Advances in Antioxidative Bioactive Macromolecules

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Abstract. Oxidative stress has been implicated in the pathogenesis of various chronic diseases such as cardiovascular disease, cancer, coronary heart disease, and arthritis. The antioxidative bioactive macromolecules, as evidenced by substantial studies, can effectively scavenge reactive oxygen species (ROS) and free radicals or mediate the immune system of the body to regulate the redox level, arousing the concern of numerous researchers on their antioxidative activities. An overview was carried out in this paper emphasizing on the types, antioxidant activities, application fields, and preparation methods of antioxidative biomacromolecules, which is expected to provide theoretical basis for the development and utilization of antioxidative biomacromolecules, as well as their applications in the fields of biomedicine, functional foods and skin care products.

1. Introduction
In addition to be a byproduct of the normal metabolism in vivo, reactive oxygen species (ROS) can also be inevitably produced owing to environmental stimuli such as UV light exposure and smoking. ROS encompasses a variety of diverse species such as hydroxyl radicals (·OH), superoxide anions (O₂⁻), hydrogen peroxide (H₂O₂), nitrogen oxide radicals (NO⁻), and peroxy radicals (ROO⁻)[1-2]. The generation of ROS is the normal physiological response in vivo to certain stimuli, It enables the self-regulation of the body with its own redox regulation system to eliminate free radicals and keep the body in sound and stable conditions. However, excessive ROS can result in impaired physiological function through cellular damage of lipids, proteins and DNAs, which may further induce human pathologies such as cancers, diabetes, neurodegenerative diseases, inflammation, aging, and immune system damage [3-4]. Moreover, the development of disease or the growth of age may lead to the gradual accumulation of ROS, resulting in a declined antioxidant capacity in the repair capacity for oxidative damage in the body. when the repair capacity of the body itself is insufficient to maintain the redox homeostasis, supplementation of exogenous antioxidants is considered to be a promising and necessary approach to maintain human health.

Antioxidants can remove ROS from the body and prevent the body from being attacked by excessive free radicals. According to massive published studies, antioxidants play an important role in the prevention and treatment of various diseases including cardiovascular diseases, cancers, coronary heart disease, and arthritis [5-11]. Consequently, antioxidants are of serious concern in human health research. Chemical synthesis of antioxidants pollutes the environment and long-term use of chemically synthesized antioxidants poses a potential threat to human health owing to its accumulated toxicity. Therefore, increasingly more attention has been paid to the research and development of natural antioxidants. At present, natural bioactive micromolecule compounds are more concerned in the study
of natural antioxidants, such as carotenoids, vitamins, and polyphenols. They are extensively used in medicine, cosmetics, and food industries due to their favorable antioxidant activities[12-13].

A large number of bioactive macromolecules are found to have excellent antioxidation property with good stability in the gastrointestinal tract than bioactive micromolecules. Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (GPx) are well-documented endogenous antioxidases, and non-enzymatic compounds include metallothioneins [14-17]. Several exogenous bioactive macromolecules are discovered to possess antioxidative activities that exist widely in plants, animals, and microorganisms[18]. These macromolecules (proteins, peptides, polysaccharides and glycoproteins) can regulate the redox state and serve as important organic components of the body. Our study was designed as an overview to summarize the types, antioxidative activities, effects on human health, and preparation methods of antioxidative biomacromolecules, aiming at providing a theoretical basis for the development and utilization of antioxidative biomacromolecules, as well as their applications in the fields of biomedicine, functional foods and skin care products.

