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Research Article
Systematic Pharmacology Mechanisms of Starfish in the Treatment of Peptic Ulcer

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Abstract

Background and Objective: Starfish has been used to treat gastrointestinal diseases since ancient times. Marine gastric medicine with starfish as the main pharmacodynamic component has been used in the clinic for many years. To explore the pharmacological mechanisms of starfish as a traditional medicine in the treatment of peptic ulcer, this study explored the molecular targets and signal networks of starfish in the treatment of peptic ulcer based on systems pharmacology. **Materials and Methods:** On basis of the PubChem database and SWISS ADME database, four active compounds from Japanese starfish *Aphelasterias japonica* were selected for protein targets prediction and 38 potential protein targets were matched for peptic ulcer. **Results:** Results based on Metascape and ClueGo showed that the pharmacological action of starfish towards peptic ulcer mainly involved in the IL-17 signalling pathway and the process of Helicobacter pylori infecting epithelial cells, Mitogen-Activated Protein Kinases (MAPKs) were the key action targets, such as MAPK1 that could be stably bound by the four active compounds. **Conclusion:** The mechanisms of starfish treating peptic ulcer involves multiple targets, signalling pathways and biological processes, which provides a theoretical basis for developing marine gastric medicine and a direction for further research.

Key words: Starfish, peptic ulcer, systems pharmacology, IL-17 signalling pathways, *Helicobacter pylori*, marine gastric medicine, antioxidant capacity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Starfish firstly recorded in "Medicinal Fauna of China" has been used as medicine for treating hyperacidity, stomachache, diarrhoea, gastric ulcers and duodenal ulcers[1]. Starfish has been reported to have many important bioactive substances, such as polyhydroxysterols, lipids, amino acids and polypeptides but steroids are the most commonly found[1,2]. In northeastern Brazil, dried starfish, such as *Oreaster reticulates*, *Luidia senegalensis* and *Echinaster* sp., were used in traditional medicines to treat asthma, bronchitis, diabetes and heart disease[3,4]. Marine gastric medicine (Hai Yang Wei Yao), a traditional Chinese medicine formula prepared with starfish as the primary ingredient, has been used clinically for many years and can be used for the treatment of spleen and stomach weakness, chills and pain caused by excess gastric acid and ulcer of stomach and duodenum[5,6]. Modern pharmacology proves that starfish has anti-cancer, anti-bacterial, anti-inflammatory, antiviral and other physiological and pharmacological activities[7,8]. Li *et al.*[9] detected the antioxidant capacity and anticancer activity of different solvent extracts from *Acanthaster planci* starfish and found that ethanol fraction of starfish contained better antioxidant effects, while butanol fraction showed the maximum cytotoxicity on human malignant melanoma A375.S2 cells through inducing apoptosis. The study found that starfish are promising candidates for chemoradiation therapy that can reduce the number and size of the colonies of human colorectal carcinoma cells[10].

The strong reproduction and regeneration ability of starfish has caused great loss to coral reefs and marine aquaculture[11] but the marine animal has a great value of medical development and has attracted more attention. Presently, starfish-based drugs and functional preparation, such as starfish nutrient solution and other health products are appearing in the market[12]. However, it is still unknown what targets and associated mechanisms are employed by the main effective constituents of starfish to play a curative effect. Based on network biology and multiple pharmacology, this work explored the potential association between drugs and diseases by constructing active compounds-targets, diseases-targets and their related pathways and biological processes via systems pharmacology[13]. Molecular docking is used to predict the interaction between active components and targets through simulating and calculating the binding strength between small molecules and biological macromolecules[14]. Based on systematic pharmacology method and molecular docking technology, this study explored the targets and signal transduction pathways of starfish in the treatment of peptic ulcer, to provide a theoretical basis for the development of the medicinal value of starfish.

MATERIALS AND METHODS

Study area: The study was carried out at the College of Chemistry and Environmental Science, Guangdong Ocean University, Zhanjiang, China from September, 2020-March, 2021.

Screening of active compounds: The compounds of starfish are not recorded in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database, we refer to some steroid compounds in the reviews[7,15] and download the 2D structure from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The bioavailability score and drug-likeness were used as parameters to screen the candidate active compounds by the Swiss ADME database (http://swissadme.ch/)[16].

Targets prediction of active compounds and diseases: According to the conditions of drug-likeness (Yes of one or more of the five drug-likeness evaluation indicators) and bioavailability score (>0.3), 2D structures of active compounds were subjected to predict the protein targets through PharmMapper database (http://www.lilab-ecust.cn/pharmmapper/)[17]. Searching and screening the protein targets of diseases was conducted in the GeneCards database using the keywords “Peptic Ulcer”, “Duodenal Ulcer”, “Esophagitis Peptic” and “Stomach Ulcer” that were searched from the NCBI’s Medical Subject Headings (MeSH) related to peptic ulcer[18]. The retrieved results were matched with active compounds targets after the removal of duplicates and the Venny plot of active compounds-disease targets was drawn (https://bioinfogp.cnb.csic.es/tools/venny/). Common protein targets of active compounds and disease were classified by Protein Analysis Through Evolutionary Relationships (PANTHER) classification system (http://pantherdb.org/about.jsp)[19]. The active compounds-disease targets was imported into the Reactome database https://reactome.org/ to draw a pathways preview diagram[20].

Biological function and pathway analysis: Biological processes and signalling pathways related to potential targets enrichment analysis of active compounds in starfish were conducted by Metascape (https://metascape.org/)[21]. The top 20 reliability pathways were selected according to the significant difference of enrichment analysis. Determine

Targets distribution in the pathways was determined by KEGG Mapper tools (http://www.genome.jp/kegg/)[22]. Cytoscape's ClueGo plugin was used to analyze the relationship between targets/pathways and biological processes[23].

