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Polysaccharides from Marine *Enteromorpha*: Structure and function

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ABSTRACT

Background: Marine *Enteromorpha* polysaccharides (EPPs) have been reported to present various physiological and bioactivities based on their specific chemical structures, such as monosaccharide compositions and glycosidic linkages.

Scope and approach: However, the structure-activity relationship of *Enteromorpha* polysaccharides is mostly unknown. This review aims to provide a brief summary of structural, functional, and chemical characteristics of *Enteromorpha* polysaccharides.

Key findings and conclusions: Modified *Enteromorpha* polysaccharides have several powerful bioactive functions. Sulfated EPPs with lower molecular weights were found to exhibit excellent immune-relevant and antioxidant activities. Carboxymethylated, hydroxamated, phthaloyl, and acetylated derivatives also showed significantly enhanced antioxidant and antibacterial activities as well as moisture absorbing capacities. EPPs were considered as novel natural agents, exhibiting outstanding druggable effects compared with ordinary drugs. However, the structure-function relationships of them are yet to be clearly established. The purpose of this overview is focusing on the current advances in the structural characterization, biological activities and mechanisms of action of *Enteromorpha* polysaccharides.

1. Introduction

Green algae have attracted increasing attention for their important functional properties and potential biological applications (Alves, Caridade, Mano, Sousa, & Reis, 2010). Macroalgal blooms in coastal areas worldwide have become increasingly common in recent years, due to water eutrophication and global climate change. The world's largest green macroalgal blooms in the Yellow Sea of China have exceeded 10 million tons in 2015 alone, with *Enteromorpha* (Li et al., 2016) as the main species. Green algae *Enteromorpha* belongs to the

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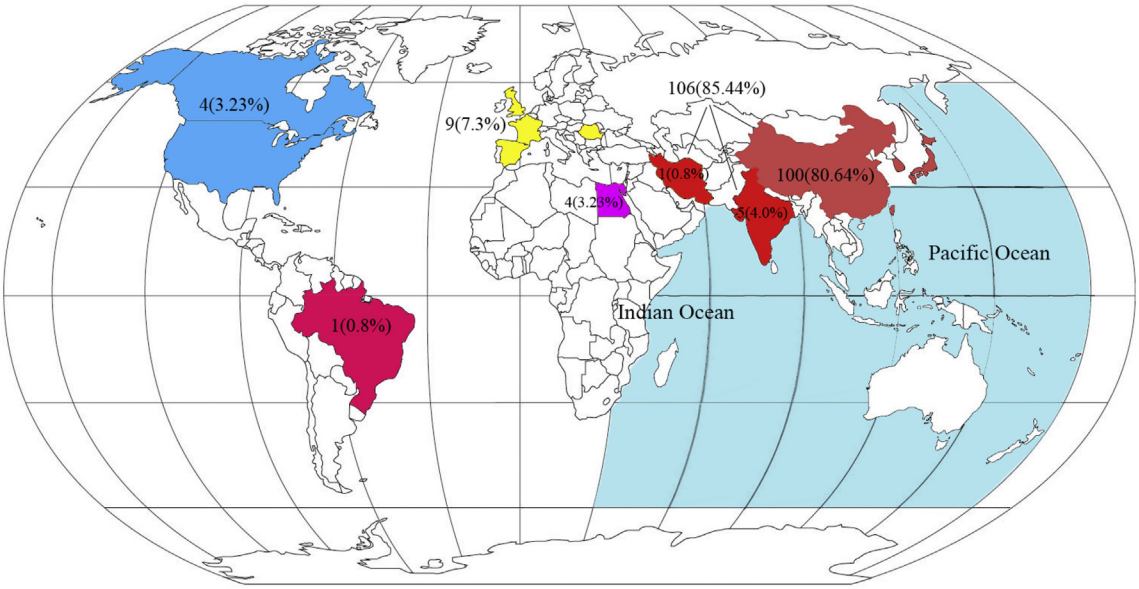

Fig. 1. The research distribution diagram of *Enteromorpha* polysaccharides.

Ulvaceae family of Chlorophyta, which primarily includes *E. prolifera*, *E. linza, E. compressa*, *E. intestinalis*, and *E. clathrata*. The classifications of genera *Ulva* and *Enteromorpha* were incompletely defined because of different evolutionary entities and earlier traditional culture (Hayden et al., 2012). Until now, a clear and effective standard which can completely separate *Enteromorpha* from *Ulva* specimens has not been reported. There would be one set of *E. prolifera* to unify *E. prolifera* and *U. prolifera* for the entire review. The green algae *Enteromorpha* has been widely used in different areas including as foods and drug candidates, especially in Asia.

