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Preparation, structure and application of polysaccharides from *Poria cocos*

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Poria cocos polysaccharides (PCPs) are fungal polysaccharides derived from the traditional Chinese medicine *Poria cocos*. They are considered an important active ingredient for their pharmacological activity. Herein, the extraction, separation and purification, structure, and application of PCPs are reviewed. Additional research is necessary to fully understand the advanced structure of PCPs, which has implications for their structure–activity relationship. Their application mostly involves the medical industry, with less involvement in other fields. This article highlights the current research status on PCPs in the above-mentioned areas and some problems that need to be solved in future research. Additionally, it points the way for further studies on PCPs in the hopes that they will be more widely and realistically used in various industries.

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1. Introduction

Poria cocos, an important and therapeutic fungus belonging to the Poria family, is commonly found in southern and southwestern China. It is a saprophytic fungus that causes wood degradation. It can parasitize citrus, eucalyptus, and oak trees.[1] *P. cocos* is used for clinical purposes in a wide range of chemical compositions, and it is also considered a functional herbal food.[2] In traditional Chinese medicine (TCM), *P. cocos* is frequently used in conjunction with other herbs to treat a variety of diseases.[3] For example, the DangGui-Shaoyao-San formula has been demonstrated to improve cognitive dysfunction in Alzheimer's disease patients.[3] In Asian nations, including China, Japan, and South Korea, *P. cocos* has been used for medical treatment for several centuries.[4,5] *P. cocos* is mainly sold as a nutritional health product that is easy to obtain and widely added to beverages, including soup, tea, and alcoholic beverages, for mixed consumption.[6] Conventional Chinese medicine practitioners often use *P. cocos* to treat kidney disease,[7] chronic gastritis,[6] phlegm, swelling, restlessness, intestinal damage[8] and other diseases as a result of its diuretic,[9] sedative, and spleen-strengthening abilities. Its considered to be one of the highest ranked medicines in a variety of medical books on herbal medicine.[10] *P. cocos* is traditionally considered by the Chinese to be a beneficial drug with life-extending properties,[11] and most of its main active ingredients have been intensively studied.[12] Triterpenes,[13] polysaccharides,[14] and steroid chemicals[9] are among the many and rich chemical components of *P. cocos*. Aside from potassium salts, histidine, choline, and amino acids, there are other secondary compounds,[15–17] The multiple pharmaceutical activities of *P. cocos* are related to the presence of several triterpenes and polysaccharides.[18]

P. cocos polysaccharides (PCPs) are the most prevalent, helpful, and plentiful part of the fungus, making up around 90% of its fungal nuclei.[19] Several components of PCPs have significant differences in their monosaccharide composition and molecular weight. PCPs can be divided into water-soluble polysaccharides and alkali-soluble polysaccharides according to their solubility; water-soluble polysaccharides are the main active ingredients of *P. cocos* used in clinical medicine. *P. cocos* alkali-soluble polysaccharides have almost no bioactivity, and chemical modification to improve their bioactivity and means of application are a main focus of research today.[20] Studies have shown that alkali-soluble PCPs prevents the growth of cancer cells and affects the activity of a range of enzymes involved in apoptosis and senescence in living organisms.[21] Pharmacological researchers have shown a variety of medicinal activities of PCP,[22,23] such as immune regulation,[24,25] anti-inflammatory effects,[26,27] antioxidant effects,[28] anti-tumor effects,[21] hepatoprotective effects,[29–31] and kidney protection,[32] In addition, it is also one of the main ingredients in health supplements, such as *P. cocos* polysaccharide oral solution.[11] The biological activity and mechanism of action of PCPs have been widely studied by researchers for many years, but their potential applications still need to be developed. Recent relevant literature on the application of PCPs in food packaging and feed additives indicates that PCPs are being investigated for use in industries other than the healthcare industry. With the continuous improvement of polysaccharide extraction, isolation and purification, structural identification, and other techniques, the study of PCPs is becoming more and more comprehensive; their structures and mechanisms of bioactivity are becoming more and more

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accurate; their application is expanding; and the past reviews may need new and additional descriptions. Herein, the extraction, isolation, purification, structure and application of PCPs are reviewed and summarized.

2. Preparation of PCPs

Nowadays, polysaccharides are primarily obtained from natural materials, and the majority of our research on polysaccharides begins with extraction. PCPs can be classified into two categories, extracellular polysaccharides and intracellular polysaccharides, according to different extraction locations. Extracellular polysaccharides are those obtained through liquid fermentation, while intracellular polysaccharides are those found in the fruiting bodies and mycelium.[33,34] According to the solubility of PCPs in different solvents, they can be divided into water-soluble PCPs, which are biologically active, and alkali-soluble PCPs, which are mostly biologically inactive. The structure of an alkali-soluble polysaccharide is shown in Fig. 1, and it is mainly composed of linear β-(1 → 3)-D-glucan, containing a small amount of β-(1 → 6) glycosidic side chains, and its conformation is generally a triple helix conformation. There are three main methods used by researchers to extract PCPs: solvent extraction, enzyme-assisted extraction, and physically assisted extraction. In addition to the above extraction methods, deep eutectic solvent (DES) extraction and others are available in the recent literature. Table 1 summarizes the advantages and disadvantages of different PCP extraction methods. In order to obtain more PCPs, researchers sometimes use multiple extraction methods to extract polysaccharides at the same time, for example, using both enzyme extraction and physically assisted extraction to extract polysaccharides. The research on the extraction methods of PCPs is relatively mature, and future researchers should focus on improving the extraction methods or discovering new, high-yield, and cost-effective extraction methods.