2. Antioxidative Biomacromolecules

2.1 Proteins

Proteins are the material basis of life and important components of cells and tissues in living organisms, which function significantly in various biological and physiological processes. Antioxidative activity among others is an essential representative. Some antioxidative proteins, such as SOD, Prxs, and GPxs, can maintain the redox state of the body by preventing the production of intracellular ROS or promoting the elimination of ROS. Three subtypes of SOD are identified, including extracellular SOD3, cytoplasmic SOD1, and mitochondrial SOD2. These enzymes can break down superoxide radicals into harmless molecules. Prxs are a family of thioredoxin-dependent peroxidases, with 6 subtypes identified in mammals, which are expressed mainly in cells except for GPx3. Prxs can catalyze the redox reaction of intracellular H₂O₂, peroxynitrite and organic oxides to protect cells against oxidative stress. Non-enzymatic metal chelating proteins, including transferrin, transcalcgin, and selenoprotein, strictly control the levels of metal ions and prevent their excessive accumulation in the body. In addition, the DNA repair enzymes and proteolytic enzymes are responsible for repairing the free radical-induced damage to cells and rebuilding damaged cell membranes. Besides, these enzymes can contribute to identifying, cleaning, decomposing and eliminating oxidized or damaged macromolecules, such as proteins, DNAs, and lipids, to prevent the accumulated damage to the body. These enzymes mainly include DNA polymerases, glucoamylases, nucleases, proteases, proteasomes and peptidases. They are generally located in the cytoplasm and mitochondria in mammals, and constitute the essential components of the antioxidant defense system in the body [19]. Additionally, some antioxidant metalloproteins are involved in various brain functional activities. For instance, 25 selenoproteins are identified with selenocysteine in the catalytic site, most of which are expressed in the cerebral cortex and hippocampal neurons with protective effects on neural mechanisms. Glutathione peroxidase I-IV and VI (GPx1-4, 6), thioredoxin reductase I-III (TrxR1-3), and methionine sulfoxide reductase B (MrsB) have been found to be members of the selenoprotein family[20-26].

2.2 Polypeptides

Various peptides released by animals and plants are reported to have the capability to serve as nutrients for human growth and development. Meanwhile, they can also provide important physiological functions to the human body, including neural, hormonal and immunol regulation, as well as antithrombotic, antihypertensive, anticholesterol, anti-bacterial, anti-viral, anti-cancer, antioxidation, anti-aging and other physiological activities, and antioxidation is a major one[27-29]. Despite an incomplete understanding of the antioxidative mechanism of antioxidative peptides, enormous studies have shown that these peptides can scavenge free radicals and metal transition chelates or inhibit lipid peroxidation. Antioxidative activities of these peptides are related to their
structural features (amino acid compositions, molecular size, hydrophobicity, etc.) [30]. It has been hypothesized that hydrophobic amino acids at the N-terminus of the peptide chain can increase the antioxidative activity of the peptide as the affinity between the peptide chain and hydrophobic cell targets is increased by hydrophobicity [31]. Some peptides are also active in signal transduction, which protects the body from oxidative damage by inducing the transcription of genes encoding antioxidative enzymes. For example, Orsini Delgado et al. [32] found that many natural antioxidative peptides in the seeds of Amaranthus plants can scavenge free radicals and inhibit linoleic acid oxidation. These antioxidative peptides are also found in many proteins, such as albumin, globulin and mucin. In order to further prove the relationship between the antioxidative activity and its structural characteristics of antioxidant peptides in Amaranth, 4 antioxidative peptides were isolated from Amaranth and their antioxidative activities were analyzed comprehensively in simulated gastrointestinal digestive condition. The results showed that all the 4 peptides contained at least one large aromatic residue and were part of the acidic subunit of 11S globulin, with antioxidative activities as AWEEREQGSR> YLAGKPQQEH≈ IYIEQGNGITGM≈TEVWDSNEQ.