Enteromorpha contains large amount of sulfated polysaccharides, among numerous other bioactive substances (Alves et al., 2010; Lin et al., 2019a; Yan et al., 2019). *Enteromorpha* polysaccharides (EPPs) have been widely studied within explosive growth as one of the important green algal phytochemicals. From 1961 to 2020, there have been around 130 papers published and indexed in Scopus and ScienceDirect databases with the keywords "*Enteromorpha* polysaccharides". Their specific digital data were shown in Fig. 1. Most of these researches have been performed in China, India, and Pacific oceans, which correspond to the 85.4% of all studies and about 7.3% of the studies were from Europe. However, in North America, South America, and Canada, few reports have been identified. EPPs have been proved to show various physiological and biological activities, including antioxidant (Xu et al., 2015), immunomodulation (Wei et al., 2014), antibacteria (Lu, Gao, Shan, & Lin, 2014), anti-hyperlipidemia (Teng, Qian, & Zhou, 2013), anti-tumor (Jiao et al., 2009), anti-cancer (Li, He, Liu, Wang, & Feng, 2018), anti-viral (Lopes et al., 2017), and anti-coagulant (Qi et al., 2012) properties, and regulate gut microbiota (Kong, Dong, Gao, & Jiang, 2016). Moreover, these bioactive properties are dependent on their specific compositional characteristics, such as molecular weights (MWs) and chemical functional structures modifications with carboxyl, hydroxyl, acetyl, and sulfate groups (Chi et al., 2018; Li et al., 2018c). However, the structure-activity relationship of EPPs is mostly unknown. This review aims to provide a brief summary of structural, functional, and chemical characteristics of EPPs (Table 1).

2. *Enteromorpha* polysaccharides compositions

2.1. Monosaccharide compositions

The monosaccharide compositions of EPPs have been analyzed by hydrolysis, derivatization, and gas chromatography-mass spectrometry detection (Li et al., 2018c; Wang, Xie, Shen, Nie, & Xie, 2018). The monosaccharide composition is affected by the source of the *Enteromorpha* as shown in Table 1. Most of EPPs were mainly composed of rhamnose, xylose, and glucose, with galactose, mannose, and glucuronic acid being rare and varying with different molar ratios (Liu et al., 2019). Different raw materials, growing conditions, collection seasons, and purification and degradation of backbone in the sulfation process, may lead to different monosaccharide compositions and molar ratios. Li, Chi, Yu, Jiang, and Liu (2017) have reported that the xylose of sulfated and non-sulfated *Enteromorpha* polysaccharides were at a ratio of 0.85:0.30. It was also revealed that the monosaccharide compositions of WE, WE-1, WE-2, WE-3 and WE-4 from *E. intestinalis* via different purified conditions have a significant change (Li et al., 2017; Li et al., 2018b).

2.2. Molecular weights and bio-effects

Molecular weight (MW) is a key factor to influence antioxidant and immune activities. The MWs of EPPs are closely correlated with their functional bioactivities and mostly range from 10^3 to 10^6 Da identified by high-performance gel permeation chromatography analysis. The variation in MWs of the EPPs might be due to species, purification processes, and analysis methods (Cho, Yang, Kim, & You, 2010). *E. intestinalis* was separated into four different MWs fractions via DEAE-Cellulose column with the stepwise gradient elution of NaCl (0, 0.1, 0.3, 0.5 M) (Li et al., 2018b). In the study of other green seaweeds, including *E. prolifera*, *E. compressa*, *E. clathrata*, and *E. intestinalis*, the MWs of these polysaccharides ranged from 8.1 to 620.3 KDa, showing the significant variations (Chi et al., 2018; Qi et al., 2012; Ray, 2006; Tabarsa, You, Dabaghian, & Surayot, 2018). As a result of its lower MWs, fraction F_1 separated from *E. intestinalis* showed better immune responses for proliferating RAW264.7 macrophage than fraction F_2 (Tabarsa et al., 2018). Degraded polysaccharides from *E. prolifera* with lower MWs exhibited more obvious antioxidant activities on chelating effects, reducing powers, and hydroxyl radicals. (Li et al., 2013; Zhang, Wang, Mo, & Qi, 2013a). Sulfated modifications can improve the solution properties of polysaccharides and enhance the negative charge on the surface of polysaccharides, thereby promoting the antioxidant, antitumor, and immune activities of polysaccharides (Wang, Zhang, Yao, Zhao, & Qi, 2013). The degradation of high MW polysaccharides through sulfation and hydrolysis could yield stronger effects. Sulfated derivatives from *Enteromorpha* polysaccharides are commonly obtained

Table 1
Effects of composition and structure on the biological activities of *Enteromorpha* polysaccharides.

