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Review

Chemical constituents, pharmacological activities and quality evaluation methods of genus *Hippocampus*: A comprehensive review

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ABSTRACT

The genus *Hippocampus* is a multi-origin animal species with high medicinal and healthcare values. About 57 species of *Hippocampus* spread worldwide, of which about 14 species can be used as medicine, showing anti-oxidation, anti-inflammation, anti-depressant, anti-hypertension, anti-prostatic hyperplasia, antivirus, anti-apoptotic, antifatigue, and so on. And those pharmacological effects are mainly related to their active ingredients, including amino acids, abundant proteins (peptides and oligopeptides), fatty acids, nucleosides, steroids, and other small molecular compounds. The main means of authentication of *Hippocampus* species are morphological identification, microscopic identification, thin layer chromatography method, fingerprint method and genomics method. This review will provide useful insight for exploration, further study and precise medication of *Hippocampus* in the future.

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1. Introduction

Marine biological resources, with their diversity, complexity and specificity, have become an important source of lead compounds for the development of new drugs (Gao et al., 2021; Kumaravel, Ravichandran, Balasubramanian, & Sonneschein, 2012; Stuart, Welsh, Walker, & Edrada-Ebel, 2020). In addition to being a rich source for pharmaceutical drugs, marine natural products are increasingly recognized as a source in the discovery of functional foods and dietary supplements, and provide a useful exploration for breakthroughs in various scientific fields.

Hippocampus, commonly known as the seahorses, is a kind of precious marine medicine with high medicinal and healthcare values. In the traditional Chinese diet, it is often used to make medicated-cuisine and medicated-wines, such as Haima soup, Haima wine et al. (Chen, Shen, Chen, Gao, & Yang, 2015), which have the function of warming kidney to strengthen *yang*, dispersing nodules and detumescence, and relieving cough and asthma (Chinese Pharmacopoeia Commission, 2020). It is known as 'South ginseng' in traditional Chinese medicine (TCM) culture. Pharmacological studies have shown that *Hippocampus* have anti-oxidation (Chen et al., 2010; Kim, Kim, Fernando, & Sanjeewa, 2019; Zheng et al., 2012), anti-inflammation (Chen, Wang, & Huang, 2015; Tharuka, Bathige, & Oh, 2019; Wu et al., 2020), anti-depressant (Li et al., 2020), anti-hypertension (Je et al., 2020), anti-prostatic hyperplasia (Xu et al., 2014), anti-virus (Sandamalika, Samaraweera, Yang, & Lee, 2021; Tharuka, Priyathilaka, Yang, Pavithiran, & Lee, 2019; Udayantha et al., 2021), anti-apoptotic (Kodagoda et al., 2022; Sellaththurai et al., 2020; Wijerathna et al., 2022), anti-fatigue (Guo et al., 2017; Zhang et al., 2019), and other functions (Pangestuti, Ryu, Himaya, & Kim, 2013; Sellaththurai et al., 2020; Yuan et al., 2018). And those pharmacological action are mainly related to their active ingredients, including amino acids (Ge, Gu, & Xu, 2019; Sari, Nurilmala, & Abdullah, 2017; Zhao, 2018), abundant proteins (peptides and oligopeptides) (Je et al., 2020; Pangestuti, Ryu, Himaya, & Kim, 2013), fatty acids (Huang & Xu, 2016; Shen, Dai, Huang, & Cheng, 2016; Su & Xu, 2015), nucleosides (Wei, Xu, Wei, Gao, & Wang, 2015; Zhao et al., 2011), steroids (Wu et al., 2017; Zhao, 2018), and other small molecular compounds (Si, Ge, Xu, & Wang, 2018; Zhao, 2018).

The *Hippocampus* is a multi-origin species with the majority of its distribution in tropical, subtropical and temperate seas, of which 70% is found in the Indian, Pacific and Atlantic Ocean (Lourie, Pollom, & Foster, 2016). The *Chinese Pharmacopoeia* of 2020 edition includes five species of *Hippocampus*, namely *H. kelloggi* Jordan et Snyder, *H. hitrix* Kaup, *H. kuda* Bleeker, *H. trimaculatus* Leach, and *H. japonicus* Kaup (Chinese Pharmacopoeia Commission, 2020). The high economic value of *Hippocampus* has led to a large international trade (Jiang et al., 2018; Marín et al., 2021), which has led to the use of many similar species as commercial *Hippocampus* in TCM market. On the other hand, the identification of *Hippocampus* in the *Chinese Pharmacopoeia* (2020) only has morphological and microscopic methods, without content determination indicators, which poses a great challenge for the market supervision of *Hippocampus* herbs (Chinese Pharmacopoeia Commision, 2020). Therefore, it is crucial to develop an among-species identification method for distinguishing different species of *Hippocampus*.

The literature retrieved was conducted from a number of databases (e.g., PubMed, China National Knowledge Infrastructure (CNKI), Web of Science, Baidu Scholar, Elsevier, Scopus, Springer) to review the biological characterization, chemical constituents, pharmacology, and quality control methods of *Hippocampus* species, which is helpful to establish a more scientific and perfect quality control standard and provide references for the exploitation for new drug of this species. Even it is significant for finding reasonable substitutes species of medicinal *Hippocampus* based on this review.

2. Biological characterization

Genus *Hippocampus*, a kind of marine teleost fish, belongs to the Syngnatidae family, which also includes pipefish and seadragons (Vitturi & Catalano, 1988), and mainly distributed in the Indian, Atlantic and Pacific oceans (Perera, Dahanayaka, & Udagedara, 2017). *Hippocampus* generally inhabits shallow waters above 30 m in tropical and temperate regions, favoring seagrasses and macroalgal (Pereira, Silveira, & Abilhoa, 2018), which have a suite of unusual biological characteristics shared by these species including male pregnancy and monogamy (Holt, Fazeli, & Otero-Ferrer, 2021). In turn, these uncommon characteristics render them extremely vulnerable to environmental impacts, including climate change on the coral reef and bottom trawling causes to seabed habitats destruction (Scales, 2010; Wei, Estalles, Pollom, & Luzzatto, 2017).

The lifespans of *Hippocampus* species range from approximately one year of the species with small niche to approximately 3–5 years for the large niche. The *Hippocampus* species have similar shapes with unique body morphology includes a grasping, finless tail, the head positioned at a right angle to their trunk, a brood pouch sealed along the midline, and a raised dorsal fin base (Aylesworth, Loh, Rongrongmuang, & Vincent, 2017). More than 50 species of the genus *Hippocampus* distributed worldwide (Jiang et al., 2018), and Fig. 1 shows several common *Hippocampus* species in the TCM market.

3. Chemical constituents

With the upgrading and updating of analytical technology, and the continuous innovation of analytical instruments, the complex chemical components in TCM can be well separated and determined, which is of great help in elucidating their pharmacological mechanism of action (Shen, He, & Shi, 2021). Recent studies show

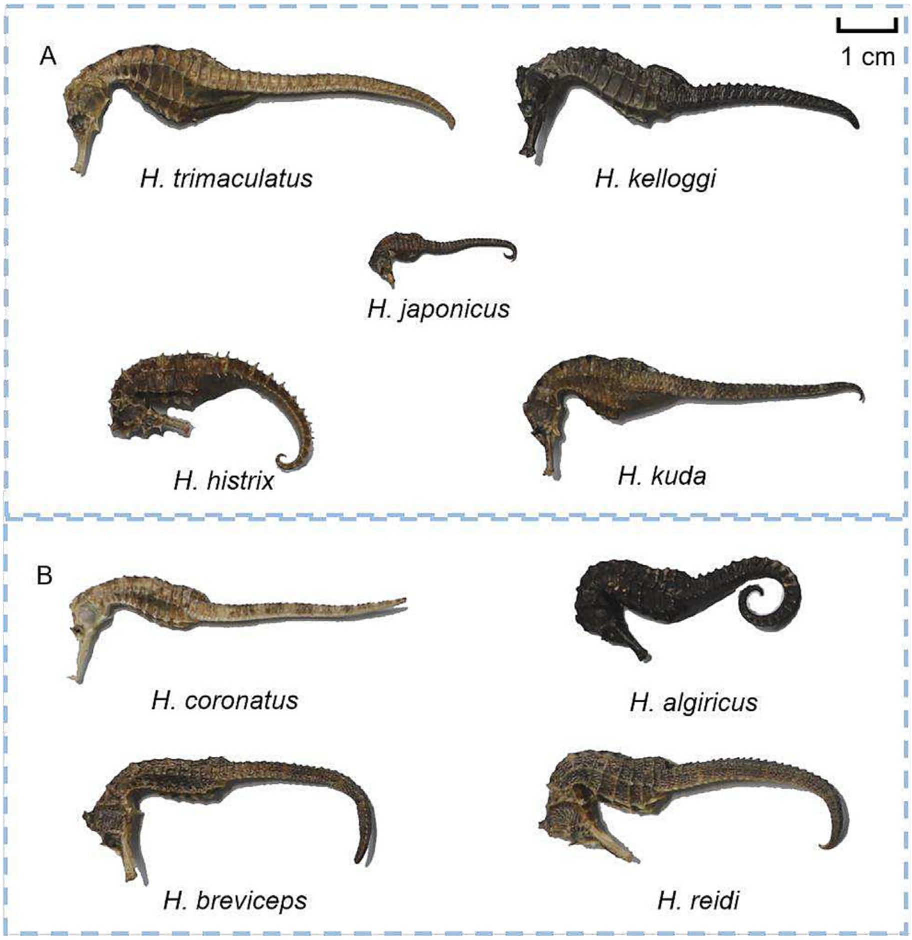

Fig. 1. Several common *Hippocampus* species (A, medicinal; B, adulterants) in TCM market.

that the main chemical constituents of *Hippocampus* include amino acids, polypeptides, steroids, phospholipids, fatty acids, nucleosides, and other compounds.

3.1. Amino acids and polypeptides

Amino acids are natural compounds that are involved in the regulation of various immune activities in the human body and can enhance the immunity of the organism (Bongioanni, Bueno, Mezzano, Longhi, & Garnero, 2021). Zhao et al. (2018) identified 33 compounds including 17 of amino acids and 16 other small molecules from three different *Hippocampus* (*H. kelloggi*, *H. kuda* and *H. trimaculatus*) using ^{1}H NMR (nuclear magnetic resonance) technique. Lin et al. (2008) analyzed the chemical composition of six species of *Hippocampus* from the China coastal and found that the major amino acids (> 5% of the total) in *Hippocampus* were arginine, aspartic acid, glutamic acid, alanine, and glycine. Sari, Nurilmala, & Abdullah, 2017 identified 15 amino acids from the *Hippocampus* including nine essential and six non-essential amino acids. Je et al. (2020) isolated three angiotensin-converting enzyme (ACEs) inhibitory peptides from *Hippocampus* extract, and identified as Ala-Pro-Thr-Leu, Cys-Asn-Val-Pro-Leu-Ser-Pro-Pro-Leu, Pro-Trp-Thr-Pro-Leu with Q-TOF-MS. Kim, Kim, Fernando, & Sanjeewa, 2019 used quadrupole time-of-flight (Q-TOF) mass spectrometer combined with ESI source to study the chemical components with antioxidant activity in *Hippocampus*. The results showed that the tripeptide (Ala-Gly-Asp) had strong antioxidant activity. The chemical structures of amino acids and polypeptides in *Hippocampus* are shown in Table 1 and Fig. 2.