Crude PCPs extracted directly from *P. cocos* usually contain impurities such as proteins, oligosaccharides and pigments, which may have an impact on the biological activity and structure, thus affecting the structural analysis of PCPs and experiments on their activity.[37,41] Removal of these impurities is needed to avoid affecting the studies.[37] The process of isolating and purifying PCPs is essentially similar to that for other naturally occurring polysaccharides;[37,38] it primarily entails the elimination of lipids, proteins, and pigments as well as the separation of PCPs with varying molecular weights and physical characteristics. Commonly used protein removal methods include hydrochloric acid, ellagic acid, trichloroacetic acid, and the Sevag method.[42] The most commonly used of these is the Sevag method,[37] in which polysaccharides are mixed with Sevag reagent[43] (*n*-butanol and chloroform in the ratio of 1 : 5 or 1 : 4 by volume), and the mixture is centrifuged after being shaken. This removes proteins from the polysaccharides. Based on the principle of similar solubility, a common method of PCP degreasing used by researchers is refluxing using a Soxhlet extractor and an organic solvent such as ethanol. Lipid impurities that are not soluble in the organic solvent will be separated from the PCPs. Common methods of removing pigments in the laboratory include H_2O_2 decolorization, activated carbon decolorization, and large-pore adsorption resin methods.

Common polysaccharide separation and purification methods include partition precipitation, salt precipitation, quaternary ammonium precipitation, metal complexation, and column chromatography. Column chromatography is now commonly used by researchers to isolate and purify polysaccharides due to its ease of operation and wide selection for polysaccharide purification.[41] Gel chromatography columns[39,44] and ion exchange chromatography[45,46] are commonly used for the separation and purification of PCPs.

2.1 Solvent extraction

2.1.1 Water extraction method. Since most of the polysaccharides are highly polar and soluble in water, water is regularly used as a solvent for the extraction of PCPs. The extraction process mainly involves grinding *P. cocos* into powder, reflux extraction with hot water, concentrating to obtain the aqueous extract, and then using ethanol for preliminary purification, precipitation, centrifugation, and drying, to finally obtain crude polysaccharides from *P. cocos*.[47–49] However, although the process of extracting PCPs with hot water is straightforward, simple to use, and cheap, the yield is low, the extraction time is lengthy, and the extraction efficiency

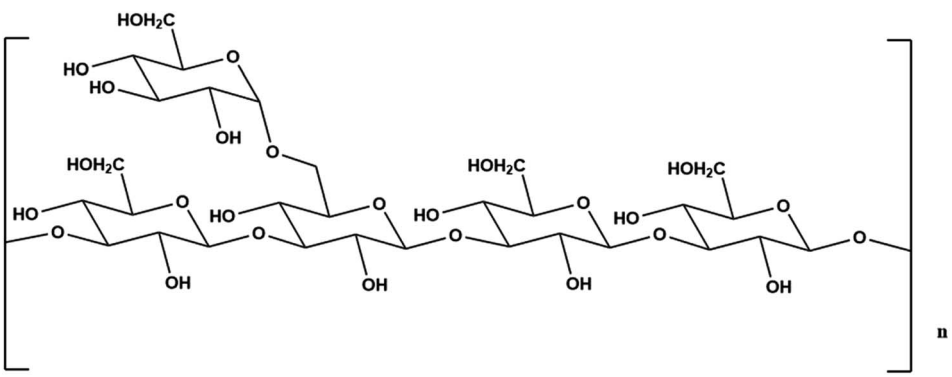

Fig. 1 The structure of an alkali-soluble *P. cocos* polysaccharide.

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Table 1 Advantages and disadvantages of different *P. cocos* polysaccharide extraction methods[a]

Extraction methods		Advantages	Disadvantages	Extraction rate	Reference
Solvent method	Water extraction method	Low cost and easy to operate	Long extraction times and low yields	5.40%	35
	Alkaline extraction	High polysaccharide yield and low cost, easy to operate	Alkali may disrupt polysaccharide structure	—	36
Physically assisted extraction method	Ultrasound-assisted extraction	Simple operation, fast extraction rate and good applicability	May disrupt polysaccharide structure, leading to unpredictable loss of activity	1.38%	1
	Microwave-assisted extraction	Short extraction time, low power consumption and high polysaccharide activity obtained	May disrupt polysaccharide structure, leading to unpredictable loss of activity	9.95%	37
	SCF extraction	Low temperature, high efficiency and high penetration	Costly and cumbersome to operate	3.209%	38
Enzyme-assisted extraction		High yield, easy to operate and green	Expensive, enzymes have the potential to degrade polysaccharides	12.8%	39
DES extraction		Low cost, simple preparation, biodegradable, well-defined structure and low toxicity	May lead to disruption of the complex structure of polysaccharides and some DESs with high viscosity and toxicity	46.24%	35 and 40

[a] Annotation: supercritical fluid (SCF); deep eutectic solvent (DES); not detected (—).

is low. In addition, the high temperature brought by prolonged extraction may have an irreversible effect on the polysaccharide structure, and this method has been gradually replaced by new milder and more efficient extraction methods.