Species	Name	Monosaccharide compositions	MW (KDa)	Structure and composition characterization	Function	Effects	References
E. prolifera	F1-3	Rha (57.1–87.6%), Glu (3.6–39.1%), Xyl (2.4–8.8%)	37–1218	Carbohydrates (57.6–62.5%), sulfates (14.5–18.8%), uronic acid (13.8–16.6%); F2 (11.3%) has higher proteins than F1 and F3 (1.0–2.8%)	Immunomodulation	Produce NO in Raw 264.7 cells	Cho et al. (2010)
	PEP	Rha:Glc:Gal:Xyl:Ara = 1.48:1:0.13:0.3:0.06	148	/	Antioxidant	Enhance hydroxyl and DPPH radical scavenging activity	Li et al. (2017)
	SPEP	Rha:Glc:Gal:Xyl:Ara = 1.49:1.00:0.16:0.85:0.07	176	/	Antioxidant Anti-aging	Improve hydroxyl and DPPH radical scavenging activity	
	LEP	Rha:Glc:Gal:Xyl: Ara = 1.65:1.00:0.09:0.57:0.17	44.8	/	Antioxidant	Improve superoxide, hydroxyl and DPPH radicals scavenging activity; Enhance reducing power	
	SLEP	Rha:Glc:Gal:Xyl:Ara = 1.09:1.00:0.06:0.10:0.11	59.9	/	Antioxidant Anti-aging	Improve radical scavenging ability, hydrogen donating ability, high electron withdrawing ability; Enhance reducing power, moisture absorption and retention properties	
	DEP1-6	Rha + Glc	3.1–445.6	/	Antioxidant	Enhance inhibitory effects on superoxide radical, hydroxyl radical; Enhance metal chelating effects	Li et al. (2013)
	EPF2	Rha:Xyl:Man:Gal:Glu = 3.64:1.08:0.21:0.75:0.27	103.51	53.2% carbohydrates, 11.5% proteins, 18.6% sulfate, 12.4% uronic acid	Hypolipidemic Antioxidant	Enhance antioxidant enzymes; MDA reduction in serum; Inhibit against serum TC, TG, LDL-c accumulation	Tang et al. (2013)
	ACP	Rha:GlcA:Glc:Xyl = 1:0.37:1.16:0.23	41.1	16.2% sulfate	Iron supplements	ACP-iron (III) complex has iron content of 9.14%	Chi et al. (2018)
	PE	Rha:GlcUA:Xyl = 3.2:1.1:1	620.3	D-GlcUA*p*-α-(1 → 4)-3-sulfate-l-Rha*p*-β-(1 → 4)-D-Xyl *p*-β-(1 → 4)-3-sulfate-l-Rha*p* units	Iron supplements	immunostimulators	Yu et al. (2017)
E. intestinalis	DAEB	Rha:Xyl:Gal:Glu = 5.36:1.00:0.57:0.64	46.8	(1 → 4)-β-l-Rha and (1 → 4)-Xyl with sulfate groups at the C-3 rhamnose	Anti-tumor immunomodulation	Stimulate ConA-induced lymphocyte proliferation; Increase spleen and thymus weight; Activate macrophages and release TNF-α, NO	Jiao et al. (2009)
	WE-11	Rha:Xyl = 0.71:0.29	223	47.57% carbohydrate	Antioxidant	Increase reducing power; Stronger scavenging ability of hydroxyl radicals, DPPH, and superoxide anion	Li et al. (2018b)
	WE-21	Man:GlcA = 0.75:0.25	142	48.54% carbohydrate, 16.03% sulfate	Antioxidant	Increase reducing power; scavenge hydroxyl, DPPH, and superoxide anion	
	WE-31	Man:GlcA = 0.97:0.03	142	52.16% carbohydrate, 2.75% sulfate	Antioxidant	Increase reducing power; scavenge hydroxyl, DPPH, and superoxide anion	
	WE-32	Rha:GlcA = 0.40:0.60	26.2	52.76% carbohydrate, 11.84% sulfate	Antioxidant	Increase reducing power; scavenge hydroxyl, DPPH, and superoxide anion	
	WE-42	Rha:GlcA = 0.88:0.12	80.9	56.08% carbohydrate, 13.38% sulfate	Antioxidant	Increase reducing power; scavenge hydroxyl, DPPH, and superoxide anion	
	WE-41	Rha:GlcA:Glc:Xyl:Ara = 7.7:19.0:3.7:1.0:2.0	223	53.87% carbohydrate 12.83% sulfate	Antioxidant	Increase reducing power; Stronger scavenge ability of	

(*continued on next page*)

Table 1 (*continued*)

Species	Name	Monosaccharide compositions	MW (KDa)	Structure and composition characterization	Function	Effects	References
						hydroxyl, DPPH, and superoxide anion	
	WEA	Rha:Xyl:Man:Glc = 1.39:1.00:0.13:3.23	72.03	92.19% total sugars	Antitumor Immunomodulation	Inhibit tumor growth; Increase spleen and thymus weight; Increase TNF-α expression in serum; Produce NO in macrophages	Jiao et al. (2010)
	WEB	Rha:Xyl:Gal:GlcA = 7.32:1.00:0.51:1.28	60.12	19.98% sulfate 80.99% total sugars	Antitumor Immunomodulation	Inhibit tumor growth; Increase spleen and thymus weight; Increase TNF-α expression in serum; Produce NO in macrophages	
	F1	Rha:Xyl:Glu:Gal = 0.39:0.1:0.39:0.11	28.7	(1 → 2)-Rha (1 → 2)-Glu residues with random branches at C-4, 6.25% sulfate	Immunomodulation	Induce cell proliferation; Release NO, IL-1b, TNF-α, IL-6, IL-10 and IL-12 cytokines from RAW264.7 cells	Tabarsa et al. (2018)
	F2	Rha:Ara:Xyl:Glu = 0.35:0.05:0.11:0.48	87.15	24.5% sulfates with no proteins	Immunomodulation	Release NO by inducing macrophage proliferation	
E. compressa	WE	Rha:Xyl:Gal:Glc = 5.7:1.5:1.0:1.8	55	8% protein and abundant amino acids (glutamic acid/glutamine and aspartic acid/asparagine)	/	/	Chattopadhyay et al. (2007)
E. clathrata	FEP	Ara:Rha:Gal:Glc = 20.1:2.7:1.2:1.0	511	(1 → 4)-linked β-L-Ara*p* residues with sulfate at C-3 position	Anticoagulant	Inhibit both the intrinsic and/or common pathways of coagulation and thrombin activity or conversion of fibrinogen to fibrin	Qi et al. (2012)

via the attachment of sulfate groups to the hydroxyl groups of polysaccharides, which could improve bio-effects including antioxidant, antibacterial, and immunity activities (Li et al., 2017). On the other hand, the higher MW polysaccharides from other algae were found to activate NF-κB pathway, leading to the increased immune cytokine mRNA levels (Pugh, Ross, ElSohly, ElSohl, & Pasco, 2001; Qi & Kim, 2018).