3.2. Fatty acids

Fatty acid components include saturated fatty acids, unsaturated fatty acids, essential fatty acids, triglyceride oils, phospholipids and many other categories, which are the basic substances of life and have the functions of lowering serum cholesterol, improving blood circulation, and preventing cardiovascular diseases (Dyall et al., 2022; Ibarguren, López, & Escribá, 2014). Shen, Dai, Huang, & Cheng, 2016 established a new technology for extraction, visualization and quantitative analysis of phospholipids in *Hippocampus*. After being extracted and purified using solid phase extraction (SPE) technology, the samples were analyzed by hydrophilic interaction liquid chromatography (HILIC) coupled with Q-TOF-MS, and 50 kinds of phospholipid molecules were isolated and identified, including 15 kinds of PCs, 14 kinds of PEs, 12 kinds of PIs and nine kinds PSs. Su & Xu (2015) extracted the fatty acids from *Hippocampus* by CO_2 supercritical fluid extraction technique, and the composition and distribution of fatty acids were determined by gas chromatography-mass spectrometry (GC–MS). Huang & Xu (2016) compared the extraction efficiency of fatty acids from *Hippocampus* under different extraction methods, and analyzed their fatty acid composition by GC–MS. Zhao (2018) used GC–MS analytical techniques combined with chemometric methods to compare the chemical composition of different species of *Hippocampus*. By comparing the standard substances, a total of 42 compounds were identified. It was found that fatty acids were the main factor affecting the difference in chemical compound between the *H. histrix* and the *H. kuda*. The chemical structures of fatty acids in *Hippocampus* are shown in Table 2 and Fig. 3.

Table 1
Amino acids and polypeptides isolated from *Hippocampus*.

No.	Compounds	Analytical techniques	*Hippocams* species	References
1	Isoleucine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
2	Leucine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
3	Valine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
4	Alanine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
5	Arginine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
6	Glutamic acid	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
7	Methionine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
8	Hydroxyproline	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
9	Proline	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
10	Aspartic acid	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
11	Ornithine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
12	Taurine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
13	Lysine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
14	Glycine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
15	Cysteine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
16	Tyrosine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
17	Phenylalanine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
18	Threonine	HPLC	*H. barbourin*, *H. comes* Cantor, *H. kuda*	Sari, Nurilmala, & Abdullah, 2017
19	Serine	HPLC	*H. barbouri*, *H. comes*, *H. kuda*	Sari, Nurilmala, & Abdullah, 2017
20	Histidine	HPLC	*H. barbouri*, *H. comes*, *H. kuda*	Sari, Nurilmala, & Abdullah, 2017
21	Ala-Pro-Thr-Leu	Q-TOF-MS/MS	*H. abdominalis*	Je et al., 2020
22	Cys-Asn-Val-Pro-Leu-Ser-Pro-Pro-Leu	Q-TOF-MS/MS	*H. abdominalis*	Je et al., 2020
23	Pro-Trp-Thr-Pro-Leu	Q-TOF-MS/MS	*H. abdominalis*	Je et al., 2020
24	Ala-Gly-Asp	Q-TOF-MS/MS	*H. abdominalis*	Kim, Kim, Fernando, & Sanjeewa, 2019

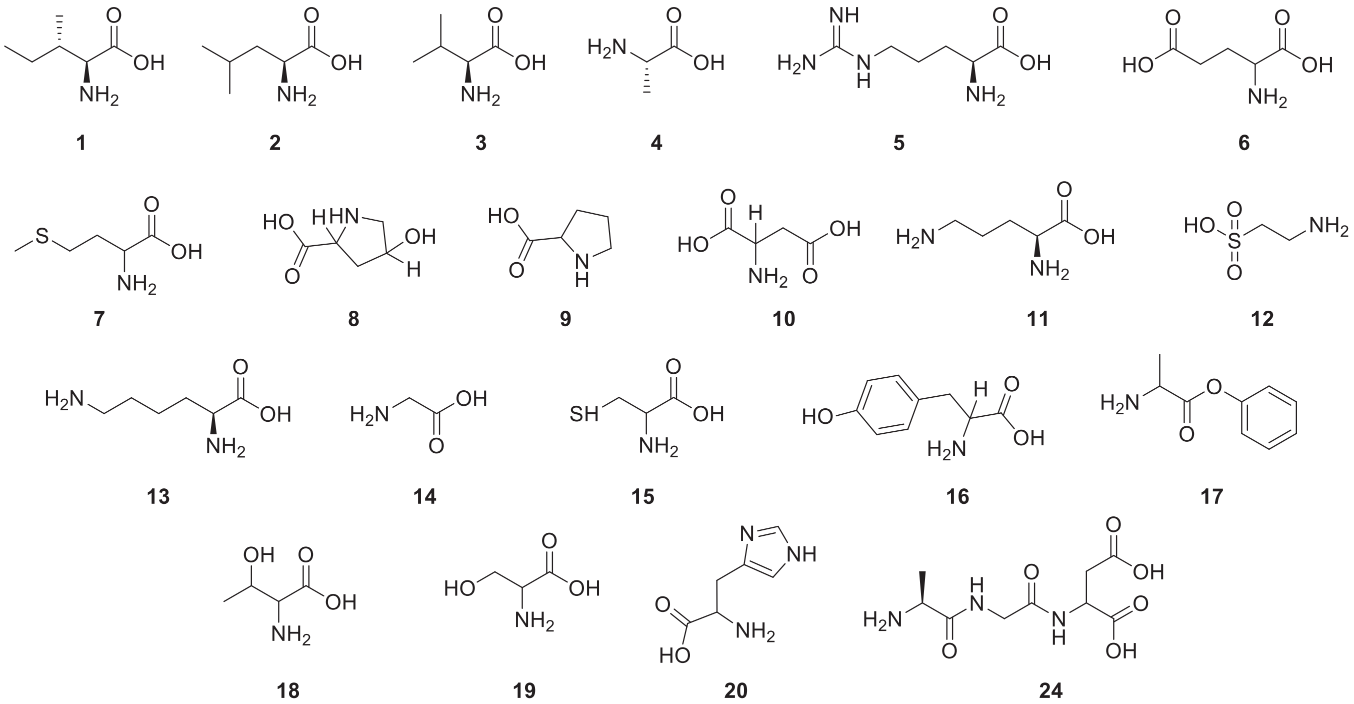

Fig. 2. Chemical structures of amino acids and polypeptides isolated from *Hippocampus* (compounds **21**–**23** are not listed in figure due to compound **24** sampling them).

3.3. Steroids

Steroids are a class of natural chemical components that are widely found in nature. Wu et al. (2017) isolated nine compounds from *H. trimaculatus*, including four steroids. Zhao (2018) found that the content of androst-4-ene-3,17-dione in *H. histrix* was significantly different from that of the other four species of *Hippocampus* in the *Chinese Pharmacopoeia*, and was more suitable for its aphrodisiac effect. The chemical structures of steroids in *Hippocampus* are shown in Table 3 and Fig. 4.

3.4. Nucleosides

Nucleosides are involved in mediating various physiological activities in the body and have a variety of biological activities. Zhao et al. (2011) developed a new method for the simultaneous determination of 16 nucleosides and nucleobases in various marine organism extracts based on ultrasound-assisted extraction (UAE), HILIC and ESI-TOF/MS. All 16 compounds were detected in *Hippocampus* extracts. Yan, Zhang, & Lin, 2019 investigated the nutritional and functional components of *Hippocampus* and identi-

Table 2
Fatty acids isolated from *Hippocampus*.

No.	Compounds	Analytical techniques	*Hippocams* species	References
25	Sphingomyelin	HILIC-Q-TOF-MS/MS	*H. histrix*, *H. trimaculatus*, *H. japonicus*, *H. kelloggi*, *H. spinosissimus*	Shen, Dai, Huang, & Cheng, 2016
26	Phosphatidyl cholines	HILIC-Q-TOF-MS/MS	*H. histrix*, *H. trimaculatus*, *H. japonicus*, *H. kelloggi*, *H. spinosissimus*	Shen, Dai, Huang, & Cheng, 2016
27	Phosphatidyl ethanolamines	HILIC-Q-TOF-MS/MS	*H. histrix*, *H. trimaculatus*, *H. japonicus*, *H. kelloggi*, *H. spinosissimus*	Shen, Dai, Huang, & Cheng, 2016
28	Phosphatidyl inositols	HILIC-Q-TOF-MS/MS	*H. histrix*, *H. trimaculatus*, *H. japonicus*, *H. kelloggi*, *H. spinosissimus*	Shen, Dai, Huang, & Cheng, 2016
29	Phosphatidyl serines	HILIC-Q-TOF-MS/MS	*H. histrix*, *H. trimaculatus*, *H. japonicus*, *H. kelloggi*, *H. spinosissimus*	Shen, Dai, Huang, & Cheng, 2016
30	Diphosphatidylglycerol	HILIC-Q-TOF-MS/MS	*H. histrix*, *H. trimaculatus*, *H. japonicus*, *H. kelloggi*, *H. spinosissimus*	Shen, Dai, Huang, & Cheng, 2016
31	Phosphatidic acid	HILIC-QTOF/MS	*H. histrix*, *H. trimaculatus*, *H. japonicus*, *H. kelloggi*, *H. spinosissimus*	Shen, Dai, Huang, & Cheng, 2016
32	Myristic acid	GC–MS	*H. kelloggi*	Su & Xu, 2015
33	Palmitic acid	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao, 2018
34	6-Hexadecenoic acid	GC–MS	*H. kuda*	Su & Xu, 2015
35	9-Hexadecenoic acid	GC–MS	*H. kuda*	Su & Xu, 2015
36	6,9-Hexadecadienoic acid	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao, 2018
37	Heptadecanoic acid	GC–MS	*H. kuda*	Huang et al., 2016
38	Stearic acid	GC–MS	*H. kuda*	Huang et al., 2016
39	9-Octadecenoic acid	^{1}H NMR	*H. trimaculatus*	Wu et al., 2017
40	12-Octadecenoic acid	GC–MS	*H. kuda*	Su & Xu, 2015
41	8,11-Octadecadienoic acid	GC–MS	*H. kuda*	Su & Xu, 2015
42	9,12-Octadecadienoic acid	GC–MS	*H. kuda*	Su & Xu, 2015
43	6,9,12-Octadecatrienoic acid	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao, 2018
44	Arachidic acid	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao, 2018
45	11-Eicosenoic acid	GC–MS	*H. kuda*,	Su & Xu, 2015
46	5,8-Eicosadienoic acid	HPLC	*H. kuda*, *H. trimaculatus*, *H. kelloggi*, *H. spinosissimus*, *H. histrix*, *H. comes*	Lin et al., 2008
47	5,8,11,14-Eicosatetraenoic acid	GC–MS	*H. kuda*	Su & Xu, 2015
48	Docosanoic acid	GC–MS	*H. kuda*	Su & Xu, 2015
49	13-Docosenoic acid	GC–MS	*H. kuda*	Su & Xu, 2015
50	Docosahexaenoic acid	GC–MS	*H. kuda*	Su & Xu, 2015
51	Tetracosanoic acid	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao, 2018