2.1.2 Alkaline extraction. Extraction with alkaline water gives more PCPs than using water or ethanol as extractant. The experimental procedure was to first grind *P. cocos* into powder, extract it with alkaline solution, neutralize it after extraction and dry it with ethanol to obtain crude PCPs. Chen *et al.*[36] selected NaOH solution as the extractant and used ultrasound to assist with the extraction of PCPs. The optimal extraction process parameters obtained by the response surface methodology (RSM) method are as follows: extraction time of 2.44 min, NaOH concentration of 0.789 mol L^{-1}, and ratio of NaOH aqueous solution to raw material of 53 : 1, giving a PCP yield of 82.3%.[36] The advantage is that the experiments can be carried out at low temperatures with higher yields. The disadvantage is that alkaline and high-temperature environments may destroy the structure of PCPs, and care must be taken when using this method.

2.2 Physically assisted extraction method

2.2.1 Ultrasound-assisted extraction. Since the application of ultrasound may enhance mass transfer, injure cells, improve permeability, and improve capillary action, it is frequently used for polysaccharide extraction.[50] Chen *et al.*[1] used an orthogonal design to optimize the ultrasound-assisted PCP extraction process. At ideal extraction conditions (extraction time of 75 min, extraction size of 70 mesh, and extraction temperature of 90 °C), PCP yield was 1.38%.[1] The advantages are that the ultrasonic extraction technique is short and requires less solvent. The disadvantage is that the structure of polysaccharides extracted by the ultrasonic extraction technique is often destroyed during the process, resulting in an unexpected loss of polysaccharide activity. This is why ultrasonic extraction techniques are often utilized to extract polysaccharides for research purposes requiring strict control of experimental conditions to prevent impacts on yield.

2.2.2 Microwave-assisted extraction. Target compound extraction is facilitated by microwave extraction because it breaks down hydrogen bonds and enhances dissolved ion migration, increasing solvent penetration into the matrix.[51] Wang *et al.*[37] looked into the way various extraction techniques affected PCP antioxidant activity, and in order to retain the maximum antioxidant activity, microwave-assisted extraction is the best choice for extracting PCPs. The yield of microwave extraction of PCPs in their experiments was 9.95%.[37] Zhao *et al.*[52] optimized the optimal process for microwave extraction of PCP as follows: temperature at 80 °C, extraction for 40 min, solid/solvent ratio 1 : 30, microwave power 600 W. Under these conditions, the average yield of PCP was 7.22%, which was not only much higher than that of the conventional reflux extraction, but the time was also substantially decreased.[52] This extraction method is rapid and the polysaccharides obtained are highly active; the downside is low yield. In addition, microwaves may cause structural damage to polysaccharides, leading to loss of their biological activity.

2.2.3 Supercritical fluid (SCF) extraction. SCF extraction, as a common method for extracting active substances from natural products, has also been applied to the extraction of fungal polysaccharides such as PCPs.[53] SCF extraction method involves manipulating the fluid's temperature and pressure at the critical point of its solvency capacity to extract, separate, and purify the material. This method often uses CO_2 as the solvent. It offers several benefits, including easy operation, safe and pollution-free product, quick extraction rate, and fast extraction

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speed. But the operation is cumbersome, and the running cost of the experiment is high, so it is less utilized.[38]

2.3 Enzyme-assisted extraction

The enzymatic extraction method still relies on the use of appropriate enzymes to disrupt the structure of the *P. cocos* mycelium and increase the solubility of the polysaccharides in the solvent. It is the focus of current research on polysaccharide extraction methods.[54] Tang *et al.*[39] isolated β-glucosidase enzyme from fermented *Aspergillus niger* HS-5 using an enzymatic extraction method; it was used to assist in the extraction of PCPs. Response surface methods and single-factor trials were used to identify the ideal enzymatic hydrolysis conditions for PCPs, as follows: hydrolysis temperature of 60 °C, time of 120 minutes, pH of 5.0, and volume of 20 mL. Under these conditions, the extraction rate of PCP can reach up to 12.8%.[39] Khaskheli *et al.*[55] used an enzyme extraction method to extract PCP. They found that a liquid/feed ratio of 1 : 50, temperature of 40 °C, time of 3.0 hours, and pH of 5 were the ideal extraction parameters. The maximum percentage of crude polysaccharides extracted was 4.14%.[55] Compared with the traditional extraction technology, it has the advantages of high extraction rate, good extraction activity, low energy consumption and investment cost, high reproducibility in a short period of time, simple operation and green environmental protection. However, enzymes may still degrade polysaccharides during the extraction process, reducing the yield and affecting the results. In addition, the high cost of the enzyme extraction method is also a problem, making it unsuitable for large-scale production of PCPs.[56]