2.3. Polysaccharides
There is a gradual increase in the incidence of chronic diseases (e.g., diabetes, hypertension, and cardiovascular diseases) with the rapid process of urbanization, excessive intake of high-fat and high-sugar diet, insufficient physical exercise and lack of self-discipline, as well as increasing population ageing and greater living pressure. Long-term use of chemically synthesized drugs may produce toxicities to human bodies that may cause various adverse effects such as gastrointestinal disorders, liver disease, and obesity [33-35]. It speeds up the development of natural bioactive compounds as an effective alternative. Polysaccharides are one of the important bioactive alternative macromolecules. They are natural high molecular polymers, generally linear or branched molecules composed of at least 10 monosaccharide molecules connected by glycosidic bonds, with a molecular weight ranging from thousands to millions of kD. Similar to proteins and lipids, polysaccharides are essential for life and play an important role in the growth and development of organisms. Polysaccharides are found to be widely distributed in plants, fungi, algae bacteria, and marine animals. The structure of natural polysaccharides is complicated and the main chain, however, is generally composed of monosaccharides, including fructan, dextran, galactan, and mannan, or formed by two or more different monosaccharide molecules. The complexity of the branched chain is a reflection of the structural diversity of polysaccharides [36]. Various studies have demonstrated the beneficial pharmacological effects of polysaccharides, including anti-oxidation, anti-inflammation, immune regulation, anti-tumor, and lowering blood lipids. Their biological effects have also been established in the aspects of promoting the synthesis of proteins and nucleic acids and regulating cell proliferation, growth and death. Consequently, there is an extensive application of polysaccharides in clinical medicine and functional food development [37].

2.4. Glycoproteins
Glycoproteins (GPs) are glycconjugates composed of a polypeptide backbone to which one or more carbohydrate units are conjugated via covalent bonds, usually N- and O-glycosidic bonds [37-38]. GPs are present in a large number of plants, and have been also found in animals, fungi, molds and algae. GPs are active with many biological functions such as antitumor, antioxidant, antiviral, and lowering blood glucose. GPs are also involved in the maintenance of protein structure and stability, regulation of protein processing and transport, cell adhesion, and cell surface or intracellular recognition [39].

Biological functions of GPs are affected by the glycosylation sites, amino acid sequences, monosaccharide composition, and glycoprotein linkage. Accelerated development of biotechnology promotes extensive studies concerning the relationship of the biological activity function, and structure of GPs. For example, Dai et al. [41] determined the optimal conditions using Box-Behnken design to extract a GP (SPMG) from the muscle of Pharaoh Cuttlefish. Two fractions, SPMG-I and SPMG-II, were obtained by isolation and purification of the crude extract using DEAE-Nevis 52 ion
exchange column chromatography and Sephadex G-100 gel chromatography, with molecular weights of 42.5 and 36.3 kDa, respectively. Both fractions were rich in Glu, Asp, Lue, Arg, and Lys, and the main monosaccharide molecules were glucose. SPMG-I was linked via O-glycosidic bonds and SPMG-II obtained by β-elimination reaction. SPMG, SPMG-I and SPMG-II exhibited excellent antioxidative activity, with the highest activity found in SPMG-I. In addition, Li et al. [42] isolated and purified an antioxidative GP of 27.2 kDa in molecular weight from the ethanol-soluble protein fraction of Mustelus griseus. A total of 17 amino acids were identified from this GP with serine as the dominant one. The ratio of trehalose, arabinose, galactose, glucose and mannose was 1.00:1.53:7.27:9.07:2.09. The structure of the obtained GP was characterized by typical features of polysaccharides and proteins that were linked by N- and O-glycosidic bonds. The clearance of 1,1-diphenyl-2-picrylhydrazol (DPPH) by this GP reached up to 96.73 ±2.33%, which was higher than that of ascorbic acid at the concentration of 5.0 mg/mL.

3. Applications
Antioxidative biomacromolecules can effectively reduce oxidative stress and prevent the development of various chronic diseases. Being safety and non-toxic with no potential threat to human health, these macromolecules are a great source for biomedicine, functional foods and skincare product development.