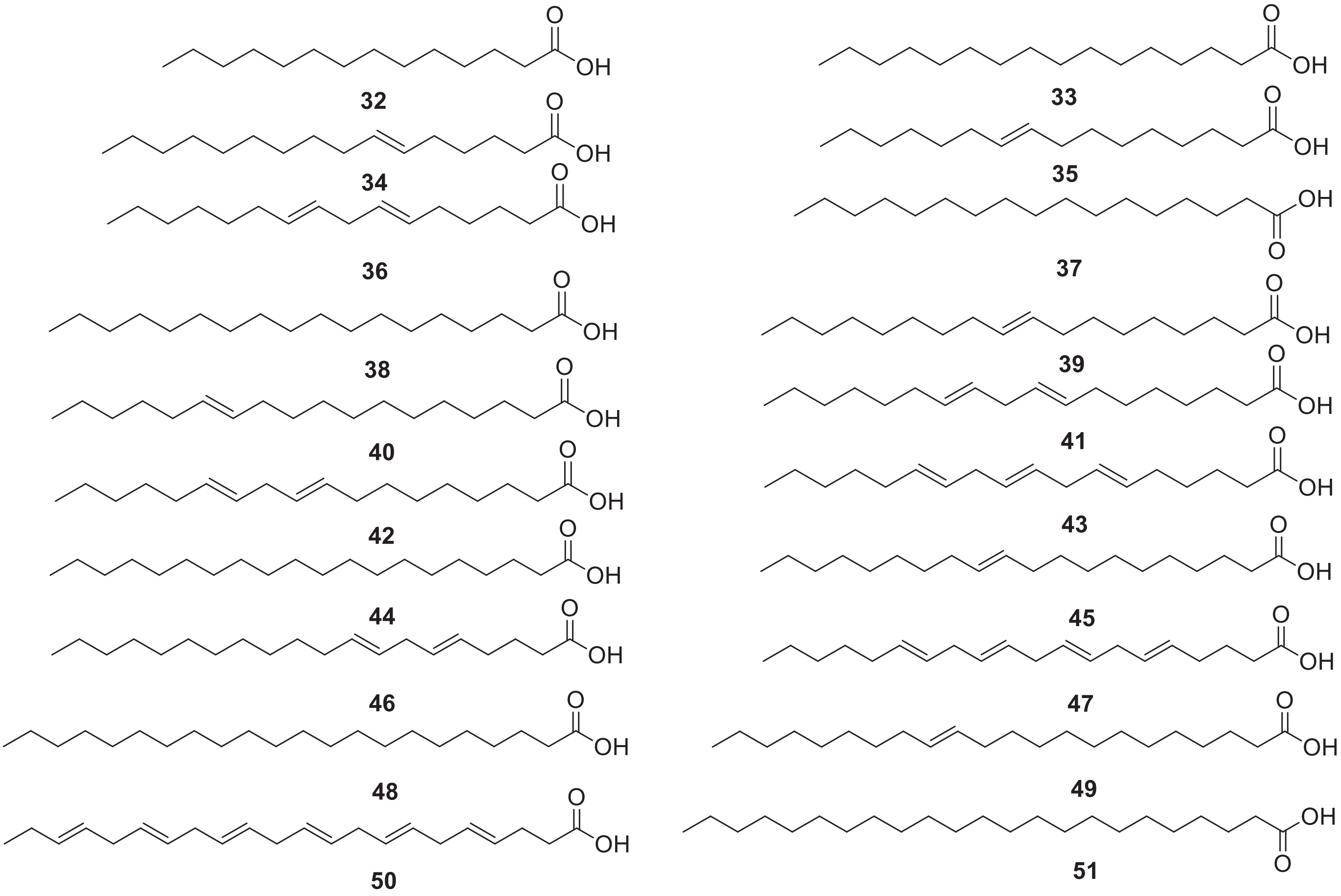

Fig. 3. Chemical structures of fatty acids isolated from *Hippocampus*.

Table 3
Steroids isolated from *Hippocampus*.

No.	Compounds	Analytical techniques	*Hippocams* species	References
52	Cholest-4-en-3-one	^{1}H NMR	*H. trimaculatus*	Wu et al., 2017
53	3β-Hydroxycholest-5-en-7-one	^{1}H NMR	*H. trimaculatus*	Wu et al., 2017
54	Cholest-5-ene-3β, 7β-diol	^{1}H NMR	*H. trimaculatus*	Wu et al., 2017
55	Cholest-5-ene-3β, 7*a*-diol	^{1}H NMR	*H. trimaculatus*	Wu et al., 2017
56	Cholesterol	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao, 2018
57	Cholestane-3,6-dione	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao, 2018
58	Cholesteryl stearate	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao, 2018
59	3β, 5α, 9α-Trihydroxy-ergosterol-7, 22-dien-6-one	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao, 2018
60	24-Methyl-5α-cholest-7,22-dien-3β, 5, 6β-triol	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao, 2018
61	3β-Hydroxy-7-methoxy-cholesta-5-en	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao, 2018
62	Androst-4-ene-3,17-dione	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
63	Cholest-4-ene-3, 6-dione	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
64	1, 2-Epoxycholestan-3-one	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
65	β-Sitosterol	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
66	Cholesta-3, 5-dien-7-one	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018
67	Cholesta-4, 6-dien-3-one	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculatus*	Zhao et al., 2018

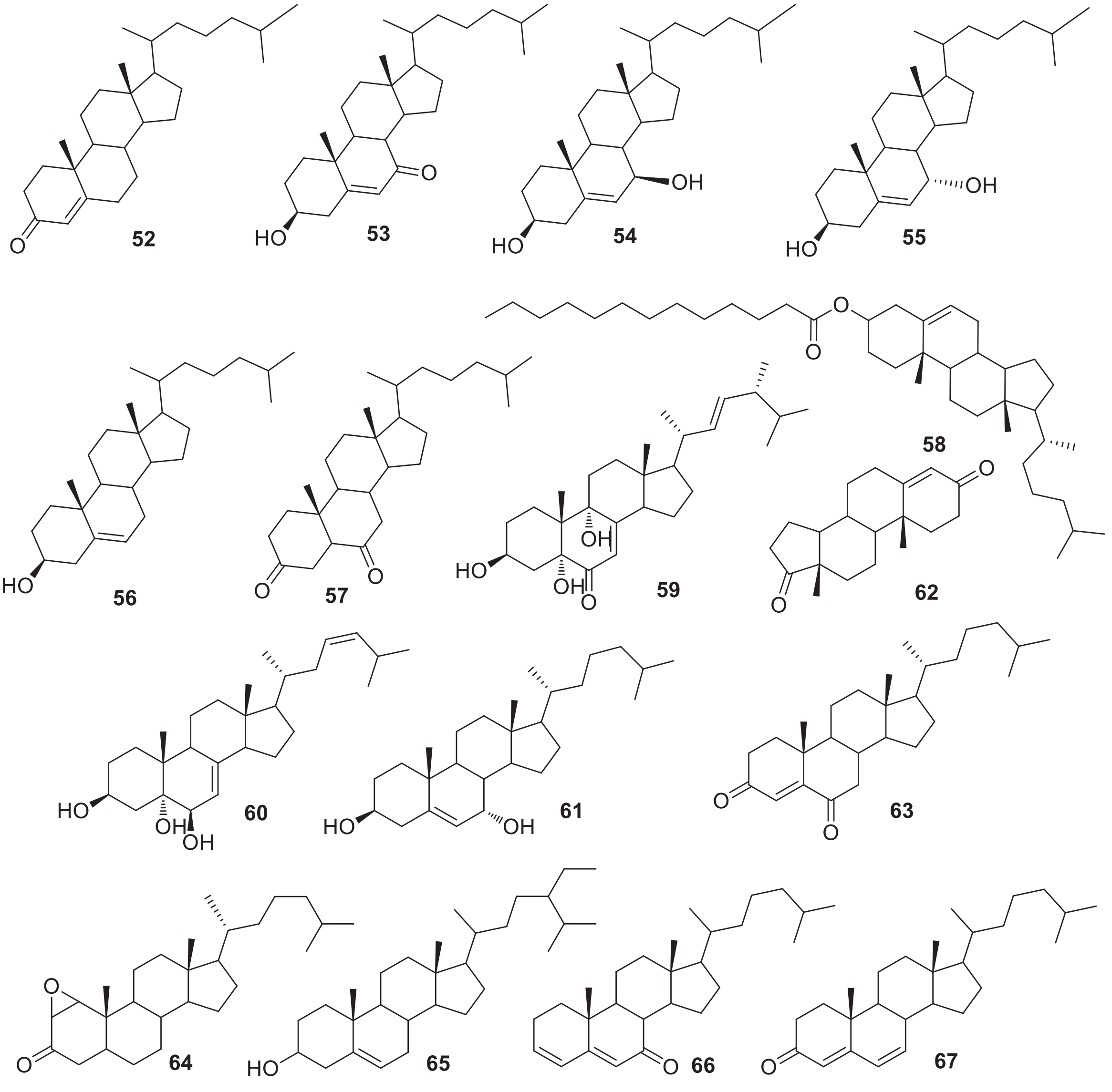

Fig. 4. Chemical structures of steroids isolated from *Hippocampus*.

fied a variety of components including amino acids, nucleosides, and fatty acids. Wei, Xu, Wei, Gao, & Wang, 2015 established a method for simultaneous determination of five nucleosides in *Hippocampus*, which provides assurance for their quality control. The chemical structures of nucleosides in *Hippocampus* are shown in Table 4 and Fig. 5.

3.5. Others

In addition, compounds **85**–**102** (Table 5, Fig. 6) also were isolated from *Hippocampus* (Si, Ge, Xu, & Wang, 2018; Wu, Liu, Su, Pan, & Song, 2017; Zhao et al., 2018).

4. Pharmacological activities

Hippocampus is a kind of tonic herb with the effects of warming kidney to strengthening *yang*, dispersing nodules and detumescence, relieving cough and asthma, relaxing muscles and activating collaterals, relieving pain and inflammation, calming nerves (Chen, Shen, Chen, Gao, & Yang, 2015). Modern pharmacological studies have shown that *Hippocampus* have anti-oxidation, anti-inflammation, anti-depressant, anti-hypertension, anti-prostatic hyperplasia, anti-virus, anti-apoptotic, anti-fatigue, and other functions (Fig. 7).

Table 4
Nucleosides isolated from *Hippocampus*.

