



# Advances in the Global Distribution, Chemical Constituents, and Pharmacology of *Hippocampus*, a Traditional Marine Chinese Medicine

Xinhai Cui<sup>1,2</sup>, Xiangfeng Zhao<sup>3</sup>, Jintong Li<sup>2,3</sup>, Ziwei Li<sup>2</sup>, Xia Ren<sup>2</sup>, Yangang Zhao<sup>2</sup>, Xianjun Fu<sup>1,2,4\*</sup> and Xiuxue Li<sup>2\*</sup>

<sup>1</sup> College of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan, China, <sup>2</sup> Marine Traditional Chinese Medicine Research Center, Qingdao Academy of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Qingdao, China, <sup>3</sup> Institute of Traditional Chinese Medicine Literature and Culture, Shandong University of Traditional Chinese Medicine, Jinan, China, <sup>4</sup> Shandong Engineering and Technology Research Center of Traditional Chinese Medicine, Jinan, China

## OPEN ACCESS

### Edited by:

Bin Wu,  
Zhejiang University, China

### Reviewed by:

Yueying Li,  
Texas A&M University, United States

Caisheng Wu,  
Xiamen University, China

Qihao Wu,  
Yale University, United States

### \*Correspondence:

Xianjun Fu  
xianxiu@hotmail.com  
Xiuxue Li  
lixixuelixixue@126.com

### Specialty section:

This article was submitted to  
Marine Biotechnology,  
a section of the journal  
Frontiers in Marine Science

**Received:** 13 September 2021

**Accepted:** 30 October 2021

**Published:** 06 December 2021

### Citation:

Cui X, Zhao X, Li J, Li Z, Ren X, Zhao Y, Fu X and Li X (2021) Advances in the Global Distribution, Chemical Constituents, and Pharmacology of *Hippocampus*, a Traditional Marine Chinese Medicine. *Front. Mar. Sci.* 8:774927. doi: 10.3389/fmars.2021.774927

*Hippocampus* is an important traditional marine Chinese medicinal resource that has been used to warm and tonify kidney yang in the clinic for a long time in China. Modern pharmacological studies show that its active ingredients display a wide range of pharmacological activities associated with the kidney, such as anti-inflammation, antioxidation, antitumor, and neuroprotective effects. Herein, we systematically summarize and analyze the research progress on the resource distribution, active ingredients, pharmacological activities, and clinical application of *Hippocampus*. First, the species and worldwide distribution of *Hippocampus* were assessed to clarify the existing resources, and the results showed that 44 species of *Hippocampus* have been found in 159 countries and regions worldwide. Then, based on the analysis of 16 kinds of active ingredients and extraction methods, the relationship between the ingredient extraction and pharmacological activities of *Hippocampus* was revealed. This review may provide a foundation for further research on the potential active ingredients and mechanisms of *Hippocampus*. In addition, the research status of traditional prescriptions containing *Hippocampus* was evaluated. The results implied that research on *Hippocampus* is still in its infancy, and the mechanism and material basis of its efficacy have not been clarified. This paper should provide directions for further studies on *Hippocampus*.

**Keywords:** *Hippocampus*, traditional marine Chinese medicine, resources, active ingredients, pharmacological activities

## INTRODUCTION

The ocean is rich in biodiversity, and many organisms have evolved unique morphological and physiological characteristics to adapt to challenging environments (Conte et al., 2021). Marine organisms further produce marine natural products (MNPs) with chemical diversity and interesting structures, including peptides, terpenoids, steroids, polyphenols, alkaloids, etc., (Sable et al., 2017; Murray et al., 2018; Wu et al., 2018; Carroll et al., 2019; Althagbi et al., 2020; Ermolenko et al., 2020). Currently, thousands of new MNPs are being reported every year (Carroll et al., 2020, 2021). These compounds appeared to be promising therapeutics for the treatment of a variety of human diseases, such as cancers and cardiocerebrovascular diseases (Dayanidhi et al., 2021; Liang et al., 2021). In

addition, studies have shown that the molecular weight and clog *P* of MNPs isolated since 1957 were particularly similar to that of approved drugs (Blunt et al., 2018). MNPs have always been an important source of new medicines (Khalifa et al., 2019). Currently, there are seven FDA-approved medicines and more than 20 medicine candidates from 25,000 NMPS; thus, MNPs have a bright future compared with synthetic molecules (Newman and Cragg, 2016). Marine biodiversity may afford potential resources for new NMPS.

Organisms of the genus *Hippocampus* (Syngnathidae) are widely distributed in marine ecological systems. As a traditional Chinese medicine (TCM), *Hippocampus* was first recorded in a Supplement to Materia Medica (Bencao Shiyi), which has been clinically applied for thousands of years (Chen, 1983). There are five medicinal *Hippocampus* organisms recorded in the Chinese Pharmacopoeia: *Hippocampus kuda* Bleeker, *Hippocampus trimaculatus* Leach, *Hippocampus kelloggi* Jordan et Snyder, *Hippocampus histrix* Kaup, and *Hippocampus japonicus* Kaup (Chinese, 2020). In TCM, the main efficacy of *Hippocampus* is warming and tonifying of the kidney yang. Modern pharmacological studies have reported that *Hippocampus* shows various biological activities, including antifatigue, anti-inflammation, antioxidation, antitumor, and antimicrobial activities (Kumaravel et al., 2012). Peptides, steroids, and fatty acids are the main active ingredients of *Hippocampus*. In addition, nucleosides, phthalates, ketones, etc., have also been isolated and identified (Chen et al., 2015). These results supported the great therapeutic potential of *Hippocampus*.

In this study, the research progress on the resource distribution, active ingredients, pharmacological activities, and prescriptions of *Hippocampus* was systematically analyzed and summarized. According to the literature, websites, and databases, we reviewed 44 *Hippocampus* species and their distributions worldwide, and resources for medicinal *Hippocampus* were emphasized. Sixteen kinds of ingredients of *Hippocampus* were described, and possible *Hippocampus* factors were investigated, such as the species, growth stage, wild or culture, and bait. In addition to traditional efficacy, more attention has been given to modern pharmacological agents, especially neuroprotective agents. Moreover, studies on the relationship among ingredients, extraction methods, and pharmacological activities may help to identify new active ingredients to supplement the compound-constituent groups of *Hippocampus*, which may further provide a scientific basis for explaining its mechanism in treating diseases. In addition, the clinical application of traditional prescriptions containing *Hippocampus* also may provide new ideas for the development and utilization of *Hippocampus* in the future.

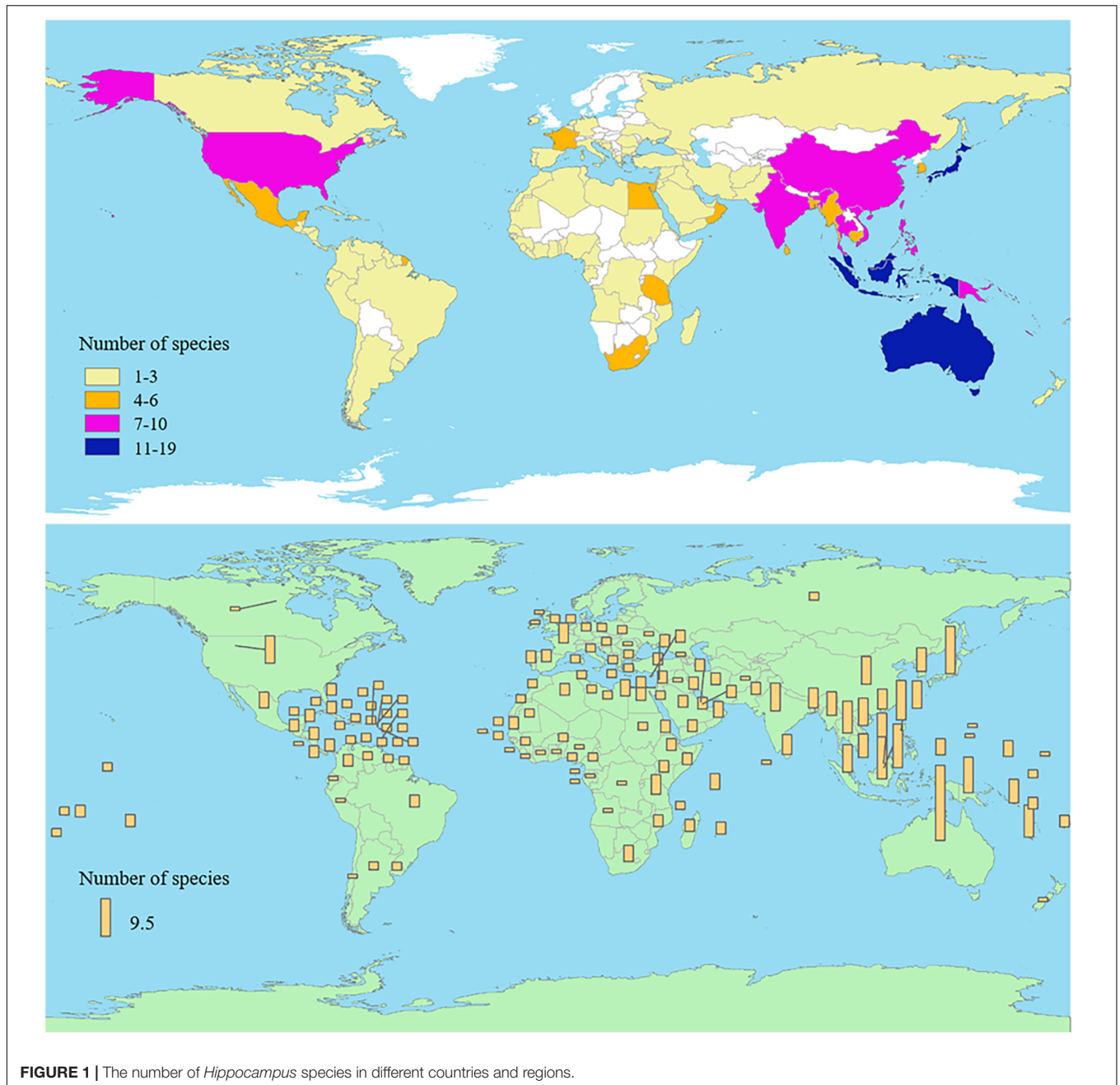
## STATUS OF HIPPOCAMPUS RESOURCES

### *Hippocampus* Distribution and Species Worldwide

At present, 44 species of *Hippocampus* have been found in 159 countries and regions worldwide (Figure 1 and

Supplementary Table 1; CITES, 2021b; FishBase, 2021), with the highest diversity of species observed in Australia (Figure 2A). Oceania has the largest number of species on the continent, followed by Asia and Africa (Figure 2B). *Hippocampus* is primarily found in the Indo-Pacific Ocean and distributed in shallow waters with a depth of less than 30 m in temperate, subtropical, and tropical regions (Foster and Vincent, 2004). On the west coast of peninsular Malaysia, *Hippocampus kelloggi* Jordan et Snyder was collected by trawlers in deepwater below 65 m (Choo and Liew, 2003). The habitat of *Hippocampus* includes seagrass, coral reefs, mangroves, and seaweeds, with coral reefs representing the main habitat constituents of tropical *Hippocampus* (Foster and Vincent, 2004). *Hippocampus coronatus* Temminck and Schlegel and *Hippocampus mohnikei* Bleeker generally inhabit seagrass in the southern coastal waters of Korea. Temperate *Hippocampus* tends to inhabit seagrass beds, which provide food for a variety of flora and fauna (Choi et al., 2012). Among the five medicinal *Hippocampus* in China, only *Hippocampus japonicus* Kaup is mainly distributed in northern China. The other four species were mainly found in the tropical and subtropical regions of China, such as the East China Sea and South China Sea (Lin et al., 2008; Han, 2013).

The ease of identification of a *Hippocampus* specimen varies from species to species. *Hippocampus* specimens were generally identified using preparation tools (ruler, magnifying glass, calculator, etc.) by identifying characteristics, using measured and recorded data about an unidentified specimen, and using standards (Supplementary Tables 1–6 from a guide of *Hippocampus* identification) to determine possible species and identify specimens. The data mainly included the head length (HL) and snout length (SnL), length of the snout in relation to the length of the head (HL/SnL), number of tail rings (TaR), trunk rings (TrR), etc. (Lourie et al., 2004). The characteristics and morphological identification based on distinguishing characteristics are appropriate when performed by investigators who are experienced with *Hippocampus* identification. However, some *Hippocampus* specimens are particularly similar, and new species may be encountered; thus, deoxyribonucleic acid (DNA) barcoding technology may need to be applied for *Hippocampus* identification. The species phylogenetic tree is constructed based on the alignment results of the complete mitochondrial genome or mitochondrial cytochrome b gene from different species (Casey et al., 2004; Ge et al., 2018). The phylogenetic tree with the Cytochrome Oxidase I (COI) gene as a primer showed that different species of *Hippocampus* were in different branches with reasonable distances between them, and the accuracy was higher than that of other mitochondrial genes (Hou et al., 2016). Liu et al. (2018) used a multiplex polymerase chain reaction (PCR) method to simultaneously identify five medicinal *Hippocampus* species by judging the absence and size of DNA bands on the gel map in a single assay. DNA barcoding provides an accurate technique for the identification of *Hippocampus* specimens. A rapid detection kit should be developed in the future to reduce the complicated and time-consuming aspects of this technology. Fast and effective identification methods can be used to protect endangered *Hippocampus*