2.3. Chemical structures

The methods adopted to investigate the structures of polysaccharides include high-performance liquid chromatography, nuclear magnetic resonance, methylation analyses, high-performance gel-permeation chromatography, gas chromatography, and infrared spectroscopy (Li et al., 2017; Lin et al., 2019b; Qi et al., 2012; Yu, Li, Du, Mou, & Wang, 2017). However, only a few studies have been reported on the detailed chemical structures of EPPs with their monosaccharide composition and biological activities (Kim, Cho, Karnjanapratum, Shin, & You, 2011; Yu et al., 2017). *Enteromorpha* polysaccharides are usually glucuronic acid, xylose, and rhamnose polymers with major repeating disaccharide units of α-L-Rha*p*-(1 → 4)-D-Xyl and (→4)-βD-Glc*p*A-(1 → 4)-α-L-Rha*p*. Sulfate groups were also found at O-3 of the ulvan backbones from *E. compressa* (Lahaye & Robic, 2007; Ray, 2006). According to Yu et al. (2017), the backbone of *E. prolifera* polysaccharides contained D-GlcUA*p*-α-(1 → 4)-3-sulfate-l-Rha*p*-β-(1 → 4)-D-Xyl*p*-β-(1 → 4)-3-sulfate-L-Rha*p* units. Polysaccharides from *E. clathrata* mainly consisted of (1 → 4)-linked *β*-L-Ara*p* residues with partial sulfate groups at the C-3 position (Qi et al., 2012). *E. compressa* polysaccharide was branched and composed of (1 → 4)- and (1 → 2,4)-linked-Rha*p*, (1 → 4)-linked Xyl*p*, and (1 → 4)- and terminally linked-glucuronosyl residues (Ray, 2006). Monosaccharides such as (1 → 4)-*β*-L-rhamnose and (1 → 4)-linked xylose with sulfate groups linked on rhamnose at the C-3 position were observed in *E. intestinalis*, *E. compressa*, and *E. prolifera* polysaccharides (Jiao et al., 2009). These sulfated rhamnose-rich polysaccharides played a crucial part in their immunomodulatory function. Besides, few articles have reported that the conformational features of EPPs, such as xyloglucan from *E. compressa* with a linear configuration. However, the local regularity of *Ulvan* polysaccharides contained ordered helical conformations with homogeneous sequences (Lahaye & Robic, 2007). The diverse glycosidic linkages of various monosaccharides led to a complexity in the carbohydrate polymers and made it hard to analyze the chemical structures of EPPs. To determine the chemical structures of EPPs, further studies are required using advanced technologies, such as gas chromatography-mass spectrometry and high performance liquid chromatography-mass spectrometry (Chen et al., 2019; Qin et al., 2019).

2.4. Modified EPPs and bio-effects

It has been reported that sulfation and its position in the polysaccharides enhanced their antioxidant, anti-coagulant, and antiviral properties (Kim et al., 2011; Wang et al., 2012). A similar situation occurs for EPPs, with their sulfated groups stimulating the hydrogen atom of the anomeric carbon with stronger hydrogen atom-donating capacities. The sulfated group distribution in *E. linza* polysaccharide influences anti-coagulant activities, owing to its sulfated derivation (Wang et al., 2013). The sulfated polysaccharides from *E. prolifera* have presented increased free radical scavenging activity (Li et al., 2017). Moreover, the high contents of sulfate and glucuronate groups in a water-soluble polysaccharide from *E. intestinalis* provided higher antioxidant capacity *in vitro* (Li et al., 2018b). Besides the sulfate contents, monosaccharide components also affected the biological activities of EPPs, with high mannose showing the strongest free radical scavenging ability (Xu et al., 2015).

3. Bioactivities of *Enteromorpha* polysaccharides

3.1. Immunomodulatory activity

Polysaccharides in plant, fungi, and alga have immunomodulatory activities and regulate the innate immune functions. The immune system includes nonspecific and specific immunity. Nonspecific immunity can immediately respond to invaders without encountering previous pathogen, and gives signals to subsequently activate adaptive specific immunity (Fearon & Locksley, 1996). Specific immunity involves B- and T-lymphocytes and its function is activated immediately after the initial antigenic stimulus (Ferreira, Passos, Madureira, Vilanova, & Coimbra, 2015). Polysaccharides mainly activate the immune response or control immune cells, such as macrophages and lymphocytes. These immunologically active polysaccharides could also regulate the levels of natural killers (NK) including tumor necrosis factor (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), and interferon-c (IFN-c) (Meng, Liang, & Luo, 2016; Wang, Wang, Wu, & Liu, 2014; Wu et al., 2016). More specifically, polysaccharides have increased the productions of reactive oxygen species (ROS), IL-6, and TNF-α through regulating the expressions of iNOS, IL-6, and TNF-α (Ren, Liu, Gamallat, Zhang, & Xin, 2017). In addition, polysaccharides can strengthen the macrophage phagocytic activity, activate NK cells, increase thymus and spleen indices, and delay neutrophil apoptosis (Fig. 2) (Zhang, Oda, Yu, & Jin, 2015; Zou, Chen, Sun-Waterhouse, Zhang, & Li, 2018).