No.	Compounds	Analytical techniques	*Hippocampus* species	References
68	Thymine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
69	Uracil	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
70	Thymidine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
71	2′-Deoxyadenosine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
72	Adenine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
73	Uridine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
74	Adenosine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
75	Hypoxanthine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
76	Xanthine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
77	Cytosine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
78	Inosine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
79	2′-Deoxycytidine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
80	Guanine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
81	2′-Deoxyguanosine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
82	Cytidine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
83	Guanosine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011
84	2′-Deoxyinosine	HPLC-TOF/MS	*H. japonicus*	Zhao et al., 2011

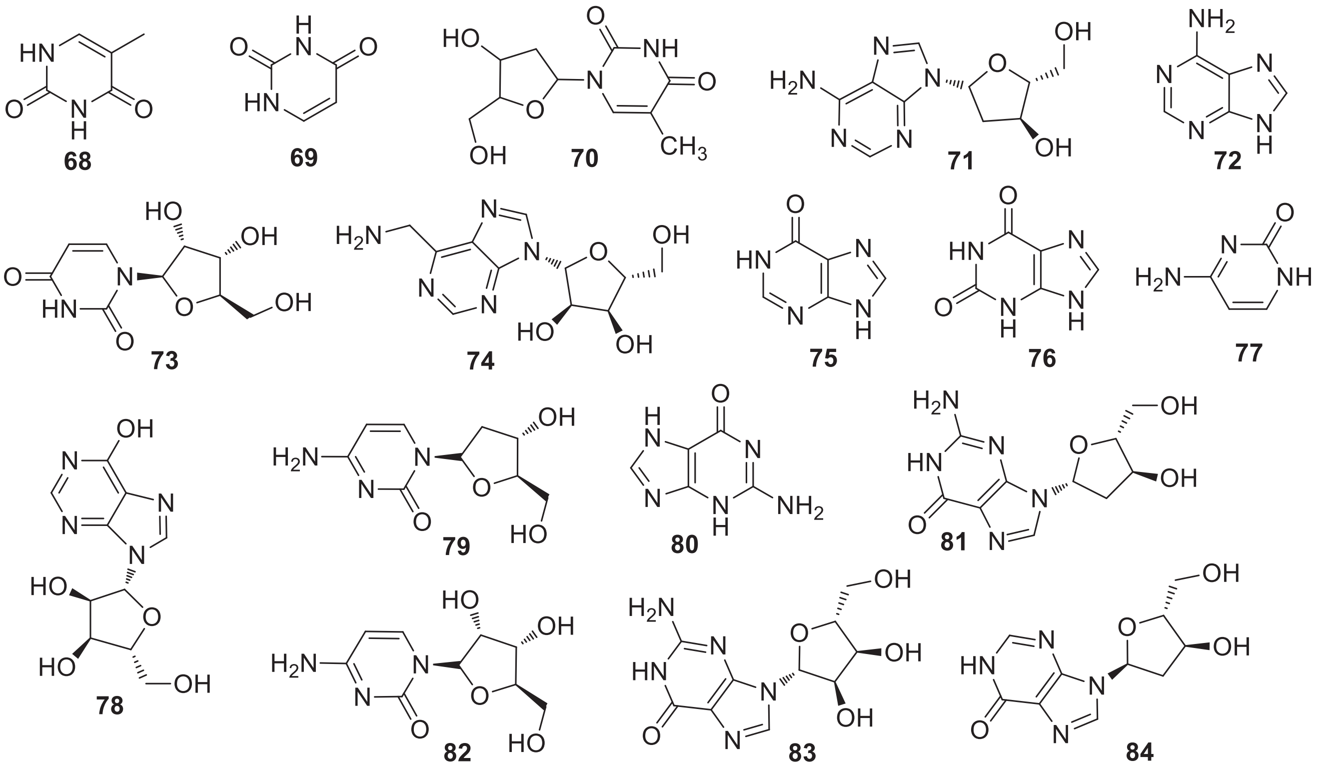

Fig. 5. Chemical structures of nucleosides isolated from *Hippocampus*.

Table 5
Others compounds isolated from *Hippocampus.*

No.	Compounds	Analytical techniques	*Hippocampus* species	References
85	Creatinine	HS-SPME-GC–MS	*H. kuda*	Si, Ge, Xu, & Wang, 2018
86	Bis(2-ethylhexyl) phthalate	^{1}H NMR	*H. trimaculatus*	Wu et al., 2017
87	Dibutylphthalate	^{1}H NMR	*H. trimaculatus*	Wu et al., 2017
88	Glycerol	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao, 2018
89	Xylitol	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao, 2018
90	Inositol	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao, 2018
91	Glucitol	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao, 2018
92	Lactic acid	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao et al., 2018
93	Amber acid	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao et al., 2018
94	Citric acid	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao et al., 2018
95	Creatine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao et al., 2018
96	Choline	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao et al., 2018
97	Betaine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao et al., 2018
98	Taurine	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao et al., 2018
99	Lactose	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao et al., 2018
100	Fumaric acid	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao et al., 2018
101	2-Hydroxy-4-methoxyacetophenone	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao et al., 2018
102	2-Ethyl-11-methylhexadecyl phthalate	^{1}H NMR	*H. kelloggi*, *H. kuda*, *H. trimaculats*	Zhao et al., 2018

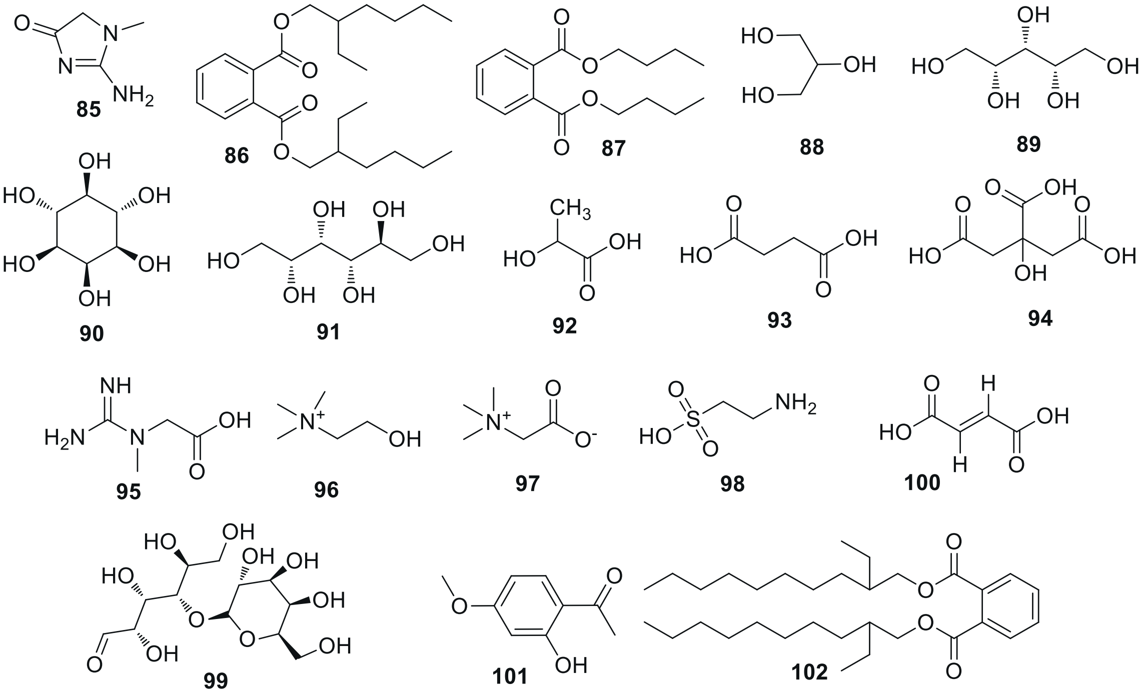

Fig. 6. Chemical structures of other components isolated from *Hippocampus.*

4.1. Anti-oxidation

The active components in *Hippocampus* have scavenging free radicals scavenging and antioxidation effects. Ge, Gu, & Xu, 2019 investigated the antioxidant activity of different types of amino acids. It was found that polar and nonpolar amino acids synergistically increased the scavenging efficiency by increasing the effective concentration of free radical scavenging, and the antioxidant capacity per unit of polar amino acids was about 1.2 times higher than that of nonpolar amino acids. In addition, the antioxidant capacity per unit of aromatic amino acids was about two times higher than that of aliphatic amino acids. In assessing the antioxidant activity of *Hippocampus*, Kim, Kim, Fernando, & Sanjeewa, 2019 found that increasing the protein hydrolysate (HPH) of *H. abdominalis* was effective in reducing the level of reactive oxygen species (ROS) and cell death induced by 2,2-azobis hydrochloride (AAPH) in zebrafish embryo cells. In the study on the change of antioxidant activity of polypeptide compounds in *Hippocampus* extracts during enzymatic hydrolysis, Guo et al. (2017) found that under the optimal conditions, the peptide induced by papain has high antioxidant activities. In the study of the protective and inhibitory effects of alkaline protease on low-density lipoprotein (LDL) oxidation in *Hippocampus* hydrolysate, Oh et al. (2018) found that *H. abdominalia* hydrolysates (SHAH) showed high antioxidant capacity. Nadarajapillai et al. (2021) analyzed the antioxidant properties of glutathione *S*-transferases alpha-4 from *H. abdominalis* (HaGSTA-4). Recombinant HaGSTA-4 was discovered to significantly protect cells from ROS inducers stress. In addition, overexpression of HaGSTA-4 protected cells from H_2O_2-induced oxidative stress. Thioredoxin domain-containing protein 17 (TXNDC17), which contains the structural domain of thioredoxin (Trx), can be involved in maintaining cellular redox homeostasis through thiol-disulfide reductase activity. Liyanage et al. (2019) identified TXNDC17 from *H. abdominalis* to determine free radical

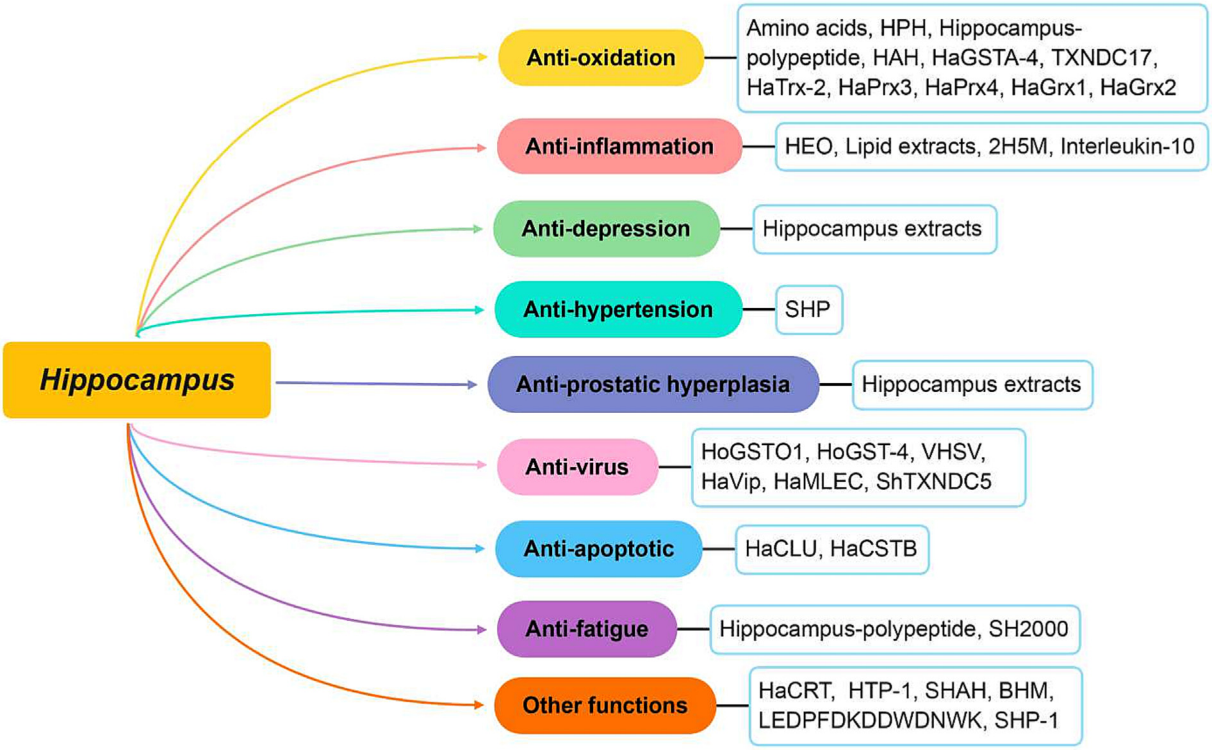

Fig. 7. Health care function and active ingredients of *Hippocampus*.