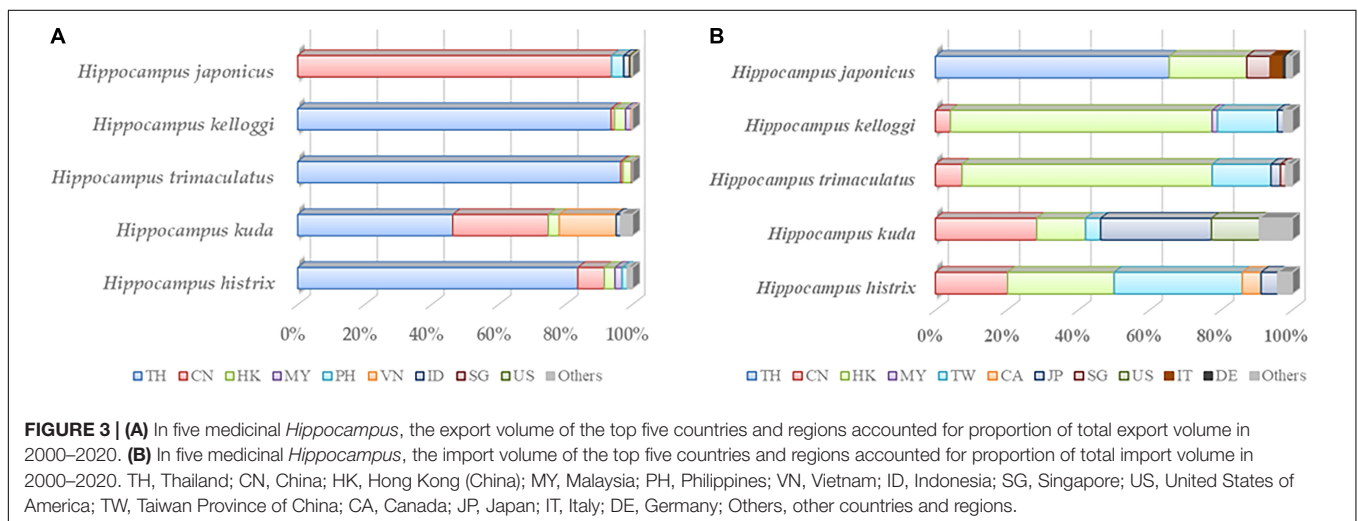
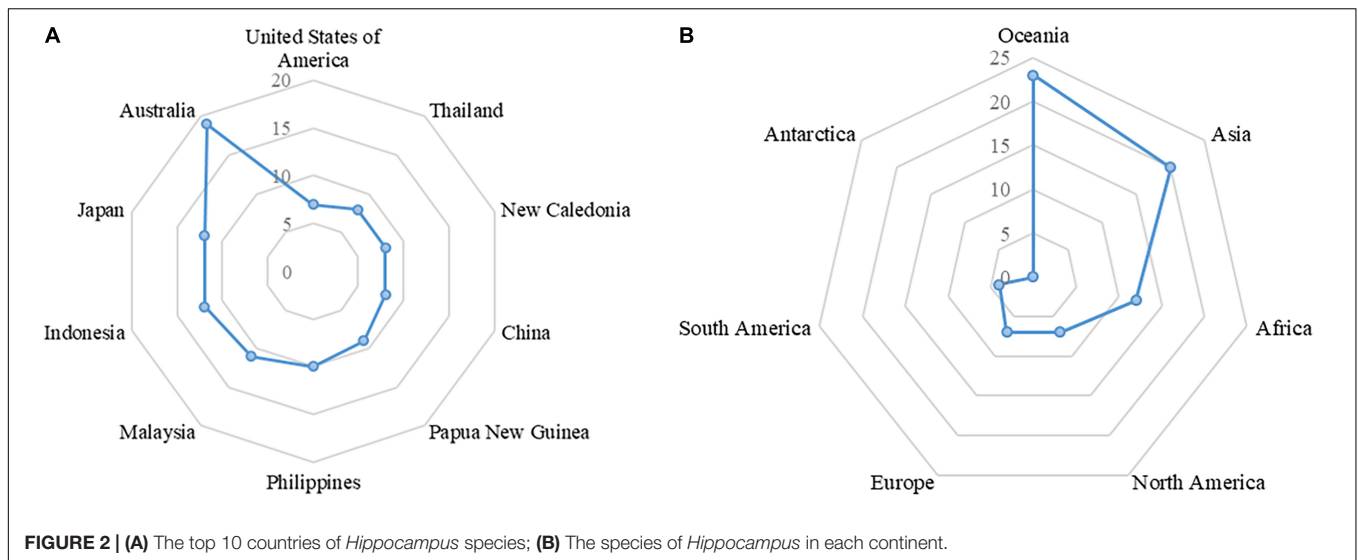


species and monitor the *Hippocampus* trade (Luo et al., 2013). In TCM, metabolomics technology has been used to study differences in metabolites for various species and find metabolic markers, and such work has provided references for the identification of multisource medicinal materials (Fan, 2012). This may provide new ideas for the identification of *Hippocampus*.

### Global Trade Volume of *Hippocampus*

Recent studies have revealed that the number of *Hippocampus* individuals has continuously declined over the past 20 years. The trade demand for *Hippocampus* has increased significantly

because of its value for medicine, aquarium displays, and collectibles (Vincent et al., 2011). The export volume of medicinal *Hippocampus* is concentrated in Southeast Asian countries, with Thailand being the largest exporter (Figure 3A and Supplementary Table 2; CITES, 2021a). The export volume of *Hippocampus kuda* Bleeker, *Hippocampus trimaculatus* Leach, and *Hippocampus kelloggi* Jordan et Snyder accounted for 88% of the global export of dried *Hippocampus* (Loh et al., 2016). Regarding importing countries, China and its affiliated Hong Kong Special Administrative Region and Taiwan were the main importers of dried *Hippocampus* due to the influence of TCM (Figure 3B and Supplementary Table 3; CITES, 2021a).

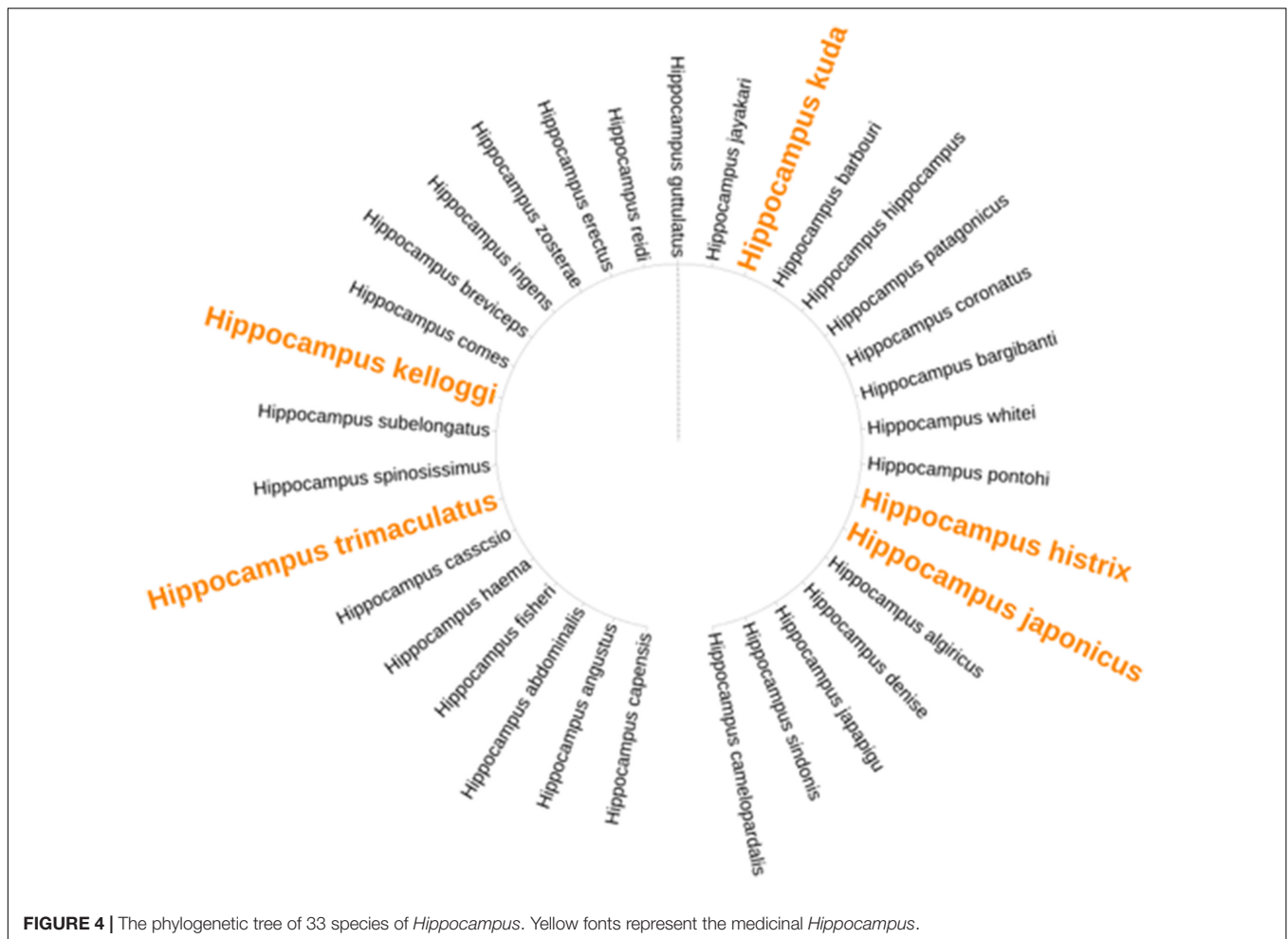


Exports of other *Hippocampus* species were mainly concentrated in Australia and some countries in the Americas (Convention on International Trade in Endangered Species of Wild Fauna and Flora, 2020). A large global trade volume is mainly associated with wild *Hippocampus* caught by trawl by catch. Huge fishing pressure has made *Hippocampus* populations unsustainable (Vincent et al., 2011). Moreover, the *Hippocampus* population is also threatened by habitat reduction and environmental pollution (Rosa et al., 2005). The International Union for Conservation of Nature (IUCN) listed *Hippocampus* on the red list, with five medicinal *Hippocampus* species listed as vulnerable species (IUCN, 2021). In addition, breeding techniques for *Hippocampus* similar to wild medicinal species are required. With the gradual solution to problems associated with rearing and bacterial infection in the breed, an increasing number of countries have tried to breed *Hippocampus*, such as Australia, Brazil, China, Sri Lanka, the United Kingdom, the United States, and Vietnam (Koldewey and Martin-Smith, 2010). The amount of wild *Hippocampus* cannot meet market needs, and artificial breeding

is an important way to meet market demand and protect wild *Hippocampus* resources. In addition, we may seek alternative species. The phylogenetic tree of *Hippocampus* listed eight species of *Hippocampus* that were similar to the medicinal *Hippocampus* (Figure 4 and Supplementary Table 4; NCBI, 2020). As a next step, we may identify *Hippocampus* species with a large trade volume to study their ingredients and pharmacological activities and strive to find supplemental medical *Hippocampus* species.

## TRADITIONAL MEDICINAL USE

The traditional usage of *Hippocampus* was recorded in the Chinese Materia Medica (Zhonghua Bencao), and it indicates that *Hippocampus* was extracted with water to make a decoction, in which the dosage of *Hippocampus* was 3–9 g. The decoction was taken for its ability to warm and tonify kidney yang and reduce swelling. In addition, *Hippocampus* was further processed into a powder for external use to treat furunculosis



(Song, 1999). The Chinese Pharmacopoeia also included this traditional extraction method and dosage (Chinese, 2020). At present, relevant reports have not indicated that *Hippocampus* is harmful to the human body. However, few studies have performed safety assessments of *Hippocampus* (Zhu, 2004).

In the Ming dynasty, “warming kidney, tonifying yang, eliminating lumps, and curing carbuncle” were the efficacies of *Hippocampus* recorded in The Compendium of Materia Medica (Bencao Gangmu; Li, 1982). The traditional efficacy of warming and tonifying kidney yang is not only reflected in its ability to improve reproductive function but also in its ability to regulate organs related to the kidney (Zhao et al., 2021). Kidney and yang have unique meanings in terms of TCM theory. The kidney may be understood from two aspects. First, in addition to referring to the organ in Western medicine, the kidney is also associated with a series of internal organs, namely, the kidney, bladder, bone, brain, ear, etc. Second, the kidney has effects on reproduction and operates in conjunction with other organs to complete the metabolism of the body (Wang et al., 2012). Yin and yang theory has been used to explain diseases that occur in the body (Ji and Yu, 2020). Yang has effects of warming, exciting, pushing, dispersing, and lifting in the human body (Zhang,

2021). Therefore, kidney yang deficiency causes the human body to have symptoms that include sperm deficiency, soreness and weakness of the waist and knees, dizziness, and tinnitus. These symptoms correspond to kidney disease and reproductive, immune, nervous, and other diseases in modern medicine (Qi et al., 2012; Reheman et al., 2019; Tian et al., 2021). In modern studies, the pharmacological activities of *Hippocampus*, such as its antihypertensive, antifatigue, and neuroprotection activities, have been reported and are consistent with traditional theory (Chen et al., 2015).

Traditional prescriptions containing *Hippocampus* were commonly made into pills, capsules, plasters, and wines for medicinal usage. The main clinical efficacies of the pill and capsule forms were warming and tonifying kidney yang, such as *Haimabushen pill*, *Haimaduobian pill*, and *Hippocampus capsule* (Supplementary Table 5). In TCM, pills have delayed release in the gastrointestinal tract to achieve stable and long-lasting efficacy and reduce toxicity and adverse reactions (Zhang et al., 2017). Pills containing *Hippocampus* might be suitable for chronic diseases of kidney deficiency. *Haimabushen pill* enriches Yin and tonifies the kidney and improves fatigue, soreness, and weakness in the waist and knees. Zhi et al. (2019a) found

that *Haimabushen pill* improved the testosterone content level and had androgen-like effects in castrated or young mice by regulating the hypothalamus-pituitary-gonad axis. Additionally, *Haima Zhuangyang soft capsule* significantly shortened the incubation period for the erection of the penis and increased the prostate and semen sac coefficients of kidney yang-deficient mice (Lu et al., 2001). Capsules with controllable quality have been made by modern advanced preparation technology. Compared with traditional pills, the reproductive organ index of ovariectomized mice treated with low-dose *Haima* capsules (0.045 g/kg) was significantly higher than that of ovariectomized mice treated with middle-dose *Haimabushen pill* (0.28 g/kg) (Mei et al., 2005). In addition to the traditional effect of warming and tonifying kidney yang, *Hippocampus* in the plaster form was often compatible with medicines for dispelling wind and drying dampness. In particular, plasters are preferred formulations to treat local symptoms and quickly relieve the pain to improve the treatment comfort of the patient (Jia et al., 2003). For example, *Shexiang Haima Zhuifeng Gao* has been used to treat wind and dampness impediments and lumbago and leg pain (Liu and Lin, 2006). Medicinal wine may exert the biological effects identified in the trace elements in *Hippocampus* better than a decoction. Research has shown that *seahorse* medicinal liquor contains amino acids and mineral elements, has antifatigue effects, and improves immunity (Huang et al., 1997).

Traditional prescriptions containing *Hippocampus* are widely used in the clinic, and their pharmacological effects have gradually attracted more attention. Traditional prescriptions containing *Hippocampus* have been further explored using animal experiments to identify other effects, such as in learning, memory, and tumors. In a mouse model of memory impairment induced by scopolamine and sodium nitrite, *Haimabushen pill* effectively reduced the impairment of learning and memory ability of mice through its acetylcholinesterase inhibitory ability (Zhi et al., 2019b). *Haimashengshui pill* was also found to inhibit S180 tumor-bearing mice and promote tumor cell apoptosis (Han, 2002). The formulation of traditional prescriptions containing *Hippocampus* has become an urgent issue. New formulations are another main method of expanding the clinical application of traditional prescriptions containing *Hippocampus*. For example, nanotechnology has the ability to cross various biological barriers (De Jong and Borm, 2008). The nanoparticle formulation of *Haimabushen pill* may play a greater role in crossing the blood-brain barrier and entering the brain. In addition, the *Hippocampus* species used in traditional prescriptions are not fully understood, which may have a certain impact on clinical efficacy.