Glucans having β-1,3- linkages and β-1,6- linkages were significant for the cell immune function (Lin et al., 2008). EPPs have received widespread attention in recent times because of their effective impact on the immune system, without side effects (Li et al., 2018). They have induced splenocyte proliferation and influenced the immune-related enzymes of spleen and thymus, such as acid phosphatase, superoxide dismutase, alkaline phosphatase, and lactate dehydrogenase. Delayed-type hypersensitivity responses and serum hemolysin levels were increased following the treatment with EPP, indicating enhanced cellular and humoral immunities. Moreover, the level of transcription factor NF-κB in thymus and spleen was also found to increase extraordinarily (Wei et al., 2014). Water-soluble sulfated EPPs stimulated macrophage cells to produce NO and diverse cytokines by up-regulating the expressions of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK), while these pathways were directly promoted by PI3K/Akt (Kang et al., 2011; Qi & Kim, 2018). One *E. prolifera* polysaccharide fraction, strengthened concanavalin A-induced splenocyte proliferation *in vivo* and stimulated T cells by promoting IFN-γ and IL-2 secretions and T helper(h)1 (Th-1) response (Kim et al., 2011). *E. intestinalis* polysaccharides significantly stimulate concanavalin A-induced lymphocyte proliferation, enhance phagocytosis, and increase the secretion of NO and TNF-α in peritoneal macrophages of mice (Jiao et al., 2009). FSP30, a polysaccharide of *E. intestinalis* induced the secretion of pro-inflammatory cytokines in macrophage J774A.1 cells, including NO, IL-1β, and TNF-α (Peasura, Laohakunjit, Kerdchoechuen, Vongsawasdi, & Chao, 2016). Water- and alkali-extracted *E. linza* polysaccharides showed excellent activities in enhancing the proliferation of B- and T-lymphocytes (Zhang, Wang, Zhao, Yu, & Qi, 2013b). Moreover, phthaloyl modification of polysaccharides was found to be more efficient and accurate in immune regulations (Chen et al., 2016). Sulfated polysaccharides from *G. lemaneiformis* further strengthened the iNOS expression, promoted the production of serum cytokines, and strengthened the activities of acid phosphatase and lactate dehydrogenase in mice treated with cyclophosphamide (Ren, Zheng, You, Wen, Li, Fu et al., 2017). Previous studies reported that polysaccharides possess immune activities on Th17 and Treg cells and improve the expression of Treg cell-specific cytokines (TGF-β1 and IL-10) and transcription factor Foxp3. Similarly, the production of Th17 cell-specific cytokines (IL-17A and IL-6) and transcription factors (STAT3 and RORγt) were also found to be increased in cyclophosphamide-induced

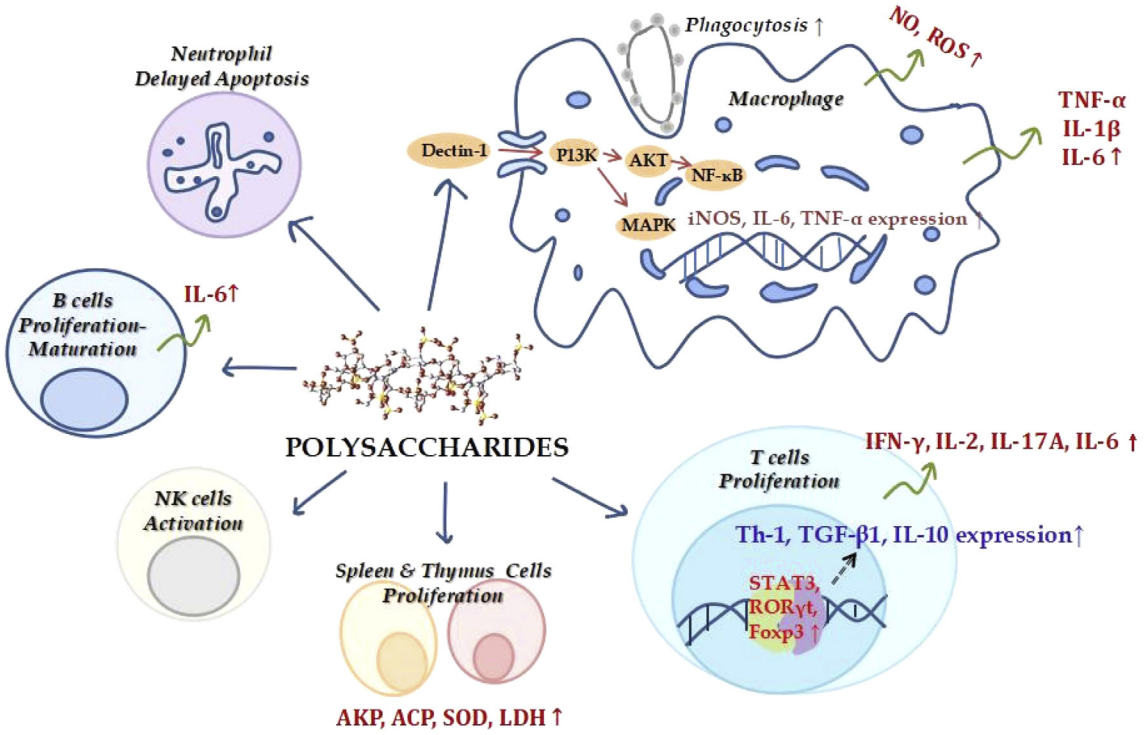

Fig. 2. A diagram of the immune system triggered by polysaccharides on molecular and cellular level.

immunosuppressed mice (Xiang et al., 2018). Further investigations are required for the modified EPPs to obtain better immunomodulatory activities and reveal their molecular immune-stimulatory mechanisms.