scavenging ability, antioxidant activity and cell viability. The study results indicate that HaTXNDC17 is involved in the immune mechanism of genus *Hippocampus* and has a role in maintaining cellular redox homeostasis. Trx plays a crucial role in the antioxidant defense system. Nadarajapillai, Sellaththurai, Liyanage, Yang, & Lee, 2020 characterized the mitochondrial Trx protein (HaTrx-2) from *H. abdominalis*, and the study results indicate that rHaTrx-2 had the ability to scavenge the free radicals. Peroxiredoxins (Prxs) are ubiquitously expressed antioxidant proteins that can protect aerobic organisms from oxidative stress. The studies have found that the recombinant *H. abdominalis* peroxiredoxins (HaPrx3, HaPrx4) protein exhibited insulin disulfide reduction activity, peroxidase activity and cell survival ability (Samaraweera et al., 2020; Samaraweera et al., 2021). Glutaredoxins (Grx) are redox enzymes conserved in viruses, eukaryotes, and prokaryotes. The studies have found that the recombinant *H. abdominalis* glutaredoxins (HaGrx1, HaGrx2) exhibited redox activity and cytoprotective activity (Omeka, Liyanage, Priyathilaka, et al., 2019; Omeka, Liyanage, Yang, & Lee, 2019).

4.2. Anti-inflammation

Inflammation is a defense response of the body to stimulation, characterized by redness, swelling, heat and pain. Wu et al. (2017) studied the anti-inflammation activity of 3β-hydroxycholesterin-5-en-7-one (HEO), which was isolated from *H. trimaculatus*. The results showed that HEO can significantly inhibit the expression of inflammatory factors of nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), and interleukin-1β (1L-1β), leading to achieve the purpose of anti-inflammation. On the other hand, Wu et al., (2020) showed that HEO exerted an anti-inflammatory effect via miR-98-5p. Cellular signaling analyses demonstrated that the HEO downregulated the nuclear factor κB (NF-κB) and extracellular signal-regulated kinase (ERK) of mitogen-activated protein kinase (MAPK) signaling pathways. These results lay a foundation for the development of new drug targets. IL-10 is a pleiotropic cytokine involved in the regulation of innate immunity and acquired immunity. In the study of *Hippocampus* IL-10, Tharuka, Priyathilaka, Yang, Pavithiran, & Lee, 2019 found that *H. abdominalis* IL-10 can significantly reduce the protein expression of inducible nitric oxide synthase and cyclooxygenase-2 in raw-264.7 cells induced by lipopolysaccharide (LPS). In the study of anti-inflammation active components in the extract of *H. Trimaculatus* Leach, Chen et al. (2015) found that lipid extracts of *Hippocampus* could inhibit the release of IL-6, IL-1β and TNF-α. Zhang et al. (2019) successfully isolated 2′-hydroxy-5′-methoxyacetophenone (2H5M) from *Hippocampus* extract, and observed the anti-inflammation effect of the compound in BV-2 cells and RAW264.7 cells stimulated by LPS. Molecular docking studies showed that 2H5M could form an active site with NF-κB, indicating that 2H5M has anti-inflammation effect.

4.3. Anti-depression

Hippocampus is an herb with sedative and sleep improving properties. In the study of the main mechanism of antidepressant effect on *Hippocampus*, Li et al. (2020) measured the concentrations of serum corticosterone, glial fibrillary acidic protein (GFAP), brain-derived neurotrophic factor (BDNF), IL-1β, and monoamine neurotransmitters in CUMS-exposed mice after feeding hippocampus diet. It was found that feeding *Hippocampus* could increase the concentrations of neurotransmitters, BDNF, IL-1β and ROS significantly, while the concentrations of IL-10, antioxidant superoxide dismutase and glutathione peroxidase were reduced, and that dietary *Hippocampus* were effective in reversing anxiety and depression-like behaviors.

4.4. Anti-hypertension

Je et al. (2020) elucidated the vasodilation mechanism caused by the inhibition of ACE in *Hippocampus*. It was found that the peptides isolated from *Hippocampus* extracts could cause vasodilation through ACE inhibition, thus reducing the blood pressure of SHR (spontaneously hypertensive rat). Seahorse was hydrolyzed by Protamex (SHP) inhibits angiotensin-converting enzyme secretion and increases nitric oxide production, showing anti-hypertension effect.

4.5. Anti-prostatic hyperplasia

Hippocampus can enhance male function by dilating blood vessels. Xu et al. (2014) investigated the effects of *Hippocampus* spp. extracts in a rat model of benign prostatic hyperplasia (BPH) and oligospermia. It was found that *Hippocampus* extracts reduced the prostate index, increased penile NOS activity, decreased acid phosphatase (ACP) activity and prostatic proliferating cell nuclear antigen (PCNA) and basic fibroblast growth factor (bFGF) expression, and restored sperm viability and motility. *Hippocampus* extract may be a candidate marine drug for BPH.

4.6. Antivirus

Glutathione *S*-transferases (GSTs) are important enzymes involved in phase II detoxification. Udayantha et al. (2021) isolated an omega class GST from *H. abdominalis* (HaGSTO1) to study the defense ability against viral and bacterial infections. It was found that GSTO1 expression is significantly elevated after attack by bacteria and PAMPs, which indicated that HaGSTO1 is involved in the host defense mechanism in *Hippocampus*. Viperin is an antiviral protein. Tharuka, Priyathilaka, Yang, Pavithiran, & Lee, 2019 isolated a viperin homolog from *H. abdominalis* (HaVip) to determine its antiviral activity *in vitro*. It was found that HaVip could trigger antiviral and antibacterial responses, upon viral and bacterial pathogenic infections. Sandamalika, Samaraweera, Yang, & Lee, 2021 identified thioredoxin domain containing from *Hippocampus abdominalis* (ShTXNDC5), which was shown to have a potentially protective role against bacterial and viral invasions.

4.7. Antiapoptotic

Clusterin (CLU) is a glycoprotein that functions in different cell signaling pathways that are associated with various diseases. Wijerathna et al. (2022) investigate the bioactivity of CLU from *H. abdominalis* (HaCLU) on oxidative stress-induced cell death. The study found that HaCLU has anti-apoptotic function, inhibiting H_2O_2-induced oxidative stress and subsequent cell death. Cystatins play a crucial role in diverse pathophysiological conditions in animals. Kodagoda et al. (2022) showed that cystatin B from *H. abdominalis* (HaCSTB) increased the cell viability and reduced cell apoptosis upon VHSV infection. Sellaththurai et al. (2020) identified and characterized malectin from *H. abdominalis* (HaMLEC). It was found that over expression of HaMLEC can down regulate the viral transcription *in vitro*.

4.8. Antifatigue

In the study of anti-fatigue activity of *Hippocampus*, Guo et al. (2017) found that *Hippocampal* polypeptide could prolong the swimming time of mice by 33%–40%, stabilize blood glucose concentration, increase liver glycogen level and reduce blood lactate and blood urea nitrogen level. Zhang et al. (2019) purified and isolated a new peptide (SH200) from *H. abdominalis*. It was found that SH200 can prolong the time of cerebral ischemia–reperfusion in rats, which was a candidate natural preparation for alleviating body fatigue.

4.9. Other functions

Calreticulin (CRT) is a multi-functional, ubiquitous protein known for a variety of cellular functions. Sellaththurai et al. (2020) identified and characterized CRT from *H. abdominalis* (HaCRT) and analyzed the functional properties. The recombinant HaCRT demonstrated the detectable wound-healing ability. Pangestuti, Ryu, Himaya, & Kim, 2013 isolated and identified neuroprotective peptides from *H. trimaculatus* (HTP-1). HTP-1 has the ability to protect PC_{12} cells from $A\beta_{42}$-induced neuronal death, which has the potential to be used in treatment of neurodegenerative diseases, particularly alzheimer disease. Oh et al. (2018) found that SHAH exhibited anti-atherogenic effects in oxidative stress-mediated human umbilical vein endothelial cell. Yuan et al. (2018) isolated active compound 1-(5-bromo-2-hydroxymethoxyphenyl)-ethenone (BHM) from *Hippocampus* and loaded it on TiO_2/Sr doped hydroxyapatite (TiO_2/Srha) composite scaffold to study the controlled release kinetics of BHM. The results showed that the TiO_2/Srha/BHM composite exhibited good biocompatibility at a certain concentration of BHM (20 mol/L). The phenolic compound BHM mediated by the TiO_2/Srha composite scaffold could be used for bone tissue repair. Ryu et al., (2010) found that a peptide (LEDPFDKDDWDNWK, 1821 Da) isolated from genus *Hippocampus* has the function of inducing the differentiation of osteoblast MG-63 and chondrocyte SW-1353 cells. In a study of collagen release in arthritis, Ryu et al., (2010) found that a novel peptide of *Hippocampus* hydrolysis product (SHP-1) has the function of inhibiting collagen and glycosaminoglycan (GAG) release.

5. Quality control methods

5.1. Market analysis of Hippocampus

As a multi-origin species, there are about 57 species of *Hippocampus* distributed worldwide, 14 of which are used as medicine. The high economic value of *Hippocampus* has led to a large international trade of it, and many similar morphological species are used as medicinal *Hippocampus*. For example, *H.* giraffe, which is difficult to distinguish by morphological identification, is often traded as a kind of adulterant of *Hippocampus* (Chen, 2015; Lai et al., 2019). Jiang et al. (2018) identified 23 different species from more than 1 000 *Hippocampus* samples collected from the TCM market, and the five species included in the pharmacopoeia accounted for 44.22% of the total number of *Hippocampus*, among which *H. trimaculatus* and *H. japonicus* accounted for the largest proportion, while the *H. histrix* was the rarest. Among the adulterants, *H. spinosa* and *H. pacific* are most commonly seen, accounting for 18.26% and 11.95% of the total, respectively.