Molecular docking: MAPK1, the core target with the highest degree in ClueGo, was selected and the crystal structure of MAPK1 (PDB ID: 2OJI)[24] was downloaded from the Protein Data Bank (PDB) database (http://www.rcsb.org/)[25]. PyMoL and Autodocking were used to conduct molecular docking with the active compounds. Then, the interaction between starfish and MAPK1 binding sites was analyzed by LigPlot+v.2.2 software.[26]

RESULTS

Active compounds of starfish: According to the papers and PubChem database search, 110 steroid compounds of starfish were obtained (Table 1). According to the potential druggability, 9 active compounds with bioavailability >0.3 were selected, including methasterone, 3-O-sulfoasterone, 3-O-sulfothornasterol A, forbeside E3, aphelaketotriol, thornasterol asterasterol A, asterasterol B and asterasterol C. Among them, four compounds came from the Japanese starfish *Aphelasterias japonica* (3-O-sulfoasterone, 3-O-sulfothornasterol A, forbeside E3 and aphelaketotriol). To ensure the scientificness and accuracy of the prediction results, Japanese starfish *Aphelasterias japonica* was selected for subsequent experiments.

Identification and classification of common protein targets: The 2D structures of four active compounds from starfish *Aphelasterias japonica* were imported into the Pharmmapper database and 62 protein targets were obtained after removing the duplicates (Fig. 1). In the GeneCards database, targets related to peptic ulcer were searched and compared with the 62 targets of starfish and 38 potential protein targets of starfish for the treatment of peptic ulcer were obtained

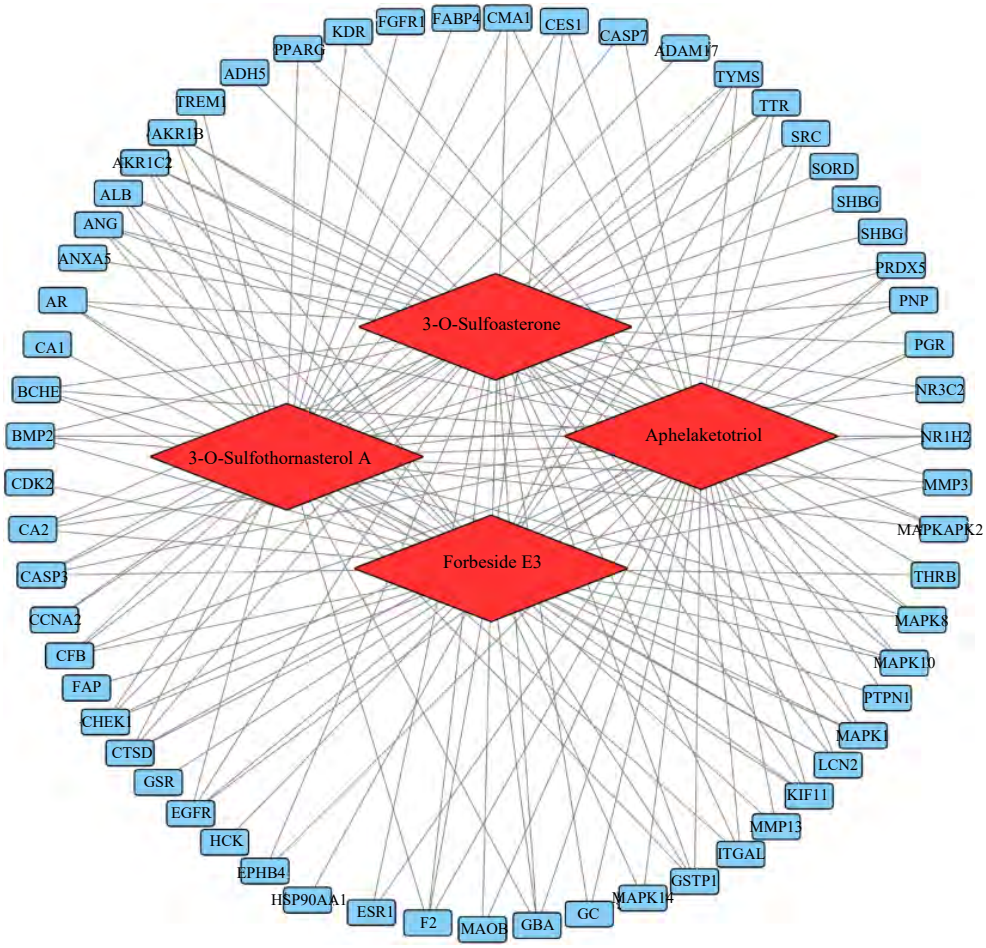

Fig. 1: Targets of active compounds of *Aphelasterias japonica* starfish

Table 1: Chemical constituents from starfish

Sea area	Sources	Compounds names	Drug-likeness					Bioavailability score
			Lipinski	Ghose	Veber	Egan	Muegge	
Sanya Bay, South China Sea	*Anthenea chinensis*	Anthenoside B	NO	NO	NO	NO	NO	0.17
		Anthenoside C	NO	NO	NO	NO	NO	0.17
		Anthenoside D	NO	NO	NO	NO	NO	0.17
		Anthenoside F	NO	NO	NO	NO	NO	0.17
		Anthenoside H	NO	NO	NO	NO	NO	0.17
		Anthenoside I	NO	NO	NO	NO	NO	0.17
		Anthenoside J	NO	NO	NO	NO	NO	0.17
		Anthenoside K	NO	NO	NO	NO	NO	0.17
		CHEMBL1097916	NO	NO	NO	NO	NO	0.17
		CHEMBL1097917	NO	NO	NO	NO	NO	0.17
		CHEMBL1097918	NO	NO	NO	NO	NO	0.17
		CHEMBL1097919	NO	NO	NO	NO	NO	0.17
	Culcita novaeguineae	Novaeguinoside A	NO	NO	NO	NO	NO	0.17
		Novaeguinoside C	NO	NO	NO	NO	NO	0.17
		Novaeguinoside D	NO	NO	NO	NO	NO	0.17
		Novaeguinoside I	NO	NO	NO	NO	NO	0.17
		Novaeguinoside II	NO	NO	NO	NO	NO	0.17
		Marthasterone	YES	NO	YES	YES	NO	0.55
Khuan Lan Island, South China Sea	*Anthenea aspera*	CHEMBL4063624	NO	NO	NO	NO	NO	0.17
		Anthenoside L	NO	NO	NO	NO	NO	0.17
		Anthenoside M	NO	NO	NO	NO	NO	0.17
		Anthenoside N	NO	NO	NO	NO	NO	0.17
		Anthenoside O	NO	NO	NO	NO	NO	0.17
		Anthenoside P	NO	NO	NO	NO	NO	0.17
		Anthenoside Q	NO	NO	NO	NO	NO	0.17
		Anthenoside R	NO	NO	NO	NO	NO	0.17
		Anthenoside S	NO	NO	NO	NO	NO	0.17
		Anthenoside T	NO	NO	NO	NO	NO	0.17
		Anthenoside U	NO	NO	NO	NO	NO	0.17
Tu Long Bay near Khuan Lan Island in the Vietnamese	*Anthenea aspera*	Anthenoside A	NO	NO	NO	NO	NO	0.17
Van Phong Bay off the coast of Vietnam, South China Sea	*Asteropsis carinifera*	Cariniferoside A	NO	NO	NO	NO	NO	0.17
		Cariniferoside B	NO	NO	NO	NO	NO	0.17
		Cariniferoside C	NO	NO	NO	NO	NO	0.17
		Cariniferoside D	NO	NO	NO	NO	NO	0.17
		Cariniferoside E	NO	NO	NO	NO	NO	0.17
		Cariniferoside F	NO	NO	NO	NO	NO	0.17
	Echinaster luzonicus	Luzonicoside A	NO	NO	NO	NO	NO	0.11
Quang Ninh, Vietnam	*Archaster typicus*	Archasteroside A	NO	NO	NO	NO	NO	0.17
		Archasteroside B	NO	NO	NO	NO	NO	0.17
Cat Ba, Haiphong, Vietnam	*Astropecten monacanthus*	Astrosterioside A	NO	NO	NO	NO	NO	0.17
		Astrosterioside B	NO	NO	NO	NO	NO	0.17
		Astrosterioside C	NO	NO	NO	NO	NO	0.17
		Astrosterioside D	NO	NO	NO	NO	NO	0.17
Coast of Komun Island, Korea	*Certonardoa semiregularis*	Acodontasteroside A	NO	NO	NO	NO	NO	0.11
		acodontasteroside B	NO	NO	NO	NO	NO	0.11
		Acodontasteroside C	NO	NO	NO	NO	NO	0.11
		Acodontasteroside D	NO	NO	NO	NO	NO	0.17
		Acodontasteroside E	NO	NO	NO	NO	NO	0.17