3.2. Antioxidant activity

Free radicals/reactive oxygen species (ROS) regulate cell growth *in vivo* and inhibit bacterial and viral infections in human bodies. However, excessive reactive oxygen species such as superoxide anion or hydrogen peroxide may cause several human chronic diseases (Chen et al., 2016), and ROS are generated during aerobic cell metabolic processes but may be cytotoxic and can cause diseases (Weydert & Cullen, 2010). Antioxidant activities can protect the living organisms from oxidative injuries (Cho, Lee, Kang, Won, & You, 2011). Polysaccharides have aroused the increased interest from scientists as effective reducing agents, free radical scavengers, and ferrous chelators *in vitro* (Wang, Hu, Nie, Yu, & Xie, 2016; Yu, Shen, Song, & Xie, 2018). *E. prolifera* polysaccharides have been found to have high antioxidant activities as free radical scavengers, including DPPH (1, 1-diphenyl-2-picrylhydrazyl), HO•, and O2•− (Xu et al., 2015). They have also been demonstrated to improve the activities of endogenous antioxidant enzyme, such as catalase, glutathione peroxidase, and superoxide dismutase, which have been viewed as the major defense system against ROS during oxidative stress. Moreover, *E. prolifera* polysaccharide could reduce the content of maleic dialdehyde (MDA) in serum. The low MDA levels result in lower oxidant stress and lipid peroxidation (İnal, Kanbak, & Sunal, 2001; Padmavathi, Senthilnathan, Chodon, & Sakthisekaran, 2006; Tang, Gao, Wang, Wen, & Qin, 2013). Furthermore, degraded polysaccharides from *E. linza* had stronger reducing powers due to their higher water-solubility and specific surface area in contact with radicals (Zhang, Wang, Zhao, et al., 2013b). Most low MW EPP derivatives possess better anti-oxidative capacities by directly scavenging the free radicals, stimulating enzymatic activities, and ferric reducing power (Fig. 3). Hydroxamated and carboxymethylated polysaccharides of *E. prolifera* have enhanced the total antioxidant ability by measuring the ferric reducing ability of plasma (Shao et al., 2017; Shi et al., 2017). Sulfated derivatives from *E. prolifera* with low MWs were observed to have distinct antioxidant activities, which might be attributed to their high hydrogen donating ability, efficient electron withdrawing ability, and strong radical scavenging ability (Li et al., 2017; Luo, Li, & Kong, 2012). Phthaloyl and acetylated derivatives of low MW *E. linza* polysaccharides also exhibited excellent radical-scavenging abilities (Wang, Zhang, Zhao, & Qi, 2014; Zhang, Wang, Zhao, & Qi, 2014). It is worth mentioning that EPPs derivatives can markedly improve antioxidant activities and promote their applications in the food, cosmetic, and pharmaceutical industries. However, further and systematic research is required to investigate the anti-oxidant mechanisms involved.

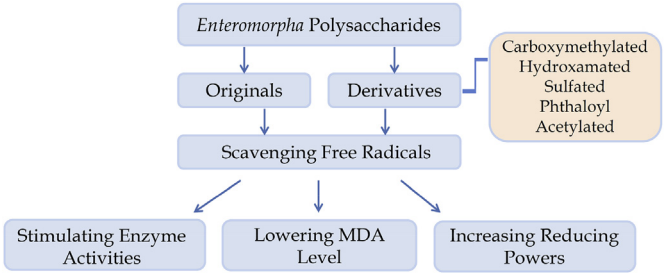

Fig. 3. The anti-oxidant mechanism of mice as motivated by *Enteromorpha* polysaccharides.

3.3. Anti-tumor activity

Chemotherapy and radiotherapy treatments for cancer and malignant tumor are widely used, with many side effects such as injury of normal cells (Chen et al., 2016). Hence, natural anticancer agents with low toxicity and high efficiency are desperately required. Marine algae polysaccharides have been reported to show anti-tumor and anti-cancer activities (Wang, Wang, et al., 2014). These isolated polysaccharides were found to significantly suppress the proliferation of cultured human cancer cells such as prostate cancer, alveolar carcinoma, cervical

cancer, and hepatocellular carcinoma. They have also induced human gastric adenocarcinoma cell apoptosis via inhibiting of the IGF-IR and PI3K/AKT signaling pathways (Kwon & Nam, 2007; Synytsya et al., 2010). Polysaccharides from *E. intestinalis* have shown anti-tumor activity, increasing the relative weights of spleen and thymus and inhibiting tumor growth in S180 tumor-bearing mice. They increased the expression of TNF-α in serum and macrophages, induced the proliferation of lymphocyte, and stimulated NO production by up-regulating inducible NO synthase (NOS) activity in a dose-dependent manner (Jiao, Jiang, Zhang, & Wu, 2010). EPPs motivated modulation of the immune system to indirectly inhibit tumor cells without direct cytotoxicity. However, the mechanisms of this anti-tumor action of EPPs are still unclear, and need to be investigated further.