3.1. Biomedicine
ROS and oxidative stress increase in the course of malignant tumor and myeloproliferative disease development. It is hence necessary to improve the redox homeostasis by altering the expression of related antioxidative proteins. Antioxidative activities of bioactive macromolecules are of great significance for regulating the redox state of the body and reducing the damage caused by diseases or drugs. Bhattacharyya et al. [43] isolated an antioxidative protein from Phyllanthus niruri. By stimulating the activation of PI3k/Akt signaling pathway, the isolated protein could inhibit liver weight loss, enhance the phosphorylation of the transcription factor p65 subunit, and improve aspirin-induced oxidative damage and apoptosis, so as to reduce the adverse reactions of aspirin. Meanwhile, Sun et al. [44] found a biologically active polypeptide from Pleurotus eryngii mycelium. According to the results, at a concentration of 0.05-2 mg/ml, the polypeptide could inhibit tumor cell proliferation, promote the proliferation of macrophages, the release of tumor necrosis factor (TNF)-α and interleukin 6 (IL-6), and the expression of TLR2 and TLR4, and enhance the phagocytic capacity of macrophages via the release of NO and H2O2. While at a concentration of 0.2-1mg/ml, the discovered polypeptide could efficiently reduce DPPH, O2′, and OH′. The favorable antioxidative and immunostimulatory activities of this polypeptide make it a potential biologically active candidate for antiviral drugs.

DJ-1 protein, encoded by the PARK7 gene, is a redox protein with multiple biological functions. It is activated under oxidative stress to act as a transcriptional regulator of antioxidative proteins. Trivedi et al. [45] reported in their study that Caki-2 cells were treated with cisplatin, ROS continued to accumulate in dose and time dependent manners, eventually leading to cell apoptosis. Proteomic analysis found that the expression of DJ-1 protein gradually decreased along with the presence of Caki-2 cell apoptosis. Cell apoptosis slowed down when DJ-1 was overexpressed and increased when the PARK7 gene was knocked out. These findings proved that DJ-1 protein can eliminate the ROS accumulated in kidney cells and reduce cellular oxidative stress. However, it is not the truth that a higher expression of DJ-1 is always associated with significant effect. The reason lies in that besides a protective function of transformed cells against increased ROS, redox adaptation mechanism of malignant cells can also induce decreased cell apoptosis, increased DNA repair capacity and enhanced drug resistance. Kim et al. [46] observed a bidirectional change in the expression of DJ-1 in a mouse model of mastocytosis, i.e., DJ-1 degraded and decreased due to reduction of ROS in mice with mild mastocytosis and significantly increased in a malignant mouse model of mastocytosis. In the latter case, IL-6 mediated the transcription of the PARK7 gene, which enhanced the expression of DJ-1 to make up the loss of DJ-1 caused by oxidation and reduce oxidative stress, providing a favorable
physiological environment for mast cell proliferation. While the use of anti-IL-6 antibody blocked IL-6 receptor signals, resulting in inhibited ROS increment and DJ-1 expression, and effectively reduced number of mast cells in tissue and blood at the same time. Therefore, decreasing in DJ-1 expression via blocking the IL-6 signal may serve as an effective method for adjuvant treatment of patients with advanced mastocytosis.

3.2 Functional foods

Traditional Chinese medicinal materials rich in various bioactive macromolecules are abundantly available in China and can be used as important raw materials for functional food development. Wang et al. [47] extracted a polysaccharide from Gynostemma pentaphyllum. In vitro tests showed that this polysaccharide eliminated DPPH free radicals, superoxide anions, and ABTS free radicals in a dose-dependent manner. Meanwhile, animal experiments demonstrated that this polysaccharide effectively reduced body weight and fasting blood glucose as well as ALP, ALT, AST and BUN in blood. Antioxidative macromolecules from marine organisms have special structures due to their unique living environment, i.e., low temperature, high pressure, low oxygen, and high salt, and are active in regulating the immune function of the human body. Therefore, bioactive functions of marine organisms are gradually discovered and utilized for the development and production of functional foods. Qi et al. [48] extracted sea cucumber polysaccharide (PESCPL) from processing liquid of sea cucumber as a raw material by proteolysis and electrophoresis. The main component of PESCPL is mannose, with some glucose and fucose as well. PESCPL can effectively remove DPPH, hydroxyl free radicals and superoxide anion free radicals, enhance the activities of catalase and SOD, reduce serum malondialdehyde, cholesterol and triglyceride, and increase the content of high-density lipoprotein cholesterol. Therefore, it is a natural antioxidants that can be used as a dietary supplement for dyslipidemia.