Table 1: Continue

Sea area	Sources	Compounds names	Drug-likeness					Bioavailability score
			Lipinski	Ghose	Veber	Egan	Muegge	
		Acodontasteroside F	NO	NO	NO	NO	NO	0.17
		Acodontasteroside G	NO	NO	NO	NO	NO	0.17
		Acodontasteroside H	NO	NO	NO	NO	NO	0.17
		Acodontasteroside I	NO	NO	YES	NO	NO	0.17
		Certonardoside A	NO	NO	NO	NO	NO	0.17
		Certonardoside B	NO	NO	NO	NO	NO	0.17
		Certonardoside B2	NO	NO	NO	NO	NO	0.17
		Certonardoside B3	NO	NO	NO	NO	NO	0.17
		Certonardoside C	NO	NO	NO	NO	NO	0.17
		Certonardoside D	NO	NO	NO	NO	NO	0.17
		Certonardoside E	NO	NO	NO	NO	NO	0.17
		Certonardoside F	NO	NO	NO	NO	NO	0.11
		Certonardoside H	NO	NO	NO	NO	NO	0.17
		Certonardoside H2	NO	NO	NO	NO	NO	0.17
		Certonardoside H3	NO	NO	NO	NO	NO	0.17
		Certonardoside H4	NO	NO	NO	NO	NO	0.17
		Certonardoside I	NO	NO	NO	NO	NO	0.11
		Certonardoside J2	NO	NO	NO	NO	NO	0.17
		Certonardoside J3	NO	NO	NO	NO	NO	0.17
		Certonardoside K	NO	NO	NO	NO	NO	0.17
		Certonardoside O1	NO	NO	NO	NO	NO	0.17
		Certonardoside P1	NO	NO	NO	NO	NO	0.17
Pohang Coast, Korea	*Anaster asminuta*	Anasteroside B	NO	NO	NO	NO	NO	0.17
Posyet Bay, Sea of Japan	*Aphelasterias japonica*	3-O-Sulfoasterone	YES	YES	YES	YES	YES	0.55
		3-O-Sulfothornasterol A	YES	NO	YES	NO	YES	0.55
		Forbeside E3	YES	NO	NO	NO	NO	0.55
		Aphelaketotriol	YES	NO	YES	NO	YES	0.55
		Aphelasteroside C	NO	NO	NO	NO	NO	0.17
		Cheliferoside L1	NO	NO	NO	NO	NO	0.17
Troitsa bay of the Possiet bay, Sea of Japan	*Distolasterias nipon*	(24s)-24-O-(β-d-Xylopyranosyl)-5α-cholestane-3β, 6α, 8, 15β,16β, 24-hexaol	NO	NO	NO	NO	NO	0.17
		(25S)-5α-Cholestan-3β, 4β, 6α, 7β, 8β, 15α,16β, 26-octol	NO	NO	NO	NO	NO	0.17
		Echinasteroside C	NO	NO	NO	NO	NO	0.17
Zampa, Okinawa	*Nardoa tuberculata*	Halityloside A 6-O-sulfate	NO	NO	NO	NO	NO	0.17
		Halityloside A 6-O-sulfate	NO	NO	NO	NO	NO	0.11
		Halityloside A	NO	NO	NO	NO	NO	0.17
		Halityloside B	NO	NO	NO	NO	NO	0.17
		Halityloside D	NO	NO	NO	NO	NO	0.17
		Halityloside E	NO	NO	NO	NO	NO	0.17
		Halityloside F	NO	NO	NO	NO	NO	0.17
Kuril Islands, Sea of Okhotsk	*Hippasteria kurilensis*	Echinasteroside C 15-O-sulfate	NO	NO	NO	NO	NO	0.17
		Kurilensoside D	NO	NO	NO	NO	NO	0.17
		Kurilensoside E	NO	NO	NO	NO	NO	0.17
		Kurilensoside F	NO	NO	NO	NO	NO	0.17