2.4 Deep eutectic solvent (DES) extraction

Polysaccharides have a number of drawbacks as a result of their typical solvent extraction process, including low extraction rates, structural degradation, toxicity toward experimenters, and the operational environment. To address these issues, a novel solvent is becoming more and more necessary. DESs are very beneficial for PCP extraction, which is facilitated by linking hydrogen bonded acceptors and donors. Zhang *et al.*[35] used DESs, a novel green extractant, to successfully extract PCPs. They discovered that a molar ratio of 1 : 2 between choline and oxalic acid, an extraction temperature of 100 °C, and a 15 minutes extraction period were the optimal extraction conditions. After six cycles, the PCP extraction rate was 38.40%, indicating that DESs can be reused and have commercial value. The yield was significantly higher than that of the traditional hot water extraction, reaching as high as 46.24%. The outcomes of the experiment demonstrated that DESs are efficient solvents for extracting polysaccharides from *P. cocos*.[35] Guo *et al.*[40] employed the novel ternary DESs. The polysaccharide yield of this method was 55.02% under the optimized conditions of 300 mesh sieve, 30 mL g^{-1} liquid–solid ratio, 300 W mean fluorescence intensity ratio (MFIR) and 80 °C circulating air temperature. The yield was significantly higher compared to the traditional extraction of PCPs with hot water solvent and alkaline water.[40] DESs can be regarded as the best method for PCP extraction at present due to its low cost, simple preparation, definable structure and biodegradability. The disadvantage is that DESs may also lead to the disruption of the complex structure of polysaccharides, resulting in macromolecular decomposition, and should be handled with caution.[44]

3. Structure, structure–activity relationship and techniques for the structural analysis of PCPs

The structure of polysaccharides is strongly associated with their biological activity.[57] Structural research is significant for understanding the bioactive expression process and conformational relationships of polysaccharides. PCPs, considered complex macromolecules in chemical research, are difficult to finely obtain at multiple levels of their structure.[58] As a heteropolysaccharide, PCPs comprise a blend of many types of polysaccharides, and distinct polysaccharide fractions can be produced by employing various extraction techniques.[59] Based on the literature, it is inferred that the main component of PCPs is a water-insoluble polysaccharide that is a linear (1 → 3)-β-D-glucan,[52] which acts as a nonspecific bioregulator and contains approximately more than 700 β-(1 → 3) glycosidic bonds.[60,61] PCPs are mainly composed of ribose (Rib), arabinose (Ara), xylose (Xyl), mannose (Man), glucose (Glc) and galactose (Gal), of which Glc has the highest content, followed by Man content.[62,63] Their monosaccharide component is mainly D-glucose. β-Glucan is the primary component of PCPs.[64] Its structure has a β-(1 → 3)-linked glucose main chain and β-(1 → 6)-linked glucose side chains.[12] At present, the research on the structure of PCPs is almost exclusively concerned with their molecular mass and monosaccharide composition, and their structural parameters and spatial configurations, such as functional groups and glycosidic bonding, need to be further investigated.[52]

The chain conformation has an effect on the activity of PCPs. Many β-glucans in nature have a triple helix conformation, such as lentinan and split-leaf polysaccharides.[65] Generally speaking, when polysaccharides have a triple-helical structure, their antitumor activity is stronger compared to other conformations.[66] However, the β-glucan in *P. cocos* does not have a triple helix conformation, but exists in an irregular line group conformation, resulting in the aggregation of polysaccharides with relatively large molecular weights and that are insoluble in water. These characteristics make it difficult for PCPs to express biological activity. Researchers found that carboxymethyl *P. cocos* polysaccharides (CMP) with triple-helical conformation showed good immunomodulatory ability and hypothesized that altering the structure of alkali-soluble PCPs could lead to better pharmacological activity.[67] Huang *et al.*[68] found that a semi-rigid chain form had better anti-cancer activity compared to its natural counterpart, which has a flexible chain.[68]

Relative molecular mass has a significant effect on the activity of PCPs. Some researchers have found that PCPs with relatively small molecular weight show better biological activity and speculate that because of their relatively small molecular

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weight, they can more easily enter cells and express activity. Zhang *et al.*[69] found that PCPs with a relatively low molecular mass were more capable of exhibiting better anti-tumor activity *in vitro*.[69] Tang *et al.*[48] degraded alkali-soluble PCPs to obtain degraded polysaccharides with low relative molecular mass, increased solubility and improved antioxidant activity.[48]

Past studies have shown that the primary structures of PCPs are better understood through existing techniques. Most experimenters have chosen methylation analysis (MA), high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), ion chromatography (IC), gel permeation chromatography (GPC), infrared spectroscopy (IR) and other methods to analyze the primary structure of PCPs.[70] However, due to the complex structure of polysaccharides, it is difficult to analyze the high-level structure of PCPs by existing analytical techniques, which affects the functional understanding of PCPs. Referring to the analytical methods of other polysaccharides, it is hypothesized that the advanced structure of polysaccharides can be investigated using atomic force microscopy (AFM) in subsequent experiments.[71] Based on the above methods of polysaccharide analysis, a variety of PCPs were found to have different structures, some of which were characterized as shown in Table 2. Regarding the determination of PCP content, most experimenters chose the phenol-sulfuric acid method.

4. Application of PCPs

The application of PCPs is still mainly related to the medical industry, with a small number of applications in the aquaculture industry.[6,83–86] PCPs have rich and effective pharmacological activities on the one hand, and on the other hand, they have the advantages of being nontoxic, easy to chemically modify, and chemically stable; they can be made into a series of medicines, vaccines, and other healthcare products; they can also be used as a drug carrier material, such as nanoparticles and hydrogels.