5.2. Morphological identification

The unique body morphology of *Hippocampus* includes a grasping, finless tail, the head positioned at right angle to their trunk, a brood pouch sealed along the midline, and a raised dorsal fin base. He, Zhang, & Huang, 2021 identified different species of *Hippocampus* using the morphological identification method. This method was effective in distinguishing different species of *Hippocampus* circulating in the market, and was simple and fast. The morphological characteristics and geographical origins distribution of the five medicinal *Hippocampus* in *Chinese Pharmacopoeia* (2020) is shown in Table 6.

Table 6
Morphological characteristics and geographical origins distribution of five medicinal *Hippocampus* in *Chinese Pharmacopoeia*.

Names	Geographical origins	Main morphological characteristics	References
H. kelloggi Jordan et Snyder	America, Australia, Caribbean, Cuddalore Coastal Water, Southeast Coastal of India, Coastal of China	Body length 25–30 cm, yellowish white or dark brown	Balasubramanian & Murugan, 2017; Chen, 2015; He, Zhang, & Huang, 2021; Harasti, 2017
H. histrix Kaup	Japan, Singapore, Red Sea, Coastal of China, Hawaiian Islands, French Polynesia, Australia	Body length 12–20 cm, Yellowish white or dark	Chen, 2015; He, Zhang, & Huang, 2021; Stocks, Foster, & Bat, 2017; Wen, Li, Wan, Ren, & Guo, 2013
H. kuda Bleeker	North Korea, Japan, Philippines, Northern Australia, Eastern Africa, Red Sea and Coastal of China	Body length 12–25 cm, yellowish white or dark brown	Chen, 2015; Harasti, 2015; He, Zhang, & Huang, 2021; Wang, 2012
H. trimaculatus Leach	Coastal of China, East Africa and the Indian	Body length 8–16 cm, yellowish white or dark brown	Chen, 2015; Choo & Liew, 2006; He, Zhang, & Huang, 2021
H. japonicus Kaup	Japan, North Korea, Western Pacific, Thailand and Cambodia, Coastal of China, Vietnam	Body length 5–8 cm, dark brown	Chen, 2015; He, Zhang, & Huang, 2021; Thangaraj, Lipton, & John, 2012

Morphological identification can distinguish most species of *Hippocampus*, while a few species of *Hippocampus* are difficult to distinguish, for example, both *H. histrix* and *H. spinosissimus* have many spines on their bodies, which often cause confusion. Moreover, after processing, the appearance characteristics of the *Hippocampus* will change, it is difficult to identify the species by morphological identification alone (Jiang et al., 2017). Therefore, it is necessary to strengthen the basic research on *Hippocampus*, establish its quality control standards, and improve the confusing situation of *Hippocampus* sales.

5.3. Thin layer chromatography (TLC) method

TLC methods use the adsorbent's different adsorption capacity for each component of the sample to achieve the separation of each component (Kucherenko et al., 2019), and is always used for the quality control of TCM, which can reflect their internal quality directly. Wang (2015) used TLC method to study the differences of different *Hippocampus* samples. The results showed that individual variability existed between different *Hippocampus* samples, and for the problem of confusion in the sale of *Hippocampus* in the TCM market, the physical and chemical identification by TLC could achieve the purpose of differentiation, but to identify the variability of different species of *Hippocampus* needs to be studied in combination with other analytical methods.

5.4. Fingerprint method

Fingerprint of TCM is a comprehensive and quantifiable identification method, mainly including chromatographic fingerprints and spectral fingerprints (Wang et al., 2021). Among which, infrared spectroscopy is a qualitative and quantitative analysis method based on the absorption characteristics of infrared radiation (Simbizi, Gahungu, & Nguyen, 2020). Wang (2012) used Fourier transform infrared (FT-IR) combined with cluster analysis to perform spectral scanning of 33 batches of *Hippocampus* raw herbs and their extracts. The results of clustering analysis showed that the IR fingerprint spectra of the raw herbs showed unsatisfactory clustering results and the alcoholic extracts showed better results. High performance liquid chromatography (HPLC) method can separate the complex compound system and form a characteristic chromatogram (Staniak et al., 2020). Wang et al. (2009) established the HPLC fingerprints method for the analysis of *H. japonicus*, and used the similarity calculation software of Chinese medicine fingerprint profile to identify the authenticity and quality evaluation of *H. japonicus*. Li et al. (2009) established an HPLC fingerprints method to authenticity identification of *H. trimaculatus*.

5.5. Genomics method

Genomics technology refers to the combination of DNA sequences with a variety of molecular technical tools, thus elucidating the relationship between the structure and function of the whole genome and the interaction between genes (Gupta, 2008). Among them, DNA barcoding technology has great application potential in wildlife conservation (Galimberti et al., 2019). Sun, Fang, & Lai, 2019 systematically studied the *Hippocampus* by combining the morphological characteristics of *Hippocampus* with DNA barcodes. The results showed that CO-I and ATP6 barcodes could be used as indicators for studying the geographical ecology of vertebrates, which laid a foundation for rapid and accurate identification of medical *Hippocampus* species. Liu et al. (2018) developed a multiplex polymerase chain reaction (mPCR) method to identify the biological origin of *Hippocampus*. Based on the DNA sequence of mitochondria, specific primers for *Hippocampus* were designed. The multiplex PCR technology can be used for the simultaneous identification of complex multi-origin samples, which provides a new idea for quality control of *Hippocampus*. Hou, Wen, Peng, & Guo, 2018 identified nine different species of *Hippocampus* using the *CO-I* gene, and the results showed that the DNA barcode technology based on *CO-I* identify different *Hippocampus* species accurately. Lai et al. (2019) used morphological identification combined with DNA barcodes to conduct a biopharmacological study on *H. japonicus*, which provided support for the identification technology development and genetic diversity study of it. Chen et al. (2019) established a DNA barcoding database of CO-I, 16S rRNA and ATP6 sequences of *H. trimaculatus* and identify *H. trimaculatus* and other adulterants quickly and accurately. Lai et al. (2019) report the complete mitochondrial genome of *H. camelopardalis* Bianconi 1854, which provided essential and important molecular data for evolutionary analysis of genus *Hippocampus*.

Through reviewing the documents, it was found that the quality control methods of animal drugs are not complete, and the relevant literature only has the trait identification and microscopic identification items, lacking quantitative indicators, which may be related to the fact that the material basis of the pharmacological effect of animal drugs is still unclear, it will be an interesting research direction. The *Chinese Pharmacopeia* of 2020 edition stipulates that adenosine can be used as a quantitative indicator of Cordyceps (Qian et al., 2021). Some scholars have similarly determined nucleosides in a variety of marine organisms, which points to a direction for quality control of animal drugs (Zhao et al., 2011).

6. Conclusion

Marine biological resources have become an important source of leading compounds in new drug research and development due to their diversity, complexity and specificity. As a precious marine animal medicine, *Hippocampus* have high medicinal and economic value. However, there is no comprehensive quality evaluation method for *Hippocampus* yet, which seriously affects the further processing of it. Therefore, it is necessary to strengthen the basic research of *Hippocampus*, establish its quality control standards, and improve the confusion status of *Hippocampus* sales. In the *Chinese Pharmacopoeia* of 2020 edition, there are only morphological and microscopic ways for the identification of *Hippocampus*, which requires high artificial experience and operation technology. This paper reviews the biological characterization, distribution, active ingredients, pharmacological actions, species identification and quality analysis methods of *Hippocampus*, which is helpful to establish a more scientific and perfect quality control standard and provide references for the exploitation for new drug of this species. And also, rapid analysis means of *Hippocampus* are needed, such as machine vision technology, spectroscopy and imaging technology, combined with artificial intelligence system, which can realize the rapid identification and automatic sorting of *Hippocampus* medicinal materials.

CRediT authorship contribution statement

Zhiyong Zhang: Conceptualization, Methodology, Software, Writing – original draft. **Xiaoyang Zhang:** Data curation, Software. **Xin Gao:** Data curation, Software. **Xinhao Yan:** Data curation. **Xuting Guo:** Data curation. **Zheng Li:** Writing – review & editing. **Wenlong Li:** Writing – review & editing. Funding acquisition.

Declaration of Competing Interest

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

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References

Aylesworth, L., Loh, T. L., Rongrongmuang, W., & Vincent, A. C. J. (2017). Seahorses (*Hippocampus spp.*) as a case study for locating cryptic and data-poor marine fishes for conservation. *Animal Conservation, 20*(5), 444–454.

Balasubramanian, R., & Murugan, A. (2017). Length-weight relationship of the great seahorse, Hippocampus kelloggi (Jordan and Snyder 1902), inhabiting coromandel coast, southeast coast of India. *Indian Journal Marine Sciences, 46* (6), 1193–1197.

Bongioanni, A., Bueno, M. S., Mezzano, B. A., Longhi, M. R., & Garnero, C. (2021). Amino acids and its pharmaceutical applications: A mini review. *International Journal of Pharmaceutics, 613*, 121375.

Chen, L. (2015). *Study on identification and quality standard of Hippocampus. China (SH): Second Military*. Medical University. Thesis of Master Degree.

Chen, L., Wang, X. Y., & Huang, B. K. (2015). The genus *Hippocampus*–a review on traditional medicinal uses, chemical constituents and pharmacological properties. *Journal of Ethnopharmacology, 162*, 104–111.

Chen, L. P., Shen, X. R., Chen, G. H., Gao, X. Y., & Yang, J. (2015). A comparative study of the effects upon LPS induced macrophage RAW264.7 inflammation *in vitro* of the lipids of *Hippocampus trimaculatus* Leach. Journal of Oleo. *Science, 64*(12), 1273–1281.

Chen, M., Zhu, L. Y., Huang, Z., Ge, Y. Q., Zhang, G. J., & Cheng, R. B. (2019). Molecular identification of *Hippocampus trimaculatus* and its adulterants based on DNA barcoding. *China Traditional and Herbal Drugs, 50*(22), 5554–5562.

Chen, J., Zhao, H., Shi, Q., Zhang, D., Cheng, H., Wang, X., & Lee, S. C. F. (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. *Journal of Separation Science, 33*(4–5), 672–677.

Chinese Pharmacopoeia Commission (2020). *Pharmacopoeia of the People's Republic of China*. Beijing: China Medical Science and Technology Press.

Choo, C. K., & Liew, H. C. (2006). Morphological development and allometric growth patterns in the juvenile seahorse *Hippocampus kuda* Bleeker. *Journal of Fish Biology, 69*(2), 426–445.

Dyall, S. C., Balas, L., Bazan, N. G., Brenna, J. T., Chiang, N., da Costa Souza, F., & Taha, A. Y. (2022). Polyunsaturated fatty acids and fatty acid-derived lipid mediators: Recent advances in the understanding of their biosynthesis, structures, and functions. *Progress in Lipid Research, 86*, 101165.

Galimberti, A., Casiraghi, M., Bruni, I., Guzzetti, L., Cortis, P., Berterame, N. M., & Labra, M. (2019). From DNA barcoding to personalized nutrition: The evolution of food traceability. *Current Opinion in Food Science, 28*, 41–48.

Gao, Y., Xu, Q. N., Cai, M. G., Hu, X. C., Tian, W. J., Chen, H., & F. (2021). Development and utilization of marine-derived medicine and food homologous products. *Chinese Herbal and Traditional Drugs, 52*(17), 5455–5464.