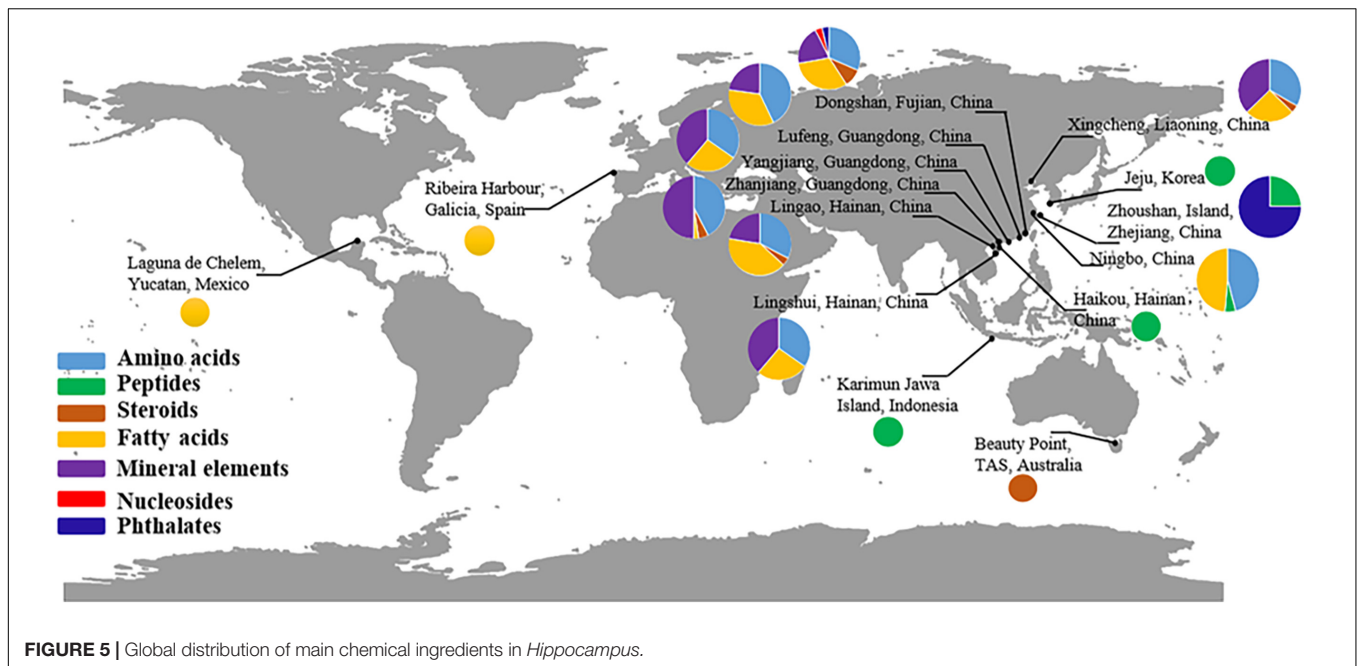
## ACTIVE INGREDIENTS OF HIPPOCAMPUS

Many *Hippocampus* species in China have been chemically investigated. Peptides were identified from *Hippocampus* located in Indonesia, South Korea, and China. Steroids were mainly obtained from species in China and Australia. Although the main source of fatty acids is China, these ingredients were

also found in Mexico and Spain (Figure 5). To date, a total of 329 ingredients in 16 categories have been identified, including acids, steroids, amino acids, mineral elements, nucleosides, phospholipids, phthalates, peptides, glycoproteins, volatile ingredients (hydrocarbons, alcohols, aldehydes, ketones, esters, amines), and others (Supplementary Figure 1 and Supplementary Table 6).

## Amino Acids and Peptides

Amino acids play vital roles in regulating metabolism, growth, and development. Among the 20 types of amino acids constituting human proteins, essential amino acids cannot be synthesized by humans (Mitsuhashi, 2014). Twenty-three types of amino acids have been found in *Hippocampus* (Supplementary Table 6). Lin et al. (2008) found that the total amount of amino acids was as high as 50–60% (based on a comparison of their retention times with those of amino acid standards) in six species of *Hippocampus*, and eight types of essential amino acids accounted for 17–20% of the total amino acid content, which indicated that the *Hippocampus* is a high-quality protein source. The content of glycine was the highest (83.98 mg/g) among the 15 types of amino acids in *Hippocampus kuda* Bleeker and *Hippocampus trimaculatus* Leach (Sun et al., 2020). In addition, *Hippocampus* is also rich in glutamic acid, aspartic acid, alanine, and arginine (Lin et al., 2009). Jia and Yao (1990) reported 22 types of amino acids in *Hippocampus kuda* Bleeker and *Hippocampus japonicus* Kaup, including taurine, a special amino acid with high medicinal value. The type and the content of amino acids in *Hippocampus erectus* Perry were similar to those in medicinal *Hippocampus*, and even the content of essential amino acids in *Hippocampus erectus* Perry was higher than that in medicinal *Hippocampus* (Yan Z. Z., 2019). Peptides are important marine natural products that widely exist in vast marine organisms. Most peptides extracted from *Hippocampus* have various pharmacological activities and can be classified as angiotensin-converting enzyme (ACE) inhibitory peptides, anti-inflammatory peptides, neuroprotective peptides, and antimicrobial peptides. Shi et al. (2020) isolated ACE inhibitory peptides with the amino acid sequence Pro-Ala-Gly-Pro-Arg-Gly-Pro-Ala from the enzymatic hydrolysate of *Hippocampus trimaculatus* Leach. Circular dichroism analysis showed that the secondary structure was mainly composed of a random coil. Three ACE inhibitory peptides were also isolated from cultured *Hippocampus abdominalis* Lesson. The amino acid sequence of Cys-ASN-Val-Pro-Leu-Ser-Pro was more stable in molecular docking with ACE, which may become the preferred candidate for antihypertensive peptides (Je et al., 2020). Ryu et al. (2010) isolated an anti-inflammatory peptide composed of 15 types of amino acids (including five aspartic acids) from *Hippocampus*. Recently, a novel neuroprotective peptide (HTP-1) rich in hydrophobic amino acids and a molecular weight of less than 1 kDa have been isolated from *Hippocampus trimaculatus* Leach. The N-terminal amino acid sequence of HTP-1 is similar to that of insulin-like growth factor-1 (IGF-1) and may have neuroprotective effects (Pangestuti et al., 2013). Sun et al. (2012) identified an antimicrobial peptide (HKPLP) from the brooding pouch of male *Hippocampus Kuda* Bleeker.



Bioinformatics analyses of available genomic and transcriptomic data allowed us to find the distribution of antimicrobial peptides closely related to the male brooding pouch (Chen et al., 2019). In conclusion, the activity of low molecular weight peptides is affected by the composition and sequence of amino acids. In terms of favorable structure-activity relationships for high ACE-inhibitory activity, aromatic amino acids at the C-terminus end and hydrophobic amino acids at the N-terminus end were most potent (Xu, 2015). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging results indicated that low molecular weight (43–278 kDa) peptides from *Hippocampus erectus* Perry showed higher activity than peptides over 278 kDa (Chen et al., 2018). Elucidating the structure-activity relationships of these peptides may help develop peptides with enhanced bioactivity.

## Steroids

Steroids are among the major ingredients of *Hippocampus*, and 38 types of steroids have been isolated and identified to date (Supplementary Table 6). Gas chromatography–mass spectrometry (GC–MS) analysis identified 16 types of steroids from seven species of *Hippocampus*. Cholesterol and cholest-4-en-3-one were the major ingredients in the total steroids (Chen, 2015). Sun et al. (2020) reported that the average content of steroids in *Hippocampus kuda* Bleeker was 6.16 mg/g, and cholesterol accounted for 0.55 mg/g. The average ratios of cholest-4-en-3-one and cholest-3,6-dione in *Hippocampus trimaculatus* Leach, *Hippocampus histrix* Kaup, and *Hippocampus japonicus* Kaup were 0.0301 and 0.0096%, respectively (Zhang et al., 1998). In addition, Wu et al. (2017b) extracted 3 $\beta$ -hydroxycholest-5-en-7-one and cholest-5-en-3 $\beta$ ,7 $\beta$ (7 $\alpha$ )-diol from *Hippocampus trimaculatus* Leach. Cholesterol stearate and cholest-5-en-3 $\beta$ ,7 $\alpha$ -diol were also extracted from *Hippocampus histrix* Kaup (Wang et al., 1998). Yang et al. (2014)

supplemented steroids of *Hippocampus erinaceus* Gunther, and six ingredients were identified: cholesterol, cholest-4-en-3-one, (22E,24R)-3 $\beta$ ,5 $\alpha$ ,9 $\alpha$ -trihydroxyergosta-7,22-dien-6-one, 24-methyl-cholesta-7,22-diene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol, 3 $\beta$ -hydroxy-7-methoxy-cholesta-5-en, and 3 $\beta$ -hydroxycholest-5-en-7-one. In addition, brassicasterol, which has antitumor activity, has been recently obtained from *Hippocampus abdominalis* Lesson (Xu et al., 2020). Although a large number of steroids have been isolated, marker ingredients have not been identified from *Hippocampus* quality evaluation standards. It is urgent to establish a stable and controllable method to scientifically evaluate and control the quality of *Hippocampus*.

## Fatty Acids

*Hippocampus* contains 82 types of fatty acids, including 35 types of saturated fatty acids and 47 types of unsaturated fatty acids (Supplementary Table 6). Lin et al. (2008) found that there were 12 types of fatty acids distributed widely among six species of *Hippocampus*, and hexadecanoic acid, octadecanoic acid, oleic acid, 5,8,11,14,17-eicosapentaenoic acid (EPA), and 4,7,10,13,16,19-docosahexaenoic acid (DHA) were the main ingredients. The ratios of unsaturated fatty acids and EPA and DHA to total fatty acids were 48–59% and 14–35%, respectively. Studies have shown that EPA and DHA not only reduce the risk of cardiovascular diseases, such as atherosclerosis, arrhythmia, and hyperlipidemia (Watanabe and Tatsuno, 2017), but also improve sperm quality and sperm motility when used as food supplements (Hosseini et al., 2019). Chen (2015) identified 26 types of fatty acids from seven species of *Hippocampus*; among benzenepropanoic acids, 3,5-bis(1,1-dimethylethyl)-4-hydroxy was found for the first time. Tetradecanoic acid, hexadecanoic acid, octadecanoic acid, 9(Z)-octadecenoic acid, 5,8,11,14-eicosatetraenoic acid, and DHA were the main fatty acids in

the five medicinal *Hippocampus* species. Zhang and Wang (1996) isolated 42 fatty acid peaks of *Hippocampus japonicus* Kaup by a rapid fat extraction method, among which EPA and DHA accounted for 14.1%. Amazingly, there were many other types of fatty acids in *Hippocampus erectus* Perry, such as heptadecanoic acid, tetracosenoic acid, 11-octadecenoic acid, 15-tetracosenic acid, and 7,10,13,16-docosatetraenoic acid (Nicolás et al., 2014).

## Mineral Elements

Mineral elements play an important role in growth because they help to improve immunity and anti-fatigue (Sherman, 1992). Twenty-four types of mineral elements were found in five *Hippocampus* medicinal species. The contents of P, K, Na, and Mg were all above 100 mg/g, while the contents of mineral elements, including Al, Fe, Mn, Sr, Zn, etc., were at the level of a microgram. Lin et al. (2009) reported that the contents of Mn and Zn in *Hippocampus trimaculatus* Leach were 21.73 and 31.44 µg/g, respectively. The total contents of mineral elements in *Hippocampus Kuda* Bleeker were 84,873 µg/g, which also contained Cr, Co, Se, and Pb (Jia and Yao, 1990). The effect of *Hippocampus* on warming and tonifying kidney yang also benefited from mineral elements (Zhang et al., 1997). Clinical studies have shown that higher levels of mineral elements may reduce the likelihood of declines in cognitive function over 5 years (Gerardo et al., 2020). In the brain, various nervous activities require the participation of essential mineral elements, such as Fe and Zn. Some neurodegenerative diseases are related to an imbalance in the homeostasis of these cations (Bohic et al., 2011), which indicates that *Hippocampus* may have a therapeutic effect on neurodegenerative diseases.

## Phospholipids and Nucleosides

Studies on *Hippocampus* mainly focus on amino acids, mineral elements, and fatty acids, while relatively few reports have focused on the analysis technologies and ingredient activities, such as that of phospholipids and nucleosides. Phospholipids are the main component of biomembranes. A study of the contents and types of phospholipids in five medicinal *Hippocampus* was carried out using molybdenum blue colorimetry and thin layer chromatography (TLC) scanning. The results showed that the total contents ranged from 3.28 to 7.82 mg/g, including LPC, SM, PC, and PE (Xu et al., 1994). Shen et al. (2016) identified 50 phospholipid molecules by hydrophilic interaction chromatography-quadrupole time of flight-mass spectrometry (HILIC-QTOF/MS). In addition to PC and PE, there were 12 PIs and 9 PSs. PC and PE accounted for most of the total phospholipids at 1.91–4.89 mg/g and 1.52–2.64 mg/g, respectively. Marine nucleosides were found to have antiviral, anticancer, and hypertensive activities (Huang et al., 2014). Zheng et al. (2012) reported that the contents of hypoxanthine extracted from *Hippocampus Kuda* Bleeker and *Hippocampus japonicus* Kaup were 0.73 and 0.52 mg/g, respectively. The average contents of nucleosides and nucleotides in the *Hippocampus japonicus* Kaup were 1.974 and 0.79 mg/g, respectively. However, uridine, cytosine nucleotides, and guanine nucleotides were not found in *Hippocampus japonicus* Kaup (Wei et al., 2015). Yan Z. Z. (2019)

found six nucleosides in *Hippocampus erectus* Perry, more types and contents than those in *Hippocampus japonicus* Kaup.

## Other Ingredients

Apart from the abovementioned ingredients, other ingredients have also been reported. Six phthalate derivatives have been isolated from ethanol extracts of *Hippocampus Kuda* Bleeker and *Hippocampus trimaculatus* Leach, including dibutyl phthalate, bis(2-ethylhexyl)phthalate (Wu et al., 2017b), bis(2-ethylheptyl)phthalate, bis(2-ethyl-dodecyl) phthalate, 2-ethyldecyl 2-ethylundecyl phthalate, and 2,12-diethyl-11-methylhexadecyl 2-ethyl-11-methylhexadecylphthalate (Li et al., 2008). As a part of ongoing research on extraction and isolation, paeonol was incidentally isolated from *Hippocampus Kuda* Bleeker (Himaya et al., 2012). The flavor profile was identified based on a set of volatile ingredients that rendered the characteristic taste and smell of *Hippocampus*. Based on headspace solid-phase microextraction combined with GC-MS, 84 potential volatile ingredients were identified, including acids, hydrocarbons, alcohols, aldehydes, ketones, esters, and amines. The main flavor ingredients with contributions over 70% were (E,E)-3,5-octadiene-2-one, trimethylamine, hexanoic acid, 2-nonone, etc., (Si et al., 2018). Glycoproteins of *Hippocampus* have rarely been reported because of their relatively complex composition. Box-Behnken and ultrasonic extraction technology were applied to separate glycoproteins HG-11 and HG-21 (Su and Xu, 2015a). Glycoproteins included in marine organisms are the main active ingredients and deserve additional attention in future research. In addition, the nuclear magnetic resonance (NMR) and GC-MS metabonomics methods (Zhao, 2018) and the Traditional Chinese Medicines Integrated Database (TCMID) (TCMID, 2020) have provided a large amount of information regarding the chemical ingredients of *Hippocampus*.