In addition, many micromolecules have antioxidative activity. However, the expected efficacy of these molecules is poor because of their low bioavailability and unstability in the gastrointestinal environment after oral administration. Studies have shown that this issue can be effectively solved when bioactive micromolecules and polypeptides are prepared into nanoparticles by nanotechnology. Tang et al. [49] successfully prepared chitosan/poly(γ-glutamic acid) (γ-PGA) self-assembled nanoparticles (CS/γ-PGA). Meanwhile, the discovered nanoparticles were found to have the ability to effectively scavenge free radicals and reversibly unlock the tight connection between Caco-2 cells to enhance the delivery of catechins across the monolayers of Caco-2 cells. The above method of preparing nanoparticles provides new ideas for the development of novel peptide-based antioxidant functional foods.

3.3 Skincare products

Antioxidative activities of biological macromolecules can effectively remove ROS and free radicals to protect skin cells, slow down aging, and inhibit melanin production. Chen et al. [50] found that squid ink polysaccharides effectively reduced oxidative damage to fibroblasts mediated by upregulated NADPH oxidase and connexin 43, and inhibited the ROS-induced upregulation of matrix metalloproteinase (MMP) 1 and MMP9, slowing down the MMP9 mediated skin aging. Hou et al. [51] extracted collagen peptide from cod skin and found that this collagen peptide can promote antioxidant activity, reduce water and lipid loss, repair endogenous collagen and elastin fibers, and maintain the ratio of type III to type I collagen. Furthermore, the extracted collagen polypeptide can reduce the skin damage caused by UV light exposure with excellent moisture absorption and retention capacity. Wang et al. [52] prepared a conjugate from mulberry polysaccharide and whey protein to coat fish oil. The obtained fish oil emulsion with decreased particle size improved the emulsification ability and stability of whey protein, and consequently increased the antioxidative activity of fish oil significantly. Therefore, antioxidant peptides are becoming popular in the skincare industry.
3.4 Other bio-products

Except as an ingredient of the productions in biomedical, skincare and functional foods, the antioxidant biological macromolecules in the food and drug packing materials also has great application value as people in the pursuit of better high quality of life and environmental protection consciousness enhancement. Therefore, it has become the research and development direction of new food packaging materials, such as safety, non-toxic, bioactive, biodegradable and recyclable. The new functional materials with biological activity can be developed by a variety of techniques from antioxidant bioactive macromolecules with special structure, and which will be the raw materials for the development of food packaging materials in the future[53]. Spizzirri et al. [54]prepared a conjugates composed of catechin-alginate and catechin-inulin by adopting free radical-induced grafting procedure. The functional materials showed good antioxidant activity, and it could be very useful in the optimization of food preservation. This method mentioned above provides an idea for designing new packaging materials.