Table 1: Continue

Sea area	Sources	Compounds names	Drug-likeness					Bioavailability score
			Lipinski	Ghose	Veber	Egan	Muegge	
		Kurilensoside G	NO	NO	NO	NO	NO	0.17
		Kurilensoside H	NO	NO	NO	NO	NO	0.17
		Kurilensoside J	NO	NO	NO	NO	NO	0.17
	Henricia leviuscula	Henricioside H2	NO	NO	NO	NO	NO	0.17
Eastern coast, Hokkaido	*Asterias amurensis*	Asterosaponin-1	NO	NO	NO	NO	NO	0.17
		Asteriacerebroside A	NO	NO	NO	NO	NO	0.17
Sendai, Japan/Northern Gulf, Mexico	*Luidia quinaria/Psilaster cassiope*	Psilasteroside	NO	NO	NO	NO	NO	0.17
		Luidiaquinoside	NO	NO	NO	NO	NO	0.17
Northern Gulf, Mexico	*Henricia downeyae*	Downeyoside C	NO	NO	NO	NO	NO	0.11
		Downeyoside D	NO	NO	NO	NO	NO	0.11
		Downeyoside E	NO	NO	NO	NO	NO	0.11
		Downeyoside F	NO	NO	NO	NO	NO	0.11
		Downeyoside G	NO	NO	NO	NO	NO	0.11
		Downeyoside H	NO	NO	NO	NO	NO	0.11
		Downeyoside I	NO	NO	NO	NO	NO	0.11
		Downeyoside J	NO	NO	NO	NO	NO	0.11
		Downeyoside K	NO	NO	NO	NO	NO	0.11
		Downeyoside L	NO	NO	NO	NO	NO	0.11
Patagonian coast, Argentina	*Cosmasterias lurida*	Forbeside H	NO	NO	NO	NO	NO	0.17
		Ophidianoside F	NO	NO	NO	NO	NO	0.17
		Thornasterol A	YES	NO	YES	YES	YES	0.55
Golfo San Jorge near Comodoro Rivadavia, Chubut Province, Argentina	*Anasterias minuta*	Minutoside A	NO	NO	NO	NO	NO	0.17
		Minutoside B	NO	NO	NO	NO	NO	0.17
		Pycnopodioside B	NO	NO	NO	NO	NO	0.11
Philippine sea	*Mediaster murrayi*	Mediasteroside M1	NO	NO	NO	NO	NO	0.17
		Mediasteroside M2	NO	NO	NO	NO	NO	0.17
Tethys Bay	*Asteriidae*	Asterasterol A	YES	NO	YES	NO	NO	0.56
		Asterasterol B	YES	NO	YES	NO	NO	0.56
		Asterasterol C	YES	NO	YES	NO	YES	0.56

Table 2: Classification of common targets of starfish-peptic ulcer

Types	Protein name	Target name
Calcium-binding protein	Annexin A5	ANXA5
Cell adhesion molecule	Integrin alpha-L	ITGAL
Chaperone	Heat shock protein HSP 90-alpha	HSP90AA1
	Peptidyl-prolyl cis-trans isomerase A	PPIA
Gene-specific transcriptional regulator	Peroxisome proliferator-activated receptor gamma	PPARG
Intercellular signal molecule	Bone morphogenetic protein 2	BMP2
Metabolite interconversion enzyme	Alcohol dehydrogenase 1C	ADH1C
	Thymidylate synthase	TYMS
	Transthyretin	TTR
	Glutathione reductase, mitochondrial	GSR
	Glutathione S-transferase P	GSTP1
Protein modifying enzyme	Stromelysin-1	MMP3
	Disintegrin and metalloproteinase domain-containing protein 17	ADAM17
	Mitogen-activated protein kinase 10	MAPK10
	Collagenase 3	MMP13
	Serine/threonine-protein kinase Chk1	CHEK1
	Cyclin-dependent kinase 2	CDK2
	Mitogen-activated protein kinase 1	MAPK1
	Mitogen-activated protein kinase 8	MAPK8
	Caspase-3	CASP3
	Caspase-7	CASP7
	Mitogen-activated protein kinase 14	MAPK14
	Prothrombin	F2
Protein-binding activity modulator	Cyclin-A2	CCNA2
Transfer/carrier protein	Albumin	ALB
	Neutrophil gelatinase-associated lipocalin	LCN2
Transmembrane signal receptor	Epidermal growth factor receptor	EGFR
	Fibroblast growth factor receptor 1	FGFR1
	Vascular endothelial growth factor receptor 2	KDR
Other	Carbonic anhydrase 2	CA2
	Cathepsin D	CTSD
	Complement factor B	CFB
	Estrogen receptor	ESR
	Lysosomal acid glucosylceramidase	GBA
	Mineralocorticoid receptor	NR3C2
	Progesterone receptor	PGR
	Proto-oncogene tyrosine-protein kinase Src	SRC
	Triggering receptor expressed on myeloid cells 1	TREM1

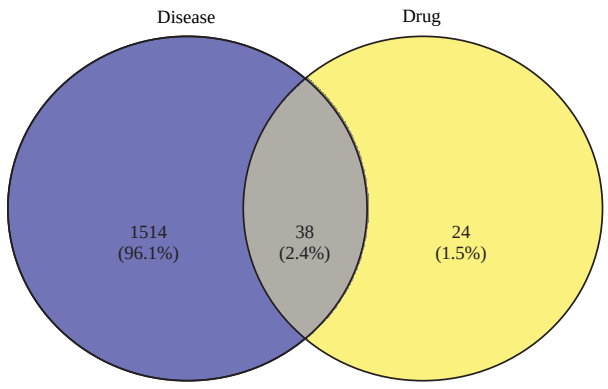

Fig. 2: Venny plot of starfish-peptic ulcer targets

(Fig. 2), accounting for about 61.3% (38/62) of starfish targets and the protein targets were classified (Table 2), most of them were protein-modifying enzymes, metabolite interconversion enzymes and transmembrane signal receptors, indicating that starfish could play a therapeutic effect on peptic ulcer through different targets. According to the pathway preview diagram of potential targets (Fig. 3), these targets are mainly involved in multiple biological processes such as the immune system, signal transduction, cell cycle, programmed cell death and developmental biology.

Bioinformatic annotation of common protein targets: The top 20 pathways of reliability were selected (Fig. 4). Among the 38 potential targets of starfish, 14 targets were involved in the cancer-related pathway. However, the potential targets of starfish were highest significantly correlation with the IL-17 signalling pathway and had the highest enrichment, with the log P value of-14 and the rich factor of 0.096, such as MAPK1, MAPK8, MAPK10, MAPK14, CASP3, LCN2, HSP90AA1, MMP3 and MMP13 that were presented in Fig. 5.