## INFLUENCING FACTORS OF DIFFERENT INGREDIENT CONSTITUENTS

The ingredient constituents of *Hippocampus* are affected by multiple factors. Different species of *Hippocampus* have significant differences in ingredient constituents (Lin et al., 2008). Studies on the influence of sex and wild and cultured growth stages on *Hippocampus* ingredients provided a basis for medicinal research and breeding technology (Su and Xu, 2015b). Based on the connection of factors and constituents, *Hippocampus* species may be chosen according to the needs of the research. We may adjust the proportion of baits in breeding according to the difference in ingredient constituents. In contrast, such studies have also provided references for species identification. The ingredient constituents in the samples may also provide information on the *Hippocampus* species, genders, growth stage, etc.

## Species and Genders

Lin et al. (2008) compared different ingredient constituents of six species of *Hippocampus*. The crude protein content was the most abundant in *Hippocampus kelloggi* Jordan et Snyder, while,



in *Hippocampus histrix* Kaup, was the lowest. Notably, more than half of the fatty acids in *Hippocampus comes* Cantor were unsaturated fatty acids, resulting in approximately 1.25–1.8 times as much EPA and DHA in *Hippocampus comes* Cantor compared to those in medicinal *Hippocampus* species. Mg was the most abundant mineral element in *Hippocampus trimaculatus* Leach and *Hippocampus histrix* Kaup, and the contents were 1125.44 and 1076.57  $\mu\text{g/g}$ , respectively. Chen (2015) compared steroids in seven species of *Hippocampus*. The most abundant steroids content was found in *Hippocampus japonicus* Kaup, and the least abundant steroid content was found in *Hippocampus histrix* Kaup and *Hippocampus comes* Cantor. Xu et al. (1994) reported the total phospholipid contents of five medicinal *Hippocampus* species. The highest phospholipids content was observed in *Hippocampus japonicus* Kaup (7.82 mg/g), but PEs were hardly detected compared with that of any other *Hippocampus* species.  $^1\text{H-NMR}$  metabolomics studies showed that the contents of glutamic acid, aspartic acid, glycerol, taurine, and acetic acid in *Hippocampus trimaculatus* Leach and *Hippocampus kelloggi* Jordan et Snyder were significantly higher than those in *Hippocampus kuda* Bleeker, while the contents of isoleucine and leucine were significantly low (Zhao, 2018). TCM theory about medicinal *Hippocampus* emphasizes that both male and female *Hippocampus* should be used together (Xu et al., 2002). Whether there was a difference in medicinal value between males and females was not clear. At the age of 6 months, female *Hippocampus* had a slightly higher proportion of steroids and unsaturated fatty acids than males (Sun et al., 2020). The fatty acid extraction rate of male *Hippocampus* was slightly higher than that of females (Su and Xu, 2015b). There was no significant difference in ingredient constituents between male and female *Hippocampus erectus* Perry and *Hippocampus trimaculatus* Leach, although the contents of cholesterol and hypoxanthine in female *Hippocampus* were significantly higher than that in males, and the content of uridine in female *Hippocampus erectus* Perry was significantly lower than that in males (Yan Z. Z., 2019). Such findings may explain why male and female *Hippocampus* would be used together. In conclusion, gender had a limited effect on the ingredient constitutions.

## Growth Stage

Eggs of *Hippocampus guttulatus* Cuvier are round and transparent, and the yolk sac provides nutrition for embryo development. Eggs and newborn *Hippocampus* represent different growth stages, and their ingredient constituents are different. Taking fatty acids as an example, the fatty acid contents in newborn *Hippocampus* were much lower than those in eggs. The content of unsaturated fatty acids in eggs was more than four times higher than that in newborn *Hippocampus*, and DHA and EPA were rich in eggs. Omega-3 unsaturated fatty acids were consumed during embryonic development, which explained the difference in fatty acid contents between newborn *Hippocampus* and eggs (Faleiro and Narciso, 2010). Sun et al. (2020) analyzed the ingredients of wild *Hippocampus* at the ages of 6 months, 1 year, and 2 years. It was obvious that crude protein content was higher in the ration of 1-year-old *Hippocampus* compared with the ration of 6- and 2-year-old. The Fe, Cu, and

Zn contents increased with age, whereas unsaturated fatty acid contents decreased significantly. Contents of proteins and fatty acids were more abundant in 1-year-old *Hippocampus*; therefore, *Hippocampus* in this stage is proper for TCM applications.

## Wild Types and Cultured Types

The qualities of wild and cultured *Hippocampus* differed due to the different growth environments. The contents of essential amino acids and unsaturated fatty acids were much higher in cultured *Hippocampus kuda* Bleeker than in wild *Hippocampus*. In terms of mineral elements, Se, Fe, Cu, and Ca were much more abundant in wild *Hippocampus* (Sun et al., 2020). Lin et al. (2009) showed that the contents of DHA and EPA in cultured *Hippocampus trimaculatus* Leach were nearly two times as high as those in wild *Hippocampus*, and the proportions of polyunsaturated fatty acids in total fatty acids were 31.89 and 21.39%, respectively. The cultured *Hippocampus* was rich in polyunsaturated fatty acids, but there were some deficiencies in protein. In addition to Pb, the contents of Cd, Cr, Zn, and Mn in cultured *Hippocampus* were higher than those in wild *Hippocampus*. Additional nutritional supplements were added to the bait as required. Understanding the difference in nutritional characteristics between wild and cultured *Hippocampus* is helpful for optimizing the breeding technology and supplementing wild *Hippocampus*.

## Baits

For cultured *Hippocampus*, different baits had a great influence on the ingredient constituents of *Hippocampus*. Hexadecanoic acid, octadecenoic acid, octadecatrienoic acid, eicosapentaenoic acid, etc., are rich in *Artemia* and rotifers. Several differences in fatty acid types have been observed for different baits, and obvious variations have been observed for EPA and DHA. Otero-Ferrer et al. (2010) fed *Hippocampus hippocampus* Linnaeus *Artemia* and rotifers, and, after 5 days of feeding, the rotifer group showed a higher level of omega-3 unsaturated fatty acids, and the fatty acid types were more abundant than those in the *Artemia* group. The contents of 9,12-octadecadienoic acid and 9,12,15-octadecatrienoic acid in the *Artemia* group were more prominent. After 34 days of feeding, fatty acids in the *Artemia* group were gradually enriched, and the contents of DHA and EPA in the *Artemia* group were higher than those in the rotifer group. In addition, the *Artemia* group also showed lower mortality and a higher growth rate. Segade et al. (2016) further compared the differences among *Hippocampus*-fed *Artemia*, frozen *Artemia*, and Mysis. The results showed that the crude protein content of the frozen *Artemia* group was the lowest among the three groups. The content of polyunsaturated fatty acids in the *Artemia* group was the highest, while that in the Mysis group was the lowest. Frozen bait may not fully meet the nutritional needs of *Hippocampus*. In addition, the Mysis group had the most abundant nucleosides, and thymine was not detected in the frozen Mysis group or the *Artemia* group (Yan Z. Z., 2019). In the selection of baits, mixing of different baits may meet different nutritional needs.

**TABLE 1** | The optimized enzyme hydrolysis conditions.

Enzyme	Temperature (°C)	Hydrolysis time (h)	pH	Enzyme to substrate (E/S) ratio (%)	References
pronase E	36.7	20.0	7.3	2.0	Pangestuti et al., 2013
Protamex	40.0	NA*	6.0	1.0	Je et al., 2020
Alcalase	54.7	6.0	9.0	4.0	Jiang et al., 2014
Trypsin	45.0	4.0	8.8	4.0	Jiang et al., 2014
Papain	59.0	7.5	7.7	5.0	Gu and Xu, 2016
Pepsin	50.0	6.0	2.6	1.0	Yuan et al., 2011
Alcalase + Trypsin	45.0–54.7	4.0–6.0	8.8–9.0	4.0	Jiang et al., 2014

The optimized enzyme hydrolysis conditions. \*NA: Not Available.

## EXTRACTION STRATEGIES OF HIPPOCAMPUS

Extraction is a method of obtaining effective ingredients from medicinal materials through chemical processes, such as solvent treatment, or by mechanical processes, such as supercritical fluid extraction and microwave-assisted extraction (Chaves et al., 2020). Extraction of *Hippocampus* active ingredients is the basis for the study of its pharmacological activity. The potential targets and mechanisms of action of *Hippocampus* in disease treatment must be clarified. Extraction methods for different ingredients should be further researched to find a high-efficiency method that presents a shorter extraction time.

### Extraction Strategies of Hydrophilic Ingredients

The hydrophilic ingredients of *Hippocampus* are mainly amino acids, peptides, glycoproteins, nucleosides, etc., Chen et al. (1997) compared amino acids in water and ethanol extracts of *Hippocampus*. The results showed that the *Hippocampus* water extract contained 17 types of amino acids, while the ethanol extract contained only 10 types of amino acids. The essential amino acid content of the *Hippocampus* water extract was also higher than that of the ethanol extract. *Hippocampus* peptides were obtained by enzyme hydrolysis and chromatography technologies. For bioactive peptides from marine organisms, the first step was the trituration of the tissues, followed by initial treatment (organic extraction, concentration, and/or partitioning). Under the guidance of activity assays, chromatographic technology was used for prepurification, and purified peptides were finally obtained by high-performance liquid chromatography using reversed-phase (RP-HPLC). Alternatively, after tissue separation, enzymatic hydrolysis could be employed to identify bioactive peptides (Macedo et al., 2021). Enzymatic hydrolysis is a commonly used method to extract *Hippocampus* peptides. Commonly selected enzymes include pronase E, alcalase, protamex, trypsin, papain, and pepsin (Table 1). Enzymes and substrates at a ratio of 1:100 (w/w) were mixed under certain conditions, and then the mixture was inactivated at 100°C for 10 min and stored at –80°C (Pangestuti et al., 2013). In addition, *Hippocampus* was defatted with ethyl acetate or CO<sub>2</sub> supercritical fluid extraction technology and then mixed with enzyme (Jiang et al., 2014). Jiang et al. (2014) studied the condition of compound enzyme

hydrolysis and found that the hydrolysis rate of alcalase and trypsin was 2–3% higher than that of single enzyme hydrolysis. We summarized the enzyme reaction under different conditions to obtain optimized reaction conditions (Table 1). Enzymes were selected according to the different needs of research. For example, *Hippocampus trimaculatus* Leach protein was hydrolyzed by four different proteases (trypsin, pepsin, papain, and alcalase) to prepare peptides, and, compared with other hydrolysates, alcalase hydrolysates exhibited the highest ACE inhibitory activity (Xu, 2015). Nucleosides of *Hippocampus* are generally extracted with 50% ethanol or water, followed by HPLC analysis (Wei et al., 2015; Yan Z. Z., 2019). Chen et al. (2010) developed an online rapid method for the identification of hypoxanthine, and the antioxidant hypoxanthine was identified from a water extract of *Hippocampus japonicus* Kaup by HPLC-ESI-TOF/MS combined with an ABTS free radical scavenging online detection system. Zheng et al. (2012) used different antioxidant evaluation experiments for DPPH free radical scavenging, and an online detection system also identified hypoxanthine from water extracts of *Hippocampus kuda* Bleeker and *Hippocampus japonicus* Kaup. The online detection system combines biological activity and chemical separation, and the target ingredient may be quickly separated.

### Extraction Strategies of Hydrophobic Ingredients

More reports have focused on *Hippocampus* hydrophobic ingredients than other types, and these hydrophobic ingredients include fatty acids, steroids, and phospholipids. The extraction of fatty acids in *Hippocampus* is performed by the traditional solvent extraction method. Nicolás et al. (2014) directly soaked *Hippocampus* powder in chloroform:methanol (2:1) for 24 h to extract total lipids. The extraction solvent of chloroform:methanol (2:1) was also used for ultrasonic extraction (Xu et al., 1994). The results showed that there were more types of fatty acids in the ultrasonic extraction. Extraction methods using ether as the extraction solvent include Soxhlet extraction (Zhang et al., 1997), rapid fat extraction (Zhang and Wang, 1996), and ultrasonic extraction (Chen, 2015). Among them, the rapid fat extraction method greatly shortened the extraction period and effectively reduced the oxidation of *Hippocampus* unsaturated fatty acids (Zhang and Wang, 1996). In summary, ultrasonic extraction and rapid fat extraction are generally used in the extraction of fatty acids. Compared with traditional

solvent extraction methods with the problems of solvent residue and heating damage to fatty acids, CO<sub>2</sub> supercritical fluid extraction can extract total lipids at low temperatures and without organic solvents (Jiang et al., 2013). Huang and Xu (2016) compared the effects of chloroform-methanol extraction, absolute ethanol extraction, petroleum ether Soxhlet extraction, and CO<sub>2</sub> supercritical fluid extraction on the lipid and fatty acid constituents of *Hippocampus*. The results showed that the types of fatty acids by the four methods were almost the same while the contents of unsaturated fatty acids in chloroform-methanol extraction and absolute ethanol extraction were the highest, with saturated fatty acids in CO<sub>2</sub> supercritical fluid extraction the highest. The extraction of *Hippocampus* steroids has generally been performed by ethanol extraction followed by organic solvent (n-butanol, ethyl acetate, and petroleum ether) extraction. The fractions were then repeatedly chromatographed with silica gel column chromatography using a stepwise gradient elution of different solvents (Wu et al., 2017b). The total lipids of the *Hippocampus* were extracted by chloroform-methanol (2:1), 75% ethanol, and water, and the content of total phospholipids was identified by molybdenum blue colorimetry. Studies have shown that the chloroform-methanol (2:1) extraction method has the highest content of total phospholipids (Xu et al., 1994; Sheng et al., 2011).