Gupta, P. K. (2008). Single-molecule DNA sequencing technologies for future genomics research. *Trends in Biotechnology, 26*(11), 602–611.

Ge, X. M., Gu, W., & Xu, Y. J. (2019). Relationship of amino acids composition and antioxidative capacity of hydrolyzed protein from *Hippocampus kuda* Bleeker. *Journal of Nuclear Agricultural Sciences, 33*(2), 322–329.

Guo, Z., Lin, D., Guo, J., Zhang, Y., & Zheng, B. (2017). *In vitro* antioxidant activity and *in vivo* anti-fatigue effect of Sea Horse (*Hippocampus*) peptides. *Molecules, 22*(3), 482.

Harasti, D. (2015). Range extension and first occurrence of the thorny seahorse *Hippocampus histrix* in New South Wales. *Australia. Marine Biodiversity Records, 8*, e49.

Harasti, D. (2017). Southwards range extension of the great seahorse (*Hippocampus kelloggi* Jordan & Snyder, 1901) in Australia. *Journal of Applied Ichthyology, 33*(5), 1018–1020.

He, X. H., Zhang, C. Z., & Huang, B. K. (2021). Based on microscopic characteristic authentication for systematic zoology of *Hippocampus*. *Journal of Marine Drugs, 40*(4), 18–26.

Holt, W. V., Fazeli, A., & Otero-Ferrer, F. (2021). Sperm transport and male pregnancy in seahorses: An unusual model for reproductive science. *Animal Reproduction Science, 246*, 106854.

Hou, F. X., Wen, L. L., Peng, C., & Guo, J. L. (2018). Identification of marine traditional Chinese medicine dried seahorses in the traditional Chinese medicine market using DNA barcoding. *Mitochondrial DNA PartA Mapping Sequencing and Analysis, 29*(1), 107–112.

Huang, D., & Xu, Y. J. (2016). Different extraction process for *Hippocampus kuda* lipid and its analysis of the fatty acid composition. *China Journal of Marine Drugs, 35* (2), 35–40.

Jiang, C., Liu, F. Y., Jin, Y., Yuan, Y. Y., Zhao, Y. Y., & Huang, L. Q. (2018). Zoological origin survey of commercial hippocampus in Chinese herbal markets by morphological and DNA sequencing identification. *China Journal of Chinese Materia Medica, 43*(23), 4553–4561.

Jiang, C., Shan, F., Yuan, Y., Liu, Y., Zhan, Z. L., Jin, Y., ... Huang, L. Q. (2017). Exploration and thoughts about zoological origin and macroscopical identification of Chinese material medica "Haima" in *China Pharmacopoeia. China Journal of Chinese Materia Medica, 42*(19), 3836–3842.

Ibarguren, M., López, D. J., & Escribá, P. V. (2014). The effect of natural and synthetic fatty acids on membrane structure, microdomain organization, cellular functions and human health. *Biochimica et Biophysica Acta Biomembranes, 1838*(6), 1518–1528.

Je, J. G., Kim, H. S., Lee, H. G., Oh, J. Y., Lu, Y. A., Wang, L., & Jeon, Y. J. (2020). Low-molecular weight peptides isolated from seahorse (*Hippocampus abdominalis*) improve vasodilation via inhibition of angiotensin-converting enzyme *in vivo* and *in vitro*. *Process Biochemistry, 95*, 30–35.

Kucherenko, E., Kanateva, A., Pirogov, A., & Kurganov, A. (2019). Recent advances in the preparation of adsorbent layers for thin-layer chromatography combined with matrix-assisted laser desorption/ionization mass-spectrometric detection. *Journal of Separation Science, 42*(1), 415–430.

Kim, H. S., Kim, S. Y., Fernando, I. P. S., & Sanjeewa, K. K. A. (2019). Free radical scavenging activity of the peptide from the Alcalase hydrolysate of the edible aquacultural seahorse (*Hippocampus abdominalis*). *Journal of Food Biochemietry, 43*(7), 12833.

Kodagoda, Y. K., Liyanage, D. S., Omeka, W. K. M., Kwon, H., Hwang, S. D., & Lee, J. (2022). Molecular characterization, expression, and functional analysis of cystatin B in the big-belly seahorse (*Hippocampus abdominalis*). *Fish Shellfish Immunology, 124*, 442–453.

Kumaravel, K., Ravichandran, S., Balasubramanian, T., & Sonneschein, L. (2012). Seahorses - A source of traditional medicine. *Natural Product Research, 26*(24), 2330–2334.

Liyanage, D. S., Omeka, W. K. M., Yang, H., Godahewa, G. I., Kwon, H., Nam, B. H., & Lee, J. (2019). Identification of thioredoxin domain-containing protein 17 from big-belly seahorse *Hippocampus abdominalis*: Molecular insights, immune responses, and functional characterization. *Fish Shellfish Immunology, 86*, 301–310.

Liu, F. Y., Yuan, Y., Jin, Y., Qin, W., Jiang, W., Zhao, Y. Y., & Huang, L. (2018). Multiplex PCR to simultaneous identification of five traditional Chinese medicinal seahorses. *China Journal Chinese Materia Medica, 43*(23), 4562–4568.

Li, K., Yan, L., Zhang, Y., Yang, Z., Zhang, C., Li, Y., & Song, C. (2020). Seahorse treatment improves depression-like behavior in mice exposed to CUMS through reducing inflammation/oxidants and restoring neurotransmitter and neurotrophin function. *Journal of Ethnopharmacology, 50*, 112487.

Lai, M., Sun, S., Chen, J., Lou, Z., Qiu, F., Zhang, G., & Cheng, R. (2019). Complete mitochondrial genome sequence for the seahorse adulteration *Hippocampus camelopardalis* Bianconi 1854. *Mitochondrial DNA Part B Resources, 4*(1), 432–433.

Lai, M. R., Sun, S. Y., Chen, J. Z., Lou, Z. H., Qiu, F. M., Zhang, G. J., & Cheng, R. B. (2019). Pharmacognosy study of *Hippocampus japonicus* based on the combination of morphological characteristics and DNA barcodes. *Journal of Chinese Medicinal Materials, 42*(10), 2253–2259.

Lin, Q., Lin, J., Lu, J., & Li, B. (2008). Biochemical composition of six seahorse species, *Hippocampus sp.*, from the Chinese coast. *Journal of the World Aquaculture Society, 39*(2), 225–234.

Li, R., Tang, X. L., Li, G. Q., Zhang, M., Wang, C. Y., & Guan, H. S. (2009). Study on series of HPLC fingerprint II: *Hippocampus trimaculatus*. *Periodical of Ocean University of China, 39*(4), 729–734.

Lourie, S. A., Pollom, R. A., & Foster, S. J. (2016). A global revision of the seahorses *Hippocampus rafinesque* 1810 (Actinopterygii: Syngnathiformes): Taxonomy and biogeography with recommendations for further research. *Zootaxa, 4146*(1), 1–66.

Marín, A., Alfaro, R., Villegas-Llerena, C., Reyes-Flores, L. E., Alvarez-Jaque, I. B., Robles, C., & Zelada-Mázmela, E. (2021). Molecular tools against the illegal exploitation of the threatened Pacific seahorse *Hippocampus ingens* Girard, 1858. *Journal of Nature Conservation, 62* 126030.

Nadarajapillai, K., Liyanage, D. S., Sellaththurai, S., Jeong, T., Lee, S., & Lee, J. (2021). Glutathione-S-transferase alpha-4 in *Hippocampus abdominalis* (big-belly seahorse): Molecular characterization, antioxidant properties, and its potent immune response. *Comparative Biochemistry & Physiology Toxicology Pharmacology, 240* 108917.

Nadarajapillai, K., Sellaththurai, S., Liyanage, D. S., Yang, H., & Lee, J. (2020). Molecular and functional explication of thioredoxin mitochondrial-like protein (Trx-2) from big-belly seahorse (*Hippocampus abdominalis*) and expression upon immune provocation. *Fish Shellfish Immunology, 99*, 495–504.

Oh, Y., Ahn, C. B., Yoon, N. Y., Nam, K. H., Kim, Y. K., & Je, J. Y. (2018). Protective effect of enzymatic hydrolysates from seahorse (*Hippocampus abdominalis*) against H_2O_2-mediated human umbilical vein endothelial cell injury. *Biomedicine Pharmacotherapy, 108*, 103–110.

Omeka, W. K. M., Liyanage, D. S., Priyathilaka, T. T., Godahewa, G. I., Lee, S., Lee, S., & Lee, J. (2019). Glutaredoxin 1 from big-belly seahorse (*Hippocampus abdominalis*): Molecular, transcriptional, and functional evidence in teleost immune responses. *Fish Shellfish Immunology, 90*, 40–51.

Omeka, W. K. M., Liyanage, D. S., Yang, H., & Lee, J. (2019). Glutaredoxin 2 from big belly seahorse (*Hippocampus abdominalis*) and its potential involvement in cellular redox homeostasis and host immune responses. *Fish Shellfish Immunology, 95*, 411–421.

Pangestuti, R., Ryu, B., Himaya, S. W. A., & Kim, S. K. (2013). Optimization of hydrolysis conditions, isolation, and identification of neuroprotective peptides derived from seahorse *Hippocampus trimaculatus*. *Amino Acids, 45*, 369–381.

Pereira, L. F., Silveira, R. B., & Abilhoa, V. (2018). Feeding habits of the seahorse *Hippocampus patagonicus* (Actinopterygii: Syngnathiformes: Syngnathidae) on the southern coast of Brazil. *Acta Ichthyologica Piscatoria, 48*(3), 267–271.

Perera, N., Dahanayaka, D. D. G. L., & Udagedara, S. (2017). Habitat preference and population structure of two data deficient seahorse (Syngnathidae) species. *OUSL Journal, 12*(2), 75–97.

Qian, Z. M., Zi, W. U., Huang, Q., Wang, C. X., Tan, G. Y., & Li, W. J. (2021). Development of an eco-friendly and fast HPLC method for quantitative analysis of four nucleosides in Cordyceps and related products. *Chinese Journal of Natural Medicines, 19*(12), 954–960.

Ryu, B., Qian, Z. J., & Kim, S. K. (2010a). Purification of a peptide from seahorse, that inhibits TPA-induced MMP, iNOS and COX-2 expression through MAPK and NF-B activation, and induces human osteoblastic and chondrocytic differentiation. *Chemico Biological Interactions, 184*, 413–422.

Ryu, B., Qian, Z. J., & Kim, S. K. (2010b). 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- κB/p38 kinase. *Peptides, 31*, 79–87.

Samaraweera, A. V., Liyanage, D. S., Omeka, W. K. M., Yang, H., Priyathilaka, T. T., & Lee, J. (2020). Molecular insights into peroxiredoxin 4 (HaPrx4) from the big-belly seahorse (*Hippocampus abdominalis*): Molecular characteristics, functional activity and transcriptional responses against immune stimulants. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 250*, 110481.