4. Preparation stages and Methods

Antioxidative biological macromolecules are widely distributed in higher plants, fungi, molds, algae, and bacteria. Methods for extraction, isolation, and purification are of vital importance to maintaining the biological activities of these macromolecules[55-60]. At present, many studies have been conducted to develop and optimize extraction methods. Due to the complex composition and structure of these bioactive macromolecules, extraction methods need to be optimized based on the characteristics of extracted bioactive substances constantly. Moreover, the recovery efficiency and safety shall be improved under the condition that the composition, structure and biological activity remain unchanged to ensure the accuracy of biological activity test, so as to evaluate the development and application value of these antioxidative macromolecules in human health.

| Products source | Products | Pre-treatment | Extraction | Purification | Yield | Characterization analysis | Results | Reference |
|-----------------|----------|---------------|------------|--------------|-------|--------------------------|---------|-----------|
| Phyllanthus niruri protein (PNP) | Homogenized in 50 mM phosphate buffer (pH 7.4) | Centrifugation at 15,000 g, 60% ammonium sulphate saturation | Dialed against 50 mM phosphate buffer, DEAE cellulose column (0–1 M NaCl) / Heat treatment and enzymatic digestion | / | Four peptide fragments of nominal mass 2128 Da, 2392 Da, 2533 Da and 2719 Da. None of these fragments possess similarity with any peptide sequences. PNP could be a safe antidote against aspirin induced detrimental complications | [43] |
| Protein extracts | Ganoderma lucidum | Washed with distilled water and homogenized (0.01 M HCl containing 0.15 M NaCl) in a proportion of 1 g of mycelia to 2 ml of extraction solution. | After filtering through cheesecloth, the filtrate was centrifuged at 3,904 × g, 4°C for 30 min. Ammonium sulphate precipitation (85% saturation) | Diethyl aminoethyl (DEAE)- Sepharose column and Sulfopropyl (SP)- Sepharose column | / | Soluble protein content was determined by the Bradford method | IC50 of the mycelia protein and fruiting bodies protein extracts against ABTS⁺ were 2.47±0.01 and 2.77±0.01 μg protein/ml and against DPPH⁺ were 2.5 ±0.01 and 3.42±0.01 μg protein/ml, respectively. FRAP values of those samples were 1.73±0.01 and 2.62±0.01 μmole trolox/μg protein respectively. | [62] |
| The polypeptide extracts | Homogenised with phosphate buffered saline (PBS, Ph 8.2) for 4 h, centrifuged at 4000×g for 20 min, fractional precipitation by saturated (30%, 75%, 100%) (NH₄)₂SO₄ at 4°C(4000×g, 0.2 h) then dialysed at 4°C for 24 h using 30 kDa dialysis membranes, then centrifuged at 4000×g for 20 min, fractional precipitation by saturated (30%, 75%, 100%) (NH₄)₂SO₄ at 4°C, then dialysed at 4°C for 24 h using 30 kDa dialysis membranes, then centrifuged at 4000×g for 20 min, fractional precipitation by saturated (30%, 75%, 100%) (NH₄)₂SO₄ at 4°C. | Protein content determined by the Bradford method using bovine serum albumin as the standard, / | PEMP was a good antioxidant with antitumor and immunostimulatory activities that was concentration dependent. | [44] |