## PHARMACOLOGICAL ACTIVITIES OF HIPPOCAMPUS

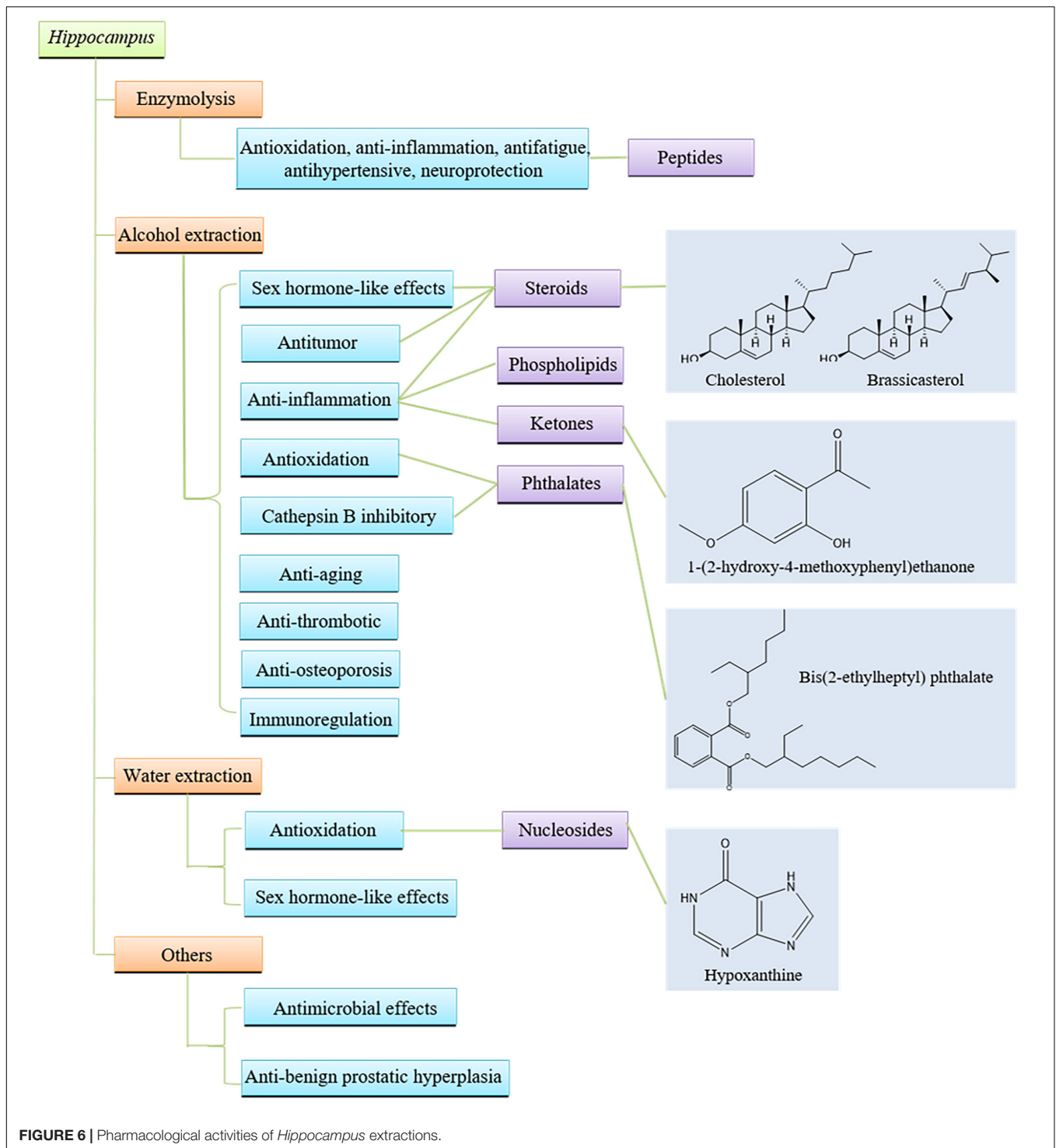
The pharmacological activities of *Hippocampus* were sorted according to the different extraction methods (enzymolysis, alcohol extraction, water extraction, and other extraction methods). Modern pharmacological studies have revealed that *Hippocampus* shows various biological activities, such as sex hormone-like effects and antioxidation, anti-inflammation, antitumor effects, neuroprotection, and antimicrobial activities. Different extraction portions of *Hippocampus* exhibited particular pharmacological activities, which may be related to the ingredient types. For example, the antihypertensive and neuroprotective activities of enzymolysis portions were based on the peptides extracted. The alcohol extraction portions mainly showed sex hormone-like effects and anti-inflammation, which were closely related to steroids, ketones, etc. In **Figure 6**, the extraction methods, ingredients, and pharmacological activities are summarized.

### Enzymolysis

The pharmacological activities of *Hippocampus* peptides include antihypertensive, antioxidation, and anti-inflammation activities (**Figure 6**). The ACE inhibitory peptide obtained from *Hippocampus abdominalis* Lesson induced an increase in NO content in blood vessels through the expression of phosphor-endothelial nitric oxide synthase (p-eNOS) to promote vasodilation and lower blood pressure (Je et al., 2020). ACE is part of the renin-angiotensin system and plays a vital role in regulating blood pressure, and ACE inhibitors are effective in the treatment of hypertension (Li et al., 2014). In addition, free

radicals are in a special state of balance in the human body, and when this balance is disrupted, they may cause oxidative stress, which can lead to the damage of blood vessels and cardiovascular diseases. Interestingly, Gu and Xu (2016) found that *Hippocampus* ACE inhibitory peptide showed DPPH radical scavenging activity. One of the medicinal candidates for the treatment of hypertension may be bifunctional peptides with both ACE inhibitory and antioxidant activity. The therapeutic effects of peptide SHP-1 isolated from *Hippocampus kuda* Bleeker on arthritis were observed with human chondrocyte-like SW-1353 and human osteoblast-like MG-63 cells. The inhibition rates of SHP-1 on collagen release in SW-1353 and MG-63 cells were 40.35–59.96% and 60.82–85.82%, respectively. SHP-1 inhibited the expression of collagenase 1,13 and NO induced by the proinflammatory cytokine 12-O-tetradecanoylphorbol-13-acetate (TPA) and blocked NF-κB/p38 kinase (Ryu et al., 2010). Ryu (2010) further isolated a *Hippocampus* peptide with alkaline phosphatase activity and showed that it not only promoted the differentiation of MG-63 osteoblasts and SW-1353 chondrocytes but also inhibited the expression of the inflammatory factors cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). A peptide showed remarkable anti-inflammatory activity by regulating the NF-κB and MAPK signaling pathways. Patients with arthritis have persistent joint swelling and restricted movement due to pain (Mandl, 2019). The anti-inflammatory effect of peptides may be associated with the traditional efficacy of *Hippocampus* in reducing swelling.

*Hippocampus* also presents an anti-fatigue effect (Hu et al., 2000). Kang et al. (2017) found that enzymatic hydrolysates of *Hippocampus abdominalis* Lesson promoted the proliferation of C2C12 myoblasts and increased the contents of physical activity markers, such as ATP and glycogen. *Hippocampus* enzymatic hydrolysates not only promoted the elimination of lactic acid and urea nitrogen during exercise but also strengthened the antifatigue activity with increasing exercise intensity. Compared with the antifatigue effect of the one-time fatigue model, the incremental exercise fatigue model was more effective. For normal mice, *Hippocampus* enzymatic hydrolysates improved the survival time under hypoxia and cold conditions (Peng and Chen, 2005). Accordingly, supplementation with *Hippocampus* enzymatic hydrolysates may be a natural health product that has an improved effect on the human body. For nervous diseases, *Hippocampus* enzymatic hydrolysates promoted the recovery of neurological function and reduced the volume of cerebral infarction and brain edema in animal models of ischemia-reperfusion brain damage (Feng et al., 2005). The peptide HTP-1 had a protective effect on Aβ<sub>42</sub>-induced PC12 cells and may be an active ingredient of *Hippocampus* for the treatment of neurodegenerative disease (Pangestuti et al., 2013). However, the neuroprotective mechanisms of *Hippocampus* require further investigation. In a coculture system with PC12 cells and BV-2 cells induced by Aβ<sub>42</sub>, HTP-1-protected PC12 cells were not subjected to the neurotoxicity of BV-2 cells. HTP-1 attenuated the neurotoxic mediators released by BV-2 cells and activated PI3K/Akt by inducing transforming growth factor-β (TGF-β),

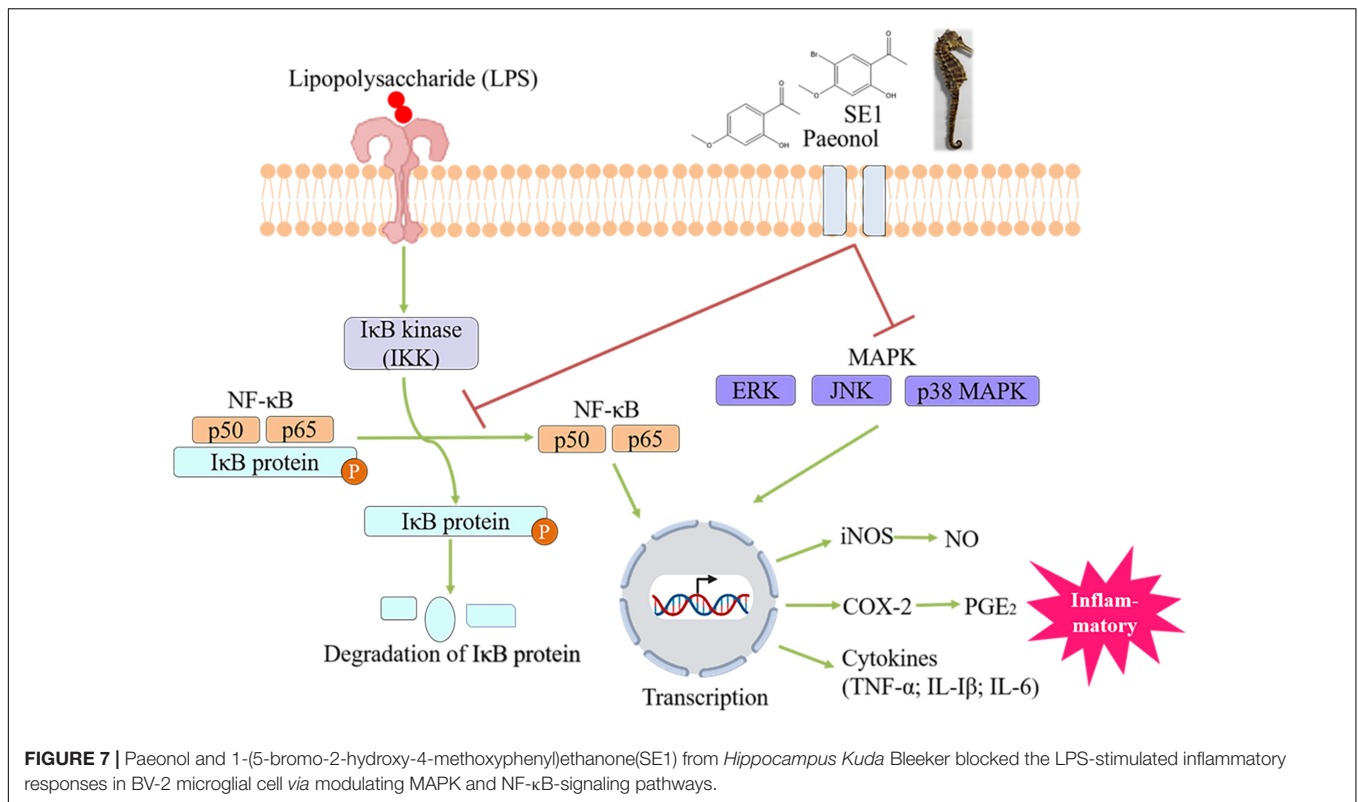


which is the main signaling pathway of neuronal survival (Pangestuti and Kim, 2015).

## Alcohol Extraction

The *Hippocampus* activity of warming and tonifying kidney yang was closely related to the alcohol extract of *Hippocampus*. Chen et al. (2009) studied the effect of the n-butanol extract from

*Hippocampus* on a mouse model of kidney yang deficiency. After 15 days of continuous administration, the results showed that the n-butanol group improved the rectal temperature, grip strength, testis index, and seminal vesicle index of the model mouse. The ethanol extract of *Hippocampus* was orally administered to normal and cyclophosphamide model mice, and it was found to increase the overall sperm number and



viability. From the perspective of modern science, *Hippocampus* improves the symptoms of kidney deficiency, such as weakness at the waist and knees, frigidity, chilliness, and cold limbs (Zhang et al., 1995). The total sterol content in *Hippocampus kuda* Bleeker not only increased the content of testosterone *in vivo* but also changed the structure of the seminiferous duct and promoted the formation of sperm. However, long-term high-dose intake of total sterols might change the shape of the kidney and testis (Sun, 2015). Over thousands of years, traditional Chinese clinical practices have supported the effect of *Hippocampus* on improving sexual function, although the material basis and concrete mechanism of efficacy were not clear. The enzymatic hydrolysate of *Hippocampus* had a certain therapeutic effect on arthritis, while the alcohol extract was more effective in the treatment of neuroinflammation. Himaya et al. (2011, 2012) isolated 1-(5-bromo-2-hydroxy-4-methoxyphenyl)ethanone (SE1) and paeonol from *Hippocampus kuda* Bleeker and showed that it inhibited the expression of proinflammatory cytokines in BV-2 microglial cells induced by lipopolysaccharide (LPS). This compound played an anti-inflammatory role by inhibiting the phosphorylation of JNK and p38 in the MAPK pathway and nuclear heterotrophs in the NF-κB pathway (Figure 7). Yan et al. (2019) further found that SE1 and paeonol improved the morphology of activated microglia with enlarged cell bodies and shorter synapses. Recent studies have shown that neurodegenerative diseases are closely related to neuroinflammation caused by activated microglia (Sarlus and Heneka, 2017). SE1 and paeonol extracted from *Hippocampus*-modulated neuroinflammation and may be

potential candidates for treating neurodegenerative diseases, such as Alzheimer's disease (AD). To verify the effects and mechanisms of SE1 and paeonol on AD, *in vitro* or *in vivo* experiments with pharmacological approaches are needed in the future. Recently, patients with schizophrenia have been shown to have an increased inflammatory response, and autophagy dysfunction caused by inflammation might continue to increase the inflammatory response. Studies have shown that the alcohol extract of *Hippocampus kelloggi* Jordan et Snyder exerts anti-inflammatory effects by improving autophagy, thus revealing a new possible target for *Hippocampus*-based therapeutics to combat schizophrenia (Yan L., 2019). In addition, the methanol extract of *Hippocampus* increased the concentration of neurotransmitters and the expression of brain-derived neurotransmitters by inhibiting the secretion of proinflammatory factor interleukin-1 $\beta$  (1L-1 $\beta$ ), which improved depressive behavior in mice and restored normal neurotransmitter function (Li et al., 2020). The *Hippocampus* anti-inflammatory effect is associated with the regulation of the MAPK and NF-κB pathways, which are primarily associated with inflammation (Wu et al., 2017a). Wu et al. (2020) identified miR-98-5p from the cellular RNA library, and 3 $\beta$ -hydroxycholest-5-en-7-one isolated from *Hippocampus* regulated the expression of the miR-98-5p target gene TNFAIP3 to inhibit the excessive secretion of proinflammatory factors and produce an anti-inflammatory effect. The screening of antitumor active ingredients from TCM has become a hot field. Studies have shown that the alcohol extract of *Hippocampus* has an inhibitory effect on tumor cells. In a study of dihydrotestosterone-induced LNCaP cells of prostate

cancer, dual-targeting AKT and AR signaling showed that brassicasterol from *Hippocampus abdominalis* Lesson exerted an anticancer effect (Xu et al., 2020). Sphingoid bases were purified from *Hippocampus* using lipid extraction, saponification, and acid hydrolysis (Yu, 2018). The IC<sub>50</sub> of human chronic myeloid leukemia cells treated with sphingoid bases for 24 h was 37.17 µg/ml. Further investigation of the mechanisms by Western blot and RT-PCR analysis revealed that sphingoid bases exerted their antitumor activity by inducing apoptosis via both the mitochondrial apoptosis pathway and the death receptor pathway. In addition, sphingoid bases also inhibited the formation of neovascularization and promoted cell apoptosis by inhibiting the expression of COX-2. The antitumor activity of *Hippocampus* is mainly caused by low- and medium-polarity ingredients and has great research prospects in cancer therapy.