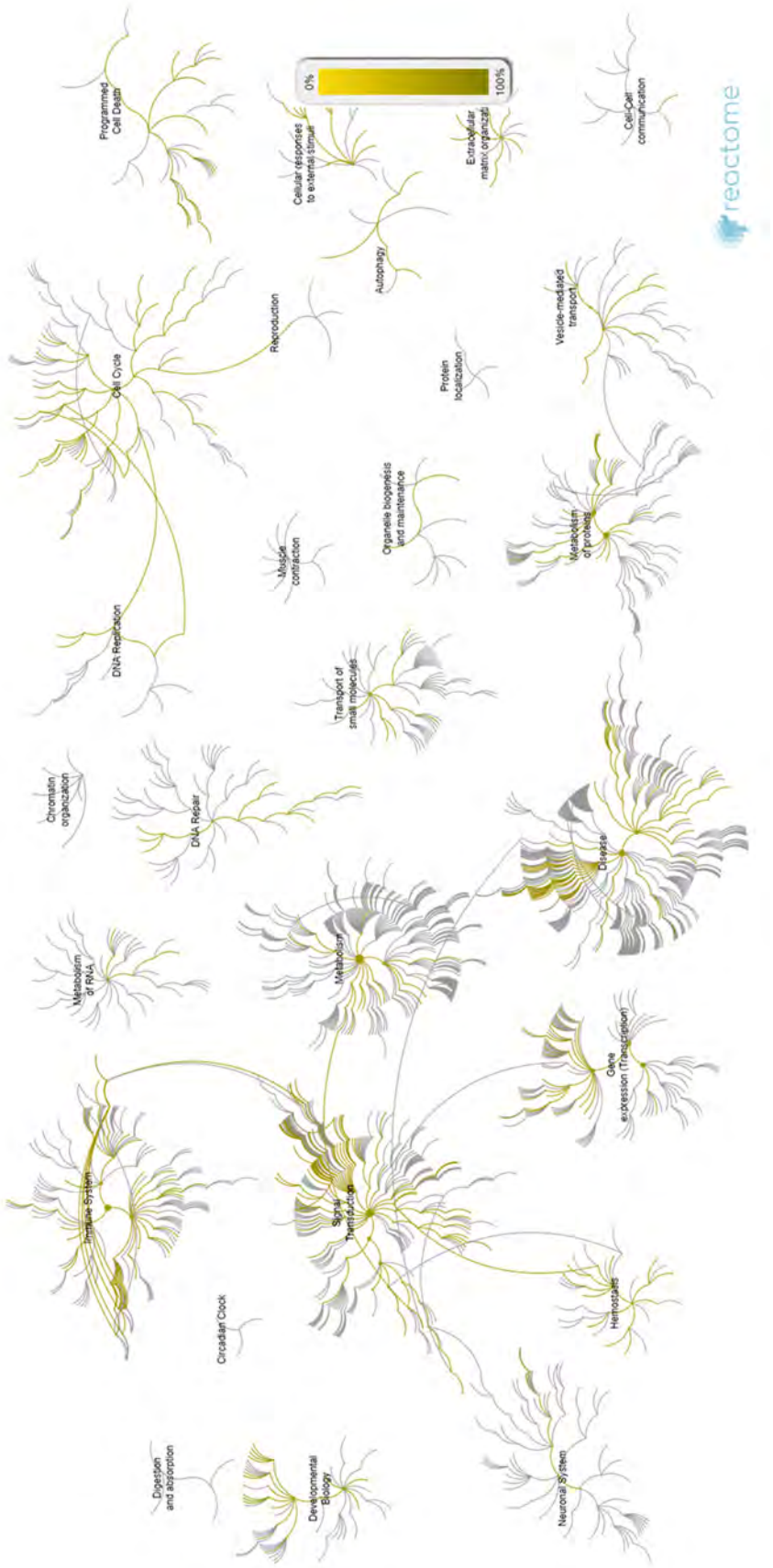

Fig. 3: Pathways preview of starfish-peptic ulcer targets

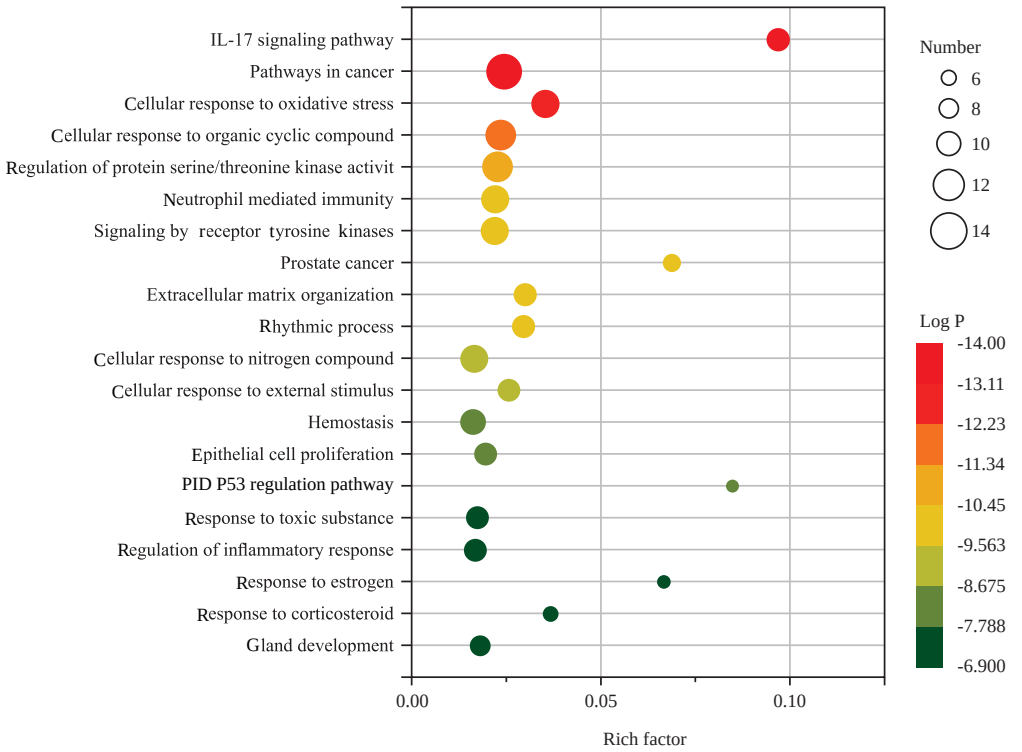

Fig. 4: Bioinformatic analyses