4.1 Application of PCPs as drugs

Among today's treatments, the application of polysaccharides, a natural substance, as a form of medication is seen as a very promising therapeutic approach.[87] The natural fungal polysaccharides derived from *P. cocos*, PCPs and their derivatives, contain intricate structures and a variety of biological functions. They are used in many different medical applications, including as immune modulators, antioxidants, antibacterial agents, and anticancer medication. However, the clinical application of PCPs is still uncommon; it is mainly regarded as an anticancer drug and an immunomodulator. Nowadays, in China, a Chinese patented medicine based on PCPs[88] known as "*Poria cocos* polysaccharides oral liquid" is used as a health supplement that can strengthen the spleen, invigorate qi, and prevent aging. It promotes tumor cell apoptosis and inhibits tumor growth by decreasing the content of Bcl-2 protein and increasing the content of caspase-3 and caspase-9 protein.[88] Fucose-containing mannoglucan polysaccharide (FMGP) in *P. cocos* greatly inhibits the migration of human lung cancer cell line CL1-5 cells by reducing TGFβRI production and FAK and AKT activation. This implies that FMGP may find application in the production of anticancer medications.[89]

Additionally, PCPs have been demonstrated efficacious in the treatment of certain inflammatory diseases, including enteritis and prostatitis. Through an investigation into PCPs' mechanism of action in treating chronic nonbacterial prostatitis (CNP), it was discovered that PCPs can treat CNP through a number of different mechanisms, including the improvement of histologic damage in the inflammatory prostate, inhibition of TNF-α, IL-2, IL-8, and androgen production, and modulation of the intestinal microbiota.[88] In particular, modulation of the gut microbiota provides a therapeutic target for the treatment of CNP.[6] PCPs inhibit mRNA expression of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and by regulating the intestinal microbiota, PCPs are protective against colitis.[90]

Oral administration of water-insoluble PCPs to mice with alcoholic hepatic steatosis (ALD) effectively ameliorated inflammatory damage and fat accumulation by modulating the gut microbiota and inhibiting ethanol-induced fungal overgrowth, and the results of the above experiments suggest that it is an effective treatment for and alleviates ALD.[91] Studies have shown that PCPs can prevent atherosclerosis (AS) by inhibiting the elevation of serum inflammatory mediators and lipid levels in high-fat mice, and it is speculated that PCPs can be applied to future drugs for the prevention and treatment of atherosclerosis.[27] PCPs exhibit potential for the treatment of depression in the pharmaceutical industry by inhibiting ConA-stimulated T cell proliferation and suppressing LPS-induced B cell proliferation.[19]

In comparison to veterinary rabies vaccine adjuvant aldehyde hydrogel, PCP-II in combination with rCVS-11-G enhances and promotes the elevation of virus-neutralizing antibody (VNA) titers in mice. Moreover, PCP-II activated multiple immune cells in the lymph nodes and blood, and induced proliferation of splenic T-lymphocytes and high levels of cytokine secretion from splenic cells. These results indicate that PCP-II has good adjuvant activity and is an excellent candidate for inactivated rabies vaccine.[92] PCP-II increased the level of antigen-specific antibodies in mice immunized with influenza vaccine and significantly increased the titer of anti-hepatitis B surface antigen (HBsAg) antibodies and produced stronger and longer-lasting immunity. The experimental results indicate that PCP-II is an effective vaccine adjuvant worthy of research and development.[93]

In terms of application, PCPs not only rely on their own good drug activity to make drugs and functional foods, but studies have also shown that they can be used in combination with other drugs to play a better role. It is assumed that the synergistic effect with other drugs changes their structural characteristics to increase their activity. Low amounts of lotus root oligomeric procyanidins (LSPC) showed significant synergistic effects with CMP to modulate antimicrobial activity. It is hypothesized that the mixture can be applied as a natural antimicrobial agent.[94] This offers fresh concepts for using PCPs in pharmaceutical development.

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Table 2 Structural characteristics of *P. cocos* polysaccharides[a]