The antioxidant activity of *Hippocampus* was also explored. Three phthalic acid derivatives isolated by Qian et al. (2012) from the methanol extract of *Hippocampus kuda* Bleeker had antioxidant activity, among which 2,12-diethyl-11-methylhexadecyl 2-ethyl-11-methylhexadecylphthalate had the strongest antioxidant activity and exhibited dose-dependent effects; moreover, it is a safe and effective antioxidant that inhibited reactive oxygen in mouse macrophages (RAW264.7) and had no toxic effect on cells. The ethanol extract of *Hippocampus* had a certain immunomodulatory effect. On the one hand, it increased the spleen weight of mice and promoted lymphocyte proliferation and phagocyte function (Chen et al., 1995). On the other hand, it inhibited the delayed-type hypersensitivity induced by dinitrochlorobenzene in mice (Zhu, 2005). Brain monoamine oxidase B (MAO-B) increases during aging, which is considered a sign of aging. The alcohol extract of *Hippocampus Kuda* Bleeker had a significant inhibitory effect on MAO-B activity in the mouse brain, suggesting that the *Hippocampus* delayed aging (She et al., 1995). The methanol extract of *Hippocampus* had an obvious inhibitory effect on the experimental common carotid artery and cerebral thrombosis in mice. The inhibition rate of 200 mg/kg extract on cerebral thrombosis was 46.8%. The main active ingredients were unsaturated fatty acids, and the content of 9,12-octadecadienoic acid accounted for 18.5% (Xu and Xu, 1997). In addition, the ethanol extracts from five species of *Hippocampus* promoted the differentiation of osteoblasts and the formation of bone nodules and had a certain therapeutic effect on osteoporosis (Wang, 2015).

## Water Extraction

Antioxidant activity was found in both the water extracts of *Hippocampus japonicus* Kaup and *Hippocampus Kuda* Bleeker (Chen et al., 2010; Zheng et al., 2012), suggesting that the water extract of *Hippocampus* had antioxidant activities. The most important key factor affecting the degree of pharmacological activity was the extraction time, followed by the extraction temperature (Yi et al., 2006). Qian et al. (2008) compared the antioxidant activities of the methanol extract, ethanol extract, and water extract. The antioxidant activity degree of the methanol extract was higher than that of the other two extracts, and its activity degree was between that of  $\alpha$ -tocopherol and vitamin C.

In general, moderately and less hydrophilic ingredients showed strong antioxidant activity. The warming and tonifying kidney yang effect has been associated with the alcohol extract of *Hippocampus*. However, the water extract of *Hippocampus* also improved the ability of the testicles in the mice model with kidney yang deficiency, and the testis index was higher than that observed for the petroleum ether extract at the same dose (Chen et al., 2009).

## Other Extraction Methods

Sun et al. (2012) synthesized the antimicrobial peptide HKPLP from the brooding pouch cDNA library of *Hippocampus kuda* Bleeker. The results indicated that the most sensitive bacterium of HKPLP was *Staphylococcus aureus*. In addition, HKPLP appeared to have a minor inhibitory effect on fungi. Ko et al. (2016) identified a goose-type lysozyme (ShLysG) from *Hippocampus abdominalis* Lesson that had a certain inhibitory effect against gram-positive/negative bacteria and a potential immune-protecting effect against pathogens. Bacterial pathogen factors are one of the important factors affecting the survival of marine organisms. Isolation of marine microorganisms with antimicrobial activity from the intestinal contents and skin mucus of *Hippocampus guttulatus* Cuvier promoted the development of breeding and rearing of *Hippocampus* (Balcazar et al., 2010). In addition, the ethyl acetate extract from *Hippocampus* significantly improved benign prostatic hyperplasia in castration and testosterone mouse models (Xu et al., 2014).

## CONCLUSION

The increasing demand for safe and effective medicines has led to increased attention on marine natural products. This review mainly focused on the ingredients, extraction methods, pharmacological activities, and traditional prescriptions containing different species of *Hippocampus*. *Hippocampus* is mainly distributed in the Pacific region, with the largest species number in Australia. *Hippocampus* trade in China and surrounding Southeast Asian countries is high. The active ingredients of *Hippocampus* include hydrophobic ingredients, such as steroids and fatty acids, and hydrophilic ingredients, such as peptides and glycoproteins. Studies have shown that anti-inflammation, antioxidation, antitumor, and neuroprotective effects are the important key functions of *Hippocampus*. The alcohol extract of *Hippocampus* not only warms and tonifies kidney yang but also blocks neuroinflammation by regulating the MAPK and NF- $\kappa$ B pathways. In particular, *Hippocampus* peptides reduce the toxicity of A $\beta$ <sub>42</sub> to nerve cells. The neuroprotective activity of *Hippocampus* indicates its ability to treat nervous system diseases. In addition, an animal experiment involving traditional prescriptions containing *Hippocampus* also showed its antitumor value and treatment of benign prostatic hyperplasia. In the future, quality evaluation standards for *Hippocampus* should be established, and the targets and mechanisms of the pharmacological activity, active ingredients, and pharmacological activities of *Hippocampus* need to be further studied. The kidney-brain axis may be the key point

at which *Hippocampus* impacts neurodegenerative diseases, and *Hippocampus* peptides may have broader application prospects.

## AUTHOR CONTRIBUTIONS

XL, XF, and XC conceived and proposed the idea, designed the study, and prepared the manuscript. XZ contributed to **Supplementary Table 6**. JL contributed to **Figure 4**. XF was the project organization leader. XL and XF guided the manuscript writing. All authors contributed to the article and approved the submitted version.

## REFERENCES

- Althagbi, H. I., Alarif, W. M., Al-Footy, K. O., and Abdel-Lateff, A. (2020). Marine-derived macrocyclic alkaloids (MDMAs): chemical and biological diversity. *Mar. Drugs* 18:368. doi: 10.3390/md18070368
- Balcazar, J. L., Loureiro, S., Da Silva, Y. J., Pintado, J., and Planas, M. (2010). Identification and characterization of bacteria with antibacterial activities isolated from seahorses (*Hippocampus guttulatus*). *J. Antibiot. (Tokyo)* 63, 271–274. doi: 10.1038/ja.2010.27
- Blunt, J. W., Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., and Prinsep, M. R. (2018). Marine natural products. *Nat. Prod. Rep.* 35, 8–53. doi: 10.1039/c7np00052a
- Bohic, S., Ghersi-Egea, J. F., Gibon, J., Paoletti, P., Arnaud, J., Hunot, S., et al. (2011). [Biological roles of trace elements in the brain with special focus on Zn and Fe]. *Rev. Neurol. (Paris)* 167, 269–279. doi: 10.1016/j.neurol.2010.07.035
- Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., and Prinsep, M. R. (2019). Marine natural products. *Nat. Prod. Rep.* 36, 122–173. doi: 10.1039/c8np00092a
- Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., and Prinsep, M. R. (2020). Marine natural products. *Nat. Prod. Rep.* 37, 175–223. doi: 10.1039/c9np00069k
- Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., and Prinsep, M. R. (2021). Marine natural products. *Nat. Prod. Rep.* 38, 362–413. doi: 10.1039/d0np00089b
- Casey, S. P., Hall, H. J., Stanley, H. F., and Vincent, A. C. (2004). The origin and evolution of seahorses (genus *Hippocampus*): a phylogenetic study using the cytochrome b gene of mitochondrial DNA. *Mol. Phylog. Evol.* 30, 261–272. doi: 10.1016/j.ympev.2003.08.018
- Chaves, J. O., de Souza, M. C., da Silva, L. C., Lachos-Perez, D., Torres-Mayanga, P. C., Machado, A., et al. (2020). Extraction of flavonoids from natural sources using modern techniques. *Front. Chem.* 8:507887. doi: 10.3389/fchem.2020.507887
- Chen, C. Q. (1983). *A Supplement to Materia Medica*. Wuhu: Academic Research of Wannan Medical College.
- Chen, D., Chen, J., Gao, J., and Cao, X. Y. (2018). Evaluation of the antioxidant activity of *Hippocampus erectus* peptides. *Food Res. Technol.* 39, 10–15. doi: 10.3969/j.issn.1005-6521.2018.08.002
- Chen, J., Zhao, H., Shi, Q., Zhang, D., Cheng, H., Wang, X., et al. (2010). Rapid screening and identification of the antioxidants in *Hippocampus japonicus* Kaup by HPLC-ESI-TOF/MS and on-line ABTS free radical scavenging assay. *J. Sep. Sci.* 33, 672–677. doi: 10.1002/jssc.200900544
- Chen, L. (2015). *Study on Identification and Quality Standard of Hippocampus*. Master's thesis. Shanghai: The Second Military Medical University.
- Chen, L., Wang, X., and Huang, B. (2015). The genus *Hippocampus*—a review on traditional medicinal uses, chemical constituents and pharmacological properties. *J. Ethnopharmacol.* 162, 104–111. doi: 10.1016/j.jep.2014.12.032
- Chen, S. H., Lv, G. Y., Fan, J., Yan, M. Q., Ye, H., Fang, Z., et al. (2009). Effects of different extracts from *Hippocampus japonicus* on kidney-yang deficiency mice induced by extradiol. *China Tradit. Herb. Drugs* 40, 258–262.
- Chen, W. N., Xu, L. Z., Gao, E., Li, H. Z., and Miao, N. F. (1995). The pharmacological effects of extracts of hippocampus. *Acta Acad. Med. Weifang* 17, 105–109.

## FUNDING

This work was supported by the National High Technology Research and Development Program of China (863 Program) (No. 2013AA093001).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.774927/full#supplementary-material>

- Chen, W. N., Yu, S. M., Li, Y. H., Xu, L. Z., and Zhang, W. (1997). Assaying of amino acids and trace elements in the extract of hippocampus. *Acta Acad. Med. Weifang* 19, 25–26. doi: 10.1530/eje.0.1360316
- Chen, X., Yi, Y., You, X., Liu, J., and Shi, Q. (2019). High-throughput identification of putative antimicrobial peptides from multi-omics data of the lined seahorse (*Hippocampus erectus*). *Mar. Drugs* 18:10030. doi: 10.3390/md18010030
- Chinese, P. C. (2020). *Pharmacopoeia of the People's Republic of China*. Beijing: China Medical Science Press.
- Choi, Y.-U., Rho, S., Park, H.-S., and Kang, D.-H. (2012). Population characteristics of two seahorses, *Hippocampus coronatus* and *Hippocampus mohnikei*, around seagrass beds in the southern coastal waters of Korea. *Ichthyol. Res.* 59, 235–241.
- Choo, C. K., and Liew, H. C. (2003). Spatial distribution, substrate assemblages and size composition of sea horses (Family Syngnathidae) in the coastal waters of Peninsular Malaysia. *J. Mar. Biol. Assoc. U. K.* 83, 271–276. doi: 10.1017/S0025315403007069h
- CITES (2021b). *Hippocampus* [Online]. Available online at: <https://checklist.cites.org/#/en> (accessed September 10, 2021).
- CITES (2021a). *Hippocampus* [Online]. Available online at: <https://trade.cites.org/> (accessed January 20, 2021).
- Conte, M., Fontana, E., Nebbioso, A., and Altucci, L. (2021). Marine-derived secondary metabolites as promising epigenetic bio-compounds for anticancer therapy. *Mar. drugs* 19:15. doi: 10.3390/md19010015
- Convention on International Trade in Endangered Species of Wild Fauna and Flora (2020). Available online at: <https://trade.cites.org/> (accessed December 18, 2020).
- Dayanidhi, D. L., Thomas, B. C., Osterberg, J. S., Vuong, M., Vargas, G., Kwartler, S. K., et al. (2021). Exploring the diversity of the marine environment for new anti-cancer compounds. *Front. Mar. Sci.* 7:1184. doi: 10.3389/fmars.2020.614766
- De Jong, W. H., and Borm, P. J. (2008). Drug delivery and nanoparticles: applications and hazards. *Int. J. Nanomed.* 3, 133–149. doi: 10.2147/ijn.s596
- Ermolenko, E. V., Imbs, A. B., Glorizova, T. A., Poroikov, V. V., Sikorskaya, T. V., and Dembitsky, V. M. (2020). Chemical diversity of soft coral steroids and their pharmacological activities. *Mar. Drugs* 18:613. doi: 10.3390/md18120613
- Faleiro, F., and Narciso, L. (2010). Lipid dynamics during early development of *Hippocampus guttulatus* seahorses: searching for clues on fatty acid requirements. *Aquaculture* 307, 56–64. doi: 10.1016/j.aquaculture.2010.07.005
- Fan, G. (2012). *Application of <sup>1</sup>H NMR-Based Metabolomics for the Species Differentiation and Quality Evaluation of Traditional Chinese Medicine and Tibetan Medicine From Various Species*. Dissertation's thesis. Chengdu: Chengdu University of Traditional Chinese Medicine.
- Feng, X., Wu, Z. F., Yang, L., Zhou, Y., Wang, Q., Liu, Y. Z., et al. (2005). Effects of haima extraction on experimental ischemia/reperfusion brain damage. *J. Hunan Norm. Univ. (Med. Sci.)* 2, 1–4.
- FishBase (2021). *Hippocampus* [Online]. Available online at: <https://www.fishbase.org/Nomenclature/ValidNameList.php?syng=Hippocampus&syng=&vtitle=Scientific+Names+where+Genus+Equals+%3Ci%3EHippocampus%3C%2Fi%3E&crit2=CONTAINS&crit1=EQUAL> (accessed September 10, 2021).
- Foster, S. J., and Vincent, A. C. J. (2004). Life history and ecology of seahorses: implications for conservation and management. *J. Fish. Biol.* 65, 1–61. doi: 10.1111/j.0022-1112.2004.00429.x