of starfish-peptic ulcer targets

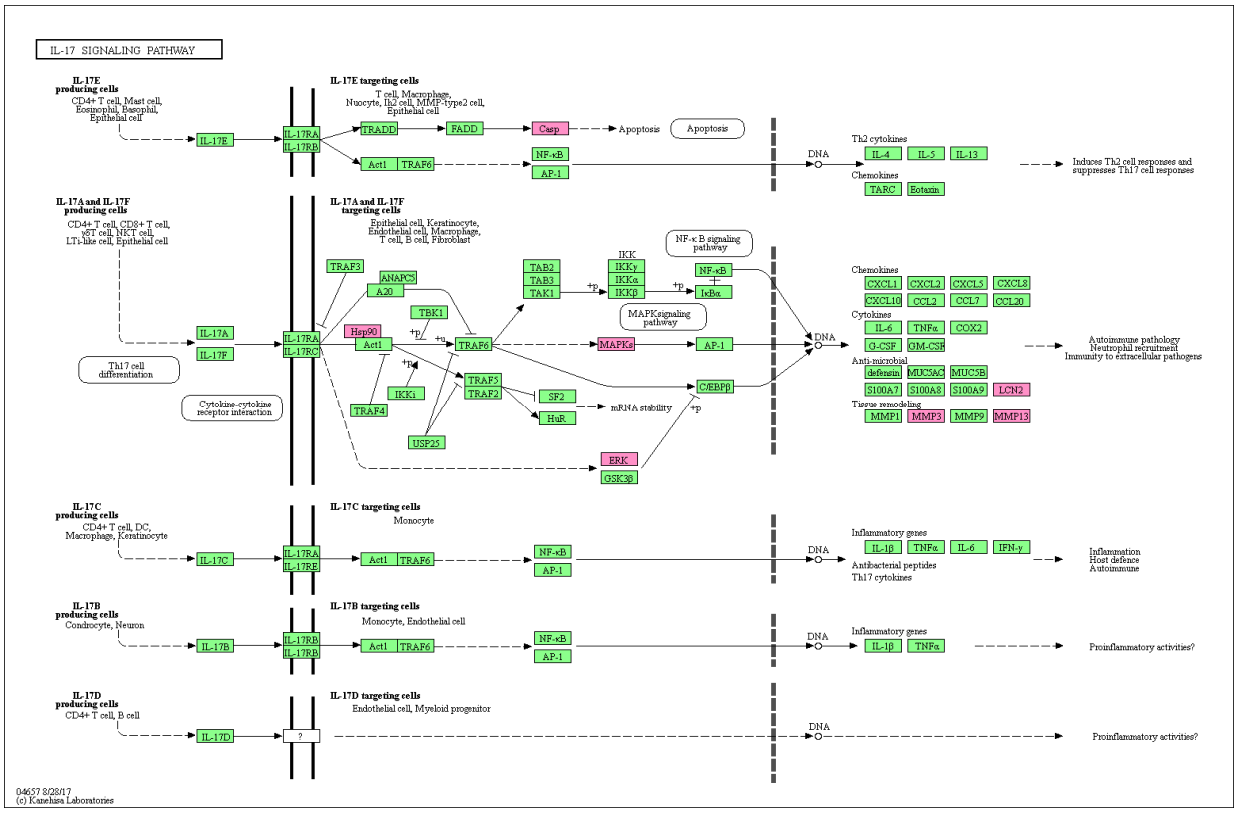

Fig. 5: KEGG annotation (the starfish potential target protein box is highlighted in pink)