Compound name	Extraction method	Methods of analysis	Carbohydrate composition	Molecular weight (kDa)	Structural characteristic	Reference
ac-PCM0	—	IR, NMR, GC	Man : Gal : Glc : Fuc : Ara = 43.00 : 27.40 : 27.20 : 1.4 : 1.00	32.9	—	69
PCP-1	DES extraction	GC-MS, HPGPC, FT-IR, NMR	Glc	3.2	(1 → 3)-β-D-glucan	44
PCP-II	Water extraction	HPGPC, CE	Fuc : Man : Glc : Gal = 10.00 : 16.30 : 1.60 : 62.90	29.0	—	72
PCP-E	Enzyme-assisted extraction	HPLC, FT-IR, GPC	Man : Gal : Glc : Ara = 1.98 : 0.36 : 81.72 : 15.93	10.6	—	37
PCP-U	Ultrasound-assisted extraction		Man : Gal : Glc : Ara = 2.18 : 2.36 : 87.27 : 8.18	21.2	—	
PCP-H	Hot water extraction		Man : Gal : Glc : Ara = 0.92 : 0.18 : 86.88 : 12.01	21.5	—	
PCP-M	Microwave-assisted extraction		Man : Gal : Glc : Ara = 4.02 : 4.93 : 79.48 : 11.57	15.1	—	
PCP-1	Alkali extraction	HPSEC, HPLC, IR	Ara : Glc = 0.02 : 1.00	2.33	Degraded polysaccharides	48
PCP-2			Ara : Glc = 0.01 : 1.00	3.20	Degraded polysaccharides	
PCP-3			Ara : Glc = 0.03 : 1.00	2.85	Degraded polysaccharides	
PCP	Hot water extraction	GC-MS, FT-IR	Rib : Ara : Xyl : Man : Glc : Gal = 1.49 : 1.17 : 0.62 : 10.34 : 86.39 : 1.31	—	(1 → 3)-β-D-glucan	62
PCP-1	Hot water extraction	—	Fuc : Man : Glc : Gal = 1.00 : 1.81 : 0.27 : 7.27	30	—	73
EPS-0M	Hot water extraction	HPSEC, UV, FT-IR, SEM, IC	Glc : Man : Gal : Fuc : Rha = 17.30 : 46.30 : 19.90 : 8.70 : 5.00	1.77	—	59
EPS-0.1M			Glc : Man : Gal : Fuc : Rha = 11.50 : 46.50 : 21.90 : 10.70 : 5.60	2.01	—	
IPS-0M			Glc : Man : Gal : Fuc : Rha = 79.70 : 8.90 : 5.50 : 1.70 : 3.10	0.03	—	
IPS-0.1M			Glc : Man : Gal : Fuc : Rha = 50.30 : 20.90 : 16.10 : 6.00 : 4.00	4.97	—	
PCAPS1	Alkali extraction	HPGPC, FT-IR, GC-MS, NMR	Glc : Man : Gal : Xyl : Fuc = 44.80 : 28.70 : 12.20 : 7.70 : 6.20	11.5	1,3-Linked D-glucan backbone with 1,4-residue and 1,6-residue branches	74
PCPP-1A	—	—	Man : GA : Glc : Gal : Fuc = 10.93 : 1.00 : 235.76 : 26.99 : 6.56	45.38	—	14
PCP	—	IC	Fuc : Ara : D-glucosamine hydrochloride : Gal : Glc : Xyl : Man = 1.00 : 1.20 : 2.50 : 5.40 : 83.90 : 1.80 : 4.20	—	—	75
PCP	Hot water extraction	HPGPC, HPLC, FT-IR, SEM, UV-vis	Man : Glc : Gal : Fuc = 13.50 : 33.00 : 40.30 : 13.20	18.67	1,6-α-D-Galp, 1,2,6-α-D-Glcp, and T-α-D-Manp	76
SEPCP			Man : Glc : Gal : Fuc = 2.10 : 90.30 : 5.80 : 1.80	6.52	1,3-β-D-Glcp backbone and T-β-D-Glcp	
PCP	Hot water extraction	IC, SEM, AFM, FT-IR, GC-MS	Man : D-glucosamine hydrochloride : Glc : Gal : Fuc = 15.31 : 0.97 : 28.72 : 31.63 : 23.37	11.583	may be t-Gal(p), 6-Gal(p) and 2,6-Gal(p)	77
Pi-PCM0	—	FT-IR, NMR, GC	Ara : Xyl : Man : Gal : Glc = 2.50 : 1.50 : 70.60 : 18.50 : 7.00	64.6	(1,3)-α-D-glucan	78
Pi-PCM1	0.9% NaCl extraction		Fuc : Ara : Xy : Man : Gal : Glc = 10.90 : 1.00 : 2.80 : 23.60 : 36.50 : 25.20	304	(1,3)-α-D-glucan	
Pi-PCM2	Hot water extraction		Fuc : Man : Gal : Glc = 1.90 : 29.60 : 38.90 : 29.70	1030	β-D-galactofuranan, (1 → 3)-α-D-glucan and mannan	
Pi-PCM3-I	0.5 M NaOH		Glc	149	Linear (1,3)-α-D-glucan	
Pi-PCM3-II	extraction			452	(1,3)-α-D-glucan	

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Table 2 (*Contd.*)