- Ge, Y. Q., Zhu, L. Y., Chen, M., Zhang, G. J., Huang, Z., and Cheng, R. B. (2018). Complete mitochondrial genome sequence for the endangered Knysna seahorse *Hippocampus capensis* Boulenger 1900. *Conserv Genet Resour* 10, 461–465. doi: 10.1007/s12686-017-0849-3
- Gerardo, B., Cabral Pinto, M., Nogueira, J., Pinto, P., Almeida, A., Pinto, E., et al. (2020). Associations between Trace Elements and cognitive decline: an exploratory 5-year follow-up study of an elderly cohort. *Int. J. Environ. Res. Public Health* 17:6051. doi: 10.3390/ijerph17176051
- Gu, W., and Xu, Y. J. (2016). Preparation of *Hippocampus* ACE inhibitory peptide and determination of antioxidant capacity. *Sci. Technol. Food Industry* 37, 201–206. doi: 10.13386/j.issn1002-0306.2016.05.031
- Han, F. J. (2002). The mechanism study on hai-ma-sheng-sui pill resisting the tumor on S<sub>180</sub> and SKOV<sub>3</sub> Mice. *J. Harb. Univ. Comm. Natur. Sci.* 18, 66–68.
- Han, S. L. (2013). *Taxonomy, Resources, Utilization and Conservation of Sea Horses in China*. Master's thesis. Guangxi: Guangxi Normal University.
- Himaya, S. W., Ryu, B., Qian, Z. J., Li, Y., and Kim, S. K. (2011). 1-(5-bromo-2-hydroxy-4-methoxyphenyl)ethanone [SE1] suppresses pro-inflammatory responses by blocking NF-kappaB and MAPK signaling pathways in activated microglia. *Eur. J. Pharmacol.* 670, 608–616. doi: 10.1016/j.ejphar.2011.09.013
- Himaya, W. A., Ryu, B. M., Qian, Z. J., and Kim, S. K. (2012). Paeonol from *Hippocampus kuda* bleeler suppressed the neuro-inflammatory responses in vitro via NF-κB and MAPK signaling pathway. *Toxicol. in Vitro* 26, 878–887. doi: 10.1016/j.tiv.2012.04.022
- Hosseini, B., Nourmohamadi, M., Hajipour, S., Taghizadeh, M., Asemi, Z., Keshavarz, S. A., et al. (2019). The effect of omega-3 fatty acids, EPA, and/or DHA on male infertility: a systematic review and meta-analysis. *J. Diet. Suppl.* 16, 245–256. doi: 10.1080/19390211.2018.1431753
- Hou, F., Wen, L., Peng, C., and Guo, J. (2016). Identification of marine traditional Chinese medicine dried seahorses in the traditional Chinese medicine market using DNA barcoding. *Mitochondr. Dna Dna Mapp. Seq. Anal.* 16, 1–6. doi: 10.1080/24701394.2016.1248430
- Hu, J. Y., Li, B. F., Li, Z. J., and Lv, P. (2000). An experimental study on anti-fatigue effects of eight marine pharmacokons. *Chin. J. Mar. Drugs* 2, 56–58.
- Huang, B. Q., Ai, C. R., and Mai, Q. F. (1997). The preparation of compound seahorse medicinal liquor and its health protection effect. *Chin. J. Mar. Drugs* 4, 44–49.
- Huang, D., and Xu, Y. J. (2016). Different extraction process for *Hippocampus kuda* lipid and its analysis of the fatty acid composition. *Chin. J. Mar. Drugs* 35, 35–40. doi: 10.13400/j.cnki.cjmd.2016.02.007
- Huang, R. M., Chen, Y. N., Zeng, Z. Y., Gao, C. H., Su, X. D., and Peng, Y. (2014). Marine nucleosides: structure, bioactivity, synthesis and biosynthesis. *Mar. Drugs* 12, 5817–5838. doi: 10.3390/md12125817
- IUCN (2021). *Hippocampus* [Online]. Available online at: <https://www.iucnredlist.org/search?query=Hippocampus&searchType=species> (accessed September 10, 2021).
- Je, J.-G., Kim, H.-S., Lee, H.-G., Oh, J.-Y., Lu, Y. A., Wang, L., et al. (2020). Low-molecular weight peptides isolated from seahorse (*Hippocampus abdominalis*) improve vasodilation via inhibition of angiotensin-converting enzyme in vivo and in vitro. *Process Biochem.* 95, 30–35. doi: 10.1016/j.procbio.2020.04.016
- Ji, W. T., and Yu, X. (2020). A reinterpretation of yin-yang theory of TCM based on chaos theory. *Liaoning J. Tradit. Chin. Med.* 47, 77–79.
- Jia, W., Gao, W. Y., Wang, T., Liu, Y. B., Xue, J., and Xiao, P. G. (2003). Cataplasma of traditional Chinese medicine. *Chin. J. Chin. Mat. Med.* 28, 7–11.
- Jia, Y. Y., and Yao, Q. Y. (1990). Comparative analysis of trace elements and amino-acids in *Hippocampus kuda* bleeker and *H. parvus*. *Chin. J. Mar. Drugs* 9, 13–15.
- Jiang, Z. Z., Xu, Y. J., and Su, Y. T. (2013). The extraction technology of fat from bone powder of sea horse by sfe and the analysis of fatty acid composition. *Chin. J. Mar. Drugs* 32, 47–52. doi: 10.13400/j.cnki.cjmd.2013.02.009
- Jiang, Z., Xu, Y., and Su, Y. (2014). Preparation process of active enzymolysis polypeptides from seahorse bone meal. *Food Sci. Nutr.* 2, 490–499. doi: 10.1002/fsn3.125
- Kang, N., Kim, S.-Y., Rho, S., Ko, J.-Y., and Jeon, Y.-J. (2017). Anti-fatigue activity of a mixture of seahorse (*Hippocampus abdominalis*) hydrolysate and red ginseng. *Fish Aquat. Sci.* 20:3. doi: 10.1186/s41240-017-0048-x
- Khalifa, S. A. M., Elias, N., Farag, M. A., Chen, L., Saeed, A., Hegazy, M. F., et al. (2019). Marine natural products: a source of novel anticancer drugs. *Mar. Drugs* 17:491. doi: 10.3390/md17090491
- Ko, J., Wan, Q., Bathige, S., and Lee, J. (2016). Molecular characterization, transcriptional profiling, and antibacterial potential of G-type lysozyme from seahorse (*Hippocampus abdominalis*). *Fish Shellf. Immunol.* 58, 622–630. doi: 10.1016/j.fsi.2016.10.014
- Koldewey, H. J., and Martin-Smith, K. M. (2010). A global review of seahorse aquaculture. *Aquaculture* 302, 131–152. doi: 10.1016/j.aquaculture.2009.11.010
- Kumaravel, K., Ravichandran, S., Balasubramanian, T., and Sonneschein, L. (2012). Seahorses – a source of traditional medicine. *Nat. Prod. Res.* 26, 2330–2334. doi: 10.1080/14786419.2012.662650
- Li, E. C., Heran, B. S., and Wright, J. M. (2014). Angiotensin converting enzyme (ACE) inhibitors versus angiotensin receptor blockers for primary hypertension. *Cochrane Database Syst. Rev.* 2014:CD009096. doi: 10.1002/14651858.CD009096.pub2
- Li, K. W., Yan, L., Zhang, Y. P., Yang, Z. Y., Zhang, C., Li, Y. J., et al. (2020). Seahorse treatment improves depression-like behavior in mice exposed to CUMS through reducing inflammation/oxidants and restoring neurotransmitter and neurotrophin function. *J. Ethnopharmacol.* 250:112487. doi: 10.1016/j.jep.2019.112487
- Li, S. Z. (1982). *The Compendium of Materia Medica*. Beijing: People's Medical Publishing House.
- Li, Y., Qian, Z. J., and Kim, S. K. (2008). Cathepsin B inhibitory activities of three new phthalate derivatives isolated from seahorse, *Hippocampus kuda* bleeler. *Bioorg. Med. Chem. Lett.* 18, 6130–6134. doi: 10.1016/j.bmcl.2008.10.016
- Liang, B., Cai, X.-Y., and Gu, N. (2021). Marine natural products and coronary artery disease. *Front. Cardiovas. Med.* 8:1135. doi: 10.3389/fcvm.2021.739932
- Lin, Q., Lin, J. D., and Wang, C. (2009). Biochemical composition of the wild and cultured seahorses, *Hippocampus kuda* bleeker and *Hippocampus trimaculatus* leach. *Aquac. Res.* 40, 710–719. doi: 10.1111/j.1365-2109.2008.02149.x
- Lin, Q., Lin, J. D., Lu, J. Y., and Li, B. J. (2008). Biochemical composition of six seahorse species, *Hippocampus* sp., from the Chinese coast. *J. World Aquacult. Soc.* 39, 225–234. doi: 10.1111/j.1749-7345.2008.00159.x
- Liu, F. Y., Yuan, Y., Jin, Y., Qin, W., Jiang, C., Zhao, Y. Y., et al. (2018). Multiplex PCR to simultaneous identification of five traditional Chinese medicinal seahorses. *Chin. J. Chin. Mater. Med.* 43, 4562–4568. doi: 10.19540/j.cnki.cjcm.20181025.012
- Liu, X., and Lin, Y. (2006). Quality standard for Shexiang Haima Zhufeng Gao. *Centr. South Pharm.* 4, 350–353.
- Loh, T. L., Tewfik, A., Aylesworth, L., and Phoonsawat, R. (2016). Species in wildlife trade: socio-economic factors influence seahorse relative abundance in Thailand. *Biol. Conserv.* 201, 301–308. doi: 10.1016/j.biocon.2016.07.022
- Lourie, S. A., Foster, S. J., Cooper, E. W., and Vincent, A. C. (2004). A guide to the identification of seahorses. *Proj. Seah. Traffic North Am.* 4:114.
- Lu, Y., Chen, W. X., Hua, Y. Q., and Meng, D. C. (2001). Pharmacological research on effect of haima zhuangyang capsule in warming kidney and strengthening Yang. *J. Nanjing. Med. Univ.* 17, 99–101. doi: 10.14148/j.issn.1672-0482.2001.02.014
- Luo, J. Y., Yan, D., Song, J. Y., Zhang, D., Xing, X. Y., Han, Y. M., et al. (2013). A strategy for trade monitoring and substitution of the organs of threatened animals. *Sci. Rep.* 3:3108. doi: 10.1038/srep03108
- Macedo, M. W. F. S., Cunha, N. B. d., Carneiro, J. A., Costa, R. A. d., Alencar, S. A. d., Cardoso, M. H., et al. (2021). Marine organisms as a rich source of biologically active peptides. *Front. Mar. Sci.* 8:889. doi: 10.3389/fmars.2021.667764
- Mandl, L. A. (2019). Osteoarthritis year in review 2018: clinical. *Osteoarth. Cartil.* 27, 359–364. doi: 10.1016/j.joca.2018.11.001
- Mei, X. T., Xu, D. H., Lin, Z. L., Xu, S. B., and Li, B. J. (2005). Strengthening and restoring effects of *Hippocampus* Capsule on kidney-deficient rats. *Chin. Tradit. Herbal Drugs* 3, 409–410.
- Mitsushashi, S. (2014). Current topics in the biotechnological production of essential amino acids, functional amino acids, and dipeptides. *Curr. Opin. Biotechnol.* 26(Suppl. 6), 38–44. doi: 10.1016/j.copbio.2013.08.020
- Murray, M., Dordevic, A. L., Ryan, L., and Bonham, M. P. (2018). An emerging trend in functional foods for the prevention of cardiovascular disease and diabetes: marine algal polyphenols. *Crit. Rev. Food Sci. Nutr.* 58, 1342–1358. doi: 10.1080/10408398.2016.1259209
- NCBI (2020). *Hippocampus* [Online]. Available online at: <https://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/> (accessed November 3, 2020).



