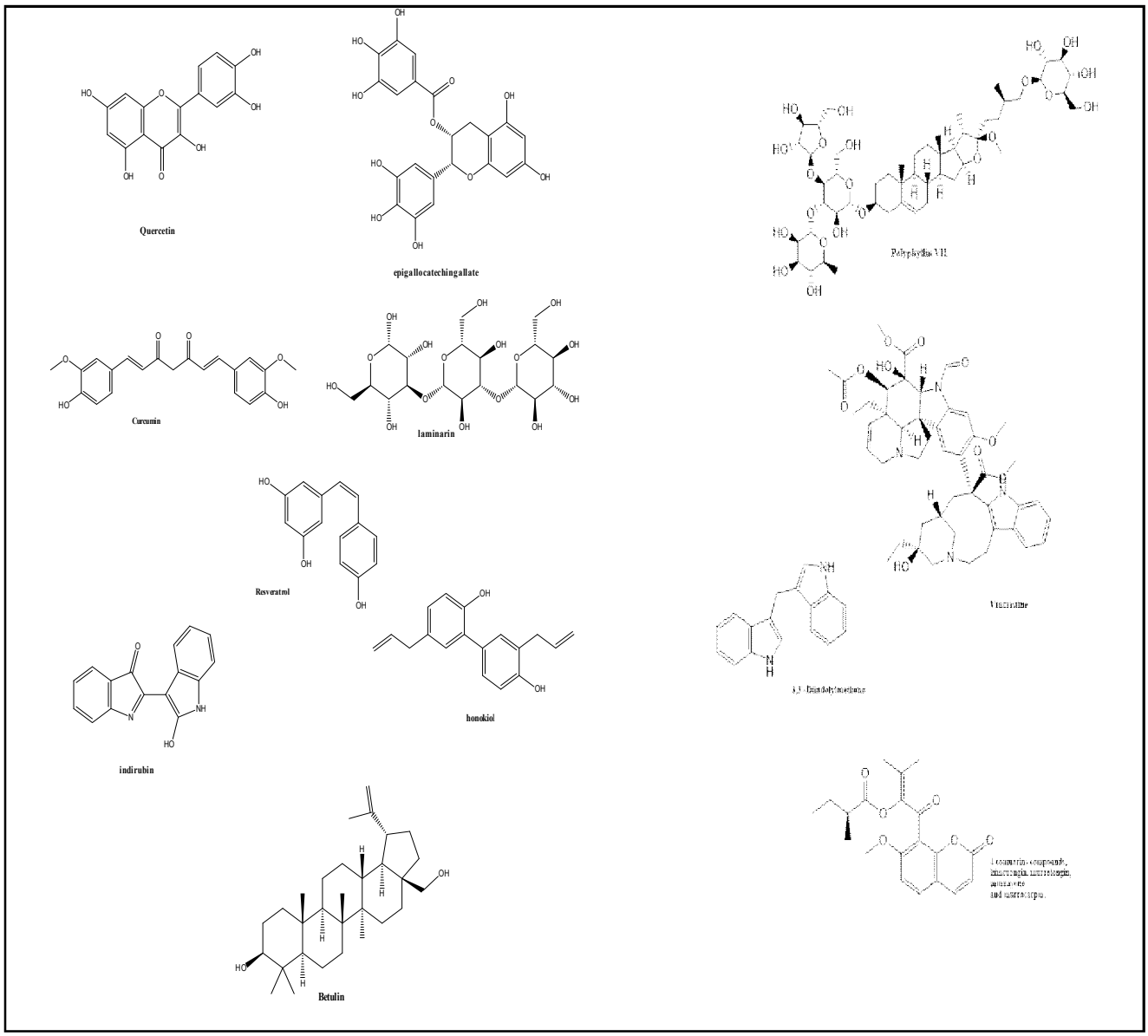

Figure 2: Chemical structure of certain anticancerous secondary metabolites.

3. Conclusion

Zebrafish xenograft models provide a valuable tool for preclinical screening of phytomedicines, the translational potential of these findings to human cancer patients is still unclear. Future research could focus on investigating the efficacy and safety of phytomedicines in zebrafish models that more closely mimic human cancer conditions, such as using patient-derived xenografts or genetically modified zebrafish models with humanized organs. There is a need for standardization and optimization of experimental protocols in zebrafish xenograft models, including dosing, timing, and route of administration of phytomedicines. Identification of novel plant based medicines have been studied through zebrafish xenograft models, there is still a vast repertoire of plant-derived compounds that remain unexplored. Future research could focus on identifying and testing novel phytomedicines in zebrafish xenograft models to expand the knowledge on their anticancer potential and uncover new leads for drug development. Ultimately, the clinical translation and validation of findings from zebrafish xenograft models to human cancer patients remain a critical gap. Clinical trials to validate the safety and efficacy of phytomedicines identified in zebrafish models in human cancer patients, and to assess their potential as complementary or alternative therapeutic options for cancer treatment. Overall, while zebrafish xenograft models have shown great promise in elucidating the anticancer potential of phytomedicines. There are still several future gaps that could be addressed through further research to enhance our understanding of these natural compounds and their potential for cancer therapy.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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