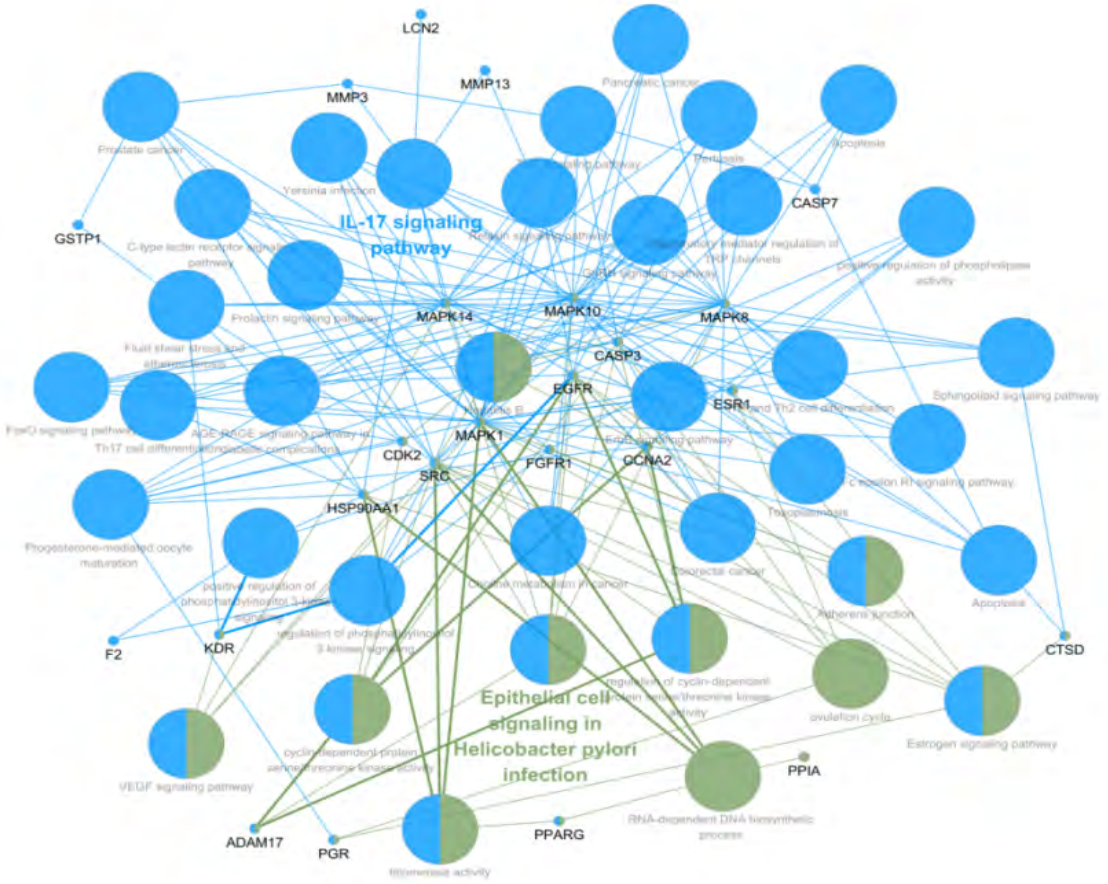

Fig. 6: ClueGo analysis of starfish-peptic ulcer targets

Table 3: Molecular docking results of MAPK1 and active components of starfish

Molecule	Binding energy (kcal mol^{-1})	Number of hydrogen bonds
3-O-Sulfoasterone	-8.6	2
3-O-Sulfothornasterol A	-7.9	5
Aphelaketotriol	-8.4	4
Forbeside E3	-9.6	5

ClueGo analyzed correlations among targeted biological processes showed that all of them were closely related to the IL-17 signalling pathway and the process of *Helicobacter pylori* infecting epithelial cells. Total 24 of 38 potential targets for starfish targeted diseases were intimately involved in the IL-17 signalling pathway or the process of *Helicobacter pylori* infecting epithelial cells and 17 targets were involved in both processes (Fig. 6), suggesting that these targets may be the core targets for starfish to play a role in efficacy, including MAPK1, MAPK10, MAPK8, MAPK14, SRC and CASP3.

Affinities between active ingredients of starfish and key targets: The key target MAPK1 with the highest degree value was molecularly docked with the four active compounds, the docking sites were shown in Fig. 7(a-d). The docking site of MAPK1 and 3-O-sulfoasterone was Met106 (Fig. 7a). The docking sites of MAPK1 and 3-O-sulfothornasterol A were Lys149, ser151 asn152, Met 106 and Asp165 (Fig. 7b). The docking sites of MAPK1 and forbeside E3 were Ala33, Glu69, Ser151, Lys52 and Lys149 (Fig. 7c). And the docking sites of MAPK1 and aphelaketotriol were Lys149, Ser151 and Met106 (Fig. 7d). The binding energy of the four active compounds with MAPK1 were all less than-5, indicating that they can combine stably to form a complex. Among them, forbeside E3 had the lowest binding energy and formed the most hydrogen bonds (Table 3).

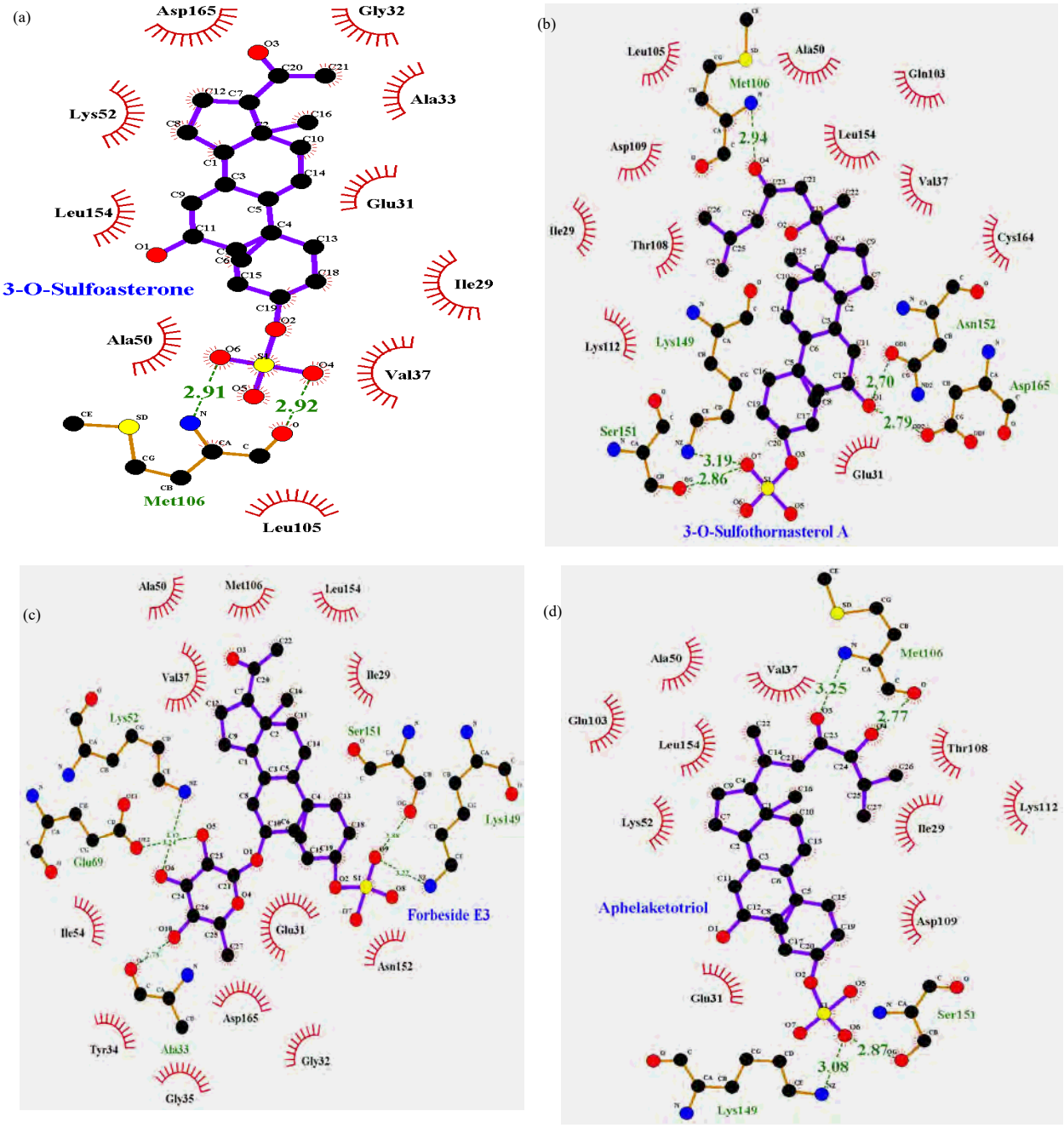

Fig. 7(a-d): Molecular docking model diagram of MAPK1 and active compounds

(a-d) Molecular docking models of MAPK1 with 3-O-sulfoasterone, 3-O-sulfothornasterol A, forbeside E3, and aphelaketotriol, respectively

DISCUSSION

A peptic ulcer is a common and frequently occurring disease that is most commonly found in the stomach and duodenum. Exposure of various pathogenic factors disrupts the equilibrium between mucosal injury factors and mucosal self-defence-repair factors, mediating mucosa to undergo inflammation, necrosis, exfoliation and then ulcer[27]. Among the starfish targets, 61% of them were potential targets for peptic ulcer. According to the classification of potential targets, they mainly belong to protein-modifying enzymes, metabolite interconversion enzymes, transmembrane signal receptors, etc., which are involved in multiple biological processes such as immune system, signal transduction and cell cycle.