Compound name	Extraction method	Methods of analysis	Carbohydrate composition	Molecular weight (kDa)	Structural characteristic	Reference
			Man : Gal : Glc = 10.90 : 21.00 : 68.10			
Pi-PCM4-I	88% formic acid extraction		Glc	2010	Linear (1,3)-α-D-glucan	
Pi-PCM4-II			Gal : Glc = 45.60 : 54.40	4360	(1,3)-β-D-glucopyranoside and β-D-galactofuranoside	
PCS0	Alkaline extraction	SEC, GC-MS	Glc	251	(1, 3)-β-D-glucans	79
PCS10			Glc	104	(1, 3)-β-D-glucans	
PCS90			Glc	43	(1, 3)-β-D-glucans	
PC-I	—	NMR	Myo-inositol : Sorbitol : Fuc : galactosamine : glucosamine : Gal : Glc : Man = :13.80 : 6.29 : 35.60 : 55.90 : 67.90 : 10 : 47.20 : 77.4	610.7	—	80
PC-II			Myo-inositol : Sorbitol : Fuc : GS : Gal : Glc : Man = 6.26 : 3.14 : 44.40 : 3.94 : 159.00 : 0.94 : 16.50	40.7	1,3-α-D-Gal main skeleton and 1,6-α-D-Gal side chains	
PC-III			—	7.9	—	
PC-IV			Myo-inositol : Fuc : Gal : Glc : Man = 9.98 : 33.10 : 35.10 : 3.69 : 3.80	1.6	—	
PC-V			Myo-inositol : Sorbitol : Fuc : galactosamine : glucosamine : Gal : Glc : Man = 18.50 : 0.67 : 17.80 : 8.22 : 6.31 : 11.00 : 2.25 : 5.36	0.3	—	
PCWPW	Hot water extraction	HPGPC, IR, HPLC	Man : Glc : Gal : Fuc = 36.90 : 7.30 : 40.48 : 15.33	37.154	—	19
PCWPS			Man : Glc : Gal : Fuc = 30.07 : 16.60 : 41.47 : 10.10	186.209	—	
IDF	—	GC, SEM	Glc : glucosamine : uronic acid = 86.80 : 1.53 : 1.95	—	—	81
SDF			Glc : glucosamine : Man : Gal : uronic acid = 6.71 : 1.01 : 13.10 : 5.31 : 1.27	—	—	
wb-PCM0	—	FT-IR, SEC-LLS, LLS, NMR, GC	Ara : Xyl : Man : Gal : Glc = 6.10 : 3.90 : 11.40 : 5.90 : 71.70	144	Mainly (1 → 3)-α-D-glucans, containing other sugar units such as β-linked D-mannose and β-linked D-galactose	34
wb-PCM1	0.9% NaCl extraction		Man : Gal : Glc = 7.70 : 19.20 : 73.10	333	—	
wb-PCM2	Hot water extraction		Man : Gal : Glc = 0.90 : 1.30 : 95.90	—	—	
wb-PCM3-I	0.5 M NaOH extraction		Xyl : Man : Glc = 1.00 : 2.20 : 95.60	231	(1 → 3)-α-D-glucans	
wb-PCM3-II			Ara : Xyl : Man : Gal : Glc = 2.60 : 2.00 : 1.20 : 2.00 : 91.40	113	(1,3)-β-D-glucan	
wb-PCM4-I	88% formic acid extraction		Man : Glc = 5.80 : 94.10	1323	(1,3)-β-D-glucan	
wb-PCM4-II			Gal : Glc = 23.90 : 76.10	1211	(1,3)-β-D-glucan	
wc-PCM0	—		Fuc : Ara : Xyl : Man : Gal : Glc = 4.10 : 3.00 : 2.50 : 61.70 : 15.00 : 13.70	92	Mainly comprised of α-D-mannose residues with branching	
wc-PCM1	0.9% NaCl extraction		Fuc : Man : Gal : Glc = 10.50 : 24.50 : 27.50 : 37.50	262	—	
wc-PCM2	Hot water extraction		Fuc : Man : Gal : Glc = 3.40 : 12.50 : 13.40 : 70.70	892	—	
wc-PCM3-I	0.5 M NaOH extraction		Xyl : Man : Glc = 6.40 : 16.70 : 76.90	545	(1 → 3)-α-D-glucans	
wc-PCM3-II			Glc	89	(1,3)-β-D-glucan	
wc-PCM4-I	88% formic acid extraction		—	—	(1,3)-β-D-glucan	
wc-PCM4-II			—	—	(1,3)-β-D-glucan	

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Table 2 (*Contd.*)

Compound name	Extraction method	Methods of analysis	Carbohydrate composition	Molecular weight (kDa)	Structural characteristic	Reference
wc-PCM0	Hot water extraction	FT-IR, NMR, SEC-LLS, GC	Fuc : Ara : Xyl : Man : Gal : Glc = 4.10 : 3.00 : 2.50 : 61.70 : 15.00 : 13.70	92	—	82
wc-PCM1			Fuc : Man : Gal : Glc = 10.50 : 24.50 : 27.50 : 37.50	262	—	
wc-PCM2			Fuc : Man : Gal : Glc = 3.40 : 12.50 : 13.40 : 70.70	892	(1 → 3)-α-D-glucans	
wb-PCM0			Ara : Xyl : Man : Gal : Glc = 6.10 : 3.90 : 11.40 : 5.90 : 71.70	144	—	
wb-PCM1			Man : Gal : Glc = 7.70 : 19.20 : 73.10	333	—	
wb-PCM2			Man : Gal : Glc = 0.90 : 1.30 : 95.90	—	(1 → 3)-α-D-glucans	
ac-PCM0			Fuc : Ara : Man : Gal : Glc = 1.40 : 1.00 : 43.00 : 27.40 : 27.20	101	—	
ac-PCM1			Fuc : Man : Gal : Glc = 4.50 : 15.80 : 23.90 : 53.40	125	—	
ac-PCM2			Fuc : Man : Gal : Glc = 0.80 : 19.10 : 29.70 : 51.40	170	—	
ab-PCM0			Ara : Xyl : Man : Gal : Glc = 9.20 : 11.10 : 21.50 : 12.70 : 45.40	93	—	
ab-PCM1			Fuc : Ara : Xyl : Man : Gal : Glc = 7.90 : 4.00 : 2.60 : 10.50 : 27.60 : 47.30	57	—	
ab-PCM2			Man : Gal : Glc = 5.60 : 13.10 : 81.20	—	—	