Samaraweera, A. V., Tharuka, M. N., Priyathilaka, T. T., Yang, H., Lee, S., & Lee, J. (2021). Molecular profiling and functional delineation of peroxiredoxin 3 (HaPrx3) from the big-belly seahorses (*Hippocampus abdominalis*) and understanding their immunological responses. *Gene, 771*, 145350.

Sandamalika, W. G., Samaraweera, A. V., Yang, H., & Lee, J. (2021). A newly discovered teleost disulfide isomerase, thioredoxin domain containing 5 (TXNDC5), from big-belly seahorse (*Hippocampus abdominalis*): Insights into its molecular and functional properties and immune regulatory functions. *Development Comparative Immunology, 114*, 103827.

Sari, E. M., Nurilmala, M., & Abdullah, A. (2017). Amino acid profile and bioactive compounds of seahorse *Hippocampus comes*. *Jurnal Ilmu dan Teknologi Kelautan Tropis, 9*(2), 605–617.

Scales, H. (2010). Advances in the ecology, biogeography and conservation of seahorses (genus *Hippocampus*). *Progress in Physical Geography, 34*(4), 443–458.

Stocks, A. P., Foster, S. J., & Bat, N. K. (2017). Catch as catch can: Targeted and indiscriminate small-scale fishing of seahorses in Vietnam. *Fisheries Research, 196*, 27–33.

Si, X. D., Ge, X. M., Xu, Y. J., & Wang, R. P. (2018). Chemical composition analysis of *Hippocampus kuda* Bleeker based on odor objectification. *Journal of Nuclear Agricultural Sciences, 32*(5), 941–951.

Simbizi, R., Gahungu, G., & Nguyen, M. T. (2020). Molecular structure, IR, Raman and UV-VIS spectra of 2-cyanothiophene and 3-cyanothiophene: A comparative quantum chemical investigation. *Spectrochimica Acta Part A: Molecular Biomolecular Spectroscopy, 239*, 118393.

Staniak, M., Wójciak, M., Sowa, I., Tyszczuk-Rotko, K., Strzemski, M., Dresler, S., & Myśliński, W. (2020). Silica-based monolithic columns as a tool in HPLC-an overview of application in analysis of active compounds in biological samples. *Molecules, 25*(14), 3149.

Shen, M. R., He, Y., & Shi, S. M. (2021). Development of chromatographic technologies for the quality control of traditional Chinese medicine in the *Chinese Pharmacopoeia*. *Journal of Pharmaceutical Analysis, 11*(2), 155–162.

Sellaththurai, S., Omeka, W. K. M., Nadarajapillai, K., Shanaka, K. A. S. N., Jung, S., Lee, S., & Lee, J. (2020). Identification, molecular characterization, expression analysis and wound-healing ability of multifunctional calreticulin from big-belly seahorse *Hippocampus abdominalis*. *Fish Shellfish Immunology, 106*, 410–420.

Sellaththurai, S., Shanaka, K. A. S. N., Liyanage, D. S., Yang, H., Priyathilaka, T. T., & Lee, J. (2020). Molecular and functional insights into a novel teleost malectin from big-belly seahorse *Hippocampus abdominalis*. *Fish Shellfish Immunology, 99*, 483–494.

Shen, Q., Dai, Z., Huang, Y. W., & Cheng, H. Y. (2016). Lipidomic profiling of dried seahorses by hydrophilic interaction chromatography coupled to mass spectrometry. *Food Chemistry, 205*, 89–96.

Stuart, K. A., Welsh, K., Walker, M. C., & Edrada-Ebel, R. (2020). Metabolomic tools used in marine natural product drug discovery. *Expert Opinion on Drug Discovery, 15*(4), 499–522.

Su, Y. T., & Xu, Y. J. (2015). Study on differences of fatty acids from the farmed and wild seahorses, *Hippocampus kuda* Bleeker. *Chinese Journal of Marine Drugs, 34* (4), 7–12.

Sun, S. Y., Fang, Y., & Lai, M. R. (2019). Morphological characteristics identification and molecular DNA barcoding analysis of *Hippocampus spinosissimus*. *China Journal of Chinese Materia Medica, 44*(22), 4837–4843.

Thangaraj, M., Lipton, A. P., & John, L. (2012). Genetic diversity of three spotted seahorse, *Hippocampus trimaculatus* (Leach, 1814) in India using four microsatellite loci. *Notulae Scientia Biologicae, 4*(4), 7–13.

Tharuka, M. D. N., Bathige, S. D. N. K., & Oh, M. Y. (2019). Molecular characterization and expression analysis of big-belly seahorse (*Hippocampus abdominalis*) interleukin-10 and analysis of its potent anti-inflammatory properties in LPS-induced murine macrophage RAW 264.7 cells. *Gene, 685*, 1–11.

Tharuka, M. N., Priyathilaka, T. T., Yang, H., Pavithiran, A., & Lee, J. (2019). Molecular and transcriptional insights into viperin protein from Big-belly seahorse (*Hippocampus abdominalis*), and its potential antiviral role. *Fish Shellfish Immunology, 86*, 599–607.

Udayantha, H. M. V., Liyanage, D. S., Nadarajapillai, K., Omeka, W. K. M., Yang, H., Jeong, T., & Lee, J. (2021). Molecular characterization, immune and xenobiotic responses of glutathione S-transferase omega 1 from the big-belly seahorse: Novel insights into antiviral defense. *Fish Shellfish Immunology, 109*, 62–70.

Vitturi, R., & Catalano, E. (1988). Karyotypes in two species of the genus *Hippocampus* (Pisces: Syngnatiformes). *Marine Biology, 99*(1), 119–121.

Wu, J., Chen, X., Pan, N., Chen, B., Zhang, J., & Liu, Z. (2020). 3β-Hydroxycholest-5-en-7-one from seahorse alleviates lipopolysaccharide-induced inflammatory responses by downregulating miR-98-5p. *Life Sciences, 258*, 118176.

Wu, J., Liu, Z., & Su, J. (2017). Chemical constituents of the seahorse *Hippocampus trimaculatus* from east China sea. *Chemistry of Natural Compounds, 53*(5), 982–983.

Wu, J., Liu, Z., Su, J., Pan, N., & Song, Q. (2017). Anti-inflammatory activity of 3β-hydroxycholest-5-en-7-one isolated from *Hippocampus trimaculatus* leach via inhibiting iNOS, TNF-α, and 1L−1β of LPS induced RAW 264.7 macrophage cells. *Food Function, 8*(2), 788–795.

Wang, H., Chen, J. H., Wang, L. L., Zhang, D. L., Wang, X. R., Lee, S. C., & Yang, D. F. (2009). Evaluation of DPPH free radical scavenging capability of *Hippocampus japonicus* Kaup and their HPLC fingerprint. *Chinese Pharmaceutical Journal, 44* (16), 1221–1225.

Wen, L. L., Li, J. D., Wan, D. G., Ren, Y., & Guo, J. L. (2013). Market survey and identification of *Hippocampus* (Haima). *China Journal of Chinese Materia Medica, 38*(7), 969–972.

Wijerathna, H. M. S. M., Nadarajapillai, K., Udayantha, H. M. V., Kasthuriarachchi, T. D. W., Shanaka, K. A. S. N., Kwon, H., & Lee, J. (2022). Molecular delineation, expression profiling, immune response, and anti-apoptotic function of a novel clusterin homolog from big-belly seahorse (*Hippocampus abdominalis*). *Fish Shellfish Immunology, 124*, 289–299.

Wei, N., Xu, W. W., Wei, Q., Gao, B. M., & Wang, Y. (2015). Assay of nucleosides and nucleotides in *Hippocampus* by HPLC. *Guangzhou Chemical Industry, 43*(4), 128–131.

Wei, J., Estalles, M., Pollom, R., & Luzzatto, D. (2017). *Hippocampus patagonicus*. The IUCN Red List of Threatened Species, 2017, e. T195100A54909767.

Wang, X. Y. (2015). *Research on identification and anti-osteoporosis activity of traditional Chinese medicine Hippocampus*. Fujian University of Traditional Chinese Medicine. Thesis of Master Degree.

Wang, X., Liu, X., Wang, J., Wang, G., Zhang, Y., Lan, L., & Sun, G. (2021). Study on multiple fingerprint profiles control and quantitative analysis of multi-components by single marker method combined with chemometrics based on Yankening tablets. *Spectrochim. Spectrochimica Acta Part A: Molecular Biomolecular Spectroscopy, 253*, 119554.

Wang, Y. (2012). *Fingerprint of Syngnathus, Hippocampus and Mussel*. Zhejiang Ocean University. Thesis of Master Degree.

Xu, D. H., Wang, L. H., Mei, X. T., Li, B. J., Lv, J. L., & Xu, S. B. (2014). Protective effects of seahorse extracts in a rat castration and testosterone-induced benign prostatic hyperplasia model and mouse oligospermatism model. *Environmental Toxicology and Pharmacology, 37*(2), 679–688.

Yan, Z., Zhang, D., & Lin, T. A. (2019). Comparative analysis of functional components and nutritional values of female and male *Hippocampus erectus* and *H. trimaculatus*. *Food Science, 40*(16), 206–212.

Yuan, Q., He, L., Qian, Z. J., Zhou, C., Hong, P., Wang, Z., & Li, C. (2018). Significantly accelerated osteoblast cell growth on TiO/SrHA composite mediated by phenolic compounds (BHM) from *Hippocampus kuda* Bleeler. *ACS Applied Materials Interfaces, 10*, 30214–30226.

Zhao, H., Chen, J., Shi, Q., Li, X., Zhou, W., Zhang, D., & Lee, F. S. C. (2011). Simultaneous determination nucleosides in marine organisms using ultrasound-assisted extraction followed by hydrophilic interaction liquid chromatography-electrospray ionization time-of-flight mass spectrometry. *Journal of Separation Science, 34*(19), 2594–2601.

Zheng, L., Chen, J., Zhao, H., Shi, Q., Yang, B., Cheng, H., & Wang, X. (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. *Natural Product Research, 26*(9), 873–877.

Zhao, X. Z. (2018). *Study on the otherness of chemical constituents in Hippocampus of different commodity medicinal materials by metabolomics approach*. Shanxi University. Thesis of Master Degree.

Zhao, X. Z., Zhao, S. J., Tian, J. S., Gao, X. X., Liang, H. D., Hao, J. Q., & Qin, Z. J. (2018). Comparison of chemical constituents in Hippocampus of different breeds by ^{1}H-NMR metabonomics approach. *Chinese Traditional Herbal Drugs, 49*(3), 536–543.

Zhang, Y., Ryu, B., Cui, Y., Li, C., Zhou, C., Hong, P., & Qian, Z. J. (2019). A peptide isolated from *Hippocampus abdominalis* improves exercise performance and exerts anti-fatigue effects via AMPK/PGC-1α pathway in mice. *Journal of Functional Foods, 61*, 103489.

Zhang, Y. Y., Li, C. Y., Zhou, C. X., Hong, P. Z., Zhang, Y., Sun, S. L., & Qian, Z. J. (2019). 2'-Hydroxy-5'-methoxyacetophenone attenuates the inflammatory response in LPS-induced BV-2 and RAW264.7 cells via NF-κB signaling pathway. *Journal of Neuroimmunology, 330*, 143–151.