Enrichment analysis showed that starfish potential targets were not only involved in the IL-17 signalling pathway but also involved in neutrophil-mediated immunity, cellular response to oxidative stress, regulation of inflammatory response, hemostasis and other biological processes. An ulcer is an inflammatory response, when the gastric mucosa is damaged, a large number of inflammatory factors and mediators are released[28]. IL-17 is a T cell-derived cytokine and can induce the production of proinflammatory cytokines, such as TNF-α, IL-1β and IL-6 and various chemokines like IL-8 and monocyte chemoattractant protein 1[29]. Studies reported that IL-17 stimulates the gastric epithelial cells to release IL-8, which in turn promotes the chemotaxis of neutrophils and the increase of IL-8 levels in gastric mucosa colonized by *Helicobacter pylori*[30,31]. After *Helicobacter pylori* infection, the expression of T cell-derived IL-17 in gastric mucosa of mice was increased and the degree of damage of neutrophil infiltration in the submucosa and the lamina propria was higher than that of IL-17 gene knockout mice, suggesting that IL-17 is involved in the inflammatory response to *Helicobacter pylori* colonization and ultimately affects the development of *Helicobacter pylori*-associated disease[29]. Oxidative stress and consumption of antioxidants are related to the pathophysiology of ulcer[32]. For example, the depletion of antioxidants and the increase of Reactive Oxygen Species (ROS), which play an important role in gastric mucosal damage, have been implicated in the development of gastric ulcers[33].

Investigation shows that more than 90% of duodenal ulcers and 70~80% of gastric ulcers are caused by *Helicobacter pylori* infection[34]. *Helicobacter pylori* stimulate the secretion of gastrin, thus stimulates parietal cells and chief cells to secret gastric acid and pepsin secretion, eventually destroy the gastric mucosal barrier, resulting in ulcers or slowing ulcer healing[35]. ClueGo analysis showed that starfish was closely related to the IL-17 signalling pathway and the process of *Helicobacter pylori* infecting epithelial cells. According to the degree value of targets, the core targets were mainly MAPKs, caspase, proto-oncogene tyrosine-protein kinase src and the transmembrane signal receptor. ERK, p38 MAPK and JNK are important members of the MAPKs family. Studies have shown that down-regulation of gene and protein expression levels of JNK and p38 MAPK can inhibit abnormal secretion of inflammatory cytokines TNF-α, IL-1β and IL-6 and plays a protective role in gastric mucosa[36]. ERK is mediated by receptor tyrosine kinase, Ca^{2+} and protein kinase C[37,38], forming active p-ERK. Studies have shown that increased p-ERK content can alleviate mucosal injury of gastric ulcer and plays an important role in protecting gastric mucosa and promoting gastric mucosal proliferation and repair[39,40]. The healing of an ulcer requires the reconstruction of epithelial structure and underlying connective tissue, involving cell proliferation and angiogenesis[41,42]. EGFR, KDR and FGFR are involved in angiogenesis and play an active role in the healing process of ulcer[43]. Studies have shown that inhibition of the VEGF-KDR-ERK signal transduction pathway can inhibit gastric mucosal cell proliferation and mucosal/submucosal angiogenesis in gastric ulcer healing and delay the healing process of gastric ulcer in rats[42]. In the process of gastric mucosa repair, the proliferation and migration of mucosal epithelium lead to the re-epithelialization of ulcer, which is mainly dependent on the effect of EGF and EGFR[44]. EGF binding to EGFR activates the MAPKs system through a series of processes, which eventually leads to the increase of the transcription level of early response genes in the nucleus and promotes the proliferation, differentiation and maturation of epithelial cells[45]. The study found that level of EGFR is closely related to the recurrence of ulcerative colitis[46], chronic inflammatory stimulation induces mutations of genes related to cell proliferation regulation through oxidative stress injury, leading to accelerated proliferation and transport of colonic epithelial cells and then dysplasia and canceration[47]. And EGFR signalling pathways involved in ulcerative colitis-associated colorectal cancer development and it has a certain relationship with the progression and metastasis of colorectal cancer[47,48]. As a key effector protease in apoptosis signal transduction, down-regulation of CASP3 can prevent the apoptosis of gastric mucosal epithelial cells, thereby increasing the production of prostaglandin E2 and thus playing a protective role in the stomach[49,50]. To verify whether the active compounds of starfish target the biological process of IL-17 signalling pathway and *Helicobacter pylori* infecting epithelial cells signalling, molecular docking method was used to analyze the feasibility of starfish active compound binding to key target MAPK1 and it was found that all four active compounds could bind with MAPK1 to form a stable conformation, which supports that starfish is effective in treating peptic ulcer via regulating key protein targets.

CONCLUSION

In summary, this study preliminarily explored the potential targets and pharmacodynamic mechanism of Japanese starfish in the treatment of peptic ulcer based on systems pharmacology, speculated that the active compounds associated efficacy is closely related to IL-17 signalling pathway and epithelial cells signalling in *Helicobacter pylori* infection, This work provides a theoretical basis for the application of starfish in marine gastric medicine, explains the mechanism of traditional drug effect.

SIGNIFICANCE STATEMENT

This study exposed that the treatment of peptic ulcer by starfish was related to the IL-17 signalling pathway and epithelial cells signalling in *Helicobacter pylori* infection based on systematic pharmacology. This study will provide a theoretical basis for the application of starfish in Marine gastric medicine and will help researchers to know the pharmacological mechanism of starfish. Thus, the medicinal value of starfish will be further developed and applied.

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