[a] Methylation analysis (MA); high-performance liquid chromatography (HPLC); nuclear magnetic resonance (NMR); ion chromatography (IC); gel permeation chromatography (GPC); infrared spectroscopy (IR); gas chromatography (GS); high-performance gel permeation chromatography (HPGPC); atomic force microscopy (AFM); capillary electrophoresis (CE); Fourier transform infrared spectroscopy (FT-IR); high-performance size exclusion chromatography (HPSEC); gas chromatography-mass spectrometry (GC-MS); ultraviolet spectra (UV); scanning electron microscope (SEM); gas chromatography (GC); size exclusion chromatograph (SEC); laser light scattering (LLS); size exclusion chromatography-laser light scattering (SEC-LLS); ribose (Rib); arabinose (Ara); xylose (Xyl); mannose (Man); glucose (Glc); galactose (Gal); not detected (/).

4.2 The application of PCPs as a drug carrier

Drug carriers are capable of altering the distribution of drugs in the body, controlling the rate of drug release, and delivering drugs to target organs.[95] Since they are a naturally occurring polymer with stable chemical properties, facile chemical modification, and inexpensive processing costs, polysaccharides are frequently employed in the creation of tailored drug carriers.[96] Polysaccharides play a significant role in drug delivery systems because of their biocompatibility, which allows drug molecules to be encapsulated in their interstitial spaces to provide controlled release of cargo drug molecules.[85] The release kinetics of PCP-based nanoparticles, which were most similar to the Higuchi model, indicated diffusion-controlled release of SA (salicylic acid) in phosphate buffer (pH = 7.4). The experimental results suggested that PCPs have promising applications in the field of nanocarriers.[97] When polysaccharides are used in the manufacture of pharmaceutical carriers, they have special physical and chemical properties such as high osmotic pressure, high viscosity, and water absorption, and they easily form gels.[87] A hydrophilic spherical cellulose-based hydrogel was prepared by cross-linking water-soluble carboxymethyl pachyman (CMP) with biocompatible and biodegradable epichlorohydrin (ECH), which can be used to release model protein drugs in a controllable manner and to ensure the stability and activity of protein drugs. The experimental results showed that the substance could be used as a carrier for the delivery of protein drugs.[98]

4.3 Application of PCPs as feed additives

PCPs can be used as a feed additive in animal husbandry to treat livestock diseases, improve livestock growth and reproduction, and increase productivity.[99] PCPs have a variety of potent pharmacological activities and can be added as a feed probiotic with the aim of enhancing host growth and immune function. PCPs used as pig feed additive significantly and linearly improved the growth of weaned piglets, and also effectively improved the cellular immunity of weaned piglets and regulated and improved the microbiota in their cecum. The above

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results demonstrate the potential of PCPs as a feed additive in the pig industry.[100] PCPs were also used as a feed additive for spotted sea bass, which increased fish growth and antioxidant capacity, but they had less of an impact on the fish's lipid metabolism and nonspecific immunity. The ideal PCP addition level for spotted sea bass feed was 1.4 g kg^{-1}. For aquaculture to improve the metabolic problems and control diseases in aquatic animals, PCP adoption as a food supplement is essential. This implies that there are potential benefits to using PCP in spotted sea bass aquaculture.[101]

4.4 Application of PCPs as a food packaging material

Compared with traditional plastic packaging materials, new packaging materials developed based on polysaccharides are more environmentally friendly in terms of reducing dependence on fossil fuels and carbon emissions, due to the fact that polysaccharides, as polymeric carbohydrates, have the advantages of being nontoxic to organisms and compatible, as well as being biodegradable.[102] The PCP-preparation of silver nanoparticles (AgNPs)/chitosan (CS) membranes fabricated from PCPs became more regular and more dense due to noncovalent interactions and significantly maintained the quality and prolonged the shelf life of strawberries. Moreover, the membrane is not toxic to the neighboring. The above results indicate that the PCP-AgNPs/CS films are environmentally friendly and have the potential to be used in current packaging materials.[103]

5. Conclusion and prospects

PCPs are the main active ingredient in *P. cocos*. Years of research have shown that the extraction and purification of PCPs still face difficulties and low yields, and further improvements are needed by researchers. The most recent method related to the extraction of PCPs is the DES extraction method, which deserves continued research and improvement due to its high efficiency in extracting PCPs and its nontoxicity and ease of use, *etc.*, among other advantages. Most of the purification methods for PCPs use column chromatography. Most of the information about the structure of PCPs remains on the molecular weight and monosaccharide composition of the polysaccharides. The advanced structures of PCPs still need to be studied. This may require innovations in polysaccharide structure analysis techniques to be realized. Future studies on PCPs should use AFM or NMR to study the advanced structures. It is hoped that more studies on the advanced structures of PCPs will be carried out in the future to help understand the link between structure and activity of PCPs and to increase the application of PCPs. Currently, PCPs are used as drugs (vaccine, clinical drug, and nutraceutical), functional food, drug carriers, feed additives and in other applications. It is hoped that there will be more clinical applications involving PCPs.

Data availability

Data sharing is not applicable to this article, as no datasets were generated or analysed during the current study.

Conflicts of interest

There are no conflicts to declare.

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