3.4. Anti-bacterial activity

Due to the occurrence of bacterial resistance against commercial antibiotics, the discovery of novel natural antibiotics has become a global public health issue (Zhang et al., 2017). Some degraded *E. prolifera* polysaccharide selenides exhibited appreciable antimicrobial activity on *Escherichia coli*, *Staphylococcus aureus*, and plant pathogenic fungi (Lu et al., 2014). Some carboxymethylated degraded polysaccharides of *E. prolifera* and their modified hydroxamated polysaccharides derivatives presented marked anti-bacterial effects on tested gram-positive (*Bacillus subtilis* and *S. aureus*) and gram-negative (*Salmonella*, *Pseudomonas aeruginosa*, and *E. coli*) bacteria as shown by the inhibition zone and minimum inhibitory concentration assays (Shao et al., 2017). One possible antimicrobial mechanism of modified polysaccharides may be the iron-binding ability and electron-donating properties of hydroxamic acid moieties since iron is a basic element for bacterial growth, and the high iron (III) affinity chelating ability inhibits absorption of iron by the bacteria (Jia et al., 2014; Xu et al., 2011; Zhou et al., 2015). So, EPPs and their modified derivatives might be used as bacteriostatic and bactericidal agents in clinical treatments.

3.5. Regulation of the gut microbiota

Human gut microbiota encode the abundance and variety of carbohydrate-active enzymes degrading and fermenting the polysaccharides of plant origin to oligosaccharides, which are absorbed into body easily, producing significant therapeutic effects (Shang et al., 2018; Turnbaugh et al., 2010; Yin, Wang, Nie, & Xie, 2018). Accordingly, polysaccharides such as EPPs significantly alleviate intestinal motility function and inflammation, simultaneously improving microbiota (Ren, Liu et al., 2017). Human gut microbiota fermented with sulfated polysaccharides from *E. prolifera* has produced a large number of short-chain fatty acids (SCFAs), including butyrate, acetate, and lactic acid. *E. prolifera* polysaccharides could also exert prebiotic effect by increasing the number of intestinal *Enterobacter* and *Lactobacillus* (Kong et al., 2016), and their glycosides might change the structure of gut microbiota in the mouse (Zhang, Wang, Han, Liu, & Liu, 2018). In addition, *E. prolifera* polysaccharides have the potential to treat constipation. These results indicated that EPPs could increase the amount of beneficial bacteria and SCFAs to regulate gut microbiota.

3.6. Hypolipidemic activity

Hyperlipidemia, as a common endocrine disease, induces cerebrovascular, cardiovascular, and atherosclerosis (Chen et al., 2018; Song & Jiang, 2017; Zhao et al., 2019). While hypolipidemic drugs such as statins prevent and cure hyperlipidemia, their side effects can not be ignored (Rozman & Monostory, 2010; Zhao et al., 2018a, 2018b). Teng et al. (2013) reported that *E. prolifera* polysaccharides presented high anti-hyperlipidemic activities, which inhibited the body weight gain and also decreased triacylglycerol (TG), the total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) levels of plasma and liver. They also inhibited the expressions of sterol regulatory element-binding protein-1c (SREBP-1c) and hepatic acetyl-CoA carboxylase (ACC) in high-fat diet rats. SREBP-1c enhances the transcription of the required genes for fatty acid synthesis. ACC, as the rate-limiting enzyme in *de-novo* lipogenesis, controls β-oxidation of fatty acids in the mitochondria (Horton, Goldstein, & Brown, 2002; Ren et al., 2018). Moreover, *E. prolifera* polysaccharides showed pancreatic lipase inhibition activity (Yuan et al., 2018). One *E. prolifera* polysaccharide, namely EPF2, exhibited stronger hypolipidemic effect than simvastatin. EPF2 also enhanced endogenous antioxidant enzymes and decreased MDA content and lipid peroxidation in serum. This was linked with its antioxidant potential, and it likely reduced oxidative stress as a risk element for coronary artery diseases (Jiang et al., 2013; Tang et al., 2013).

3.7. Anticoagulant activity

Some anticoagulants used in clinical treatment, such as heparin sodium, have side effects such as bleeding, causing thrombocytopenia, and thrombosis syndrome (Yu et al., 2018). Some sulfated polysaccharides are universally recognized as natural anticoagulant agents (Melo, Pereira, Foguel, & Mourão, 2004). Polysaccharides from green alga have been investigated, showing stronger anticoagulant activities than those from brown and red alga (Shanmugam, Ramavat, Mody, Oza, & Tewari, 2001). The sulfated *E. linza* polysaccharide exhibited remarkable anticoagulant activities *in vitro* by effectively prolonging activated partial thromboplastin and thrombin times, which inhibited the formation of thrombin-mediated fibrin and the intrinsic coagulation pathway (Wang et al., 2013; Yang, Du, Huang, Wan, & Li, 2002). A high arabinose containing sulfated polysaccharide of *E. clathrata* also showed anticoagulant activity by prolonging thromboplastin and thrombin times (Qi et al., 2012).