| Enzymic polypeptide | Preparation of de-oiling powder by petroleum ether and de-sugar with boiling water reflux hydrolyzed with the proteases (pH was adjusted to 8.0 for alkaline protease and trypsinase, and pH 7.0 for papain, neutral protease and proteinase, and pH 2.5 for pepsin) | / | / | 19.7% | The enzymatic polypeptide from alkaline protease method had good antioxidant activity and physiological activity. | [63] |
| Gelatin Polypeptides | Rinsed, cleaned and treated with sodium hydroxide solution and sulphuric acid solution respectively. Homogenized and extracted with distilled water, the double-enzyme hydrolysis includes the progressive and mixed hydrolysis of two selected enzymes, then centrifuged at 5200×g for 20 min. MMCO (10, 6 and 2 kDa) membranes | / | / | Amino acid composition analysis, antioxidant activities using superoxide anion and hydroxyl radical scavenging assays. | Three series of gelatin polypeptides were obtained. Each fraction exhibited significant antioxidant effects and free radical scavenging activities. | [64] |
| Collagen Peptides | Scales of crocei ne croaker (ASC-C) remove non-collagenous proteins and denaturalized soaked in 0.5 M acetic acid and filtered with two layers of cheesecloth, then precipitated by adding NaCl, dialysed, double-Enzyme Hydrolysis. | / | / | / | Three antioxidant peptides (ACH-P1, ACH-P2, and ACH-P3) showed good radicals scavenging activities and abilities of inhibiting the autoxidation in linoleic acid model system | [65] |
| Squid Ink Polysaccharides (SIP) | Kill the squid to obtain fresh ink sacs, collected ink, resuspended in PBS(pH 6.7), then ground and sonicated enzymolysis with 1% papain in PBS, precipitated with absolute ethanol and lyophilized under vacuum. | / | / | / | Squid ink polysaccharides effectively reduce the fibroblast oxidative damage mediated by the upregulation of NADPH oxidase and Connexin43. | [50] |
| Glycoprotein (SPMG) | Muscles of Sepia pharaonis | Dried in a vacuum freeze drier for 24 h, and pulverized by the disintegrator. | DEAE-Cellulose 52 ion-exchange chromatography and Sephadex G-100 gel chromatography | Automated amino acid analyser, UV spectrometer, FT-IR and GC-MS | SPMG-I and SPMG-II were rich in Glu, Asp, Leu, Arg and Lys with the molecular weight of 42.5 and 36.3 kDa, respectively. SPMG, SPMG-I and SPMG-II exhibited a certain antioxidant activity. | [41] |
|---|---|---|---|---|---|---|
| Glycoprotein (COG2a) | Mustelus griseus muscle | Homogenized for 60s in 90% ethanol solution | DEAE-cellulose-52 anion-exchange column, Sephadex G-100 gel filtration column | Proteins and carbohydrates were 24.23 ± 0.48 % and 9.25 ± 1.30 %, respectively | COG2a was O-linked glycoprotein with a molecular mass of 25.9 kDa. COG2a enhanced the SOD and GSH-PX activities and reduced the MDA and total carbonyl contents in blood and liver tissues of D-galactose-induced mice. | [68] |
| Proteoglycan (CFPS-11) | Corbicula fluminensis | Defatted, removed sasamquasinapin and other impurities by using 90% (v/v) ethanol, freeze-drying | Column Chromatography, ultrafiltration, gel chromatography, AKTA Pure Chromatography | SDS-PAGE, HPGPC, DSC, GC, UV-spectrophotometer, FTIR, CD, HPLC-Q-TOF-MS/MS | CFPS-11 had an average molecular weight of 807.7 kDa and consisted of D-glucose and D-glucosamine, and possessed remarkable antioxidant activity. | [69] |