- Newman, D. J., and Cragg, G. M. (2016). Drugs and drug candidates from marine sources: an assessment of the current "state of play". *Planta Med.* 82, 775–789. doi: 10.1055/s-0042-101353
- Nicolás, V.-G., Arjona, O., Morales-Bojorquez, E., Mascaro, M., Simoes, N., and Palacios, E. (2014). Assessment of lipid classes and fatty acid levels in wild newborn seahorses (*Hippocampus erectus*) (Perry 1810): implications for survival and growth in aquarium culture. *Mar. Behav. Physiol.* 47, 401–413. doi: 10.1080/10236244.2014.959368
- Otero-Ferrer, F., Molina, L., Socorro, J., Herrera, R., Fernández-Palacios, H., and Izquierdo, M. S. (2010). Live prey first feeding regimes for short-snouted seahorse *Hippocampus hippocampus* (Linnaeus, 1758) juveniles. *Aquac. Res.* 41, e8–e19. doi: 10.1111/j.1365-2109.2010.02505.x
- Pangestuti, R., and Kim, S.-K. (2015). Peptide-derived from seahorse exerts a protective effect against cholinergic neuronal death in vitro model of Alzheimer's Disease. *Proc. Chem.* 14, 343–352. doi: 10.1016/j.proche.2015.03.047
- Pangestuti, R., Ryu, B., Himaya, S., and Kim, S. K. (2013). Optimization of hydrolysis conditions, isolation, and identification of neuroprotective peptides derived from seahorse *Hippocampus trimaculatus*. *Amino Acids* 45, 369–381. doi: 10.1007/s00726-013-1510-4
- Peng, W. D., and Chen, Q. L. (2005). Anti-fatigue effect of enzymatic extract of hippocampus. *Chin. J. Food Hygiene* 17, 404–407. doi: 10.13590/j.cjfh.2005.05.006
- Qi, D., Qiao, Y., Zhang, X., Yu, H., Cheng, B., and Qiao, H. (2012). A $\beta$  damages learning and memory in Alzheimer's disease rats with kidney-yang deficiency. *Evid. Based Compl. Alt. Med.* 2012:132829. doi: 10.1155/2012/132829
- Qian, Z. J., Kang, K. H., and Kim, S. K. (2012). Isolation and antioxidant activity evaluation of two new phthalate derivatives from seahorse, *Hippocampus kuda* Bleeker. *Biotechnol. Bioprocess Eng.* 17, 1031–1040. doi: 10.1007/s12257-012-0115-1
- Qian, Z. J., Ryu, B., Kim, M. M., and Kim, S. K. (2008). Free radical and reactive oxygen species scavenging activities of the extracts from seahorse, *Hippocampus kuda* bleeker. *Biotechnol. Bioprocess Eng* 13, 705–715. doi: 10.1007/s12257-008-0093-5
- Reheman, A., Gao, Z. Y., Tursun, X., Pu, X. P., Wu, T., He, F., et al. (2019). Optimization of extraction technology of majun mupakhi ela and its effect on hydrocortisone-induced kidney yang deficiency in mice. *Sci. Rep.* 9:4628. doi: 10.1038/s41598-019-41006-6
- Rosa, I. M., Alves, R. R., Bonifácio, K. M., Mourão, J. S., Osório, F. M., Oliveira, T. P., et al. (2005). Fishers' knowledge and seahorse conservation in Brazil. *J. Ethnobiol. Ethnomed.* 1:12. doi: 10.1186/1746-4269-1-12
- Ryu, B. (2010). Purification of a peptide from seahorse, that inhibits TPA-induced MMP, iNOS and COX-2 expression through MAPK and NF- $\kappa$ B activation, and induces human osteoblastic and chondrocytic differentiation. *Chem. Biol. Interact.* 184, 413–422. doi: 10.1016/j.cbi.2009.12.003
- Ryu, B., Qian, Z.-J., and Kim, S.-K. (2010). SHP-1, a novel peptide isolated from seahorse inhibits collagen release through the suppression of collagenases 1 and 3, nitric oxide products regulated by NF- $\kappa$ B/p38 kinase. *Peptides* 31, 79–87. doi: 10.1016/j.peptides.2009.10.019
- Sable, R., Parajuli, P., and Jois, S. (2017). Peptides, peptidomimetics, and polypeptides from marine sources: a wealth of natural sources for pharmaceutical applications. *Mar. Drugs* 15:124. doi: 10.3390/md15040124
- Sarlus, H., and Heneka, M. T. (2017). Microglia in Alzheimer's disease. *J. Clin. Invest.* 127, 3240–3249. doi: 10.1172/JCI90606
- Segade, A., Robaina, L., Novelli, B., Otero-Ferrer, F., and Dominguez, L. M. (2016). Effect of the diet on lipid composition and liver histology of short snout seahorse *Hippocampus hippocampus*. *Aquacult. Nutr.* 22, 1312–1319. doi: 10.1111/anu.12341
- She, M., He, G. X., Chen, H., and Jin, Z. J. (1995). An experimental study of 5 kinds of halibios on antiaging action. *Chin. J. Mar. Drugs* 2, 30–34. doi: 10.13400/j.cnki.cjmd.1995.02.008
- Shen, Q., Dai, Z., Huang, Y. W., and Cheung, H. Y. (2016). Lipidomic profiling of dried seahorses by hydrophilic interaction chromatography coupled to mass spectrometry. *Food Chem.* 205, 89–96. doi: 10.1016/j.foodchem.2016.02.151
- Sheng, L., Luo, H. Y., Zhang, J. Q., and Liu, M. S. (2011). Study on processing method of *Hippocampus* preparation. *Chin. Med. J. Res. Prac.* 25, 55–57. doi: 10.13728/j.1673-6427.2011.02.023
- Sherman, A. R. (1992). Zinc, copper, and iron nutrition and immunity. *J. Nutr.* 122(Suppl. 3), 604–609. doi: 10.1093/jn/122.suppl\_3.604
- Shi, J., Su, R. Q., Zhang, W. T., and Chen, J. (2020). Purification and the secondary structure of a novel angiotensin I-converting enzyme (ACE) inhibitory peptide from the alkalase hydrolysate of seahorse protein. *J. Food Sci. Technol.* 57, 3927–3934. doi: 10.1007/s13197-020-04427-0
- Si, X. D., Ge, X. M., Xu, Y. J., and Wang, R. P. (2018). Chemical composition analysis of *Hippocampus kuda* bleeker based on odor objectification. *J. Nuc. Agricult. Sci.* 32, 941–951.
- Song, L. R. (1999). *Chinese Materia Medica (Selected Edition)*. Shanghai: Shanghai Scientific & Technical Publishers.
- Su, Y. T., and Xu, Y. J. (2015a). Study on the extraction and purification of glycoprotein from the yellow seahorse, *Hippocampus kuda* Bleeker. *Food Sci. Nutr.* 3, 302–312. doi: 10.1002/fsn3.219
- Su, Y. T., and Xu, Y. J. (2015b). Study on differences of fatty acids from the farmed and wild seahorses, *Hippocampus kuda* bleeker. *Chin. J. Mar. Drugs* 34, 7–12. doi: 10.13400/j.cnki.cjmd.2015.04.002
- Sun, D., Wu, S., Jing, C., Zhang, N., Liang, D., and Xu, A. (2012). Identification, synthesis and characterization of a novel antimicrobial peptide HKPLP derived from *Hippocampus kuda* bleeker. *J. Antibiot. (Tokyo)* 65, 117–121. doi: 10.1038/ja.2011.120
- Sun, H. H. (2015). *The Effect and Mechanism of Sterol in Hippocampus kuda on Mice Reproductive System*. Master's thesis. Hangzhou: Zhejiang Gongshang University.
- Sun, J. H., Xia, S. D., Xie, S. D., Yang, Y. Q., Cui, P., Shao, P., et al. (2020). Biochemical composition of wild and cultured seahorses (*Hippocampus kuda* bleeker). *Aquac. Res.* 51, 1542–1550. doi: 10.1111/are.14502
- TCMID (2020). *Hippocampus* [Online]. Available online at: <http://47.100.169.139/tcmid/herb/7468> (accessed December 18, 2020).
- Tian, J., Huang, Y., Wu, T., Huang, H. D., Ko, K. M., Zhu, B. T., et al. (2021). The use of chinese Yang/Qi-invigorating tonic botanical drugs/herbal formulations in ameliorating chronic kidney disease by enhancing mitochondrial function. *Front. Pharmacol.* 12:622498. doi: 10.3389/fphar.2021.622498
- Vincent, A. C., Foster, S. J., and Koldewey, H. J. (2011). Conservation and management of seahorses and other syngnathidae. *J. Fish. Biol.* 78, 1681–1724. doi: 10.1111/j.1095-8649.2011.03003.x
- Wang, G. F., Dong, J. C., and Duan, X. H. (2012). Concept of "kidney" in TCM and mechanism, method, prescription, drug & application of kidney-invigoration. *Chin. J. Tradit. Chin. Med. Pharm.* 27, 3112–3115.
- Wang, Q., Zhang, Z. H., Zang, X. X., and Xu, G. J. (1998). Chemical studies on *Hippocampus histrix* kaup. *J. Chin. Pharm. Univ.* 29, 3–5.
- Wang, X. Y. (2015). *Research on Identification and Anti-Osteoporosis Activity of Traditional Chinese Medicine Hippocampus*. Master's thesis. Fuzhou: Fujian University of Traditional Chinese Medicine.
- Watanabe, Y., and Tatsuno, I. (2017). Omega-3 polyunsaturated fatty acids for cardiovascular diseases: present, past and future. *Exp. Rev. Clin. Pharmacol.* 10, 865–873. doi: 10.1080/17512433.2017.1333902
- Wei, N., Xu, W. W., Wei, Q., Gao, B. M., and Wang, Y. (2015). Assay of nucleosides and nucleotides in *Hippocampus* by HPLC. *Guang. Chem. Industry* 43, 128–130.
- Wu, J. N., Liu, Z. Y., Su, J., Lu, H. X., Liao, D. Y., and Song, Q. L. (2017b). Chemical constituents of the seahorse *Hippocampus trimaculatus* from East China Sea. *Chem. Nat. Compd.* 53, 982–983. doi: 10.1007/s10600-017-2178-x
- Wu, J., Chen, X., Pan, N., Chen, B., Zhang, J., and Liu, Z. (2020). 3beta-Hydroxycholest-5-en-7-one from seahorse alleviates lipopolysaccharide-induced inflammatory responses by downregulating miR-98-5p. *Life Sci.* 258:118176. doi: 10.1016/j.lfs.2020.118176
- Wu, J. N., Liu, Z., Su, J., Pan, N., and Song, Q. (2017a). Anti-inflammatory activity of 3beta-hydroxycholest-5-en-7-one isolated from *Hippocampus trimaculatus* leach via inhibiting iNOS, TNF-alpha, and IL-1beta of LPS induced RAW 264.7 macrophage cells. *Food Funct.* 8, 788–795. doi: 10.1039/c6fo01154c
- Wu, Q., Sun, J., Chen, J., Zhang, H., Guo, Y. W., and Wang, H. (2018). Terpenoids from marine soft coral of the genus *lennalia*: chemistry and biological activities. *Mar. Drugs* 16:320. doi: 10.3390/md16090320
- Xu, D. H., and Xu, S. B. (1997). The extract from *hippocampus trimaculatus*(eht): its effect of antithrombosis in rats and ingredients analysis. *Chin. J. Mar. Drugs* 1, 11–13. doi: 10.13400/j.cnki.cjmd.1997.01.004
- Xu, D. H., Wang, L. H., Mei, X. T., Li, B. J., Lv, J. L., and Xu, S. B. (2014). Protective effects of seahorse extracts in a rat castration and testosterone-induced benign

- prostatic hyperplasia model and mouse oligospermism model. *Environ. Toxicol. Pharmacol.* 37, 679–688. doi: 10.1016/j.etap.2014.02.001
- Xu, K. H. (2015). *Study on ACE-Inhibitory and Antioxidant Activities of Three-Spot Hippocampus Hydrolysis Peptides*. Master's thesis. Haikou: Hainan University.
- Xu, S. B., Xu, D. H., Lv, J. Y., Mei, X. T., Wu, Z. F., and Li, B. J. (2002). Prospect of research and development for Chinese medicinal material *Hippocampus* in China. *Chin. Tradit. Herbal Drugs* 33, 82–84.
- Xu, Y. M., Chen, J. W., and Guo, R. (1994). Studies on the phospholipids and fatty acids in traditional chinese medicinal materials *hippocampus* and *syngnathus*. *Chin. J. Mar. Drugs* 4, 14–18. doi: 10.13400/j.cnki.cjmd.1994.01.003
- Xu, Y., Ryu, S., Lee, Y. K., and Lee, H. J. (2020). Brassicasterol from edible aquacultural hippocampus abdominalis exerts an anti-cancer effect by dual-targeting AKT and AR signaling in prostate cancer. *Biomedicines* 8:370. doi: 10.3390/biomedicines8090370
- Yan, L. (2019). *The Relationship Between Inflammation and Autophagy and Autophagy Related mTOR Signaling Pathway in Schizophrenia and the Anti-Neuroinflammatory Effect of Hippocampus erectus Ethanol Extracts*. Master's thesis. Zhanjiang: Guangdong Ocean University.
- Yan, L., Yang, Z. Y., Huang, W. Z., Zhang, Y. P., Yang, W. C., Liu, Y. Y., et al. (2019). Anti-inflammatory and Nitric Oxide(NO) Release Effects of *Hippocampus erectus* Ethanol Extracts on Lipopolysaccharide-induced BV2 Microglia. *J. Guang. Ocean Univ.* 39, 90–96. doi: 10.3969/j.issn.1673-9159.2019.01.014
- Yan, Z. Z. (2019). *Preliminary Studies on Chemical Ecology of Biochemical Composition in Two Seahorses, Hippocampus erectus and H. trimaculatus*. Master's thesis. Shanghai: Shanghai Ocean University.
- Yang, Y., Wang, Z., Gu, Y. L., Xu, Q. P., and Kong, Y. (2014). Study on chemical constituents of *Hippocampus erinaceus*. *J. China Pharm.* 25, 1780–1782. doi: 10.6039/j.issn.1001-0408.2014.19.17
- Yi, M. H., Li, G. Q., Xiao, H., Lin, Z. S., and Zeng, D. Z. (2006). Study on the antioxidative activities of extracts of *Hippocampus trimaculatus* leach and *syngnathus* on fat and oil. *China Trop. Med.* 6, 1784–1785.
- Yu, M. M. (2018). *Study on Preparation and Anti-Tumor Activity of Sphingoid Bases from Hippocampus Trimaculatus Leach*. Master's thesis. Haikou: Hainan University.
- Yuan, X. H., Chen, C. B., Chen, Z., and Yi, M. H. (2011). Study on optimization of enzymatic and the antioxidant activity to DPPH of liquid protein of Three-spot hippocampus. *Sci. Technol. Food Industry* 32, 291–293. doi: 10.13386/j.jssn1002-0306.2011.09.032
- Zhang, D. B. (2021). The meaning and significance of the strict regularity of the concept of "Yin and Yang". *Tradit Chin. Med. J.* 20, 1–3. doi: 10.14046/j.cnki.zyytb2002.2021.01.002
- Zhang, Q., and Wang, Y. L. (1996). Extraction and analysis of fat of *S. acus* linnaeus and *H. japonicus* kaup. *Chin. J. Anal. Chem.* 24, 139–143.
- Zhang, Z. H., Xu, G. J., Xu, L. S., and Wang, Q. (1995). Hormonic effects of the ethanol extracts of *syngnathus*. *J. Chin. Med. Mater.* 18, 197–199. doi: 10.13863/j.issn1001-4454.1995.04.018
- Zhang, Z. H., Xu, G. J., Xu, L. S., and Wang, Q. (1997). Physical and chemical analysis of medicinal animals of *syngnathidae*. *J. Chin. Med. Mater.* 20, 140–144. doi: 10.13863/j.issn1001-4454.1997.03.012
- Zhang, Z. H., Xu, G. J., Xu, L. S., and Wang, Q. (1998). Analysis of steroids in crude drugs by capillary gas chromatography. *J. Chin. Med. Mater.* 21, 81–83. doi: 10.13863/j.issn1001-4454.1998.02.013
- Zhang, Z., Gao, T. H., Fu, C. M., Zhang, J. M., Shi, J. F., He, Y., et al. (2017). Analysis on dosage form theory and current application situation of traditional Chinese medicine pill. *Chin. J. Chin. Mater. Med.* 42, 2408–2412. doi: 10.19540/j.cnki.cjmm.20170416.001
- Zhao, X. F., Xiang, G. X., Shi, C. W., Wang, M. Y., Fu, X. J., and Teng, J. L. (2021). Exploration of marine traditional chinese medicine experience in the use of hippocampus through the ages based on knowledge discovery and associative network construction of formulary composition information. *Modern. Trad. Chin. Med. Mat. Med. World Sci. Technol.* 23, 335–343.
- Zhao, X. Z. (2018). *Study on the Otherness of Chemical Constituents in Hippocampus of Different Commodity Medicinal Materials by Metabolomics Approach*. Master's thesis. Taiyuan: Shanxi University.
- Zheng, L., Chen, J., Zhao, H., Shi, Q., Yang, B., Cheng, H., et al. (2012). Rapid finding and quantification of the major antioxidant in water extracts of three marine drug organisms from China by online HPLC-DAD/MS-DPPH. *Nat. Prod. Res.* 26, 873–877. doi: 10.1080/14786419.2011.565285
- Zhi, D. G., Pang, J. P., Jin, Z. X., Chen, F. R., Wang, Y. J., Fan, D., et al. (2019a). Effect of haima bushen pills on nourishing yin and reinforcing kidney. *China Tradit. Herb Drugs* 50, 2632–2638. doi: 10.7501/j.issn.0253-2670.2019.11.019
- Zhi, D. G., Pang, J. P., Jin, Z. X., Chen, F. R., Wang, Y. J., Fan, D., et al. (2019b). Improvement of haima bushen pills on learning and memory impairment in mice. *Drugs Clin.* 34, 1290–1293. doi: 10.7501/j.issn.1674-5515.2019.05.005
- Zhu, A. M. (2004). Study of toxicity of ethanol extracts from *Hippocampus*. *China Pharm.* 10, 822–824.
- Zhu, A. M. (2005). Pharmacologic researches on ethanol extracts from *Hippocampus*. *Chin. Pharm. Aff.* 19, 23–24. doi: 10.16153/j.1002-7777.2005.01.010

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Cui, Zhao, Li, Li, Ren, Zhao, Fu and Li. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.