3.8. Other bioactivities

EPPs have shown various biological activities and potential health benefits, such as respiratory burst activities, anti-virus, and anti-cancer. They have also been treated as iron fortifiers and mutant inhibitors. In addition, *Enteromorpha* polysaccharides-based nanoparticles showed anti-cancer ability by enhancing the bioavailability, solubility, and stability of hydrophobic curcumin and they might be treated as a hydrophobic drug in cancer therapy (Li et al., 2018a; Sun et al., 2018). Some sulfate modified polysaccharides from *E. compressa* revealed anti-virus activity by inhibition of herpes simplex virus replication, and reached 100% virus inhibitory rate at a dose of 100 μg/mL (Chen & Huang, 2018; Lopes et al., 2017). Moreover, low MW phthaloyl derivatives of *E. linza* polysaccharide have outstanding moisture absorption and retention capacities in living cells and tissues (Wang, Zhang, et al., 2014). Castro, Zarra, and Lamas (2004) reported that EPPs could control the respiratory burst activities of turbot phagocytes. Some low MW *Enteromorpha* polysaccharide-iron (III) complexes were considered as a new iron fortifier to treat iron deficient anemia disease (Cui et al., 2018). In addition, *E. linza* polysaccharides acting as mutant inhibitor have decreased genetic damage and inhibited the formation of micronuclei (Zhang, Wang, Li, Liu, & Zhang, 2016). The above findings indicate that *Enteromorpha* polysaccharides have an important biological potential (Fig. 4).

4. Future outlooks

In this review, recent advances in the study of the composition and biological activities of *Enteromorpha* polysaccharides have been investigated and summarized. The structural characterization and biological function of *Enteromorpha* polysaccharides have been reviewed. However, there are still many points that need to be improved in the related work including: (*i*), the high-order structural features of *Enteromorpha* polysaccharides have not been clearly studied beyond

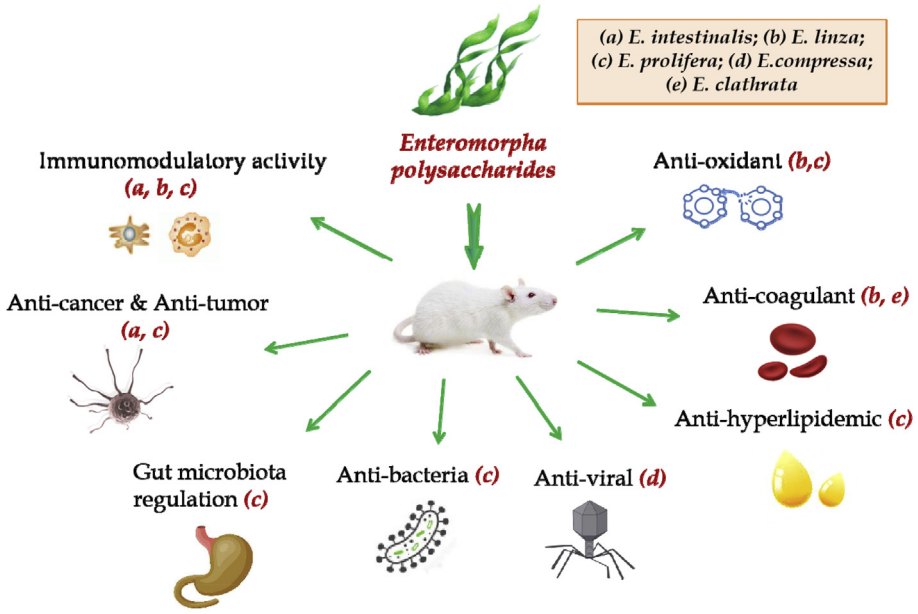

Fig. 4. Biological effects of *Enteromorpha* polysaccharides. The letters represent related polysaccharides, with bioactivity in a mouse model.

their bioactivities, with the relationship between the structures themselves and the resultant bioactivities of *Enteromorpha* polysaccharides still lacking in detailed description due to complicated molecules and diverse structural types. Further in-depth systematic research on the chemical structures of *Enteromorpha* polysaccharide is required, applying advanced technologies such as gas chromatography-mass spectrometry and high performance liquid chromatography-mass spectrometry (Chen et al., 2019; Qin et al., 2019). (*ii*), structural modifications of *Enteromorpha* polysaccharides, including sulfated, carboxymethylated, phosphorylated, and acetylated forms, exhibited better bioactivities. However, the accurate estimation of the degree of derivatization of the derivative functional groups and their safety need to be further investigated. (*iii*), the composition characteristics and molecular weight on the pharmacological action of *Enteromorpha* polysaccharides are still unknown. Big-data analysis and statistical practices in polymers should be developed to explore their interior mechanisms. (*iv*), compared with the chemicals and synthetic drugs, *Enteromorpha* polysaccharides as natural drugs are perceived as more efficient, less toxic, and fewer side-effects. For instance, the effect of administrating oral *Enteromorpha* polysaccharide was almost the same as the common lipid-lowering drug on the whole animal level. However, preclinical and clinical researches are necessary to confirm the real efficacy of *Enteromorpha* polysaccharides to aid further in disease prevention.

In conclusion, a significant interest has been generated over *Enteromorpha* polysaccharides due to their numerous beneficial effects. These important functions can promote their applications in food, cosmetic, and pharmaceutical industries. Their excellent performance was due to their chemical structures and glycosidic linkages. However, the structure-activity relationships of *Enteromorpha* polysaccharides remain largely unclear. The opportunities and challenges coexist. It is reasonable to state that *Enteromorpha* polysaccharides appear to have great developing potential in modern medicine and healthy food area.

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