**Table:**

| Polysaccharides (CPs) | Comfrey (Symphytum officinale L.) | Liquid to solid ratio 25 mL/g, extraction temperature 89°C, and extraction time 155 min | DEAE-52 cellulose and Sepharose CL-6B columns | Gel filtration chromatography, HPLC, composition, UV, FT-IR, Congo red experiment | CPs-1.2 consisted of galacturonic acid, arabinose, glucose, and galactose. CPs-1.2 showed notable DPPH and ABTS radical scavenging abilities. | [66] |
|---|---|---|---|---|---|---|
| Fucoidan | Marine macroalgae Turbina conoides | Hot water extraction | Anion-exchange chromatography | Colorimetric assays, GC-MS, agarose gel electrophoresis, FT-IR, NMR | Profound antioxidant and anti-inflammatory activities of fucoidan | [67] |

**Legend:**

- DEAE: DEAE-52 cellulose and Sepharose CL-6B columns
- HPLC: High-performance liquid chromatography
- SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
- GC-MS: Gas chromatography-mass spectrometry
- FT-IR: Fourier transform infrared spectroscopy
- GC: Gas chromatography
- UV: Ultraviolet
- COG2a: Camelina oleifera Abel seeds
- CFPS-11: Corbicula fluminensis
spectrometry; NMR: Nuclear magnetic resonance; SEM: Scanning electron microscope; AFM: Atomic force microscope; IR: Infrared spectrum; GSH-Px: Glutathione peroxidase; CPs: Polysaccharides from comfrey; SPMG: Glycoproteins from Sepia pharaonis muscle; BHT: Butylated hydroxytoluene; BHA: Butylated hydroxyanisole; TBHQ: Tertbutylhydroquinone; DPPH: 1,1-diphenyl-2-picrylhydrazyl; SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; LC-MS/MS: Liquid chromatography-mass spectrometry; HPLC-Q-TOF-MS/MS: High performance liquid chromatography of quadrupole time of flight-tandem mass spectrometry.

Preparation of bioactive macromolecules generally consists of two major stages, crude extraction and purification. In the first stage (crude extraction), bioactive macromolecules are isolated from the prepared sample, while the products usually contained a lot of impurities. Methods commonly used for crude extraction include solvent extraction, precipitation, acid/alkali extraction, enzymatic hydrolysis, ultrasonic extraction, microwave extraction and supercritical fluid extraction. In the second stage (purification stage), impurities are removed to improve the purity of the bioactive materials. Common methods are gel filtration chromatography, ion exchange chromatography, column chromatography, ultrafiltration, reversed-phase high-performance liquid chromatography, and capillary electrophoresis. Each method has its own advantages and drawbacks, and proposed method(s) shall be selected based on the source, structure, molecular weight, charge or hydrophobicity of the bioactive molecules of interest. Importantly, different methods need to be used in combination to achieve the desired purity. For instance, zeng et al.[61] purified the crude extract of polysaccharides by dialysis, column chromatography, and High Performance Liquid Chromatography, and identified the main components and molecular structure adopting methylation, GC-MS, and NMR technology. Finally, one new water-soluble non-starch polysaccharidewas obtained from Truffle Tuber sinense. The common extraction, purification and characterization methods of bioactive macromolecules were summarized in table1.

4. Summary and prospects
ROS is a chemically active group that regulates various physiological and pathological processes of normal and tumor cells in the human body. Appropriate ROS levels can promote the growth and differentiation of cells. However excessive ROS may induce oxidative stress in cells, leading to damaged lipids, proteins, and nucleic acids and even apoptosis. Therefore, maintaining the dynamic balance of ROS is critical to the survival and growth of cells as well as human health. When carcinogenesis occurs, the level of intracellular ROS is higher than that of normal cells, and the imbalanced redox state makes cells more sensitive to oxidative stress. Appropriate readjustment of ROS level can promote tumor cell apoptosis. Furthermore, antioxidative bioactive macromolecules are featured by wide distribution, high bioactivity, specificity and safety, which can effectively regulate the redox level of the body. These features make these macromolecules to be potential drug candidates for the prevention and treatment of various diseases with promising development and application prospect in the future. However, there are insufficient standars currently available for evaluating the antioxidative activities of bioactive macromolecules, which restricts corresponding comparison consequently and market standardization. Therefore, it is urgent to develop evaluation standars for the antioxidative activity of bioactive macromolecules, so as to provide a theoretical basis for the development of these macromolecules.

Antioxidative bioactive macromolecules possess heterogeneous structure, and their biological activities are largely affected by the preparation methods. Simultaneously, different preparation methods have their pros and cons, and scope of application. Therefore, specific methods shall be determined based on the physicochemical properties or bioactivities of the target molecules to keep the bioactivities unchanged. Furthermore, the separation and purification methods of bioactive macromolecules shall be optimized with the continuous innovation of technologies. In this way, it is expected to explore the functions of more bioactive macromolecules and accelerate their application in biomedicine, clinical diagnosis and treatment, and functional foods, so as to promote the rapid and efficient development of human health ultimately.
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